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Ecophysiology of introduced *Impatiens* species

A thesis submitted for the degree of Doctor of Philosophy

2011

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DECLARATION

This thesis is a record of my original work and has not been accepted in any previous application for a degree. All sources of information have been appropriately acknowledged.

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SUMMARY

This thesis aims to improve knowledge of invasion processes through investigation of the invasive strategies of congeneric *Impatiens* species that vary in invasive status in Ireland. Since a range of physiological traits promote plant fitness, many studies have attempted to compare these traits among invasive and non-invasive species. Meta-analysis and desk-based reviews offer the advantage of highlighting which traits are consistently associated with plant invasiveness. However, comparisons among studies are not straightforward due to the heterogeneity of the approaches used by different authors. In addition, many comparisons have so far chosen unrelated species, a method that is potentially confounded by phylogenetic differences. The most direct approach to identifying the determinants of invasiveness appears to be to make comparisons between related invasive and non-invasive, or less invasive, introduced species.

Knowledge of the invaders' reproductive biology is important when attempting to manage them since it determines the continued survival of invasive populations even after eradication of the mother plants. I compared germination rates in different environmental conditions among three *Impatiens*: the invasive *I. glandulifera*; the naturalized *I. parviflora*; the casual *I. balfourii*. Greater seed mass and seed production rates, lower mortality, earlier germination, a shorter stratification period and the capacity to germinate under a wider range of conditions are factors that may contribute to the greater invasiveness of *I. glandulifera*. Similarly, lower seed mass and seed production rate, and higher mortality could contribute to explaining the lack of success of *I. balfourii* as an invader. The lack of success of *I. parviflora* (which has a very limited distribution in Ireland but which is invasive in central and eastern Europe) may be due, in terms of germination, to its requiring a longer and colder stratification period in order to break dormancy.

This thesis further explored the ecophysiology of these three introduced species. In particular, I assessed and compared traits that have been frequently suggested as contributing to plants' invasiveness. From the characterisation of traits in a common high-resource environment, it emerged that *I. glandulifera* and *I. balfourii* have similar ecological needs. They also showed similar leaf-morphological and photosynthetic traits. I then assessed and compared these two species in terms of traits related to growth, biomass allocation, leaf morphology, photosynthesis and efficiency, and in terms of plasticity: in one case, growing seedlings under two different light intensities; in another, growing plants under three different water regimes. Across the set of experiments, the invasive *I. glandulifera* showed consistently better performances than *I. balfourii* for the growth-related traits. I observed less consistency in terms of the leaf-morphological, photosynthetic and use-efficiency traits, and in terms of plasticity. These characteristics were generally not significantly different between species, or were even found to be inferior in the invader.

Finally, I assessed the potential for hybridization between *I. glandulifera* and *I. balfourii*. The need for investigation into the potential for this cross is due to the fact that hybridization is suggested as a factor that may enhance invasiveness. The possibility of natural pollen transfer between the two species exists since they co-occur, the flowering time overlaps, and the pollinators switch from one species to the other. Moreover, hybridizations within the genus *Impatiens* are reported to occur between wild populations. I found that the heterospecific crosses produced seeds. However, even if the possibility of hybridization is excluded (due to the lack of germination of the hybrids), the fact that the heterospecific cross produced seeds represents the possible presence of incomplete reproductive barriers.

The results of this thesis highlight traits, such as reproductive capacity and growth characteristics, which are possibly involved in determining the greater invasive capacity of *I. glandulifera*. However, the possibility of *I. balfourii* also becoming invasive in Ireland cannot be excluded. This species showed a very high germination rate in some of the experimental conditions, suggesting that, in the right environments, it has great reproductive potential. In addition, on the basis of its ecophysiological characteristics, it could become invasive, in particular in disturbed high-light environments. Under climate warming, *I. balfourii* could possibly experience the favourable conditions required to invade cooler regions such as Ireland as well as more northern regions of Europe.

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To my family

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To my family

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ABBREVIATIONS

A	Net photosynthetic rate	(µmol CO_2 $m^{-2}s^{-1}$)
A_{max}	Light saturated photosynthetic rate	(µmol CO_2 $m^{-2}s^{-1}$)
BxB	Homospecific artificial pollination of *Impatiens balfourii*	
BxG	Heterospecific artificial pollination where *I. balfourii* is the mother and *I. glandulifera* the pollen donor	
Bx0	Pollinator exclusion in *I. balfourii*	
CO_2	Carbon dioxide	(µmol)
ET	Transpiration rate	(mmol $m^{-2}s^{-1}$)
GxB	Heterospecific artificial pollination where *I. glandulifera* is the mother and *I. balfourii* the pollen donor	
GxG	Homospecific artificial pollination of *I. glandulifera*	
Gh	Height growth rate	(cm day^{-1})
GS	Stomatal conductance	(mmol $m^{-2}s^{-1}$)
Gx0	Pollinator exclusion in *I. glandulifera*	
LAR	Leaf area ratio	(m^2 leaf g^{-1} plant)
LA:RM	Leaf area:root mass ratio	(m^2 leaf g^{-1} root)
LCP	Light compensation point	(µmol photon $m^{-2}s^{-1}$)
LSP	Light saturation point	(µmol photon $m^{-2}s^{-1}$)
LWR	Leaf weight ratio	(g leaf g^{-1} plant)
PAR	Photosynthetically active radiation	(µmol photon $m^{-2}s^{-1}$)
Rd	Dark respiration	(µmol CO_2 $m^{-2}s^{-1}$)
RDPI	Relative distance plasticity index	
RE	Respiration efficiency	
RGR	Relative growth rate	(gg^{-1} $week^{-1}$)
RWR	Root weight ratio	(g root g^{-1} plant)
SLA	Specific leaf area	(m^2 leaf g^{-1} leaf)
SWR	Stem weight ratio	(g stem g^{-1} plant)
WUE	Water use efficiency	(µmol mol^{-1})
Ψ_{Leaf}	Leaf water potential	(MPa)
ϕ	Apparent quantum yield	(µmol CO_2 µmol^{-1} PAR)

1 General introduction

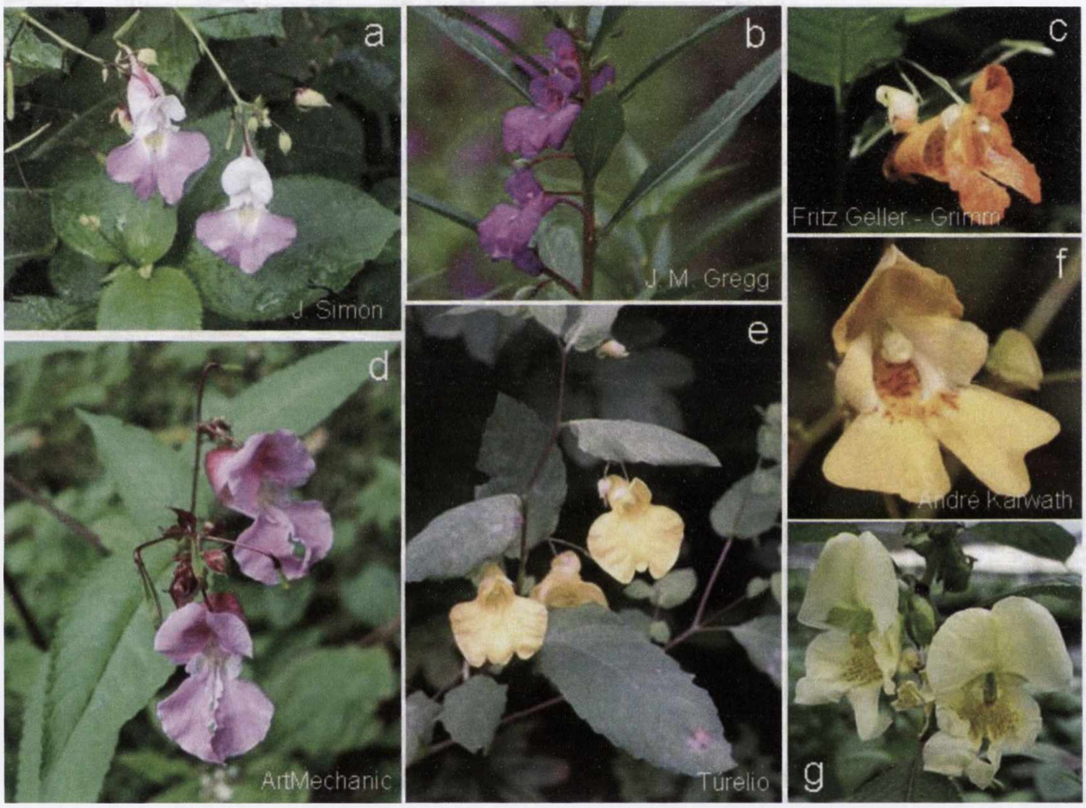

Plate 1.1 Flowers of the *Impatiens* species present in Europe. *I balfourii* (a), *I. balsamina* (b), *I. capensis* (c), *I. glandulifera* (d), *I. noli-tangere* (e), *I. parviflora* (f) and *I. scarbida* (g).

1.1 INTRODUCTION

Human activities are responsible for the introduction of many organisms which have invaded and continue to invade new regions (Vitousek *et al.*, 1997; Sala *et al.*, 2000; Davis, 2003). It is intriguing that only a small proportion of the introduced organisms are able to persist and subsequently spread in their new environment. What makes a species invasive is a critical question in ecology. A large number of recent comparative studies have investigated the relationship between species' functional traits and invasiveness. Of the comparative studies that considered introduced plants, only a few of them compared introduced invasive with introduced non-invasive species while the majority chose a native counterpart to the introduced invader (van Kleunen *et al.*, 2010b). However, comparing introduced species that differ in their invasive capacity is the most direct way to identify the determinants of invasiveness (van Kleunen *et al.*, 2010a). Even if, so far, a number of ecophysiological traits have been shown to be associated with species invasiveness, it has not been possible to achieve general conclusions on what determines species invasiveness (Kolar and Lodge, 2001; Daehler, 2003).

This thesis assesses and compares functional traits of *Impatiens* species which vary in their invasiveness in Ireland with the aim of increasing the knowledge of the relationship between species traits and invasiveness. Gaining an understanding of which species traits are determinants of invasiveness contributes to predictions of future invasions, and it is significant for weed risk assessment (van Kleunen *et al.*, 2010b).

This chapter introduces concepts and definitions of biological invasions, in particular in relation to introduced plant species. It then examines which traits have been associated with plant performance and invasive capacity, with particular emphasis on phenotypic plasticity. In the last section of this chapter, the study species will be described and their distribution and invasive capacity examined.

1.2 BIOINVASION

The introduction of species in a new area is frequently the intentional or accidental result of various human activities and its magnitude is currently increasing at an extraordinary rate (van Kleunen *et al.*, 2010a). The ways, or vectors, by which a new organism is able to overcome geographical barriers include several anthropogenic activities such as horticulture, agriculture, aquaculture, shipping, biological control, etc. (Hulme *et al.*, 2008).

Introduced species are also referred to as non-native, alien, exotic and non-indigenous species (Richardson *et al.*, 2000b). If they are able to persist and spread in the introduced range, they can have a negative impact on the native biota (Davis, 2003), since they can directly affect the long-established native species and alter the invaded ecosystem. Numerous local extinctions of native species caused by introduced predators or pathogens have been documented (e.g. Kaufman, 1992; Fritts and Rodda, 1998). However, Davis (2003) attributed the threat of extinction largely to intertrophic (predation) and not intratrophic (competition) interactions. Gurevitch and Padilla (2004) conclude that the role of the invaders in causing extinction can not be generalized and remains unproven. Introduced species are therefore a major threat to biodiversity (Wilcove *et al.*, 1998). This is exacerbated by virtue of their potential to hybridize with the native biota. Hybridization between native and introduced species has been observed both for plants and animals (Stokes *et al.*, 2006). A well-known example in Ireland is represented by the hybridization between the Sika deer which hybridizes with the native red deer to produce viable offspring (Stokes *et al.*, 2006). Hybridization has been suggested to contribute towards enhancing further invasiveness (Ellstrand and Schierenbeck, 2000).

Biological invasions also represent a considerable threat to an ecosystem's economic value. Surprisingly large economic costs can be inflicted by introduction of species to agriculture and forestry. Born *et al.* (2005), in a survey of economic cost caused by biological invasion, reported several studies which included quantitative analysis on the cost of biological invasions. Among these, the review of Pimentel *et al.* (2001) estimated that invasive species achieved a total worldwide cost of US$ 336 billion per year. Pimentel *et al.* (2005) updated the estimated costs due to biological invasions and reported a cost of US$ 120 billion per year in the Unites States alone. In addition, human health can be directly affected by biological invasion since infectious disease agents can also be considered invasive species when they are able to spread across their range of occurrence (Vitousek *et al.*, 1997).

Mooney and Hobbs (2000) suggested that the magnitude of the threat represented by biological invasion will increase in the future, since climate change could favour invasion (Mooney and Hobbs, 2000). However, climate change, in same cases, might limit the distribution of invasive species. For instance, since one of the main effect of climate change is a reduction in rainfall, plants that require high water availability might negatively be affected by climate change.

1.2.1 Alien plants

Richardson *et al.* (2000b) give clear and unambiguous definitions of plant introduction, naturalization and invasion that help us to clarify the field of invasion biology. Their definitions are reported in Table 1.1.

However, this approach should not overlook the fact that invasiveness is a continuous variable, not a categorical one, and it changes through time (Muth and Pigliucci, 2006). In addition, invasiveness is strictly related to specific geographic context; consequently a species may occur as invasive in a particular region and only as casual elsewhere (Alpert *et al.*, 2000).

Following the terminology of Richardson *et al.* (2000b), Milbau and Stout (2008) characterized the factors which possibly allow introduced plants to move from casual to naturalized and from naturalized to invasive in Ireland. Using two multiple logistic regressions, they discriminate among factors which contribute to the transition from casual to naturalized (such as clonal growth, native range, residence time); and factors that distinguished invasive from non-invasive species (such as ornamental introduction, hermaphroditic flowers, pollination mode, being invasive elsewhere, onset of flowering season, native range, and residence time). In addition, mainly based on Reynolds (2002), they created a database (comprehensive of species invasive status) of alien plants which can be a useful tool in invasion biology research in Ireland and increase the available information on introduced species at European-scale.

Table 1.1 Definition of the different invasive statuses of introduced plants species according to Richardson *et al.* (2000b).

Alien or exotic or non-native plants	Plant taxa in a given area whose presence there is due to intentional or accidental introduction as a result of human activity
Casual alien plants	Alien plants that may flourish and even reproduce occasionally in an area, but which do not form self-replacing population, and which relay on repeated introductions for their persistence
Naturalized plants	Alien plants that reproduce consistently and sustain population over many life cycles without direct intervention by humans and do not necessarily invade natural, semi natural or human made ecosystems
Invasive plants	Naturalized plants that produce reproductive offspring, often in very large numbers, at considerable distances from parent plants
Transformers	Invasive plants which change the character, condition, form or nature of ecosystem over substantial area relative to the extent of that ecosystem
Weeds	Plants (not necessary alien) that grow in sites where they are not wanted and which usually have detectable economic or environmental effects

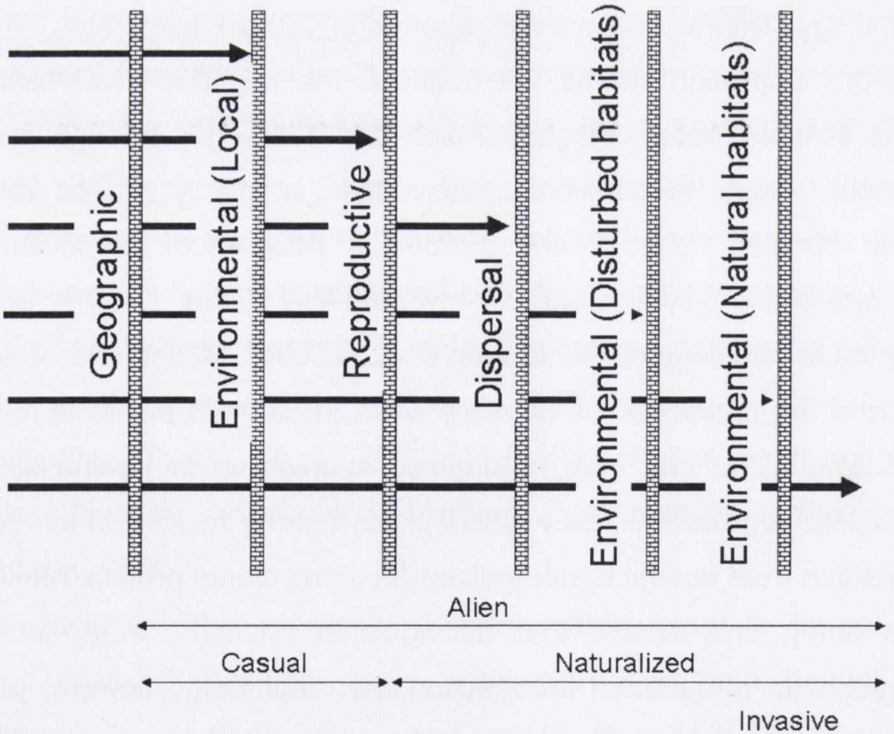

Figure 1.1 Representation of the biotic and abiotic barriers that a plant must overcome to move from one stage to the next of the invasion process (after Richardson *et al.*, 2000b; Richardson and Pysek, 2006).

The current list (1987-2001) of introduced plants in Ireland contains 645 alien plant taxa (Reynolds, 2002). Reynolds (2002) reported that almost 50% of the Irish flora is represented by introduced species. Only 30% of introductions were accidental with most of the species deliberately introduced for horticulture or as crop plants (Reynolds, 2002).

Forty-five percent of the introduced plants are present in Ireland as casuals, 25% persisting and 30% established. Over 90 taxa are considered established in natural or semi-natural areas, of which only a small proportion (65 alien plant taxa) (Milbau and Stout, 2008) are invasive and may have an impact on the invaded ecosystem. Mainly casual aliens are found in manmade and disturbed habitats (e.g. in urban areas, on roadsides, at ports, on waste ground). A small number of introduced plants escaped from cultivation, mainly ornamental plants, and are now able to invade natural habitats where they compete with native vegetation and may prevent regeneration of native plants (Reynolds, 2002; Milbau and Stout, 2008).

1.2.2 How do introduced species become invasive?

The majority of the species which are introduced in a new area fail to establish (Richardson and Pysek, 2006). Williamson's 'tens rule' predicts that 10% of species introduced into a new region can afterwards be found in the wild, 10% of those in the wild can establish themselves well enough to self-sustain populations, 10% of which (therefore only 0.1% of all introduced species) become invasive (Williamson, 1996). The mortality rate of the introduced species in the new environment can be attributed to when plants are not adapted to an unsuitable environment, to biotic resistance of the native community (competition, predation and pathogens), and to chance (Lonsdale, 1999; Levine and D'Antonio, 2003; Peterson, 2003; Richardson and Pysek, 2006). The climatic characteristics of the new environment strongly determine restrictions to the distribution of introduced species and may be responsible for a species' immediate failure after introduction (Sakai *et al.*, 2001; Theoharides and Dukes, 2007). Species that show a wider distribution in their native range may have a broader climatic tolerance and, as a result, they might have an enhanced survival probability in their introduced range (Goodwin *et al.*, 1999). Propagule pressure also plays a central role in determining the probability of the invasion success of introduced species (Rejmánek, 2000; Kolar and Lodge, 2001; Kühn *et al.*, 2004; Colautti *et al.*, 2006; Richardson and Pysek, 2006; Catford *et al.*, 2009). Propagule pressure is a product of the number of individuals introduced during a single introduction event (propagule size), multiplied by the number of introduction events (propagule number) (Eppstein and Molofsky, 2007). It can be considered in space (widespread introductions) and in time (long cultivation) (Richardson and Pysek, 2006). Therefore, propagule pressure might be very difficult to estimate or quantify (Richardson and Pysek, 2006 but see McKinney, 2002).

The question, however, is not simply "whether a species will invade" but also "when a species will invade". It is possible that introduced species which initially do not show an invasive character may become invasive. The invasive potential of a species increases with time since introduction (residence time) and the distribution of an introduced species is often directly proportional to its residence time (Pyšek and Jarošík, 2005 and references therein). Introduced species, therefore, even when they are shown to persist in natural habitats, can often remain non-invasive for long periods of time (lag-phase) before aggressively beginning to spread (Ewel *et al.*, 1999; Alpert *et al.*, 2000; Ellstrand and Schierenbeck, 2000). Species which become successful invaders must therefore be able to overcome the initial lag-phase. In its introduced range, a new estabilished population encounters a loss of genetic variation since it is established only by a small number of individuals (i.e. founder effect). Therefore introduced populations, whose genetic variation has been reduced by founder effects, should have limited ability to persist and adapt in new conditions (Sakai *et al.*, 2001). Invasive species must have a multi-purpose genotype that allows sufficient levels of physiological adaptability (phenotypic plasticity) to enable them to spread out from a restricted area with specific environmental conditions and to invade a wider range of habitats, characterized by various environmental conditions. Phenotypic plasticity or genetic variability may allow introduced plant species to adapt to less favourable and heterogeneous environmental conditions and therefore to overcome the lag-phase (Sakai *et al.*, 2001).

Subsequently, the invasion process takes place in a series of successive stages (Richardson *et al.*, 2000b; Colautti and MacIsaac, 2004; Milbau and Stout, 2008). The transition from one stage to the next requires a species to overcome a combination of geographical, biotic and abiotic barriers (Williamson and Fitter, 1996; Richardson *et al.*, 2000b; Richardson and Pysek, 2006) (Figure 1.1). Habitat characteristics, species traits, invasion history and native distribution may all contribute to the success of an invasion (Goodwin *et al.*, 1999; Dehnen-Schmutz *et al.*, 2005; Pyšek and Jarošík, 2005; Milbau and Stout, 2008). Level of invasion can vary considerably from one habitat to another even within a particular region, suggesting that some habitats are more susceptible to invasion than others (Chytrý *et al.*, 2008). Habitats with fewer introduced species are generally those with persistently low nutrient availability, and those with the greatest proportion of aliens are generally anthropogenic habitats and costal, littoral and riverine habitats (Chytrý *et al.*, 2008). Species traits potentially responsible for invasiveness have been extensively investigated since Baker's attempt to characterise "ideal weeds" (Baker, 1965; 1975). Among traits considered responsible for invasiveness are fast growth, efficient resource use, competitive ability, high seed production and high germination rate under different environmental conditions. Self-compatibility and cross pollination are also considered to be traits that enhance competitive performance (Sakai *et al.*, 2001; Lloret *et al.*, 2005; Dietz and Edwards, 2006). Biotic interactions with herbivores, parasites, pathogens, mutualistic soil biota, pollinators, and dispersal agents also influence introduced plant species establishment, spread and invasion success (Richardson *et al.*, 2000a; Theoharides and Dukes, 2007).

Cataford *et al.* (2009) summarized the multitude of hypotheses which form the foundation of invasion biology studies. With the aim of reducing redundancy, they unified hypotheses and they suggested that invasion is a function of (1) propagule pressure, (2) the abiotic character of the area of introduction and (3) the biotic characteristics of the invaded community and of the introduced species (Catford *et al.*, 2009). Among the biotic components, several hypothesis are related to invader traits as is, for example, the "ideal weed" hypothesis that predicts that invasive species possess particular traits that enable them to outcompete native species (Baker, 1965; Rejmánek and Richardson, 1996; Sutherland, 2004), or the "novel weapon" hypothesis which explains invasiveness through the fact that invasive species might release allelopathic substances, to which native species are not adapted, to repress potential competitors (Callaway and Ridenour, 2004; Hierro, 2005). Biotic components also include enemies, and the "enemy release" hypothesis attributes invader-fitness to the lack of natural enemies in the new environment (Keane and Crawley, 2002; Colautti *et al.*, 2004; Joshi and Vrieling, 2005). Additionally, the "evolution of an increased competitive ability (EICA)" hypothesis suggests that enemy release enables invaders to re-allocate resources to enhancing their competitive ability (Blossey and Notzold, 1995; Callaway and Ridenour, 2004; Joshi and Vrieling, 2005). Moreover, mutualism and commensalism are also biotic components important in the concept of "invasional meltdown", which suggests that the co-occurrence of several introduced species might favour each other's invasions (Simberloff and Von Holle, 1999; Mack, 2003) (for a detailed review on invasion biology hypothesis see Cataford *et al.* 2009).

1.2.3 Phenotypic plasticity

Phenotypic plasticity is defined as the ability of a genotype to express different phenotypes in response to different environmental conditions (Bradshaw *et al.*, 1965; Pigliucci, 2005; Ghalambor *et al.*, 2007). Phenotypic plasticity has been repeatedly proposed as being among the traits associated with invasion (e.g. Marshall and Jain, 1968; Williams *et al.*, 1995; McDowell, 2002; Geng *et al.*, 2007; Caño *et al.*, 2008), since plasticity in traits that contribute to fitness increases the probability of persistence in a larger range of new environments (Baker, 1965; Price *et al.*, 2003). Greater adaptive phenotypic plasticity is therefore likely to confer greater invasiveness (Brown and Marshall, 1980; Gray, 1986; Schierenbeck *et al.*, 1994; Williams *et al.*, 1995). Introduced species populations may have a higher risk of extinction because of reduced genetic variation due to founder effects and to small population size (Allendorf and Lundquist, 2003). Nevertheless, many non-native species, establish themselves well enough to displace locally adapted, long-established native species (Mack *et al.*, 2000). It has been suggested that introduced individuals must have a multi-purpose genotype which allows sufficient levels of phenotypic plasticity to be able to spread in the new environment (Richardson and Pysek, 2006). However, phenotypic plasticity must not be seen as an alternative to genetic variation but as a proper characteristic of a genotype, and it is specific to a particular trait in definite environments (Richards *et al.*, 2006). Natural selection will also act toward favouring individuals that exhibit high adaptive plasticity, and therefore fitness, across different environments. As a result, plasticity in fitness traits might evolve rapidly in introduced species in the new environment and contribute to overcoming the lag-phase and subsequently to increasing invasiveness (Richards *et al.*, 2006).

It has been suggested that plasticity can favour invasion in two ways. It can allow a species to maintain fitness across a wide range of environmental conditions (general purpose genotype). Thus, the invader shows "robustness" in unfavourable environments; or the invader might benefit from plasticity in an "opportunistic" way, drastically increasing fitness in favourable conditions (Richards *et al.*, 2006). An interesting framework to assess phenotypic plasticity has been provided by Richards *et al.* (2006). They suggested three scenarios in which the successful invader benefits from phenotypic plasticity: i) a "Jack-of-all-trades", where the invaders have the ability to maintain fitness (fitness homeostasis) in unfavourable environmental conditions as, for istance, low-resources environments; ii) a "master-of-some", where the invaders have the ability to highly increase fitness when the environmental condictions become favourable (e.g. high availability of resources or low predation); or iii) a "Jack-and-master" that combines both the abilities, so that the invader is able to maintain fitness in stressfull environments as well as to increase fitness in favourable environments (Figure 1.2).

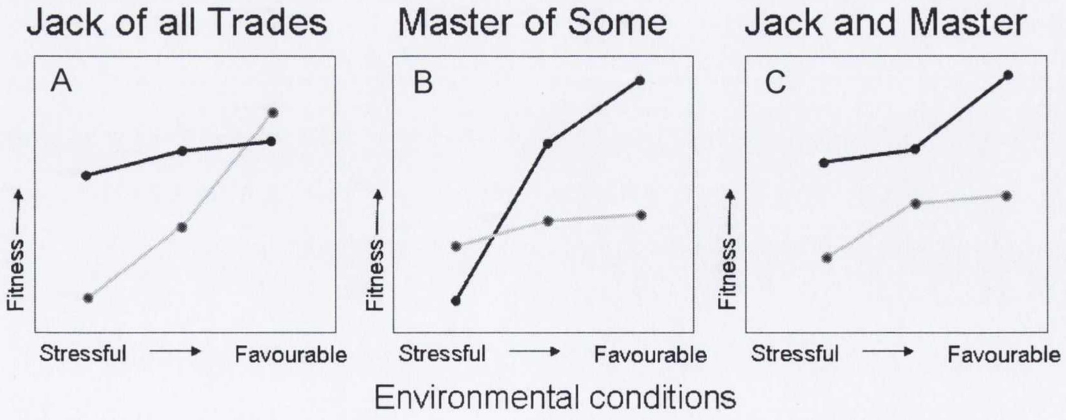

Figure 1.2 Fitness norm-of-reactions of invasives (black line) and non-invasives (grey line). Invasives might benefit from plasticity in fitness traits as described by the three different patterns (A, B and C). Invasives might be able to maintain fitness in adverse environmental conditions (fitness homeostasis) (A), or might be able to greatly increase fitness as a response to favourable environmental conditions (B), or might shown a combination of the two abilities (C) (after Richards *et al.*, 2006).

A number of studies that compare plasticity in traits associated with fitness have been performed between pairs or small sets of alien invasive species and native species or alien invasives and alien non-invasives (Bossdorf et al., 2005; Burns and Winn, 2006; McAlpine et al., 2008). Generally they were conducted as controlled experiments in common-gardens or greenhouses with the manipulation of one or more abiotic factors. With the increase in the number of these comparative studies that consider phenotypic plasticity, the need to quantify phenotypic plasticity, beyond simply comparing slopes of the norms of reaction emerged. Norms of reaction graphically describe patterns of phenotypic expression for fitness related traits of a genotype or a species across a range of environments (Brock *et al.*, 2005; Geng *et al.*, 2007). Valladares *et al.* (2006), after having examined a multitude of indices used to quantitatively assess phenotypic plasticity in comparative studies, suggested a different approach based on "relative distance plasticity indexes" (RDPIs), which consider distances among individuals of a species growing in different environments and which allow for statistical comparisons (Valladares *et al.*, 2006). Reaction norms are useful tools to explore and describe fitness responses to different environmental conditions. Differences in plasticity between species can be detected by observing the ANOVA's interaction effect between species and environment. Moreover, RDPIs represent a quantitative approach to plasticity estimation and allow the ranking of species according to their plasticity (Valladares *et al.*, 2006). However, there are limitations. The RDPI analyses depend on, for example, whether the environments are balanced according to the reaction norm of a species: if more than one experimental environment is above "saturation", the relative distances between two individuals growing in two different saturated environments might be zero (or very low), and RDPI would be underestimated (Valladares *et al.*, 2006).

1.2.4 Assessing traits in different environmental conditions

"*We are becoming increasingly aware that the individual cannot be considered out of the context of its environment. The way in which it reacts to different environments is as much part of its characteristics as its appearance and qualities in a single environment.*"

(Bradshaw et al., 1965)

Some traits contribute to fitness and they can consequently contribute to species invasiveness only in particular conditions. Therefore it is necessary to compare the traits of invasive and non-invasive species growing under several environment conditions. In this way, it might be possible to characterize which traits confer invasiveness under which circumstances. In addition, phenotypic plasticity is a characteristic that represents a competitive advantage (and can be evaluated) only in a changing environment.

In this thesis, traits are assessed in introduced species firstly in a high-resource environment, the kind of environment most susceptible to invasion. Afterwards, the experimental environmental conditions were changed to provide a view of the same traits in more stressful conditions. Low-light resource habitats represent situations where close (native) vegetation offer resistance to the introduced species' invasions.

Biological invasions, climate change and their effect on biodiversity have usually been considered separately, even though it is expected that climate change would have an effect on the rate of bioinvasion (Walther *et al.*, 2009). Climate change, as it affects native species, affects also the probability of success of a new introduced organism in the new environment. In a climate change scenario, native species might become progressively less adapted to the changed environment and the introduced species might encounter less biotic resistance from the native community to their invasion (Walther *et al.*, 2009). Alterations induced by climate change are particularly intense at more northerly latitudes (including Ireland) where low temperatures have represented a limiting factor. Walther *et al.* (2009) describe how climate change can favour each of the steps of the invasion process, increasing the probability of survival in the earlier stages of the process, then contributing to a successful establishment and reproduction, and lastly, enhancing the suitable areas for invaders' spread. Generally studies aimed at evaluating the effects of climate change on invasion are focused on temperature increase. However, lower water availability is an important constituent of global climate change, and can represent a major limiting factor to plant growth, but its effects on biological invasion have been poorly investigated (Walther *et al.*, 2009). The experiment described in Chapter 5 of this thesis aimed to provide a preliminary insight into the correlation between traits and invasiveness under low water availability, which could be the case with global warming.

1.3 A CASE STUDY: THE GENUS *IMPATIENS*

In the European flora, the family *Balsaminaceae* is represented only by the genus *Impatiens* (Cigić *et al.*, 2003). About 850 - 1000 species of flowering plants constitute the genus that is widely distributed in the world. The majority of the species of this genus occur in tropical or subtropical regions (Grey-Wilson, 1980). In Europe, the genus *Impatiens* is mostly found in the central part of the continent and it is represented by the following species: *Impatiens balfourii* (Hooker f.), *I. balsamina* (L.), *I. capensis* (Meerb.), *I. glandulifera* (Royle), *I. noli-tangere* (L.), *I. parviflora* (DC.) and *I. scabrida* (DC.) (Moore, 1968; Cigić *et al.*, 2003) (Plate 1.1). Their invasive status in Europe, Britain and Ireland, their native range and their habitats are listed in Appendix 1.1.

According to the National Biodiversity Network Gateway (NBN) (http://data.nbn.org.uk), the genus *Impatiens* is represented in Britain and Ireland by five species: *Impatiens balfourii*, *I. capensis*, *I. glandulifera*, *I. noli-tangere* and *I. parviflora*. All the species have a native temperate range (Tabak and von Wettberg, 2008). The distribution of the *Impatiens* species present in Britain and Ireland is shown in Figure 1.3.

Impatiens noli-tangere is the only *Impatiens* species native to central Europe and Britain, although it is not present in Ireland. This annual plant can be found in wet woodlands, streams and lakesides. In Britain it is common in mid-Wales and the English Lake District (Watson, 1883; Hatcher, 2003). In recent years, populations of this species have decreased in number and size (Preston *et al.*, 2002). All others *Impatiens* species have been introduced from Asia and North America (Moore, 1968; Cigić *et al.*, 2003).

Figure 1.3 Distribution of *I.balfourii* (a), *I. capensis* (b), *I. glandulifera* (c), *I. noli-tangere* (d) and *I. parviflora* (e) in Britain and Ireland. *Maps developed for National Biodiversity Network by CEH & JNCC, 2004.*

Comparing congeneric species which vary in their invasive status minimize trait differences associated with inter-specific variation in unrelated species (McDowell, 2002; Burns and Winn, 2006; Muth and Pigliucci, 2006; Richards *et al.*, 2006). A great number of ornamental plants belong to the genus *Impatiens* and the gardening business is continuously supplying new *Impatiens* species. For this reason, it is of particular interest to understand the variability in potential of invasive behaviour in the genus *Impatiens* (Tabak and von Wettberg, 2008).

This thesis is based on comparative studies which consider three *Impatiens* species. I originally aimed to include all the *Impatiens* species reported to occur in Britain and Ireland but, due to low seed availability or to poor seed germination rates, the comparative studies presented in this thesis include only *I. glandulifera*, *I. parviflora* and *I. balfourii*. These three species represent excellent examples of introduced congenerics which appear to vary in their invasive capacity and in their distribution in Britain and Ireland (Figure 1.3 a, c and d). However, even if comparing introduced invasive species and introduced non-invasive species represented the most direct approach to testing the determinants of invasiveness, this approach has limitations. These limitations are due to the fact that non-invasive introduced species might not have yet achieved their full invasive potential, and so the intrinsic invasiveness of the invasive and of the non-invasive might not differ. Consequently it is important to also consider the introduction history of each species (van Kleunen *et al.*, 2010a).

1.3.1 *Impatiens glandulifera*

Impatiens glandulifera, also known as Himalayan or Indian Balsam, is the tallest naturalized annual herb in Europe, and can reach up to 2.5 m in height (Beerling *et al.*, 1994). It produces flowers with a colour that ranges from white to dark purple (Plate 1.1 c). Flowers are self-compatible but self pollination is limited by protandry (Valentine, 1978). *I. glandulifera* can produce between 700 and 800 seeds plant^{-1} which are dispersed to a distance of up to 5 m with an explosive dehiscence of the capsule (Beerling and Perrins, 1993).

I. glandulifera is currently one of the most dominant invasive plant species in Britain, Ireland and continental Europe (Perrins *et al.*, 1993; Pyšek and Prach, 1995). *I. glandulifera* is a large annual plant native to the Himalayas, where it grows from 1800 to 4000 m altitude (Polunin and Stainton, 1984). This species was first introduced to Europe (to Kew Gardens) in 1839 as an ornamental and nectar-producing plant for beekeepers (Beerling and Perrins, 1993). First records of naturalization are from Middlesex and Hertfordshire in 1855 (Britten, 1900). It has now colonized most parts of mainland Britain, and much of Ireland, as well as more isolated localities in the UK (Beerling and Perrins, 1993) (Figure 1.3 c).

In continental Europe, it began spreading in 1900, almost half a century later than in England (Helmisaari, 2006). *I. glandulifera* is now widespread, mostly along Europe's river systems, in nearly all European countries (Pyšek and Prach, 1995). The "Delivering Alien Invasive Species Inventories for Europe" database (DAISIE), reports *I. glandulifera* as established in Ireland, Britain and 20 other European countries (Austria, Belgium, Czech Republic, Denmark, Finland, France, Germany, Hungary, Italy, Latvia, Liechtenstein, Lithuania, Luxembourg, Netherlands, Norway, Poland, Romania, Slovakia, Sweden, Switzerland). *I. glandulifera* was introduced in the United States at the beginning of 19th century and is now widespread both on the East and West Coast of North America (USDA, 2009). *I. glandulifera* is also a problematic invasive species in New Zealand (ISSG, 2005) but, maybe due to the warmer climate, it seems absent or "not-of-concern" in Australia and it is not listed among the national weeds on the Australian weeds website (2009).

I. glandulifera grows in a wide range of soil textures and structures. To establish successfully it generally requires limited amounts of disturbance and bare ground (Beerling and Perrins, 1993). *I. glandulifera* is often observed growing on river banks and other wet, nutrient-rich natural habitats where it forms dense monocultures and promotes erosion when it dies back in the winter (Beerling and Perrins, 1993; Helmisaari, 2006). *I. glandulifera* is considered to have a negative effect on co-occurring native plants because of its ability to strongly compete for aerial space and nutrients, and because of its stronger ability to attract pollinators (Chittka and Schürkens, 2001; Nienhuis, 2009). Hulme and Brenmer (2006) predicted that extensive *I. glandulifera* stands may reduce species-richness by as much as 25% on riparian habitats.

The spread of *I. glandulifera* may be limited by frost and soil moisture, and its northern distribution limits in Europe seem to be dependent on the length of the growing season (Beerling and Perrins, 1993).

I. glandulifera has been studied extensively, because of its invasive capacity, in studies from Ireland and Britain (e.g. Perrins *et al.*, 1990; Beerling and Perrins, 1993; Perrins *et al.*, 1993; Willis and Hulme, 2002; Hulme *et al*, 2006; Nienhuis and Stout, 2009), from continental Europe (e.g. Pyšek and Prach, 1995; Cigić *et al.*, 2003; Hejda and Pyšek, 2006) and from North America (e.g. Tabak and von Wettberg, 2008).

1.3.2 *Impatiens parviflora*

Impatiens parviflora is an annual herb with a stem length that reaches between 20 and 100 cm. Flowers are pale yellow (Plate 1.1 f) and they reach a size of 1.5 cm (Coombe, 1956). Seed production is very variable and depends on habitat and plant density (Coombe, 1956).

The native range of *I. parviflora* is in temperate central Asia where it grows near rivers or streams, ravines, stony slopes and moist shady environments (Coombe, 1956). In Europe the species is widely naturalized and it is considered invasive in the damp shady forests of central and northern Europe (Chmura and Sierka, 2007). It was first introduced to Geneva Botanic Garden in 1831 and, within a few decades, it occurred spontaneously in many places in Europe, often near botanic gardens (Coombe, 1956). It has been hypothesized that this species was introduced with buckwheat grown for pheasants (Druce, 1897 in Coombe, 1956). When first naturalized in Europe, it occupied disturbed habitats, but thanks to its ability to grow and reproduce in low light levels, it has been able to invade undisturbed forests.

I. parviflora is reported as established in Ireland, Britain (Figure 1.3 e) and 20 other European countries as well (Austria, Belgium, Czech Republic, Denmark, Estonia, Finland, France, Germany, Hungary, Italy, Latvia, Lithuania, Luxembourg, Netherlands, Norway, Poland, Romania, Slovakia, Sweden, Switzerland) (DAISIE, 2008). It has not yet been recorded as naturalized from any state in the United States (Tabak and von Wettberg, 2008).

1.3.3 *Impatiens balfourii*

Impatiens balfourii, or Kashmir balsam, is an annual plant which can grow 1.2 m tall. Flowers are white and pink, or white and lavender (Plate 1.1 a) of a size of ca. 2.5 – 4 cm (Adamowski, 2009). *I. balfourii*, like *I. glandulifera*, is from the Himalayas where it grows at altitude between 1500 and 2500 m, which is narrower than the altitude range of *I. glandulifera* (Nasir, 1980).

I. balfourii was introduced at the beginning of the 20th century simultaneously in Montpellier Botanic Garden in France and in Edinburgh Botanic Garden in Scotland. From the beginning of last century, it started to be cultivated in most of the European countries and it became more popular also in the United States (Adamowski, 2009 and references therein). The first record of *I. balfourii* occurring spontaneously is from Montpellier, in 1906. It now occurs as a casual plant in southern and central Europe (Adamowski, 2009). From DASIE database *I. balfourii* is reported as established in 4 European countries (Belgium, France and Corsica, Italy, Portugal) but not as established in Britain or Ireland (DAISIE, 2008) (Figure 1.3 a). Adamowski (2009) suggested *I.*

balfourii as a potential invader in Europe and reported this species as listed in the watch list in Switzerland, and considered invasive in some parts of France (Adamowski, 2009). However, this species occurs only as casual in countries where it has long been cultivated (e.g. Hungary) (Adamowski, 2009). *I. balfourii* is established in a few states of the United States, mainly in the West Coast (USDA, 2009) but naturalization and spread is probably limited most by its relatively low frost tolerance (Tabak and von Wettberg, 2008).

1.4 AIMS OF STUDY

This study focuses on the most frequently investigated growth characteristics and ecophysiological traits as well as on some less frequently investigated traits. Among the traits that have been repeatedly associated with invasion, growth rate is widely considered to be a measure of plant fitness at least in hight resources environments, since it is important for both survival and reproduction (Shipley, 2006; Feng, 2008). High growth rates which are often correlated with high maximum photosynthetic rates (A_{max}) and with morphological traits that promote light capture efficiency, such as leaf area ratio (LAR), leaf weight ratio (LWR) and specific leaf area (SLA), are all generally considered to be directly proportional to invasive capacity (Poorter, 1999; Feng *et al.*, 2007c). In addition, less investigated ecophysiological traits, such light compensation point (LCP), light saturation point (LSP), dark respiration (Rd) and apparent quantum yield (ϕ), can potentially contribute to plant fitness and can enhance plant invasiveness and consequently were considered in this study. This comparative study might therefore identify new traits that can be related to invasion.

In particular, the specific aims of this thesis are:

1. to investigate and compare germination characteristics in *Impatiens glandulifera*, *I. parviflora* and *I. balfourii* (Chapter 2);
2. to examine growth and ecophysiological characteristics of *Impatiens glandulifera*, *I. parviflora* and *I. balfourii* growing in a high-resource environment (Chapter 3);

3. to compare growth and ecophysiology of the invasive *I. glandulifera* and of the non-invasive *I. balfourii* growing in two different light environments (Chapter 4);
4. to compare growth-related and ecophysiological traits of *I. glandulifera* and *I. balfourii* growing at different water regimes and to assess plasticity of the considered traits in the different environments (Chapter 5);
5. to investigate the potential for hybridization between I. *glandulifera* and *I. balfourii* (Chapter 6).

2 On the ecology and germination of seeds of introduced *Impatiens*

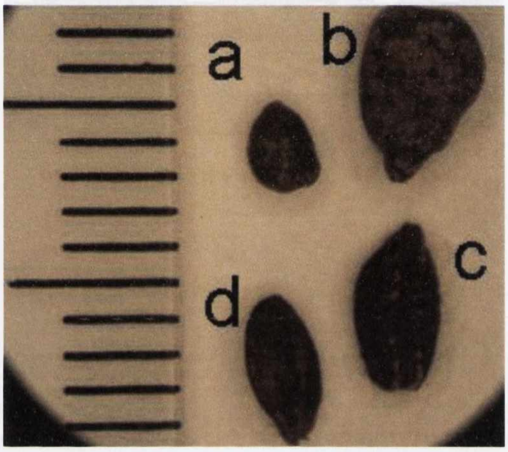

Plate 2.1 Seeds of a) *I. balfourii*, b) *I. glandulifera*, c) *I. parviflora*, d) *I. noli-tangere*. The scale is in millimetres.

SUMMARY

Since the sixties, with Backer's characteristics of "ideal" weeds, the reproductive biology of invasive plants has been the subject of many studies that investigate traits potentially responsible for invasion. Seed production, dispersal and germination are important factors that determine persistence and spread of invasive plant populations, even after eradication of the mother plants, as seed bank dynamics may be responsible for a future re-infestation. In addition, germination trials that allow the identification of the environmental conditions that promote seed germination may help to understand which habitats are more at risk of invasion.

In this chapter germination characteristics of successful invaders and closely related non-invasive or less-invasive *Impatiens* species were compared with the aim of understanding the invasive strategy of the most aggressive species and the lack of invasiveness of the non-invasive one.

I. glandulifera, the most invasive, *I. parviflora*, invasive in Central and Northern Europe but only established in Ireland, and *I. balfourii*, naturalized in central and southern Europe but not reported in Ireland, were germinated in different environmental conditions. In a laboratory experiment seeds were pre-treated with different periods of cold in order to break seed dormancy. In addition, in a common-garden experiment, seeds were placed either on the soil surface or buried.

In laboratory conditions, *I. glandulifera* and *I. balfourii* showed significantly higher germination rates than *I. parviflora*. Additionally, increasing the length of the cold period before germination increased germination rates. In the common-garden experiment *I. glandulifera* showed overall the highest germination rates. All species showed highest germination rates when buried. Mortality of seedling varied depending on treatment and species. Germination started earliest in *I. glandulifera*, then in *I. balfourii* and last in *I. parviflora*.

Higher seed mass, seed production, lower mortality, early germination and the capacity to germinate under a wider range of conditions are factors that

may contribute to *I. glandulifera*'s superior invasive capacity. On the other hand, lower seed mass and seed productivity may be partially responsible for *I. balfourii*'s lack of success. *I. parviflora* has a very limited distribution in Ireland. One of the factors that may contribute to limiting the invasion of this species in Ireland, although it is invasive elsewhere, may be the stratification required to break dormancy.

2.1 INTRODUCTION

The negative effects of alien introduced species on the composition and ecology of native communities have been widely reported, as have their impacts on human health and economy (Collingham *et al.*, 2000; Davis, 2003). Much effort has been spent attempting to identify traits that contribute to the success of invaders. Invasive species are often reported to show better performance than native or non-invasive ones, and germination traits are no exception. H. G. Baker (1965; 1975; 1991), starting in the sixties, underlined the importance of seed ecology and germination characteristics as indicators of plant invasive capacity. For over 40 years, the reproductive biology of invasive plants has been the subject of many studies that investigate traits potentially responsible for invasion with the aim of understanding the relationship between reproductive potential and invasiveness, and to attempt to predict which species have the potential to become invasive. (Baker, 1975; Rejmánek, 1996; Milbau and Nijs, 2004; Milbau and Stout, 2008).

For invasive plants which reproduce mainly via seeds, understanding seed ecology is crucial to their control. Seed production, dispersal and germination are important factors that determine subsistence and spread of invasive plant populations, even after eradication of the mother plants, as seed bank dynamics may be responsible for a future re-infestation (Cochard and Jackes, 2005). Germination is a particularly crucial stage of plant success and invasion in annual species. Identification of the environmental conditions that promote seed germination may help to understand which habitats are more at risk of invasion and can help to inform effective prevention measures.

Among the traits that may potentially promote germination, seed size and seed production (number of seeds produced per flower) have been the most widely investigated and many authors found that larger seed mass represents an advantage in the process of seedling establishment (Rees, 1995; Bonfil, 1998; Hewitt, 1998; Bond *et al.*, 1999; Seltmann *et al.*, 2007). Jakobsson and Eriksson (2000) describe a trade off between seed abundance and seed mass that reflect a trade-off between the quantity of attempts at germination and quality or probability that the attempt will be successful (Jakobsson and Eriksson, 2000). Generally seed size has been positively correlated to seedling fitness and probability of survival (Smith and Fretwell, 1974). However, Gomez (2004) identified circumstances in which seed size is negatively correlated with seedling survival rate (i.e. the risk of predation increase for larger seeds and seedlings).

In comparative studies, trait differences associated with inter-specific variation in unrelated species can make it difficult to determine which differences are associated with invasiveness and which are purely coincidental (McDowell, 2002; Burns and Winn, 2006; Muth and Pigliucci, 2006; Richards *et al.*, 2006; Funk, 2008). In this study, to overcome these difficulties, I compared germination characteristics of introduced closely related species of the genus *Impatiens* that vary in their distribuion in Ireland. *Impatiens glandulifera* is currently one of the most aggressive invasive plant species in Britain, Ireland and continental Europe (Perrins *et al.*, 1993; Pyšek and Prach, 1995). *I. parviflora* is considered invasive in the damp shady forests of Central and Northern Europe (Chmura and Sierka, 2007). In Ireland, *I. parviflora* is reported only as naturalized (Reynolds, 2002; Milbau and Stout, 2007). *I. balfourii* is naturalized in disturbed habitats in central and southern Europe (Moore, 1968) but is not considered to be invasive there. *I. balfourii* is not reported as established in Britain and Ireland from the DASIE database but has been reported in a few sites in England by the National Biodiversity Network Gateway maps (NBN, 2008). Since *I. glandulifera* is an aggressive invasive species in Europe, its ecology (Britten, 1900; Beerling and Perrins, 1993; Perrins *et al.*, 1993; Pyšek and Prach, 1995; Hulme *et al.*, 2006) and reproductive characteristics (Mumford, 1988; Nienhuis and Stout, 2009; Perglová *et al.*, 2009) as well as its impact on invaded ecosystems (Pyšek and Prach, 1995; Hulme *et al*, 2006) have been widely investigated. In particular, Perrins *et al.* (1993), as well as comparing the rate of spread in three introduced *Impatiens* species, also compared germination, seedling establishment and seed production *of I. balfourii*, *I. glandulifera* and *I. parviflora* and recorded frost resistance in these species. With their common-garden experiment in the UK, they were able to explain the more invasive nature of *I. glandulifera* being a result of higher germination rates (at least in disturbed conditions) and a greater frost tolerance compared to *I. parviflora* (Perrins et al. 1993). However, their results did not explain the lack of success of *I. balfourii* as an invader in terms of reproductive capacity and frost tolerance, since this species showed both higher germination and greater frost tolerance than its invasive congener *I. parviflora*. Only recently has *I. balfourii* been suggested as a potential invader (Adamowski, 2009).

In another comparative experiment of *Impatiens* germination characteristics conducted in the Czech Republic, Perglová *et al.* (2009) investigated seed and seedling characteristics in *I. glandulifera, I. parviflora*, *I. capensis* and *I. noli-tangere*, but *I. balfourii* was not included. Unlike Perrins *et al.* (1993), they found that *I. capensis* showed high germination rates and they suggested this species has the potential to become an invader. Furthermore, in a laboratory experiment, they observed earlier germination in *I. glandulifera* than in the other species, although this trend was not confirmed by their common-garden experiment.

The *Impatiens* species present in Europe, in common with many species from temperate regions, have seeds that show dormancy which can be alleviated by temperatures lower than 4ºC; although, in the case of *I. glandulifera*, the length of the chilling period required to break dormancy varies significantly depending on seed age and storage conditions (Mumford, 1988). The process of administering to seeds a period of cold in order to simulate the natural winter conditions necessary to break seed dormancy is known as stratification. According to Mumford (1988), *I. glandulifera* seeds about 4 months old, stored at 15ºC need less than 40 days of cold at 4 ºC to break dormancy. Germination rate can therefore be affected by differences in dry seed storage. As a consequence, studies on germination on *Impatiens* species might be difficult to compare.

Seed dormancy was defined by Vleeshouwers *et al.* (1995) as "a seed characteristic, the degree of which defines what condition should be met to make the seed germinate". Dormancy is a mechanism which prevents germination when seeds are dispersed into favourable conditions which would allow their germination before the winter (Vleeshouwers *et al.*, 1995). Dormancy can also avoid other types of fatal germination. One of the causes of fatal germination is germination at depths too deep for the seedling to reach the surface and survive (Fenner and Thompson, 2005). The depth from which a seed can emerge depends on the nature of the substrate but also on the energy stored in the seed which is necessary for the extension growth in the dark and for the penetration of the soil itself, and this is usually considered proportional to seed mass (Fenner and Thompson, 2005).

It has been suggested that the length of seed dormancy period in *Impatiens* species increases going northwards with respect to species distribution, so that dormancy periods are *I. balfourii* < *I. glandulifera* < *I. parviflora* < *I. noli-tangere* (Jouret, 1977; Beerling and Perrins, 1993). The need for a longer period of cold at more northerly latitudes might protect seedlings against longer winter conditions and allow seeds to germinate when there is a greater availability of daylight. The aims of the experiments reported in this chapter are to compare germination rates between species and between populations. In a laboratory experiment I pre-treated seeds with different periods of cold to simulate different natural winter conditions (i.e. the process known as stratification) in order to break dormancy and in a common-garden experiment I sowed seeds at two different depths. Three introduced *Impatiens* species were used: *I. glandulifera*, *I. parviflora* and *I. balfourii*. A comparison of the germination characteristics of successful invaders and closely related non-invasive or less-invasive species should improve our understanding of the invasive strategy of the most aggressive species and will allow the evaluation of the possible invasive potential of the non-invasive species.

2.2 METHODS

Study species

In this study I originally included all the *Impatiens* species reported to occur in Britain and Ireland: *Impatiens balfourii* Hooker f., *I. capensis* Meerb., *I. glandulifera* Royle, *I. noli-tangere* L. and *I. parviflora* DC. (NBN, 2008). Only *I. noli-tangere* is native to Britain and continental Europe (but not Ireland) while the other species have all been introduced from Asia and North America. Since no germination occurred in the seeds of *I. capensis* and in *I. noli-tangere*, these two species were excluded from the data analysis. Perrins *et. al.* (1993), also observed no seedling emergence for these species.

Seed production

I. glandulifera and *I. balfourii* seed production (number of seeds per pod) was measured in pods from hand-pollinated flowers from plants grown at Trinity College Botanic Garden during summer 2009. Seed production was also measured in five open pollinated plants per species of *I. glandulifera*, *I. balfourii* and *I. parviflora* grown at Trinity College Botanic Garden in 2008.

Germination and stratification

Of the 13 populations of all the *Impatiens* species from which seeds were collected, 9 were wild populations. All seeds were collected in late summer and autumn 2008 and were stored dry in paper bags at room temperature (approximately between 15 and 20 °C) for between 4 and 6 months before the beginning of the stratification period. Seeds were either personally collected or received from several Botanic Gardens in Europe and the United States (Table 2.1). Seeds were air-dried and stored in paper bags before stratification.

Before starting the germination experiments, mean seed size was determined. Three randomly selected seeds per population of *I. glandulifera*, *I. noli-tangere* and *I. parviflora* were weighed. Since the number of available populations was lower in *I. balfourii*, eight randomly selected seeds (collected from the TCD population) and eight randomly selected seeds (collected from a wild population growing in Vallouise, France) were weighed.

Table 2.1 Provenance and providers of *Impatiens*' seeds received from botanic gardens and personally collected. All seeds were collected in 2008. (* = wild population). Information on *I. noli-tangere* seeds are not reported in the table since this species was not included in the experiments. Seeds of *I. noli-tangere* were personally collected from wild populations in Sumava, Czech Republic; from wild population in Nötle-Berg, Saerbeck and Altenberge Hohenhorst, Germany (received from Munster University); and they were collected in the National Plantentuin of Maise, Belgium.

Provenance	**Provider**
I. glandulifera	
TCD Botanic Garden (Dublin, Ireland)	Personally collected
Chapelizod (Dublin, Ireland)*	Personally collected
Palmerstown (Dublin, Ireland)*	Personally collected
Golden Acre Path (Edinburgh, UK)*	Personally collected
Dulmen (Germany)*	University of Munster
Boothbay, Maine (US)*	Coastal Maine Botanical Gardens
Plancher – Bas (France)*	MNHN Département des jardins botaniques et zoologiques. Jardin des plantes, Arboretum national de Chévreloup (Paris)
Werkendam (The Netherlands)*	De Hortus. Hortusatbotanicus Amsterdam
I. parviflora	
Trinity College (Dublin, Ireland)*	Personally collected
TCD Botanic Garden (Dublin, Ireland)	Personally collected
Sumava (Czech Republic)*	Personally collected
Chevreloup (Paris, France)	MNHN Département des jardins botaniques et zoologiques. Jardin des plantes, Arboretum national de Chévreloup (Paris)
I. balfourii	
Trinity Botanic Garden (Dublin)	Personally collected

All seeds were placed on moist filter paper in 90 mm diameter Petri dishes in batches of 20 seeds per dish.

To compare among species, seeds of three species: *I. glandulifera*, *I. balfourii* and *I. parviflora* were used. All seeds were collected from three plants per species grown at Trinity College Botanic Garden in 2008. Seeds were placed in the refrigerator at intervals of 20 days to receive 90, 70 or 50 days at 4°C.

Due to the abundance of seeds, for this comparison six replicates of 20 seeds per plant per species for every stratification treatment were used. An additional six replicates of 20 seeds per plant per species did not receive any cold period;

they were directly placed on moist filter paper at 20°C. The germination trial started for all seeds at the same time.

Separately, to investigate population level differences, seeds from six *I. glandulifera* populations (from Trinity College Botanic Garden, Chapelizod - Dublin, Edinburgh, United States, France and the Netherlands) and three *I. parviflora* populations (from Trinity College - Dublin, France and Czech Republic) were used. Seeds again received a stratification of 90, 70 or 50 days. During the stratification, seeds were checked weekly and kept moist.

Seeds were all germinated at 20ºC, and the germination trial was started for all seeds at the same time. Moisture was kept constant by watering as required. Germinated seeds were removed daily from the Petri dishes.

Germination and burial

At the beginning of December 2009, seeds of *I. glandulifera*, *I. balfourii* and *I. parviflora* from Northern Italy (for exact coordinate see Appendix 2.1) were sown in 10 cm diameter pots and placed outside at Trinity College Botanic Garden (53°18'44"N, 6°15'34"W). Half of the seeds were sown on the soil surface and half of the seeds were sown at between 1 and 2 cm depth from the soil surface. Five pots with 20 seeds for each species for each treatment were left outside during the winter.

From the middle of February, pots were checked weekly for seedling emergence. After the first seedling emergence, germination rate was recorded every three days. In this experiment, as well as percentage of final germination rate, mortality and day of first seed emergence was recorded. Seeds were not removed after germination and seedling mortality was recorded for approximately two months after the germination.

During the winter and the spring, pots did not have the opportunity to dry out because of the low temperatures and the frequent precipitation so they did not receive any additional water but only natural rainfall.. Weather data (precipitation and temperature) were recorded from a weather station placed at the Botanic Garden (Figure 2.1).,

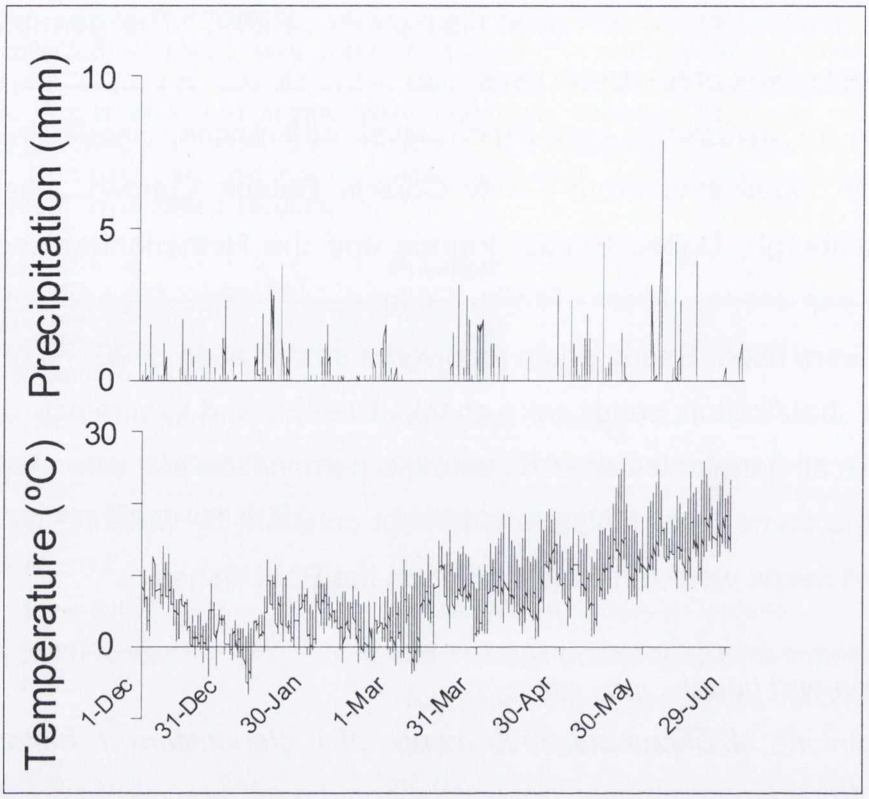

Figure 2.1 Weather data recorded from Trinity College Botanic Garden's weather station. Precipitation and temperature recorded from the beginning of December 2009 to the end of June 2010.

2.2.1 Data analysis

Germination and stratification

Analyses of the data were done in SPSS 16.0. Germination proportions were arc-sin transformed since proportions tend to be binomially distributed and this transformation has the effect of removing heterogeneity of variance (Sokal and Rohlf, 1995; Underwood, 1997).

Univariate analysis of variance (ANOVA) with a nested design, where species and stratification were fixed factors and plant (3 plants per species) was nested in species, was used to compare species, plant and stratification period effects on final percentage of germination.

Univariate ANOVA, with species and stratification as fixed factors and provenance nested in species, was used to estimate species, population and stratification period effect on final germination in six populations of *I. glandulifera* and 3 populations of *I. parviflora.*

Even when transformations failed to remove heterogeneity of variances, ANOVAs were used for the data analyses since large, balanced ANOVAs are robust against breaches of this assumption (Box, 1953; Underwood, 1981) However, the nested factors were not balanced in the second comparison and significant results were treated with caution.

The full factorial model was chosen to evaluate the effect of the main factors and of all their possible interactions.

Once it had been determined that differences existed between the germination proportion means, *post hoc* range tests and pair-wise multiple comparisons were performed to determine which means differed.

Germination and burial

Percentage of final germination, mortality and first day of germination were compared among species and between treatments (depth) with a 2-way ANOVA with species and treatment as fixed crossed factors.

Where significant differences were found, if variances were homogeneous, Bonferroni's *post-hoc* test was used to compare species and stratifications pair-wise. Bonferroni's test was chosen because it is considered more adequate for a small number of pair-wise comparisons (when there are >6-7 comparisons, Tukey's test is more powerful than Bonferroni's test) (Soliani, 2005). When variances were not homogeneous, Tamhane's *post-hoc* test, which does not assume equal variance, was used for the pair-wise comparisons.

2.3 RESULTS

Pods from the hand pollinated *I. balfourii* plants, contained the largest mean (± SE) seed set with 5.62 ± 0.2 seeds pod^{-1}. *I. glandulifera* pods contained a mean (± SE) seed set of 3.48 ± 0.45. In the open pollinated plants, the mean number of seeds (± SE) counted per pod for *I. glandulifera* and *I. balfourii* was respectively 3.67 ± 0.32 and 5.99 ± 1.44 seeds pod^{-1}. *I. parviflora* pods contained a mean (± SE) seed set of 3.03 ± 0.32.

I. glandulifera showed a higher mean (± SE) seed mass of 0.0149 ± 0.0009 grams, followed by the native *I. noli-tangere* of 0.0092 ± 0.0003 g and by *I. parviflora* of 0.0066 ± 0.0002 g. *I. balfourii* had the smallest seed mass of 0.0042 ± 0.0002 g (Plate 2.1 and Figure 2.2).

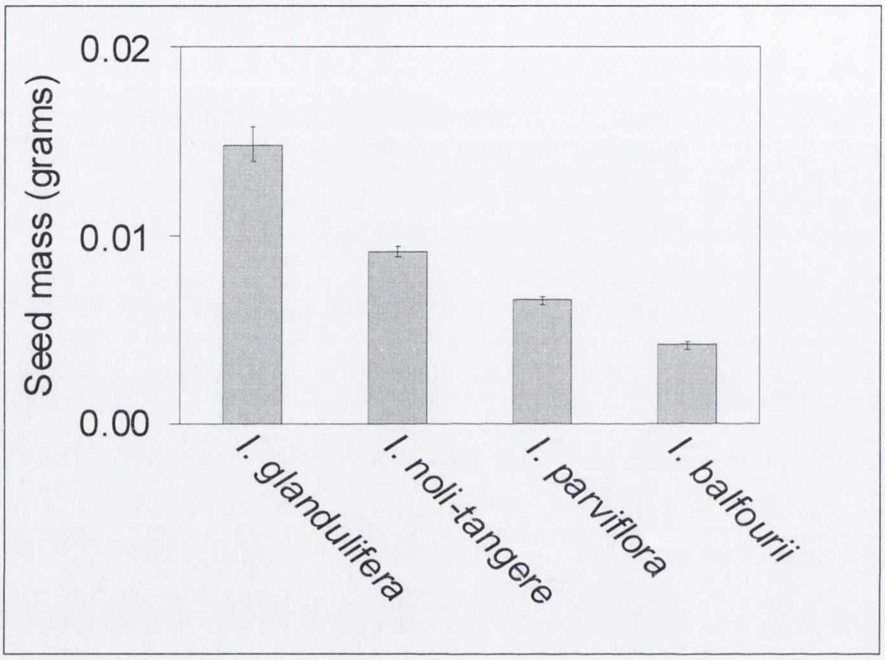

Figure 2.2 Mean seeds mass ± SE for eight population of *I. glandulifera* (n = 3), three population of *I. noli-tangere* (n = 3), five population of *I. parviflora* (n = 3) and two population of *I. balfourii* (n = 8).

Germination and stratification

Comparing seed germination rates of *I. glandulifera*, *I. balfourii* and *I. parviflora* from plants from Trinity College Botanic Garden, there were highly significant differences among species and among stratification treatment and no interaction between them. Germination rates appear consistently higher in *I. balfourii* than in *I. glandulifera* but the pair-wise comparisons faild to detect statistical significant difference. The pair-wise comparisons showed significantly higher germination rates in *I. glandulifera* and in *I balfourii* than in *I. parviflora*. No significant difference was found between *I. glandulifera* and *I. balfourii* (Table 2.2 and Figure 2.3).

Table 2.2 Comparisons in percentage of final germination. Significant values are in bold. Pair-wise comparisons among species and among treatment. Tamhane's *post-hoc* test, that does not assume homoscedasticity, was used for pair-wise comparisons. *I. g.* = *I. glandulifera*, *I. p.* = *I. parviflora* and *I. b.* = *I. balfourii*. * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$.

I. glandulifera, I. parviflora, I. balfourii	d.f.	F	p
Species	2, 6	**57.758**	**< 0.001**
Stratification	3, 198	**196.653**	**< 0.001**
Interaction (Sp.X St.)	6, 198	**50.821**	**< 0.001**
Plant (species)	6, 198	**8.276**	**< 0.001**

Pair-wise comparisons (mean differences (±SE))					
Species		Treatment			
I.b. – I.g.	0.113±0.0556	90 – 70 days	-0.021±0.071	70 - 50 days	0.105±0.0670
I.b. – I.p.	**0.575±0.040*****	90 – 50 days	0.083±0.069	70 - 0 days	**0.484±0.051*****
I.g. – I.p.	**0.463±0.039 *****	90 - 0 days	**0.4623±0.050*****	50 - 0 days	**0.379±0.048*****

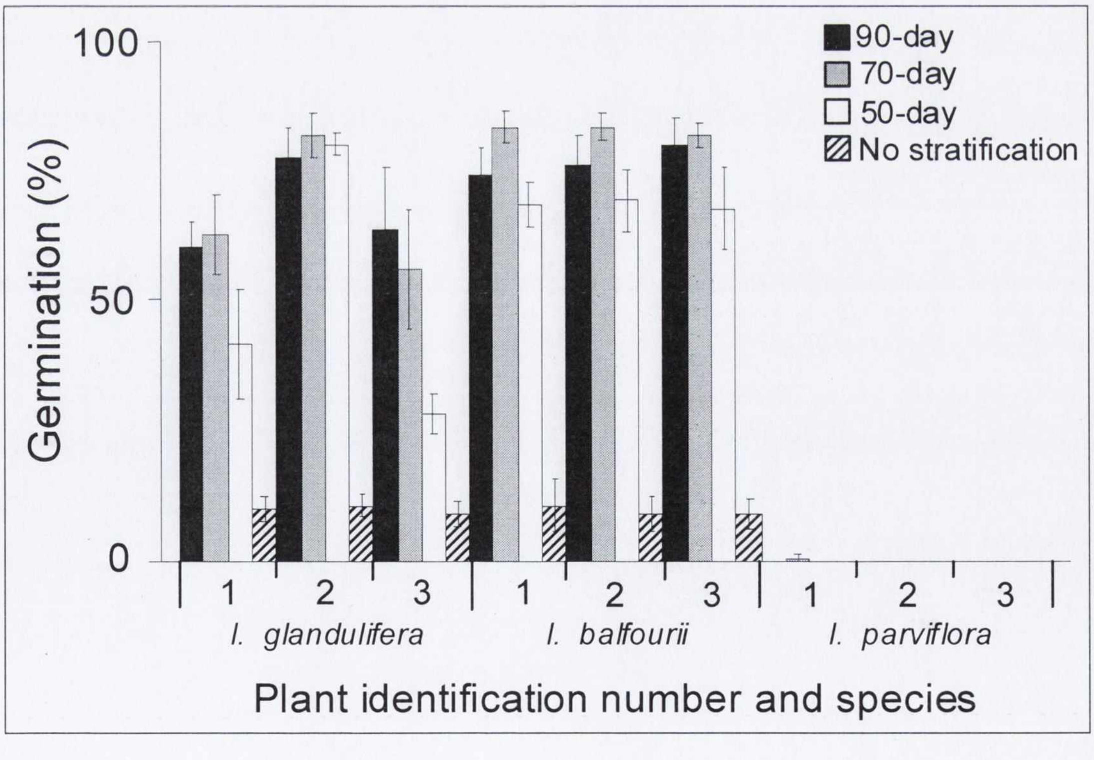

Figure 2.3 Mean ± SE of the percentage of final germination. Comparison among 3 plants of *I. glandulifera,* 3 plants of *I. balfourii* and 3 plants of *I. parviflora*. "Ninety day", "70 day", "50 day" and "No stratification" refer to the stratification treatment.

Pair-wise comparisons between stratification treatments showed no difference between 90 and 70 and 50 day cold period. Germination rate was significantly lower when seeds did not receive any cold period than for seeds receiving any of the stratified treatments.

By comparing germination percentages in populations of *I. glandulifera* and in populations of *I. parviflora*, I again found highly significant differences due to the effects of species, stratification period and population within species. The *post-hoc* test failed to resolve differences between pairs of treatments. No significant interaction between species and stratification was found (Table 2.3).

Table 2.3 Comparisons in percentage of final germination. Significant values are in bold.

***I. glandulifera* and *I. parviflora* populations**	d.f.	F	p
Species	1, 7.027	**41.753**	**< 0.001**
Stratification	2, 68	**7.148**	**0.002**
Interaction (Sp.X St.)	2, 68	1.021	0.366
Provenience (species)	7, 68	**19.463**	**< 0.001**

I. glandulifera had a significantly higher germination rate than *I. parviflora* (Figure 2.4).

Among *I. glandulifera* populations, lower germination occurred in the two non-wild populations, from France and Trinity College Botanic Garden. The other populations did not differ significantly and they showed germination percentages of, on average, between 89% and 100% (Figure 2.4). *I. parviflora* seeds from Dublin did not germinate. The other two populations of this species had a higher germination rate when they received the longest stratification period (Figure 2.4).

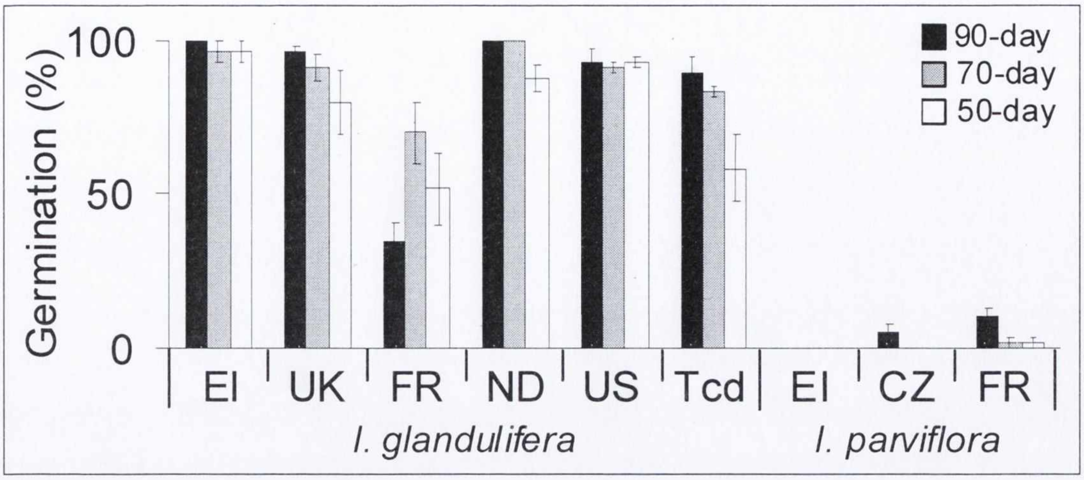

Figure 2.4 Mean ± SE of the percentage of final germination. Comparison among 6 populations of *I. glandulifera* (EI = Dublin, Ireland; UK = Edinburgh, UK; FR = France; ND = Netherlands; US = United States; Tcd = Trinity College Botanic Garden) and 3 population of *I. parviflora* (EI = Dublin, Ireland; CZ = Czech Republic and FR = France. "Ninety day", "70 day", and "50 day" refer to the stratification treatment.

Germination and burial

Germination patterns of the three species at the two depth treatments are shown in Figure 2.5.

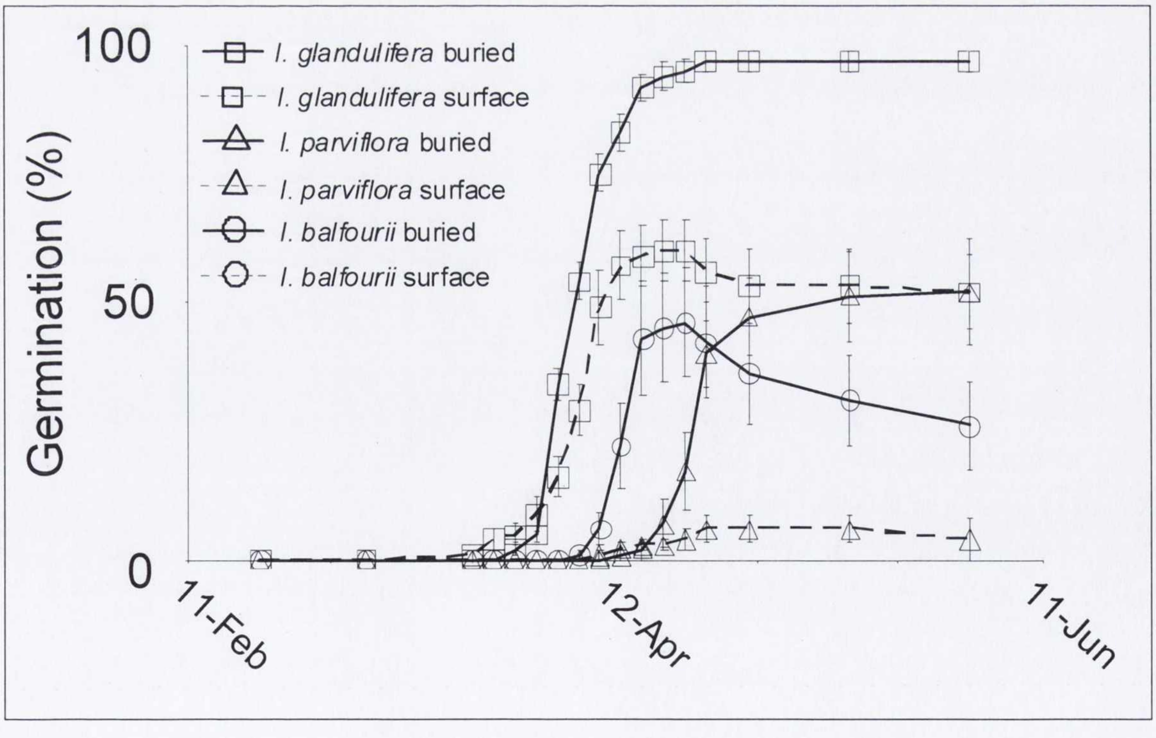

Figure 2.5 Mean ± SE of cumulative seed germination percentage per pot in *I. glandulifera, I. parviflora* and *I. balfourii*. Seeds were sown at 1 ÷ 2 cm depth and on soil surface.

I. glandulifera seeds sown between 1 and 2 cm deep in the soil had the highest germination rate. *I. parviflora* seeds, germinated at the same depth, reached a germination rate of around 50%. Seeds of *I. parviflora* started to germinate much later than I. *glandulifera* seeds. At the same soil depth *I. balfourii* had a germination rate that almost reached 50%, but this species showed the highest seedling mortality. All species had a lower germination rate when seeds were sown on the soil surface. Although no germination occurred in *I. balfourii*, *I. glandulifera* reached a germination rate of over 50%. However seedling mortality was higher in this species.

Comparing final germination percentage and seedling mortality percentage, there was a highly significant difference among species and between depth treatments (Table 2.4).

Table 2.4 Comparisons in percentage of final germination, percentage of seedling mortality and day of first seed germinated recorded among species (*I. g.* = *I. glandulifera*, *I. p.* = *I. parviflora* and *I.b.* = *I. balfourii*). * = p < 0.05; ** = p < 0.01; *** = p < 0.001.

Germination	d.f.	F	p
Species	2, 24	**42.502**	**< 0.001**
Depth	1, 24	**67.274**	**< 0.001**
Interaction (Sp. – D)	2, 24	0.324	0.643
Pair-wise comparisons	*I. g. –I. p.*	*I. g. –I. b.*	*I. p. –I. b.*
(mean difference (± SE))	**0.480±0.066*** **	**0.560±0.066*** **	0.080±0.066
Mortality	**d.f.**	**F**	**p**
Species	2, 24	**7.458**	**0.004**
Depth	1, 24	**12.119**	**0.002**
Interaction (Sp. – D)	1, 24	**5.162**	**0.035**
First day of germination	**d.f.**	**F**	**p**
Species	2, 24	**44.495**	**0.000**
Depth	1, 24	2.304	0.145
Interaction (Sp. – D)	1, 24	0.000	1.000
Pair-wise comparisons	*I. g. –I. p.*	*I. g. –I. b.*	*I. p. –I. b.*
(mean difference (± SE))	**-18.767±1.970*** **	**-12.300±1.970*** **	**6.467±1.970** **

I. glandulifera showed the highest germination percentage (Figure 2.5). All species had significantly higher germination rates and significantly lower mortality when seeds were sown between 1 and 2 cm deep in the soil. *I. glandulifera* and *I. parviflora* had lower mortality when seeds were buried. Seedling mortality could not be calculated in *I. balfourii*'s seedlings on soil surface because no germination occurred for this species in this treatment (Figure 2.6).

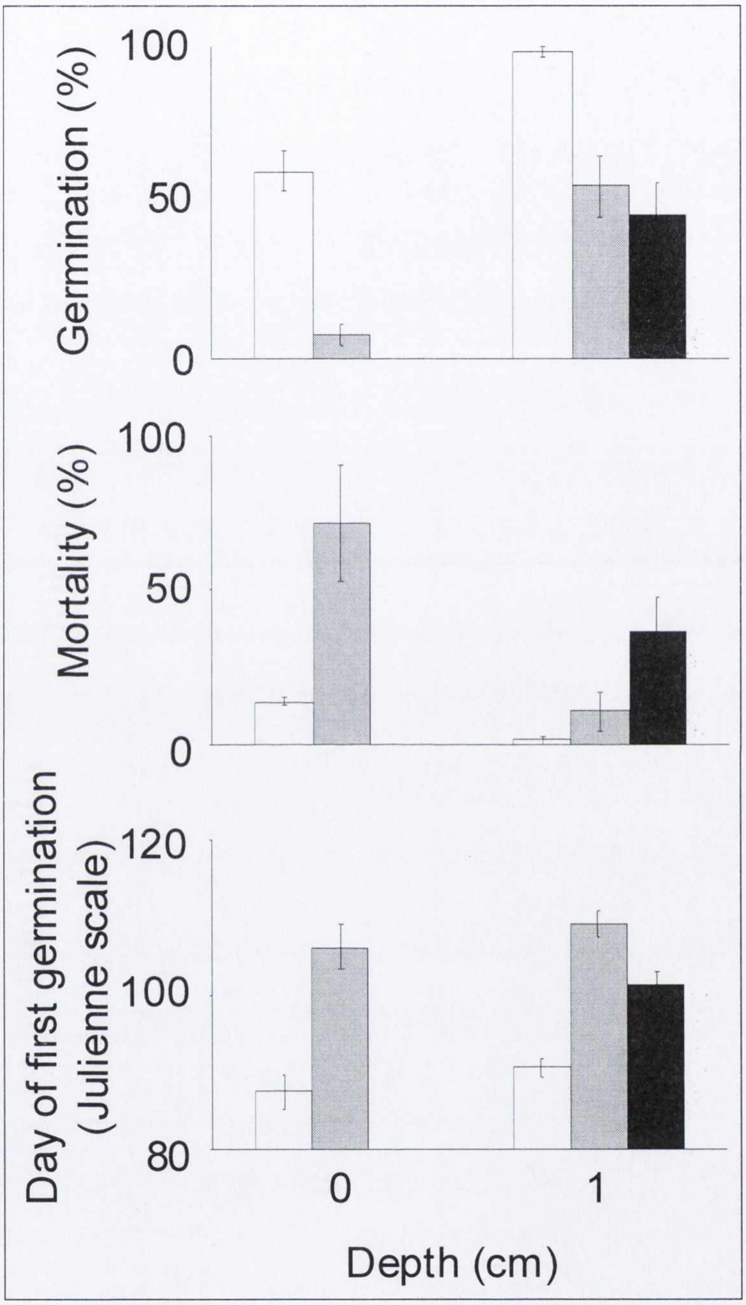

Figure 2.6 Total seed germination, mortality and day of first germinated seed recorded in *I. glandulifera* (white), *I. parviflora* (grey) and *I. balfourii* (black). Seeds were sown at 1 cm depth and at 0 cm depth on soil surface.

The day of first seed emergence differed significantly among species. *I. glandulifera* started to germinate significantly earlier than the other species at both depths. *I. balfourii,* when sown 1 cm deep in the soil, started to germinate significantly earlier than *I. parviflora*. No significant difference was found between depth treatments in terms of day of first seed emergence.

2.4 DISCUSSION

This study focused on seed germination and seedling survival of three introduced *Impatiens* species. All *Impatiens* species introduced in Europe and Ireland (along with the native *I. noli-tangere*) are annuals, and thus seed ecology and early stages of growth have a primary importance in the establishment of and invasion dynamics of these species. High germination rates have not always been associated with the process of colonizing new open areas, and consequently with the invasion success of a species, but in the case of closely related species or genotypes, the invasives usually outperform the non-invasive in reproductive capacity and germination performances (Radford and Cousens, 2000; Erfmeier and Bruelheide, 2005).

Reproductive capacity

The mean number of seeds per pod in the open pollinated plants was consistent with the values in the hand pollinated plants. For *I. glandulifera*, Beerling and Perris (1993) reported a higher number of seeds (5 – 7 seeds pod^{-1}) and suggested the number of seeds per plant decreases with an increase in plant density. Nienhuis and Stout (2009) also recorded a much higher seed production (6 – 9 seeds pod^{-1}) in wild populations of *I. glandulifera* in Ireland, but Lopezaraiza-Mikel (2006) in open-pollinated *I. glandulifera* plants in southwest England, found a lower seed set of 4.9 ± 0.4 seeds pod^{-1} . The lower number of seeds per pod in this study, compared to the others, may be due to the fact that measurements were made on plants grown in pots, not on wild populations.

I. balfourii showed the smallest seed mass and the highest seed number per pod, but Perrins *et al.* (1993), who estimated the total seed productivity in *I. balfourii*, *I. parviflora* and *I. glandulifera*, found that the number of pods per plant was much lower *in I. balfourii* than in *I. glandulifera* and *I. parviflora*. Additionally, when measuring the number of seeds plant^{-1}, they observed the lowest total seed set per plant in *I. balfourii* and the highest in *I. glandulifera*. The superior reproductive capacity in *I. glandulifera*, which produces larger seeds and larger numbers of seeds compared to the other two *Impatiens*, is assumed to contribute to *I. glandulifera's* invasive capacity.

Germination

Seeds used in the current study were all collected approximately 7 months before they were germinated. Consequently, seed age should not have had an influence on germination rate in particular because *I. glandulifera* and *I. parviflora* are reported to have non-persistent seed banks (Perglová *et al.*, 2009), which is why I selected seeds less than one year old.

In this study, the most invasive species, *I. glandulifera*, was the one that showed overall the highest germination and seedling emergence rates and the lowest mortality rate. These results are consistent with the findings of Perrins *et al.* (1993) and Perglová *et al.* (2009). However, while *I. glandulifera* shows higher germination rates than *I. parviflora*, they were not significantly different to *I. balfourii* in the laboratory conditions.

I. parviflora showed very low germination in the laboratory comparisons. Germination conditions in this study might not have been optimal for *I. parviflora* as this species, which is invasive in central and eastern Europe, experiences winter temperatures which often fall below 0ºC for long periods (Coombe, 1956; Pysek *et al.*, 2002). This supposition is confirmed by the fact that *I. parviflora* reached a much higher germination rate in the common-garden experiment where seeds experienced colder temperatures. The winter 2009-2010 was particularly cold in Ireland and temperatures during the experiment fell below -6ºC.

The non-invasive *I. balfourii,* showed a high germination rate, similar to *I. glandulifera*, in the laboratory comparison, but this was not repeated in the garden experiment where this species showed the lowest germination and the highest mortality rates. Seed performance in a common-garden experiment might differ considerably from seed performance in natural conditions since, in nature, seeds are exposed to a greater variety of factors. The effects of such factors and of interactions between factors can not all be simultaneosly considered and reproduced by garden experiments. However, the results of common-garden experiments are likely to be more realistic than laboratory results.

Stratification

The length of the cold period administered to the seeds in the laboratory experiment had a significant effect on the final germination rate. Lack of stratification drastically reduced the germination percentage from an average of 56% – 77% (70 and 90 days stratification) to an average of under 0.03% in *I. glandulifera* and from 71% – 87% to under 0.05% in *I. balfourii*. Increasing the cold period from 50 to 70 or 90 days also resulted in an increase in germination, at least in some populations of both species. Mumford (1998) concluded that a period of 40 days is enough to break dormancy in *I. glandulifera* seeds stored in conditions similar to those used here. The data suggests that germination success of *I. glandulifera* and *I. balfourii* increases with the length of the cold period (from 50-70 days), up to a maximum threshold after which increasing the length of the cold period has little effect (there was no difference between 70 and 90 days). However, these trends were not statistically significant and would require further experimentation. Under laboratory conditions, although *I. parviflora* showed very low germination rates overall, the highest rate occurred following the longest cold period which suggests that *I. parviflora* needs a longer cold stratification than the other two species.

Depth

When seeds were sown between 1 and 2 cm under the soil surface, *I. balfourii* showed a germination rate of almost 50% while seeds that were placed on the soil surface did not germinate. However, they did not decay either. Seeds may avoid a fatal germination in adverse environmental conditions and wait until more favourable conditions are available to germinate via a mechanism of secondary dormancy (Crocker, 1916; Vleeshouwers *et al.*, 1995; Benvenuti *et al.*, 2001). *I. balfourii* has been much less investigated than its more invasive congener *I. glandulifera* and *I. parviflora* since it is not considered an aggressive unwanted weed (yet). Information on the characteristics and ecology of this species is therefore more difficult to find in the published literature and, to my knowledge, no studies of its dormancy characteristics and of the persistence of its seed bank have been conducted prior to this. Only a few recent studies suggest this species as a potential invader (e.g. Adamowsky, 2009).

All three species showed lower germination when seeds were on the soil surface. This finding would suggest that light might be a factor involved in the mechanism of secondary dormancy to avoid germination when seeds are situated on the soil surface in the presence of light. However, from previous personal observations, both *I. balfourii* and *I. glandulifera* were found to germinate in full light in the laboratory. Bewley and Black (1994) reported interaction between temperature and light. Light requirements for germination can be dependent on temperature, and as a consequence, seeds of some species might be dormant either in the dark or in the light only below certain temperatures. It has been observed that also the Red-Far Red ratio (R:FR) can be responsible for breaking dormancy. Red wavelengths are more effective in breaking seed dormancy, while far red light has the ability to re-induce dormancy (Bewley and Black, 1994). However, further investigations, in experiments with controlled light conditions, are necessary to be able to understand the light requirements of these species.

Moisture was not controlled in this experiment but it was assumed that in the soil it remained close to field capacity. Soil moisture deficit may reduce germination. Yet more experiments would be needed to investigate in detail the interactions between seed germination and the soil environment, with the aim of understanding how and why seed depth affects germination. Soil relocation or seed coverage by organic matter might favour germination in these three invasive species, even if *I. glandulifera* seems to be the less affected. This species, in fact, still shows a germination rate of over 50% and does not greatly increase mortality when the seeds are placed on the soil surface. In the management and control of weeds, burial of seeds by means of tillage practices has been used to reduce germination potential (Gardarin *et al.*, 2010). The current results suggest that, in the case of *Impatiens*, burying the seed to depths of about 2 cm would not reduce the germination and the invasive potential of these species; on the contrary it may promote it.

Mortality

Seedling mortality (or survival) is a relevant measure of the fitness of early-stage seedlings and it contributes to explaining the subsistence and spread of invasive species. Mortality rate in my experiment was affected, in the same way as germination, by the seed depth. *I. glandulifera* and *I. parviflora*, which emerged when seeds were on the soil surface, showed higher mortality for this treatment than for buried seeds. This may possibly be due to an easier penetration and anchorage of the roots in the soil. The general conclusions and implications are the same as for the germination rate. Likewise in this case more experiments are needed to understand the effects of seed depth in the soil on seedling mortality.

Species showed differences in mortality. *I. glandulifera* outperformed the other species showing nearly no mortality in buried seeds and significantly lower mortality then *I. parviflora* on the soil surface. Predation is a factor that was not considered in this experiment but that could possibly have affected seedling mortality differently in each species.

Germination time

In these experiments, seedlings of *I. glandulifera* emerged earlier than seedlings of the other species. Perglová *et al.* (2009) found the same results in laboratory conditions. Their results were not confirmed by their common-garden experiment where all the species emerged simultaneously. In their field study in the UK, Perrins *et al.* (1993) also observed a contemporaneous emergence of seedlings of different *Impatiens* species. Perglová *et al.* (2009) attributed the later emergence of *I. glandulifera* to an exceptionally cold winter and concluded that less severe weather conditions would have resulted in an early emergence of *I. glandulifera*. In the present study, conducted in Ireland, temperatures during the experiment were lower than the seasonal averages but certainly higher in comparison to the Czech Republic winter temperature. In addition spring 2010 temperatures were mild in Ireland. *I. glandulifera* might have taken advantage of the favourable weather conditions which resulted in an early emergence of seedling of this species. An early seedling emergence would confer on *I. glandulifera* an advantage in the competition for colonizing open areas even if it would expose its seedling to the risk of frost damage.

2.5 CONCLUSIONS

Higher seed mass, higher seed production (Perrins *et al.*, 1993), lower mortality, early germination, shorter stratification period (than *I. parviflora*) and the fact that *I. glandulifera* seeds seem to germinate under a wider range of environmental conditions are characteristics that appear to contribute to *I. glandulifera* having greater invasion potential.

The lack of success of *I. balfourii* as an invasive species in Europe may be explained by the co-occurrence of several factors. Historically, this species was introduced much later than *I. glandulifera* and *I. parviflora* and consequentially it started its spread more recently (Adamowski, 2009). Moreover, *I. glandulifera* and *I. parviflora* were extensively cultivated in many countries, while *I. balfourii* was not, and did not benefit from the same propagule pressure (Perrins *et al.*, 1993; Adamowski, 2009). The results of the current laboratory and common-garden experiments could support the hypothesis that a lower seed production (Perrins *et al.*, 1993) and a higher mortality contribute to the lack of success of this species. As opposed to that, *I. balfourii* showed a very high germination rate in the laboratory suggesting that, in the right conditions, this species has the potential to produce a great number of seedlings.

In Britain and Ireland the lack of success of *I. balfourii* as an invader may be due to the cooler weather (which might also have contributed to seedling mortality) but, with climate warming, *I. balfourii* does not appear to require a long stratification, and could possibly experience the favourable conditions required to invade more northern regions of Europe.

I. parviflora is an introduced species able to sustain populations without human intervention but which has a very limited spread in Ireland (Reynolds, 2002). From the results reported here it seems that one of the factors that may contribute to limiting the invasion of this species in Ireland is the stratification required to break dormancy. As shown by these experiments, *I. parviflora* germinated better outside, where it was exposed to lower temperatures (but still only about the 50% of the seeds germinated). Additionally this species is invasive in Central and Eastern Europe where winters are colder than in Ireland.

However, the laboratory and common-garden experiments did not only differ in terms of temperature conditions. Many more factors besides reproductive biology must be considered before it is possible to bring forward a firm explanation of why this species, which is extremely widespread in some regions, has not, as yet, spread in Ireland. In addition, habitat characteristics and water availability in particular might affect the capacity of spread of these three water-requiring species.

3 Characterisation of ecophysiological traits of introduced *Impatiens* species

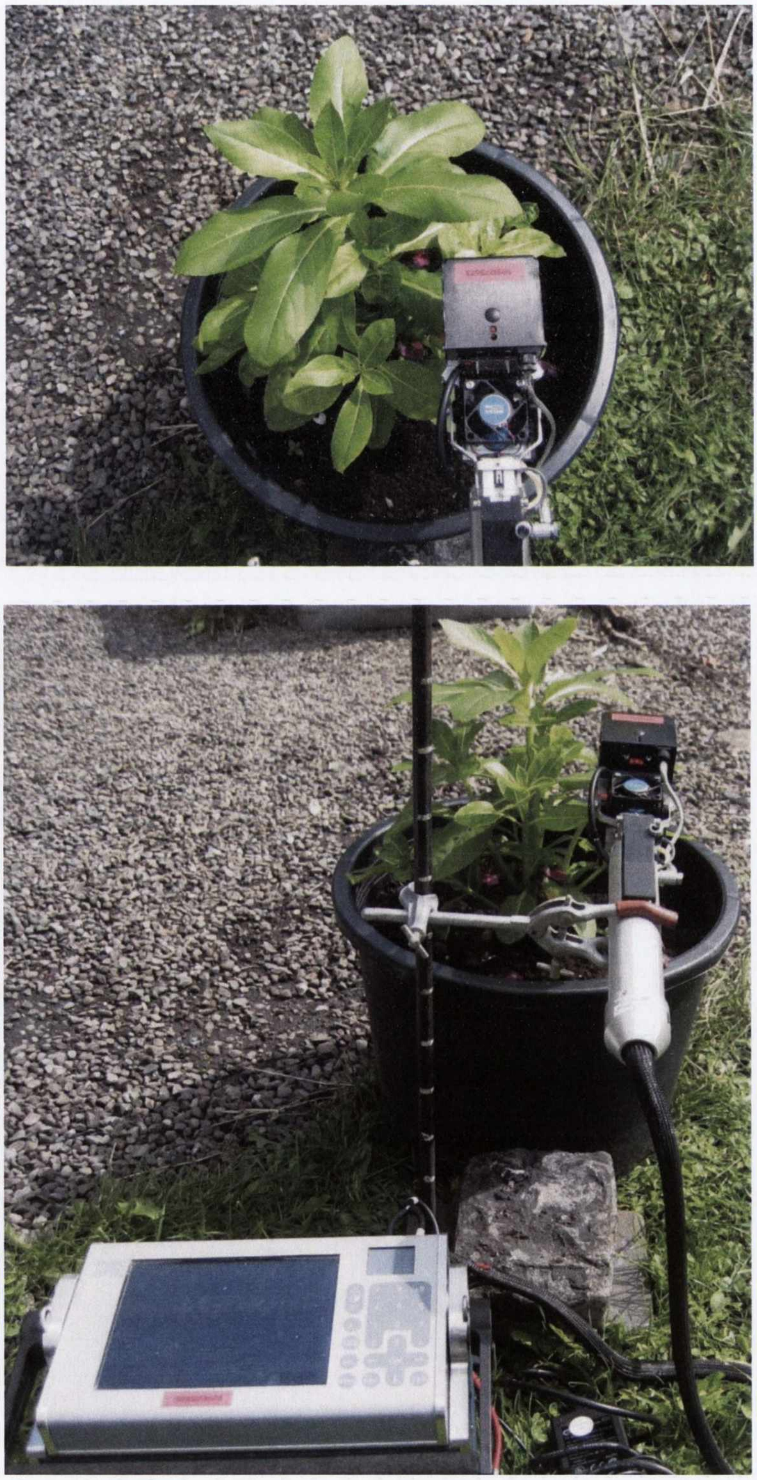

Plate 3.1 Measurement of photosynthesis using an infrared gas analyzer (Ciras 2, PP System).

SUMMARY

Biomass allocation parameters and leaf attributes are ecophysiological traits that can discriminate between plant species which exploit different light resource environments. Disturbed and high-light environments provide easy access for invasive species which exhibit high growth rates, light capture and light use efficiency. A comparison of these characteristics among invasive and non-invasive species could help to predict a species' invasive potential and identify which habitat would be most susceptible to its invasion.

I examined ecophysiological traits of species within the genus *Impatiens* that vary in their ecological needs and in their invasive status. I compared growth and photosynthetic characteristics of three introduced species in a common-garden experiment: *Impatiens glandulifera,* one of the most dominant invasive plants in Ireland and Europe; *I. parviflora*, a species naturalised in Ireland and invasive in shady forests in central Europe; and *I. balfourii*, absent from Ireland but established in a few European countries.

I observed differences in plant allometry and leaf characteristics between the light-demanding *I. glandulifera* and the shade-tolerant *I. parviflora*. *I. glandulifera* had the highest growth rate, whilst *I. parviflora* displayed the highest leaf area ratio and leaf weight ratio.

The selected ecophysiological traits illustrate the contrasting light interception characteristics and light-use strategies of *I. glandulifera* and *I. parviflora*. Since *I. balfourii* was able to grow, flower and produce seeds under Irish climatic conditions, and on the basis of the measured performance, I suggest that *I. balfourii* has the potential to become invasive, in particular in disturbed high-light environments.

3.1 INTRODUCTION

Organisms introduced into novel environments as a result of human activities are variously referred to as non-native, introduced, exotic or alien species (Richardson et al., 2000). They must possess certain ecophysiological characteristics in order to become established (i.e. able to sustain populations without human intervention), and subsequently to invade (i.e. spread widely and rapidly, with environmental and economic effect on the invaded ecosystem) (Richardson et al., 2000). Since introduced invasive species can have negative impacts on both global economy and ecosystems (EEA, 2003), there have been numerous attempts to identify biological characteristics that promote invasion (e.g. Rejmánek 1996, 2000, Milbau and Stout 2008). Ecophysiological traits that promote invasion are likely to be dependent both on the characteristics of the introduced species and on the habitat into which it is introduced (Rejmanek, 1996), as well as other variables related to the introduction event (Milbau and Stout 2008). High growth rates, linked to allocation of a greater proportion of biomass to leaf area and high net photosynthetic rates which increase light capture and efficiency of light use in photosynthesis, are expected to confer success on invaders (Grime and Hunt, 1975; Pattison *et al.*, 1998; Baruch and Goldstein, 1999; Durand and Goldstein, 2001a; b; McDowell, 2002; Feng *et al.*, 2007b; Zheng *et al.*, 2009). Highly disturbed environments are often the entry point for introduced invasive species, which exhibit high growth rates, high efficiency of light capture and efficiency of light use. (Grime and Hunt, 1975; Pattison *et al.*, 1998; Baruch and Goldstein, 1999; Durand and Goldstein, 2001a; b; McDowell, 2002; Feng *et al.*, 2007b; Zheng *et al.*, 2009).

Several comparative studies of ecophysiological characteristics of invasive plants have compared introduced invasive species and native species (Pattison *et al.*, 1998; Baruch and Goldstein, 1999; Durand and Goldstein, 2001b; a; Nagel and Griffin, 2001; Daehler, 2003; Bossdorf *et al.*, 2005; Feng *et al.*, 2007b; Kudoh *et al.*, 2007; Leishman *et al.*, 2007; Feng, 2008; Funk, 2008; McAlpine *et al.*, 2008). This approach permits the investigation of invasion mechanisms, since invasive exotics directly compete with natives, but does not facilitate the identification of traits which make an introduced species a potential invader (Nijs *et al.*, 2004). Fewer studies have compared invasive with less- or non-invasive introduced species (but see (Bossdorf *et al.*, 2005; Burns and Winn, 2006; Muth and Pigliucci, 2006; Feng *et al.*, 2007a; Feng *et al.*, 2007c; McAlpine *et al.*, 2008). Many introduced species are able to successfully establish but do not outperform native species and therefore do not become invasive (Rejmánek, 2000). Comparing successful and unsuccessful introduced invaders may therefore represent a more appropriate approach to identifying the traits that determine variation in invasiveness (Milbau and Nijs, 2004; Nijs *et al.*, 2004; Milbau and Stout, 2008). This is not straightforward however, and problems arise because of the criteria used to characterize invasive status: invasiveness is a continuous variable, not a discrete one, which changes through time (Muth and Pigliucci, 2006), and is strictly related to specific geographic areas so that a species may occur as invasive in a particular region and only as casual elsewhere.

I chose to compare congeneric species which vary in their invasive status in Ireland, to minimize trait differences associated with inter-specific variation in unrelated species (McDowell, 2002; Burns and Winn, 2006; Muth and Pigliucci, 2006; Richards *et al.*, 2006; Funk, 2008). The genus *Impatiens* contains a large number of ornamental plants, and horticulture is responsible for continuously distributing new species of *Impatiens* (Jerardo, 2005; Tabak and von Wettberg, 2008). Therefore, understanding the potential invasiveness and the target habitat of a species in the genus becomes extremely important in preventing the invasion of new habitats (Tabak and von Wettberg, 2008). I compared germination rate, growth, biomass-allocation parameters and photosynthetic characteristics of three species in a single genus: *Impatiens balfourii*, *I. glandulifera* and *I. parviflora*.

Impatiens glandulifera, a large annual plant originally introduced to Europe as an ornamental and nectar-producing plant, is currently one of the most dominant invasive plant species in Britain, Ireland and continental Europe (Perrins *et al.*, 1993; Pyšek and Prach, 1995). *I. parviflora* is widely naturalized in Europe, including Ireland, and invasive in the damp shady forests of Central and Northern Europe (Chmura and Sierka, 2007). In addition, another introduced species was used in this study: *I. balfourii*, which is also naturalized in disturbed habitats in central and southern Europe (Moore, 1968) but is not considered to be invasive, and not reported as established in Britain or Ireland (DAISIE, 2008). Both *I. balfourii* and *I. glandulifera* are from the Himalayas, although *I. glandulifera* can grow at higher altitudes than *I. balfourii* (Nasir, 1980; Polunin and Stainton, 1984) which suggests that this species may be better adapted to lower temperatures. Furthermore, the distribution of their introduced ranges suggests that *I. glandulifera* is able to spread and invade further north while the *I. balfourii* range is limited to the southern and central parts of Europe (DAISIE, 2008).

In my study, I grew the three species in a common-garden experiment and compared 11 traits related to plant growth and allometry and leaf physiology. The aims were (1) to detect the differences among species in biomass allocation related to light capture, (2) to investigate leaf ecophysiological differences (rate of respiration, photosynthetic light saturation point, light compensation point) and (3) to determine if the measured ecophysiological traits are indicative of fitness and to attempt to relate performances in these traits to species-invasiveness.

3.2 MATERIALS AND METHODS

3.2.1 Establishment

Seeds of *I. glandulifera*, *I. balfourii* and *I. parviflora*, received from several botanic gardens across Europe (see Appendix 3.1), were stratified at 4 °C for about 50 days to break dormancy (Mumford, 1988) and then germinated on moist filter paper in 90 mm diameter Petri dishes in a growth chamber under optimal conditions for *Impatiens* species: 25°C during the day and 19°C during night (Elias, 1975; Herrera and Alizaga, 1995) with a 12 hour photoperiod (Souza and Pereira, 1994). Germinated seeds were removed daily and placed in 76 mm diameter pots filled with Shamrock Ready Mix (pH range between 5.2 and 5.7, nutrients added: N = 300, P = 90, K = 330 mg/l and fertilizer with 50% slow release nitrogen). Since *I. parviflora* showed a low germination rate, additional plants of this species were collected from a wild population in the grounds of Trinity College in Dublin. Plants were moved to a greenhouse at Trinity College Botanic Garden in Dublin (53°18'44"N, 6°15'34"W), kept inside for 3 weeks to avoid frost damage to the seedlings, and then moved outside on the first week of June 2008 and re-potted in 20 litre pots filled with the same growing mix. One seedling was planted per pot and a total of 14 plants per species were potted. Pots were randomly positioned approximately 1 m apart on gravel, in an open, sunny area and watered daily to maintain optimum soil moisture. Temperature data for the growing period were obtained from the 'Daily dataset of 20th-century surface air temperature and precipitation series for the European Climate Assessment' (Tank, 2002). Maximum and minimum daily temperatures for summer 2008 are reported in Figure 3.1. Temperatures were measured by a weather station in Phoenix Park, Dublin (53°21'50"N, 06°19'09"W).

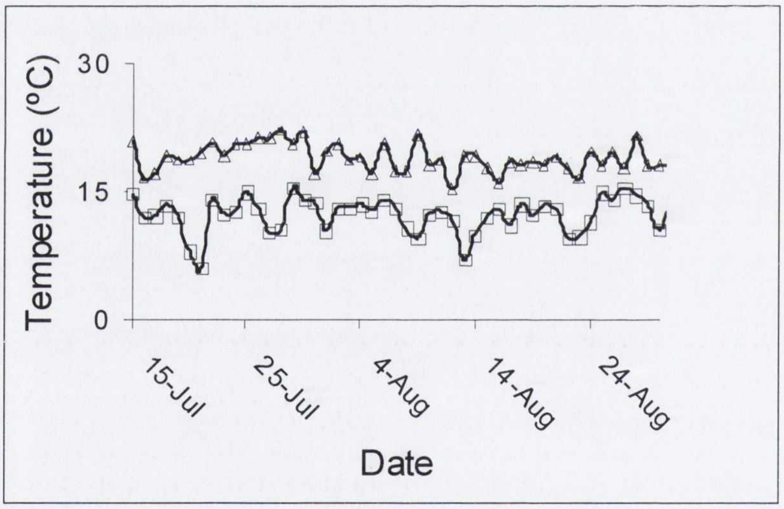

Figure 3.1 Daily minimum (squares) and maximum (triangles) temperatures recorded for July and August 2008 by Phoenix Park weather station in Dublin.

3.2.2 Plant Growth Analysis

Stem length was measured as the distance between soil surface and apical growing tip on all 14 plants per species. Measurements were taken weekly for 8 weeks, from the beginning of July, when seedlings were 6 weeks old, to the end of August 2008, when plants reached senescence. The relative stem growth rate (Gh) between each measurement was calculated according to Hunt (1990) as:

$$\textbf{(3.1)}\; Gh_1 = \frac{\ln H_2 - \ln H_1}{t_2 - t_1}$$

where H_2 and H_1 represents the stem length at sequential times t_1 and t_2 respectively.

At the end of the growth season (beginning of September), five randomly selected plants per species were harvested. Leaf area was scanned and measured with WinDIAS (Delta – T Devices) software for all leaves except senescent ones. Flowers and seeds were removed. Leaves and stems were separately dried at 80°C for two days. Roots were manually washed and dried in the same way. Dry leaves, stems and roots were weighed separately in order to determine leaf weight ratio (LWR), stem weight ratio (SWR) and root weight ratio (RWR), as the ratio between respectively leaf, stem and root weight, and total plant weight. Leaf area ratio (LAR) was calculated as the ratio between leaf area and total plant weight and specific leaf area (SLA) was calculated as the ratio between leaf area and leaf weight (Hunt, 1990) (Table 3.1). LAR, LWR, and SLA are related by the equation:

$$\textbf{(3.2)}\; LAR = SLA \times LWR$$

Table 3.1 The 11 traits compared among *I. glandulifera*, *I. balfourii* and *I. parviflora*.

Trait	Description	Units
Plant growth and allometry		
Gh	Growth rate in height	cm day^{-1}
LWR	Leaf weight ratio	g leaf g^{-1} plant
SWR	Stem weight ratio	g stem g^{-1} plant
RWR	Root weight ratio	g root g^{-1} plant
LAR	Leaf area ratio	m^2 leaf g^{-1} plant
Leaf physiology		
SLA	Specific leaf area	m^2 leaf g^{-1} leaf
A_{max}	Maximum photosynthetic rate	µmol CO_2 $m^{-2}s^{-1}$
Rd	Dark respiration rate	µmol CO_2 $m^{-2}s^{-1}$
ϕ	Apparent quantum yield	µmol CO_2 µmol PAR
LCP	Light compensation point	µmol photon $m^{-2}s^{-1}$
LSP	Light saturation point	µmol photon $m^{-2}s^{-1}$

3.2.3 Photosynthetic characteristics

The photosynthetic capacity of 10 individual plants per species was assessed using a CIRAS-2 portable infra-red gas analyzer (IRGA). Each measurement assessed the photosynthetic response to 9 levels of photosynthetically active radiation (PAR) (0, 50, 100, 200, 500, 800, 1200, 1600, 1800 µmol m^{-2} s^{-1}) (Joesting *et al.*, 2007) with a constant CO_2 concentration set at 380 ppm, and ambient air temperatures that ranged between 17 and 25 °C during measurements. To follow the leaf developmental phases, photosynthetic capacity was assessed on the same leaf, in 10 plants per species, weekly for 3 weeks.

Light response curves were fitted using *Photosyn Assistant* 1.2 (Parson and Ogstone, 1997). The net photosynthesis (A) in response to light level (Q) was described by a non-rectangular hyperbola where the initial slope of the curve represents the Apparent Quantum yield (ϕ). The light compensation point (LCP) and apparent respiration (Rd) were estimated from axis intercepts and the light saturated maximum rate of photosynthesis (A_{max}) was estimated as the upper asymptote of the curve (Parson and Ogstone, 1997). The value of the light saturation point (LSP) was calculated by extrapolating the initial linear function to its intersection with A_{max} (Walker, 1989; Parson and Ogstone, 1997).

An additional parameter, k, a convexity factor was required to describe the progressive rate of bending between the linear gradient and value of maximum photosynthetic rate (Parson and Ogstone, 1997). All of these parameters were determined by fitting data to the model function, expressed by the equation of Prioul and Chartier (1977) where:

$$\textbf{(3.3)}\ A = \frac{\phi Q + A_{\max} - \sqrt{(\phi Q + A_{\max})^2 - 4\phi Q k A_{\max}}}{2k} - Rd$$

The best fit for this curve to the experimental data was obtained by varying the parameters and minimizing the sum of squares (Parson and Ogstone, 1997).
Mass based values of whole plant photosynthesis where calculated for each species by multiplying the average of the maximum rate of photosynthesis, measured over the three weeks, and the average of leaf area ratio calculated for each species. Total standard deviation was calculated as the square-root of the sum of the squares of the ratio between each standard deviation and its corresponding mean.

3.2.4 Statistical analysis

Statistical analyses to compare measured traits among species were performed using SPSS 16.0. Normality and variance homogeneity of the data were tested with Kolmogorov-Smirnov and with Levene's tests respectively. Rd was arcsine transformed and LCP was Ln-transformed to obtain normality and homogeneity of variance.

Repeated measures analysis of variance was used when there was more than one measurement or derived value over the season (stem growth rate, maximum rate of photosynthesis, quantum yield, dark respiration rate, light compensation point, light saturation point). One way analysis of variance was used to compare means among species for single measurements over the season (leaf weight ratio, stems weight ratio, root weight ratio and leaf area ratio).

Bonferroni *post hoc* tests were used for species pairwise comparisons for all the measured traits with the exception of Gh and LAR where Tamhane's *post hoc* tests, that do not assume equal variance, were used.

3.3 RESULTS

Significant differences in stem growth rate were found among species and over time (Table 3.2). The stem growth rate was significantly higher in *I. glandulifera* than in the other species (Figure 3.2). *I. glandulifera* reached a growth rate of 3 cm day^{-1}. Significant differences were found between *I. glandulifera* and *I. parviflora*, and between *I. glandulifera* and *balfourii* (Table 3.2). The interaction between species and time was also highly significant. At the beginning of the season, *I. glandulifera* showed the fastest growth and it reached a maximum around the middle of July. A similar pattern was followed by *I. balfourii*. From the middle of July both the species had a reduced growth rate, and toward the end of the growing season, *I. balfourii* had a growth rate slightly higher than *I. glandulifera*. *I. parviflora* showed a different pattern from the other two species (Figure 3.2) having the lowest growth rate at the beginning of the season but by the end of the season its growth rate was the highest. Seasonal patterns of growth were different over a period of relatively stable air temperatures (Figure 3.1).

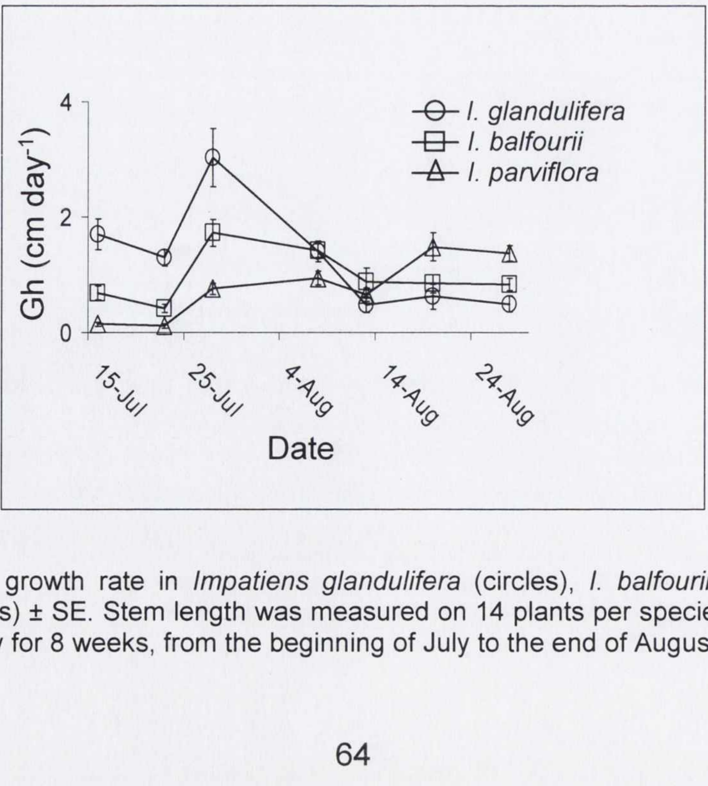

Figure 3.2 Stem growth rate in *Impatiens glandulifera* (circles), *I. balfourii* (squares) and *I. parviflora* (triangles) ± SE. Stem length was measured on 14 plants per species. Measurements were taken weekly for 8 weeks, from the beginning of July to the end of August 2008.

I. parviflora showed a significantly higher leaf weight ratio (LWR) than *I. glandulifera* (Figure 3.3 a). No significant differences were found between the other pairs. Similarly, *I. parviflora* showed a significantly higher leaf area ratio (LAR) than *I. glandulifera* (Figure 3.3 b). No significant differences were found among the other pairs. No significant differences in stem weight ratio (SWR), root weight ratio (RWR) or specific leaf area (SLA) were found among species (Figure 3.3 c-e).

Table 3.2 Significance of difference among species means and pairwise comparisons using Bonferroni *post hoc* test or Tamhane's *post hoc* test for Gh and LAR. I.g. = *Impatiens glandulifera*, I.b. = *Impatiens balfourii*, I.p. = *Impatiens parviflora*, *n. s.* = non-significant, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$. Numbers subscripted represent the degree of freedom.

Trait	**Species**	**Weeks**	**Species - Week**	**Pairwise comparisons**		
				I.g.- I.b.	**I.g.- I.p.**	**I.b. – I.p.**
Gh	$F_{2,39} = 12.603^{***}$	$F_{6,234} = 22.394^{***}$	$F_{12,234} = 7.664^{***}$	$p < 0.001$	$p = 0.011$	*n.s.*
LWR	$F_{2,12} = 5.305^{*}$			*n. s.*	$p = 0.016$	*n.s.*
SWR	*n. s.*					
RWR	*n. s.*					
LAR	$F_{2,12} = 4.636^{*}$			*n. s.*	$p = 0.045$	*n.s.*
SLA	*n. s.*					
A_{max}	$F_{2,27} = 11.315^{***}$	$F_{2,54} = 1.080$ *n. s.*	$F_{4,54} = 1.947$ *n. s.*	*n. s.*	$p < 0.001$	$p = 0.006$
Rd	$F_{2,27} = 21.302^{***}$	$F_{2,54} = 1.453$ *n. s.*	$F_{4,54} = 4.616^{**}$	*n. s.*	$p < 0.001$	$p < 0.001$
ϕ	$F_{2,27} = 8.022^{**}$	$F_{2,54} = 0.144$ *n. s.*	$F_{4,54} = 0.243$ *n. s.*	*n. s.*	$p = 0.002$	$p = 0 .002$
LCP	$F_{2,27} = 14.846^{***}$	$F_{2,54} = 1.140$ *n s.*	$F_{4,54} = 3.820^{**}$	*n. s.*	$p < 0.001$	$p < 0.001$
LSP	$F_{2,27} = 11.456^{***}$	$F_{2,54} = 1.263$ *n. s.*	$F_{4,54} = 3.154^{*}$	*n. s.*	$p < 0.001$	$p = 0.015$

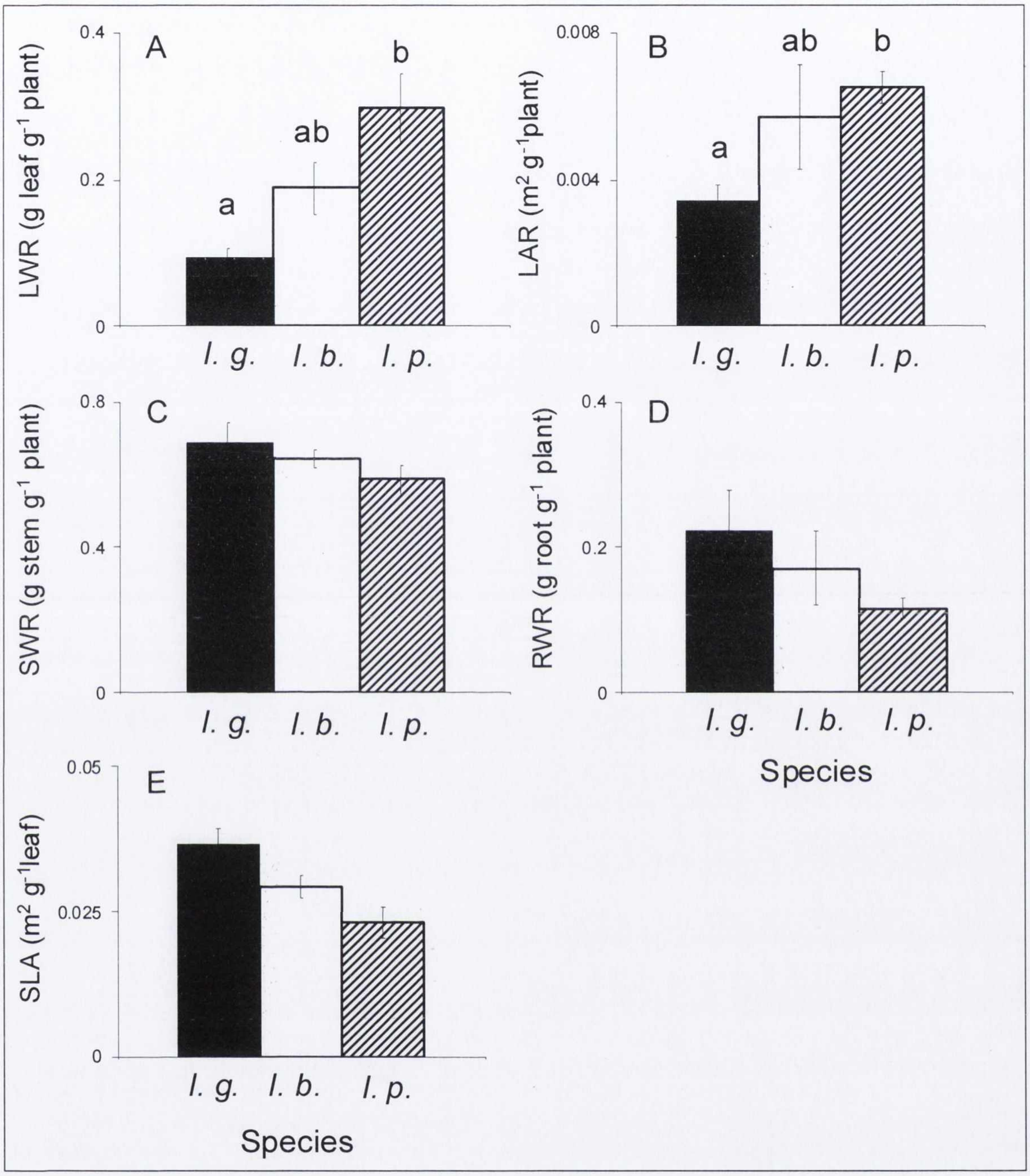

Figure 3.3 Species means ± SE for: A) final leaf weight ratio, B) final leaf area ratio, C) final stem weight ratio, D) final root weight ratio and E) final specific leaf area in *I. glandulifera (I.g.)*, *I. balfourii (I.b.)*, *I. parviflora (I.p.)*. These parameters were measured by harvesting five randomly selected plants per species at the end of the growth season. Different letters above columns in the same graph indicate differences among species (Bonferroni or Tamhane's test, $p < 0.05$).

Maximum rate of photosynthesis (A_{max}) was significantly higher in both *I. glandulifera* and *I. balfourii* than in *I. parviflora* (Table 3.2). However, when the maximum rate of photosynthesis was multipliy by the leaf area ratio, the estimate of mass based whole plant photosynthesis was very similar in *I. glandulifera* and *I. parviflora* and slightly higher in *I.balfourii* (mean ± SD = 0.074 ± 0.038 μmol g^{-1} s^{-1} for *I. glandulifera*; 0.082 ± 0.044 μmol g^{-1} s^{-1} for *I. parviflora* and 0.111 ± 0.075 μmol g^{-1} s^{-1} for *I. balfourii*).

Light response curves of the three *Impatiens* species in the second week of measurement are shown in Figure 3.4.

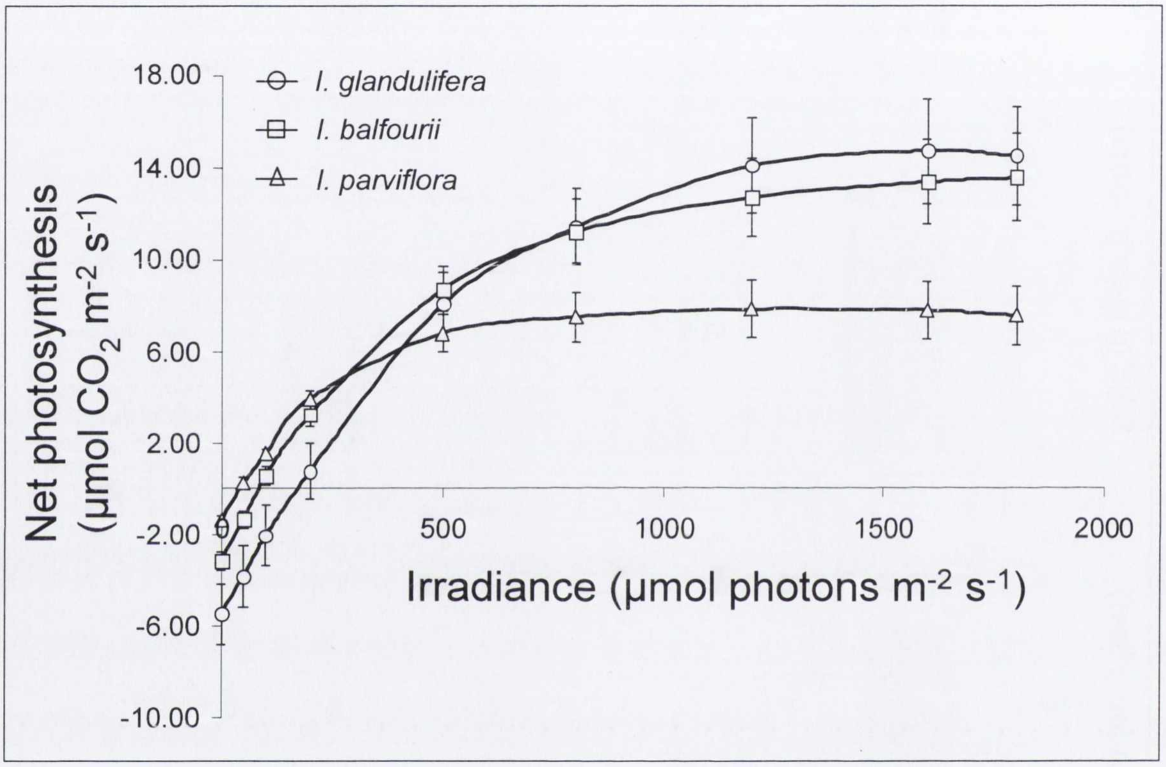

Figure 3.4 Light response curves for *I. glandulifera* (circles), *I. balfourii* (squares) and *I. parviflora* (triangles). The photosynthetic capacity of 10 individual plants per species was assessed at 9 levels of photon irradiance: 0, 50, 100, 200, 500, 800, 1200, 1600, 1800 μmol m^{-2} s^{-1}. Photosynthesis was measured on the same leaf weekly for 3 weeks. The curves represent the average of the measurements over the ten plants per species ± SE in the second week of measurement.

The quantum yield (ϕ), as calculated by the model, was significantly lower in *I. parviflora* than in the other species (Table 3.2). From Figure 3.4, however, the initial slope of the curve, which represents the quantum yield, appears similar in all three species. It has been suggested that *Photosyn Assistant* does not predict the initial part of the slope very accurately (Leverez 1988). Dark respiration (*Rd*) was also significantly different among species. Post hoc tests revealed this difference was due to significantly lower *Rd* in *I. glandulifera* compared with *I. parviflora* and in *I. balfourii* compared with *I. parviflora* (Table 3.2). No significant difference was found between *I glandulifera* and *I. balfourii*. For all three variables (A_{max}, *Rd*, ϕ), no significant differences due to time were found and time-species interaction was significant only for *Rd* (Table 3.2). Patterns followed by A_{max}, *Rd* and ϕ during the time of measurements for the three species are reported in Figure 3.5 a, b, and c. Highly significant differences among species were also found in light compensation point (LCP) and light saturation point (LSP) (Figure 3.5 d-e). LCP was significantly lower in *I. parviflora* than in the other species (Table 3.2). The interaction between species and time was significant, with the lowest LCP in *I. parviflora* during the first week and an increase over time in this species. Conversely, the LCP in *I. glandulifera* decreased with the age of the leaf. LCP in *I. balfourii* was highest in the second week of measurements but differences were not significant between *I. balfourii* and *I. glandulifera* (Figure 3.5 d). LSP was higher in *I. glandulifera* than in the other species and significantly higher than in *I. parviflora* (Table 3.2). The interaction between species and time was also significant with *I. glandulifera*'s LSP highest at the beginning of the season and decreasing over time. *I. balfourii* showed the highest LSP values in the second week of measurements and then they decreased following the same pattern as *I. glandulifera* (Figure 3.5 e). A completely different pattern was followed by *I. parviflora* where LSP increased with the age of the leaf. No significant difference in LCP and LSP was found among weeks (Figure 3.5 d and e).

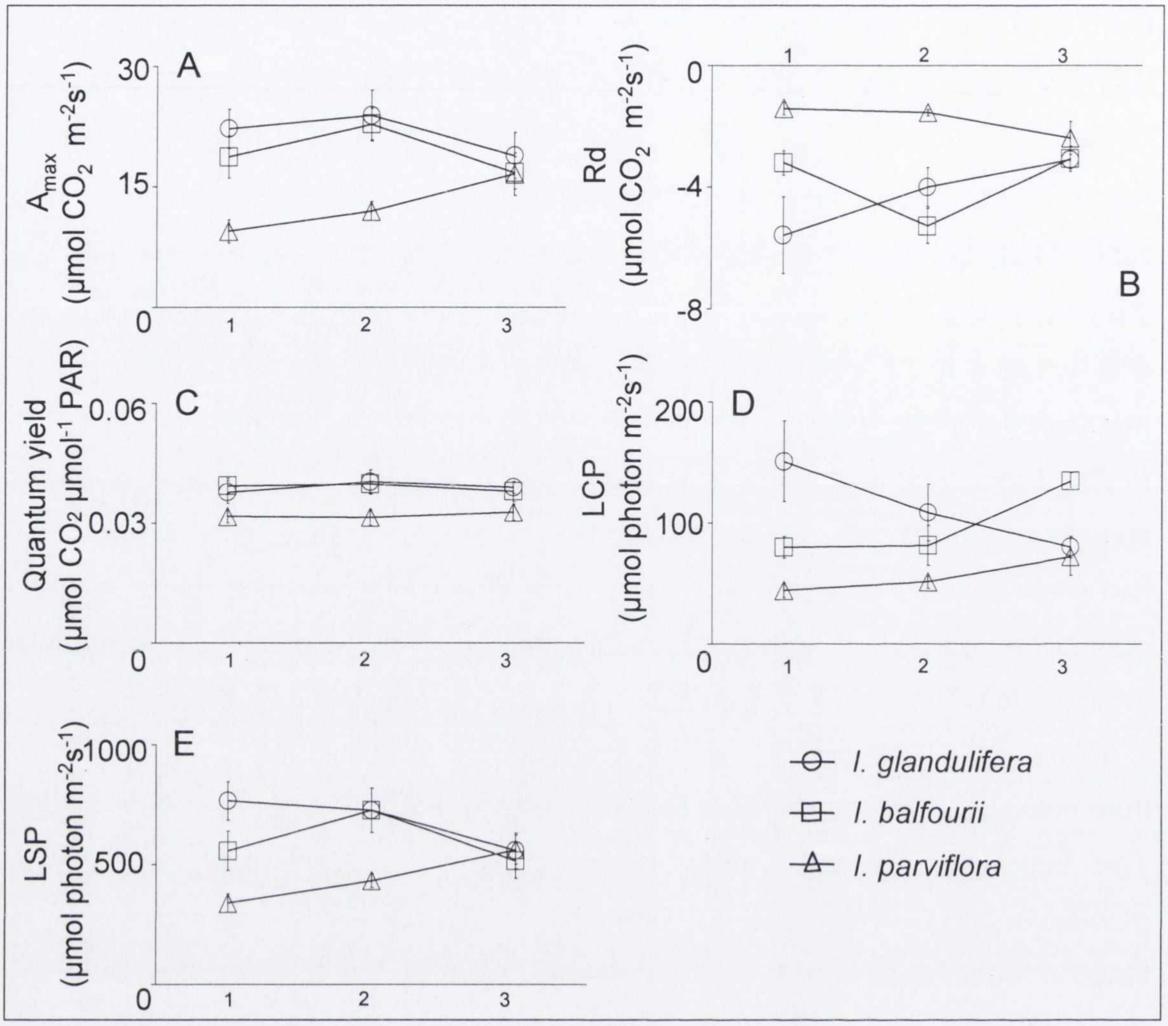

Figure 3.5 Species mean ± SE for: A) maximum photosynthetic rate, B) dark respiration, C) quantum yield, D) light compensation point and E) light saturation point in *I. glandulifera* (circles), *I. balfourii* (squares) and *I. parviflora* (triangles). All the parameters were determined from the light response curves. Shown is the average of the three weeks' measurements.

3.4 DISCUSSION

Impatiens species that have been introduced in UK and Ireland show different distributions. The mechanism behind their success in their introduced range has not been well understood yet. A comparison of growth, reproductive and leaf-physiological traits was aimed at increasing the understanding of their ecology and of their invasive strategies, particularly in a high-resource environment.

The ecophysiological traits considered in my studies have often been reported among those traits that may contribute to plant fitness and consequently confer success in establishing and colonizing (therefore invading) new areas. Of the 11 traits that were measured, eight showed significant differences between species.

In the common-garden experiment *I. glandulifera* showed the highest stem growth rate. Stem growth may reflect the ability of a plant to compete for aerial space and its capacity to intercept light resources. *I. glandulifera* was the species that grew fastest and tallest. The interaction between species and week shows that species do not follow the same pattern of growth throughout the season. *I. glandulifera* grows quickly at the beginning of the summer, rapidly reaching its maximum size. In the experimental environment, characterized by high-light and high-water availability, traits which would contribute to plant invasiveness should therefore be the ones directly related to plant growth since plants have no need to conserve resources nor to improve their resource acquisition systems.

Since high growth rates are a consequence of a whole plant high photosynthetic rate, I expected greater A_{max}, in *I. glandulifera* than in the other species (Baruch and Goldstein, 1999; Durand and Goldstein, 2001a). However, the photosynthetic characteristics of *I. glandulifera* were not significantly different from those of *I. balfourii*. These two species though, showed significantly higher A_{max} than *I. parviflora*. In addition, light saturation was reached at a higher light intensity in *I. glandulifera* and *I. balfourii* than in *I. parviflora*. This trait is characteristically strong in light-demanding, fast-growing plants (Mc Donald, 2003). In particular, *I. glandulifera*'s LSP was highest at the beginning of the growing season and decreased over time, reflecting once more the fast initial growth of this species and its ability to quickly colonize new open areas.

Except for the absence of significant differences in SWR, RWR and SLA among species, I found *I. parviflora* to be the species with the most favourable ecophysiological traits with respect to efficient capture of light resources. LAR and LWR were significantly higher in *I. parviflora* than in *I. glandulifera* but no significant differences were found between *I. parviflora* and *I. balfourii*, and between *I. balfourii* and *I. glandulifera*. *I. parviflora* compensates for the lower photosynthetic capacity with a higher light-harvesting capacity expressed by more "leafiness" of the plant (i.e higher LWR and higher LAR), which is typical of "shade" species (Boardman, 1977; Givinish, 1988). Therefore, photosynthetic capacity at whole-plant basis does not differ between *I. glandulifera* and *I. parviflora*.

The non-invasive species, *I. balfourii* showed values of LAR and LWR between the values measured for *I. glandulifera* and *I. parviflora* and is the species that showed higher whole-plant photosynthetic capacity.

Looking simultaneously at the values measured for all three variables (LAR, LWR, SLA), we can observe that the more leafy nature of *I. parviflora* is due to the productive investment of this species in leaf area while there is no significant difference in leaf thickness or density (no significant difference in SLA). A higher SWR would also represent an advantage since support organs contribute to photosynthesis and sustain higher leaf area. At the same time, a lower RWR would mean increased carbon assimilation due to a reduction in root respiration and an increase of biomass allocation to leaves and support organs.

I. parviflora showed the lowest light compensation point, which reflects, in part, the shade tolerance of this species. LCP represents the lowest light intensity at which a plant can survive. A low LCP combined with a high LWR and LAR reveal the ability of *I. parviflora* for successfully establish and invade the shady forests of Central and Northern Europe. Moreover the opposing strategies of *I. glandulifera* and *I. parviflora* to light resource acquisition reflect the differences in light resource availability of the habitats invaded by these species.

I. balfourii, which has so far not invaded Ireland and has a restricted European distribution, was able to grow, flower and produce seeds in Irish climatic conditions. Furthermore, measurements of morphological and physiological traits in this species ranged between the measurements in the two congeneric invasives but often showed no significant difference to *I. glandulifera*. The high photosynthetic capacity shown by *I. balfourii*, which performed similarly to its most invasive congeners, may also be associated with carbon gain and is a characteristic often reported among aggressive aliens (Williams and Black, 1994; Pattison *et al.*, 1998; McDowell, 2002; Zou *et al.*, 2007). My results lend support to the hypothesis that *I. balfourii* has the potential to become an invader, in particular in disturbed high light environments - the same habitats already susceptible to *I. glandulifera* invasion. Some authors have already proposed *I. balfourii* as a future invader mainly on the basis of its history and distribution (Adamowski, 2009).

The lack of success as an invasive species and the limited spread in Europe (along with the absence in Ireland) that have so far characterized *I. balfourii* may be explained, at least partially, by the fact that this species was introduced in Europe much later than *I. glandulifera* and *I. parviflora* (Adamowski, 2009) and from the fact that these last two species were extensively cultivated in many countries, while *I. balfourii* was not, and consequently did not benefit from the same propagule pressure (Perrins *et al.*, 1993; Adamowski, 2009). In Britain and Ireland the lack of success of *I. balfourii* as an invader may be due to the cooler weather, but under climate warming, *I. balfourii* could possibly experience the favourable conditions required to invade Ireland and Britain as well as more northern regions of Europe.

3.5 CONCLUSIONS and OUTLOOK

The use of ecophysiological characteristics to predict invasive potential of introduced species in a particular habitat might therefore become a useful tool, and afterwards assist in the decision to limit the introduction of a new, potentially invasive, species. In my study, high-light growing conditions were chosen to represent the accessible habitat for invasive species in man-made environments. Therefore, ecophysiological traits such as LCP and quantum yield, which are important at low loght levels, may not contribute to plant fitness and invasive capacity in high-light conditions. It would be useful to fully evaluate invasiveness by assessing ecophysiological traits in a range of environmental conditions, since invasive capacity of a species is strongly dependent on the environment. Varying abiotic factors would also allow us to evaluate species plasticity. The capacity of plastic responses to different habitat conditions may increase the probability of persistence in a larger range of new environments; therefore conferring greater invasiveness (Brown and Marshall, 1980; Gray, 1986; Schierenbeck *et al.*, 1994; Williams *et al.*, 1995). Plasticity could be investigated with further experiments meant to compare these and other ecophysiological traits in species growing under different environmental conditions (e.g. irradiances, water levels, etc). Nevertheless, in any attempt to predict invasion potential or habitat invasibility, other factors such as invasion history, native distribution and other biological and ecological traits may need to be investigated to provide a robust explanation for invasiveness.

4 Growth and photosynthetic traits of invasive *Impatiens glandulifera* and its non-invasive congener *I. balfourii* at two light levels

SUMMARY

Impatiens glandulifera is one of the most dominant invasive alien plants in Europe while its congeneric *Impatiens balfourii* is reported as casual in Britain, not present in Ireland and established in a restricted number of European countries. The mechanism underlying the greater invasive capacity of *I. glandulifera* is not fully understood. In invasion biology, it has not been possible to achieve general conclusions on what determines species invasiveness. This is probably because traits generally associated with invasion vary with environmental conditions and resource availability.

I compared growth, plant allometry, leaf morphology and photosynthetic characteristics of seedlings of these two introduced congenerics growing at two light levels. The ecophysiological traits considered in this study are generally reported to be among the functional traits that may facilitate invasion so I expected the invasive *I. glandulifera* to out-perform the non-invasive *I. balfourii* in these resource capture-, use- and growth-related traits. In addition, the aim was to assess whether the two species adjust their growth rates, light capture and light use efficiency in the different light environments.

Results show that both species are similarly able to adjust their leaf morphological traits to improve light capture efficiency in different light environments. It was observed that the invasive *I. glandulifera* out-performs the non-invasive *I. balfourii* in growth-related traits which are less negatively affected by the low-light environment in particular, showing a “jack of all trades” invasive strategy. *I. glandulifera* also shows better performance than its non-invasive congener in photosynthetic traits, especially in high-light environments which could be associated with a “master of some” strategy for those traits.

4.1 INTRODUCTION

A large number of exotic plant species are able to successfully establish themselves outside their natural range but can not outperform native species and therefore do not become invasive (Williamson and Fitter, 1996). Only a few of them turn out to be able to spread and can subsequently invade, with negative consequences for the native biota (Rejmánek, 2000). It is important to enhance knowledge of the mechanisms that underlie the invasion process to be able to predict future invasion and to be able to provide valuable tools for the management and control of current invasive species (Pyšek and Richardson, 2007; Funk, 2008). Growth rate is widely considered a measure of plant fitness since it is important for both survival and reproduction (Shipley, 2006; Feng, 2008). Invasive species generally show higher relative growth rates (Durand and Goldstein, 2001a) than native species and this implies a large demand for resources. High maximum photosynthetic rates associated with morphological traits that promote light capture efficiency, such as leaf area ratio (LAR), leaf weight ratio (LWR) and specific leaf area (SLA), are considered to be directly proportional to growth and therefore proportional to invasive capacity (Poorter, 1999; Feng *et al.*, 2007c).

However, even though a number of ecophysiological traits have been shown to be associated with invasiveness, it has not been possible to achieve general conclusions as to what determines species invasiveness (Kolar and Lodge, 2001; Daehler, 2003). The inconsistencies that prevent an unambiguous association between traits and invasiveness might be due to the fact that traits vary according to the environmental conditions and resources available (Alpert *et al.*, 2000; Dietz and Edwards, 2006; Feng, 2008). In addition, some traits might be coincidentally associated with invasiveness because many invasive species grow in high-resource habitats and such traits are characteristic of these environments (Poorter, 1999; Schumacher *et al.*, 2009). However, several studies have demonstrated that invasion does not occur only in high-resource environments but also in relatively undisturbed low-resource habitats (Stohlgren *et al.*, 1999; Martin and Marks, 2006; Funk and Vitousek, 2007).

I. glandulifera and its congener *I. balfourii* have both been introduced to Europe from The Himalayas. *I. glandulifera* is one of the most dominant invasive plant species in Britain, Ireland and continental Europe (Perrins *et al.*, 1993; Pyšek and Prach, 1995) while *I. balfourii*, which is naturalized in disturbed habitats in central and southern Europe (Moore, 1968), is not considered to be invasive and is not reported as established in Britain or in Ireland (DAISIE, 2008). These closely-related species (Janssens *et al.*, 2006) differ in invasive potential and therefore represent an excellent case study, since comparing congenerics minimizes trait differences associated with inter-specific variation and facilitates discrimination of which traits determine invasiveness and which are purely coincidental (McDowell, 2002; Burns and Winn, 2006; Muth and Pigliucci, 2006; Richards *et al.*, 2006; Funk, 2008). Characterization of traits and comparisons between introduced successful invaders and introduced not-invaders also represents a more appropriate approach to identifying those traits that determine variation in invasiveness than that of simply comparing invasive and native species (Milbau and Nijs, 2004; Nijs *et al.*, 2004; Milbau and Stout, 2008).

Richards *et al.* (2006), in their work on phenotypic plasticity, hypothesized that a successful invader may behave as either a “jack of all trades”, where its success is due to its ability to maintain fitness in unfavourable conditions such as low-resource environments; “a master of some”, where success is due to the ability to increase fitness in high-resource environments; or a “jack and master”, where the invader succeeds both in poor and resource-rich environments (Richards *et al.* 2006). If the invasive *I. glandulifera* out-performed the non-invasive *I. balfourii* under low-resource conditions, according to the definition of Richards *et al.* (2006) this could be attributed to a “jack of all trades” invasive strategy in this species (Richards *et al.*, 2006).

Chapter 3 of this thesis described a common-garden experiment in which the ecophysiology of *Impatiens* species was compared. From this chapter it emerged that both *I. glandulifera* and *I. balfourii*, unlike the invasive shade-tolerant *I. parviflora*, are light-demanding species. In high-light environments, which are characteristic of disturbed habitats (and are often the entry point for introduced invasive species), *I. glandulifera* and *I. balfourii* showed similar results regarding several fitness-related traits. These results could be explained by several new hypotheses: 1) the experiment might not be able to detect differences that exist; 2) other factors might be involved in determining invasive capacity, and the species characteristics chosen to explain invasions might not be the ones actually responsible for *I. glandulifera*'s greater invasiveness; 3) *I. balfourii* might have the potential to spread and become as invasive as *I. glandulifera*; 4) some of the selection traits might vary across resource gradients.

In this study, with a full-factorial experiment in controlled conditions, I assessed and compared 15 traits in seedlings of *I. glandulifera* and *I. balfourii* growing at two different light levels. I aimed to evaluate invasiveness by measuring traits considered responsible for growth, resource-capture efficiency and resource-use efficiency. I hypothesized a higher efficiency in capture and utilization of light in *I. glandulifera*. In addition, I aimed to investigate whether the two exotic species adjusted their growth rate, biomass-allocation parameters and photosynthetic characteristics in the two different light environments.

4.2 MATERIALS AND METHODS

4.2.1 Establishment

For the current study, seedlings of *I. glandulifera* and *I. balfourii* were germinated from seeds collected in 2008 from experimental plants grown in Trinity College Botanic Garden. Seeds were germinated on moist filter paper after 50 days stratification at 4 °C (Chapter 2). Seedlings were placed in 76 mm diameter pots filled with Shamrock Ready Mix. The growing media had a pH range between 5.2 and 5.7, nutrients added: N = 300, P = 90, K = 330 mg/l and fertilizer with 50% slow release nitrogen. All seedlings were kept together in a growth chamber (PGR15 - Conviron, Winnipeg, Canada) at a photosynthetically active radiation (PAR) of 300 µmol photon m^{-2} s^{-1} for approximately 2 weeks.

Light saturation points were measured for *I. glandulifera* and *I. balfourii* in a common-garden experiment at the Trinity College Botanic Garden in 2008. Plants of both species growing in an open sunny area had a light saturation point that ranged on average between 500 and 800 µmol photons m^{-2} s^{-1} (Chapter 3). In a growth chamber, light was varied during the day using fluorescent lights for day light and incandescent lights for dawn and dusk light (dawn between 6.00 and 7.30, full light between 7.30 and 20.00, dusk between 20:00 and 21.30 and dark from 21.30 to 6.00) to approximately reproduce the external conditions. Temperatures were 15 °C during the day and 10 °C during the night. Afterwards, seedlings were divided into two irradiances: 100 µmol photon m^{-2} s^{-1} (lower light) and 300 µmol photon m^{-2} s^{-1} (higher light). The light was varied during the day according to the same cycle mentioned above. Both light levels in the experiment were low with respect to the light saturation of photosynthesis of these two species measured in high-light conditions.

The amount of light required for optimum plant growth has been reported to be a function of irradiance and of the length of time during which the irradiance is supplied (Tibbitts and Langhans, 1993). Tibbitts and Langhans (1993) suggested that a 400 µmol photons m^{-2} s^{-1} for 12 hours is sufficient for cool-season crops.

The light conditions were produced by using a wooden frame covered with white muslin cloth set over the low-light seedlings. Photosynthetically active radiation was measured with a quantum sensor *(*LI190 – LI-COR, Lincoln, Nebraska, USA*)* at seedling leaf level. As seedlings grew, the lamps used for irradiation were moved progressively away from seedlings (thanks to the growth chamber adjustable ceiling) in an attempt to keep a near constant PAR on seedlings' upper leaves.

Pots were randomly positioned far enough from each other to avoid shading and position was changed weekly to avoid any edge effect. Seedlings were watered with tap water every two days to maintain the soil well watered.

Plants were harvested 1 week after they were placed in the new light environment, and again after 3 weeks and after 5 weeks. Nine seedlings per species per treatment were harvested each time.

Before the final harvest, the photosynthetic capacity of all the remaining seedlings was assessed using a CIRAS-2 portable infra-red gas-analyzer. Each measurement assessed the photosynthetic response to 9 different irradiances: 0, 50, 100, 200, 500, 800, 1200, 1600, 1800 µmol m^{-2} s^{-1} (Joesting *et al.*, 2007), starting with the highest irradiance (Feng *et al.*, 2007c; Joesting *et al.*, 2007). Ambient CO_2 concentration was set to 380 ppm and cuvette temperature was 28.5 ± 0.5°C.

4.2.2 Plant growth and allometry and leaf morphology

Seedling height was measured as the distance between soil surface and apical growing tip. Height was measured on all the seedlings of each harvest. Leaf area was scanned and measured with WinDIAS software (Delta – T Devices) for all leaves except senescent ones. Leaves and stems were separately dried at 80°C for two days. Roots were manually washed and dried in the same way. Dry leaves, stems and roots were weighed separately in order to determine leaf weight ratio (LWR), stem weight ratio (SWR) and root weight ratio (RWR) as the ratio between, respectively, leaf, stem, and root weight and total plant weight. Leaf area ratio (LAR) was calculated as the ratio between leaf area and total plant weight and specific leaf area (SLA) was calculated as the ratio between leaf area and leaf weight (Hunt, 1990). Leaf area: root mass ratio (LA:RM) was calculated as the ratio between leaf area and root weight. Total biomass was calculated as the sum of leaf, stem and root dry weights. The measured traits are shown in Table 4.1.

Table 4.1 The 15 traits compared among *I. glandulifera* and *I. balfourii* growing under two different irradiances..

Trait	Description	Units
Plant allometry		
LWR	Leaf weight ratio	g leaf g^{-1} plant
SWR	Stem weight ratio	g stem g^{-1} plant
RWR	Root weight ratio	g root g^{-1} plant
Leaf morphology		
LAR	Leaf area ratio	m^2 leaf g^{-1} plant
SLA	Specific leaf area	m^2 leaf g^{-1} leaf
LA:RM	Leaf area:root mass ratio	m^2 leaf g^{-1} root
Plant growth		
Height	Distance between soil and apical growing tip	cm
Biomass	Dry weight of the whole plant	g
Photosynthesis characteristics		
A_{max}	Light saturated photosynthetic rate	µmol CO_2 $m^{-2}s^{-1}$
Rd	Dark respiration rate	µmol CO_2 $m^{-2}s^{-1}$
ϕ	Apparent quantum yield	µmol CO_2 $µmol^{-1}$ PAR
LCP	Light compensation point	µmol $m^{-2}s^{-1}$
LSP	Light saturation point	µmol $m^{-2}s^{-1}$
Utilization efficiency		
WUE	Water use efficiency (Photosynthesis/Transpiration)	µmol mol^{-1}
RE	Respiration efficiency (Amax/Rd)	

In order to estimate relative growth rate, biomass data were natural-logarithm-transformed (Hunt, 1990) and plotted versus time using SPSS 16.0 (SPSS, 2006) (log_e(biomass) on the y-axis and time on the x-axis for each species and each treatment). Relative growth rates were calculated as the slopes of the lines. Nine plants per species per treatment were harvested each time so that every line was fitted using 27 data points.

4.2.3 Photosynthetic characteristics and utilization efficiency

Light response curves were fitted using *Photosyn Assistant* 1.2 (Parson and Ogstone, 1997). The rationale for the curve fitting and the parameters involved are described in Chapter 3.

Water use efficiency (WUE) was calculated as the ratio of photosynthesis to transpiration at 1800 µmol m^{-2} s^{-1} (Xu and Hsiao, 2004; Funk, 2008). Respiration efficiency (RE) was calculated as the ratio between the light saturated photosynthetic rate (A_{max}) and the value of dark respiration (Rd) (Feng *et al.*, 2007b).

4.2.4 Data analysis

Analyses of the data were run in SPSS 16.0. Normality was tested with Kolmogorov-Smirnov's test. Variances were tested for heterogeneity using Levene's test and data were transformed where necessary to homogenize variances. When transformations failed to reduce heterogeneity of variances, analyses were carried out on untransformed data. Large, balanced ANOVAs are robust to breaches of this assumption, but significant results were treated with caution (Box, 1953; Underwood, 1981). Univariate analysis of covariance with species (*I. glandulifera* and *I. balfourii*) and treatments (high- versus low-irradiance) as fixed factors and harvests (1-3) as random factor was used to compare traits when there was more than one measurement over time (LWR, SWR, RWR, LAR, SLA, LA:RM and height). Univariate ANCOVA with species and treatments as fixed factors was used to compare traits when there was a single measurement over the time (A_{max}, ϕ, Rd, LCP, LSP, WUE and RE). Natural logarithm of biomass was included in the analysis as a covariate for both growth-related and photosynthesis-related traits. Species and treatment effects could thus be assessed by comparing plants of similar biomass. Univariate ANOVA with species and treatment as fixed factors and harvest as a random factor was used to compare biomass. The full-factorial model was chosen to evaluate the effect of the main factors and of all their possible interactions.

4.3 RESULTS

4.3.1 Plant allometry

Significant differences due to species and treatment effects were found in stem weight ratio (SWR). *I. glandulifera* allocated more biomass to the support organs than *I. balfourii*. SWR also showed significant variation over time, and increased with the age of the seedling in both species for both treatments. No interactions between species and harvest or treatment and harvest were found in SWR. In contrast, the interaction between species and treatment was significant. *I. glandulifera* increased drastically the proportion of biomass allocated to support organs in the lower irradiance environment, while the light conditions caused only a very slight reduction of SWR in *I. balfourii* at the lower irradiance level (Table 4.2). Neither leaf weight ratio (LWR) nor root weight ratio (RWR) showed significant differences due to the main factors or to their interactions (Figure 4.1).

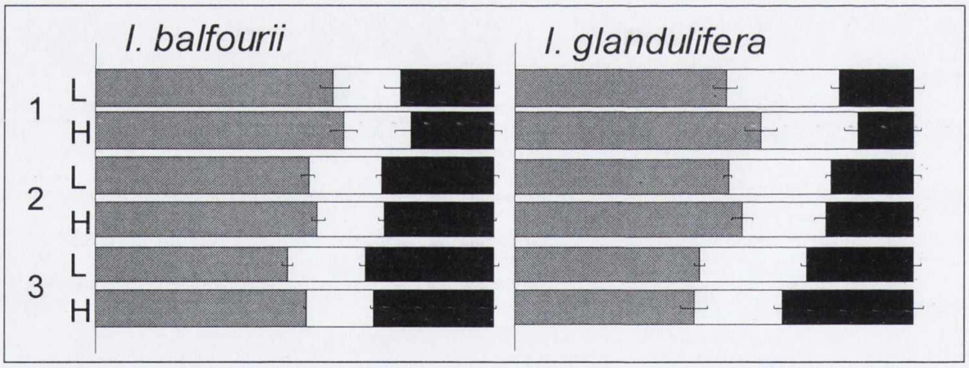

Figure 4.1 Biomass-allocation in *I. balfourii* and *I. glandulifera*. Mean ± SE of LWR (grey), SWR (white) and RWR (black) for the first, second and third harvests (1, 2 and 3 respectively). Each time 9 plants per species per light-treatment were harvested. L = lower light treatment and H= higher light treatment. Significant differences in SWR were found between treatments (p = 0.04).

Table 4.2 F-values and p-values of the full factorial univariate ANCOVA and ANOVA. S = species, T = treatment, H = harvest. * indicate interactions between factors. Significant p-values are reported in bold.

Plant allometry		**S**	**T**	**S * T**	**H**	**S * H**	**T * H**	**S * T * H**
	df	*1, 2*	*1, 8*	*1, 6*	*2, 21*	*2, 1*	*2, 0.165*	*2, 94*
LWR	F	5.410	3.021	0.322	12.453	1.232	0.996	0.917
	p.	*0.138*	*0.202*	*0.626*	*0.064*	*0.450*	*0.504*	*0.403*
SWR	F	**116.376**	**12.737**	**92.757**	**5.216**	28.971	18.744	0.017
	p.	***0.004***	***0.008***	***<0.001***	***0.014***	*0.218*	*0.639*	*0.983*
RWR	F	7.994	0.079	0.945	3.632	1.133	1.372	2.334
	p.	*0.103*	*0.803*	*0.432*	*0.261*	*0.469*	*0.423*	*0.102*
Leaf morphology		**S**	**T**	**S * T**	**H**	**S * H**	**T * H**	**S * T * H**
LAR	F	0.188	**44.740**	2.545	0.792	2.421	0.299	0.762
	p.	*0.705*	***0.003***	*0.247*	*0.560*	*0.295*	*0.771*	*0.469*
SLA	F	4.387	**101.737**	1.251	19.165	0.536	0.061	1.151
	p.	*0.158*	***<0.001***	*0.377*	*0.841*	*0.652*	*0.942*	*0.321*
LA:RM	F	2.968	2.651	0.681	0.548	5.397	3.114	0.632
	p.	*0.225*	*0.234*	*0.560*	*0.534*	*0.160*	*0.249*	*0.534*
Plant growth		**S**	**T**	**S * T**	**H**	**S * H**	**T * H**	**S * T * H**
Height	F	**178.728**	**67.611**	0.315		0.352	0.188	**3.444**
	p.	***0.004***	***0.006***	*0.631*		*0.740*	*0.842*	***0.036***
Biomass	F	**4.209**	**9.341**	3.003	214.251	0.573	0.275	1.700
	p.	**0.043**	**0.003**	0.086	**<0.001**	*0.636*	*0.784*	*0.188*
Photosynthesis		**S**	**T**	**H**				
	df	*1, 31*	*1, 31*	*1, 31*				
Amax	F	4.105	4.179	**6.386**				
	p.	*0.051*	*0.050*	***0.017***				
Rd	F	3.414	**31.731**	0.305				
	p.	*0.074*	***<0.001***	*0.585*				
□	F	**6.035**	0.063	1.930				
	p.	***0.020***	*0.803*	*0.175*				
LCP	F	0.379	**20.039**	0.819				
	p.	*0.542*	***<0.001***	*0.373*				
LSP	F	**12.756**	**10.197**	**11.487**				
	p.	***0.001***	***0.003***	***0.002***				
Utilization efficiency		**S**	**T**	**H**				
WUE	F	0.018	0.378	0.307				
	p.	*0.893*	*0.543*	*0.583*				
RE	F	**4.336**	1.282	1.844				
	p.	***0.046***	*0.266*	*0.184*				

4.3.2 Leaf morphology

Highly significant differences were found in the leaf-related traits. Differences in leaf area ratio (LAR) and specific leaf area (SLA) were due to the treatment effect (Table 4.2). Both species showed significantly higher LAR and SLA in the low-light environment. LAR and SLA were consistently higher at the low irradiance in all three harvests (Figure 4.2 a and b). No significant differences were found between species and no differences due to the interaction between factors were found in LAR and SLA.

No differences in leaf area:root mass ratio (LA:RM) were found (Figure 4.2 c).

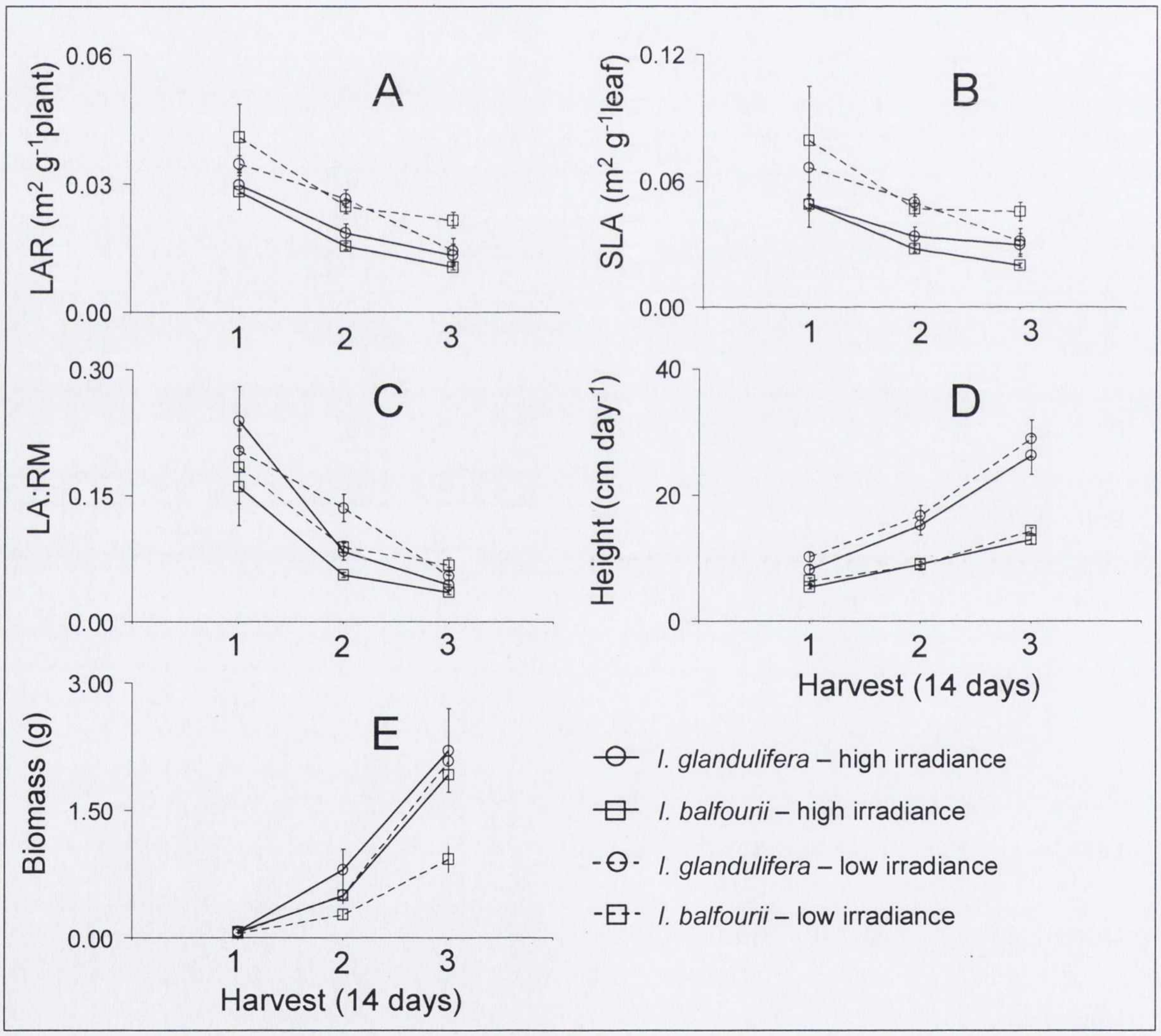

Figure 4.2 Leaf-morphological traits (LAR, SLA and LA:RM) and growth traits (height and biomass). Traits were measured on harvesting the plants 3 times: one, three and five weeks after plants were placed in the light new environment. Means ± SE for *I. balfourii* (squares) and *I. glandulifera* (circles) at lower irradiance (dashed line) and at higher irradiance (solid line).

4.3.3 Plant growth traits

Significant differences in height were found between species and between treatments (Table 4.2). Stem length was significantly greater in *I. glandulifera* than in *I. balfourii* and that was largely consistent across treatments and over time, except in *I. balfourii* at the second harvest (resulting in the significant three-way interaction) (Figure 4.2 d).

Differences due to species and treatment were also found in the total biomass (Table 4.2). *I. balfourii* in the low irradiance environment produced significantly less biomass than *I. glandulifera* in both treatments and than *I. balfourii* at the higher irradiance level (Figure 4.2 e). Consistently, *I. balfourii* growing at low irradiance showed the lowest RGR (Table 4.3). No significant interactions between factors were found for the biomass (Table 4.2). Relative growth rate for the two species at the two irradiances are reported in Table 4.3.

Table 4.3 Relative growth rate as the slope of the line given by biomass (on y-axis) plotted against time of harvest (on x-axis). Nine plants per species per treatment were harvested each time. Every line was fitted using 27 data points.

		RGR	R^2
I. balfourii	Lower light	0.593	0.814
	Higher light	0.750	0.826
I. glandulifera	Lower light	0.790	0.904
	Higher light	0.715	0.717

4.3.4 Photosynthetic traits

Significant differences were also found between the photosynthesis-related traits (Table 4.2). Significant differences in the maximum rate of photosynthesis (A_{max}) were due to treatment effects and to species and treatment interactions (Table 4.2). A_{max} was significantly higher in *I. glandulifera* at the higher irradiance (Figure 4.3 a). Significant differences in respiration (Rd) were due to treatment effects (Table 4.2). Both species showed a significantly lower Rd at higher irradiance (Figure 4.3 b). *I. balfourii* showed significantly higher apparent quantum yield (ϕ) at both light levels (Figure 4.3 c) but this difference was only significant at the high irradiance. The light compensation point (LCP) was significantly different between the light treatments (Table 4.2). Both *I. balfourii* and *I. glandulifera* showed the lowest LCP at lower light intensity (Figure 4.3 d). Species, treatment and interaction effects were all significant for the light saturation point (LSP) (Table 4.2). *I. glandulifera* showed a significantly higher LSP at the higher light intensity (Figure 4.3 e). A less severe variation in LSP between treatment occurred in *I. balfourii* and that caused the significant interaction between treatments and species. Light response curves of the two *Impatiens* species growing at the two irradiances are shown in Figure 4.4.

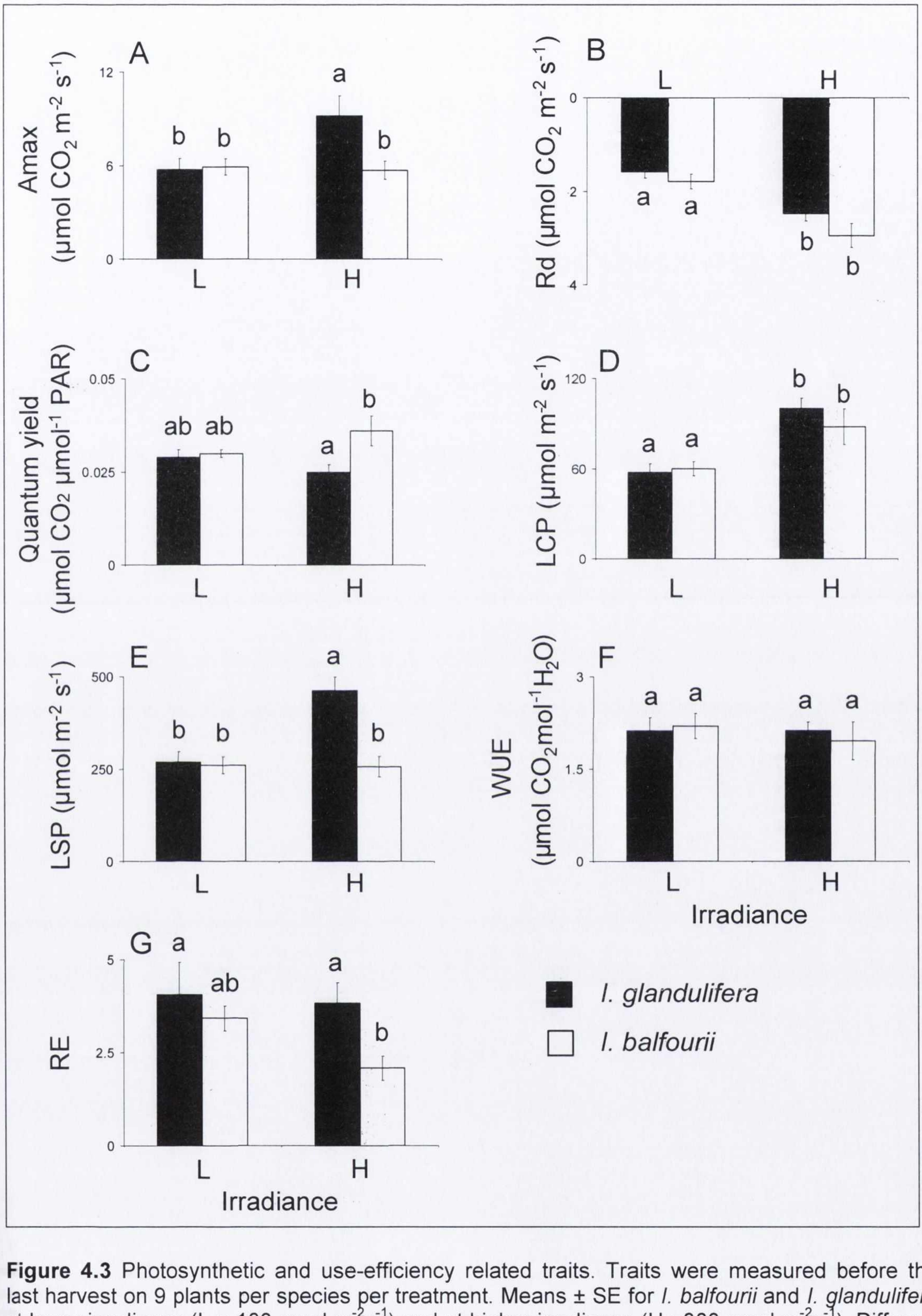

Figure 4.3 Photosynthetic and use-efficiency related traits. Traits were measured before the last harvest on 9 plants per species per treatment. Means ± SE for *I. balfourii* and *I. glandulifera* at lower irradiance (L = 100 µmol $m^{-2}s^{-1}$) and at higher irradiance (H =300 µmol $m^{-2}s^{-1}$). Different letters above columns in the same graph indicate significant differences ($p < 0.05$).

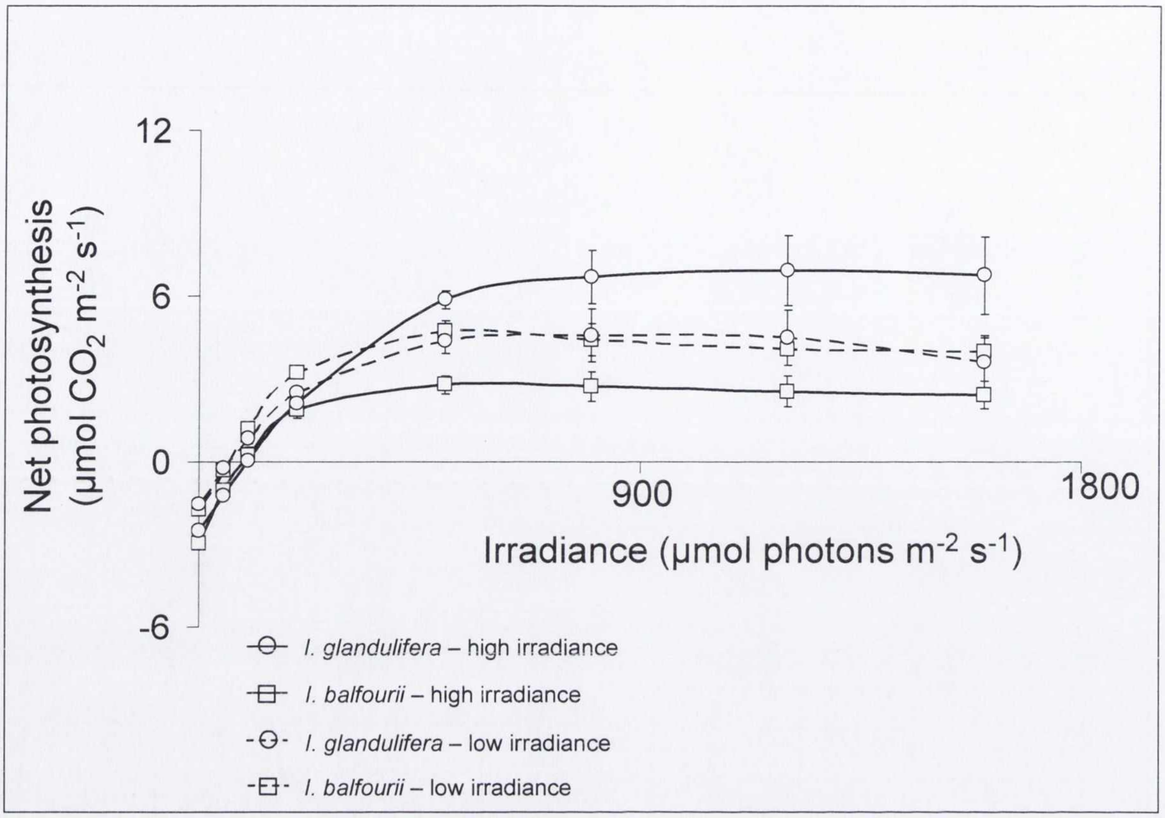

Figure 4.4 Light response curves for *I. glandulifera* (circles), *I. balfourii* (squares) at lower irradiance (dashed line) and higher irradiance (solid line). The photosynthetic capacity of 9 individual plants per species per treatment was assessed at 9 levels of photon irradiance: 0, 50, 100, 200, 500, 800, 1200, 1600, 1800 µmol m^{-2} s^{-1}. The curves represent the average of the measurements ± SE.

4.3.5 Utilization efficiency

No significant differences due to species, treatment, or their interaction were found in water use efficiency (WUE) (Table 4.2 and Figure 4.3 f). Significant differences between species were found in respiration efficiency (RE) (Table 4.2). *I. glandulifera* showed higher RE in both light environments and this difference was significant at the higher irradiance (Figure 4.3 g). No significant difference due to species and treatment was found for RE (Table 4.2).

4.4 DISCUSSION

Physiological traits that allow a plant to efficiently capture and utilize light have frequently been shown to be associated with competitive ability and plant fitness (Chazdon *et al.*, 1996; Pattison *et al.*, 1998; Baruch and Goldstein, 1999; Durand and Goldstein, 2001a). However it is to date unknown which traits determine invasiveness of introduced species since characterization of traits is strongly dependent on the environmental conditions in which traits are assessed, and their contribution to plant fitness also differs according to environmental resource availability (Daehler, 2003; Richards *et al.*, 2006).

4.4.1 Plant adjustments to lower light levels

In this study, both *I. glandulifera* and *I. balfourii* adapted their leaf morphological traits in order to exhibit more leafiness at low irradiance as is revealed by the increased leaf area ratio and specific leaf area in both species. Since LWR does not change significantly between the two light environments, the much leafier nature of the plants of both species growing at low irradiance was due entirely to an increase in leaf area that caused a reduction in leaf density which can be observed through the variation in SLA. The relationship between LWR, LAR and SLA is reported in equation 3.2. Feng *et al.* (2007b), in a comparison among two invasive and one non-invasive introduced species growing at four irradiances, also found that variation in leaf morphological traits such as LAR and SLA was primarily due to the light treatments, while other traits, for example plant height, were shown to be species-specific (Feng *et al.*, 2007c). Consistent with this study and with Feng *et al.* (2007b), Shumacher *et al.* (2009), comparing the effect of light level on growth and morphology of invasive and native tropical tree seedlings, observed an increased SLA when radiation was reduced, which is a common response. Daehler (2003) reviewed 119 papers containing comparisons between invasive and native species, and he found that invasive species showed higher leaf area ratio then natives. Van Kleunen *et al.* (2010), in their recent review, considered 117 published studies that assessed (in addition to the comparisons between alien and native) the comparisons between alien invasive and alien non-invasive. However, they found no differences in leaf area allocation between invasive and non-invasive aliens (van Kleunen *et al.*, 2010b). In my study, in agreement with van Kleunen et al (2010), the non-invasive and the invasive did not differ in their capacity to vary their leaf morphological traits in order to maximise light resource capture at different irradiances.

Both species also exhibited a lower LCP at the lower light level. Similar trends are reported from other authors (Wang *et al.*, 2006; Joesting *et al.*, 2007). A generally low LCP indicates the adaptation of a species to growth in low-light environments. Deng *et al.* (2004) compared LCP in invasive and native congeners of *Mikania* in South China and they also found a similar shade-tolerance in the two species reflected by no differences in LCP (Wang *et al.*, 2006).

4.4.2 Growth related traits

In terms of total biomass, I found that plant growth was greater under higher light for both species. However, *I. balfourii* appeared to be more negatively affected than *I. glandulifera* by the low-light environment. Even if the two species showed similar leaf-photosynthetic rate, *I. balfourii* produced less biomass at low irradiance. These results suggest that early growth of *I. balfourii* might be strongly limited by low-light environments, such as under closed canopies in undisturbed habitats or in the presence of faster-growing competitors. Generally, comparisons which involve size related traits consistently find higher size measures in invasive species, both when compared with native and with other introduced species (van Kleunen *et al.*, 2010b).

The invasive *I. glandulifera*, compared to the non-invasive *I. balfourii*, also allocated more biomass to the support organs when it grew at the lower light level. This behaviour is reflected by the significant differences in SWR and height due to species and treatment effect. These patterns agree with existing evidence which suggests that rapid growth allows an invasive species to out-perform slower-growing species in the competition for aerial space. As a consequence, invasive species have an increased capacity for light-resource interception, which is particularly important when light is scarcely available (Grime and Hunt, 1975; Schierenbeck *et al.*, 1994; Wilsey and Polley, 2006). Light competition is considered a crucial mechanism that brings changes after invasion as invaders can create unfavourable conditions by shading slow-growing native plants (Feng *et al.*, 2007c).

4.4.3 Plant allometry

A higher SWR, besides sustaining a higher leaf area, would also contribute to light-capture capacity since support organs are, similar to leaves, photosynthetically active parts of the plant and therefore also contribute to maintaining a positive carbon balance. In this study, I found higher biomass allocation in the support organs in the invasive species, in contrast with Feng *et al.* (2007) who found a higher shoot allocation in the native non-invasive species. Van Kleunen *et al.* (2010) found differences in shoot allocation in comparisons of invasive versus native, but not in comparisons of introduced invasive versus non-invasive species.

Biomass allocation to root has also been considered in several studies. Feng *et al.* (2007) found lower root biomass allocation in the non-invasive species. Daehler (2003) found great inconsistencies among the studies that considered this trait. There is also disagreement on how the RWR contributes to plant fitness. A low RWR would increase carbon assimilation because it would reduce root respiration but, on the other hand, it would decrease the water- and nutrient-uptake; consequently a broad inconsistency characterizes the studies that aim to compare RWR of invasive and native, or of invasive and non-invasive species (Pattison *et al.*, 1998; D'Antonio *et al.*, 2001). The contribution of a greater biomass allocation to roots to general plant fitness might be even more related to environmental conditions than the contribution of other allometric traits to fitness. Variation in RWR could be better evaluated and compared between invasives and non-invasives in a situation of plant stress due to low-water or low-nutrient conditions, when, for plants, it is essential to maximize uptake.

4.4.4 Photosynthetic traits

The photosynthetic characteristics of *I. glandulifera* and *I. balfourii* seedlings growing at two low-light levels show that the maximum photosynthetic rate was affected by light level; however the light level did not affect the maximum photosynthetic rate in the same way in both species. Differences in A_{max} were mostly due to a high maximum photosynthetic rate in *I. glandulifera* at high irradiance. When irradiance in *I. glandulifera* was increased, it drastically increased A_{max}. *I. balfourii*, grown at two different light intensities, does not show such a strong adjustment in A_{max}. *I. glandulifera* showed therefore a higher plasticity in A_{max} than *I. balfourii* in response to light intensity. Previous studies have linked this trait - or plasticity in this trait - to increased carbon gain in hight light environment and often they reported it among the characteristics of aggressive, introduced species since it might be important to colonize new areas (Williams and Black, 1994; Pattison *et al.*, 1998; McDowell, 2002; Zou *et al.*, 2007). Following the same pattern as A_{max}, the LSP was significantly higher in *I. glandulifera* at higher irradiance. Light saturation point is characteristically high in fast growing plants (Mc Donald, 2003).

Contrary to expectations, it is not possible to explain the greater invasion capacity of *I. glandulifera* in terms of apparent quantum yield (ϕ). This trait has been less investigated then other photosynthesis-related traits and there is less published literature available on it (but see Joesting *et al.,* 2007 or Deng *et al.*, 2004). Several studies failed in detecting a relationship between light level and apparent quantum yield (Ellsworth and Reich, 1992; Beaudet *et al.*, 2000; Aranda *et al.*, 2004; Joesting *et al.*, 2007) while others failed to detect differences in apparent quantum yield between native and introduced species (Deng *et al.*, 2004; McAlpine *et al.*, 2008).

Daehler (2003) compared performances related to photosynthesis and did not find a significant difference between invasive and native species. Van Kleunen *et al.* (2010), comparing “physiology”-related traits, found that invasive outperform native but did not differ from non-invasive introduced species.

4.4.5 Efficiency traits

The respiration efficiency was consistently higher in the invasive *I. glandulifera* across light treatments. This trait might also contribute to plant fitness as an increased RE means a reduction in carbon cost (Pattison *et al.*, 1998; McDowell, 2002; Feng *et al.*, 2007b). RE represents the carbon use efficiency and is an important parameter in estimating growth rate from photosynthesis data or models. It is a highly significant determinant of plant growth. No significant differences between species or light treatments were found in WUE. However, water conservation may not represent an advantage to a plant growing in well-watered conditions consequently the contribution of this trait to plant fitness (therefore to plant invasiveness) may be better evaluated in conditions of low water availability.

4.5 CONCLUSIONS

Of the 15 traits considered in this study, 6 of them did not show differences between species (LWR, RWR, LAR, SLA, LA:RM, Rd, LCP and WUE). The two species showed similar acclimatization to the lower light level. They similarly adjusted their leaf characteristics (LAR and SLA) and their LCP in order to cope with the different light resources available.

Species differed mainly in photosynthesis- and growth-related traits. *I. glandulifera* out-performed *I. balfourii* in high-light environment for two photosynthetic traits (A_{max} and LSP) and in both light environments for one trait (RE). High A_{max} and LSP might reflect the fact that *I. glandulifera* is more of a "sun" plant species than *I. balfourii*. Respiration efficiency might be an important depterminant of plant fitness since it is related to whole-plant carbon gain. *I. balfourii*, on the contrary, out-performed *I. glandulifera* in high-light environment in 1 photosynthetic trait (apparent quantum yield). *I. glandulifera* also showed better growth performances than *I. balfourii* (higher biomass, height and SWR in both light environment conditions). Overall, the invasive species performed better than the non-invasive in traits considered responsible for growth, resource-capture efficiency and resource-use efficiency, this can at least partially explain *I. glandulifera*'s greater invasive potential. The invasive strategy of *I. glandulifera* might be identified as "jack of all trades" in terms of growth (total biomass, height, SWR) since this species is much less negatively affected than the non-invasive by low resource-availability and it still can maintain a fitness homeostasis in the less favourable light conditions. At the same time it might be possible to recognize a "master of some" scenario in photosynthetic traits such as A_{max} and LCP, where the invader take advantage of the high availability of resources

5 Trait plasticity of invasive *Impatiens glandulifera* and non-invasive *I. balfourii* growing under three water regimes

SUMMARY

Phenotypic plasticity has been repeatedly suggested among the traits associated with plant invasiveness in view of the fact that it permits introduced species to colonize and persist in a larger range of new environments.

The invasive *Impatiens glandulifera* and its non-invasive congener *I. balfourii* have similar reproductive and ecophysiological characteristics but they differ in their invasive status.

The aim of this study was to investigate how the two species adjust their characteristics to cope with water deficits and to compare trait plasticity between species. Growth, plant allometry and leaf morphology were assessed in well-watered and water deficit conditions. Relative distance plasticity indices were calculated for each of the measured traits for both species.

Following a similar pattern, both species lower their leaf water potential and their stomatal conductance when exposed to severe water deficit, to cope with the drier environment. For almost all the measured traits, little variation due to the varying water stress was found, suggesting “elasticity” instead of “plasticity” for the measured traits. *I. glandulifera* showed higher plasticity then *I. balfourii* in relation to leaf weight ratio (LWR) and to water use efficiency (WUE), which are both important traits in a changing water availability environment. However, no differences in plasticity were found in most of the compared traits. Differences in plasticity might be more difficult to detect when comparing invasives and introduced non-invasives than when comparing native and invasive species, because the non-invasive introduced population might have originated from individuals with greater adaptive capacity.

5.1 INTRODUCTION

Phenotypic plasticity is defined as the ability of a genotype to express different phenotypes in response to different environmental conditions (Ghalambor et al., 2007). The increased interest in species plasticity comes from the need to forecast species' responses to environmental change. Both bioclimate models and population dynamics models, which are used to predict responses to environmental change, tend not to consider species plasticity, which results in an overestimation of species loss (Valladares *et al.*, 2006). Furthermore invasion biologists, since the late sixties (Marshall and Jain, 1968), have shown interest in species plasticity, repeatedly suggesting plasticity among the traits associated with invasion (e.g. Baker, 1965; Williams *et al.*, 1995; McDowell, 2002; Geng *et al.*, 2007; Caño *et al.*, 2008). The importance of phenotypic plasticity in invasion biology appears particularly clear if we think that introduced populations, whose genetic variation has been reduced by founder effects, should have limited ability to persist and adapt under new conditions. An introduced population is expected to be less genetically varied than the population from its native range (Sakai *et al.*, 2001). Yet, many non-native species are able to persist in the new environment and to establish themselves sufficiently to displace locally adapted native species. Phenotypic plasticity might allow introduced populations to overcome difficulties in adaptation due to a reduced genetic difference.

In a number of invasion biology studies, phenotypic plasticity has been shown to permit introduced alien species to colonize different habitats (Brown and Marshall, 1980; Gray, 1986; Schierenbeck *et al.*, 1994; Williams *et al.*, 1995). In the last decade, there has been a marked increase in the number of studies suggesting that greater adaptive phenotypic plasticity is likely to confer greater invasiveness (Sexton *et al.*, 2002; Parker *et al.*, 2003; Peperkorn *et al.*, 2005; Sharma *et al.*, 2005; Feng *et al.*, 2007c; Caño *et al.*, 2008; Funk, 2008).

Richards *et al.* (2006) provide an interesting framework to assess phenotypic plasticity both at the genotype level - focused on evolutionary aspects and using known genetic lines - and at the species level - focused on species or population in their ecological contexts. Moreover, Richards *et al.* (2006) suggested three scenarios in which the successful invader benefits from phenotypic plasticity: i) "jack of all trades", where the invader has the ability to maintain fitness in unfavourable conditions (fitness homeostasis); ii) "master of some", where the invader has the ability to increase fitness in favourable environments; or iii) "jack and master", that combines both the abilities (Figure 1.2). The second scenario proposed by Richards *et al.* (2006) might also be ascribed to the fluctuating resources availability theory proposed by Davis et al. (2000).

The invasive *Impatiens glandulifera* and its non-invasive congener *I. balfourii* differ greatly in their capacity to invade Ireland and Europe (see Chapter 1 for species distribution). Both *I. balfourii* and *I. glandulifera* are from the same native range, the Himalayas. *I. glandulifera* is currently one of the most dominant invasive plant species in Britain, in Ireland and in continental Europe (Perrins *et al.*, 1993; Pyšek and Prach, 1995). *I. balfourii* is only naturalized in disturbed habitats in central and southern Europe (Moore, 1968) where it is not considered to be invasive. *I. balfourii* is not reported as established in Britain or Ireland (DAISIE, 2008).

In the previous chapters, the ecophysiology of these introduced species has been investigated under optimal growing conditions (Chapter 3) and sub-optimal light conditions (Chapter 4) with the aim of identifying which traits might be responsible for *I. glandulifera*'s greater invasiveness. The implications of these traits for plant fitness have been described previously in Chapters 3 and 4. In this chapter, the same traits related to plant growth, plant allometry, leaf morphology and photosynthesis were measured and compared in plants growing under three water regimes. The ability of invasive species to tolerate stress, especially since climate change will most likely result in more low-water resource environments, will largely affect plant fitness and, subsequently, the competition between plants (Funk and Zachary).

The two *Impatiens* species appear to vary in their soil moisture requirements with *I. glandulifera* reported as requiring high soil moisture (Beerling and Perrins, 1993) while *I. balfourii* prefers fresh soil of moderate dampness (Schmitz and Dericks 2008). However, the two species have also been observed growing together in intermediate soil moisture conditions (Adamowski, 2009). Beerling and Perris (1993) reported that *I. glandulifera* is sensitive to drought and that, in its normal habitat, enough water is available even in very dry years (Beerling and Perrins, 1993).

In this experiment, the values of fitness-related traits and plasticity, measured using a relative-distance plasticity index (Valladares *et al.*, 2006), were compared between the two *Impatiens* species growing under three water regimes. In line with other studies to detect plasticity, I did a controlled experiment in a greenhouse with the manipulation of one abiotic factor. Plants of both species were grown under three water regimes: in well-watered conditions and at two different levels of water shortage. The traits measured were related to plant growth, biomass allocation, leaf morphology and photosynthetic characteristics.

The aims of the study were: (i) to compare trait differences between an invasive and a non-invasive species growing in great water-availability conditions and in conditions of different levels of stress; (ii) to investigate how the invasive and the non-invasive species respond their characteristics to cope with the water deficit; and (iii) to measure and compare between species the relative distance plasticity index.

5.2 MATERIALS AND METHODS

5.2.1 Choice of growing media

Samples of soil were taken from three locations near Dublin. Soil was sampled in two sites along the river Liffey where wild populations of *I. glandulifera* grow. The third site where soil was sampled was the garden of the Zoology Department in Trinity College, where a wild population of *I. parviflora* grows. Since *I. balfourii* does not grow in the wild in Ireland it has not been possible to select a location for this species. In each location, soil was sampled randomly in three different points and then mixed. Soil was sieved through a 2 mm sieve. The pH was measured with a pH meter; a 50 ml beaker was filled one third with soil, an equal volume of distilled water was added, the mixture was stirred, and pH was measured after allowing it to equilibrate for 10 minutes. Soil texture was examined by an analysis of soil particle size using a Bouyocos Hydrometer.

Fresh soil samples were dried in an oven at 105°C and weighed. The samples were then heated at 450°C and the organic matter was estimated as the percentage of weight loss ("loss on ignition") (Allen, 1989). The same analyses were run on samples of 1) "Shamrock Ready Mix"; 2) "Shamrock Ready Mix" mixed with soil excavated from Trinity College Botanic Garden in a proportion 1:1; and 3) a mix of "Shamrock Ready Mix", soil and lake sand in the proportion 2:2:1. Comparing pH, soil texture and organic content (Table 5.1), I decided to use the third mixed described (ready mix, soil and sand) as the growing media since its characteristics appeared most similar to the characteristics of the sampled soil.

Table 5.1 Characteristics of the soil (pH, soil texture and organic content) in three sites where wild *Impatiens* population grow and characteristics of three prepared mixes.

Soil	**pH**	**Texture**			**Organic content**
		clay	**silt**	**sand**	
Samples					
Wren's nest	7.91	4%	9%	87%	0.19
Palmerstown	8.47	7%	12%	81%	0.11
Zoology garden	8.36	2%	2%	96%	0.06
Mix					
1	5.50				0.92
2	6.91				0.40
3	7.30				0.10

During the winter 2009-2010, seeds of *I. glandulifera* and *I. balfourii*, collected during the previous summer in one area in northern Italy (further details on the source are reported in Appendix 2.1), were stratified and germinated as described in Chapter 2. With the comparison of plants originating from the same ecological conditions I aimed to minimize intra-specific genetic differences in order to identify which differences were due to phenotypic plasticity. Germinated seeds were removed daily and placed in 76 mm diameter pots filled with "Shamrock Ready Mix" for the first two weeks, then repotted in five-litre pots filled with a soil-sand-compost mix (Shamrock Ready Mix, with a pH range between 5.2 and 5.7, nutrients added: N = 300, P = 90, K = 330 mg/l and fertilizer with 50% slow release nitrogen) and placed in a heated greenhouse at Trinity College Botanic Garden in Dublin (53°18'44''N, 6°15'34''W).

5.2.2 Water manipulation

At the end of January 2010, 36 seedlings of *I. glandulifera* and 36 seedlings of *I. balfourii* were potted in five-litre pots (one plant per pot) filled with the selected mix and placed in a heated greenhouse at the Trinity College Botanic Garden. After a 2-week period of acclimatization, each pot was randomly assigned to one of the following water supply regimes: well-watered (water potential in the soil > -0.015 MPa); mild water-deficit (soil water potential could reach as low as -0.15 MPa); severe water-deficit (soil water potential could reach as low as -0.30 MPa). Water potential for each treatment was adjusted, according to visible signs of wilting of plants. For soil water potential of -0.15 MPa, plants showed moderate signs of stress. When soil water potential reached -0.30 MPa, plants showed high signs of water stress, leaves were very wilted, rolled and of a darker green colour. Water potential was not reduced below -0.30 MPa. From previous observations it has been noticed that lower soil water potentials could result in stem break, which could bring to plant death. Twelve plants per species received each treatment. Pots assigned to the same treatment were connected with an irrigation system made with a polyethylene pipe with an internal diameter of 17 mm. Dripper stakes joined to the pipe, which allowed a water loss of 2 litres per hour, were driven into the soil in each pot. Ten dielectric water potential sensors (MPS 1, Decagon Devices, Pullman, WA) connected to dataloggers (CR10-series, Campbell Scientific Inc.) were used to monitor the soil water potential. When the first sensor for each treatment indicated a soil water potential below the limit set for each treatment, plants were abundantly re-watered. To achieve this, each of the three irrigation systems was connected to the tap for the amount of time necessary to the pots to drip water.

Temperature data for the growing period were obtained measuring maximum and minimum temperatures in the greenhouse every second day using a maximum-minimum thermometer.

5.2.3 Leaf water potential

The water potential of leaves (Leaf ψ_W) was measured on three plants per species per treatment with a portable Plant Moisture Vessel (SKPM 1400 Series, Skye Instruments Ltd.). A freshly cut leaf per plant was placed in the vessel with the petiole exposed to the atmosphere. The pressure in the body of the vessel was slowly increased until water appeared at the cut end of the petiole. Such pressure corresponds to the water potential in the leaf. Measurements were repeated on the same plants three times throughout the experiment as reported in Figure 5.1. Water potential was measured on the second fully developed leaves. Measurements were taken between 12 am and 2pm to assess the midday Leaf ψ_W

5.2.4 Plant growth and allometry and leaf morphology

At the end of the acclimatization period, before assigning the plants to the different water regimes, nine plants per species were harvested to determine initial plant growth and allometry and leaf morphological traits.

Plants were then harvested, after 6 weeks and after 12 weeks after they were placed in the new water conditions (Figure. 5.1). Three plants per species per treatment were harvested each time. The measured traits are shown in Table 5.2.

Table 1.2 Traits related to plant growth and allometry and leaf morphology compared between *I. glandulifera* and *I. balfourii* growing under three different water regimes.

Trait	Description	Units
Plant growth		
Final height	Distance between soil and apical tip	cm
Leaf area	Total plant leaf area	m^2
Biomass	Total plant biomass	g
RGR	Relative growth rate	gg^{-1} $week^{-1}$
Plant allometry		
LWR	Leaf weight ratio	g leaf g^{-1} plant
SWR	Stem weight ratio	g stem g^{-1} plant
RWR	Root weight ratio	g root g^{-1} plant
Leaf morphology		
LAR	Leaf area ratio	m^2 leaf g^{-1} plant
SLA	Specific leaf area	m^2 leaf g^{-1} leaf
LA:RM	Leaf area-root mass ratio	m^2 leaf g^{-1} root

Final plant height

When plants reached their maximum size, the stem length, as the measure between the soil surface and the apical tip, was assessed in three random plants per species per treatment.

Leaf area

All healthy leaves of the harvested plants were scanned and measured with WinDIAS (Delta – T Devices) software.

Biomass

Leaves and stems were dried separately at 80°C for two days. Roots were manually washed and dried in the same way. Dry leaves, stems and roots were weighed separately and total biomass was calculated as the sum of these three components. Flowers were removed prior to weighing.

Relative growth rate

Relative growth rate was calculated according to Hunt (1990) as:

$$\textbf{(5.1)}\ RGR = \frac{\ln W_2 - \ln W_1}{t_2 - t_1}$$

where $\ln W_2$ and $\ln W1$ represents the natural logarithm of the biomass at sequential times t_2 and t_1 respectively.

Relative growth rate was calculated by plotting ln(biomass) on the y-axis and the corresponding measurement time (expressed in weeks) on the x-axis for each species and each treatment. Relative growth rates were calculated as the slopes of the lines using SPSS 16.0 (SPSS, 2006).

Plant-allometric and leaf-morphological traits

On the three plants per species per treatment, which were harvested after 6 and after 12 weeks from when plants were placed in the new water environment, measurements of plant allometry and leaf morphology were taken and ratios were calculated as described in Chapters 3 and 4.

5.2.5 Photosynthetic characteristics and utilization efficiency

Photosynthetic measurements were made using a CIRAS-2 portable infra-red gas analyzer (IRGA) (PP systems, Amesbury, Massachusetts). Each measurement assessed the photosynthetic response to 9 levels of irradiance (0, 50, 100, 200, 500, 800, 1200, 1600, 1800 μmol m^{-2} s^{-1} (Joesting *et al.*, 2007) with a constant CO_2 concentration set at 380 ppm, as described in the previous chapters. Measurements on the same plant were repeated three times as shown in Figure. 5.1. The first set of measurements was taken before plants were assigned to the different water regimes and photosynthesis was assessed in 12 plants, six plants per species. In the successive measurements, the same photosynthetic parameters, which are reported in Table 5.3, were measured in three plants per species per treatment.

Table 5.3 Traits related to leaf photosynthetic characteristics and resource-use efficiency compared between *I. glandulifera* and *I. balfourii* growing under three different water regimes.

Trait	Description	Units
Photosynthesis characteristics		
A_{max}	Maximun photosynthetic rate	μmol CO_2 $m^{-2}s^{-1}$
Rd	Dark respiration rate	μmol CO_2 $m^{-2}s^{-1}$
ϕ	Apparent quantum yield	
LCP	Light compensation point	μmol photon $m^{-2}s^{-1}$
LSP	Light saturation point	μmol photon $m^{-2}s^{-1}$
GS	Stomatal conductance	mmol $m^{-2}s^{-1}$
Utilization efficiency		
WUE	Water use efficiency (Photosynthesis/Transpiration)	
RE	Respiration efficiency (Amax/Rd)	

Stomatal conductance

In addition, stomatal conductance (GS) was measured using the CIRAS-2 portable infra-red gas analyzer.

Photosynthesis in leaves of different age

Measurements of photosynthetic capacity are usually taken on the most recently fully expanded leaves or, in general, on leaves located at the top of the plant. Measuring the photosynthesis in young healthy leaves allows evaluation of the full photosynthetic capacity of the plant. However, leaves of different age show different photosynthetic capacity and contribute in different ways to the plant carbon gain/cost balance. During this experiment I also assessed the photosynthetic capacity of three leaves of different ages and from different heights on the stem. In *I. glandulifera*, the choice of the leaves is straightforward since this species often has a simple stem and produces leaves in whorls of three with a lifespan of about 4-5 weeks (personal observations). On the other hands, *I. balfourii* has a branched stem that makes it more difficult to identify the leaf development order. Thus, leaves of plants of both species were labelled to keep track of leaf age. The photosynthetic response to a PAR of 1800 µmol photon m^{-2} s^{-1} was measured on the first, second and third fully-developed leaf in six plants per species per treatment.

5.2.6 Trait phenotypic plasticity and plasticity indexes

Phenotypic plasticity for a given trait (x) can be related, with respect to the environmental variable, to the difference in x among two individual of the same species grown in different environments (Valladares et al., 2006).

Calculation of phenotypic plasticity can be extended to a whole data set by compute all the differences across individual and environments (Valladares et al., 2006).

In this study, for each species and each trait over the whole growing season, a relative distance plasticity index (*RDPI*) was calculated according to Valladares *et al.* (2006) as:

$$\textbf{(5.2)}\quad RDPI = \frac{\sum d_{ij} \rightarrow i'j'/(x_{i'j'} + x_{ij})}{n}$$

Where $d_{ij} \rightarrow i'j'$ is the distance among trait values for all pairs of individual for which *i* is the environment variable different from *i'*. Such distance represents the difference for a given trait (x) among two individuals (*j* and *j'*) of the same species growing in different environments and it is calculated as the absolute value of the difference $(x_{i'j'} - x_{ij})$. To obtain the relative distance $rd_{ij} \rightarrow i'j'$, $d_{ij} \rightarrow i'j'$ is divided by $(x_{i'j'} + x_{ij})$. In equation (5.2) *n* is the number of distances considered.

Environmental standardized plasticity index (*ESPI*), as described by Valladares *et al.* (2006), was not calculated because it considers environmental values that can be measured only when environment is taken as a continuous variable and the environmental values for each individual are known (Valladares *et al.*, 2006).

5.3 DATA ANAYSIS

All data analysis was run in SPSS 16 (SPSS, Inc. Chicago IL).

All measured variables were tested for normality with Kolmogorov-Smirnov tests and transformed as required to meet the normality assumptions for parametric tests. Homogeneity of variance was tested as well in all measured variables with Levene's test. When multi-comparisons showed significant differences among treatments, Bonferroni's *post-hoc* test was used to detect differences between each pair of treatments. When transformations failed to reduce heterogeneity of variances, analyses were carried out on untransformed data. Large, balanced ANOVAs are robust to breaches of this assumption, but significant results were treated with caution (Box, 1953; Underwood, 1981).

5.3.1 Leaf water potential

Leaf water potential data showed normality and homoscedasticity. Leaf water potential was analyzed with a repeated measures general linear model where species and water regimes were entered as fixed factors.

5.3.2 Plant growth and allometry and leaf morphology

Plant final height

Plant final height was analyzed with a univariate general model where species and treatments were fixed factors.

Relative growth rate

Relative growth rates were compared as the slopes of the regression lines with ln(biomass) as response variable and the time of the measurements as the independent variable. First I tested the null hypothesis H_0: $\beta_g = \beta_b$, where β_g is the regression coefficient and relative growth rate for *I. glandulifera* and β_b is the regression coefficient for *I. balfourii* for each treatment. Afterwards, for each species, I tested the null hypothesis H_0: $\beta_1 = \beta_2$ and H_0: $\beta_1 = \beta_3$ where β_1 is the regression coefficient and relative growth rate for the first water regime (well-watered), β_2 is the regression coefficient for the second water regime (mild water deficit) and β_3 is the regression coefficient for the third water regime (severe water deficit).

Plant-allometric and leaf-morphological traits

Means of allometry and morphological traits, measured before assigning plants to the water treatment, were compared between species. Leaf weight ratio and specific leaf area were natural-logarithm transformed to achieve normality. For the other variables no transformation was needed. Species were compared with a 2-tailed independent samples t-test.

Moreover, all plant-allometric and leaf-morphological parameters measured at different water regimes were normally distributed except LAR and LA:RM. Following the Box-Cox Method I chose the best transformation for these parameters: LAR was square root transformed and LA:RM was Box-Cox transformed with a $\lambda = 0.30$. Leaf area, LWR, SLA and total biomass were normally distributed but did not show equal variance even if transformed. Analyses of these variables were carried out on untransformed data. Large, balanced ANOVAs are robust to breaches assumptions (Box, 1953; Underwood, 1981). LA:RM, after transformation, showed normal distribution but did not show equal variance; data analysis was carried out on transformed data. Univariate analysis of covariance, with species, water regimes and time as factors, was used to compare plant-allometric and leaf-morphological data (LWR, SWR, RWR, LAR, SLA, LA:RM and height). Natural logarithm of biomass was included in the analysis as a covariate. Species and treatment effects could thus be assessed by comparing plants of similar biomass (Feng *et al.*, 2007c). Univariate ANOVA with species and treatments as fixed factors and harvest as a random factor was used to compare biomass. The full factorial model was chosen to evaluate the effect of the main factors and of all their possible interactions.

5.3.3 Photosynthetic characteristics and utilization efficiency

Light response curves were fitted using *Photosyn Assistant* 1.2 (Parson and Ogstone, 1997). The rationale for the curve-fitting and the parameters involved are described in Chapters 3 and 4.

Water-use efficiency (WUE), at the leaf level, corresponds to the ratio of energy captured by photosynthesis per unit of water transpired and was calculated as:

$$\textbf{(5.3)}\ WUE = \frac{A}{ET}$$

Where A is the net photosynthetic rate and ET the evapotranspiration measured at a PAR of 1800 µmol photon m^{-2} s^{-1} (Xu and Hsiao, 2004; Funk, 2008).

Respiration efficiency (RE) was calculated as the ratio between the maximum photosynthetic rate (A_{max}) and the absolute value of dark respiration (Rd). Both parameters were estimated by the fitted light response curves (Feng *et al.*, 2007b).

The means of the photosynthetic traits, which were measured before assigning plants to the water treatments, were compared between species. Stomatal conductance (GS) was lambda transformed ($\lambda = -0.80$) to achieve normality. For the other variables no transformation was needed. Species were compared with a 2-tailed independent samples t-test.

Once plants were subjected to the different water regimes, repeated-measures analysis of variance was used to compare photosynthesis-related and efficiency-related traits since there was more than one measurement or derived value over the season on the same plant.
Data were transformed to meet the ANOVA's assumptions if required. Apparent quantum yield, LCP and LSP were Box-Cox transformed ($\lambda = 1.60$ for ϕ and $\lambda = 0.50$ for LCP and LSP). Absolute values of the dark respiration and the respiration efficiency (RE), both characterized by all-negative values, were Box Cox transformed with $\lambda = 1.10$ and $\lambda = 0.30$ respectively.

Photosynthesis in leaves of different age
Photosynthesis in different leaves was compared with a univariate general linear model. Data fulfilled the normality assumption. Data did not fulfil homoscedasticity assumption but they were not transformed. Tamhane's test that does not assume equal variance was used for *post-hoc* comparisons between each pair of water regimes and between each pair of leaves.

5.3.4 Trait phenotypic plasticity and plasticity indexes

Relative distances ($rd_{ij} \rightarrow i'j'$) were calculated for each trait. Since distances range between 0 (no plasticity) and 1 (maximal plasticity) (Valladares *et al.*, 2006), the values were arc-sine-transformed (Underwood, 1997) so that:

$$\textbf{(5.4)}\quad X' = \sin^{-1}\sqrt{X}$$

Normality of the data was tested with Kolmogorov-Smirnov's test. When plasticity data for a trait showed normal distribution they were compared between species with an independent-samples t-test. If data did not show normal distribution, they were compared between species with a non-parametric two-independent-samples Mann-Whitney Z test.

5.4 RESULTS

Soil water-potential in the well-watered pots showed little variation. For the mild and severe water deficit treatments, sensors recorded respectively four and two cycles throughout the experiment. Soil water potential data recorded during the experiment are reported in Figure 5.1.

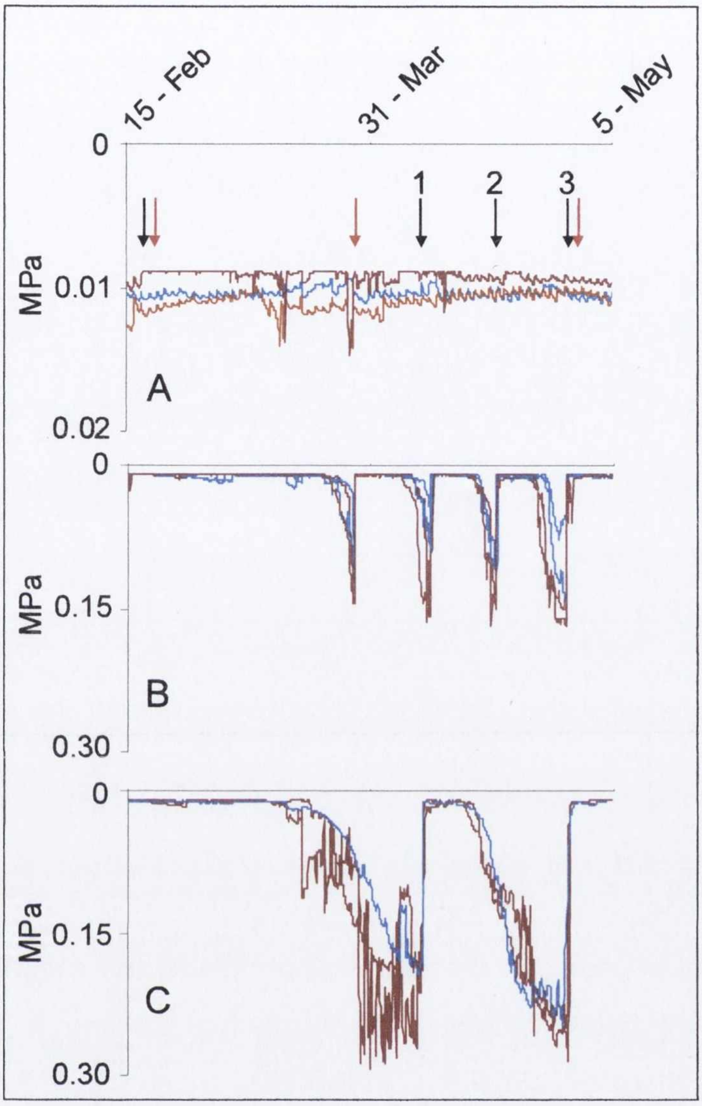

Figure 5.1 Soil water potential measured in ten pots. Red lines refer to water potential measured in *I. glandulifera* pots and the blue lines in *I. balfourii* for the three water regimes: well watered (A); water-deficit (B); severe water-deficit (C). Red arrows show the time of the harvests and black harrows show the time of each photosynthesis measurement. Leaf Ψ_w measurements were taken at times 1, 2 and 3, at the same time as the last three photosynthetic measurements.

The maximum temperatures measured during the experiment were on average 22 ± 0.8°C and they ranged between 12 and 34°C. The minimum temperatures were on average 12 ± 0.7°C and they ranged between 4 and 25°C.

Maximum and minimum temperatures registered in the greenhouse during the experiment are reported in Figure 5.2.

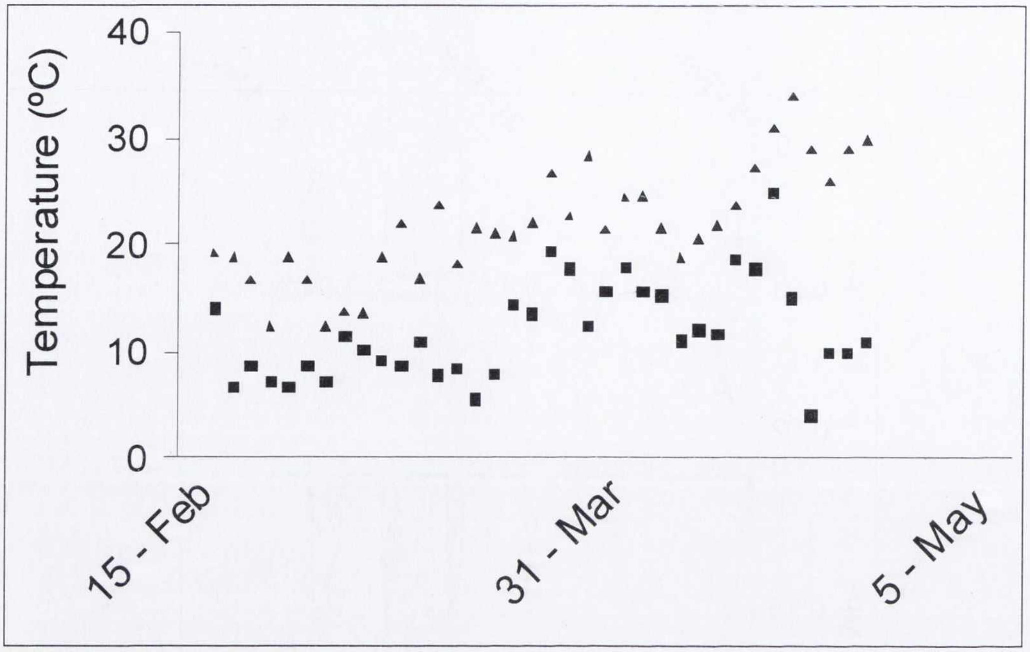

Figure 5.2 Maximum (triangles) and minimum (squares) temperatures measured in the greenhouse during the experiment. Temperature readings were taken every second day during the experiment.

5.4.1 Leaf water potential (Leaf ψ_W)

Leaf ψ_W decreased significantly with soil moisture content in both species (Figure 5.3). Leaf water potential of the plants showed a linear positive correlation with the water potential measured in the soil (R^2= 0.736) (Figure 5.3). Differences between species were not significant within each moisture level. As shown in Table 5.4, significant differences were found between each pair of treatments. Both species showed the lowest leaf ψ_W in severe stress condition and the highest leaf ψ_W when grown well watered.

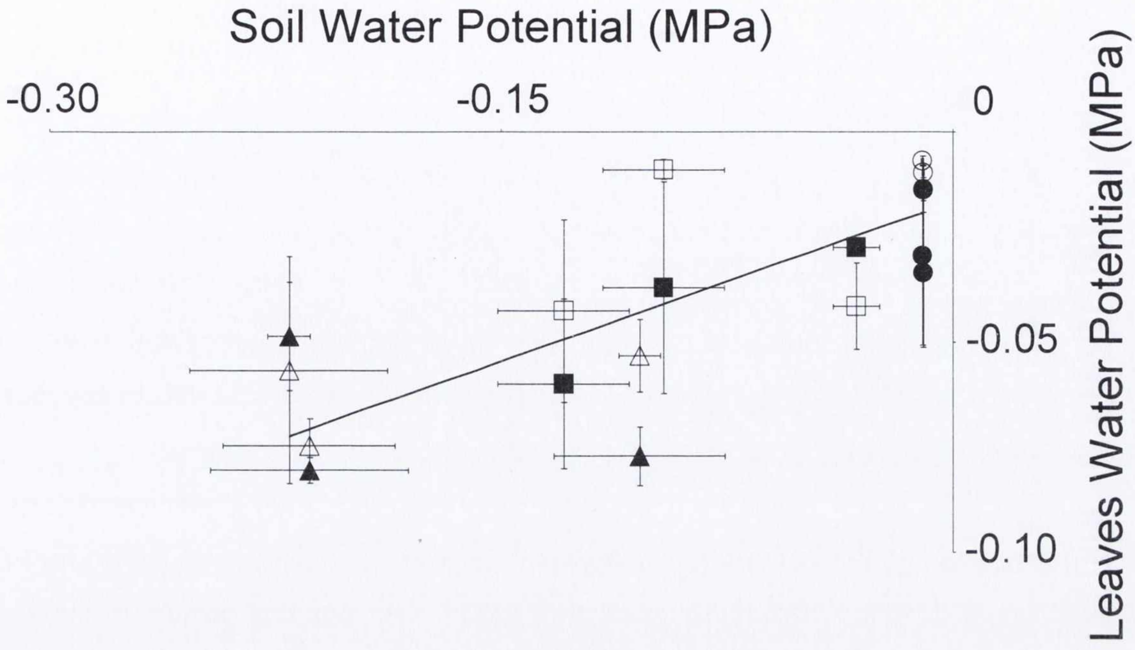

Figure 5.3 Water potential measured in leaves (±SE) of *I. glandulifera* (black) and *I. balfourii* (white) as a function of soil water potential. Plants were considered under three water regimes: well-watered (circles), mild water deficit (squares) and severe water deficit (triangles). Measurements were repeated three times during the experiment as shown in Figure 5.1.

Table 5.4 Repeated measures ANOVA table for the leaf water potential and *post-hoc* comparison Bonferroni's test (equal variance) between each pair of water treatments. * indicates interactions between factors. Mean differences given in the lower part of the table are in bar. Significant p-values are reported in bold.

Leaf water potential	*df*	F	*p*
Species	1,12	4.707	0.051
Water level	2,12	**32.966**	**<0.001**
Species * Water level	2,12	0.378	0.693
Post-hoc comparisons	*Mean difference*		*p*
Well-watered vs. Mild water deficit	0.7549		**0.007**
Well-watered vs. Severe water deficit	1.5927		**<0.001**
Mild water deficit vs. Severe water deficit	0.8378		**0.003**

5.4.2 Plant growth and allometry and leaf morphology

Plant final height

The final height of the plants at the end of the experiment was significantly different between species. *I. glandulifera* always grows taller than *I. balfourii*. Once more, comparing the treatment effect on final plant size we found significant differences. Plants of both species grew higher when well-watered and at mild water deficit than at severe water deficit (Table 5.5). No interaction between species and water level was found.

The reduction in final height as a result of growth in mild water deficit conditions, compared to plants growing well-watered, was on average 7.6% for *I. glandulifera*, and this reduction reached 27.7% as a result of growth in severe water stress conditions. The final height of *I. balfourii* was reduced by 19% in plants growing in severe water stress conditions compared to plants growing well-watered, but final height increased by 13.1% from well-watered to mild water deficit (Figure. 5.4).

Table 5.5 Univariate analysis of variance table for the final plant height and *post-hoc* comparison using Bonferroni's test (equal variance) between each pair of water treatments. Mean differences are in cm. * indicate interactions between factors. Significant p-values are reported in bold.

Final plant height	*df*	F	*p*
Species	1,12	103.649	**<0.001**
Water level	2,12	5.569	**0.019**
Species * Water level	2,12	1.481	0.266
Post-hoc comparisons	*Mean difference*		*p*
Well-watered vs. Mild water deficit	2.333		1.000
Well-watered vs. Severe water deficit	34.833		**0.034**
Mild water deficit vs. Severe water deficit	32.500		**0.049**

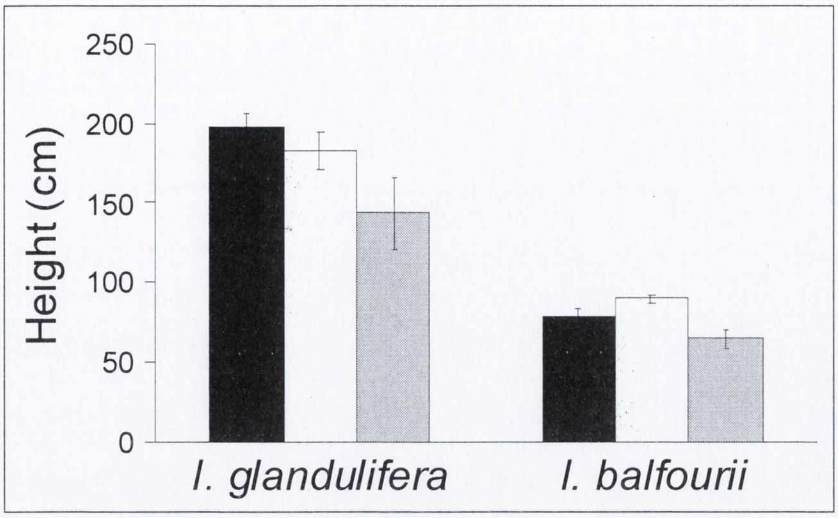

Figure 5.4 Mean (±SE) of final height measured in three plants per species per treatment: well-watered (black bars), mild water deficit (white bars) and severe water deficit (grey bars). Measurements were taken when plants reached their maximum size.

Relative growth rate

Relative growth rate for the two species at the three water regimes are reported in Table 5.6.

Table 5.6 Relative growth rate as the slope of the line given by biomass (on y-axis) plotted against time of harvest (on x-axis). Every regression line was fitted using 15 data points.

		RGR	R^2
I. balfourii	Well-watered	0.147 ± 0.018	0.844
	Mild water deficit	0.181 ± 0.019	0.871
	Severe water deficit	0.195 ± 0.026	0.816
I. glandulifera	Well-watered	0.381 ± 0.032	0.915
	Mild water deficit	0.355 ± 0.042	0.857
	Severe water deficit	0.388 ± 0.049	0.830

Relative growth rate was significantly higher in *I. glandulifera* under all water regimes. For both species, no differences in RGR were found among water regimes (Table 5.7 and Figure 5.5).

Table 5.7 Comparison of RGR between *I. balfourii* and *I. glandulifera* growing under three different water regimes. Relative growth rates were calculated as the slopes of the regression lines and compared with a two-tailed independent-samples t-test. For each comparison the t-value and the p-value are reported.

Relative growth rate	t	*p*
I. glandulifera vs. *I. balfourii* – Well-watered	12.502	**<0.001**
I. glandulifera vs. *I. balfourii* – Mild water deficit	8.794	**<0.001**
I. glandulifera vs. *I. balfourii* – Severe water deficit	8.721	**<0.001**
I glandulifera		
– Well-watered vs. Mild water deficit	1.484	0.150
– Well-watered vs. Severe water deficit	0.603	0.552
I. balfourii		
– Well-watered vs. Mild water deficit	1.075	0.292
– Well-watered vs. Severe water deficit	0.540	0.593

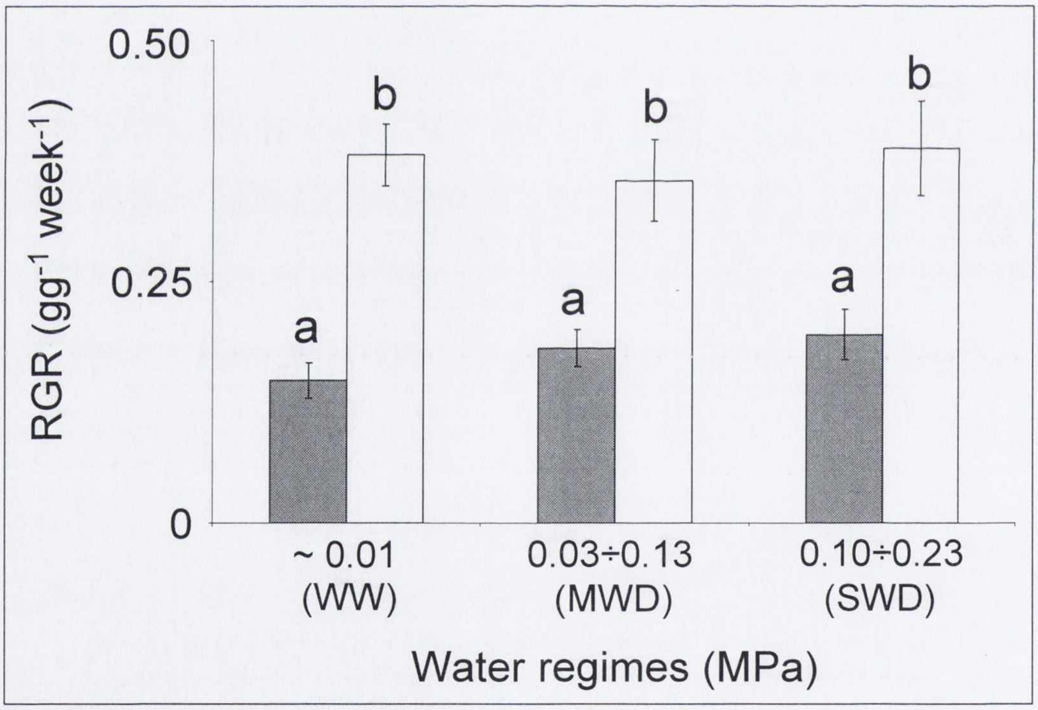

Figure 5.5 Relative growth rate ± SE in *I. balfourii* (grey bars) and *I. glandulifera* (white bars) growing under three water regimes (WW = well-watered; MWD = mild water deficit; SWD = sever water deficit). For each water treatment it is indicated the range of soil water potential measured before the last harvest. Different letters above bars indicate significant differences (Independent t-test, p<0.05).

Plant-allometric and leaf-morphological traits

From the initial comparison of growth, allometry and morphological data on seedlings of the two species growing in the same water conditions I found significant differences between species in all the traits except for SWR.

I. glandulifera showed higher height, LWR, LAR, SLA and LA:RM while *I. balfourii* showed higher biomass and RWR (Table 5.8).

Table 5.8 Comparison of growth allometry and morphological traits between *I. balfourii* and *I. glandulifera* before plants were assigned to the different water regimes. For each trait is reported the mean difference between species ± SE, the t-value and p-value of a two-tailed independent-samples t-test.

Trait	***df***	**Mean difference (*I. gand.- I. balf*)**	**t**	***p***
Height	*16*	7.444 ± 1.168	6.374	**<0.001**
Leaf area	*16*	0.006 ± 0.003	-1.886	0.078
biomass	*16*	-0.507 ± 0.124	-4.079	**0.001**
LWR	*16*	0.355 ± 0.098	3.616	**0.002**
SWR	*16*	-0.019 ± 0.016	-1.202	0.247
RWR	*16*	-0.137 ± 0.039	-3.500	**0.003**
LAR	*16*	0.020 ± 0.004	5.464	**<0.001**
SLA	*16*	0.564 ± 0.152	3.723	**0.002**
LA:RM	*16*	0.121 ± 0.020	5.947	**<0.001**

Comparing data collected from plants growing well-watered and under mild and severe water deficit, differences in plant allometry were found between species. In particular, *I. glandulifera* showed higher SWR and RWR than *I. balfourii* while the later showed higher LWR (Table 5.9 and Figure. 5.6 a, b and c). LWR and SWR were affected also by the factor "Time". Both species decrease their LWR and increased their SWR over the growth season (Figure. 5.6 a and b). Species also showed significant differences in leaf-morphological traits (Table 5.9). *I. balfourii* had a higher LAR, SLA and LA:RM than *I. glandulifera* (Figure. 5.6 d, e and f). Both species decreased their LAR significantly over the time. *I. glandulifera* increased SLA whilst *I. balfourii* decreased its SLA, which resulted in a significant interaction effect between species and time for this trait. Species also showed differences in total biomass and in total leaf area. *I. glandulifera* reached a bigger size and produced greater total leaf area per plant then *I.*

balfourii (Figure 5.6 g). Both total biomass and total leaf area increased for both species over time. No significant differences due to the water availability conditions were found in any of the measured traits.

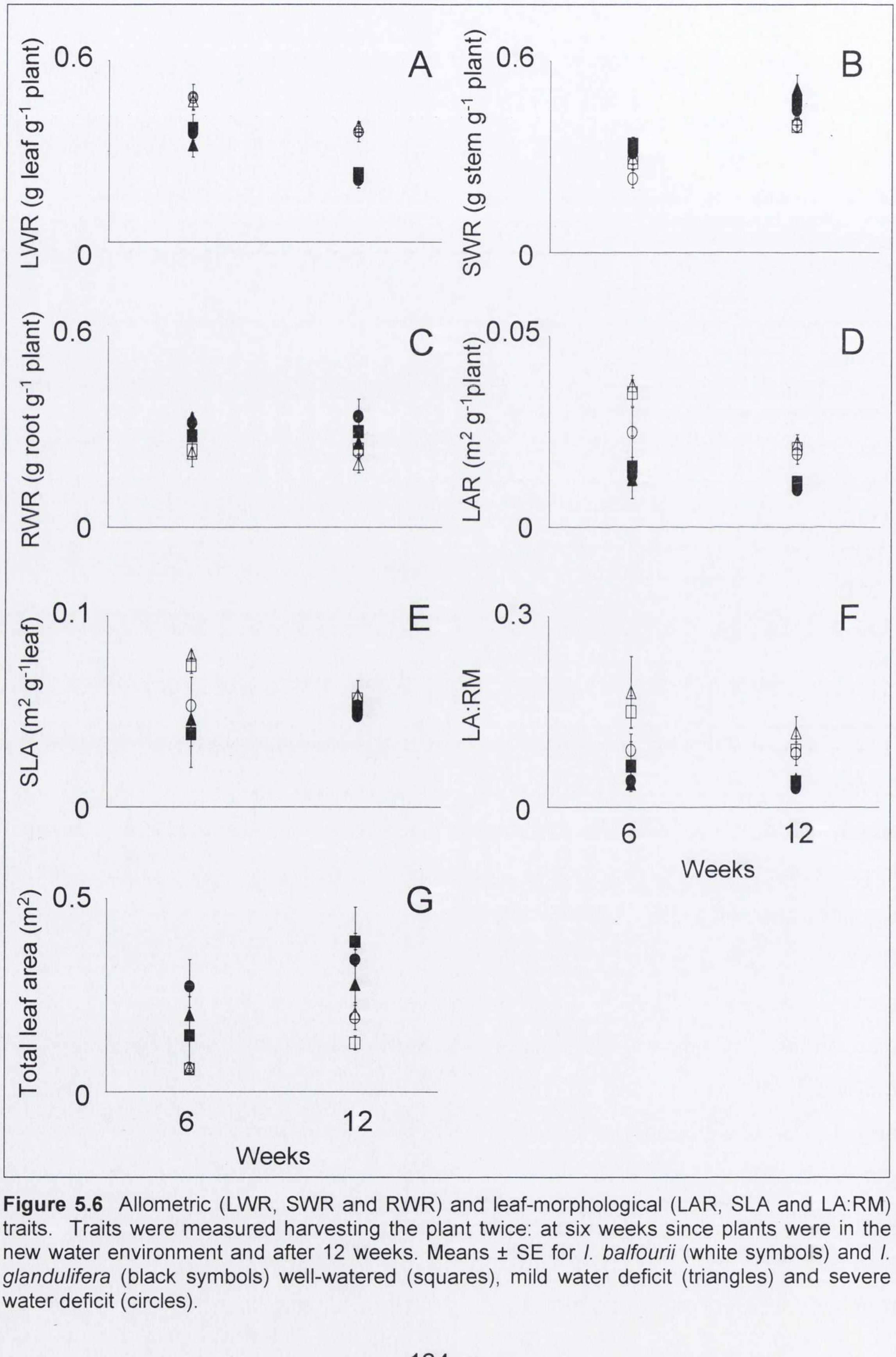

Figure 5.6 Allometric (LWR, SWR and RWR) and leaf-morphological (LAR, SLA and LA:RM) traits. Traits were measured harvesting the plant twice: at six weeks since plants were in the new water environment and after 12 weeks. Means ± SE for *I. balfourii* (white symbols) and *I. glandulifera* (black symbols) well-watered (squares), mild water deficit (triangles) and severe water deficit (circles).

Table 5.9 F-values and p-values of the full factorial univariate ANCOVA and ANOVA. S = species, WT = water treatment, Time = time of the measurement (Figure 5.1). * indicate interactions between factors. Significant p-values are reported in bold.

Plant allometry		**Species**	**WT**	**Time**	**S*WT**	**S*T**	**WT*T**	**S*WT*T**
	df	*1, 24*	*2, 24*	*1, 24*	*2, 24*	*1, 24*	*2, 24*	*2, 24*
LWR	F	73.022	0.200	55.833	0.373	0.783	0.676	0.062
	p.	***<0.001***	*0.820*	***<0.001***	*0.693*	*0.385*	*0.519*	*0.940*
SWR	F	15.423	2.296	69.685	0.005	0.199	0.246	0.581
	p.	***0.001***	*0.123*	***<0.001***	*0.995*	*0.660*	*0.784*	*0.567*
RWR	F	13.435	1.654	1.214	0.313	0.119	0.908	0.739
	p.	***0.001***	*0.213*	*0.282*	*0.735*	*0.733*	*0.417*	*0.488*
Leaf morphology								
LAR	F	33.260	0.822	6.057	0.526	2.139	0.081	0.600
	p.	***<0.001***	*0.452*	***0.022***	*0.598*	*0.157*	*0.922*	*0.557*
SLA	F	12.976	1.871	0.133	0.537	5.772	0.575	0.531
	p.	***0.001***	*0.176*	*0.719*	*0.591*	***0.024***	*0.570*	*0.595*
LA:RM	F	29.172	1.259	1.252	0.709	0.536	0.086	0.618
	p.	***<0.001***	*0.273*	*0.305*	*0.408*	*0.592*	*0.918*	*0.548*
Plant growth								
Leaf area	F	24.435	0.564	15.366	0.593	0.079	0.306	1.587
	p	***<0.001***	*0. 576*	***0.001***	*0.561*	*0.781*	*0.739*	*0.225*
Biomass	F	119.404	2.299	51.715	0.104	6.451	0.489	1.457
	p.	***<0.001***	*0.123*	***<0.001***	*0.901*	*0.018*	*0.619*	*0.254*

5.4.3 Photosynthetic characteristics and utilization efficiency

From the initial comparison of photosynthesis-related traits in seedlings of the two species growing in the same water condition, we found significant differences between species in LCP and in RE only.

I. glandulifera showed significantly lower LCP and significantly higher RE than *I. balfourii* (Table 5.10).

Table 5.10 Comparison of photosynthesis-related traits between *I. balfourii* and *I. glandulifera* before plants were assigned to the different water regimes. For each trait is reported the mean difference between species ± SE and the t-value and p-value of a two-tailed independent-samples t-test. Stomatal conductance was lambda transformed ($\lambda = -0.80$) to achieve normality. For the other variables no transformation was needed since untransformed data showed normal distribution.

Photosynthetic trait	*df*	**Mean difference** **(*I. gand.- I. balf*)**	**t**	*p*
Rd	*10*	0.634 ± 0.289	2.197	0.053
ϕ	*10*	0.001 ± 0.003	0.217	0.832
A_{max}	*10*	1.668 ± 1.393	1.197	0.259
LCP	*10*	-18.333 ± 6.981	-2.626	**0.025**
LSP	*10*	21.333 ± 45.342	0.471	0.648
WUE	*10*	-0.246 ± 0.318	-0.774	0.457
lnRE	*10*	1.055 ± 0.385	2.739	**0.021**
GS	*10*	0.005 ± 0.003	1.540	0.159

After plants were exposed to the different water stress treatments, significant differences were found in apparent quantum yield (ϕ). *I. balfourii* showed a higher ϕ then *I. glandulifera*. Differences in ϕ were also found among water treatments (Table 5.11). In the mild water deficit regime, ϕ was significantly higher then in the severe water deficit regime. No differences were found between well-watered and severe water deficit.

Light compensation point also varied according to the water availability (Table 5.11). Lower LCP was observed in plants growing in the severe water deficit regime compared with the other two water regimes.

Table 5.11 Repeated measures ANOVA table for the photosynthetic traits and for the utilization efficiency. * indicate interactions between factors. Significant p-values are reported in bold.

Photosynthesis characteristics		**Species**	**WT**	**S*WT**
	df	*1, 12*	*2, 12*	*2, 12*
A_{max}	F	0.356	0.272	1.073
	p.	0.563	0.767	0.375
Rd	F	0.878	0.277	2.428
	p.	0.369	0.763	0.134
ϕ	F	14.973	6.050	1.497
	p.	**0.002**	**0.015**	0.263
LCP	F	0.000	5.086	3.470
	p.	0.995	**0.033**	0.076
LSP	F	0.413	0.291	1.394
	p.	0.541	0.756	0.309
Utilization efficiency				
WUE	F	0.023	3.151	0.980
	p.	0.883	0.083	0.406
RE	F	0.033	0.185	0.994
	p.	0.860	0.834	0.404

Stomatal conductance (GS)

Figure 5.7 shows the stomatal conductance of the two species growing at different water availabilities. *I. glandulifera* appears to shows higest variation then I. balfourii. In particular, this species consistently shows the lowest stomatal conductance at the lowest water availability. Nevertheless, no significant statistical difference in stomatal conductance was found between species. However it has been possible to detect significant differences in GS among treatments. Bonferroni *Post-hoc* test revealed significantly higher GS in plants growing in abundant water compared to plants growing in severe water deficit conditions. No differences in stomatal conductance were found between the intermediate water regime and well-watered or severe water deficit (Figure 5.7).

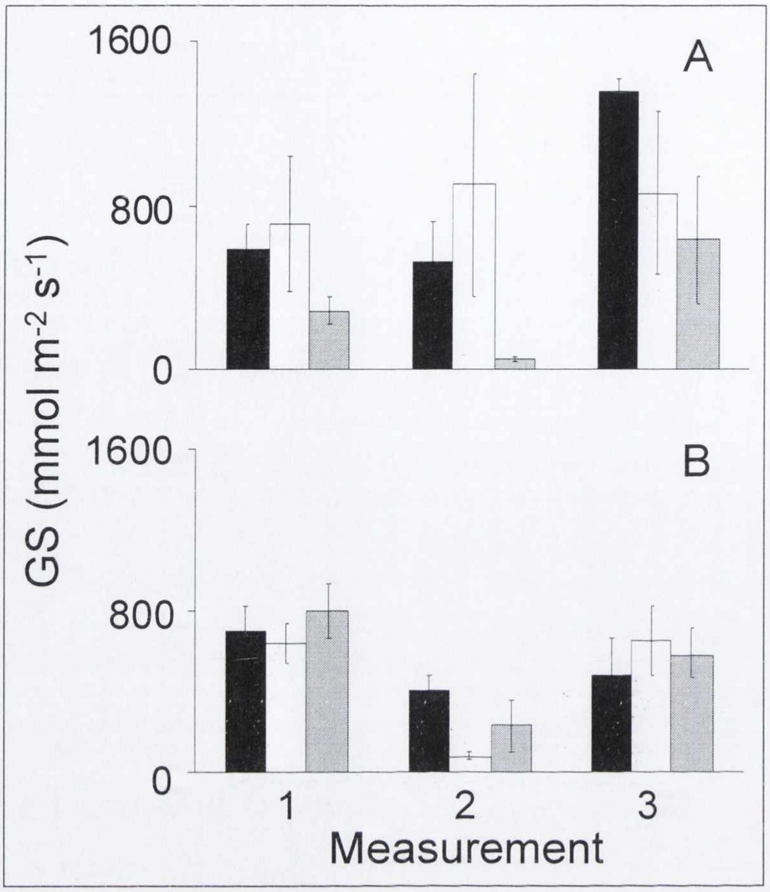

Figure 5.7 Stomatal conductance in leaves of *I. glandulifera* (A) and *I. balfourii* (B) plants under three water regimes: well-watered (black), mild water deficit (white) and severe water deficit (grey). Measurements were repeated three times during the experiment (1, 2 and 3).

Table 5.12 Repeated measures ANOVA table for the stomatal conductance and *post-hoc* comparison using Tamhane's test between each pair of water treatments. Mean differences are mmol $m^{-2}s^{-1}$. Data were square-root transformed and a normal distribution was obtained. Transformation did not improve homogeneity of variance. * indicates interactions between factors. Significant p-values are reported in bold.

Stomatal Conductance	*df*	F	*p*
Species	1, 12	6.679	0.134
Water level	2, 12	2.588	**0.011**
Species * Water level	2, 12	1.058	0.377
Post-hoc comparisons			*p*
Well-watered vs. Mild water deficit			1.000
Well-watered vs. Severe water deficit			**0.014**
Mild water deficit vs. Severe water deficit			0.056

Photosynthesis in leaves of different age

We did not find significant differences in net photosynthesis (A) between species however we found significant differences due to the leaf age and to the water regimes in which plants were growing. A was significantly higher in the first and second upper leaves than in the third upper leaf. Net photosynthetic rate (A) was also higher in the well-watered plants than in plants growing in severe water deficit regimes. No differences were found between the well-watered and the mild water deficit treatment and between mild water deficit and severe water deficit treatment (Table 5.13 and Figure. 5.8).

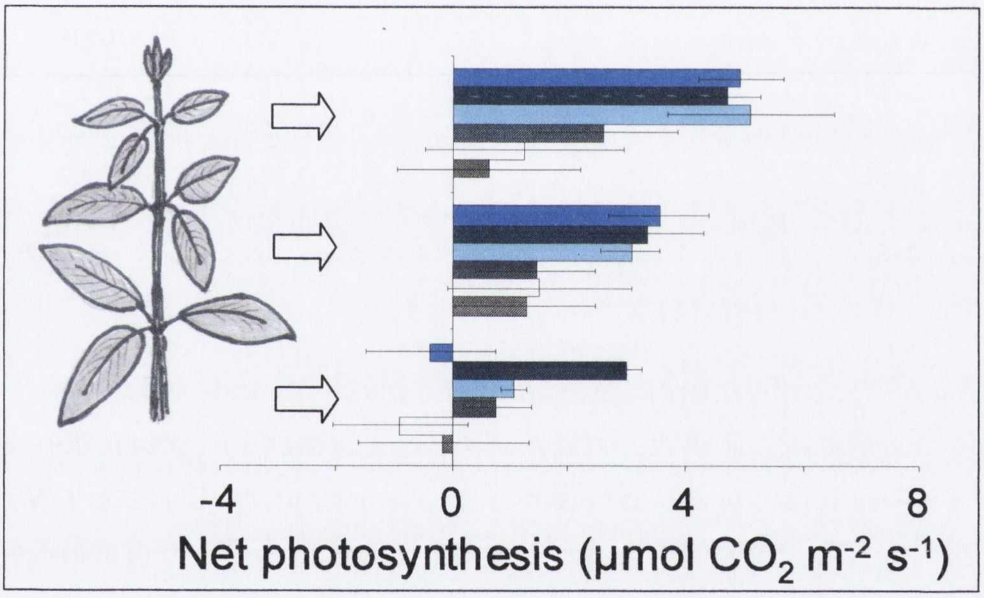

Figure 5.8 Light saturated net photosynthesis (± SE) in three different leaves per plant (first, second and third fully developed leaf from the apical tip of the plant; N = 6) of *I. glandulifera* (open bars) and *I. balfourii* (dashed bars) under three water regimes: well-watered (dark blue), mild water deficit (light blue) and severe water deficit (white).

Table 5.13 Univariate ANOVA table for the photosynthetic response in leaves of different age and *post-hoc* comparison Tamhane's test (which does not assume equal variance, but normality of data) between each pair of water treatments and between pairs of leaves with different age. * indicates interactions between factors. Mean differences are in μmol CO_2 $m^{-2}s^{-1}$. Significant p-values are reported in bold.

Photosynthesis in leaves of different age	*df*	F	*p*
Species	1, 87	0.113	0.738
Water level	2, 87	8.264	**0.001**
Leaf age	2, 87	8.626	**<0.001**
Species * Water level	2, 87	1.769	0.177
Species * Leaf	2, 87	1.957	0.147
Water level * Leaf	4, 87	0.682	0.606
Species * Water level * Leaf	4, 87	0.222	0.926
Post-hoc comparisons	*Mean difference*		*p*
Well-watered vs. Mild water deficit	0.891		0.375
Well-watered vs. Severe water deficit	2.633		**0.001**
Mild water deficit vs. Severe water deficit	1.742		0.053
First leaf vs. Second leaf	0.829		0.600
First leaf vs. Third leaf	2.560		**0.002**
Second leaf vs. Third leaf	1.731		**0.017**

5.4.4 Trait phenotypic plasticity and plasticity indexes

From the comparison of all the relative distances between *I. glandulifera* and *I. balfourii*, we found significant differences in plasticity in WUE and in LWR. For both traits *I. glandulifera* showed higher plasticity then *I. balfourii* (Table 5.14).

Table 5.14 Relative distance plasticity index (RDPI) calculated for *I. balfourii* and *I. glandulifera*. Comparison of trait plasticity between *I. balfourii* and *I. glandulifera*. All values were arc-sin transformed. For each trait the t-value and p-value of a two-tailed independent-samples t-test are reported. Plasticity value of LAR, SLA, ϕ and GS were compared between species with a non-parametric two-independent-samples Mann-Whitney Z-test.

Trait	**RDPI**				
	I. glandulifera	***I. balfourii***	***df***	**t**	***p* (2-tailed)**
Leaf Ψ_W	0.446	0.541	*52*	-1.333	0.187
Biomass	0.252	0.188	*32*	0.987	0.331
H	0.118	0.115	*16*	0.057	0.955
LWR	0.088	0.053	*32*	2.037	**0.050**
SWR	0.101	0.098	*32*	-0.276	0.785
RWR	0.098	0.133	*32*	-1.662	0.106
LA:RM	0.226	0.270	*32*	-0.968	0.340
Rd	0.172	0.169	*34*	-0.019	0.985
A_{max}	0.229	0.247	*34*	-0.353	0.726
LCP	0.296	0.179	*34*	1.604	0.118
LSP	0.123	0.175	*34*	-1.365	0.181
WUE	0.314	0.154	*34*	2.131	**0.040**
RE	0.274	0.251	*34*	0.246	0.807
				Z	**Asymp. Sig. (2-tailed)**
Leaf area	**0.306**	**0.141**		2.499	**0.012**
LAR	0.173	0.149		0.207	0.836
SLA	0.186	0.142		0.475	0.635
ϕ	0.154	0.034		1.392	0.164
GS	0.462	0.490		0.285	0.775

The reaction norm for *I. glandulifera* and *I. balfourii* for the total leaf area (Figure 5.9) shows fitness responses, in terms of total leaf area, to the different environmental conditions (represented by the water regimes) (Richards *et al.*, 2006).

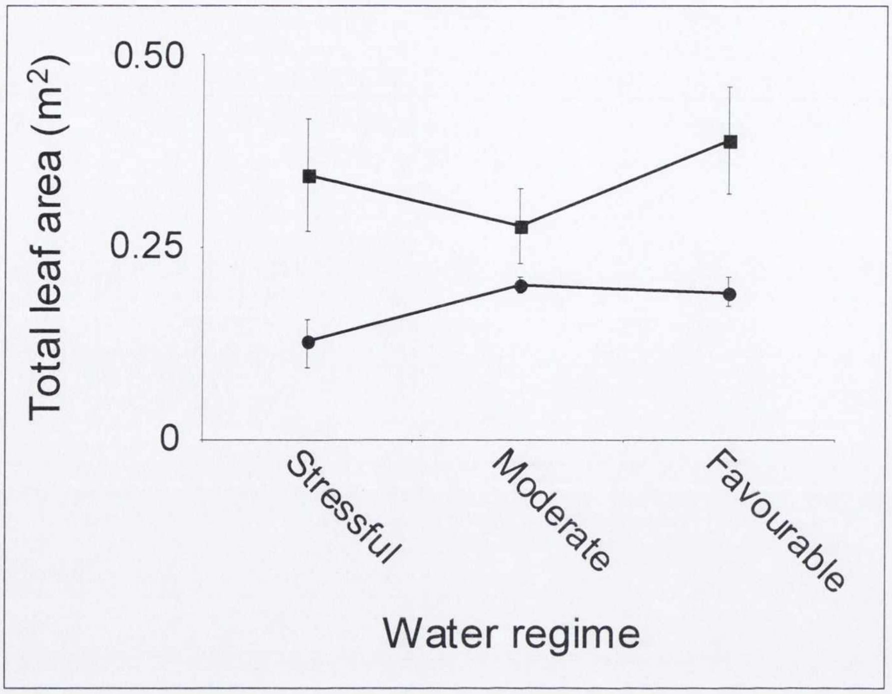

Figure 5.9 Reaction norm for *I. glandulifera* (squares) and *I. balfourii* (circles) for the total leaf area as an example of reaction norm according to Richards *et al.* (2006) Points represent means and bracket ± SE.

5.5 DISCUSSION

5.5.1 Leaf water potential (Leaf ψ_W) and stomatal conductance (GS)

It is vital for plants which grow in dry conditions to be able to conserve water. If plants lose sufficient water to fall below their wilting point they are not able to recover and they inevitably die (Kirkham, 2005). Plants, in a dry environment, in order to reduce water loss due to transpiration and to actively maintain active the water uptake from the soil, need to be able to lower their leaf water potential (ψ_W) to create a gradient between plant and soil. The mechanism to reduce water loss includes a reduction of the size of the open stomata which leads to a reduction of the stomatal conductance. Decreasing stomatal conductance and leaf water potential however reduces plant metabolism and consequently affects plant biomass production (Adejare and Umebese, 2007).

Both species were observed to lower their leaf ψ_W when exposed to severe water deficit. In addition, the two species reduced their stomatal conductance in drying soil, as a mechanism to reduce transpiration and water loss. Even if the statistical analysis did not detect an overall significant difference between the two species, from Figure 5.7 it is possible to observe a higher variation in stomatal conductance in *I. glandulifera*. This species consistently showed the lowest stomatal conductance at the lower water availability. Decreasing stomatal conductance reduces plant metabolism and consequently it should affect plant biomass production (Adejare and Umebese, 2007) but a reduction in the size of the open stomata which leads to a reduction in the stomatal conductance represents a mechanism to reduce water loss.

5.5.2 Trait performances across water treatment

Geng *et al.* (2006), in a similar experiment, compared phenotypic plasticity in genotypes of native and introduced invasive congeners of *Althernanthera* growing in different conditions of water availability. They found general similarity in the patterns of biomass allocation in response to the water regimes. Both invasive and native species under drought conditions allocated more biomass to roots, and they decreased their specific leaf area and their internode length. These functional adjustments enable the plants to increase their effectiveness of water uptake via the root system and to minimize the total water transpiration by reducing leaf area. Consistent with the study of Geng *et al.* (2006), we found that water stress in both species causes a reduction in stem length. This study, on the other hand, did not detect differences for other allometry and leaf-morphological traits due to the effect of the water treatments.

Some authors who have investigated water stress in plants reached lower leaf water potentials in the stressed treatments (e.g. Gomes *et al.*, 2004) but the differences in species' water requirements and in the ways to quantify water soil moisture make comparisons extremely difficult. In my experiment, both species showed visible signs of stress in both the mild and sever water deficit regimes but it might be possible that the stress caused by the water deficit was not intense enough to produce permanent adjustments in plant biomass allocation, leaf morphology and photosynthetic characteristics. Relative growth rate and total leaf area, which are traits particularly sensitive to stress, did not show variation due to the water treatments, suggesting that a stronger stress would have been needed to investigate the response of these two species.

The only photosynthetic traits that were an exception, which consequently showed variations due to the availability of water, were ϕ and LCP. Apparent quantum yield (ϕ), consistent with the results presented in the Chapter 4, was significantly higher in *I. balfourii* than in *I. glandulifera*. In addition, the highest ϕ was observed in plants growing in mild water deficit conditions and that may be due to *I. balfourii* which largely increased its ϕ at mild water deficit (but it decreases ϕ again in severe water deficit condition). Schmitz and Dericks (2008) assessed the photosynthetic characteristics of *I. balfourii* at different temperatures and light intensity and its growth in different soil moisture conditions but did not measure photosynthesis in relation to soil moisture. Their results show that *I. balfourii* shows better growth performances under "dry to humid soil conditions" (Schmitz and Dericks, 2008). Therefore, *I. balfourii* might exhibit higher fitness in less moist soil conditions and might possibly be able to perform better in terms of photosynthesis in drier environments.

Light compensation points also showed significant differences under water stress. The importance of this trait is usually associated with the light environment and it indicates the ability of plants to grow in shady habitats. From the experiment presented in Chapter 4, it is possible to observe how the two species lower their LCP to cope with the lower light resource environment. It was not anticipated that water availability would affect this trait. On the contrary, it was expected that water stress would affect the efficiency-related traits such as RE and WUE. However, no differences due either to species or water regimes were found for these traits. Differences in RE were found between species only before plants were assigned to the different water regimes. *I. glandulifera* seedlings showed a significantly higher RE than *I. balfourii* seedlings growing in the same condition. Respiration efficiency is a trait considered to contribute to plant fitness as an increase in RE causes a reduction in carbon cost (Pattison *et al.*, 1998; McDowell, 2002; Feng *et al.*, 2007b). Failing to detect differences in RE and WUE might be due to the low level of replication.

5.5.3 Light saturated net photosynthesis in leaves of different age

Over time, as the position of a leaf in the canopy became progressively lower, the photosynthetic capacity of the leaf was reduced. The correlation between photosynthetic activity of a leaf and leaf age and position, which had been already assessed (e.g. Kikuzawa *et al.*, 2009; Kositsup *et al.*, 2010), was confirmed in this study. In the two *Impatiens* species, the photosynthetic activity, and consequently the carbon assimilation and plant growth (Grime and Hunt, 1975), were reduced when leaves were older than three weeks. Such leaves can represent a carbon cost instead of a carbon gain and negatively affect the whole plant carbon balance. Leaf age and/or position in the canopy are leaf characteristics which should be included among the parameters used in modeling canopy photosynthetic activity (Kositsup *et al.*, 2010).

In addition, in this study, it has also been possible to observe that a severe water deficit might affect the photosynthetic capacity of leaves. The net photosynthetic rate is significantly lower in the severe water stress regime as a consequence of the stomata closure. Stomatal conductance follows the same pattern as the light saturated net photosynthetic rate across water treatments. Kositsup *et al.* (2010) found that, among the leaf gas exchange parameters that they measured, GS showed the best correlation with net photosynthetic rate.

However, the invasive and non-invasive species did not show any significant differences in how leaf age and water regimes affect their light saturated net photosynthetic rate. Both *I. glandulifera* and *I. balfourii* showed significant reductions in net photosynthetic rate in older leaves and at lower water availability.

5.5.4 Differences in traits and trait plasticity between the invasive and non-invasive species

Consistent with other studies which considered growth-related traits (van Kleunen *et al.*, 2010b) and with the findings presented in previous chapters (Chapter 3 and 4), I found that the invasive *I. glandulifera*, compared to the non-invasive *I. balfourii* reached a bigger size (greater biomass and height) and grew faster (higher relative growth rate) in all water treatments. It also produced greater total leaf area per plant which entails greater total carbon assimilation per plant. Once more, as in the previous chapters, *I. glandulifera* allocated more biomass to the support organs which suggests the capacity for rapid growth that allows the invasive to out-perform slower-growing species. Additionally, in this study I found a higher biomass allocation to roots (higher RWR) in *I. glandulifera* than in *I. balfourii*. Daehler (2003), which considered several comparisons between invasives and non-invasives, found that a broad inconsistency characterizes the studies that aim to compare RWR of invasive and non-invasive species. Inconsistency might be due to the fact that biomass allocation to roots can contribute to fitness in different ways. A low RWR would contribute to fitness by reducing root respiration and consequently increasing the whole-plant carbon assimilation. On the other hand, a high RWR would increase the water and nutrient uptake (Pattison *et al.*, 1998; D'Antonio *et al.*, 2001). In my particular study, given the experimental environmental conditions characterized by limited water availability, greater biomass allocation to roots might contribute to plant fitness because it increased water uptake. The invasive, *I. glandulifera*, also showed higher biomass allocation to root in the control treatment. The warm greenhouse temperatures might have made it necessary to maximize water uptake even when plants were grown in abundant water availability.

Differences between species were found also in leaf-related traits. While *I. glandulifera* showed higher total leaf area, *I. balfourii* exhibited higher LWR and LAR due to a significantly higher SLA which is directly proportional to the two previous traits (equation 3.2). Generally leaf attributes are considered to be related to plant invasiveness (Daehler, 2003 but see; van Kleunen *et al.*, 2010b). In my study however, it is the non-invasive species that exhibits those leaf morphological traits that enable it to maximise light-resource capture. Nevertheless, when the same traits were compared in seedlings of the two species that had not been subjected to any water deficit treatment, an opposite trend was observed and the invasive *I. glandulifera* showed higher LWR, LAR, SLA and LA:RM than the non-invasive. Adjustments in leaf morphology and biomass allocation could possibly be due to the variation in water regimes and warmer temperatures.

In my study, I did not find significant differences in performances trait values across the water regimes. However, when trait plasticity was compared, significant differences were found for three traits. *I. glandulifera* showed higher plasticity than *I. balfourii* in relation to the total leaf area produced per plant, in relation to the leaf weight ratio (LWR) and in relation to the water use efficiency (WUE). Biomass allocation to leaves (total leaf area and LWR) and water use efficiency (WUE) are fundamental aspects of plant physiology and both traits are extremely important in plants which have to cope with changing water availability. However, very little variation was shown for these trait across water treatment and no differences in plasticity were found in other efficiency–, or performance–related traits that might also be extremely important in water deficit conditions (as for example RWR or SLA). Once again, the lack of variation, and consequently tha lack of plasticity, that these species showed in respect to the changes in water availability might be due to the fact that water potential in the soil never fell under 0.3 MPa and therefore it was always relatively high.

Van Kleunen *et al.* (2010b) reviewed 117 studies that compare invasive and non-invasive species. They found more trait differences in invasive versus native comparisons than in invasive versus non-invasive introduced comparisons. In their review, however, only six studies compared invasive and non-invasive introduced species. The studies which hypothesized greater phenotypic plasticity in introduced species than in native species have generally been able to confirm such hypotheses (e.g. Schweitzer and Larson, 1999; Sultan, 2001; Daehler, 2003; Geng *et al.*, 2007), with the exception of some studies which did not find differences in plasticity between native and introduced species (Gonzalez and Gianoli, 2004; Brock *et al.*, 2005). Among the studies that chose a non-invasive introduced counterpart to the invasive, Burns *et al.* (2006) found higher plasticity in the invasive species compared with the non-invasive species in performance-related traits (such as total biomass) but not in competitive ability-related traits (such as SLA and plant-allometry traits). In addition, Feng *et al.* (2007), for instance, did not find any differences in plasticity between invasive and non-invasive *Gynura* species growing at different light intensities.

Such disparity in the results might be due to the large number of studies focused on native versus introduced, or it could also be due to a genetic differentiation of the introduced population in the introduced range from the sources population in the native range (Sakai *et al.*, 2001; Caño *et al.*, 2008). The strong selection pressure involved in the evolution of introduced species in their new range might favour individuals with greater adaptive capacities, which are more plastic (Richards *et al.*, 2006). Consequently, comparisons between native and introduced species will more often show the native as more specialized and the introduced as more plastic. Studies that include populations of invasive species both from the native and introduced range might be necessary to investigate the evolution of phenotypic plasticity (Bossdorf *et al.*, 2005).

5.6 CONCLUSION

With the findings presented in this chapter, we can not explain *I. glandulifera*'s greater invasive potential as being because of greater phenotypic plasticity since this species, like the non-invasive *I. balfourii*, showed little variation in the measured traits across the various water availability environments. The experimental water conditions, however, might have not been stressfull enough to cause variations in the measured traits. Therefore species plasticity might have been underestimated in this study and further investigation would be needed to explain the role of plasticity in determining the invasivenesso of *I. glandulifera*.

With this study it has been possible to observe that both species, showed similar adjustments to cope to the limited water availability: they decreased the leaf water potential and the stomatal conductance, and they reduced stem length in stressful conditions. Across water conditions, the invasive species outperformed the non-invasive in growth-related traits, showing consistently higher growth rate, height and total leaf area. The present study, which is focused on low water availability conditions, also found the characteristics of the invasive to be a higher biomass allocation to roots and a lower biomass allocation to leaves. More comparative experiments between invasive and non-invasive (preferably introduced) in water stress conditions would be needed to test the consistency of these patterns. The mechanism of invasion in water-stress conditions needs particular attention since lower water availability will be among the effects of global climate change.

6 Potential for hybridization between *Impatiens glandulifera* and *I. balfourii*

Plate 6.1 *Impatiens glandulifera* and *I. balfourii* growing together and flowering at the same time. The picture was taken (by the author) in Saint Christophe en Oisans (Rhone-Alpes, Isere, France) at the beginning of August 2010.

SUMMARY

Hybridization can result in evolutionary variations and thus increase invasiveness since it may produce new adaptive systems adjusted to various habitats. These evolutionary variations can, in theory, allow a plant to succeed in a multitude of environmental conditions.

In addition to ornamental varieties of hybrid *Impatiens*, hybridizations within this genus are reported to occur between wild populations both in temperate and in tropical regions. The invasive *I. glandulifera* and its less aggressive congener *I. balfourii* exhibit similar reproductive and ecophysiological characteristics but little is known of their hybridization potential.

With this experiment I aimed to test whether the possibility of hybridization between these two species exists. It was first considered whether the two species flower simultaneously in Ireland and whether pollinators switch from one species to the other. Afterwards, homospecific and heterospecific artificial crosses were performed between *I. glandulifera* and *I. balfourii*, and seed production and hybrid germination success was assessed.

Flowering times of *I. balfourii* and of *I. glandulifera* overlap, and the pollinators have been observed to switch from one species to the other, confirming the natural possibility of pollen transfer between the two species.

Heterospecific crosses produced seeds. This would suggest the possibility of improper pollen transfer, and could represent a competition mechanism between the two species because it could reduce the seed set produced by homospecific pollination in both species. However, due to the lack of germination of the hybrid seeds in this experiment I exclude the possibility of successful hybridization.

6.1. INTRODUCTION

Of all the species that are able to overcome geographical barriers (by means of human intervention) and arrive in an introduced range, only a very small proportion is able to persist (Williamson, 1993; Ellstrand and Schierenbeck, 2000). When a novel species arrives in a new environment, if it is able to survive, it generally goes through a time lag before it eventually spreads and becomes invasive (Ellstrand and Schierenbeck, 2000). The lag phase for *I. glandulifera*, for example, was quantified in the Czech Republic as 40 years (Pysek and Prach, 1993). To enable an introduced plant species to spread from a restricted area with specific environmental conditions to invade a wider range of habitats characterized by various environmental conditions, a multi-purpose genotype which allows sufficient levels of physiological adaptability (phenotypic plasticity) is required. If a species does not show high phenotypic plasticity it can still become invasive but it has to undergo a genetic differentiation to be able to achieve fitness in different habitats (Richardson and Pysek, 2006).

Ellenstrad and Schierenbeck (2000) suggested that hybridization might result in evolutionary variations which can increase species invasiveness. Hybridization between populations may result in new adaptive systems adjusted to new habitats (Anderson and Stebbins, 1954). The hybrids may be more versatile than parents, and be able to tolerate a wider range of environments, which includes intermediate habitats with respect to the parental species. *Spartina anglica* represents a well known example of a fertile vigorous hybrid which is aggressively colonizing Britain and Western Europe. It originates by the chromosome doubling of a sterile hybrid, *S. x townsedii*, which is the result of the cross between the European *S. maritima* and the introduced North American *S. alterniflora*. *S. anglica* has been introduced in China, Australia and New Zealand where it also became invasive (Petit, 2004; Nehring and Hesse, 2008). Furthermore, when the hybrids are fertile, they might be able to backcross with one or both of the parents, which increases the genetic variation of the natural population through introgression. Introgression is a frequent event among plants (Anderson and Stebbins, 1954), and it allows the production of various recombinations which can be functional in different habitats since the new organism will have a complex mix of parental genetic material. Introgression can occur naturally; human intervention, however, can induce introgression by bringing together new species, which were previously geographically isolated. In addition, human activity, when it creates new ecological niches, can increase the necessity for hybridization since new adaptive systems might represent a selective advantage in new man-made habitats (Anderson and Stebbins, 1954). Milne and Abbott (2000) present evidence of introgression of *Rhododendron pontium* with other *Rhododendron* species in Britain, and they suggest that, in colder regions, natural selection would have acted to favour populations of *R. pontium* with higher levels of introgression from *R. catawbiense*, which would improve cold tolerance (Milne and Abbott, 2000).

Ellenstrad and Schierenbeck (2000) reported 28 examples, across 12 families, of hybrids that had become invasive. Tiébré *et al.* (2007) investigated the sexual reproduction of the *Fallopia* complex, and they concluded that the sexual reproduction among *Fallopia* species can result in the production of new genotypes which can increase the invasive capacity of the genus. The *Fallopia* species, however, outside their native range, reproduce mainly by vegetative regeneration (Weder, 1960; Beerling *et al.*, 1994; Forman and Kesseli, 2003; Tiébré *et al.*, 2007). The genus *Impatiens*, on the contrary, contains annual species which sexually reproduce throughout their introduced range (Perrins *et al.*, 1993). Annual species of the genus *Impatiens* usually produce a large number of seeds that are dispersed with the explosive opening of the capsule (Coombe, 1956; Grey-Wilson, 1980; Beerling and Perrins, 1993; Tabak and von Wettberg, 2008). The genus *Impatiens* contains a great number of hybrids, generally created for ornamental purpose. However, besides these ornamental varieties of hybrid *Impatiens*, hybridizations in *Impatiens* are reported to occur also between wild populations both in tropical regions (Grey-Wilson, 1980; Tsukaya, 2004) and in temperate regions (Zika, 2006). According to Tabak and von Wettberg (2008), frequently co-occurring species in the north-east of the United States (*I capensis* and *I. pallida*) do not appear to hybridize, but these authors suggest a possible hybridization between *I. capensis* and the closely related native European species, *I. noli-tangere.* Ornduff (1967) reported that *I.*

capensis can hybridize with *I. ecalcarada* in the north-west of the United States. However, little information is available on species boundaries between other temperate *Impatiens* species (Tabak and von Wettberg, 2008). The most common *Impatiens* species in Ireland, Britain and continental Europe is the aggressive invader *I. glandulifera* which has been introduced from the Himalayas (DAISIE, 2008; NBN, 2008). From the same native range, with a delay of about 50 years (Beerling and Perrins, 1993; Adamowski, 2009), *I. balfourii* was also introduced to Europe. The former species is now present in Ireland and in over 20 European countries where it is considered an unwanted weed (NOBANIS; DAISIE, 2008). The latter was introduced to the south of France and it now occurs in southern and central Europe, but it is not present in Ireland (Reynolds, 2002; Milbau and Stout, 2007). In its introduced range, *I. glandulifera* has been reported to flower from July to October with a delay of 2-3 weeks when growing in shaded sites (Beerling and Perrins, 1993). For *I. balfourii*, there is little information available on its phenology in its introduced range. Although it has been reported to flower in its native range from the end of July to August (Adamowski, 2009). Wild populations of *I. glandulifera* and *I. balfourii* have been seen co-occurring and co-flowering in Saint Christophe en Oisans (Rhone-Alpes, Isere, France) (Plate 6.1). The two species present a similar flower morphology and reproductive system. Flowers are protandrous (Bell *et al.*, 1984) with the male phase prior to the female phase (Titze, 2000). The stigma becomes visible and receptive only when the androecium has completely dehisced off (Wilson and Thomson, 1991; Wilson and Thomson, 1996). *I. glandulifera* is self-compatible (via geitonogamy) but depend upon insect pollination since flowers are not capable of self-pollination because of protandry (Valentine, 1978). *I. glandulifera* has been reported to attract great numbers of bees, especially generalist *Bombus* species (Nienhuis, 2009). Accordingly, *I. glandulifera* has been suggested to have an increased competitive ability to attract bumblebees (Brink and Dewet, 1980; Cresswell, 1990) which might be due to the quality and the abundance of its nectar (Nienhuis, 2009).

I. glandulifera and *I. balfourii* have similar germination characteristics, ecophysiology, plasticity and habitat requirements (Chapter 2, 3, 4 and 5).

Hybridization and subsequent introgression would potentially be enhancing both species' gene-pools.

I. glandulifera is not reported to hybridize with other species (Beerling and Perrins, 1993) and *I. parviflora* is reported likewise (Coombe, 1956). In "Flora of Pakistan" the possibility of hybridization between *I. balfourii* and *I. bicolour* Royle is hypothesized, but the potential for crosses with *I. glandulifera* is not considered (Nasir, 1980).

The aim of this study is to test if there is the potential for hybridization between *I. glandulifera* and *I. balfourii.* In particular, with this study I try to answer the following questions.

1. Does the possibility of natural hybridization between the two *Impatiens* species exist in Ireland, in terms of flowering time and pollinator behaviour?
2. Is there seed production when *I. balfourii* and *I. glandulifera* are pollinated with the other species' pollen?
3. Are seeds produced by cross-pollination viable?

6.2. MATERIALS AND METHODS

In summer 2008, 60 plants of *I. glandulifera* and 60 plants of *I. balfourii* were grown outside in Trinity College Botanic Garden, Dublin (see Appendix 3.1 for plant provenance). To evaluate the possibility of overlapping of the early flowering period in the two species, the experimental plants were monitored from the beginning of June to the end of July and flowering was recorded as the total number of inflorescences produced by all plants. Although plants continue to flower until autumn, the interest in early flowering is due to the fact that the peak of bee activity is registered in that period (Goulson, 2003).

The two species were interspersed and the plants were randomly positioned approximately 1 meter apart from each other. Insect foraging behaviour on flowers was observed for approximately 7 minutes. Insects were identified and the number of visits to each species and foraging patterns were recorded.

In spring 2009, seeds from a population of *I. balfourii* received from the Department of “Jardins botanique and zoologiques, Arboretum national de Chevreloup” (Paris) and four populations of *I. glandulifera* from Chapelizod (Dublin), Golden Acre Path (Edinburgh), Boothbay, Main (US) and Werkendam (The Netherlands) (more details on the populations are available in table 2.1) were germinated (Chapter 2), potted in 20-litre pots and placed outside at Trinity College Botanic Garden in Dublin.

To test the potential for hybridization between *I. glandulifera* and *I. balfourii*, a total of 30 plants (15 plants per species) were selected. On each of them, 30 flower buds were bagged with bridal veil material (with a mesh < 1 mm) to exclude pollinators. The experiment was carried out from June to September 2009 at Trinity College Botanic Garden, Dublin. When experimental plants had produced over 30 flowers per plant, those in excess were removed. Five plants were randomly assigned to each of the treatments: BxG and GxB, where B = *I. balfourii* and G = *I. glandulifera*; the first letter represents the mother plant and the second letter represents the pollen donor. As a control, 30 flower buds of both *I. balfourii* and *I. glandulifera* were bagged, and not pollinated, in five plants per species (treatments B0 and G0). Additionally, 30 flowers of both *I. balfourii* and *I. glandulifera* on five plants per species received pollen from a different plant of the same species (treatments BxB and GxG). Since the flowering occurs only over two or three days (Titze 2000), flowers were checked every day. When, at the end of the male phase, the androecium fell off exposing the gynoecium (Wilson and Thomson, 1991), flowers were hand pollinated by removing the whole anthers from a non-experimental flower pollen donor and applying the pollen directly to receptive stigmas of test flowers (Nienhuis, 2009). Ramdal and Hilu (1990) reported that stigmas of both *I. capensis* and *I. pallida* are receptive from when the androecium falls off until after the perianth drops. Hand pollinated flowers were marked and re-bagged. Once mature, pods were collected and the number of seeds per pod was counted.

Collected seeds were stored dry in paper bags at 20°C for a period of 8 months, after which they received a stratification of 20 days at 4°C to break dormancy (Chapter 2) and they were subsequently placed to germinate in Petri dishes on moist filter paper at 20°C. Germination was compared among four treatments

(BxG, GxB, BxB and GxB) since the non-pollinated flowers (B0 and G0) produced a very low number of seeds.

6.2.1. Data analysis

The numbers of visits of each bee species to plants of *I. glandulifera* or *I. balfourii* were compared using a non-parametric Wilcoxon test (Z) for two related samples.

Data on the seed set (number of seeds per pod) consisted of integer counts, therefore differences between treatments were tested with a generalized linear model (GLM) with Poisson distribution and log-link function (Crawley, 1993). Since data showed overdispersion (i.e. the ratio between the value of the deviance divided by its degrees of freedom and the Pearson chi-square divided by its degrees of freedom was substantially greater than 1) an over-dispersed Poisson model was fitted, including the inverse of Pearson chi-square/df as scale weight, which increased the standard error and made the test more conservative. The type of cross (BxB, BxG, B0, GxG, GxB, G0) was included in the model as categorical predictor (factor). A likelihood ratio chi-squared was chosen as statistics. Pair-wise comparisons between each pair of crosses were performed using sequential Bonferroni's *post-hoc* tests (Crawley, 1993).

To investigate differences in the fruit set (proportion of pods containing seeds) due to the different types of cross, a GLM with Binomial distribution and logit-link function was used. The type of cross (BxB, BxG, B0, GxG, GxB, G0) was included in the model as categorical predictor (factor). A likelihood ratio chi-squared was chosen as statistics. Pair-wise comparisons between each pair of crosses were performed using sequential Bonferroni's *post - hoc* tests (Crawley, 1993).

The percentage of germinated seeds was calculated for each Petri dish and the means were calculated for each treatment. Germination rates of seeds were compared with a binomial logistic GLM with logit-link function. The type of cross (BxB, BxG, GxG and GxB) included in the model as categorical predictor (factor). A likelihood ratio chi-squared was chosen as statistics. Pair-wise comparisons between each pair of crosses were performed using sequential Bonferroni's *post-hoc* test.

All the data analyses were run using SPSS 16 (SPSS, Inc. Chicago IL).

6.3. RESULTS

6.3.1. Flowering overlap

I. balfourii began to flower slightly before *I. glandulifera* and, by the end of July, it produced a greater number of inflorescences (Figure 6.1). However, *I. glandulifera* produces inflorescences that are larger in dimension (Beerling and Perrins, 1993; Adamowski, 2009) and that have a greater number of single flowers for each inflorescence (personal observation). Plants of both species have been observed to flower until late October. When plants are killed by the first frost they occasionally still have flowers (personal observations). Therefore, the two species' flowering time overlaps in Ireland, and the overlap is, at least, from the end of June till October (Figure 6.1).

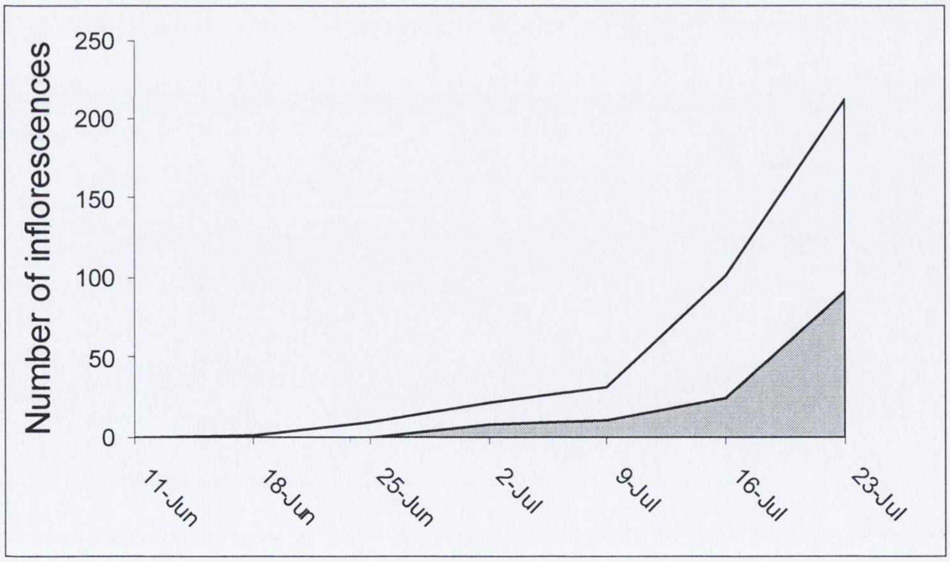

Figure 6.1 Inflorescence production observed in 60 plants of *I. balfourii* (white) and 60 plants of *I. glandulifera* (gray) during summer 2008 at Trinity College Botanic Garden.

6.3.2. Bee behaviour

By the end of the observations, visitation patterns were recorded for eight *Apis mellifera*, six *Bombus hortorum* and 42 *Bombus pascuorum*.

All three bee species visited more *I. glandulifera* flowers (Figure 6.2). This preference was significant in *A. mellifera* and *B. pascuorum* (Table 6.1).

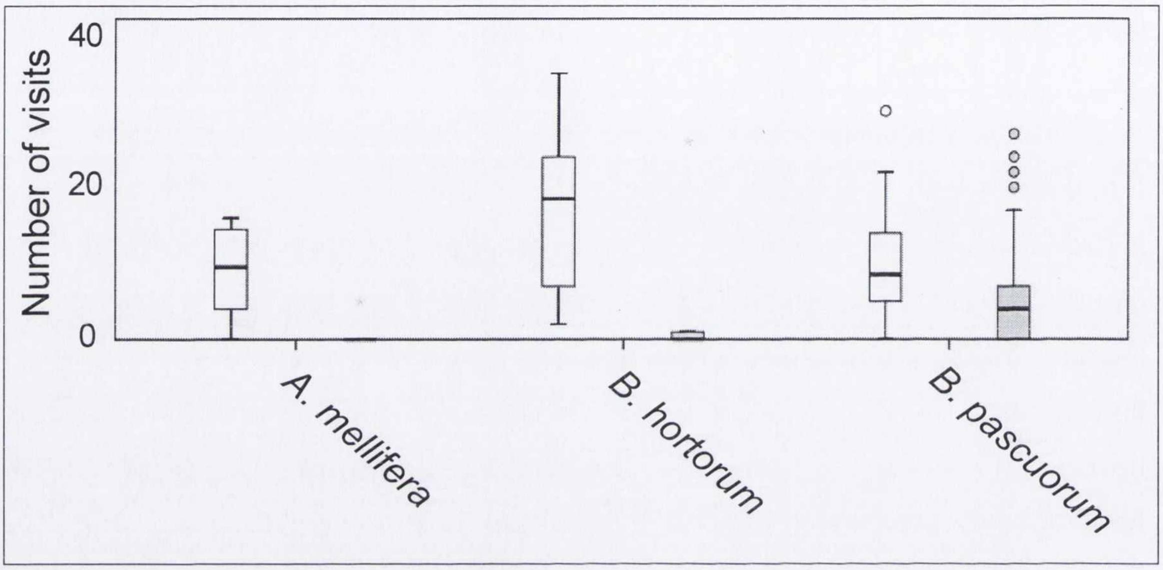

Figure 6.2 Flowers visited by *A. mellifera* (N = 8), *B hortorum* (N = 6) and *B. pascuorum* (N = 42) at TCD Botanic Garden. Bees were followed for approximately 7 minutes while foraging. Median (indicated by the horizontal black line inside the box), upper and lower quartile and adjacent values for the number of visits to *I. balfourii* flowers (gray boxes) and to *I. glandulifera* flowers (open boxes). Circles represent outliers and asterisks represent extreme cases.

Table 6.1 Average number of visits of each pollinator was compared for the two plant species with Wilcoxon test (Z) for two related samples. The table shows Z and p values. Significant differences are reported in bold.

Pollinator	**Z**	*p*
A. mellifera	2.366	**0.018**
B. hortorum	1.782	0.075
B. pascuorum	2.308	**0.021**

For all bee species, the possibility of switching between the two species exists (Figure 6.3). *A. mellifera* was observed mainly on *I. glandulifera* flowers and it was seen switching only from *I. glandulifera* to *I. balfourii*, however the other two pollinators switched between species almost 50% of the time and they both switched from *I. glandulifera* to *I. balfourii* and from *I. balfourii* to *I. glandulifera* (Figure 6.4). Overall, the bee species switched 1.50 ± 0.26 times every 7 minutes.

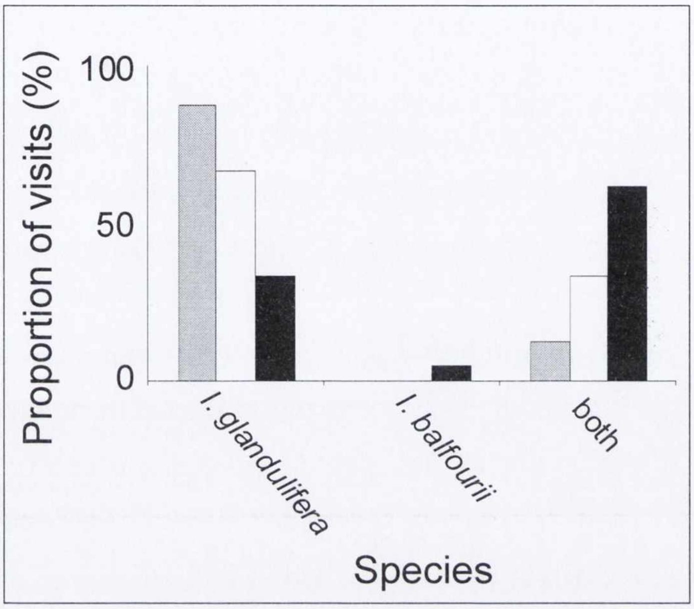

Figure 6.3 Proportion *A. mellifera* (gray bars), *B. hortorum* (white bars) and *B. pascourum* (black bars) that visited only *I. glandulifera*, only *I. balfourii* or both species during a 7 minute foraging bout.

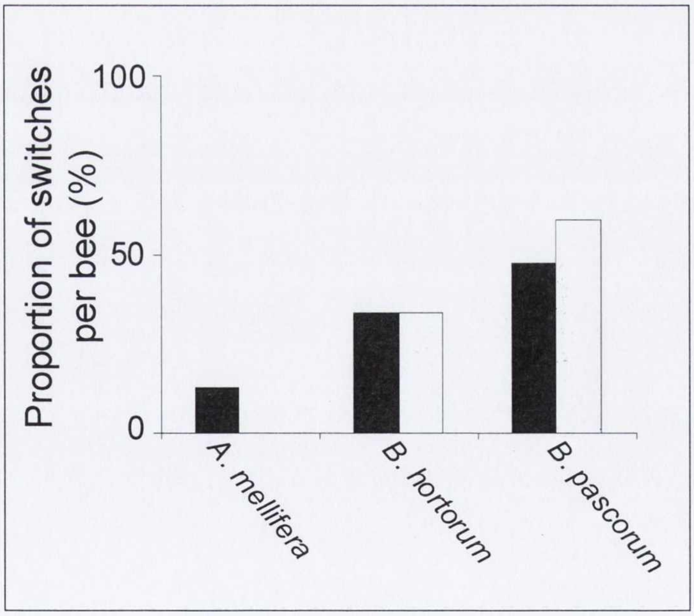

Figure 6.4 Proportion of switching between the two *Impatiens* species for *A. mellifera*, *B. hortorum* and *B. pascorum*. The black bars represent the proportion of switching from *I. glandulifera* to *I. balfourii* and the open bars represent the proportion of switching from *I. balfourii* to *I. glandulifera*.

6.3.3. Seed production

Most pods from bagged flowers, which did not receive any pollen, fell off. The ones which reached maturity contained very low numbers of seeds per pod (mean ± S.E. = 0.057 ± 0.017 and 0.167 ± 0.028 seed pod^{-1} for B0 and G0, respectively). No significant differences were found between the two species. Flowers pollinated with homospecific pollen produced significantly more seeds than flowers pollinated with heterospecific pollen (Table 6.2). Statistical differences were found between *I. glandulifera* and *I. balfourii* in the seed set of plants pollinated with homospecific pollen. Seed set was higher for *I. balfourii* (mean ± S.E. = 4.024 ± 0.139 and 2.487 ± 0.109 seed pod^{-1} for BxB and GxG, respectively). Seed set in plants pollinated with homospecific pollen was significantly higher than seed set in plants pollinated with heterospecific pollen. Differences in seed set were not significant between the two cross types (BxG and GxB), (mean ± S.E. = 0.535 ± 0. 051 and 0.726 ± 0. 059 seed pod^{-1} for BxG and GxB, respectively) (Figure 6.5 a. and Table 6. 2).

As mentioned above, pods from bagged flowers that did not receive any pollen mainly fell off before reaching maturity and so fruit set in both species was very low when flowers were bagged and not artificially pollinated (mean ± S.E. = 0.04 ± 0. 012 and 0.04 ± 0. 014 for B0 and G0, respectively). Fruit set was higher in flowers pollinated with both homospecific and heterospecific pollen. No differences between *I. glandulifera* and *I. balfourii* were found in fruit set in flowers pollinated with homospecific pollen (mean ± S.E. = 0.95 ± 0. 020 and 0.87 ± 0. 031 for BB and GG, respectively). Differences in fruit set were significant between the two hybrids types: *I. glandulifera* flowers pollinated with *I. balfourii* pollen had higher fruit set than *I. balfourii* flowers pollinated with *I. glandulifera* pollen (mean ± S.E. = 0.160 ± 0. 027 and 0.310 ± 0. 036 seed pod^{-1} for BxG and GxB, respectively) (Figure 6.5 b and Table 6. 2).

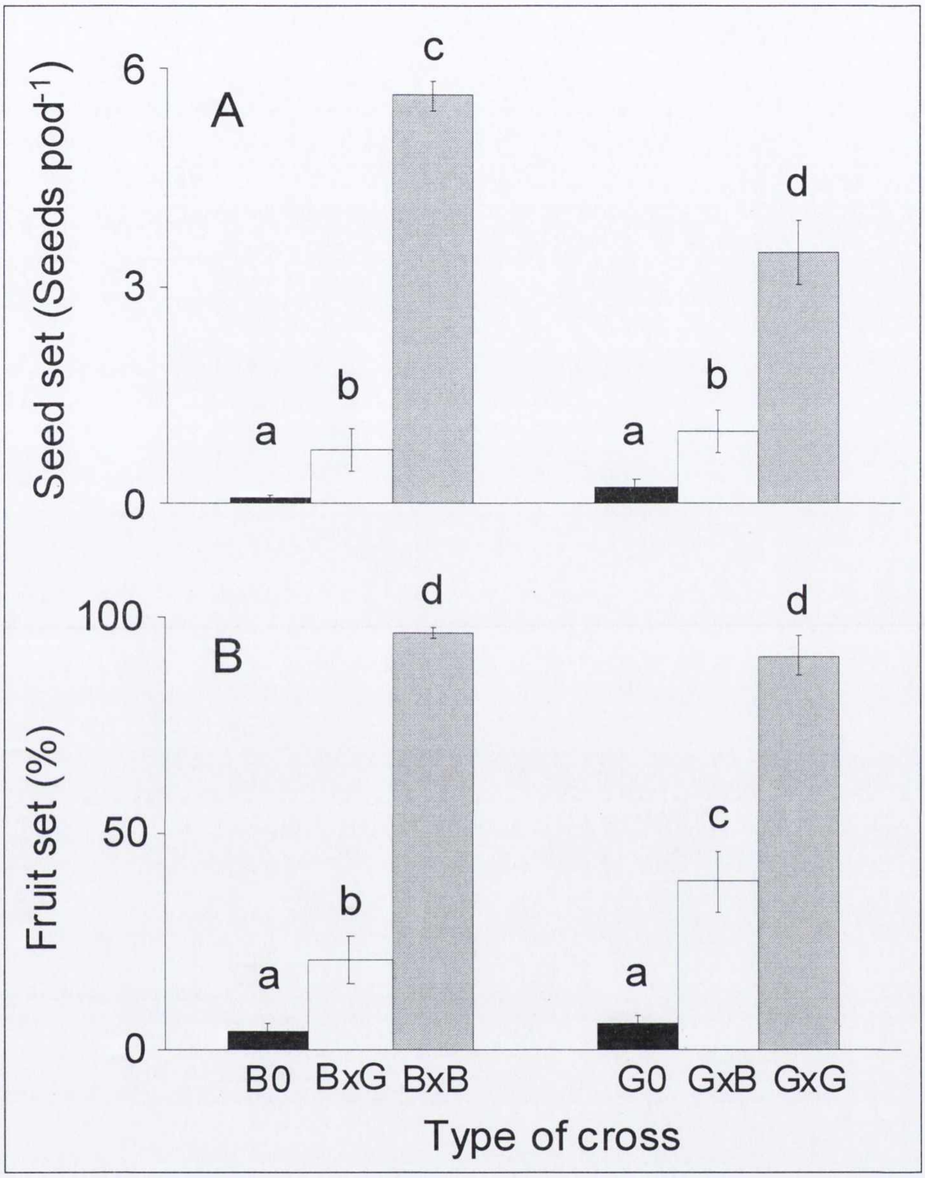

Figure 6.5 Seed production for the four types of cross (BxG, BxB, GxB and GxG) and for the non-pollinated flowers (B0 and G0). B = *I. balfourii* and G = *I. glandulifera*; the first letter represents the mother plant and the second letter represents the pollen donor. Mean number of seeds produced per pod (A) and percentage of pods containing seeds (B). Different letters above columns in the same graph indicate differences among types of cross (Sequential Bonferroni test, $p < 0.05$).

Table 6.2 GLM (Poisson errors with log-link and Binomial with logit-link) for the effect of the type of cross on seed production that was estimated as seed and fruit set (BxG, BxB, GxB, GxG, B0 and G0, where B = *I. balfourii* and G = to *I. glandulifera*; the first letter represents the mother plant and the second letter represents the pollen donor). *Post-hoc* comparisons were performed between treatments using Sequential Bonferroni tests.

	Numb. of seeds per pod			**Numb. of pods with seeds**		
Source of variation	**df**	**Wald X^2**	*p*	**df**	**Wald X^2**	*p*
Type of cross	5	930.132	**< 0.001**	5	2783.885	**< 0.001**
Pair-wise comparisons	*p*			*p*		
BxB vs. BxG	**< 0.001**			**< 0.001**		
vs. B0	**< 0.001**			**< 0.001**		
vs. GXG	**< 0.001**			0.063		
vs. GXB	**< 0.001**			**< 0.001**		
vs. G0	**< 0.001**			**< 0.001**		
BxG vs. B0	**< 0.001**			**< 0.001**		
vs. GXG	**< 0.001**			**< 0.001**		
vs. GXB	0.105			**0.001**		
vs. G0	**< 0.001**			**< 0.001**		
B0 vs. GXG	**< 0.001**			**< 0.001**		
vs. GXB	**< 0.001**			**< 0.001**		
vs. G0	0.155			0.575		
GXG vs. GXB	**< 0.001**			**< 0.001**		
vs. G0	**< 0.001**			**< 0.001**		
GXB vs. G0	**< 0.001**			**< 0.001**		

6.3.4. Hybrid seed germination

Of the seeds produced from *I. balfourii* flowers pollinated with *I. glandulifera* pollen (BxG), none germinated. Hybrid seeds with *I. glandulifera* as mother and *I. balfourii* as the pollen donor had a negligible germination (1.9% ± 1.9) (Figure 6.6). In the control seeds, produced by artificially pollinating flowers with homospecific pollen, germination was significantly different from zero for both species. Additionally, significant differences were found between BxB and GxG seeds which show a mean germination of 28.3% ± 7.5 and 14.1% ± 6.2 respectively (Figure 6.6) (Table 6.3.).

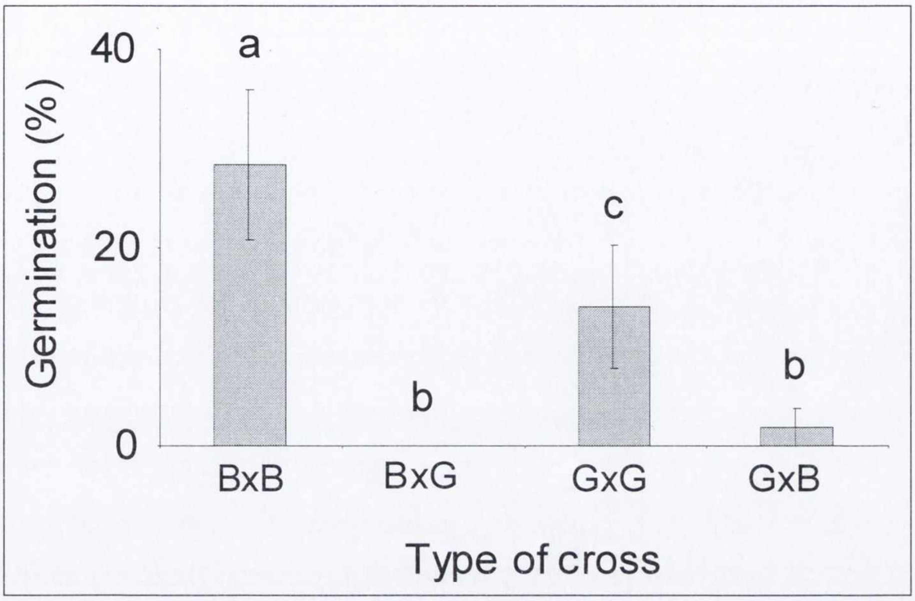

Figure 6.6 Mean percentage of germination (±SE) (n = 5) for each type of cross: BxB = homospecific artificial pollination in *I. balfourii*; BxG = heterospecific artificial pollination where *I. balfourii* is the mother and *I. glandulifera* is the pollen donor; GxG = homospecific artificial pollination in *I. glandulifera*; GxB = heterospecific artificial pollination where *I. glandulifera* is the mother and *I. balfourii* is the pollen donor. Different letters above columns indicate differences among species (Sequential Bonferroni test, p< 0.05).

Table 6.3 GLM (Binomial with logit-link) for the effect of the type of cross on seed germination (BxG, BxB, GxB, GxG, B0 and G0, where B = *I. balfourii* and G = to *I. glandulifera*; the first letter represents the mother plant and the second letter represents the pollen donor). *Post-hoc* comparisons were performed between treatments using Sequential Bonferroni tests.

		Seed germination	
Source of variation	**df**	**Pearson X^2**	***p***
Type of cross	3	159.763	**< 0.001**
Pair-wise comparisons		***p***	
BxB vs. BxG		**< 0.001**	
vs. GXG		**< 0.001**	
vs. GXB		**< 0.001**	
BxG vs. GXG		**< 0.001**	
vs. GXB		`0.315	
GXG vs. GXB		**< 0.001**	

6.4. DISCUSSION

Although *I. glandulifera* has been extensively studied because of its widespread invasive capacity, and *I. balfourii* has recently been suggested as a potential invader, no data were available until now concerning the potential for sexual reproduction between these two introduced species in their introduced range.

Unlike the perennial *Impatiens* species, characteristic of the tropical regions, the annual *Impatiens,* such *I. glandulifera* and *I. balfourii*, are not capable of vegetatively reproducing and they rely completely on sexual reproduction for continued annual cycles of growth (Grey-Wilson, 1980; Beerling and Perrins, 1993). An understanding of the hybridization potential of these species therefore becomes significant since it could be responsible for an enhanced invasive capacity of these introduced co-occurring species (Ellstrand and Schierenbeck, 2000; Richardson and Pysek, 2006).

6.4.1. Natural possibility of pollen transfer between the two species in Ireland

I. balfourii has been observed to be able to grow, flower and produce seeds in Ireland under Atlantic conditions. Flowering time of *I. balfourii* lasts longer in Ireland than in its native range (Adamowski, 2009) and it overlaps with *I. glandulifera* flowering time, which begins only slightly later. The pollinators that were observed feeding on these two *Impatiens* visited more *I. glandulifera* flowers, perhaps because of the large nectar reward of this species (Nienhuis, 2009). However, pollinators have been observed to switch from one species to the other during a single foraging bout. Even if the number of plants was the same for each species, *I. balfourii* produced a greater number of flowers. Switches were rather frequent, with an overall average of over 1 switch every 7 minutes. In addition, the flower morphology is similar in the two species, even if flower size is reported to be slightly larger in *I. glandulifera* than in *I. balfourii* (3 – 4 cm and 2.5 – 4 cm respectively) (Valentine, 1978; Adamowski, 2009). Bees contact both male and female parts of the flower with their dorsal body parts (Valentine, 1978). Nienhuis and Stout (2009), comparing the size of bumblebee body parts with the size of *I. glandulifera* floral parts, found that *B. pascuorum*, being bigger than *I. glandulifera* flower parts, is forced to touch the reproductive parts of the flower. The same consideration might be applied to other bumblebees that present similar morphology to *B. pascuorum*, and to the slightly smaller *I. balfourii* flowers which present male and female parts in an analogous position to *I. glandulifera*'s.

Although *I. glandulifera* requires higher soil moisture than *I. balfourii* (Beerling and Perrins, 1993) (Schmitz and Dericks, 2008), these two species have been observed to grow together in the same habitats (Adamowski, 2009). Therefore, there are not reproductive barriers in terms of flower phenology, of pollinators or of habitat. This study confirmed that the possibility that pollen can be naturally transferred from flowers of one species to flowers of the other exists in nature.

6.4.2. Seed production from crosses involving heterospecific pollen and hybrid seed germination

Seed production by crosses with heterospecific pollen (BxG and GxB) was significantly lower then seed production by crosses with homospecific pollen (BxB and GxG). However, the seed set was significantly higher in BxG and GxB crosses than seed set from flowers that were bagged to exclude visitors and did not receive any pollen. This result confirms the hypothesis that there is seed production when *I. glandulifera* and *I. balfourii* are crossed. In particular it has been possible to observe that both species can be either the pollen donor or receiver. However, when hybrid seeds were germinated, no germination occurred for either of the hybrids. The percentage of germination of the heterospecific crosses did not significantly differ from zero. On the other hand, the percentage of germination of homospecific crosses was significantly lower than seed germination previously observed for these two species (Chapter 2). This might be due to the fact that seeds, to be counted, were collected before they were naturally dispersed and, in some cases, they might not have reached maturity. Therefore, early seed collection might have also lowered hybrid seed germination. Alternatively, artificial pollination, in comparison with open pollination via insects, might be responsible for lower seed viability (as well as for a lower seed set, see Chapter 2).

Experimental pollen crosses between *I. capensis* and *I. pallida*, two native co-occurring species of North America, showed no seed set (Randall and Hilu, 1990). Randal and Hilu (1990) in their study suggested that improper pollen transfer might represent an important competitive mechanism between these two *Impatiens* species. *I. pallida*'s pollen was able to germinate on the stigma of *I. capensis* but when it reached the ovules was not able to fertilize them. As a result, *I. capensis*' fruit set was noticeably reduced by the co-occurrence of *I. pallida*. Reidy (2008, unpublished data) found that pollen of both *I. glandulifera* and *I. balfourii* germinate on the stigma of the other species and, following pollen tube progression, he found that over 60% of the pollen reached the ovary of the other species when heterospecific-pollinated. Based on the presented results, I exclude the possibility of hybridization between *I. glandulifera* and *I. balfourii*. However, the fact that the two species, when crossed, produced seeds represents the possibility of the presence of incomplete reproductive barriers. Additional experiments could investigate whether improper pollen transfer between these two *Impatiens* would also represent a competition mechanism that would affect the seed set of one or both species. Bell *et al.* (2005) imply that improper pollen transfer and pollinator preference represent the two mechanisms of competition for pollination. Hybridization from interspecific pollination may result in a reduction of reproductive success (Levin *et al.*, 1996) and hybrid (either viable or not) seeds could be produced at the expense of conspecific seed production (Nagamitsu *et al.*, 2006). Murphy (2009) suggested that allelopathic pollen could even be used as a biological control in weed management.

The results of this study validate the hypothesis that heterospecific pollination between *I. glandulifera* and *I. balfourii* results in seed production. The seed production is, however, lower than the seed production in homospecificly pollinated plants. In addition, the hybrid seeds produced are not viable. Therefore, hybridization between these two *Impatiens* might possibly represent a competition mechanism instead of a genetic advantage, as in the case of *I. pallida* and *I. c*apensis.

7 General discussion

In this thesis I compared introduced congeneric plants that vary in their invasive capacity in Ireland with the aim of improving the general understanding of the mechanisms of invasion. I began with a comparison of reproductive and germination characteristics in different environment conditions among three *Impatiens* species, the invasive *Impatiens glandulifera*, the naturalized *I. parviflora* and the casual *I. balfourii*. I then explored several ecophysiological traits, which have been repeatedly suggested to contribute to plant fitness. Traits were initially assessed in these *Impatiens* species growing in a high-resource environment, which is typically the entry point of invasive species. Through the characterisation of ecophysiological traits in a common high-resource environment it emerged that *I. glandulifera* and *I. balfourii* have similar ecological needs, and that they show similar leaf morphological and photosynthetic traits. It is intriguing that these two species, which have been introduced from the same native range and show ecophysiological similarity, differ dramatically in their distribution and in their spread in their introduced range, in Britain and Ireland in particular. I therefore used these two species in comparative studies aimed at assessing the same growth, biomass allocation, leaf morphological and photosynthetic traits in different environmental conditions through the manipulation of abiotic factors. Firstly, performances in seedlings of the two species were compared at two light intensities; subsequently traits were assessed and compared between species in plants growing in three water regimes. Finally, the potential for hybridization of these two congeners, which could possibly enhance invasive capacity of one or both species, was assessed. In the last chapter, I summarise the results obtained from all the experiments to present an overview of the relationships between traits and invasiveness in these *Impatiens* species. I put my findings in the context of previous research that considers traits and invasiveness, and I outline general conclusions and an outlook for further studies.

7.1 OVERVIEW OF INTRODUCED *IMPATIENS* PERFORMANCE

7.1.1 Reproductive capacity

From the germination trial it emerged that the superior reproductive capacity in *I. glandulifera*, which produces larger and more seeds compared to the other two *Impatiens*, might contribute to *I. glandulifera*'s greater invasive capacity. This, the most invasive species, was the species that showed the highest germination rate, seedling emergence and survival rate overall. In addition, it was shown to require a shorter stratification period (compared to *I. parviflora*) and demonstrated the capacity to germinate under a wider range of conditions compared to the other two less invasive *Impatiens* species. Similarly, characteristics that might explain the lack of success of *I. balfourii* as an invader in Ireland could be its lower seed mass and seed production and higher mortality. In Ireland, lack of success of *I. parviflora*, which is an aggressive invader in other European countries, might be attributed, in terms of germination, to the different stratification period required to break dormancy. This species possibly requires longer periods of cold at lower temperatures, characteristic of central and eastern European winters, where *I. parviflora* is an aggressive invasive species.

7.1.2 Ecophysiological traits

The characterisation of ecophysiological traits in a common high-resource environment demonstrated the contrasting light interception and light use strategies of the light-demanding *I. glandulifera* and the shade-tolerant *I. parviflora*. The former is a fast-growing plant that allocates a greater proportion of biomass to support organs which reflect the ability of this species to compete for aerial space and its capacity to intercept light resources. The latter showed the more favourable leaf-morphological traits with respect to efficient capture of light resources when light availability is limited.

Similarity in ecological needs and photosynthetic traits emerged from the comparison between *I. glandulifera* and *I. balfourii*, both showed photosynthetic traits typical of light-demanding plants (such as high A_{max} and LSP) in contrast to the typically shade-tolerant photosynthetic traits shown by *I. parviflora* (such as low LCP).

When traits where assessed and compared between *I. glandulifera* and *I. balfourii* in conditions where one abiotic factor was manipulated, the invasive *I. glandulifera* showed consistently higher performances than *I. balfourii* in the growth-related traits. This was also reflected in a higher allocation of biomass to support organs in *I. glandulifera*. No significant differences were observed for the leaf-morphological, photosynthetic and use-efficiency traits. Leaf-morphological traits can not unequivocally explain invasiveness in the considered species of the genus *Impatiens*, but they show how different species have dissimilar leaf-morphological strategies to achieve fitness (as in the case of *I. glandulifera* and *I. parviflora*). When plasticity was compared in plants growing under water deficit, all the traits showed little variation, suggesting low plasticity for both the invasive and non-invasive species for the measured traits. Comparing the traits' relative distances between the two species, the invasive *I. glandulifera* showed higher plasticity in three of the 18 considered traits (Table 7.1). *I. glandulifera* showed higher plasticity then *I. balfourii* in relation to the total leaf area, leaf weight ratio (LWR) and water use efficiency (WUE). Biomass allocation to leaves and water use efficiency (WUE) are characteristics which are very important in plants which have to deal with changing water availability. However, no differences in plasticity were found in other efficiency-, or performance-related traits that might also be important in water deficit conditions (as for example RWR or SLA).

7.1.3 Potential for hybridization between *Impatiens glandulifera* and *I. balfourii*

Hybridization might produce evolutionary variations which can increase invasiveness since it can generate new genotypes which are adapted to a wider range of environments. Some hybrids have shown even greater invasive capacity than the introduced parents (e.g. *Spartina anglica*). Consequently, it is important to asses the potential for hybridization between the introduced *I. glandulifera* and *I. balfourii*. To my knowledge, the potential for this cross has not been considered before.

This experiment confirms the possibility of natural pollen transfer between the two species since they can share the same habitat and they can co-occur. In addition, the flowering time in their introduced range overlaps and pollinators have been observed to switch from one species to the other during a single foraging bout. The artificial heterospecific crosses between the two species produced seeds. However, it was not possible to stimulate germination in either of the hybrids. Due to the lack of germination of the hybrid seeds the possibility of successful hybridization was discounted; on the other hand, the fact that the heterospecific cross produced seeds, represents the possible presence of incomplete reproductive barriers. Moreover, in these two species, improper pollen transfers could represent a competition mechanism that possibly affect the seed set of one or both species and it could be an interesting subject for further investigation.

Table 7.1 Qualitative representation of species' performances for each of the reproductive, growth, biomass allocation, leaf morphological and use-efficiency traits, and for plasticity. + and – in "Species" columns indicate the species which showed respectively the highest and the lowest value for that trait in the given experimental condition; ± indicates that a species does not show significant differences from the highest and lowest values; n.s. indicates no significant differences between species; / indicate that no comparison was made; + (Both), (High), (Low) indicate the highest values in respectively both, high and low light intensities; + (All) indicates the best performances in all water regimes. + and – in the "Correlation with invasiveness" column indicate respectively a positive and a negative correlation between trait and species invasiveness according to (cited besides) large multi-species comparative studies (in bold) or comparisons of pairs or small sets of invasive and non-invasive species. (*I. g.* = *I. glandulifera, I. b.* = *I. balfourii, I. p. I. parviflora*).

Trait	Species			Correlation with invasiveness
	I. g.	*I. b.*	*I. p.*	
Reproductive				
Seed mass	+	-		**- (Rejmánek and Richardson, 1996; Hamilton *et al.*, 2005)** **n.s. (Lloret *et al.*, 2005)**
Seed production (Perrins *et al.*, 1993)	+	-		- (Brock *et al.*, 2005) + (Radford and Cousens, 2000; Goergen and Daehler, 2001)
Germination rate				+ (Radford and Cousens, 2000; Goergen and Daehler, 2001; Wilson and Wilson, 2004; Cervera and Parra-Tabla, 2009)
Stratification	+	+		
No stratification	+	+		
Buried	+	-		
Surface	+	-		
Seedling mortality				- (Radford and Cousens, 2000; McAlpine *et al.*, 2008; Cervera and Parra-Tabla, 2009)
Buried	-	+		
Surface	-	/	+	
Earliest germination				
Buried	+		-	
Surface	+		-	
Growth				
Plant height				**n.s. (Goodwin *et al.*, 1999; Hamilton *et al.*, 2005; Lloret *et al.*, 2005)** + (Durand and Goldstein, 2001b)
High- resources				
Varying light environment	+ (Both)	-	/	
Varying water environment	+ (All)		/	
Total biomass				+ (Williams and Black, 1994; Zheng *et al.*, 2009)
Varying light environment	+ (Both)	-	/	
Varying water environment	+ (All)		/	
Total leaf area				**+ (Daehler, 2003; Lloret *et al.*, 2005)** + (Zheng *et al.*, 2009)
Varying water environment	+ (All)	-	/	
RGR				**+ (Baruch and Goldstein, 1999; Dawson *et al.*, 2010)** **n.s. (Daehler, 2003)** + (Pattison *et al.*, 1998; Fogarty and Facelli, 1999; Durand and Goldstein, 2001b; Wilson and

				Wilson, 2004; Grotkopp and Rejmanek, 2007; Zheng *et al.*, 2009) n.s. (Schumacher *et al.*, 2009)
Varying water environment	+ (All)	-		
Biomass Allocation				
LWR				+ (Williams and Black, 1994; Feng *et al.*, 2007c) n.s. (Zheng *et al.*, 2009)
High-resources	-	±	+	
Varying light environment	n.s.	n.s.	/	
Varying water environment	-	+ (All)	/	
SWR				+ (Zheng *et al.*, 2009) n.s. (Pattison *et al.*, 1998)
High-resources	n.s.	n.s.	n.s.	
Varying light environment	+ (Both)	-	/	
Varying water environment	+ (All)	-	/	
RWR				+ (Feng *et al.*, 2007c; Grotkopp and Rejmanek, 2007) - (Wilsey and Polley, 2006; Zheng *et al.*, 2009) n.s. (Pattison *et al.*, 1998)
High-resources	n.s.	n.s.	n.s.	
Varying light environment	n.s.	n.s.	/	
Varying water environment	+ (All)	-	/	
Leaf Morphology				
LAR				**+ (Leishman *et al.*, 2007)** + (Pattison *et al.*, 1998; Feng *et al.*, 2007c) n.s. (Zheng *et al.*, 2009)
High-resources	-	±	+	
Varying light environment	n.s.	n.s.	/	
Varying water environment	-	+ (All)	/	
SLA				**+ (Baruch and Goldstein, 1999; Hamilton *et al.*, 2005)** + (Grotkopp and Rejmanek, 2007; Schumacher *et al.*, 2009) - (Wilson and Wilson, 2004; Feng, 2008; Zheng *et al.*, 2009) n.s. (Feng *et al.*, 2007b; Feng *et al.*, 2007c)
High-resources	n.s.	n.s.	n.s.	
Varying light environment	n.s.	n.s.	/	
Varying water environment	-	+ (All)	/	
LA:RM				+ (Zheng *et al.*, 2009)
High-resources	/	/	/	
Varying light environment	n.s.	n.s.	/	
Varying water environment	-	+ (All)	/	
Photosynthesis				
A_{max}				**+ (Baruch and Goldstein, 1999; Leishman *et al.*, 2007)** + (Williams and Black, 1994; Pattison *et al.*, 1998; McDowell, 2002; Deng *et al.*, 2004; Wilson and Wilson, 2004; Feng *et al.*, 2007b; McAlpine *et al.*, 2008)
High-resources	+	+	-	
Varying light environment	+ (High)	-	/	
Varying water environment	n.s.	n.s.	/	

Rd				**n.s. (Leishman *et al.*, 2007)** - (Pattison *et al.*, 1998) n.s. (Deng *et al.*, 2004)
High-resources	-	-	+	
Varying light environment	n.s.	n.s.	/	
Varying water environment	n.s.	n.s.	/	
ϕ				+ (Pattison *et al.*, 1998) n.s. (McAlpine *et al.*, 2008)
High-resources	+	+	-	
Varying light environment	-	+ (Hight)	/	
Varying water environment	-	+ (All)	/	
LCP				n.s. (Deng *et al.*, 2004)
High-resources	+	+	-	
Varying light environment	n.s.	n.s.	/	
Varying water environment	n.s.	n.s.	/	
LSP				+ (Deng *et al.*, 2004)
High-resources	+	+	-	
Varying light environment	+ (Hight)	-	/	
Varying water environment	n.s.	n.s.	/	
GS				n.s. (McAlpine *et al.*, 2008)
Varying water environment	n.s.	n.s.		
Efficiency				
WUE				+ (McDowell, 2002; Deng *et al.*, 2004; McAlpine *et al.*, 2008) - (Blicker *et al.*, 2003)
Varying light environment	n.s.	n.s.	/	
Varying water environment	n.s.	n.s.	/	
RE				+ (McDowell, 2002; Feng *et al.*, 2007b)
Varying light environment	+ (Low)	-	/	
Varying water environment	n.s.	n.s.	/	
Plasticity				
Varying water environment	+ (3/18 Traits)	-	/	**+ (Daehler, 2003)** + (Schweitzer and Larson, 1999; Schumacher *et al.*, 2009) - (Brock *et al.*, 2005) n.s. (Williams and Black, 1994; Peperkorn *et al.*, 2005; Zheng *et al.*, 2009)

7.2 THE TRAITS OF THE INVADERS

"What makes a species invasive?" is a fundamental question in invasion biology with significant practical implications (van Kleunen *et al.*, 2010b). Accordingly, in recent years a huge amount of research has attempted to address this issue. Large multi-species comparisons generally use trait information from different sources (e.g. databases or published literature) to compare relatively simple traits (e.g. plant height) but often do not include ecophysiological traits measured in common-garden experiments (Kolar and Lodge, 2001; Pyšek and Richardson, 2007; Hayes and Barry, 2008; van Kleunen *et al.*, 2010b). Experimental studies generally consist of trait comparisons between pairs, or small sets, of invasive and native species (e.g. Dreyer *et al.*, 1987; Williams and Black, 1994; Fogarty and Facelli, 1999; Schweitzer and Larson, 1999; Radford and Cousens, 2000; Durand and Goldstein, 2001b; Blicker *et al.*, 2003; Brock *et al.*, 2005; Feng *et al.*, 2007b; Radford *et al.*, 2007; Feng, 2008; Funk, 2008; Allred *et al.*, 2010). A smaller number of studies has considered invasive and non-invasive introduced species (e.g. Mihulka *et al.*, 2003; Burns, 2004; Burns and Winn, 2006; Grotkopp and Rejmanek, 2007). These experimental studies, which assess and compare traits of invasive and non-invasive species growing in common environments in experimental-garden settings, can often consider only limited numbers of species due to logistical issues of experiment design (e.g. limited number of replicates). Comparing small number of species makes it difficult to make wider generalizations about the results. However, literature reviews might provide useful outlooks for the general relationship between traits and invasiveness.

7.2.1 The current findings in the context of invasion biology research

Table 7.1 gives a representation of this thesis' findings in a wider context of previous research in the field of biological invasion. It is possible to identify in Table 7.1 a consistency in reproductive- and growth-related traits that is reinforced by the findings presented in this thesis. High seed production and germination rates and low seed mortality are characteristics common to all invaders since, as expected, they contribute to the invaders' spread. A controversial trait among the reproductive characteristics is the seed mass. Among the *Impatiens* species that were considered in this thesis, the most invasive was the species which showed the highest seed mass. By contrast, however, two multi-species studies, by Rejmánek and Richardson (1996) and Hamilton *et al.* (2005), found that low seed mass was a characteristics that significantly contributed to discrimination between groups of native and invasive pine trees, and was a significant variable correlated with invasion, in the flora of Royal National Park in eastern Australia. However, other studies, which do not necessarily consider invasiveness, found higher seed mass associated with higher germination rates (e.g. Rees, 1995; Bonfil, 1998; Hewitt, 1998; Seltmann *et al.*, 2007). The latter is generally explained by larger seeds providing a greater nutrient supply to seedlings, which enhances establishment and growth (Hewitt, 1998). Hence, high germination rates imply high invasive potential.

In the existing studies, growth related traits are, in general, correlated with invasion capacity. An exception, though, is represented by plant height. Goodwin *et al* (1999), Hamilton *et al.* (2005) and Lloret *et al.* (2005) did not find any statistical difference between invasive and non-invasive species in plant height. However, other growth traits, such as total leaf area, total biomass and relative growth rate, seem to be good predictors of plant invasiveness, as also shown by the present study. As reported by Daehler (2003), and as appears evident from reviewing the existing literature, growth rate is also the most explored of the traits. In my experiments, relative growth rate was consistently higher in the most invasive species of the genus *Impatiens*, independent of the growing conditions. Relative growth rate is generally related to plant fitness in high resource environments. Relative growth rate has been repeatedly correlated also with other ecophysiological traits such as specific leaf area (Shipley, 2006) or dark respiration rates (Smith *et al.*, 1995). However, it is possible to find inconsistencies among the studies that try to detect the association between invasiveness, leaf attributes and photosynthetic traits.

The study of Grotkopp and Rejmanek (2007) is distinguished from other experimental studies by the size of their comparative experiment. They compared 14 invasive woody species with 12 less-invasive woody species cultivated in California, and they found that fast seedling growth (high RGR) along with an efficient resource acquisition system (high SLA and increased biomass allocation to root) were positively associated with invasion. In line with their study, I found, as well as a higher growth rate in the invasive, a higher root biomass allocation in the invasive than in the non-invasive, in the water deficit experiment. However, across experiments, the invasive never showed higher SLA than the non-invasive.

High specific leaf area is a trait which increases light-resource capture and it is also, along with RGR, among the most investigated traits that have so far been associated with invasion. Baruch and Goldstein (1999) compare 34 native and 30 invasive species occurring in the Hawaiian Archipelago and they found significantly lower SLA in the native species. Although this trait has often been reported to be associated with invasion, some exceptions have also been found (e.g. Wilson and Wilson, 2004; Feng, 2008; Zheng *et al.*, 2009). Also in this comparison, SLA was not significantly higher in the invader. In addition, in the water deficit experiment, it was found that the non-invasive species shows the highest values for the leaf morphological traits, such as SLA, LAR and LA:RM. Turning to the photosynthetic-related traits, the maximum rate of photosynthesis (A_{max}) is a direct measure of carbon uptake. Photosynthtic capacity at plant level is consistently associated with invasiveness. However, in the current comparisons, photosynthtic rates, at leaf or plant level, are not always significantly higher in the invasive species. Other photosynthetic traits (Rd, LCP and stomatal conductance), which have been intuitively associated with invasiveness, have so far not been extensively investigated. To my knowledge, no study has yet found significant differences between invasive and non-invasive species for these traits. It must be kept in mind that the contribution of some traits (for instance the use-efficiency traits) to plant fitness is largely dependent on the environmental conditions and habitit characteristics.

7.2.2 Three notable reviews on the correlation between species' traits and invasiveness

Daehler (2003), Pyšek and Richardson (2007) and van Kleunen *et al* (2010) provide interesting reviews of experimental published studies which consider respectively 119, 64 and 117 comparative papers and allowed us to summarize the existing work. The three reviews differ in the kinds of comparisons considered. Daehler (2003) considered comparative studies between invasive alien and non-invasive native. Pyšek and Richardson (2007) considered comparative studies on invasive vs. non-invasive related species, both native and introduced. The third study considered invasive and non-invasive (both native and introduced) in their introduced range. Daehler (2003) and Pyšek and Richardson (2007), consistent with the results of this thesis, found that high fecundity is often associated with invasiveness. In addition, Pyšek and Richardson (2007), again consistent with the findings presented in this thesis, found that relative growth rate was frequently associated with invasion but Daehler (2003) did not reach the same conclusion. The reviews by Daehler (2003) and Pyšek and Richardson (2007) are based on the counting of the number of studies in which invasives outperform natives/non-invasives, the number of studies in which natives/non-invasives outperform invasives and the number of studies that did not find significant differences. This approach entails an unavoidable loss of information since it does not consider the magnitude of the differences between invasive and non-invasive species in the traits that were investigated. Van Kleunen *et al* (2010) took into account the magnitude of the differences and the inconsistency in sample sizes by means of a meta-analysis. In addition, they underline the differences in the results across the different kinds of comparison (e.g. invasive vs. native or invasive vs. non-invasive; related or unrelated). In their meta-analysis they included physiological fitness traits such as leaf area, shoot allocation, growth rate, plant size and fitness. Other traits were not included because the number of experimental studies which consider them was too low. This thesis considers, besides the more frequently investigated growth characteristics and ecophysiological traits, less frequently investigated traits such as light compensation point (LCP), light saturation point (LSP), dark respiration (Rd) and apparent quantum yield (ϕ). These are generally considered to contribute to plant fitness and to enhancing plant invasiveness at low light levels since they are supposed to contribute to increasing plants' shade tolerance.

The study by van Kleunen *et al* (2010) consisted mainly of comparisons between invasive and native species; only six out of 117 studies were comparisons between invasive and non-invasive introduced species and all six of them compared congenerics. Van Kleunen *et al* (2010) found that invasive species showed higher values overall than native non-invasives in traits associated with “fitness”, “size”, “growth rate” and “shoot allocation”. On the other hand, in the comparison between invasive and non-invasive introduced species, invasive species outperformed non-invasive only in “size” and “fitness” traits. It appears clear that more differences were therefore detected comparing introduced invasive species with native species. The higher number of trait differences found with this kind of comparison could be attributed, at least partially, to the lower number of studies that involved invasive versus introduced non-invasive comparisons, and this would entail a lower statistical power in the data analysis. From van Kleunen *et al.* (2010)’s meta-analysis, it emerged that there is a need for more studies that compare invasive and non-invasive introduced species, and that there is a necessity to investigate more traits that are potentially associated with invasive capacity. Accordingly, this thesis compares non-extensively investigated traits in invasive and non-invasive introduced species, with the aim of increasing the available information on the ecophysiological differences among introduced species with different invasive statuses.

7.3 A POTENTIAL INVADER?

I. balfourii was introduced to Europe later, when compared to the other two most invasive *Impatiens* species. The first record of the presence of *I. balfourii* in the wild is from 1906 in the south of France (Chapter 1). *I. glandulifera* and *I. parviflora* have a closer history in their introduced ranges, in particular in Britain, where they were recorded in the wild within just a few years of each other (Appendix 1.1). The spread of *I. balfourii*, consequently, started much later. In addition, some authors suggest that this species did not benefit from the same propagule pressure (i.e. the product of the number of individuals introduced during a single introduction event, and the number of introduction events) as the other two, which were extensively cultivated in many countries (Perrins *et al.*, 1993; Adamowski, 2009). Therefore, shorter residence time and lower propagule pressure could be listed among the factors that might contribute to the lack of success of this species as an invader. Conseguently a comparison between *I. glandulifera* and *I. parviflora* might be more appropriate in terms of invasion history. However, comparing *I. glandulifera* and *I. balfourii* might be more appropriate in terms of species ecology. When choosing a non-invasive counterpart to compare with an invasive species in comparative studies, the chance of misclassifying the non-invasive exists, both when comparing invasive vs. native and invasive vs. non-invasive introduced. With the choice of non-invasive native species there is the possibility that the native species can be, or can become, invasive elsewhere. With the choice of a non-invasive introduced species, the possibility exists that the introduced species might not have reached its full invasive potential yet (van Kleunen *et al.*, 2010a). It is therefore not possible to be certain of the absence of invasive capacity in a species (van Kleunen *et al.*, 2010a).

As a result of the current study, the lack of success of *I. balfourii* as an invasive species in Britain and Ireland may be explained by the co-occurrence of several factors. Besides residence time and propagule pressure mentioned above, low germination in common-garden conditions followed by high seedling mortality might be responsible of the non-success of this species. In Britain and Ireland, the cooler weather (which might have possibly contributed to seedling mortality) might contribute to limiting the spread of *I. balfourii* which, in its native range, occurs only at more southerly latitudes than *I. glandulifera*. However *I. balfourii* showed a very high germination rate in some of the experimental conditions, suggesting that, in the right environment, this species has the potential to produce a great number of seedlings. Along with a high reproductive potential, *I. balfourii*, similar to its more invasive congeners, showed high photosynthetic capacity which is a characteristic often reported among aggressive aliens (Williams and Black, 1994; Pattison *et al.*, 1998; McDowell, 2002; Zou *et al.*, 2007).

In a climate change scenario, some introduced species might experience more favourable conditions for growth and survival, and they could possibly spread in areas that they have not been able to invade before. (Theoharides and Dukes, 2007). For example, it has been suggested that climatic warming could favour a northward spread of *I. glandulifera* (Beerling, 1993; Willis and Hulme, 2002). Accordingly, global warming might allow *I. balfourii* to successfully establish and subsequently invade more northern regions of Europe including Britain and Ireland.

7.4 FINAL CONCLUSION AND OUTLOOK

Some of the traits assessed in this thesis appear to be good predictors of plant invasiveness and can very often be found to be associated with invasive species across comparative studies. As opposed to that, other traits are not unequivocally correlated with invasiveness and there is great inconsistency among comparative studies. The reason for such inconsistency might be due to the fact that some traits contribute to fitness (therefore to invasiveness) only in particular habitats. Consequently, it is important to assess traits in a range of environments and any remark concerning invasiveness has to be qualified in respect to the habitat. For example efficiency traits or leaf construction cost might be important only when there is the need to optimize resources. Experimental studies might be aimed at assessing the invasive potential of a target species in a particular habitat (either a man-made disturbed habitat where invasion is more likely to occur or a natural habitat where invasion might have a higher ecological impact). When trying to explain invasiveness using species traits, it is important to consider also the geographical region where the considered species are invasive. For instance, the lack of invasiveness of *I. parviflora* in Ireland could possibly be associated with low germination rates due to warmer winters. Global climate warming or smaller scale climatic variations might also affect traits contributing to invasiveness, and therefore allow species to spread in new habitats.

Experimental comparative studies are limited in scale and are generally confined to the comparison of a few species; therefore their results do not have a general character valid for a multitude of species. However, the information provided by these studies is essential to exploring the relationship between traits and invasiveness. Further experimental comparisons that assess more traits under a wider range of detailed environmental conditions would be needed to identify the most important physiological traits that, in these environments, allow a species to successfully compete with its neighbours.

Reviews might help to discriminate among traits that can be unmistakably associated with invasion, and they might help to identify the traits that can promote invasion under particular circumstances. Having a clearer picture of the traits involved in the invasion processes could contribute to predicting which species might become invasive and, afterwards assist in the design of weed management protocols and assist in the decision to limit the introduction of a new, potentially invasive, species.

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APPENDICES

Appendix 1.1 Status, native range and date of introduction for all recorded European *Impatiens* species (Moore, 1968; Cigić *et al.*, 2003). Species highlighted in bold were selected for this thesis. * First record of spontaneous plants (Coombe, 1956; Perrins *et al.*, 1993; Clement and Foster, 1994; Pysek *et al.*, 2002; Cigić *et al.*, 2003; Milbau and Stout, 2007; NBN, 2008; Adamowski, 2009).

Species	Europe (First record*)	Britain (First record*)	Ireland (First record*)	Native range	Habitats invaded
***balfourii* Hooker f.**	**Naturalized (1906)**	**Casual**	**Casual**	**Western Himalayas 1500 – 2500 m a.s.l.**	**Water courses, alluvial forests, disturbed habitats in central and southern Europe**
balsamina L.		Casual	Not present	India and Myanmar	Disturbed areas
capensis Meerb.	Naturalized	Naturalized (1884)	Not present	Temperate eastern North America	Water courses
***glandulifera* Royle.**	**Invasive**	**Invasive (1855)**	**Invasive (1906)**	**Western Himalayas 1800 – 4000 m a.s.l.**	Moist and nutrient rich habitats, on lake and seashores, along rivers and brooks
noli-tangere L.	Native	Native	Not present	Central Europe and Britain	Streams and wet ground
***parviflora* DC.**	**Invasive (1837)**	**Naturalized (1851)**	**Naturalized**	**Pamir and Altai**	**Damp shady forests of Central and Northern Europe**
scabrida DC	Casual	Not present	Not present	Himalayas, northern India, Kashmir, Nepal and Pakistan	

Appendix 2.1 Details on the location of the populations from which the seeds used for the germination trail (Chapter 2) and for the water treatments experiments (Chapter 5) were collected.

Impatiens glandulifera Royle

Rio Bedale, near the bridge of San Domenico square, Peveragno (CN), Italy.
Altitude 570 m a.s.l., facing SE.
N 44°19’55.00”   E 7°37’2.50”

Impatiens balfourii Hook. f.

Rio Bedale, near the bridge of San Domenico square, Peveragno (CN), Italy.
Altitude 570 m a.s.l., facing SE.
N 44°19’55.85”   E 7°37’5.46”

Impatiens parviflora D.C.

Rio Bedale, near the bridge of San Domenico square, Peveragno (CN), Italy.
Altitude 570 m a.s.l., facing SE.
N 44°19’55.85”   E 7°37’5.46”

Appendix 3.1 Providers and provenance of the *Impatiens*'s seeds germinated and growth in Trinity College Botanic Garden in the summer 2008.

Provider	Provenience
I. glandulifera	
Natural History Museum of Denmark, Botanical Garden and Museum, University of Copenhagen	Lake Bagsveard (North of Copenhagen, Denmark)
Botanical Garden University of Helsinki, Finland	Salzburg, Hallein S, Gamp. Industrial area, ruderal, alluvial deposit, 440 msm. Coll. Apollonia
Jardin Botanique National de Belgique, Meise, Belgium	
MNHS Department des jardins botaniques et zoologiques, jardine des plantes, arboretum national de Chevreloup, Paris	
Hortus Botanicus Ljubljana.	Dedni Vrh (near Ljubljana, Slovenia)
I. parviflora	
Natural History Museum of Denmark, Botanical Garden and Museum, University of Copenhagen	
Garden University of Cambridge	Large Damm Weisen (Germany)
MNHS Department des jardins botaniques et zoologiques, jardine des plantes, arboretum national de Chevreloup, Paris	
Hortus Botanicus Ljubljana, Slovenia	
I. balfourii	
Natural History Museum of Denmark, Botanical Garden and Museum, University of Copenhagen	
MNHS Department des jardins botaniques et zoologiques, jardine des plantes, arboretum national de Chevreloup, Paris	