

# Impacts of the invasive *Impatiens glandulifera* on the growth of three native plants

Poppy Mynard

University of Aberdeen

Julia Sanchez Vilas (✉ [sanchezvilasj@cardiff.ac.uk](mailto:sanchezvilasj@cardiff.ac.uk))

Cardiff University

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## Research Article

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# Abstract

Himalayan balsam (*Impatiens glandulifera*) is a highly invasive annual herb that has spread rapidly throughout the UK. This species has allelopathic potential – chemicals released into the soil that can reduce seed germination and growth of neighbouring plants. Allelopathy and resource competition are key contributors to the success of this species; however, little is still known about the effects of litter of *I. glandulifera* on native species. This is important because in the invaded range, *I. glandulifera* frequently grows in monotypic stands that die back in autumn leaving large amounts of litter. Here, we aim to investigate the effects that seedlings and residues (above-ground plant matter) of *I. glandulifera* have on the chlorophyll content and growth of co-occurring native species, namely, *Trifolium pratense*, *Linum grandiflora*, and *Silene dioica*. We found reduced chlorophyll content and growth (measured as above-ground dry mass) in the three native species studied in response to growing with a *I. glandulifera* individual. However, the presence of residues of this invasive in the soil did not reduce the chlorophyll content and growth of the native species but rather had positive effects for *S. dioica* – which increased the above-ground dry mass in response to the addition of plant matter in the soil. Our results confirm the competitive superiority of *I. glandulifera* over native plants and highlight potential benefits of the litter of this invasive on the growth of natives.

## Introduction

The introduction of non-native invasive species poses a major threat to natural biodiversity (Pejchar and Mooney 2009); non-native invasive plants can negatively affect invaded ecosystems by altering species diversity, community structure and interactions between organisms, sometimes leading to local extinction of native species (Vilà et al. 2011). Invasive plants can displace native species through direct competition for light, space, nutrients, and water (Chittka and Schürkens 2001) and also have the potential to indirectly affect native flora through alterations to the local soil composition, e.g. via release of chemicals that can be toxic to native plants or via changes in soil microorganisms (Csiszár et al. 2013; Ruckli et al. 2016; Gaggini et al. 2018; Power and Sánchez Vilas 2020).

*Impatiens glandulifera* Royle (Balsaminaceae), commonly known as Himalayan balsam, is an invasive plant, which has spread rapidly throughout the UK and mainland Europe since the 19th century (Beerling and Perrins 1993). *I. glandulifera* was first recorded as a naturalised alien species in England in 1855 (Pyšek and Prach 1995). Native to the Western Himalayas, at altitudes of 2000 to 2500 m, *I. glandulifera* is the tallest European annual, commonly reaching heights of 2 m (Beerling and Perrins 1993); it is readily able to outcompete native plants, due in part to its rapid growth and high stature (Pyšek and Prach 1995). *I. glandulifera* also successfully competes with native plants for pollinators, which may reduce native plant fitness (Chittka and Schürkens 2001). At invaded sites, *I. glandulifera* commonly grows in riparian and open wooded habitats, where it forms monotypic stands, often replacing native flora (Beerling and Perrins 1993). In autumn it dies back, which leaves the ground bare increasing the risk of erosion (Helsen et al. 2021).

Some plants, including *Impatiens glandulifera*, produce allelopathic compounds, secondary metabolites capable of directly interfering with other species, for example, by decreasing germination or growth (Callaway and Aschehoug 2000; Del Fabbro et al. 2014). Allelopathy is regarded as a crucial mechanism in the context of non-native plant invasions (Del Fabbro et al. 2014). The novel weapons hypothesis suggests that plant invaders produce biochemical compounds that are novel in their non-native range and function as unusually powerful allelochemicals, providing invaders with an advantage over native plants (Callaway and Ridenour 2004). *I. glandulifera* produces naphthoquinones, allelochemicals that are leached from leaves and exuded from roots into the soil (Ruckli et al. 2014). Naphthoquinones have been found in *I. glandulifera* all throughout its annual vegetative cycle, and are known to inhibit growth of mycorrhizal fungi and the germination of seeds of native herbaceous plant species (Ruckli et al. 2016).

This study aims to investigate the effects that seedlings and residues (above-ground plant matter) of *I. glandulifera* have on the growth of co-occurring native species, namely, *Trifolium pratense*, *Linum grandiflora*, and *Silene dioica*. By growing the invasive with the native species we aim to explore the competitive ability of *I. glandulifera*, where resource competition and allelopathy will be interacting. By adding residues to the soil, we aim to mimic the natural process of decomposition at the end of the life cycle of *I. glandulifera*, and its effects on the growth of native species. Seedlings were grown in a glasshouse under controlled conditions, in individual pots containing garden soil. In previous studies comparison between invaded and uninvaded sites was made, e.g. (Hejda et al. 2009; Del Fabbro et al. 2014), which may result in bias from differences in soil properties not related to a history of *I. glandulifera*, e.g. mineral composition, soil structure, and other physical properties. Instead, here we used soil from the same site, with the addition of either a seedling of *I. glandulifera* or plant material from *I. glandulifera*.

## Materials and Methods

### Study species

Seeds of *I. glandulifera* were collected from two large established populations along the riverbanks (River Taff, Cardiff) in Autumn 2015. A random bulk sample of these seeds was used for this experiment. Seeds were sterilised using a bleach solution (20% commercial bleach + distilled water) and then placed on moist filter paper in Petri-dishes and stored in a refrigerator (ca. 4°C) in darkness for 1-month prior to the start of the experiment in order to break their dormancy requirements.

Seeds of *Silene dioica* (Caryophyllaceae) and *Trifolium pratense* (Fabaceae), were purchased from Emorsgate Seeds (Norfolk, England) and *Linum grandiflora* var *rubrum* (Linaceae) was purchased from Thompon & Morgan (Suffolk, UK), and sown into germination trays in the glasshouse.

### Experimental Setup

In November 2016, seedlings of *S. dioica*, *T. pratense* and *L. grandiflora* were transplanted into 9 cm diameter pots filled with garden soil collected at the plant growth facilities in Talybont (Cardiff) and assigned to three experimental treatments at random (N = 11 plant per species × 3 treatments = 33 plants per species). The experimental treatments consisted of: plants growing 'alone', plants growing with a neighbouring '*I. glandulifera* plant' ('IGPlant'), and plants growing in soil with '*I. glandulifera* residues' added ('IGSoil'). For the *I. glandulifera* plant treatment, seedlings of *I. glandulifera* were transplanted at the same time to the experimental pots with *S. dioica*, *T. pratense* and *L. grandiflora*. For the '*I. glandulifera* residues' treatment, fresh plants were collected from wild *I. glandulifera* populations and 3g of leaf and 5g of fresh stem were added to each pot by cutting the plant parts into small pieces (< 2cm) and mixing them with the soil prior to the transplant of seedlings. Pots were randomly placed in benches in the glasshouse under a 12-hour photoperiod, and watered every 3–4 days.

## Harvesting of plants

After approximately two months growing under the experimental conditions, on 25th January 2017, the above-ground biomass was harvested for *S. dioica*, *T. pratense* and *L. grandiflora*. Above ground dry mass was obtained by drying in an oven at 70°C for 3 days and weighed using a ± 0.0001g precision balance.

On 15th December 2016 and prior to the harvest, the leaf chlorophyll content was measured in three fully developed young leaves per plant using a hand-held chlorophyll meter (SPAD-502, Minolta Camera Co., Osaka, Japan), which calculates an index based on absorbance at 650 and 940 nm. SPAD values are well correlated with the chlorophyll content of leaves (Markwell et al. 1995).

## Data analysis

All data analyses were performed using R version 4.2.2 (R Core Team 2022). To determine the effect of the experimental treatments on the above-ground dry mass and chlorophyll content of *S. latifolia*, *T. pratense* and *L. grandiflora*, we used ANOVAs by means of the 'aov' function. We checked graphically for normality of residuals and homogeneity of variance, and the above-ground dry mass of *T. pratense* was log<sub>10</sub> transformed to meet the assumptions of the analysis of variance. We obtained P-values using the summary function of the ANOVA model. Tukey's HSD tests were used to determine the significance of differences between groups, with  $\alpha < 0.05$ .

## Results

The above-ground dry mass and the chlorophyll content of *T. pratense*, *L. grandiflorum* and *S. dioica* were significantly affected by the experimental treatments (Table 1). In particular, the above-ground dry mass of the three species was significantly reduced in response to growing in competition with an *I. glandulifera* neighbour (Fig. 1). However, when growing on its own, the presence of plant material of *I.*

*glandulifera* in the soil did not affect the above-ground dry mass of *T. pratense* or *L. grandiflora* (Figs. 1a) and 1b)), but did significantly increase the above-ground dry mass of *S. latifolia* (Fig. 1c).

Table 1  
Results of the analysis of variance (ANOVA) for the above-ground dry mass and chlorophyll content of *Trifolium pratense*, *Linum grandiflorum* and *Silene dioica* in response to the experimental treatments. P- values < 0.05 are marked in bold.

Above-ground dry mass (g)					Chlorophyll content (SPAD units)		
T. pratense							
Source of variation	<i>d.f.</i>	<i>SS</i>	<i>F</i>	<i>P</i>	<i>SS</i>	<i>F</i>	<i>P</i>
Treatment	2	3.348	53.01	< 0.001	107.3	5.489	0.009
Error	30	0.947			293.2		
L. grandiflorum							
Source of variation							
Treatment	2	0.1644	26.69	< 0.001	63.06	5.197	0.011
Error	30	0.0924			182.01		
S. dioica							
Source of variation							
Treatment	2	3.126	84.18	< 0.001	523.14	30.343	< 0.001
Error	30	0.557			258.62		

The chlorophyll content, measured as SPAD units, was significantly lower for the three species when growing in competition with an *I. glandulifera* plant (Fig. 2). No significant effect of the addition of plant material in the soil was found on chlorophyll content of the native species (Fig. 2).

## Discussion

We found reduced chlorophyll content and growth (measured as above-ground dry mass) in the three native species studied in response to growing with an *I. glandulifera* individual. However, the presence of residues of this invasive in the soil did not reduce the chlorophyll content and growth of the native species but rather had positive effects for *S. dioica* – which increased the above-ground dry mass in response to the addition of plant matter in the soil.

Our results confirm the competitive superiority of *I. glandulifera* over native plants (Bottollier-Curtet et al. 2013; Bieberichid et al. 2018; Helsen et al. 2021). Here, in our study we cannot disentangle the effects of resource competition and allelopathy; however, a combination of greater resource competition and allelopathy have been invoked before to explain the superior competitive ability of *I. glandulifera* over

native plants (Bieberichid et al. 2018; Power and Sánchez Vilas 2020). It is plausible that the magnitude of each of these factors contributing to the competitive ability of *I. glandulifera* changes over time. For example, it is known that *I. glandulifera* produces greater amount of naphthoquinones at the beginning of the growing season, during the seedling/juvenile phase, than later in life (Lobstein et al. 2001; Bieberichid et al. 2018). This could give *I. glandulifera* an advantage by interfering with the establishment and growth of native plants early in life; whilst as it grows in size, competition for resources may be stronger in shaping the interaction with co-occurring natives.

The allelopathic potential of extracts obtained from plant material of *I. glandulifera* has been tested before, negatively affecting germination and growth of other plant species (Vrchotová et al. 2011; Csiszár et al. 2013; Loydi et al. 2014; Bieberichid et al. 2018). However, the direct effect of plant residues of *I. glandulifera* on co-occurring natives has not been given much attention, but see (Loydi et al. 2014). Here, we found no detrimental effects of residues added into the soil on the chlorophyll content and growth of the three native species studied. Moreover, it enhanced the growth of *S. latifolia*. These results could appear surprising in light of the known allelochemical potential of this invasive species, with naphthoquinone potentially leaching from decomposing plant residues (Vrchotová et al. 2011). However, (Loydi et al. 2014) have also found no negative effects of added litter of *I. glandulifera* on the growth of native grasses and forb species. The lack of allelopathic effects of residues of *I. glandulifera* in our study could be due to several factors. On one hand, the developmental stage of the plants is known to affect the production of naphthoquinone, with older or senescing plants having lower content of this allelochemical in leaves and stems than seedlings or juveniles (Vrchotová et al. 2011). We (and also (Loydi et al. 2014)) collected the plant matter to add into the soil from leaves and stems of individuals at the end of the growing season, and therefore the amount of allelochemicals may have been lower than those present in plant matter of younger individuals. On the other hand, the potential allelopathic effect of plant residues could be masked by an increase in nutrients in the soil, which are released during the decomposition of the plant matter added to the soil. Alien species have been found to increase nutrient pools and fluxes in the invaded ecosystems (Liao et al. 2008), and particularly, *I. glandulifera* has a high concentration of nutrients in shoots and leaves, which will return to the soil during decomposition (Dassonville et al. 2008). It is worth to note here that the autumn dieback of large stands of *I. glandulifera* leaves the soil bare of vegetation for months during winter and spring, which may promote leaching into watercourses and increase the risk of eutrophication (Greenwood and Kuhn 2014).

In conclusion, this study confirms the competitive superiority of *I. glandulifera* over natives in the invaded range. Interestingly, this study does not find any detrimental effect of *I. glandulifera* residues on the chlorophyll content and growth of native plants. In fact, plant residues did enhance the growth of *S. latifolia*, which could be attributed to the release of nutrients from the decomposing plant material added to the soil and to a lower release of allelochemicals at the end of the growing season. This highlights that *I. glandulifera* can therefore benefit native species, likely due to an increase in nutrient availability in the soil. However, this result should be taken with caution: the soil is left bare of vegetation at the autumn dieback of *I. glandulifera*, and therefore nutrients may be lost in natural habitats. Finally, further research

is needed to elucidate the role of plant age in shaping the interaction of *I. glandulifera* with native plants, both in terms of allelopathy and resource competition.

## Declarations

### Competing Interests

*The authors have no relevant financial or non-financial interests to disclose*

## Author Contributions

All authors contributed to the conception and design of the study. Material preparation and data collection were performed by all authors. Analyses were performed by JSV. The first draft of the manuscript was written by JSV and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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## Figures

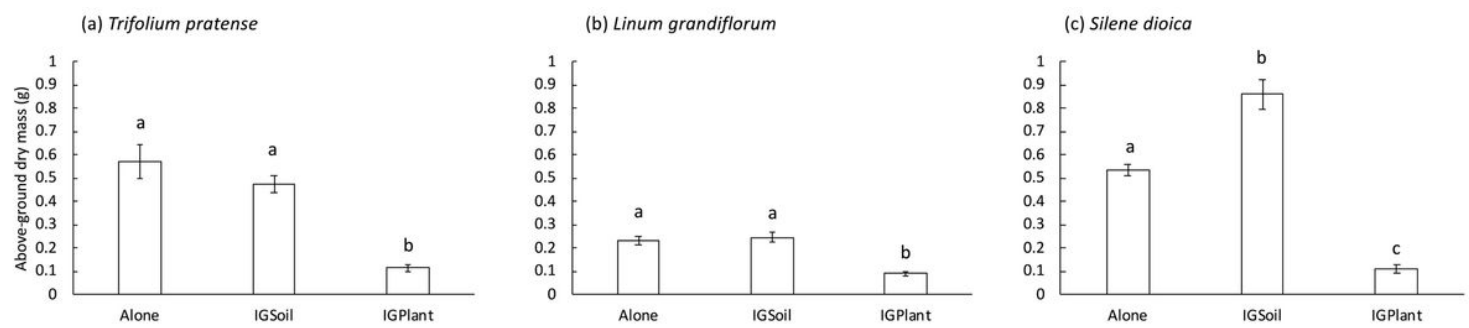


Figure 1

Above-ground dry mass for a) *Trifolium pratense*, b) *Linum grandiflorum*, and c) *Silene dioica* in response to the experimental treatments: 'Alone': plants growing 'alone', 'IGSoil': plants growing in soil with '*I. glandulifera* material' added and 'IGPlant': plants growing with a neighbouring '*I. glandulifera* plant'. Bars represent mean values (N=11), and error bars indicate standard error. Different letters above bars indicate significant differences between means of groups (Tukey HSD,  $P \leq 0.05$ ).

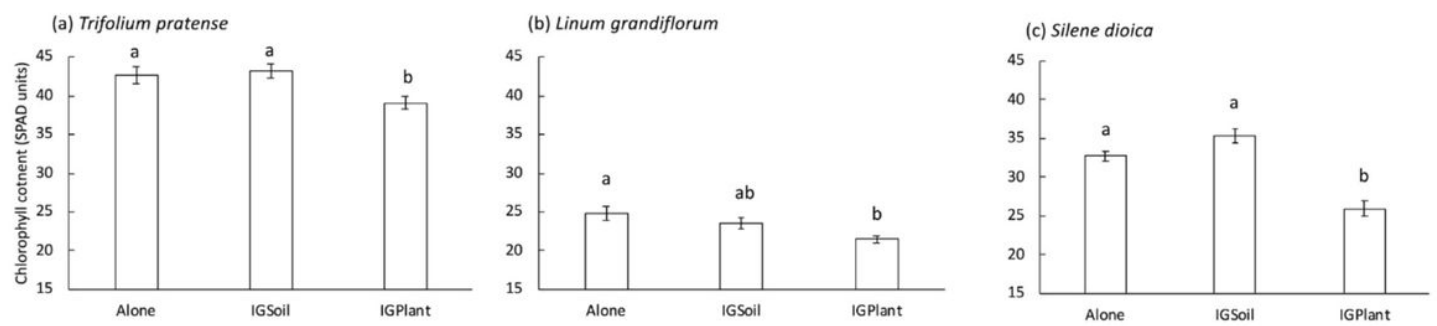


Figure 2

Chlorophyll content, estimated using SPAD measurements, for a) *Trifolium pratense*, b) *Linum grandiflorum*, and c) *Silene dioica* in response to the experimental treatments: 'Alone': plants growing 'alone', 'IGSoil': plants growing in soil with '*I. glandulifera* material' added and 'IGPlant': plants growing

with a neighbouring '*I. glandulifera* plant'. Bars represent mean values (N=11), and error bars indicate standard error. Different letters above bars indicate significant differences between means of groups (Tukey HSD,  $P \leq 0.05$ ).