Guilty in the Court of Public Opinion: Testing Presumptive Impacts and Allelopathic Potential of Ranunculus ficaria

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ABSTRACT.—Information about invasive species is often based primarily on anecdotal evidence, indicating the need for further information. *Ranunculus ficaria* is an ephemeral riparian plant species that is presumed invasive in the United States, despite the lack of any published information on its impacts. Mechanisms by which *R. ficaria* may affect native plant species include competition and allelopathy. We examined if *R. ficaria* negatively affected the growth and reproduction of the native *Impatiens capensis* and, if so, whether it is by allelopathy, nutrient competition or some combination thereof. We performed a fully-factorial field experiment, manipulating the presence of *R. ficaria*, nutrients, and allelopathy with the use of activated carbon. The presence of *R. ficaria* tended to negatively affect life span of *I. capensis*. In the absence of carbon, *R. ficaria* significantly decreased seed production, illustrating the negative impact of *R. ficaria*. In the presence of carbon, there was no effect of *R. ficaria*, suggesting that carbon may have ameliorated the negative allelopathic effect of *R. ficaria*. Nutrient competition did not show strong effects. Despite its widespread identification as an invasive species, this is the first study to demonstrate the negative impact of *R. ficaria* on a native species and the

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possible role of allelopathy in its success. Further, the negative impacts of this ephemeral species persist well beyond its early growing season, which calls into question previous widespread assumptions about *R. ficaria* exerting effects primarily on other ephemeral species.

INTRODUCTION

Invasive species threaten biodiversity on a global scale (Wilcove *et al.*, 1998; McGeoch *et al.*, 2010) and are defined as those non-native species that cause or have the potential to cause economic or environmental harm, weighed against their benefits (NISC, 2006). Most naturalized plants are introduced through the horticultural industry (Mack and Erneberg, 2002). Some horticultural species can still be purchased in some instances despite their invasive status (Harris *et al.*, 2009; Axtell *et al.*, 2010). However, only a portion of naturalized species are actually considered to be invasive (Milbau and Stout, 2008). There is much interest in characterizing the species traits that make a species invasive and the routes by which they tend to be introduced (Lambdon *et al.*, 2008; Milbau and Stout, 2008; van Kleunen *et al.*, 2010). Yet, in many of these studies the methods by which species are even categorized as invasive are vague and based on expert opinion and anecdotal evidence, with little scientific evidence (Blossey, 1999). Further, even when there is some published information, the impacts of an invasive species can be overstated by the popular press (Lavoie, 2010), which may lead to inappropriate response strategies or undue focus. The lack of information on the impact of invasive species and on the possible mechanism of impact is an obstacle to effectively prioritizing the control of invasive species during a time of dwindling resources.

Invasive plant species can negatively impact native species through a variety of mechanisms (Levine *et al.*, 2003). Invasive species can simply outcompete native species for above- and/or below-ground resources (e.g., Kueffer *et al.*, 2007; Cipollini *et al.*, 2008a; Galbraith-Kent and Handel, 2008). Enhanced nutrient acquisition can lead to invasive species success. For example, *Centaurea maculosa* acquired more phosphorus than surrounding native species which may have increased competitive success (Thorpe *et al.*, 2006). Additionally, Leishman and Thomson (2005) found that in a study testing 28 different invasive species, the 15 invasive species had greater responses on average to high nutrient soils than the 13 non-invasive species tested, thus providing a possible mechanism for why invasive species may be able to outcompete natives in nutrient-rich environments. Another mechanism by which invasive species affect native communities is allelopathy (Hierro and Callaway, 2003; Ens *et al.* 2009). Most plants produce secondary compounds (Ehrenfeld, 2006) that can affect an adjacent plant either directly (Cipollini *et al.*, 2008b) or indirectly through changing soil ecology (Stinson *et al.*, 2006; Mangla *et al.*, 2008). Some allelopathic chemicals that have no negative impact in their native environment may have negative effects in an invaded community, a mechanism coined the "novel weapons hypothesis" (Callaway and Aschehoug, 2000; Callaway and Ridenour, 2004; Callaway *et al.*, 2008; Thorpe *et al.*, 2009).

Discovering impacts due to allelopathy can be done with careful experimentation (Inderjit and Callaway, 2003). Allelopathic effects have been studied using activated carbon (e.g., Ridenour and Callaway, 2001; Cipollini *et al.*, 2008a). Activated carbon adsorbs organic compounds, including allelochemicals (Ridenour and Callaway, 2001). Addition of carbon can also has effects on soil properties and plant growth in potting soil (Lau *et al.*, 2008; Weisshuhn and Prati, 2009). Addition of nutrients is thought to help ameliorate any fertilizing effects of the addition of activated carbon (Inderjit and Callaway, 2003). Activated carbon may also serve as a restoration tool to change soil conditions in invaded soils (Kulmatiski and Beard, 2006), by a combination of absorption of allelochemicals and/or effects on microbial communities (Kulmatiski, 2010).

Ranunculus ficaria L. (Ranunculaceae), or lesser celandine, is a groundcover native to Europe (Taylor and Markham, 1978; Sell, 1994), which appears to be negatively affecting native plants in forested floodplains in many US states (Swearingen, 2005). There are five known subspecies, all of which are found in the United States (Post *et al.*, 2009). *Ranunculus ficaria* was first recorded naturalized in the United States in 1867 (Axtell *et al.*, 2010). As it is still being marketed by the nursery industry (Axtell *et al.*, 2010), it was likely imported for horticultural purposes. It was recognized as a naturalized species in the Midwest in the 1980’s (Rabeler and Crowder, 1985) and in southern states more recently (Krings *et al.*, 2005; Nesom, 2008). *Ranunculus ficaria* is currently documented in at least 21 US states, the District of Columbia, and 4 Canadian provinces (USDA, 2010). It has been identified as invasive in 9 states and the District of Columbia and is banned in Massachusetts and Connecticut as a noxious weed (Axtel *et al.*, 2010).

Ranunculus ficaria emerges before most native spring species, which may provide it with a competitive advantage. Once established, it spreads rapidly across the forest floor to form a dense monoculture, which native species seemingly cannot penetrate (Swearingen, 2005). Hammerschlag *et al.* (2002) reported that *R. ficaria* created a monoculture in the Rock Creek floodplain in Washington, DC and few native species re-colonized after its removal. It is thought that *R. ficaria*, as a spring ephemeral, has impacts primarily on other spring ephemerals (Swearingen, 2005). However, most all information on *R. ficaria* as an invasive species is primarily anecdotal in nature. Unpublished and preliminary data indicate that presence of *R.*

ficaria is associated with reduced abundance and richness of native species in spring and summer field surveys (Hohman, 2005). *Ranunculus ficaria* exhibits direct allelopathic effects on germination of some native species (Cipollini, unpublished data), indicating that *R. ficaria* may have negative effects beyond the spring time period through lingering allelopathic effects.

For our study, we examined if *R. ficaria* negatively affects the growth and reproduction of the native annual *Impatiens capensis* and, if so, whether it is by allelopathy, nutrient competition or some combination thereof. In the field we performed a fully-factorial experiment with the treatments of *R. ficaria* (present and absent), carbon (present and absent), and nutrient addition (present and absent). We expected that the presence of *R. ficaria* would have an overall negative impact, that addition of nutrients would have an overall positive impact and that addition of carbon would have no overall effect on the performance of *I. capensis*. If allelopathy were important, we expected to see a significant carbon by *R. ficaria* interaction and, if nutrient competition were important, we expected to see a significant nutrient by *R. ficaria* interaction on *I. capensis* response variables.

METHODS

We performed the study in 2009 at Hamilton County Park District's Winton Woods in Cincinnati, Ohio in an area invaded by *R. ficaria* ssp. *bulbilifer*, a subspecies that forms asexual bulbils (Post *et al.*, 2009). The study area was found in a floodplain along Westfork Mill Creek. We set up the experiment on 24 April, choosing an area with uniform 95-100% coverage of *R. ficaria*. We fenced the entire 3 x 4-m site using deer fencing to prevent trampling and/or herbivory. We used a fully factorial design with the main factors of: presence/absence of *R. ficaria*, presence/absence of activated carbon and presence/absence of additional nutrients, replicated 8 times (2 *R. ficaria* levels x 2 carbon levels x 2 nutrient levels x 8 replicates = 64 experimental units). The treatment combinations were haphazardly assigned to each 25 cm^2 plot and each plot was located approximately 25 cm apart. Treatments were planted in 5 rows and center rows could be accessed (with minimal disturbance to other plots) from the outside edge of the experimental area. We tested the effects of these treatment combinations on the target plant *Impatiens capensis* Meerb., jewelweed (Balsaminaceae). A single plant was used in each experimental plot. We chose *I. capensis* because of the overlap in habitat and distribution with *R. ficaria*. Additionally, the nearly complete lack of *I. capensis* within the *R. ficaria* monoculture at our site indicated the potential negative impact of *R. ficaria* on *I. capensis*. Another advantage is that reproduction can be measured in this annual species. Furthermore, *I. capensis* has served as a model organism in previous studies of invasive species effects (e.g., Cipollini *et al.*, 2008a; Cipollini *et al.*, 2009; Cipollini and Hurley, 2009). Any competitive effect or plot interaction would be negligible at 25 cm. This distance between plants has been used in previous studies (Cipollini *et al.*, 2008a, Cipollini *et al.*, 2009) and we have observed in this study and earlier research that above-ground competition has been absent at this distance. Another study found that the density-dependent effect on resistance to disease was not seen when plants were thinned to 15 cm apart (Lively *et al.*, 1995), suggesting negative competitive effects are minimal at this distance. *Impatiens capensis* usually grows in much higher densities; natural populations of *I. capensis* can be found at densities of more than 2,500 seedlings/m^2 (Schmitt *et al.*, 1987). All *I. capensis* seedlings were obtained in an immediately adjacent area free of *R. ficaria*.

In the *R. ficaria*-present treatments, we removed *R. ficaria* and planted them back in place (at their natural density) while transplanting *I. capensis* seedlings. Identifying how many plants of *R. ficaria* were back-transplanted in a non-destructive manner was nearly impossible in the dense monoculture of clonally-reproducing *R. ficaria*. However, the back-transplantation of *R. ficaria* was completely successful and led to nearly 100% *R. ficaria* cover in experimental plots with the *R. ficaria*-present treatment. In *R. ficaria*-absent treatments, we removed the *R. ficaria* completely in the 25 cm^2 plot before transplanting *I. capensis*. In activated carbon-present treatments, we worked 10 ml of activated carbon (Aquarium Pharmaceuticals Black Magic Activated Carbon, Chalfont, Pennsylvania) into the top 8 cm of soil of each plot. This ratio of activated carbon to soil volume has been shown to mitigate allelopathic effects in previous research (Ridenour and Callaway, 2001; Cipollini *et al.*, 2008a). In nutrient-addition treatments, we worked the manufacturer-recommended amount of 1.5 teaspoons of slow-release fertilizer (Scotts Osmocote, Scotts-Sierra Horticultural Product Co., Marysville, Ohio) into the top 8 cm of soil in each plot. We disturbed the soil in each plot in the same manner (to 8 cm deep) in every plot regardless of treatment to control for any soil disturbance effects. We noticed no movement of the large-sized carbon or fertilizer granules out of the experimental plots. No flooding occurred during the course of the experiment. On May 1 we replaced any *I. capensis* transplants that did not survive, presumably due to transplant shock. We measured the height of each seedling when transplanted. The number of fruits, number of seeds and life span (days to death) of the seedlings were recorded once each week. Height and stem diameter (measured directly beneath bottom node with a digital caliper) were measured. *Ranunculus ficaria* had lost all of its foliage by 2 June (week 6) and the leaf litter had decomposed by 12 June (week 8), leaving the bulbils exposed on the soil surface. Measurements began on 5 May and ended on 28 August (week 18).

We performed separate Analysis of Variances (ANOVAs) on the *I. capensis* response variables of life span and total number of seeds, using the full model with fixed factors of nutrients (+/-), *R. ficaria* (+/-) and carbon (+/-). We used a General Linear Model (GLM), which can be used for unbalanced designs, using the appropriate Type III sums of squares (Ryan *et al.*, 2005). We were unable to include all of the variables in a single multivariate model due to the missing values generated as plants died. For life span, we analyzed the day to death for all plants. For total number of seeds, we removed the ten plants that had died within eight weeks of transplant, as seed production was essentially non-existent up to that time. For final height and stem diameter, we used a MANCOVA (using the full model as with the ANOVAs) with starting height as a significant covariate for the 32 plants that survived to the last growth measurement on August 14 (week 16) (SAS 1999; Scheiner 2001). When significance was found in the MANCOVA using Wilk's λ, we ran separate univariate Analyses of Covariance (ANCOVAs) for each variable. For all statistical tests, model assumptions of normality and heteroskedasticity were verified prior to analysis and transformed where appropriate.

RESULTS

For life span, there was a near-significant effect of *R. ficaria* ($F_{1,55} = 3.75$, $P = 0.058$), with *I. capensis* tending to die sooner when *R. ficaria* was present (Fig. 1). For the total number of seeds produced, there was a significant effect of nutrients and a significant interactive effect between

R. ficaria-presence and carbon (Table 1). The presence of nutrients nearly tripled seed production (Fig. 2). When carbon was absent, the presence of *R. ficaria* reduced seed production. When carbon was present, seed production was similar whether *R. ficaria* was present or absent (Fig. 3). In the MANCOVA, there was a significant effect of nutrients on final height and stem diameter (Table 2; eigenvalue = 0.4002, canonical coefficient for height = 1.126 and diameter = 1.96). In the univariate ANCOVA, nutrients increased both height (Table 3) and stem diameter (Table 4) (Fig. 2).

DISCUSSION

We wanted to know if the putative invasive *R. ficaria* negatively affected the growth and reproduction of the native *I. capensis*, and if it did, whether allelopathy or nutrient competition played a causative role. Because *R. ficaria* has been identified as invasive (Axtell *et al.*, 2010) and has been associated with reduced native abundance and diversity (Hohman, 2005), we expected that the presence of *R. ficaria* would have a negative impact on the performance of *I. capensis*. Our results show that *R. ficaria* does indeed negatively affect *I. capensis* providing confirmatory evidence of its assumed impact on native species. *Ranunculus ficaria* showed a tendency to have a negative overall impact on the life span of *I. capensis* with plants dying on average ~10 days earlier in the presence of *R. ficaria*. In the absence of carbon, *R. ficaria* decreased seed production by ~50%, in part most likely through its effects on life span. As expected, nutrients showed a significant effect on growth (in terms of height and stem diameter) and seed production. If nutrient competition were a key factor in inhibition of *I. capensis* by *R.*

ficaria, we would have expected *I. capensis* to exhibit a release from competition in the presence of added nutrients. There was no significant interaction between nutrient addition and presence of *R. ficaria*, suggesting that nutrient competition was most likely not the primary mechanism of impact of *R. ficaria*. We do however acknowledge that our study does not rule out other forms of resource competition, such as competition for light or water.

In the presence of carbon, there was no effect of *R. ficaria*; carbon therefore ameliorated the negative effect of *R. ficaria* on *I. capensis.* In previous research, application of carbon to soils has mitigated the effects of invasive species (Ridenour and Callaway, 2001; Cipollini *et al.*, 2008a). Ridenour and Callaway (2001) found that the competitive ability of an invasive *Centaurea* species was greatly reduced by activated carbon and suggested that the advantage of this species, at least in part, was due to allelopathy. Similarly, one possible conclusion from our study is that the addition of carbon may have attenuated the allelopathic effect of *R. ficaria*. However, when using activated carbon as an experimental tool to test allelopathy, caution must be used in interpreting results. It has been shown that addition of activated carbon can change soil conditions in potting soil (Lau *et al.*, 2008; Weisshuhn and Prati, 2009), though it is important to note that these studies used twice as much activated carbon compared to the amount used in our study. Activated carbon can also affect root mutualisms, perhaps by absorbing signaling compounds (Wurst *et al.*, 2010). Activated carbon itself can have a direct fertilizing effect (Lau *et al.*, 2008). In our study, the mitigating effect of carbon was significant across both nutrient treatments, suggesting that direct addition of nutrients by the activated carbon was most likely not the mechanism through which carbon exerted its effects. *Ranunculus ficaria* contains a number of secondary chemicals (Texier *et al.*, 1984; Tomczyk *et al.*, 2002) and is purported to have medicinal uses in herbal medicine as a treatment for hemorrhoids and as an astringent

(Chevallier, 1996). The presence of putative bio-active secondary compounds may be a good predictor of invasive species impacts, including allelopathy (Ehrenfeld, 2006). Further, because *R. ficaria* exhibits direct allelopathy on some species in the laboratory and greenhouse (Cipollini, unpublished data), allelopathy is a likely mechanism in the field, though clearly further study is needed (see Blair *et al.*, 2009). Whatever the exact mechanism of its effect, activated carbon nevertheless was clearly able to negate the negative effect of *R. ficaria*, suggesting that, at the very least, it may serve as an effective restoration tool to modify soil conditions in the field to the benefit of native species (Kulmataski and Beard, 2006; Kulmatiski, 2010), provided it can be effectively applied in restoration practice.

Interestingly, we found that *R. ficaria* had lasting effects beyond its brief growing season. Hohman (2005) similarly found reduced diversity associated with presence of *R. ficaria* for species other than ephemeral species, though the results were correlational rather than experimental in nature. We expected that effects on *I. capensis* would not be particularly strong, as it is thought that the negative effects of this species are exerted primarily on spring ephemeral species (Swearingen, 2005). Further studies using spring ephemeral species are obviously needed to clarify the comparative effect on this set of species presumed most sensitive. Even though *R. ficaria* had completely senesced by week 6 of the experiment, it still significantly negatively impacted *I. capensis*, which lived without the presence of *R. ficaria* for about two-thirds of its life span. Other invasive species can have residual effects on native species, even after the removal of the invasive species (Conser and Conner, 2009). The effect of *R. ficaria* well past its growing season may be due to lingering effects, such as those due to allelochemicals or to other modification of soil conditions. An alternative explanation may be that the seedling stage is the most vulnerable stage of *I. capensis*, similar to the findings in Barto *et al.* (2010).

This is the first study to show the negative impact of *R. ficaria* on *I. capensis* and to point towards a possible mechanism of success – allelopathy or other modification of soil conditions. It is surprising that this is the first published study to investigate the invasive potential of *R. ficaria*, given that natural resource agencies have recognized it as a species important to control in natural areas since *at least* the year 2000 (Hammerschlag *et al.*, 2002). *Ranunculus ficaria* has already been banned in two states (Axtell *et al.*, 2010) since the year 2006. Admittedly, there is a balance between taking immediate action against an invasive species and waiting for time-consuming scientific studies (Blossey, 1999). Indeed, we support the use of the "precautionary principle" (e.g., Blossey *et al.*, 2001) in assessing invasive species. Since *R. ficaria* can form dense monocultures, the assumption that it is an invasive species is most likely a valid one. However, in the ten or more years it has been identified as a concern, there is not a single published paper documenting its impact. Even with our evidence of a negative impact of *R. ficaria*, it is still entirely possible that the impact of *R. ficaria* has been exaggerated (Lavoie, 2010), causing larger-than-needed economic losses to the horticultural industry and redirection of resources away from more important invaders. This research provides an example of the need for more basic research into invasive species impacts and mechanisms of impacts on native species for use in effective invasive species targeting, control and management.

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TABLE 1.─Results of ANOVA on total seed number of *Impatiens capensis*

Source	df	F	P
Presence of *Ranunculus ficaria*	1	0.88	0.352
Presence of Carbon	1	3.07	0.086
Nutrient Addition	1	45.30	**<0.001**
R. ficaria x Carbon	1	6.81	**0.012**
R. ficaria x Nutrients	1	0.05	0.828
Carbon x Nutrients	1	0.65	0.423
R. ficaria x Carbon x Nutrients	1	0.96	0.331
Error	47		

TABLE 2.─Results of MANCOVA on height and stem diameter of *Impatiens capensis* on August 14 (week 16)

Source	df	Wilk's λ	F	P
Starting Height Covariate	2	0.6732	5.31	**0.013**
Presence of *Ranunculus ficaria*	2	0.9004	1.216	0.315
Presence of Carbon	2	0.9075	1.121	0.344
Nutrient Addition	2	0.4247	14.904	**< 0.001**
R. ficaria x Carbon	2	0.8954	1.286	0.296
R. ficaria x Nutrients	2	0.8838	1.447	0.257
Carbon x Nutrients	2	0.9455	0.635	0.540
R. ficaria x Carbon x Nutrients	2	0.7831	3.047	0.068
Error	22			

TABLE 3.—Results of ANCOVA on height of *Impatiens capensis* on August 14 (week 16)

Source	df	F	P
Starting Height Covariate	1	9.46	**0.005**
Presence of *Ranunculus ficaria*	1	1.19	0.287
Presence of Carbon	1	0.05	0.831
Nutrient Addition	1	7.28	**0.013**
R. ficaria x Carbon	1	0.31	0.584
R. ficaria x Nutrients	1	2.19	0.153
Carbon x Nutrients	1	1.33	0.261
R. ficaria x Carbon x Nutrients	1	0.58	0.454
Error	23		

TABLE 4.─Results of ANCOVA on stem diameter of *Impatiens capensis* on August 14 (week 16)

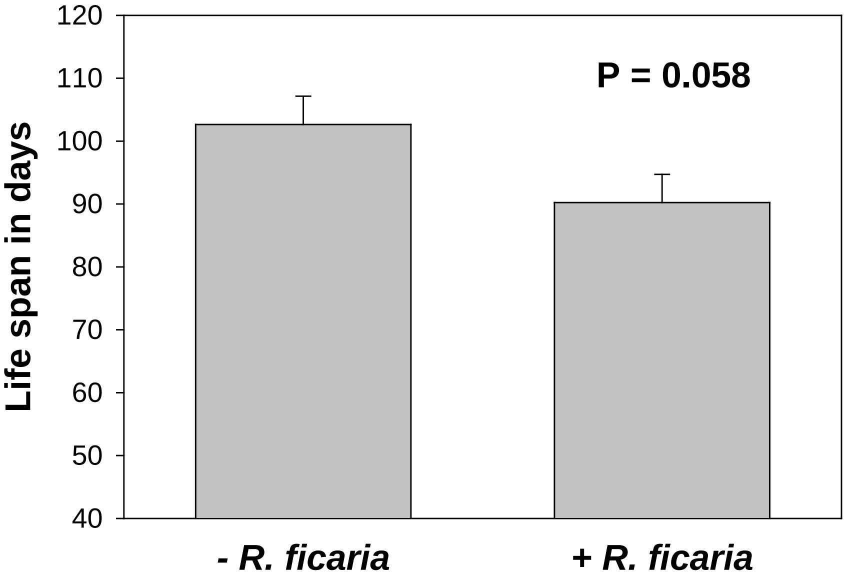
Source	df	F	P
Starting Height Covariate	1	8.59	**0.008**
Presence of *Ranunculus ficaria*	1	2.53	0.126
Presence of Carbon	1	1.12	0.302
Nutrient Addition	1	30.34	< **0.001**
R. ficaria x Carbon	1	0.75	0.395
R. ficaria x Nutrients	1	0.04	0.896
Carbon x Nutrients	1	0.52	0.479
R. ficaria x Carbon x Nutrients	1	2.01	0.170
Error	23		

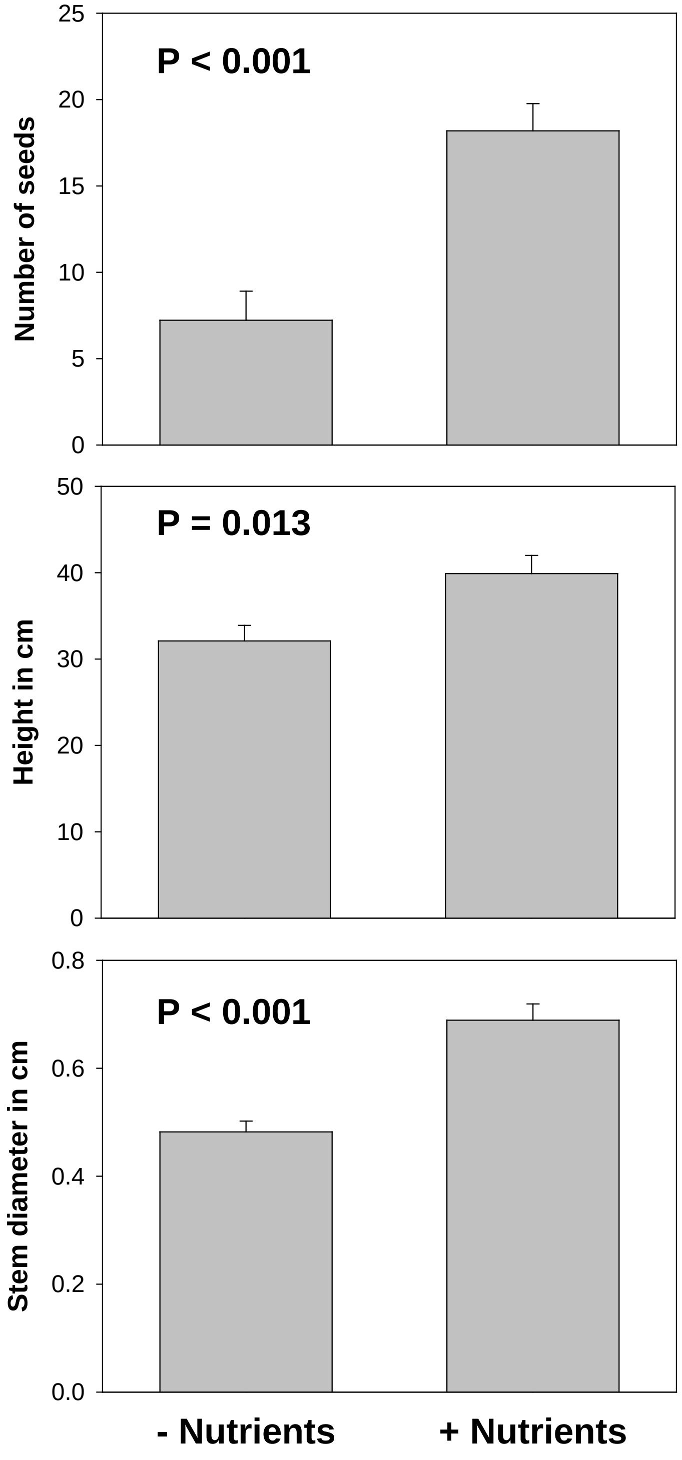
Figure captions

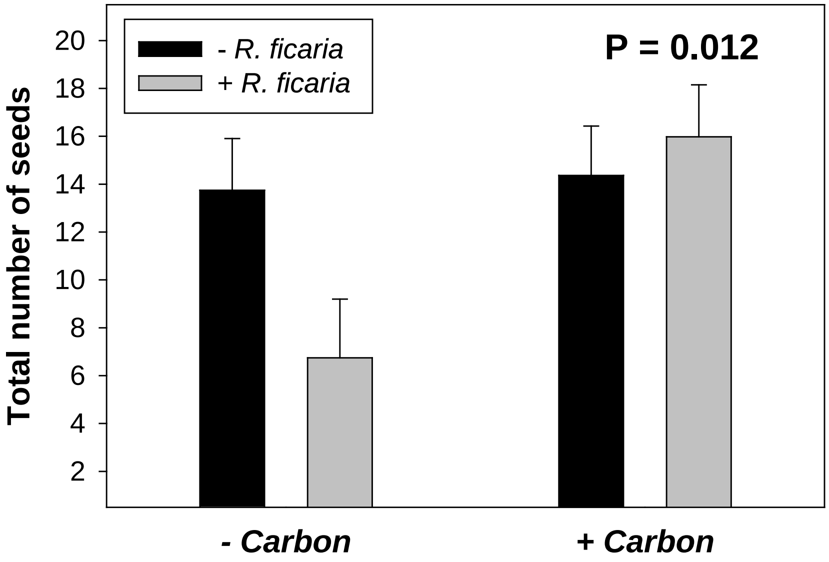
FIG. 1─Mean (± SE) life span of *Impatiens capensis* grown with and without *Ranunculus ficaria*, across nutrient and carbon treatments

FIG. 2─Mean (± SE) number of seeds, height and stem diameter of *Impatiens capensis* grown with and without nutrients, across *Ranunculus ficaria* and carbon treatments.

FIG. 3─Mean (± SE) number of seeds for *Impatiens capensis* grown with and without *Ranunuculus ficaria* in the presence and absence of carbon, across nutrient treatments


Life span in days
120
110
100
90
80
70
60
50
40
P = 0.058
- *R. ficaria*
+ *R. ficaria*


25
P < 0.001
20
15
Number of seeds
10
5
0
50
P = 0.013
40
30
Height in cm
20
10
0
0.8
P < 0.001
0.6
Stem diameter in cm
0.4
0.2
0.0
- Nutrients
+ Nutrients


Total number of seeds
20
18
16
14
12
10
8
6
4
2
- *R. ficaria*
+ *R. ficaria*
P = 0.012
- *Carbon*
+ *Carbon*