

BRIEF NOTE

Variation in Resistance of Experienced and Naïve Seedlings of Jewelweed (*Impatiens capensis*) to Invasive Garlic Mustard (*Alliaria petiolata*)

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ABSTRACT. The invasive species garlic mustard, *Alliaria petiolata*, has negative impacts on understory forest species in the Midwest. Plants that coexist with *A. petiolata* in the field may show resistance to its negative effects as a result of natural selection. In a growth room experiment, we investigated if naïve and experienced seedlings of *Impatiens capensis* vary in their response to the presence of *A. petiolata*. *Impatiens capensis* individuals from areas without *A. petiolata* (i.e., naïve plants) and from nearby areas with *A. petiolata* (i.e., experienced plants) were collected from the field and were then grown with *A. petiolata* in pots for 16 weeks. We measured height, stem diameter, reproduction and biomass of *I. capensis* and biomass of *A. petiolata*. There was a significant ($P < 0.05$) negative correlation between biomass and height of naïve *I. capensis* and biomass of *A. petiolata*, while there was no significant correlation between these variables for experienced *I. capensis*. Our results indicate the potential for the evolution of resistance to the presence of *A. petiolata* in *I. capensis* and point toward the need for further studies.

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INTRODUCTION

Invasive species have profound economic and environmental impacts (Pimentel and others 2005). Because invasive species can cause significant decreases in the abundance of native species (e.g., Collier and others 2002), they may act as selective agents on native populations. For example, Callaway and others (2005) found that grass populations that have co-existed with invasive *Centaurea maculosa* had better growth and germination when grown with *C. maculosa* than the same grass species that had never been exposed to *C. maculosa*. Similarly, Lau (2006) demonstrated that experienced *Lotus wragelianus* were better able to maintain performance when grown with invasive *Medicago polymorpha* than naïve plants. A set of loci in two native grasses have changed in populations invaded by *Acroptilon repens* (Mealor and Hild 2006) and the degree of change was related to resistance (Mealor and Hild 2007).

Alliaria petiolata (Bieb.) Cavara & Grande (Brassicaceae), or garlic mustard, is an important invasive species in the Midwest and beyond (Nuzzo 1993). *Alliaria petiolata* has been shown to reduce growth and survival of native trees and herbs (Meekins and McCarthy 1999, Carlson and Gorchoy 2004, Stinson and others 2007). Abundance, genetic diversity and reproduction of the native annual herb, *Impatiens capensis* (Meerb.) (Balsaminaceae), or jewelweed, were reduced in the presence of *A. petiolata* in the field (McCarthy 1997, Weber 2005, Cipollini and others 2008, respectively), indicating that *A. petiolata* may exert a selective force on *I. capensis* populations. *Impatiens capensis* has proven amenable for many studies on natural selection (e.g., Dudley and Schmitt 1996, Heschel and Riginos 2005) in part because it is a fast-growing annual plant. Studies have shown that it can respond quickly to selective forces such as light availability (Donohue and others 2000). The purpose of this study was to determine whether seedlings of *I. capensis* that had germinated with *A. petiolata* show greater resistance to the competitive effects of *A. petiolata* compared to *I. capensis* that had no prior experience growing with *A. petiolata*. To our knowledge, this is the first investigation of the variation in resistance of a native species to *A. petiolata*.

MATERIALS AND METHODS

During the week of 6 May 2007, we collected *I. capensis* seedlings from three sites in southwest Ohio - Cowan Lake near Wilmington (39°22'47.53"N, 83°53'48.98"W), Wright State University Woods in Dayton (39°47'14.82"N, 84° 3'24.68"W), and Sharon Woods in Sharonville (39°16'49.68"N, 84°23'50.99"W). At each site, we collected six *I. capensis* seedlings (~5-cm tall) from areas with no *A. petiolata* (i.e., naïve *I. capensis*) and six seedlings from adjacent areas (<100 m) with a high density of *A. petiolata* (i.e., experienced *I. capensis*). We do not know how long *I. capensis* had interacted with *A. petiolata* at each site. We planted each seedling in the center of a 1-L pot with potting soil (Pro-Mix BX, Premier Horticulture Inc, Quakertown, PA) and then planted four *A. petiolata* seedlings (collected from the Frank O. Hazard Arboretum at Wilmington College) along the outer edges of the pot.

The pots were haphazardly placed on benches in an air-conditioned growth room, equipped with grow lights (Tek Light 44, Sunlight Supply, Inc., Vancouver, WA) with high output fluorescent bulbs. Light levels were ~50 $\mu\text{mol m}^{-2}\text{s}^{-2}$ PAR (Li-Cor Quantum Sensor, Lincoln, NB) and set on a timer for 15 h days and 9 h nights. Growth room light intensity was similar to that of a wooded area where both species are found. We applied 140 mL of full-strength fertilizer on two occasions (Peters 20-20-20 N-P-K plus micronutrients, Grace-Sierra, Milpitas, CA) to every pot. From 24-26 August, we measured height, fruit number, flower number and stem diameter (measured between the second and third nodes with a digital caliper) of each surviving *I. capensis* plant. We separated *I. capensis* and *A. petiolata* plants from the soil, dried them for 24 h at 110°C and determined the dry biomass of above- and below-ground parts separately for *I. capensis* and total biomass per pot for *A. petiolata*. Because we were not confident on our ability to distinguish accurately between flower stalks and fruit stalks after flowers and fruits had been shed, we added them together as a measure of reproduction.

We examined pairwise correlations between *I. capensis* variables and between the *I. capensis* variables and total *A. petiolata* biomass, separately for each experience level (naïve or experienced). We found the data to be normal by using the Ryan-Joiner method (Ryan and others 2005). We performed separate correlations rather than Analysis of Variance (ANOVA) with experience level as a factor

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because the total biomass of the *A. petiolata* plants per pot varied greatly (0.2 to 4.6 g). Biomass of *A. petiolata* could not be used as a covariate in an ANOVA, since covariates should not be affected by the treatment (Neter and others 1996) and we expected the level of experience of *I. capensis* to differentially affect the growth of *A. petiolata*. We combined data from sites since there were no significant differences in growth measures of *I. capensis* among sites, determined using a one-way ANOVA.

RESULTS

In both the experienced and naïve plants, there were significant positive correlations between *I. capensis* height and biomass (Table 1). Additionally, stem diameter was significantly (or nearly significantly) positively correlated with biomass, height and reproduction in both experienced and naïve plants. In naïve plants, there was a significant positive correlation between reproduction and biomass of *I. capensis* and significant negative correlations between height and biomass of *I. capensis* and *A. petiolata* biomass. In experienced plants, the positive correlations between reproduction of *I. capensis* and *A. petiolata* biomass approached significance. In general, other correlations between *I. capensis* growth variables and *A. petiolata* biomass tended to be negative in naïve *I. capensis* and positive in experienced *I. capensis*.

Table 1

Pairwise correlation matrices for experienced and naïve *Impatiens capensis* grown with *Alliaria petiolata*. All variables are measurements of *I. capensis* until otherwise indicated

| | Height | Reproduction | Stem diameter | Root to shoot | Total biomass |
|-----------------------------------|---------------------|--------------------|--------------------|---------------|---------------------|
| Naïve plants, n = 14 | | | | | |
| Reproduction | 0.140 | | | | |
| Stem diameter | 0.522 ¹ | 0.543 ² | | | |
| Root to shoot | -0.278 | 0.300 | 0.253 | | |
| Total biomass | 0.661 ³ | 0.660 ³ | 0.684 ³ | -0.021 | |
| <i>A. petiolata</i> total biomass | -0.568 ² | -0.373 | -0.369 | -0.191 | -0.738 ⁴ |
| Experienced plants, n = 15 | | | | | |
| Reproduction | 0.357 | | | | |
| Stem diameter | 0.790 ⁴ | 0.587 ² | | | |
| Root to shoot | -0.086 | 0.206 | 0.218 | | |
| Total biomass | 0.895 ⁴ | 0.240 | 0.691 ³ | -0.228 | |
| <i>A. petiolata</i> total biomass | 0.384 | 0.535 ¹ | 0.316 | 0.223 | 0.196 |

1 = P < 0.06

2 = P < 0.05

3 = P < 0.01

4 = P < 0.005

DISCUSSION

Growth of naïve *I. capensis* was negatively impacted by the presence of *A. petiolata* as expected (Cipollini and others 2008). While only height and biomass were significantly negatively correlated, most correlations between naïve *I. capensis* growth and *A. petiolata* biomass tended to be negative. On the other hand, experienced plants failed to respond negatively to *A. petiolata*. In fact, while none of the correlations were significant at P = 0.05, there was a trend for experienced *I. capensis* growth and reproduction to correlate positively with *A. petiolata* biomass. Increased reproduction in response to stress, such as pollution, has been observed in some plants (Saikkonen and others 1998, Zereva and Kozlov 2005) and shown to be selected for in some animals (Donker and others 1993). A shift in resource allocation, under genetic control in some *I. capensis* populations (Abrahamson and Hershey 1977), may therefore be part of the strategy for resistance of *I. capensis* to *A. petiolata*.

Our results are similar to those of Callaway and others (2005), who studied a different ecological system using a similar experimental approach. Adult size can be heritable and can therefore undergo evolution by natural selection (Mitchell-Olds 1986). Height and fruit production are related in *I. capensis* in the field (Cipollini and others 2008), suggesting that growth variables such as height may be good predictors of fitness. Due to our experimental design, it is possible that the seedlings may be displaying resistance due to physiological acclimatization and maternal effects as opposed to genetic differences. In previous studies, there was genetic differentiation between *I. capensis* growing only 10 m apart (Argyes and Schmitt 1991), which suggests that differences in resistance observed between seedlings may be genetically based despite the small separation among them. It is important to note that we found differences in growth variables only; there was no significant differential effect of *A. petiolata* on reproduction in our study, which is necessary to determine if natural selection is indeed occurring. Conclusions about reproduction in our study are limited by the fact that we had a coarse measure of reproductive effort rather than a more precise measure of production of viable offspring. In addition, the experiment was concluded before plants had completed their life cycle (though siblings in the field had completed their life cycle), which may have limited the detection of differences in reproduction.

Our study therefore provides evidence that *I. capensis* can display variation in resistance to *A. petiolata*, and that seedlings germinating with *A. petiolata* in the field may have been selected for this resistance. The mechanism of resistance is unknown and depends upon the mechanism by which *A. petiolata* impacted growth. *Alliaria petiolata* is most likely an interference competitor (e.g., Cipollini and others 2008), though has been hypothesized as an effective exploitative competitor (e.g., Meekins and McCarthy 1999). Clearly, more studies are needed to investigate whether the patterns seen here are detectable across a broader range of habitats in the field, as well as the mechanism of resistance. Future studies should seek to create genetic lines of *I. capensis* to determine if resistance is heritable, a condition necessary for evolution by natural selection to occur. Nevertheless, our results are an important contribution towards the small but growing body of knowledge about evolutionary responses of native species to invasive species. Additionally, the results of this study are important in evaluating the potential for long-term persistence of *I. capensis* when challenged by the threat of *A. petiolata*, an important consideration for successful forest understory restoration efforts using this species, as resistance can vary by species (Lesica and Atthowe 2007, Meador and Hild 2007).

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