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## Comparison of allelopathic effects of five invasive species on two native species<sup>1</sup>

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**Abstract.** Recent studies have found that allelopathy can be an important mechanism of plant invasions. *Alliaria petiolata*, *Lonicera maackii*, *Ranunculus ficaria*, *Celastrus orbiculatus*, and *Microstegium vimineum* are invasive species found in the Midwestern USA. We investigated the comparative direct and indirect allelopathic effects of these five species in a laboratory setting using leaf extracts in a germination experiment and a growth experiment. Results illustrate that the effect of each invasive species varied with target species and with life stage. Extracts of *L. maackii* and *R. ficaria* had the largest overall effects (~50% reduction) on germination across both species, but effects of extracts varied by target species. Extracts of *A. petiolata* and *C. orbiculatus* had a greater effect on germination of *E. hystrix* than on *C. fasciculata*. Extracts of *L. maackii*, *M. vimineum*, and *R. ficaria* had larger inhibitory germination effects on *C. fasciculata* than on *E. hystrix*. For growth of *E. hystrix* after 8 wk, *C. orbiculatus* and *L. maackii* extracts had the largest (>80%) reduction of biomass, followed by treatment with extracts of *R. ficaria* (~20% reduction). Treatment with *L. maackii* and *C. orbiculatus* extracts reduced height, with extracts of *L. maackii* having greater effects (60% versus 40% reduction, respectively). Plants treated with *L. maackii* extracts allocated significantly less biomass to roots. We also found evidence of indirect impacts. Mycorrhizal inoculation overall was most negatively affected by treatment with *L. maackii* extracts (~70% reduction), even at low concentrations, and least by treatment with *C. orbiculatus*, with intermediate effects of *A. petiolata*, *M. vimineum*, and *R. ficaria*. Overall, *L. maackii* had the strongest effects, followed closely by *C. orbiculatus*, which was then followed in turn by *R. ficaria*. These results provide further support for the allelopathic potential of *L. maackii* and add to the growing body of evidence on the allelopathic potential of *R. ficaria* and *C. orbiculatus*. Effects of *A. petiolata* and *M. vimineum* were less strong than in other studies, suggesting the importance of variation between populations and in experimental venue.

Key words: allelopathy, germination inhibition, mycorrhizae, novel weapons hypothesis

Invasive plants are introduced to a given ecosystem, have negative impacts on the invaded community, and are a topic about which there are many active research questions (Sakai *et al.* 2001). Invasive plants can affect native ecosystems in ways such as modification of ecological function (Vitousek 1990) and reduction of diversity (McGeoch *et al.* 2010). Allelopathy is a possible explanation for the mechanism of success of many invasive plants (Hierro and Callaway 2003). Allelochemicals, released from the roots, leaves, and/or other parts of a plant, can negatively impact neighboring species (Rice 1974). The “novel weapons hypothesis” suggests that allelochemicals from invasive plants may have a negative effect on

native plants because they have not yet been able to evolve tolerance or resistance to the chemicals (Callaway and Ridenour 2004). The effects of allelochemicals can be direct or indirect. For example, germination and/or growth may be directly affected (Cipollini, Stevenson, and Cipollini 2008, Cipollini, Titus, and Wagner 2012). Indirect effects can also occur when allelochemicals modify interactions in the soil (Cipollini, Rigsby, and Barto 2012), including mycorrhizal associations (Stinson *et al.* 2006).

In order to fully understand allelopathy in a realistic setting, field studies are necessary (Inderjit and Nielson 2003). However, field studies are complex and separating direct versus indirect effects of allelopathy can be difficult. Direct effects can be more clearly demonstrated in a laboratory setting (*e.g.*, Dorning and Cipollini 2006). Simple laboratory germination and greenhouse growth experiments can also provide a screening tool to determine possible allelopathic effects before conducting more expansive and complicated field studies. Further, laboratory studies provide the opportunity to easily compare allelopathic effects using multiple species in a highly controlled context and simple aqueous plant extracts can be used as a method to study allelopathic potential

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(McEwan *et al.* 2010; Pisula and Meiners 2010; Cipollini, Titus, and Wagner 2012; Cipollini and Flint 2013).

For this study, we comparatively examined the effects of five invasive species that are currently impacting forests in the Midwest and Northeast USA. *Alliaria petiolata* (M. Bieb) Cavara & Grand, garlic mustard, is a well-studied biennial, native to Europe and now found widely in the USA and adjacent Canada (Nuzzo 1993, USDA 2015). *Alliaria petiolata* has direct (Prati and Bossdorf 2004; Cipollini, Titus, and Wagner 2012; Cipollini and Flint 2013) effects on germination and growth of native species. *Alliaria petiolata* is a nonmycorrhizal species and can have indirect effects on belowground mutualisms in other species. For example, Stinson *et al.* (2006) found less mycorrhizal colonization in roots of two native tree species grown in soils in which garlic mustard had been found. Similarly, Callaway *et al.* (2008) found that, in invaded ranges, mycorrhizal fungi exposed to soils conditioned by garlic mustard had lower infectivity and viability; growth of mycorrhizal plant species was also suppressed when grown in soils conditioned by garlic mustard. *Alliaria petiolata* has putative allelochemicals that have been identified and studied (Vaughn and Berhow 1999, Cipollini and Gruner 2007, Barto and Cipollini 2009). Cipollini, McClain, and Cipollini (2008) have demonstrated probable allelopathic effects in the field.

*Lonicera maackii* (Rupr.) Maxim, Amur honeysuckle, is a deciduous shrub from northeast Asia, now found as an invasive species in the eastern USA (Luken and Thieret 1995, USDA 2015). Reduced native plant diversity and abundance has been found to be associated with the presence of *L. maackii* (Collier, Vankat, and Hughes. 2002). *Lonicera maackii* exhibits direct allelopathic effects on seed germination of several species (Dorning and Cipollini 2006, Cipollini and Flint 2013). Though allelopathy has not been definitively demonstrated in the field (Cipollini, McClain, and Cipollini 2008), plants grown in conditioned soils or treated with extracts in field soils do exhibit allelopathic effects (Cipollini and Dorning 2008; Cipollini, Stevenson, and Cipollini 2008; Cipollini, Titus, and Wagner 2012). Negative effects of *L. maackii* extracts on mycorrhizal infection have been demonstrated for *Impatiens* and other native species in greenhouse experiments

(Shannon *et al.* 2014; Ali, Lieurance, and Cipollini 2015).

*Ranunculus ficaria* L., lesser celandine or fig buttercup, is a short-lived spring herb, imported from Europe and now invasive in the Northeast and Pacific Northwest regions of the USA and adjacent Canada (Axtell, DiTommaso, and Post 2010). While it is considered an invasive species (*e.g.*, Axtell, DiTommaso, and Post 2010), there is very little published information on its effect on native species to confirm its invasive status (see Cipollini and Schradin 2011). Cipollini and Schradin (2011) did find that *R. ficaria* had effects past its brief growing season and demonstrated possible allelopathic effects in the field. *Ranunculus ficaria* has also shown allelopathic effects on germination of native and model plant species in the laboratory (Cipollini and Flint 2013; Cipollini, Titus, and Wagner 2012).

*Celastrus orbiculatus* Thunb., Oriental bitter-sweet, is an invasive woody vine, brought from Asia and now found in native habitats throughout the eastern USA and adjacent Canada (McNab and Meeker 1987, USDA 2015). As with *R. ficaria*, *C. orbiculatus* has been identified as an invasive species and consequently there are many efforts to control it (*e.g.*, Hutchison 1992); yet little information is available on any negative ecological impacts. There are changes in successional dynamics in the presence of *C. orbiculatus* (Fike and Neiring 1999), but there was no negative effect on tree growth (Horton and Francis 2014) or bird nesting success (Schlossberg and King 2010). Soils primed with *C. orbiculatus* generally did not have any effects different from those of native vines on target species (Leicht-Young, Bois, and Silander 2015). Pisula and Meiners (2010) did find that *C. orbiculatus* exhibited strong germination inhibition in target seeds of common radish, *Raphanus sativus*, in a laboratory bioassay. *Celastrus orbiculatus* itself is mycorrhizal (Lett, DeWald, and Horton 2011) and its presence is associated with altered soil composition and nutrient dynamics (Leicht-Young *et al.* 2009).

*Microstegium vimineum* (Trin.) A. Camus, Japanese stiltgrass or Nepalese browntop, is a grass native to Asia and is now found in the eastern half of the USA from Texas to Massachusetts (Fairbrothers and Gray 1972, USDA 2015). *Microstegium vimineum* can be an important invasive species at some sites (Drake, Weltzin, and Parr 2003) and has been suggested to have a

greater potential for spread and thus for associated negative effects than *A. petiolata* (Morrison *et al.* 2007). Native species growth and density are negatively affected by *M. vimineum* (Flory, Rudgers, and Clay 2007; Oswalt, Oswalt, and Clatterbuck 2007; Flory and Clay 2010). *Microstegium vimineum* had relatively strong inhibitory effects on germination inhibition of common radish in a laboratory bioassay (Pisula and Meiners 2010), but effects on lettuce and radish were found to be less strong than from a common native understory species (Corbett and Morrison 2012). It is not known if there are effects of *M. vimineum* on mycorrhizal associations, but *M. vimineum* itself is mycorrhizal (Lee *et al.* 2014) and its leaf litter creates soil microbial conditions different from those created by native species (Elgersma *et al.* 2012).

The purpose of this study was to examine the effects of these five invasive species in a comparative context on germination, growth, and mycorrhizal infection of target species. Previous comparative studies have focused on studying the allelopathic effects of these invasive species on test species that lack ecological relevance (*e.g.*, Pisula and Meiners 2010; Cipollini, Titus, and Wagner 2012). In addition, different native species can be affected differently by different invasive species (McEwan *et al.* 2010; Cipollini, Titus, and Wagner 2012; Cipollini and Flint 2013); thus, using multiple species gives a better range of effects. Two native species, *Elymus hystrix* L., bottlebrush grass, and *Chamaecrista fasciculata* (Michx.) Greene, partridge pea, were used as target species in this study, as they can be found in overlapping habitats with the invasive species of interest and are readily available as seed.

**Materials and Methods.** **EXTRACT PREPARATION.** Leaves from *Alliaria petiolata*, *Lonicera maackii*, *Ranunculus ficaria*, and *Celastrus orbiculatus* were collected from forested areas in Cincinnati, OH (39°15'15.94"N, 84°28'27.98"W), on May 5, 2011. Leaves of *Microstegium vimineum* were collected from Laurelville, OH (39°31'36.10"N, 82°39'5.47"W), on July 19, 2011, for the germination experiment and on August 8, 2011, for the growth experiment. Different collection dates were used due to difficulties in obtaining enough leaf material from annual *M. vimineum*, which were small in spring. Leaves were washed and weighed. Extracts were made by placing 300 g of each leaf

tissue separately into 1,000 mL of distilled water and left at room temperature for 60 hr. The solutions were then vacuum filtered and frozen, except for *M. vimineum* extracts, which were used after preparation. Frozen extracts were thawed completely at room temperature before using and all extracts were stored at 4 °C during the experiment. Two extract concentrations were used. The high concentration (0.3 g fresh leaf/mL dH<sub>2</sub>O) was the extract solution without dilution. The low-concentration solution (0.15 g fresh leaf/mL dH<sub>2</sub>O) was created by halving the original extract solution with distilled water. Similar concentrations have been used for previous work and are thought to be within the range of realistic field levels (*e.g.*, Dorning and Cipollini 2006; Cipollini, Titus, and Wagner 2012; Cipollini and Flint 2013).

**GERMINATION EXPERIMENT.** The two target species, *Elymus hystrix* (Poaceae; bottlebrush grass) and *Chamaecrista fasciculata* (Fabaceae; partridge pea) were obtained from Prairie Moon Nursery (Winona, MN). The target species were chosen based on their potential to overlap in the same habitats as the extract species, as well as the availability of their seeds. The probability of overlap or degree of overlap between the target and extract species in the field is not precisely known, but it is expected that there are indeed differences in the amount of interaction between the target and extract species. There were four replicates for each treatment combination (5 species × 2 concentrations = 10 treatment combinations) and for the control. Ten seeds each of *E. hystrix* and *C. fasciculata* were placed on paper towels saturated with 20 mL of extract solution. The control replicates were each saturated with 20 mL of distilled water. The paper towels were then placed in plastic bags under growth lights with 12 hr of day length. Germination was recorded at the end of 4 wk.

Due to constraints of the fully factorial statistical model, the control treatments were not included in the analysis as a factor. The primary goal of this experiment was to compare differences between invasive species. The control serves primarily to validate the viability of the seeds. It is important to note that there are no direct statistical comparisons to the control treatment in these studies. Instead, response variables for all experiments were calculated as the percentage of the average control level. We have presented the levels in relation to a control to give an understanding of relative

Table 1. Results of ANOVA for percentage of germination (compared to control treatment) of seeds of native *Chamaecrista fasciculata* and *Elymus hystrix* (target species) treated with extracts of five invasive species (*Alliaria petiolata*, *Celastrus orbiculatus*, *Lonicera maackii*, *Microstegium vimineum*, and *Ranunculus ficaria*; extract species) at two concentrations.

Source	df	F	P
Extract species	4	11.09	< <b>0.001</b> <sup>a</sup>
Extract concentration	1	2.51	0.119
Target species	1	1.74	0.193
Extract species × extract concentration	4	1.35	0.262
Extract species × target species	4	2.59	<b>0.045</b>
Extract concentration × target species	1	1.96	0.167
Extract species × extract concentration × target species	4	0.51	0.728
Error	60		

<sup>a</sup> Bold text indicates a significant effect.

germination levels of treatments in relation to a control, but focus our discussion on comparisons between extract treatments. We performed a fully factorial ANOVA with the factors of extract species, extract concentration, and target species on the percentage germinated (out of average number germinated in the control treatments for each species). Data met model assumptions of normality. A Tukey's test was used to determine differences between factors when significance was found in the ANOVA.

**PLANT GROWTH EXPERIMENT.** Soil was collected from a natural woodlot with no invasive species present. There were four replicates of the control group and of each treatment combination (5 species × 2 concentrations = 10 treatment combinations). Soil was added to 75-mL pots and four *E. hystrix* seeds were planted in each pot on July 19. One week later the plants were thinned to one per pot. Ten milliliters of fertilizer (Peter's 20-20-20 NPK with micronutrients; Grace-Sierra, Milpitas, CA) were added each time treatment solutions were added to the soil. Four pots were watered with 10 mL of treatment solution on September 16, October 4, October 20, and November 2. All pots were watered with distilled water as needed. The pots were arranged randomly under growth lights (Tek Light 44, Sunlight Supply, Vancouver, WA) with high-output fluorescent bulbs (General Electric, 54 W, 6,500 K) with 14-hr day length. Plant height was measured weekly for 8 wk. At 8 wk, plants were removed from the soil and dried in a 100 °C oven for 24 hr; roots and shoots were then weighed separately.

Vierheilig, Schweiger, and Brundrett (2005) provide a review of techniques for staining mycorrhizae, including the following method. For our experiment, a small amount of dried root from

each plant in the growth experiment was removed, placed into separate vials, and covered with 10% KOH. The roots were allowed to soak for 3 days. Roots were then covered in 85% EtOH to remove any pigmentation. After 3–4 days, roots were rinsed with distilled water and observed under the microscope to assure root clarity. Roots were then soaked at room temperature in 5% HCl for 5 min, removed, and then covered in 0.05% Trypan Blue stain for 1 hr. Roots were covered in lactoglycerol until slide mounting. After mounting, roots were observed under a microscope and percentage of cells with mycorrhizal infection was blindly assessed by visual approximation at five locations on each slide and by then averaging the five measurements.

We performed a fully factorial multivariate ANOVA (MANOVA) with the factors of extract species and extract concentration on percentage (relative to the average of the control treatment) of final total mass, root to shoot ratio, height, and mycorrhizal inoculation. Root to shoot data were log transformed prior to analysis to meet model assumptions. All other data met model assumptions of normality and heteroscedasticity. Since significance was found in the MANOVA, separate univariate ANOVAs were then performed for each response variable. A Tukey's test was used to determine differences between factors when significance was found in the ANOVA.

**Results.** Percentage of germination of target species was significantly affected by extract species and the interaction of extract species and target species (Table 1). Percentage of germination ( $\pm$  SE) in the control treatment was  $77.5 \pm 7.5\%$  for *E. hystrix* and  $37.5 \pm 4.8\%$  for *C. fasciculata*. Across all other treatments, extracts of *L. maackii*

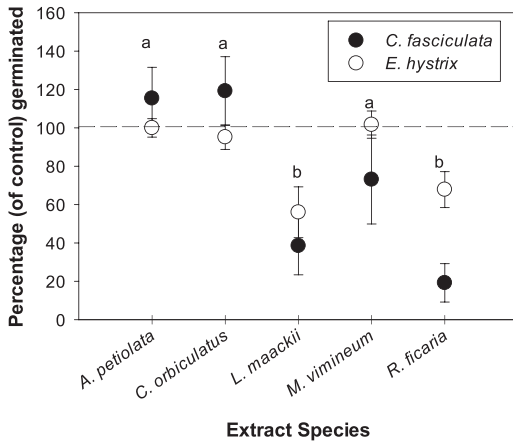


FIG 1. Mean ( $\pm$  SE) percentage (of the control treatment) germination of each target species (*Chamaecrista fasciculata* and *Elymus hystris*) versus invasive extract species (*Alliaria petiolata*, *Celastrus orbiculatus*, *Lonicera maackii*, *Microstegium vimineum*, and *Ranunculus ficaria*), averaged across concentration treatments ( $n = 8$  for each mean). Letters indicate significant differences between extract species from the Tukey's test. The dashed line indicates the average level of the control (no extract) treatment for visual comparison.

and *R. ficaria* significantly reduced the percentage of seeds germinated (Fig.1). However, effects of extract species varied by target species. Extracts of *A. petiolata* and *C. orbiculatus* had more effect on germination of *E. hystris* than on *C. fasciculata*

(Fig. 1). Extracts of *L. maackii*, *M. vimineum*, and *R. ficaria* had stronger inhibitory effects on *C. fasciculata* than on *E. hystris*. (Fig. 1).

Average  $\pm$  SE biomass, height, root to shoot ratio, and percentage of mycorrhizal inoculation in the control treatments in the *E. hystris* growth experiment were  $0.525 \pm 0.6$  g,  $26.5 \pm 1.8$  cm,  $1.15 \pm 0.13$ , and  $38.25 \pm 1.3\%$ , respectively. In the MANOVA with all growth experiment measurements included, there were significant effects of extract species, extract concentration, and their interaction (Wilk's  $\lambda$ , respectively,  $F_{16,64} = 17.48$ ,  $P < 0.001$ ;  $F_{4,21} = 3.94$ ,  $P = 0.015$ ;  $F_{16,64} = 2.21$ ,  $P = 0.013$ ). In the subsequent ANOVAs, increasing extract concentration significantly lowered biomass, root to shoot ratio, and mycorrhizal inoculation (Table 2; Fig. 2A, B, 3). Extract species affected all of these variables as well, along with height (Table 2; Fig. 2C). Biomass was most negatively affected by *L. maackii* and *C. orbiculatus* extracts and least affected by *A. petiolata* and *M. vimineum* extracts, with effects of *R. ficaria* extracts intermediate between the two groups (Fig. 2A). Height was most negatively affected by *L. maackii* extracts, followed by *C. orbiculatus* extracts, with little effect of *A. petiolata*, *M. vimineum*, and *R. ficaria* extracts (Fig. 2C). Root to shoot ratio was significantly lowered by *L. maackii* extracts only (Fig. 2B), with greater allocation to shoots compared to control treatments. Mycorrhizal inoculation was significantly

Table 2. Results of ANOVA for percentage (compared to control) of total biomass, root to shoot ratio, height, and mycorrhizal inoculation of *Elymus hystris* treated with extracts of five invasive species (*Alliaria petiolata*, *Celastrus orbiculatus*, *Lonicera maackii*, *Microstegium vimineum*, and *Ranunculus ficaria*) at two concentrations.

Source	df	F	P
Total biomass (error df = 29)			
Extract species	4	66.87	< <b>0.001</b>
Extract concentration	1	5.0	<b>0.033</b>
Extract concentration $\times$ extract species	4	1.36	0.273
Root to shoot ratio (error df = 29)			
Extract species	4	9.56	< <b>0.001</b>
Extract concentration	1	9.68	<b>0.015</b>
Extract concentration $\times$ extract species	4	1.61	0.073
Height (Error df = 29)			
Extract species	4	123.25	< <b>0.001</b>
Extract concentration	1	0.21	0.648
Extract concentration $\times$ extract species	4	0.18	0.947
Percentage of mycorrhizal inoculation (Error df = 24)			
Extract species	4	4.22	<b>0.010</b>
Extract concentration	1	9.93	< <b>0.001</b>
Extract concentration $\times$ extract species	4	5.37	<b>0.003</b>

<sup>a</sup> Bold text indicates a significant effect.

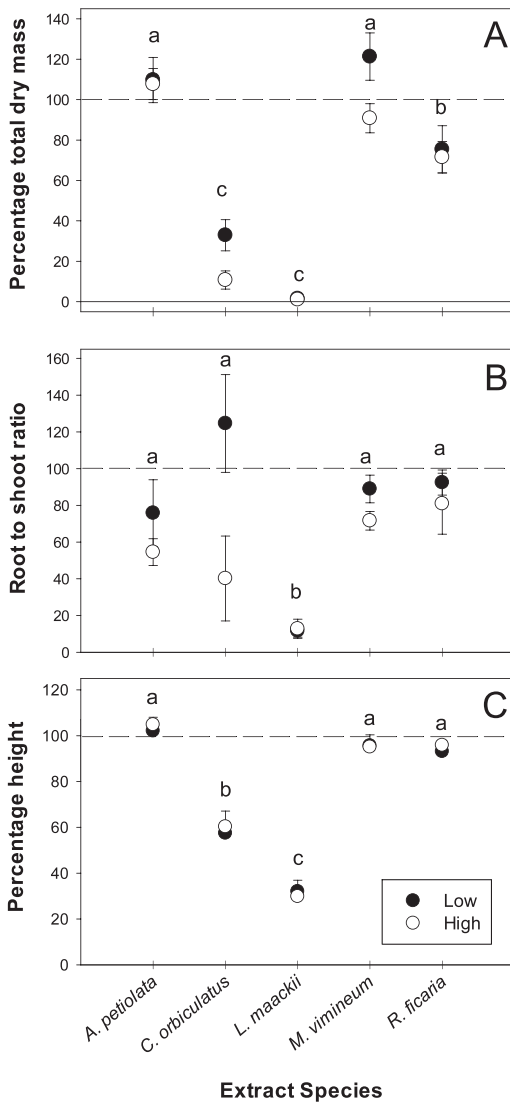


FIG. 2. Mean ( $\pm$  SE) percentage (of the control treatment) of (A) dry mass (B) root to shoot ratio, and (C) height of *Elymus hystrix* by invasive extract species (*Alliaria petiolata*, *Celastrus orbiculatus*, *Lonicera maackii*, *Microstegium vimineum*, and *Ranunculus ficaria*) and concentration ( $n = 4$  for each mean, except  $n = 3$  for *L. maackii* at high concentration). Letters indicate significant differences between extract species from the Tukey's test. The dashed line indicates the average level of the control (no extract) treatment for visual comparison.

higher in plants treated with *C. orbiculatus* extracts compared to plants treated with *L. maackii* extracts, with intermediate values for plants treated with *A. petiolata*, *M. vimineum*, and *R. ficaria* extracts (Fig. 3). Increasing concentration had

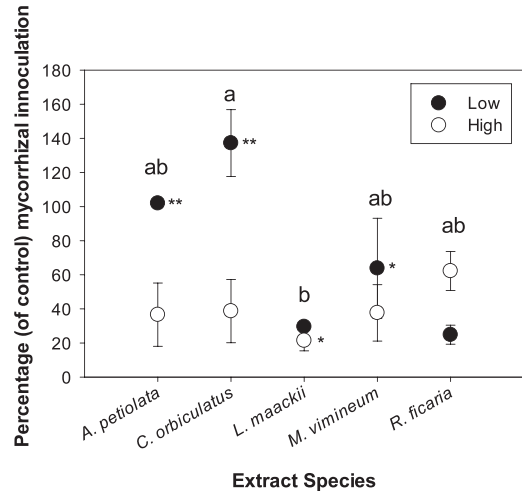


FIG. 3. Mean ( $\pm$  SE) percentage (of the control treatment) of mycorrhizal inoculation of *Elymus hystrix* by invasive extract species (*Alliaria petiolata*, *Celastrus orbiculatus*, *Lonicera maackii*, *Microstegium vimineum*, and *Ranunculus ficaria*) and concentration ( $n = 4$  for each mean, except  $n = 2$ ). Letters indicate significant differences between extract species from the Tukey's test. The dashed line indicates the average level of the control (no extract) treatment for visual comparison.

differential effects on mycorrhizal inoculation demonstrated by the significant interaction of extract concentration and extract species (Table 2). Increasing concentrations of *L. maackii* extracts had little effect. Increasing concentration of *A. petiolata*, *C. orbiculatus*, and *M. vimineum* extracts negatively affected mycorrhizal inoculation, while increasing concentration of *R. ficaria* extracts positively affected mycorrhizal inoculation (Fig. 3).

**Discussion.** Our results provide information on the relative allelopathic effects of five invasive species common in forest of the Midwest and Northeast using ecologically relevant species as target species. Overall, *L. maackii* had the greatest effects on a variety of response variables among the five invasive species studied. In comparison, *C. orbiculatus* had strong yet slightly weaker impacts. The effects of *R. ficaria* were generally smaller and with less broad impacts. Extracts of *A. petiolata* and *M. vimineum* had the least effects. Our results provide further insight into allelopathy of these species, which allows the

setting of priorities for further studies in the field and subsequent invasive species control and management.

There were strong allelopathic effects of *L. maackii* leaf extracts on germination, growth, resource allocation pattern, and mycorrhizal inoculation. Other studies have found allelopathic effects of *Lonicera maackii* on germination (Dorning and Cipollini 2006; Cipollini, Titus, and Wagner 2012; Cipollini and Flint 2013) and growth (Cipollini, Stevenson, and Cipollini 2008). McEwan *et al.* (2010) similarly found stronger allelopathic effects of extracts of *L. maackii* compared to extracts of native species and also found that the effects varied with target species. We confirmed effects of *L. maackii* on mycorrhizal infection, as found in other studies (Shannon *et al.* 2014; Ali, Lieurance, and Cipollini 2015).

Extracts of *C. orbiculatus* also had strong effects on germination and growth. Earlier studies found similar effects of leaf extracts on germination (Pisula and Meiners 2010) and of conditioned soil on growth (Leicht-Young, Bois, and Silander 2015). *Celastrus orbiculatus* had varying effects depending on life stage of the target species; germination of *E. hystrix* was not very affected by extracts of *C. orbiculatus*, yet growth of *E. hystrix* was strongly affected. Thomson (2005) and Shannon-Firestone and Firestone (2015) similarly found differences in effect of an invasive species depending on life stage. Extracts of *R. ficaria* also had weak but significant effects on germination and growth, confirming earlier work using a similar approach (Cipollini, Titus, and Wagner 2012, Cipollini and Flint 2013) and in the field (Cipollini and Schradin 2011). Clearly, further research into allelopathy in these two generally understudied species is warranted.

Extracts of *M. vimineum* had few effects on germination and growth, which conflicts with results from earlier studies. Corbett and Morrison (2012) did find allelopathic effects of *M. vimineum* on germination and seedling establishment, but the effects of *M. vimineum* on lettuce and radish were lower than those of a native species found in the same habitat. Pisula and Meiners (2010) found strong effects of *M. vimineum* on germination of radish compared to a suite of nine other invasive species. Similarly, in contrast to similar earlier studies (Pisula and Meiners 2010; Cipollini, Titus, and Wagner 2012), there were generally smaller effects of *A. petiolata* in this

study as well. While it is to be expected that different target species would respond differently (McEwan *et al.* 2010; Cipollini, Titus, and Wagner 2012), conflicting results were found for effects of *A. petiolata* and *L. maackii* on the same target species, *E. hystrix*, used in previous assays (Cipollini and Flint 2013).

Differences in responses may be attributed to differences in chemistry due to environmental or other conditions, as leaves used for extracts were collected from different populations in different months at different locations using differing techniques. For example, Cipollini (2002) found variations in chemistry of *A. petiolata* with soil conditions and Smith (2015) found variation in glucosinolates along a light gradient. Additionally, Lankau *et al.* (2009) found a pattern of reduction in putative allelochemicals and associated negative effects on native tree species with time since invasion. However, no assessment of the chemical nature of the extracts was performed in our studies. Additionally, in contrast to earlier studies (Stinson *et al.* 2006), we did not find strong effects of *A. petiolata* extracts except at high concentrations on mycorrhizal inoculation of *E. hystrix*. These results suggest that the allelochemical effects of *A. petiolata*, *M. vimineum*, and *L. maackii* can vary and these species may not be a threat to native communities under all conditions. Indeed, Morrison *et al.* (2007) found that the life history traits of *M. vimineum* may make this species a greater invasive threat than *A. petiolata*. Further, even in this well-studied species, there are still many questions as to if *A. petiolata* has ecologically relevant effects in the field (Cipollini and Cipollini 2016).

Patterns of variation of effects of extracts on growth among species were not similar to patterns of effects on mycorrhizal inoculation. Extract of *L. maackii* was the only type of extract that had effects at low concentrations. *Lonicera maackii* was the only species for which strong growth effects and strong mycorrhizal effects were observed in tandem, suggesting this may be an important mechanism of impact for this species, likely combined with direct or other indirect effects. Other species did not show similar parallel changes in growth measures and mycorrhizal inoculation. For example, extracts of *C. orbiculatus* had a low amount of effect on mycorrhizal inoculation, yet had strong effects on growth,



suggesting an alternate, perhaps direct, mechanism of impact. Conversely, extracts of *A. petiolata* had low effects on growth even at high concentrations, yet high concentrations reduced mycorrhizal inoculation by 50%. Likely, the reduction of mycorrhizae was not large enough to affect growth.

Our study provides an important screening of potential allelopathy, yet conclusions from this study are limited by the experimental venue. Field studies are necessary to fully understand allelopathic effects of invasive species *in situ* (Inderjit and Nielsen 2003). We also do not adequately consider and address the suite of indirect interactions that are possible (Cipollini, Rigsby, and Barto 2012). Additionally, the presence and strength of allelopathic effects varies with experimental venue (Bauer *et al.* 2012, Shannon-Firestone and Firestone 2015). Future studies can also be enhanced by comparing the effects of invasive species with native species in order to understand if allelopathy truly represents a novel weapon (McEwan *et al.* 2010, Corbett and Morrison 2012, Shannon-Firestone and Firestone 2015). Finally, our results confirm the necessity of using multiple target species, as effects varied among them, as in previous work (McEwan *et al.* 2010; Cipollini, Titus, and Wagner 2012; Cipollini and Flint 2013). Nevertheless, our study has the advantages of the direct comparison of a suite of invasive species, using ecologically relevant target species rather than agricultural species or model organisms. It further provides valuable information for *C. orbiculatus*, *M. vimineum*, and *R. ficaria*, three species for which less information on allelopathic potential is available.

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