

## Invasive plants can inhibit native tree seedlings: testing potential allelopathic mechanisms

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Received 19 January 2005; accepted in revised form 13 April 2005

**Key words:** Allelopathy, *Elaeagnus*, Fescue, Forest, *Lolium*, Succession

### Abstract

The mechanisms by which invasive species affect native communities are not well resolved. For example, invasive plants may influence other species through competition, altered ecosystem processes, or other pathways. We investigated one potential mechanism by which invasive plants may harm native species, allelopathy. Specifically, we explored whether native tree species respond differently to potential allelopathic effects of two invasive plant species. We assessed the separate effects of *Lolium arundinaceum* (tall fescue) and *Elaeagnus umbellata* (autumn olive) on three common successional tree species: *Acer saccharinum* (silver maple), *Populus deltoides* (eastern cottonwood), and *Platanus occidentalis* (sycamore). Tall fescue and autumn olive are widely planted and highly invasive or persistent throughout North America where they often grow in forest edges, old fields, and other sites colonized by pioneering tree species. In an exploratory greenhouse experiment, we applied aqueous extracts derived from soil, leaf litter, or live leaves to native trees. We compared these treatments to a sterile water control and also to minced leaves leached in water, a common, but potentially less realistic method of testing for allelopathy. For all tree species, minced leaves from tall fescue reduced the probability that seedlings emerged, and minced leaves of autumn olive reduced the number of days to emergence. During other demographic stages, the three native tree species diverged in their responses to the invasive plants. *Platanus occidentalis* exhibited the widest range of responses, with reduced root biomass due to minced tissue from both invasive species, reduced days to emergence and marginally reduced survival from minced tall fescue, and reduced leaf biomass from tall fescue leaf litter. *Populus deltoides* appeared insensitive to most extracts, although survival was marginally increased with application of minced or fresh leaf extracts from autumn olive. In addition, minced tall fescue shortened the time to seedling emergence for *Acer saccharinum*, potentially a positive effect. Overall, results suggest that allelopathy may be one mechanism underlying the negative impacts of tall fescue and autumn olive on other plant species, but that effects can depend strongly upon the source of allelochemicals and the tree species examined.

### Introduction

Exotic, invasive species have the potential to affect the structure of native plant communities (Vitousek 1990; Mack et al. 2000; Woitke and Dietz 2002). Impacts include changes in the diversity or relative abundance of native species and alteration

of the successional dynamics of communities over time (Wilcove et al. 1998; Parker et al. 1999; Cronk and Fuller 2001). Mechanisms underlying the effects of invasive species on communities have received much less attention than the impacts of the invaders on communities or ecosystems (Levine et al. 2003). For example, invasive species

may displace natives through competition, changes in ecosystem processes, or allelopathy, among other mechanisms (Mallik and Prescott 2001; Hierro and Callaway 2003; Levine et al. 2003), and the relative importance of these factors remains unclear.

The success achieved by many exotic plants may result from ecological advantages afforded by plant traits that are novel to the recipient community (the novel weapons hypothesis) (Callaway and Aschehoug 2000; Bais et al. 2003; Callaway and Ridenour 2004; Callaway et al. 2004; Vivanco et al. 2004). One potentially important trait, allelochemical interference (Seigler 1996), has been reported for many species, in either their native or introduced habitats, although usually not in both (e.g., Kocacaliskan and Terzi 2001; Souto et al. 2001; Bertin et al. 2003b). Testing the novel weapons hypothesis requires comparing the relative strength of effects of the invading species on co-occurring plants in the introduced habitat to effects of the invader on co-occurring plants in its native range. Stronger negative impacts on co-occurring plants in the introduced habitat than in the native habitat support the hypothesis that the invader possesses a novel weapon in its new habitat (Callaway and Ridenour 2004). A first step toward testing the novel weapons hypothesis is to determine whether the invasive species has any negative impacts on co-occurring species in its introduced habitat. Here, we test for such negative impacts of the invader via potentially allelopathic mechanisms.

Much of the prior research on allelopathy comes from agricultural or laboratory settings, limiting the ability to infer the ecological relevance of the results for native plant and soil communities (Inderjit 2001). Furthermore, methods used to extract and deliver putative allelochemicals have also often been ecologically unrealistic (Inderjit and Weston 2000). Inderjit and Callaway (2003) note, 'Allelopathy is better demonstrated through experiments in which a toxic product is shown to be released from the putative aggressor, and arrives at the putative victim in functional concentrations under reasonably natural conditions.' Despite recent efforts aimed to mitigate the shortcomings of earlier methodology (e.g., Tongma et al. 1998; Fujii 2003; Hane et al. 2003), relatively few studies have investigated allelopathic interference by using realistic sources and concentrations of putative

allelochemicals (e.g., foliar leachates or root exudates; Inderjit and Weston 2000; Inderjit and Callaway 2003). For example, compounds toxic to other plants can be artificially isolated from many plant species, but these compounds are not necessarily encountered by neighboring plants in nature (Harper 1994). Furthermore, little research has explored whether co-occurring native species differ in their responses to allelopathy (but see, Vandermaast et al. 2002; Grant et al. 2003; Renne et al. 2004), potentially resulting in a shift in species composition due to the allelopathic effects of an invader.

We explored the allelopathic potential of two common, exotic and invasive plant species, *Lolium arundinaceum* (tall fescue) and *Elaeagnus umbellata* (autumn olive). These Eurasian species have been widely planted throughout North America and are considered invasive or pest species in many regions (Hiebert 1990; Clay 2001; Clay and Schardl 2002; Raloff 2003; Yates et al. 2004). Both species often grow in old fields and along forest edges colonized by pioneering tree species. Tall fescue and related species have been reported to exhibit allelopathic potential in their introduced habitats (Peters and Zam 1981; Luu et al. 1982; Peters and Luu 1985; Preece et al. 1991; Malinowski et al. 1999; Bertin et al. 2003a). Both tall fescue and autumn olive are often found in dense monotypic stands that suggest allelopathy as a potential mechanism underlying their success (Levine et al. 2003). Tall fescue occurs commonly in old field communities and is most likely to affect native early successional tree species. In contrast, autumn olive is shade-tolerant and can dominate the understorey of closed canopy forests; however, it also readily colonizes old fields and other disturbed sites (Edgin and Ebinger 2001).

We investigated the inhibitory effects of several types of aqueous extracts of tall fescue and autumn olive on the emergence, survival and growth of important members of native plant communities – early successional tree species: *Acer saccharinum* (silver maple), *Populus deltoides* (eastern cottonwood), and *Platanus occidentalis* (sycamore). To our knowledge, our work is some of the first to test for potentially allelopathic impacts of invaders by comparing effects among native tree species (but see, Conway et al. 2002). In addition, we compared realistic treatments (aqueous extracts derived from non-sterilized soil, leaf litter, and live leaves

soaked in water) with the extracts typically used in prior studies (ground and macerated materials) that are thought to be less ecologically meaningful (Inderjit and Callaway 2003). Our experiments were not designed to distinguish between the direct effects of allelopathic compounds (Kobayashi 2004) and the indirect effects mediated through the microbial community associated with the soil, leaves or litter of exotic plants (Inderjit and Weiner 2001; Kourtev et al. 2002, 2003). Our work is exploratory in that we use only one method – aqueous extracts – to investigate allelopathic potential; therefore, a lack of inhibitory effects does not necessarily imply the absence of allelopathic potential of the invader. However, our work takes an important first step toward understanding the inhibitory potential of invasive, exotic plants on native communities by addressing the following questions: (1) Do the exotic species inhibit native tree species? (2) Does inhibition depend upon the source of the aqueous extracts, including extracts from minced live leaves, intact live leaves, leaf litter, or soil? (3) Are tree species differentially inhibited, suggesting the potential to alter community composition and succession? (4) At what life history stage (seedling emergence, seedling survival or seedling growth) were target species most affected?

## Methods

### *Natural history of the system*

*Lolium arundinaceum* (tall fescue, Poaceae) is a perennial grass with a center of origin in the Mediterranean region of northern Africa and southern Europe; it has been widely planted throughout North America and elsewhere, primarily for pasture and turf (Ball et al. 1993; Clay and Schardl 2002). More than three fourths of the tall fescue in the US is infected with a mutualistic endophyte, *Neotyphodium coenophialum* (Ball et al. 1993). The endophyte produces alkaloids and enhances host resistance to herbivores, pathogens, drought, and plant competition (Clay 1990; Elmi and West 1995; Clay 1996; Malinowski and Belesky 2000). The endophyte may also contribute to the presence of allelochemicals in tall fescue (Malinowski et al. 1999; Renne et al. 2004). Importantly, the presence of the endophyte in tall

fescue strongly slows the successional dynamics of plant communities by suppressing colonizing tree species (J.A. Rudgers, S.P. Orr, and K. Clay, unpubl. data), and one mechanism by which succession is slowed may be allelopathic effects of tall fescue on native trees.

Due to the dominance of endophyte-infected tall fescue in the US, we used endophyte-infected plants in our experiments. Tall fescue plants were located in experimental field plots (30 m × 30 m) that were planted in September 2000 approximately 6 km north of Bloomington, Indiana, USA (39°3'9" N, 086°32'29" W). The plots were surrounded (within 20 m) by secondary growth forest that included silver maple, eastern cottonwood and sycamore. During July 2003, live leaf tissue, leaf litter, and soil samples (see Aqueous extract experiment) were collected from 10 randomly chosen sub-samples within each of four field plots dominated by endophyte-infected tall fescue. In these plots, the percentage of tillers with an endophyte was 94% ± 0.04 SE (10 tillers sampled per plot).

*Elaeagnus umbellata* (autumn olive, Elaeagnaceae) is a perennial shrub that is native to Eurasia. It has been planted widely in eastern North America for erosion control, re-vegetation, and wildlife resources (Catling et al. 1997; Edgin and Ebinger 2001; Yates et al. 2004). A large stand of autumn olive was located at the Touch the Earth Nature Area, approximately 60 km east of Bloomington, Indiana, USA (39°5'7" N, 085°51'29" W). Detailed prior land use history of the preserve is unknown; however, it is likely that autumn olive was planted during the 1960s when the property was actively farmed (personal communication with D. Welch, preserve manager). At the time of collection, ~8 ha of nearly monotypic autumn olive stands were present. Live leaf tissue, leaf litter, and soil samples (see Aqueous extract experiment) were collected during July 2003 from 10 individual autumn olive shrubs chosen at random along a 50 m transect.

Silver maple, eastern cottonwood, and sycamore are all early successional tree species native to eastern North America. Seedlings readily colonize mesic and lowland sites and co-occur with both tall fescue and autumn olive. Silver maple seeds were collected from 20 trees in Bloomington, Indiana, USA during May 2003. Eastern cottonwood seeds were collected from nine trees in Beall

Woods State Park, Illinois, USA during June 2003. Sycamore seeds were collected from 10 trees in Bloomington, Indiana, USA during October 2002. All seeds were stored at 3 °C from the time of collection until planting in July 2003. Seeds from all species were collected before falling to the ground, and seeds were visually examined for damage by seed predators. Any damaged seeds were discarded and not used in the experiment.

#### *Aqueous extract experiment*

We created four aqueous extracts from each exotic plant (tall fescue or autumn olive) to test whether the invasive species may reduce emergence, survival or growth of the native tree species. For both tall fescue and autumn olive, extracts were created from (1) intact, live leaves collected from living plants (Fresh), (2) senesced leaves located in leaf litter on the ground (Old), (3) soil from the top 6 cm of soil beneath living plants (Soil), or (4) minced live leaves collected from living plants (Minced). Live leaves were minced to <3 mm using a food processor (Black and Decker Super Chopper, Hampstead, Maryland, USA) and pulverized with a mortar and pestle. In all cases, the extracts were compared to deionized water, applied as a control. Seeds of native trees were planted into 4.1 cm diameter conetainer pots (Ray Leach Conetainers, Corvallis, Oregon, USA) containing 80 ml sterilized, composted soil (193 ppm N, 13 ppm P, 188 ppm K). We used 20 replicate pots per species per treatment.

The timing of collection of invasive species materials (July) was relevant to the period of natural germination and growth of native tree seedlings (spring and early summer). Given mild winters, both invasive species can grow and shed senescing leaves throughout the year, thus resulting in a long potential period of exposure to these species by the native plant community. However, it is possible that materials collected during different times of year (e.g., winter vs. summer) could result in different potential allelopathic impacts from the invaders.

Realistic concentrations of extracts were created by determining the amount of surface area experienced by each seedling per pot and applying an extract that represented the amount of spring rainfall that would pass through same amount of

surface area of the exotic source material (live leaves, litter, or soil) in the field. More specifically, to determine the concentration of each extract, we first calculated the surface area of all the pots used for each treatment (60 pots per treatment  $\times$  12.8 cm<sup>2</sup> surface area  $\approx$  770 cm<sup>2</sup> total surface area). Then, live leaves, leaf litter, or soil was collected from this same total amount of surface area (770 cm<sup>2</sup>) in the field. The total surface area collected came from 10 sub-samples within each site (77 cm<sup>2</sup> per sub-sample), and sub-samples were bulked for each tissue or soil treatment. We then determined the average rainfall for a typical 9 week period in spring (April–June) in Bloomington, Indiana, USA ( $\sim$ 25 cm) from local weather records. Extracts were created by soaking field-collected material at room temperature in 10 l of deionized water for 24 h. Following soaking, extracts were strained through cheesecloth to remove all particles, and diluted by adding 10 l of water (amount of total surface area (770 cm<sup>2</sup>)  $\times$  average rainfall (25 cm)  $\approx$  20 l per treatment). Extracts were not sterilized so as to prevent the potential loss or alteration of putative allelochemicals through the application of heat or chemical disinfection. The large volume of extract made fine filtering impractical. Therefore, we were not able to tease apart direct effects of allelochemicals from indirect effects mediated through associated microbes.

Seeds were planted in the Indiana University greenhouse in late July 2003. For silver maple seeds, we removed the wings from the samara prior to planting. For all tree species, we pre-tested germination and viability of seeds in a growth chamber. Although we do not know how these germination rates compare to normal rates of germination under field conditions, seeds were collected from natural environments and stored at 4 °C for less than a year. To ensure at least one seedling per replicate for the two species with very small seeds prone to dessication, four eastern cottonwood or three sycamore seeds (one of which was pre-germinated in a growth chamber because germination of sycamore was low) were placed in each pot. After 18 days, surplus seedlings (>1) were weeded. Because of the large size and high emergence rates of silver maple seeds, only one seed was planted per pot. Seeds were assigned at random to the treatments and controls; therefore, there would be no differences in

seed viability or germination potential among the treatments. Individual pots were randomly arranged within the greenhouse.

After planting, each pot was exclusively hand-watered daily with 7 ml of one of the nine treatments for 3 weeks. Thereafter, 12 ml were used to water seedlings every other day, and on alternate days the pots were lightly misted with tap water to prevent drying. Seedling emergence and mortality was recorded for each pot every 2 days throughout the experiment. Plants were harvested at the end of 9 weeks. We determined the oven-dry biomass of root, shoot, and leaf tissue to the nearest 0.0001 g, and measured final shoot length to the nearest 0.1 cm.

#### *Statistical analysis: emergence*

In all analyses, the effects of each exotic species were examined separately. For whether or not a seedling emerged, data were analyzed with a log-linear model, assuming a binomial distribution (emerged or not at the end of 9 weeks) (Proc GENMOD, SAS Institute 2000). Fixed effects included treatment (with five levels: fresh, old, soil, minced, or the control, deionized water), tree species (with three levels: silver maple, eastern cottonwood, and sycamore), and the treatment  $\times$  tree species interaction. Within a tree species, orthogonal contrasts (Bonferroni corrected for 4 comparisons) were used to compare each treatment to the control. For days to first emergence, we analyzed the data using ANOVA including the fixed effects of treatment, tree species and treatment  $\times$  species (Proc GLM, SAS Institute 2000). When the effect of treatment was significant, Tukey HSD (honestly significant difference) tests were used to compare each treatment to the control within a tree species.

#### *Statistical analysis: survival*

Survival data were analyzed with a log-linear model assuming a binomial distribution (alive or dead at the end of 9 weeks) (Proc GENMOD, SAS Institute 2000). Only seedlings that had emerged were included in the analysis of survival data (i.e., survival was conditional on emergence). Fixed effects included treatment, tree species, and treatment  $\times$  tree species. Within a tree species,

orthogonal contrasts (Bonferroni corrected for 4 comparisons) were used to compare each treatment to the control.

#### *Statistical analysis: biomass*

Several measures were obtained to estimate plant growth, including the biomass of leaves, shoots and roots (g), as well as shoot length (cm). All biomass estimates were first analyzed by including them in a MANOVA with the fixed effects of treatment, tree species, and treatment  $\times$  tree species (Proc GLM, SAS Institute 2000). Univariate analyses were examined only when effects were significant in the multivariate analysis, and Tukey HSD tests were used to compare each treatment to the water control.

## **Results**

### *Seedling emergence*

For emergence, all tree species responded similarly to both the autumn olive and the tall fescue treatments. Autumn olive extracts did not affect the probability of seedling emergence for silver maple, eastern cottonwood, or sycamore combined when compared to the sterile water control (Figure 1a; treatment  $\chi^2_{(4)} = 5.11$ ,  $p = 0.28$ ; treatment  $\times$  tree species  $\chi^2_{(8)} = 7.04$ ,  $p = 0.53$ ). However, the tall fescue extract from minced leaves decreased the probability of emergence for all three tree species combined by 19% compared to the control (Figure 1b; treatment  $\chi^2_{(4)} = 11.34$ ,  $p = 0.02$ ; treatment  $\times$  tree species  $\chi^2_{(8)} = 10.27$ ,  $p = 0.25$ ).

For the number of days to emergence, the tree species responded similarly to autumn olive extracts. Minced autumn olive leaf extracts reduced the number of days to emergence for all species combined (treatment  $F_{4,239} = 11.93$ ,  $p < 0.0001$ ; treatment  $\times$  tree species  $F_{8,239} = 1.72$ ,  $p = 0.09$ ; means  $\pm$  SE: Water =  $4.16a \pm 0.26$  ( $n = 55$ ), Fresh =  $3.87a \pm 0.25$  ( $n = 52$ ), Old =  $3.94a \pm 0.27$  ( $n = 49$ ), Soil =  $3.88a \pm 0.27$  ( $n = 49$ ), Minced =  $2.96b \pm 0.20$  ( $n = 49$ ); different letters represent significant differences according to a Tukey HSD test).

In contrast, the tree species diverged in response to the tall fescue treatments for days to emergence.

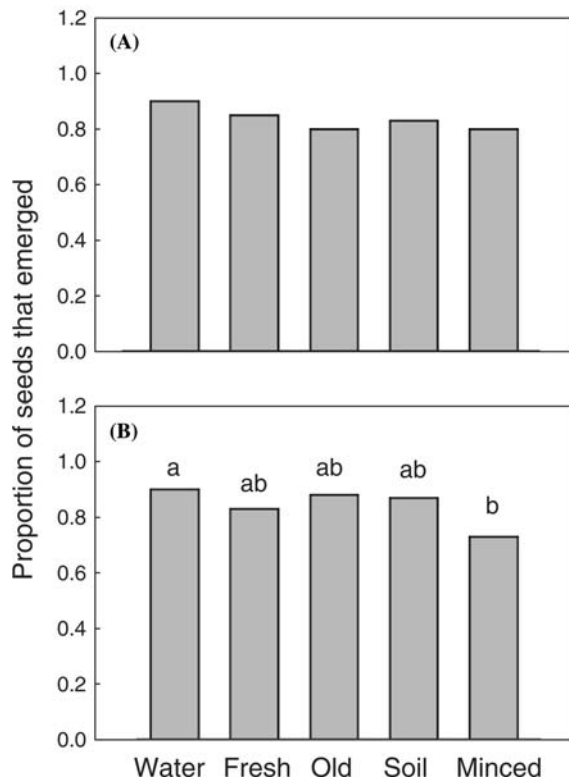


Figure 1. Effects of aqueous extracts from autumn olive (a) and tall fescue (b) on the proportion of seedlings that emerged from the soil. Treatments are Water (sterile water control), Fresh (extract from fresh live leaves), Old (extract from dead leaf litter), Soil (extract from soil), and Minced (extract from minced live leaves). Different letters indicate significant differences among treatments. Sample size = 60 plants per treatment.

The extract from minced leaves of tall fescue had no effect on the number of days to emergence for eastern cottonwood (Figure 2a). However, for sycamore, minced tall fescue reduced the number of days to emergence by 36% compared to sterile water (Figure 2b; treatment  $F_{4,244} = 8.81$ ,  $p < 0.0001$ ; treatment  $\times$  tree species  $F_{8,244} = 3.12$ ,  $p = 0.0022$ ). Similarly, for silver maple minced tall fescue reduced the number of days to seedling emergence by 34% relative to the water control (Figure 2c).

#### Seedling survival

For survival, the tree species responded differently to the invasive species extracts. Extracts from autumn olive increased the survival of eastern

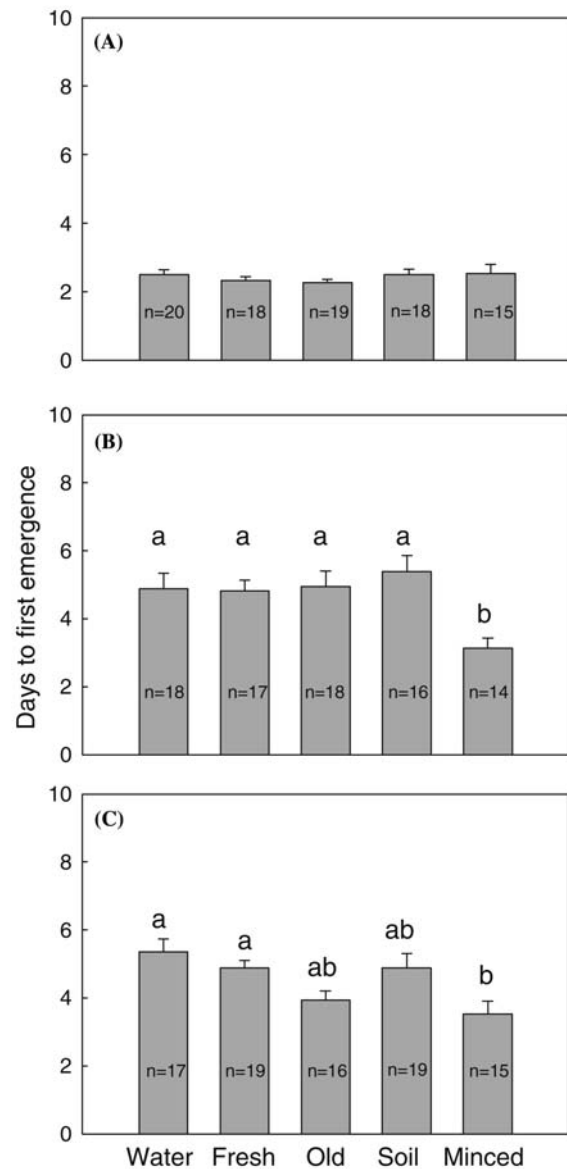


Figure 2. Effects of aqueous extracts from tall fescue on the number of days until the first seedling emerged for (a) Eastern cottonwood, (b) Sycamore, and (c) Silver maple. Treatments are Water (sterile water control), Fresh (extract from fresh live leaves), Old (extract from dead leaf litter), Soil (extract from soil), and Minced (extract from minced live leaves). Bars show means  $\pm$  SE, and different letters indicate significant differences among treatments within a species (Tukey HSD test). Sample size given on bars in graph; some plants did not emerge, reducing sample sizes.

cottonwood seedlings by 66% for minced leaves and by 60% for fresh leaves as compared to the control (Figure 3a; treatment  $\chi^2_{(4)} = 1.80$ ,  $p = 0.77$ ; treatment  $\times$  tree species  $\chi^2_{(8)} = 17.94$ ,

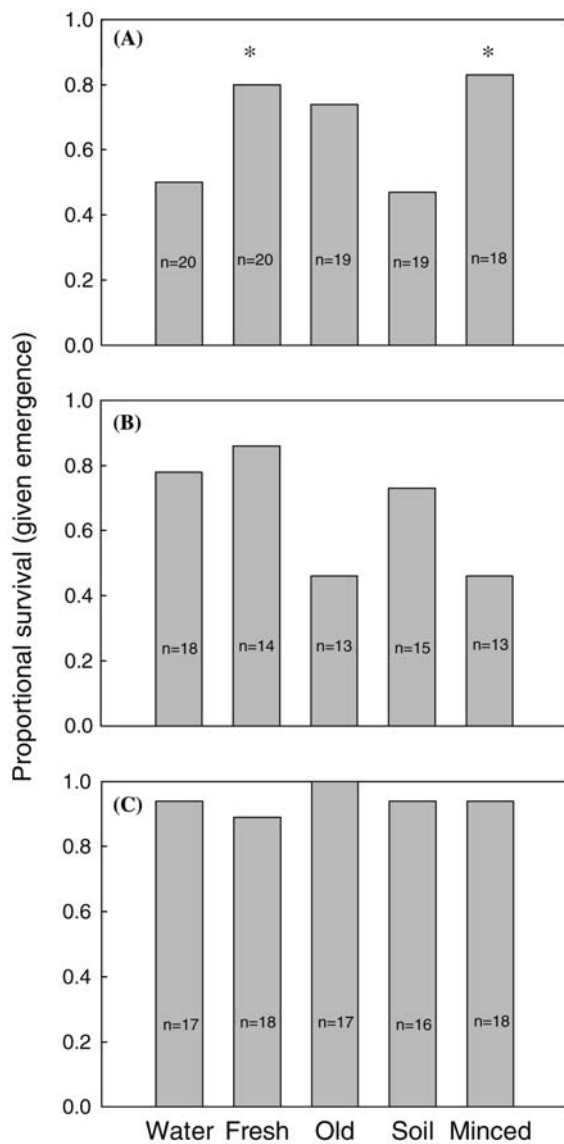


Figure 3. Effects of aqueous extracts from autumn olive on the proportion of seedlings that survived (only for seedlings that emerged) for (a) Eastern cottonwood, (b) Sycamore, and (c) Silver maple. Treatments are Water (sterile water control), Fresh (extract from fresh live leaves), Old (extract from dead leaf litter), Soil (extract from soil), and Minced (extract from minced live leaves). \*Effects on cottonwood were marginally significant following Bonferroni-correction of the  $p$ -values (corrected  $p$  must be  $< 0.0125$  for significance). Fresh vs. water control,  $p = 0.0441$ . Minced vs. water control,  $p = 0.0272$ . Sample size given on bars in graph; some plants did not emerge.

$p = 0.0217$ ). Autumn olive extracts had no significant effects on the survival of sycamore or silver maple (Figure 3b and c). In contrast to autumn olive extracts, tall fescue extracts did not affect

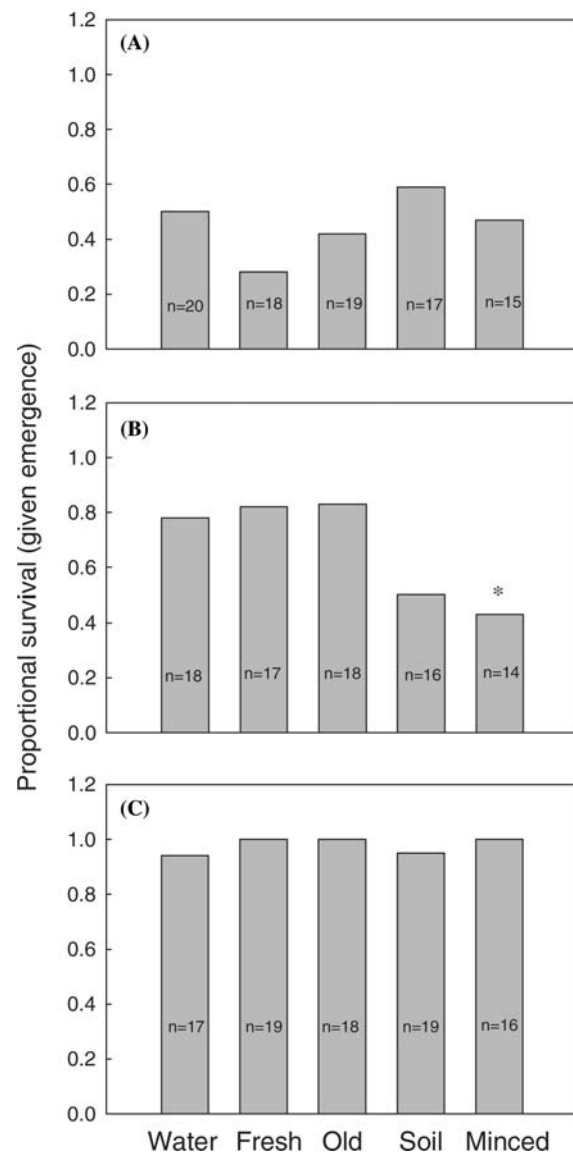


Figure 4. Effects of aqueous extracts from tall fescue on the proportion of seedlings that survived (only for seedlings that emerged) for (a) Eastern cottonwood, (b) Sycamore, and (c) Silver maple. Treatments are Water (sterile water control), Fresh (extract from fresh live leaves), Old (extract from dead leaf litter), Soil (extract from soil), and Minced (extract from minced live leaves). \*Effects on sycamore were marginally significant following Bonferroni-correction of the  $p$ -values (corrected  $p$  must be  $< 0.0125$  for significance). Minced vs. water control,  $p = 0.0417$ . Sample size given on bars in graph; some plants did not emerge.

eastern cottonwood survival (Figure 4a). However, minced extracts from tall fescue reduced the survival of sycamore by 45%, although this effect was only marginally statistically significant after

Bonferroni correction Figure 4b; treatment  $\chi^2_{(4)} = 3.08$ ,  $p = 0.54$ ; treatment  $\times$  tree species  $\chi^2_{(8)} = 15.69$ ,  $p = 0.0470$ ). No significant effect of the treatments was observed for silver maple survival (Figure 4c).

### Seedling biomass

For growth, sycamore responded most strongly to the invasive plant extracts, and most of this response occurred through changes in root biomass. Minced autumn olive significantly reduced the root biomass of sycamore relative to the sterile water control by 35% and relative to extracts from live leaves by 34%, as indicated by a Tukey HSD test (Figure 5a; Table 1). There were no significant effects of autumn olive on other measures of sycamore growth, including leaf mass, shoot mass, and shoot length (Table 1). In addition, extracts from tall fescue leaves significantly affected sycamore when compared to the control: leaf litter extract reduced leaf biomass by 26% (Figure 5b; Table 1), and minced leaf extract reduced root biomass by 35% (Figure 5c; Table 1). Sycamore shoot biomass and shoot length were not affected by tall fescue treatments (Table 1).

Across all species, neither the autumn olive nor the tall fescue treatments affected shoot mass or shoot length (Table 1). For both silver maple and eastern cottonwood, in contrast to sycamore, there were also no significant differences between the treatments and the control for leaf or root biomass (Table 2).

## Discussion

### *Do the exotic species inhibit native tree species?*

Both tall fescue and autumn olive extracts had some inhibitory effects on all three native tree species tested. However, these effects were differential, with the strongest inhibition resulting from tall fescue extracts, and relatively weaker effects mediated by autumn olive. Ultimately, our experimental design cannot determine if these effects are the direct result of allelochemicals, or result indirectly from differences in the microbial associates of soil, leaf, or litter or from differential nutrient levels among the non-sterilized extracts (Llinares

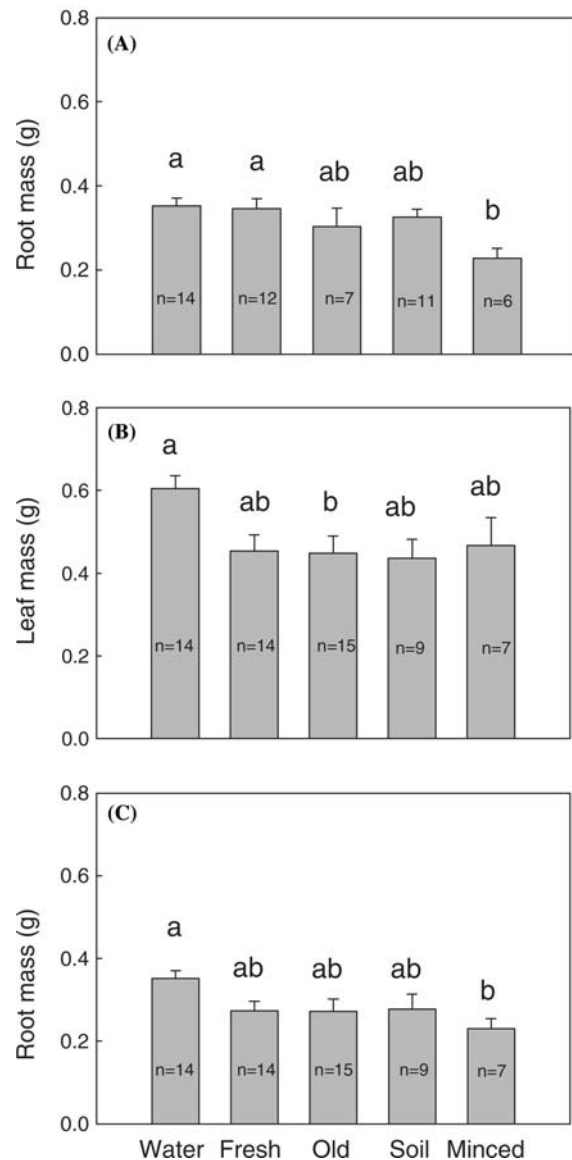


Figure 5. Effects of aqueous extracts on the biomass of sycamore the response of (a) root mass to extracts from autumn olive, (b) leaf mass to extracts from tall fescue, and (c) root mass to extracts from tall fescue. Treatments are Water (sterile water control), Fresh (extract from fresh live leaves), Old (extract from dead leaf litter), Soil (extract from soil), and Minced (extract from minced live leaves). Bars show means  $\pm$  SE, and different letters indicate significant differences among treatments within a species (Tukey HSD test). Sample size given on bars in graph; some plants did not survive until the end of the experiment.

et al. 1993; Souto et al. 2000; Inderjit and Weiner 2001). However, because any microbial or nutrient effects of our treatments would be similar to

Table 1. Statistical results from MANOVA and ANOVA examining the effects of the extract treatment, the tree species, and the treatment × tree species interaction on leaf mass, shoot mass, shoot length, and root mass.

Effect	MANOVA				ANOVA		Leaf mass (g)		Shoot mass (g)		Shoot length (cm)		Root mass (g)	
	Pillai	df	F	p	df	F	p	F	p	F	p	F	p	
<i>Autumn olive</i>														
Treatment	0.10	16,732	1.20	0.2612	4,183	1.43	0.2254	0.85	0.4937	0.19	0.9425	1.88	0.1165	
Tree	1.37	8,362	97.30	<0.0001	2,183	194.03	<0.0001	339.49	<0.0001	85.97	<0.0001	122.93	<0.0001	
Treatment × tree	0.25	32,732	1.51	<b>0.0367</b>	8,183	1.59	0.1315	1.04	0.4043	1.68	0.1048	2.84	<b>0.0053</b>	
<i>Tall fescue</i>														
Treatment	0.13	16,704	1.51	0.0906	4,176	0.99	0.4141	2.22	0.0688	0.47	0.7577	0.38	0.8233	
Tree	1.22	8,348	67.76	<0.0001	2,176	151.19	<0.0001	264.12	<0.0001	105.94	<0.0001	119.80	<0.0001	
Treatment × tree	0.35	32,704	2.14	<b>0.0003</b>	8,176	2.12	<b>0.0363</b>	0.48	0.8665	1.50	0.1587	3.93	<b>0.0003</b>	

p-values < 0.05 are shown in bold face. 'Pillai' is Pillai's Trace test statistic for MANOVA.

conditions experienced by plants in the field, our experiments provided more natural conditions to test for allelopathic potential, broadly defined (Inderjit 2001). In addition, the lack of any inhibitory effects of our treatments does not exclude the possibility for allelopathy during other times of year, with more concentrated extracts, or with a different decay rate of the plant tissues collected, although both invasive species can grow and shed leaves throughout the year in Indiana, especially during mild winters. Our extract methods were largely an exploratory first step, and other experimental methods, such as the use of activated carbon, could be used to further investigate allelopathic effects. Experiments are underway to determine if inhibition (or stimulation) is a direct consequence of allelopathy or an indirect effect of changes in nutrients and/or microbial activity.

An important next step in this system would be to test for allelopathic effects of the invasive plants on tree species that co-occur with tall fescue or autumn olive in their native habitats. A comparison of the relative strength of inhibitory effects on trees in the introduced vs. native environments would provide a clear test of the novel weapons hypothesis: that the success achieved by exotic plants results from an ecological advantage afforded by plant traits that are novel to the recipient community.

#### *Does inhibition depend upon the source of the aqueous extracts?*

Our results suggest that extracts obtained from tall fescue and autumn olive may have allelopathic

potential, but the source of the putative compounds largely determines these effects (see also Fuerst and Putnam 1983). For contrast with the three 'naturally' derived extracts (soil, leaf litter, and live leaves), a fourth treatment utilized minced live leaves to approximate the methodology of many earlier studies that have reported allelopathic inhibition (Inderjit and Weston 2000). The strongest effects were exhibited by these minced leaf extracts. For example, minced tall fescue extracts decreased emergence rates of all native species, as well as reducing seedling survival (marginally) and decreasing root biomass in sycamore. Although the effect of minced leaf extract was mostly negative (reduced survival and growth), minced tall fescue also reduced the number of days to emergence in both silver maple and sycamore. Similar stimulatory effects were found for the extracts of minced, live leaves of autumn olive, which reduced the number of days to emergence for all species and increased (marginally) the survival of eastern cottonwood. These effects are positive because emergence rates and survival were enhanced.

There are several explanations for stimulated emergence times, including a fertilizing effect due to nutrient release from damaged or decayed tissue (particularly from the nitrogen-fixing *Elaeagnus*; Simons and Seastedt 1999), a release of hormones such as gibberellin that may stimulate germination (Ritchie and Gilroy 1998; Brady and McCourt 2003), or a release of toxic compounds that could break down the seed coat (Cohn 1996). Seeds may also respond to the extracts as an environmental signal to induce early germination (Preston and Baldwin 1999), perhaps signaling the presence of

Table 2. Means  $\pm$  SE (sample size) for leaf and root biomass of eastern cottonwood and silver maple in response to both autumn olive and tall fescue extracts.

	Eastern cottonwood				Silver maple			
	Autumn olive extracts		Tall fescue extracts		Autumn olive extracts		Tall fescue extracts	
	Leaf	Root	Leaf	Root	Leaf	Root	Leaf	Root
Water	0.32 $\pm$ 0.02 (10)	0.20 $\pm$ 0.02 (10)	0.32 $\pm$ 0.02 (10)	0.20 $\pm$ 0.02 (10)	0.69 $\pm$ 0.03 (18)	0.38 $\pm$ 0.01 (18)	0.69 $\pm$ 0.03 (18)	0.38 $\pm$ 0.01 (18)
Fresh	0.35 $\pm$ 0.01 (16)	0.24 $\pm$ 0.02 (16)	0.32 $\pm$ 0.02 (5)	0.23 $\pm$ 0.02 (5)	0.70 $\pm$ 0.02 (16)	0.42 $\pm$ 0.02 (16)	0.71 $\pm$ 0.02 (20)	0.39 $\pm$ 0.02 (20)
Old	0.39 $\pm$ 0.01 (14)	0.26 $\pm$ 0.01 (14)	0.32 $\pm$ 0.02 (8)	0.20 $\pm$ 0.02 (8)	0.69 $\pm$ 0.03 (17)	0.42 $\pm$ 0.02 (17)	0.72 $\pm$ 0.02 (18)	0.45 $\pm$ 0.01 (18)
Soil	0.30 $\pm$ 0.03 (9)	0.22 $\pm$ 0.03 (9)	0.35 $\pm$ 0.02 (11)	0.18 $\pm$ 0.02 (11)	0.69 $\pm$ 0.01 (16)	0.39 $\pm$ 0.02 (16)	0.70 $\pm$ 0.02 (18)	0.43 $\pm$ 0.02 (18)
Mined	0.36 $\pm$ 0.01 (15)	0.24 $\pm$ 0.01 (15)	0.33 $\pm$ 0.02 (8)	0.27 $\pm$ 0.03 (8)	0.62 $\pm$ 0.03 (17)	0.41 $\pm$ 0.01 (17)	0.67 $\pm$ 0.03 (16)	0.42 $\pm$ 0.02 (16)

Treatments are Water (sterile water control, the same control plants were used for autumn olive and for tall fescue), Fresh (extract from fresh live leaves), Old (extract from dead leaf litter), Soil (extract from soil), and Mined (extract from mined live leaves). There were no significant differences among treatments within a species and invader extract type. Unequal sample sizes are due to differential emergence and survival among treatments.

competition from other species. However, the presence and concentrations of such compounds from minced leaf tissue are unlikely to occur in nature unless plants commonly encounter damaged plant tissues (e.g., from vole, insect, cattle, or other herbivory), which could occur, especially for heavily grazed tall fescue (Ball et al. 1993).

In contrast to minced live leaves, extracts that were potentially more natural did not have very strong effects on seedlings. Of the two exotic species, only tall fescue leaf litter had a significant inhibitory effect, and only on sycamore leaf biomass. Therefore, more ecologically realistic methodology suggests that allelopathic potential may not be as prevalent as has been reported for tall fescue (Peters and Zam 1981; Luu et al. 1982; Peters and Luu 1985; Preece et al. 1991), and possibly for other species as well.

#### *Are tree species differentially inhibited?*

Differential response of the three tree species to potential allelopathic interference may have important consequences for forest community composition and succession over time. Specifically, sycamore was affected most strongly by the treatments, with declines in survival as well as reductions in both root and leaf biomass. Eastern cottonwood appeared largely insensitive to the extracts, with the exception that the probability of emergence for all three tree species was reduced by minced live leaves of tall fescue. Furthermore, survival of eastern cottonwood was marginally enhanced by extracts from fresh leaves and minced leaves of autumn olive. These results suggest that some species (e.g., eastern cottonwood) may gain a competitive advantage when colonizing sites with one or both of the exotic plants, whereas other species (e.g., sycamore) may be suppressed.

#### *At what life history stage are target species most affected?*

In general, the strongest inhibition of native trees occurred during the germination and emergence stage. Specifically, minced tall fescue leaves significantly reduced the probability of emergence for all three native tree species by 20%. Effects during later life stages were more specific to each target

species and weaker than effects observed at early life stages. These results suggest that the strongest allelopathic inhibition by exotics may occur during germinating and early establishment. Again, the use of extracts is only one of several ways to explore potential allelopathic effects, and other techniques (e.g., activated carbon) would be useful for assessing allelopathy at different life history stages.

There was one exception to this general pattern. For sycamore, the species most sensitive to the extracts, leaf biomass declined with the application of old leaf litter extract, relative to the control. In addition, root biomass declined with the application of minced live leaves of both exotic species and overall survival was marginally reduced by minced tall fescue. Although it remains unclear whether these differences in biomass (26% and 35% reductions, respectively) would translate into reduced competitive ability, the 45% reduction in survival could have an impact on the relative abundance of sycamore in the plant community. Thus, for sycamore, the potentially allelopathic effects of exotic species were not limited to early life history stages.

### Conclusion

Few studies have investigated the impact of allelopathy on community composition and succession under ecologically relevant conditions (Callaway and Aschehoug 2000). Furthermore, we are just beginning to understand the importance of allelopathy in driving the impacts of invasive plant species (Bais et al. 2003). In our study, the smaller response of the three native tree species to ecologically realistic extracts as compared to mechanically minced leaves suggests that allelopathic potential may be overstated in some laboratory studies utilizing macerated, minced, or ground donor materials (see also, Stowe 1979). However, the differential impact of potentially allelopathic extracts on tree species (primarily the reduced performance of sycamore vs. the enhanced performance of eastern cottonwood) strongly supports the hypothesis that invasive plant species can differentially inhibit native trees through allelopathic mechanisms. Importantly, exotic plants may influence plant community dynamics by shifting the relative abundance of native plant species.

### Acknowledgements

We thank C. Burnett and P. James for helping with data collection and J. Lemons for greenhouse support. This work was supported by NSF DBI-0200485 to J.A.R., NSF DEB-9727116 to K.C., and the Howard Hughes Medical Institute Capstone Research Award to S.O. through Indiana University. Thanks to two anonymous reviewers for helpful revisions to the manuscript.

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