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Does Evolutionary Exposure Mediate Allelopathic Effects?

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Does Evolutionary Exposure Mediate Allelopathic Effects?

(TITLE)

BY

Nikki Leigh Pisula

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ABSTRACT

Allelopathy most commonly refers to chemically mediated plant interference, though the effects may range from beneficial to inhibitory. Allelopathy has been suggested as a potential mechanism for invasion success because invasive species may produce allelochemicals that are evolutionarily novel to recipient communities. To test for evolutionary mediation of allelopathy, six target species were grown in a factorial experiment with and without activated carbon or a native allelopathic species, *Solidago canadensis* (Canada goldenrod). I used activated carbon to remove allelochemicals to separate allelopathic effects from resource competition. The species represented pairs of evolutionary experienced (native - N) and evolutionarily naïve (non-native - NN) species within plant life forms: C3 grasses - *Danthonia spicata* (N) vs. *Poa pratensis* (NN), herbaceous perennials - *Eupatorium rugosum* (N) vs. *Chrysanthemum leucanthemum* (NN) and herbaceous biennials - *Aster pilosus* (N) vs. *Daucus carota* (NN). If evolutionary exposure to allelochemicals is important, I would expect non-native species to respond more to *S. canadensis* allelochemicals than native species. Generally, there were growth differences among treatments for all target species. Activated carbon significantly increased aboveground biomass for *Danthonia spicata* and *P. pratensis* in the absence of *S. canadensis*. However, in combination with *S. canadensis*, activated carbon reduced growth in half of the species. This result was opposite of my predictions as activated carbon was anticipated to reduce allelopathic activity contributed by *S. canadensis* and improve growth in susceptible plants. There was no clear pattern between natives and non-natives in their responses to experimental treatments. This pattern was confirmed by the germination responses of each target species to *S.*

canadensis extracts, where there was no systematic difference between native and non-native species. These results suggest that using activated carbon to tease apart competitive outcomes may not be an appropriate method for allelopathy studies as it may induce unintended effects. This study finds no support for evolutionary exposure playing a role in mediating allelopathic effects in this system.

DEDICATION

I would like to dedicate this manuscript to my departed grandfather, Carl "Red" Nussmeyer. He was a great man who looked after his family and he is sorely missed.

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I would like to thank my mentor and advisor, Dr. Scott Meiners for his encouragement and support for the last two years. I would also like to thank the members of my committee, Dr. Barbara Carlsward and Dr. Andrew Methven for their constructive criticism on this manuscript.

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INTRODUCTION

Allelopathy most commonly refers to chemically mediated plant interference, though the effects may range from beneficial to inhibitory (Inderjit and del Moral 1997). Allelopathic plants interfere with their neighbors directly and indirectly by dispersing chemicals derived from secondary metabolites into the soil, which generally affect neighboring plant growth in an inhibitory way. Allelochemicals are primarily dispersed via leachates from live or dead roots or leaves or emitted as volatiles (Jackson and Willemsen 1976). Theoretically, this strategy results in more available resources for the allelopathic plant, offsetting the cost of allelochemical production (Inderjit and del Moral 1997).

As many species of plants produce allelochemicals (Muller 1966), allelopathy has the capability of impacting plant communities by influencing plant distributions (Putnam and Duke 1978, Mahall and Callaway 1992, Nilsson and Zackrisson 1992, Lau et al. 2008). Despite this potential, many ecologists focus on interactions at the species level and do not place allelopathy in a community context. Pisula and Meiners (2010a) tested six species of goldenrod and linked their allelopathic potential to succession. They found that goldenrods did have allelopathic potential; however, this strategy did not impact community turnover (Pisula and Meiners 2010a). Studies have also proposed that allelopathy may contribute to the ability of non-native plant species to become dominant in their new communities (Fletcher and Renney 1963, Abdul-Wahab and Rice 1967, El-Ghareeb 1991, Ridenour and Callaway 2001, Hierro and Callaway 2003). Overall, the importance and function of allelopathy in plant communities is still in debate.

An allelopathic plant may also inhibit itself by autoallelopathy (Picman and Picman 1984, Kumari and Kohli 1987). This autoinhibition may provide beneficial effects for the plant by reducing the establishment of intraspecific competitors in dense populations (Schenck et al. 1999, Perry et al. 2005) or by delaying germination in seeds when intraspecific competition is intense (Picman and Picman 1984, Dyer 2004, Perry et al. 2005). These sorts of shifts in recruitment can have major impacts on plant communities (Warner and Chesson 1985, Perry et al. 2005).

Though allelopathy has mostly been discussed within a population and community context, allelopathy may have impacts on processes other than inhibition of neighboring plants. Secondary metabolites that have allelopathic activity may also function as herbivore defensive chemicals or affect litter decomposition and nutrient mineralization rates (Wardle et al. 1998). This sets the potential for allelopathy to regulate ecosystem functioning at a much broader scale.

Though individual species are often thought of as being allelopathic, there is some evidence that allelopathy may be environmentally mediated. Inderjit and del Moral (1997) suggested that in crop systems, shade may increase allelochemical production. Similarly, Ladwig et al. (unpublished data) found that several species of lianas were twice as toxic when grown in the shade compared to individuals growing in full sun. Changes in nutrient dynamics may also impact toxicity of allelochemicals (Inderjit and del Moral 1997). Kong et al. (2002) found that *Ageratum conyzoides* inhibited several co-occurring species in nutrient limited conditions and that herbivore damage and fungal infection reduced allelopathic activity of plant tissues. Secondary metabolite production often increases as a result of plant stress (Grime 1977, McKey et al. 1978, Gershenzon

1984, Aerts and Chapin 2000, Hierro and Callaway 2003). Likewise, the nutrient status of a plant may mitigate its response to allelopathic chemicals (Fischer et al. 1978, Inderjit and del Moral 1997). In general, physiological stress appears to alter allelopathic interactions.

Methodological approaches to allelopathy

The potential effect of allelochemicals on associated plants is most often tested through bioassays, where seeds are challenged with plant tissue extracts from potentially allelopathic species (Inderjit and Dakshini 1995). Although they allow for a consistent measure of allelopathy, laboratory bioassays can only determine the *potential* for a plant to be allelopathic. Laboratory bioassays do not mimic the environmental and biological variability found in the field, where it is often difficult to identify allelopathic interactions. Allelochemicals may be degraded or enhanced by microbial processes in the soil (Inderjit and Dakshini 1995), and their impacts are mitigated by precipitation, temperature, and the presence of fungal pathogens (Stinson et al. 2006, Callaway et al. 2008). However, laboratory bioassays are a useful first step to screen species for allelopathic potential and to determine whether further studies are warranted (Pisula and Meiners 2010a, 2010b). Allelopathy studies have been criticized for the difficulty of separating allelopathic interactions from direct resource competition (Inderjit and del Moral 1997). As allelopathy is a competitive strategy (Grime 2001), it would be expected to be associated with species that occur in communities that are heavily structured by competition.

To address the limited ability to separate allelopathy from resource competition, activated carbon has been used to neutralize allelopathic effects in field and pot experiments. Activated carbon binds with the organic compounds released by plants, removing the effects of allelochemicals while leaving root competition intact. The most well-known use of activated carbon to isolate allelopathic effects was conducted by Nilsson (1994), who found that both resource competition and allelopathy played a role of *Empetrum hermaphroditum* suppressing *Pinus sylvestris* in a large scale field study. Since then, several studies have used activated carbon to separate the effects of resource competition from allelopathy (Ridenour and Callaway 2001, Prati and Bossdorf 2004, Callaway et al. 2005, Abhilasha et al. 2008, Gómez-Aparicio and Canham 2008). More recently, some researchers have reported methodological problems with activated carbon directly affecting plant growth beyond removal of allelochemicals (Lau et al. 2008, Weißhuhn and Prati 2009). Despite these studies, the use of activated carbon to separate resource competition from allelopathic interactions has renewed interest in allelopathic studies.

Allelopathy and species invasions

Invasive non-native species threaten native plant communities by out-competing and displacing native species. One of the most cited mechanisms for the success of invasive non-natives is the escape of the natural enemies that keep plants in check, enabling them to compete in recipient communities where native species remain limited by their enemies. This phenomenon is known as the natural enemies' hypothesis (Blossey and Notzold 1995, Keane and Crawley 2002, Hierro and Callaway 2003,

Colautti et al. 2004). Due to the failure of biological controls for some species, the natural enemies' hypothesis has not accounted for invasive success in its entirety (Hierro and Callaway 2003). Because of the expansive range of invasives and their tendency to establish monocultures, allelopathy has often been invoked as an invasive plant strategy (Hierro and Callaway 2003, Inderjit et al. 2008).

Three approaches have been used to study allelopathy in the context of plant invasion (Inderjit et al. 2008). The traditional approach assesses the allelopathic potential of non-native species individually, without placing them in an ecological context (Inderjit et al. 2008). The congeneric approach, in which natives and non-natives of the same genus are studied (Inderjit et al. 2008) specifically test whether invasives are more toxic while controlling for phylogenetic constraints. Third, ecologists are beginning to test allelopathy from a biogeographical approach by comparing the allelopathic potential of a plant species from within its native and recipient communities (Abhilasha et al. 2008, Inderjit et al. 2008, Thorpe et al. 2009). If an invader has stronger allelopathic impacts within a recipient community than its native community, biological communities may be more coevolved and functionally organized than previously believed (Goodnight 1990, Wilson 1992, Thompson 1999, Callaway et al. 2005).

Invasive species may also be more successful in their introduced ranges because of the greater susceptibility of the recipient community to allelochemicals. Non-natives may bring unique species-specific biochemicals into the recipient community, a phenomenon known as the novel weapon hypothesis (Callaway et al. 2005). The novel weapons hypothesis is defined as the success of a non-native plant due to its allelochemicals or other defenses for which the recipient community has little resistance

(Callaway and Aschehoug 2000, Callaway and Ridenour 2004, Callaway et al. 2005, Abhilasha et al. 2008). Species that have evolved in association with an allelopathic species would have had the time to adapt to the specific allelochemicals and therefore buffer the effect of allelopathy. Naïve species newly subjected to these unknown allelochemicals have not yet developed resistance. In this case, the non-native species are essentially escaping from co-evolved competitors.

The obvious advantage of having an evolutionary history with an allelopathic plant, is time to evolve resistance to the allelochemicals produced (Callaway et al. 2005). In general, it has been shown that plants can evolve rapidly to human-made chemicals and adapt to neighboring chemical composition (Powles and Holtum 1994, Ehlers and Thompson 2004, Callaway et al. 2005). If plants can evolve tolerance to allelochemicals over time, then biotic resistance may be providing some balance within natural communities.

Thesis objective

Based on ecological theory, I hypothesized that naïve species, those without evolutionary exposure to an allelochemical, should be more affected by an allelopathic agent than plants that have coexisted over evolutionary time. My primary question is whether evolutionary exposure mediates allelopathic effects. In order to address this question, I tested six native and non-native target species against a native allelopathic plant, *Solidago canadensis* (Canada goldenrod). *Solidago canadensis* is invasive in Europe, and its potential for allelopathy has been cited as a potential mechanism for this success (Abrahamson et al. 2005, Bing-yao et al. 2006, Abhilasha et al. 2008). Non-

native species within North America should be naïve to the allelochemicals produced by *S. canadensis* and should be more inhibited by the chemicals than native species, which have evolved with those chemicals. To separate allelopathic effects from direct resource competition, I used activated carbon to remove allelochemicals. If allelopathy is present, then activated carbon is expected to minimize the effect of the allelopathic chemicals. In theory, the contribution of allelopathy to competition should be considerably greater for the evolutionarily naïve, non-native species.

METHODS

Study species

To assess whether evolutionary history affected species responses to allelopathic agents, I challenged six native and non-native target species with a native allelopathic species. The target species studied were natives, *Aster pilosus* Willd., *Danthonia spicata* (L.) F. Beauv. and *Eupatorium rugosum* Houttuyn. The non-native target species were *Daucus carota* L., *Poa pratensis* L. and *Chrysanthemum leucanthemum* L., all native of Eurasia (Gleason and Cronquist 1991). *Aster pilosus* and *C. leucanthemum* spread vegetatively by rhizomes (Rhoads and Block 2000). All of the species are common on roadsides and in old fields in the United States (Rhoads and Block 2000). Species were selected to represent native and non-native (evolutionary experienced vs. evolutionarily naïve) pairs of species within plant life forms (grasses, herbaceous perennials and herbaceous biennials) in a manipulative pot experiment.

These target species were challenged with a native allelopathic plant species, *Solidago canadensis* L. (Canada goldenrod), which has become invasive in Europe and China (Abrahamson et al. 2005, Abhilasha et al. 2008). *Solidago canadensis* is a native herbaceous perennial commonly found in meadows, prairies, roadsides and abandoned agricultural land in eastern North America. It is a self-incompatible species that produces numerous wind-dispersed seeds which germinate easily on a wide range of soils (Weber 2001). This species was chosen as the allelopathic agent, as it has demonstrated allelopathic effects in laboratory bioassays (Fisher et al. 1978, Butcko and Jensen 2002, Bing-yao et al. 2006, Abhilasha et al. 2008, Pisula and Meiners 2010a) and its invasive nature as a non-native in Europe (Abrahamson et al. 2005, Abhilasha et al. 2008).

Pot experiment

The six target species were grown in a 2 x 2 factorial experiment, with and without activated carbon (AC) or *S. canadensis* to determine competitive effects and the effects of allelochemical contribution. Each treatment was replicated 10 times for each target species. The target species compared were: C3 grasses - *Danthonia spicata* (N - native) vs. *P. pratensis* (NN - non-native), herbaceous perennials - *E. rugosum* (N) *vs. C. leucanthemum* (NN) and herbaceous biennials - *A. pilosus* (N) vs. *Daucus carota* (NN). Overall, there were 40 pots for each native or non-native test plant (N = 240).

Activated carbon was used in the experiment to neutralize the effects of the allelochemicals. Theoretically, the presence of *S. canadensis* and the activated carbon treatment was predicted to yield an increase in biomass of target species compared to the *S. canadensis* treatment alone as allelochemicals remain in the soil. Fifteen grams of activated carbon (AC finely ground, Fisher Scientific, Pittsburgh, PA, C272-212) were mixed in each designated pot (6″ diameter, 1.67 L), which contained a peat-based growing medium with vermiculite and perlite (Pro-Mix BX, Hummert, Topeka, Kansas), following the methods of Abhilasha et al. (2008). *Aster pilosus*, *E. rugosum* and *Danthonia spicata* seeds were bought from Prairie Moon Nursery (Winona, MN). *Chrysanthemum leucanthemum* and *Daucus carota* seeds were bought from American Meadows (Williston, VT), and *P. pratensis* seeds were bought at a local store (Rural King, Charleston, IL). The herbaceous perennials and biennials were cold-moist stratified and stored in a refrigerator at 33-38°C for 60 days. Grasses did not require stratification.

Whole *S. canadensis* plants were collected from Douglas-Hart Nature Center (Mattoon, IL) and transplanted to prepared pots. Only *S. canadensis* with rhizomes of at least 8 cm in length were planted. To maintain constant plant size, if rhizomes were longer than 8 cm they were trimmed to 8 cm. Seeds of each target species were grown in trays for 15 days (22 days for *A. pilosus* and *Daucus carota* because they were slow to emerge) before transplanting into their respective treatments. Three seedlings were transplanted into each pot. All pots were top dressed with 5 ml of 14-14-14 Osmocote Smart-Release Plant Food at first signs of chlorosis, as activated carbon has been linked to decreases in nitrogen availability (Lau et al. 2008). After a growing period of 65 days in the Eastern Illinois University greenhouse courtyard, grasses and herbaceous perennials (and their respective *S. canadensis*) were harvested. Herbaceous biennials were harvested after 40 days as they grew much more quickly. Aboveground biomass was dried at 60° C and weighed. *Solidago canadensis* above and belowground (rhizome) biomass was dried and weighed.

The relative interaction intensity (RII) was calculated for aboveground biomass (Armas et al. 2004). RII has been used in other studies to indicate the strength of interaction (Williamson and Richardson 1988, Armas et al. 2004, Gao et al. 2009). The utility of RII is the comparison of treatment values relative to the control. It transforms the data to fit in a scale ranging from -1 to 1. Positive values indicate an increase in interaction intensity relative to the control, while negative values indicate a decrease in interaction intensity relative to the control.

$$RII = \frac{[Treatment - Control]}{[Treatment + Control]}$$

Individual analysis of variances (ANOVAS) were performed for each species to evaluate competition as a function of activated carbon and *S. canadensis* (SAS 9.1; SAS Institute Inc., Cary, NC). These data were also transformed using RII to determine competitive outcomes. The effect of activated carbon on aboveground, belowground and total biomass was tested with an ANOVA.

Effects of activated carbon on S. canadensis allelopathy

Laboratory bioassays determined whether activated carbon affected allelochemical production directly. *Solidago canadensis* tissues were tested with laboratory bioassays following Butcko and Jensen (2002) as modified by Pisula and Meiners (2010a). Leaf and rhizome tissue from *S. canadensis* in each treatment (with and without activated carbon) were collected and dried at 60 ° C for two days. Extracts were made from 12.5 g of dried tissue placed in 500 ml of distilled water. Mixtures were placed on a magnetic stirrer for 24 hours at room temperature and strained through cheesecloth to remove particulate plant material. Dilutions of each extract, ranging from 0% to 100% in 10% increments, were made. Filter paper was placed in 90 mm petri plates with 20 seeds of the indicator species (*Raphanus sativus* L., radish). I used this species to indicate allelopathic potential because it germinates quickly, is commonly used in allelopathic studies, is sensitive to allelopathic inhibition, and detects differences among species' extracts (Butcko and Jensen 2002, Pisula and Meiners 2010a, 2010b). Eight trials were run for each dilution. Four ml of extract were added to each plate and incubated at 25 °C for a 12/12 h light/dark cycle. Petri plates were placed in bags to

retain moisture. Plates were removed after four days and germinated seeds were counted. An analysis of covariance (ANCOVA) was used to measure the overall effect of plant extract concentration, activated carbon and species identity on germination. Individual logistic regressions of percentage germination as a function of extract concentration, activated carbon and species identity were conducted for each species. Coefficients (β values) from these regressions were used to compare the relative strength of plant extracts on seed germination.

Germination response of target species to S. canadensis

Seeds of the target species were tested via the aforementioned methods using *S. canadensis* leaf tissue as the source of allelochemicals. The seeds were removed from incubation after most germination had occurred (*A. pilosus* and *E. rugosum* 4 days, *Danthonia spicata* 14 days, *C. leucanthemum* 8 days, *Daucus carota* 9 days and *P. pratensis* 6 days). The same statistical analysis was conducted as described for the effects of activated carbon on *S. canadensis* allelopathy.

RESULTS

Pot Experiment

Within the pot experiment, all species were influenced by *S. canadensis*, except *E. rugosum*, which had no significant effects (Table 1; Figure 1). The aboveground biomass of all other species was reduced by the presence of *S. canadensis*. Activated carbon also had some direct influences on the growth of target species. Growth of three species, *A. pilosus, C. leucanthemum* and *E. rugosum,* were unaffected by the presence of activated carbon. In contrast, activated carbon had a significant positive impact on both grass species (Table 1). Three species, *Daucus carota, Danthonia spicata* and *P. pratensis,* also had significant interactions between *S. canadensis* and activated carbon. When grown in combination with *S. canadensis*, activated carbon reduced growth in *Daucus carota* and *P. pratensis*, while activated carbon alone increased aboveground biomass. In *Danthonia spicata*, the beneficial effect of activated carbon in isolation was greatly diminished in the presence of *S. canadensis*. These results were opposite the prediction that activated carbon would reduce allelopathic activity contributed by *S. canadensis* and improve growth in susceptible plants. The only species to follow this prediction was *Danthonia spicata*. There was no clear difference between natives and non-natives in their responses to experimental treatments (Figure 1).

Activated carbon also had direct effects on the growth and toxicity of *S. canadensis*. The addition of activated carbon increased aboveground biomass of *S. canadensis* but did not affect belowground biomass (Table 2; Figure 2). When compared to the effects of activated carbon on target plant growth, the effects of activated carbon on *S. canadensis* growth were much smaller. In laboratory bioassays of *S. canadensis* tissue,

there were effects of extract concentration, *S. canadensis* tissues (rhizome vs. leaves) and interactions among extract concentration, tissue type, and activated carbon (Table 3). When analyzed as individual logistic regressions, these effects became clearer. Rhizome tissue inhibited germination less than leaf tissue (Table 4, Figure 3). The allelopathic activity of leaf tissue was unaffected by activated carbon. In contrast, activated carbon increased the toxicity of rhizomes and markedly reduced germination within the bioassays.

Germination responses of target species to S. canadensis

Laboratory bioassays that tested the toxicity of *S. canadensis* on the germination of the six target species found large differences among species (Table 5). Individual logistic regressions quantified the effect of *S. canadensis* extract on the germination of each target species (Table 6, Figure 4). The most inhibited target species were the native *Danthonia spicata* and *P. pratensis* (β = -0.0319 and β = -0.0611 respectively, Table 6). The least affected species were the native *A. pilosus* and the non-native *C. leucanthemum* (β = -0.0112 and β = -0.0059 respectively, Table 6). Overall, there was no association between evolutionary history and allelopathic response, as species varied widely in germination response in both native and non-native taxa. There also was no association between laboratory bioassay results and the pot experiment, as responsive species in one experiment were often much less responsive in the other.

TABLE 1—Individual ANOVAs of the influence of activated carbon and *Solidago canadensis* on aboveground biomass for each target species. Bolding indicates a significant *P*-value.

Factor	df	MS	F	R^2	*P*-value
Danthonia spicata					
AC	1	1.09	20.19	0.73	**<0.0001**
S. canadensis	1	3.66	67.73		**<0.0001**
AC × *S. canadensis*	1	0.55	10.25		**0.0029**
Error	36				
Poa pratensis					
AC	1	0.33	15.22	0.91	**0.0004**
S. canadensis	1	6.76	308.43		**<0.0001**
AC × *S. canadensis*	1	1.31	59.83		**<0.0001**
Error	36				
Aster pilosus					
AC	1	0.01	0.20	0.70	0.6608
S. canadensis	1	4.27	78.34		**<0.0001**
AC × *S. canadensis*	1	0.18	3.34		0.0766
Error	34				
Daucus carota					
AC	1	0.03	0.51	0.61	0.4818
S. canadensis	1	2.75	48.90		**<0.0001**
AC × *S. canadensis*	1	0.39	6.99		**0.0122**
Error	35				
Eupatorium rugosum					
AC	1	144.56	2.70	0.19	0.1111
S. canadensis	1	195.37	3.65		0.0660
AC × *S. canadensis*	1	148.46	2.77		0.1066
Error	29				
Chrysanthemum leucanthemum					
AC	1	0.25	3.25	0.52	0.0801
S. canadensis	1	2.62	33.94		**<0.0001**
AC × *S. canadensis*	1	0.04	0.56		0.4582
Error	34				

TABLE 2—Individual ANOVAs showing the effects of activated carbon on aboveground and belowground biomass of *Solidago canadensis*. Bolding indicates a significant *P*-value.

Factor	df	MS	F	R^2	*P*-value
Aboveground					
AC	1	0.157	4.16	0.0346	**0.0436**
Error	116				
Belowground					
AC	1	0.004	0.07	0.0006	0.7870
Error	116				

TABLE 3— Effects of *Solidago canadensis* tissue (categorical), extract concentration (continuous variable) and activated carbon (categorical variable). Overall ANCOVA $F_{7, 344} = 142.38$; $P < 0.0001$; $R^2 = 0.74$. Bolding indicates a significant *P*-value.

Factor	df	MS	F	*P*-value
AC	1	36.52	0.25	0.6207
Concentration	1	119751.17	804.49	**< 0.0001**
Concentration × AC	1	2219.74	14.91	**< 0.0001**
Tissue	1	925.65	6.22	**0.0131**
AC × Tissue	1	106.43	0.71	0.3984
Concentration × Tissue	1	4390.10	29.49	**< 0.0001**
Concentration × AC × Tissue	1	3371.08	22.65	**< 0.0001**

TABLE 4— Influence of *Solidago canadensis* tissue and activated carbon treatment on the strength of *S. canadensis* allelopathy effects. Data presented here are logistic regression coefficients ($\beta \pm$ standard error) of the relationship between plant extract concentration and percent germination. Bolding indicates a significant *P*-value.

Tissue	β	Standard Error	Chi-Wald	*P*-value
Leaf Control	-0.0384	0.00212	328.0797	**< 0.0001**
Leaves and AC	-0.0407	0.00230	314.5238	**< 0.0001**
Rhizome Control	-0.0225	0.00226	99.0946	**< 0.0001**
Rhizomes and AC	-0.0326	0.00195	279.4843	**< 0.0001**

TABLE 5— Effects of target species identity (categorical variable) and extract concentration (continuous variable) on target species germination. Overall ANCOVA $F_{11, 516} = 220.83$; $P < 0.0001$; $R^2 = 0.82$. Bolding indicates a significant P-value.

Factor	df	MS	F	P-value
Species	5	14244.79	98.62	**<0.0001**
Concentration	1	73278.20	507.31	**< 0.0001**
Species × Concentration	5	3041.53	21.06	**< 0.0001**
Error	516	144.44		

TABLE 6— Regression coefficients ($\beta \pm$ standard error) of the relationship between plant extract concentrations and percent germination to quantify the strength of allelopathy for each target species. Bolding indicates a significant *P*-value.

Species	β	Standard Error	Wald Chi-Square	*P*-value
Danthonia spicata	-0.0319	0.0020	264.3009	**< 0.0001**
Poa pratensis	-0.0611	0.0045	183.1813	**< 0.0001**
Aster pilosus	-0.0112	0.0016	51.0749	**< 0.0001**
Daucus carota	-0.0200	0.0022	85.7050	**< 0.0001**
Eupatorium rugosum	-0.0251	0.0018	199.5384	**< 0.0001**
Chrysanthemum leucanthemum	-0.0059	0.0017	12.6668	**0.0004**

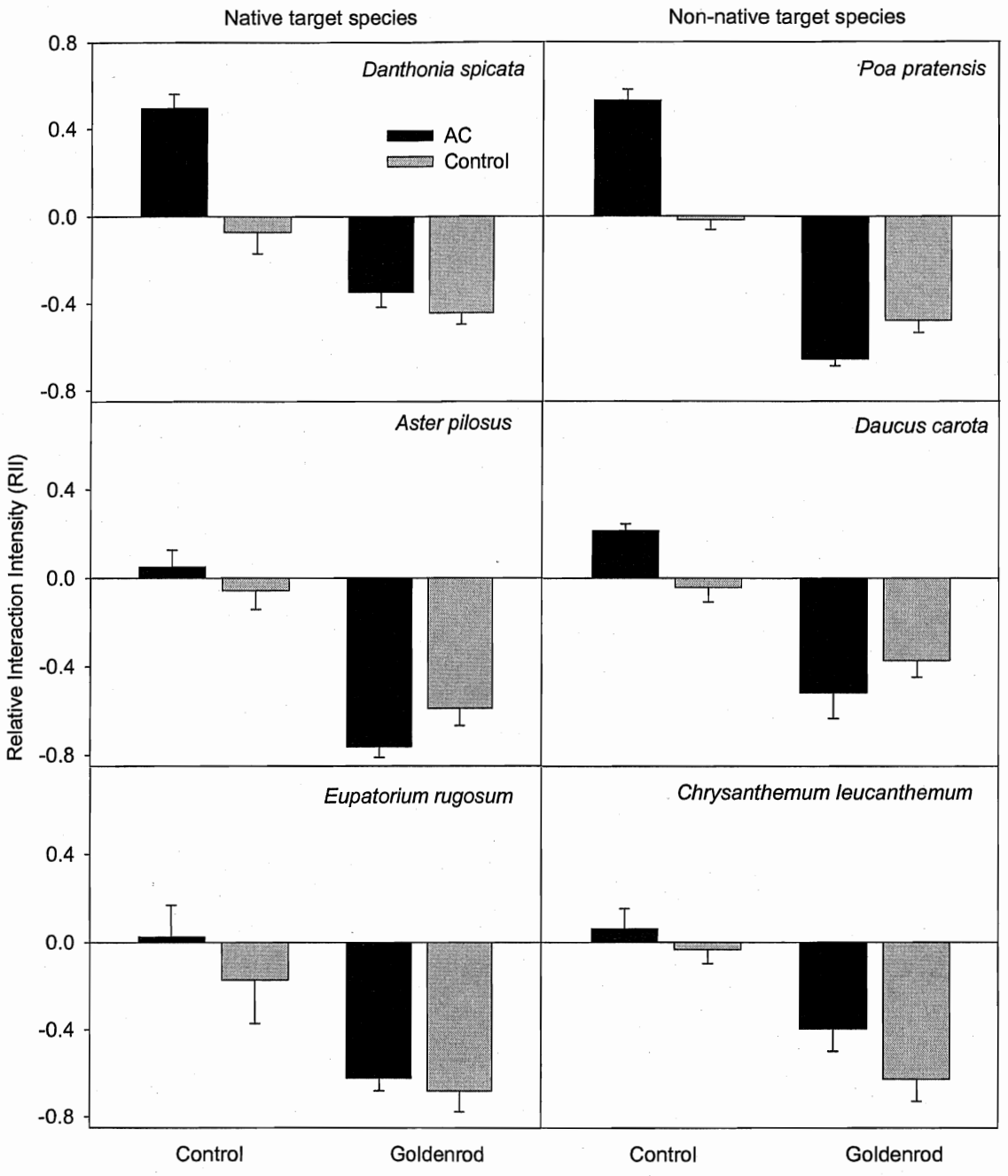

FIGURE 1— Effects of *Solidago canadensis* and activated carbon (AC) on the aboveground biomass of six target species. Data were transformed using the relative interaction intensity (RII). Values closer to zero indicate change relative to the control (no *S. canadensis* and no AC). Positive and negative values reflect an increase or decrease in intensity relative to the control respectively.

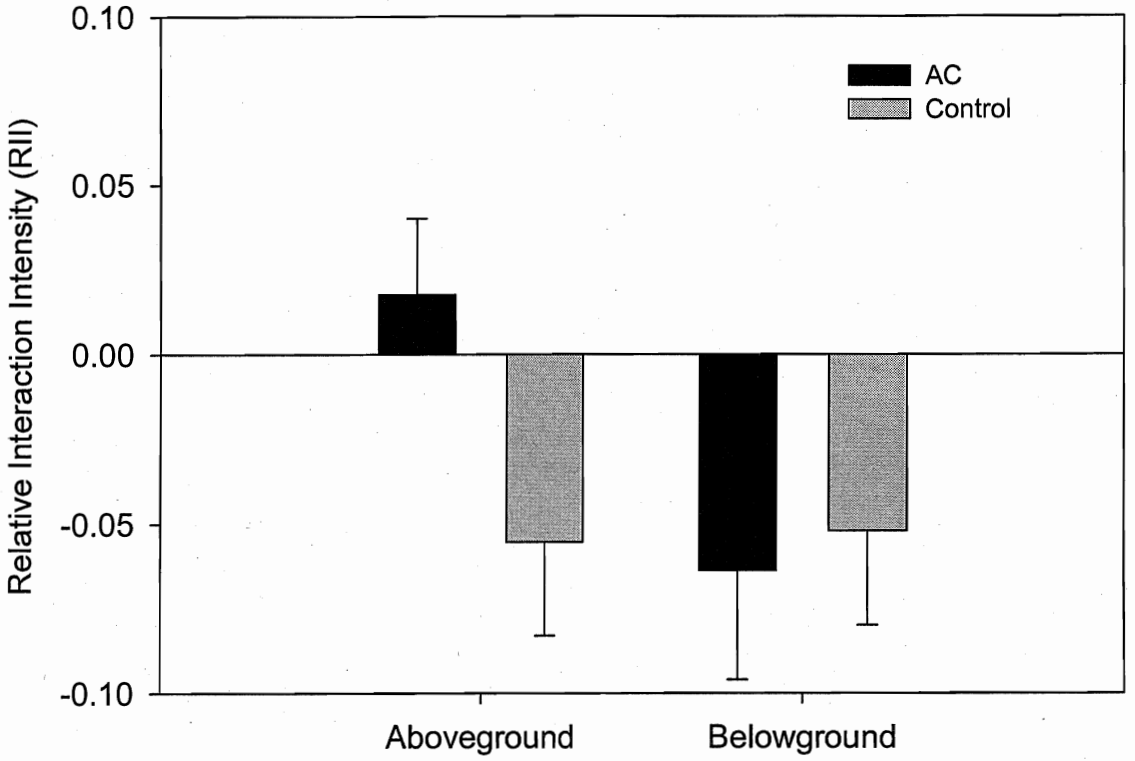

FIGURE 2—Above and belowground biomass of *Solidago canadensis* tissues from each treatment. Data were transformed using the relative interaction intensity (RII). Values closer to zero indicate change relative to the control (no AC). Positive and negative values reflect an increase or decrease in intensity relative to the control respectively.

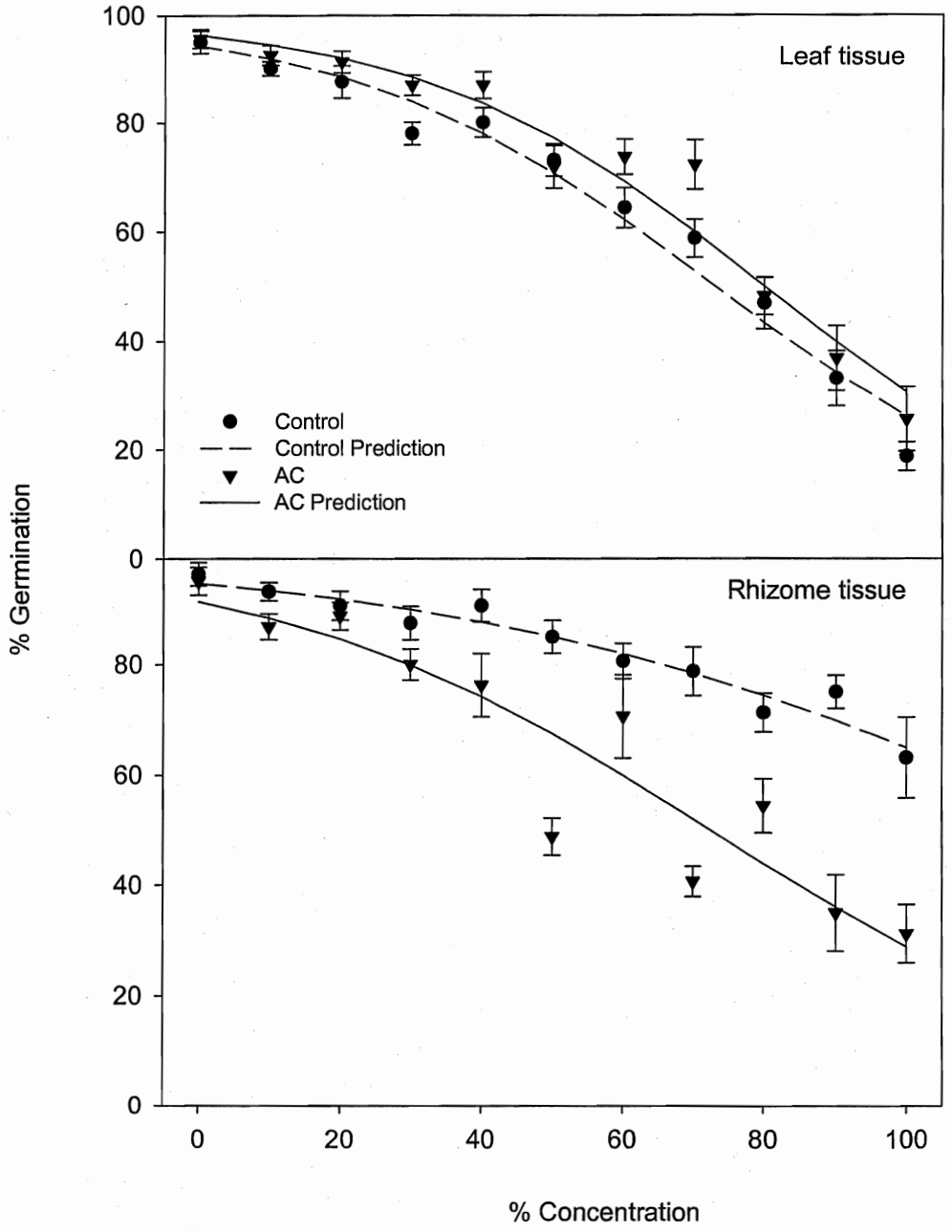

FIGURE 3— Response of radish germination to *Solidago canadensis* rhizome and leaf extracts. Data plotted are mean and standard error of the percent germination at extract concentrations (N = 8 for each tissue at each concentration). The estimated germination probability for the logistic regression is also plotted (prediction line).

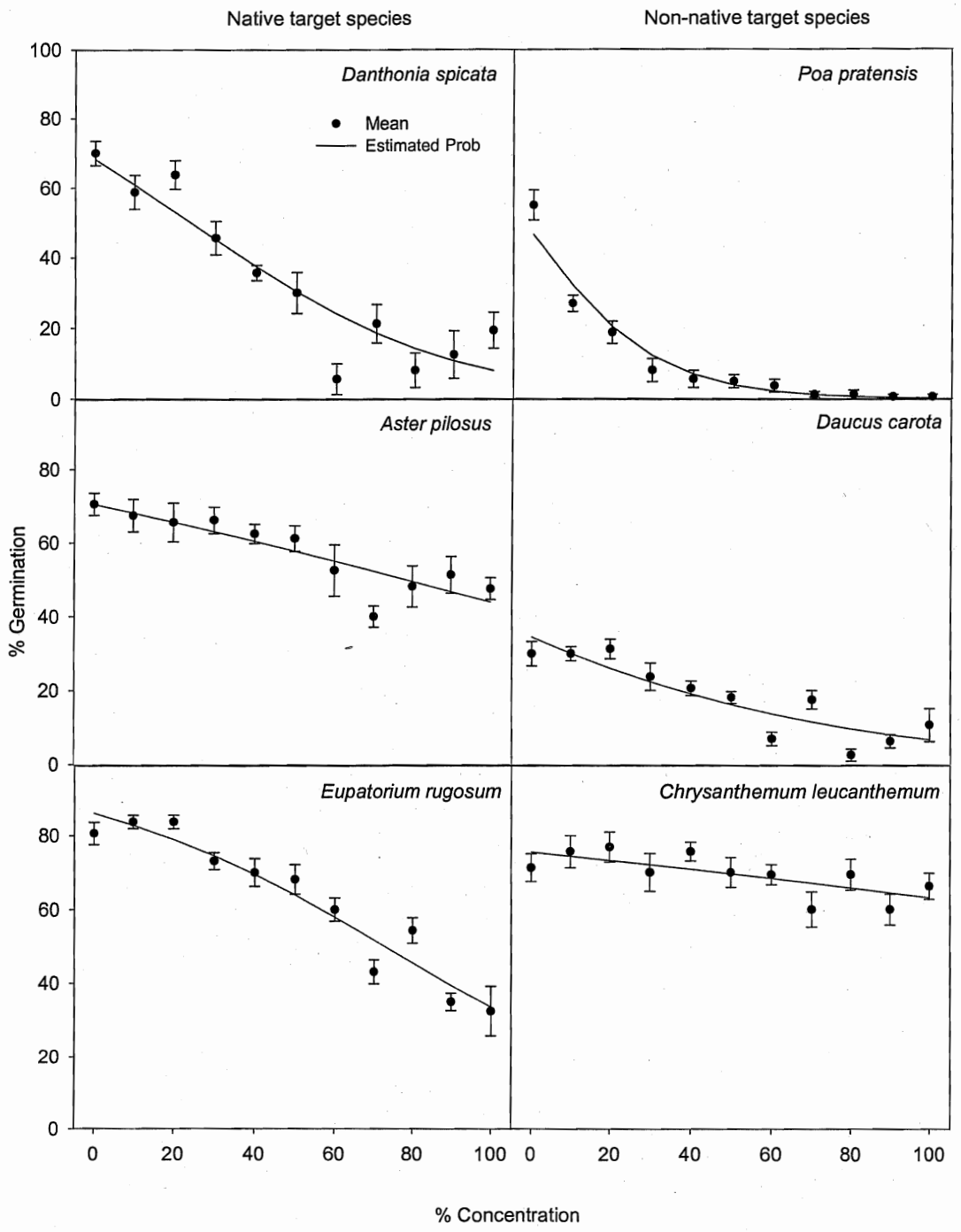

FIGURE 4— Response of radish to target species extracts. Data plotted are mean and standard error of the percentage of germination at each extract concentrations (N = 8 for each species at each concentration). The estimated germination probability for the logistic regression is also plotted (prediction line).

DISCUSSION

Allelopathy is predicted to increase a plant's ability to compete within communities, so I expected allelopathic plants to generate large negative impacts on six species tested. The pot experiment showed clear competitive effects of *S. canadensis* on other species, as growth of five of the six target species was reduced. However, there were also strong effects of activated carbon on plant growth that confounded the results of the experiment. Beyond alteration of chemical interactions in the soil, activated carbon also affected the growth and toxicity of *S. canadensis*. These unintended effects of activated carbon reduce the ability to test whether allelopathic responses are mitigated by evolutionary exposure.

Effects of activated carbon

Target species varied in their response to competition from *S. canadensis*, activated carbon and the combination of the two treatments. Target species exposed to competition all had decreased aboveground biomass (Figure 1), as was expected. Treatments with both activated carbon and *S. canadensis* ameliorated allelopathic effects in *Danthonia spicata*, but not for *Daucus carota* and *P. pratensis* whose aboveground biomass was further reduced. This result does not fit with original expectations, as activated carbon was expected to remove allelochemicals from the soil and therefore reduce the effect of competition, not increase it.

Activated carbon by itself significantly increased aboveground biomass for *Danthonia spicata* and *P. pratensis*. Activated carbon also significantly increased

aboveground biomass for *S. canadensis*, potentially altering competitive effects. Activated carbon was expected to minimally affect plant growth in the absence of allelopathic chemicals. My results suggest that activated carbon may be influencing soil chemistry, specifically nutrient availability. This is somewhat unexpected, as soil nutrients within the pots were maintained with fertilizer application. Other studies have found a similar effect of activated carbon on aboveground biomass (Lau et al. 2008, Weißhuhn and Prati 2009). Lau et al. (2008) found that the same type of activated carbon used in this study increased soil phosphorus by 60 μg for each 2.8 g added. Weißhuhn and Prati (2009) confirmed this effect for a different brand of activated carbon, as it increased available phosphorus by 94%. If activated carbon generally increased phosphorous within treated pots, it could account for the increase in aboveground tissues of *S. canadensis* and the target species. Despite applying fertilizer, activated carbon increased *S. canadensis* toxicity for some target species. This result may provide evidence that the soil fertility (by addition of nutrients) may control allelopathic interactions. However, it is not clear whether increases (Fischer et al. 1978) or decreases (Kong et al. 2002) in nutrient availability intensify allelopathic effects (Inderjit and del Moral 1997).

The microbial community may have been impacted by the addition of activated carbon, though it was not tested for in this study. Allelopathy indirectly impacts mycorrhizal fungi (Roberts and Anderson 2001, Callaway et al. 2008). Callaway et al. (2008) found that *Alliaria petiolata* inhibited the mycorrhizae from North America but not Europe. *Solidago canadensis* is mycorrhizal so it would not be expected to inhibit these associations (Medve 1984). While virtually all plants are associated with

mycorrhizal fungi the degree to which plants depend on fungi varies (Klironomos 2002, Callaway et al. 2008). In times of severe competition, the abundance of fungi could decide competitive outcomes. If allelopathy impacts these mutualisms, then allelopathy may indirectly play a role in determining plant community composition and ecosystem function. Though microbial inoculations were not added in this experiment, fertilizer applications should have minimized their importance to nutrient uptake.

Beyond the effects of activated carbon on plant growth, activated carbon also directly altered the toxicity of *S. canadensis* tissues. Laboratory bioassays revealed that rhizomes were more toxic when activated carbon was present, though toxicity was unchanged for aboveground tissues. The effect of activated carbon on the soil media may have altered nutrient availability, causing *S. canadensis* rhizomes to be more toxic. While the utility of activated carbon for separating allelopathic and competitive effects has been questioned based on altered nutrient availability (Lau et al. 2008, Weiβhuhn and Prati 2009), the potential for activated carbon to directly increase the toxicity of an allelopathic plant is even more problematic. This study is the first to document increased allelopathic potential caused by activated carbon. The prevalence of this effect must be determined across a suite of species to assess whether activated carbon should continue to be used in allelopathy studies.

Allelopathic interactions have been traditionally viewed in a unidirectional way in which one species is deemed allelopathic and the plant receiving the chemicals is not. However, allelopathy may be bidirectional. Many plants produce secondary metabolites and may utilize them for defensive purposes. This issue may have confounded the results in this study, as there may have been potential for the target species to exude allelopathic

chemicals themselves. Species of *Aster* (Jackson and Willemsen 1976, Rink and van Sambeek 1985), *Daucus* (Rink and van Sambeek 1985) and *P. pratensis* (Bosy and Reader 1995) have been suggested to be allelopathic. However, laboratory bioassays were not conducted to assess their allelopathic potential. The possibility of bidirectional interactions occurring may have confounded our system further.

Does evolutionary exposure mediate allelopathic interactions?

The novel weapons hypothesis proposes that species without evolutionary exposure to an allelochemical should be more affected by an allelopathic agent than those which have been previously exposed to these chemicals (Callaway and Ridenour 2004). Plants may adapt rapidly to the chemical composition of their neighbors, indicating that plants can evolve resistance to allelochemicals (Ehlers and Thompson 2004, Callaway et al. 2005). Therefore, recipient communities would be more susceptible to invasion by a species with novel allelochemicals, at least until selection pressures generate resistance within the invaded community.

Because of methodological issues associated with activated carbon treatments, I was not able to directly test whether evolutionary exposure mediated allelopathic effects. If plant populations can adapt to allelochemicals, then they may be able to eventually resist inhibition, providing a buffer from negative effects. This capability would potentially allow natives and non-natives to coexist. If the treatments, including activated carbon, are ignored because of the confounding effects, there is still no pattern between native and non-native target species in their response. All target species, except *E.*

rugosum, experienced a significant decline in aboveground biomass due to competition of *S. canadensis* relative to the control (Table 1; Figure 1).

The germination bioassays provide the only clear test of allelopathy response and evolutionary exposure in this project. Target species' germination responses to *S. canadensis* leaf extracts varied dramatically. All species showed some degree of inhibition by *S. canadensis*. The species most affected by the *S. canadensis* extract were *Danthonia spicata* (β = -0.0611) and *P. pratensis* (β = -0.031). In general, the laboratory bioassay results were consistent with the pot experiment, in that allelopathic responses could not be linked with the evolutionary exposure of the target plants to *S. canadensis*.

Native species, those which had evolved with *S. canadensis* were not consistently less responsive to allelochemicals than non-native species, which should be naïve to *S. canadensis*. This suggests that the non-natives have already developed a resistance to *S. canadensis* allelochemicals. This process may be common in many systems. However, it remains unclear how long it takes for a plant to evolve resistance to allelochemicals it had not been previously exposed to. This may depend on the scale at which allelopathy is being assessed. It seems more likely that local populations will evolve resistance to allelochemicals when in sustained contact with an allelopathic plant. At broader geographic scales contact between species is less consistent as is selection pressure.

Concluding Remarks

Determining whether evolutionary exposure mediates allelopathic responses was confounded due to methodological issues. Despite this, I was able to document the unintended effects of activated carbon on the growth of target plant species. Activated carbon increased plant growth for some species and interacted with *S. canadensis* competition in others. I was also able to document the effects of activated carbon on the allelopathic plant, *S. canadensis*. Activated carbon increased aboveground growth in *S. canadensis* and increased toxicity of rhizomes. These effects of activated carbon suggest that the allelopathic plant was sufficiently altered to influence experimental results. Because of this, activated carbon may not be an effective tool in allelopathy studies and should be used with caution. Although I was not able to determine if evolutionary exposure mediated allelopathic effects, placing allelopathy in a broader context is still a need in allelopathy studies.

LITERATURE CITED

Abdul-Wahab, A. S. and E. L. Rice. 1967. Plant inhibition by Johnson grass and its possible significance in old-field succession. Bulletin of the Torrey Botanical Club **94**: 486-497.

Abhilasha, D. N. Quintana, J. Vivanco and J. Joshi. 2008. Do allelopathic compounds in invasive *Solidago canadensis* s.l. restrain the native European flora? Journal of Ecology **96**: 993-1001.

Abrahamson, W. G., K. B. Dobley, H. R. Houseknecht and C. A. Pecone. 2005. Ecological divergence among five co-occurring species of old-field goldenrods. Plant Ecology **177**: 43-56.

Aerts, R. and F. S. Chapin III. 2000. The mineral nutrition of wild plants revisited: a re-evaluation of processes ad patterns. Advanced Ecological Research. **30**: 7-67.

Armas, C., R.Ordiales and F. I. Pugnaire. 2004. Measuring plant interactions: a new comparative index. Ecology **85**: 2682-2686.

Bing-yao, S., T. Jian-Zhong, W. Zhi-gang, G. Fu-gen and Z. Ming-de. 2006. Allelopathic effects of extracts from *Solidago canadensis* L. against seed germination and seedling growth of some plants. Journal of Environmental Sciences **18**: 304-306.

Blossey, B. and R. Notzold. 1995. Evolution of increased competitive ability in invasive nonindigenous plants – a hypothesis. Journal of Ecology **83**: 887-889.

Bosy, J. L. and R. J. Reader. 1995. Mechanisms underlying the suppression of forb seedling emergence by grass (*Poa pratensis*) litter. Functional Ecology **9**: 635-639.

Butcko, V. M. and R. J. Jensen. 2002. Evidence of tissue-specific allelopathic activity in *Euthamia graminifolia* and *Solidago canadensis* (Asteraceae). American Midland Naturalist **148**: 253-262.

Callaway, R. M. and E. T. Aschehoug. 2000. Invasive plants versus their new and old neighbors: a mechanism for exotic invasion. Science **292**: 521-523.

Callaway, R. M. and W. M. Ridenour. 2004. Novel weapons: invasive success and the evolution of increased competitive ability. Frontiers in Ecology and the Environment **2**: 436-443.

Callaway, R. M., W. M. Ridenour, T. Laboski, T. Weir and J. M. Vivanco. 2005. Natural selection for resistance to the allelopathic effect of invasive plants. Journal of Ecology **93**: 576-583.

Callaway, R. M., D. Cipollini, K. Barto, G. C. Thelen, S. G. Hallett, D. Prati, K. Stinson and J. Klironomos. 2008. Novel weapons: invasive plant suppresses fungal mutualists in America but not in its native Europe. Ecology **89**: 1043-1055.

Colautti, R. I., A. Ricciardi, I. A. Grigorovich and H. J. MacIsaac. 2004. Is invasion success explained by the enemy release hypothesis? Ecology letters **7**: 721-733.

Dyer, A. R. 2004. Maternal and sibling factors induce dormancy in dimorphic seed pairs of *Aegilops triuncialis*. Plant Ecology **172**: 211–18.

Ehlers, B. K. and J. Thompson. 2004. Do co-occurring plant species adapt to one another? The response of *Bromus erectus* to the presence of different *Thymus vulgaris* chemotypes. Oecologia **141**: 511-518.

El-Ghareeb. R. M. 1991. Suppression of annuals by *Tribulus terrestris* in an abandoned field in the sandy desert of Kuwait. Journal of Vegetation Science **2**: 147-154.

Fisher, R. F., R. A. Wood, and M. R. Glavicic. 1978. Allelopathic effects of goldenrod and aster on young sugar maple. Canadian Journal of Forest Research **8**: 1-9.

Fletcher, R. A. and A. J. Renney. 1963. A growth inhibitor found in *Centaurea* spp. Canadian Journal of Plant Science **43**: 475-481.

Gao, X, M. Li, Z. Gao, C. Li and A. Sun. 2009. Allelopathic effects of *Hemistepta lyrata* on the germination and growth of wheat, sorghum, cucumber, rape, and radish seeds. Weed Biology and Management **9**: 243-249.

Gershenzon, J. 1984. Changes in the levels of plant secondary metabolites under water and nutrient stress. Plenum Press, New York, New York. 320p.

Gleason, H. A. and A. Cronquist. 1991. Manual of vascular plants of northeastern United States and adjacent Canada. Second Edition. The New York Botanical Garden, New York., New York, USA. 910p.

Gómez-Aparicio, L. and C. D. Canham. 2008. Neighborhood analyses of the allelopathic effects of the invasive tree *Ailanthus altissima* in temperate forests. Journal of Ecology **96**: 447-458.

Goodnight, C. J. 1990. Experimental studies of community evolution. 1. The response to selection at the community level. Evolution **44**: 1625-1636.

Grime, J. P. 1977. Evidence for the existence of three primary strategies in plants and its relevance to ecological and evolutionary theory. Am. Nat. **111**: 1169-1194.

Grime, J. P. 2001. Plant strategies, vegetation processes, and ecosystem properties. Second Edition. John Wiley & Sons Ltd, Chichester, West Sussex, England. 417p.

Hierro, J. L. and R. M. Callaway. 2003. Allelopathy and exotic plant invasion. Plant and Soil **256**: 29-39.

Inderjit and K. M. M. Dakshini. 1995. On laboratory bioassays in allelopathy. Botanical Review **61**: 28-44.

Inderjit and del Moral. 1997. Is separating resource competition from allelopathy realistic? Botanical Review **63**: 221-230.

Inderjit, T. R. Seastedt, R. M. Callaway, J. L. Pollock and J. Kaur. 2008. Allelopathy and plant invasions: traditional, congeneric, and bio-geographical approaches. Biological Invasions **10**: 875-890.

Jackson, J. R. and R. W. Willemsen. 1976. Allelopathy in the first stages of secondary succession on the piedmont of New Jersey. American Journal of Botany **63**: 1015-1023.

Keane, R. M. and M. J. Crawley 2002. Exotic plant invasions and the enemy release hypothesis. Trends in Ecology and Evolution **17**: 164-170.

Klironomos, J. 2002. Feedback with soil biota contributes to plant rarity and invasiveness in communities. Nature **417**: 67-70.

Kong, C., F. Hu and X. Xu. 2002. Allelopathic potential and chemical constituents of volatiles from *Ageratum conyzoides* under stress. Journal of Chemical Ecology **28**: 1173-1182.

Kumari, A. and R. K. Kohli. 1987. Autotoxicity of ragweed parthenium (*Parthenium hysterophorus*). Weed Science **35**: 629-632.

Lau, J. A., K. P. Puliafico, J. A. Kopshever, H. Steltzer, E. P. Jarvis, M. Schwarzlander, S. Y. Strauss, and R. A. Hufbauer. 2008. Inference of allelopathy is complicated by effects of activated carbon on plant growth. New Phytologist **178**: 412-423.

Mahall, B. E. and R. M. Callaway. 1992. Root communication mechanisms and intracommunity distributions of two Mojave Desert shrubs. Ecology **73**: 2145-2151.

McKey, D. P., G. Waterman, C. N. Mbi, J. S. Gartlan and T. T. Struhsaker. 1978. Phenolic content of vegetation in two African rain forests: ecological implications. Science **202**: 61-63.

Medve, R. J. 1984. The mycorrhizae of pioneer species in disturbed ecosystems in western Pennsylvania. American Journal of Botany **71**: 787-794.

Muller, C. H. 1966. The role of chemical inhibition (allelopathy) in vegetational composition. Bulletin of the Torrey Botanical Club **93**: 332-351.

Nilsson, M. 1994. Separation of allelopathy and resource competition by the boreal dwarf shrub *Empetrum hermaphroditum* Hagerup. Oecologia **98**: 1-7.

Nilsson, M. and O. Zackrisson 1992. Inhibition of Scots pine seedling establishment by *Empetrum hermaphroditum*. Journal of Chemical Ecology **18**: 1857-1870.

Perry, L. G., G. C. Thelen, W. M. Ridenour, T. L. Weir, R. M. Callaway, M. W. Paschke and J. M. Vivanco. 2005. Dual role for an allelochemical: (±) –catechin from *Centaurea maculosa* root exudates regulates conspecific seedling establishment. Journal of Ecology **93**: 1126-1135.

Picman J. and A. K. Picman. 1984. Autotoxicity in *Parthenium hysterophorus* and its possible role in control of germination. Biochemical Systematics and Ecology **12**: 287–92.

Pisula, N. L. and S. J. Meiners. 2010a. Allelopathic effects of goldenrod species on turnover in successional communities. American Midland Naturalist **163**: 161-172.

Pisula, N. L. and S. J. Meiners. 2010b. Relative allelopathic potential of invasive plant species in a young disturbed woodland. Journal of the Torrey Botanical **137**: 81-87.

Powles, S. B. and J. A. M. Holtum. 1994. Herbicide resistance in plants. First Edition. CRC-Press. Lewis, Boca Raton, Florida. 353p.

Prati, D. and O. Bossdorf. 2004. Allelopathic inhibition of germination by *Alliaria petiolata* (Brassicaceae). American Journal of Botany **91**: 285-288.

Putnam, A. R. and W. B. Duke. 1978. Allelopathy in agrosystems. Annual Review of Phytopathology **16**: 421-451.

Rhoads, A. F. and T. A. Block. 2000. The plants of Pennsylvania. University of Pennsylvania Press, Philadelphia., Pennsylvania, USA. 1061p.

Ridenour, W. M. and R. M. Callaway. 2001. The relative importance of allelopathy in interference: the effects of an invasive weed on a native bunchgrass. Oecologia **126**: 444-450.

Rink, G. and J. W. van Sambeek. 1985. Variation among black walnut seedling families in resistance to competition and allelopathy. Plant and Soil **88**: 3-10.

Roberts, K. J. and R. C. Anderson. 2001. Effect of garlic mustard [*Alliaria petiolata* (Beib. Cavara & Grande)] extracts on plants and arbuscular mycorrhizal (AM) fungi. American Midland Naturalist **146**: 146-152.

Schenck, J. B., E. Mahall and R. M. Callaway. 1999. Spatial segregation of roots. Advances in Ecology **28**: 145-180.

Stinson, K. A., S. A. Campbell, J. R. Powell, B. E. Wolfe, R. M. Callaway, G. C. Thelen, S. G. Hallett, D. Prati, and J. N. Klironomos. 2006. Invasive plant suppresses the growth of native tree seedlings by disrupting belowground mutualisms. PLoS Biology **4**: 727-731.

Thompson, J. N. 1999. Specific hypotheses on the geographic mosaic of coevolution. American Midland Naturalist **153**: 1-14.

Thorpe, A. S., G. C. Thelen, A. Diaconu and R. M. Callaway. 2009. Root exudate is allelopathic in invaded community but not in native community: field evidence for the novel weapons hypothesis. Journal of Ecology **97**: 641-645.

Wardle, D. A., M. C. Nilsson, C. Gallet and O. Zackrisson. 1998. An ecosystem-level perspective of allelopathy. Biological Reviews **73**: 305-319.

Warner, R. R. and P. L. Chesson. 1985. Coexistence mediated by recruitment fluctuations – a field guide to the storage effect. American Naturalist **125**: 769-787.

Weber, E. 2001. Current and potential ranges of three exotic goldenrods (*Solidago*) in Europe. Conservation Biology **15**: 122-128.

Weißhuhn, K. and D. Prati. 2009. Activated carbon may have undesired side effects for testing allelopathy in invasive plants. Basic and Applied Ecology **10**: 500-507.

Williamson, G. B. and D. Richardson. 1988. Bioassays for allelopathy measuring treatment responses with independent controls. Journal of Chemical Ecology **14:** 181-188.

Wilson, D. S. 1992. Complex interactions in metacommunities with implications for biodiversity and higher levels of selection. Ecology **73:** 1984-2000.