

CONTRASTING EFFECTS OF ALLELOCHEMICALS FROM TWO INVASIVE PLANTS ON THE PERFORMANCE OF A NONMYCORRHIZAL PLANT

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In addition to resource competition, allelopathy is believed to contribute to the invasiveness and impact of several plant invaders of North America. In this study, we examined whether aqueous leaf extracts of *Alliaria petiolata* and *Lonicera maackii*, two invaders of deciduous forests in North America, affected growth and reproduction of a target nonmycorrhizal plant and whether effects varied across a soil fertility gradient. While nutrient addition substantially improved the performance of *Arabidopsis thaliana* grown in field soils in pots in a growth room, addition of *A. petiolata* extracts to these soils had no significant independent or interactive effects on growth or reproduction. In contrast, addition of *L. maackii* extracts both directly reduced growth and reproduction of *A. thaliana* and greatly constrained increases in growth and reproduction of *A. thaliana* in response to increasing nutrient availability. Use of a nonmycorrhizal target plant revealed that effects of *L. maackii* were independent of allelopathic effects on mycorrhizae, an effect attributed to *A. petiolata* in other studies.

Keywords: allelopathy, *Alliaria petiolata*, *Lonicera maackii*, nutrients.

Introduction

After receiving a great deal of scrutiny for several decades, allelopathy has received renewed attention in the literature, in part because of its potential to explain the dramatic success of some invasive plants (Hierro and Callaway 2003). Allelochemicals produced in the tissues of such plants may enter soils as leaf leachates or root exudates or during tissue decomposition (Inderjit and Duke 2003). There is even evidence for airborne allelopathy mediated by volatile allelochemicals (Matsuyama et al. 2000). Impacts of putative allelochemicals produced by plants on other organisms can be direct, mediated through their acute or chronic toxicity to physiological processes in target organisms (Bais et al. 2003). Impacts can also be indirect, where putative allelochemicals modify the environment for other organisms in some way, such as through alterations in soil microbial communities, nutrient availability, or pH (Blum et al. 1993).

Alliaria petiolata (Bieb.) Cavara and Grande (garlic mustard, Brassicaceae) and *Lonicera maackii* (Rupr.) Maxim. (Amur honeysuckle, Caprifoliaceae) are two potentially allelopathic invaders of eastern and midwestern North America. Each species is known to have negative effects on individual plants or plant communities mediated by competition for space or resources (McCarthy 1997; Collier et al. 2002; Miller and Gorchoy 2004). Furthermore, experiments using tissue extracts or conditioned soils indicate that each of these plants can inhibit seed germination of other species (Trisel 1997; McCarthy and Hanson 1998; Prati and Bossdorf 2004; Dorning and Cipollini 2006), which is presumably a direct allelopathic ef-

fect. In addition, there is some evidence from experiments using tissue extracts or conditioned soils that *A. petiolata* (a nonmycorrhizal plant) may affect neighboring plants indirectly through allelopathic effects on mycorrhizae (Roberts and Anderson 2001; Stinson et al. 2006; Callaway et al. 2008). However, despite assertions about allelopathic potential, few data exist on the direct or indirect allelopathic effects of these species or their extracts, including variation in allelopathic effects across a wider range of target plant life-history stages or across environmental gradients.

The potential of an allelopathic plant to exert direct and indirect effects depends in large part on the chemistry of the plant and whether putative allelochemicals reach meaningful levels in the environment surrounding the plant. Chemicals in *A. petiolata* leaves with the potential to be active in allelopathy have been studied to some extent and include flavonoid and other glycosides and their aglycones, phenolic acids, cyanide, and glucosinolates (Cipollini et al. 2005; Cipollini and Gruner 2007). Although nothing has been published on the chemistry of *L. maackii*, leaves of other *Lonicera* species are known to contain flavonoid glycosides and aglycones (similar to those in *A. petiolata*), phenolic acids, and iridoids (Flamini et al. 1997; Skulman et al. 2004), each of which we have confirmed to be present in *L. maackii* (D. Cipollini, unpublished data). The suite of compounds possessed by each species has the potential to have both direct and indirect allelopathic effects, assuming that they can reach bioactive levels in the environment outside of the plant. For example, phenolic acids are noted for their direct toxicity to some organisms (Chon and Kim 2002) and are also capable of interacting with nutrients in soils (Blum et al. 1993), altering their availability to target organisms. The importance of such indirect effects probably varies across environmental gradients in the field.

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In this study, we examined whether aqueous leaf extracts of *A. petiolata* and *L. maackii* affected growth and reproduction of a target plant and whether effects varied across a soil fertility gradient. Soil fertility varies greatly in the field and is one characteristic that can influence allelochemical behavior (Inderjit 2001). We chose *Arabidopsis thaliana* (L.) Heynh (Brassicaceae) as a target-response species because that was one of the species whose germination was sensitive to *L. maackii* extracts (Dorning and Cipollini 2006). Although it possesses general allelochemical detoxification mechanisms, as most plants do (Baerson et al. 2005), *A. thaliana* has been used in several studies of allelopathy because of its general sensitivity to allelochemicals (Bais et al. 2003; Pennacchio et al. 2005). Its range overlaps with those of *A. petiolata* and *L. maackii* throughout their native and introduced ranges in North America, Europe, and Asia (USDA, NRCS 2007). All three species can be found growing in close proximity in the same field sites in North America (D. Cipollini, personal observation). Like all mustards, *A. thaliana* is nonmycorrhizal, thus excluding the possibility of indirect allelopathic effects on mycorrhizae, an effect attributed to *A. petiolata* that has yet to be studied in *L. maackii*. As members of the Brassicaceae, the physiology and biochemistry of *A. thaliana* is similar to *A. petiolata* in many respects, including the production of glucosinolates (Cipollini et al. 2004, 2005). However, biochemical differences are known to exist, including the production of cyanide by *A. petiolata* (Cipollini and Gruner 2006). We predicted that while extracts of both species would directly reduce performance of *A. thaliana*, *A. petiolata* would exert fewer effects owing to its closer evolutionary relationship (and presumed similarity in physiology) to *A. thaliana*. We also predicted that allelopathic effects of each of these species would vary across a gradient in soil fertility, being more noticeable at low fertility, where allelopathic effects on nutrient availability would greatly constrain plant performance.

Material and Methods

Leaves for extracts were randomly collected from several individuals of *Alliaria petiolata* and *Lonicera maackii* from the Wright State University Woods, Dayton, Ohio, in July 2006. Separate aqueous leachates of fresh leaves of both species were prepared without mechanical homogenization as in Dorning and Cipollini (2006), using a tissue-to-volume ratio of 1 g of leaf material to 5 mL of water (Roberts and Anderson 2001). After extraction and filtering, aqueous extracts were stored at 4°C throughout the experiment. Field soils used in this experiment were collected from a forested section of the Hazard Arboretum, Wilmington College, Wilmington, Ohio. Field soils were homogenized and added to 75-mL pots. Several *Arabidopsis thaliana* seeds (ecotype Columbia) were sown in each pot and were subsequently thinned to one seedling per pot after germination. The Columbia ecotype of *A. thaliana* was used by Dorning and Cipollini (2006) to demonstrate allelopathic effects of *L. maackii* extracts on seed germination; thus, we consider it a “sensitive” ecotype. Whether variation in sensitivity to allelochemicals among *A. thaliana* ecotypes exists is unknown. However, the response in seed germination of the Columbia ecotype to extracts of *L. maackii* was

reflected entirely in the response of seeds from two populations of *A. thaliana* collected from the wild in Ohio (D. Cipollini, unpublished data).

For each invasive species, an experiment was conducted using a fully factorial design with three levels of plant extracts and three levels of nutrients. The experiment using *A. petiolata* extracts was run separately (but simultaneously) from the experiment using *L. maackii* extracts. The three nutrient levels were distilled water with no added nutrients and 0.2 g L⁻¹ and 0.4g L⁻¹ fertilizer (Peters 20-20-20 N-P-K plus micronutrients; Grace-Sierra, Milpitas, CA) dissolved in distilled water. The three extract levels were distilled water with no extract and 0.1 and 0.2 g leaf equivalent mL⁻¹ extract in distilled water. There were four replicate pots per treatment combination. The first treatments were applied on July 7 immediately after seeds were sown and consisted of 10 mL extract treatment and 10 mL nutrient treatment applied to each pot. Additional treatments were applied in the same fashion on July 19, July 26, August 7, and August 22, except that the nutrient treatments were not applied on July 26. Between treatments with nutrients and extracts, pots were given distilled water as needed. Pots were randomly located on benches in an air-conditioned growth room with grow lights (Tek Light 44, Sunlight Supply, Vancouver, WA) with high-output

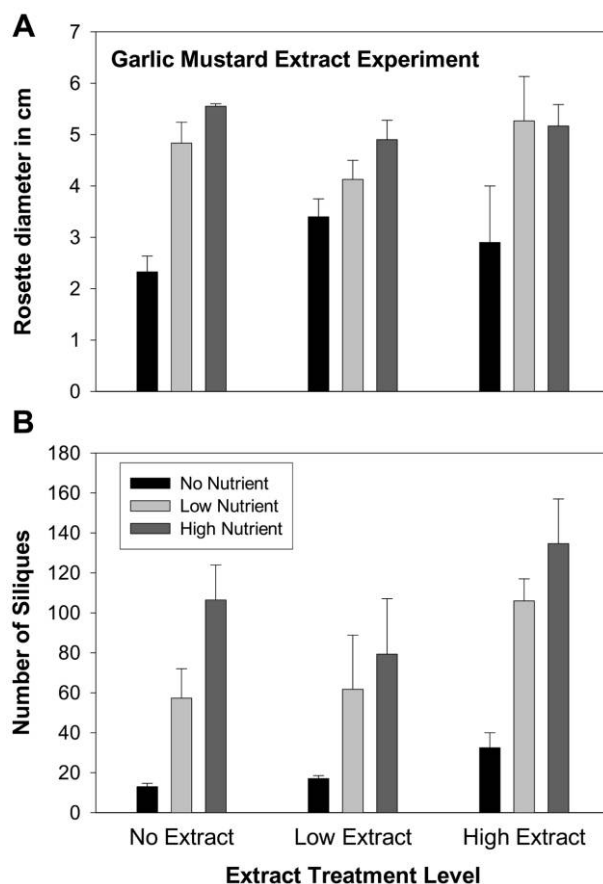


Fig. 1 Response of *Arabidopsis thaliana* to increasing concentrations of aqueous leaf extracts of *Alliaria petiolata* across a soil fertility gradient. A, Rosette diameter. B, Silique number.

Table 1

F Values from ANOVA of the Effects of Leaf Extracts from *Lonicera maackii* and *Alliaria petiolata*, Nutrients, and Their Interaction on Growth and Reproduction of *Arabidopsis thaliana*

Source	<i>L. maackii</i> extracts		<i>A. petiolata</i> extracts	
	Rosette diameter	Silique number	Rosette diameter	Silique number
Nutrient	$F_{2,19} = 32.23^{***}$	$F_{2,19} = 40.34^{***}$	$F_{2,18} = 16.91^{***}$	$F_{2,18} = 14.31^{***}$
Extract	$F_{2,19} = 4.14^*$	$F_{2,19} = 10.91^{***}$	$F_{2,18} = .27$	$F_{2,18} = 3.27$
Nutrient \times extract	$F_{4,19} = .56$	$F_{4,19} = 5.81^{**}$	$F_{4,18} = 1.4$	$F_{4,18} = .39$

* $P \leq 0.05$.

** $P \leq 0.01$.

*** $P \leq 0.001$.

fluorescent bulbs (General Electric, 54 W, 6500 K). Light levels were ca. $180 \mu\text{mol m}^{-2} \text{s}^{-1}$ PAR (Li-Cor Quantum Sensor, Lincoln, NB) and set on a timer for 14-h days and 10-h nights. On September 21, when nearly all plants had finished flowering, the rosette diameter of plants in each pot was measured to the nearest 0.1 cm, and the total silique number produced by each plant was recorded. Since *A. thaliana* almost exclusively self-pollinates, fruit and seed production is an excellent measure of both male and female fitness in this plant. At this time, one plant in the *L. maackii* experiment and three in the *A. petiolata* experiment had not flowered. These plants were removed from the statistical analyses, which did not change the results. In addition, the total seed mass produced by 30 haphazardly collected siliques from haphazardly selected plants in each experiment was measured.

For each species, variation in rosette diameters and total silique number were statistically analyzed by ANOVA with the two fixed factors of extract treatment and nutrient treatment and their interaction (Ryan et al. 2005). When significance was found in the ANOVA, a Tukey's test was used to compare means. To assess the relationship between plant size and reproductive output, regressions were performed between rosette diameter and total silique number for each experiment.

Results and Discussion

In the experiment using *Alliaria petiolata* extracts, rosette diameters and total silique number of *Arabidopsis thaliana* increased with nutrient concentration (fig. 1A, 1B; table 1). This indicates that, in the absence of nutrient addition, the field soil used in our experiments had suboptimal levels of fertility to support *A. thaliana* growth and reproduction. However, addition of *A. petiolata* extracts to this soil had no significant independent or interactive effects on growth or reproduction of *A. thaliana*. Although we expected *A. thaliana* to respond weakly to extracts of *A. petiolata* based on their evolutionary relationship and shared physiology, the lack of any effect on growth was surprising. As some studies have suggested, the primary allelopathic effect of *A. petiolata* on other plants in the field may not be direct (McCarthy and Hanson 1998) but may be mediated through mycorrhizae (Stinson et al. 2006), which was excluded as a possibility in this study. It is also possible that particular allelochemicals in *A. petiolata* extracts are not stable in storage or are quickly

degraded in soils. Extracts of *A. petiolata* induced morphological alterations in newly germinated seedlings reminiscent of the triple response induced by ethylene (Hopkins and Hüner 2004), but this effect did not persist beyond the first extract treatment. Several potential allelochemicals in *A. petiolata* are volatile, including isothiocyanates (derived from glucosinolates) and cyanide, which may have only transient effects in soils and which may be lost in storage of the liquid extracts. There have been no studies on the stability or per-

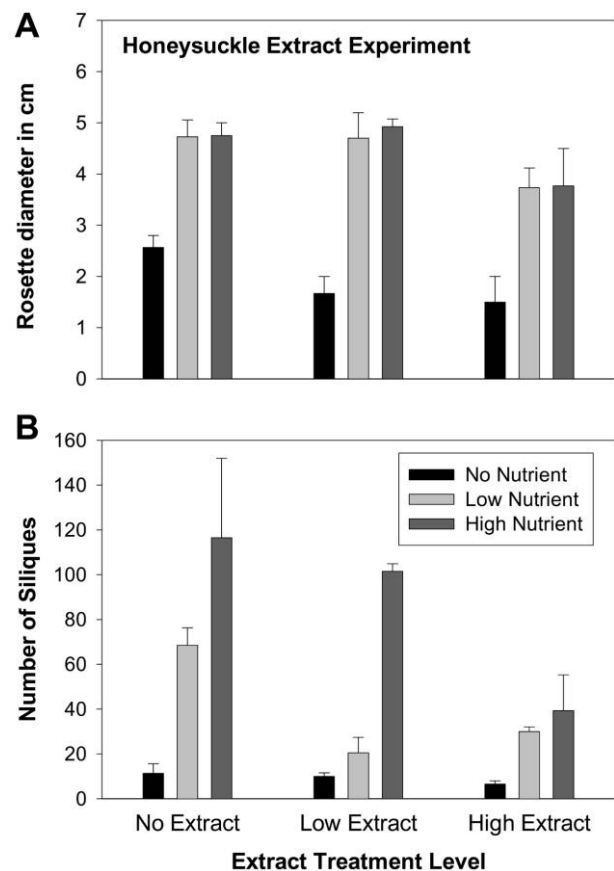


Fig. 2 Response of *Arabidopsis thaliana* to increasing concentrations of aqueous leaf extracts of *Lonicera maackii* across a soil fertility gradient. A, Rosette diameter. B, Silique number.

sistence of *A. petiolata* allelochemicals, but it is known that degradation of phenolic acids and glucosinolates in soils can quickly reduce their allelopathic effects (Ohno 2000). Across treatments, there was a significant relationship between rosette diameter of target plants and their total silique number in this experiment ($R^2 = 0.53$, $F_{1,25} = 28.6$, $P < 0.001$). Rosette diameter is a proxy for total leaf area and is a well-known predictor of fitness in *A. thaliana* (Mauricio 1998). Thus, our results indicate that effects of the treatments on reproduction were mediated largely through changes in plant size induced by the treatments.

In the experiment using *Lonicera maackii* extracts, increasing nutrient addition also increased rosette diameters and total silique number of *A. thaliana* (fig. 2A, 2B; table 1). However, *L. maackii* extracts had both independent and interactive effects on total silique number. Across nutrient treatments, extracts reduced total silique number, an effect that may have been due to direct toxic effects of extracts or indirect effects mediated by changes in soil characteristics. This effect could have resulted from a lack of evolutionary relationship of *A. thaliana* with *L. maackii* that prevented tolerance or detoxification of allelochemicals. Although *A. thaliana* possesses some general mechanisms to detoxify xenobiotic allelochemicals (Baerson et al. 2005), allelochemicals from *L. maackii* were still capable of affecting growth. Extracts of *L. maackii* may have also contained higher levels of “active ingredients” than extracts of *A. petiolata* because of their extractability from leaves or their stability in storage or in soils. Using *A. thaliana* as a target plant also revealed that allelochemicals from *L. maackii* can directly suppress growth of neighboring plants independently of effects on mycorrhizae, which would broaden the implications of allelopathic effects of this plant in the field. Importantly, *L. maackii* extracts also suppressed the growth and reproductive responses of *A. thaliana* to increases in nutrient availability, especially at the highest concentration (fig. 2; table 1). This finding was in contrast to our prediction that allelochemical effects would be most noticeable at low nutrient levels, where allelochemical addition would inhibit already resource-limited plants. Instead, allelopathic effects were more pronounced in high-fertility soils, where untreated plants had a much higher growth potential than untreated plants in low-fertility soils. This effect may have been due to interactions between *L. maackii* allelochemicals (particularly phenolics) and nutrients that indirectly affected growth (Blum et al. 1993) or effects of extracts on the physiology of *A. thaliana* that inhibited its ability to acquire nutrients (Bais et al. 2003). Regardless of the mechanism, our results indicate that allelochemicals from *L. maackii* leaves can directly affect target plant species and

may constrain plastic plant responses to enhanced nutrient availability in the field, such that allelochemical effects may be most important in highly productive environments. While there was also a relationship between rosette diameter and total silique number in this experiment ($F_{1,26} = 14.55$, $P < 0.001$, $R^2 = 0.36$), the relationship was weaker than in the experiment with *A. petiolata*. Examination of the treatment means reveals that effects of *L. maackii* extracts on silique number were not mediated solely through effects on rosette diameter.

Although results from these two experiments cannot be statistically compared, it is clear that allelochemicals from *L. maackii* had a greater effect on performance of *A. thaliana* than allelochemicals from *A. petiolata*. Total seed mass in 30 siliques was 29.5 mg for *A. thaliana* exposed to *A. petiolata* extracts and 24.5 mg for *A. thaliana* exposed to *L. maackii* extracts. Thus, not only did *L. maackii* extracts affect the number of siliques produced by *A. thaliana*, but they also reduced the average mass of seeds produced per silique. Assuming that the response of *A. thaliana* adequately represents the response of other allelochemical-sensitive herbaceous species, such effects indicate that *L. maackii* is more likely to have meaningful allelopathic effects than *A. petiolata* in the field (whose seed germination is, incidentally, strongly inhibited by aqueous extracts of *L. maackii* [Dorning and Cipollini 2006]). *Lonicera maackii* is a large shrub with a much larger leaf area index than the densest *A. petiolata* patch, and *L. maackii* has a longer leafing season than any native deciduous tree or shrub of forests in northeastern and midwestern North America (Trisel 1997). Although never studied, the input of *L. maackii* allelochemicals from leaf leachates, root exudates, or decomposition is probably far greater than that of *A. petiolata*. This does not preclude *A. petiolata* as a potentially important allelopathic plant, but its allelopathic effects may be more localized or transient and may be mediated primarily through mycorrhizae of neighboring plants, an effect not yet examined for *L. maackii*. *Alliaria petiolata* also displays a higher degree of shade tolerance than *L. maackii*, suggesting that it may differentially affect sensitive forest understory communities, where *L. maackii* is unlikely to invade.

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