

Exploring the mechanisms of allelopathic interaction in the invasive annual plant, *Phyllanthus urinaria*

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Abstract

The belowground mechanisms facilitating invasion and proliferation of non-native plant species into natural communities are of critical importance for understanding plant invasions. Research on allelopathy suggests that some exotic plant species produce compounds via root exudates that may suppress or inhibit the growth of neighboring plants, but the specific mechanisms and consequences of these plant-chemical interactions remain elusive. In an effort to understand the abiotic and biotic factors governing allelopathic activity, a two-part greenhouse experiment was designed to examine plant-soil interactions of the invasive euphorb, *Phyllanthus urinaria*, and the residual soil effects of these interactions on subsequent growth of a target species, *Brassica rapa*. Our results confirmed that plant-soil feedback from *Phyllanthus* negatively influenced growth of *Brassica* and the effect was proportional to the *Phyllanthus* biomass. However, it is unclear whether the mechanism responsible for this negative effect is associated with chemical suppression (i.e., allelopathy) or with depletion of soil nutrients (i.e., competition). In addition to screening for phyto-toxic compounds, soil analysis of macro- and micro- nutrient levels may be necessary to differentiate between plant strategies that create chemical interference vs. those that influence resource availability. Description and definition of these plant mechanisms may provide useful insight into understanding the factors that enable alien species to invade and successfully coexist in natural communities.

Introduction

Among the thousands of non-native plants that humans have introduced to new ecosystems, only a small number of these species have become dominant invaders of their new habitats (Murrell et al. 2011). Recognizing that the occurrences of non-native dominance in natural communities are infrequent and often associated with specific environmental factors, our focus was directed toward understanding the mechanisms of plant-plant interactions that might result in dominance and, more specifically, the potential abiotic and biotic factors which may facilitate these interactions.

The broad concept that plants produce chemicals in above- or below-ground tissues that suppress or inhibit the growth of neighboring plants is a phenomenon recognized as *allelopathy* (Rice 1979, Casper et al. 2007). Inderjit et al. (2011) suggested that the production, release, and the fate of allelochemicals are fundamental mechanisms of plant behavior which influence most aspects of plant ecology. However, the concept is often used very generally and experimenters should specify whether the chemically-mediated plant-plant interactions are occurring above- or below-ground; whether they result from leaf litter, root exudates, or aromatic compounds; and whether the interactions occur within a growing season or represent legacy effects spanning multiple growing seasons (Inderjit 1996). Regardless of where allelopathic interactions occur, in most instances, the specific abiotic and biotic cues responsible for eliciting and regulating them are not well understood.

The production of allelopathic compounds comes at a physiological cost to the plant because they represent energy

diverted to purposes other than growth and reproduction (Bloom et al. 1985). If allelopathic activity results in a positive effect on fitness, this would be perceived as adaptive and these traits should persist and spread through the population. However, not all phyto-chemicals have allelopathic potential and when phyto-chemicals are present, their allelopathic activity must be verified (Inderjit et al. 2011). For example, in the rhizosphere, root exudates may be used to attract symbiotic fungi or bacteria, may act as chelating agents for sequestering micronutrients, or may create negative interactions with roots of neighboring.

Invasive plant species have provided an opportunity to test hypotheses relating to phyto-chemical production and allelopathic interactions (Bais et al. 2003). Invasive species have the ability to successfully enter, establish, and spread into environments to which they have not previously been exposed. Because they are new to the environments they invade, invasive species should not possess adaptations that are specific to these territories due to a lack of evolutionary history, yet a large number of these species eventually dominate the new habitats both in terms of numerical abundance and biomass. Callaway and Ridenour (2004) have suggested that “invaders” may be successful because they produce allelopathic chemicals to which the native species are not adapted (i.e., the “novel weapons” hypothesis). These phyto-chemicals may inhibit germination, growth, or reproduction of the natives and may persist in the soils for many years. In addition, once established, invasive species may create long-lasting soil signatures that have been broadly termed “legacy effects” (Casper et al. 2007).

To explore the environmental cues and residual soil effects potentially associated with the production of allelochemicals

in the rhizosphere by a non-native plant, we tested abiotic and biotic factors in a two-part greenhouse experiment. We investigated the strength of plant-soil feedbacks mediated by intra- and interspecific interactions of an invasive plant, *Phyllanthus urinaria* (Euphorbiaceae), in amended and un-amended soil treatments using a target species, *Brassica rapa*. The effects of plant-soil feedback on target plant growth were examined by planting *Brassica* in soil treatments previously occupied by *Phyllanthus*. Intra- and inter-specific density treatments tested whether biotic cues stimulated plant-soil feedback responses. Soil amendments (fertilizer or activated carbon) were used as treatments to test whether these abiotic factors were correlated with plant-soil feedback. We predicted *Brassica* would exhibit a greater negative effect when *P. urinaria* was grown in situations of interspecific competition compared to intra-specific competition. We did not differentiate whether this response was due to a legacy effect caused by allelopathic compounds in the soil or as a consequence of resource limitation.

Materials & Methods

Phyllanthus urinaria L. is an herbaceous plant native to Southeast Asia and is a member of the Euphorbiaceae family. Characteristic of most of the Euphorbiaceae, *P. urinaria* produces a toxic milky sap in the aboveground tissues that acts to deter herbivory (Casper et al. 2007). Knowledge of these existing toxic compounds in the aboveground biomass, coupled with the invasive nature of the plant, led us to investigate allelopathic potential of *P. urinaria* beneath the surface of the soil. Soil legacy effects caused by *P. urinaria* were examined using the target species, *Brassica rapa*. We used a rapid-cycling cultivar of *B. rapa* (Wisconsin Fast Plants®) because of its sensitivity to environmental influences, which makes it a useful indicator of abiotic and biotic stress.

A two-part greenhouse experiment was designed to investigate the effect of *P.urinaria* on soil via root interactions. Part 1 examined the abiotic and biotic factors that influence the ability of *P. urinaria* to alter soil conditions. Part 2 investigated residual soil effects of *P. urinaria* on the growth of a target species. Part 1 of the experiment was devoted to “soil conditioning” and allowed *P. urinaria* seedlings to grow to maturity in eight separate soil treatments of either amended or un-amended soils (Voorde et al. 2011). These same pots were used in Part 2 to grow single *B. rapa* plants to evaluate their response to the soil conditions created in Part 1. A total of 120 pots were used in the experiment with 15 replicates for each of the eight treatments (Table 1).

Part 1. Conditioning the soil

The objective of the abiotic and biotic soil treatments was to create conditions that might induce allelopathic responses by the *P. urinaria* target plants. Abiotic factors were represented by two treatments containing amended soils: (1) NPK amended (MiracleGro® 10-10-10 slow-release pelleted fertilizer) and (2) soil amended with activated carbon pellets

(Seachem™ matrix carbon). Activated carbon (AC) has been used to bind to and nullify the effects of phyto-toxic chemicals (Ridenour et al. 2001) and was used as a soil additive to help detect (by reducing) chemical suppression by the non-native. *P. urinaria* seedlings were transplanted into six of the eight treatments, at densities of 1, 3, and 6 to create intra- and interspecific competition treatments. The remaining two treatments corresponded to soil controls, one of which had no plants in it and was unaffected by the invader or soil amendments, while the other contained a single plant of *Brassica rapa*. *P. urinaria* was grown to maturity over the course of eight weeks in a greenhouse environment. All soil treatments were kept moist throughout the conditioning period, and their locations were randomized on trays to reduce error associated with environmental heterogeneity in the greenhouse. After eight weeks the aboveground biomass in all treatments, including the *B. rapa* only treatment, were harvested and weighed. All of the treatments used to elicit possible plant-soil feedbacks produced by the non-native are shown in Table 1.

Part 2. Testing for soil legacy effects

After conditioning the soil with *P. urinaria* to create soil legacy effects, the same soils were used to grow single specimens of *B. rapa*. Several seeds of *B. rapa* were sown directly into each of the pots of the six *P. urinaria* treatments, then thinned to one individual, and kept well watered (Table 1). *B. rapa* was also sown into the two control soils (previously *B. rapa* alone and the empty control). Above ground biomass of the target plants was harvested after eight weeks, dried, and weighed.

Results

The mean per pot biomass of *P. urinaria* from the first greenhouse experiment (Part 1) was the highest when grown alone or in intra-specific neighborhoods (Fig. 1). Per capita biomass was similar across all treatments except when *P. urinaria* was grown with no competition or soil manipulation. The AC treatment reduced biomass the most and inter-specific competition with *B. rapa* had the least effect on *P. urinaria* growth. *B. rapa* biomass in the second experiment (Part 2) was highest when growing in pots not previously occupied by *P. urinaria* or which had *P. urinaria* but had been treated with fertilizer (Fig. 2). Per pot biomass was highest for *B. rapa* in the soil control (no previous plants) and in soil occupied previously by *B. rapa*.

When performance of the two species was compared using biomass production from the same pots, a significant correlation ($p=0.022$, $r=0.88$, $n=6$) suggested a strong negative legacy effect of *P. urinaria* on the growth of *B. rapa* (Fig. 3). The six data points included *B. rapa* in pots with and without previous *P. urinaria*, but excluded those treatments that influenced the abiotic growing conditions (i.e., soil amendments).

Table 1. Abiotic and biotic treatments used to influence *P. urinaria* growth and subsequent strength of soil legacy effects in Part 1 and Part 2 of the experiment. Each treatment was replicated 15 times (n = 120 pots).

Type of Factor	Treatment	Number of Plants			
		Experiment 1		Experiment 2	
		<i>P. urinaria</i>	<i>B. rapa</i>	<i>P. urinaria</i>	<i>B. rapa</i>
Abiotic effects	Soil control	0	0	0	1
	Target control	0	1	0	1
	Fertilizer (NPK)	1	0	0	1
	Carbon (AC)	1	0	0	1
Biotic effects	Intra-specific	1	0	0	1
		3	0	0	1
		6	0	0	1
	Inter-specific	1	1	0	1

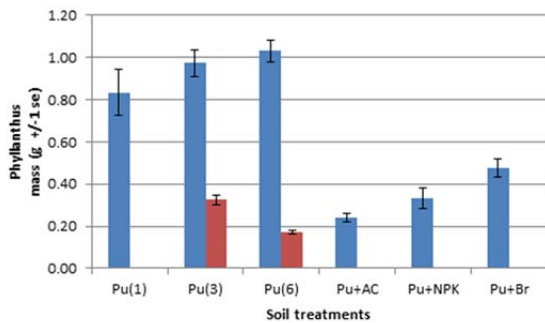


Figure 1. Mean *Phyllanthus urinaria* above ground biomass (g +/- 1 se) per pot in each of the treatments. Treatments are one, three, and six plants per pot; activated charcoal; 10-10-10 fertilizer pellets; one *Brassica* neighbor. Red bars indicate mean values of per plant biomass for pots containing densities of three and six *P. urinaria*.

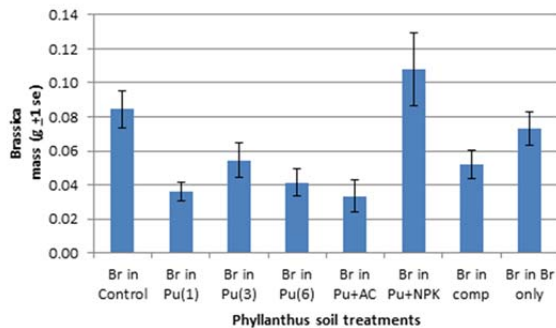


Figure 2. Mean *Brassica rapa* biomass (g +/- 1 se) when grown in pots previously occupied by *Phyllanthus urinaria* subjected to different density and amendment treatments. No additional amendments were added when the *Brassica*

Discussion

We demonstrate in this study that the presence of the invasive euphorb, *Phyllanthus urinaria*, produces a legacy effect in the soil that has a negative influence on the growth of the target species, *Brassica rapa*. The decrease in the biomass of the target was proportional to increase of the biomass of the non-native, however, we are uncertain whether the cause of this negative influence is a consequence of chemical suppression (allelopathy) or a reduction of soil nutrients by *P.*

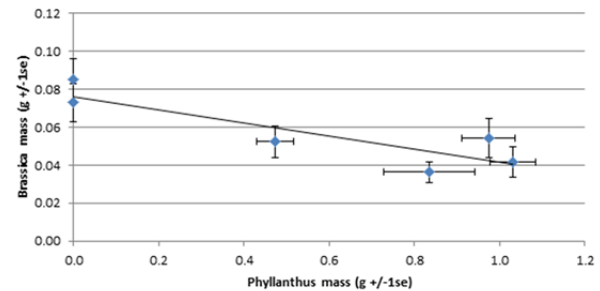


Figure 3. *Phyllanthus urinaria* biomass (mean +/- 1 se) plotted against *Brassica rapa* biomass. *B. rapa* were grown in soil previously occupied by varying densities of *P. urinaria*. The significant negative correlation ($p=0.022$, $r=0.88$) suggests a legacy effect of non-native presence on the biomass of the target species. Only data from pots with unamended soil (no carbon or fertilizer) were used.

urinaria.

Previous studies recognized that plant-soil feedbacks change abiotic and biotic components in the soil that may influence plant-plant and plant-microbe interactions (Kardol et al. 2007). Some studies have provided support for the hypothesis that intra-specific soil conditioning via plant-soil feedback may facilitate growth of that species and may positively influence its survival and reproduction (Casper et al. 2007). If the mechanism of plant-soil feedback has a role in altering soil quality, we may infer that changes made within the soil environment may also influence the fitness of other species inhabiting the environment (i.e., a negative inter-specific effect).

In this study, we investigated the possibility that negative effects of *P. urinaria* on *B. rapa* were likely related to root allelopathy, but separating allelopathic interactions from the simple negative effect of competition for soil resources is difficult. Here, and in our other associated experiments, it appears that *P. urinaria* is negatively affected by the addition of nutrients (Fig. 1). However, *B. rapa* performed best in the fertilizer amended soil (Fig. 2) despite the previous presence of *P. urinaria* which had a demonstrated negative effect on *B. rapa* (Fig. 3). Our interpretation is that either the NPK addition reduced the strength of soil legacy effects by *P. urinaria* or that the additional nutrients alleviated the negative soil legacy effect on *B. rapa*.

We propose a possible mechanistic link between the apparent inability of *P. urinaria* to take up supplemental soil nutrients and its potential ability to produce soil allelochemicals. Previous studies have shown that some plants may take up needed nutrients using root exudates that act as chelating compounds and bind to soil micro- and macro-nutrients (Ridenour et al. 2001). The action of such compounds may impoverish the soil of micronutrients thereby increasing the competitive effect of the plant. However, if the compounds persist in the soil into the next growing season, their presence may cause effects resembling a negative allelopathic interaction on other species. In the current study, the positive response of *B. rapa* in the NPK treatment despite the previous occupation of the soil by *P. urinaria* and the negative response in all other soils previously occupied by *P. urinaria* suggests that the negative effects may be present only when *P. urinaria* must use root exudates for taking up soil nutrients. This mechanism provides a possible explanation for the invasive success of *P. urinaria* across different habitat types, particularly those with low soil nutrient availability.

Future studies will examine soil micro- and macro-nutrient levels in greenhouse experiments before and after soil impoverishment by the invasive species. Differences among treatments in the effect on nutrient levels would suggest that the invasive species uses strategies which allow it to obtain soil resources more efficiently than its competitors. In the broader discussion of plant use of allelopathic compounds, it will be useful to discriminate between those situations where plants actively suppress neighbors and competitors vs. those situations where suppression is an indirect result of the production of root exudates that facilitate nutrient sequestration. Experiments are needed to identify the soil conditions that stimulate strong phyto-toxic interactions and what relationship those interactions have with changes in nutrient levels from one season to the next. Such information on soil nutrient levels will help us distinguish between strategies for chemical interference and resource competition. However, analysis of organic compounds in the rhizosphere is necessary for us to more clearly define the boundaries defining allelopathic interactions.

References

1. Bais HP, Vepachedu R, Gilroy S, Callaway RM, Vivanco JM. 2003. Allelopathy and exotic plant invasion: from molecules and genes to species interactions. *Science* 301:1377-1380.
2. Bloom AJ, Chapin FS III, Mooney HA. 1985. Resource limitation in plants- an economic analogy. *Annual Review of Ecology and Systematics* 16:363-392.
3. Callaway RM, Ridenour WM. 2004. Novel weapons: a biochemically based hypothesis for invasive success and the evolution of increased competitive ability. *Frontiers in Ecology and the Environment* 2:436-433.
4. Callaway R, Ridenour W, Laboski T, Weir T, Vivanco J. 2005. Natural selection for resistance to the allelopathic effects of invasive plants. *Journal of Ecology* 93:576-583.
5. Casper B, Castelli J. 2007. Evaluating plant-soil feedback together with competition in a serpentine grassland. *Ecology Letters* 10:394-400.
6. Inderjit. 1996. Plant phenolics in allelopathy. *The Botanical Review* 62:186-202.
7. Inderjit, Evans H, Crocoll C, Bajpai D, Kaur R, Feng Y, Silva C, Carreón JT, Valiente-Banuet A, Gershenzon J, Callaway RM. 2011. Volatile chemicals from leaf litter are associated with invasiveness of a neotropical weed in Asia. *Ecology* 92:316-324.
8. Kardol P, Cornips N, Van Kempen M, Bakx-Schotman J. 2007. Microbe-mediated plant-soil feedback causes historical contingency effects in plant community assembly. *Ecological Monographs* 77(2):147-162.
9. Murrell C, Gerber E, Krebs C, Parepa M, Schaffner U, Bossdorf O. 2011. Invasive knotweed affects native plants through allelopathy. *American Journal of Botany* 98(1):38-43.
10. Rice, E. L. 1979. Allelopathy: an update. *The Botanical Review* 45:15-109.
11. Ridenour W, Callaway R. 2001. The relative importance of allelopathy in interference: the effects of an invasive weed on a native bunchgrass. *Oecologia* 126:444-450.
12. Voorde T, Putten W, Bezemer T. 2011. Intra- and interspecific plant-soil interactions, soil legacies and priority effects during old-field succession. *Journal of Ecology* 99:945-95.