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Seed extracts impede germination in *Brassica rapa* plants

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Abstract

Allelopathy is an important mechanism of interference competition in some plants, but little is known about whether compounds exuded from seeds influence the germination of neighbors. We treated seeds of multiple lines of *Brassica rapa* (field mustard) with aqueous extracts of *Brassica rapa* Fast Plant seeds and extracts of seeds of the invasive, allelopathic plant *Alliaria petiolata* (garlic mustard). Germination patterns differed significantly among populations, with Fast Plants germinating earliest. As predicted, *Alliaria* seed extracts significantly delayed germination compared to controls. Surprisingly, Fast Plant extracts reduced germination probability and delayed germination more than both controls and *Alliaria* extract-treated seeds. These results suggest that compounds in the seeds of both species appear to be allelopathic, and indicate a potentially important and unrecognized role for allelopathy in intra- and interspecific competition at the seed stage.

Introduction

Allelopathy occurs when plants exude compounds that have a negative impact on growth or performance of conspecific or heterospecific neighbors.[1] The Novel Weapons Hypothesis proposes that some introduced species may become invasive because they contain allelopathic compounds that are particularly effective against competitors in the new environment, which lack coevolved defenses.[2] Allelopathy has been demonstrated for many invasive plants, including *Centaurea maculata*,[3] *Lolium arundinaceum*,[4] *Elaeagnus umbellata*,[4] and *Alliaria petiolata*.[5] Most studies of allelopathy have investigated the effects of compounds exuded through the roots or litter of mature plants, and very few have examined whether seeds could contain allelopathic compounds. One study found that extracts of newly germinated *Abutilon theophrasti* seeds appeared to inhibit germination of *Brassica rapa* seeds,[6] and another study found that extracts of four wetland seeds also seemed to inhibit initial germination in *B. rapa* and two other wetland species.[7]

In contrast to allelopathic effects of seed exudates, it is also possible that seeds could release compounds that enhance germination in neighboring seeds. Many compounds, including the plant hormones abscisic acid and gibberellins, as well as nitrate, have been shown to promote germination.[8,9] Some of these compounds could be released by seeds and affect germination of neighboring seeds, possibly synchronizing germination.

In this study, we examined the effects of aqueous seed extracts on seed germination. The extracts were from two species in the Brassicaceae (mustard) family: *Alliaria petiolata* (garlic mustard) and *Brassica rapa* (field mustard) rapid cycling Fast Plants (Carolina Biological Supply, Burlington, NC). Adult *A. petiolata* plants have been shown to be allelopathic,[5] and we predicted that that seed extracts from this species would inhibit germination. *Brassica rapa* Fast Plants have been selected for rapid germination and the absence of dormancy,[10] and we predicted that seed extracts from this species would enhance germination.

Materials and Methods

Study species

Alliaria petiolata (M.Bieb.) Cavara & Grande (garlic mustard), is a biennial plant native to Europe and introduced in North America.[11-13] It is considered one of the most problematic invasive plant in the U.S.[14,15] Leaf and root tissue of *A. petiolata* has been shown to contain compounds with allelopathic effects on other species, and it is thought that allelopathy may be one reason for the success of this species in its introduced range.[5] Seeds of A. *petiolata* require cold wet stratification for germination.[16] *Brassica rapa* (L.) syn. campestris (field mustard) is an important crop plant species (turnip, rapini, bok choi). Originally from Eurasia and now distributed throughout North America, it occurs not only as a crop but also as escaped or wild varieties and is considered a noxious weed.[17]

Extracts

Seeds of *Alliaria petiolata* (n=100) were collected from Fordham University's Louis Calder Center in Armonk, NY and stratified on moist filter paper at 1-2°C for 30 days. The seeds were then placed on new moist filter paper in petri plates and kept at room temperature for 24 hours. *B. rapa* Fast Plant seeds (n=100) were placed on moist filter paper in separate petri plates at room temperature for 24 hours. The seeds of each species were then crushed with a mortar and pestle in 30 mL distilled water, with the extracts left at room temperature for an additional 24 hours. The extracts were then filtered through a 1 mm screen mesh and then filter paper.

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Contributions: KB and SJF conceived of and designed the experiment, KB collected the data, KB and SF analyzed the data and wrote the manuscript.

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Treated seed sources

Seeds of 4 groups of *B. rapa* plants were treated with the extracts. The groups include B. rapa Fast Plants, obtained from Carolina Biological Supply, *B. rapa* biennials derived from crops in the Netherlands and obtained from the USDA National Plant Germplasm System, and two populations in coastal central California: Oxford Tract, on the campus of UC Berkeley in Alameda County, and Shell Beach, in Sonoma Coast State Park on Bodega Bay in Sonoma County. These populations were chosen because they vary in life-history rates and characteristics.

Experimental design

The seeds were placed in 96-well bubble plates with one seed per well, in a randomized block design. With this design and the use of the 96 well plates, each seed represented a true replicate rather than pseudoreplicates, which would be the case if the seeds were germinated together in petri plates. Fifty µL of the designated extract or distilled water (controls) was added to each well, and the plates were sealed with parafilm. All seeds were checked daily for germination, with germination defined as the breaking of the seed coat and the emergence of the radicle. Data was collected until there had been no germination of any seeds for 7 days.

Analyses

The effect of seed extract on probability of germination was analyzed with a logistic regression. Time to germination was analyzed with failure time analysis. Differences among the extracts in germination probability and time to germination were assessed with planned orthogonal contrasts in general linear models.

Results

Percent germination varied among *B. rapa* seed populations, ranging from 83% in one California population (SB) to 100% in the other natural population (OT), with germination rates 94.4% for Fast Plants and 95.8% for the biennials. According to logistic regression, there was a significant effect of extract on germination rates (Wald χ^2=6.17, d.f.=2, P=0.0457). Germination was highest in distilled water, second highest (but not significantly different) in the *Alliaria* extract and lowest in the Fast Plant extract (Figure 1).

Failure time analysis showed that time to germination varied significantly among seed populations (Wald χ^2=40.05, d.f.=3, P<0.0001), with Fast Plants germinating in the least time (1.2 days), OT populations in 2.0 days, biennials in 2.7 days and SB in 3.0 days, on average. There was a significant effect of extract on time to germination (Wald χ^2=21.26, d.f.=2, P<0.0001). Seeds in distilled water germinated in the shortest time, with *Alliaria* extract significantly delaying germination compared to controls and Fast Plant extract significantly delaying germination compared to controls and more than, but not significantly different from, *Alliaria* extract treated seeds (Figure 2). There was no significant statistical interaction between source population and extract (Wald χ^2=5.12, d.f.=6, P=0.5279).

Discussion

The results of this study clearly indicate a negative, inhibitory effect of extracts of both *B. rapa* Fast Plant seeds and *A. petiolata* seeds on germination of *B. rapa*. The Fast Plant extracts significantly reduced the probability of germination, and both Fast Plant and *Alliaria* extracts significantly delayed germination compared to distilled water controls. These results suggest that the seeds of both species contain compounds that are allelopathic. While allelopathy has been demonstrated for many plants, including a number of invasive species, this is one of only a few studies to demonstrate allelopathic effects of seed extracts on seed germination and the first to demonstrate the potential for intraspecific allelopathic inhibition at the seed stage.[6,7]

While it is not known if the inhibitory effects of the seed extracts we found under laboratory conditions would occur in the field, it seems likely that they could influence competition at the seed stage under natural conditions. The extracts were water-soluble so would presumably dissolve in rainwater and diffuse through the soil. Compounds released from a seed would likely have the greatest impact on other seeds that were very close by, which would also be the strongest resource competitors with the target seedlings after germination. Thus the inhibitory effects of the compounds could offer a strong competitive advantage. Even a brief delay of germination by a neighbor could substantially favor the target seedling, since the cotyledons of the target could shade out the neighbor and the emerging roots gain more water and nutrients. In plant interactions in general, competition at the seedling stage is well known to be particularly intense, resulting in self-thinning.[18] This study indicates that interference competition via allelopathic compounds exuded from seeds could at least potentially be one mechanism of self-thinning.

While previous studies have shown compounds exuded through the leaves and roots of mature *Alliaria* plants to be allelopathic,[5] this is the first study to demonstrate an inhibitory effect of *Alliaria* seeds on germination of another species. In contrast, there is no previous evidence that *B. rapa* plants are allelopathic at any life stage. We had expected that com-

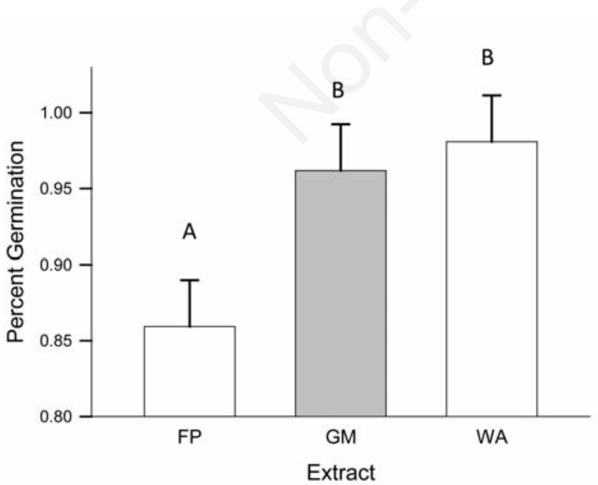

Figure 1. Percent germination as a function of seed extract. The seed extracts are *Brassica rapa* Fast Plants (FP), *Alliaria petiolata* - Garlic mustard (GM), or distilled water only (WA) controls. Bars with different letters indicate means significantly different from each other at P<0.05 based on planned orthogonal contrasts.

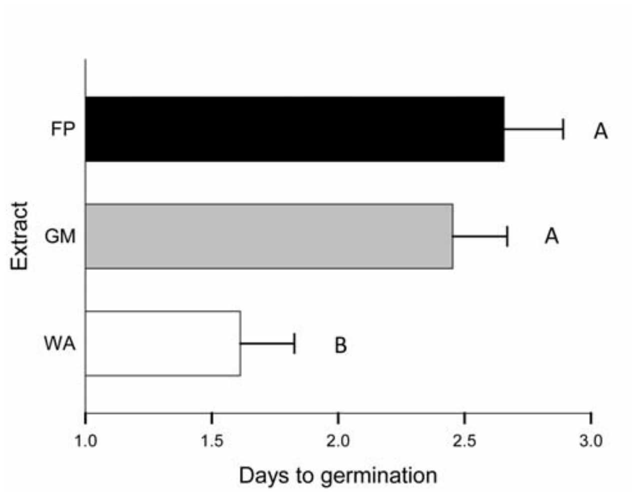

Figure 2. Days to germination as a function of seed extract. The seed extracts are *Brassica rapa* Fast Plants (FP), *Alliaria petiolata* - Garlic mustard (GM), or distilled water only (WA) controls. Bars with different letters indicate means significantly different from each other at P<0.05 based on planned orthogonal contrasts.

pounds in *B. rapa* Fast Plant seeds might enhance germination, since these have been selected for very rapid germination and the absence of dormancy.[10] However, the results showed that Fast Plant extracts inhibited germination. This result is consistent with previous findings that extracts of leaves, flowers, stems and roots of the related plant *Brassica nigra* impede germination of *Avena fatua*.[19]

The identity of the compounds that inhibited germination in this study remain unknown, since a chemical analysis was beyond the scope of this study. We can infer, however, that the compounds responsible must occur in the seeds and be water-soluble and stable at room temperature upon extraction. Many different compounds, including organic acids, quinines, coumarins, flavonoids, tannins, terpenoids, and phenolics can be allelopathic, with phenolics like –catechin especially implicated in allelopathy.[3,20] Future studies examining the chemical composition of the seed extracts as well as the effects of specific compounds in the extracts on seed germination would be useful.

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