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Allelopathic Leachates from Two Native Plant Species Reduce Germination Success and Alter Germination Timing of Two Invasive Plant Species

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Abstract

Globally invasive plants are responsible for major alterations to ecosystem structure and function, leading to a loss of biodiversity across systems. At the same time, some native plants utilize allelopathy as a means to compete with other native plants. The allelochemicals produced by these natives have been shown to reduce germination, growth, and development of other native plants. Yet, little is known about how invasive plants respond to exposure to native allelopathic compounds. In this study, the effects of three concentrations of leachates (1 g/L, 10 g/l, and 100 g/L) separately derived from dried leaf litter from two allelopathic plants native to New York State, black walnut (*Juglans nigra*) and giant goldenrod (*Solidago* gigantea) were tested on seeds from two plants considered invasive in New York State as well as in many areas within North America, Japanese stiltgrass (*Microstegium vimineum*) and garlic mustard (*Alliaria petiolata*). We found strong suppression of germination in garlic mustard across both native allelopathic treatments and at all three concentration levels. Japanese stiltgrass only showed significant declines in germination at the highest concentration levels for both native allelopathic leachates. Germination timing was also affected, with the greatest delay of germination for both garlic mustard and Japanese stiltgrass being observed primarily at the highest allelopathic concentrations (100g/L) for both of the native plant leachates. Overall, our findings suggest that in the preemergent stage, native allelopathy may have the potential to serve as a novel source for biocontrol agents for some invasive plant species.

INTRODUCTION

Invasive plant species are among the most powerful biological drivers of ecological change, as they can alter ecosystem structure, biological functions, and interactions among native species [1, 2]. The success of these species is often attributed to traits that facilitate ecological release, such as efficient and prolific reproduction, rapid growth, and tolerance of varying abiotic conditions. Ecological release describes the phenomenon in which an invasive species, freed from the limitations imposed by biotic interactions in its native range, rapidly expands in population size. For invasive plants, this release often means they can outcompete native plant species, particularly during germination and early establishment. [3]. Ecological release is commonly explained by the enemy release hypothesis, which proposes that introduced species may experience reduced pressure from specialist herbivores, pathogens, or competitors that regulate populations in their native ranges [3, 4].

However, not all non-native plants become invasive; those that do tend to possess traits that enhance competitive ability, including rapid growth, high reproductive output, phenotypic plasticity, and tolerance to disturbed environments [1, 5]. During early establishment, even small advantages in germination timing, seedling survival, or resource capture can strongly influence whether invasive plants become dominant within a community. These advantages may be further strengthened when invasive plants encounter native species that lack effective resistance mechanisms, or when invasive plants alter soil conditions, which can suppress neighboring plants, or change microbial communities in ways that favor invader establishment [6–8].

Current control methods for invasive plants often rely on mechanical removal or synthetic post-emergent herbicides. These processes are often ineffective, expensive, or hazardous to the surrounding environment [9], as broad-spectrum herbicides are non-selective and often remove desirable native vegetation alongside invasive species, potentially creating open niches that facilitate reinvasion [10]. Mechanical control techniques, such as hand harvesting, are costly and laborious. Generally, unless implemented repeatedly over long periods, harvesting will yield only temporary results, as seed banks and robust belowground structures (e.g., rhizomes of Reynoutria spp.) allow invasive species to reestablish [11]. Glyphosate is one of the most widely used herbicides globally, and glufosinate is a widely used broad-spectrum herbicide increasingly important for managing glyphosate-resistant weeds [12, 13]. Both herbicides are highly water-soluble, allowing them to travel long distances from the applied site via runoff to nearby waterways. Both can also leach into and contaminate groundwater [14]. Once introduced into an aquatic system, glyphosate can persist for weeks to months, remaining in sediments and posing a risk to aquatic organisms. [15]

Given these limitations with current control methods, there is growing interest in finding management strategies with less intensive impacts on the surrounding area. One potential mechanism is allelopathy, an ecological phenomenon in which plants produce chemicals that influence the germination, reproduction, or development of neighboring species. Allelochemicals can be released through root exudation, leaching from foliage, or decomposition of plant litter. Once in the environment, these compounds can alter membrane permeability, disrupt photosynthesis and respiration, or impair nutrient uptake [6, 7]. Allelopathic species can suppress competitors and influence patterns of plant succession and development in nearby areas. In fact, many invasive species have also been shown to possess allelopathic properties, enhancing their competitive ability. However, allelopathic compounds are also produced by several native plant species, and they are increasingly recognized as potential natural mechanism of biological control [16].

Black walnut (Juglans nigra) and giant goldenrod (Solidago gigantea), both native to New York State, exhibit well-documented allelopathic effects and serve as the focal species of this study. Black walnut (Juglans nigra) is one of the most widely recognized and well-documented allelopathic species, primarily due to its production of juglone, or 5-hydroxy-1,4-naphthoquinone, a naphthoquinone derivative concentrated in the leaves, fruit hulls, bark, and roots [17–19]. In living tissues, juglone occurs primarily in a reduced, non-toxic form known as hydrojuglone; however, once released into the soil environment and exposed to oxygen, hydrojuglone oxidizes into its toxic form. Juglone enters surrounding soils through root exudation, rainfall leaching from fallen leaves, and the decomposition of plant debris, where it can accumulate and exert phytotoxic effects on neighboring plants [17, 18]. When competing plant species are exposed to juglone, seed germination and seedling growth may be inhibited through disruption of cellular respiration, photosynthesis, membrane function, enzyme activity, and water uptake [20, 21]. These physiological disruptions can reduce plant establishment and contribute to black walnut's strong allelopathic influence in both natural and managed ecosystems.

In contrast, giant goldenrod (Solidago gigantea) exhibits a more diverse suite of allelopathic compounds, including phenolic acids, saponins, terpenoids, and flavonoids such as chlorogenic acid, rutin, quercitrin, and quercetin, which are released into the surrounding environment through root exudates and decomposing litter [22–26]. These compounds can suppress germination and early seedling growth of neighboring species by interfering with cell division, hormonal regulation during germination, root elongation, and nutrient uptake [23–25, 27]. In addition to direct phytotoxic effects, Solidago species may indirectly enhance competitive dominance by altering soil microbial communities and nutrient cycling processes, further reinforcing their allelopathic impact in invaded ecosystems [6, 8].

Although allelopathic compounds have known properties that can disrupt germination, growth, and development of native plant species, few studies have investigated their potential as biocontrol agents for invasive plants. Here, the effects of leachates derived from dried leaf litter of the native allelopathic plants, black walnut and giant goldenrod, on seeds from the invasive plants Japanese stiltgrass (*Microstegium vimineum*) and garlic mustard (*Alliaria petiolata*) were examined. Specifically, we conducted a controlled laboratory experiment in which seeds of both invasive species were exposed to aqueous leachates separately derived from black walnut and giant goldenrod leaf litter at varying concentrations, along with a distilled water control. Germination was monitored under standardized environmental conditions to quantify differences in germination rate and timing among treatments. This design allowed the effects of allelopathic sources and concentrations on early life-stage performance of invasive plants to be isolated, and the potential of native allelopathic species as an environmentally sustainable biological control agents for the preemergent invasive plant stage to be assessed.

METHODS

Seeds of Japanese stiltgrass (*Microstegium vimineum*) and garlic mustard (*Alliaria petiolata*), which henceforth will be referred to using common nomenclature, were respectively collected from established invasive populations in a public park and a private property within central New York, with permission from park authorities and landowners (Table 1). To potentially capture natural population-level variation, seeds were gathered from plants distributed across each site rather than from a single concentrated area.

After collection, all non-seed material and tissues were removed from seeds and they were subsequently stored under species-specific conditions until experimental use. Garlic mustard exhibits physiological dormancy [28]; therefore, before the start of the experiment, garlic mustard seeds were subjected to moist-cold stratification to break dormancy and promote synchronous germination. Seeds were stored for approximately three months at 4°C under moist conditions by placing them in sterile, damp paper towels within sealed containers to prevent desiccation throughout the stratification period. Seeds were monitored daily for initial signs of germination. Once dormancy break was observed, all seeds were evaluated to identify viable, non-germinated individuals for use in subsequent experimental trials. In contrast, Japanese stiltgrass seeds do not require stratification for germination and were therefore

stored dry at 4°C until the start of the experiment, without requiring any additional steps to break dormancy.

Allelopathic plant material was obtained from native plants, black walnut (Juglans nigra), and giant goldenrod (Solidago gigantea), which henceforth will be referred to using common nomenclature, at two sites in central New York (Table 1). As previously described, these species were selected due to their well-documented production of allelopathic compounds that can influence plant establishment and competitive dynamics [29]. Collection methods were designed to reflect the natural pathways through which these species release allelochemicals into the environment. For black walnut, dried leaves were collected from beneath mature trees, representing leaf litter that naturally decomposes and releases juglone into surrounding soils through leaching. For giant goldenrod, whole plants were harvested during daylight hours, with rhizomes intact, to capture both above and belowground tissues associated with allelopathic activity (Table 1).

Table 1

Collection locations of invasive plant seeds and native plant litter used in experimental treatments. Geographic coordinates (latitude, °N; longitude, °W) of collection sites for invasive species seeds, Japanese stiltgrass and garlic mustard, and native species leaf litter, black walnut and giant goldenrod.

Species Collected	Latitude (°N)	Longitude (°W)
Japanese stiltgrass *(Microstegium vimineum)*	42.46398	-75.05385
garlic mustard *(Alliaria petiolata)*	42.07637519404556,	-75.9139539759865
Black walnut *(Juglans nigra)*	42.461435	-75.070094
Giant goldenrod *(Solidago gigantea)*	42.722771	-74.78663

Giant goldenrod rhizomes were included because rhizome decomposition is a known pathway by which this species introduces allelopathic compounds into the soil. All native plant material was collected from private properties with landowner permission. Samples were collected from multiple individual plants to reduce bias arising from chemical variation among plants. Collected tissues were transported to the laboratory, air-dried at room temperature, and stored until aqueous leachates were prepared. This collection strategy was intended to approximate natural sources of allelochemical inputs while ensuring sufficient concentrations of inhibitory compounds for experimental treatments.

The germination experiment included seed species (Japanese stiltgrass or garlic mustard), allelopathic source species (black walnut or giant goldenrod), and allelopathic concentration (1 g/L, 10 g/L, or 100 g/L) as experimental variables. A control treatment consisting of distilled water was also included to provide a baseline for germination in the absence of allelopathic compounds. Leachates were prepared

by soaking the dried plant material in distilled water for seven days to achieve the target concentrations, allowing allelochemicals to diffuse into solution before application. The differing concentrations of leachate were created by soaking either 1, 10, or 100 grams of the dried plant material in 1 liter of water. After the seven days, the leachates were strained, and all solid matter was removed from the liquid with a fine-mesh strainer and cloth. For each treatment combination, twenty seeds were placed in a sterile 9.0 cm petri dish lined with Fisherbrand™ qualitative P8 filter paper circles, also 9.0 cm in diameter (Catalog no. 09-795C). Five milliliters of the appropriate leachate solution were added to each dish to moisten the filter paper and expose seeds to the treatment solution. Each treatment group consisted of twenty-five replicate Petri dishes, yielding twenty seeds × twenty-five replicates per treatment combination (Fig. 1).

Petri dishes were maintained underneath a thick covering to simulate underground conditions and germinated at room temperature throughout the germination trial. They were left on a level surface, covered with cardboard to prevent excess light from altering seed germination and to help replicate natural environments. Because garlic mustard has a relatively longer germination period than Japanese stiltgrass, moisture levels in the dishes were monitored regularly to prevent desiccation. To maintain consistent exposure to the treatment solutions, 1 mL of the appropriate solution was added to each dish every four days as needed to maintain moisture levels. Petri dishes were monitored daily for germination. A seed was considered germinated when the radicle had emerged to a length of at least 1 mm. The number of germinated seeds in each dish was recorded throughout the experiment, and the day of first germination was also noted to capture differences in germination timing across treatments. At the conclusion of the experiment, any seeds that had not germinated were subjected to a cut test to assess viability.

All statistical analyses for this chapter were performed using R Statistical Software [30]. Data were imported, cleaned, and organized using the packages readxl [31], readr [32], tidyverse [33], forcats [34], stringr [35], and tibble [36]. Germination responses were analyzed using generalized linear models and generalized linear mixed models fitted with the glmmTMB package [37]. Model diagnostics and assumption checks were conducted using the DHARMa [38] and performance [39] packages. Estimated marginal means, pairwise comparisons, and compact letter displays were generated using the emmeans [40], multcomp [41], and multcompView [42] packages. Figures were produced using ggplot2 [43], with additional formatting and figure assembly completed using patchwork [44], and scales [45]. Germination data were analyzed to evaluate the effects of seed species, leachate species, and concentration on both germination success and germination timing. Germination success was analyzed using a binomial generalized linear model (GLM) with a logit link function. The response variable was specified as the number of germinated and ungerminated seeds per dish using a two-column format. Fixed effects included seed species (Japanese stiltgrass, garlic mustard), leachate species (black walnut, goldenrod, water control), concentration (1, 10, 100 g/L), and all interaction terms among these factors. This model structure was used because germination was measured as a proportion of successes out of a fixed number of trials. Germination timing (days to first seed germination) was analyzed using a linear model (LM) with the same fixed effect structure (seed species, leachate species, concentration, and their interactions). Germination timing was treated as a continuous response variable. To further examine

species-specific responses, follow-up models were conducted separately for each species. For each species, germination success was analyzed using a binomial GLM, and germination timing was analyzed using a linear model, with leachate species, concentration, and their interaction as predictors. For binomial models, the significance of predictors was assessed using analysis of deviance with chi-square tests. For linear models, significance was evaluated using analysis of variance (ANOVA). When significant effects were detected, estimated marginal means were calculated, and pairwise comparisons were performed using Tukey-adjusted tests.

RESULTS

Germination success

Germination success was strongly influenced by seed species, leachate species, and concentration, with multiple significant interactions. The analysis of deviance for the binomial model showed significant main effects of seed species ($\chi^2 = 852.94$, $p < 0.001$), leachate species ($\chi^2 = 1252.58$, $p < 0.001$), and concentration ($\chi^2 = 781.54$, $p < 0.001$). Significant two-way interactions were detected for seed species × leachate species ($\chi^2 = 70.47$, $p < 0.001$), seed species × concentration ($\chi^2 = 52.17$, $p < 0.001$), and leachate species × concentration ($\chi^2 = 26.45$, $p < 0.001$). The three-way interaction among seed species, leachate species, and concentration was not significant ($\chi^2 = 4.57$, $p = 0.102$). (Table 2.)

For garlic mustard, germination was highest in the water control at 0 g/L (estimated probability = 0.978, 95% CI: 0.961–0.988). Germination declined sharply under all allelopathic leachates and generally decreased further as concentration increased. Under black walnut leachate, estimated germination probabilities were 0.233 at 1 g/L, 0.142 at 10 g/L, and 0.125 at 100 g/L. Under giant goldenrod leachate, estimated probabilities were 0.208 at 1 g/L, 0.155 at 10 g/L, and 0.082 at 100 g/L (Fig. 2). Therefore, germination was strongly suppressed by both leachates across all concentrations.

For Japanese stiltgrass, germination was also high in the water control at 0 g/L (0.972, 95% CI: 0.953–0.983). At low concentrations, Japanese stiltgrass maintained high germination under both black walnut and goldenrod leachates, with estimated probabilities of 0.944 and 0.950 at 1 g/L black walnut and giant goldenrod, respectively. At 10 g/L, germination remained high under giant goldenrod (0.954) but declined somewhat under black walnut (0.838). At 100 g/L, germination dropped markedly for both leachates, reaching 0.450 under black walnut and 0.444 under giant goldenrod (Fig. 2). Overall, Japanese stiltgrass was much more tolerant of low and moderate leachate concentrations than garlic mustard, but both species experienced reduced germination at the highest concentration (Table 3).

Table 2

Model effect results of seed species Japanese stiltgrass (Microstegium vimineum) and garlic mustard (Alliaria petiolata), under leachate species black walnut (Juglans nigra), giant goldenrod (Solidago gigantea), and water control, and concentration (0, 1, 10, and 100 g/L) on germination proportion and germination timing (days to first germination). Germination proportion was analyzed using a binomial generalized linear model, while germination timing was analyzed using a linear model. Test statistics are reported as χ^2 for germination proportion and F-values for germination timing. Significant effects ($p < 0.05$) indicate factors influencing germination success or germination rate across treatments.

Model Effect	df	Statistic	Test	p-value
Germination proportion				
Seed Species	1	χ^2	852.935	< 0.001
Leachate Species	2	χ^2	1252.579	< 0.001
Concentration	2	χ^2	781.542	< 0.001
Seed species x Leachate	2	χ^2	70.473	< 0.001
Seed species x Concentration	2	χ^2	52.173	< 0.001
Leachate x Concentration	2	χ^2	26.448	< 0.001
Seed Species x Leachate x Concentration	2	χ^2	4.569	0.1019
Germination Timing				
Seed Species	1	F	0.020	0.8886
Leachate Species	2	F	46.480	< 0.001
Concentration	2	F	133.796	< 0.001
Seed species x Leachate	2	F	50.539	< 0.001
Seed species x Concentration	2	F	46.699	< 0.001
Leachate x Concentration	2	F	3.484	0.0322
Seed Species x Leachate x Concentration	2	F	4.886	0.0083

Germination timing

Time to germination varied significantly with leachate species and concentration, but not with seed species alone. The linear model explained a substantial portion of variation in germination timing (R^2 = 0.613, adjusted R^2 = 0.594; overall model F = 31.7, $p < 0.001$). ANOVA showed significant main effects of leachate species ($F_{2,240} = 46.48$, $p < 0.001$) and concentration ($F_{2,240} = 133.80$, $p < 0.001$), but the main effect of seed species was not significant ($F_{1,240} = 0.020$, $p = 0.8886$). Significant interactions were detected for seed species × leachate species ($F_{2,240} = 50.54$, $p < 0.001$), seed species × concentration

($F_{2,240}$ = 46.70, $p < 0.001$), leachate species × concentration ($F_{2,240}$ = 3.48, $p = 0.0322$), and seed species × leachate species × concentration ($F_{1,240}$ = 4.89, $p = 0.0083$) (Table 2).

For garlic mustard, the fastest germination occurred in black walnut leachate at 10 g/L (mean = 2.09 days), followed by the water control at 0 g/L (3.24 days), black walnut leachate at 1 g/L (4.33 days), and giant goldenrod leachate at 10 g/L (4.90 days). The slowest germination occurred in black walnut leachate at 100 g/L (9.25 days) and giant goldenrod leachate at 1 g/L (6.23 days) (Table 3 and Fig. 3). Pairwise comparisons indicated many significant differences among these treatment combinations, including substantially later germination at 100 g/L black walnut leachate relative to lower-concentration treatments and the water control.

For Japanese stiltgrass, germination timing was relatively stable at low and moderate concentrations, with mean germination times of 4.00–4.80 days across water 0 g/L, black walnut leachate 1 g/L, giant goldenrod leachate 1 g/L, black walnut leachate 10 g/L, and giant goldenrod leachate 10 g/L. Germination was delayed at 100 g/L for both leachates, increasing to 6.04 days under black walnut leachate and 6.20 days under giant goldenrod leachate. Pairwise contrasts confirmed that 100 g/L treatments germinated significantly later than most lower-concentration treatments (Table 3 and Fig. 3). Taken together, the timing analysis indicates that both species showed treatment-dependent shifts in germination speed, but the pattern differed by species. Garlic mustard exhibited stronger and more variable timing responses across leachate types and concentrations, whereas Japanese stiltgrass was more resistant.

Table 3

Model-estimated germination proportion and germination timing (mean ± SE) for Japanese stiltgrass (Microstegium vimineum) and garlic mustard (Alliaria petiolata) across leachate species black walnut (Juglans nigra), giant goldenrod (Solidago gigantea), and water control. Germination proportion represents the predicted probability of seed germination, while germination timing represents the estimated mean number of days to first germination. Higher germination timing values indicate delayed germination under treatment conditions.

Species	Leachate	Concentration (g/L)	Germination proportion	SE	Germination timing (days)	SE
A. petiolata	*J. nigra*	1	0.233	0.024	4.333	0.262
A. petiolata	*J. nigra*	10	0.142	0.022	2.091	0.306
A. petiolata	*J. nigra*	100	0.125	0.037	9.250	0.507
A. petiolata	*S. gigantea*	1	0.208	0.025	6.231	0.281
A. petiolata	*S. gigantea*	10	0.155	0.026	4.900	0.320
A. petiolata	*S. gigantea*	100	0.082	0.018	9.273	0.306
A. petiolata	Water	0	0.978	0.007	3.240	0.203
M. vimineum	*J. nigra*	1	0.944	0.010	4.160	0.203
M. vimineum	*J. nigra*	10	0.838	0.016	4.320	0.203
M. vimineum	*J. nigra*	100	0.450	0.022	6.040	0.203
M. vimineum	*S. gigantea*	1	0.950	0.010	4.000	0.203
M. vimineum	*S. gigantea*	10	0.954	0.009	4.800	0.203
M. vimineum	*S. gigantea*	100	0.444	0.022	6.200	0.203
M. vimineum	Water	0	0.972	0.007	4.800	0.203

DISCUSSION

This study demonstrates that allelopathic leachates derived from native plant species can significantly influence the germination success and timing of invasive plants, but that these effects are species-

specific and concentration dependent. Germination responses differed markedly between garlic mustard and Japanese stiltgrass, indicating that susceptibility to allelochemicals is not uniform across invasive species and may be mediated by differences in seed physiology, dormancy mechanisms, and tolerance to chemical stress. This pattern is consistent with broader work showing that allelopathy can play an important role in plant invasion dynamics, but its effects vary substantially among species and ecological contexts [3, 6, 8].

The most pronounced effect observed was the strong suppression of germination in garlic mustard across all allelopathic treatments. Even at the lowest concentrations, both black walnut and giant goldenrod leachates substantially reduced germination probability relative to the control, with further declines at higher concentrations. This pattern suggests that garlic mustard is highly sensitive to allelochemicals, particularly juglone and phenolic compounds, which are known to disrupt cellular respiration, membrane integrity, hormonal regulation, enzyme activity, and water uptake during early developmental stages [7, 17–21]. However, prolonged or repeated exposure to allelopathic compounds may also create selective pressure favoring individuals with greater physiological tolerance or resistance. Over longer time scales, resistant individuals may persist and reproduce despite chemical inhibition, potentially resulting in populations with reduced sensitivity to allelochemicals. Such tolerance could arise through mechanisms including altered detoxification pathways, reduced membrane permeability, shifts in microbial associations, or changes in germination and growth timing that minimize exposure to phytotoxic compounds [3, 6, 8]. Consequently, while allelopathic suppression may initially reduce establishment and growth, long-term exposure could favor the survival of more tolerant individuals, potentially decreasing the effectiveness of allelopathic control strategies over time. More broadly, allelopathic compounds have been shown to influence germination and seedling performance across a wide range of systems, including litter- and extract-based studies in which inhibitory effects were strongest at higher concentrations [16, 29]. Although garlic mustard is widely recognized as a successful invader with vast ecological impacts, including effects on native diversity and belowground interactions, the results of this study indicate that it may remain vulnerable during germination when exposed to strong biochemical interference from neighboring species [46, 47].

In contrast, Japanese stiltgrass exhibited considerable tolerance to allelopathic leachates at low and moderate concentrations. Germination remained high under both leachates up to 10 g/L, with only a substantial decline at the highest concentration. This suggests that Japanese stiltgrass may possess either physiological tolerance mechanisms or reduced sensitivity to the compounds present in these leachates. However, differences in dormancy-breaking methods between species may also have contributed to variation in germination responses observed in this study. Unlike garlic mustard, which required an extended moist-cold stratification period prior to experimentation, Japanese stiltgrass seeds did not undergo the same prolonged dormancy treatment. Cold stratification can alter seed coat permeability, metabolic activity, and hormonal regulation associated with germination, potentially influencing seed responses to environmental stressors such as allelopathic compounds [48, 49]. Additionally, stratification has been shown to modify germination sensitivity and synchrony in many temperate plant species by regulating dormancy-related physiological pathways [50]. Therefore, species-

specific dormancy requirements and pretreatment conditions should also be considered when interpreting differences in allelopathic sensitivity between the two invasive species. Such tolerance may contribute to its invasive success, allowing it to establish in environments where allelopathic interactions might otherwise suppress less-resistant competitors. This interpretation is consistent with studies showing that Japanese stiltgrass can strongly affect invaded communities and suppress native vegetation and successional processes once established [51, 52]. However, the sharp decline in germination at 100 g/L indicates that even relatively tolerant species can be inhibited under sufficiently high allelochemical concentrations, suggesting a threshold-dependent response rather than complete resistance.

Germination timing further revealed contrasting strategies between the two species. Garlic mustard exhibited highly variable and treatment-dependent germination timing, with both accelerated and delayed germination under different conditions. The delayed germination observed at high concentrations likely reflects physiological stress and inhibition of metabolic processes, including disruptions to respiration, hormone regulation, and water or nutrient uptake [53]. In contrast, faster germination at certain intermediate treatments may indicate a hormetic or stress-induced response, in which low or sublethal allelochemical exposure stimulates germination or early growth. At the same time, higher concentrations become inhibitory [54]. In contrast, Japanese stiltgrass maintained relatively consistent germination timing across most treatments, with delays occurring only at the highest concentrations. This stability indicates that Japanese stiltgrass germination is less sensitive to environmental chemical variation, which may confer an advantage in heterogeneous or chemically complex environments. Such species-specific differences are in agreement with the broader allelopathy literature, which has repeatedly shown that the strength and direction of allelopathic effects depend on both the identity of the chemical donor and the biological traits of the target species [3, 8].

Together, these findings indicate that allelopathic effects are not universally suppressive but instead operate along a gradient influenced by species identity and chemical concentration. The differential sensitivity observed here has important ecological and management implications. While allelopathic compounds from native species may be effective in suppressing certain invasive plants, such as garlic mustard, they may be less effective against more tolerant species such as Japanese stiltgrass unless concentrations are sufficiently high. This highlights the importance of species-specific management strategies when considering allelopathy as a biological control mechanism, a conclusion that aligns with studies proposing allelopathy as a potentially useful but context-dependent component of invasive plant control [8, 55].

Several limitations should be considered when interpreting these results. The use of aqueous leachates in a controlled laboratory setting isolates chemical effects but does not fully replicate the natural conditions and complexities of field conditions. For example, in the soil, microbial degradation, adsorption to soil particles, litter composition and structure, and environmental variability can alter allelochemical availability and persistence [7, 16, 56]. In natural systems, invasive plants and their native plant neighbors also interact through changes in soil microbial communities and nutrient cycling. Taken

together, the responses we observed under laboratory conditions may differ from those expressed in the field [1, 56].

Laboratory allelopathy studies commonly use aqueous extract concentrations that are much higher than those expected in natural field conditions, as these studies are designed to isolate potential chemical effects under controlled exposure conditions. For example, previous studies on giant goldenrod have tested aqueous extracts at 1%, 5%, and 10%, which are approximately equivalent to 10, 50, and 100 g/L when expressed as a weight/volume extract concentration and reported stronger inhibitory effects at higher concentrations [57]. Similarly, Gala-Czekaj et al. [59] applied goldenrod aqueous extracts at 5% and 10% (approximately 50 and 100 g/L) and observed reductions in growth and biomass, indicating that these concentrations are commonly used in experimental allelopathy bioassays rather than as direct estimates of field exposure. By contrast, naturally occurring allelochemical concentrations in soils are often much lower and are usually reported on a soil-mass basis rather than as g/L leachate concentrations, making direct comparison difficult. For black walnut, field studies have measured juglone in soil and found that concentrations vary spatially with distance from the tree and decline with soil depth, with Jose and Gillespie [17] reporting that soil juglone decreased by as much as 80% as distance from the tree row increased to 4.25 m [17, 58]. Because these field values are typically reported as mass of juglone per mass of soil, they cannot be directly converted to g/L without additional information about soil bulk density, soil moisture, and extraction volume. Therefore, the leachate concentrations used in this study should be interpreted as experimentally relevant exposure levels that allow comparison among treatments, rather than as precise simulations of naturally occurring soil concentrations.

Overall, this study supports the hypothesis that native allelopathic species can influence the early life-stage performance of invasive plants. However, effectiveness may vary with concentration and species. In particular, the strong response of garlic mustard and the relative tolerance of Japanese stiltgrass at low leachate concentrations suggests that native plant-derived allelochemicals at environmentally relevant concentrations are more likely to function as species-specific suppressive agents. In contrast, at high concentrations, beyond what might be observed naturally, they might be considered universally effective control agents. Future research should focus on integrating these findings into field conditions and examining how soil processes, microbial communities, litter-mediated effects, and environmental variability influence allelopathic interactions during germination and establishment [16, 55].

CONCLUSION

Overall, these findings support the potential role of native allelopathic species as biological control agents during germination, particularly for species highly sensitive to chemical inhibition. However, the effectiveness may vary depending on species-specific tolerance and concentration thresholds. Future research is needed to examine additional native plant allelochemicals and to determine how the findings presented here translate to field conditions, where environmental factors and soil processes may alter the availability and effectiveness of allelochemicals as suppressive agents.

Declarations

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Not applicable.

Plant Ethics Declaration

All plant material for this study was obtained ethically from wild populations on privately or publicly owned land located at the coordinates provided in the methodology. All plant species were identified by the authors and collected with full landowner permission and following local guidelines. This was a lab-based study wherein all native plant material used in the lab was harvested from robust populations. Goldenrod and black walnut are two very common species in the study area and the harvesting process left roots intact. The invasive plant seeds from Japanese stiltgrass and garlic mustard were collected from established local populations where sexual reproduction was naturally occurring. No other plant material was collected aside from the seeds. At the conclusion of the research, any remaining invasive plant seeds were destroyed through a process of desiccation followed by bagged disposal in municipal landfill waste.

Consent for Publication

Not applicable.

Author Contributions

L.A.M.: Conceptualization, Methodology, Investigation, Formal Analysis, Data Curation, Visualization, Writing – original draft, Writing – review & editing.

M.A.K.: Conceptualization, Methodology, Resources, Supervision, Investigation, Formal Analysis, Writing – review & editing.

Conflict of Interest

The authors declare no conflict of interest.

Data Availability

The authors confirm that the data supporting the findings in this study are available within the article. Raw data that support the findings of this study are available from the corresponding author, upon reasonable request.

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Figures

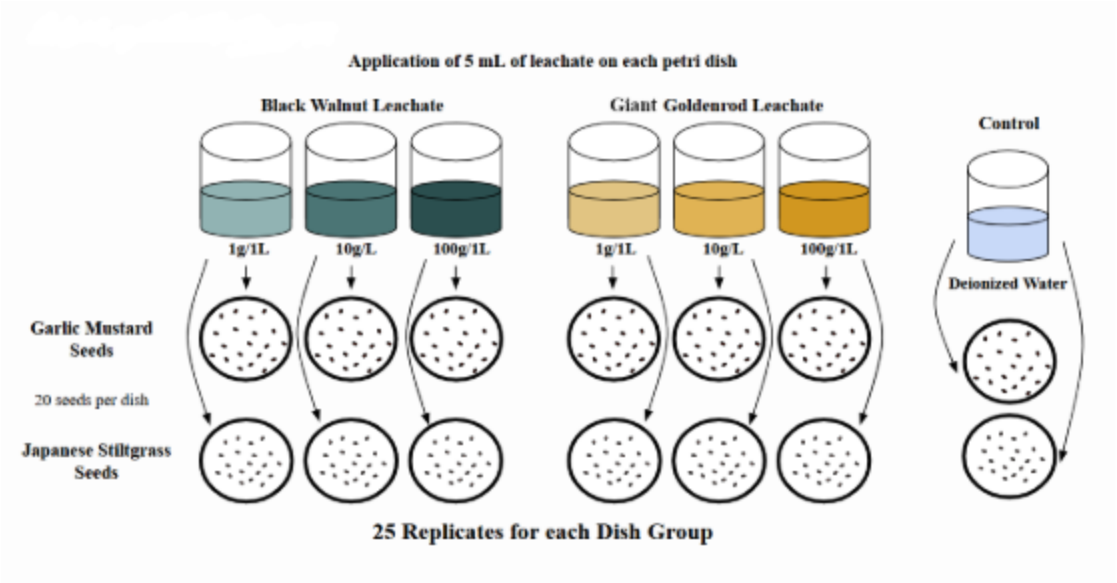

Figure 1

Experimental design for laboratory germination study. Petri dishes containing 20 seeds of either garlic mustard or Japanese stiltgrass were treated with 5 mL of solution. Treatments included black walnut leachate and giant goldenrod leachate at three concentrations (1, 10, and 100 g/L), as well as a deionized water control. Each treatment was applied to both species, and each dish group was replicated 25 times

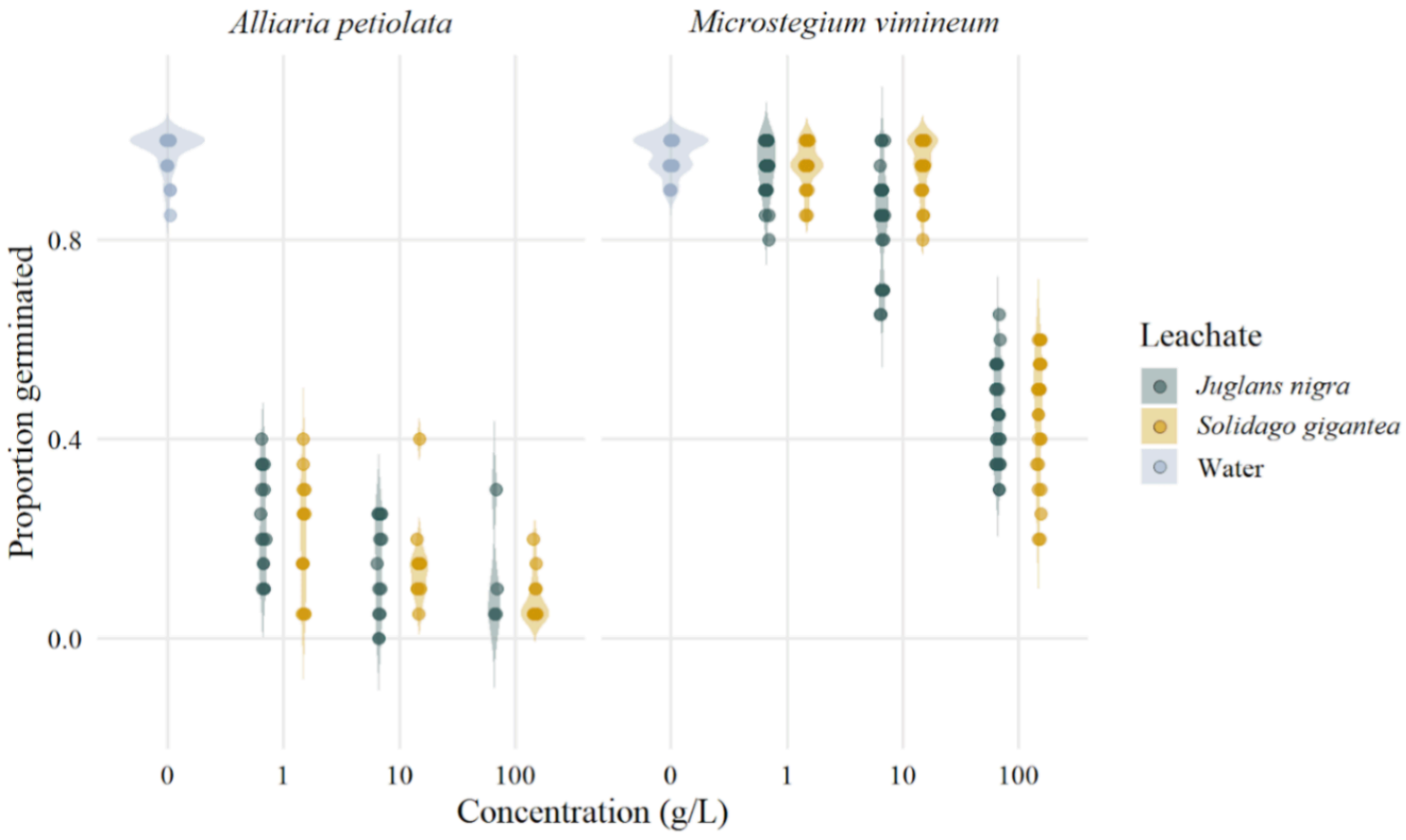

Figure 2

Proportion of seeds germinated for garlic mustard (Alliaria petiolata) and Japanese stiltgrass (Microstegium vimineum) exposed to water control (blue), black walnut (Juglans nigra) leachate (green), and giant goldenrod (Solidago gigantea) leachate (gold) across four concentrations (0, 1, 10, and 100 g/L). Points represent individual observations and violin plots show the distribution of germination proportions within each treatment group

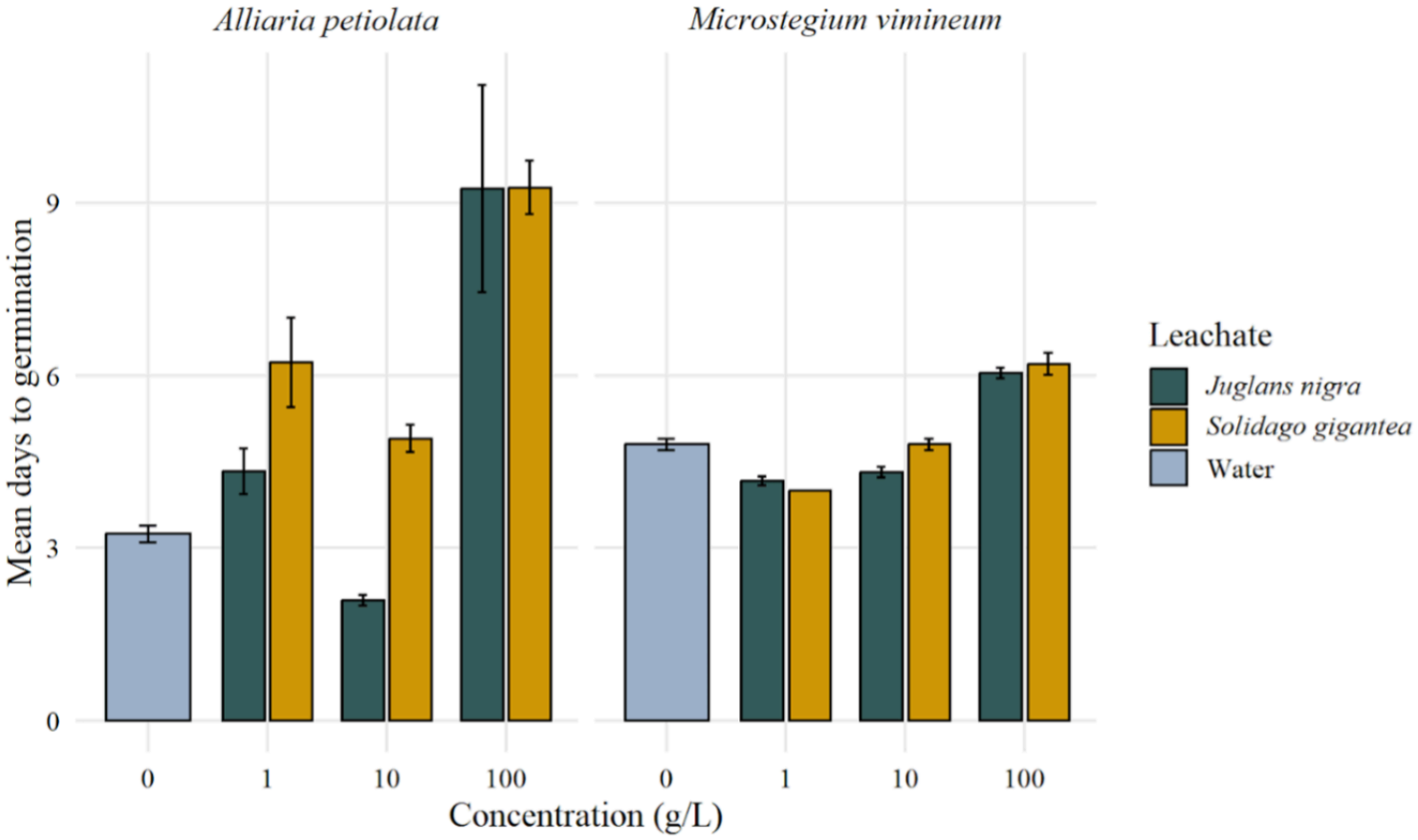

Figure 3

Bar graph showing mean days to first germination ±1 SE for garlic mustard (Alliaria petiolata) and Japanese stiltgrass (Microstegium vimineum) under water control (blue bars), black walnut (Juglans nigra) leachate (green bars), and giant goldenrod (Solidago gigantea) leachate (gold bars) across four concentrations (0, 1, 10, and 100 g/L)