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Experimental approaches to test allelopathy: A case study using the invader *Sapium sebiferum*

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ABSTRACT

Allelopathy occurs when plants release chemicals that inhibit neighboring plants. Invaders can have particularly effective allelochemicals which facilitate invasions. Allelopathy tests often compare the leaf extracts to water controls or employ activated carbon to neutralize allelochemicals. Each has limited power to detect the relative allelopathic effects of native versus exotic species. Here we use these approaches, combined with a new approach using interspecific leaf extract mixtures with all but one leaf extract treated with activated carbon, to test the allelopathic role of Chinese tallow tree (*Sapium sebiferum*) in invasions. We used foliar leaf extracts of *Sapium* and three native tree species and seeds of *Sapium*, two other exotic species and two native species. We measured seed germination and seedling growth. Results indicated that allelopathy does not contribute to *Sapium*'s invasive success. Effects of *Sapium* leaf extracts were within the range of native species of activated carbon on *Sapium* leaf extracts. The effect of non-carbon treated *Sapium* leaf extract was within the range for native species and statistically indistinguishable in the mixtures. Together these results demonstrate the variety of experimental designs that can be used to investigate allelopathy and invasions.

Keywords: Allelopathy, biological invasions, facilitation, inhibition, Novel Weapons Hypothesis, *Sapium sebiferum*

INTRODUCTION

Allelopathy, the effect of one plant on another by chemicals released into the environment (18), has been implicated as an important factor in the spread of invasive plants (19). This may be a result of unusually allelopathic successful invaders (Allelopathy Hypothesis), which are highly competitive in their introduced range (4,9), or invaders which release chemicals toxic to plants in the introduced range but not toxic to plants in their native range (Novel Weapons Hypothesis; 1,5). For example, the invasive *Centaurea diffusa* was more inhibitory to neighbours in its introduced North American range than neighbours in its native Eurasian range (4). Despite the existence of such striking cases, our knowledge of the importance of allelopathy in plant invasions is limited and may be hindered in part by experimental approaches that provide only indirect or weak information about the importance of allelopathy in determining invasive plant success.

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Traditionally, allelopathic experiments have tested the effects of leaf extracts on plant performance versus distilled water controls. This type of experimental design limits assessment of allelopathic potential because it is difficult to distinguish between potential positive resource or solute effects and negative effects of allelochemicals (23). The effect of allelochemicals may be offset by correlated, often antagonistic effects of other chemicals (12). For instance, a foliar leachate may elevate the concentration of nitrogen in solution compared to distilled water, increasing germination and growth (10,16), but simultaneously increase concentrations of allelochemicals that reduce germination and growth.

A slight improvement to such an experimental design would be one with a solute concentration gradient that allows for the visualization of allelopathic effects at increasing concentrations. We will refer to this type of design as a "dose response experiment." In such a design, allelopathic effects would be indicated by increasingly poorer performance of test species at increasingly higher concentrations of extracts. However, if resource and allelochemical effects are tightly correlated, a concentration gradient design may still do a poor job of estimating the magnitude of allelopathic effects.

Additionally, activated carbon absorbs many types of allelochemicals, providing a way to distinguish between resource and solute effects versus effects of allelochemicals (23). Allelopathy is indicated when addition of activated carbon to a foliar solution (or other materials) increases plant performance. We will refer to this type of design as a "neutralization experiment." It is possible that conclusions from a dose response experiment and a neutralization experiment differ in terms of the magnitude of allelopathy. For instance, in a case with strong positive resource effects and strong allelopathic effects, a dose response experiment would conclude that allelopathy is weak, whereas, application of activated carbon would cause a strong positive effect on plant performance. Such chemical facilitation may stimulate plant performance, especially germination. Regardless of the direction of effect, however, demonstrating a strong allelopathic effect on a single test species may help little in understanding the role of allelopathy in determining invasion success.

To understand the role of allelopathy in invasions, we need to know the relative strengths of allelopathic effects of exotic and native species on each other. A common way to do this is to use extracts of a number of species and test their effects on the performance of many species in a crossed design. If activated carbon is used, it may be possible to separate resource effects from allelopathic effects. If carbon is not used then only the net effect of resources and allelopathy can be determined. By examining the magnitudes of effects in different pairings of source (plant contributing allelochemicals) and response species (plant responding to allelochemicals), it is possible to infer the role of resources and allelopathy in facilitating or inhibiting invasions If there is a strong allelopathic effect of exotic plant extracts compared to native plant extracts on the performance of an assortment of exotic and native test species, allelopathy will facilitate initial invasion (Allelopathy Hypothesis). If there is only a strong allelopathic effect of an exotic plant on native species but not on exotic species from the same range, this would indicate that the novelty of the chemicals contributes to this effect (Novel Weapons Hypothesis; 5). In this case or one in which conspecific seedlings are weakly affected by allelochemicals compared to heterospecifics, allelopathy may facilitate invasion as well as contribute to the persistence of the exotic species via a positive feedback.

A simpler experimental design for determining the relative strengths of allelopathic effects of different species while controlling for resource effects is to use a mixture of extracts of different species, in which all but one species' extract has been treated with activated carbon ("mixture experiment"). This type of design has been frequently used to study plant -soil biota interactions (for example 2; 6). In this type of design, the relative strengths of allelopathy can simply be inferred by the performances of test species in mixtures with different species' extracts not treated with carbon. We do not know any study that has used this type of experimental design for the study of allelopathy despite the potential advantages in terms of making the link between allelopathy and community level effects.

To investigate the ability of different designs to determine allelopathy, we used Chinese tallow tree (*Sapium sebiferum* (L.) Roxb., Euphorbiaceae, synonyms include *Triadica sebifera*, "*Sapium*" hereafter) as a focal exotic species. This species is extremely invasive and has been reported in the popular press as strongly allelopathic;however, tests to date using a variety of dose response experimental designs have shown a mixture of negative, to neutral or facilitative effects of *Sapium* extracts. Therefore, it is an ideal species to examine the strengths and weaknesses of different experimental approaches to the study of allelopathy.

MATERIALS AND METHODS

Since its introduction from China in the late 1700's, *Sapium* has become naturalized across the southeastern United States (3). In Texas, *Sapium* has converted coastal prairies, mesic to hydric forests and wetlands into monospecific woodlands (3). To date, three studies have investigated the allelopathic potential of *Sapium*. Gresham (8) reported in a forestry proceeding that experiments conducted with "cold water leaf leachates of the tallowtree" produced "a significant reduction of loblolly pine germination and seedling growth" but he presented no data, no description of the experimental design, no statistics, no description of the methodology or even what baseline condition this reduction was being compared. But, together with the phenomenological observation that *Pinus taeda* saplings were rare, where *Sapium* saplings are abundant in a single *Pinus taeda* stand, he to concluded that *Sapium* is allelopathic. His *Sapium* study has never been formally published. Keay *et al.* (13) compared the germination and growth of little bluestem (*Schizachyrium scoparium* L.) in aqueous extracts of *Sapium* fresh foliage versus that in aqueous extracts of a mixture of fresh coastal prairie vegetation. They found that *Sapium* leaf extracts facilitated *Schizachyrium* growth, possibly due to resource effects. Conway *et al.* (7) found that *Sapium* foliage and litter and soil of *Sapium* stands had a range of effects from neutral to weakly positive on *Sapium*, bald cypress (*Taxodium distichum*) and black willow (*Salix nigra*) germination and growth compared to that in water controls.

Experimental species

Sapium seeds were collected in the spring of 2005 (Hardin County, Texas) and stored at room temperature until use. Prior to germination, seeds were washed with Alconox brand detergent and agitated for 2.5 h at room temperature to remove the waxy

seed covering. We bought seeds for King ranch bluestem (*Bothriochloa ischaemum* L.Keng, from Turner Seed Company, Breckenridge, TX, USA), little bluestem (*Schizachyrium scoparium*, from Turner), and gulf annual ryegrass seed (*Lolium multiflorum* Lam., from Pennington Seed Inc., Lebanon, OR, USA). Seeds were stored at room temperature until use. Sweetgum (*Liquidambar styracilflua* L.) seeds were obtained from Louisiana Forest Seed Co. (Lecompte, LA, USA) and stored at 4° C until use. For convenience, we will refer to the response species and leaf source species (below) by their genus names hereafter. *Sapium*, *Bothriochloa*, and *Lolium* are of Eurasian origin. *Schizachyrium* and *Liquidambar* are native to North America.

Experimental material

Leaves from *Liquidambar*, silver maple (*Acer saccharinum* L.), sycamore (*Platanus occidentalis* L.) and *Sapium* trees were hand collected from living trees in May 2005 from 20 year old monoculture plots, at the University of Houston Coastal Center, about 50 km southeast of Houston, Texas, USA (see 20,21). Leaves from each species were combined to form composite samples, placed in plastic bags, and refrigerated at 4° C for three days. *Acer*, *Liquidambar*, and *Platanus* are native to North America.

I. Neutralization Experiment

The experiment consisted of 3 factors: (i) leaf source (*Acer*, *Liquidambar*, *Platanus*, *Sapium*, or distilled water), (ii) Carbon (treated with carbon or no carbon treatment), and (iii) response species (*Bothriochloa*, *Liquidambar*, *Lolium*, *Schizachyrium*, or *Sapium*) with 5 replications (5 x 2 x 5 x 5=250 standard Petri dishes).

To make four types of leaf solutions, 50 g fresh leaves of each leaf source were suspended with clothes pins from a plastic test-tube basket. One liter of deionized water was slowly poured over the leaves and re-captured in a tray holding the test-tube basket until a noticeable color change was present in the solution. This is equivalent to 5% tissue concentration, common for plant extract bioassays (17, 22). The distilled water control solution was not poured over any leaves.

To distinguish resource effects from allelopathy, portions of each of the five leaf source solutions were treated with activated carbon. Two grams of finely powdered activated carbon (Carbochem, Philadelphia, PA, USA) per 100 mL leaf extract was added to the solution, manually stirred and then filtered though Whatman no. 2 filter paper. All solutions were stored at 4° C.

Soil texture is an important factor in plant-plant interactions and can influence the qualitative and quantitative availability of allelochemicals (11,12). We used an artificial soil mix containing 2:1:1 peat, perlite, and coarse sand by volume. In all three experiments, each Petri dish received 25 mL of this mix. Ten seeds of each response species were placed into randomly arranged Petri dishes. *Sapium* seeds were placed on heating mats and subjected to a heat cycling treatment of 8 h 32° C and 16 h 16° C to induce germination (15). All experiments were conducted in a 27° C laboratory with light from windows and full spectrum indoor lights.

In all experiments, dishes were treated identically in terms of solution added and data collection. Dishes initially received 25 mL of solution followed by 10 mL solutions three times per week, until two weeks after germination at which time seedlings were collected. *Sapium* dishes on heating mats received an additional 10 mL of deionized water

on alternate days to prevent desiccation. All other treatments received 25 mL deionized water once per week to prevent dryness. Germination rates were recorded daily. Cumulative germination was calculated as the total number of seeds that germinated in a dish regardless of survival. Germination timing was calculated as the average number of days from planting to germination for seeds that germinated in a dish. Two weeks after germination, the length of the primary root and shoot of each seedling was measured. Shoot and root growth rates were calculated as the mean of shoot or root length in a petri dish.

II. Dose response experiment

This experiment consisted of three factors: (i) leaf source (*Acer*, *Liquidambar*, *Platanus*, *Sapium*), (ii) extract concentration (1.25%, 2.5%, 3.75%, 5%) and (iii) response species (*Bothriochloa*, *Liquidambar*, *Lolium*, *Schizachyrium*, or *Sapium*) with 5 replications (4 x 4 x 5 x 5=400 dishes). The one-hundred dishes which received the 5% concentration extracts were the same ones used in the neutralization experiment.Solutions with 5% extract concentrationwere prepared as per the methods used in the neutralization experiment. Then solutions were diluted with distilled water to prepare the lower concentrations. Petri dish set-up and response variable data collection followed the protocols in the neutralization experiment.

III. Mixture experiment

This experiment consisted of 3 factors: (i) leaf preparation (rinse or soaking of leaves), (ii) leaf source (*Acer*, *Liquidambar*, *Platanus*, or *Sapium* untreated with carbon) and (iii)response species (*Bothriochloa*, *Liquidambar*, *Lolium*, *Schizachyrium*, or *Sapium*) with 5 replicates (2 x 4 x 5 x 5=200 dishes).

For the rinse method of preparation, 5% solutions were prepared using the same methods as the neutralization experiment. For the soak preparation method, 5% extract solutions were prepared by soaking 50 g leaves per 1 L of distilled water for 60 h at room temperature.

Each mixture was composed of leaf extract solutions from each of the four source species. Three of the solutions were treated with activated carbon following the methods in the neutralization experiment. The other solution was not treated with carbon. For example, a mixture designated as "*Sapium* leaf source" was composed of one quarter each of carbon treated *Acer*, *Liquidambar* and *Platanus* solutions and one quarter untreated *Sapium* solution. Presumably, only *Sapium* allelochemicals would be present in that solution as the activated carbon should bind potentially allelochemicals present in the other solutions.

Petri dish set-up and response variable data collection followed the protocols in the neutralization experiment.

Statistical Analysis

For the neutralization experiment a three-way ANOVA was used to analyze the effects of leaf source, carbon, and response species on cumulative germination using Statview version 5.0 (SAS Institute Inc. Cary, N.C., USA). Due to sparse germination of some species in some conditions, ANOVAs for germination timing, shoot growth and root growth did not include a three way interaction term. Data met the assumptions of

normality and homogeneity of variances. For all experiments, Fisher's LSD post hoc tests were used to test for differences among treatment levels for significant main effects. We used single leaf source type analyses to examine the effects of carbon treatments. Key terms for detecting allelopathy in this design are the carbon terms (overall allelopathic effect across leaf sources), leaf source by carbon interaction terms (overall allelopathic effect across leaf sources if water is different from leaves [Fig 1a] or variation in allelopathy among species if leaves vary [Fig 1b]), and leaf source by carbon by response species terms (variation in effect of allelochemicals depending on pairing of source and response species). Key terms for resource or solute effects are the leaf source terms and leaf source by response species interaction terms.

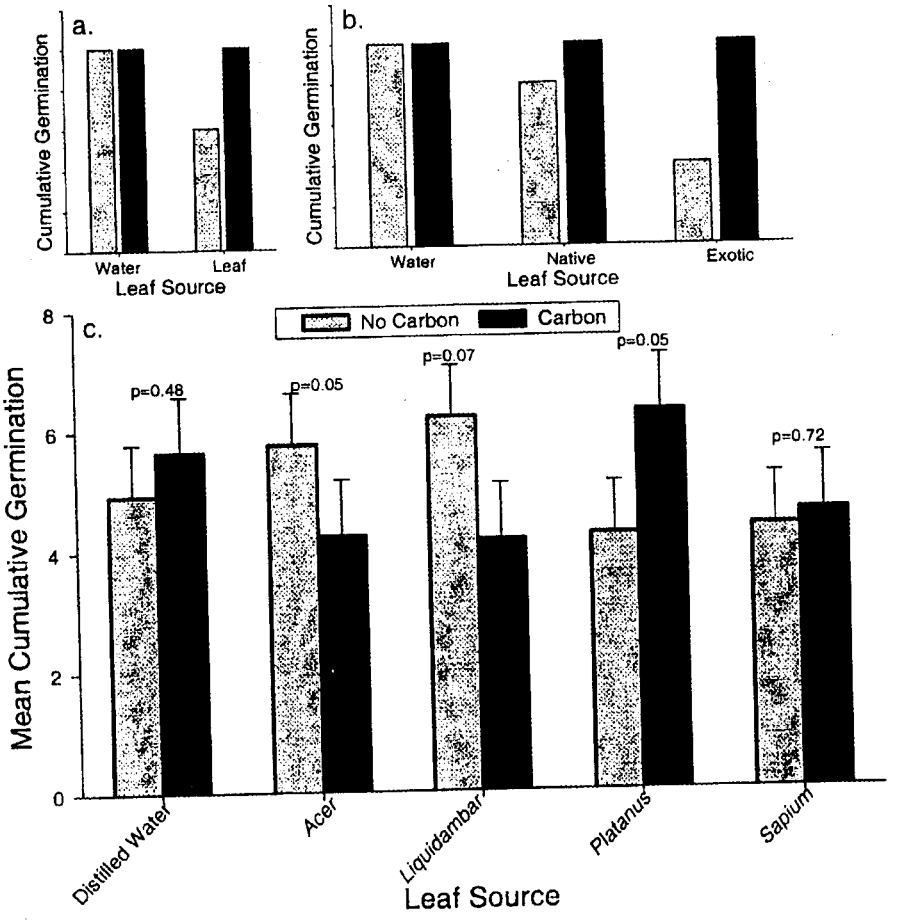

Figure. 1. Patterns of allelopathy in a neutralization experiment.

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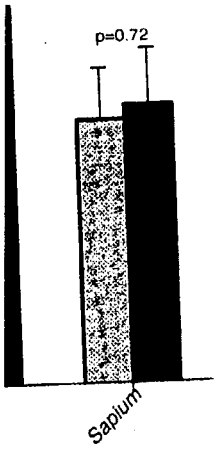

For the dose experiment a three-way ANOVA was used to analyze the effects of leaf source, concentration, and response species on cumulative germination using Statview. Because we could not assume linear relationships between concentration and response variables, concentration was treated as a four-level categorical variable. Due to sparse germination of some species in some conditions, ANOVAs for germination timing, shoot growth and root growth did not include a three way interaction term. Data met the assumptions of normality and homogeneity of variances.

For the mixture experiment a three-way ANOVA was used to analyze the effects of leaf preparation, leaf source, and response species on cumulative germination using Statview. Due to sparse germination of some species in some conditions, ANOVAs for germination timing, shoot growth and root growth did not include a three way interaction term. Data met the assumptions of normality and homogeneity of variances. The key term for detecting differences in the strengths of allelopathy for different species is the leaf source main effect. The leaf source by response species interaction term is the key term for detecting variation in effect of allelochemicals depending on pairing of source and response species.

RESULTS AND DISCUSSION

I. Neutralization experiment

Platanus had inhibitory allelopathic effects, *Acer* and *Liquidambar* had stimulatory effects and *Sapium* was neutral. Response species varied significantly in their germination rates, germination timing, shoot growth and root growth (Table 1). In general the grasses, especially *Lolium*, germinated quickly and at a high rate compared to the trees. Leaf source and carbon treatment were never significant as main effects. The interaction of leaf source and carbon had a significant effect on germination rates. This was mainly due to a significant allelopathic effect of *Platanus* (48% increase with carbon) and facilitative effects of *Acer* (24% decrease with carbon) and *Liquidambar* (33% decrease with carbon) (Figure 1c). Germination rates in *Sapium* solutions were on average slightly higher with carbon application (increase by 6%) but the magnitude of difference was smaller than that for carbon addition to water (increase 14%) and the effect was not significant (p=0.72). Root growth rates depended on the interaction of leaf source and response species but this was an apparently idiosyncratic result with *Schizachyrium* root growth unusually high in water and *Acer* solutions and *Sapium* root growth unusually high in *Platanus* and *Sapium* solutions.

II. Dose response experiment

Only *Platanus* showed a pattern consistent with allelopathy. Response species varied significantly for every response variable with the same qualitative differences among these species as in the neutralization experiment (Table 2). No other factor influenced germination rate. Leaf source significantly affected root growth rate with root growth greater in *Acer* and *Liquidambar* leaf extracts than in *Sapium* and *Platanus* leaf extracts (Table 2). The interaction of leaf source and response species was significant for

Table 1. Neutralization experiment ANOVA results showing the effects of leaf source (distilled water [dw], *Acer [A], Liquidambar[Li], Platanus [P], Sapium [Sa]*), activated carbon treatment (yes or no), response species ((*Lolium [Lo], Bothriochloa [B], Schizachyrium [Sc], Liquidambar[Li], Sapium [Sa]*), and their interactions on cumulative germination, days until maximum number of seeds germinated, mean shoot length, and mean root length.

Term	Germination Rate		Germination timing		Shoot growth rate		Root growth rate	
	Df	p-value	Df	p-value	df	p-value	df	p-value
Leaf Source	4	0.7304	4	0.9721	4	0.4565	4	0.4850
Carbon treatment	1	0.7884	1	0.7665	1	0.6997	1	0.4107
Response Species	4	**<0.0001**	4	**<0.0001**	**4**	**<0.0001**	**4**	**0.0003**
	Lo>B>Sc>Sa≥Li		*Lo≥B≥Sc>Li>Sa*		*Lo>Sc≥Li>Sa≥B*		*Sc≥Sa≥Li≥Lo>B*	
Leaf source x carbon treatment	4	**0.0140**	4	0.4443	4	0.5661	4	0.6947
	P>dw>Sa>0>A>Li							
Leaf source x response species	16	0.3730	16	0.9374	16	0.5659	16	**0.0438**
Carbon treatment x response species	4	0.5832	4	0.8745	4	0.7560	4	0.8529
Source x carbon x response species	16	0.3944						
Error df	200		179		148		148	
Model significance		**<0.0001**		**<0.0001**		**<0.0001**		**<0.0001**

Significant results are indicated in bold. Ordering of means and whether means were different in means contrast tests are shown for response species. For timing, larger means indicate faster germination. For the leaf source by carbon interaction term, the relative magnitude of the carbon effect is shown (no statistical information). Low germination for some species prevented inclusion of the three-way interaction term in some analyses.

Table 2. Dose response experiment ANOVA results showing the effects of leaf source (*Acer [A], Liquidambar[Li], Platanus [P], Sapium [Sa]*), concentration (0.125, 0.250, 0.375, 0.500) , response species ((*Lolium [Lo], Bothriochloa [B], Schizachyrium [Sc], Liquidambar[Li], Sapium [Sa]*), and their interactions on cumulative germination, days until maximum number of seeds germinated, mean shoot length, and mean root length.

Term	Germination Rate		Germination timing		Shoot growth rate		Root growth rate	
	Df	p-value	Df	p-value	df	p-value	df	p-value
Leaf Source	3	0.9841	3	0.2232	3	0.0802	**3**	**0.0031**
							A~Li>Sa~P	
Carbon treatment	3	0.8903	3	0.0990	3	0.2931	3	0.2140
Response Species	4	**<0.0001**	4	**<0.0001**	**4**	**<0.0001**	4	**0.0001**
	Lo>B>Sc≥Sa≥Li		*Lo≥B>Sc>Li>Sa*		*Lo>Sc≥Li>Sa≥B*		*Sc≥Sa≥Li≥Lo≥B*	
Leaf source x carbon treatment	9	0.2465	9	0.8676	9	0.3521	9	0.6435
Leaf source x response species	12	0.8434	12	0.2319	**12**	**0.0027**	**12**	**0.0021**
Carbon treatment x response species	12	0.9571	12	0.5700	12	0.6483	12	0.0149
Source x carbon x response species	36	0.3818						
Error df	320		302		231		231	
Model significance		**<0.0001**		**<0.0001**		**<0.0001**		**<0.0001**

Significant results are indicated in bold. Ordering of means and whether means were different in means contrast tests are shown for concentration and response species. For timing, larger means indicate faster germination. Low germination for some species prevented inclusion of the three-way interaction term in some analyses.

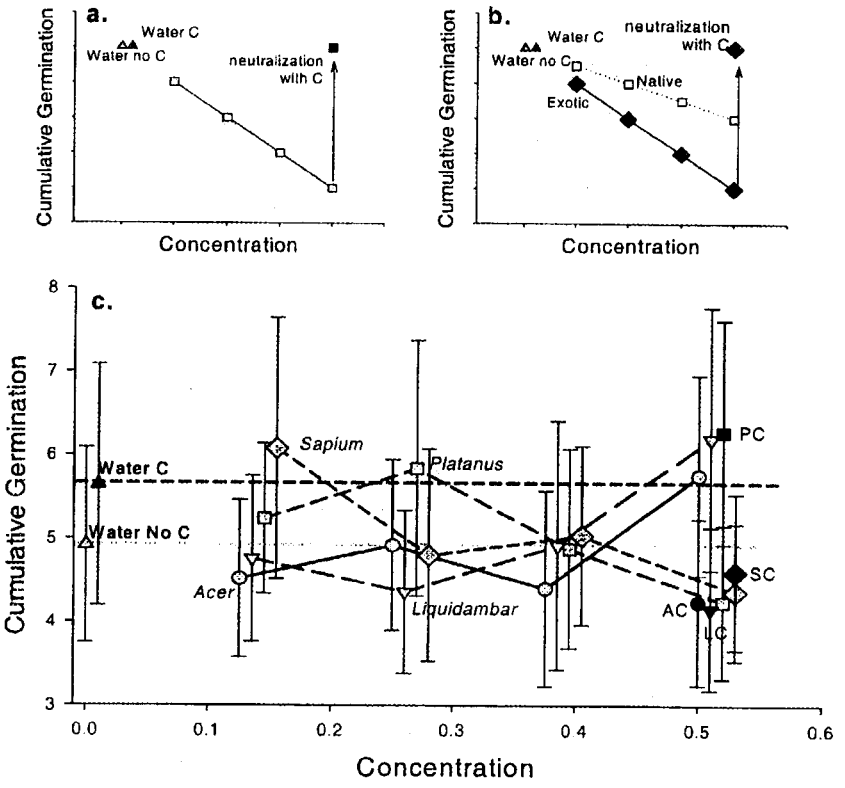

Figure. 2. Patterns of allelopathy in a dose response experiment.

both shoot and root growth. The faster shoot growth of *Schizachyrium* in *Acer* and *Platanus* leaf extracts and faster root growth of *Schizachyrium* in *Acer* leaf extracts appeared to be driving these results. Neither of these patterns is easily explained.

III. Mixture experiment

Leaf process affected germination rate and timing (Table 3) with soaked leaf extracts causing each to be lower compared to rinse leaf extracts (Figure 3a). Response species varied for each response variable with the same qualitative patterns as for the previous two experiments. Each interaction term had a significant effect on at least one response variable (Table 3). Only two of these terms are relevant to allelopathy studies. The interaction of leaf source and response species had a significant effect on root growth rate. The pairings of species that likely caused this result were rapid growth of *Schizachyrium* in *Acer* leaf extracts and of *Sapium* in *Liquidambar* leaf extracts

(distilled water [dw], *Acer* or no), response species , and their interactions on oot length, and mean root

rate	Root growth rate	
alue	df	p-value
4565	4	0.4850
6997	1	0.4107
0001	**4**	**0.0003**
Sa≥B	*Sc≥Sa≥Li≥Lo>B*	
5661	4	0.6947
5659	16	**0.0438**
7560	4	0.8529

	148	
.0001		**<0.0001**

different in means contrast ation. For the leaf source by tatistical information). Low some analyses.

f leaf source (*Acer [A]*,), 0.375, 0.500) , response *Li], Sapium [Sa]*), and their eds germinated, mean shoot

h rate	Root growth rate	
-value	df	p-value
0.0802	**3**	**0.0031**
		A~Li>Sa~P
0.2931	3	0.2140
0.0001	4	**0.0001**
>Sa≥B	*Sc≥Sa≥Li≥Lo≥B*	
0.3521	9	0.6435
0.0027	**12**	**0.0021**
0.6483	12	0.0149

	231	
0.0001		**<0.0001**

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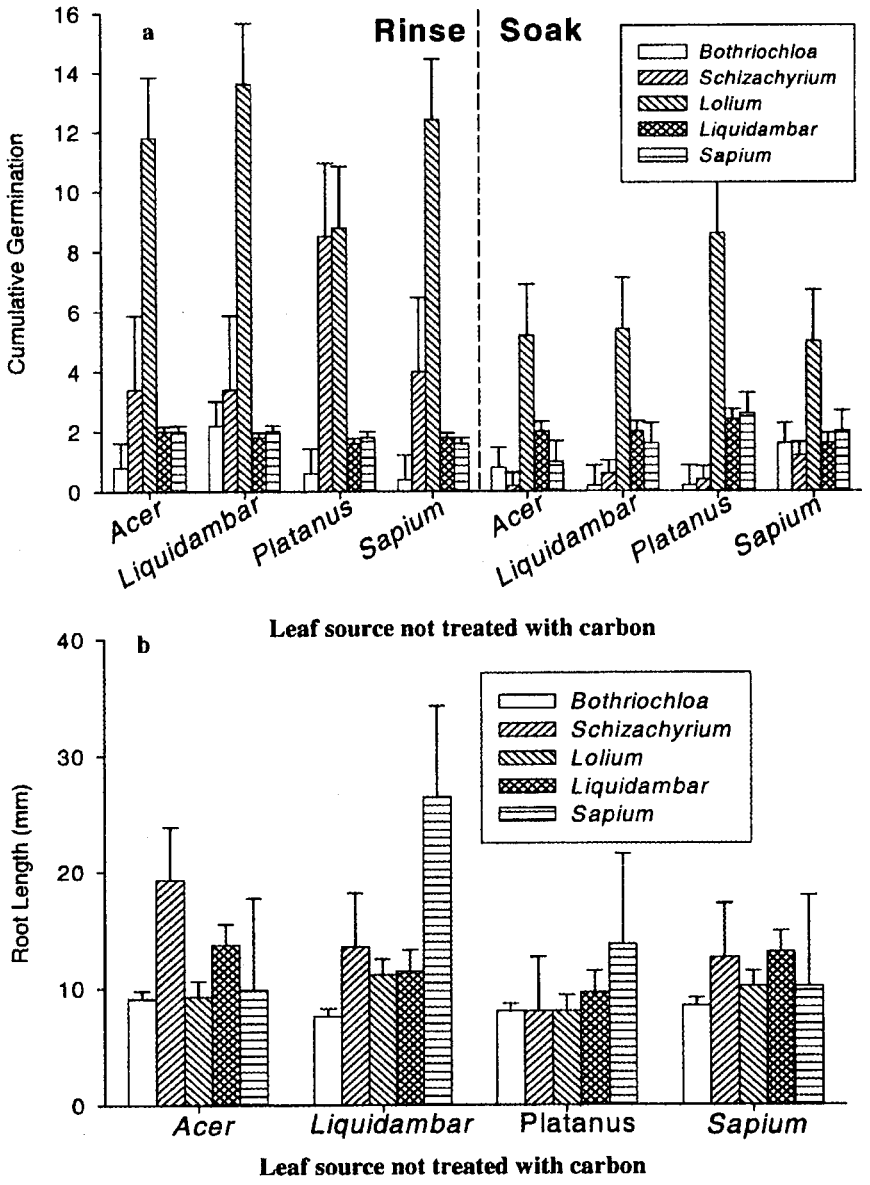


Figure. 3. Germination and root growth in the mixture experiment.

Table 3. Mixture experiment ANOVA results showing the effects of leaf process (soaking or rinse), leaf source (*Acer [A], Liquidambar[Li], Platanus [P], Sapium [Sa]*), response species ((*Lolium [Lo], Bothriochloa [B], Schizachyrium [Sc], Liquidambar[Li], Sapium [Sa]*), and their interactions on cumulative germination, days until maximum number of seeds germinated, mean shoot length, and mean root length.

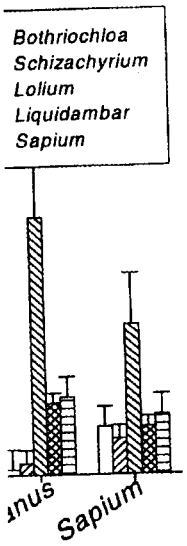
Term	Germination Rate		Germination timing		Shoot growth rate		Root growth rate	
	Df	p-value	Df	p-value	df	p-value	df	p-value
Leaf Source	**1**	**<0.0001**	**1**	**<0.0001**	1	0.6092	1	0.5988
Carbon treatment	3	0.5669	3	0.3142	3	0.8911	3	0.0742
Response Species	**4**	**<0.0001**	**4**	**<0.0001**	**4**	**<0.0001**	**4**	**<0.0001**
	Lo>B≥Li>Sa>Sc		*B≥Lo≥>Sc>Li>Sa*		*Lo>Sc≥Li>Sa≥B*		*Sc≥Sa>Li>Lo≥B*	
Leaf source x carbon treatment	3	0.0555	**3**	**0.0212**	3	0.1685	3	0.1766
Leaf source x response species	**4**	**<0.0001**	**4**	**0.0058**	**4**	**0.0003**	4	0.1828
Carbon treatment x response species	12	0.9145	12	0.0642	12	0.2350	**12**	**<0.0001**
Source x carbon x response species	**12**	**0.0208**						
Error df	160		125		100		100	
Model significance		**<0.0001**		**<0.0001**		**<0.0001**		**<0.0001**

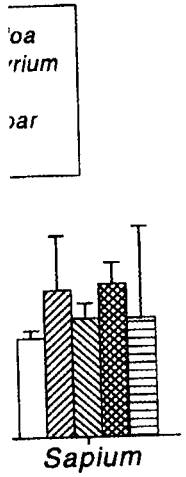
Significant results are indicated in bold. Ordering of means and whether means were different in means contrast tests are shown for concentration and response species. For timing, larger means indicate faster germination. Low germination for some species prevented inclusion of the three-way interaction term in some analyses.

(Figure 3b). Germination rate depended on the three-way interaction term with *Lolium* and *Schizachyrium* decreasing sharply in the soaked solutions compared to rinse solutions, especially for *Schizachyrium* in *Platanus* leaf extracts (Figure 3a).

CONCLUSIONS

Sapium leaf extracts did not have allelopathic effects on natives when tested in a variety of experimental designs. In the dose response experiment, *Sapium* leaf extracts had no net effect on germination or growth and were within the range of those of native species leaf extracts. Similarly, in the neutralization experiment, the effect of activated carbon on *Sapium* leaf extracts was within the range of responses seen for natives and comparable to water controls. Finally, in the mixture experiment the effect of non-carbon treated *Sapium* leaf extract was between that of *Platanus* and the other two native species but was statistically indistinguishable from them. This supports prior research indicating that *Sapium* does not have an allelopathic effect on native species in its introduced range (13;7). Furthermore, it indicates that the limitations of the approaches in these two studies did not cause allelopathy to be obscured by strong correlated non-allelopathic effects. However, the native tree species *Plantanus* appeared to have an allelopathic effect on mean germination rates and root length of plant species in the neutralization experiment (Figure 1) and on germination in the dose response experiment (Figure 2). These results are consistent with previous experiments which show the allelopathic effect of *Plantanus* on other understory plant species in lowland tree forests (14). However, these results do not explain the invasion success of *Sapium*.





The ability of a non-native species to invade new ecosystems may be determined by a variety of positive and negative feedback effects. In order to demonstrate that allelopathy contributes to invasion, response species performance should be lower and decrease with increasing concentrations of the invader's leaf extract compared with distilled water controls and should be rescued with activated carbon application. If an invader is predicted to have a stronger allelopathic effect than native species, the performance of response species should be much lower in invader compared to native leaf extracts without activated carbon and the rescue effect for response species performance should be much higher in invader compared to native leaf extracts with the application of activated carbon.

Alternatively, positive feedbacks are indicated when the response species performance is higher and increases at increasing concentrations of the invader's leaf extract compared with distilled water controls and should decrease with the application of activated carbon. In our experiment this can be seen with the tree species *Acer* and *Liquidambar*. Depending on the relative responses of species to such positive effects, this could also have an effect on the outcome of plant competition although it is due to a different mechanism than allelopathy. The variety of resource effects and allelopathic effects we observed in these experiments together with the potential for the independent effect of each to be obscured in traditional experimental approaches, reinforces the need for caution in designing experiments and using their results to infer the role of allelopathy in plant invasions.

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