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EFFECTS OF LITTER ON AQUATIC MACROPHYTE GERMINATION AND GROWTH

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ABSTRACT

In Arkansas, during seasonal drought periods, lake water levels drop leaving exposed mudflats which are primary sites for seedling establishment. Commonly, these exposed mudflats are partially covered with decomposing litter from previous stands of aquatic plants. In this study, aqueous leachates from the litter of four macrophytes, *Eleocharis quadrangulata, Justicia americana, Polygonum lapathifolium,* and *Potamogeton nodosus,* were used to test for chemical interference with germination and seedling growth. Only *Justicia americana* leachate was found to affect the growth of several of the test species. The potential for chemical interference by *Justicia* litter was evaluated further using extracts of acidified leachate. Resulting bioassays demonstrated significant effects on growth but not on seed germination. Bioassays of chromatographically separated fractions again revealed significant effects on seedling growth. These results suggest that interference by *Justicia americana* leachate on growth of seedlings is in part allelochemic.

INTRODUCTION

A central problem in plant ecology is determining how individuals interfere with each other's growth and reproduction (Barbour et al., 1980; Harper, 1978; Rice, 1974, 1979). There are various means by which interference may be mediated including the deposition of plant litter. The physical and chemical conditions associated with litter deposition, movement, and decomposition on saturated and/or submersed substrates are substantially different from those on terrestrial substrates (Ponnamperuna, 1972). In wetland habitats, the typically high rates of productivity (and litter production) combine with slow and incomplete decomposition to produce conditions conducive to both mechanical and chemical effects by litter on plant growth. A few studies have shown a potential for chemical interference by litter of certain aquatic and wetland macrophyte species (Bonasera et al., 1979; Szczepanska, 1977; Barko and Smart, 1983). These studies have shown that the litter from different plant parts can have different effects and that receiver species can differ in their responses to litter leachates. The objectives of the present study were: (1) to determine the potential for chemical interference on germination and growth by litter among four species of semi-aquatic plants common in northwest Arkansas, and (2) to characterize the chemical fractions of litter that could be responsible for any observed effects from the litter. The semi-aquatic species examined were *Eleocharis quadrangulata* (Michx.) R. & S., *Justicia americana* (L.) Vahl, *Polygonum lapathifolium* L., and *Potamogeton nodosus* Poir.

METHODS

General Bioassay of Plant Litter

The chemical effect of litter on growth was evaluated by applying leachate from dried aboveground material to plants growing in plastic pots. Leachate was obtained from living plants harvested in July 1982 from lakes Fayetteville and Elmdale in Washington County, Arkansas. Collected material was dired at 90° C for 48 hrs, cut into 5 cm segments, and leached in tubs containing tap water. The control treatment was a tub of tap water without the addition of plant material. The amount of dried material used in the tubs was based on preliminary studies to determine the mean dry weight / unit area of wetland surface for each of the four species. The ratios then were used to determine the amount of litter needed to provide leachate for pots used in each treatment. The concentration of litter used in each greenhouse tub was as follows: *E. quadrangulata,* 14.50; *J. americana,* 18.99; *P. lapathifolium,* 21.73; and *P. nodosus,* 4.65 gms/liter.

The total volume of litter and water in each tub was maintained at 14 liters. The leachate, remove daily to water recipient plants, was filtered through a nylon mesh to exclude coarse particulate material. Plants of the four species were established from rhizome segments, stolons, or stem fragments in six-inch plastic pots lined with plastic bags and filled with greenhouse soil (⅓ sand: ⅓ peat moss: ⅓ top soil). Propagules were selected to be of uniform size and grown in greenhouse conditions for several weeks before treatments were begun. Leachate from the four test species was used to water six plants of that species and six plants of each of the other three species. For controls, six plants of each species were watered with tap water from the control tub. Positions of pots on the greenhouse bench and treatments assigned were random.

Treated and control plants were harvested when weekly measurements of the control plant height growth began to level. Harvested plants were divided into roots and shoots and dried for 48 hours at 90° C. Any treatments showing significant differences from the controls were repeated with more replication and the ash-free weight was determined by drying plants for 48 hours at 90° C and then ashing them at 550° C for 24 hours.

Effects of *JUSTICIA* Leachate on Germination and Growth

Leachate was obtained from *Justicia americana* by soaking 6.65 grams of dried plant material in 280 ml of distilled water for 48 hours. This quantity of material was equivalent to the quantity of material used in the first set of experiments on a per pot basis. Leachate was suction filtered through Whatman #1 filter paper and diluted to 1.66, 3.31, 6.62, 13.53, and 27.66 gm/1 with osmotic potentials of −0.23, −0.31, −0.42, −0.58, −0.96, and −1.70 bars respectively. The pH of the leachate dilutions was not significantly different. Four milliliters of each dilution was added to petri plates which contained 50 seeds of either *Polygonum lapathifolium, Plantago psyllium,* or *Typha latifolia* on filter paper. The control treatment was 4 ml of distilled water added to seeds of each species. Three replicates were used for each species-dilution combination. *Polygonum lapathifolium* and *T. latifolia* were chosen because they are found in the same habitat as *Justicia americana* and because their seeds germinate readily. Seeds of *P. psyllium,* a terrestrial species, were used because of their rapid germination and because this species has been shown to be sensitive to allelochemics (Abdul-Wahab and Rice, 1967). *Polygonum lapathifolium* and *P. psyllium* seeds were germinated in the dark but *T. latifolia* seeds were germinated in the light due to a light requirement for germination. Seeds were considered to be germinated upon emergence of the radical. Growth in this and subsequent experiments was determined by measuring the seedling length from the tip of the radical to the tip of the shoot with a millimeter ruler.

A second experiment was conducted to compare the effects of mannitol on germination and growth with those of *Justicia* leachate. Mannitol was used to evaluate the osmotic effects of leachates by growing seeds in mannitol solutions having osmotic potentials similar to those found in the *Justicia* leachate (Del Moral and Cates, 1971). The dilutions had the following molarities: 0.0, 0.01, 0.25, 0.5, 1.0, and 2.0 with osmotic potentials of −0.15, −0.31, −0.66, −1.09 −2.14, and −4.29 bars, respectively. Since the pH of the varying dilutions was similar, no bioassay was used to determine the effect of pH on the growth and germination of the seedlings.

Chromatographic Separation of Inhibitors

Leachate, obtained from the germination and growth bioassays described above was, acidified to 1N HC1, refluxed for 1.5 hours, and extracted with 5 ml of diethyl ether per 25 ml of leachate. Each sample was extracted sixteen times with the optical density measured at 280 nm. A regression line with a 99% significant correlation, using extract number vs. optical density, indicated that virtually all desired material had been removed by the time of the sixteenth extraction. The extracted fractions then were combined and allowed to evaporate to dryness under a hood. A 25 ml volume of 95% ethanol was used to dissolve the residue to obtain the hydrolyzed extract.

As a preliminary test of inhibition, fifty seeds of *P. psyllium* were treated with 4 ml of water which had been added to different concentrations of the hydrolyzed extract. Four milliters of water was used as a control treatment. There were three replicates for each concentration of extract. The number of germinated seeds was counted after 1 week and the seedling size measured. Osmolarity of these different concentrations was determined by freezing point depression with an Advanced Wide Range Osmometer.

Once it had been established that inhibition did occur in the presence of different extract concentrations, 200 ul of the hydrolyzed extract were used to spot Whatman #1 chromatography paper. Material present in this quantity of hydrolyzed leachate is approximately equivalent to a concentration of 0.66 gm of dried leaf material per liter of the original leachate. Three different solvent systems were used to make descending chromatograms: isopropanol:butanol:water (70:10:20 v/v, IBW); 2% acetic acid, AA; and butanol:acetic acid:water (60:10:20 v/v, BAW). Once chromatographed, the chromatogram were divided into two groups. The first group was used in a bioassay to test for inhibition of growth and germination. To do this, chromatograms were divided into .1 Rf units. Each .1 Rf unit was placed in a petri plate, fifty seeds of either *P. lapathifolium* or P. psyllium were added along with 4 ml of distilled water, and the plates were placed in the dark. At the end of seven days, the number of seedlings was counted and the length of each was measured. The control consisted of chromatography paper that had been washed by one of the three solvents to which no hydrolyzed leachate had been added; otherwise, it was treated in the same manner as above. There were three replicates used for each of the ten .1 Rf units and the control. The second group of chromatograms was viewed under short- and long-wave ultraviolet light both in the presence and absence of concentrated ammonium hydroxide. The color of the spots under these conditions was noted and the Rf for each spot determined. There were six replicates for each solvent type. These characteristics were compared with 15 commonly reported allelochemics. The data in this and all the previous experiments were analyzed by using an analysis of variance procedure to compare treatment means with that of the control.

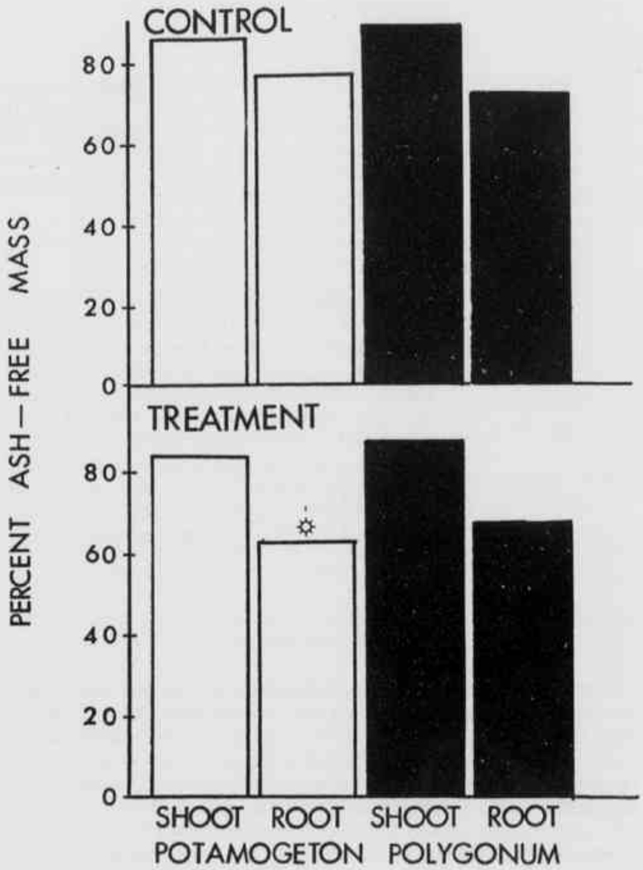

Figure 1. The effect of *Justicia americana* leachate on the growth of *Potamogeton nodosus* and *Polygonum lapathifolium*. Comparison of the mean percent ash-free mass of plants divided into root and shoot biomass. Root growth of *Potamogeton nodosus* was significantly different from control growth at p = .05 level, n=20.

RESULTS

General Bioassay of Plant Litter

Initial bioassay results showed *J. americana* leachate to significantly inhibit the root growth of *P. nodosus* and the shoot growth of *P. lapathifolium*. Leachate from any of the other plant species did not significantly affect the growth of any of the test plants. The second bioassay reexamined the effects of *J. americana* leachate on the growth of *P. nodosus* and *P. lapathifolium* using twenty replications. As shown in Figure 1, the ash-free, dry weight of *P. nodosus* roots was significantly reduced again though shoot weight was not significantly affected. There was no significant effect on the growth of *P. lapathifolium*.

Effect of *JUSTICIA* Leachate on Germination and Growth

Germination of *P. lapathifolium, P. psyllium,* and *T. latifolia* was not significantly affected by *Justicia* leachate. Only *Typha* germination was significantly reduced and then only at the highest osmotic potential with 22% germination compared to a control germination of 67%.

Seedling growth by *P. psyllium, P. lapathifolium,* and *T. latifolia* in the *Justicia* leachate and in the mannitol dilutions is indicated in Figure 2. Growth is expressed as mean percent difference from mean control growth.

$$\text{Mean Percent Difference} = \frac{\text{Sum}\left\{\dfrac{(\text{Mean control growth} - \text{Treatment growth})}{\text{Mean control growth}}\right\}}{N}$$

Negative values indicate a stimulation of growth while positive values indicate an inhibition of growth. Growth by *P. psyllium* was significantly inhibited at all osmolarities (Figure 2). None of the values for *P. psyllium* growth in mannitol solutions was significantly different from the control values. When the osmotic effect on growth is subtracted from the *Justicia* leachate effect, there is a slight increase in inhibition by the *Justicia* leachate. In the case of *P. lapathifolium*, values for the leachate were significantly different from the control at −0.31, −0.96, and −1.7 bars. All values for growth in mannitol were significantly different from that of the control. The osmotic potential of the mannitol solutions inhibited growth while *Justicia* leachate, at similar osmotic potentials, stimulated the growth of *Polygonum*. When osmotic effects were subtracted from effects of the *Justicia* leachate an even stronger stimulation of growth by the leachate was indicated. Growth by *T. latifolia* in *Justicia* leachate resulted in no significant differences from the control values. Values for growth in mannitol were significantly different from the control but only at −2.14 and −4.29 bars, far beyond the range of osmolarities encountered in the *Justicia* leachate. Subtracting the osmotic effects from the leachate effects brought the initial growth values even closer to control values.

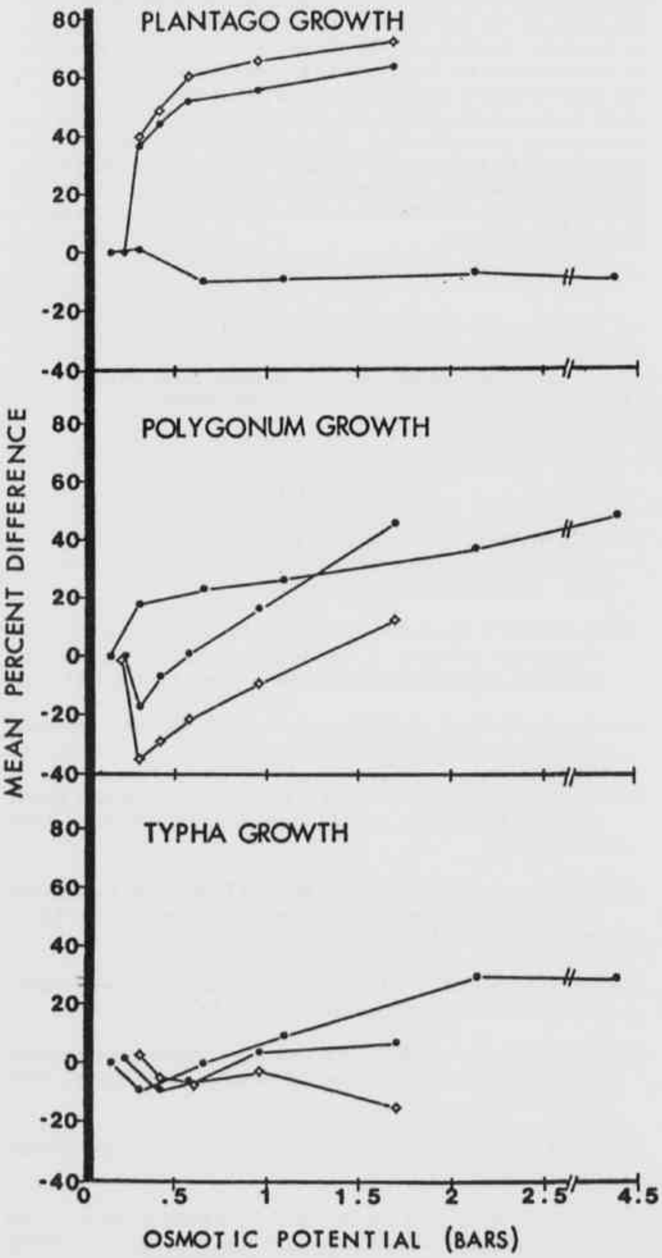

Figure 2. Comparison of *Plantago psyllium, Polygonum lapathifolium*, and *Typha latifolia* seedling growth when seedlings were germinated in *Justicia americana leachate and in mannitol solutions. Growth is expressed as mean percent differences from mean control growth,* * *growth in Mannitol,* ● growth in *Justicia* leachate, ◇ non-osmotic effect, difference between the osmotic curve and the leachate curve.

Chromatographic Separation of Inhibitors

Germination of *P. lapathifolium* or *P. psyllium* on any of the chromatography strips was not significantly affected at any of the .1 Rf subdivisions. Seedling growth of *P. lapathifolium* was not significantly affected by the hydrolyzed leachate that had been chromatographed in acetic acid (Figure 3). Values varied within 5% of the control mean. *P. psyllium* growth on the acetic acid chromatograph was similar to that of *P. lapathifolium* with no significant differences from the control mean (Figure 3). *P. lapathifolium* growth on the chromatogram run in the BAW solvent was significantly affected at rfs .21-.3 and .91-1.0. Growth at both of these rfs was inhibited by 11% and 17% respectively. *P. psyllium* growth was not significantly affected, but

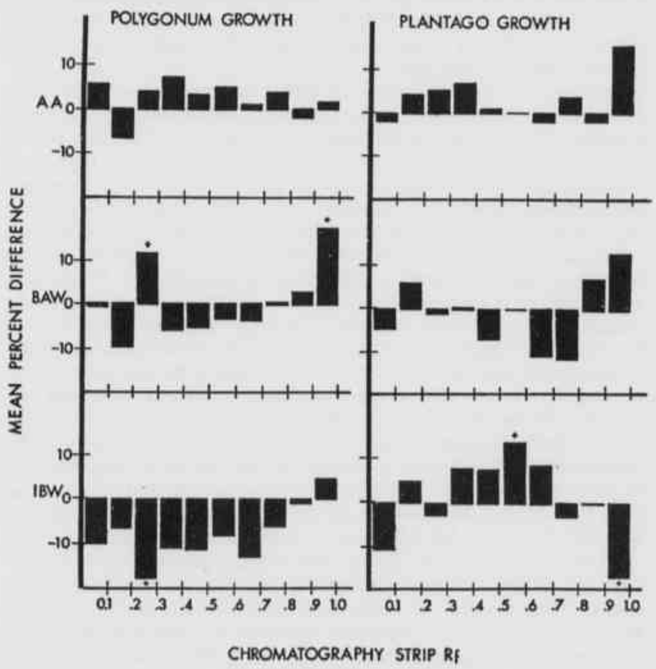

Figure 3. Growth by *Polygonum lapathifolium* and *Plantago psyllium* seedlings on chromatography strips divided into .1 Rf units. Growth is expressed as mean percent difference from mean control growth. AA = Acetic acid chromatogram; BAW = Butanol/Acetic acid/Water chromatogram; and IBW = Isopropanol/Butanol/Acetic acid chromatogram; + = significantly different from control values at p = .05 level.

growth by both *P. lapathifolium* and *P. psyllium* was affected by some of the Rf divisions on the IBW chromatography strip (Figure 3). *P. lapathifolium* growth was stimulated 17% above that of the control at Rf .21-.3 while *P. psyllium* growth was significantly inhibited by 13% at Rf .51-.6 and stimulated by 16% at Rf .91-1.0. Fluorescence characteristics and rf's of the hydrolyzed leachate spots chromatographed in the three solvents were not similar to any of the fifteen known allelochemics to which they were compared.

DISCUSSION

The effect by the *J. americana* litter leachate on the growth of *P. nodosus* indicates that there is a potential for chemical interference by *J. americana* litter on the growth of at least some plant species. Such an effect can result from several sources: 1) it can be purely allelochemic (Rice, 1974 and 1979); 2) it can result from some alteration of osmotic potential or pH (Del Moral and Cates, 1971); 3) it can result from a stimulation of microbial growth through the release of nutrients from the litter (Kimber, 1973); or 4) it can be a result of some combination of the above factors (Kimber, 1973). The observation that *Justicia* leachate inhibited the root growth of *P. nodosus* without affecting the shoot growth was surprising. If the leachate affected nutrient availability through the stimulation of soil microbial growth with a consequent reduction in nutrient availability, a stimulation of root growth rather than decreased root growth would be expected (Chapin, 1980). If *Potamogeton* could absorb nutrients from the water column, it would possibly explain reduced root growth. However, if this were the case the leachate in effect would be acting as a fertilizer through the addition of nutrients, and if the leachate were acting as a fertilizer why was there not an increase in shoot biomass along with the consequent decrease in root biomass? The presence of an allelochemic, inappropriate pH, or osmotic potential would be predicted to affect growth through a decrease in both shoot and root growth (Alsaadawi and Rice, 1982; Abdul-Wahab and Rice, 1967; Barko and Smart, 1983). The results clearly do not fit any of these possibilities and no clear interpretations can be offered at this time.

Because *Justicia* leachate had no effect on germination and growth of *T. latifolia*, there is not evidence that the *Justicia* leachate represents a source of chemical interference for this species. The effect of the *Justicia* leachate on growth of the other two species does indicate a potential source of chemical interference. In the case of *Plantago psyllium* osmotic effects on growth do not appear to be involved; thus, the effects on growth appear to be due to allelochemics (pH effects are ruled out due to non-significant differences between the dilutions). The case for *P. lapathifolium* is somewhat more complicated. *Polygonum* growth in the presence of the *Justicia* leachate was stimulated while mannitol at the same osmotic potential inhibited growth. We hypothesize, therefore, the osmotic effects were acting to negate the stimulatory effects of the leachate. That those solutions with similar pH had significantly different growth indicates an allelochemic was present. In addition, the growth by *Plantago psyllium* was inhibited in dilutions of the hydrolyzed extract of the leachate, strongly suggesting the presence of an allelochemic. In view of the fact that the osmotic potential of these dilutions could not affect *Plantago* growth and the fact that the pH of all the dilutions did not differ significantly, both osmotic and pH effects can be dismissed as contributing to these results.

The growth results for *Plantago* and *Polygonum* seedlings during the bioassay of the different chromatographs do not entirely substantiate the allelochemic hypothesis. For example, in the case of the acetic acid chromatographs no significant inhibition or stimulation of growth was found while significant affects did occur for the chromatograms run in the other two solvents. Similar results were found by Murthy and Nagodra (1977) who reported the inhibition of *Rhizobium* in only one of the four solvent systems they used. They did not address why this sould occur. It is noteworthy that, for our study, the maximum separation of compounds occurred in the acetic acid solvent. It is possible that the lack of significant effects on growth by the acetic acid chromatogram is due to the separation of potentially inhibitory compounds that have acted synergistically. Synergism between allelochemics has been documented in a few instances (Wilson and Rice, 1968; Rasmussen and Einhellig, 1977 and 1979). However, this synergistic hypothesis should be viewed with caution since it is dependent upon comparability of the migration of chemicals in the different solvents. Without identification of the chemicals on each of the chromatographs, an absolute comparison of the migration characteristics is impossible.

As a whole, litter from most of the plants used in this study *(E. quadrangulata, P. lapathifolium,* and *P. nodosus)* did not demonstrate any potential for chemical interference, indicating that allelochemic interactions may be uncommon among semi-aquatic plants. Nonetheless, the results for the *Justicia* litter indicate an allelochemic component to that plant's interference. The allelochemic effect by the *Justicia* litter did not simply act to prevent the growth of the susceptible species but either reduced or stimulated it. These facts coupled with the effect of the leachate being greater on seedlings, when susceptible, than on other types of propagules (e.g., *P. lapathifolium* seedling growth was stimulated by the leachate while the same leachate had no effect on cuttings) suggests that the effectiveness of the allelochemic component of *Justicia americana* litter may be highly dependent on both the species and age of neighbors.

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