l could not be accounted for by differences in water quality (light, temperature, nutrients) between the ponds (Table 1). While the split treatment conducted in Pond 2 initially produced a sustained high concentration, the split treatment did not prolong the longevity of the herbicide since the time required for it to decline to a concentration of 1 ppb was nearly the same in both ponds (161 days, Pond 1; 167 days, Pond 2) Figure 1.

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# Allelopathic Potential of Sixteen Aquatic and Wetland Plants


STELLA D. ELAKOVICH AND JEAN W. WOOTEN[1]



## ABSTRACT

Aqueous extracts of 17 selected hydrophytes were tested for allelopathic activity using lettuce seedling and duckweed assay systems. Results from the lettuce seedling method were: all plant extracts showed statistically significant growth inhibition at the highest extract concentration tested; 15 extracts showed inhibition at the mid concentration tested; six extracts showed inhibition at the lowest concentration tested. Duckweed frond growth was inhibited by 12 of the 16 plant extracts at the highest extract concentration and by five of 16 at the mid concentration. Comparisons of the six most inhibitory plant extracts showed three of the six are common to both assays.



*Key words:* Duckweed, lettuce seedling assay, growth inhibition, hydrophytes, aqueous extracts, bioassays, chlorophyll.


## INTRODUCTION

Allelopathy plays a role in determining the distribution and growth of higher plants (Rice, 1979). There are numerous reports of the allelopathic interactions of terrestrial plants, but much less is known about these relationships among hydrophytes. Dwarf spikerush plants have frequently been reported as being allelopathic Yeo, 1980; Frank and Dechoretz, 1980; Yeo and Thurston, 1984; Ashton *et al.*, 1985; Nichols and Shaw, 1983) as has cattail (Szczepanski, 1977; Bonasera *et al.*, 1979; McNaughton, 1968; Szczepanska, 1971) (See Table 1 for listings of common and scientific names). Some publications report allelopathic activity based on field observations (dwarf spikerush and spikerush) (Nichols and Shaw, 1983) while others are based on plant extract activity (dwarf spikerush, water shield) (Frank and Dechoretz, 1980; Ashton *et al.*, 1985; Elakovich and Wooten, 1987) making it difficult to draw conclusions as to the most promising allelopathic hydrophytes. Szczepanska (1971) investigated the allelopathic interactions of roseacane, cattail, bullrush, and horsetail. She found plant production different in mixed cultures than in monospecific cultures, but the extent of influence


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Table 1. Common and Scientific Names of Species. The plant numbers are used to identify species in the figures.

| Plant Number | Name | |
|---|---|---|
| | Common | Scientific |
| 1 | Water shield | Brasenia schreberi Gmel. |
| 2 | Fanwort | Cabomba caroliniana Gray |
| 3 | Coontail | Ceratophyllum demersum L. |
| 4 | Least spikerush | Eleocharis acicularis (L.) R.&S. |
| 5 | Blunt spikerush | Eleocharis obtusa (Willd.) Schultes |
| 6 | Hydrilla | Hydrilla verticillata (L.f.) Royle |
| 7 | Creeping rush | Juncus repens Michx. |
| 8 | Frogbit | Limnobium spongia (Bosc.) Steud. |
| 9 | Parrot-feather | Myriophyllum aquaticum (Vell.) Verdc. |
| 10 | Eurasian water-milfoil | Myriophyllum spicatum L. |
| 11 | Southern naiad | Najas guadalupensis (Spreng.) Magus |
| 12 | Fragrant water-lily (leaves + petioles) | Nymphaea odorata Ait. |
| 13 | Fragrant water-lily (rhizomes + roots) | Nymphaea odorata Ait. |
| 14 | Little floating-heart | Nymphoides cordata (Ell.) |
| 15 | Leafy pondweed | Potamogeton foliosus Raf. |
| 16 | American burreed | Sparganium americanum Nutt. |
| 17 | Eelgrass | Vallisneria americana Michx. |
| | Dwarf spikerush | Eleocharis coloradoensis (Britt.) Gilley |
| | Cattail | Typha latifolia L. |
| | Small spikerush | Eleocharis parvula (R. & S.) Link |
| | Roseacane | Phragmites australis (Cav.) Trin. ex Steud. |
| | Soft-stem bulrush | Schoenoplectus lacustris (L.) Palla |
| | Horsetail | Equisetum limosum L. |
| | Sedge | Carex hudsonii Bennett |
| | Lettuce | Lactuca sativa L. var. black seeded Simpson |

of one plant on another depended on soil type. A 4-year study of interactions between roseacane and a sedge showed that the sedge competed successfully with the reed and suggested that the frequently observed field succession of sedge communities displacing rush communities is, at least in part, allelopathic in nature (Szczepanska, 1977). Szczepanska (1977) suggested the use of allelopathy to control water weeds. The allelopathic nature of hydrilla toward coontail was investigated by Kulshreshta and Gopal (1983).

This study was initiated with the objective of screening 16 hydrophytes for allelopathic activity. Two bioassays were used to compare the results and to provide data for comparisons with information in the literature.

## MATERIALS AND METHODS

Plant species selected for allelopathic activity testing are listed in Table 1. Field collected, fresh plants were washed free of debris and drained of excess water. In all cases except one, entire plants, including roots were extracted. Fragrant water-lily plants were divided into two portions: leaves and petioles; rhizomes and roots. These two portions were treated as separate samples in all assays. A 200 g fresh weight aliquot of each sample was blended with 200 ml of distilled, deionized water, and the resulting pulpy mixture was refrigerated for 24 to 72 hours. The mixture was filtered through cheese cloth to remove the majority of the celluosic material, through filter paper to remove smaller particulate matter, and finally through a 0.45 μm millipore filter to render the solution sterile. The sterile solution was either assayed immediately or stored frozen.

In the lettuce seedling bioassay, three plant extract concentrations were used for each sample: 0 (control), 25, 125, and 250 parts per thousand (ppt) of extract per test plate. These concentrations were selected to be in the same range as those of published data (Cheng and Riemer, 1988). Each test plate contained 30 ml of 0.5% agar and the appropriate amount of distilled water. Osmotic potentials and pH of the extracts were not adjusted since Cheng and Reimer (1988) have shown that osmotic potentials of less than 70 milliosmols per kilogram and pH of plant extracts have no effect on lettuce growth. The controls contained 30 ml of 0.5% agar and 10 ml of distilled water. Tests were run in 9 cm disposable sterile Petri dishes. Lettuce seeds were germinated on 0.5% agar in a growth chamber set at 22 C, 16 hour days and 18 C, 8 hour nights. Germinated seedlings were transferred to the prepared Petri dishes and were incubated under the same light and temperature conditions for three to four days or until the control plate (no extract) showed good growth. Each extract was assayed in two replicate Petri dishes of 20 seedlings each. The lengths of lettuce seedling radicles (primary roots) were measured to the nearest millimeter. Replicate assay results were combined and percentages of the controls were calculated.

Common duckweed (strain 5), obtained from Dr. Gerald R. Leather, USDA, ARS, Frederick, Maryland, was cultured with axenic techniques. Plants were grown in E medium, a complete medium containing inorganic macro- and micro-nutrients, the organic tartaric acid, and EDTA as a chelating agent (personal communication, Dr. G. R. Leather). Instructions for preparation of this medium are available from the authors.

To 1.5 ml of E medium in 24-well, sterile, disposable tissue culture dishes were added aqueous plant extracts and distilled water to achieve concentrations of 0 (control), 20, 100, and 200 ppt of plant extract in a design which ensured that each treatment occupied one corner and three edge wells. Concentrations were selected to allow comparison with the lettuce seedling assay. Each extract concentration was tested twice (2 replicates) using six wells per trial (n = 6 per replicate) according to the method of Einhellig *et al.* (1985). The E medium provided an excess of nutrients; pH of the aqueous extracts was not adjusted as common duckweed growth is not sensitive to pH in the range of 4.0 to 6.0 (Einhellig *et al.*, 1985).

Counted fronds were placed in a small test tube and 1.5 ml of 95% ethanol was added to extract chlorophyll. The tubes were allowed to stand at room temperature for 24 hours or refrigerated for 48 to 72 hours at which time 1.5 ml of water was added to each tube. The fronds were removed, air dried, stored in a dessicator for at least two

days, and then weighed on a 5-place analytical balance. The absorbance of the chlorophyll extracts was read at 649 nm and 665 nm. Total chlorophyll concentration was calculated from the following formula (G. R. Leather, personal communication):

$$\frac{\text{g chlorophyll}}{\text{ml solution}} = (5.10) \times (\text{A665 nm}) + (20.02) \times (\text{A649 nm})$$

The initial frond number was subtracted from each counted number. The data were subjected to analysis of variance using SPSSX software (1983). If the results indicated the existence of added variance components, the data were transformed to base 10 logarithms and reanalyzed. If transformed data results did not indicate that the assumptions for analysis of variance were met, the data were not statistically examined. If interaction between replicate tests was not present, the replicates were combined; the remaining replicates for each tested extract were thereafter treated separately. To compare treatment means with the control, Dunnett's test was applied (Steel and Torrie, 1960). Results were graphed as percentages of the controls.

## RESULTS AND DISCUSSION

Seven plant extracts, representing six species, showed inhibition greater than 77% of the control lettuce seedling radicle growth (Figure 1). Of these, fragrant water-lily leaves and petioles extract was the most active with 95% inhibition of lettuce radicle growth by 250 ppt of aqueous extract. Coontail extracts brought about the greatest inhibition (66%) at the 25 ppt concentration. Water shield, eelgrass, and fragrant water-lily rhizomes and roots were strongly inhibitory at 250 ppt but were stimulatory at 25 ppt, although the growth stimulation was not significant according to Dunnett's test (Steel and Torrie, 1960). Rice (1984) has suggested that many, perhaps most, plant growth inhibitors may be growth stimulators at some much lower concentrations.

Analysis of variance of the results from the common duckweed frond reproduction assay showed no interaction between replicate tests except for blunt spikerush; the replicates were combined and Dunnett's test was applied to the results (Figure 2). The replicate data from blunt spikerush tests were too variable to consider valid; they were excluded from further consideration. Five extracts inhibited 68% or greater of common duckweed frond reproduction. Fragrant water-lily leaves and petioles were the most inhibitory followed by parrot-feather, fragrant water-lily rhizomes and roots, fanwort, and water shield.

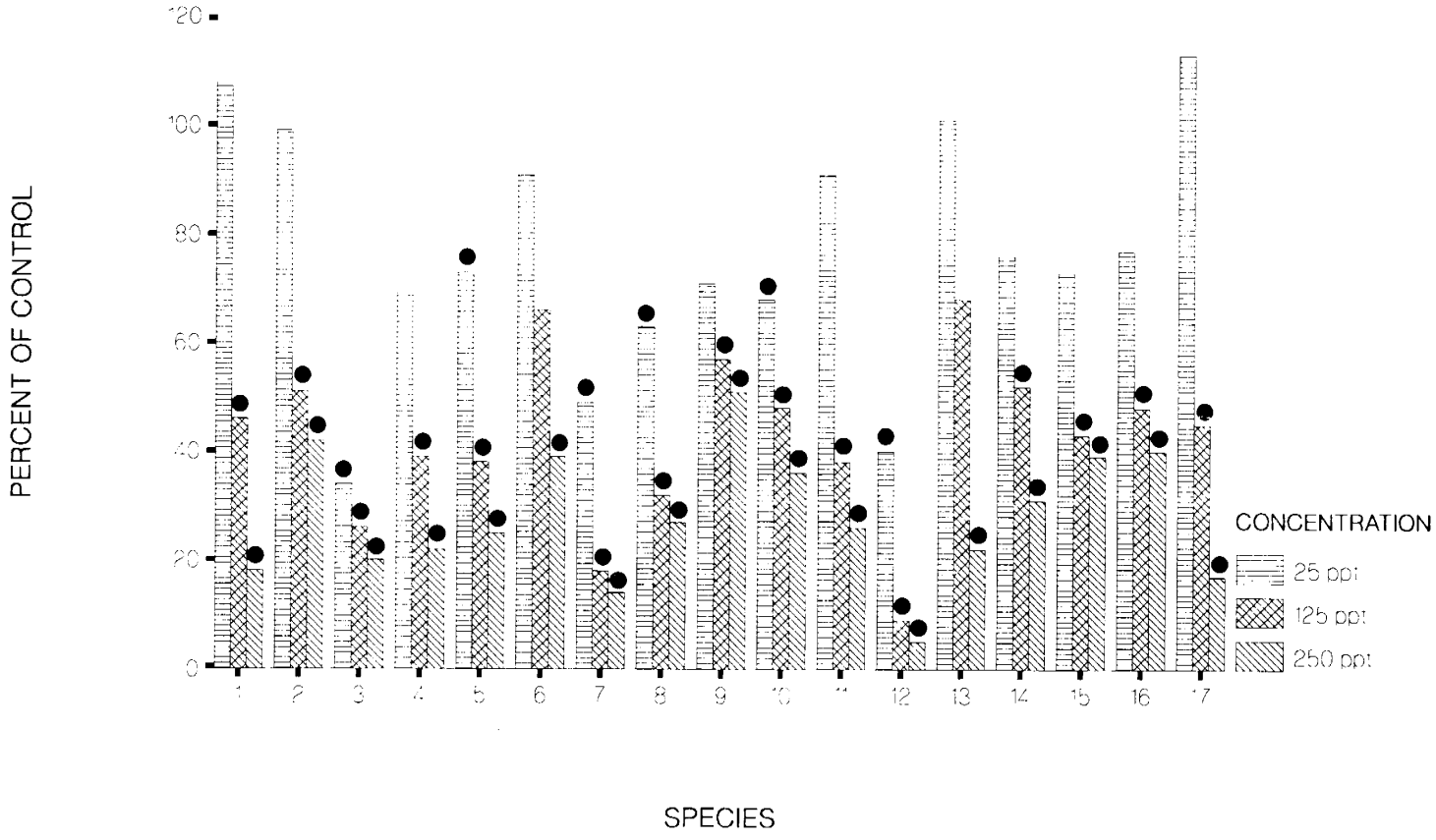


Figure 1. Lettuce seedling radicle bioassay of aqueous extracts of selected hydrophytes. See Table 1 for numeric identification of species. Bars with a dot indicate means significantly different from the control at $P \leq 0.05$ according to the Dunnett's test.

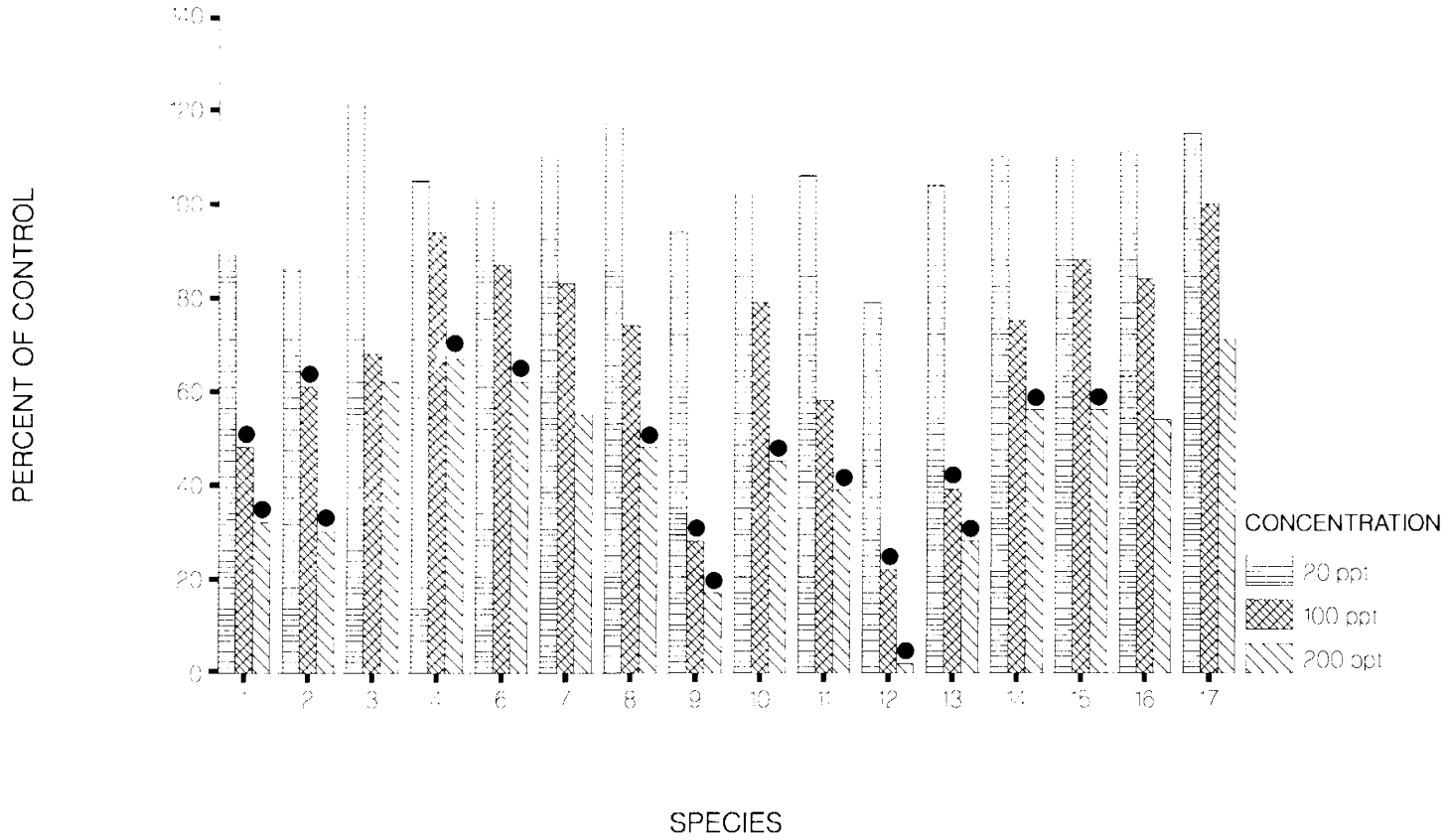


Figure 2. Common duckweed frond growth bioassay of aqueous extracts of selected hydrophytes. See Table 1 for numeric identification of species. Bars with a dot indicate means significantly different from the control at $P \leq 0.05$ according to the Dunnett's test.

Analysis of variance of the results from the chlorophyll measurments showed no interaction between replicate tests. The replicates were combined and Dunnett's test was applied to the results (Figure 3). The chlorophyll results are largely confirmatory of the duckweed frond results—the same five plant extracts reduced chlorophyll content by almost 60% or more as compared to the control. Fragrant water-lily leaves and petioles and parrot-feather were almost equally inhibitory, followed by fanwort. Water shield, parrot-feather and fragrant water-lily rhizomes and roots follow with almost equal (60%) inhibition. Thus, the correspondence between results of these two methods is good.

Analysis of variance of the results from the dry weight measurments (including log transformed data) indicated the existence of added variance components in four species. These results were not statistically analyzed but percentages of controls were calculated and each replicate was graphed separately (Figure 4). Replicate results for all other species were combined and Dunnett's test was applied to the results (Figure 5).

Measurement of plant dry weight should give an accurate measure of biomass, comparable to the frond area measurement (Einhellig *et al.*, 1985). However, only milligram and sub-milligram amounts of common duckweed dry weight were produced; thus, small errors in transfer of material were critical. The plant dry weight reduction results (Figures 4 and 5) show lack of differences in many cases due to the variance in the data. However, the five plants which showed greatest frond number reductions and chlorophyll reductions are among the seven plants with greatest dry weight reduction. Fanwort extract caused a 50% reduction in dry weight; extracts of fragrant water-lily leaves and petioles, parrot-feather, fragrant water-lily rhizomes and roots, and water shield brought about a 32-28% reduction. Hydrilla and creeping rush extracts also fell in this range at 33% and 36% reduction, respectively. Overall comparison of frond number inhibition and dry weight reduction suggests that plant dry weight is not as sensitive a measurement of growth inhibition as is frond number.

## COMPARISON OF LETTUCE SEEDLING AND COMMON DUCKWEED BIOASSAY RESULTS

Fragrant watter-lily (leaves and petioles) was the most inhibitory extract tested by lettuce seedling assay, inhibiting 95% of seedling radicle growth (Figure 1) at the highest (250 ppt) extract concentration. This was also the most inhibitory plant extract tested by common duckweed assay, inhibiting 98% of frond reproduction, 38% of plant biomass, and 72% of total chlorophyll. Water shield extract inhibited 82% of lettuce seedling radicle growth and also inhibited 68% of frond reproduction in the common duckweed assay.

The lettuce seedling assay is a widely used, experimen-

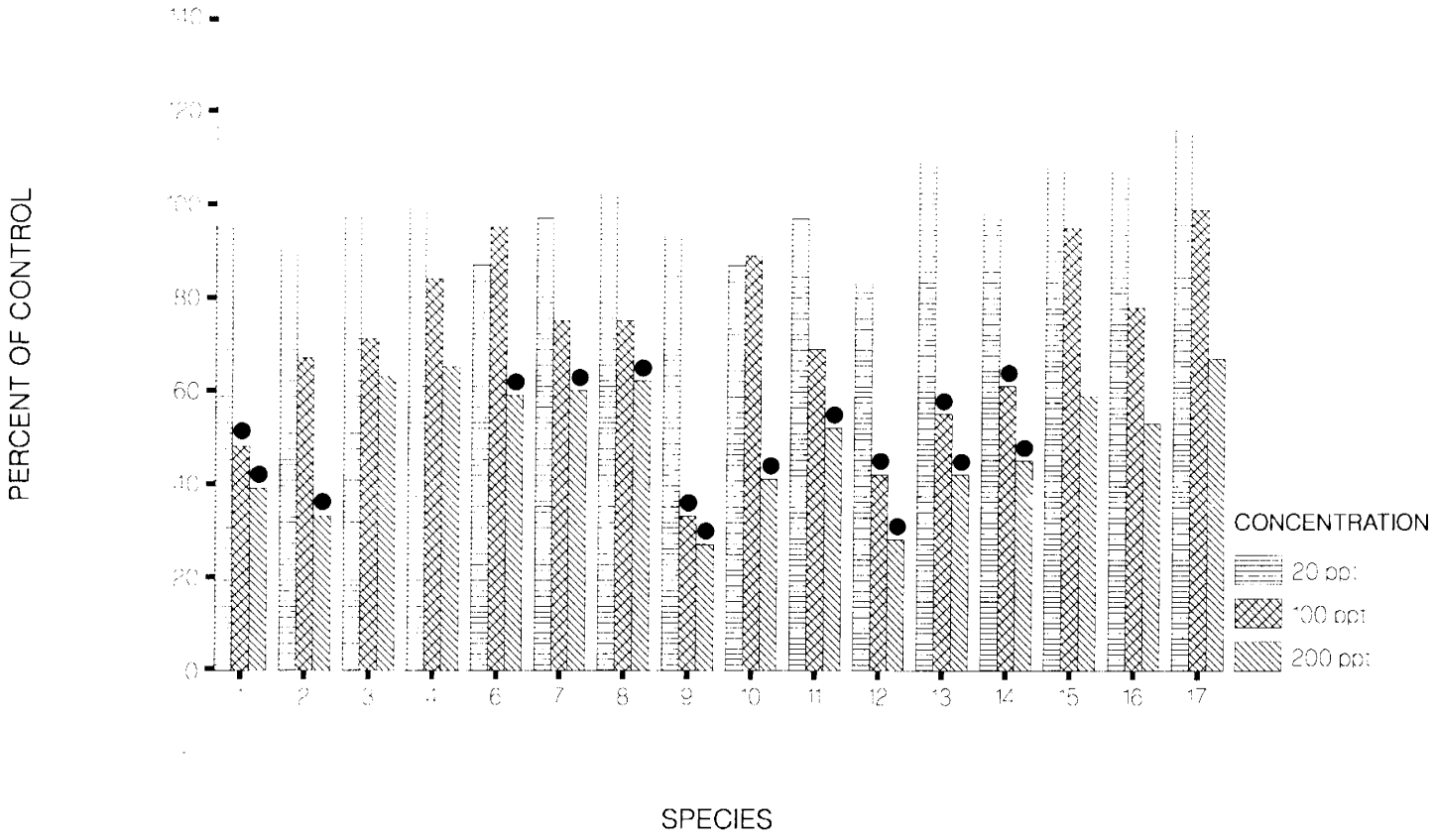


Figure 3. Common duckweed chlorophyll bioassay of aqueous extracts of selected hydrophytes. See Table 1 for numeric identification of species. Bars with a dot indicate means significantly different from the control at $P \leq 0.05$ according to the Dunnett's test.

tally simple way to determine allelopathic (growth inhibition or stimulation) activity. However, it uses lettuce, a terrestrial plant, as the target species, and thus may be less appropriate for use with hydrophytes. The common duckweed assay involves an aquatic plant as the target species and so may be more appropriate for extracts from hydrophytes, but is experimentally more complex and time consuming.

Comparison of the six most inhibitory plant extracts identified by the lettuce seedling and common duckweed (frond) assays shows three of the six are common to both assays. These are fragrant water-lily leaves and petioles, water shield, and fragrant water-lily rhizomes and roots. Fragrant water-lily leaves and petioles was the most inhibitory extract in both assays. Both fragrant water-lily and water shield extracts are thus highly allelopathically active, regardless of the selected assay system. That only three plant extracts are in common among the six most inhibitory plant extracts of both assay systems suggests that the two assays are measuring different effects. The two *least* active plant extracts as measured by the lettuce seedling assay, parrot-feather and fanwort, are among the five *most* active by common duckweed assay whether one examines frond number, total chlorophyll, or dry weight. One would expect root formation in the lettuce seedling assay to respond to different stimulation and/or inhibition than does whole plant growth as measured in the common duckweed assay. Ideally, a plant growth bioassay should have as its target species the plant whose growth inhibition/stimulation is desired, but such a bioassay is by definition very specific. These results show that the lettuce seedling assay is useful in identifying inhibitory plants, but the correspondence with the common duckweed assay is not complete.

Results of this work showed that the lettuce seedling assay was more sensitive than the common duckweed assay. All 17 plant extracts showed significant growth inhibition at the highest concentration (250 ppt) tested. Fifteen of the 17 plant extracts showed significant growth inhibition at the mid concentration (125 ppt), six showed inhibition at the lowest (25 ppt) concentration tested. This contrasts with common duckweed frond growth inhibition in which 12 of 16 plant extracts showed significant inhibition at the highest extract concentration (200 ppt). At the lowest concentration, six plant extracts inhibited lettuce seedling growth; none reduced common duckweed frond growth. The small (20%) differences in extract concentrations used in the two assays would not account for the observed differences in activity.

Allelopathic activity as measured by common duckweed frond growth inhibition closely parallels that measured by total chlorophyll reduction. Common duckweed dry weight reduction proved to be an ineffective measure of growth inhibition due to the variance in the data.

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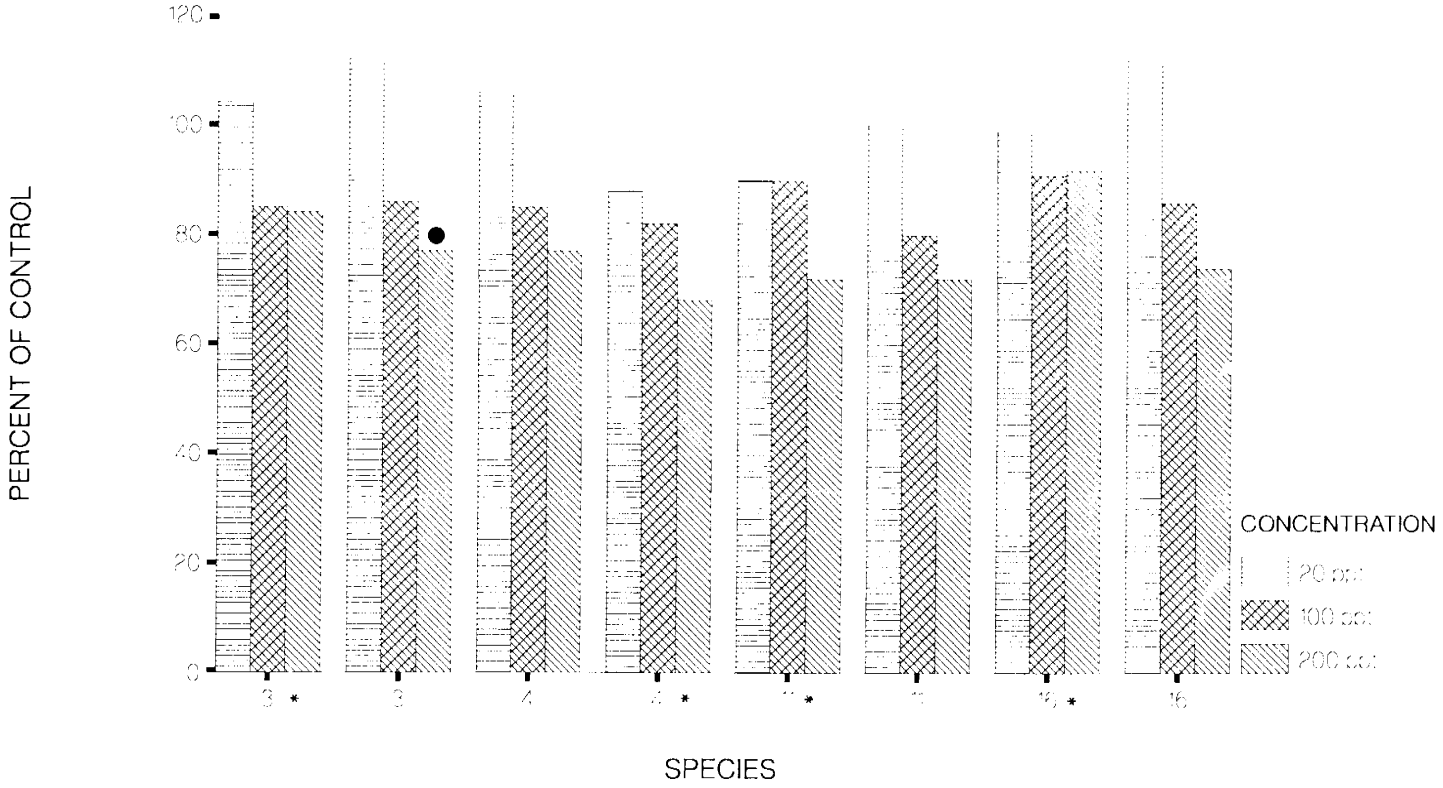


Figure 4. Individual replicates of common duckweed dry weight bioassay of aqueous extracts of selected hydrophytes. See Table 1 for numeric identification of species. Replicates with an asterisk after the species number were not statistically analyzed. Bar with a dot indicates a mean significantly different from the control at $P \leq 0.05$ according to the Dunnett's test.

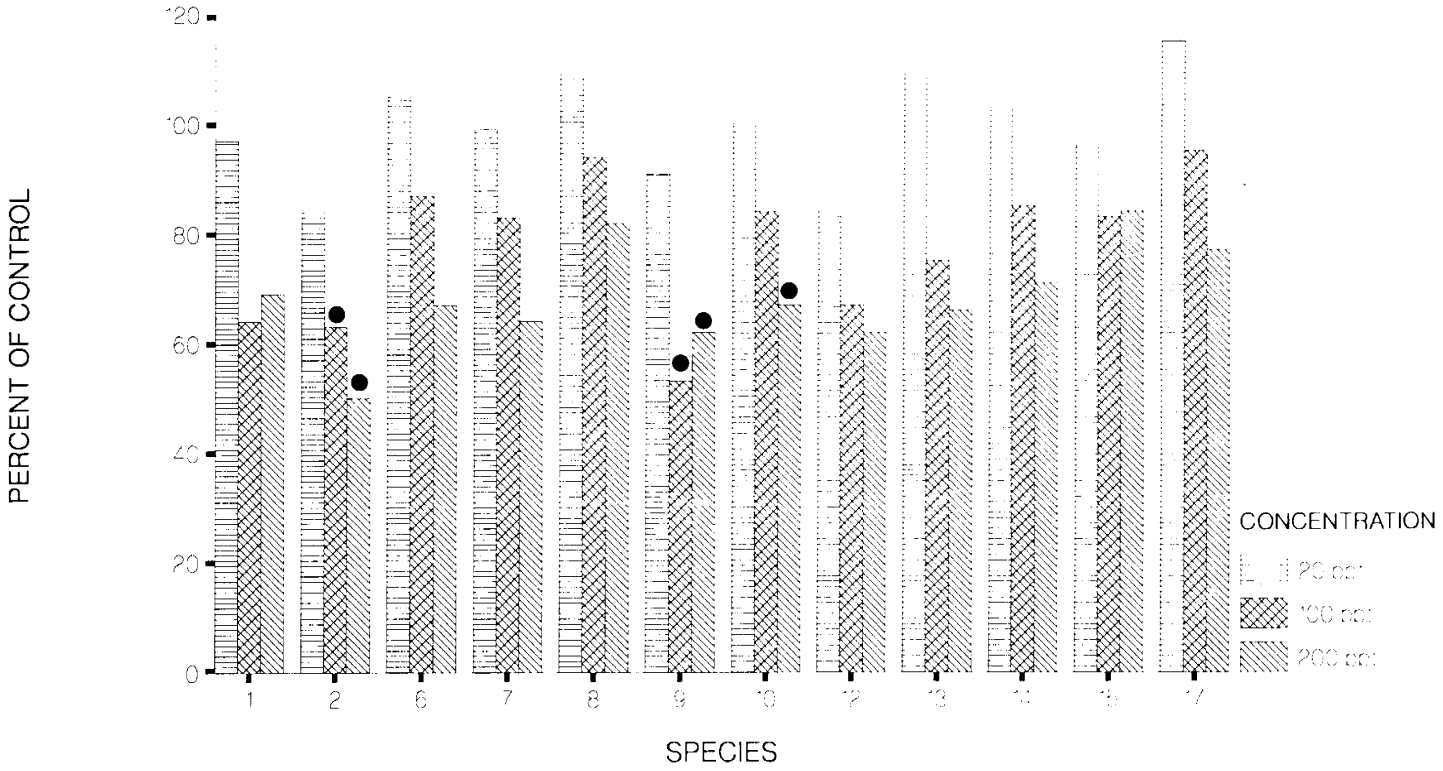


Figure 5. Combined replicates of common duckweed dry weight bioassay of aqueous extracts of selected hydrophytes. See Table 1 for numeric identification of species. Bars with a dot indicate means significantly different from the control at $P \leq 0.05$ according to the Dunnett's test.

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*J. Aquat. Plant Manage.* 27: 84-89

# Characterization of Allelochemicals in American Eelgrass[1]

TAI-SHENG CHENG AND D. N. RIEMER[2]

## ABSTRACT

Water extracts of American eelgrass (*Vallisneria americana* Michx.) were partitioned into neutral, acidic, and basic fractions by adjusting the pH of the aqueous phase and extraction into ethyl ether:ethyl acetate (1:1 v/v). The neutral fraction was shown to inhibit both seed germination and seedling growth. This fraction was applied to a preparative high performance liquid chromatographic (HPLC) column to separate it into two fractions (F-1 & F-2). At high concentration, F-1 reduced common duckweed (*Lemna minor* L.) chlorophyll *a* production and reduced the growth rate as measured by frond number. The frond size was very small in F-1 treated plants as compared to the controls, the fronds were darker green in color than the controls and no roots were produced. Gallic, vanillic, p-coumaric, and ferulic acids were identified in the neutral fraction by means of an analytical HPLC. Gallic and vanillic acids were the main components, whereas p-coumaric and ferulic acids were present, but in much lower concentrations.

*Key words:* allelopathy, phenolic acids, *Vallisneria*, gallic acid, vanillic acid.

## INTRODUCTION

American eelgrass was shown to have allelopathic properties when applied as a mulch and when extracts were tested by a bioassay technique using lettuce (*Lactuca sativa* L. var. "Buttercrunch") as the test organism (Cheng and Riemer, 1988). In addition to demonstrating the existence of allelopathy, characterization of the allelochemicals is also important.

An approach to the characterization of allelochemicals in plant tissues is to isolate the chemicals with water or organic solvents and then to identify the compounds with certain chromatographic methods (Einhellig, 1985; Putnam and Tang, 1986). The isolation of allelochemicals was recently accomplished by an Amberlite XAD-4 nonpolar resin column (Tang and Young, 1982). The isolated chemicals were then identified by means of gas liquid chromatography-mass spectroscopy (GLC-MS). The combination of these two methods provided an efficient technique to quantify phytotoxins in allelopathy studies. This technique has been utilized to isolate phytotoxins from roots and root exudates (Young, 1979; Tang and Young, 1982) and soil samples (Young and Tang, 1984; Young, 1984; Young and Chou, 1985). Amberlite XAD-16 and Amberlite XAD-4 are very similar (Krepelka, personal communication, 1987). The major differences between the two are that XAD-16 has a larger surface area (850 $m^2/g$) than XAD-4 (725 $m^2/g$) and XAD-16 is more easily and rapidly prepared for use than XAD-4 (Young, personal communication, 1987).

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