

Influence of Allelochemicals and Aqueous Plant Extracts on Growth of Duckweed¹

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ABSTRACT

Gallic acid, followed by hydroquinone, was the least toxic of four allelochemicals evaluated at concentrations of 10^{-6} , 10^{-4} , and 10^{-2} M on growth of duckweed (*Lemna paucicostata* Hegelm. 6746) under axenic and controlled environmental conditions. After 10 days of growth both catechol and salicylic acid at 10^{-6} and 10^{-4} M reduced the number of plants by an average of 34 and 57%, respectively, as compared to the control plants. Stimulation of duckweed growth occurred with extracts based on concentrations of 1.0 g of plant material per liter or lower from azolla (*Azolla caroliniana* Willd.) plants and 0.1 g per liter from shoots of Illinois pondweed (*Potamogeton illinoensis* Morong) but concentrations higher than these reduced growth of the duckweed plants. Aqueous extracts from shoots of dried spikerushes, *Eleocharis interstincta* (Vahl) R. & S. and *Eleocharis cellulosa* Torr., exhibited growth retarding effects on the number of duckweed plants. Extracts from green (alive when collected) and brown (dead when collected) Casuarina needles (*Casuarina* sp.), and leaves of lantana (*Lantana camara* L.) and St. Augustine grass [*Stenotaphrum secundatum* (Walt.) Kuntze.], were also found to inhibit growth of the duckweed plants.

Key words: azolla, *Casuarina*, cattail, hydrilla, Illinois pondweed, lantana, spikerush, St. Augustine grass, *Lemna paucicostata*.

INTRODUCTION

Allelopathy, biochemical interactions among plants as a result of one or more chemical compounds being produced by a plant plays a major role in the growth and development of some plant communities (Rice, 1984). The nature and extent to which these chemicals regulate plant growth varies according to species.

The feasibility of using allelochemicals to regulate growth of certain agricultural crops has received considerable attention in recent years (Rice, 1984). Also, allelopathy is being considered for its potential in the management of weed species (Muller, 1969; Putnam, 1988) including aquatic plants (Szczepanski, 1977); however, little information is available on the manner in which allelochemicals may regulate aquatic plants in natural systems.

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In one of the first studies with aquatic plants involving possible allelochemicals, pondweeds (*Potamogeton* spp.) were eliminated from cultures planted with the spikerush [*Eleocharis acicularis* (L.) R. & S.] or with dwarf arrowhead [*Sagittaria subulata* (L.) Buch.] during a 2-year period under greenhouse conditions (Oborn *et al.*, 1954). Since the spikerush and dwarf arrowhead produce shoots of only a few centimeters in height as compared to the pondweeds which may grow 2 to 4 m in height in the water column, competition for light did not appear to have been a factor resulting in the poor growth of the pondweeds.

Frank and Dechoretz (1980) used the leachate from containers planted with cultures of dwarf spikerush (*Eleocharis coloradoensis* Britt. Gilly) to reduce the production of new shoots of American (*Potamogeton nodosus* Poir.) and sago pondweed (*Potamogeton pectinatus* L.). Additional evidence of allelopathy with the spikerushes was produced by the study of Ashton, *et al.* (1985) when a leachate was collected from axenic cultures of dwarf spikerush. This leachate contained a compound, or perhaps several compounds, with a molecular weight between 600 and 1000, that was phytotoxic to excised parts of sago pondweed and hydrilla (*Hydrilla verticillata* Royle).

Allelopathic materials can be inhibitory to neighboring species as well as to offspring of the species producing the allelochemicals (Szczepanski, 1977). Extracts from cattail leaves (*Typha latifolia* L.) were found to be toxic to seedlings of the same species (McNaughton, 1968; Grace, 1983). Hydrilla was found to exhibit allelopathic responses on *Ceratophyllum demersum* L. and *Ceratophyllum muricatum* Chamisso (Kulshreshtha and Gopal, 1983). Aqueous leachates from litter of *Justicia americana* (L.) Vahl. were found to inhibit seedling growth of *Polygonum lapathifolium* L. to a greater degree than the influence of the leachates on either germination of the seeds or growth of established plants of *P. lapathifolium* (Carter and Grace, 1986).

Phenolic acids, alkaloids, coumarins, and quinones elicit allelopathic responses from some terrestrial plants (McNaughton, 1968; Muller, 1969; Rice, 1984), but little is known of their influence on aquatic species. A study was initiated therefore with the objective of evaluating several of these compounds for their allelopathic potential using duckweed (*Lemna paucicostata* Hegelm. 6746) in an aquatic plant bioassay test. The second part of the study involved the use of aqueous extracts from dried aquatic and terrestrial plants to determine their influence on duckweed growth.

MATERIALS AND METHODS

Duckweed plants used in this study were from cultures

of plants maintained since September 1978³ under axenic culture conditions with continuous transfers in one-third strength of the nutrient solution developed by Hutner (1953). The media was amended with 10 g of sucrose per liter as a carbon source. This nutrient solution is termed 'HS' hereinafter.

Fronds from stock cultures were transferred into plates with 24 small wells containing 1 ml of HS solution. A single frond was placed in each well. After 4 days of growth, the plants were inspected under a microscope and only those fronds in the L4 and R4 stage of development were selected for use (Datko, *et al.*, 1980). In this study, a single duckweed plant consisted of all fronds attached to each other and floating separate of other fronds.

All experiments were conducted under axenic and controlled environment conditions of 25 C with a photoperiod of 14 hours of light at 28 E/m²·s⁻¹. These same conditions were used to maintain the stock cultures of plants.

Commercially available allelochemicals¹ evaluated were catechol, hydroquinone, gallic acid, and salicylic acid at concentrations of 10⁻⁶, 10⁻⁴, and 10⁻² M. First a 10⁻¹M concentration was prepared with HS solution, filtered through a 0.2 µm disposable Nalgene filter, and diluted with additional HS to obtain the desired concentrations.

Each treatment concentration consisted of 25 ml of the appropriate solution contained in a Petri dish to which three duckweed plants were added. Controls consisted of duckweed plants in HS nutrient solution only. Mixing and preparation of all allelochemicals was conducted under subdued light to avoid potential degradation of the compounds. The dishes were wrapped with aluminum foil and placed in the controlled environmental chamber for 48 hours after which the foil was removed and the plants exposed to light. The number of plants were counted after 3, 7, and 10 days of growth. This experiment was repeated three times with four dishes per allelochemical concentration, and the results were combined for statistical analysis.

For this portion of the study, shoots of the spikerushes *E. interstincta* and *E. cellulosa*, cattail (*Typha latifolia* L.), Illinois pondweed, and hydrilla, and entire azolla plants were collected from populations growing in Lake Okeechobee. Leaves of lantana and St. Augustine grass, and brown and green Casuarina needles were collected from plants growing on the grounds of the Fort Lauderdale Research and Education Center (FLREC). Brown casuarina needles were dead needles collected from beneath the tree and the green casuarina needles were picked from live branches. All plant material was washed with tap water, dried at 60 C, and ground to pass through a 20 mesh screen.

Procedures for the extraction of water soluble compounds from the dried plant material were adapted from those described by Barnes and Putnam (1986). Briefly, 50 g of ground, dried plant tissue were placed in 1.0 liter of

distilled water. The mixture was placed in a controlled temperature chamber maintained at 4 C and stirred for 48 hours after which the mixture was filtered through cheese cloth and the liquid centrifuged for 30 minutes at 14,000 rpm. The supernatant was filtered through a 0.45 µm disposable Nalgene Filter and held at 4 C until used for culturing of duckweed.

Each treatment consisted of 25 ml of HS nutrient solution with the appropriate amount of aqueous plant extract contained in Petri dishes. Controls consisted of duckweed in HS nutrient solution only. Three duckweed plants were added to each Petri dish. The filtered supernatant was added to sterile HS solution at a rate which resulted in solutions based on 0.1, 0.2, 0.4, 1.0, 2.0, and 4.0 g of dry plant material per liter. An additional treatment of 0.04 g of plant material per liter was used for the extract from lantana. Treatments were repeated an average of nine times and the data were combined for statistical analysis. All dishes which had obvious contamination were discarded and the treatment repeated. The Petri dishes were not wrapped with aluminum foil as with the allelochemicals. The duckweed was allowed to grow for 10 days after which the number of plants was counted.

The influence of the allelochemicals and plant extracts on growth of the duckweed was evaluated using general linear models (GLM) procedures. Analyses were performed on numbers of duckweed plants in each treatment group. Means separation for the allelochemical portion of the study was accomplished using the Duncan-Waller Empirical Bayes LSD procedure (Petersen 1985). For the plant extract data, a response value was obtained for the treatment groups by subtracting the number of plants in the associated control. The response values and plant concentrations for the plant extract study were transformed to natural logarithms prior to use of the GLM procedures, and the effect of concentration was quantified using linear regression analysis.

RESULTS AND DISCUSSION

Influence of Allelochemicals on Growth of Duckweed. Growth of control duckweed plants during the 10-day culture period for the tests with the allelochemicals is shown in Figure 1. The number of control plants increased from 3 to an average of 163 which represents a 55-fold increase showing the rapid growth obtained under the culture conditions of this study for the duckweed plants.

Gallic acid was the least toxic of the four allelochemicals used in this study. Only the 10⁻² M concentration of gallic acid inhibited growth of the duckweed plants (Figure 2). The average number of plants in the 10⁻⁶ M and 10⁻⁴ M concentrations of gallic acid were not significantly different from the controls.

No significant differences between catechol and salicylic acid were noted for the number of duckweed plants for the 3, 7, and 10-day culture periods exposed to the 10⁻⁶ M, 10⁻⁴ M, and 10⁻² M concentrations of these chemicals. Plant growth was completely inhibited with the 10⁻² M concentrations but the average number of duckweed plants after 10 days of growth was 108 and 70 for the 10⁻⁶ M and 10⁻⁴ M concentrations, respectively,

³Plants originally obtained from Dr. W. S. Hillman (deceased) of the Brookhaven National Laboratories.

⁴Compounds obtained from either Fisher Scientific, Fort Lauderdale, FL 33308 or Sigma Chemical Co., St. Louis, MO 63178. Mention of a trademark or a proprietary product does not constitute a guarantee or warranty of the product by the University of Florida and does not imply its approval to the exclusion of other products that also may be suitable.

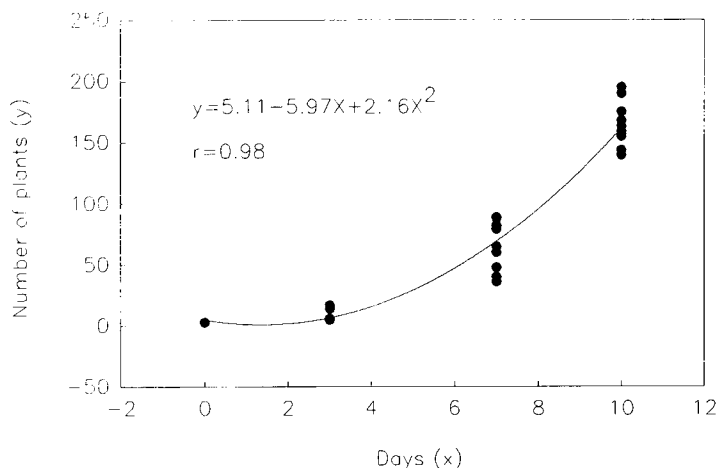


Figure 1. Growth of duckweed (*Lemna paucicostata*) in HS nutrient solution held under axenic and controlled environment conditions.

which represents a reduction of 34 and 57% as compared to the number of control plants.

Plant number was completely inhibited for duckweed exposed to the 10^{-2} M concentration of hydroquinone. After 10 days of growth however, the number of plants in the 10^{-4} M concentration of hydroquinone was 32% lower than the control, but the number in the 10^{-6} M concentration was not significantly different from the control.

These results agree with those of Patterson (1981) who found allelochemicals to exhibit varying degrees of effectiveness on growth of soybean [*Glycine max* (L.) Merr 'Tracy']. Likewise, salicylic acid at concentrations of 10^{-4} M or higher was the most effective of 11 allelopathic chemicals tested in inhibiting sprouting of hydrilla tubers and subsequent growth; however, catechol inhibited sprouting only at the 10^{-2} M concentration (Sutton 1986). Furthermore, Martin and Martin (1988) evaluated 11 substituted phenols at 5.0×10^{-5} M, which corresponds to the concentrations of allelopathic compounds that have been isolated from soils (Whitehead 1964), and found this concentration of salicylic acid did not influence fresh weight of hydrilla sprigs and it was necessary to increase the amount of this chemical to 5.0×10^{-3} M to obtain a 13% weight loss.

Influence of Aqueous Plant Extracts on Growth of Duckweed.

All of the aqueous extracts, except cattail, produced significant results when GLM procedures were used to evaluate the relationship of the log response (independent variable) to the log concentration (the dependent variable) (Table 1). The F-values ranged from a high of 107.08 ($p = .0001$) for the brown Casuarina needles to a low of 4.79 ($p = .0337$) for cattail. Likewise, the Root MSE values were 0.50 and 0.96 for the brown Casuarina needles and cattail, respectively. However, the Root MSE ranged from a low of 0.34 for the green Casuarina needles to a high of 1.04 for *E. interstincta*.

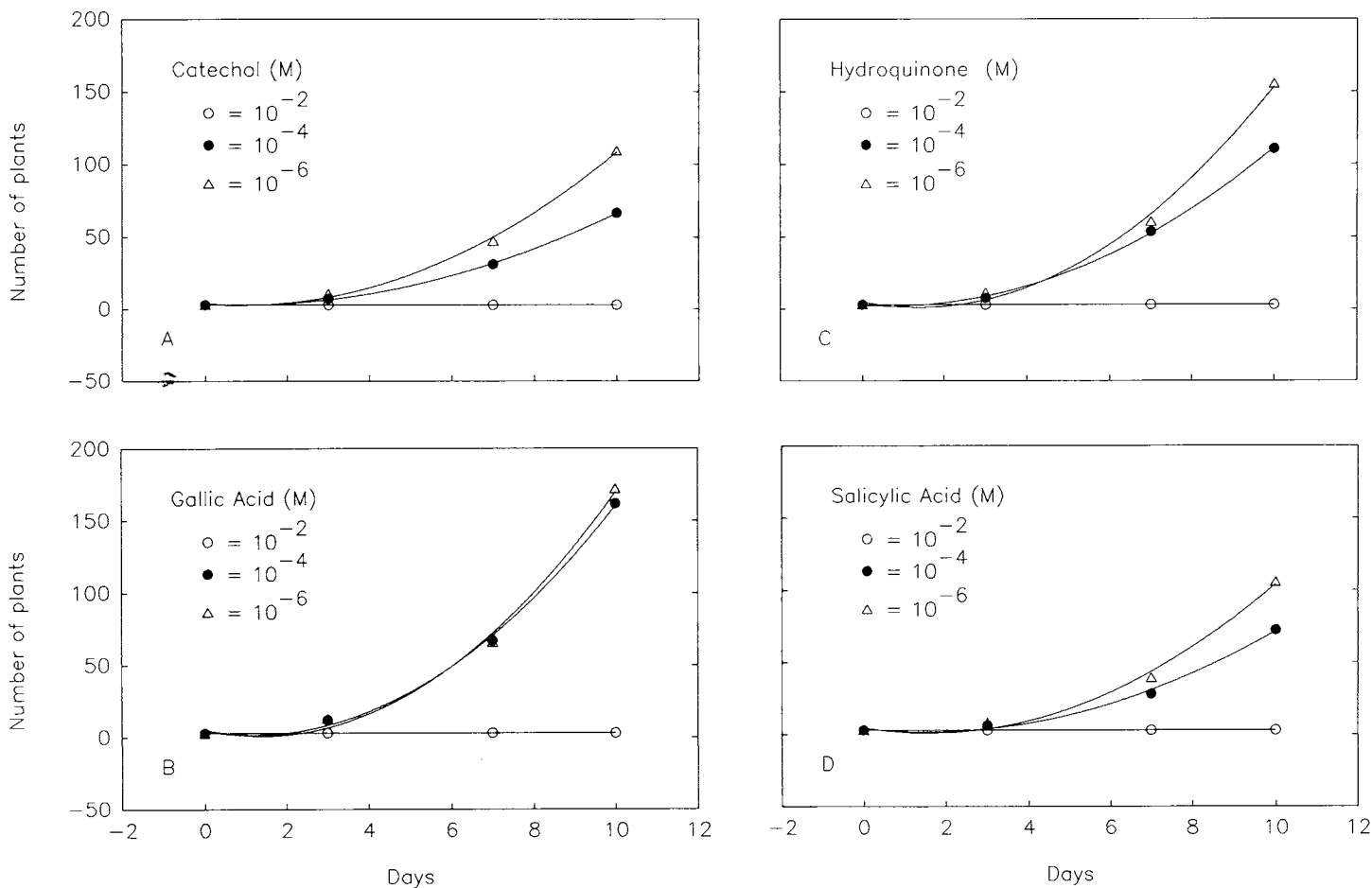


Figure 2. Influence of (A) catechol, (B) gallic acid, (C) hydroquinone, and (D) salicylic acid on growth of duckweed (*Lemna paucicostata*).

TABLE 1. SUMMARY OF SELECTED TEST STATISTICS USED IN THE EVALUATION OF THE RELATIONSHIP OF LOG RESPONSE (y) TO LOG CONCENTRATION (x) AS A MEASURE OF THE INFLUENCE OF AQUEOUS PLANT EXTRACTS ON GROWTH OF DUCKWEED (*LEMNA PAUCICOSTATA*).

Plant extract	Root MSE	F-value	(prob.>F)
<i>E. cellulosa</i>	0.57	16.97	(0.0001)
<i>E. interstincta</i>	1.04	16.59	(0.0002)
Azolla	0.92	60.66	(0.0001)
Hydrilla	0.79	51.26	(0.0001)
Cattail	0.96	4.79	(0.0337)
Illinois pondweed	0.72	64.92	(0.0001)
St. Augustine grass	0.57	83.18	(0.0001)
Lantana	1.01	21.65	(0.0001)
Green Casuarina needles	0.34	38.64	(0.0001)
Brown Casuarina needles	0.50	107.08	(0.0001)

Plots of the log response (y) and log concentration (x) along with r-values and linear regression equations for the influence of the aqueous extracts on growth of the duckweed are shown in Figures 3 to 5. Linear regressions are shown in all cases because the predominant trend is linear although in certain cases, such as with azolla and cattail, a higher order polynomial equation would better

describe the response. However, these plots were developed to show general trends and not predictive equations.

The two spikerushes, *E. cellulosa* and *E. interstincta*, (Figure 3) produced similar results. Azolla, hydrilla, and Illinois pondweed were more toxic than cattail to duckweed (Figure 4). Some stimulation of growth was noted for the lower concentrations of azolla and Illinois pondweed. For the terrestrial plants, considerable variability was noted for lantana resulting in a poor relationship for the log response to log concentration over the range of plant amounts used in this study as compared to St. Augustine grass and the Casuarina needles (Figure 5).

Achhireddy and Singh (1984) reported that lantana shoots incorporated into the soil produced yellowing of the foliage of milkweedvine (*Morrenia odorata* Lindl.). Allelopathic activity of lantana residues was still strong even after decomposition of lantana residues for 4 weeks prior to the planting of milkweedvine seeds suggesting the compound(s) involved in the allelopathic effect are very stable and remain active for a period of time. This may help explain in part why both the green (living) and brown (dead) Casuarina needles resulted in the same growth response from the duckweed. It must be assumed that rain fall would cause natural leaching of materials from the dead needles under the tree to occur.

Results from this study agree with other published information showing allelopathic effects of aqueous extracts of various aquatic plants. Recently, Cheng and Riemer (1988), using a bioassay technique with lettuce (*Lactuca sativa* L. var. "Buttercrunch") seeds, found a reduction in germination percentage and radicle length when various concentrations of water extracts were made of dried threesquare burreed (*Sparganium americanum* Nutt.) and American eelgrass (*Vallisneria americana* Michx.). Also, the low molecular weight fraction of extracts from *Chara tomentosa* L. exhibited strong inhibitory effects on shoot growth and leaf development of plants germinated from seeds of *Lepidium sativum* L. while extracts from *C. demersum* L. showed only small inhibitory effects on shoots of the test plants and no effects on leaf development (Kleiven and Szczepanski 1988); furthermore, extracts from *Myriophyllum verticillatum* L. were not inhibitory to the *L. sativum* seeds.

These data show the potential of allelochemicals and extracts from various plants to influence growth of duckweed. Additional studies are needed to evaluate the implications of these results to growth of aquatic plants under field conditions. And finally, identification of a compound or compounds in the plant extracts would be necessary to properly evaluate their potential to regulate aquatic plant growth in various bodies of water.

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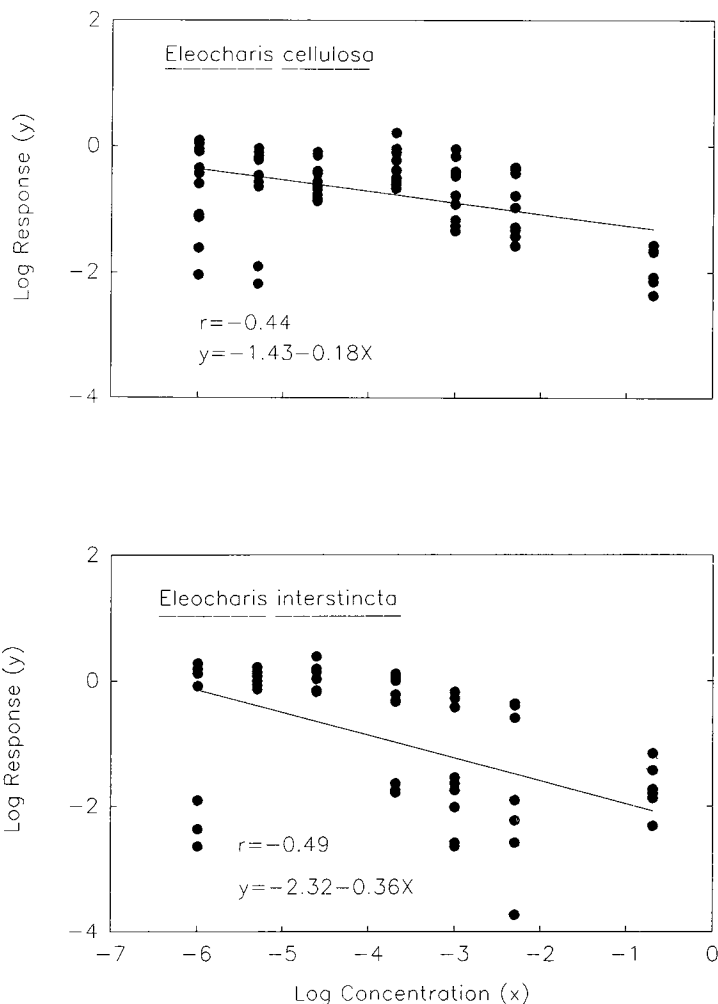


Figure 3. Influence of aqueous extracts of the spikerushes *Eleocharis cellulosa* and *Eleocharis interstincta* on growth of duckweed (*Lemna paucicostata*).

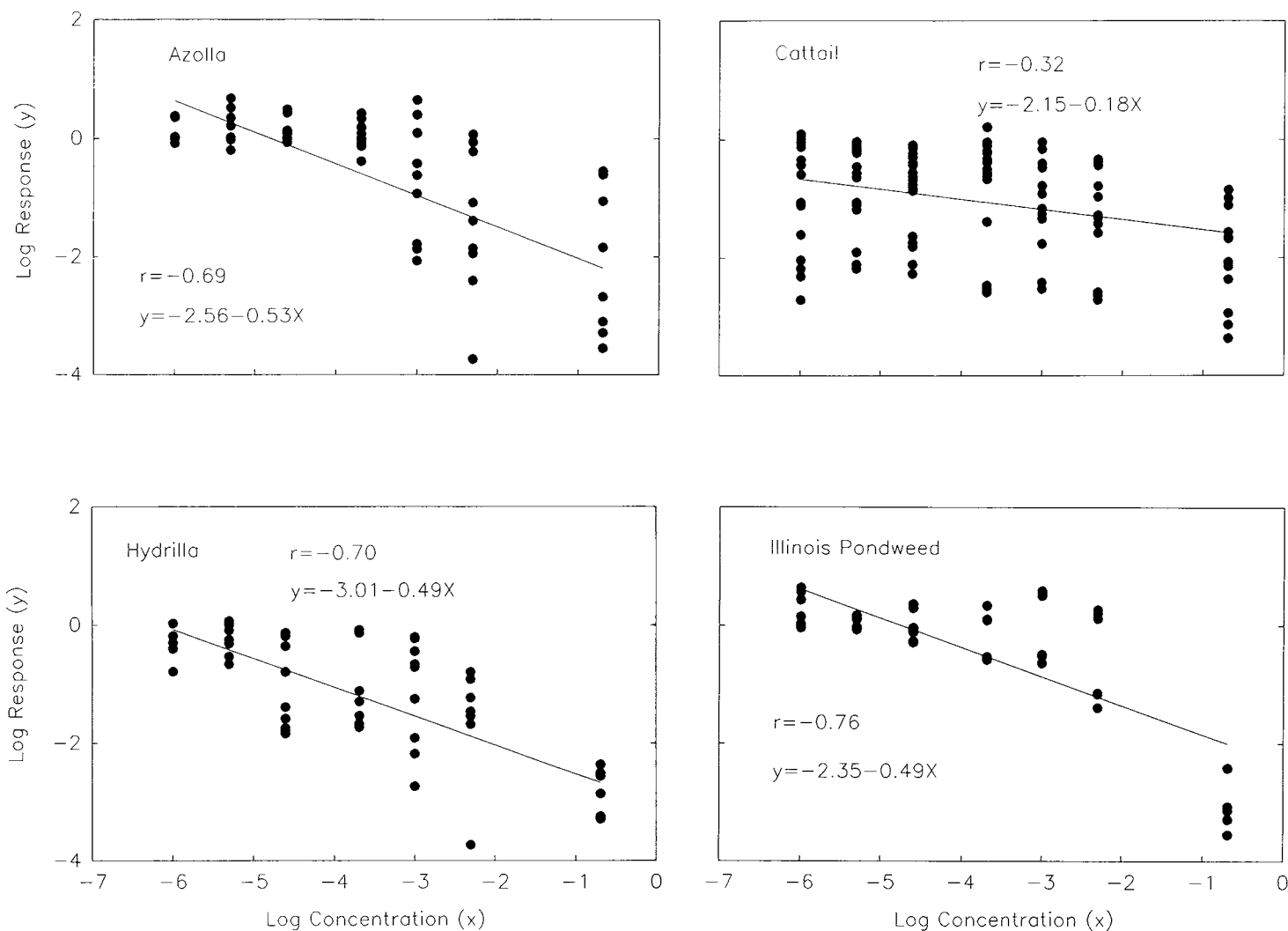


Figure 4. Influence of aqueous extracts of azolla, cattail, hydrilla, and Illinois pondweed on growth of duckweed (*Lemna paucicostata*).

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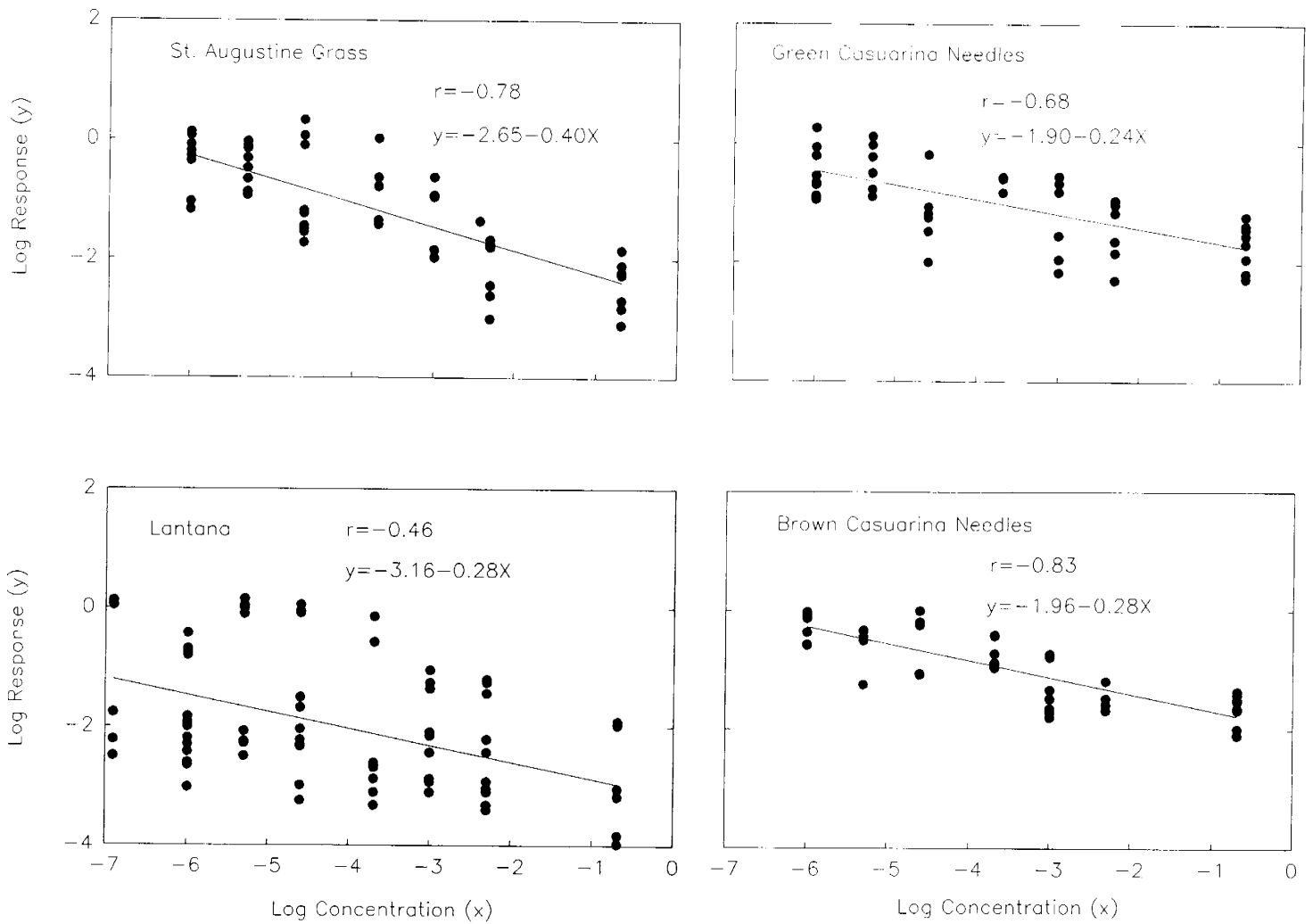


Figure 5. Influence of aqueous extracts of St. Augustine grass, lantana, green and brown Casuarina needles on growth of duckweed (*Lemna paucicostata*).

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