

Mississippi under contract DACA 39-86-K-0002. The technical assistance of Bonnie Sanders, Amanda Letchworth and L. Tillis is gratefully acknowledged.

LITERATURE CITED

- Ashton, F. M., J. M. DiTomasco, and L. W. J. Anderson. 1985. Spikerush (*Eleocharis* spp.): A source of allelopathics for the control of undesirable aquatic weeds. *J. Aquat. Plant Manage.* 22:52-56.
- Ashton, F. M., J. M. DiTomasco, and L. W. J. Anderson. 1985. Spikerush (*Eleocharis* spp.): A source of allelopathics for the control of undesirable aquatic plants. *ACS Symp. Ser.* 268:401-414.
- Bonaser, J., J. Lynch, and M. A. Leck. 1979. Comparison of the allelopathic potential of four marsh species. *Bull. Torrey Bot. Club* 106:217-222.
- Cheng, T. S. and D. N. Riemer. 1988. Allelopathy in threesquare burreed (*Sparganium americanum* and American eelgrass (*Valisneria americana*). *J. Aquat. Plant Manage.* 26:50-55.
- Einhellig, F. A., G. R. Leather, and L. L. Hobbs. 1985. Use of *Lemna minor* L. as a bioassay in allelopathy. *J. Chem. Ecol.* 11:65-72.
- Elakovich, S. D. and J. W. Wooten. 1987. An examination of the phytotoxicity of the water shield, *Brasenia schreberi*. *J. Chem. Ecol.* 13:1935-1940.
- Frank, P. A. and N. Dechoretz. 1980. Allelopathy in dwarf spikerush *Eleocharis coloradoensis*. *Weed Sci.* 28:499-505.
- Kulshreshtha, M. and B. Gopal. 1983. Allelopathic influences of *Hydrilla verticillata* (L.f.) Royle on the distribution of *Ceratophyllum* species. *J. Aquat. Bot.* 16:207-209.
- McNaughton, S. J. 1968. Autotoxic feedback in relation to germination and seedling growth in *Typha latifolia*. *Ecology* 49:367-369.
- Nichols, S. A. and B. H. Shaw. 1983. Physical, chemical and biological control of aquatic macrophytes. *Lake Restoration, Protection and Management*. U.S. E.P.A., 181-192.
- Rice, E. L. 1979. Allelopathy-An update. *Bot. Rev.* 45:15-109.
- Rice, E. L. 1984. *Allelopathy*, 2nd Ed. Academic Press, N.Y. p. 1.
- SPSSX. 1983. SPSS, Inc. Chicago, Ill.
- Steel, R. G. D. and J. H. Torrie. 1960. *Principles and Procedures of Statistics*, McGraw-Hill, New York, pp. 111-112.
- Szczepanski, A. J. 1977. Allelopathy as a means of biological control of water weeds. *Aquat. Bot.* 3:193-197.
- Szczepanska, W. 1977. Interactions of *Phragmites communis* Trin. and *Carex hudsonii* Bennett. *Ekol. Pol.* 24:431-436.
- Yeo, R. R. 1980. Life history and ecology of dwarf spikerush (*Eleocharis coloradoensis*). *Weed Science* 28:263-272.
- Yeo, R. R. and J. R. Thurston. 1984. The effect of dwarf spikerush (*Eleocharis coloradoensis*) on several submersed aquatic weeds. *J. Aquat. Plant Manage.* 22:52-56.

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

Characterization of Allelochemicals in American Eelgrass¹

TAI-SHENG CHENG AND D. N. RIEMER²

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.

¹N.J. Agr Expt Sta. Pub. No. D-15181-1-89, supported by State and Hatch Act Funds. Received for publication February 7, 1989 and in revised form May 11, 1989.

²Former Research Asst. and Assoc. Prof., respectively, Dept. of Soils and Crops, Rutgers Univ., Agr Expt. Sta., New Brunswick, NJ 08903. The senior author is currently Grad. Asst., Dept. of Plant Sciences, Univ. of Rhode Island, Kingston, RI 02881.

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 m²/g) than XAD-4 (725 m²/g) and XAD-16 is more easily and rapidly prepared for use than XAD-4 (Young, personal communication, 1987).

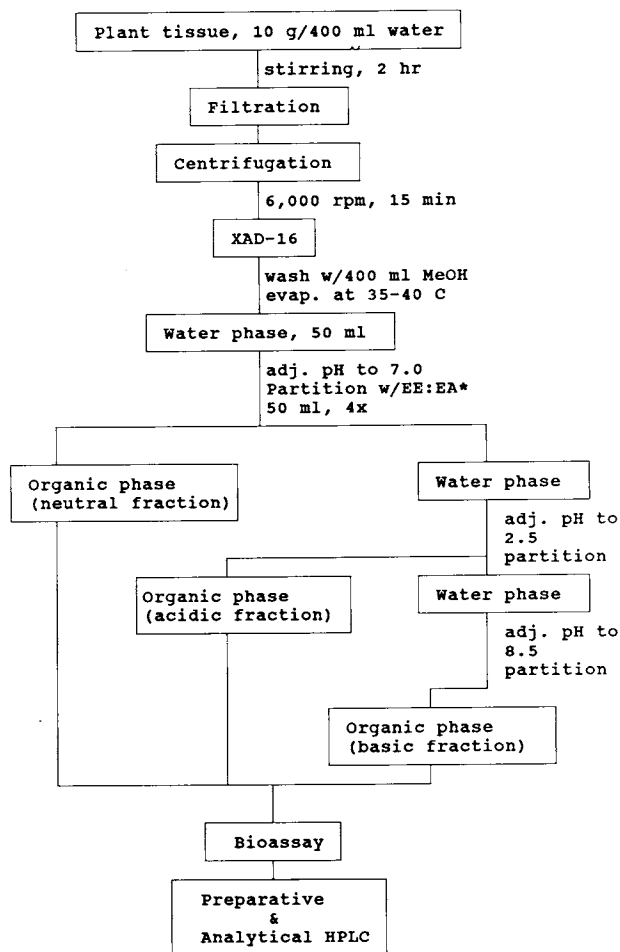
Organic compounds such as aliphatic and aromatic acids, coumarins, quinones, flavonoids, tannins, alkaloids, terpenoids, steroids, and toxic gasses have been identified as allelopathic agents (Einhellig, 1985; Mandava, 1985; Putnam, 1985; Rice, 1984). Aqueous plant extracts were chosen for study because dead and decomposed plant tissues commonly release toxins into the environment through rainfall. Under natural conditions, those toxins may be transformed into other compounds that are more effective on the target species (Guenzi and McCalla, 1962; Patrick, 1971). Furthermore, most allelochemicals that have been identified are phenolic or lipophilic compounds which are secondary metabolites (Rice, 1984). The objectives of this study were to isolate and characterize some of the allelochemicals in water extracts of American eelgrass.

MATERIALS AND METHODS

Isolation of allelochemicals. American eelgrass plants were collected in central New Jersey on 9 August 1987. They were washed with tap water to remove soil and debris, cut into sections approximately 2 cm in length, and freeze-dried for two days. The freeze-dried plant tissues were then ground in a Wiley mill and stored at -20°C .

The general procedures for the isolation and characterization of allelochemicals from American eelgrass are outlined in Figure 1. Ten grams of finely ground plant tissue were extracted with 400 ml (1:40 w/v) of deionized distilled water at room temperature for 2 h. The extracts were filtered through four layers of cheesecloth and centrifuged at 6,000 rpm for 15 min. The supernatant liquid was decanted and added to 50 g of Amberlite XAD-16, a hydrophobic and nonpolar resin, to adsorb relatively low molecular weight and non-polar organic compounds from the aqueous system. The mixture of XAD-16 and plant extract was stirred at room temperature for 1 h then poured into a glass column and the remaining water was removed under vacuum. The column was then eluted with 400 ml of methanol which was evaporated to dryness in a rotary evaporator at 35 to 40 C. The residue was washed out of the evaporator flask with deionized distilled water, brought to 50 ml volume, and adjusted to pH 7.0 with 2 N NaOH and extracted four times with 50 ml ethyl ether:ethyl acetate (EE:EA = 1:1 v/v). The organic phases, designated as the neutral fraction, were combined and dried under reduced pressure at 30 to 35 C. The concentrate was taken up in 3 ml of methanol and passed through a 0.45 μm filter (Acro LC 13, Gelman).

The acidic fraction was obtained in a similar manner by acidifying the remaining aqueous fraction to pH 2.5 with 2 N HCl and extracting with EE:EA. The basic fraction was obtained by adjusting the acidified residue to pH 8.5 with 2 N NaOH and extracting with EE:EA. Both acidic and basic fractions were dried and taken up in 3 ml of methanol and passed through a 0.45 μm filter. The biological activity of each of these three fractions (neutral, acidic, and Riemer (1988). Briefly, the assays were conducted by counting 10 seeds into plastic petri dishes (15 by 90 mm) with two layers of filter paper on the bottom, and adding 500 μl of either neutral (N), acidic (A), or basic (B) fraction. In order to get this small amount of plant extract evenly



*ethyl ether : ethyl acetate (1:1v/v)

Figure 1. General procedures for the isolation and characterization of allelochemicals from American eelgrass.

and basic) as well as the aqueous residue from the XAD-16 resin separation procedure was assayed by means of a lettuce seed germination test (see section on bioassays, following). The experiment was repeated twice and there were four replications per experiment.

Preliminary separation. Based on the results of lettuce seed germination tests, the neutral fraction was chosen for further study. Samples were injected into a preparative HPLC system (Laboratory Data Control; Consta Metric Metering Model I & III Pumps; Rheodyne 7125 injector with a 2-ml sample loop; Pharmacia Fine Chemicals Single Path Monitor UV-1). The columns (reversed phase C18 preparative column) were eluted isocratically with 40% alcohol (Fisher HPLC grade alcohol reagent) in 1% acetic acid at a flow rate of 4 ml/min with a UV detector at 254 nm. Two fractions were collected from the columns and their biological activity was tested by means of a duckweed growth assay (see section on bioassays, following). The experiment was repeated twice and there were four replications per experiment.

Bioassays. Lettuce seed germination tests were conducted in a manner similar to those described by Cheng

distributed on the filter paper (63 by 63 mm), each sample was mixed with 1.5 ml of methanol before adding it to the filter paper. Thus, methanol was used as one control and compared to either N, A, or B fractions or deionized distilled water. Each experiment was repeated four times and there were four replications per experiment.

Growth rate and frond number of common duckweed (*Lemna minor*), herein after referred to as duckweed, have been used to measure toxicity of various types of compounds (Offord, 1946). This species has been used as a test organism for antibiotics, plant growth inhibitors, phytohormones, and many other toxins (Nickell and Finlay, 1954; Nickell and Celmer, 1965; Offord, 1946; Hillman, 1961). Duckweed was first utilized in allelopathy studies by Einhellig et al. (1985). The plant proved to be very sensitive as an indicator of allelopathic activity with small amounts of inhibitors (50 to 1,000 μM).

Bioassays were conducted with preparative HPLC fractions to determine their effects on growth rate and chlorophyll production in duckweed. An axenic duckweed culture was used. Stock cultures of duckweed were grown in cotton-stoppered 500 or 1,000 ml Erlenmeyer flasks with 200 to 350 ml of FNO growth medium (Frick, 1985). The stock flasks were maintained in a growth chamber at 29 ± 1 C under constant light ($120 \mu\text{E}/\text{sec}/\text{m}^2$).

The FNO medium was prepared, autoclaved, and pipetted into each well (2 ml) of a 24-well tissue culture plate (Falcon). One plant with three visible fronds was placed in each well in a laminar flow hood. Each treatment was repeated four times. The culture plates were then placed in the growth chamber where high humidity was maintained. The fronds were counted daily and the growth rate was calculated according to Hillman (1961):

$$\frac{\log [\text{final frond \#}] - \log [\text{initial frond \#}]}{\# \text{ days (d)}}$$

On day six, the plants were harvested from each well separately, the frond numbers were determined, and they were ground in 80% acetone with a polytron. The chlorophyll *a* and *b* contents were measured according to Arnon (1949).

Analytical HPLC to determine allelochemicals. The neutral fraction was analyzed on an analytical HPLC system. The column (Zorbax ODS column, 250 x 4mm, 6 μm , Du Pont) was eluted isocratically with 40% methanol in 1% acetic acid at a flow rate of 1 ml/min. The peak height was used for quantitative analysis of phytotoxins in the samples. Samples were spiked with various phenolic acids in order to identify the major peaks on the HPLC tracing.

RESULTS AND DISCUSSION

Isolation of allelochemicals. The allelopathic activities of the neutral, acidic, and basic fractions were assayed by the lettuce seed germination and growth test (Table 1). Methanol-treated filter paper did not inhibit seed germination or radicle growth but there was inhibition of germination and/or radicle growth by all three extract fractions. The neutral fraction appeared to contain more potent in-

hibitors or higher concentrations of inhibitors than the acidic or basic fractions. Germination was reduced by more than 50% and radicle growth was strongly inhibited by the neutral fraction. The non-germinated seeds in the N-fraction at the end of the 72-h growth period were shown to be able to germinate after re-incubation in deionized distilled water. The germinated seeds in all three fractions had fewer root hairs than those of the controls and brown root tips. In some cases dead seedlings were observed. The results appearing in Table 1 show that all three fractions inhibited radical growth significantly. Because both seed germination and radical growth were significantly reduced by the neutral fraction, this fraction was chosen for further study. Inhibition of lettuce seed germination and radicle growth was also detected in the aqueous residues from the XAD-16 resin separation procedure and from the partitioning procedures (data not shown). This observation indicates that there is more than one group of allelochemicals in American eelgrass.

Preliminary separation. In order to characterize the phytotoxins isolated from American eelgrass, further purification of the neutral fraction was accomplished with a preparative HPLC column. One major peak at retention time (RT) of 8.6 min and one or two minor peaks at retention time of 14-18 min were found in this fraction (Figure 2). They were collected separately in two fractions (F1: from RT 6.4 to 13.2 min and F2: from RT 13.2 to 24.2 min). The phytotoxicity of these two fractions was tested by a duckweed bioassay.

Effects of preparative HPLC fractions (F-1 & F-2) on growth rate and chlorophyll content of duckweed are shown in Figure 3 and Table 2. Figure 3 shows that the growth rate of duckweed as measured by frond number was reduced by F-1. Daily observation revealed that F-1 treated plants were small in size and dark in color. There were no roots produced on newly formed fronds. The F-1 fraction at the highest concentration (20 μl) significantly reduced chlorophyll *a* production (Table 2). However, chlorophyll *a/b* ratio was not affected by any treatment,

TABLE 1. EFFECTS OF NEUTRAL, ACIDIC, AND BASIC FRACTIONS FROM AMERICAN EELGRASS EXTRACT ON GERMINATION AND GROWTH IN LETTUCE. RADICLE GROWTH MEASURED AT THE END OF 72-H GROWTH PERIOD.¹

Treatment	Germination %			Radicle length mm	Percent of radicle suppression %
	24 hr	48 hr	72 hr		
Neutral fraction	2.5b	16.2b	41.2b	1.0c	97.8a
Acidic fraction	93.8a	97.5a	98.8a	3.3c	88.6a
Basic fraction	92.5a	100.0a	100.0a	15.6b	47.3b
MeOH	100.0a	100.0a	100.0a	28.9a	2.9c
Control (dist. water)	100.0a	100.0a	100.0a	29.7a	0c

¹Means in each column followed by the same letter are not significantly different at the 5% level of probability according to Duncan's Multiple Range Test. Each value is the mean of 8 replications.

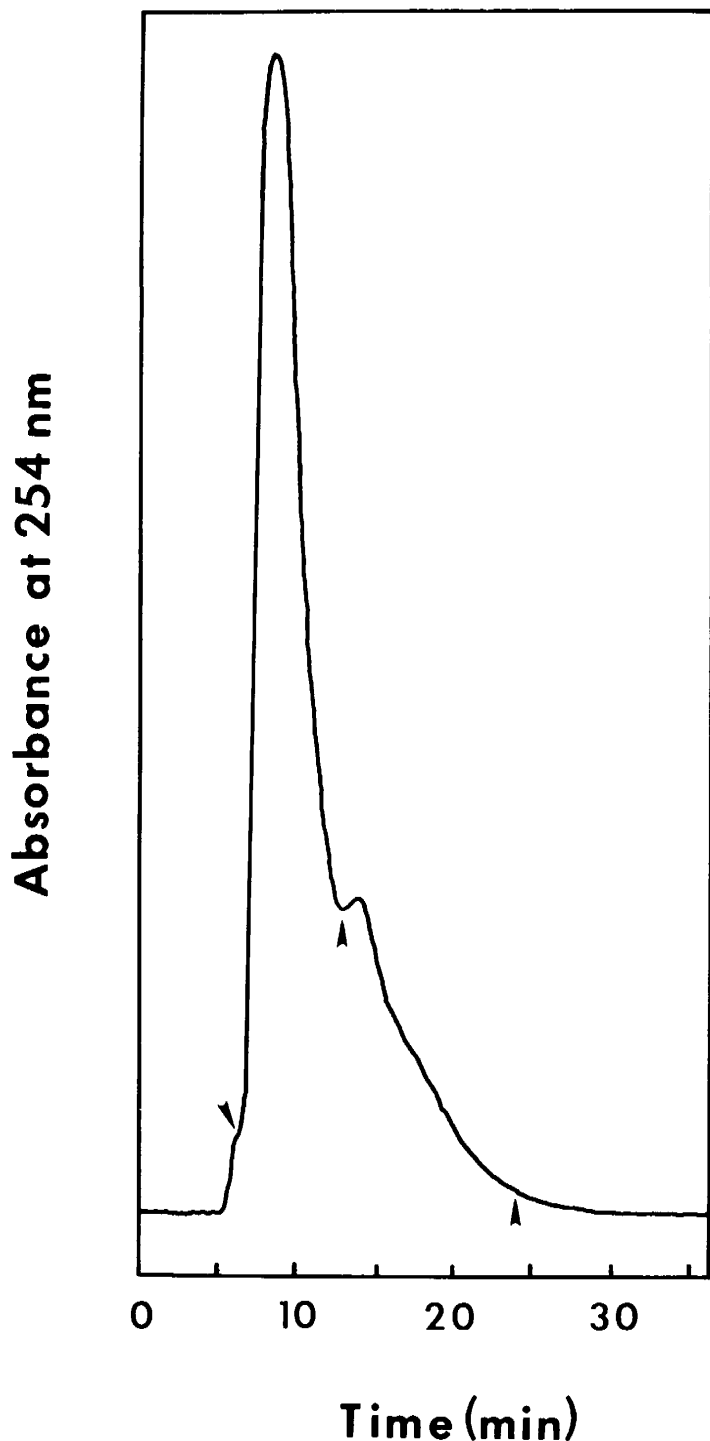


Figure 2. Preparative HPLC analysis of neutral fraction from American eelgrass. Two fractions (F1: from RT 6.4 to 13.2 min & F2: from 13.2 to 24.2 min as indicated by arrows) were collected and assayed for allelopathic activity with duckweed growth test.

which is similar to results reported by Ramirez Toro *et al.* (1988). Suppression of duckweed growth and chlorophyll production have been reported by Einhellig *et al.* (1985) and Leather and Einhellig (1985). The concentration threshold of allelochemicals tested by them was 50 to 1,000 μM . Many compounds have been shown to reduce frond size, including phenolic acids (Ramirez Toro *et al.*, 1988),

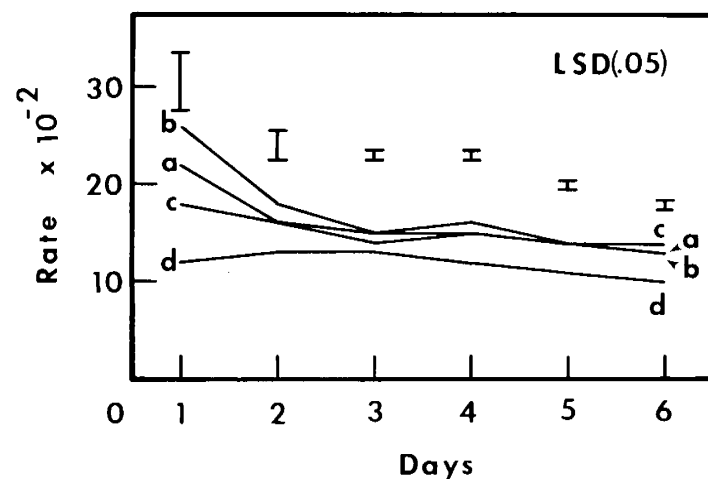


Figure 3. Effects of preparative HPLC fractions from the neutral fraction of American eelgrass on the growth of duckweed as measured by frond number. a: Control, b: Fraction 2, c: Blank collection from preparative HPLC, and d: Fraction 1. Twenty μl of each sample was added to 1.5 ml FNO medium in each well and repeated 4 times.

abscisic acid (McLaren and Smith, 1976; Newton, 1974; van Overbeek *et al.*, 1968), and herbicides (Offord, 1946). Evidence from this preliminary separation work indicated that F-1 contained potent inhibitors.

Because Amberlite XAD-16 adsorbs relatively low molecular weight and non-polar compounds from aqueous plant extracts, it was suspected that the allelochemicals in the neutral and F-1 fractions were phenolic compounds. Therefore, an attempt was made to identify commonly occurring phenolic acids in these fractions by means of an analytical HPLC system.

Analytical HPLC to determine allelochemicals. Figure 2 and Table 2 show that the neutral fraction from American eelgrass could be further fractionated by preparative HPLC into two fractions (F-1 & F-2) and that biological activity as determined by a duckweed growth test was associated with the F-1 fraction. A preliminary experiment with an analytical HPLC showed that 9 compounds were found in both

TABLE 2. EFFECTS OF PREPARATIVE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY FRACTIONS FROM AMERICAN EELGRASS EXTRACT ON CHLOROPHYLL CONTENT OF DUCKWEED.¹

Treatment	Chlorophyll content ($\mu\text{g/ml}$)		a/b ratio
	a	b	
F-1			
5 (μl)	4.8abc	1.7ab	2.8a
10	4.7abc	1.7ab	2.8a
15	4.2bc	1.5ab	2.8a
20	3.7c	1.4b	2.8a
F-2			
20	5.9a	2.0ab	3.0a
Blank	5.9a	2.2a	2.9a
Control (dist. water)	5.3ab	1.8ab	2.9a

¹Means in each column followed by the same letter are not significantly different at the 5% level of probability according to Duncan's Multiple Range Test. Each value is the mean of 8 replications.

the neutral and F-1 fractions without differences except in concentration. However, these 9 chemicals were not found in the F-2 fraction (data not shown). Thus, the neutral fraction was used to demonstrate the existence of some commonly occurring phenolic acids and the amount of each identified compound was also estimated.

Three benzoic acid derivatives or monophenols; p-coumaric (4-hydroxycinnamic acid), ferulic (4-hydroxy-3-methoxycinnamic acid), and vanillic (4-hydroxy-3-meth-

oxybenzoic acid); one hydrolyzable tannin (gallic acid or 3,4,5-trihydroxybenzoic acid); and one cinnamic acid derivative (chlorogenic acid or 3-[3,4-dihydroxycinnamate]) were used to spike the samples to demonstrate their existence in the neutral fraction. Figures 4 and 5 show the existence of large amounts of two compounds identified as gallic and vanillic acids at RT = 4.8 min and 8.6 min respectively. Ferulic acid (RT = 12.4 min) and p-coumaric acid (RT = 11 min) were present in very low concentra-

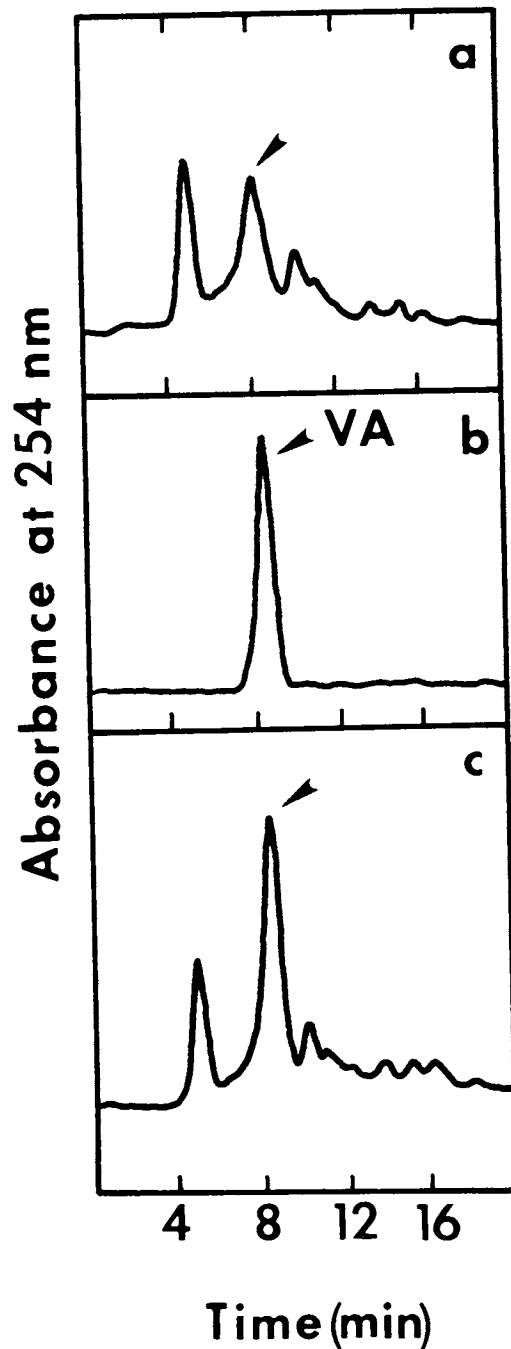
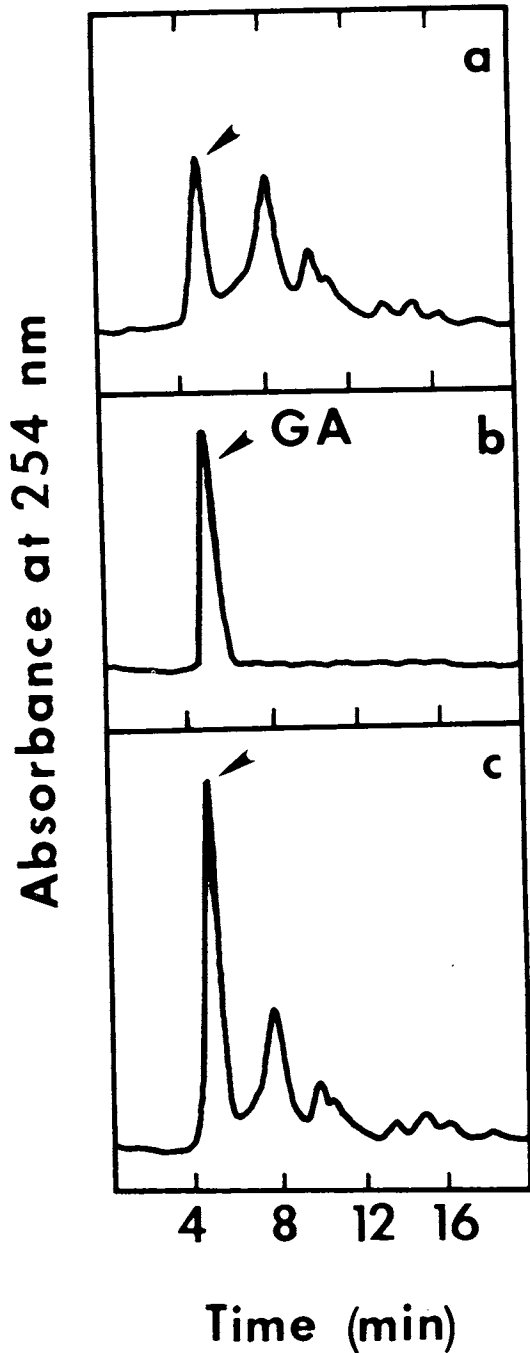


Figure 4. Analytical HPLC of the neutral fraction of aqueous extract from American eelgrass. Peak one (indicated by arrow) was spiked and identified by authentic gallic acid at retention time of 4.8 min. For conditions, see materials and methods. a: unspiked sample. b: gallic acid standard. c: sample spiked with gallic acid.

Figure 5. Analytical HPLC of the neutral fraction of aqueous extract from American eelgrass. Peak two (indicated by arrow) was spiked and identified as authentic vanillic acid at retention time of 8.6 min. For conditions, see Materials and Methods. a: unspiked sample. b: vanillic acid standard. c: sample spiked with vanillic acid.

TABLE 3. MAJOR PHENOLIC ACID COMPONENTS IN THE NEUTRAL FRACTION OF AMERICAN EELGRASS EXTRACT.

Phenolic acid	mg/100 gm D.W. (dry wt.)	mg/100 gm F.W. (fresh wt.)
Gallic acid	3.62	0.29
Vanillic acid	2.98	0.24

tions. The existence of these four phenolic compounds in American eelgrass has not been reported before, but they have been found in residues of many plants (particularly wheat, corn, sorghum, and oats) as soil toxins (Guenzi and McCalla, 1962,1966). Chlorogenic acid (RT = 6 min) was not found in the neutral fraction. In order to quantify the two major phenolic acids in this fraction, authentic gallic and vanillic acid standards of 10 mM were prepared for determining the linear correlation equation of peak height and concentration. The equations and coefficients of correlation (r) are $Y = 0.4656 + 0.0298 X$ ($r = 0.9998$) and $Y = 5.7336 + 0.0532 X$ ($r = 0.9948$) respectively. The content of gallic and vanillic acids in the neutral fraction were estimated and are presented in Table 3.

In general, phenolic compounds affect physiological processes such as photosynthesis, chlorophyll production, plant-water relations, protein synthesis, respiration, membrane permeability, and seed germination (Duke *et al.*, 1983; Einhellig and Rasmussen, 1979; Leather and Einhellig, 1985; Mersie and Singh, 1988; Patterson, 1981; Williams and Hoagland, 1982). Vanillic acid, a derivative of benzoic acid, at 10 mM was shown to have more effect than p-coumaric or ferulic acids on soybean (*Glycine max* (L.) Merr.) growth by Einhellig and Rasmussen (1979). Gallic acid is a derivative of dehydroshikimic acid. Both compounds were found to reduce net photosynthetic rate and stomatal conductance of single, fully expanded leaves of soybean at concentrations of 10 mM (Patterson, 1981). It is likely, therefore, that these two phenolic acids, abundant in American eelgrass, played a major role in the inhibition of seed germination, and seedling growth, and chlorophyll production in the bioassays reported on herein. The presence of p-coumaric and ferulic acids in American eelgrass was also proven (data not shown), however the concentrations of these two benzoic acid derivatives was not estimated because they are not major components of the plant.

ACKNOWLEDGMENT

The authors would like to thank Dr. Hugh Frick, Plant Science Dept., University of Delaware, Newark, DE 19717-1303, for providing axenic duckweed cultures for this research.

LITERATURE CITED

- Arnon, D. I. 1949. Copper enzyme in isolated chloroplasts. Polyphenoloxidases in *Beta vulgaris*. Plant Physiol. 24:1-15.
- Cheng, T. S. and D. N. Riemer. 1988. Allelopathy in threesquare burreed (*Sparganium americanum*) and American eelgrass (*Vallisneria spiralis*). J. Aquat. Plant Manage. 26:50-55.
- Duke, S. O., R. D. Williams, and A. H. Markhart, III. 1983. Inhibition of moisture stress and three phenolic compounds on lettuce seed germination. Ann. Botany. 52:923-926.
- Einhellig, F. A. and J. A. Rasmussen. 1979. Effects of three phenolic acids on chlorophyll content and growth of soybean and grain sorghum seedlings. J. Chem. Ecol. 5:815-824.
- Einhellig, F. A. 1985. Allelopathy—A natural protection, allelochemicals. In: Handbook of Natural Pesticides: Methods, Theory, Practice, and Detection. N.B. Mandava (ed.), CRC Press, Boca Raton, FL. 1:534 pp.
- Einhellig, F. A., G. R. Leather, and L. L. Hobbs. 1985. Use of *Lemna minor* L. as a bioassay in allelopathy. J. Chem. Ecol. 11:65-72.
- Frick, H. 1985. Boron tolerance and accumulation in the duckweed, *Lemna minor*. J. Plant Nutrition. 8:1123-1129.
- Guenzi, W. D. and T. M. McCalla. 1962. Inhibition of germination and seedling development by crop residues. Soil Sci. Soc. Am. Proc. 26:456-458.
- Guenzi, W. D. and T. M. McCalla. 1966. Phenolic acids in oats, wheat, sorghum, and corn residues and their phytotoxicity. Agron. J. 58:303-304.
- Hillman, W. S. 1961. The Lemnaceae, or duckweeds. Bot. Rev. 27:221-287.
- Leather, G. R. and F. A. Einhellig. 1985. Mechanisms of allelopathic action in bioassay. In: The Chemistry of Allelopathy. A. C. Thompson (ed.), American Chem. Soc., Washington, D.C. 470 pp.
- Mandava, N. B. 1985. Chemistry and biology of allelopathic agents. In: The Chemistry of Allelopathy. A. C. Thompson (ed.), American Chem. Soc., Washington, D.C. 470 pp.
- McLaren, J. S. and H. Smith. 1976. The effect of abscisic acid on growth, photosynthetic rate and carbohydrate metabolism in *Lemna minor* L. New Phytol. 76:11-20.
- Mersie, W. and M. Singh. 1988. Effects of phenolic acids and ragweed parthenium (*Parthenium hysterophorus*) extracts on tomato (*Lycopersicon esculentum*) growth and nutrient and chlorophyll content. Weed Sci. 36:278-281.
- Newton, R. J. 1974. Abscisic acid effects on growth and metabolism in the roots of *Lemna minor* L. Physiol. Plant. 30:108-112.
- Nickell, L. G. and A. C. Finlay. 1954. Antibiotics and their effects on plant growth. J. Agr. Food Chem. 2:178-182.
- Nickell, L. G. and W. D. Celmer. 1965. Structure-phytoactivity relationships in the penicillins. Nature. 206:302-304.
- Offord, H. R. 1946. Rapid estimation of the phytocidal action of chemicals. Science. 103:474-476.
- Patrick, Z. A. 1971. Phytotoxic substances associated with the decomposition in soil of plant residues. Soil Sci. 111:13-18.
- Patterson, D. T. 1981. Effects of allelopathic chemicals on growth and physiological responses of soybean (*Glycine max*). Weed Sci. 29:53-59.
- Putnam, A. R. 1985. Weed Allelopathy. In: Weed Physiology. D. O. Stephen (ed.), CRC Press, Boca Raton, FL. 1:165 pp.
- Putnam, A. R. and C. S. Tang. 1986. The Science of Allelopathy. John Wiley & Son, New York, NY. 317 pp.
- Ramirez Toro, G. I., G. R. Leather, and F. A. Einhellig. 1988. Effects of three phenolic compounds on *Lemna gibba* G3. J. Chem. Ecol. 14:845-853.
- Rice, E. L. 1984. Allelopathy. Academic Press, Orlando, FL, 422 pp.
- Tang, C. S. and C. C. Young. 1982. Collection and identification of allelopathic compounds from the undisturbed root system of bigalita limpograss (*Hemarthria altissima*). Plant Physiol. 69:155-160.
- van Overbeek, J., M. Iona, and M. I. R. Mason. 1968. Dormin and cytokinin: Growth regulation of *Lemna*. Acta Bot. Neerl. 17:441-444.
- Williams, R. D. and R. E. Hoagland. 1982. The effects of naturally occurring phenolic compounds on seed germination. Weed Sci. 30:206-212.
- Williams, R. D. 1984. Non-polar macroreticular resin to recover phenolic acids from a subtropical latosol. Soil Biol. Biochem. 16:377-380.
- Williams, R. D. and C. S. Tang. 1984. Recovery of phenolic acids by Amberlite XAD-4 resin from tropical soils. Proc. Natl. Sci. Council. B. ROC. 8:26-29.
- Williams, R. D. and T. C. Chou. 1985. Auto-intoxication in residues of *Asparagus officinalis* L. Plant and Soil. 85:385-393.