J. Aquat. Plant Manage. 24: 76-79

Germination Suppression By Extracts of Aquatic Plants[1]

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ABSTRACT

Water extracts of five species of aquatic plants were shown to suppress seed germination to varying degrees in three of four species of terrestrial plants tested. The terrestrial plants included two monocots and two dicots. In some instances seeds germinated, but the seedlings died or became grossly deformed after a few days. Evidence is presented which indicates that the germination effects were not merely the result of salts leaching from the aquatic plant tissues.

Key words: allelopathy, biocontrol, leachates, *Cabomba, Elodea, Spirodela, Myriophyllum, Vallisneria.*

INTRODUCTION

It has long been known that some plants release substances into the environment that have detrimental effects on other species of plants. This phenomenon is known as allelopathy and allelopathic substances have been reported from a wide range of plant taxa including crop species (Shilling *et al.,* 1985), terrestrial weeds (Fischer and Quijano, 1985; Nicollier *et al.,* 1985), aquatic angiosperms (Ashton *et al.,* 1985) and algae (Moon and Martin, 1985). The list of plants detrimentally affected by allelopathic compounds is equally diverse. Among the aquatic plants studied, there have been reports of algae affecting other species of algae, algae affecting submersed angiosperms, aquatic angiosperms affecting algae, and aquatic angiosperms affecting other aquatic angiosperms.

While conducting field experiments on the value of harvested aquatic weeds as mulch, the junior author observed that soil which had been briefly covered with aquatic plants in the spring and then uncovered for the remainder of the summer, remained nearly weed-free for the entire growing season (Riemer, unpublished data). This observation raised the question of whether or not leachates from the dried aquatic plants had accumulated in the soil and prevented the establishment of normal weed populations.

The present research was designed and conducted to determine if water-extractable substances from aquatic plants had the ability to prevent, suppress, delay, or in any way affect the germination of seeds of terrestrial plants or to affect the seedlings after they had germinated. The terrestrial plants were chosen to represent as wide a spectrum as possible, and included two monocots and two dicots as well as two crop species and two weed species. They were also chosen to represent some variation in seed size and hardness of seed coat.

MATERIALS AND METHODS

Cabomba (*Cabomba caroliniana* Gray), western elodea [*Elodea nuttallii* (Planch.) St. John], watermilfoil (*Myriophyllum* sp.), giant duckweed [*Spirodela polyrhiza* (L.) Schleid.], and American eelgrass (*Vallisneria americana* Michx.) plants were collected on 2 August 1985 and transported directly to the laboratory where they were oven-dried at 80°C to constant weight. They were then ground in a Wiley mill to pass through a 2 mm seive and 100 g of ground tissue were extracted in 1.5 l of distilled water at room temperature for 48 hr. The extract was filtered through Whatman 2V filter paper and stored in a refrigerator.

The terrestrial plants used as indicators were lettuce (*Lactuca sativa* L. var. "Buttercrunch"), wheat (*Triticum aestivum* L. var. "Pike"), barnyardgrass [*Eichinochloa crus-galli* (L.) Beauv.], and ivyleaf morningglory [*Ipomea hederacea* (L.) Jacq.). All germination tests were conducted by counting 25 seeds into a plastic, disposable petri dish with a double layer of filter paper on the bottom and adding 10 ml of distilled water (control), 10 ml of aquatic plant tissue extract (1X treatment), or 5 ml of distilled water and 5 ml of aquatic plant tissue extract (0.5X treatment). Four replications were used in all experiments.

The dishes were kept in a germinator with 12 hr of light and 12 hr of darkness. The temperature during the light period was 30°C and the temperature during the dark period was 25°C. Seeds were examined after specific periods of time, depending upon species, and the germinated seeds were removed from the dishes and their numbers recorded. Seeds were considered to have germinated only when the radicle and/or the epicotyl equaled or exceeded the length of the seed.

For three species, the length of the radicle was measured and recorded at the same time that the first germination counts were made. For ivyleaf morningglory, this was impossible because the radicle was too brittle and convoluted for accurate measurement.

In order to estimate the amounts of inorganic salts present in the tissue extracts, the concentrations of five ions were measured in each of them. The ions measured were K, Ca, Mg, P, and Na. Inorganic salt solutions were then made, having approximately the same ionic content as the extract with the highest salt concentration (American eelgrass) and the extract with the lowest salt concentration (western elodea). Germination tests were conducted using

[1]N.J. Agr. Expt. Sta. Pub. No. D-15180-1-86, supported by State and Hatch Act Funds.

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these two salt solutions in the same way that tests had been conducted with the plant extracts.

All data were transformed into "percent of control" in order to eliminate the effects of inherent differences in germination percentage and radicle size among the four species of terrestrial plants used as indicators. The data were then subjected to standard analysis of variance procedures and a least significant difference (LSD) was calculated for each data set.

RESULTS AND DISCUSSION

The concentrations of ions found in the aquatic plant extracts are shown in Table 1 and the compositions of the two salt solutions are shown in Table 2. Germination results and radicle length (where applicable) for the tissue extracts are presented in Tables 3 through 6. Lettuce (Table 3) was affected to a much greater extent than the other three species. The only extract failing to have a significant effect on lettuce germination was the extract of western elodea. All of the treatments had a highly significant effect on radicle growth.

Barnyard grass (Table 4) was much less severely affected. Only the extracts of western elodea at the .5X concentration, cabomba at the 1X concentration, and American eelgrass at the 1X concentration significantly affected germination. As with lettuce, however, all treatments resulted in a highly significant reduction in radicle length. Ivyleaf morningglory germination was not significantly affected by any of the treatments, although American eelgrass extract reduced the percent germination to approximately one half that of the controls (Table 5).

TABLE 1. CONCENTRATIONS OF FIVE IONS IN WATER EXTRACTS OF FIVE SPECIES OF AQUATIC PLANTS.

	Ion Concentration (ppm)				
Aquatic Plant	K	Ca	Mg	P	Na
Giant Duckweed	748	257	220	114	246
Western Elodea	469	282	108	32	253
Cabomba	802	404	171	107	1039
American Eelgrass	2556	570	254	109	954
Watermilfoil	800	343	184	103	522

TABLE 2. CONCENTRATIONS OF FIVE IONS IN SALT SOLUTIONS PREPARED TO APPROXIMATE THE WATER EXTRACTS OF AMERICAN EELGRASS AND WESTERN ELODEA.

	Ion Concentration (ppm)				
Solution	K	Ca	Mg	P	Na
# 1 (Approximating Western Elodea)	451	280	96	31	253
# 2 (Approximating American Eelgrass)	2604	600	240	100	506

TABLE 3. GERMINATION AND RADICLE LENGTH OF LETTUCE (VAR. BUTTERCRUNCH) IN WATER EXTRACTS OF FIVE SPECIES OF AQUATIC PLANTS. ALL DATA ARE PRESENTED AS PERCENT OF CONTROL AND REPRESENT THE MEAN OF FOUR REPLICATIONS.

	Germination		Radicle Length
Treatment	3 days	5 days	3 days
Control	100	100	100
Giant Duckweed (.5X)	73**	79**	53**
Giant Duckweed (1X)	0**	24**	0**
Western Elodea (.5X)	85NS	86*	39**
Western Elodea (1X)	36**	89NS	34**
Cabomba (.5X)	54**	70**	70**
Cabomba (1X)	0**	0**	0**
American Eelgrass (.5X)	2**	33**	8**
American Eelgrass (1X)	0**	0**	0**
Watermilfoil (.5X)	60**	86**	48**
Watermilfoil (1X)	0**	24**	0**
LSD .05	15.949	11.185	10.210
LSD .01	21.478	15.062	13.750

** = Significantly different from Control at .01 level.
* = Significantly different from Control at .05 level.
NS = Not significantly different from Control at .05 level.

TABLE 4. GERMINATION AND RADICLE LENGTH OF BARNYARD GRASS IN WATER EXTRACTS OF FIVE SPECIES OF AQUATIC PLANTS. ALL DATA ARE PRESENTED AS PERCENT OF CONTROL AND REPRESENT THE MEAN OF FOUR REPLICATIONS.

	Germination		Radicle Length
Treatment	3 days	5 days	3 days
Control	100	100	100
Giant Duckweed (.5X)	95NS	95NS	69**
Giant Duckweed (1X)	94NS	94NS	41**
Western Elodea (.5X)	88*	88*	53**
Western Elodea (1X)	94NS	94NS	34**
Cabomba (.5X)	91NS	93NS	72**
Cabomba (1X)	86**	88*	34**
American Eelgrass (.5X)	93NS	95NS	26**
American Eelgrass (1X)	80**	85**	0**
Watermilfoil (.5X)	91NS	92NS	46**
Watermilfoil (1X)	90NS	96NS	32**
LSD .05	10.210	9.017	4.789
LSD .01	13.750	12.144	6.449

** = Significantly different from Control at .01 level.
* = Significantly different from Control at .05 level.
NS = Not significantly different from Control at .05 level.

TABLE 5. GERMINATION OF IVYLEAF MORNINGGLORY IN WATER EXTRACTS OF FIVE SPECIES OF AQUATIC PLANTS. ALL DATA ARE PRESENTED AS PERCENT OF CONTROL AND REPRESENT THE MEAN OF FOUR REPLICATIONS.

	Germination	
Treatment	10 days	15 days
Control	100	100
Giant Duckweed (.5X)	121NS	131NS
Giant Duckweed (1X)	112NS	119NS
Western Elodea (.5X)	107NS	113NS
Western Elodea (1X)	133NS	142NS
Cabomba (.5X)	75NS	79NS
Cabomba (1X)	66NS	65NS
American Eelgrass (.5X)	95NS	94NS
American Eelgrass (1X)	47NS	51NS
Watermilfoil (.5X)	61NS	68NS
Watermilfoil (1X)	148NS	144NS
LSD .05	68.063	58.510
LSD .01	91.662	78.796

NS = Not significantly different from Control at .05 level.

Wheat was intermediate in its response (Table 6). Three of the extracts (cabomba, American eelgrass, and watermilfoil, all at the 1X concentration) significantly reduced the percentage of germination in this species. As with the lettuce and barnyard grass, all treatments resulted in a highly significant reduction in the length of the radicle.

The American eelgrass extract at the 1X concentration resulted in the lowest percentage of germination and the shortest radicles in all experiments. This species can clearly be identified as the most inhibitory of all of the extracts tested. No one extract can similarly be identified as the least inhibitory, however. In different tests with different indicator species, different extracts resulted in the lowest levels of inhibition.

Table 7 presents the results of the germination trials with the two inorganic salt solutions and lettuce. This table also includes comparable germination percentages with western elodea extract and American eelgrass extract, from table 3. It can be seen that the weaker salt solution had no effect on germination at either 3 or 5 days. At both times the percent germination was identical to the control. The 1X western elodea extract, which the weak salt solution was approximating, caused a highly significant reduction at 3 days but no significant reduction at 5 days. This indicates that some factor or factors other than inorganic salts were functioning to suppress germination in the tissue extract.

The more concentrated salt solution, which was intended to approximate the extract of American eelgrass, suppressed lettuce germination to 58% of the control at both 3 and 5 days. While these percentages are significantly below the controls, they are well above the 0% germination observed with the American eelgrass extract, again indicating that the tissue extract was exerting its suppression through factors in addition to salts dissolved from the tissue. Radicle length was significantly reduced by both salt solutions, but the degree of reduction was not nearly as severe as it was with the extracts of the two aquatic plants.

Table 8 presents the results of the germination trials with the two inorganic salt solutions and wheat. As in Table 7, germination percentages from trials with plant extracts

TABLE 6. GERMINATION AND RADICLE LENGTH OF WHEAT (VAR. PIKE) IN WATER EXTRACTS OF FIVE SPECIES OF AQUATIC PLANTS. ALL DATA ARE PRESENTED AS PERCENT OF CONTROL AND REPRESENT THE MEAN OF FOUR REPLICATIONS.

Treatment	Germination		Radicle Length
	5 days	8 days	5 days
Control	100	100	100
Giant Duckweed (.5X)	101NS	98NS	83**
Giant Duckweed (1X)	93NS	93NS	46**
Western Elodea (.5X)	103NS	100NS	80**
Western Elodea (1X)	99NS	101NS	59**
Cabomba (.5X)	94NS	99NS	38**
Cabomba (1X)	77**	90NS	21**
American Eelgrass (.5X)	96NS	95NS	30**
American Eelgrass (1X)	44**	66**	13**
Watermilfoil (.5X)	102NS	99NS	69**
Watermilfoil (1X)	83*	87*	47**
LSD .05	15.144	11.277	6.126
LSD .01	20.395	15.187	8.250

** = Significantly different from Control at .01 level.
* = Significantly different from Control at .05 level.
NS = Not significantly different from Control at .05 level.

TABLE 7. RESULTS OF LETTUCE GERMINATION TRIALS WITH 2 INORGANIC SALT SOLUTIONS COMPARED TO THE PLANT TISSUE EXTRACTS THEY APPROXIMATE. ALL DATA ARE PERCENTS OF CONTROL.

Treatment	Germination				Root Length	
	3 days	3 days[1]	5 days	5 days[1]	3 days	3 days[1]
Control	100	100	100	100	100	100
Salt Solution #1	100		100		62**	
Western Elodea		36**		89NS		34**
Salt Solution #2	58**		58**		26**	
American Eelgrass		0**		0**		0**
LSD .05	26.1		16.3		8.5	
LSD .01	39.5		24.7		12.8	

[1]These data are extracted from Table 3 and are included here for purposes of comparison.

TABLE 8. RESULTS OF WHEAT GERMINATION TRIALS WITH 2 INORGANIC SALT SOLUTIONS COMPARED TO THE PLANT TISSUE EXTRACTS THEY APPROXIMATE. ALL DATA ARE PERCENTS OF CONTROL.

Treatment	Germination				Root Length	
	5 days	5 days[1]	8 days	8 days[1]	5 days	5 days[1]
Control	100	100	100	100	100	100
Salt Solution #1	108NS		104NS		59**	
Western Elodea		99NS		101NS		59**
Salt Solution #2	97NS		96NS		38**	
American Eelgrass		44**		66**		13**
LSD .05	13.2		10.4		5.2	
LSD .01	20.0		15.7		7.9	

[1]These data are extracted from Table 6 and are included here for purposes of easy comparison.

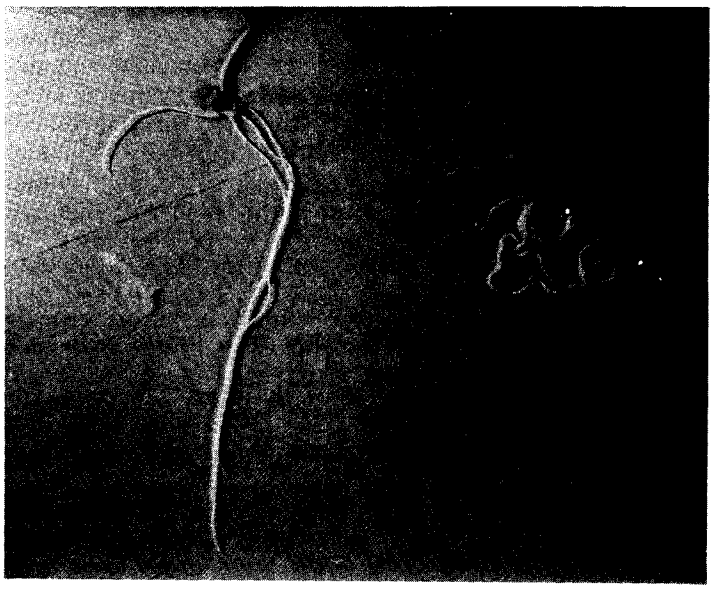

Figure 1. Five day old wheat seedlings germinated in distilled water (left) and in a water extract of dried watermilfoil tissue (right).

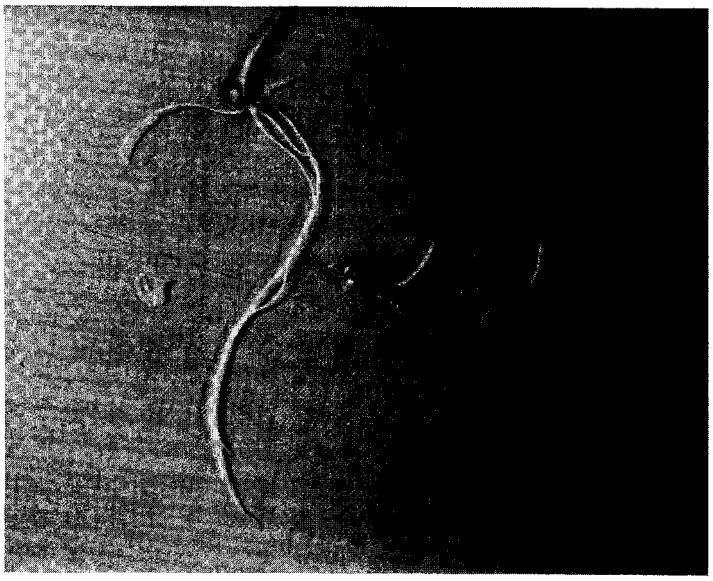

Figure 2. Five day old wheat seedlings germinated in distilled water (left) and in a water extract of dried American eelgrass tissue (center and right).

are included for easy comparison. The data in this table further indicate that salts alone cannot account for all of the effects manifested by the tissue extracts. The high salt concentration, for example, had no significant effect on the percentage of wheat germination while the American eelgrass extract at 1X concentration had a highly significant effect.

In addition to the quantitative measurements discussed in the preceding paragraphs, various epinastic effects and discolorations were observed in seedlings germinating in the aquatic plant extracts. Ivyleaf morningglory and wheat appeared to be particularly susceptible to these effects. Several ivyleaf morningglory plants emerged from the seed coat but the radicle turned black and died within a day or two of emergence. Radicles of other plants of this species became twisted and convoluted to an extreme degree but did not turn black or die.

In wheat, the radicles exhibited pronounced epinasty in the aquatic plant extracts but did not show any discoloration. Figure 1 shows the degree of radicle epinasty resulting from the 1X concentration of the watermilfoil extract. Figure 2 shows the extreme reduction in radicle length caused by the 1X concentration of American eelgrass extract.

The results of these experiments provide strong evidence that aquatic angiosperm leachates contain substances that prevent, inhibit, or otherwise have a negative impact upon germination and seedling development in some terrestrial plant species. The sensitivity of all terrestrial plants to these substances is not equal. The chemical nature of the substances is unknown at the present time but it appears that they consist of more than just the inorganic salts which are present. Additional work needs to be done to isolate and identify the allelopathic compound or compounds responsible for the observed effects.

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