Johnstone, I. M., B. T. Coffey, and C. Howard-Williams. 1985. The role of recreational boat traffic in interlake dispersal of macrophytes: A New Zealand case study. J. Environ. Manage. 20:263-279.

Kimbel, J. C. 1982. Factors influencing potential intralake colonization by *Myriophyllum spicatum*. Aquat. Bot. 14:295-307.

Madsen, J. D. and C. W. Boylen. 1988. The physiological ecology of Eurasian watermilfoil (*Myriophyllum spicatum*) and a native macrophyte (*Potamogeton praelongus*): Depth distribution of biomass and photosynthesis. Rensselaer Fresh Water Institute Report 88-5, Troy, New York. In preparation.

Ogden, E. C., J. K. Dean, C. W. Boylen, and R. B. Sheldon. 1976. Field guide to the Aquatic Plants of Lake George, New York. New York State Museum Bull. 426, Albany, New York. 65 pp.

Rensselaer Fresh Water Institute. 1986. A survey of Eurasian watermilfoil in Lake George. Fresh Water Institute Report 86-3, Troy, New York. 8 pp.

Rensselaer Fresh Water Institute. 1988. Report on the Lake George Chemical Monitoring Program January 1987-December 1987. Fresh Water Institute Report 88-2. Troy, New York. 20 pp.

Rensselaer Fresh Water Institute, New York State Department of Environmental Conservation, and Adirondack Park Agency. 1988. Lake George Aquatic Vegetation Survey 1987 Interim Report. New York Department of Environmental Conservation Report, Albany, New York. March 30, 1988.

Rogers, K. H. and C. M. Breen. 1980. Growth and reproduction of *Potamogeton crispus* in a South African lake. J. Ecol. 68:561-571.

Sculthorpe, C. D., 1967. The biology of aquatic vascular plants. William Clowes, London. 610 pp.

Weber, J. A. 1972. The importance of turions in the propagation of *Myriophyllum exalbescens* (Haloragidaceae) in Douglas Lake, Michigan. Mich. Bot. 11:115-121.

J. Aquat. Plant Manage. 26: 50-55

Allelopathy In Threesquare Burreed (*Sparganium americanum*) and American Eelgrass (*Vallisneria americana*)

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

ABSTRACT

Water extracts of dried threesquare burreed (*Sparganium americanum* Nutt.) shoots, threesquare burreed roots, and American eelgrass (*Vallisneria americana* Michx.) plants were shown to have allelopathic properties when tested by a bioassay technique using lettuce (*Lactuca sativa* L. var. "Buttercrunch") as the test organism. Allelopathy was manifested as a reduction in germination percentage and in radicle growth and it showed a concentration response. Osmotic potentials of less than 70 milliosmols per kilogram (mOs/kg) did not affect lettuce germination or gowth. The pH of the plant extracts was shown to have no effect on germination or growth. Extract of burreed roots caused the lettuce hypocotyls to be short and bulbous but the same phenomenon was not observed with extract of burreed shoots

Key words: allelopathy, aquatic plants, burreed, eelgrass, leachates, *Sparganium, Vallisneria.*

INTRODUCTION

The existence of allelopathy has been well documented over the past few decades. A myriad of weed species have been reported to have allelopathic properties (Putnam, 1985; Rice, 1984). Most of these reports pertain to terrestrial weeds but a few workers did report on allelopathy in aquatic species (Ashton, *et al.,* 1985; Szczepanski, 1977). Documenting the extent of allelopathy in aquatic weeds and isolating and identifying the allelochemicals which occur in them is an important undertaking. It could lead to the use of these compounds in the control of terrestrial or aquatic weeds. It could also lead to the eventual commercial extraction of these compounds from mechanically harvested aquatic weeds which would provide a market for the harvested biomass, thereby reducing the disposal problem and the cost of harvesting to the consumer. The commercial use of natural products is not without precedent. At least 17 natural products have already been developed for commercial use as pesticides or plant growth regulators (Rice, 1983; Misato, 1982).

To prove the existence of allelopathy, several procedures must be undertaken. Among the most important of these are bioassays. Although no standardized and universally accepted bioassays have been developed, they are still useful and necessary tools in the study of allelopathy (Leather and Einhellig, 1986). However, many non-allelopathic factors can affect seed germinanation when conducting bioassays to study allelopathy. Among these are the osmotic potential, ionic strength, and pH of the plant extracts being studied (Duke *et al.,* 1983; Chou and Young, 1974; Anderson and Loucks, 1966; Bell, 1974; Leather, 1983a, b; Leather and Einhellig, 1985; Chou and Chung, 1974; El-Ghazal and Riemer, 1986 Reynolds, 1975). The studies reported on herein were conducted to determine if crude water extracts of aquatic angiosperms had the ability to inhibit or delay seed germination in terrestrial plants or to affect radicle growth after germination. The effects of osmotic potential and pH of the water extracts were also considered.

[1]N.J. Agr. Expt. Sta. Pub. No. D-15180-1-87, supported by State and Hatch Act Funds. Received for publication June 3, 1988.

[2]Research Asst. and Assoc. Prof., respectively, Dept. of Soils and Crops, Rutgers Univ., Agr. Expt. Sta., New Brunswick, NJ 08903.

MATERIALS AND METHODS

Preliminary Screening of Species. Six species of aquatic plants were collected from wild populations in New Jersey to be screened for possible allelopathic properties. The species chosen were variable watermilfoil (*Myriophyllum heterophyllum* Michx.), purple cabomba (*Cabomba caroliniana* Gray), American eelgrass, giant duckweed (*Spirodela polyrhiza* (L) Schleid.), threesquare burreed, and common cattail (*Typha latifolia* L.). The threesquare burreed plants were separated into shoots and roots for individual testing.

The plants were oven dried, water extracts of the dried tissues were prepared, and lettuce seed germination tests were conducted with the extracts as described in the following section. Based on these screening tests, threesquare burreed shoots, threesquare burreed roots, and American eelgrass were selected for more detailed testing.

Detailed Studies. Threesquare burreed and American eelgrass were collected from natural populations in central New Jersey on 26 September 1986. They were washed with tap water to remove soil and debris and air-dried at 80°C for three days. The burreed roots were then separated from the shoots and all plants were cut into sections approximately 2 cm in length and stored in the dark at room temperature.

Twenty gm (dry wt.) of each of the three types of tissues were extracted in 300 ml (1:15 W/V) of deionized water at room temperature for 2 hr. The extracts were filtered through Fisher's quantitative filter paper (9-795-C IPK) and serially diluted with deionized water into five concentrations (5, 25, 50, 75, and 100%) They were then used immediately in bioassays without storage. The osmotic potential of each dilution was measured with a Wescor 5500 Vapor Pressure Osmometer and the pH was measured with a glass electrode pH meter. The germination tests were conducted by counting 12 lettuce seeds into a plastic petri dish (15 x 150 mm) with four layers of filter paper on the bottom, and adding 7 ml of aquatic plant extract or deionized water as a control. The lettuce seeds were presoaked in deionized water for 1 hr and only seeds which sank to the bottom during this time were used in the germination trials. Each experiment was repeated three times and there were four replications per experiment.

The dishes were kept in a germinator with 24 hr of darkness, a constant temperature of 28 ± 1°C, and 100% relative humidity. Seeds were examined every 24 hr and the germination percentage was determined and recorded. Germination was defined as the emergence of the radicle 2 mm or more beyond the seed coat. At the end of 72 hr, the final germination percentage was determined and the length of each radicle and the pH of the solution in each dish were measured.

In order to study the effects of osmotic potentials on germination, mannitol solutions with osmotic potentials of 34, 64, 105, 150, and 208 mOs/kg were prepared. Germination tests were conducted using these mannitol solutions in exactly the same way that tests had been conducted with the plant extracts These experiments were repeated four times with four replications per experiment.

RESULTS AND DISCUSSION

Preliminary Screening of Species. Figure 1 shows the percent germination of lettuce seed in water extracts of the species used in the preliminary screening test. It is apparent from this graph that threesquare burreed shoot (E) and American eelgrass (C) extracts depressed germination percentages far below the controls and the other extracts tested, including those of threesquare burreed roots (F).

Figure 2 shows the lengths of the radicles and coleoptiles of the lettuce seedlings from the same tests at the end of 72 hr. It can be seen that extracts of threesquare burreed shoots (E) and American eelgrass (C) inhibited growth of both radicles and coleoptiles more than any other extracts except that of purple fanwort. As a result of these preliminary trials, American eelgrass, burreed shoots, and burreed roots were selected for further study.

Detailed Studies. The effects of osmotic potential on radicle growth in the mannitol experiments are shown in Figure 3. It can be seen from this figure that radicle growth

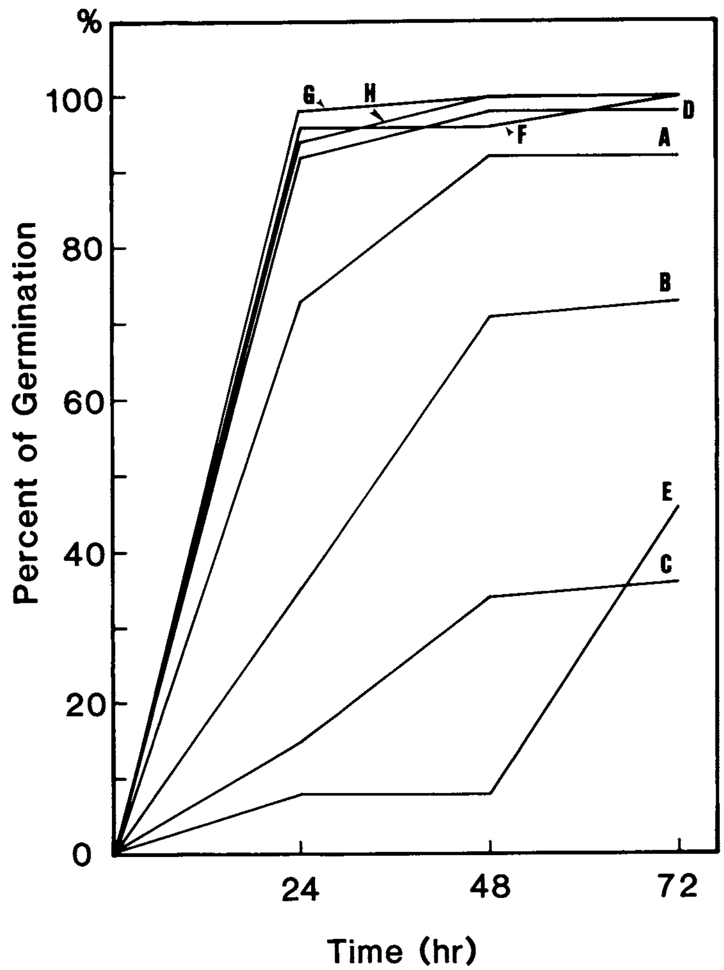

Figure 1. Percent germination of lettuce seed in water extracts of various aquatic plants. A = variable watermilfoil, B = purple fanwort, C = American eelgrass, D = giant duckweed, E = threesquare burreed shoots, F = threesquare burreed roots, G = common cattail leaves, and H = controls.

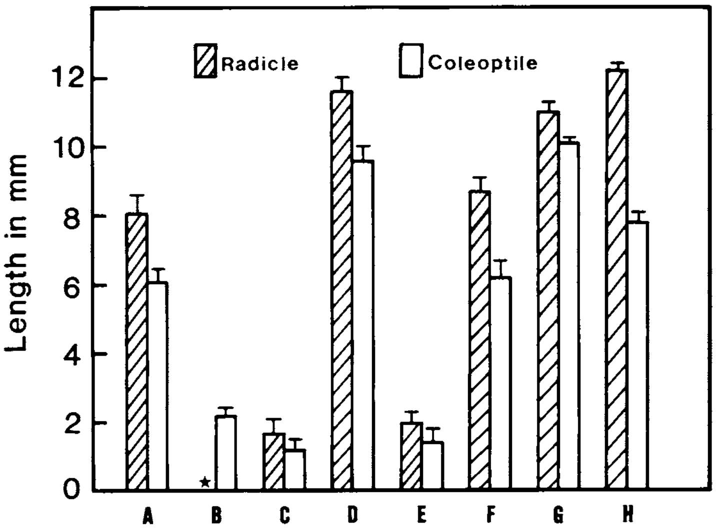

Figure 2. Radicle and coleoptile lengths of lettuce seedlings germinated in water extracts of various aquatic plants. A = variable watermilfoil, B = purple fanwort, C = American eelgrass, D = giant duckweed, E = threesquare burreed shoots, F = threesquare burreed roots, G = common cattail leaves, and H = controls. *Seeds germinated but radicle died before measurement.

was not affected by osmotic potentials below 70 mOs/kg. Table 1 shows the effect of osmotic potential on the germination percentage of lettuce. It is obvious that no supression of germination occured at any of the osmotic potentials used in these experiments. These results are similar to those of Bell (1974) who demonstrated that osmotic potentials greater than 75 mOs/kg inhibited early radicle growth. Delayed germination at osmotic potentials above 150 mOs/kg was reported by Leather (1983a) and Leather and Einhellig (1985) but was not observed in the experiments being reported on herein

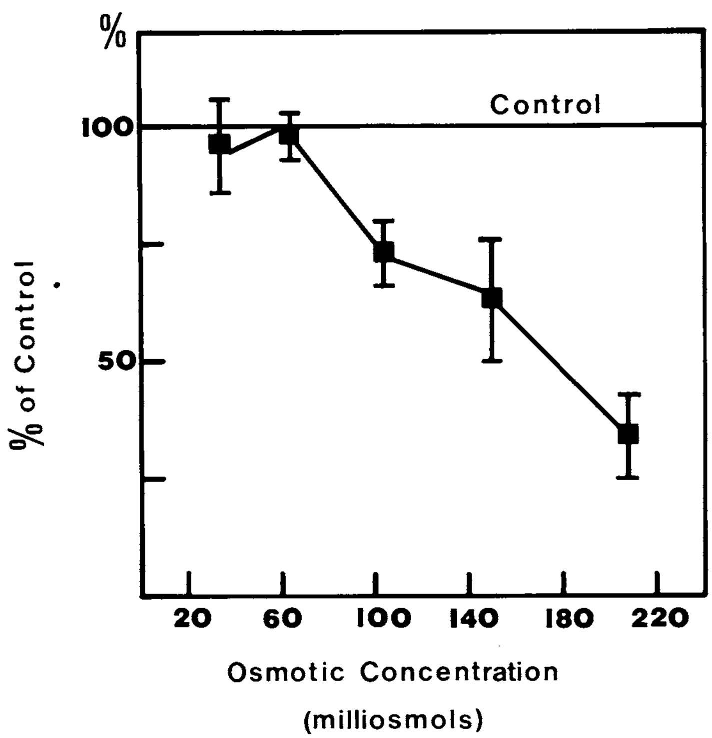

Figure 3. Effect of osmotic concentration of mannitol solutions on growth of lettuce radicles. Data are presented as percent of growth of radicles in control plants.

TABLE 1. EFFECTS OF OSMOTIC CONCENTRATION ON GERMINATION AND RADICLE GROWTH IN LETTUCE. RADICLE GROWTH MEASURED AT THE END OF 72-H GROWTH PERIOD.

Treatment (milliosmols)	*Germination % 24 hr	48 hr	72 hr	*Radicle Length (mm)
Control				
(22)	99a	100a	100a	21a
Mannitol				
(34)	96a	98a	98a	20a
(64)	97a	99a	99a	20a
(105)	98a	98a	98a	15b
(150)	95a	97a	97a	13c
(208)	83b	90b	90b	7d

*Means 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.

Table 2 presents the results of the germination trials with water extracts of threesquare burreed shoots, threesquare burreed roots, and American eelgrass. It can be seen from this table that the half-strength burreed shoot extract (osmotic potential of 70 mOs/kg) resulted in a highly significant reduction in germination percentage and radicle growth at 72 hr. Figure 4 shows lettuce seedlings from

TABLE 2. EFFECTS OF WATER EXTRACTS (1:15 W/V) OF THREESQUARE BURREED AND AMERICAN EELGRASS ON GERMINATION AND RADICLE GROWTH IN LETTUCE. RADICLE GROWTH MEASURED AT THE END OF 72-H GROWTH PERIOD.

Treatment	Osmotic Concentration (milliosmols)	*Germination % 24 hr	48 hr	72hr	*Radicle length (mm)
Control	22	96a	98a	99a	18b
Threesquare Burreed Shoot					
5	26	96a	98a	99a	19a
25	55	90a	94a	96a	13d
50	70	33c	50c	60c	5f
75	88	17e	18e	23e	1g
100	108	8e	8f	8f	1g
American Eelgrass					
5	27	95a	100a	100a	19a
25	55	94a	98a	98a	15c
50	72	76b	94a	95a	12d
75	91	36c	78b	80b	8e
100	113	28c	30d	35d	2g
Control	22	99a	99a	100a	19a
Threesquare Burreed Root					
100	50	98a	100a	100a	14b

*Means 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. Comparisons can be made only above the dotted line or below the dotted line.

Figure 4. Comparison of radicle length of lettuce seedlings germinated in various concentrations of a water extract of three- square burreed shoots. The concentrations are, from left to right, 100%, 75%, 50%, 25%, 5%, and control.

each of the concentrations of threesquare burreed shoot extract.

The half-strength American eelgrass extract (osmotic potential of 72 mOs/kg) resulted in a significant reduction in radicle length, but not in germination percentage. Comparing these results to the mannitol test results presented in Table 1 and Figure 3, shows that the reductions caused by the plant extracts were not due to osmotic potential. A comparison of lettuce radicle growth in extracts of shoots of threesquare burreed and American eelgrass is presented in Figure 5.

Reynolds (1975) found that pH had no effect on lettuce seed germination in the light or in the dark within the range of pH 2.6 to 10.6. Chou and Young (1974) suggested that the pH of germination media be adjusted to 6.0 for use in lettuce seed germination trials. All of the plant extracts used in our experiments had pH values between 5.75 and 6.00 and we therefore believe that the observed effects were not caused by pH. We did observe, however, that the pH of the American eelgrass extract increased from 6.0 to 8.0 during the 72 hr course of the trials. The senior author observed similar pH changes in earlier experiments and they were frequently associated with thin, dark colored radicles (Cheng, unpublished data). These symptoms are frequently caused by ammonia and other alkaline compounds.

The results of lettuce seed germination trials with undiluted threesquare burreed root extract are presented in

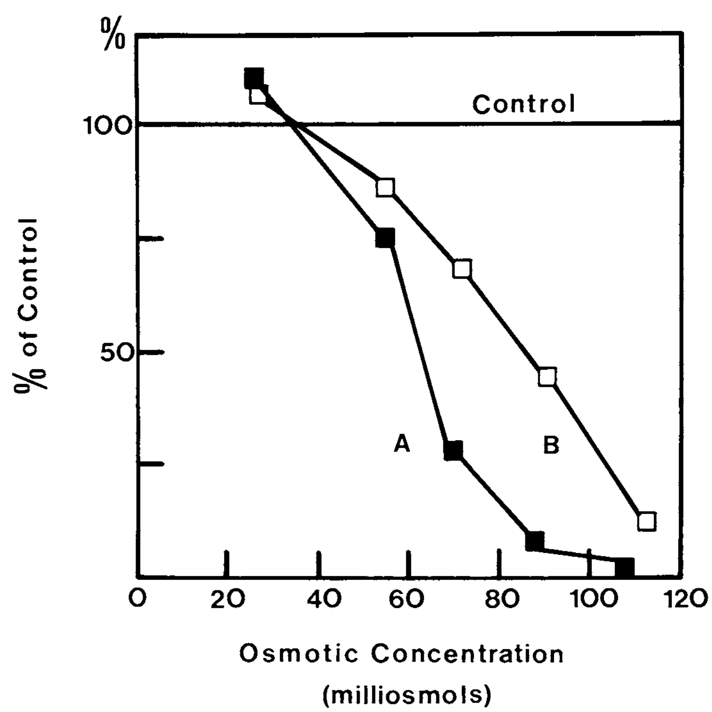

Figure 5. Effect of osmotic concentration on growth of lettuce radicles in water extracts of threesquare burreed (A) and American eelgrass (B). Data are presented as percent of growth of radicles in control plants.

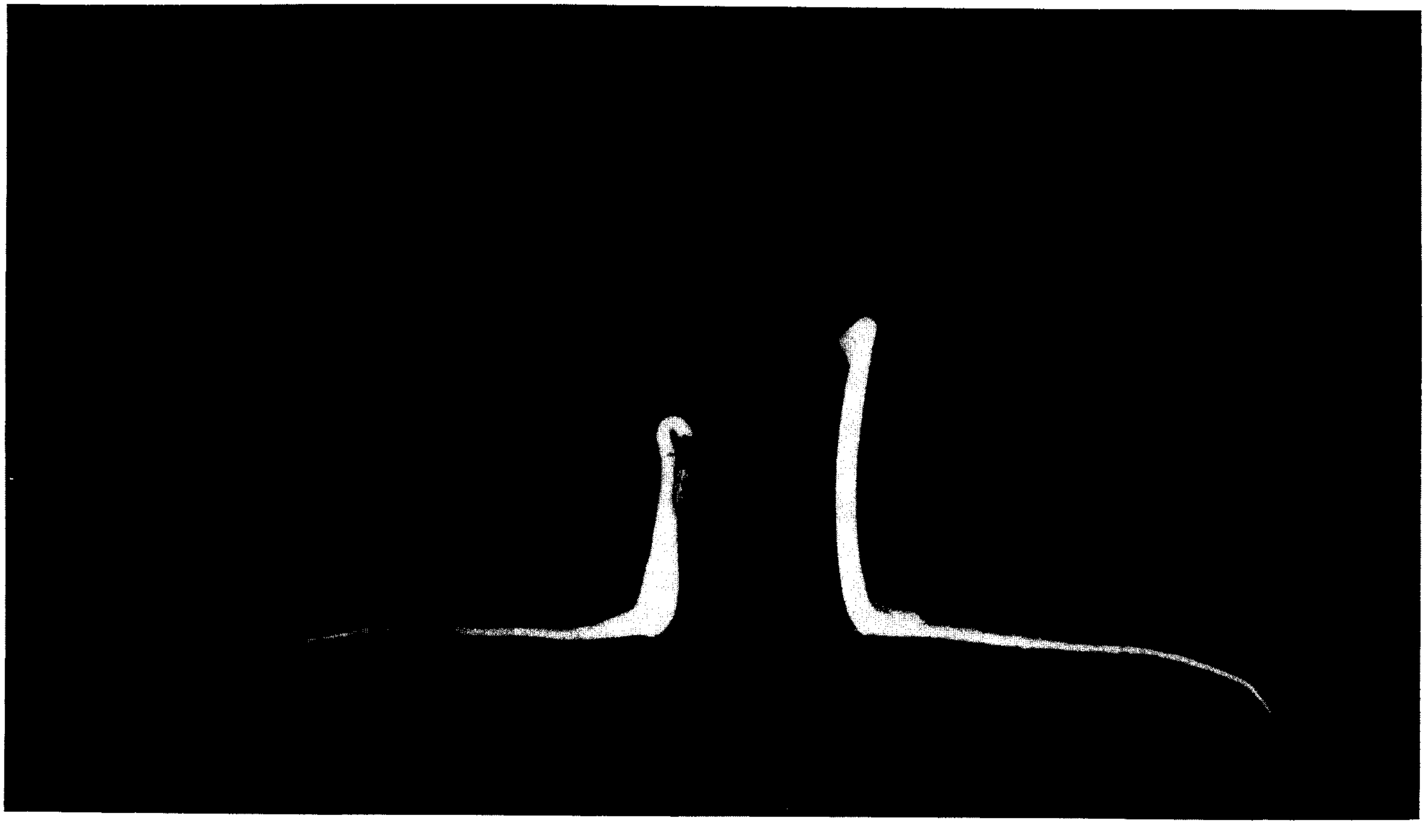

Figure 6. Photograph of shortened, bulbous hypocotyl on lettuce seedling germinated in a water extract of threesquare burreed roots (left), compared to a control plant (right), germinated in distilled water.

Table 2. This extract results in radicles that are approximately 70% as long as the controls but there is no reduction in germination percentage. It is interesting to note that this extract produces bulbous, shortened hypocotyls on the lettuce seedlings as shown in Figure 6. The mechanism of this phenomoenon is unknown at the present time. Lurssen and Konze (1985) suggested that ethylene analogues shorten cereals by suppression of cell elongation and enhancement of cell expansion. There is no direct evidence that ethylene or its analogues were instrumental in causing the shortened, bulbous hypocotyls we observed, but we believe it may play some role.

CONCLUSIONS

Crude water extracts of threesquare burreed and American eelgrass at various concentrations were shown to have the ability to decrease germination percentage and radicle growth in lettuce. It was demonstrated that the observed effects were not due entirely to osmotic potential or pH and it was therefore concluded that both species of aquatic plants contain allelopathic chemicals. Work is presently underway to isolate and identify these compounds.

LITERATURE CITED

1. Anderson, R. C. and O. L. Loucks. 1966. Osmotic pressure influence in germination tests for antibiosis. Science 152:771-773.
2. Ashton, F. M., J. M. Ditomaso, and L. N. J. Anderson. 1985. Spikerush (*Eleocharis* spp.): A source of allelopathics for the control of undesireable aquatic plants. *In:* The Chemistry of Allelopathy. A. C. Thompson (ed). American Chem. Soc., Washington, DC. 470 pp.
3. Bell, D. T. 1974. The influence of osmotic pressure in tests for allelopathy. Trans. Ill. State Acad. Sci. 67:312-317.
4. Chou, C. H. and C. C. Young. 1974. Effects of osmotic concentration and pH on plant growth. Taiwania. 19:157-165.
5. Chou, C. H. and Y. T. Chung. 1974. The allelopathic potential of *Miscanthus floridulus*. Bot Bull. Acad. Sin., New Ser. 15:14-27.
6. Duke, S. O., R. D. Williams, and A. H. Markhart, III. 1983. Interaction of moisture stress and three phenolic compounds on lettuce seed germination. Annals of Botany. 52:923-926.
7. El-Ghazal, R. A. K. and D. N. Riemer. 1986. Germination suppression by extracts of aquatic plants. J. Aquat. Plant Manage. 24:76-79.
8. Leather, G. R. and F. A. Einhellig. 1986. Bioassays in the study of allelopathy. *In:* The Science of Allelopathy. A. R. Putnam and C. S. Tang (eds). John Wiley & Sons. New York, NY. 317 pp.
9. ———. 1985. Mechanisms of allelopathic action in bioassay. *In:* The Chemistry of Allelopathy. A. C. Thompson (ed). American Chem. Soc. Washington, DC. 470 pp.
10. Leather, G. R. 1983a. Sunflowers (*Helianthus annuus*) are allelopathic to weeds. Weed Sci. 31:37-42.
11. ———. 1983b. Weed control using allelopathic crop plants. J. Chem. Ecol. 9:983-989.
12. Lurssen, K. and J. Konze. 1985. Relationship between ethylene production and plant growth after application of ethylene releasing growth regulators. *In:* Ethylene and Plant Development. J. A. Roberts and G. A. Tucker (eds). Proc. 39th Univ. of Nottingham Easter School in Agric. Sci. Robert Hartnoll Press. Sutton, Bonington, England. 416 pp.
13. Misato, T. 1982. Human welfare and the environment. *In:* Pesticide Chemistry. Proc. 5th Int. Congr. Pest. Chem. Kyoto. 231 pp.

14. Putnam, A. R. 1985. Weed allelopathy. *In:* Weed Physiology. D. O. Stephen (ed). CRC Press. Boca Raton, FL. 1:165 pp.
15. Rice, E. L. 1983. Pest control with nature's chemicals Univ. Okla. Press. Norman, OK. 224 pp.
16. Rice, E. L. 1984. Allelopathy. 2nd ed. Academic Press. Orlando, FL. 422 pp.
17. Reynolds, T. 1975. pH restraints on lettuce fruit germination. Annals of Botany. 39:797-805.
18. Szczepanski, A. J. 1977. Allelopathy as a means of biological control of water weeds. Aquatic Botany. 3:193-197.

J. Aquat. Plant Manage. 26: 55-58

Mineral Deficiency Symptoms of Waterhyacinth[1]

S. NEWMAN AND W. T. HALLER[2]

ABSTRACT

Healthy waterhyacinth (*Eichhornia crassipes* (Mart.) Solms) ramets of known size and weight were selected from a monoclonal population and transplanted to polyethylene tubs containing culture media deficient in either N, P, K, S, Mg, Ca, or Fe. The plants were observed daily, photographed after 4 weeks, and the visual mineral deficiency symptoms recorded. The most apparent symptom of the various deficiencies was the change in color; all plants grown in N-, P- and S-deficient media exhibited blue roots. Foliar chlorosis was observed in both N- and S-deficient plants, while P-deficient plants exhibited dark green leaves. Magnesium-deficiency resulted in a necrotic area first appearing at the apex of the leaves and then chlorosis spreading basipetally. Iron-deficient plants exhibited interveinal chlorosis. Potassium-deficient plants displayed brown bands across the mid to upper leaf region. Calcium-deficient plants exhibited brown spots on the leaves and petioles and then subsequent necrosis. The deficiency symptoms of waterhyacinth are discussed and presented in a tabular form similar to other mineral deficiency keys.

Key words: Eichhornia crassipes, nutrition, nitrogen, phosphorus, potassium, sulfur, magnesium, iron, calcium.

INTRODUCTION

Recently, interest in waterhyacinth (*Eichhornia crassipes* (Mart.) Solms) has increased as their potential for use in removing nutrients from water bodies, particularly in wastewater treatment facilities, has become more widely recognized. Although the plant has not been studied as extensively as traditional crop plants, scientists have studied the ability of waterhyacinth to remove N and P. Phosphorus reduction values from 32 to 61% and N reduction values of 75 to 94% are reported (Clock, 1968; Sheffield, 1967; Reddy et al., 1982). However, the extent of nutrient removal is dependent on the growth rate of the plants, the detention time of the effluent in wastewater retention ponds, the water depth (Cornwell et al., 1977) and the nutrient content of the growth media. Apart from N, P and K, research conducted on other nutrients has emphasized correlations between plant tissue and the growth media nutrient concentrations (Gossett and Norris, 1971; Wolverton and McDonald, 1979; Boyd, 1976; Haller et al., 1970). Cooley et al. (1978) and Cooley and Martin (1980) used radioisotopes to conduct a more indepth study of waterhyacinth nutrition. They observed a positive correlation between root retention of various nutrients and the solubility of sparingly soluble salts such as metal carbonates. El-Sharkawi et al. (1980) investigated the nutrient fluxes of roots of waterhyacinth stressed by reduced water potentials. This was one of few studies which have examined S nutrition of waterhyacinth.

There are a number of potential problems associated with using waterhyacinth for nutrient removal. In a wastewater treatment system growing conditions for waterhyacinth are variable, and it is not known what conditions would maximize nutrient uptake and subsequent plant growth. For years agronomists have been able to diagnose plant nutritional problems related to soil fertility by observing symptoms plants display when nutrient stressed. Several comprehensive guides have been written which describe these symptoms (Chapman, 1966; Sprague, 1964). A key describing nutrient deficiency symptoms of waterhyacinth would be a useful tool not only as a means to rapidly determine possible nutrient imbalances in wastewater effluent, but also, it could be used to give an indication of the nutrient status of natural lakes within which waterhyacinth may be growing.

Desougi (1984) studied the growth and reproduction of waterhyacinth that were deprived of N, P, K, Ca, or Mg. The total nutrient concentration of these solutions varied considerably, and therefore it was not valid to make quantitative comparisons, and qualitative comparisons were not described. This paper qualitatively describes the symptoms displayed by waterhyacinth which were grown in nutrient deficient media.

MATERIALS AND METHODS

Healthy waterhyacinth plants approximately 4 weeks old were selected on the basis of uniform size and weight from a monoclonal population maintained in outdoor culture tanks. The stock plants were maintained in 900 l

[1]Florida Agric. Experiment Station Journal Series No. 9072. Received for publication November 9, 1987 and in revised form March 21, 1988.
[2]Graduate Assistant and Professor, Department of Agronomy, University of Florida, Gainesville, Fl 32611.