INTERFERENCE OF AQUATIC PLANTS ASSOCIATED WITH WILD RICE (*Zizania palustris* L.)

By

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ABSTRACT

The objective of this thesis was to determine the interference potential of aquatic plants to wild rice (*Zizania palustris* L.) through field and laboratory studies. Three companion papers describe the influence of aquatic plants on growth of wild rice; the allelopathic potential of aquatic plants and the isolation and identification of allelochemicals from two selected plants.

The growth of wild rice in the presence and in the absence of different plant species, namely *Eleocharis smallii, Scirpus acutus, Equisetum fluviatile, Nymphaea odorata, Nuphar variegatum, Sparganium fluctuans, Myriophyllum verticillatum and Potamogeton natans* was studied. Number of wild rice plants and their growth decreased in the presence of different plant species. Environmental factors had no influence on the growth of wild rice, either in the presence or absence of different species. It is suggested that the adverse effects of different species on wild rice may be due to either their early growth habit, shading the wild rice plant, or to their allelopathic effects.

The allelopathic potential of the above mentioned plant species was examined using lettuce and wild rice seedling bioassays. The root length of lettuce and the total root length of wild rice seedlings were significantly reduced by aqueous extracts of these plant species. The lettuce seedling bioassay was more responsive than that of wild rice. Shoot growth was less affected. Bioassay with an aqueous extract of lake sediments associated with these species had little inhibitory effect on growth of wild rice seedling. The use of target species as a bioassay material and further studies on phytotoxic effect of lake sediments have been emphasized for evaluating their ecological significance.

The phytotoxic compounds from water extracts of the rhizome of *Scirpus acutus* and the shoot of *Eleocharis smallii* were isolated by ethyl acetate extraction and identified by GC-Mass spectroscopy. The ethyl acetate organic fraction of water extract of *Scirpus* rhizomes contained lactic, succinic, fumaric, 2-hydroxy succinic, 2-phenyl lactic, m-hydroxy benzoic, p-hydroxy benzoic, protocatechuic, dehydroabietic and ferulic acids; p-hydroxy benzyl alcohol, p-hydroxy phenyl ethanol and a dye, catechin. The organic fraction of the extract of *Eleocharis* shoots contained 4-methoxy phenol, benzofuran, benzene acetic acid, 1-hydroxy-5-methyl acetophenone and 1,3,4-dimethoxy phenyl ethanone. The growth inhibiting properties of some of these compounds under field conditions are discussed.

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GENERAL INTRODUCTION

Wild rice (*Zizania palustris* L.) is the only indigenous cereal crop of North America. It occurs in a variety of aquatic habitats in shallow lakes and river margins. However, wild rice grows best along the shores of rivers, streams and near the inlets and outlets of lakes at 0.3 to 1.0 meter water depth with soft organic bottom (Moyle, 1944; Thomas and Stewart, 1969). Water depth, soil type and presence of other aquatic macrophytes are generally considered to be important factors affecting wild rice productivity (Aiken *et al*, 1988). In Canada all commercial wild rice is grown in natural lakes where a number of aquatic perennial macrophytes can interfere with its growth (Aiken *et al*, 1988). For example, dwarf spikerush (*Eleocharis smallii* Britton), big bul-rush (*Scirpus acutus* Muhlenb. ex Durand), horsetail (*Equisetum fluviatile* L.), white water lily (*Nymphaea odorata* Dryander ex Aiton), yellow water lily (*Nuphar variegatum* Engelm. ex Durand), bur reed (*Sparganium fluctuans* (Morong) Robinson), *Potamogeton* spp., *Ceratophyllum* spp., and *Myriophyllum* spp. have been reported to occur with wild rice in northwestern Ontario (Lee, 1986b; Aikens *et al*, 1988). Weed plants may affect the growth of crop plants either by competition for nutrients, water, space and light (Zimdahl, 1993) or by allelopathy (Rice, 1984). The effects of aquatic plants on wild rice have not been studied adequately. Ransom and Oelke (1982) reported that common waterplantain (*Alisma triviale* Pursh) significantly reduced the grain yield of wild rice.

Rice (1984) distinguished allelopathy from competition as "any direct or indirect harmful or beneficial effects by one plant (including microorganism) on another through production of chemical compounds that escape into the environment". Elakovitch and Wooten (1995) reported on 97 aquatic macrophytes as allelopathic to other plants. *Eleocharis* is the most frequently cited genus with 11 species reported as allelopathic (Oborn *et al*, 1954; Yeo, 1980; Frank and Dechoretz, 1980; Nichols and Shaw, 1983; Yeo and Thruston, 1984; Ashton *et al*, 1985; Sutton, 1986 and 1990 ; Sutton and Portier, 1989 and 1991, Wooten and Elakovitch, 1991). *Equisetum fluviatile*, *Myriophyllum aquaticum*, *Nymphaea odorata*, *Nuphar lutea*, *Potamogeton* spp., and *Sparganium americanum* have also been reported as allelopathic to other aquatic macrophytes (Elakovich and Wooten, 1995). However, no literature is available on allelopathic effects of these weeds on wild rice.

Plants achieve their allelopathic properties by synthesizing allelochemicals in the plant body and by releasing these compounds into the environment. Organic compounds such as aliphatic and aromatic acids, coumarins, quinones, flavonoids, tanins, alkaloids, terpenoids and steroids have been identified from terrestrial plants as allelopathic compounds (Rice, 1984). Few studies have been conducted to isolate and identify the allelochemicals responsible for allelopathy in aquatic plants. Cheng and Reimer (1989) isolated the phenolic compounds, vanillic and gallic acid, from American eelgrass (*Vallisneria americana*) and suggested that the compounds play a major role in allelopathy in this species.

The objectives of the present study were:

i) to determine if the presence or absence of certain aquatic plants in wild rice stands can influence its growth;

ii) to determine the allelopathic potential of the aquatic plants on early growth of wild rice and

iii) to isolate and identify the phytotoxic compounds from two of the aquatic plants that showed the maximum growth inhibition in the earlier experiments.

The thesis was completed in three parts by addressing the above objectives separately.

CHAPTER 1

1. The influence of aquatic plants on the growth of wild rice (*Zizania palustris* L.)

1.1 Abstract

Growth of wild rice plants in lakes was examined inside and outside areas of *Eleocharis smallii* Britton, *Scirpus acutus* Muhlenb. ex Bigelow, *Equisetum fluviatile* L., *Nymphaea odorata* Dryander ex Aiton, *Nuphar variegatum* Engelm. ex Durand, *Sparganium fluctuans* (Morong) Robinson, *Myriophyllum verticillatum* L. and *Potamogeton natans* L. Number of wild rice plants per square meter (density) and their individual dry weights were lower when these plants were present. An analysis of covariance revealed that environmental factors such as water depth, soil bulk density, loss on ignition, pH, nitrogen, potassium, phosphorus, calcium, magnesium, iron, manganese, zinc, and copper had no significant effects on dry weight of wild rice with or without other plant species. I suggest that the detrimental effect of different aquatic plants on wild rice growth may be due to growth inhibition of the seedlings caused by shading from the plant species or their allelopathic effects.

1.2 Introduction

Wild rice (*Zizania palustris* L.) is the only indigenous cereal of North America. It grows primarily in the shallow areas of lakes.

The growth of wild rice is affected by the depth of water (Moyle, 1944; Lee, 1979), type of lake sediments and the presence of other aquatic macrophytes (Aiken *et al*, 1988). Water depth of about 0.3 to 1.0 m with soft alluvial organic sediments is considered as an ideal situation for wild rice growth (Aiken *et al*, 1988). Aquatic macrophytes which adversely affect the growth of wild rice are perennial in nature (Moyle, 1944) and include such emergent macrophytes as *Eleocharis* spp., *Scirpus* spp., *Equisetum* spp., *Sagittaria rigida* and *Pontederia cordata* (Lee, 1982; Lee and Stewart, 1981; Coltas, 1983) and floating leaved submerged species as *Nymphaea* spp., *Nuphar* spp., *Brasenia* spp., *Sparganium* spp., *Ceratophyllum* spp. and *Myriophyllum* spp. (Aiken *et al*, 1988).

Weeds affect the growth of a crop through competition for light, space, water and nutrients (Zimdahl, 1993) or by chemical interference (Rice, 1984). The life cycles of aquatic plants associated with wild rice begin in the spring before the germination of wild rice. By the time wild rice starts to grow in late spring, these plants are already well established, growing above the water surface and competing for light and available nutrients with the wild rice plants (Lee, 1986a).

Little information is available on the effects of aquatic plants on the growth of wild rice. Ransom and Oelke (1882) reported that common waterplantain (*Alisma trivale*) reduced rice yield by more than 90% and caused a reduction in the number of wild rice panicles even at low weed densities (5 plants/m2). High densities of this weed (11 plants/m2) reduced the number of wild rice seeds per plant. Giant burreed (*Sparganium eurycarpum*) is a another common perennial aquatic affecting wild rice production in Minnesota. Clay and Oelke (1987) reported that yield and panicle number of wild rice was reduced by 60% at 40 /m^2 burreed shoot density. They also found that the weed interferes with the penetration of photosynthetically active radiation (PAR) to the wild rice canopy.

The objectives of the present study were to determine (i) if the growth of wild rice was affected by the common aquatic plant species; and (ii) the habitat factors responsible for this effect.

1.3 Materials and methods

Eleocharis smallii L., *Scirpus acutus* Muhlenb, *Equisetum fluviatile* L., *Nymphaea odorata* Aiton, *Nuphar variegatum* Engelm, *Sparganium fluctuans* Robinson, *Myriophyllum verticillatum* L., and *Potamogeton natans* L. were collected from Whitefish (Lat. 49 °06′ N, Long. 90 °09′ W), Ricestock (Lat. 48 °13′ N, Long. 90 °00′ W) and Chisamore (Lat. 49 °20′ N, Long. 89 °44′ W) Lakes in Northwestern Ontario during August 1 - 20, 1994, when wild rice plants were in the flowering stage. Above ground components of wild rice plants and associated aquatic plants were clipped from ten 50 X 50 cm randomly placed quadrates within and outside (at least 2 m away) the aquatic plants. Sediment samples of the top 20 cm sediment layer were collected with a core sampler from each quadrat and water depth from the water surface to the sediment was determined in the centre of the quadrat. The samples were put in plastic bags, stored in portable coolers and brought back to the laboratory for analyses.

The number of wild rice and aquatic plant species per quadrat was counted. All the rice plants and aquatic plants were oven dried at 80°C until there was no change in dry weight. The mean dry weight per wild rice plant was determined by dividing the combined weight of all wild rice plants from each quadrat by the total number of wild rice plants in each quadrat. The mean dry weight of each aquatic plant species was also determined by the same procedure.

The sediment samples were analyzed for loss on ignition, pH and extractable phosphorus, iron, manganese, zinc, copper, calcium, magnesium, and potassium following the procedure of Lee (1986a) and the nitrogen content was determined by Kjeldhal method.

1.4 Data analysis

Using the procedure of Lee and Stewart (1981), density and dry weight variables of wild rice were transformed into percent increase or decrease from the respective means in the presence and absence of aquatic plants. This transformation was necessary to make the data relative and comparable between the three lakes.

The SPSSX statistical package (SPSS Inc., 1988) was used to examine the data in three steps; i) Analyses of variance (ANOVAs) determined if wild rice growth performance (weight and number of wild rice plants per square meter) differed in the presence or absence of the aquatic plants. ii) Since sediment variables are known to be highly intercorrelated (Lee, 1979), a principal component analysis (PCA) generated new uncorrelated variables based on water depth and sediment variables that summarized the environmental data. iv) Analysis of covariance (ANCOVA) was performed using weight per plant and number per square meter of wild rice as univariates and PCA factors as covariates to determine which factor(s) were responsible for the significant difference of weight of wild rice in the presence or absence of aquatic plants.

1.5 Results

1.5.1 Wild rice growth

The mean dry weight of wild rice plants was higher in competing aquatic plant-free stands than in stands with competing aquatic plants, except wild rice with *Nuphar variegatum* (Figure 1.1). The lowest per plant dry weight of wild rice was recorded in the presence of *Scirpus acutus*. The densities of wild rice plants (number/m^2) were also lower in the presence of competing plants (Figure 1.2). The lowest density of wild rice was obtained in the presence of *Myriophyllum verticillatum*. In general, density of wild rice plants was affected more than their dry weights by the plant species. Analysis of variance revealed that the difference in dry weight of wild rice per plant inside and outside the plant species was significant ($P < 0.01$). Dry weight and density of plant species were different with different plant species. The maximum dry weight was recorded in *Scirpus acutus* and the minimum was in *Sparganium fluctuans* (Figure 1.3). The densities of plant species were highest in *Equisetum fluviatile* and the lowest was in *Spargenium fluctuans*

(Figure 1.4).

1.5.2 Environmental variables

Water depth and sediment variables examined inside and outside the different plant species in wild rice populations are presented in Table 1.1. Water depth in areas with aquatic plants varied from 50.8 to 96.1 cm, and from 63.5 to 104.2 cm in areas without aquatic plants. The sediments with aquatic plants had low nitrogen content except with *Nymphaea odorata*. The phosphorus content of sediments varied. Sediments with *Nuphar variegatum, Equisetum fluviatile, Nymphaea odorata* and *Sparganium fluctuans* had lower phosphorus content than sediments with *Scirpus acutus* and *Eleocharis smallii*. The concentration of extractable cations such as iron, manganese, zinc, copper, calcium, magnesium and potassium varied among the sediments in the presence or absence of different plant species. In many sediment samples, the cation contents was low with aquatic plants. The organic matter content was similar in presence or absence of different plant species except in sediments with *Scirpus acutus*, *Eleocharis smallii* and *Myriophyllum verticillatum* which had lower value. Mean pH values ranged from 5.84 to 6.23 in the presence and the absence of aquatic plants.

The PCA of water depth and sediment variables generated thirteen uncorrelated groups of data, the first four of which accounted for 69.5 percent of the variance. The remaining groups, each accounted for less than 6.5 percent of the total variation and were not considered for the remaining analysis.

A summary of the principal component analysis on water depth and sediment variables, for the four most important factors generated by the PCA are presented in Table 1.2. Variables with the highest coefficients contribute the most to that factor. The first factor explained 27.1 percent of the variance. It had a positive loading on bulk density and iron content and negative loading on loss of ignition. Thus the first factor can be interpreted as a function of organic matter. The second factor explained 22.1 percent of the variation which was mainly due to manganese, calcium and magnesium and can be regarded as mineral component. The third and fourth factors accounted for 18.5 and 8.1 percent of the variance, respectively, and are primarily the functions of Zinc and phosphorus.

The analysis of covariance revealed that these four environmental variables did not significantly affect the wild rice growth. Therefore, the observed variations in wild rice weights and densities might be due to other factors.

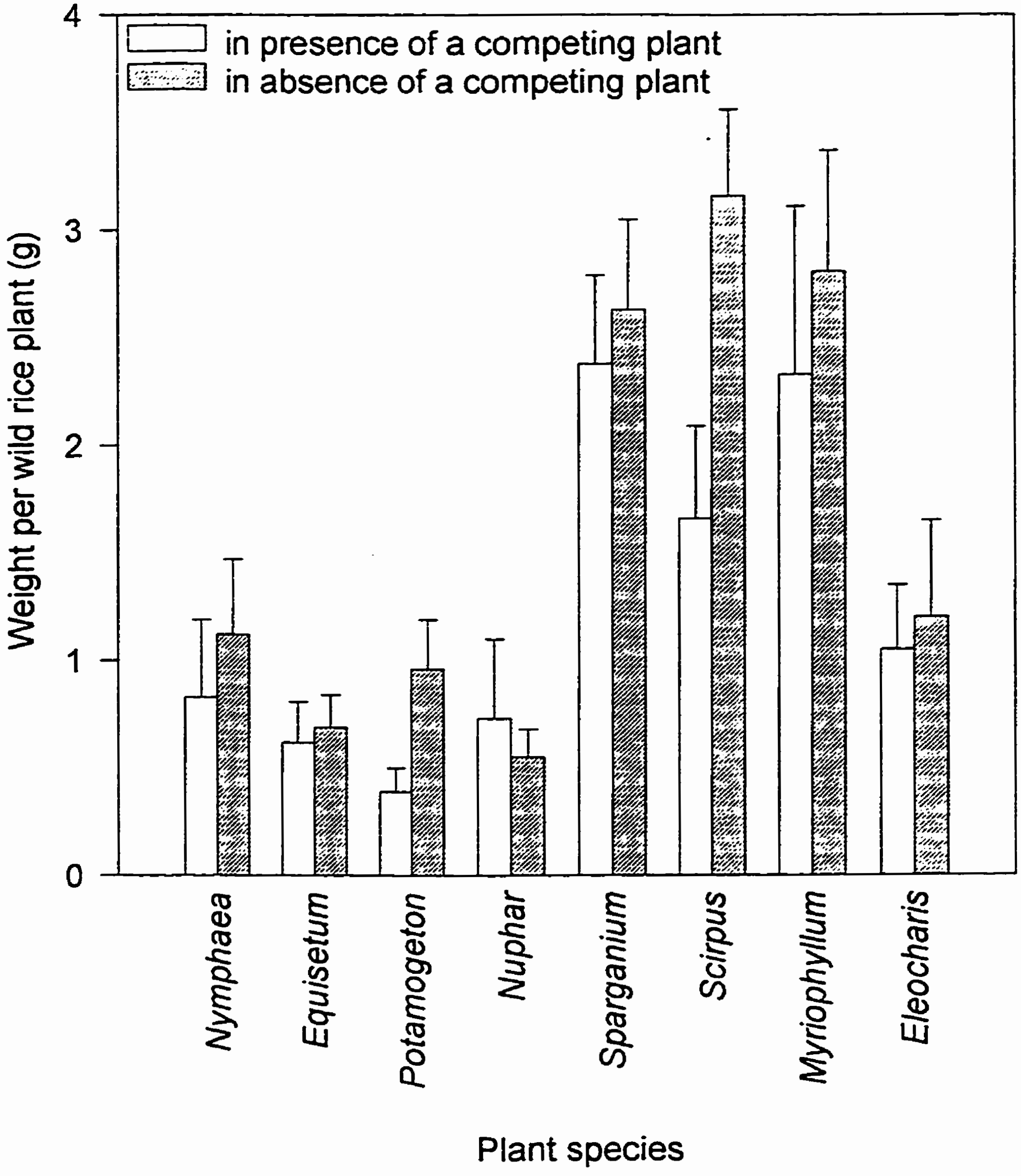

Figure 1.1 Mean dry weight of wild rice plants in presence and in absence of different plant species

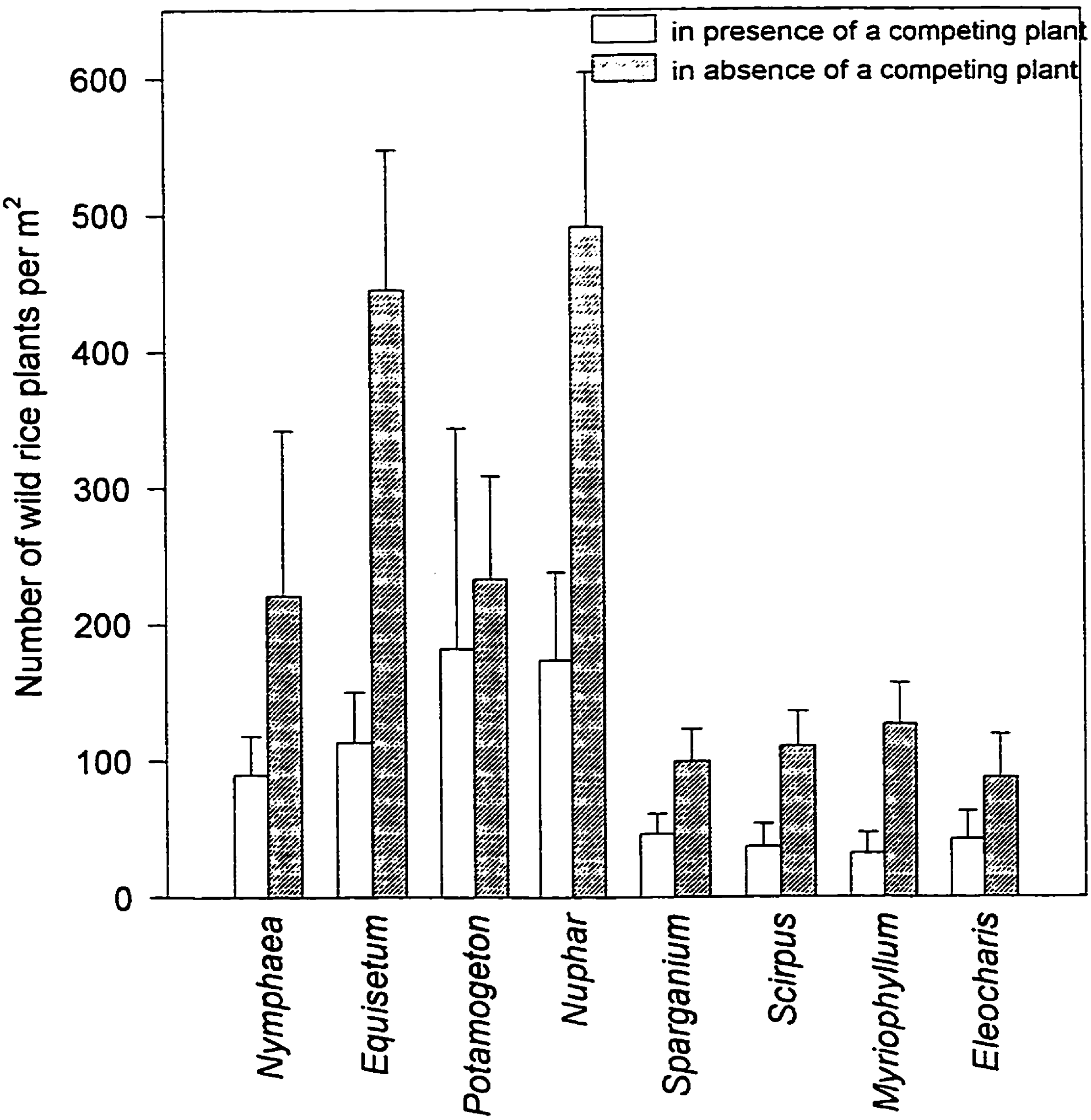

Figure 1.2 Mean density of wild rice plants in presence and in absence of different plant species

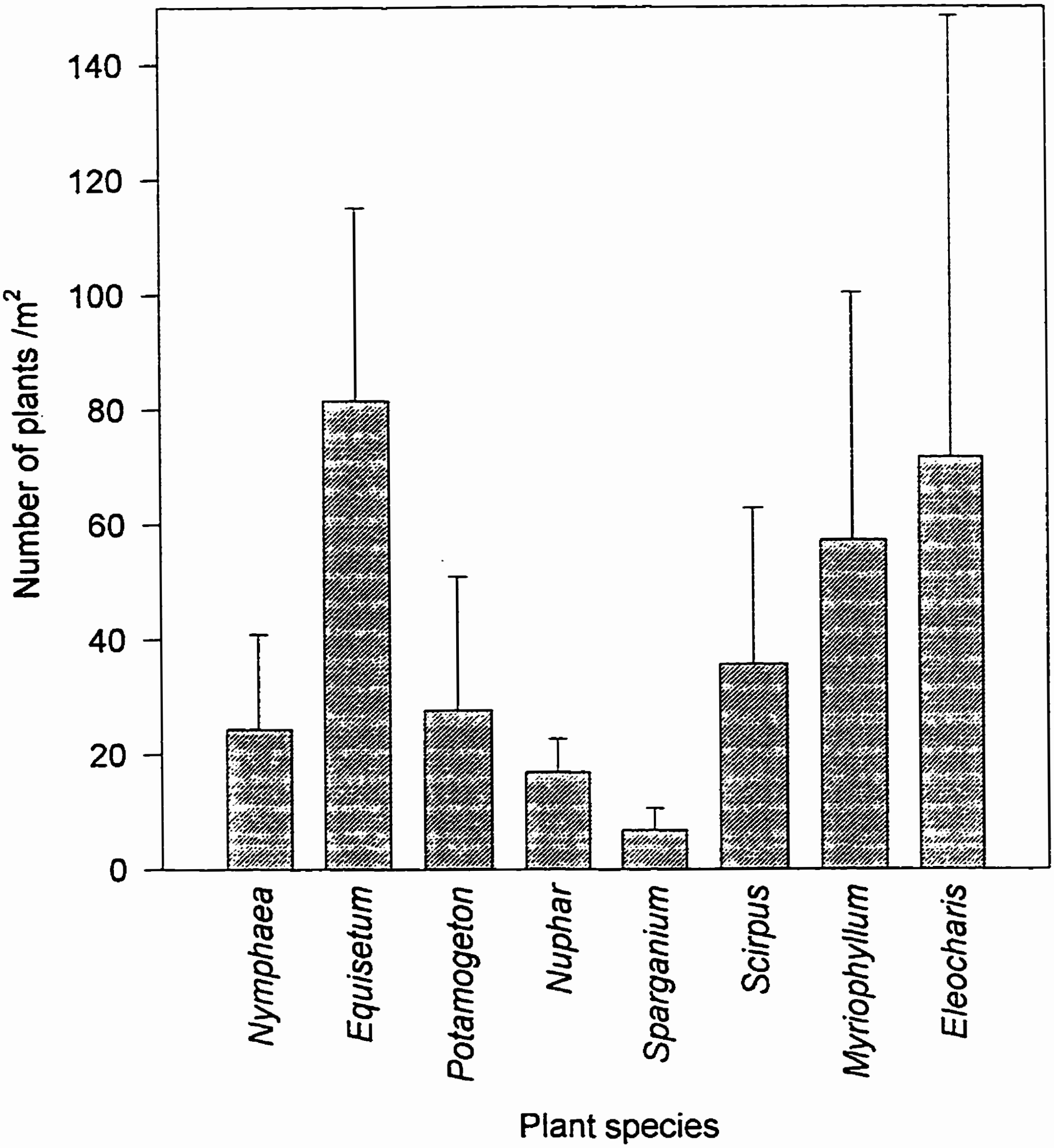

Figure 1.3 Mean density (no./m^2) of different plant species within wild rice stands

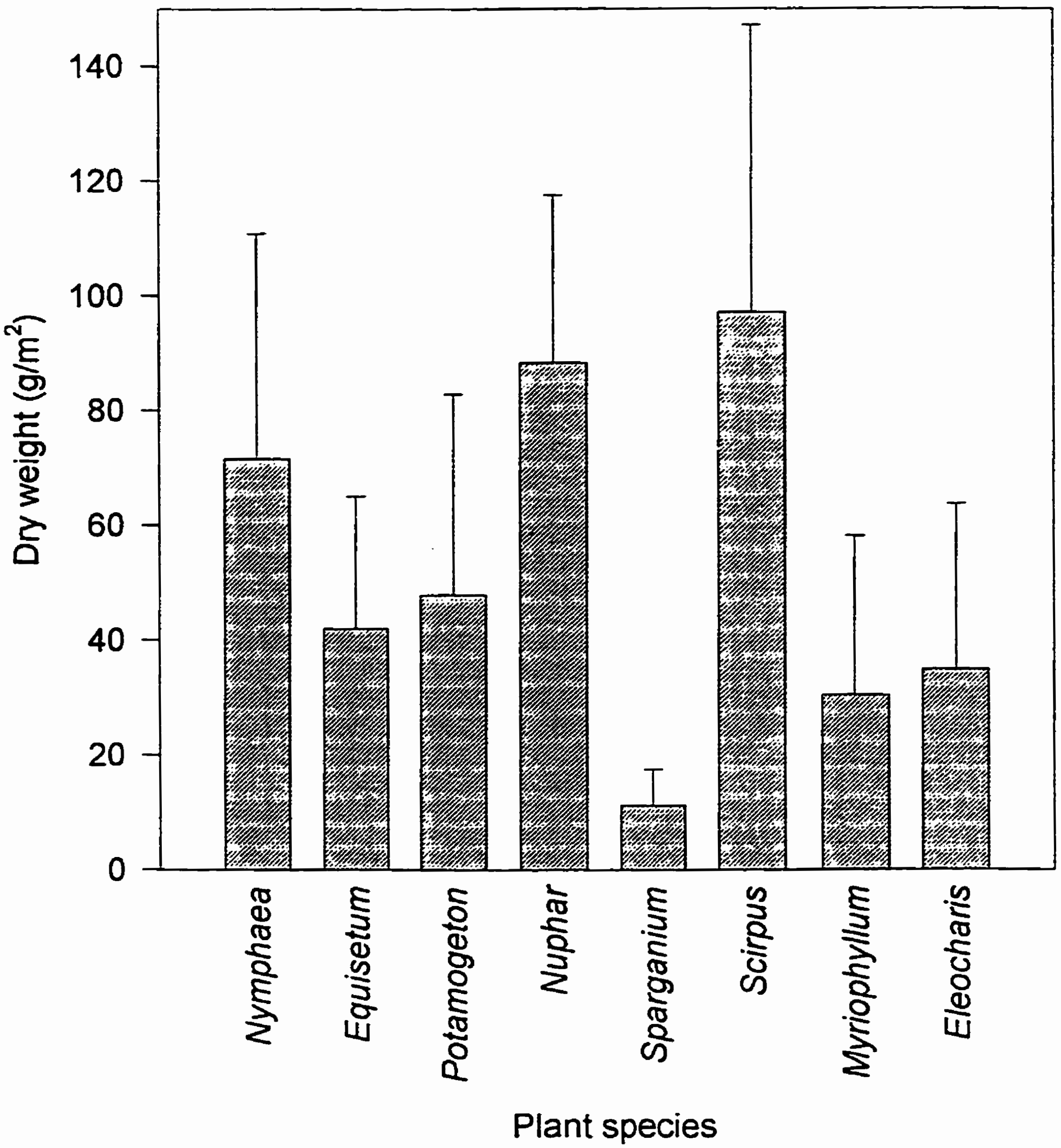

Figure 1.4 Mean dry weight of different plant species within wild rice stands

Table 1.1. Means and standard deviation (±) for water depth and sediment variables in wild rice population absence of different aquatic plants (n = 160).

Variables	Nuphar		Potamogeton		Equisetum		Nymphaea		Scirpus		Eleocharis	
	Present	Absent	Present	Absent	Present	Absent	Present	Absent	Present	Absent	Present	Absent
Depth (cm)	88.40 ± 2.72	76.10 ± 4.71	68.40 ± 12.08	74.90 ± 8.32	92.50 ± 7.17	77.00 ± 5.87	92.70 ±7.35	83.00 ± 11.35	69.80 ± 21.30	80.00 ± 3.16	50.80 ± 4.24	63.50 ± 5.23
Bulk denisty (g.cm^{-3})	0.14 ± 0.03	0.17 ± 0.07	0.12 ± 0.1	0.09 ±0.03	0.13 ± 0.01	0.13 ± 0.02	0.17 ± 0.18	0.12 ± 0.02	0.4 ± 0.15	0.21 ± 0.07	0.84 ± 0.19	0.60 ± 0.23
Loss on ignition (%)	38.61 ± 3.31	35.27 ± 6.94	37.37 ± 1.44	36.58 ± 2.24	39.16 ± 1.62	41.03 ± 3.22	31.96 ± 11.02	36.65 ± 1.40	21.96 ± 19.19	36.54 ± 8.25	5.75 ± 1.96	11.65 ± 4.62
pH	6.03 ± 0.19	6.06 ± 0.09	6.01 ± 0.15	5.90 ± 0.13	5.88 ± 0.06	5.79 ± 0.07	5.98 ± 0.17	5.84 ± 0.13	6.15 ± 0.26	6.00 ± 0.10	6.12 ± 0.19	6.05 ± 0.09
Phosphorus (g.m^{-2})	0.50 ± 0.18	0.73 ± 0.21	0.99 ± 0.08	1.00 ± 0.12	0.57 ± 0.11	0.67 ± 0.22	0.75 ± 0.24	0.84 ± 0.08	0.93 ± 0.15	0.48 ± 0.22	0.73 ± 0.72	0.52 ± 0.23
Nitrogen (g.m^{-2})	0.33 ± 0.11	0.79 ± 0.35	2.81 ± 0.70	2.94 ± 1.23	0.53 ± 0.78	0.91 ± 0.50	2.31 ± 1.90	2.10 ± 1.7	1.29 ± 0.78	1.97 ± 0.32	2.54 ± 1.2	3.27 ± 0.60
Iron (g.m^{-2})	177.91 ± 41.61	179.96 ± 42.24	45.48 ± 10.25	67.67 ± 17.09	99.67 ± 17.33	103.99 ± 13.53	76.38 ± 25.11	92.08 ± 24.46	163.22 ± 73.52	283.68 ± 18.35	407.62 ± 147.13	443.88 ± 89.84
Manganese (g.m^{-2})	10.31 ± 3.31	12.28 ± 2.89	1.39 ± 0.34	1.52 ± 0.54	7.24 ± 2.68	7.58 ± 2.64	1.97 ± 0.44	2.36 ± 0.47	2.47 ± 1.19	3.81 ± 0.48	4.65 ± 2.47	6.78 ± 3.02
Zinc (g.m^{-2})	0.63 ± 0.06	0.45 ± 0.09	0.33 ± 0.09	0.57 ± 0.09	0.41 ± 0.07	0.44 ± 0.09	0.48 ± 0.15	0.50 ± 0.18	0.59 ± 0.16	0.66 ± 0.03	0.43 ± 0.04	0.44 ± 0.06
Copper (g.m^{-2})	0.04 ± 0.01	0.02 ± 0.005	0.06 ± 0.04	0.09 ± 0.03	0.06 ± 0.03	0.04 ± 0.02	0.18 ± 0.05	0.11 ± 0.07	0.04 ± 0.02	0.05 ± 0.02	0.04 ± 0.01	0.05 ± 0.02
Calcium (g.m^{-2})	161.72 ± 22.08	161.42 ± 25.22	91.95 ± 9.13	79.31 ± 25.46	129.81 ± 12.12	133.11 ± 14.67	84.34 ± 11.08	100.36 ± 11.25	138.75 ± 11.44	141.56 ± 11.50	84.06 ± 16.11	123.70 ± 37.0
Magnesium (g.m^{-2})	17.35 ± 1.61	17.73 ± 2.73	13.63 ± 1.25	11.98 ± 3.25	15.60 ± 1.65	15.26 ± 1.69	12.36 ± 1.66	14.29 ± 1.73	21.32 ± 0.96	19.25 ± 1.61	13.96 ± 2.94	13.69 ± 3.44
Potassium (g.m^{-2})	2.48 ± 0.49	2.45 ± 0.43	2.44 ± 0.27	2.40 ± 0.88	1.45 ± 0.26	2.29 ± 0.38	3.18 ± 0.51	3.73 ± 0.80	3.51 ± 0.82	2.83 ± 0.29	5.96 ± 3.7	3.08 ± 0.97

l deviation (+) for water depth and sediment variables in wild rice populations in presence and in plants (n = 160).

Potamogeton		Equisetum		Nymphaea		Scirpus		Eleocharis		Myriophyllum		Sparganium	
Present	Absent	Present	Absent	Present	Absent	Present	Absent	Present	Absent	Present	Absent	Present	Absent
68.40 ± 12.08	74.90 ± 8.32	92.30 ± 7.17	77.00 ± 5.87	92.70 ±7.35	83.00 ± 11.35	69.80 ± 21.30	80.00 ± 3.16	50.80 ± 4.24	63.50 ± 5.23	96.10 ± 1.79	71.20 ± 1.87	109.40 ± 1.26	104.20 ± 1.99
0.12 ± 0.1	0.09 ±0.03	0.13 ± 0.01	0.13 ± 0.02	0.17 ± 0.18	0.12 ± 0.02	0.4 ± 0.15	0.21 ± 0.07	0.84 ± 0.19	0.60 ± 0.23	0.35 ± 0.05	0.25 ± 0.04	0.20 ± 0.04	0.23 ± 0.02
37.37 ± 1.44	36.58 ± 2.24	39.16 ± 1.62	41.03 ± 3.22	31.96 ± 11.02	36.65 ± 1.40	21.96 ± 19.19	36.54 ± 8.25	5.75 ± 1.96	11.65 ± 4.62	13.38 ± 2.25	26.40 ± 2.60	25.85 ± 4.37	25.36 ± 2.20
6.01 ± 0.15	5.90 ± 0.13	5.88 ± 0.06	5.79 ± 0.07	5.98 ± 0.17	5.84 ± 0.13	6.15 ± 0.26	6.00 ± 0.10	6.12 ± 0.19	6.05 ± 0.09	6.23 ± 0.13	6.16 ± 0.05	5.99 ± 0.14	5.84 ± 0.07
0.99 ± 0.08	1.00 ± 0.12	0.57 ± 0.11	0.67 ± 0.22	0.75 ± 0.24	0.84 ± 0.08	0.93 ± 0.15	0.48 ± 0.22	0.73 ± 0.72	0.52 ± 0.23	0.75 ± 0.11	0.71 ± 0.11	0.80 ± 0.16	1.30 ± 0.28
2.81 ± 0.70	2.94 ± 1.23	0.53 ± 0.78	0.91 ± 0.50	2.31 ± 1.90	2.10 ± 1.7	1.29 ± 0.78	1.97 ± 0.32	2.54 ± 1.2	3.27 ± 0.60	2.42 ± 0.51	3.58 ± 0.65	0.93 ± 0.49	2.70 ± 0.83
45.48 ± 10.25	67.67 ± 17.09	99.67 ± 17.33	103.99 ± 13.53	76.38 ± 25.11	92.08 ± 24.46	163.22 ± 73.52	283.68 ± 18.35	407.62 ± 147.13	443.88 ± 89.84	306.11 ± 54.74	312.86 ± 29.49	107.18 ± 17.08	157.70 ± 10.77
1.39 ± 0.34	1.52 ± 0.54	7.24 ± 2.68	7.58 ± 2.64	1.97 ± 0.44	2.36 ± 0.47	2.47 ± 1.19	3.81 ± 0.48	4.65 ± 2.47	6.78 ± 3.02	4.68 ± 0.70	4.79 ± 0.36	3.62 ± 0.36	6.0 ± 0.32
0.33 ± 0.09	0.57 ± 0.09	0.41 ± 0.07	0.44 ± 0.09	0.48 ± 0.15	0.50 ± 0.18	0.59 ± 0.16	0.66 ± 0.03	0.43 ± 0.04	0.44 ± 0.06	0.62 ± 0.15	0.71 ± 0.12	0.32 ± 0.04	0.48 ± 0.06
0.06 ± 0.04	0.09 ± 0.03	0.06 ± 0.03	0.04 ± 0.02	0.18 ± 0.05	0.11 ± 0.07	0.04 ± 0.02	0.05 ± 0.02	0.04 ± 0.01	0.05 ± 0.02	0.06 ± 0.03	0.05 ± 0.02	0.06 ± 0.03	0.05 ± 0.04
91.95 ± 9.13	79.31 ± 25.46	129.81 ± 12.12	133.11 ± 14.67	84.34 ± 11.08	100.36 ± 11.25	138.75 ± 11.44	141.56 ± 11.50	84.06 ± 16.11	123.70 ± 37.0	120.06 ± 27.51	142.06 ± 9.3	111.36 ± 3.47	109.60 ± 5.2
13.63 ± 1.25	11.98 ± 3.25	15.60 ± 1.65	15.26 ± 1.69	12.36 ± 1.66	14.29 ± 1.73	21.32 ± 0.96	19.25 ± 1.61	13.96 ± 2.94	13.69 ± 3.44	15.93 ± 2.29	20.96 ± 1.45	15.87 ± 1.35	16.20 ± 0.85
2.44 ± 0.27	2.40 ± 0.88	1.45 ± 0.26	2.29 ± 0.38	3.18 ± 0.51	3.73 ± 0.80	3.51 ± 0.82	2.83 ± 0.29	5.96 ± 3.7	3.08 ± 0.97	3.69 ± 0.75	3.79 ± 0.44	1.88 ± 0.20	3.03 ± 1.06

Table 2.2 Summary of the principal-components analysis on water depth and sediment variables: coefficients of the rotated factor matrix and interpretation.

Designation	Factors 1 Organic matter	2 Mineral	3 Zinc	4 Phosphorous
water depth(WD)	0.553	0.135	0.144	0.109
Bulk density (BD)	0.866	-0.199	-0.304	0.000
Loss on ignition (LOI)	-0.812	0.236	0.183	-0.141
pH	0.598	0.161	0.130	-0.015
Phosphorous (P)	-0.155	-0.476	0.177	0.746
Nitrogen (N)	0.415	-0.464	0.480	0.145
Iron (Fe)	0.846	0.208	-0.056	-0.179
Manganese (Mn)	0.034	0.746	-0.301	0.063
Zinc (Zn)	0.222	0.304	0.745	-0.199
Copper (Cu)	-0.233	-0.512	0.424	-0.513
Calcium (Ca)	0.039	0.929	0.140	0.130
Magnesium (Mg)	0.217	0.720	0.419	0.292
Potassium (K)	0.624	-0.191	0.221	0.032
Percentage variance explained	27.1	22.1	18.5	6.9
Important variables	BD,LOI, Fe,	Mn,Ca,Mg,	Zn	P

1.6 Discussion

The growth of wild rice plants was better in areas of the lakes free from aquatic plants. However, the magnitude of growth inhibiting effects of different plant species on wild rice was variable. The differential growth inhibiting effects of different plant species on wild rice may be due to their different morphological features and growth habit and/or their effect on nutrient availability to the wild rice. Morphology and growth habit of these plants could affect the growth of wild rice by shading the developing seedlings. These aquatic plants are perennial and start to grow earlier in the spring than wild rice (Aiken *et al*, 1988). Therefore, they shade the growing wild rice plant by occupying the space on the water surface especially large leaved *Nymphaea odorata* and *Nuphar variegatum*. Miller (1994) reported that the presence of water hyacinth (*Eichhornia speciosa* Kunth) and mosquito fern (*Azolla caroliniana* Willd.) on the water surface caused high mortality of young wild rice plants. Giant burreed (*Sparganium eurycarpum*) was found to reduce the penetration of photosynthetically active radiation (PAR) about 35 percent into the rice canopy at the flowering stage and reduced yield up to 60 percent (Clay and Oelke,1987). In white rice (*Oryza sativa* L.), the rate of photosynthesis has been found to be directly related to the light intensity at which it is grown (Yoshida, 1981).

The number of wild rice plants per square meter was always lower in the presence of competing plants. The density of wild rice plants depends on the survival of young seedlings as well as on the production of new tillers. New tillers develop from tiller primordia present at the lower nodes of the rice plant during the vegetative growth phase (Aiken *et al*, 1988). Development of new tillers in white rice depends on the supply of carbohydrate from the culm as well as the amount of nitrogen supplied to the growing tiller primordium (Tanaka and Garcia, 1965). Reduced light in the presence of different plant species may cause decreased accumulation of carbohydrate in the culm, which eventually may reduce the number of tillers of wild rice. Shaded conditions may also increase seedling mortality of rice by reducing the light intensity needed for normal growth (Lee, 1986b).

Wild rice grow best in soft organic soils (Moyle, 1944). Decomposition of organic matter releases nitrogen as well as other soil nutrients. Relatively low amounts of nitrogen, phosphorous and metals have been reported in wild rice sediments (Lee, 1982; Lee, 1983; Lee and Stewart, 1984), which are considered to be important factors affecting wild rice productivity. The four most important variables generated by the PCA were: bulk density, loss on ignition and iron in factor 1; manganese, calcium and magnesium in factor 2; zinc in factor 3 and phosphorous in factor 4. These factors explained the majority of the variation present in the sediment variables and could certainly influence the wild rice productivity. However, the analysis of covariance revealed that the environmental variables had little influence on the dry weight of wild rice in the presence or absence of competing plant species.

Some plants like *Eleocharis smallii*, *Scirpus acutus* and

Equisetum fluviatile, have much lower leaf area and biomass than the wild rice which suggests that allelopathy might be a possible mechanism of growth inhibition in wild rice. Several studies conducted elsewhere reported the presence of allelopathy in a wide variety of aquatic plants (Elakovich and Wooten, 1995). Seven other species of *Eleocharis* have been reported as allelopathic (Wooten and Elakovich, 1991). Cheng and Reimer (1988) tested the water extracts of dried tissue of six aquatic plants for their allelopathic activity by using a lettuce seedling bioassay. The extracts of *Sparganium americanum* root and shoot and entire plant of *Vallisneria americana* showed allelopathic properties. Elakovitch and Wooten (1989) screened sixteen hydrophytes for allelopathic potential using lettuce (*Lactuca sativa*) and duckweed (*Lemna minor*) bioassay. Aqueous extracts of leaves plus petioles, and rhizomes of *Nymphaea odorata*, entire plant extracts of *Cabomba caroliliana*, *Brasenia schreberi*, *Myriophyllum spicatum* and *Vallisneria americanum*, were most inhibitory. Later, Elakovitch and Wooten (1991) found that *Nuphar luteum* ssp. *macrophyllum* was found to be far more inhibitory to both lettuce seedlings and duckweed growth than that reported earlier.

The exact mechanism/s by which the aquatic plants affect the growth of wild rice can not be ascertained from this study. However, shading and/or allelopathy may be the probable causes which requires further investigation.

CHAPTER 2

2. Allelopathic potential of aquatic plants on wlid rice (*Zizania palustris* L.)

2.1 Abstract

The allelopathic potential of eight aquatic plants on wild rice was investigated using lettuce and wild rice seedling bioassays. Rhizome aqueous extracts of *Scirpus acutus* Muhlenb. ex Bigelow, *Potamogeton natans* L., *Nymphaea odorata* Dryander ex Aiton, *Nuphar variegatum* Engelm. ex Durand, shoot extract of *Eleocharis smallii* Britton, whole plant extract of *Myriophyllum verticillatum* L., and leaf extract of *P. natans* L. significantly reduced the root length of lettuce and total root length of wild rice seedlings. The lettuce seedling bioassay was more sensitive than the wild rice bioassay. Shoot growth was less affected than root growth. Aqueous extract of sediments associated with competing plants had little growth inhibitory effect on wild rice. The study emphasized the importance of using the target species as a bioassay material. Further studies on allelopathic effects of lake sediments associated with the competing plants of wild rice is necessary to evaluate their ecological significance.

2.2 Introduction

Wild rice (*Zizania palustris* L.), an annual aquatic cereal crop grows in lakes throughout eastern and north-central North America. A number of other aquatic plants have been reported to grow within the natural stands of wild rice, and are thought to affect its growth (Aiken *et al*, 1988). Lee (1982) reported that emergent species such as spike-rush (*Eleocharis smallii* Britton.), big bul-rush (*Scirpus acutus* Muhlenb. ex Bigelow) and horsetail (*Equisetum fluviatile* L.) displace the wild rice from shallow water bodies. Free-floating and submerged species such as white water lily (*Nymphaea odorata* Dryander ex Aiton), yellow water lily (*Nuphar variegatum* Engelm. ex Durand) and bur reed (*Sparganium fluctuans* (Morong) Robinson, *Ceratophyllum* spp. and *Myriophyllum* spp. are also reported to be detrimental to wild rice (Aiken *et al*, 1988).

The adverse effects of aquatic plants on wild rice may result from competition for light, nutrients and space or by allelopathic interference. However, the exact mechanisms of growth inhibition of wild rice in the presence of these plants are not known. Poor growth of wild rice in the presence of spike-rush was reported by Aiken *et al*, (1988). Since spike-rush and big bulrush have a much lower leaf area and biomass than that of wild rice, competition for light and nutrients seem unlikely. It is hypothesized that allelopathy is involved in this growth inhibition process.

Allelopathic interactions in terrestrial plants are reported by many authors (Rice,1984; Mallik, 1987; Putnam and Tang, 1986;

Rizvi and Rizvi, 1992; Mallik, 1992; Zhu and Mallik, 1994; Mallik and Roberts, 1994; Inderjit *et al*, 1995a) but relatively few studies have been reported involving aquatic plants.

Different species of spikerush have been reported as allelopathic to other aquatic plants (Oborn *et al*, 1954; Yeo, 1980; Frank and Dechoretz, 1980; Nichols ans Shaw, 1983; Yeo and Thurston, 1984; Ashton *et al*, 1985; Sutton, 1986 and 1990; Sutton and Portier, 1989 and 1991; Wooten and Elakovich, 1991). Elakovitch and Wooten (1989) tested sixteen hydrophytes for their allelopathic potential using duckweed (*Lemna minor*) and Lettuce (*Lactuca sativa*) as bioassay materials. Among all the extracts, aqueous extracts of *Nymphaea odorata* leaves and petioles, *Nymphaea odorata* rhizomes, entire plants of *Cabomba caroliniana*, *Brasenia schreberi*, *Myriophyllum spicatum* and *Vallisneria americanum*, were most inhibitory as determined by both the bioassays. Recently, Jones (1993) reported that aqueous extracts of *Cobomba caroliana*, *Ceratophyllum demersum*, *Myriophyllum aquaticum*, *Myriophyllum spicatum*, *Nymphaea odorata*, *Potamogeton nodosus*, *Sagittaria lancifolia*, *Pondtederia lanceolata*, *Vasslisneria americana* affect the growth of *Hydrilla verticillata*. A review of the literature since 1970 shows 67 genera and 97 species of herbaceous vascular hydrophytes reported to be allelopathic (Elakovich and Wooten, 1995). However, no work has been reported on the allelopathic potential of *Eleocharis smallii* and some other common aquatic plants associated with northern wild rice.

Lee (1987) reported dramatic growth enhancement of wild rice in the presence of *Potamogeton robinsii*. The mechanism of this growth enhancement is not known. It was assumed that *P. robinsii* increased phosphorus availability by adding organic matter to clay sediments. Aqueous extracts of water hyacinth (Sircar and Kundu, 1959); *Carex alata* Torrey and *Glyceria maxima* (Hartman) O. Holmb. (Szezepanska, 1977) and *Woolfia arrhiza* L.(Wolek, 1979) at low concentration was found to be stimulatory to several aquatic plants. Growth inhibitory properties of the aquatic plants as well as growth enhancement effects of *Potamogeton robinsii* on wild rice strongly suggest that allelopathy may be a possible mechanism.

The objective of the present study was to determine the allelopathic potential of several aquatic plants associated with northern wild rice and their lake sediments using lettuce (*Lactuca sativa*) seed and wild rice bioassays.

2.3 Materials and methods

2.3.1 Screening of wild rice plants for allelopathic potential

2.3.1.1 Preparation of water extracts:

Eight aquatic plants, namely, *Eleocharis smallii*, *Scirpus acutus*, *Equisetum fluviatile*, *Nymphaea odorata*, *Nuphar variegatum*, *Sparganium fluctuans*, *Myriophyllum verticillatum* L., and *Potamogeton natans* L. were collected from Whitefish (Lat. 49° 06′ N, Long. 90° 09′ W), Rice stock (Lat. 48°13′ N, Long. 90°00′ W) and Chisamore (Lat. 49° 20′ N, Long. 89. 44′ W) lakes of northwestern Ontario and were tested for their allelopathic potential. The freshly collected plants were separated into leaves and stems/rhizomes. One hundred grams of each sample were blended in 1 L distilled water. The mixture was filtered through Whatman Number 1 filter paper using a vacuum pump. The water extracts were either assayed immediately or were stored in a freezer at -15°C. The pH of the extracts was determined immediately. The filtrates considered as 100% (v/v) water extracts were used for the bioassay experiments. The aqueous plant extracts have been chosen based on the assumption that the dead and decomposed plant materials release toxin into the environment which may also be transformed into other more toxic compounds (Patrick, 1971; Rice, 1984).

Leachates of the shoot of *Eleocharis smallii* and the rhizomes of *Scirpus acutus* and *Potamogeton natans* were prepared by soaking 100 g plant material in 1 L distilled water for 72 hours at 4°C and then filtering through Whatman Number 1 filter paper.

2.3.2 Preparation of water extracts of sediments associated with competing plants of wild rice.

Lake sediment samples below the wild rice plants were collected from contiguous plots with and without competing plant species. Five samples were randomly collected from each of the paired plots with and without *Eleocharis smallii*, *Scirpus acutus*, *Nymphaea odorata*, *Nuphar variegatum*, *Myriophyllum verticillatum* and *Potamogeton natans*. The sixty sediment samples were air dried at room temperature (25°C), ground, sieved (2 mm) and stored in paper bags. Two hundred and fifty grams of air dry sediments were soaked for 12 hours with 625 ml distilled deionized water at 4°C and then shaken for 2 hours at room temperature. The sediment extracts were filtered through Whatman No. 1 filter paper and were stored at -15 °C. The pH of the extract was measured. The filtrates considered as 100% (v/v) sediment extracts were used for the bioassay tests.

2.3.3 Total phenols

Phenolic compounds have been reported to be involved in allelopathic interactions (Rice, 1984). The total phenolic content of the aqueous extract of the plants and their associated lake sediments was determined by the Folin-Chiocalteau reagent method (Swain and Hills, 1959).

2.3.4 Bioassays:

2.3.4.1 Phytotoxicity of water extracts

The lettuce seedling bioassay was conducted with water extracts of different plant parts of individual plant species to determine their allelopathic potential. Twenty lettuce seeds were placed in a disposable sterile Petri-dish (9 cm diameter) lined with Whatman Number 3 filter paper. Four ml of the extract was added to each Petri-dish. Control Petri-dishes received 4 ml distilled deionized water. The Petri-dishes were sealed with parafilm to prevent water loss and were stored in the dark at room temperature (22 - 24°C) for five days. Treatments were allotted in completely randomized design with five replications. Five days after incubation, percent germination and length of radicles (primary root) and plumules (primary shoot) were recorded.

2.3.4.2 Bioassay with wild rice germinants

Aqueous extracts of the plants that exhibited strong growth inhibitory effects in the previous study were used to test their allelopathic potentials on wild rice. Ten uniform pre-germinated wild rice seeds were placed in a disposable Petri-dish (9 cm diameter) lined with one layer of Whatman Number 3 filter paper. Ten ml aqueous extract was added to each Petri-dish. These were placed in a growth chamber at 14°C for one week, followed by another week at 16°C, with 70 % relative humidity and 16 hours photoperiod. The control germinants received 10 ml distilled deionized water. The treatments were replicated five times. Fifteen days after the incubation, root and shoot lengths were recorded. Root and shoot dry weights were determined by oven drying at 70°C for 48 hours.

2.4 Data analysis

Analyses of variance (ANOVAs) determined if the growth variables (root and shoot length and root and shoot dry weight) of lettuce and wild rice seedlings were affected by aqueous extracts of different plant species. Significant differences among the means were determined by Tukey's test.

2.5 Results

2.5.1 Phytotoxicity of water extracts of wild rice plants

2.5.1.1 Lettuce seedling bioassay:

Germination of lettuce seeds was slightly inhibited by the water extracts with the highest significant decrease (79.0%

compared to control) due to *Scirpus acutus*. The other species inhibited germination by between 82.0 to 99.0 percent of the control. Ten water extracts of the six weed species significantly reduced the primary root growth of lettuce seedlings (Table 2.1). However, there were marked differences among the extracts regarding the inhibition of root growth. The aqueous extracts of rhizome of *Nymphaea odorata*, *Potamogeton natans* and the whole plant extract of *Myriophyllum verticillatum* were most inhibitory, causing nearly 75 percent reduction in lettuce root length. Root growth of lettuce was not inhibited by the whole plant water extracts of *Equisetum fluviatile*, *Sparganium fluctuans* and *Potamogeton robbinsii*. In contrast to root length, the shoot length of lettuce seedlings was stimulated by several plant extracts except the rhizome extract of *Nymphaea odorata* (Table 2.1) where shoot length was significantly reduced.

2.5.1.2 Wild rice bioassay.

Total root length of wild rice seedlings in rhizome extracts of *Scirpus acutus*, *Potamogeton natans*, *Nymphaea odorata* and *Nuphar variegatum* and shoot extracts of *Eleocharis smallii* and *Myriophyllum verticillatum* was significantly less than that of the control. The total root length of wild rice in extracts of other plant species did not differ significantly from that of the control (Table 2.2). Shoot length of wild rice was inhibited significantly only in the rhizome extract of *Nymphaea odorata* (Table 2.2).

Root dry weight of wild rice seedlings was significantly less in those extracts of plant species that inhibited the total root length (Table 2.2). Root dry weight in other extracts was not significantly different from the control. Shoot dry weight of wild rice seedlings was significantly reduced in rhizome extracts of *Potamogeton natans* and shoot extracts of *Eleocharis smallii* (Table 2.2). Shoot growth was stimulated in rhizome extracts of *Scirpus acutus*, *Nuphar variegatum* and whole plant extract of *Potamogeton robinsii*. Shoot dry weight of wild rice in presence of the other extracts were not significantly different from control.

2.5.2 Effect of lake sediment extracts

The total root length, shoot length and root and shoot dry weights of wild rice seedlings did not differ significantly due to water extract of lake sediments with or without aquatic plants (Table 2.3). However, there was a trend of reduced growth of rice seedlings in extracts of sediments with aquatic plants.

2.5.3 Total phenols and pH

The total phenolic contents of aqueous extracts of different plants varied greatly (Figure 2.1). The rhizome extract of *Nuphar variegatum* had the highest amount of total phenol followed by the rhizome and whole plant extracts of *Nymphaea odorata* and *Myriophyllum verticillatum*. The rhizome extract of *Scirpus acutus* had more phenol content than the shoot extract of *Eleocharis smallii*. Among all the extracts, the rhizome extract of *Potamogeton natans* had the lowest amount of total phenol. Shoot leachate of

Eleocharis palustris and rhizome leachates of *Scirpus acutus* and *Potamogeton natans* had relatively small amounts of total phenol compared to their respective water extracts.

The total phenol content of the lake sediment extracts was very low compared to the total phenol content of the extract of plant parts (Figure 2.2). However, the phenol content of sediments associated with the competing plants was always higher than that from the competing plant-free sediments.

The pH of the water extract of different competing plants varied from 4.2 to 6.4 (Table 2.4) while that for the sediments were between 5.7 and 7.0 (Table 2.5).

Table 2.1. Effects of aqueous extract of different plant species on percent germination, root and shoot length of lettuce.

Plant species	Plant parts	Length of root (mm)	shoot (mm)	Germination (%)
Control		33.4 a	30.1 cd	100.0 a
Scirpus acutus	Rhizome	14.2 cdef	28.7 cd	79.0 c
S. acutus	Shoot	22.1 cd	25.0 de	99.0 a
Eleocharis smallii	Rhizome	25.9 bc	36.5 b	93.0 bc
E. smallii	Shoot	15.1 cdef	36.6 b	82.0 bc
Potamogeton natans	Rhizome	7.7 fg	26.4 d	98.0 a
P. natans	Leaves	17.8 cde	26.1 d	95.0 b
Nuphar variegatum	Rhizome	18.7 cde	28.6 cd	95.0 b
Nymphaea odorata	Rhizome	10.0 fg	19.8 e	96.0 a
N. odorata	Shoot	17.2 cde	35.3 b	97.0 a
Equisetum fluviatile	shoot	29.4 ab	46.0 a	91.0 bc
M. verticillatum	shoot	8.6 fg	35.6 b	86.0 bc
Sparganium fluctuans	Whole plant	29.7 a	29.3 cd	90.0 bc
P. robinsii	Whole plant	33.0 a	32.1 bc	90.0 bc

Note: Unlike letter(s) in a column indicate the mean values significantly different (p = 0.05) as determined by the Tukey's - HSD test.

Table 2.2. Effects of aqueous extracts of different plant species on total root and shoot length, root and shoot dry weight of wild rice seedlings.

Plant species	Plant parts	Total root length (cm)	Shoot length (cm)	Root dry weight (mg/ plant)	Shoot dry weight (mg/ plant)
Control		27.6 a	8.0 a	5.9 a	6.8 b
Scirpus acutus	Rhizome (WE)	18.7 bcd	7.4 a	4.7 b	7.0 a
S. acutus	Rhizome (L)	22.5 abc	8.1 a	5.3 a	7.6 a
Eleocharis palustris	Shoot (WE)	18.3 bcde	6.6 a	4.1 b	4.7 c
E. palustris	Shoot (L)	23.7 ab	7.8 a	5.7 a	6.7 b
Potamogeton natans	Rhizome (WE)	18.5 bcd	7.1 a	4.8 b	5.2 c
P. natans	Rhizome (L)	25.9 a	8.0 a	6.4 a	6.6 b
Myriophyllum verticillatum	Whole plant (WE)	19.2 bcd	6.7 a	4.4 b	6.2 b
Nymphea odorata	Rhizome (WE)	12.3 e	5.5 b	3.4 b	5.3 c
Nuphar variegatum	Rhizome (L)	15.4 de	7.1 a	4.6 b	7.8 a
Potamogeton robbinsii	Whole plant (WE)	26.6 a	7.7 a	6.1 a	7.1 a

Note: Unlike letters in a column indicate values significantly different at 0.05 level as determined by the Tukey,s test.

WE = Water extract, L = Leachate

Table 2.3. Effects of water extract of sediments associated with aquatic plants of wild rice on total root and shoot length and root and shoot dry weight of wild rice seedlings

	S. acutus		*E. smallii*		*P. natans*		*N. odorata*		*N. variegatum*		*M. verticillatum*	
Growth parameters	Present	Absent	Present	Absent	Present	Absent	Present	Absent	Present	Absent	Present	Absent
Total root length (cm)	13.7±1.9	19.3±6.9	15.6±5.1	17.5±1.9	13.7±2.7	14.5±3.6	11.0±2.8	13.4±4.5	12.1±2.1	16.6±2.2	14.7±1.7	14.9±3.7
Shoot length (cm)	5.8±0.6	6.3±1.0	6.7±o.4	6.8±0.6	5.8±0.6	7.8±0.5	7.21.4	7.2±0.8	6.5±0.5	7.7±0.9	6.3±0.7	7.1±1.2
Root dry weight (mg/plant)	4.7±0.5	4.8±1.0	4.6±0.4	4.8±0.5	4.0±0.8	4.9±1.2	3.6±1.0	4.6±0.3	4.2±0.1	4.8±0.4	4.2±0.5	4.9±0.5
Shoot dry weight (mg/plant)	6.8±0.8	6.9±1.6	7.4±1.4	7.5±0.7	5.6±0.6	7.9±1.0	7.3±0.5	7.7±1.3	7.1±1.1	8.3±1.2	7.0±0.8	7.0±0.9

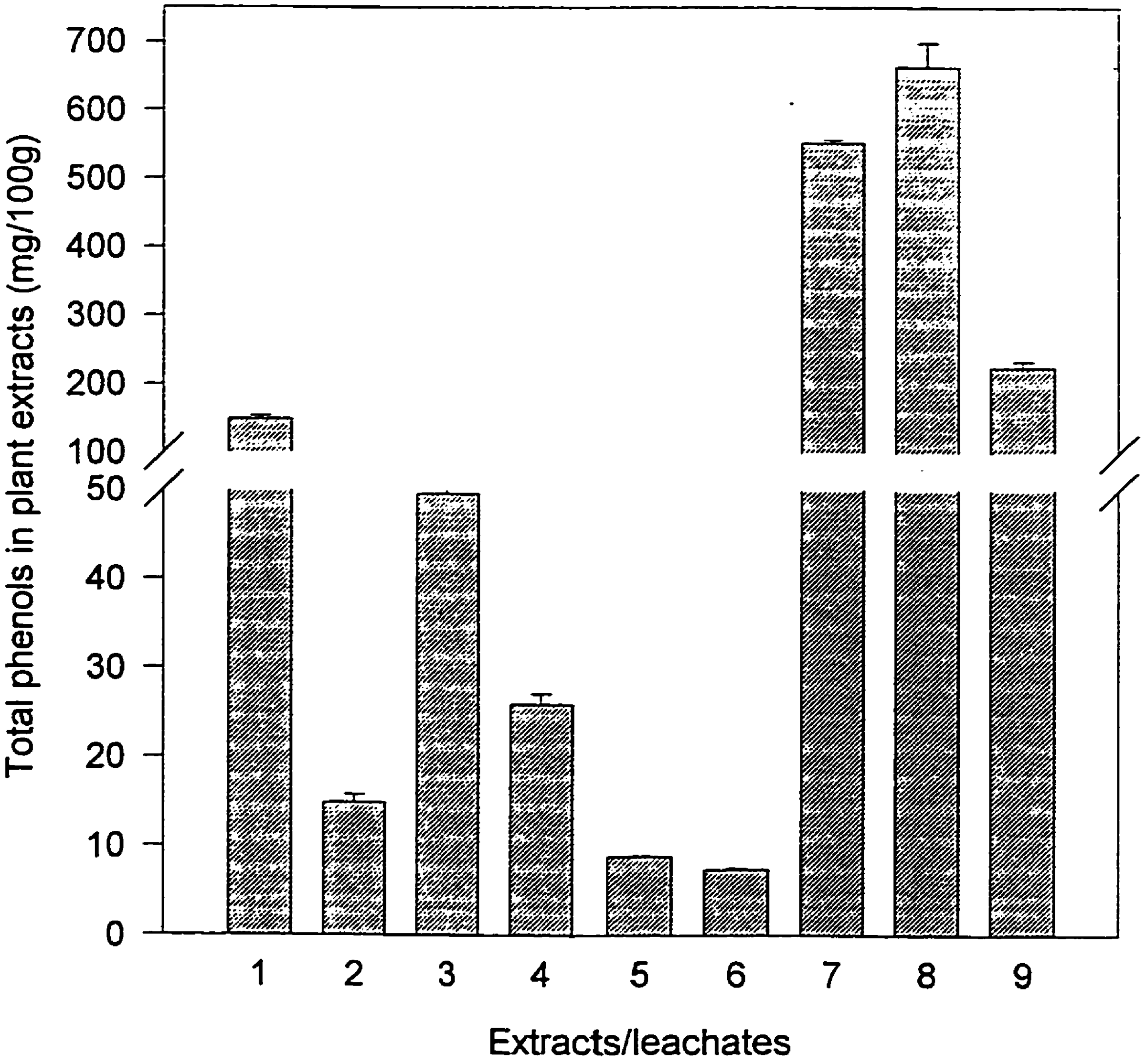

Figure 2.1 Total phenolic content of plant extracts and leachates

1. *Scirpus* rhizomes extract
2. *Scirpus* rhizomes leachate
3. *Eleocharis* shoot extract
4. *Eleocharis* shoot leachate
5. *Potamogeton* rhizomes extract
6. *Potamogeton* rhizomes leachate
7. *Nymphaea* rhizomes extract
8. *Nuphar* rhizomes extract
9. *Myriophyllum* whole plant extract

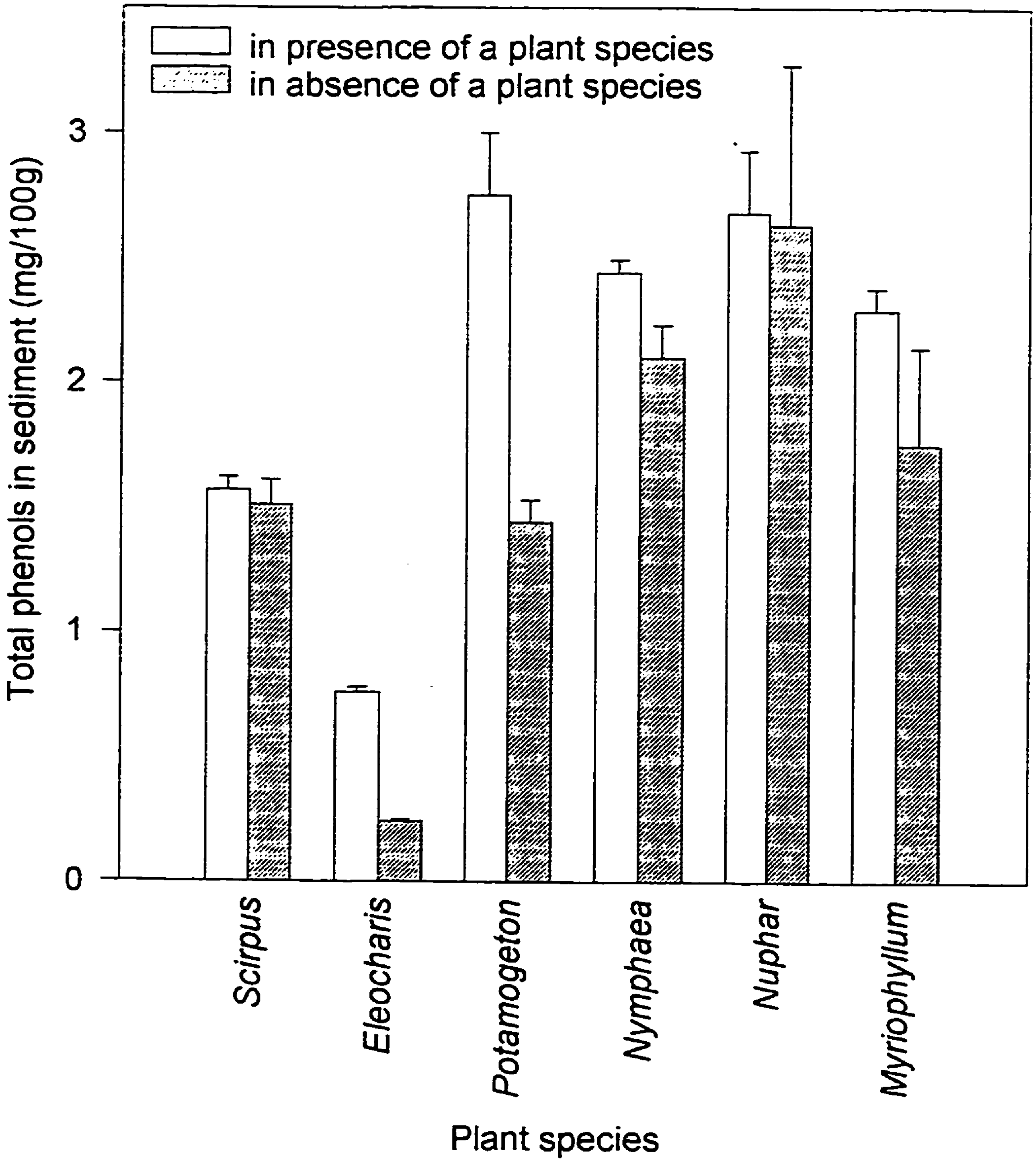

Figure 2.2 Total phenol content of sediment in presence and in absence of different plant species

Table 2.4. pH of water extracts of different plant species.

Plant species	Plant parts	pH
1. *Scirpus acutus*	Rhizome (WE)	5.5
2. *S. acutus*	Rhizome (L)	5.6
3. *Eleocharis smallii*	Shoot (WE)	5.1
4. *E. smallii*	Shoot (L)	5.8
5. *Potamogeton natans*	Rhizome (WE)	5.3
6. *P. natans*	Rhizome (L)	5.4
7. *Nymphea odorata*	Rhizome (WE)	4.5
8. *Nuphar lutea*	Rhizome (WE)	4.3
9. *Myriophyllum verticillatum*	Whole plant (WE)	5.1
10. *Potamogeton robbinsii*	Whole plant (WE)	6.4

WE = water extract; L = Leachate

Table 2.5. pH of the water extracts of sediments associated with different plant species of wild rice.

Sedimemt extracts	pH
With *Scirpus smallii*	5.7
Without *S. smallii*	5.9
With *Eleocharis smallii*	5.8
Without *E. smallii*	5.6
With *Potamogeton natans*	6.5
Without *P. natans*	6.9
With *Nymphea odorata*	7.0
Without *N. odorata*	6.6
With *Nuphar lutea*	6.7
Without *N. lutea*	6.6
With *Myriophyllum verticillatum*	6.8
Without *M. verticillatum*	6.8

2.6 Discussion

Water extracts of some aquatic plants inhibited the primary root growth of lettuce as well as total root length of wild rice seedlings. Lettuce seedlings were more sensitive to aqueous plant extracts than wild rice. The high sensitivity of lettuce seedlings to plant extracts limits their wider use as a standard bioassay technique for evaluation of allelopathic plants. The importance of using target species as a bioassay material was suggested by Anderson (1985) and Inderjit and Dakshini (1995b). Other investigators also used the target species as the bioassay material (Mallik, 1987; Mallik and Newton, 1988 and Zhu and Mallik, 1994).

Inhibition of root growth by aqueous extracts was considered an authentic parameter for the evaluation of allelopathic interactions in plants (El-Ghazal and Reimer, 1986; Elakovich and Wooten, 1991 and Zhu and Mallik, 1994). The reduction of root growth, both in lettuce and wild rice, indicates the presence of water soluble growth inhibitory substances in aqueous extracts of some plant species. Phenolic compounds are frequently reported to be growth inhibitory compounds due to their phytotoxicity and water-solubility. A number of phenolic compounds have been reported from allelopathic terrestrial plants (Rizvi and Rizvi, 1992; Zhu and Mallik, 1994; Inderjit and Dakshini, 1995a). However, only a few reports are available suggesting allelopathic effects of aquatic plants. The total phenol content of the extract of allelopathic plants was higher except the rhizome aqueous extract of *Potamogeton natans*. Cheng and Reimer (1989) isolated gallic, vanillic, p-coumeric and ferulic acids from american eelgrass (*Vallisneria americana*) and suggested that these phenolics are responsible for the alleged allelopathic properties of this weed. The rhizome of *P. natans* may contain other water soluble allelochemicals such as organic acids, some water soluble fatty acids, steroids and sugars which are also considered potential allelochemicals (Rice, 1984; Putnam and Tang, 1986). The stimulatory effects of some aqueous extracts to shoot growth of lettuce and wild rice suggest the differential response of root and shoot growth to aqueous extracts. Both the inhibitory and stimulatory responses of plants are significant for assessing allelopathic properties (Rice , 1986). Lower concentration of total phenols in the leachates may be the possible cause of its less inhibitory effects on seedling bioassay compared to the effect of their water extracts. Grinding of plant materials in the extraction method released higher amount of soluble phenols as opposed to the leachates.

The pH of all the aqueous extracts of plants was above 4. Reynolds (1979) found that pH within the range of 2.6 to 6.4 had no effect on lettuce seed germination. Therefore, it is believed that growth inhibition of lettuce and wild rice by some extracts was not caused by pH of the extracts.

The trend of reduction of growth parameters in sediment extracts indicate the presence of water soluble allelochemicals. The total phenol content of sediment was much lower than the total phenol content in plant parts. Therefore, it is necessary to develop bioassays suitable for lower concentrations of allelochemicals. Dornbos and Spencer (1990) have developed such a method.

It has been reported by many investigators that the allelopathic effects are the result of additive or synergistic activity of allelochemicals (Rice, 1984; Williamson, 1990). Under field conditions, these activities are high even at low concentrations (Einhellig *et al*, 1982; Williamson and Hoagland, 1982). Phenolic acids from living plants could accumulate in soil by root exudation; by decomposition of plant residues or by microbial transformation into other toxic chemicals (Rice, 1984; Putnam , 1985). The accumulation of allelochemicals in soil varies with the seasons. Jalal and Read (1983) reported that the levels of aromatic and aliphatic acids in *Calluna* and spruce soil were higher in the summer months. The inhibitory potential and amount of released phytotoxin from *Empetrum hermaphroditum* were highest during spring (Nilsson, 1992). Nilsson and Zackrisson (1992) also reported that phytotoxic substances accumulate in soil under conditions of ground ice. In the present study sediments associated with competing plants were collected once only during the month of August. As bioactive concentrations of phenolics in soil largely determined the visible allelopathic effects, it is necessary, therefore, to determine the level of total phenolics in soil during different months of the year.

Chemicals with allelopathic potential are present in most of the plant parts. Whether these chemicals are released into the environment, particularly to the soil and remain as a bioactive concentration for a certain period affecting the neighbouring plants is the major concern in the study of allelopathic interactions. Most of the investigators used aqueous plant extracts and lettuce and duck weed bioassays for evaluating allelopathic potential of aquatic plants (Cheng and Reimer, 1988; Elakovich and wooten, 1989, 1991). However, the inhibition of growth of bioassay materials by aqueous plant extracts does not truly represent the allelopathic interactions in an ecosystem. Therefore, aqueous plant extracts as well as associated sediment extracts should be used with target species as a bioassay material for evaluating allelopathic interactions among plants.

CHAPTER 3

3. Isolation and identification of allelochemicals from *Scirpus acutus* Muhlenb. ex Bigelow and *Eleocharis smallii* Britton.

3.1 Abstract

Aqueous extracts of rhizomes of *Scirpus acutus* and shoots of *Eleocharis smallii* were analyzed to isolate and identify the phytotoxic compounds using ethyl acetate extraction and GC-Mass spectroscopy. The organic fraction of the extract of *Scirpus* rhizomes contained lactic, succinic, fumaric, 2-hydroxy succinic, 2-phenyl lactic, m-hydroxy benzoic, p-hydroxy benzoic, protocatechuic, dehydroabietic and ferulic acids; p-hydroxy benzyl alcohol, p-hydroxy phenyl ethanol and catechin. The ethyl acetate organic fraction of *Eleocharis* shoots contained 4-methoxy phenol, benzofuran, benzene acetic acid, 1-hydroxy-5-methyl acetophenone and 1,3,4-dimethoxy phenyl ethanone. The potential allelopathic effect of these compounds on wild rice under field conditions was discussed.

3. 2 Introduction

Scirpus acutus (hereafter *Scirpus*) and *Eleocharis smallii* (hereafter *Eleocharis*) are common aquatic plants associated with wild rice (*Zizania palustris* L.) in north western Ontario, Canada. These plants start their life cycles in lakes before the germination of wild rice and attain a competitive advantage over wild rice. Aiken *et al* (1988) reported that *Eleocharis* gradually displaces wild rice from shallow water areas of the lakes. They also noted that the growth of wild rice was severely reduced when it occurs in presence of *Scirpus*. A component of this study showed that aqueous extracts of rhizomes of *Sirpus* and shoots of *Eleocharis* inhibited the root length of lettuce wild rice seedlings (Chapter 2). The aqueous extracts of *Eleocharis* and *Scirpus* causing the root growth inhibition were suspected to contain phytotoxic substance(s).

Phenolic acids have been reported frequently as potential allelochemicals due to their high water solubility and plant growth inhibitory properties (Rice,1984; Putnam and Tang, 1986; Zhu and Mallik, 1994). Phenolic acids are known to affect photosynthesis, protein synthesis, mineral uptake, chlorophyll synthesis, membrane permeability and water balance of plants (Rice, 1984). The phenolic compounds may be released from plants by leaching through fog drip or rain, exudation from roots, or by degradation of plant parts (Hale and Orcutt, 1987). The two competing aquatic plants of wild rice were selected for characterization of allelochemicals due to their wide distribution and adverse effects on wild rice. It was suspected that the decomposed organic matter of the plants release phenolic substances in the rhizosphere of wild rice causing its root growth inhibition.

The objective of the present study was to isolate and identify the water soluble allelochemicals, particularly phenolics, from the extracts of *Scirpus acutus* rhizomes and *Eleocharis smallii* shoots.

3.3 Methods

3.3.1 Extraction of phytotoxic compounds by fractionation

The aqueous extract of rhizomes of *Scirpus acutus* and shoots of *Eleocharis smallii* (100 g/litre) were fractionated according to Zhu and Mallik (1994) with some modification (Figure 1). A 250 ml water extract (fraction 1) was made alkaline to pH 12 with 2 M NaOH for conversion of phenolic acids into salts which dissolve in water. The alkaline extract was centrifuged at 12,000 g for 10 minutes. The supernatant was transferred to a separating funnel and was washed three times with 200 ml hexane to remove lipids (fraction 2). The pH of the resulting aqueous solution (fraction 3) was adjusted to pH 3 with 2 M HCl. The acidified fraction was washed four times with 200 ml ethyl acetate. The ethyl acetate fraction containing phenolic compounds (fraction 4) was first dried over anhydrous sodium sulphate and then evaporated to dryness in a rotary evaporator at 39°C. The lower aqueous fraction containing sugars and inorganics was separated (fraction 5).

Both organic and aqueous fractions of water extracts of *S. acutus* and *E. smallii* inhibited germination and the primary root growth of lettuce seedlings. These fractions were further analyzed to identify the growth inhibitory compounds. The aqueous fraction of ethyl acetate aqueous phase was lyophilized.

3.3.2 Gas Chromatography - Mass Spectroscopy (GC-MS)

Both the dried sample of ethyl acetate organic and aqueous fractions were dissolved in 4 ml methanol and passed through Whatman Number 1 filter paper. One ml of the methanol solution was transferred to a micro vial and evaporated to dryness under low air flow. The samples were silylated with N,O-bis(Trimethylsilyl)trifluoroacetamide (BSTFA) and then analyzed by GC high resolution mass spectroscopy (GC-MS)(VG Auto Spec) at 50°C, isothermal for 2 minutes and then 80 -350°C, over 5 minutes at the instrumentation laboratory of Lakehead University. The injection point temperature was 280°C. Spectral scans from total ion chromatograms were observed and a library search was done on each spectrum using the National Institute for Standard and Technology (NIST) library compilation series.

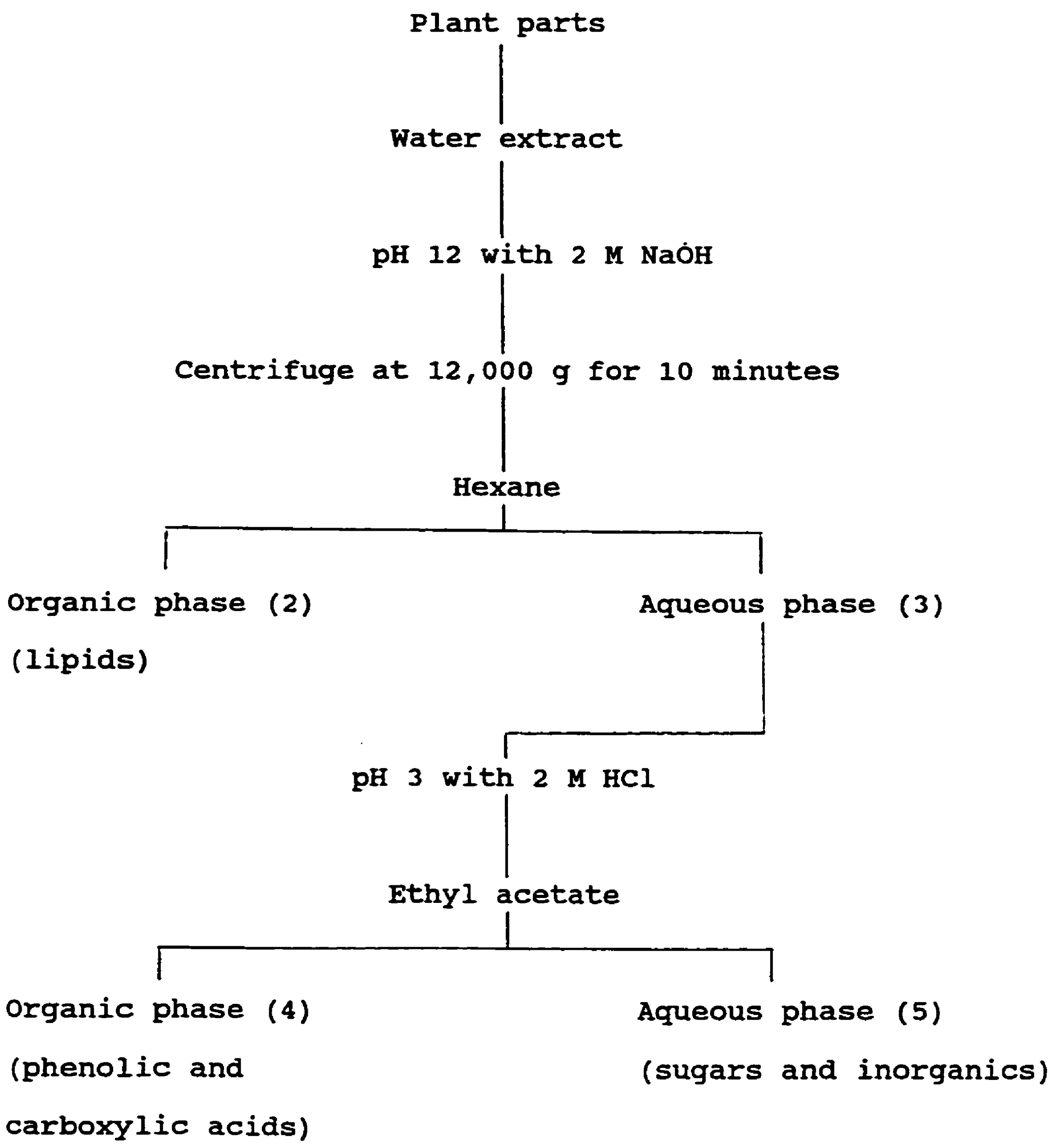

Figure 3.1. Extraction of phenolic compounds from plant parts.

3.3.3 Bioassay with water extract fractions

Phytotoxicity of the various fractions of water extract of *S. acutus* and *E. smallii* was determined by lettuce seedling bioassay. One circle of Whatman Number 3 filter paper was placed in a Petri-dish (9 cm diameter) and was wetted with 4 ml solutions of the fractions. After the solvent was evaporated from the hexane and ethyl acetate organic fractions, 4 ml deionized distilled water was added to each Petri-dish. The control plates received 4 ml of distilled water. Twenty lettuce seeds were placed on the filter paper of each petri-dish. The Petri-dishes were sealed with parafilm to prevent water loss and were placed in a laboratory drawer at room temperature (22 - 25°C) for five days. Each extract was assayed in five replicate petri-dishes. Five days after incubation, percent germination and lengths of radicles (primary root) and plumules (primary shoot) were recorded.

3.4 Data analysis

Data of root and shoot length and percent germination were subjected to one way analysis of variance and significant differences among means were determined by Tukey's test.

3.5 Results

3.5.1 Isolation and identification of allelopathic compounds

Lettuce seedling bioassays of the different fractions of *Scirpus* rhizome extracts exhibited different degrees of root growth inhibition (Table 3.1). Fractions 1, 4, and 5 were more inhibitory than fractions 2 and 3. The reduced root growth in the hexane organic fraction (fraction 3) indicates that the partition of allelochemicals between organic and aqueous phases was incomplete. The shoot length of lettuce seedlings was not inhibited by the fractions of water extracts. The germination of lettuce seeds was inhibited by different fractions of water extracts of *Scirpus*. The ethyl acetate aqueous phase showed the highest inhibition followed by water extract, ethyl acetate organic and hexane aqueous phases (Table 3.1).

The germination of lettuce seeds was inhibited by 1,2 and 4 fractions of the *Eleocharis* extracts (Table 3.2). The germination inhibition was highest in the water extract. Large variation of germinations in different replications was observed in hexane organic and ethyl acetate organic fractions. Lettuce seeds did not germinate in hexane aqueous and ethyl aectate aqueous fractions.

The primary root length of lettuce was inhibited in fraction 1 and 4 (Table 3.2). Again large variations in root length were observed in different replications of fraction 4. Fraction 2 did not inhibit root length. Shoot length was inhibited only in fraction 4 (Table 3.2).

When evaluated by GC-MS, the ethyl acetate organic fraction of *Scirpus* revealed the presence of more than 40 compounds (Figure 3.2). Among them 15 peaks were identified as lactic acid, succinic acid, fumaric acid, 2-hydroxy succinic acid, p-hydroxy benzyl alcohol, p-hydroxy phenyl ethanol, 2-phenyl lactic acid, 3-hydroxy benzoic acid (m-hydroxy benzoic acid), 4-hydroxy benzoic acid (p-hydroxy benzoic acid), protocatechuic acid, ferulic acid, dehydroabietic acid and a dye, catechin, by comparison with the mass spectral data base. A common contaminant of glassware, phthalate was identified in 26 peaks.The ethyl acetate aqueous fraction of water extract of *Scirpus* revealed the presence of several sugar moieties when evaluated by GC-MS (data not shown).

The ethyl acetate organic fraction of the water extract of *Eleocharis* showed the presence of more than 14 compounds when analyzed by GC-MS (Figure 3.3). Five peaks were identified as 4-methoxy phenol, Benzofuran, benzene acetic acid, 1-hydroxy-5-methyl acetophenone and 1,3,4-dimethoxy phenyl ethanone; four peaks were sugar moieties and others were contaminants. The ethyl acetate aqueous fraction of *Eleocharis* revealed several peaks and most of them were sugar moieties identified by the GC-MS (data not shown).

Table 3.1. Effect of different fractions of *Scirpus* rhizome extract on germination and root and shoot lengths of lettuce seedlings.

Fractions	Germination (%)	Length (mm)	
		Root	Shoot
Control	96.0 ± 3.6 a	29.0 a	28.5 a
1. Water extract	56.0 ± 12.8 b	15.7 d	30.0 a
2. Hexane organic phase	88.0 ± 9.4 a	23.8 b	28.2 a
3. Hexane aqueous phase	54.0 ± 14.2 b	18.2 c	27.9 a
4. Ethyl acetate organic phase	48.0 ± 20.5 b	17.0 cd	28.2 a
5. Ethyl acetate aqueous phase	18.4 ± 18.3 c	16.5 cd	26.9 a

In a column, means followed by a common letter are not significantly different at 0.01 percent level by Tukey's test.

Table 3.2. Effect of different fractions of *Eleocharis* shoot extract on germination, root and shoot length of lettuce seedlings.

Fractions	Germination (%)	Length (mm)	
		Root	Shoot
Control	98.0 ± 4.5	41.0 ± 2.8	28.6 ± 1.4
1. Water extract	26.0 ± 11.4	12.1 ± 0.8	26.4 ± 0.6
2. Hexane organic phase	72.0 ± 21.7	38.7 ± 5.6	29.0 ± 3.4
3. Hexane aqueous phase*			
4. Ethyl acetate organic phase	40.0 ± 31.6	9.1 ± 8.3	7.3 ± 6.7
5. Ethyl acetate aqueous phase*			

* Seeds did not germinate in these fractions.
± Standard deviation

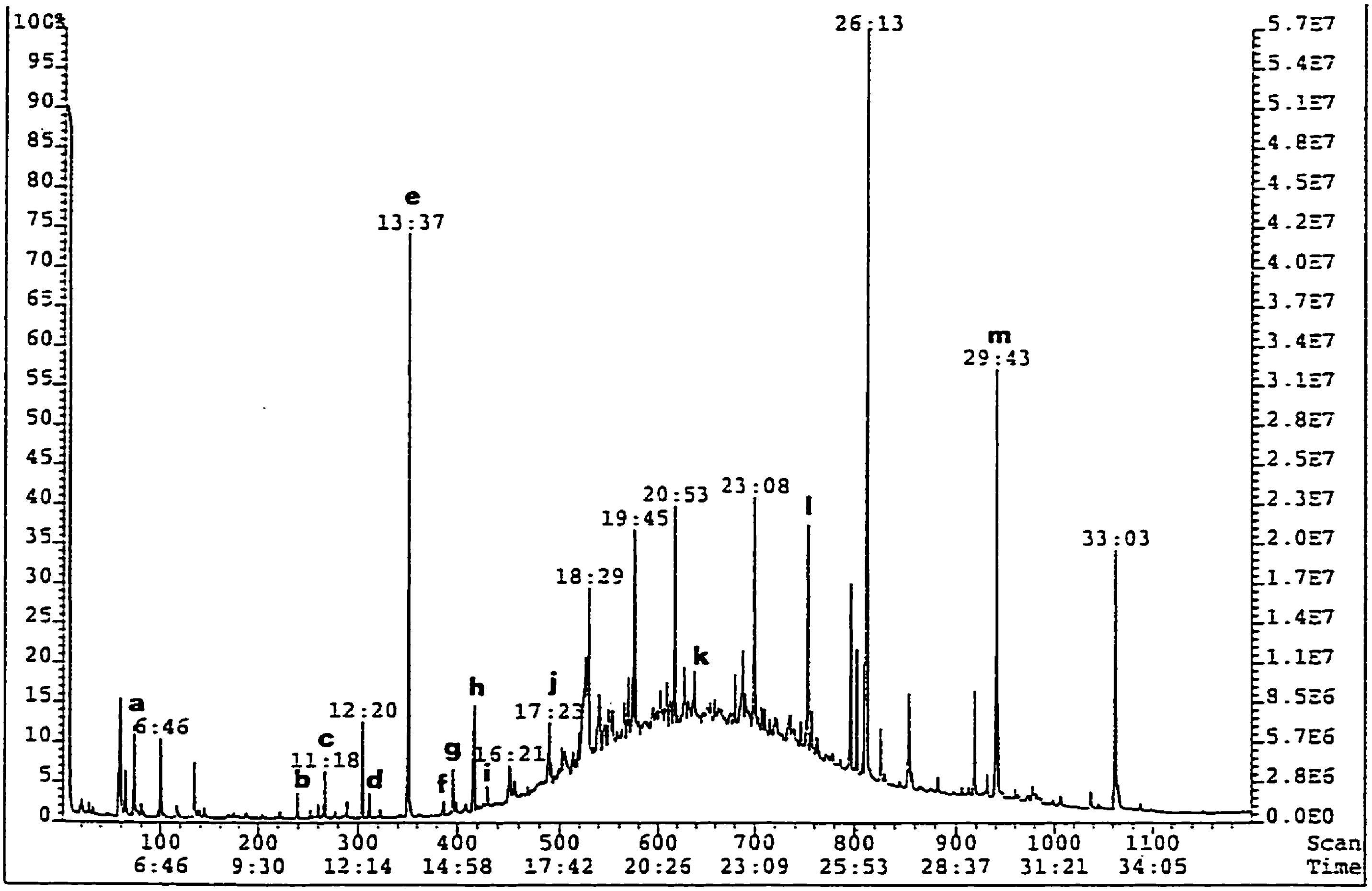

Figure 3.2. Total ion current chromatogram of GC-MS of ethyl acetate organic fraction of *Scirpus* rhizome extract. A = lactic acid, B = succinic acid, C = fumaric acid, D = p-hydroxy benzyl alcohol, E = 2-hydroxy succinic acid, F = p-hydroxy phenyl ethanol, G = 2-phenyl lactic acid, H = m-hydroxy benzoic acid, I = p-hydroxy benzoic acid, J = protocatechuic acid, K = ferulic acid, L = dehydroabietic acid and M = catechin.

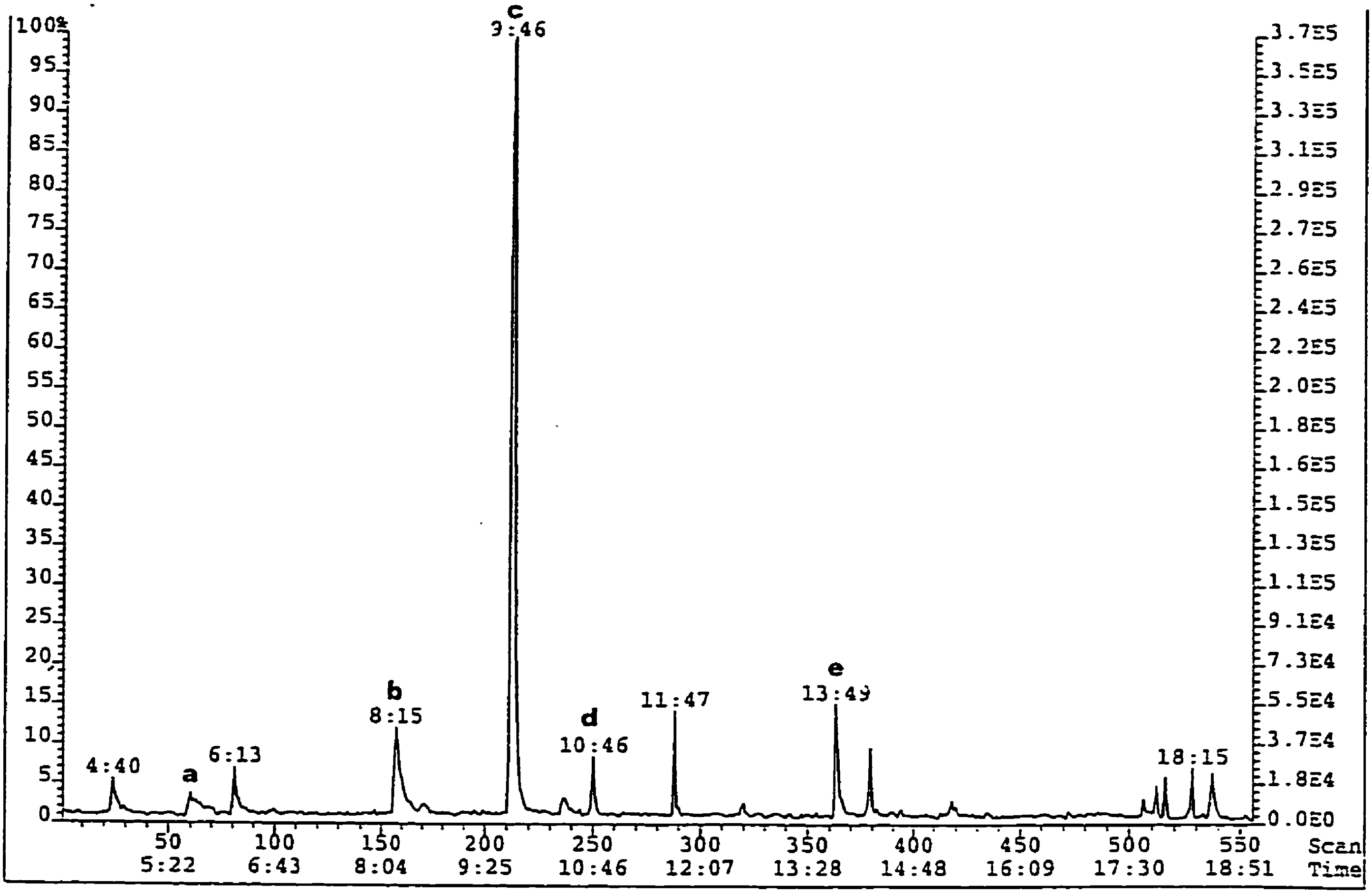

Figure 3.3. Total ion current chromatogram of ethyl acetate organic fraction of *Eleocharis* shoot extract. A = 4-methoxyphenol, B = Benzofuran, C = 1-hydroxy-5-methyl acetophenone, D = benzene acetic acid, E = 1,3,4-dimethoxyphenyl ethanone.

3.6 Discussion

Fifteen compounds were identified from the ethyl acetate organic fractions of the water extract of *Scirpus* rhizomes. Fumaric acid is a well known microbial toxin (Mirocha *et al.*, 1966) and is also exuded from seeds of *Pinus resinosa* which inhibits the growth of *Pythium afertile*. M-Hydroxy benzoic and p-hydroxy benzoic acids are benzoic acid derivatives and they have been isolated from many plants, decomposed plant parts and soil as potential allelochemicals. P-hydroxy benzoic acid is one of the most common benzoic acid derivatives involved in allelopathy (Rice,1984). It has been identified from branch leachate of *Arctostaphylos glandulosa* (Chu and Muller, 1972), subtropical grasses (Chou and Young, 1975), decomposing rye and corn residue in soil (Chou and Patrick, 1976), bottom land forest soil (Lodhi, 1976) and leaf leachate of *Kalmia angustifolia* (Zhu and Mallik, 1994). Protocatechuic acid has also been reported as an allelopathic agent. In plants, it originates directly from dehydroshikimic acid (Neish, 1964). It has been isolated from leachate of *Arctostaphylos glandulosa*, water extracts of *Erica* spp. and its associated soil (Carballeira, 1980) and from *Leucaena leucocephala* (Chou and Kuo, 1986). Ferulic acid is a cinnamic acid derivative and it has been isolated from aqueous extracts of different plant parts, crop residues and soil under allelopathic plants (Rice,1984; Chou and Kuo, 1986; Zhu and Mallik, 1994). No reports are available on allelopathic properties of other compounds identified from the aqueous extract of *Scirpus* rhizomes. Further study is necessary to determine their allelopathic properties.

Five compounds such as 4-methoxy phenol, benzofuran, benzene acetic acid, 1-hydroxy-5-methyl acetophenone and 1,3,4-dimethoxyphenyl ethanone were identified from shoot extracts of *Eleocharis*. There are no reports of these being allelochemicals. However, I believed that as *Eleocharis* shoots extract inhibited the root growth of lettuce seedlings, these compounds might have phytotoxic properties. Further work is also necessary to determine their growth inhibitory properties.

Ethyl acetate aqueous fractions of the water extracts of *Scirpus* rhizomes and that of *Eleocharis* shoots contained only sugar moieties. Sugars, at low concentration, generally do not inhibit germination and growth of plants. However, at higher concentrations they may affect plant growth by increasing the osmotic potential of the growth medium. High osmotic concentrations induce stress in plants, primarily by causing dehydration of plant material (Slayter, 1967; Waring and Cleary, 1967). However, we believe that sugar concentrations of these aqueous fractions were not high enough to induce osmotic stress. The exact mechanism by which these two extracts inhibited the growth of lettuce seedlings can not be explained.

As these weeds are perennial, they would maintain a sufficient supply of phytotoxic compounds in the soil through exudation or by decomposition of plant parts and might affect the growth of wild rice seedlings.

GENERAL DISCUSSION

Interference of aquatic plants with wild rice was studied by focusing on three aspects, 1) growth response of wild rice in the presence or absence of different species of aquatic plants, 2) The allelopathic potential of these plants on test species, and 3) isolation and identification of allelochemicals from two plants, *Scirpus acutus* and *Eleocharis smallii*.

A significant reduction in density and dry biomass of wild rice was obtained when the plant was associated with other aquatic species. Similar reduction in growth and yield of wild rice in the presence of common water plantain (*Alisma trivale*) (Ranson and Oelke, 1982) and giant burreed (*Sparganium eurycarpum*) (Clay and Oelke, 1987) has been reported. Neighbouring species associated with crop plants affect their growth by competing for light, space, nutrients and water (Zimdahl, 1993). In an aquatic environment competition for water is irrelevant. Therefore, other habitat factors, such as water depth, soil bulk density, pH and sediment nutrients such as nitrogen, phosphorus, potassium, magnesium, calcium, iron, manganese, zinc and copper, may be responsible for this growth inhibition. However, covariance analysis between the wild rice growth variables and the environmental factors was found to be not significant.

One possible mechanism by which these plants, particularly broad leaved floating species such as *Nymphaea odorata* and *Nuphar variegatum*, can affect the growth of wild rice is through shading. Since many of the aquatic plants develop from perennial rhizomes, they generally start their life cycle before the wild rice. These plants occupy the water surface by producing large foliage and, therefore, may reduce the amount of light avaiable to the developing wild rice seedlings. Miller (1994) reported very high seedling mortality of southern wild rice (*Zizania aquatica* L.) in the presence of floating species like water hyacinth (*Eichhornia speciosa* Kunth) and mosquito fern (*Azolla caroliniana* Willd.). These broad leaved floating plants also push the wild rice plants between the leaves and therefore, reduce the total leaf area for light attenuation. Clay and Oleke (1987) reported that giant burreed inhibits wild rice growth by reducing the amount of photosynthetically active radiation.

Another possible mechanism of wild rice growth inhibition in the presence of associated plants may be due to allelopathic interactions. In particular, for plants such as *Eleocharis smallii* and *Scirpus acutus*, which have much lower leaf area and biomass than wild rice, competition for light and nutrients seems less likely. Indeed, bioassay experiments with aqueous extracts of these plants showed significant root growth inhibition of lettuce and wild rice germinants. Further analyses of rhizomes of *Scirpus acutus* and shoots of *Eleocharis smallii* showed the presence of fifteen phytotoxic compounds, seven of which are phenolic in nature and are water soluble. It is possible that these compounds may be released from the plants by root exudation, leaching or by decomposition of the dead tissue and can affect the growth of wild rice. Cheng and Reimer (1989) reported the presence of four phenolic compounds, gallic, vanillic, p-coumaric and ferulic acids in American eelgrass. These compounds are known to be phytotoxic. Several other studies have claimed that allelopathy occurs fairly widely in aquatic plants (Elakovitch and Wooten, 1995). However, one major weakness in most if not all of these studies is that the ecological significance of these compounds in inducing growth inhibition has not been conclusively proven. Mere presence of allelopathic compounds in plant tissue does not prove allelopathy (Inderjit and Dakshini, 1995b). Identification of growth inhibitory compounds is only the first step. If the compounds are present in bioactive concentration in the habitat, only then perhaps, one could assume allelopathy as a growth inhibitory mechanism.

Indeed, the present study showed clearly that, although a number of phenolic compounds that are known to be phytotoxic are present in the aquatic plants associated with wild rice, the bioassay with the water extracts of wild rice soil showed no significant inhibitory effect. Concentration of total phenols in the soil was also very low. However, one can not rule out the possibility of allelopathic interaction by analyzing soils just once. It has been shown that concentrations of allelopathic substances in soil vary widely, depending on the season (Jalal and Read, 1983; Nilsson, 1992). Quantitative determination of phytotoxic compounds in the soil, roots and rhizomes of plants associated with wild rice plants several times in the growing season is required to get a better understanding of the involvement of allelopathy in growth reduction of wild rice.

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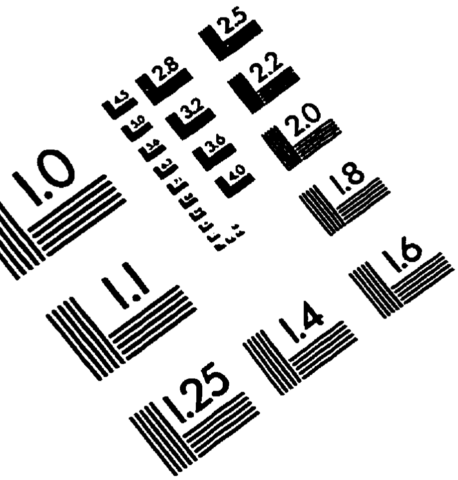
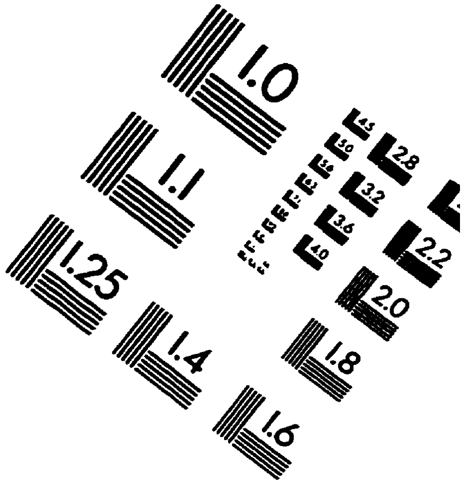
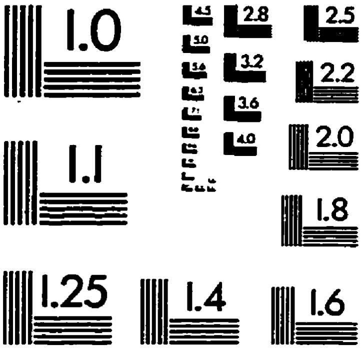
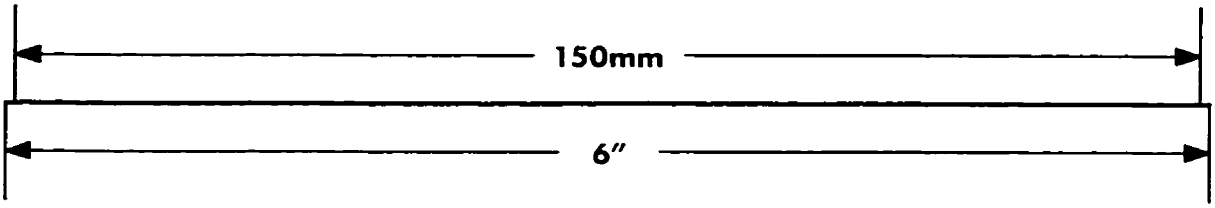
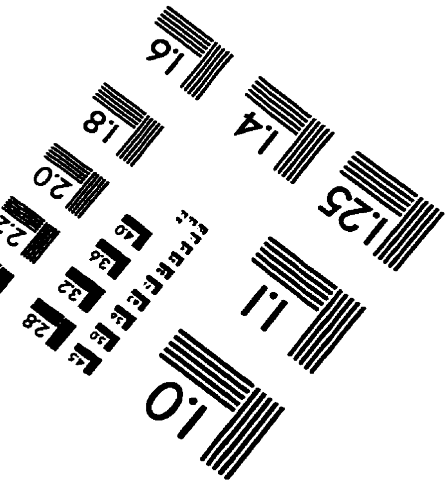
IMAGE EVALUATION TEST TARGET (QA-3)

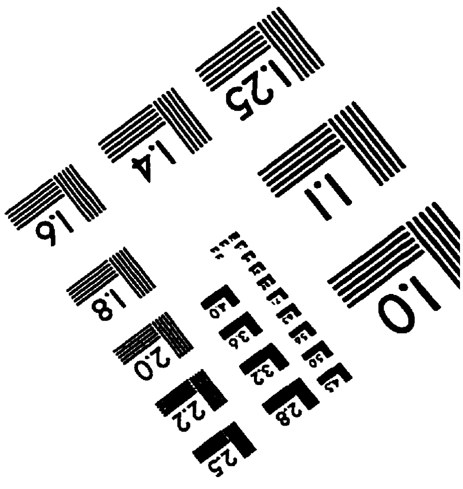












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