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Influence of External Iron Concentration on Active Iron for Four Species of Aquatic Macrophytes

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ABSTRACT

Four species of aquatic plants were grown in cultures with various levels of iron in the water phase and tissue levels of total iron and 'active' iron (Fe^{2+}) were determined. Active iron accounted for 29% of the total tissue iron. The relationship between active iron and the external iron concentration was described by a rectangular hyperbola. Estimates of the external iron concentration at which the active iron fraction was one-half of the maximum 'active' iron concentration indicate that the species could be separated into two groups. Values for monoecious and dioecious hydrilla were similar to that for variable pondweed. Sago pondweed and American pondweed had values which were similar to each other but higher than for the first group. These results may be useful for predicting the effi-

cacy of fluridone, (1-methyl-3-phenyl-5-[3-(trifluoromethyl)phenyl]-4(1H)-pyridinone), treatments.

Key words: herbicide susceptibility, iron nutrition, fluridone.

INTRODUCTION

The aquatic herbicide, fluridone, inhibits plant growth by disrupting the synthesis of photosynthetic pigments (Bartels and Watson 1978; Berard et al. 1978). Studies with wheat seedlings (Bartels and Watson 1978) and cell-free carotogenic systems from algae suggest that fluridone interferes with carotenoid synthesis. Specifically, fluridone appears to interfere with the desaturase enzyme complex which converts phytoene to gamma-carotene (Sandmann and Boger 1986). Desaturase enzyme complexes have iron as a critical component. Experimental results indicate that aquatic plants can recover from fluridone treatment (Van and Steward 1986). Recently, we have demonstrated that the extent of recovery, for hydrilla, is directly related to the tissue level of "available" or "active" iron (Spencer and

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Ksander 1989). Based on research with terrestrial plants, it appears that active iron is Fe²⁺. By definition active iron is that portion of total iron (in the tissue) which is available for, or participating in, metabolic reactions. The remainder of the total iron is probably precipitated and unavailable for plant use (Pierson and Clark 1984). Active iron has been shown to be more closely correlated with leaf chlorophyll content than total iron for some species of terrestrial plants (Katyal and Sharma 1980). To our knowledge, active iron levels have only been reported for one species of aquatic macrophyte (Spencer and Ksander 1989). Considering the influence that active iron has on hydrilla's ability to recover from fluridone treatment, understanding the relationship between active iron and the external iron supply is important. Understanding this relationship may enhance the ability to predict the response of natural populations to fluridone treatment. This paper reports the results of an experiment in which four species of aquatic macrophytes were grown at a range of iron concentrations and the level of total and active iron measured.

MATERIALS AND METHODS

Monoecious hydrilla (*Hydrilla verticillata* (L.f.) Royle) tubers (150-200 mg), dioecious hydrilla tubers (300-350 mg), sago pondweed (*Potamogeton pectinatus* L.) tubers (150-200 mg), variable pondweed (*P. gramineus* L.) winter buds (150-200 mg), and American pondweed (*P. nodosus* Poir.) winter buds (300-350 mg) were allowed to germinate for 10 days in a growth chamber at 24 C under a 14:10 h L:D cycle. All tubers were from stock cultures maintained at the USDA Aquatic Weed Facility (Davis, CA). Propagules within selected size ranges were used to minimize the effects of differences in starting propagules size on plant growth (Spencer 1986, 1987; Spencer *et al.* 1989) Variable pondweed winter buds were collected from the Weyend Canal (Solano Irrigation District, CA) while American pondweed winter buds were from the Richvale Canal (Richvale Irrigation District, CA). Taxonomic placement of the *Potamogeton* spp. follows Fassett (1957). Sprouted propagules were planted in individual plastic pots (125 ml) filled with 10% UC mix (Spencer and Anderson 1986), and placed in 3.78-l glass jars containing well water. The plants grew for 1 more week before they were treated with iron (as Fe-EDTA) for 3 weeks. We used the following iron concentrations: 0, 0.1, 0.5, 1.0, 2.5, 5.0, and 7.5 mg/l Fe. The background levels of iron in the well water were low (0.046 ± 0.027 mg/l; mean ± standard deviation; N = 5). (The mean background level was added to the treatment level and this value used in Figure 1 and the calculations.) At each iron level there were 3 replications per species. At harvest, we measured the dry weights (80 C for 48 h) of leaves plus stems, and roots plus rhizomes. We also measured chlorophyll a, b, and carotenoids in fresh leaves for individual plants. Pigments were extracted with DMSO (Hiscox and Israelstam 1979; Filbin and Hough 1984; Spencer and Ksander 1987). Dried leaf and stem material from each plant was ground to a fine powder by hand using a mortar and pestle in preparation for iron analysis. Total iron was measured on 50-100 mg of oven-dried plant tissue, digested in 5 ml of concentrated sulfuric acid and

30% hydrogen peroxide at 380 C on a heating block. Total iron was determined by atomic absorption. Active iron was determined by the method of Pierson and Clark (1984). In this procedure 100 mg of finely ground dried tissue is extracted with 2.0 ml of 1,10-o-phenanthroline, three times. We measured the absorbance at 510 nm for each extract. Active iron was calculated as the sum of the three extractions (Pierson and Clark 1984). All statistical tests were performed using SAS (SAS Institute Inc. 1987). The effects of iron on growth responses were evaluated by analysis of variance (using PROC GLM). Treatments were judged to be significant if the F-statistic had a P < 0.05. Active iron was related to the iron concentration in the medium (adjusted to include the background iron in the well water) using PROC NLIN to fit the following equation which describes a rectangular hyperbola:

$$ACFE = (ACFE_{max} \times FE) / (K_s + FE) \quad (1)$$

TABLE 1. LEVELS OF TOTAL IRON AND ACTIVE IRON (µg/g) FOR AQUATIC MACROPHYTES GROWN AT VARIOUS IRON CONCENTRATIONS. VALUES ARE THE MEAN ± THE STANDARD ERROR ROUNDED TO THE NEAREST WHOLE NUMBER (N = 3). SOMETIMES INSUFFICIENT PLANT MATERIAL WAS AVAILABLE AND NO DETERMINATION WAS POSSIBLE (ND). THE PROPORTION OF TOTAL IRON THAT IS ACTIVE IRON IS GIVEN IN THE COLUMN LABELED '%'.

SPECIES	IRON (MG/L)	TOTAL IRON	ACTIVE IRON	%
American pondweed	0.05	68 ± 24	5 ± 0	8
	0.15	52 ± 11	8 ± 1	15
	0.55	89 ± 2	12 ± 1	14
	1.05	64 ± 18	12 ± 1	18
	2.55	57 ± 11	15 ± 1	25
	5.05	81 ± 27	23 ± 0	29
	7.55	87 ± 10	35 ± 5	40
Variable pondweed	0.05	61 ± 25	7 ± 0	11
	0.15	37 ± 0	7 ± 0	18
	0.55	ND	9 ± 1	ND
	1.05	82 ± 12	10 ± 2	13
	2.55	104 ± 27	9 ± 1	9
	5.05	98 ± 37	16 ± 4	16
	7.55	107 ± 16	21 ± 1	20
Hydrilla (dioecious)	0.05	28 ± 8	3 ± 0	10
	0.15	44 ± 22	4 ± 1	10
	0.55	35 ± 11	8 ± 1	22
	1.05	24 ± 15	11 ± 2	46
	2.55	22 ± 4	10 ± 2	44
	5.05	81 ± ND	14 ± 1	18
	7.55	26 ± 4	21 ± 6	82
Hydrilla (monoecious)	0.05	32 ± 10	5 ± 1	14
	0.15	52 ± 23	6 ± 1	11
	0.55	58 ± 19	13 ± 2	22
	1.05	45 ± 24	12 ± 2	26
	2.55	40 ± 8	17 ± 3	42
	5.05	103 ± 31	23 ± 2	23
	7.55	89 ± 11	44 ± 14	49
Sago pondweed	0.05	28 ± 7	4 ± 1	14
	0.15	ND	7 ± 1	ND
	0.55	21 ± ND	10 ± 1	47
	1.05	17 ± 1	9 ± 1	54
	2.55	ND	18 ± 3	ND
	5.05	41 ± 10	25 ± 3	59
	7.55	34 ± 8	35 ± 5	105

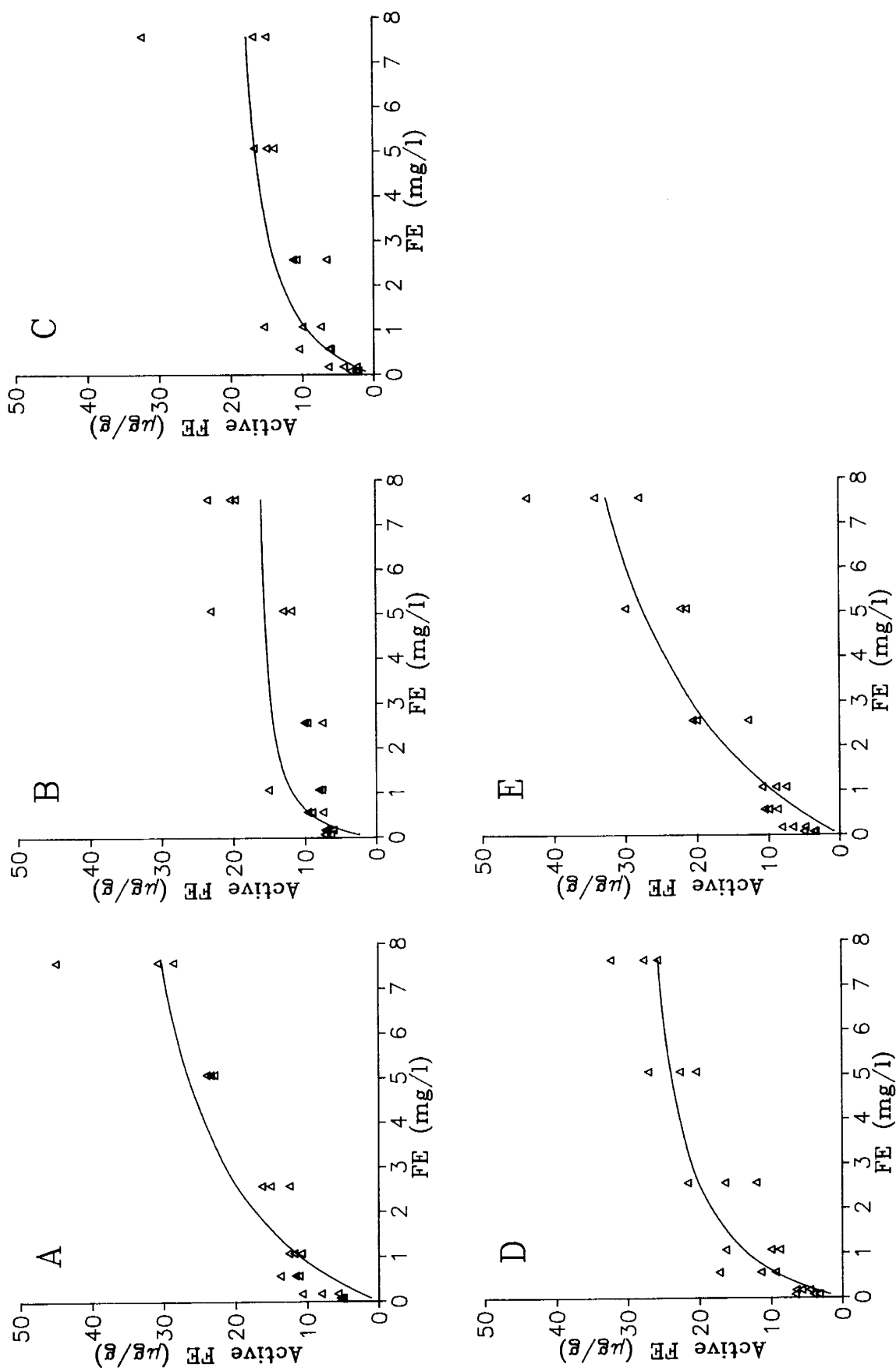


Figure 1. Relationship between active iron and the concentration of iron in the growth medium for four species of aquatic plants. The line was fit by nonlinear regression techniques. The parameters for the equation describing each line are given in Table 2. A is American pondweed; B is variable pondweed; C is hydrilla (dioecious); D is hydrilla (monoecious); and E is sago pondweed.

Where, $ACFE$ = the level of active iron in $\mu\text{g/g}$
 FE = the initial iron concentration in the medium
in mg/l
 K_s = the calculated value at which $ACFE$ is one
half of the maximum $ACFE$
 $ACFE_{\text{max}}$ = the calculated maximum tissue level
of active iron.

RESULTS AND DISCUSSION

Plant dry weight was not significantly affected by the level of iron in the external medium. Pigment concentrations were also not affected, except that carotenoid levels in variable pondweed increased with increasing external iron. Basiouny *et al.* (1977a) reported that the dry weight of hydrilla increased significantly when the iron level in the external medium was 6 mg/l . Basiouny *et al.* (1977a) also reported that the chlorophyll content of the leaves "corresponded to some extent to the variation of Fe levels in the nutrient solution." The highest external iron level used in the present experiments was 7.5 mg/l , so we might have expected to see similar results at least for hydrilla. However, there were at least two important differences in the procedures used by Basiouny *et al.* (1977a) and those employed in the present study. The results reported by Basiouny *et al.* (1977a) were for plants grown for 7 weeks under the iron treatments while those reported here are for plants that received the iron treatments for only 3 weeks. Another important difference is that we used hydrilla grown from tubers, while Basiouny *et al.* (1977a) studied hydrilla grown from 10 cm apical sections of shoots. Hydrilla grown from tubers may have more stored nutrient reserves to draw from than those grown from shoot sections. Total iron levels for the plants (Table 1) studied are comparable to values reported in the literature for plants collected from natural habitats (Hutchinson 1975; page 340). Iron is probably acquired by aquatic macrophytes directly from the sediment (Barko and Smart 1986; Smart and Barko 1985), however there is also evidence for iron absorption by the shoot system (Basiouny *et al.* 1977b; Gentner 1977; DeMarte and Hartman 1974). The similarity between the shoot iron levels reported here and those reported from field-collected plants suggest that the results of this green house study (using plants allowed to take up iron via the shoot system) may be applicable to plants from natural habitats as well (that probably received their iron via the roots). The proportion of tissue active iron compared to tissue total iron ranged from 7 to 105%. The general mean was 29% for all species at all iron concentrations. These results suggest that iron was not limiting for growth under the conditions examined, and support the hypothesis that aquatic plants can accumulate more iron than is required for immediate growth (Basiouny *et al.* 1977a).

The level of active iron in the plants varied and was directly related to the external supply (Figure 1). The parameters for the equations describing the relationship between external iron concentration and active iron for each species are listed in Table 2. Both types of hydrilla had similar values for K_s . K_s values for the three pondweed

TABLE 2. PARAMETERS FOR THE EQUATION, $ACFE = ACFE_{\text{max}} \times FE / (K_s + FE)$, RELATING ACTIVE IRON TO THE EXTERNAL IRON CONCENTRATION IN THE MEDIUM. VALUES WERE CALCULATED BY NONLINEAR REGRESSION. THE ASYMPTOTIC 95% CONFIDENCE INTERVALS ARE IN PARENTHESIS.

SPECIES	$ACFE_{\text{max}}$ ($\mu\text{g/g}$)	K_s (mg/l)
Hydrilla (dioecious)	20.5 (13.1-28.0)	1.19 (-0.17-2.55)
Hydrilla (monoecious)	30.0 (22.7-37.2)	1.25 (0.33-2.18)
Variable pondweed	17.0 (12.6-21.4)	0.45 (-0.05-0.95)
American pondweed	41.5 (24.8-58.1)	2.80 (0.06-5.54)
Sago pondweed	52.1 (29.0-75.1)	4.47 (0.42-8.52)

species covered a wider range. Variable pondweed had the lowest value, with American pondweed intermediate, and sago pondweed the greatest. There was considerable overlap among the asymptotic 95% confidence intervals for K_s . The calculated maximum tissue levels of active iron ($ACFE_{\text{max}}$) displayed a similar pattern. The 95% confidence limits for $ACFE_{\text{max}}$ for both strains of hydrilla and variable pondweed overlapped. The confidence limits for American pondweed and sago pondweed overlapped with each other but not with hydrilla and variable pondweed. American pondweed and sago pondweed have higher saturation levels of active iron than hydrilla or variable pondweed.

Since K_s is the external iron level at which active iron is one half of the maximum or saturation value, twice K_s gives an estimate of the external iron level at which the plants should have maximum levels of active iron. Plants growing in aquatic systems with iron concentrations near this value should be able to recover from fluridone treatments. For hydrilla this value is roughly 2.2 to 2.5 mg/l iron and for the pondweed species from 0.9 to 8.9 mg/l . Soluble iron concentrations range from 0.1 to 3 mg/l in most waters (Goldman and Horne 1983; page 153) and vary seasonally with higher values present in the spring. Extensive information on soluble iron levels in lakes in the United States is available in Overton *et al.* (1986) and Eilers *et al.* (1987). In temperate regions aquatic herbicides, such as fluridone, are applied in the spring or early summer. The plants are thought to be more susceptible at this stage and there is less biomass than later in the season (Ross and Lembi 1985; page 300). However, in lakes with high iron levels spring fluridone applications may coincide with the time that plants are most able to recover from the treatment. This would be especially true for herbicides with short water column half-lives such as fluridone under many circumstances (West *et al.* 1979 and 1983; Muir *et al.* 1980). Spencer and Ksander (1989) have proposed that measuring the active iron level in plants before fluridone application is one way to predict the extent of recovery following the treatment. The relationship between tissue active iron and external iron suggest that knowledge of the iron concentrations in the water column or sediments for

a few weeks before treatment may also be a useful predictor of the likelihood of recovery from fluridone treatment.

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Photolytic Degradation of Fluridone¹

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ABSTRACT

The wavelengths of light involved in the photodegradation of fluridone [1-methyl-3-phenyl-5-[3-(trifluoromethyl)-phenyl]-4-(1H)-pyridinone] were characterized by exposing the herbicide to different radiation sources and

filters (delineating separate parts of the light spectrum) to determine the wavelengths active in photolysis. The half-life of fluridone exposed to mercury radiation between 310-380 nm was 212 hr. Fluridone exposed to the full spectrum of natural sunlight (between 1.4-2.9 mW·cm⁻²) exhibited a half-life ranging from 15 to 36 hr, but radiation above 400 nm did not degrade fluridone. Fluridone exposed to radiation between 300-400 nm exhibited a half-life of 64 hr. Further studies using filters were performed to divide the range of sunlight between 297 nm and above

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