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Phytoene and Carotene Response of Aquatic Plants to Fluridone Under Laboratory Conditions

SUSAN. L. SPRECHER[1], M. D. NETHERLAND[1] AND A. B. STEWART[2]

ABSTRACT

The herbicide fluridone (1-methyl-3-phenyl-5-[3-(trifluoromethyl)phenyl]-4(1*H*)-pyridinone) disrupts the carotenoid biosynthetic pathway in plants by inhibiting phytoene desaturase. The subsequent rapid accumulation of the carotene precursor pigment phytoene above negligible pretreatment levels is diagnostic for fluridone exposure in terrestrial plants. To determine whether this symptom characterized fluridone exposure in target and nontarget aquatic plants, beta-carotene and phytoene were monitored under a range of concentrations and exposure times in nine species: American pondweed (*Potamogeton nodosus* Poir.), coontail (*Ceratophyllum demersum* L.), egeria (*Egeria densa* Planch.), Eurasian watermilfoil (*Myriophyllum spicatum* L.), hydrilla (*Hydrilla verticillata* (L.f.) Royle), sago pondweed (*P. pectinatus* L.), vallisneria (*Vallisneria americana* Michx.), water marigold (*Bidens beckii* L.), and water stargrass (*Heteranthera dubia* (Jacq.) MacMill.). Significant increase in phytoene

[1]US Army Engineer Waterways Experiment Station, 3909 Halls Ferry Rd., Vicksburg, MS 39180.

[2]AScI Corporation, 1365 Beverly Road, McLean, VA 22101. Received for publication November 18, 1997 and in revised form July 7, 1998.

above pretreatment levels and reversal of the normal carotene:phytoene ratio were seen with fluridone exposure in all species, whether susceptible, intermediately susceptible, or tolerant to this herbicide. Effects occurred as early as 2 d with exposure to 2 µg fluridone L^{-1}. With removal of fluridone following 10 d exposures to ≤25 µg L^{-1}, pigments returned to pretreatment levels in 10 to 30 d, with duration and magnitude of phytoene elevation being related to treatment rate and exposure time. The specificity of this response to fluridone was supported by 1) lack of phytoene increase following treatment with other aquatic herbicides, and 2) characteristic phytoene increase when fluridone was co-applied with a fungal pathogen. The diagnostic response of phytoene to fluridone exposure in all aquatic species examined indicates that the selective activity of this herbicide is primarily rate dependent. While these pigment responses have potential for monitoring and characterizing fluridone effects in target and nontarget aquatic plants in laboratory evaluations and operational treatments, further evaluations are needed to indicate whether they can be useful for indicating herbicide levels during treatment or predicting efficacy and weed control.

Key words: aquatic herbicide, *Bidens beckii*, *Ceratophyllum demersum*, *Egeria densa*, *Heteranthera dubia, Hydrilla verticillata, Myriophyllum spicatum*, physiological monitoring, plant stress, *Potamogeton nodosus*, *P. pectinatus*, *Vallisneria americana*.

INTRODUCTION

Fluridone, the active ingredient (ai) of the aquatic herbicide Sonar®, controls nuisance populations of a number of submersed plant species with application rates ranging to the USEPA registered maximum labeled rate of 150 µg fluridone L^{-1} in aquatic systems[3]. This bleaching herbicide functions by inhibiting phytoene desaturase, an enzyme that transforms the colorless pigment phytoene into carotene, via intermediaries that include phytofluene and lycopene, in the carotenoid biosynthetic pathway (Bartels and Watson 1978, Sandmann and Fraser 1993, Sandmann 1994). The subsequent decrease in carotenoids, which normally protect chlorophyll from photooxidation under high light/low photosynthesis conditions, produces chlorosis in new growth and results in the eventual death of the plant. Since phytoene desaturation is a rate-limiting step in carotenoid biosynthesis, only trace amounts of phytoene are found in plants when this pathway is functioning normally (Chamovitz et al. 1993, Sandmann 1994). The rapid accumulation of significant levels of phytoene, determined by ultra-violet (UV) spectrophotometric measurements of organic solvent extracts of leaf tissue, is well-described as an easily-monitored diagnostic symptom in terrestrial plants for exposure to fluridone and certain other bleaching herbicidal compounds with the same mode of action (Bartels and Watson 1978, Sandmann and Böger 1983, Duke et al. 1985, Duke and Kenyon 1986, Sandmann and Böger 1989, Sandmann and Albrecht 1990, Sandmann 1993, Misawa et al. 1994).

Fluridone is widely used to treat aquatic weeds in large areas of lakes and reservoirs. Concentrations of 5 to 40 µg ai L^{-1} (significantly lower than the maximum rate) are able to control the exotic aquatic weeds Eurasian watermilfoil ("milfoil") and hydrilla when exposure is maintained for 60 to 90 or more days (Hall et al. 1984, Netherland 1992, Haller et al. 1990, Netherland et al. 1993, Netherland and Getsinger 1995). The selective ability of fluridone at 5 to 10 µg L^{-1} to maintain desirable nontarget and native species while controlling milfoil has been demonstrated in mesocosm studies and the field (Netherland et al. 1997, Smith and Pullman 1997, Nelson et al. 1998). Maintenance of these minimal aqueous herbicide concentrations during extended periods can be accomplished by repeat or sequential block applications (Getsinger 1993, Getsinger et al. 1996), or use of the slow release fluridone pellet formulation. However, decision-making to guide these treatment regimes during the one to three months when plants may maintain appreciable biomass[3] would be aided by some physiological indication that efficacy or a lethal dose had been achieved in the target species.

Fluridone concentration in water can be quantified from water residue analyses via high performance liquid chromatography (West and Day 1981) or immunoassay[4], but these methods do not directly assess the plant's physiological status resulting from herbicide uptake. Herbicide concentration in plant tissue (tissue burden) measurements are possible (Marquis et al. 1981), but the levels of fluridone that must be attained or maintained within aquatic plants to produce control have not been determined (Netherland et al. 1993). In treated hydrilla or milfoil, chlorophyll loss is proportional to concentration and exposure time, and negative net photosynthesis in shoot apices is associated with biomass decrease below pretreatment levels, but these parameters have not been directly correlated to effective herbicide control and plant death (Westerdahl and Hall 1987, Netherland et al. 1993, Netherland and Getsinger 1995). Using fluridone's disruption of the carotenoid biosynthetic pathway to evaluate physiological status of treated plants, Doong et al. (1993) showed that a 2-wk exposure to as little as 5 µg fluridone L^{-1} significantly decreased carotene production in mature hydrilla; however, carotene response was not predictive of reduction in biomass.

The response of phytoene in terrestrial plants suggests that this carotene-precursor has potential to provide an indicator of fluridone effect in aquatic plants. As fluridone is the only bleaching herbicide labeled for aquatic use, the effect would also be expected to be compound-specific. We therefore investigated pigment response to fluridone in aquatic plants during and after exposure to determine the relevance of phytoene as an indicator of physiological response of target and nontarget plants to this herbicide.

MATERIALS AND METHODS

Analytical Methods. Pigments of the carotenoid pathway (phytoene, phytofluene, and carotene) were extracted from aquatic plants and quantified according to a protocol based

[3]SePRO Corp. 1994. Sonar A.S. and Sonar SRP: Specimen Label. SePRO, Carmel, IN.

[4]SePRO Corp. 1996. *Fas*TEST Brochure. SePRO, Carmel, IN.

on techniques of Sandmann and Böger (1983) and Duke et al. (1985). Approximately 0.25 to 0.5 g fresh apical shoot tissue was weighed and mechanically homogenized (generator and 7 × 150 mm blade, PRO Scientific, Monroe, CT) in 5 ml of a freshly-made solution of 6% (w/v) KOH in MeOH in a 15 ml disposable glass test tube. Tubes were stoppered, and the homogenate was centrifuged 5 min at 2000 g. The supernatant was decanted into a fresh tube, mixed with 2 ml of petroleum distillate ('light petrol' or 'petroleum benzin': Fluka, Ronkonkoma, NY, #85100, b.p. 80-110 C), and the pellet was discarded. The tube was then capped and shaken vigorously until thoroughly mixed. The organic solvent epiphase was allowed to separate for approximately 5 min, and an aliquot was transferred to a disposable 1.5 ml semimicro UV cuvet (methacrylate: Dynalon, Rochester, NY; optical transmission approx. 65% at 287 nm, approx. 95% at 445 nm) using a glass transfer pipet. Samples were covered to avoid light and were allowed to stand up to 30 min to yield a completely clear solution.

Sample absorbance (A) was measured spectrophotometrically (Beckman DU-640) at characteristic wavelengths of 287 nm (*cis*-phytoene), 347 nm (phytofluene), and 445 nm (β-carotene) (Sandmann and Böger 1983). Spectral analyses of extracts were measured at 1 nm intervals from 250 to 475 nm. Fresh weight (FW) concentrations of pigments were calculated based on the weight of the tissue sample and a 2-ml epiphase, using extinction coefficients (E) of 1108 for *cis*-phytoene or 2500 for β-carotene (Sandmann and Böger 1983):

$$\mu g\ g^{-1}\ FW = ((A/(1\ cm \times E) \times 2\ ml/100\ ml)/g\ FW) \times 10^6$$

Chlorophyll content was analyzed in 1 g FW of apical shoot per replicate subsample extracted in 10 ml dimethylsulfoxide, using the method of Hiscox and Israelstam (1979).

Statistical methods were carried out on pigment concentration and other data using analysis of variance to test for treatment effects, and linear regression to test for linear response, with $p \leq 0.05$. Comparison of effects among treatments and untreated references were carried out by pairwise multiple comparisons using the Student-Nueman-Kuels method; comparisons of individual treatments with associated references were made using Dunnett's method for least significant difference.

Laboratory Evaluations. In laboratory assessments, pigment changes following 2 hr to 50 d of exposure to between 0.5 and 25 µg fluridone L^{-1} were evaluated in the monocot species egeria, hydrilla, water stargrass, American pondweed, sago pondweed, and vallisneria, and in the dicot species coontail, milfoil, and water marigold. Plants were originally acquired as tubers, turions or winter buds (American pondweed, sago pondweed, vallisneria) or unrooted apical shoots (egeria, hydrilla, water stargrass, coontail, milfoil, watermarigold) from a commercial nursery (Wildlife Nurseries, Inc., Oshkosh, WI) or outdoor ponds (Lewisville, TX). Growth conditions in controlled environment chambers were as for herbicide efficacy studies (Netherland et al. 1993, Netherland and Getsinger 1995), and comprised 14L:10D daylength at a photosynthetic photon flux density of 437 ± 11.3 (standard error: s.e.) µE m^{-2} sec^{-1} provided by 400-W high pressure sodium and GE multi-vapor lamps, and a water temperature of 24 ± 2 C. Previously untreated plant tissue was exposed to herbicide as either unrooted 15-cm apical shoots incubated hydroponically in 5% Hoaglands solution (Hoagland and Arnon 1938) in 1-L glass jars, or as rooted plants cultured in 47-L volumes of a low-nitrogen, high alkalinity aquatic plant culture solution (Smart and Barko 1984) in aquaria for at least four weeks prior to treatment. Fluridone treatments were applied as liquid Sonar A.S., with concentrations based on the 41.7% ai formulation, to three replicate experimental units except where noted. Treatments of coontail and egeria with other aquatic herbicides were to three replicates via liquid formulations with concentrations as noted. Herbicide removal from treated jars or aquaria following exposure periods was accomplished by draining and refilling whole-container volumes three times in succession; untreated experimental units underwent the same process. Plant material was monitored visually for herbicide-induced chlorosis.

RESULTS AND DISCUSSION

Pigment Changes in Aquatic Plants. Absorption spectra comparing extracts from untreated American pondweed with similar tissue exposed to 5 µg fluridone L^{-1} for 20 days of exposure (DOE) (Figure 1) illustrate the relative changes in carotenoid pathway pigments that were seen in all aquatic species evaluated. Phytoene increased significantly from low levels with treatment, while carotene decreased from initial concentrations. Phytofluene, an intermediate metabolite in the carotenoid biosynthetic pathway, was initially similar in concentration to phytoene, but little change was seen with

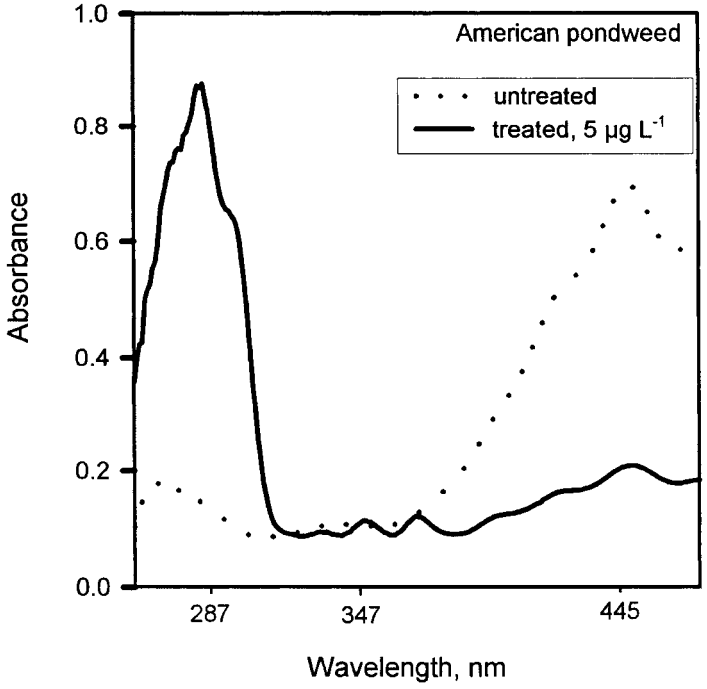

Figure 1. Spectral analyses, 250 to 475 nm, of organic extracts of American pondweed leaf tissue from plants exposed to 5 µg fluridone L^{-1} for 20 d and from untreated references. Characteristic wavelengths given by Sandmann and Böger (1983) for *cis*-phytoene (287 nm), phytofluene (347 nm), and β-carotene (445 nm) are indicated.

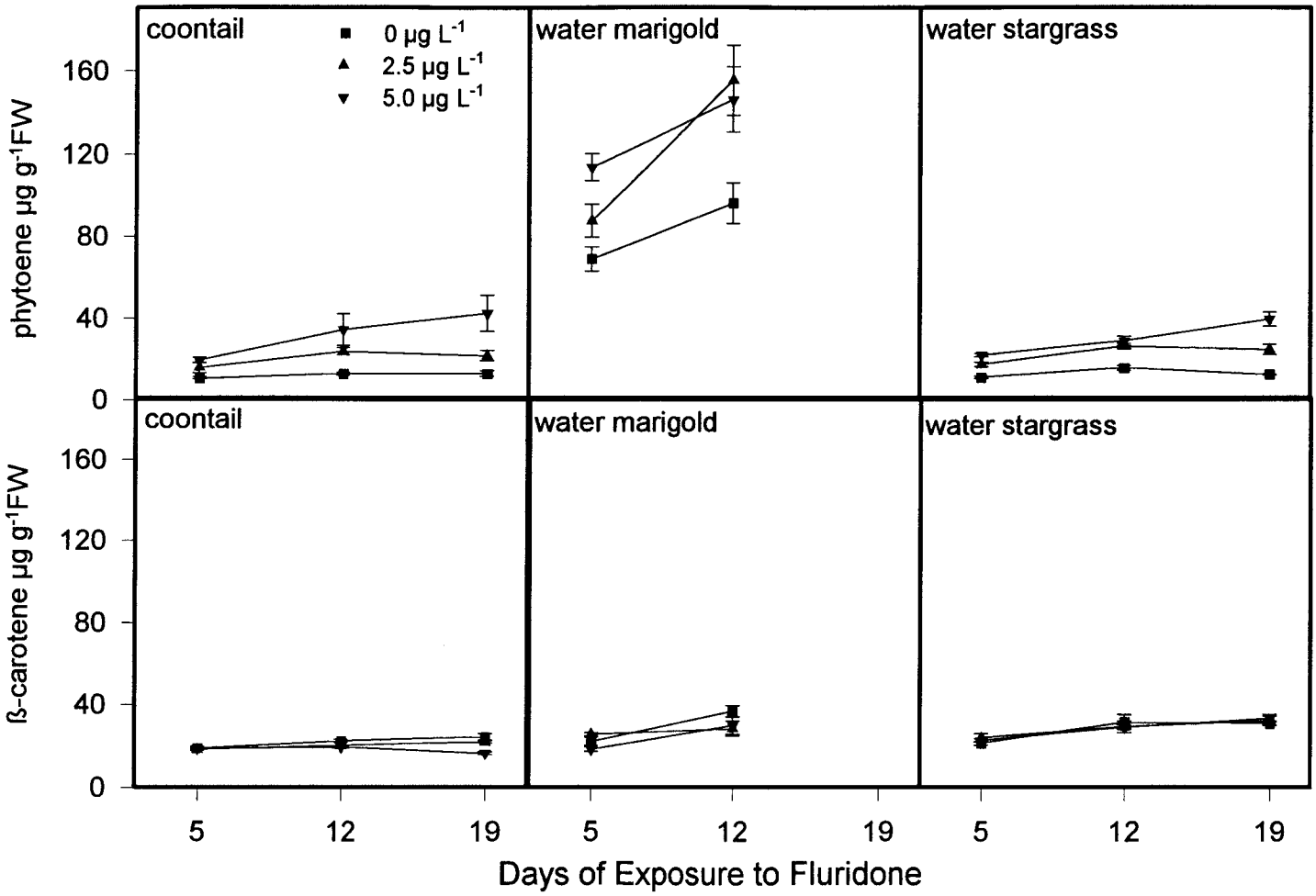

Figure 2. Phytoene and β-carotene concentrations, µg g^{-1} fresh weight (FW), in apices of unrooted shoots of coontail, watermarigold, water stargrass during 19 d of exposure to 0, 2.5 or 5.0 µg fluridone L^{-1}. Means and standard errors (bars) of three replicates, each sub-sampled twice. Insufficient material for water marigold sampling at 19 d.

fluridone exposure. Although phytofluene data were collected in all analyses, they were not reported here.

This pattern of pigment response was seen in apical shoot segments of coontail, water marigold, water stargrass, hydrilla, milfoil, and egeria, where exposure to 2.5 and 5 µg fluridone L^{-1} in hydroponic culture for 19 d increased phytoene from three- to ten-fold compared to untreated reference material (Figures 2 and 3). Background pigment levels in most of these species changed little through this period, ranging from approximately 10 to 20 µg phytoene and from 20 to 45 µg carotene g^{-1} FW. In water marigold, however, phytoene concentrations ranged between 68.8 ± 5.9 (s.e.) and 96.0 ± 9.9 µg g^{-1}, the highest we encountered in untreated tissue. By 5 DOE to 5 µg fluridone L^{-1}, phytoene had increased significantly above untreated references in all species, and in all except coontail and water marigold with exposure to 2.5 µg L^{-1} ($p \leq 0.032$). Phytoene concentrations remained elevated through 19 DOE in all cases ($p \leq 0.019$: no data available for water marigold). No significant changes in carotene were seen in water marigold or water stargrass with treatment, but by 19 DOE to 5 µg L^{-1} carotene was significantly below untreated references in coontail, hydrilla, milfoil, and egeria ($p \leq 0.013$). The decreases in carotene, up to 57% in hydrilla, were relatively minor in comparison to phytoene accumulation. Response of both pigments was similar in monocot and dicot species.

These alterations and pigment levels are comparable to those seen in other terrestrial and aquatic species. In mature hydrilla, carotene concentrations of 207 µg g^{-1} FW were reduced 78% with 2-week exposure to 5 µg fluridone L^{-1}, while in young hydrilla, untreated material contained 84 µg carotene g^{-1} FW and the same treatment did not affect this pigment (Doong et al. 1993). Agronomic treatment rates of fluridone in wheat (*Triticum aestivum* L.) and morningglory (*Ipomoea lacunosa* L.) increased phytoene to ≥72 µg g^{-1} FW and phytofluene to ≥5 µg g^{-1} FW, from undetectable levels in untreated references (Bartels and Watson 1978, Duke et al. 1985).

Response Thresholds. The minimum herbicide dose rate required to produce an elevation in phytoene was examined in two studies. Egeria apices were monitored during 72 hr exposure of unrooted shoots to 0, 0.5, 1, 2, 5, and 10 µg fluridone L^{-1} in hydroponic culture (Figure 4). Examination of means and standard errors showed that exposure to 0.5 and 1 µg L^{-1} did not alter phytoene from pretreatment levels over

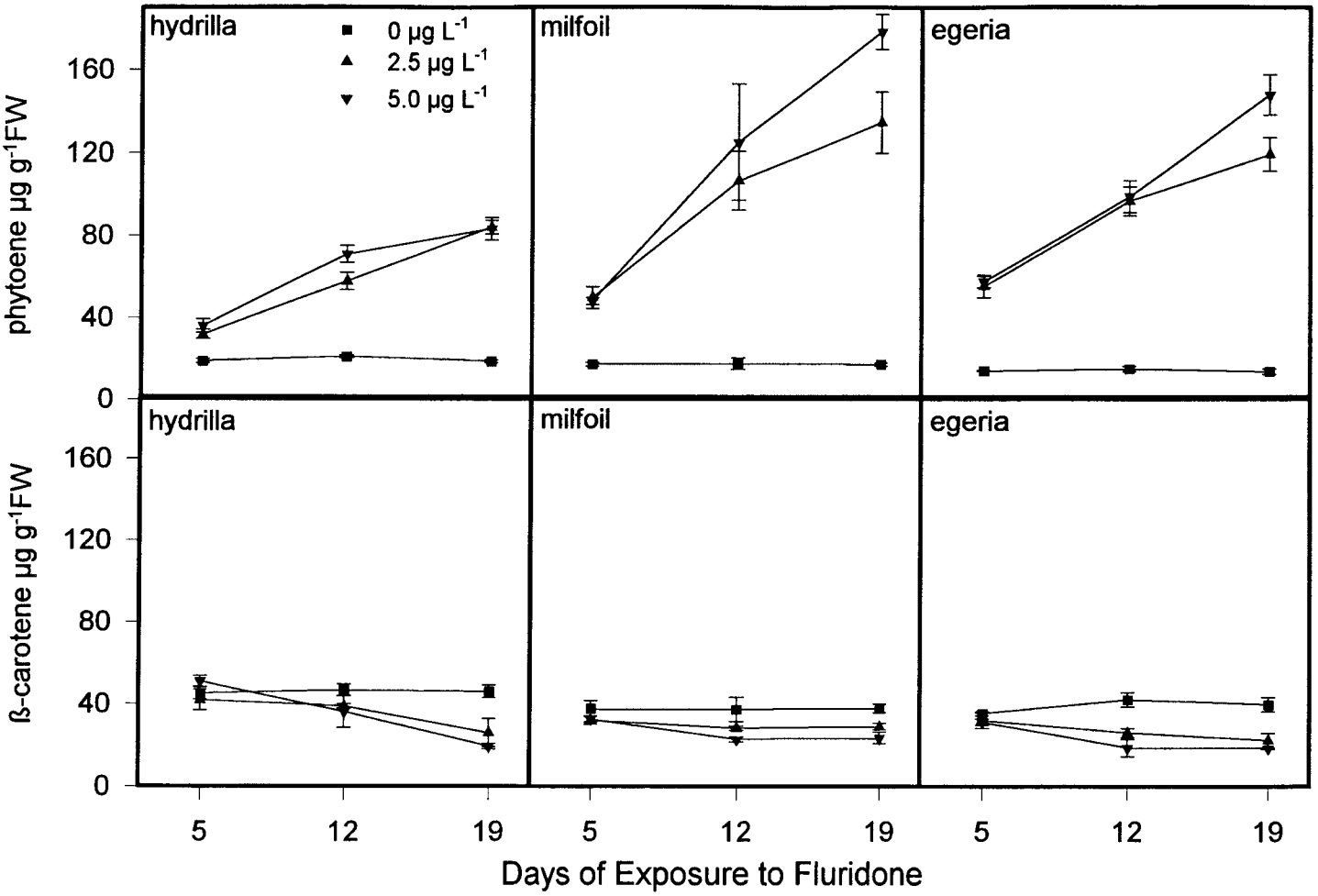

Figure 3. Phytoene and β-carotene concentrations, µg g^{-1} fresh weight (FW), in apices of unrooted shoots of egeria, hydrilla, and Eurasian watermilfoil during 19 d of exposure to 0, 2.5 or 5.0 µg fluridone L^{-1}. Means and standard errors (bars) of three replicates, each sub-sampled twice.

this time, but that a significant increase occurred with 48 hr exposure to 2 µg L^{-1}; the effect was not consistent at 5 and 10 µg L^{-1} due to high variability in the response. The trend of decreasing carotene and increasing phytoene with fluridone treatment of ≥2 µg L^{-1} indicated that a reversal of the normal ratio of carotene > phytoene occurred with 72 or more hr of exposure to these concentrations.

The inflection point at which phytoene increased above decreasing levels of carotene in treated tissue provided an easily-visualized indication of significant effect during the exposure period. The physiological response denoted by this reversal of pigment ratios was seen at a lower concentration in rooted milfoil and hydrilla plants with longer exposure time, and in certain cases before or without chlorosis (Figures 5 and 6). From inspection of means and standard errors of two samples per treatment of shoot tips from aquarium-grown plants, increase in phytoene above decreasing carotene occurred in milfoil and hydrilla with 9 DOE to 1 µg fluridone L^{-1}. There was no visible chlorosis in this plant material at 9 DOE, and a test for linear response showed significant reduction in chlorophyll content over 15 DOE only in milfoil at 1 and 2 µg fluridone L^{-1} (Table 1). Comparable time-dependent pigment response to low dose fluridone have been seen in hydrilla, where carotene decreased significantly with 8 wk exposure to 0.5 µg L^{-1} and with 2 wk exposure to 5 µg L^{-1} in mature plants (Doong et al. 1993).

In rooted plants of American and sago pondweeds and of vallisneria, long-term exposures to 2 µg fluridone L^{-1} produced characteristic pigment changes even when visible chlorosis, reduction in biomass, or deleterious effects on development did not occur (Table 2). By 8 to 9 DOE, applications of ≥2 µg fluridone L^{-1} had produced the characteristic increase in phytoene above that in untreated references in both pondweeds, although this effect occurred only with 10 µg L^{-1} in vallisneria. In American pondweed exposed to 2 µg L^{-1}, phytoene reached 431 ± 64.7 µg g^{-1} FW by 31 DOE, the highest concentration of this pigment we have encountered to date. In all three species, chlorophyll and carotene concentrations were affected by fluridone, but carotene remained higher through 5 wk of exposure to 2 µg L^{-1} than to the other treatment levels. All plants at 2 µg fluridone L^{-1} remained unbleached and underwent normal development through 90 DOE, flowering and setting seed, and a negative effect on dry weight biomass was seen only in sago pondweed (see also Sprecher 1995). In contrast, almost all biomass was eliminated in all species with 90 DOE to 10 and 25 µg fluri-

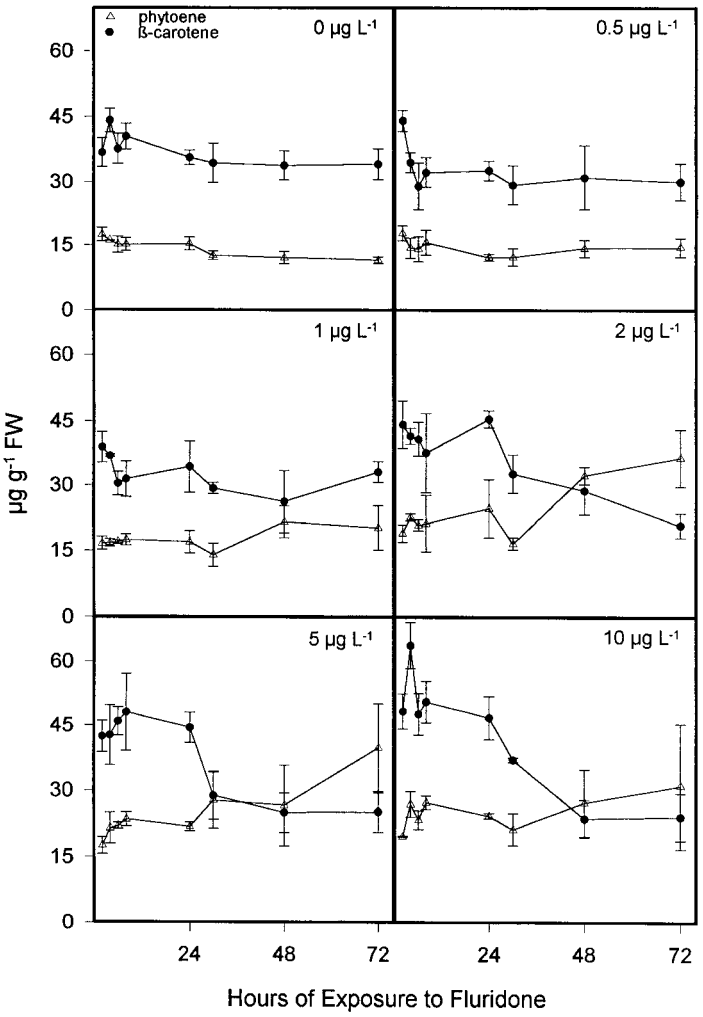

Figure 4. Phytoene and β-carotene concentrations, µg g^{-1} fresh weight (FW), in apices of unrooted egeria shoots during 72 hr exposure to 0, 0.5, 1, 2, 5, or 10 µg fluridone L^{-1}. Means and standard errors (bars) of three replicates.

done L^{-1}. Phytoene concentrations at 31 to 36 DOE did not correlate to treatment rate, perhaps because accumulation of this pigment decreased as plant health deteriorated. Status of carotene was more predictive here: while loss of ≤39% carotene by 31 to 36 DOE supported normal growth, reduction of this pigment by ≥62% at this sampling period was associated with loss of 85% or more biomass by 90 DOE.

These responses show that phytoene desaturase was inhibited by treatment in both fluridone-tolerant and susceptible species, and by fluridone concentrations lower than those recommended for weed control in the field. The manufacturers of Sonar describe sago pondweed as being susceptible, American pondweed as intermediately susceptible, and vallisneria as tolerant to fluridone[5], although constant exposure to static concentrations in the relatively controlled environments of the laboratory or mesocosm have been found to be

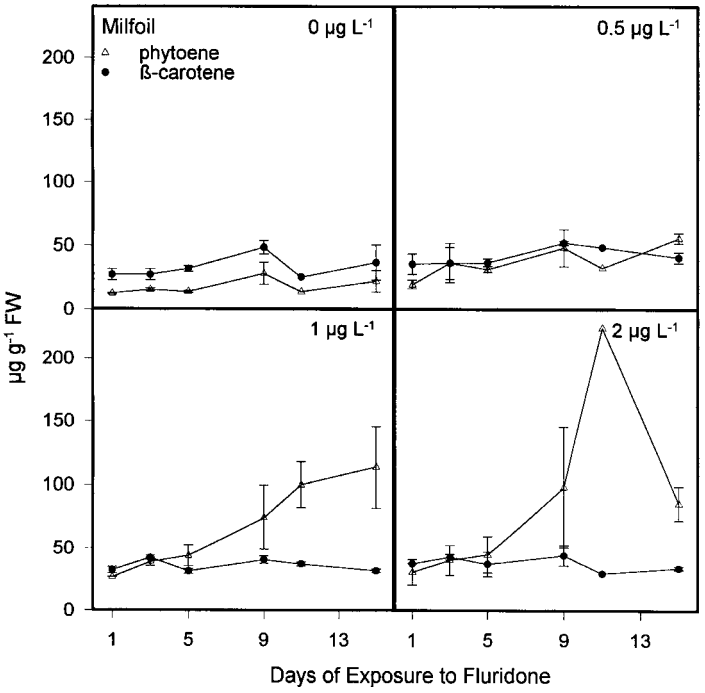

Figure 5. Phytoene and β-carotene concentrations, µg g^{-1} fresh weight (FW), in shoots apices from rooted plants of Eurasian watermilfoil during 15 d of exposure to 0, 0.5, 1, or 2 µg fluridone L^{-1}. Means and standard errors (bars) of two replicates; one replicate at 11 d.

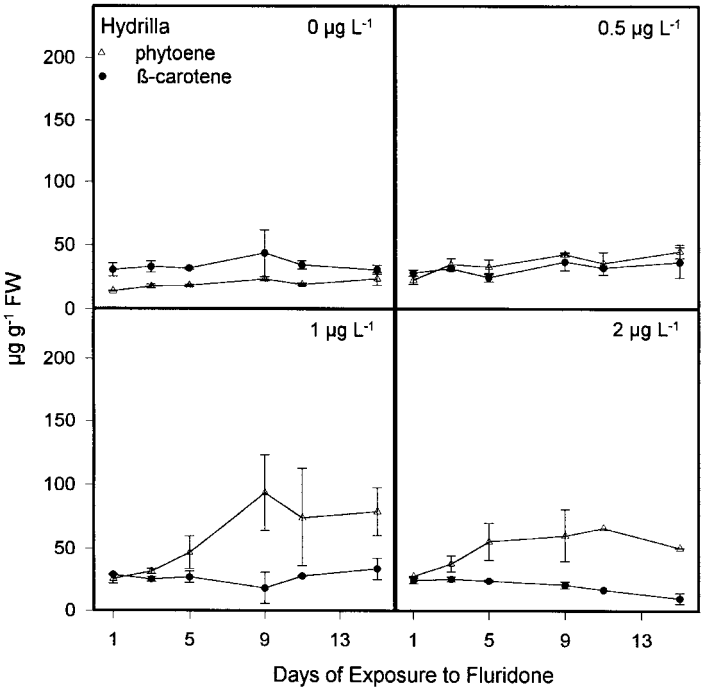

Figure 6. Phytoene and β-carotene concentrations, µg g^{-1} fresh weight (FW), in shoots apices from rooted plants of hydrilla during 15 d of exposure to 0, 0.5, 1, or 2 µg fluridone L^{-1}. Means and standard errors (bars) of two replicates; one replicate at 11 d.

[5]SePRO Corp. n.d. Sonar® Guide to Water Management. SePRO, Carmel, IN.

TABLE 1. CHLOROPHYLL CONCENTRATIONS IN EURASIAN WATERMILFOIL AND HYDRILLA APICAL SHOOTS SAMPLED BEFORE (PRE) AND DURING 15 DAYS OF EXPOSURE TO FLURIDONE. N = 2 (N = 1 AT 11 DAYS).

Species/ Fluridone (µg L^{-1})	Days of exposure to fluridone treatment							
	Chlorophyll content (mg g^{-1} fresh weight)							Linear response[1]
	Pre	1	3	5	9	11	15	
Hydrilla								
0	1.02	0.98	1.02	1.07	1.07	1.77	1.15	NS
0.5	1.08	0.95	1.04	0.91	1.15	1.27	1.01	NS
1.0	1.00	1.10	1.15	0.96	0.53	1.06	1.02	NS
2.0	0.98	1.11	0.85	1.23	0.71	1.29	1.06	NS
Milfoil								
0	1.55	1.55	1.62	1.55	1.43	1.65	1.43	NS
0.5	1.43	1.42	1.48	1.32	1.46	1.27	1.15	NS
1.0	1.86	1.67	1.48	1.19	1.50	1.23	1.30	0.04
2.0	2.41	1.73	1.83	1.73	1.53	1.34	1.29	0.03

[1]Test for linear response of chlorophyll content over sampling time within each treatment. NS = not significant, $p > 0.05$.

more detrimental to vallisneria than dissipating exposures under field conditions (Sprecher et al. 1995, Netherland et al. 1997). Thus, the pigment changes seen here indicate that the mechanism of fluridone tolerance and selectivity in aquatic plants is not due to an absence of inhibitory effect on phytoene desaturase, but is more probably related to rate and length of exposure, as indicated by Netherland et al. (1997). This pigment response to levels of fluridone too low to provide weed control is consistent with other studies. Levels of 0.05 µg fluridone L^{-1} significantly inhibited carotenoid production in mature hydrilla with 12 wk exposure (Doong et al. 1993), and 0.5 µg L^{-1} reduced chlorophyll content in hydrilla and milfoil by 30 DOE (Netherland and Getsinger 1995).

Response to Removal of Fluridone. Removal of fluridone following short-term exposures was associated with a decrease in elevated phytoene concentrations and a return to the original carotenoid: phytoene ratio (Figures 7 through 10). Following 10 DOE of rooted hydrilla, milfoil, egeria, and water stargrass plants to 2, 10, and 25 µg fluridone L^{-1}, herbicide was removed from aquaria and plants were maintained for an additional 40 d in untreated medium. Phytoene and carotene were monitored in shoot apices throughout the exposure and recovery periods. Although chlorosis was visible by 10 DOE on upper shoots in plants treated at the two higher herbicide rates, recovery from this non-efficacious exposure period was evident in all species. Healthy new growth with non-chlorotic tissue was produced in all aquaria, and sam-

TABLE 2. PIGMENT CONCENTRATIONS AT UP TO 50 DAYS OF EXPOSURE (DOE) AND BIOMASS AT 90 DOE IN THREE AQUATIC PLANT SPECIES TREATED WITH FLURIDONE AT 0, 2, 10 AND 25 µG L^{-1}. LETTERS INDICATE SIGNIFICANT DIFFERENCES AMONG TREATMENTS WITHIN INDIVIDUAL PARAMETER ON DATE OF ANALYSIS, $p \leq 0.05$, N = 3. NA = NO PLANT MATERIAL AVAILABLE TO SAMPLE.

	Chlorophyll mg g^{-1}		Carotene µg g^{-1}		Phytoene µg g^{-1}		Biomass g DW*
Plant/Fluridone	8-9 DOE	44-50 DOE	8-9 DOE	31-36 DOE	8-9 DOE	31-36 DOE	90 DOE
Sago pondweed							
Untreated	0.62 a	0.79 a	14.3 ab	23.1 a	14.5 a	20.0 a	9.33 a
2.0 µg L^{-1}	0.53 a	0.70 b	16.7 a	14.1 b	77.2 b	147 b	7.07 b
10 µg L^{-1}	0.45 b	0.23 c	10.4 bc	6.59 c	104 c	66.4 c	0.18 c
25 µg L^{-1}	0.31 c	NA	5.84 c	8.72 bc	111 c	78.8 c	0.03 c
American pondweed							
Untreated	2.44 a	1.58 a	12.9 a	44.6 a	7.23 a	48.7 a	3.50 b
2.0 µg L^{-1}	1.60 b	1.12 b	11.1 a	28.2 b	22.0 b	431 b	5.44 a
10 µg L^{-1}	1.44 b	1.16 b	13.2 a	10.7 c	23.1 b	368 b	0.06 c
25 µg L^{-1}	1.43 b	1.03 b	12.6 a	4.84 c	30.1 b	195 ab	0.20 c
Vallisneria							
Untreated	0.84 a	0.74 a	10.4 a	24.5 a	18.4 a	16.0 a	5.36 a
2.0 µg L^{-1}	0.63 b	0.66 ab	5.72 b	17.9 b	25.5 a	59.8 b	5.19 a
10 µg L^{-1}	0.56 bc	0.44 bc	1.77 c	8.26 c	51.4 b	87.3 c	0.78 b
25 µg L^{-1}	0.42 c	0.28 c	3.48 c	5.11 c	38.1 ab	91.3 c	0.57 b

*Total of three planted beakers per aquarium.

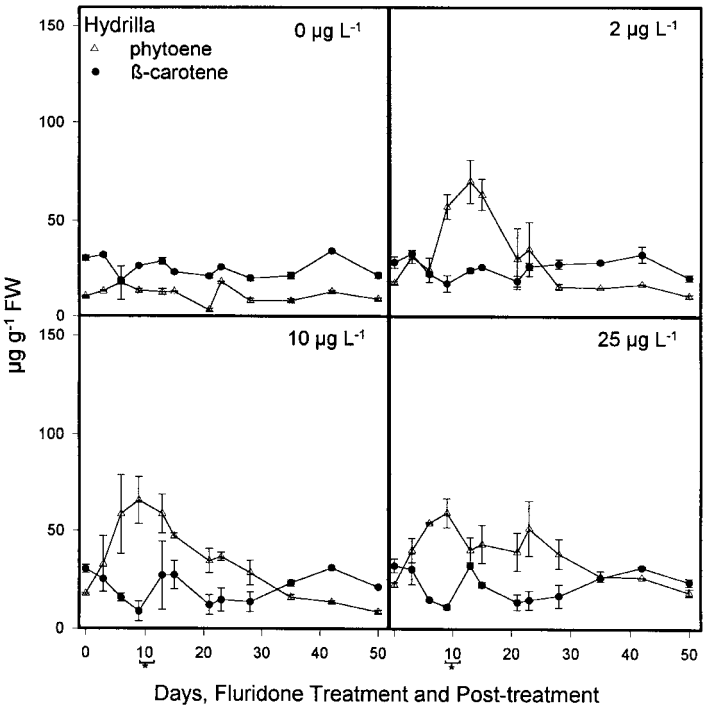

Figure 7. Phytoene and β-carotene concentrations, µg g^{-1} fresh weight (FW), in shoot apices from rooted plants of hydrilla during an initial 10 d exposure to 0, 2, 10 or 25 µg fluridone L^{-1}, and during 40 d following herbicide removal. Asterisk indicates removal of treatment.Means and standard errors (bars) of three replicates.

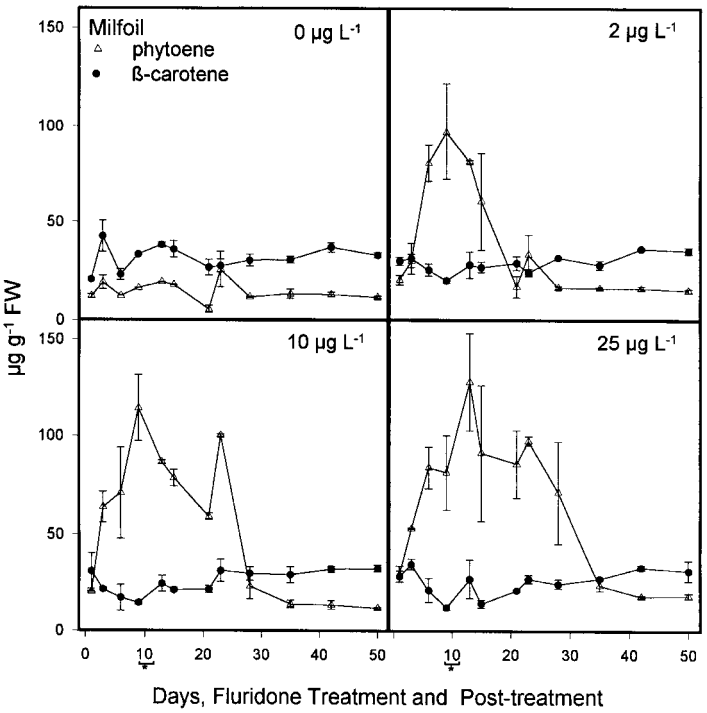

Figure 8. Phytoene and β-carotene concentrations, µg g^{-1} fresh weight (FW), in shoot apices from rooted plants of Eurasian watermilfoil during an initial 10 d exposure to 0, 2, 10 or 25 µg fluridone L^{-1}, and during 40 d following herbicide removal. Asterisk indicates removal of treatment. Means and standard errors (bars) of three replicates.

pling of shoot tips ensured that physiological status and response to current system conditions were being monitored.

From inspection of means and standard errors, it is seen that all four species exhibited similar trends in pigment response to herbicide treatment and removal (Figures 7 through 10). Phytoene reached levels significantly above carotene at each fluridone concentration, with imaxima at 6 to

TABLE 3. PHYTOENE AND CAROTENE CONCENTRATIONS (µG G^{-1} FRESH WEIGHT) IN APICAL SHOOT TISSUE OF COONTAIL AND EGERIA FOLLOWING VARIOUS HERBICIDE TREATMENTS. ASTERISK INDICATES SIGNIFICANT DIFFERENCE FROM THE UNTREATED REFERENCE WITHIN SPECIES AND PIGMENT (DUNNETT'S METHOD, $p \leq 0.05$).

Plant/Herbicide	Herbicide Conc.	Exposure Time	Phytoene µg g^{-1} FW	Carotene µg g^{-1} FW
coontail				
2,4-D	2 mg ae L^{-1}	24 hr	11.37	16.84*
diquat	0.5 mg ai L^{-1}	2 hr	10.6	25.23
fluridone	150 µg ai L^{-1}	7 d	40.09*	1.95*
triclopyr	2.5 mg ae L^{-1}	24 hr	12.09	21.98
reference	—	—	13.64	26.87
egeria				
copper	1 mg Cu L^{-1}	24 hr	3.74	6.09*
2,4-D	2 mg ae L^{-1}	24 hr	11.0	34.6
diquat	0.5 mg ai L^{-1}	2 hr	9.73	32.0
endothall, K_2	2 mg ae L^{-1}	24 hr	10.1	30.0
endothall, amine	2 mg ae L^{-1}	24 hr	9.17	26.7
fluridone	150 µg ai L^{-1}	7 d	63.6*	6.09*
triclopyr	2.5 mg ae L^{-1}	24 hr	8.56	27.8
reference	—	—	10.3	31.1

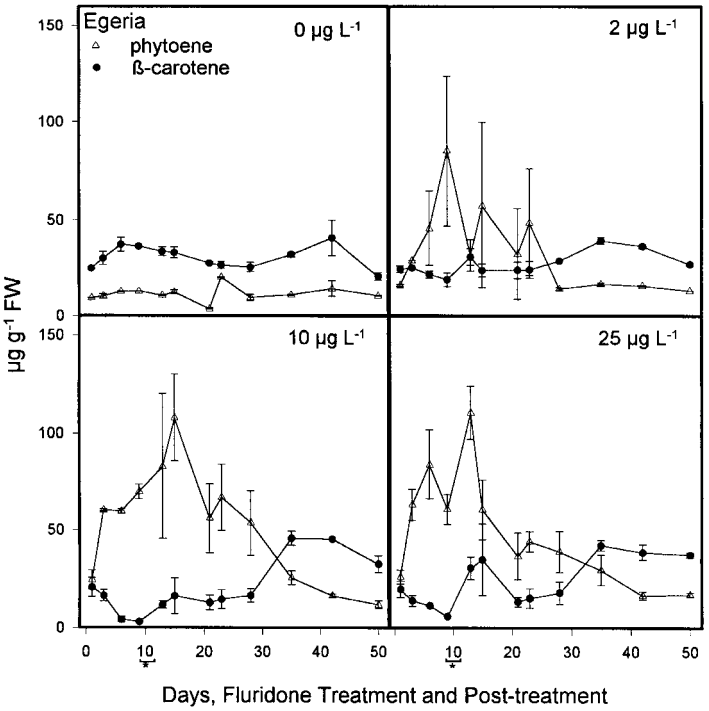

Figure 9. Phytoene and β-carotene concentrations, µg g^{-1} fresh weight (FW), in shoot apices from rooted plants of egeria during an initial 10 d exposure to 0, 2, 10 or 25 µg fluridone L^{-1}, and during 40 d following herbicide removal. Asterisk indicates removal of treatment. Means and standard errors (bars) of three replicates.

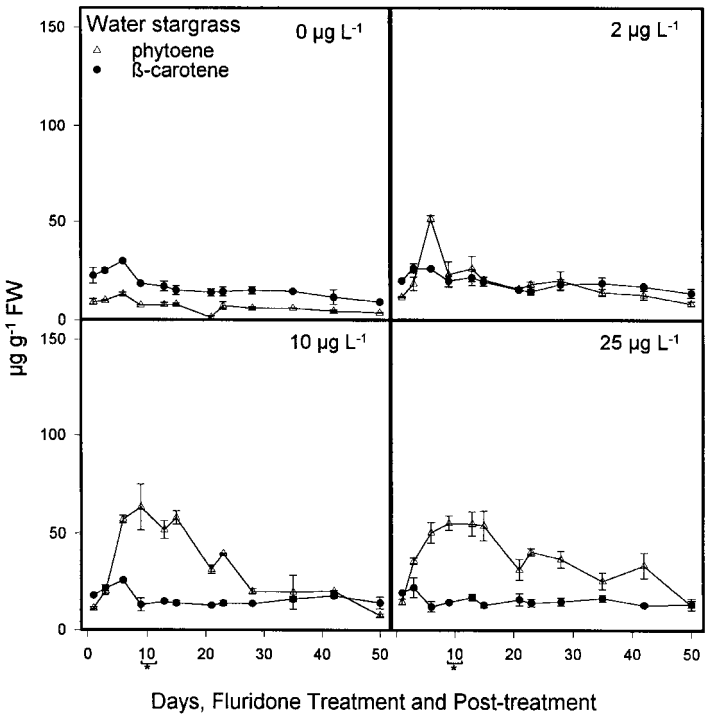

Figure 10. Phytoene and β-carotene concentrations, µg g^{-1} fresh weight (FW), in shoot apices from rooted plants of water stargrass during an initial 10 d exposure to 0, 2, 10 or 25 µg fluridone L^{-1}, and during 40 d following herbicide removal. Asterisk indicates removal of treatment. Means and standard errors (bars) of three replicates.

15 days after treatment (DAT) began, and decreased to near-background levels by 28 to 50 DAT. In egeria, hydrilla and milfoil, carotene minima were reached at approximately 10 DAT, and levels began to increase with removal of fluridone; water stargrass underwent little change in carotene. While maximum levels of phytoene were similar among treatments in the same species, phytoene generally remained elevated above carotene longer with increasing fluridone concentration. For example, in milfoil (Figure 8), phytoene fell below carotene at 23, 28, and 35 DAT with increasing concentrations of fluridone treatment. This suggests, firstly, that above some certain effective level the enzyme inhibition causing phytoene accumulation is not enhanced by higher treatment concentration; and secondly, that higher concentrations of fluridone maintain a treatment effect longer because more herbicide has accumulated in plant tissue and takes longer to dissipate. By 50 DAT, carotene and phytoene were similar to pretreatment levels, and healthy green apices were present in all treatment units.

Pigment Response to Other Plant Control Measures. To determine how specific these pigment responses were for fluridone exposure, phytoene and carotene were measured in unrooted apical shoots of coontail (a dicot) and egeria (monocot) after treatment with various aquatic herbicides in hydroponic culture. Exposures were to 1 mg Cu L^{-1} copper ethanolamine complexes, 2 mg acid equivalent (ae) L^{-1} 2,4-D (2,4-dichlorophenoxy) acetic acid), 0.5 mg ai L^{-1} diquat (6,7-dihydrodipyrido[1,2-α:2',1'-c] pyrazinediium ion), 2 mg ae L^{-1} of the dipotassium and amine salt formulations of endothall (7-oxabicyclo[2.2.1]heptane-2,3-dicarboxylic acid), and 2.5 mg ae L^{-1} triclopyr ([(3,5,6-trichloro-2-pyridinyl)oxy]acetic acid), as well as fluridone, for from 2 hr to 7 d (Table 3). Coontail tissue was not available for analysis afterexposure to endothall or copper. Comparison with untreated references showed that phytoene did not increase with exposure to any herbicide except fluridone ($p \leq 0.05$), and that carotene decreased only with exposure to fluridone and copper in egeria, and fluridone and 2,4-D in coontail ($p \leq 0.05$).

To determine if the observed pigment response resulting from fluridone treatment could be confounded by the presence of a biocontrol organism, we analyzed apical tissue from rooted hydrilla exposed to 2 and 5 µg fluridone L^{-1} amended with 50, 100, or 200 colony-forming units L^{-1} of the hydrilla-specific fungal pathogen *Mycoleptodiscus terrestris* (Gerd.) Ostazeski (Netherland and Shearer 1996). Phytoene increased in all fluridone-treated plants (data not shown) to levels characteristic of unamended applications of this herbicide. This indicates that the activity of this pathogen under co-treatment conditions produced no physiological interference in the mode of action of the herbicide.

These results show that, in a similar response to that in terrestrial plants, elevation in phytoene provides a sensitive marker for exposure of submersed aquatic plants to rates as low as 1 µg fluridone L^{-1} under laboratory conditions. Changes in pigment ratios were associated with the onset of fluridone-induced physiological change, maintenance of herbicide exposure, and recovery following herbicide removal. Preliminary results from dissipating field exposures in large-scale operational fluridone treatments have indicated that tissue concentrations of phytoene remain elevated above those of carotene for as long as 160 DOE in both target and nontarget plants (unpubl. data). However, determining whether phytoene, alone or in combination with carotene and chlorophyll, can predict fluridone efficacy and control in target species remains the goalof future work.

The specificity of these pigment responses has application in research and operational activities. The presence of fluridone at levels too low to produce chlorosis can be monitored by phytoene activity. A failure in expected pigment changes following exposure suggests hindrance of fluridone activity by interfering agents or conditions, while the presence of plant chlorosis without any increase in phytoene indicates an effect due to an agent other than fluridone. These results support the rate-dependent nature of fluridone selectivity (Netherland et al. 1997) rather than the absence of phytoene desaturase inhibition following application. They provide a basis for continued examination of fluridone's effects on individual species and can contribute to an increased understanding of selective activity in this herbicide.

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