J. Aquat. Plant Manage. 31: 55-59

Effect of Fluridone on Chlorophyll, Carotenoid and Anthocyanin Content of Hydrilla[1]

R. L. DOONG, G. E. MACDONALD AND D. G. SHILLING[2]

ABSTRACT

Hydrilla (mature and young plants) were exposed to 0.05, 0.5, 5.0, and 50 ppb of fluridone for 2, 4, 6, 8 and 12 wk and monitored for changes in chlorophyll, carotenoid and anthocyanin content. Fluridone decreased carotenoid and chlorophyll content of mature hydrilla plants. As fluridone exposure times and rates increased, chlorophyll and carotenoid content decreased concomitantly. Regardless of time, 50 ppb fluridone reduced carotenoid and chlorophyll content by 80 to 95%. In younger plants, 50 ppb fluridone lowered carotenoids and chlorophyll by at least 50 and 65%, regardless of time, respectively. Fluridone at 50 ppb caused an increase in anthocyanin content (5X the control) in mature hydrilla but did not affect anthocyanin content in young hydrilla. However, both plant types became pink in color after exposure to fluridone. Apparently, anthocyanins were simply unmasked after chlorophyll photooxidation in young hydrilla while an increase in anthocyanin biosynthesis occurred in the mature plants. The differential response in anthocyanin content of hydrilla to fluridone could be related to physiological stage of development and/or light intensity.

Key words: plant pigments, stress, herbicide, photooxidation.

INTRODUCTION

Fluridone (1-methyl-3-phenyl-5-[3-(trifluoromethyl)phenyl]-4(1H)-pyridinone) is often used in Florida to control hydrilla (*Hydrilla verticillata* (L.f.) Royle)(21), a submersed aquatic macrophyte that is a major problem throughout Florida and many other areas (7,9). Fluridone is frequently referred to as a "bleaching herbicide," due to the characteristic white coloration of treated tissue (17). Fluridone blocks the synthesis of carotenoids which are pigments essential for normal plant growth (16). Carotenoids function to protect the photosynthetic system from photodynamic damage by quenching triplet-state chlorophyll and singlet oxygen (1,5,6). In the absence of carotenoids, chlorophyll would photooxidize resulting in bleached, white tissue.

Plants grown under high light conditions will normally produce more carotenoids to offset the increase in light-

[1]Published with the approval of the Florida Agricultural Experiment Station as J. Series No. R-02812.

[2]Postdoctoral Associate, Graduate Assistant, and Associate Professor, respectively, Department of Agronomy, University of Florida, Institute for Food and Agricultural Sciences, Gainesville, FL 32611.

generated oxidative stress. Therefore, the effect of a carotenoid-inhibiting compound will be exacerbated under high light intensity due to a higher rate of photooxidation (1,5,15) and growth (*i.e.*, higher demand for *de novo* synthesis of carotenoids). However, hydrilla and many other submersed aquatic macrophytes persist in areas of very low light intensity yet fluridone provides good control.

Generally, apical meristems and new growth are the first to display the characteristic changes in pigmentation resulting from fluridone treatment. These symptoms are followed by a deterioration of the tissue and eventual plant death. However, hydrilla and certain other aquatic plants produce a pink coloration at the growing tips after exposure to fluridone. Pink coloration in plant tissue is generally attributed to anthocyanins (10), but little research has been conducted on the effect of bleaching herbicides on these pigments.

Control of hydrilla with fluridone is highly dependent upon adequate exposure time and concentration (8). Pigment levels are a measure of plant viability, and knowledge concerning the effect of fluridone on these levels could be used to improve management. Furthermore, the effects of fluridone on chlorophyll and carotenoid content in hydrilla have never been fully documented. Therefore, the objective of this study was to evaluate the effect of fluridone on the chlorophyll, carotenoid and anthocyanin content in hydrilla over time.

MATERIALS AND METHODS

Plant culture. Hydrilla was planted (2/22/91) from apical stem segments in 10- by 10-cm^2 pots (4 segments/pot) filled with organic potting medium. The medium was amended with a slow-release fertilizer and a 1-cm-thick sand cap added to prevent floating. The plants were allowed to grow under greenhouse conditions (16 hr light/8 hr dark photoperiod, 1000 $\mu mol \cdot m^{-2} sec^{-1}$(PAR) average light intensity at noon, 30C day/20C night) for approximately 7.5 months in a 900-L plastic-lined tank (150 by 100 by 60 cm). Hydrilla rapidly formed a dense mat at the water surface which persisted until the experiment was initiated.

Plants (15 pots/vault - 5 harvest dates x 3 replications) were transferred to 900-L concrete vaults (217 by 76 by 55 cm) on 10/11/91. At the same time 10-cm apical stem segments (young plants) were established in a manner similar to that described for mature plants (2 segments/pot) and grown concomitantly with the mature plants. On 10/16/91 the following concentrations of fluridone were established in the vaults: 0.0, 0.05, 0.5, 5.0, or 50 ppb. The plants were maintained outdoors under natural conditions (short-day photoperiod). After 2, 4, 6, 8 or 12 wk of exposure to fluridone, two apical shoot segments from each treatment and age group (approximately 0.1 g fresh weight each) were excised and analyzed for chlorophyll, carotenoid and anthocyanin content.

Chlorophyll/carotenoid analysis. Apical meristems were homogenized in 15 ml of chloroform/methanol (2:1, v/v) for 3 min on ice. The homogenate was filtered and the crude extract then dried under a stream of nitrogen at room temperature under dim light (<5 $\mu mol \cdot m^{-2} \cdot sec\text{-}1$). The residue was resuspended in 2 ml of 80% acetone and absorbances at 663, 646 and 470 nm were measured spectrophotometrically. Total chlorophyll concentration was calculated utilizing the following formula: ($17.32 \times A_{646} - 7.18 \times A_{663}$). Total carotenoid content was calculated using the following formula: ($1000 \times A_{470} - 3.27 \times Ca - 104 \times Cb$)/229, where $Ca = 12.21 \times A_{663} - 2.81 \times A_{646}$ and $Cb = 20.13 \times A_{646} - 5.03 \times A_{663}$. Ca and Cb are the concentrations of chlorophyll *a* and *b* in µg/ml (13).

Anthocyanin analysis. Apical meristems were homogenized in 15 ml of methanol containing 1% HCl (v/v) for 3 min on ice. The homogenate was then filtered with suction and the absorbance of the extract determined at 530 and 657 nm, spectrophotometrically. Chlorophyll has some absorbance at 530 nm in acidic methanol. Therefore, corrections were made by subtracting the absorbance contributed by chlorophyll under acidic conditions at 657 nm using the formula $A_{530} - 0.25 \times A_{657}$ as described by Mancinelli (14) which assumes an extinction coefficient of 34,000 $M^{-1}cm^{-1}$ (11).

Statistical analysis. Data were initially analyzed by analysis of variance to test for treatment effects and interactions. Age by rate interactions were significant ($P < 0.05$) and data are presented accordingly. Treatment means within a harvest interval were compared to the untreated control utilizing Dunnett's 'T' test at the 0.05 level of significance.

RESULTS

Mature plants. Carotenoid concentration in the mature plants was significantly reduced by fluridone with higher rates causing a greater decrease (Table 1). Carotenoid content was reduced to near zero by 50 ppb fluridone at 2, 6 and 12 wk. Fluridone at 5.0 ppb caused a 75% reduction at 2 and 6 wk, and by 12 wk carotenoid levels were near zero.

Fluridone at 5.0 and 50 ppb reduced chlorophyll by at least 65 and 80%, respectively, at all weeks (Table 1). Between 6 and 12 wk after treatment 0.5 ppb fluridone caused reductions of 44 to 57%. Conversely, chlorophyll content was significantly increased at 0.05 ppb fluridone 2, 8 and 12 wk after initial exposure.

Fluridone at the 50-ppb concentration increased anthocyanin content 322, 293, 275, 132, and 40% after 2, 4, 6, 8, and 12 wk of treatment, respectively. Concentrations of 0.05 and

TABLE 1. EFFECT OF FLURIDONE (ppb) ON CAROTENOID, CHLOROPHYLL AND ANTHOCYANIN CONTENT IN MATURE HYDRILLA. PIGMENT VALUES ARE PRESENTED AS μg/g FRESH WEIGHT.

Pigment	Weeks after Treatment	Fluridone				
		0	0.05	0.5	5.0	50
Carotenoid	2	207	363	241	46*[1]	0*
	4	—	—	—	—	—
	6	201	188	130	50*	0*
	8	—	—	—	—	—
	12	194	85*	0*	7*	5*
Chlorophyll	2	1571	3014*	2082	523*	304*
	4	1053	970	900	329*	186*
	6	1183	1094	660*	376*	148*
	8	1311	1340*	709*	446*	186*
	12	1178	1380*	504*	423*	356*
Anthocyanin	2	116	166	171	236	490*
	4	111	119	151	156	436*
	6	138	100	119	111	517*
	8	148	51	65	248	344*
	12	124	195*	196*	147	171*

[1]Values within a week followed by * are significantly different from the control (Dunnett's 't' test at the 0.05 level).

0.5 ppb fluridone also increased anthocyanin content but only after 12 wk of treatment.

Young plants. Carotenoid content in young, untreated plants was about 50% that of the mature plants, and the drop in carotenoid content in response to fluridone was much less than that of the mature plants (Tables 1 and 2). Concentrations of 0.5, 5.0 and 50 ppb fluridone significantly reduced carotenoid content 8 wk after treatment (Table 2). Chlorophyll content was also much lower (40%) in the young hydrilla plants as compared to the mature hydrilla and was reduced by 50 ppb of fluridone to about 65% of the control for all times of treatment (Table 2). Fluridone at 5.0 ppb reduced chlorophyll content by 54 to 68% for exposure times longer than 2 wk. The 0.5-ppb concentration caused a reduction in chlorophyll content 4 and 8 wk after treatment. A significant increase in chlorophyll at 6 and 8 wk was observed at 0.05 ppb fluridone. Anthocyanins in young plants did not significantly change in response to fluridone.

DISCUSSION

Carotenoid content of the mature plants declined rapidly followed by a decrease in chlorophyll. Chlorophyll content was lower in the younger plants, similar to the findings of Van et al. (20). They suggested that this was due to the greater amount of stem versus leaf tissue at the lower depths. Carotenoids were also lower, presumably due to the lower chlorophyll content. Interestingly, in the young plants fluridone caused less decrease in carotenoid content than in the mature plants (*i.e.* 50% or more relative to the control). Chlorophyll content also decreased in response to fluridone to a lesser extent in the young plants. Both Devlin *et al.* (5), and Bartels and Watson (1) demonstrated that a reduction in chlorophyll was dependent on light intensity, with a greater reduction under high light. The younger plants in this study were approximately 0.5 m below the surface of the water and probably received less light than the mature plants which were on the surface.

The younger hydrilla plants were exposed to a lower light intensity, decreasing the level chlorophyll photooxidation as carotenoid content decreased in response to fluridone. This decrease in carotenoid content in response to fluridone could have resulted from lower biosynthetic rates at the lower light intensity, ultimately lowering susceptibility.

When hydrilla was treated with fluridone at 5.0 or 50 ppb the apical meristems became pink in color. Further characterization indicated the pink pigments were anthocyanin as characterized by an absorption peak at 530 nm and a colormetric change at higher pH values (3). In the mature plants treated with 50 ppb fluridone, anthocyanin content was four to five times higher. There was also a significant increase in anthocyanin content in response to fluridone at the 0.5-ppb rate 12 wk after initial exposure. Anthocyanin content did not change in response to fluridone in the younger plants.

Many aquatic plants possess anthocyanins (18,19). These pigments are produced in response to various stress-

TABLE 2. EFFECT OF FLURIDONE (ppb) ON CAROTENOID, CHLOROPHYLL AND ANTHOCYANIN CONTENT IN YOUNG HYDRILLA. PIGMENT VALUES ARE PRESENTED AS μg/g FRESH WEIGHT.

Pigment	Weeks after Treatment	Fluridone				
		0	0.05	0.5	5.0	50
Carotenoid	2	84	84	89	98	46
	4	101	93	69	57	61
	6	84	122	114	65	59
	8	126	121	84*[1]	79*	64*
	12	106	87	55	55	49
Chlorophyll	2	688	733	700	683	194*
	4	693	739	511*	222*	233*
	6	604	889*	691*	277*	293*
	8	845	894*	540*	354*	300*
	12	801	668	494	267*	220*
Anthocyanin	2	202	130	83	182	176
	4	146	160	161	140	161
	6	129	107	108	166	98
	8	325	86	72	96	110
	12	100	110	85	133	96

[1]Values within a week followed by * are significantly different from the control (Dunnett's 't' test at the 0.05 level).

related factors including high light, low water temperature, and nutrient limitation (10,18). Work by Spencer and Ksander (19) showed that a decrease in chlorophyll content (due to high light intensities) in *Potamogeton gramineus* L. was responsible for the apparent anthocyanin increase. Although they suggested this phenomenon was an unmasking due to the loss of chlorophyll, they did report a slight increase in anthocyanin pigment production under high light intensities. Hydrilla is known to lose chlorophyll (12) and produce anthocyanins (2) during the fall as a result of leaf senescence. Therefore, the increase in anthocyanins in hydrilla treated with fluridone could have been due to a loss of chlorophyll. Young plants did not respond to fluridone by increasing anthocyanin content but a pink coloration was still evident. This could have resulted from an unmasking of the anthocyanins as was the case in *P. gramineus*. In addition, anthocyanin production in some plants has been correlated with high carbohydrate status (4) and the younger developing shoots could be deficient in reserves required for anthocyanin production.

Fluridone causes many changes in the pigment composition of hydrilla. As expected, carotenoid and chlorophyll levels were dramatically reduced, ultimately causing the death of the plant. Fluridone also stimulated the production of anthocyanins, albeit in mature plants only, but the exact mechanism and cause(s) remains equivocal.

ACKNOWLEDGMENTS

Support for this project was provided by the Florida Department of Natural Resources, the Center for Aquatic Plants and the Agronomy Department at the University of Florida. The assistance of Cindy Ragland, Sean Ragland, Brian Smith and Jamie Carter was greatly appreciated.

LITERATURE CITED

1. Bartels, P. G. and C. W. Watson. 1978. Inhibition of carotenoid synthesis by fluridone and norflurazon. Weed Sci. 26:198-203.
2. Berg, R. H. 1977. Annual decline of the aquatic macrophyte *Hydrilla verticillata* (L.F.) Royle. Ph.D. Dissertation, Agronomy Dept., University of Florida. 104 pp.
3. Brouillard, R. 1983. The *in vivo* expression of anthocyanin color in plants. Phytochem. 22:1311-1323.
4. Creasy, L. L. 1968. The significance of carbohydrate metabolism in flavonoid synthesis in strawberry leaf disks. Phytochem. 7:1743-1749.
5. Devlin, R. M., C. N. Saras, M. J. Kisiel and A. S. Kostusiak. 1978. Influence of fluridone on chlorophyll content of wheat (*Triticum aestivum*) and corn (*Zea mays*). Weed Sci. 26:432-433.
6. Gamble, P. E. and J. E. Mullet. 1986. Inhibition of carotenoid accumulation and abscisic acid biosynthesis in fluridone-treated dark-grown barley. Eur. J. Biochem. 160:117-121.
7. Haller, W. T. 1976. Hydrilla: A new and rapidly spreading aquatic weed problem. I.F.A.S. Circular S-245. 13 pp.
8. Haller, W.T., A.M. Fox and D.G. Shilling. 1990. Hydrilla control program in the upper St. Johns River, Florida, USA. Proc. EWRS Symposium on Aquatic Weeds. 8:111-116.

9. Haller, W. T. and D. L. Sutton. 1975. Community structure and competition between hydrilla and vallisneria. J. Aquat. Plant Manage. 13:48-50.
10. Hrazdina, G. 1982. Anthocyanins. *In*: J.B. Harborne and T.J. Mabry (eds.), The Flavenoids: Advances in Research. Chapman and Hall, London. Pp 135-188.
11. Jonsson, L. M., W. E. Donker-Koopman and A. W. Schram. 1984. Turnover of anthocyanins and tissue compartmentation of anthocyanin biosynthesis in flowers of *Petunia hybrida*. J. Plant Physiol. 115:29-37.
12. Kar, R. K. and M. A. Choudhuri. 1987. Possible mechanisms of light-induced chlorophyll degradation in senescing leaves of *Hydrilla verticillata*. Physiol. Plant. 70(4):729-34.
13. Lichtenthaler, H. K. and A. R. Wellburn. 1983. Determinations of total carotenoids and chlorophylls 'a' and 'b' of leaf extracts in different solvents. Biochem. Soc. Trans. 11:591-592.
14. Mancinelli, A. L. 1990. Interaction between light quality and light quantity in the photoregulation of anthocyanin production. Plant Physiol. 92:1191-1195.
15. Mayer, M. P., D. L. Bartlett, P. Beyer and H. Kleinig. 1989. The *in vitro* mode of action of bleaching herbicides on the desaturation of 15-*cis*-phytoene and *cis*-ζ-carotene in isolated daffodil chromoplasts. Pestic. Biochem. Physiol. 34:111-117.
16. Ragolsky, E. and T. A. Thorpe. 1989. Physiological effects of fluridone on shoot cultures of *Brassica napus* L. and *Beta vulgaris* L. J. Plant Physiol. 134:613-618.
17. Sandman, G., A. Schmidt, H. Linden and P. Böger. 1991. Phytoene desaturase, the essential target for bleaching herbicides. Weed Sci. 39:474-479.
18. Spence, D. H. N. 1974. Light and plant response in fresh water. *In*: G.C. Evans, R. Bainbridge and O. Rackham (eds.), Light as an Ecological Factor: Blackwell Scientific, London. 616 pp.
19. Spencer, D. F. and G. G. Ksander. 1990. Influence of temperature, light and nutrient limitation on anthocyanin content of *Potamogeton gramineus* L. Aquatic Bot. 38:357-367.
20. Van, T. K., W. T. Haller, G. Bowes and L. A. Garrard. 1977. The effects of light quality on growth and chlorophyll composition in hydrilla. J. Aquat. Plant Manage. 15:29-31.
21. Westerdahl, H. E. and Getsinger, K. D., eds. 1988. Aquatic Plant Identification and Herbicide Use Guide; Volume I: Aquatic Herbicides and Application Equipment, Technical Report A-88-9, U.S. Army Engineer Waterways Experiment Station, Vicksburg, MS. 130 pp.