

# Effects of Fluridone on Hydrilla Growth and Reproduction<sup>1</sup>

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## ABSTRACT

The effects of fluridone on hydrilla growth and reproduction were evaluated over time. Newly established (young) and 8-month-old (mature) hydrilla plants were exposed to 0.0, 0.05, 0.5, 5.0, or 50 ppb fluridone and maintained outdoors under ambient short-day conditions. An untreated long-day control was also included. Fresh and dry weight, number of flowers, and axillary and subterranean turions were determined 0, 2, 4, 6, 8, and 12 weeks after fluridone treatment. Short-day conditions promoted flower and axillary and subterranean turion production in mature plants. Low concentrations of fluridone (0.05 and 0.5 ppb) caused transient increases in the number of both subterranean and axillary turions by mature hydrilla, but higher concentrations (5 and 50 ppb) inhibited development of these tissues. Growth (shoot dry weight) of young plants treated with low concentrations of fluridone (0.05 and 0.5 ppb) was not affected. The 5.0 ppb-fluridone treatment did not affect the growth of young plants until after 6 weeks of exposure. The 50 ppb fluridone treatment prevented any significant change in young plant shoot dry weight over the 12-week study. There was no significant change in shoot dry weight of mature plants regardless of the treatment.

**Key words:** subterranean turion, axillary turion, herbicide, tubers, turion, photoperiod, abscisic acid.

## INTRODUCTION

Hydrilla (*Hydrilla verticillata* (L.f.) Royle) is an exotic submersed aquatic macrophyte that infests fresh water ecosystems throughout the world (12,26). Hydrilla was introduced to Florida from Asia in the late 1950s by the aquarium industry (5,7) and is currently Florida's most serious aquatic weed problem (7). Hydrilla possesses unique photosynthetic characteristics such as C<sub>4</sub> metabolism and a low light compensation point that allow this species to out-compete other

aquatic macrophytes (1,6,19,22). In addition, hydrilla can reproduce through several vegetative mechanisms including fragmentation (11), and specialized structures [axillary turions (turions) and subterranean turions (tubers)](7,24). Tubers are formed at the end of positively geotropic rhizomes, while turions are found in leaf axials or occasionally at the end of shoots (21).

Fluridone (1-methyl-3-phenyl-5-[3-(trifluoromethyl)phenyl]-4(1H)-pyridinone) was registered for aquatic use in 1986 and usually provides good control, but results are often unpredictable in terms of area treated and area controlled (14,20). Fluridone was the first herbicide to provide long-term hydrilla control; however, it was difficult to produce consistent results. In addition, methods to best utilize several unique characteristics of fluridone to improve control have not been completely developed.

Fluridone, applied at normal use rates, decreases carotenoid levels sufficiently to cause chlorophyll decomposition and plant death (4,17). Because abscisic acid is a carotenoid metabolite, fluridone is also used in physiological studies to regulate abscisic acid (ABA) levels (16,18). Abscisic acid has been associated with turion formation (initiation) in green milfoil (*Myriophyllum verticillatum* L.) (25) and giant duckweed (*Spirodela polyrrhiza* L.) (13). Therefore, fluridone could potentially prevent the production of turions in hydrilla.

Carotenoids protect chlorophyll from decomposition and are essential to the survival of all plants. The ability to regulate the reproduction of hydrilla without killing the plant would be dependent on a critical concentration of fluridone. A growth-regulating concentration of fluridone could potentially lower carotenoids (but not enough to cause a critical decrease in chlorophylls) enough to reduce ABA below a critical concentration thus preventing vegetative reproduction. These studies were conducted to evaluate the relationship between fluridone concentration and regulation of reproduction and growth in hydrilla.

## MATERIALS AND METHODS

On February 22, 1991, four 10-cm apical stem segments of hydrilla were planted in 10-cm square pots. These pots were filled with potting media (Metro-mix 200 amended with fertilizer) and a 1.3-cm deep sand cap was added to prevent

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floating of the media. These plants were grown in a greenhouse for 8 months under the following environmental conditions: 16 hr light/8 hr dark photoperiod, day temperature of  $30 \pm 5^\circ\text{C}$ , night temperature of  $25 \pm 5^\circ\text{C}$  with a mean light intensity at noon of  $900 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  (PPFD). Under these conditions the hydrilla quickly reached the surface of the water and formed a dense mat.

On October 10, 1991, plants (mature) were transferred outdoors to 900-L concrete vaults. In addition, two 10-cm hydrilla apical stem segments per pot were established in the vaults in the same manner described previously. Segments were taken from the mature plants. All plants were treated on October 16, 1991, with fluridone at the following rates: 0.0, 0.05, 0.5, 5.0, and 50 ppb and maintained outdoors under ambient short-day conditions (<12-hr daylength) in Gainesville, FL. In addition, control groups representing both age groups of plants were maintained outdoors but supplied with floodlights timed to extend the photoperiod to 16 hr. Representative plants ( $n = 4$ ) were harvested from both age groups at this time to establish the status of the plants at the time of treatment.

Plants were harvested at intervals of 2, 4, 6, 8, and 12 weeks after treatment beginning October 31, 1991. Three groups of plants from each age group were harvested from each vault at each sampling period. Parameters evaluated were fresh weight (g) and the numbers of flowers, turions, and tubers. After measurements were taken, plant material was dried at  $60^\circ\text{C}$  for 72 hr and dry weights were determined on a whole plant basis.

Data are the average of three replicates for both mature (4 plants/pot) and young (2 plants/pot) plants. Data were initially analyzed by analysis of variance to test for photoperiod and rate effects and interactions. Time was considered a repeated measure. There was a significant ( $P < 0.05$ ) time by rate interaction for all parameters, therefore data are presented for individual harvest dates. Dunnett's "t" test ( $\alpha = 0.05$ ) was used to separate the effect of fluridone rate on tubers and turions. Regression analysis was used to determine the relationship between dry weight and time as a function of fluridone concentration in the young plants. Means are presented with standard errors.

## RESULTS

Tuber production in mature hydrilla occurred only under short-day conditions (Table 1). Fluridone at concentrations of 5.0 and 50 ppb reduced tuber formation by hydrilla grown under short days. Fluridone at 0.05 and 0.5 ppb caused an initial stimulation (2 weeks after treatment) in tuber production of 49 and 61% compared to the untreated short-day plants. Flowers were produced under short-day conditions

TABLE 1. THE INFLUENCE OF FLURIDONE AND PHOTOPERIOD ON TUBER PRODUCTION (number of tubers/plant) BY MATURE HYDRILLA.

Photo-period <sup>1</sup>	Fluridone (ppb)	Time after treatment (weeks)			
		2	4	8	12
LD	0	0	0	0	
SD	0	$2.6 \pm 0.6^{*2}$	$2.7 \pm 1.0^*$	$3.2 \pm 0.5^*$	$3.2 \pm 0.5^*$
SD	0.05	$3.8 \pm 0.8^*$	$4.3 \pm 0.6^*$	$3.7 \pm 1.4^*$	$3.4 \pm 0.8^*$
SD	0.5	$4.1 \pm 0.8^*$	$2.2 \pm 0.3$	$2.7 \pm 0.2^*$	$3.6 \pm 0.7^*$
SD	5.0	$0.1 \pm 0.1$	$0.2 \pm 0.2$	$0.3 \pm 0.3$	$0.2 \pm 0.2$
SD	50	$0.5 \pm 0.5$	0	0	0

<sup>1</sup>LD = long day (16 hr light/8 hr dark).

SD = ambient short day (<12 hr light).

<sup>2</sup>Means are followed by standard errors. Values followed by \* are significantly different from the untreated long-day control within each week (Dunnett's "t" test at the 0.05 level).

but variability precluded any definitive conclusions to be drawn (data not shown).

Mature hydrilla produced fewer turions than tubers in response to short-day conditions with the exception of plants exposed to 0.5-ppb fluridone for 12 weeks (Table 2). Long photoperiod and fluridone at 5.0 and 50 ppb inhibited turion production. The lower rates of fluridone (0.05 and 0.5 ppb) caused an increase in the number of turions with the 0.5-ppb rate causing a five-fold increase after 12 weeks of treatment.

Tuber and turion production by young plants was too low and variable to draw any conclusions (data not shown). In addition, no flowers were produced by the young plants regardless of treatment.

Fluridone did not cause any change ( $P > 0.05$ ) in the dry weight of mature plant (data not shown). Young untreated

TABLE 2. THE INFLUENCE OF FLURIDONE AND PHOTOPERIOD ON TURION PRODUCTION (number of turions/plant) BY MATURE HYDRILLA.

Photo-period <sup>1</sup>	Fluridone (ppb)	Time after treatment (weeks)	
		8	12
LD	0	0	0
SD	0	$1.6 \pm 0.1$	$1.4 \pm 0.7$
SD	0.05	$2.4 \pm 0.8$	$3.0 \pm 0.8$
SD	0.5	$4.5 \pm 2.2^{*2}$	$8.8 \pm 1.6^*$
SD	5.0	0	0
SD	50	0	0

<sup>1</sup>LD = long day (16 hr light/8 hr dark).

SD = ambient short day (<12 hr light).

<sup>2</sup>Means are followed by standard errors. Values followed by \* are significantly different from the untreated long-day control within each week (Dunnett's "t" test at the 0.05 level).

plants and plants treated with fluridone at 0.05 and 0.5 ppb grew linearly over the study period (Figure 1). Young plants exposed to 5.0-ppb fluridone also grew linearly ( $y = 0.02x + 0.06$ ;  $R^2 = 0.91$ ) for 6 weeks, but subsequently stopped growing. Plants treated with 50-ppb fluridone did not produce any significant increase in biomass.

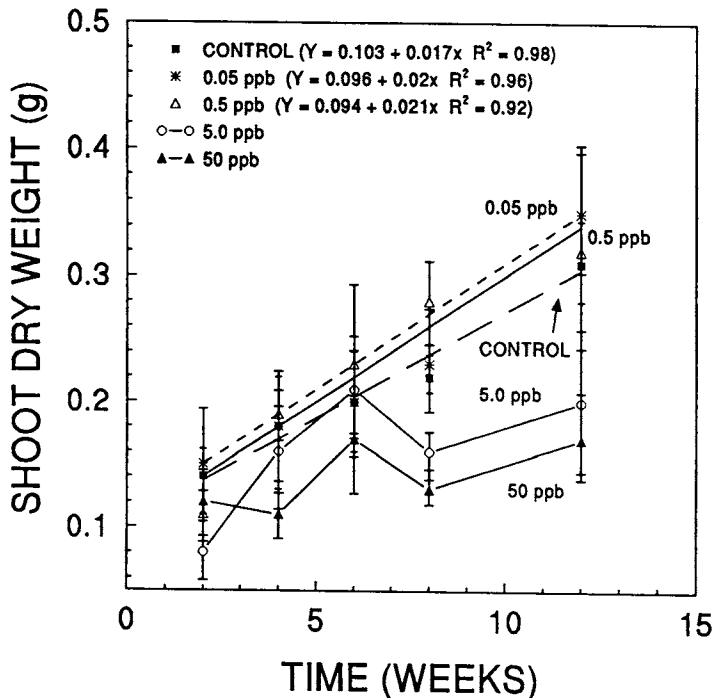


Figure 1. The effect of time and fluridone on the shoot dry weight of young hydrilla plants over 12 weeks.

## DISCUSSION

Plants that have been treated with a lethal dose of fluridone generally become bleached white or pink, due to a loss of carotenoids and chlorophyll, and a cessation of growth occurs (2,4). This effect is exacerbated in young plants due to the critical dependency of newly emerging photosynthetic tissue to provide carbohydrate (photosynthate) for the plant and the lack of pre-existing carotenoids at the time of treatment. This could explain the lack of a growth response in mature hydrilla to fluridone over the 12-week period of the experiment. The lethal concentration of fluridone on hydrilla under field conditions is believed to be between 5 and 10 ppb (8). Significant growth inhibition by 5-ppb fluridone took 6 weeks in young plants, but was immediate at 50 ppb. This latter response has been reported previously, but the importance of contact time and concentration have not been fully characterized.

Tuber and turion production in hydrilla has been reported to be a photoperiodic response (23,24), regulated via phyto-

chrome (10). Research has shown that exogenously applied ABA caused tuber and turion formation under long days (24) which indicated that high levels of ABA were involved (either directly or indirectly) in the formation of these reproductive propagules under short days. During this study, fluridone at 5 and 50 ppb inhibited growth and reproduction (tuber and turion formation). Growth inhibition occurred due to the lack of carotenoids but was probably not the cause of inhibiting reproduction. There was no significant tuber initiation at 5- and 50-ppb fluridone, even though vegetative growth occurred for 6 weeks at 5 ppb. Reproduction can occur in the absence of photosynthesis *in vitro*, when sucrose is supplied exogenously (Kane, unpublished data), and sugars required for tuber formation should not have been lacking in the mature plants. Fluridone is often utilized to study the effect of ABA, because of its ability to block ABA biosynthesis (through an inhibition of carotenoid biosynthesis) (15,16). Therefore, it is possible that fluridone at 5.0 ppb was inhibiting tuber and turion formation by lowering ABA to levels below that required for tuber and turion initiation.

At concentrations of 0.05- and 0.5-ppb fluridone, a transient stimulation was observed for young but not mature plants in tuber production. Many plants respond to stress by an elevation of ABA levels (3,9,27) and an increase in or sudden shift toward reproduction. This was probably the case for the 0.05- and 0.5-ppb treated hydrilla plants, where tuber and turion production was initially high due to an increase in the level of stress induced by the herbicide.

In conclusion, regulation of tuber and turion formation in hydrilla may be possible with sublethal rates of fluridone due to the separate mechanisms by which fluridone inhibits growth and reproduction. The critical concentration of fluridone required to potentially cause this effect remains to be determined but appears to be between 0.5 and 5.0 ppb. Due to the mechanism-of-action of fluridone, contact time, light intensity, and carotenoid levels would be very critical to obtaining a growth-regulating versus growth-inhibiting effect.

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