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Response of Hydrilla and American Pondweed to Flurprimidol

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ABSTRACT

An outdoor mesocosm system was used to evaluate the growth regulator flurprimidol ([a-(1-methylethyl)-a-[4-(trifluoromethoxy)phenyl]-5-pyrimidine-methanol]) as a potential management tool on hydrilla (*Hydrilla verticillata* (L. f.) Royle) and American pondweed (*Potamogeton nodosus* Poiret). Treatments included single doses of flurprimidol at 100 and 200 µg/L, a split application of 100 µg/L, and untreated controls. Increasing contact time by applying a split treatment of flurprimidol was more effective for suppressing plant growth than applying flurprimidol as a single dose. The split application reduced shoot length and biomass by 69 and 50%, respectively, for hydrilla, and 34 and 86%, respectively, for American pondweed, at 12 weeks after treatment (WAT). Flurprimidol-inhibited American pondweed shoots had 84% lower total nonstructural carbohydrates (TNC) than untreated plants at 6 WAT, whereas hydrilla had 80% less shoot TNC at 12 WAT. This information will be useful for assessing the susceptibility of target and nontarget aquatic plants to flurprimidol.

Key words: aquatic plant management, carbohydrates, *Hydrilla verticillata*, plant growth regulator, *Potamogeton nodosus*, TNC.

INTRODUCTION

The use of synthetic plant growth regulators (PGRs) has been suggested as a potential management tool for nuisance aquatic weed species (Klaine and Knowles 1988, Nelson and Van 1991, Netherland and Lembi 1992, Lembi and Chand-Goyal 1994). Of the numerous growth regulating compounds assayed for activity on aquatic plants, flurprimidol is one of the most effective (Netherland and Lembi 1992, Lembi and Chand-Goyal 1994). Flurprimidol is a gibberellin synthesis inhibitor currently registered by the U.S. Environmental Protection Agency for turf and horticultural applications, that is also effective for reducing main stem growth of nuisance submersed plants such as hydrilla and Eurasian watermilfoil (*Myriophyllum spicatum* L.) (Lembi and Chand 1992, Netherland and Lembi 1992, Nelson 1996). Flurprimidol also shows selectivity among aquatic plant species. Studies have documented that the growth of desirable non-target species, such as wild celery (*Vallisneria americana* Michx.) and coontail (*Ceratophyllum demersum* L.), was not affected by flurprimidol at rates sufficient to inhibit hydrilla (Lembi and Chand-Goyal 1994, Nelson 1993). Thus the potential exists to regulate the growth of noxious species without affecting native species.

While concentration and contact time are both critical factors for the effectiveness of many aquatic herbicides and PGRs, increasing flurprimidol contact time was more effective than increasing concentration on growth of Eurasian watermilfoil (Nelson 1996). Low concentrations of flurprimidol (25 and 100 µg/L) were effective for suppressing stem

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height of Eurasian watermilfoil but longer contact times (28 days) were required to maintain efficacy (Nelson 1996). Application methods that extend the contact time (controlled-release formulations and multiple or sequential applications), may be useful to maximize chemical efficacy with flurprimidol.

Although several studies have demonstrated that PGRs that inhibit gibberellin synthesis had no detrimental effect on physiological processes, such as net photosynthesis and respiration (Nelson 1993, Netherland and Lembi 1992), little is known about the carbohydrate distribution in aquatic plants after treatment with these compounds. Changes in carbohydrate allocation may be important for determining the recuperative or regrowth potential of PGR-treated plants. Understanding the physiological changes induced by PGRs is essential for evaluating the use of these compounds as potential aquatic plant management tools. It should be noted that to date none of the chemical manufacturers that produce synthetic PGRs such as flurprimidol, have pursued aquatic registration for these products.

The objectives of this study were: to identify the effects of flurprimidol on the growth of hydrilla, the target weed, and American pondweed, a desirable native plant species, grown under field conditions; to determine how treatment with flurprimidol influences plant carbohydrate status; and to compare the effectiveness of single versus split applications of flurprimidol.

MATERIALS AND METHODS

This experiment was conducted in a mesocosm system at the Lewisville Aquatic Ecosystem Research Facility (LAERF), Lewisville, Texas, which consists of large, outdoor tanks (1.4 m tall by 2.6 m in diameter) that hold approximately 6500 L of water. Each tank was individually plumbed to regulate water flow as needed and was equipped with air flow for water circulation. A holding pond adjacent to the mesocosm system provided a water source for the tanks. Further description of the mesocosm system can be found in Dick et al. (1993).

For this study, each mesocosm tank (12 total) was divided into two equal sections with netting to accommodate both plant species in each tank. The netting allowed water flow between the divided areas but restricted plant growth to each section. Plants were grown in plastic pots (19.7 cm tall by 19.7 cm in diameter) filled with nutrient-enriched soil (1 Woodace briquette (14-3-3) plus 10 g ammonium sulfate per pot). Ten pots of each plant species (4 plants per pot) were placed in each tank section. Hydrilla (dioecious biotype) was propagated from 10-cm apical cuttings and planted 4 to 5 cm into the soil. Hydrilla was supplied by Suwannee Laboratories, Lake City, Florida. American pondweed was initiated from tubers collected at the LAERF. All plants were allowed to establish in the mesocosm tanks for 2 weeks prior to flurprimidol treatment. Established plants were treated on 6 May 1993 with static exposures of either 0, 100, 100 × 2 (split treatment with a second dose applied on 17 June, 6 weeks after the initial dose), and 200 µg/L flurprimidol. Results of residue analyses showed that under similar experimental conditions, flurprimidol had a half-life in water of approximately 8 to 10 days (Chand and Lembi 1994). Therefore, it was estimated for this study that most (95%) of the initial dose of 100 µg/L flurprimidol would have dissipated by the time the second dose was applied.

Prior to the initial treatment, one pot of each plant species was removed from each mesocosm tank and pretreatment shoot length and total plant (shoot and root) biomass was measured. The estimated pretreatment biomass for each tank (the mean dry weight multiplied by the number of pots remaining in each tank ± 1 S.D.) was 40 ± 5 and 5 ± 1 g dry weight for hydrilla and American pondweed, respectively. Average shoot length (± 1 S.D.) at the time of treatment was 18 cm ± 5 for hydrilla and 18 cm ± 3 for American pondweed.

Six weeks after treatment (WAT), 4 randomly selected pots of each plant species were removed from each mesocosm tank and shoot length and shoot and root biomass were measured. Shoot length was measured from the soil surface to the top of the longest leaf or shoot apex. For each pot, shoots and roots were separated, washed to remove algae and debris, and dried to a constant weight at 60 C. Shoot and root biomass were recorded as g dry weight per pot. Stolons and stem bases were included as root biomass. At the conclusion of the experiment, 12 WAT, the remaining 5 pots were removed from the tanks and measured and harvested similarly.

After recording dry weights, shoot and root biomass samples (from each pot) were ground separately using a Cyclone Sample Mill (Udy Corporation, Boulder, CO) to pass through a 1-mm screen. Two 50-mg subsamples from each biomass sample were extracted via autoclaving, and each subsample was analyzed in duplicate for carbohydrate content. Total non-structural carbohydrate (TNC; includes starch, hydrolyzed sugars, and reducing sugars) were determined using a modification of Swank et al. (1982). Extracts were incubated for 15 min at 55 C with one unit of amyloglucosidase (Sigma A-3042) per 0.4 mg dry plant sample to completely hydrolyze starch before assaying for reducing sugars (Nelson 1944). Free reducing sugars was determined on extracts not incubated with amyloglucosidase. Samples were quantified spectrophotometrically at 540 nm (Beckman DU 640) against a standard curve based on starch and sucrose. Starch content was calculated from TNC and free sugars. The concentrations of carbohydrates were calculated as percent dry weight.

Treatments were randomly assigned to mesocosm tanks and were replicated 3 times. Data were subjected to analysis of variance procedures. When significant treatment effects were found, means were separated using the Bonferroni method of multiple comparisons at the 0.05 level of significance.

RESULTS AND DISCUSSION

Both American pondweed and hydrilla showed reduced shoot lengths in response to flurprimidol treatment 6 WAT (Figure 1). Hydrilla was more sensitive at lower rates than American pondweed, with all treatment rates showing the same response. Shoot lengths of treated hydrilla plants were 73% shorter than untreated controls. Shoot lengths of American pondweed decreased as the treatment rate increased. A single dose of flurprimidol at 100 µg/L reduced shoot

lengths by 54%, whereas doubling the treatment rate (200 µg/L) decreased American pondweed shoot lengths by 73%.

Data collected from the final harvest (12 WAT) showed that applying a second dose of flurprimidol prolonged shoot length suppression of both plant species. Compared to untreated plants, hydrilla and American pondweed subjected to a second flurprimidol application had shoot lengths that measured 69 and 34% shorter than untreated plants, respectively. Plants exposed to a one-time application of 100 and 200 µg/L flurprimidol also exhibited reduced shoot lengths compared to untreated plants, however the treatments were not statistically different from one another.

Shoot biomass also was affected by flurprimidol. Flurprimidol-treated hydrilla and American pondweed produced approximately 50% less shoot biomass than untreated plants 6 WAT (Figure 2). There were no differences between treatment rates. Although a second dose of flurprimidol prolonged inhibitory effects on hydrilla shoot length, it did not further affect shoot biomass. Shoot biomass of hydrilla was 50% less than that of untreated plants for all flurprimidol treatments at 12 WAT. In contrast, a differential response among flurprimidol treatments was noted with American pondweed at 12 WAT. Compared to untreated plants, shoot biomass was 86% less with the repeated split treatment of 100 µg/L and averaged 60% less with the 100 and 200 µg/L single dose flurprimidol treatments.

Effects on root biomass were observed only with American pondweed (Figure 2). All flurprimidol treatments showed the same response 6 WAT, with reductions in root biomass averaging 63% that of untreated plants. Inhibitory effects dissipated by the end of the study in plants treated with single doses of 100 and 200 µg/L, but persisted on plants treated with a second dose of 100 µg/L flurprimidol. In fact, there was little change in root biomass from 6 to 12 WAT with plants subjected to second growth regulator application. Statistically significant differences in root biomass were not observed among treatments with hydrilla.

Hydrilla and American pondweed responded differently to flurprimidol treatment with respect to effects on carbohydrate concentration and distribution. For hydrilla, flurprimidol did not influence TNC, free sugar, or starch content in shoots or roots 6 WAT (Table 1). TNC and starch levels were more than three times higher in roots than in shoots for both treated and untreated plants at the first harvest date, which was typical for Texas-grown hydrilla sampled in June (Madsen and Owens 1996). By 12 WAT, shoot TNC and starch content had more than tripled (from 4 to 15%) in untreated hydrilla, whereas levels in treated plants remained low. Starch, TNC, and free sugar in treated shoot tissues averaged 87, 80, and 46% lower, respectively when compared with untreated plants. Statistically, there were no differences

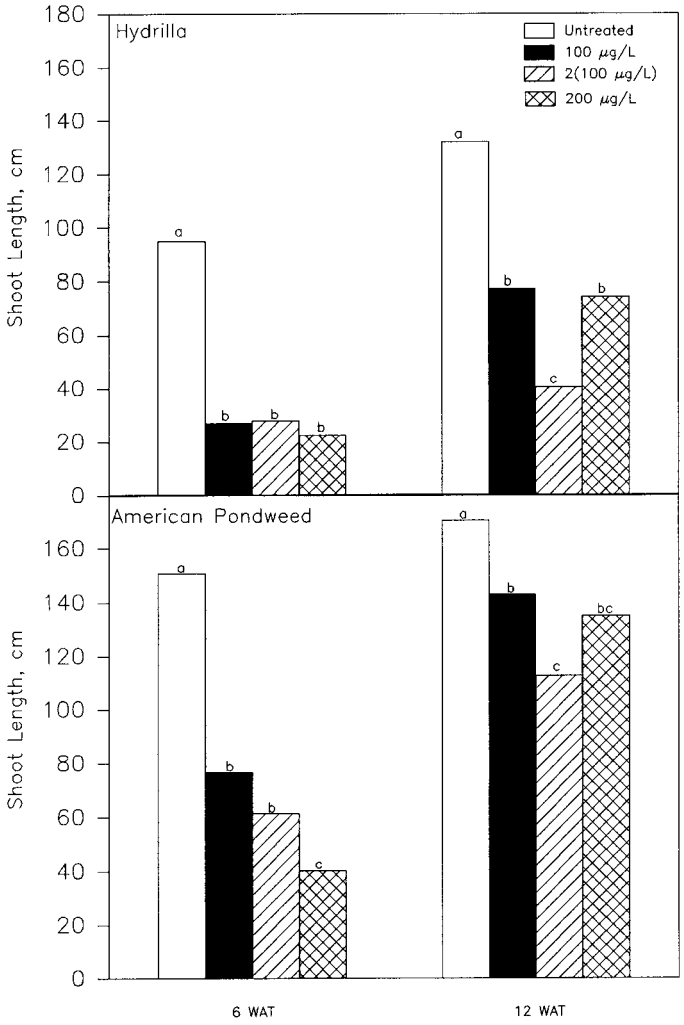

Figure 1. Effect of flurprimidol on shoot length of hydrilla and American pondweed, 6 and 12 weeks after treatment (WAT). Within each sample time, letters above bars indicate significant differences (Bonferroni T test, $P \leq 0.05$).

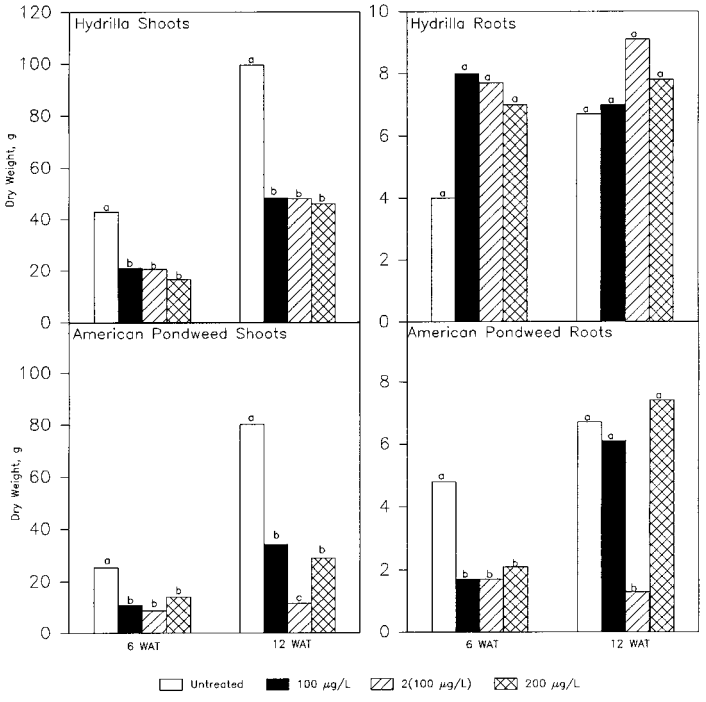

Figure 2. Effect of flurprimidol on shoot and root biomass of hydrilla and American pondweed, 6 and 12 weeks after treatment (WAT). Within each sample time, letters above bars indicate significant differences (Bonferroni T test, $P \leq 0.05$).

Table 1. Effect of flurprimidol treatment on total nonstructural carbohydrate (TNC), free sugar, and starch levels in shoot and root tissues of hydrilla, 6 and 12 weeks after treatment.

	6 Weeks[a]					
	Shoots			Roots		
Flurprimidol	TNC	Free	Starch	TNC	Free	Starch
(µg/L)	(%)	(%)	(%)	(%)	(%)	(%)
Untreated	4.0	1.3	2.5	13.0	2.8	9.2
100	4.3	1.4	2.6	15.3	2.6	11.4
200	2.7	1.4	1.2	18.5	3.5	13.4
100 × 2	2.9	1.2	1.6	15.4	3.2	11.0
	12 Weeks					
	Shoots			Roots		
Flurprimidol	TNC	Free	Starch	TNC	Free	Starch
(µg/L)	(%)	(%)	(%)	(%)	(%)	(%)
Untreated	15.0 a	2.6 a	11.2 a	26.4 a	2.5	21.6 a
100	2.2 b	1.4 b	0.7 b	13.2 b	2.3	9.8 b
200	2.5 b	1.5 b	1.0 b	10.4 b	1.9	7.6 b
100 × 2	4.1 b	1.3 b	2.6 b	27.8 a	2.7	22.6 a

[a]Within a column, means followed by different letters are significantly different (Bonferroni T test, $P \leq 0.05$).

among flurprimidol treatments. Low carbohydrate levels in shoot tissues may reflect carbon utilization or energy expenditure for plant growth. These results were surprising since flurprimidol-treated plants also exhibited significantly reduced shoot lengths and biomass 6 WAT, suggesting decreased plant growth. One would expect high rather than low levels of carbohydrates in tissues with reduced growth demands. This was the observed response in studies by Nelson (1996) in which flurprimidol-inhibited Eurasian watermilfoil accumulated starch in shoots and roots as a result of a reduced growth demand. Carbohydrate accumulation during growth suppression has also been reported for several turfgrass species following PGR treatment (Watschke 1989, Cooper et al. 1988, Hanson and Branham 1987).

Morphological differences between treated and untreated plants noted throughout this study may explain low shoot carbohydrate levels in flurprimidol-inhibited hydrilla. Plants treated with flurprimidol were green and healthy-looking and extremely dense, with an extensive proliferation of stolons; thus plant growth was evident. This horizontal or stoloniferous growth habit has been observed by other researchers following flurprimidol treatment (Netherland and Lembi 1992, Lembi and Chand 1992, Nelson 1993). Flurprimidol-treated plants appeared to utilize carbohydrates for stolon growth, while untreated plants grew to the water surface and produced a full canopy. High carbohydrate levels in shoots and roots of untreated plants indicate resource storage and coincide with topped-out growth conditions. Titus and Adams (1979) reported similar carbohydrate accumulations in Eurasian watermilfoil shoot and root tissues following maximum biomass production (full canopy) in summer.

While differences in root carbohydrates were not observed for hydrilla harvested 6 WAT, significant differences were measured 12 WAT. With treatments of 100 and 200 µg/L flurprimidol, root TNC and starch content was as much as 60% lower than in untreated plants. Root carbohydrate levels were the same for untreated plants as for those subjected to two 100 µg/L applications. There were no differences in percent free sugars among treatments.

Changes in the concentration of carbohydrates as a result of flurprimidol application were observed 6 WAT for American pondweed (Table 2). All flurprimidol treatments reduced shoot TNC, free sugar, and starch content by averages of 84, 65, and 92%, respectively, when compared with untreated plants. Statistically, American pondweed root carbohydrate concentrations were not affected by flurprimidol treatment. By the final harvest, American pondweed treated with 100 and 200 µg/L flurprimidol showed significantly higher shoot TNC and starch levels than untreated plants, whereas plants exposed to a second dose of 100 µg/L flurprimidol had low carbohydrate levels. Shoot carbohydrates of untreated plants changed slightly between harvests. Final root carbohydrate levels were the same for all treatments.

In American pondweed, a heterophyllus plant, submersed leaves are produced first from germinating tubers followed by the production of floating leaves (3 to 4 weeks after tuber germination) that eventually form a surface canopy (Anderson 1982). Typically, once a surface canopy has developed, submersed leaves degenerate at a high rate, in part due to self-shading (Madsen 1991). It was observed in this study that

Table 2. Effect of flurprimidol treatment on total nonstructural carbohydrate (TNC), free sugar, and starch levels in shoot and root tissues of American pondweed, 6 and 12 weeks after treatment.

	6 Weeks[a]					
	Shoots			Roots		
Flurprimidol	TNC	Free	Starch	TNC	Free	Starch
(µg/L)	(%)	(%)	(%)	(%)	(%)	(%)
Untreated	16.0 a	4.6 a	10.3 a	8.9	6.0	2.6
100	3.2 b	1.8 b	1.2 b	9.2	2.9	5.7
200	2.2 b	1.3 b	0.8 b	16.2	4.1	10.9
100 × 2	2.3 b	1.7 b	0.5 b	8.7	3.1	5.1
	12 Weeks					
	Shoots			Roots		
Flurprimidol	TNC	Free	Starch	TNC	Free	Starch
(µg/L)	(%)	(%)	(%)	(%)	(%)	(%)
Untreated	16.4 b	6.4 a	9.1 b	5.7	3.8	1.7
100	23.9 a	6.9 a	15.3 a	9.2	5.8	3.1
200	27.4 a	6.7 a	18.6 a	7.7	4.7	2.7
100 × 2	3.3 c	2.0 b	1.2 c	4.6	3.1	1.4

[a]Within a column, means followed by different letters are significantly different (Bonferroni T test, $P \leq 0.05$).

treatment with flurprimidol delayed the development of floating leaves and the subsequent decline of lower, submersed leaves in this plant species. Although submersed and floating leaf biomass was not separately weighed, it was visually noted that treated American pondweed had more submersed leaf tissue and less surface or floating leaf tissue than untreated plants. Visual estimates of percent canopy cover showed that untreated American pondweed developed a canopy of floating leaves that covered 90 to 95% of the water surface by the end of the study, but plants treated with two applications of 100 µg/L flurprimidol had very few floating leaves, resulting in a canopy cover of only 5%. American pondweed treated with 100 and 200 µg/L flurprimidol had surface canopies of 80 and 70%, respectively by 12 WAT. Lembi and Chand-Goyal (1994) also reported that American pondweed treated with flurprimidol (200 µg/L) had more submersed leaves than untreated plants. Since shading of lower growing species through canopy formation is one strategy for the survival for American pondweed, delayed formation of floating leaves may reduce its competitive edge when growing in a mixed plant community.

The changes in shoot carbohydrate levels of American pondweed after flurprimidol treatment can be explained by the aforementioned changes in submersed and floating leaf production. Low shoot carbohydrates in flurprimidol-treated plants 6 WAT, reflect the plants' expenditure of energy reserves for production of more photosynthetic surface area. Under the influence of flurprimidol, these plants did not produce surface leaves but rather expended energy for submersed leaf production. On the other hand, untreated plants show high levels of TNC and starch at this time. Untreated plants with surface leaves were likely producing more photosynthate than was needed for plant maintenance, thus were storing excess carbohydrates. By 12 WAT, plants treated with 100 and 200 µg/L flurprimidol had overcome most of the initial growth inhibitory effects and had formed a substantial surface canopy (70 to 80% surface area) with which to maximize photosynthesis. These plants were then storing excess photosynthate as were untreated plants. It appears that shoot carbohydrate storage and utilization patterns parallel flurprimidol-induced growth suppression as well as post-inhibition growth patterns in American pondweed.

It is evident from this and other studies (Lembi and Chand-Goyal 1994, Netherland and Lembi 1992) that flurprimidol can affect the growth of many aquatic plants; both target and nontarget species. Wherever maintaining a vegetative component is important for community structure, PGRs could be effective management tools. Further studies to determine how flurprimidol affects the competitive ability of target and nontarget plant species grown together as a mixed community are needed. Furthermore, results of this and other studies (Nelson 1996) indicated that plant carbohydrates can be manipulated with flurprimidol treatment. Additional studies should be conducted to identify whether or not reduced carbohydrate storage in growth-inhibited nuisance plants such as hydrilla could impact overwintering survivability, i.e. effects on tuber production. Tuber production is critical for survival and persistence of hydrilla and, to this point, is resistent to control efforts.

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