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Growth Regulation of Eurasian Watermilfoil with Flurprimidol

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Abstract. Studies were conducted in 55-liter aquariums under controlled environment conditions to evaluate growth regulator effects of flurprimidol [α-(1-methylethyl)-α-[4-(trifluoromethoxy)phenyl]-5-pyrimidinemethanol] on Eurasian watermilfoil (*Myriophyllum spicatum* L.). Treatments included flurprimidol concentrations ranging from 0 to 500 μg L^{-1}, with exposure times varying from 0.25 to 28 days. Extending the flurprimidol contact time increased the growth inhibitory response. Flurprimidol-treated shoots were 14–64% shorter than untreated plants at 14 DAT (days after treatment). Growth inhibition persisted 56 DAT for plants exposed to 25 and 100 μg L^{-1} flurprimidol for 28 days or 200 μg L^{-1} flurprimidol for 10 days. Growth-inhibited plants accumulated starch in shoots and roots, whereas plants showing little or no growth suppression utilized available assimilate for growth. Treatments that most effectively suppressed shoot length accumulated up to 68% more total nonstructural carbohydrate compared with untreated plants. Shoot and root dry weight biomass were unaffected by flurprimidol.

Key Words. Flurprimidol—*Myriophyllum spicatum* L.—Aquatic plant management—Growth regulation

Eurasian watermilfoil (*Myriophyllum spicatum* L.) is considered a nuisance plant species in many lakes, rivers, and reservoirs in the United States because of its rapid growth rate; ability to form dense, monotypic stands with extensive surface canopies; and aggressiveness in displacing desirable native vegetation. This submersed aquatic macrophyte is rooted in the sediment and can be found in waters from 1 to 10 m deep (Grace and Wetzel 1978, Smith and Barko 1990). Problems arise once an impenetrable surface canopy is formed, and navigation and recreational activities are hindered.

In most aquatic systems, maintaining some degree of vegetative cover is important for habitat structure, sediment stabilization, oxygen production, and community diversity. However, when plant growth is excessive, as described previously, management strategies must be implemented. Many of the herbicides currently available for use in aquatic environments are nonselective and result in severe reduction or elimination of most vegetation in the area of treatment. Recent evidence that plant growth regulators (PGRs) can effectively suppress the growth of nuisance aquatic plants without affecting overall plant function has stimulated interest in the potential use of these compounds as an alternate management technique (Klaine and Knowles 1988, Lembi and Chand 1992, Lembi and Chand-Goyal 1994, Nelson and Van 1991, Netherland and Lembi 1992).

Studies have shown that PGRs that inhibit gibberellic acid synthesis, including flurprimidol, paclobutrazol [(2*RS*,3*RS*)-1-(4-chlorophenyl)-4,4-dimethyl-2-(1,2,4-triazol-1-yl)pentan-3-ol], uniconazole [(*E*)-1-(4-chlorophenyl)-4,4-dimethyl-2-(1,2,4-triazol-1-yl)-1 penten-3-ol], and chlormequat chloride [2-chloroethyltrimethyl ammonium chloride], can significantly reduce plant height of Eurasian watermilfoil (Kane and Gilman 1991, Netherland and Lembi 1992). In laboratory bioassay experiments, main stem length of Eurasian watermilfoil was reduced by 67% after a 4-week exposure to 0.75 μg L^{-1} flurprimidol (Netherland and Lembi 1992). Studies conducted in outdoor tanks on rooted plants showed that flurprimidol concentrations higher than those predicted under bioassay conditions (200 vs 0.75 μg L^{-1}) were necessary to inhibit Eurasian watermilfoil (Lembi and Chand 1992). Under these experimental conditions, a lower flurprimidol concentration (75 μg L^{-1}) was effective only when the length of chemical exposure was

Abbreviations: PGR(s), plant growth regulator(s); TNC, total nonstructural carbohydrate; DAT, days after treatment; PVC, polyvinyl chloride; DW, dry weight; BOD, biological oxygen demand; DMSO, dimethyl sulfoxide; LSD, least significant difference.

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extended for 28 days, indicating the importance of a long contact time. Understanding the relationship between concentration and exposure or contact time is critical for achieving chemical efficacy on submersed vegetation (Netherland and Getsinger 1992, Netherland et al. 1991). Further research is needed to develop flurprimidol concentration and exposure time requirements for suppression of Eurasian watermilfoil.

Because the effects of growth regulation are not permanent (length of response varying with chemical and plant species), the potential for regrowth following inhibition must be considered. Several studies on turfgrass report a postinhibition growth surge following application of PGRs (Clapham et al. 1969, Cooper et al. 1988, Dernoeden 1984, Spak et al. 1993, Watschke 1976, 1989). This growth surge often accompanied a change in carbohydrate distribution in the plant. Cooper et al. (1988) found that the carbohydrate content of mefluidide-treated [*N*-[2,4-dimethyl-5[[(trifluoromethyl)sulfonyl]amino]phenyl]acetamide] annual bluegrass (*Poa annua* L.) decreased substantially in leaf, stem, and root tissue following growth inhibition. Field studies by Watschke (1976) reported that Kentucky bluegrass (*Poa pratensis* L.) treated with mefluidide (MBR-12325) had accumulated a higher percentage of total nonstructural carbohydrate (TNC) than untreated turf at 14 days after treatment (DAT). As growth inhibition dissipated, differences in TNC were no longer apparent. Watschke (1989) speculated that temporary storage and reallocation of photosynthates were occurring. Clapham et al. (1969) also concluded that an increased growth rate following inhibition of maleic hydrazide-treated [1,2-dihydro-3,6-pyridazinedione] turf was attributed to the subsequent utilization of carbohydrates that accumulated during growth suppression. Although several studies have shown that PGRs that inhibit gibberellin synthesis had no detrimental effect on physiologic 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 Eurasian watermilfoil. Understanding the physiologic changes induced by PGRs is essential for evaluating the use of these compounds as potential aquatic plant management tools.

The objectives of this study were to identify the effects of flurprimidol concentration and exposure time treatments on the growth of Eurasian watermilfoil and to determine how these treatments influence plant carbohydrate status.

Materials and Methods

The study was conducted at the Waterways Experiment Station in a walk-in environmental chamber equipped with 42 55-liter aquariums. Each aquarium (0.75 m tall by 0.8 m^2) was independently supplied with a simulated hard water solution (Smart and Barko 1984) via peristaltic pumps that when activated, provided a complete water volume exchange every 6 h. Overflow drains maintained the water level at 52 liters. Aquariums were also fitted with separate PVC drain and fill lines to expedite water exchange during the posttreatment drain and refill procedure described later. Air was bubbled through aquariums as a source of carbon dioxide and as a means for mixing the water column. A combination of 400-watt, mercury vapor lamps and 250-watt, high pressure sodium lamps provided overhead lighting. The mean photosynthetically active radiation measured at the water surface was 540 ± 50 $\mu E\ m^{-2}\ s^{-1}$, with a photoperiod (light:dark) cycle of 14:10 h. Water temperature was maintained at 23 ± 3°C throughout the study.

Eurasian watermilfoil was supplied by Suwannee Laboratories, Lake City, FL. Plants were separated into 10-cm apical segments and planted 5 cm deep into 300-mL, sediment-filled glass beakers (four plants per beaker). The sediment was collected from Brown's Lake, Vicksburg, MS, and was amended with commercial fertilizers (Ra-pid-gro 20-15-15 and Osmocote 14-14-14) to avoid nutrient limitations. A thin layer (1–2 cm) of silica sand was added to the sediment surface of each beaker to prevent suspension of sediment during water exchange periods. Ten beakers with plants were placed in each aquarium and were allowed to grow for 9 days before chemical treatment. Each aquarium received a daily exchange of water (via the peristaltic pumps) during the pretreatment growth period.

Immediately before chemical treatment, one randomly selected beaker of plants was removed from each aquarium. Shoot length and total plant (shoot and root) biomass were measured. Shoot length was measured from the sediment/silica sand surface to the top of the longest shoot for each beaker of plants. The estimated pretreatment plant biomass (the mean dry weight multiplied by the number of beakers remaining in the aquarium) and average shoot length (±1 S.D.) for each aquarium were 2.91 ± 0.11 g dry weight (DW) and 20.04 ± 0.23 cm, respectively.

A flurprimidol stock solution was prepared from the commercial formulation Cutless (50% a.i. wettable powder, DowElanco Products Company, Indianapolis, IN) and used for all treatments. At the time of treatment, the flowthrough water system was deactivated (peristaltic pumps turned off), and calculated volumes of the flurprimidol stock solution were added to the aquariums to provide the desired treatment concentrations. A treatment is defined as a flurprimidol concentration ($\mu g\ L^{-1}$) coupled with an exposure or contact time (days). Following the designated exposure times, each aquarium was completely drained and refilled three times to remove flurprimidol-treated water, after which the peristaltic pumps were reactivated to provide a daily water exchange for the duration of the experiment.

After flurprimidol application, visual ratings of plant appearance and vigor were recorded on a weekly basis for the duration of the study (56 DAT). Growth measurements included shoot length and shoot and root biomass. Shoot length was measured per beaker as described previously at 14, 28, and 56 DAT. Plant biomass was harvested at 28 and 56 DAT. For the first harvest, four randomly selected beakers of plants were removed from each aquarium. Shoots were clipped at the sediment surface, rinsed to remove algae, and dried at 70°C for 48 h. The remaining five beakers of plants were harvested for shoot and root biomass at the conclusion of the study. Shoots were harvested as described previously and included autofragments. Roots, including root crowns, were washed to remove sediment and dried as described above.

After recording dry weights, shoot and root biomass samples were ground using a Cyclone Sample Mill (Udy Corporation, Boulder, CO) to pass through a 1-mm screen. Two 50-mg subsamples from each field sample were extracted via autoclaving, and each subsample was analyzed in duplicate for carbohydrate. TNC (starch, hydrolyzed sugars, and reducing sugars) were determined using a modification of the procedure by Swank et al. (1982). Extracts were incubated for 15 min

at 55°C with 1 unit of amyloglucosidase (Sigma A-3042) per 0.4 mg of dry plant sample to completely hydrolyze starch before assaying for reducing sugars (Nelson 1944). Free reducing sugars were 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 was calculated as the difference between TNC and free sugars, multiplied by a factor of 0.9 to account for nonstarch components. The concentrations of carbohydrates were calculated as percent dry weight.

Net photosynthesis and chlorophyll content were also recorded at 14 and 56 DAT. Net photosynthesis, expressed as oxygen evolution (mg of O_2 g^{-1} of fresh weight min^{-1}), was measured using a digital pH meter equipped with a dissolved oxygen electrode (Selim et al. 1989). Two 8-cm apical plant segments from each aquarium were placed in 300-mL biological oxygen demand (BOD) bottles filled with fresh culture medium (one plant segment per bottle) at a known oxygen concentration. The bottles were allowed a 30-min incubation period and were subsequently measured for dissolved oxygen content. Following oxygen readings, the plant segments were removed from the BOD bottles, blotted dry, weighed, and placed in a vial with 10 mL of dimethyl sulfoxide (DMSO) for chlorophyll extraction and later, quantified spectrophotometrically (Hiscox and Israelstam 1979). Total chlorophyll (chlorophyll *a* and *b*) is expressed as mg of chlorophyll g^{-1} fresh weight.

Treatments were arranged in a randomized complete block design with three replicates. Data were subjected to analysis of variance procedures using SAS (SAS Institute 1982). When significant treatment effects were found, means were separated using a protected least significant difference (LSD) test at the 0.05 level of significance.

Results and Discussion

Fourteen DAT, all plants exposed to flurprimidol showed reduced shoot lengths compared with untreated plants (Table 1). Reductions in length ranged from 14 to 64%. For each concentration with various exposure times, extending the exposure period increased the growth inhibitory response. By 28 DAT, all aquariums had been drained and rinsed of flurprimidol-treated water. Five treatments remained as effective inhibitors of shoot length, with the greatest decrease measuring 51% that of untreated plants. Plants exposed to flurprimidol for 28 days grew very little (≈10 cm) from 14 to 28 DAT, whereas plants exposed for less time often doubled in shoot length. By the end of the study, only the 28-day exposures to 25 and 100 μg L^{-1} and a 10-day exposure to 200 μg L^{-1} were significantly different from untreated plants. All three treatments were statistically similar, averaging 29% shorter shoot lengths.

Results were similar to those reported by Lembi and Chand (1992) in that low flurprimidol concentrations required long exposure times to inhibit stem length of Eurasian watermilfoil, but they differed in that growth suppression was not sustained once flurprimidol-treated water was removed from the test system. This was most evident for treatments with short exposure times (less than 10 days). In this study, once flurprimidol was flushed from the aquarium, plant growth resumed, suggesting that Eurasian watermilfoil readily metabolized and/or did not sequester the active ingredient in plant tissues. In contrast, studies by Lembi and Chand (1992) showed that growth regulatory effects persisted for 28 days after a 2-h exposure to 200 μg L^{-1} flurprimidol. Differences in experimental conditions between these two studies may explain the disparity in results. The dissipation characteristics of flurprimidol indicate that this compound has a relatively short half-life in both Eurasian watermilfoil plant tissues and water (8.8 and 9.8 days, respectively) but a long half-life in aquatic sediments (178 days) (Chand and Lembi 1994). As a result of a long sediment half-life, it is likely that flurprimidol remains available for plant uptake after it dissipates or is otherwise removed from the water column. However, the experimental conditions of this study (four plants/300-mL sediment-filled beaker with a layer of silica sand between the water and sediment interface) may have limited the amount of sediment contact with flurprimidol. It is likely that flurprimidol uptake from the sediment played an important role in maintaining growth suppression in studies by Lembi and Chand (1992), as experimental conditions (larger sediment surface area and fewer plants per tank) allowed for maximum water-sediment contact. Although the route by which submersed aquatic plants take up flurprimidol has not been determined, uptake does occur through both leaves and roots of terrestrial plants (Lilly Research Laboratories 1983). Moreover, in turf trials when growth-inhibited plants were removed and washed free of soil treated with either flurprimidol or paclobutrazol, they readily grew to normal size (Watschke 1989).

Although shoot length was reduced by flurprimidol treatment, there were no differences in shoot and root dry

Table 1. Effect of flurprimidol treatments (concentration and exposure time) on shoot length of Eurasian watermilfoil.

Flurprimidol treatment (μg L^{-1}, days)	Shoot length[a] (cm)		
	14 DAT	28 DAT	56 DAT
Untreated	72.2 a	98.1 abc	122.7 ab
25, 28	40.3 ef	50.9 f	92.3 de
100, 0.5	62.1 b	100.8 a	122.3 ab
100, 1	52.6 cd	96.3 a–d	115.7 abc
100, 3	45.8 de	87.8 cd	130.1 a
100, 10	32.7 fg	71.6 e	105.8 bcd
100, 28	32.3 fg	41.3 f	74.5 e
200, 0.25	56.4 bc	98.8 ab	129.3 a
200, 0.5	50.1 cd	93.9 a–d	118.4 abc
200, 1	46.2 de	93.2 a–d	127.2 ab
200, 3	38.7 ef	86.4 d	132.5 a
200, 10	26.0 g	51.7 f	96.0 cde
500, 0.25	51.4 cd	88.3 bcd	118.6 abc
500, 0.5	40.2 ef	88.0 cd	121.9 ab
LSD (0.05)	9.2	10.6	22.8

[a] Within columns, means followed by different letters are significantly different (LSD test, $p \leq 0.05$); DAT, days after treatment.

weight biomass (data not shown). The average harvested biomass (±1 S.D.) at 28 DAT was 4.56 ± 0.27 g for shoots and at 56 DAT, was 13.00 ± 1.51 g and 1.84 ± 0.01 g for shoots and roots, respectively. No measurable differences in biomass may be explained by changes in the growth habits of treated plants. Visual observations recorded throughout the study indicated that those plants most affected by flurprimidol treatment (25 and 100 μg L^{-1} at 28 days and 200 μg L^{-1} at 10 days) had darker green leaves, thickened stems, and appeared more densely foliated due to shortened internodes. Affected plants also developed shortened lateral shoots or branches along both upper and lower stem nodes, which may account for the biomass results. Many of these secondary branches later developed roots and fragmented from the main stem at 35–42 DAT. In contrast, untreated plants grew the height of the water column with additional shoot growth from root crowns (rather than from lower stem nodes) and branched at nodes near the water surface forming a dense canopy by 21 DAT. Once a canopy was formed, lower leaves were sloughed; this did not occur with the aforementioned flurprimidol-treated plants. Although some fragmentation was observed in untreated tanks after canopy formation, the phenomenon was more prevalent on growth-inhibited plants. Self-generated fragments (autofragmentation) are a means of vegetative propagation in Eurasian watermilfoil and normally occur late in the growing season after flowering and maximum biomass production and are the predominant method of intra- and interlake dispersal (Grace and Wetzel 1978, Madsen et al. 1988, Smith and Barko 1990). The potential for premature fragmentation as a result of flurprimidol treatment may have serious implications concerning undesirable dispersion of this plant species and should be further researched. In laboratory bioassay experiments, an increase in lateral bud formation along stem nodes was observed on Eurasian watermilfoil; however, shoot development from these buds did not occur (Netherland and Lembi 1992). Several researchers have reported increased vegetative propagation (extensive stolon proliferation) of hydrilla (*Hydrilla verticillata* (L.f.) Royle) as a result of flurprimidol treatment (Lembi and Chand 1992, Nelson 1993, Netherland and Lembi 1992). Similarly, in turfgrass trials tiller production was enhanced after flurprimidol application (Dernoeden 1984).

Treatment effects on total chlorophyll content and net photosynthesis are summarized in Table 2. Plants exposed to 25 μg L^{-1} flurprimidol for 28 days had significantly higher (23%) chlorophyll levels compared with untreated plants at 14 DAT. Initial effects on net photosynthesis were also observed. Treatment with 100 μg L^{-1} at 28 days and 200 μg L^{-1} at 10 days resulted in lower (41%) photosynthetic rates than untreated plants at 14 DAT. However, plants treated with the same flurprimidol concentrations, and higher (up to 500 μg L^{-1}), at all other exposure times had no effect on photosynthesis, suggesting that the effects are short term. In addition, carbohydrate levels for these treatments were significantly higher than untreated plants at 28 DAT, indicating that the initial decrease in photosynthesis did not negatively affect subsequent photosynthate production. Both parameters measured at 56 DAT showed no significant differences among treatments. Other research shows varying effects of flurprimidol on photosynthesis and chlorophyll content. Netherland and Lembi (1992) reported that flurprimidol had no effect on net photosynthesis, respiration, or chlorophyll content of Eurasian watermilfoil and hydrilla. Nelson (1993) measured higher chlorophyll and net photosynthesis in hydrilla 6 weeks after treatment with 50 and 100 μg L^{-1} flurprimidol. Overall, the response of flurprimidol on net photosynthesis and chlorophyll content of Eurasian watermilfoil does not appear to affect plant viability.

Table 2. Effects of flurprimidol treatments on total chlorophyll content and net photosynthesis of Eurasian watermilfoil.

Flurprimidol treatment (μg L^{-1}, days)	Total chlorophyll[a] (mg chl g^{-1} f wt)		Net photosynthesis (mg O_2 g^{-1} f wt min^{-1})	
	14 DAT	56 DAT	14 DAT	56 DAT
Untreated	1.09 b–e	1.36	0.056 a	0.029
25, 28	1.34 a	1.48	0.045 abc	0.041
100, 0.5	0.95 cde	1.48	0.056 a	0.022
100, 1	0.84 e	1.21	0.056 a	0.029
100, 3	1.04 cde	1.57	0.050 a	0.026
100, 10	1.15 abc	1.32	0.057 a	0.034
100, 28	1.31 ab	1.40	0.034 bc	0.023
200, 0.25	1.10 a–d	1.43	0.051 a	0.027
200, 0.5	1.07 b–e	1.13	0.047 ab	0.025
200, 1	0.88 de	1.32	0.046 abc	0.029
200, 3	0.96 cde	1.53	0.060 a	0.031
200, 10	1.16 abc	1.38	0.032 c	0.022
500, 0.25	1.05 cde	1.82	0.057 a	0.028
500, 0.5	0.87 de	1.38	0.060 a	0.029
LSD (0.05)	0.25	N.S.	0.016	N.S.

[a] Within columns, means followed by different letters are significantly different (LSD test, $p \leq 0.05$); DAT, days after treatment; N.S., not significant.

Results of carbohydrate analyses showed that flurprimidol treatment did affect the concentration and distribution of plant carbohydrates in Eurasian watermilfoil (Table 3). At 28 DAT, TNC content was significantly higher in shoots of plants treated with 25 and 100 μg L^{-1} flurprimidol at 28 days and 200 μg L^{-1} flurprimidol at 10 days. Differences ranged from 45 to 68% that of untreated plants, with plants exposed to flurprimidol for 28 days showing the most effect. The predominant form of TNC was as starch with no change in percent free sugars (data for free sugar not shown). High levels of starch in shoot tissues would be expected for these treatments because of a reduced growth demand. Low

Table 3. Effect of flurprimidol treatment on total nonstructural carbohydrate (TNC) and starch levels in shoot and root tissues of Eurasian watermilfoil.

Flurprimidol treatment ($\mu g\ L^{-1}$, days)	28 DAT[a]		56 DAT			
	Shoots		Shoots		Roots	
	TNC (%)	Starch (%)	TNC (%)	Starch (%)	TNC (%)	Starch (%)
Untreated	5.8 de	3.1 de	20.7	16.3	9.2	4.9 c
25, 28	14.5 ab	10.5 ab	36.3	29.9	17.8	10.6 ab
100, 0.5	5.4 e	2.8 e	24.1	19.5	10.4	5.9 bc
100, 1	6.3 cde	3.4 de	24.5	19.6	9.5	5.1 c
100, 3	9.0 cde	5.8 cde	21.5	16.5	10.5	5.3 c
100, 10	7.1 cde	4.2 cde	29.0	23.2	9.9	5.1 c
100, 28	18.0 a	13.7 a	29.9	24.4	18.5	12.6 a
200, 0.25	8.0 cde	5.1 cde	19.2	15.2	8.0	3.7 c
200, 0.5	7.5 cde	4.4 cde	23.5	18.5	10.3	5.8 bc
200, 1	6.5 cde	3.8 cde	18.0	14.0	11.0	5.6 bc
200, 3	10.3 bcd	6.9 bcd	24.6	19.4	10.2	5.3 c
200, 10	10.6 bc	7.5 bc	27.9	22.3	18.1	12.0 a
500, 0.25	7.7 cde	4.4 cde	21.7	17.5	15.3	8.0 abc
500, 0.5	9.4 cde	6.2 cde	23.3	18.7	9.2	5.0 c
LSD (0.05)	4.6	3.9	N.S.	N.S.	N.S.	5.1

[a] Within columns, means followed by different letters are significantly different (LSD test, $p \leq 0.05$); DAT, days after treatment; N.S., not significant.

levels of starch for all other treatments including untreated plants reflect carbohydrate utilization.

Shoot TNC for all treatments was not significantly different at 56 DAT. Treated plants still measuring significantly shorter shoot lengths at this time continued to display elevated TNC levels. However, TNC levels of uninhibited plants had increased substantially from 28 to 56 DAT. This increase was presumably in response to crowded or topped-out growth conditions. All but two flurprimidol treatments, 25 and 100 $\mu g\ L^{-1}$ at 28 days, had formed a canopy layer (topped-out) at the water surface and flowered by the end of the study. Once Eurasian watermilfoil becomes space limited, such as late in the growing season when plants have formed a full canopy, TNC levels will begin to increase, indicating a shift from assimilate consumption to that of storage (Titus and Adams 1979). Percent starch in shoot tissues also showed a substantial increase from 28 to 56 DAT. Titus and Adams (1979) concluded that a conversion from consumption to storage after maximum biomass production reflects resource accumulation before a season of stress (winter).

Although root TNC was not significantly different among treatments at the 0.05 level ($p = 0.0579$), levels were higher for plants treated with 25 and 100 $\mu g\ L^{-1}$ at 28 days and 200 $\mu g\ L^{-1}$ at 10 days and did translate to significantly higher ($p = 0.0153$) starch concentrations. Treated roots had an average of 58% more starch compared with untreated plants. The percent starch in roots was not different among all other treatments. Overall, results of carbohydrate analyses showed that growth-inhibited plants stored carbohydrate as starch in shoots and roots, whereas plants showing little or no inhibitory effects utilized available assimilate for growth. Accumulation of TNC during growth suppression and subsequent utilization once growth inhibition subsides have also been reported for various turfgrass species following application of flurprimidol and other growth retardants that inhibit gibberellin synthesis (paclobutrazol and mefluidide) (Cooper et al. 1988, Hanson and Branham 1987, Watschke 1976).

In summary, results of this study indicate that flurprimidol can regulate the growth of Eurasian watermilfoil. Low concentrations (25 and 100 $\mu g\ L^{-1}$) suppressed stem height, but longer contact times (28 days) were required to maintain efficacy. Moreover, increasing the flurprimidol concentration was not as effective as increasing the exposure time. Inhibitory effects were temporary. Under these experimental conditions, when flurprimidol-treated water was removed from the system (after designated exposure time periods), plant growth resumed. This suggests either that Eurasian watermilfoil does not sequester flurprimidol or that metabolism of this compound is rapid. Steffens (1988) speculated that rapid growth after inhibition may be related to a buildup of gibberellic acid precursors that become available for gibberellin biosynthesis once inhibitors, such as flurprimidol, are no longer present or have become diluted or inactivated. In addition, our data show that plants affected by flurprimidol accumulate TNC, which also be-

comes accessible for renewed growth. Further research is needed to characterize regrowth potential under field conditions and to determine the effects of flurprimidol on autofragmentation.

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References

Chand T, Lembi CA (1994) Dissipation of gibberellin synthesis inhibitors in small-scale aquatic systems. J Aquat Plant Management 32:15–20

Clapham AJ, Wakefield RC, Bell RS (1969) The effect of maleic hydrazide on the growth of turf grasses. University of Rhode Island Agri Exp Sta Bull 399, pp 1–22

Cooper RJ, Street JR, Henderlong PR, Koski AJ (1988) An analysis of the carbohydrate status of mefluidide-treated annual bluegrass. Agron J 80:410–414

Dernoeden PH (1984) Four-year response of a Kentucky bluegrass-red fescue turf to plant growth retardants. Agron J 76:807–813

Grace JB, Wetzel RG (1978) The production of Eurasian watermilfoil (*Myriophyllum spicatum* L.): A review. J Aquat Plant Management 16:1–11

Hanson KV, Branham BE (1987) Effects of four growth regulators on photosynthate partitioning in "Majestic" Kentucky bluegrass. Crop Sci 27:1257–1260

Hiscox JD, Israelstam G (1979) A method for the extraction of chlorophyll from leaf tissue without leaf maceration. Can J Bot 57:1332–1334

Kane ME, Gilman EF (1991) In vitro propagation and bioassay systems for evaluating growth regulator effects on *Myriophyllum* species. J Aquat Plant Management 29:29–32

Klaine SJ, Knowles BJ (1988) Plant growth regulators: Strategy for use in aquatic plant management schemes. In: Kaufman JE, Westerdahl HE (eds) Chemical vegetation management. Plant Growth Regulator Society of America, pp 102–115

Lembi CA, Chand T (1992) Response of hydrilla and Eurasian watermilfoil to flurprimidol concentrations and exposure times. J Aquat Plant Management 30:6–9

Lembi CA, Chand-Goyal T (1994) Plant growth regulators as a potential tool in aquatic plant management: Efficacy and persistence in small-scale tests. Contract Report A-94-1, US Army Engineer Waterways Experiment Station, Vicksburg, MS

Lilly Research Laboratories (1983) Technical report on EL-500. Eli Lilly and Company, Indianapolis, IN

Madsen JD, Eichler LW, Boylen CW (1988) Vegetative spread of Eurasian watermilfoil in Lake George, New York. J Aquat Plant Management 26:47–50

Nelson LS (1993) Plant growth regulator effects on four aquatic plant species. Proceedings of the 27th Annual Meeting, Aquatic Plant Control Research Program, miscellaneous paper A-93-2, US Army Engineer Waterways Experiment Station, Vicksburg, MS

Nelson LS, Van TK (1991) Growth regulation of Eurasian watermilfoil and hydrilla using bensulfuron methyl. Aquatic Plant Control Research Program, Bulletin A-91-1, US Army Engineer Waterways Experiment Station, Vicksburg, MS

Nelson N (1944) A photometric adaptation of the Somogyi method for determination of glucose. J Biol Chem 153:375–380

Netherland MD, Getsinger KD (1992) Efficacy of triclopyr on Eurasian watermilfoil concentration and exposure time effects. J Aquat Plant Management 30:1–5

Netherland MD, Green WR, Getsinger KD (1991) Endothall concentration and exposure time relationships for the control of Eurasian watermilfoil and hydrilla. J Aquat Plant Management 29: 61–67

Netherland MD, Lembi CA (1992) Gibberellin synthesis inhibitor effects on submersed aquatic weed species. Weed Sci 40:29–36

SAS Institute (1982) SAS user's guide. SAS Institute, Inc., Cary, NC

Selim SA, O'Neil SW, Ross MR, Lembi CA (1989) Bioassay of photosynthetic inhibitors in water and aqueous soil extracts with Eurasian watermilfoil (*Myriophyllum spicatum*). Weed Sci 37: 810–814

Smart RM, Barko JW (1984) Culture methodology for experimental investigations involving rooted submersed aquatic plants. Miscellaneous paper A-84-6, US Army Engineer Waterways Experiment Station, Vicksburg, MS

Smith CS, Barko JW (1990) Ecology of Eurasian watermilfoil. J Aquat Plant Management 28:55–64

Spak DA, DiPaola JM, Lewis WM, Anderson CE (1993) Tall fescue sward dynamics. II. influence of four plant growth regulators. Crop Sci 33:304–310

Steffens GL (1988) Gibberellin biosynthesis inhibitors: Comparing growth-retarding effectiveness on apple. J Plant Growth Regul 7:27–36

Swank JC, Below FE, Lamber RJ, Hageman RH (1982) Interaction of carbon and nitrogen metabolism in the productivity of maize. Plant Physiol 70:1185–1190

Titus JE, Adams MS (1979) Comparative carbohydrate storage and utilization patterns in the submersed macrophytes, *Myriophyllum spicatum* and *Vallisneria americana.* Am Midland Naturalist 102:263–272

Watschke TL (1976) Growth regulation of Kentucky bluegrass with several growth retardants. Agron J 68:787–791

Watschke TL (1989) PGRs for turfgrass management. PGRSA Q 17: 5–13