Response of Hydrilla and Eurasian Watermilfoil to Flurprimidol Concentrations and Exposure Times

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ABSTRACT

Hydrilla and Eurasian watermilfoil grown out-of-doors in 67-L barrels with bottom sediment were exposed to different concentrations and exposure times of the gibberellin synthesis inhibitor, flurprimidol ([a-(1-methylethyl)-a-(4-trifluoromethoxy) phenyl]-5 pyrimidinemethanol). Two-hour exposures to the compound significantly reduced vertical main stem length at concentrations of 75 and 750 μg L^{-1} for hydrilla and 200 μg L^{-1} for Eurasian watermilfoil for at least 28 days post-treatment. Treated hydrilla plants produced more stolons per plant than untreated plants resulting in a "rug-like" carpet on the bottom of the barrel. Based on our results, field studies on the reduction of vertical plant height by gibberellin synthesis inhibitors appear to be warranted.

Key words: gibberellin synthesis inhibitors, *Hydrilla verticillata, Myriophyllum spicatum*, plant growth regulation, PGRs, submersed aquatic macrophytes.

INTRODUCTION

Laboratory bioassays (Netherland and Lembi 1991) have shown that inhibitors of gibberellin synthesis such as flurprimidol, paclobutrazol ([2RS,3RS)-1-(4-chlorophenyl)-4-dimethyl-2-(1,2,4-triazol-1-yl) pentan-3-ol]), and uniconazole ((E)-1-(4-chlorophenyl)-4,4-dimethyl-2-(1,2,4-triazol-1-yl)-1 penten-3-ol) can reduce plant height while maintaining physiological competence in hydrilla [*Hydrilla verticillata* (L. f.) Royle] and Eurasian watermilfoil (*Myriophyllum spicatum* L.). This finding suggests the potential for a new strategy in aquatic plant management: the use of plant growth regulators to keep these plants short (i.e. no longer weedy) while allowing them to retain their useful functions in the aquatic environment such as oxygen production and fish habitat.

Here we report on exposure of hydrilla and Eurasian watermilfoil to flurprimidol when plants were grown out-of-doors in 67-L barrels with bottom sediment. We monitored plant heights, dry weights, and in the case of hydrilla, the number of stolons produced. We were also interested in determining the exposure time required to achieve successful stem length reduction.

MATERIALS AND METHODS

Metal barrels (67-L capacity) were lined with plastic liners and set in an unshaded outdoor area. Loam soil (free from plant growth regulators, herbicides and other pesticides) was added to a 10-cm depth in each barrel. Approximately 55 L of well water was added, and the suspended soil was allowed to settle. Stem apices (10 cm length) without roots of healthy milfoil (from Martel pond, Tippecanoe Co., Indiana) and dioecious hydrilla (from laboratory culture, originally supplied by Dr. Stephen J. Klaine) were planted in separate barrels (two stems per barrel) and allowed to acclimate for 1 week prior to flurprimidol treatment. Flurprimidol (50% WP, Elanco Products Company, Indianapolis, IN) was applied by diluting the compound in approximately 10 ml of water and then stirring the solution into the barrel, without disturbing the soil, to insure even dispersal. Flurprimidol concentrations were 0, 75 and 750 μg L^{-1} for hydrilla and 0, 7.5, 75 and 200 μg L^{-1} for milfoil.

Treatment dates in 1989 were June 5 for milfoil and July 31 for hydrilla. Treatment dates in 1990 were June 1 for milfoil and July 31 for hydrilla. Each treatment date for each species was a separate experiment, and barrels within an experiment were arranged in a randomized complete block. The plants were exposed to flurprimidol for 2 hours, 1, 3, 7, 14, and 28 days, although the hydrilla exposure times in 1990 were limited to 1 and 2 hours, and 1 and 3 days. Flurprimidol concentrations in the 1990 milfoil test were monitored by gas chromatography and decreased by 89% over the 28 day period (unpubl. data). In a previous study (Chand and Lembi 1991) approximately 88% of the flurprimidol had dissipated from a similar test system. Therefore, the plants were not exposed to a constant flurprimidol concentration during the test period; however, we will use the term exposure time to indicate the time interval between treatment and the removal of the treated water. After exposure, the water was removed from the barrels (including untreated controls) by siphoning, and new untreated water was added in a manner to minimize sediment disturbance. After four weeks in untreated water, the plants were harvested. Therefore, plants that had been exposed to flurprimidol (including the 0 μg L^{-1} concentration) for 2 hours, 1 and 3 days were harvested at approximately 4 weeks after treatment. The plants that had been exposed to flurprimidol (including the 0 μg L^{-1} concentration) for 7, 14, and 28 days were harvested at 5, 6, and 8 weeks, respectively, after treatment.

Stem lengths of harvested plants were taken, using a cm ruler, on vertical main stems only. Stolon length was not measured since our major interest was in vertical length, but the number of stolons was counted in the 1990 hydrilla experiment. The plants were then dried at 70 C for 48 hours and weighed.

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Each exposure time/concentration combination consisted of two replicates. Plant lengths and weights in the untreated barrel replicates from the 2 hour, 1 and 3 day exposure times were combined and are referred to as 4 week-old controls; plant lengths and weights in untreated barrel replicates from the 7, 14 and 28 day exposure times represent 5, 6, and 8 week-old controls, respectively. The data were analyzed using analysis of variance, and the means of each parameter measured at each date were separated using the Student-Newman-Keuls multiple range test at the 95% confidence level (Zar 1974).

RESULTS AND DISCUSSION

Untreated hydrilla grew best in 1989. Main stem lengths ranged from 42-45 cm in all control barrels. This compared with a stem length of only 20 ± 1 (SE) cm in the 4-week old controls in 1990. In contrast, the untreated milfoil plants grew best in 1990, with main stem lengths averaging 59 ± 8 cm in 1990 versus 35 ± 7 cm in 1989 in the 4-week-old controls and 86 ± 2 cm in 1990 versus 53 ± 3 cm in 1989 in the 8-week-old controls. This difference may have been due to the growing conditions for those years. The summer of 1989 was dry and warm, conditions well suited for hydrilla growth. In contrast, the summer of 1990 was cool and very cloudy, conditions that may have been better suited for milfoil growth and perhaps somewhat detrimental to hydrilla growth, even though we always initiated our milfoil experiments early in the summer while it was still relatively cool and hydrilla later in the summer when it was warmer. In general, the hydrilla did not elongate as much as the milfoil but did produce more biomass, mostly due to extensive lateral branching. Only the data for the years with the better growing conditions, 1989 for hydrilla and 1990 for milfoil, will be presented here except where noted.

Main stem lengths in hydrilla were significantly reduced at all exposure times at both concentrations of flurprimidol (75 and 750 μg L^{-1}) (Figure 1A). After 2 h exposure and 4 weeks recovery, main stem length at 75 μg L^{-1} was 64% of the main stem length of the 4-week-old control; at 750 μg L^{-1} main stem length was 43% of that of the 4-week-old control. At exposure periods of 3 days and longer, main stem lengths at 75 and 750 μg L^{-1} were approximately 40-47% and 30-42%, respectively, of the main stem lengths of the controls. In 1990, flurprimidol exposure for only 1 h resulted in significantly reduced stem lengths compared to the controls (75 and 60% of the control length at 75 and 750 μg L^{-1}, respectively). Almost all treated plants elongated at least a little from their initial 10 cm length. The two flurprimidol concentrations also were effective in the laboratory bioassay on hydrilla (Netherland and Lembi 1991).

Flurprimidol appeared to cause a decrease in hydrilla dry weights when compared to the untreated controls (Figure 1B). The effects were greatest at the 750 μg L^{-1} concentration and at 1 day or longer exposure. In general, biomass did not seem to be affected by flurprimidol as much as main stem length; for example, the main stem length of plants exposed for 28 days to 75 μg L^{-1} was reduced by 54% but dry weight was reduced by only 34%.

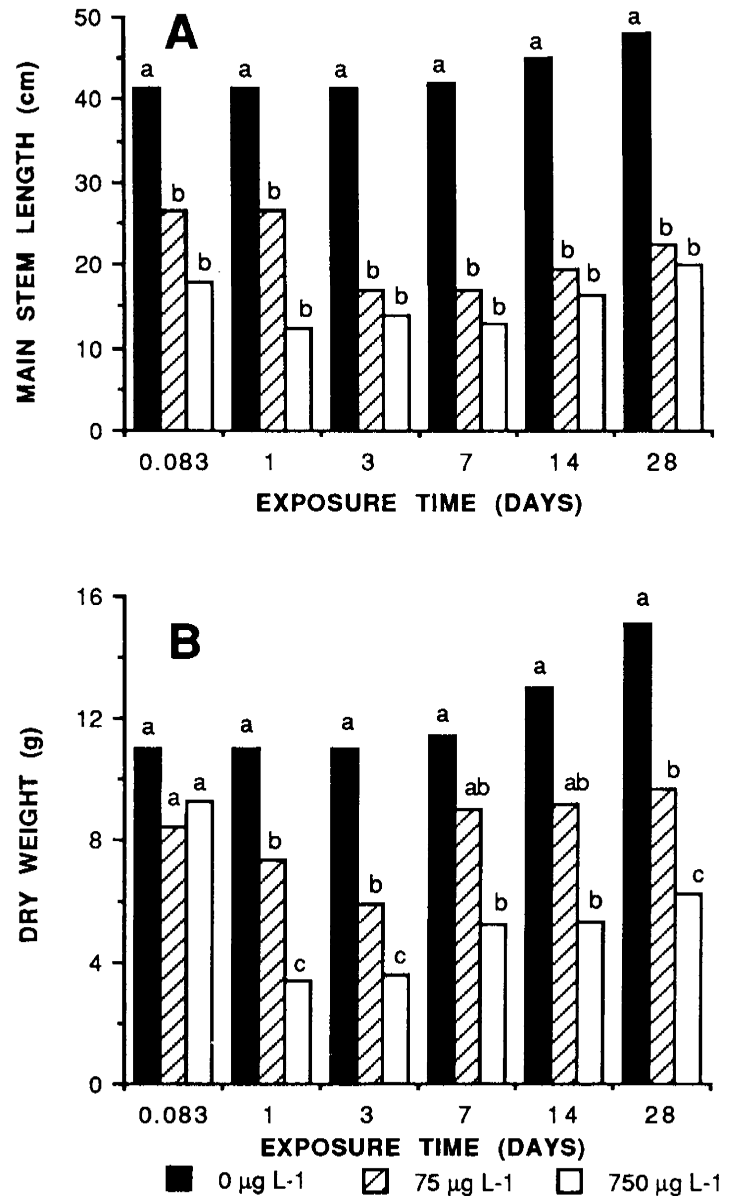

Figure 1. Effect of exposure time of flurprimidol on (A) mean main stem length and (B) mean dry weight in hydrilla in 1989. The plants were allowed to recover in untreated water for four weeks following exposure. Water in barrels with untreated (0 μg L^{-1}) plants was replaced in exactly the same way as barrels with treated plants. 0.083 days = 2 hours. Bars with similar letters within an exposure time are not significantly different ($P>0.05$) based on an SNK test.

The reason for this was probably due to the proliferation of stolons on the treated plants. In 1990, treated plants had an average of 2 ± 0.5 and 4 ± 0.75 stolons at 75 and 750 μg L^{-1}, respectively, compared to only 1 ± 0 stolon per untreated plant. Because of the stoloniferous growth, treated hydrilla formed a "rug-like" carpet on the bottom of the barrel. This was in contrast to the untreated plants which produced the typical elongated stems with a surface canopy. In addition to stolons, treated hydrilla tended to produce more erect (but shortened) vertical main stems at the points of rooting (Figure 2). Although we did not count the number of main stems in this study, Netherland (1989)

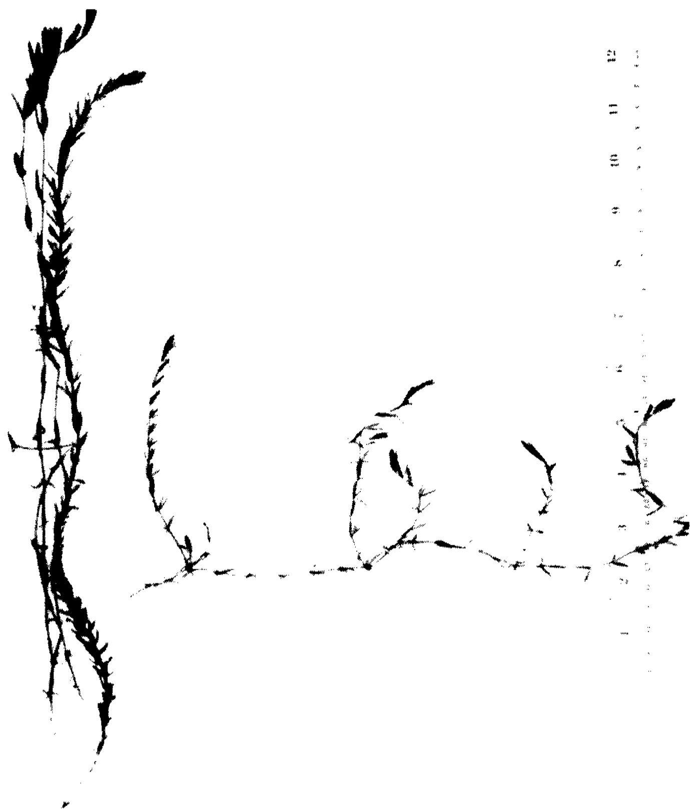

Figure 2. Hydrilla plant treated with 75 $\mu g\ L^{-1}$ flurprimidol on untreated hydrilla plant on the left. Note difference in overall heig the stolon production in the treated plant. The plants are orienteu similarly with respect to the substrate.

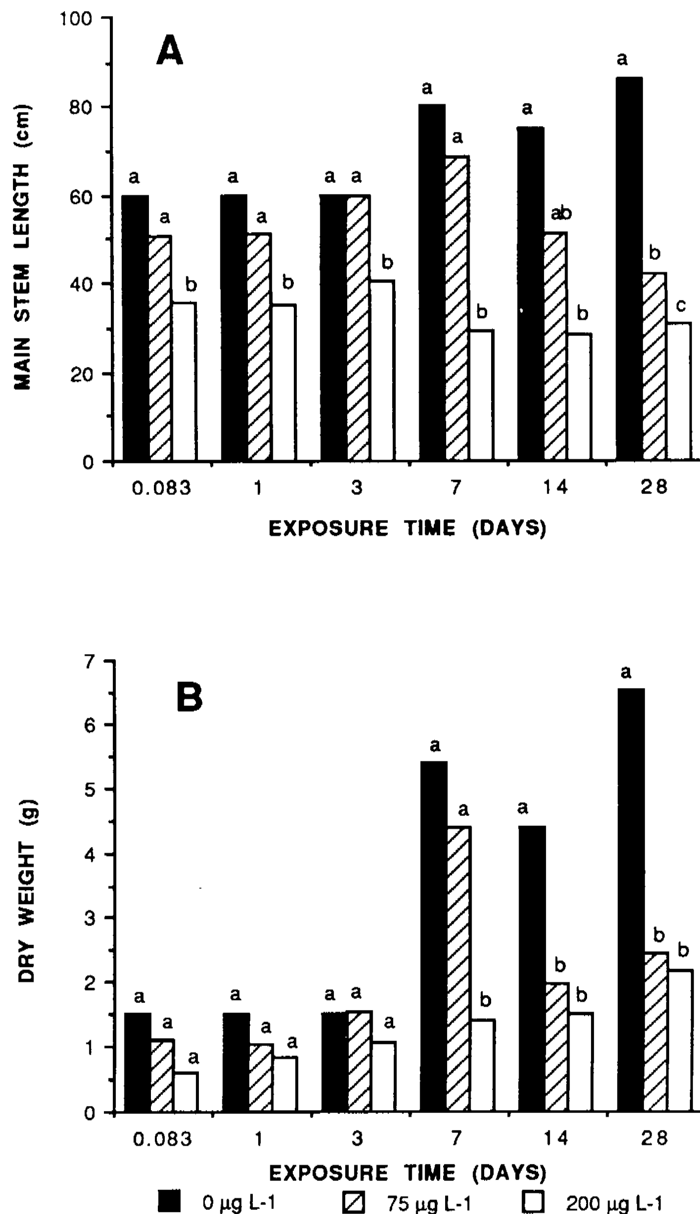

Figure 3. Effect of exposure time of flurprimidol on (A) mean main stem length and (B) mean dry weight in milfoil in 1990. The plants were allowed to recover in untreated water for four weeks following exposure. Water in barrels with untreated (0 $\mu g\ L^{-1}$) plants was replaced in exactly the same way as barrels with treated plants. 0.083 days = 2 hours. Bars with similar letters within an exposure time are not significantly different ($P>0.05$) based on an SNK test.

found significantly more main stems produced by hydrilla grown in barrels and exposed to uniconazole than by untreated plants.

In 1989, a poor growth year for milfoil in this study, there was a trend toward decreased stem lengths with increased exposure times. However, except for the 14-day exposure, the means were not significantly different between treated and untreated plants at the two concentrations tested, 7.5 and 75 $\mu g\ L^{-1}$. In 1990, we used flurprimidol concentrations of 75 and 200 $\mu g\ L^{-1}$. At all exposure times, milfoil showed reduced stem lengths at 200 $\mu g\ L^{-1}$ (Figure 3A). Only after the 28 day exposure did flurprimidol at 75 $\mu g\ L^{-1}$ begin to produce a significant reduction in stem length. Main stem lengths at 200 $\mu g\ L^{-1}$ were 37-65% of the main stem lengths of the untreated controls. Dry weights were significantly different from untreated controls at 7, 14 and 28 day-exposures for the 200 $\mu g\ L^{-1}$ treatments and at 14 and 28 day-exposures for 75 $\mu g\ L^{-1}$ treatments (Figure 3B). Stolons were seldom produced by milfoil although treated plant stems had a tendency to lie on the sediment surface. The lack of stolons and prolific lateral branching is probably the reason that, compared to hydrilla, dry weight appeared to be more reduced than main stem length.

The effective concentration of 200 $\mu g\ L^{-1}$ for milfoil was considerably higher than the concentrations predicted by the laboratory bioassay. The bioassay suggested that concentrations as low as 0.75 $\mu g\ L^{-1}$ would be effective in reducing milfoil stem growth. The reason for this difference may have been the source of the milfoil. In the laboratory bioassay, milfoil plants grown in culture medium were used. In this study, the milfoil was collected from the field and probably differed considerably from the cultured material in terms of its physiological condition. The field-collected plants seemed to be more robust than the plants grown in culture and were also lightly encrusted with calcium carbonate so that flurprimidol uptake may have been reduced. However, even 200 $\mu g\ L^{-1}$ is a relatively low concentration, and these studies plus the laboratory bioassay suggest that milfoil may be sensitive to a wide range of

concentrations depending on its growth status and ambient environmental conditions. Hydrilla plants grown in culture were used for both the bioassay and barrel studies (since hydrilla is not yet present in Indiana waters). Therefore, under natural growing conditions, hydrilla may require higher concentrations of flurprimidol than those suggested by this study.

Our results indicate that flurprimidol can reduce main stem lengths of hydrilla and Eurasian watermilfoil under outdoor culture conditions and that only short exposure times of 1 to 2 h may be required for significant stem length reduction. Further studies are needed to determine if the plants that still show reduced main stem lengths even after exposure to untreated water for 4 weeks retain the flurprimidol in their tissues or take it up from the sediments over the 4 week recovery period. When flurprimidol is added to a plant-sediment-water barrel system, approximately 88% of the flurprimidol dissipates after 4 weeks; however, the majority of the remaining flurprimidol is present in the water and the top 5 cm of sediment (Chand and Lembi 1991). This suggests that when the water is flushed from the system, flurprimidol in the sediment may still be available for plant uptake. We plan to continue our studies on flurprimidol efficacy and dissipation in small ponds in 1992.

ACKNOWLEDGMENTS

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Growth of Monoecious Hydrilla on Different Soils Amended with Peat or Barley Straw

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ABSTRACT

Monoecious hydrilla (*Hydrilla verticillata* (L. f.) Royle) was grown in six soil types amended with two levels of barley straw or peat to test the hypothesis that substrate organic matter would cause reduced growth. Soil type significantly influenced hydrilla dry weight and weight of tubers produced during 8 weeks of growth under outdoor conditions. Also, increased organic matter content (measured as loss on ignition) of the substrate over the range of 1.5 to 27.2% was associated with increased growth of hydrilla. Of 14 substrate properties, multiple regression revealed that the square root of Kjeldahl N and the square root of soil conductivity were the best predictors of hydrilla weight. These results suggest that variability in the responses of rooted aquatic plants to substrate organic matter content reported previously may be partially explained by considering properties of the organic matter, especially nutrient content.

Key words: Hydrilla verticillata (L. f.) Royle., submersed aquatic macrophyte, tubers, Kjeldahl N, organic matter, conductivity, nutrients.

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INTRODUCTION

Hydrilla is an introduced plant that has caused serious problems in many aquatic systems in the United States. Growth requirements and capabilities of the monoecious strain of hydrilla appear to differ in important ways (i.e., responses to photoperiod and perhaps temperature, and allocation of dry matter to tubers and turions) from those of the dioecious strain (Spencer and Anderson 1986; Spencer *et al.* 1987; Steward and Van 1987; Van 1989). These differences may influence its response to management techniques developed for plants of the dioecious strain.

One management approach involves altering the substrate or rooting medium. Sediment covers such as sand, gravel, or plastic sheeting and dredging have been used to this end (Barko *et al.* 1986). Altering organic matter content of sediments also has been proposed as a potential method for managing aquatic plants (Gunnison and Barko 1989). In green house experiments, increased organic matter content of sediments was identified as the cause of reduced growth of Eurasian watermilfoil (*Myriopyyllum spicatum* L.) and dioecious hydrilla (Barko and Smart 1983, 1986). McFarland and Barko (1987) compared growth of monoecious and dioecious hydrilla on sediments with two