

Laboratory Evaluation of Mefluidide Effects on Elongation of Hydrilla and Eurasian Watermilfoil

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ABSTRACT

The potential of mefluidide (N-(2,4-dimethyl-5[[trifluoromethyl] sulfonyl] amino] phenol) acetamide) to act as a submersed aquatic plant growth regulator was evaluated using a laboratory bioassay system. Main stem elongation of hydrilla (*Hydrilla verticillata* (L.f.) Royle) and Eurasian watermilfoil (*Myriophyllum spicatum* L.) was effectively reduced by mefluidide at low concentrations. The lowest effective concentration of mefluidide that reduced stem length in Eurasian watermilfoil (100 µg a.i./L) was 5 times lower than that

for hydrilla (500 µg a.i./L). Short-term net photosynthetic rates of these plants were not affected by mefluidide at concentrations as high as 1000 µg a.i./L. The minimum exposure time required to maintain an inhibitory effect for at least 28 days at a concentration of 500 µg a.i./L was 3 to 7 days for Eurasian watermilfoil and 7 to 14 days for hydrilla. The results suggest that mefluidide is a more effective growth regulator for Eurasian watermilfoil than hydrilla. Exogenously applied gibberellic acid (GA) did not completely overcome the inhibitory effect of mefluidide even when GA was added at a high concentration (10⁻⁵ M). In addition, the internodal lengths of stems treated with mefluidide were not reduced as they were when treated with gibberellin synthesis inhibitors. The reduction of main stem elongation by mefluidide appeared to be due to the inhibition of new cell and tissue development at the stem tip rather than from inhibition of GA biosynthesis.

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INTRODUCTION

Two of the most invasive weedy species in the United States are the submersed plants hydrilla and Eurasian watermilfoil. The major method for their control is the use of aquatic herbicides. Although aquatic herbicides are effective in most cases, there is always the potential for unintended results, such as the depletion of oxygen in the water due to vegetation kill, the lack of selectivity, and the excessive loss of vegetation. Too little vegetation can result in sediment erosion, loss of oxygen production, and lack of habitat for fish and fish food organisms.

Lembi (1988) proposed a strategy to manage excessive growth of hydrilla and Eurasian watermilfoil yet retain the beneficial aspects of aquatic vegetation by using plant growth regulators (PGRs). Plant growth regulators are synthetically produced compounds, some of which alter the development and growth of terrestrial species at very low concentrations by inhibiting endogenous plant hormone biosynthesis. Many of the negative impacts of invasive submersed species such as hydrilla and Eurasian watermilfoil result from their ability to form a canopy of vegetation at the surface of the water (Madsen et al. 1991). If PGRs can reduce the vertical growth rate of nuisance submersed plants, canopy formation could be prevented and the beneficial roles provided by underwater vegetation could be retained.

Mefluidide is registered worldwide as a PGR (Li 1991). It inhibits plant stem elongation and floral initiation and increases maximum root length (Baron 1989, Cooper 1988). Mefluidide is currently used commercially to reduce vertical growth and suppress seedhead formation of turfgrasses, but it also has been used to inhibit fruit set and vegetative growth of ornamental shrubs, hedges, trees, and groundcovers (WSSA 1994). It has several characteristics that might make it attractive for use in aquatic plant management such as rapid absorption into stem and leaf tissue, rapid degradation in soils (half-life is 2 days), and low mammalian and fish toxicity (WSSA 1994). The mode of action of mefluidide at cellular and molecular levels is not clearly known (Li 1991), although growth and development of meristems appear to be inhibited (WSSA 1994).

Another group of PGRs, the gibberellin synthesis inhibitors, is effective in reducing stem length in hydrilla and Eurasian watermilfoil (Netherland and Lembi 1992, Lembi and Chand 1992). These compounds inhibit plant elongation by preventing cell elongation in the internodal regions of the stem. In an effort to determine if other types of PGRs have similar properties, we evaluated the potential of mefluidide to inhibit hydrilla and Eurasian watermilfoil elongation under laboratory conditions.

MATERIALS AND METHODS

Plant culture. A laboratory bioassay system developed by Netherland and Lembi (1992) was used to determine the effective concentration of mefluidide to reduce stem elongation in hydrilla and Eurasian watermilfoil. Algal-free dioe-

cious hydrilla and Eurasian watermilfoil were maintained in stock cultures by transferring plants to fresh, liquid media every 20 to 25 days. The media used for hydrilla and Eurasian watermilfoil were 10% Hoagland's medium and inorganic Gerloff's solution, respectively. After the media were autoclaved (25 min at 250 C), NaHCO₃ solution (4 g/100 ml) was added at 6.7 ml/L and 5.0 ml/L to the hydrilla and Eurasian watermilfoil media, respectively. The NaHCO₃ solution was pre-filtered through a 0.45 µm membrane filter (Gelman metrical membrane filter) to remove contaminants. The plants were grown under constant conditions of 300 µmol photons/m²/s, 25 ± 1 C and 16 hr light : 8 hr dark in a controlled environment growth chamber.

Dose response. Single 4-cm length lateral stems were excised from stock culture plants and placed in 500-ml Erlenmeyer flasks containing 300 ml media. Mefluidide was added at concentrations of 0, 10, 100, 500, 1000, 5000 and 10,000 µg/L of active ingredient (a.i.) to the flasks containing hydrilla and Eurasian watermilfoil. The plants were grown under the same conditions as the stock cultures for four weeks prior to measurement.

Length of exposure. Based on the results of the dose response experiments, a concentration of 500 g a.i./L mefluidide was chosen to determine how long the plants had to be exposed to the chemical in order for inhibitory effects to be retained for 28 days after exposure. Hydrilla and Eurasian watermilfoil were treated for 1, 3, 7, 14 and 28 days. After plants had been exposed to the mefluidide for the desired time, they were rinsed carefully and thoroughly with about 50 ml fresh mefluidide-free culture media. The plants were then transferred to mefluidide-free fresh media (300 ml media per 500 ml flask) and returned to the growth chamber for another 28 days. A set of untreated plants was handled in the same way and for the same length of time. Plants that were treated (or untreated) for 1 day, transferred to fresh medium, and then grown for another 28 days had a total growing time of 31 days. Plants that were treated (or untreated) for 28 days, transferred to fresh medium, and then grown for another 28 days had a total growing time of 56 days. These differences in total growing times resulted in significant differences in stem lengths of untreated plants (Figure 3).

Effects of mefluidide on net photosynthetic rate. Hydrilla and Eurasian watermilfoil were treated with 0 and 1000 µg a.i./L mefluidide in order to determine the effect of mefluidide on short-term photosynthetic rates. Net photosynthetic rates were measured by monitoring oxygen evolution with a digital pH meter after plants had been exposed to mefluidide for 1, 3, 7, or 14 days. For these measurements, treated and untreated plants were transferred to a cylindrical Plexiglas chamber (volume about 340 ml) filled with fresh medium, which had been bubbled with 0.5% CO₂ for 1 min before measurement to prevent CO₂ limitation. The chamber was divided internally by a perforated plate so that the plant placed in one side of the chamber was physically separated from the electrode which was placed in the other side of the chamber. The chamber top was sealed with a circular Plexiglas plate coated with stopcock grease, and a small hole in the plate allowed the electrode to be inserted into the chamber. The chamber was placed on a magnetic stir plate at 300 µmol photons/m²/s ± 10% (the same as the growth chamber) and 25 ± 1 C to measure oxygen evolu-

tion. Oxygen concentration was measured at the beginning and at 5-min intervals for 20 min in order to determine net photosynthetic rate ($\text{mg O}_2/\text{g fr.wt./hr}$). The fresh weight of each plant was taken using a 0.001 gram precision electrical balance after excess water on the plant was wiped off with a paper towel.

Effects of mefluidide/GA mixture. To determine whether exogenous gibberellic acid (GA) can reverse the inhibitory effect of mefluidide when GA and mefluidide were supplied at the same time, four treatment sets for both species were used: control, mefluidide only, GA only, and a GA/mefluidide mixture. A gradient of GA concentrations were used in either the GA only or GA/mefluidide mixture: 10^{-8} , 10^{-7} , 10^{-6} , 10^{-5} M. Mefluidide was added at a concentration of $1000 \mu\text{g a.i./L}$ for the mefluidide only treatment. For the GA/mefluidide mixture, mefluidide at $1000 \mu\text{g a.i./L}$ was added first, and then GA was added to the desired concentration. After treatment, the plants were allowed to grow for 17 days prior to measurement.

Parameters measured and statistical analysis. At the end of each experiment, main stem length, stem internode number, lateral stem number and lateral stem length were measured. All length measurements were taken with a centimeter ruler, and plant dry weights were taken after plants were dried in an oven at 70 C for 48 hr. Internode lengths were estimated by dividing the stem length by the stem internode number.

All experiments were repeated at least once. The data were pooled and subjected to ANOVA analysis with the SuperAnova software package (Abascus Concepts, Inc. 1992). All data were checked for their variance homogeneities and distribution normalities before ANOVA analysis was conducted. Means of each parameter measured were separated by using the Student-Newman-Keul (SNK) multiple range test at a 95% confidence interval. The level of significance (α) is 0.05 unless otherwise stated.

RESULTS AND DISCUSSION

Plant growth. The bioassay system used in these experiments provided a rapid method for analyzing the effects of mefluidide on both hydrilla and Eurasian watermilfoil. The nutrient and bicarbonate supply in the media appeared to be sufficient for growth of both plants for 28 days. Untreated plants showed normal morphology and color. They produced lateral stems and roots but did not flower or, in the case of hydrilla, produce tubers. Untreated hydrilla and Eurasian watermilfoil stems grew at an average rate of 0.38 cm/day and 0.22 cm/day , respectively, over the 28-day period.

Dose response. Mefluidide inhibited stem elongation of both plant species (Figure 1). Visually, the inhibitory effects of mefluidide became more obvious as concentration increased. Symptoms of mefluidide-treated plants included shorter main stems and more lateral stems than the untreated plants.

Mefluidide concentrations of 10 and $100 \mu\text{g a.i./L}$ had no significant effect on main stem length of hydrilla (Figure 2). A significant reduction (37%) in average main stem length was obtained at a mefluidide concentration of $500 \mu\text{g a.i./L}$. At $1000 \mu\text{g a.i./L}$, plants were still green and had a normal stem and leaf structure (Figure 1A) except that the leaves were brittle to the touch. At $5000 \mu\text{g a.i./L}$ or higher, the main stems had

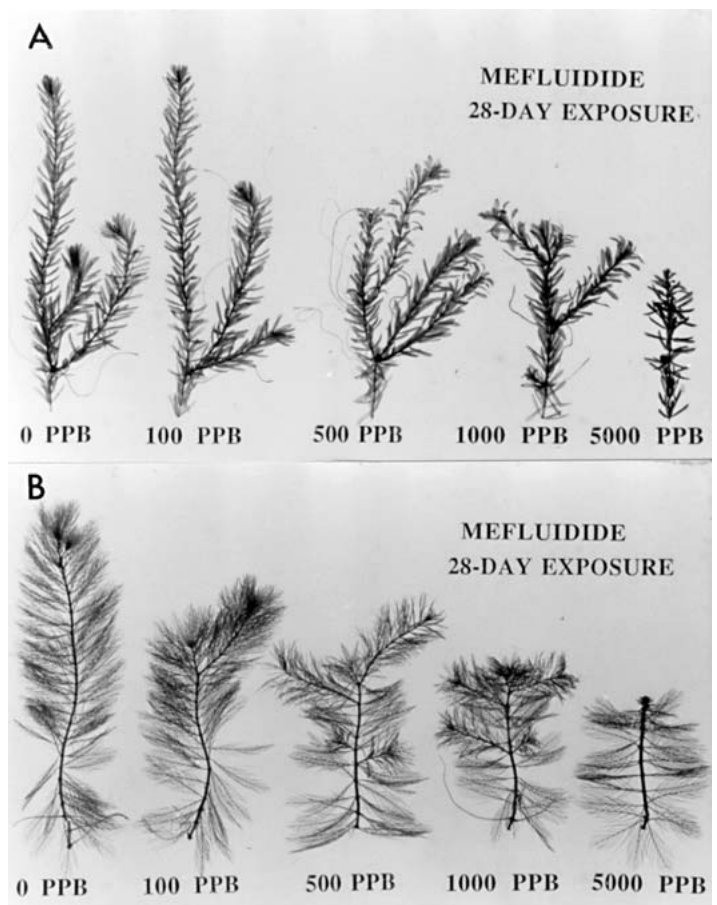


Figure 1. Mefluidide effects on hydrilla (A) and Eurasian watermilfoil (B).

grown less than 2 cm after 4 weeks and the plants were brittle, red in color, and lacked lateral stem and root production.

In contrast to hydrilla, a mefluidide concentration of $100 \mu\text{g a.i./L}$ significantly reduced average main stem length of Eurasian watermilfoil (Figure 2). Lengths were reduced by 20%, 35.4%, and 43.1% at 100, 500, and $1000 \mu\text{g a.i./L}$ mefluidide, respectively. At $1000 \mu\text{g a.i./L}$, the main stem had elongated less than 2 cm after 28 days growth, but the plants retained a relatively normal morphology (Figure 1B). At concentrations of 5000 and $10,000 \mu\text{g a.i./L}$, Eurasian watermilfoil plants also became red and lacked lateral stem and root production.

Internode lengths of untreated and treated hydrilla (range of 0.29 to 0.31 cm per internode) were almost the same. Similarly, internode lengths of untreated and treated Eurasian watermilfoil (range of 0.33 to 0.38 cm per internode) were also approximately the same. No significant difference in internode length was found between untreated and treated plants at any mefluidide concentration (data not shown). Thus, the basic difference between the main stems of untreated and mefluidide-treated plants was that, after an initial period of growth from the original 4-cm length, the treated plants stopped producing new nodes or internodes at the stem apices. The main stems of treated plants looked as though they had been clipped off at the apical meristem (Figure 1). This is in contrast to the effects of gibberellin synthesis inhibitors,

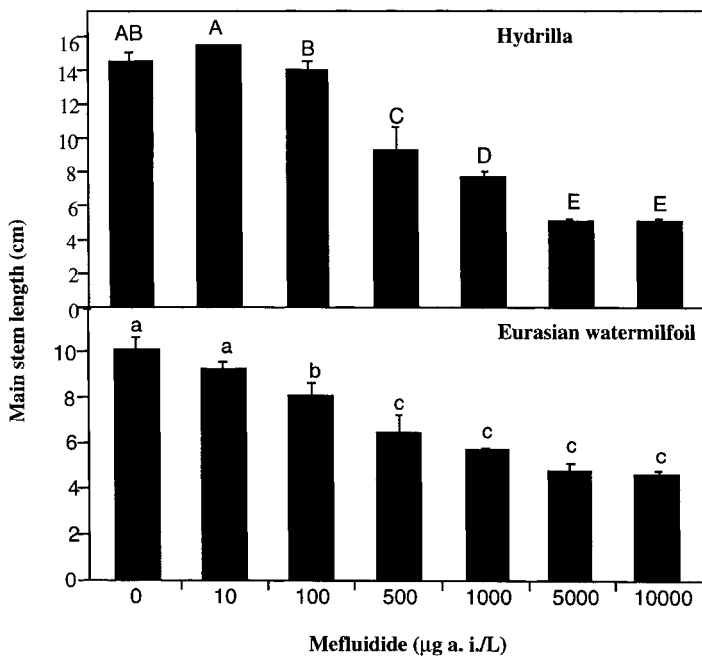


Figure 2. Mean main stem lengths of hydrilla and Eurasian watermilfoil treated with different concentrations of mefluidide. Initial length was 4 cm. Bars with similar letters within each species are not significantly different.

in which new nodes and internodes are produced throughout the treatment period (Netherland and Lembi 1992). Overall plant height is reduced in gibberellin-synthesis-treated plants because cell elongation is inhibited, resulting in much shorter internode lengths than those of untreated plants. Our results suggest that mefluidide inhibits main stem elongation by preventing new growth at the apical meristem instead of inhibiting cell elongation at the internodes.

Although mefluidide appeared to inhibit new growth at the apex of the main stem it did not appear to inhibit either the formation of lateral stems or their elongation (Figure 1). Lateral stem numbers of both plants were significantly higher at mefluidide concentrations of 500 and 1000 µg a.i./L than those of untreated plants. Lateral stem number per untreated hydrilla plant averaged 1.7 ± 0.3 (\pm SE) compared to 3.0 ± 0.6 among plants treated with 500 µg a.i./L. Lateral stem number per untreated Eurasian watermilfoil plant averaged 0.2 ± 0.1 compared to 4.7 ± 0.7 among plants treated with 500 µg a.i./L. Interestingly, the lengths of these treated lateral stems ($5.2 \text{ cm} \pm 1.0$ for hydrilla; $1.5 \text{ cm} \pm 0.1$ for Eurasian watermilfoil) at 500 µg a.i./L were not significantly different from the lateral stem lengths of the untreated plants. The number of lateral stems per plant sharply decreased to less than one in hydrilla at 5000 µg a.i./L, and no lateral stems were produced in Eurasian watermilfoil at 5000 and 10,000 µg a.i./L.

Treatment of hydrilla with gibberellin synthesis inhibitors (Netherland and Lembi 1992) also resulted in increased lateral stem production, but in contrast to mefluidide-treated plants, the lateral stem lengths were much reduced due to the inhibition of cell elongation in the internodes. Gibberellin synthesis inhibitor treatment of Eurasian watermilfoil resulted either in no lateral bud and stem production or in

the production of lateral buds that never elongated, depending on the specific inhibitor used and dosage.

The apparent difference in effects between main stem elongation versus lateral stem number/elongation in mefluidide-treated plants may be due to one or more causes. Mefluidide may accumulate at the main stem tip, thus inhibiting its development. McWhorter and Wills (1978) showed that mefluidide moves acropetally in terrestrial plants and accumulates at actively growing sites. Unfortunately, we know nothing about the distribution of mefluidide in submersed plants that are bathed in a mefluidide/water solution, only that differential effects of the compound suggest an uneven distribution of mefluidide in the plant after it enters. Lower concentrations of mefluidide further down the hydrilla or Eurasian watermilfoil stem may actually stimulate lateral stem formation and have little if any effect on lateral stem length. Low concentrations of mefluidide were reported to stimulate tillering in grasses (WSSA 1994) and to enhance growth in general (Li 1991). One effect of the differential distribution of mefluidide may be on indoleacetic acid (IAA) activity. Glenn and Rieck (1985) showed that mefluidide affected IAA transport in corn (*Zea mays* L.) coleoptiles and soybean (*Glycine max* [L.] Merr.) hypocotyls. High mefluidide concentrations (10^{-3} M and higher) inhibited IAA transport while low concentrations (10^{-4} M and lower) stimulated transport. The production and basipetal transport of IAA from the stem tip is known to inhibit the elongation of lateral stems (Salisbury and Ross 1992). High mefluidide concentrations in the main stem tips of hydrilla and Eurasian watermilfoil may reduce basipetal IAA transport so that lateral stems are produced and elongate.

Clearly, a point was reached when mefluidide concentrations appeared to be herbicidal. Both main stem tip growth and lateral stem production were inhibited at 5000 and 10,000 µg a.i./L in both species. Further, although photosynthetic rates were not monitored at these high concentrations, the plants appeared unhealthy. Mefluidide was once sold as a terrestrial herbicide at concentrations higher than those currently used when applied as a terrestrial plant growth regulator.

Another possible reason for differences in responses between the main stem apex and nodal areas further down the stem is the possibility that different parts of the shoot may have differing sensitivities to mefluidide; i.e. the main stem tip may be more sensitive to mefluidide than nodal areas. Glenn and Rieck (1985) suggested that differences in species responses to mefluidide could be due to differing rates of metabolism of the compound. The possibility that differences in metabolism could account for differences either in tissue susceptibility or in submersed species susceptibility needs to be explored.

Length of exposure. The final main stem lengths of treated and untreated plants of both species were not significantly different until after 7 to 14 days and 3 to 7 days exposure to 500 µg a.i./L mefluidide for hydrilla and Eurasian watermilfoil, respectively (Figure 3). These results indicate that, in order to maintain an inhibitory effect for at least 28 days following treatment, the exposure time for Eurasian watermilfoil was shorter than that for hydrilla. This result, plus the fact that Eurasian watermilfoil was affected by lower mefluidide concentrations than hydrilla, suggest that mefluidide is more effective as a growth regulator on Eurasian watermilfoil than on hyd-

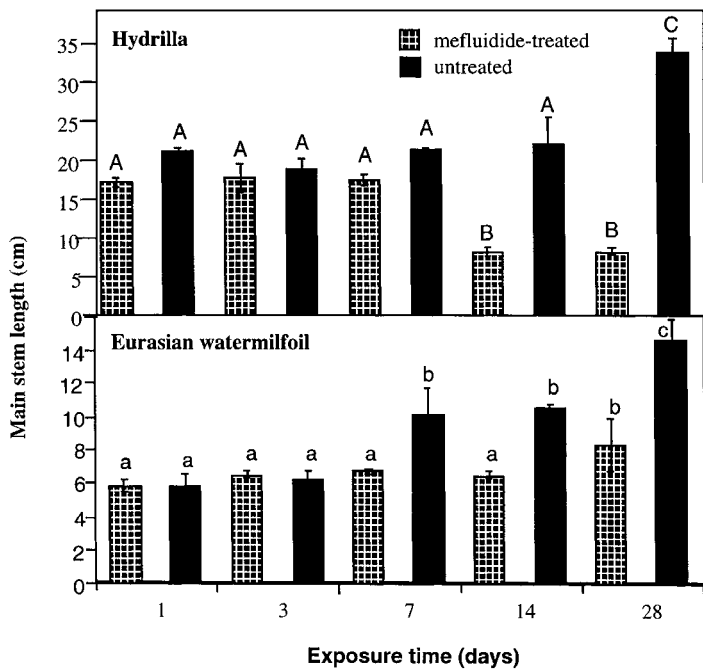


Figure 3. Effect of exposure time to mefluidide (500 μg a.i./L) on main stem lengths of hydrilla and Eurasian watermilfoil. Plants were allowed to recover in untreated media for 4 weeks following exposure. Bars with similar letters within an exposure time are not significantly different.

rilla. As a terrestrial herbicide, mefluidide was more effective for the control of weedy grasses (monocots) than broadleaved plants (dicots); however, when used in terrestrial systems as a plant growth regulator, mefluidide affects a wide variety of both terrestrial monocot and dicot species. Thus, the greater sensitivity of Eurasian watermilfoil, a dicot, to mefluidide, in contrast to that of hydrilla, a monocot, probably cannot be ascribed to differences in dicot/monocot characteristics.

Effects of mefluidide on net photosynthetic rate. Net photosynthetic rates of untreated and treated hydrilla were slightly reduced when measured 1 day following treatment whereas net photosynthetic rates of untreated and treated Eurasian watermilfoil appeared to be stimulated 1 day after treatment (Figure 4). After this initial exposure period, net photosynthetic rates of the treated and untreated plants of both species appeared to level off and were essentially the same at the end of the 14-day incubation period. Statistical comparison of the combined data for the treated plants at all measurement times versus the untreated plants at all measurement times for each species revealed no significant differences.

These results suggest that the plants remained healthy and metabolically active at a concentration of mefluidide (1000 μg a.i./L) that clearly inhibits main stem elongation. The net photosynthetic rates monitored in this study were similar to those monitored when hydrilla and Eurasian watermilfoil were exposed to non-toxic doses of gibberellin synthesis inhibitors that also inhibited stem elongation (Netherland and Lembi, 1992).

Effects of mefluidide/GA mixture. Wilkinson (1982) reported that mefluidide blocks the conversion of kaurene to kaurenol during the biosynthesis of GA. If this is the case, treat-

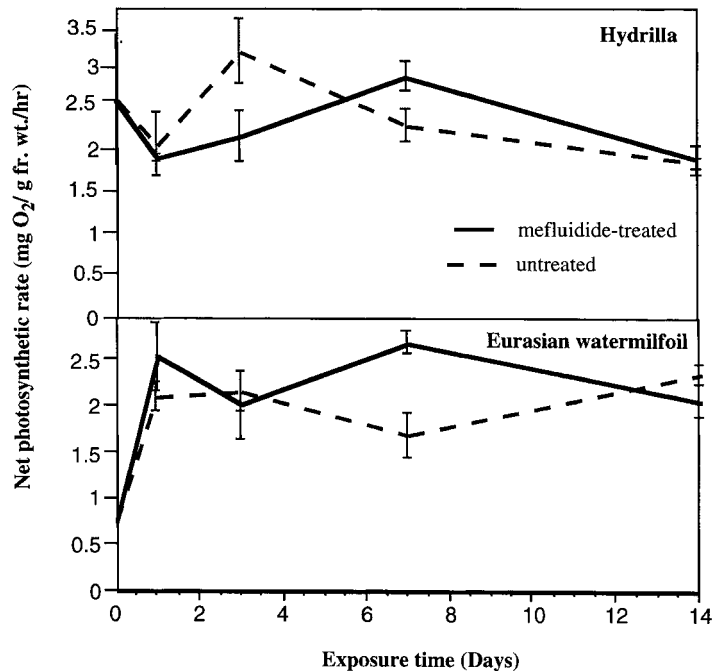


Figure 4. Effect of mefluidide (1000 μg a.i./L) on net photosynthetic rates of hydrilla and Eurasian watermilfoil after 1, 3, 7, and 14 days exposure.

ment with mefluidide should reduce the synthesis of GA, resulting in short internodes. Furthermore, the addition of GA to mefluidide-treated plants should overcome the mefluidide effects because the added GA could compensate for the loss of GA biosynthesis. To test the latter hypothesis, hydrilla and Eurasian watermilfoil plants were exposed to various GA/mefluidide combinations.

Compared with the untreated plants, 1000 μg a.i./L mefluidide reduced the main stem length of hydrilla by 31% (Figure 5: compare "untreated" with "mef"). Mefluidide did not significantly reduce main stem length of Eurasian watermilfoil, perhaps because the 17 day incubation period was too short for the untreated plants to elongate sufficiently (overall growth of untreated Eurasian watermilfoil plants is slower in culture than that of hydrilla, see 0 μg a.i./L data in Figure 2). Stem elongation of GA-only treated plants and GA/mefluidide treated plants was stimulated in both species at 10^{-6} M and 10^{-5} M GA (Figure 5). When treated with GA only, 10^{-6} M GA caused an increase in main stem length of hydrilla and Eurasian watermilfoil by 28% and 101%, respectively, over untreated controls; at 10^{-5} M GA, the increase in main stem length of hydrilla and Eurasian watermilfoil was 134% and 461%, respectively. However, when plants were treated with a mixture of GA (10^{-6} M)/mefluidide, the main stem length of hydrilla remained unchanged and the main stem length of Eurasian watermilfoil increased by 36% compared to untreated plants. When plants were treated with a mixture of GA (10^{-5} M)/mefluidide, the main stem length of hydrilla still remained unchanged and the main stem length of Eurasian watermilfoil increased by 203% compared to untreated plants (Figure 5).

If mefluidide only inhibits an enzyme in the pathway of endogenous GA biosynthesis, plants should completely recover from the inhibitory effect of mefluidide with the addition of

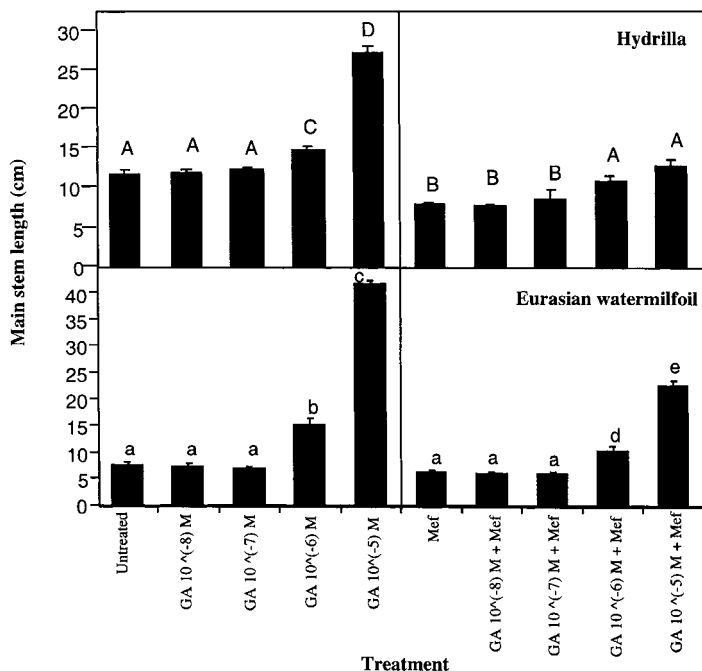


Figure 5. Mean main stem lengths of hydrilla and Eurasian watermilfoil grown under different treatment combinations. Mef denotes mefluidide at 1000 $\mu\text{g a.i./L}$. Bars with the same letter within each species are not significantly different.

high concentrations of exogenously applied GA. In our study, the stem and internode length of GA only-treated plants increased as expected over those of untreated plants. The number of internodes on GA-treated and untreated plants was similar, suggesting that length increase was due to cell elongation, not the production of more internodes. However, when plants were treated with a GA/mefluidide mixture, even when the GA concentration was 10^5 M in the mixture, both plant main stem lengths and internode lengths of hydrilla and Eurasian watermilfoil were shorter than those of GA-only treated plants. Thus, exogenously applied GA was not able to completely overcome the inhibitory effect of mefluidide even when GA was at a very high concentration (10^5 M). These results support those of Truelove et al. (1971) who also noted that addition of GA did not overcome mefluidide effects in terrestrial plants.

Interestingly, the main stem lengths of GA/mefluidide treated plants at 10^6 and 10^5 M GA concentrations were longer than those of mefluidide-treated plants, suggesting that GA may be able to partially overcome mefluidide effects. However, the GA/mefluidide results taken as a whole, plus the observation that internode lengths of mefluidide-treated plants are not reduced as they are when plants are treated with gibberellin synthesis inhibitors, suggest that mefluidide has some role other than inhibiting GA biosynthesis or activity. The lack of normal tissue development at the main stem tip indicates that mefluidide affects cell division or tissue development rather than internode elongation.

Li (1991) suggested that mefluidide may have an effect on membrane structure. Plants treated with mefluidide showed an increase in the amount of phospholipid produced in cell membranes. Zhang and Chen (1991) also reported that the amount of unsaturated fatty acids in biomembranes from seedlings

treated with mefluidide was higher than that from untreated plants. Thus, mefluidide may facilitate the biosynthesis of certain species of phospholipids, which leads to changes in membrane composition and function. Such changes in membrane structure could lead to differences in cell metabolism or in the uptake or transport of a hormone such as IAA, which in turn can have an impact on how plant tissues react to mefluidide.

Mefluidide appears to have potential as a plant growth regulator for submersed aquatic plants. The inhibition of main stem tips and the increased production of lateral stems in both hydrilla and Eurasian watermilfoil may translate into stoloniferous growth that is characteristic of hydrilla treated with gibberellin synthesis inhibitors (Lembi and Chand 1992). The production of a carpet-like growth on the sediments would be excellent for erosion control. However, a number of questions must be answered with larger-scale and field tests, including the potential for laterals to elongate rather than remain short; the effect on propagule production; the duration of effect when water residence times, and consequently mefluidide concentration, vary; and species selectivity.

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