

TA7  
W34c  
no. A-94-1  
c. 3

Contract Report A-94-1  
January 1994

US Army Corps  
of Engineers

Waterways Experiment  
Station

*Aquatic Plant Control Research Program*

# **Plant Growth Regulators as Potential Tools in Aquatic Plant Management: Efficacy and Persistence in Small-Scale Tests**

*by Carole A. Lembi, Tara Chand-Goyal  
Purdue University*

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by Carole A. Lembi, Tara Chand-Goyal

Department of Botany and Plant Pathology  
Purdue University  
West Lafayette, IN 47907-1155

**Final report**

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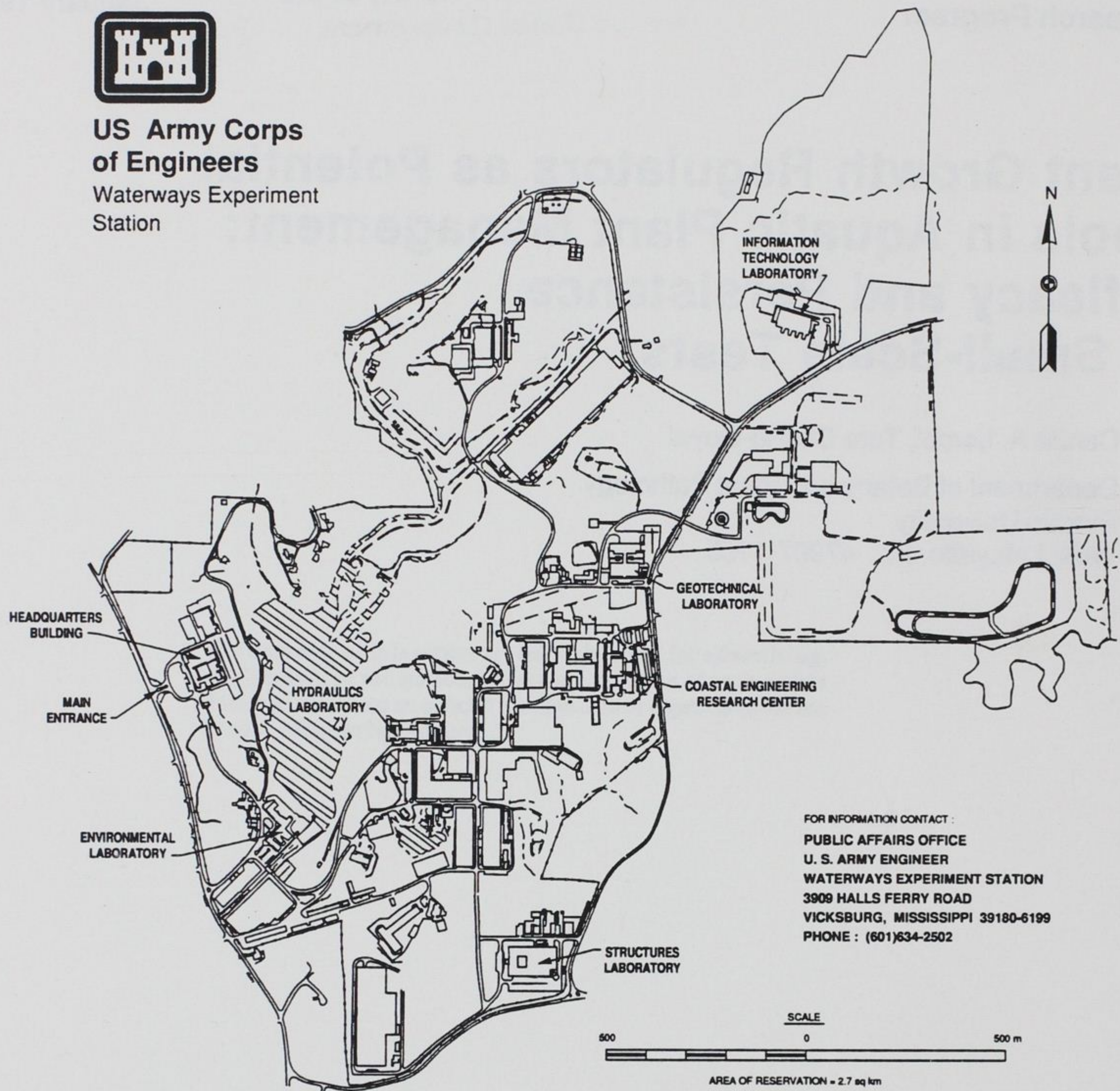
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U.S. Army Engineer Waterways Experiment Station  
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FOR INFORMATION CONTACT :  
PUBLIC AFFAIRS OFFICE  
U. S. ARMY ENGINEER  
WATERWAYS EXPERIMENT STATION  
3909 HALLS FERRY ROAD  
VICKSBURG, MISSISSIPPI 39180-6199  
PHONE : (601)634-2502

### Waterways Experiment Station Cataloging-in-Publication Data

Lembi, Carole A.

Plant growth regulators as potential tools in aquatic plant management : efficacy and persistence in small-scale tests / by Carole A. Lembi, Tara Chand-Goyal ; prepared for U.S. Army Corps of Engineers ; monitored by Environmental Laboratory, U.S. Army Engineer Waterways Experiment Station.

66 p. : ill. ; 28 cm. — (Contract report ; A-94-1)

Includes bibliographical references.

1. Aquatic plants — Control. 2. Plant regulators. 3. Vegetative management. 3. Aquatic weeds — Control. I. Chand-Goyal, Tara.  
II. United States. Army. Corps of Engineers. III. Environmental Laboratory (U.S. Army Engineer Waterways Experiment Station) IV. Aquatic Plant Control Research Program (U.S. Army Engineer Waterways Experiment Station) V. Title. VI. Series: Contract report (U.S. Army Engineer Waterways Experiment Station) ; A-94-1.

TA7 W34c no.A-94-1



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# Preface

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The work reported herein was conducted as part of the Aquatic Plant Control Research Program (APCRP), Work Unit 32578. The APCRP is sponsored by the Headquarters, U.S. Army Corps of Engineers (HQUSACE), and is assigned to the U.S. Army Engineer Waterways Experiment Station (WES) under the purview of the Environmental Laboratory (EL). Funding was provided under Department of the Army Appropriation No. 96X3122, Construction General. The APCRP is managed under the Environmental Resources Research and Assistance Programs (ERRAP), Mr. J.L. Decell, Manager. Mr. Robert C. Gunkel was Assistant Manager, ERRAP, for the APCRP. Technical Monitor during this study was Ms. Denise White, HQUSACE.

The Principal Investigator for the study was Dr. Carole A. Lembi, Department of Botany and Plant Pathology, Purdue University, West Lafayette, IN. The study was conducted and the report prepared by Dr. Lembi and Dr. Tara Chand-Goyal, Purdue University.

This research was monitored by Dr. Kurt D. Getsinger, Ecosystem Processes and Effects Branch (EPEB), Environmental Processes and Effects Division (EPED), EL, WES. Reviews of the report were provided by Ms. Linda S. Nelson and Dr. John D. Madsen, EPEB, and Drs. Thomas Jordan and Ronald Coolbaugh, Purdue University. Technical assistance was provided by Mr. A. Ekanayake, Mr. J. Hazebroek, Mr. T. Clark Reed, Mr. M. Alkire, Mrs. D. Lubelski, Ms. S. Fritschie, Dr. Coolbaugh, and Dr. P. Nelson, Purdue University, and Dr. S. West, Eli Lilly Research Laboratories (ELRL). Flurprimidol was provided by ELRL.

This investigation was performed under the general supervision of Dr. John Harrison, Director, EL; Mr. Donald L. Robey, Chief, EPED; and Dr. Richard E. Price, Acting Chief, EPEB.

At the time of publication of this report, Director of WES was Dr. Robert W. Whalin. Commander was COL Bruce K. Howard, EN.

The authors gratefully acknowledge the support of the Waterways Experiment Station and Drs. Howard Westerdahl and Kurt Getsinger as this research was being conducted. Flurprimidol was provided by Lilly Research Laboratories. The technical assistance of A. Ekanayake, J. Hazebroek, T. Clark Reed,



M. Alkire, D. Lubelski, and S. Fritschie is gratefully acknowledged. We also thank R. Coolbaugh and P. Nelson (Purdue University) for the use of their analytical equipment and S. West (Eli Lilly Research Laboratories) for technical advice. We thank Thomas Jordan and Ronald Coolbaugh for their critical reading of portions of this report.

This report should be cited as follows:

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



# 1 Introduction

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The purpose of this project was to study a potentially important new strategy for aquatic plant management. That strategy involves the use of compounds with plant growth regulating properties. Rather than kill submersed aquatic weeds with herbicides, our goal is to reduce their height. Height reduction renders the plants “non-weedy” and yet allows them to remain viable and functional in the aquatic environment, i.e. to provide oxygen, habitat, and sediment stabilization.

Previous research (Netherland 1989, Lembi and Netherland 1990, Netherland and Lembi 1992) showed that the gibberellin synthesis inhibitors flurprimidol ([ $\alpha$ -(1-methylethyl)- $\alpha$ -(4-trifluoromethoxy) phenyl]-5 pyrimidine-methanol), uniconazole ((E)-1-(4-chlorophenyl)-4,4-dimethyl-2-(1,2,4-triazol-1-yl)-1 penten-3-ol), and paclobutrazol ([2RS,3RS)-1-(4-chlorophenyl)-4-dimethyl-2-(1,2,4-triazol-1-yl) pentan-3-ol]) were effective in reducing plant height of hydrilla (*Hydrilla verticillata* Royle) and Eurasian watermilfoil (*Myriophyllum spicatum* L.) without reducing viability in laboratory bioassays.

The overall goals of the study reported here were to investigate the potential of compounds other than the gibberellin synthesis inhibitors to regulate plant height in hydrilla and Eurasian watermilfoil and to continue studying the efficacy, dose response, and environmental persistence of the gibberellin synthesis inhibitors. The specific goals were as follows:

- a. To test bensulfuron methyl (methyl 2-[[[[(4,6-dimethoxy-2-pyrimidinyl)amino]carbonyl]amino]sulfonyl]methyl]benzoate) for growth regulating properties on Eurasian watermilfoil and to test imazapyr ((+)-2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1H-imidazol-2-yl]-3-pyridinecarboxylic acid), triclopyr ([3,5,6-trichloro-2-pyridinyl]oxy]acetic acid), and amidochlor (N-[acetylamino]methyl]-2-chloro-N(2,6-diethylphenyl)acetamide) for growth regulating properties on Eurasian watermilfoil and hydrilla.
- b. To compare dose response effects of the gibberellin synthesis inhibitors on hydrilla and Eurasian watermilfoil at varying light irradiances.
- c. To test several submersed aquatic species other than hydrilla and Eurasian watermilfoil for sensitivity to the gibberellin synthesis inhibitors.



- d. To determine appropriate dosage/exposure times of gibberellin synthesis inhibitors on hydrilla and Eurasian watermilfoil.
- e. To develop procedures for detecting residues of gibberellin synthesis inhibitors in water, plant, and soil.
- f. To generate preliminary information on the dissipation characteristics of gibberellin synthesis inhibitors in the aquatic environment.



## 2 Bioassay of Compounds Other than Gibberellin Synthesis Inhibitors

---

### Introduction

Several compounds other than the gibberellin synthesis inhibitors show at least some ability to reduce stem length without killing the plant. Bensulfuron methyl is a rice herbicide used for the control of broadleaves and sedges with some suppression of grasses. At concentrations above  $2.5 \mu\text{g L}^{-1}$  it significantly reduced shoot lengths in hydrilla, Eurasian watermilfoil, sago pondweed (*Potamogeton pectinatus*), and American pondweed (*Potamogeton nodosus*) when applied pre- or postemergence (Anderson and Dechoretz 1988). Fourteen-day exposures to  $1\text{--}10 \mu\text{g L}^{-1}$  under greenhouse conditions resulted in height-reduced hydrilla plants without obvious signs of necrosis (Anderson 1988). Bensulfuron methyl is a member of the sulfonylurea class of herbicides. These compounds are ALS inhibitors (Beyer et al. 1988; Blair and Martin 1988); i.e. they inhibit the activity of acetolactate synthase which is the key enzyme in the formation of essential amino acids such as leucine, isoleucine, and valine. With the cessation of protein formation and subsequent cell division, growth essentially ceases.

Another compound with similar protein and growth inhibiting properties is imazapyr. This compound is a member of the imidazolinone class of herbicides. It is currently being used for control of annual and perennial weeds on industrial sites and rights-of-way. No research has been conducted on its potential as an aquatic herbicide or growth regulator, but in combination with imazethapyr ((+)-2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1H-imidazol-2-yl]-5-ethyl-3-pyridinecarboxylic acid) it has been reported to suppress vertical growth in tall fescue (Welterlen 1988). Both the sulfonylureas and imidazolinones have several environmentally benign features: they are effective at extremely low concentrations and do not leach appreciably through soil. Bensulfuron methyl has a relatively short half-life in soil of 4-8 weeks; however, imazapyr can persist at significant levels for up to 2 years (Herbicide Handbook 1989).



Triclopyr is being tested for herbicide use on Eurasian watermilfoil (Netherland and Getsinger 1992) under an experimental use permit. The compound has an auxin-type mode of action on broadleaved weeds, similar to that of 2,4-D ((2,4-dichlorophenoxy)acetic acid). It is readily metabolized in the environment and has low toxicity to nontarget, aquatic organisms. Although shoot length reduction has not been observed with this compound, its potential as a growth regulator at low concentrations needs to be investigated since there is a good chance that triclopyr will be labeled for aquatic use.

Amidochlor is an EPA-registered plant growth regulator for use on turfgrasses. It is an effective suppressant of seedhead development in cool-season grasses in turf and also provides some height reduction of the plant (DiPaola 1988). It suppresses growth for 6 weeks, is degraded microbially, and has a soil half-life of less than 1 week. No research has been conducted on the potential growth regulating properties of this compound on submersed aquatic plants.

The potential of bensulfuron methyl to inhibit stem elongation in Eurasian watermilfoil and of imazapyr, triclopyr, and amidochlor to inhibit stem elongation in Eurasian watermilfoil and hydrilla without affecting selected physiological parameters was tested using a laboratory bioassay.

## Materials and Methods

### Plant cultures

Algal-free cultures of Eurasian watermilfoil and the dioecious strain of hydrilla were originally obtained from Drs. John H. Andrews of the University of Wisconsin and Stephen J. Klaine of Memphis State University, respectively. Hydrilla was grown in 10 percent Hoagland's solution, and watermilfoil was grown in a modified Gerloff's solution (Andrews 1980) in 3-L round-bottomed flasks. Both media were buffered after autoclaving with 10 ml L<sup>-1</sup> of a 2-g/100-ml stock solution of NaHCO<sub>3</sub>. Stock cultures of both plants were maintained in controlled environment chambers at 25 ± 1 °C, 400 μE m<sup>-2</sup> sec<sup>-1</sup>, and a 16:8 hr light-dark cycle. The plant cultures were routinely checked for algal contamination, and only noncontaminated cultures were used for experiments.

### Bioassay conditions

Apical shoot segments 4 cm long were excised from parent plants and transferred to 250-ml flasks (one shoot per flask) with 150 ml of the appropriate culture medium and desired concentration of the compound. The formulations of the compounds used were as follows: bensulfuron methyl, 60 percent wettable powder; imazapyr, 2 lb/gal aqueous solution; triclopyr, 3 lb/gal TEA salt; and amidochlor, 4 lb/gal flowable. Experimental flasks were placed under the same growing conditions as stock cultures.



All dose response experiments were conducted for a 4-week period. Neither new medium nor test compound was added after the initial treatment.

### **Growth parameters**

Growth parameters measured included main stem length, lateral stem length and number, root length and number, internode number, and fresh and dry weights. Length measurements were taken with a centimeter ruler. Dry weights were taken on plants dried at 70 °C for 48 hr.

### **Physiological parameters**

Chlorophyll analyses were conducted on fresh tissue using a dimethylsulfoxide extraction according to the method of Hiscox and Israelstam (1979). Chlorophyll was calculated as milligrams of chlorophyll per gram of fresh weight. Photosynthetic rates were determined using a digital pH meter (Orion Model 701A/Digital, Orion Research, Inc., Cambridge, MA) equipped with a dissolved oxygen (DO) electrode (Orion Model 97-08). Plant segments were placed in a 300-ml biochemical oxygen demand (BOD) bottle with fresh medium at a known DO concentration. The bottles were placed on a shaker table in an environmental growth chamber under the same growth conditions as previously described. Bottles were allowed to shake gently for 60 to 90 min and were then removed from the chamber and measured for DO. Dissolved oxygen evolution is expressed per unit fresh weight per unit time (fr wt/min).

### **Experimental design and statistical analysis**

All dosages for testing were predetermined by a preliminary screening. Therefore, any value above the range of concentrations reported here was toxic to the plant (discoloration or bleaching, necrosis, complete lack of growth) and any value below the range of concentrations reported had no detectable effect on growth. Each treatment consisted of three replicates, and experiments were repeated at least once unless otherwise noted. Flasks within an experiment were randomized on the growth chamber shelf. Measurements were taken in the following sequence: plants were first monitored for photosynthesis. Growth parameters were then measured, and fresh weights were taken. After these measurements, the apical 4- to 6-cm of the plant was removed and used for chlorophyll analysis. Dry weight was taken on the remaining portion of the stem.

Graphs show results from one of two experiments; experiments in which results varied significantly are noted. All  $\pm$  values are standard errors. Statistical analysis consisted of ANOVA and separation of means using the Student-Newman-Keuls test. Significance was set at  $P < 0.05$ .



## Results

We considered detrimental effects on the test plants as decreases in photosynthetic rate, adventitious root initiation, and chlorophyll content. Unless noted otherwise, statistically significant decreases in photosynthetic rates were rates that were at least 33 percent less than the rates of untreated controls (mean of the untreated controls: milfoil [n=12] =  $0.020 \pm 0.002$  mg O<sub>2</sub>/g fr wt/min; hydrilla [n=9] =  $0.024 \pm 0.003$  mg O<sub>2</sub>/g fr wt/min); the minimum concentration that showed a statistically significant decrease in photosynthesis is marked as P on the graphs. Statistically significant decreases in chlorophyll (chl) were those that were at least 30 percent less than untreated control values (mean of the untreated controls: Eurasian watermilfoil [n=12] =  $0.744 \pm 0.059$  mg chl/mg fr wt; hydrilla [n=9] =  $1.137 \pm 0.064$  mg chl/mg fr wt); the minimum concentration that produced a statistically significant decrease in chlorophyll content is marked as C on the graphs. We marked the minimum concentration that resulted in no root initiation with an R. Untreated plants averaged  $4.9 \pm 0.4$  roots per plant in Eurasian watermilfoil (n=12) and  $6.8 \pm 0.6$  roots per plant in hydrilla (n=9).

### Bensulfuron methyl

This compound was tested only on Eurasian watermilfoil (Figure 1). Milfoil plants showed a gradual main stem length reduction over the range of concentrations from  $0.6 \mu\text{g L}^{-1}$  to  $300 \mu\text{g L}^{-1}$ . The minimum effective concentration that significantly reduced stem length was  $6 \mu\text{g L}^{-1}$ . Plant height was reduced approximately 36 percent at that concentration. Percent reduction in stem length at  $300 \mu\text{g L}^{-1}$  was 67 percent.

The minimum concentration of bensulfuron methyl that resulted in a significant decline in photosynthetic rate (51 percent) and completely inhibited root initiation was  $60 \mu\text{g L}^{-1}$ . Photosynthesis completely ceased at  $100 \mu\text{g L}^{-1}$ . Chlorophyll at  $6 \mu\text{g L}^{-1}$  was 36 percent of the untreated control. However, chlorophyll content remained the same at higher concentrations; chlorophyll at  $300 \mu\text{g L}^{-1}$  was still 37 percent of the untreated control.

Plants treated at  $0.6$  and  $6 \mu\text{g L}^{-1}$  were morphologically similar to untreated plants (Figure 2). However, lateral shoot initiation was induced at  $6 \mu\text{g L}^{-1}$  (not visible in Figure 2). For example, the number of lateral shoots per treated plant was 15 compared to 1.7 in untreated plants. However, the lateral shoots on the treated plants were extremely short, averaging less than 0.5 cm in length. New leaf formation on treated plants sometimes seemed abnormal, with the leaflets coalesced or fused together rather than separating.

At  $300 \mu\text{g L}^{-1}$  no new main stem growth was initiated (Figures 1, 2); i.e. the main stems of the treated plants at 4 weeks posttreatment were approximately the same length as the initial plant segments (4 cm). At all other concentrations, stems did increase in length by at least 1 cm.



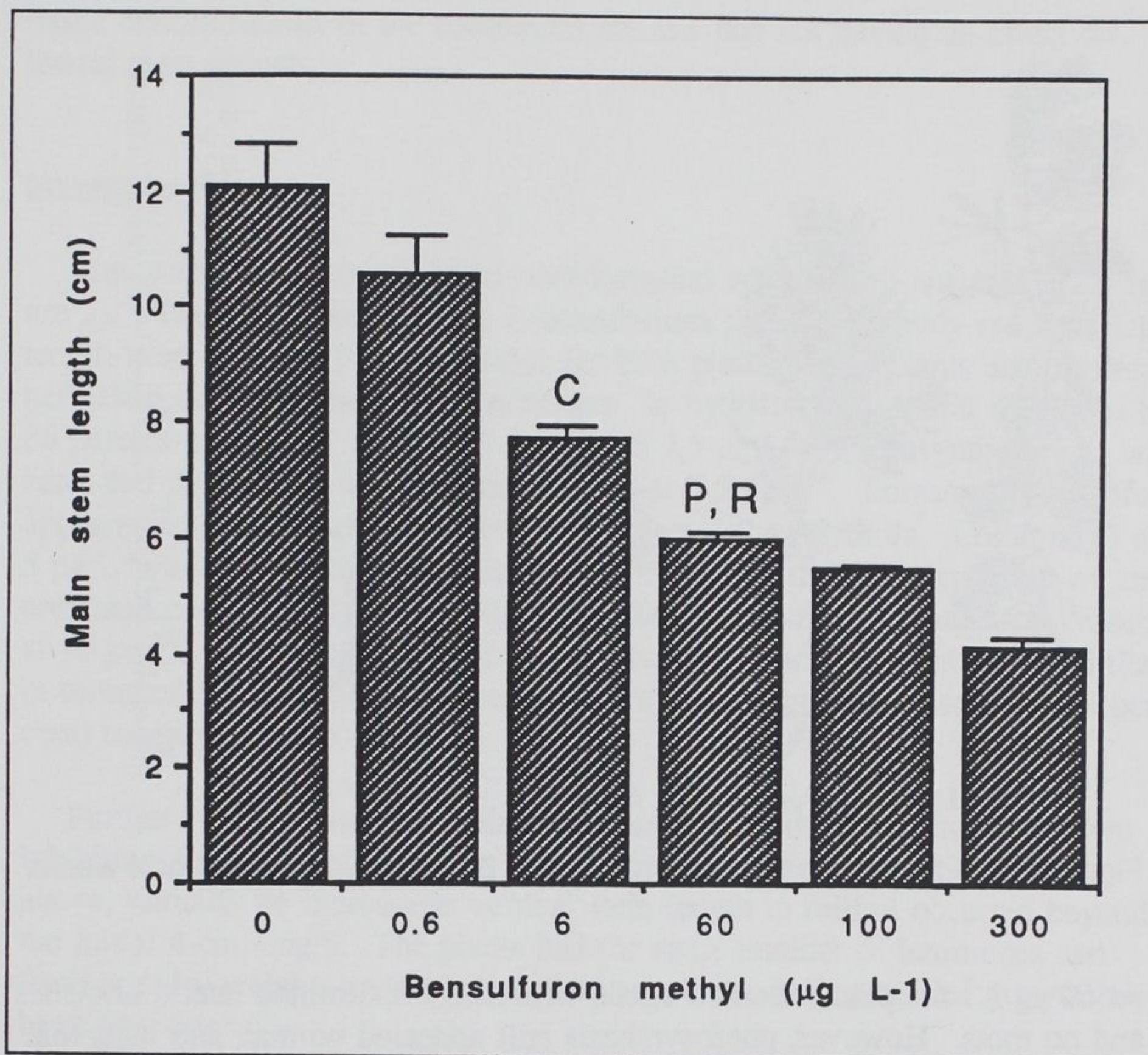


Figure 1. Effect of bensulfuron methyl on main stem length of Eurasian watermilfoil in the bioassay system at 4 weeks. C = minimum concentration that resulted in statistically significant decrease in chlorophyll content; P = minimum concentration that resulted in statistically significant decrease in photosynthetic rate; R = minimum concentration that resulted in no root initiation

We conducted a single long-term exposure experiment with bensulfuron methyl using the laboratory bioassay. Eurasian watermilfoil was exposed to 0, 0.6, 6.0, and 60  $\mu\text{g L}^{-1}$  for 4, 6, and 8 weeks. A concentrated source of inorganic nutrients was added at 2-week intervals to ensure that the plants had sufficient nutrients for growth during the long-term exposure. At 4 weeks, reduction in main stem length was 15 percent at 6  $\mu\text{g L}^{-1}$  and 42 percent at 60  $\mu\text{g L}^{-1}$  (data not shown). No effects were observed at 0.6  $\mu\text{g L}^{-1}$ . Roots again were missing at 60  $\mu\text{g L}^{-1}$ . These results generally confirmed previous 4-week results although the reduction in main stem length at 6  $\mu\text{g L}^{-1}$  was somewhat less than that in the dose response experiments. At 6 weeks, main stem lengths at 6 and 60  $\mu\text{g L}^{-1}$  were 20 and 52 percent, respectively, of those of the untreated plants. A bushy growth form with many abnormally appearing lateral branches was observed at 60  $\mu\text{g L}^{-1}$ . Photosynthetic rates at this concentration, however, were not significantly different from those of the untreated controls. At 8 weeks, main stem reduction was still apparent with 33 percent reduction at 6  $\mu\text{g L}^{-1}$  and 66 percent reduction at 60  $\mu\text{g L}^{-1}$ .



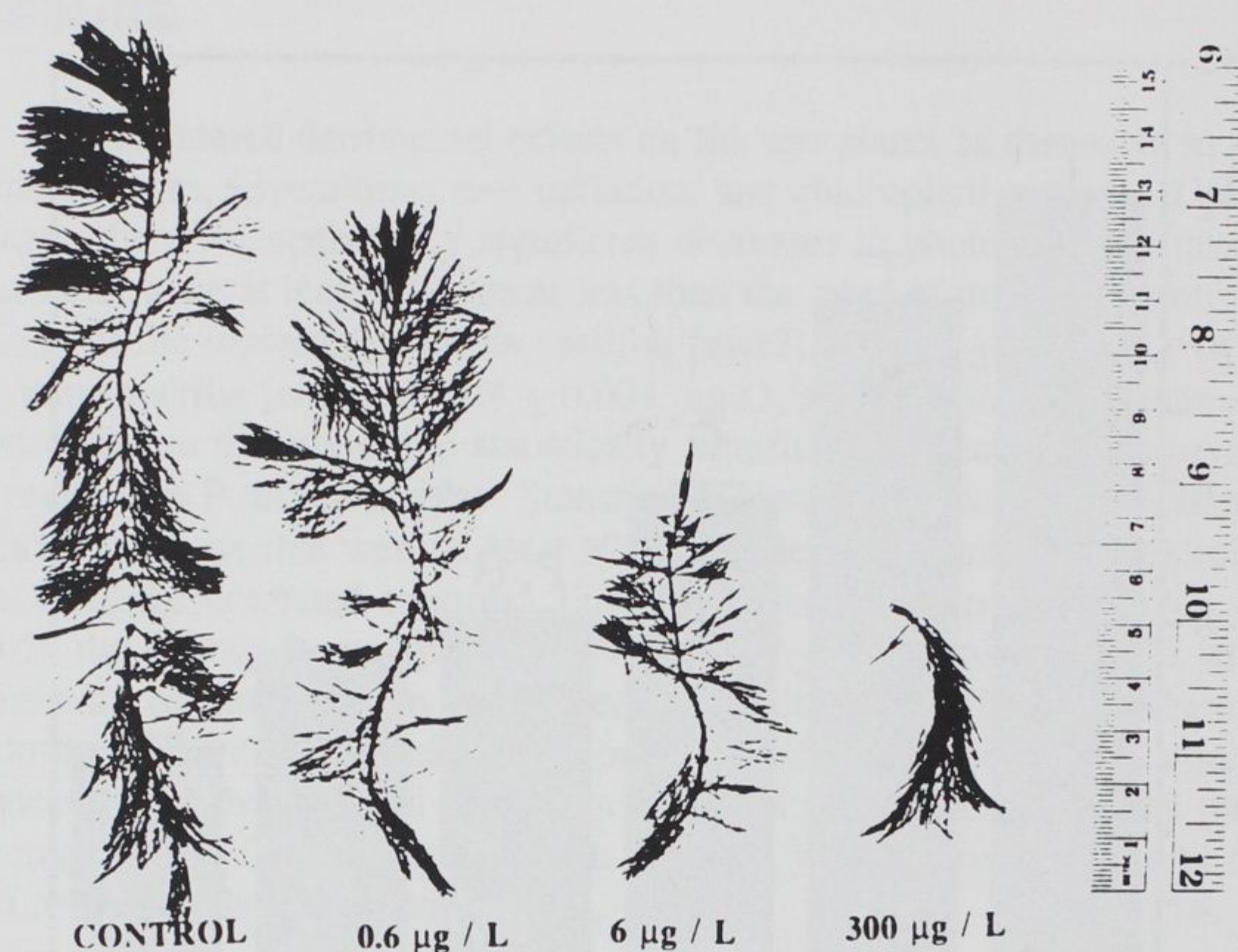


Figure 2. Effect of bensulfuron methyl on Eurasian watermilfoil at 4 weeks

At  $60 \mu\text{g L}^{-1}$  the plants seemed brittle with many malformed lateral branches and no roots. However, photosynthesis still appeared normal, and buds that were removed and transferred to fresh, untreated medium sprouted normally after another 6 to 8 weeks. No growth reduction effects were ever observed at  $0.6 \mu\text{g L}^{-1}$ . In general, milfoil appeared to be relatively tolerant to bensulfuron methyl even during long-term exposures, with concentrations in the 6 to  $60 \mu\text{g L}^{-1}$  range giving growth regulator effects (stem length reduction).

We also conducted one duration-of-exposure experiment using the laboratory bioassay. Eurasian watermilfoil was exposed to  $60 \mu\text{g L}^{-1}$  for 2, 12, and 24 hr and 3, 7, 14, and 28 days. At the end of each exposure period, the plants were removed from the treatment, rinsed thoroughly, and placed in fresh, untreated medium for a 4-week recovery period. Exposures of 2 hr did not have any effect on the plants. After 12-hr exposures, main stem lengths of treated plants were 29 percent (a statistically significant reduction in this case) less than those of untreated plants at the end of the recovery period. A 24-hr and longer exposure resulted in stem length reductions of 50 percent. At all exposure times that caused stem length reduction the plants became bushy in appearance due to lateral stem proliferation. It appeared that the main stem tip turned pale and died and that this stimulated the production of numerous lateral buds and shoots. Bensulfuron methyl appears to move rapidly (within 12 hr) into the plant under the growing conditions used in these experiments, and the effects on main stem length are retained during the recovery period, possibly because the tip has been killed. However, the sprouting of laterals and resulting bushiness of the plant during recovery may be an indication that



tissue concentrations of the compound are low and not having an effect on lateral stem growth.

### **Imazapyr**

This compound was tested on both Eurasian watermilfoil and hydrilla (Figure 3). The minimum effective concentrations that significantly reduced stem length were in the 5-10  $\mu\text{g L}^{-1}$  range for both plants. Both plants also showed herbicidal effects at these concentrations. In hydrilla, chlorophyll decreased by 36 percent at 5  $\mu\text{g L}^{-1}$  and by 87 percent at 7.5  $\mu\text{g L}^{-1}$ . Photosynthetic rate was zero, and root initiation completely ceased at 7.5  $\mu\text{g L}^{-1}$ . Eurasian watermilfoil appeared to be somewhat less severely impacted than hydrilla. Chlorophyll at 5  $\mu\text{g L}^{-1}$  was 30 percent that of the untreated control and 46 percent that of the untreated control at 10  $\mu\text{g L}^{-1}$ . Root initiation of Eurasian watermilfoil ceased at 10  $\mu\text{g L}^{-1}$ . Eurasian watermilfoil was somewhat more tolerant than hydrilla in terms of effect on photosynthesis, with the first significant decrease (38 percent) monitored at 100  $\mu\text{g L}^{-1}$ .

Further evidence that these plants are extremely susceptible to the growth inhibiting properties of imazapyr is that at concentrations of 10  $\mu\text{g L}^{-1}$  and above, virtually no increase in vertical stem length in milfoil occurred beyond the initial 4-cm length. The plants had the same number of internodes and fresh and dry weights as the initial plant segments, indicating that growth had been shut down.

### **Triclopyr**

This compound was tested on both Eurasian watermilfoil and hydrilla (Figure 3). Eurasian watermilfoil seemed very sensitive to this compound at dosages as low as 0.5 to 1  $\mu\text{g L}^{-1}$ . Main stem length and root initiation were significantly reduced at 1  $\mu\text{g L}^{-1}$ . At 0.5  $\mu\text{g L}^{-1}$  photosynthetic rate and chlorophyll were reduced by 33 percent and 53 percent, respectively.

Unique formative effects were noted on Eurasian watermilfoil. The number of roots produced per plant more than tripled at 0.5 and 1.0  $\mu\text{g L}^{-1}$  although the length per root was about half that of the untreated control. There was a slight increase in numbers of lateral shoots, but those that were produced were elongated and deformed (Figure 4). Similar effects were noted at 10  $\mu\text{g L}^{-1}$  in one of the two experiments.

Higher triclopyr dosages were required to affect hydrilla. In both experiments on this plant, the 50  $\mu\text{g L}^{-1}$  concentration produced a slight, but nonstatistical, increase in main stem length over the untreated controls. Main stem length was significantly reduced at 250  $\mu\text{g L}^{-1}$ . Chlorophyll was reduced by 36 percent at 100  $\mu\text{g L}^{-1}$  and by 64 percent at 250  $\mu\text{g L}^{-1}$ , and photosynthesis was reduced by 65 percent at 500  $\mu\text{g L}^{-1}$ . However, triclopyr did not seem to have inhibitory effects on root induction in hydrilla. In another experiment



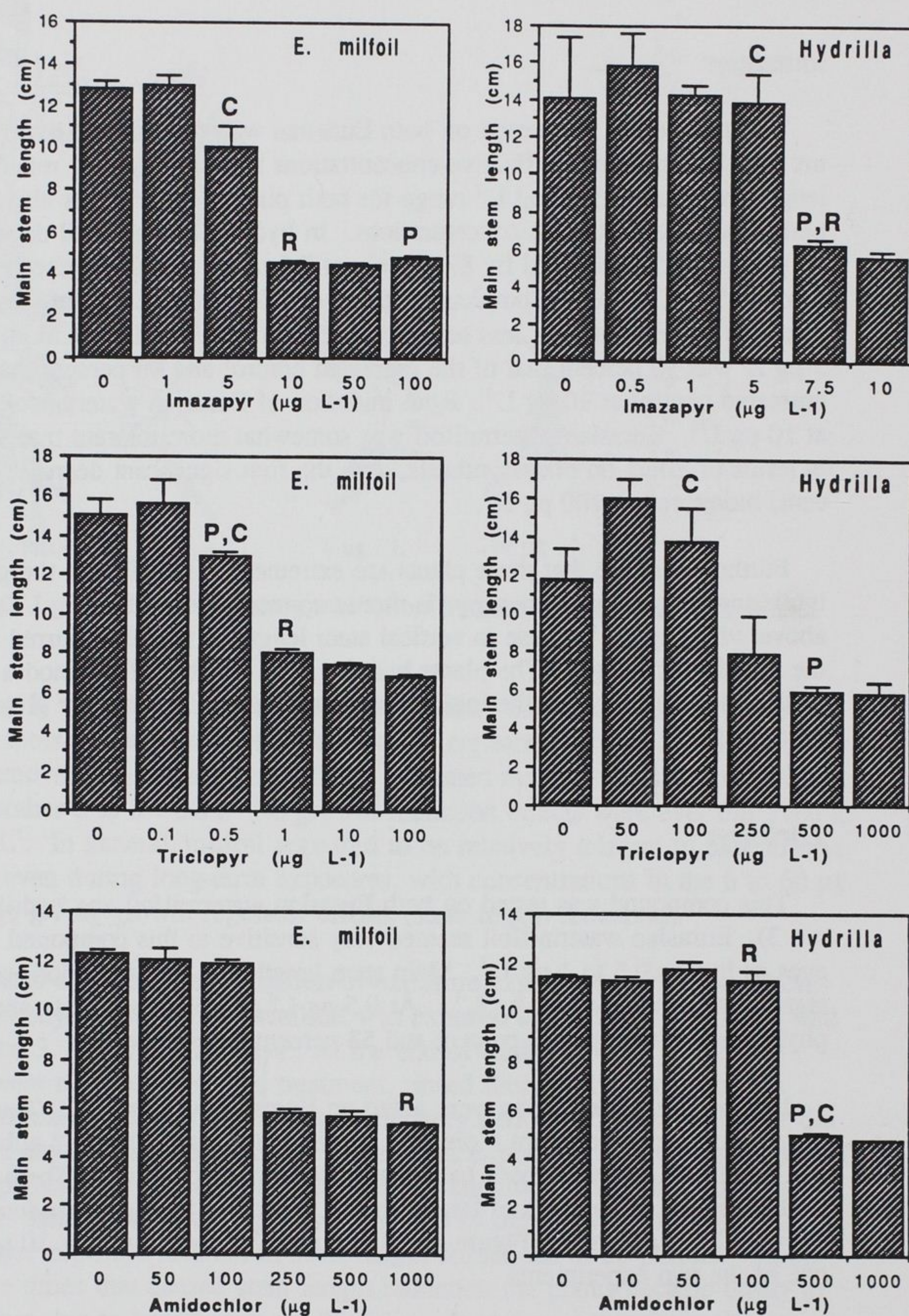


Figure 3. Effect of imazapyr, triclopyr, and amidochlor on main stem length of Eurasian watermilfoil and hydrilla in the bioassay system at 4 weeks. C, P, R as defined in Figure 1



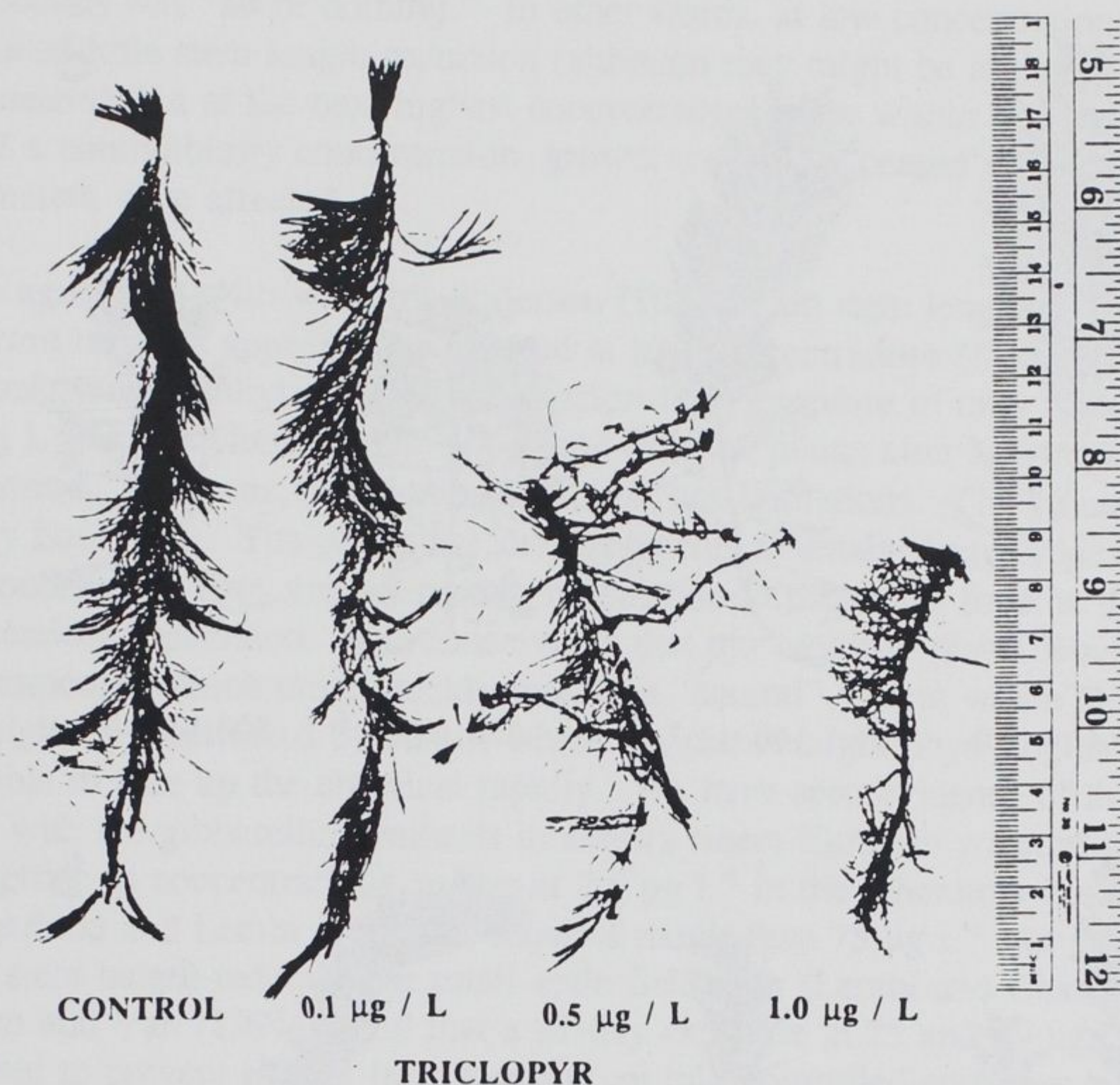


Figure 4. Effect of triclopyr on Eurasian watermilfoil at 4 weeks

(data not shown) roots were inhibited at  $1,000 \mu\text{g L}^{-1}$ , but this is still a very high concentration. The relative tolerance of hydrilla to this compound compared to Eurasian watermilfoil is probably due to the fact that monocots, including hydrilla, are more tolerant to the 2,4-D-like compounds than dicots.

### Amidochlor

Amidochlor was tested on both Eurasian watermilfoil and hydrilla (Figure 3). Significant reduction in main stem length in Eurasian watermilfoil was obtained at concentrations between  $250$  and  $1,000 \mu\text{g L}^{-1}$ . Very few adverse effects were noted on the plants at these concentrations. The only parameter negatively affected was root initiation at  $1,000 \mu\text{g L}^{-1}$ . No unusual or aberrant morphological features were observed at any of these concentrations (Figure 5a).

Amidochlor appeared to be more toxic to hydrilla than to Eurasian watermilfoil. Although concentrations between  $500$  and  $1,000 \mu\text{g L}^{-1}$  caused significant reductions in main stem length, photosynthesis, chlorophyll content, and root initiation were also inhibited. Root initiation ceased at  $100 \mu\text{g L}^{-1}$ , and photosynthesis and chlorophyll content decreased by 100 percent and 59 percent, respectively, at  $500 \mu\text{g L}^{-1}$ . At  $500 \mu\text{g L}^{-1}$ , the plants had grown



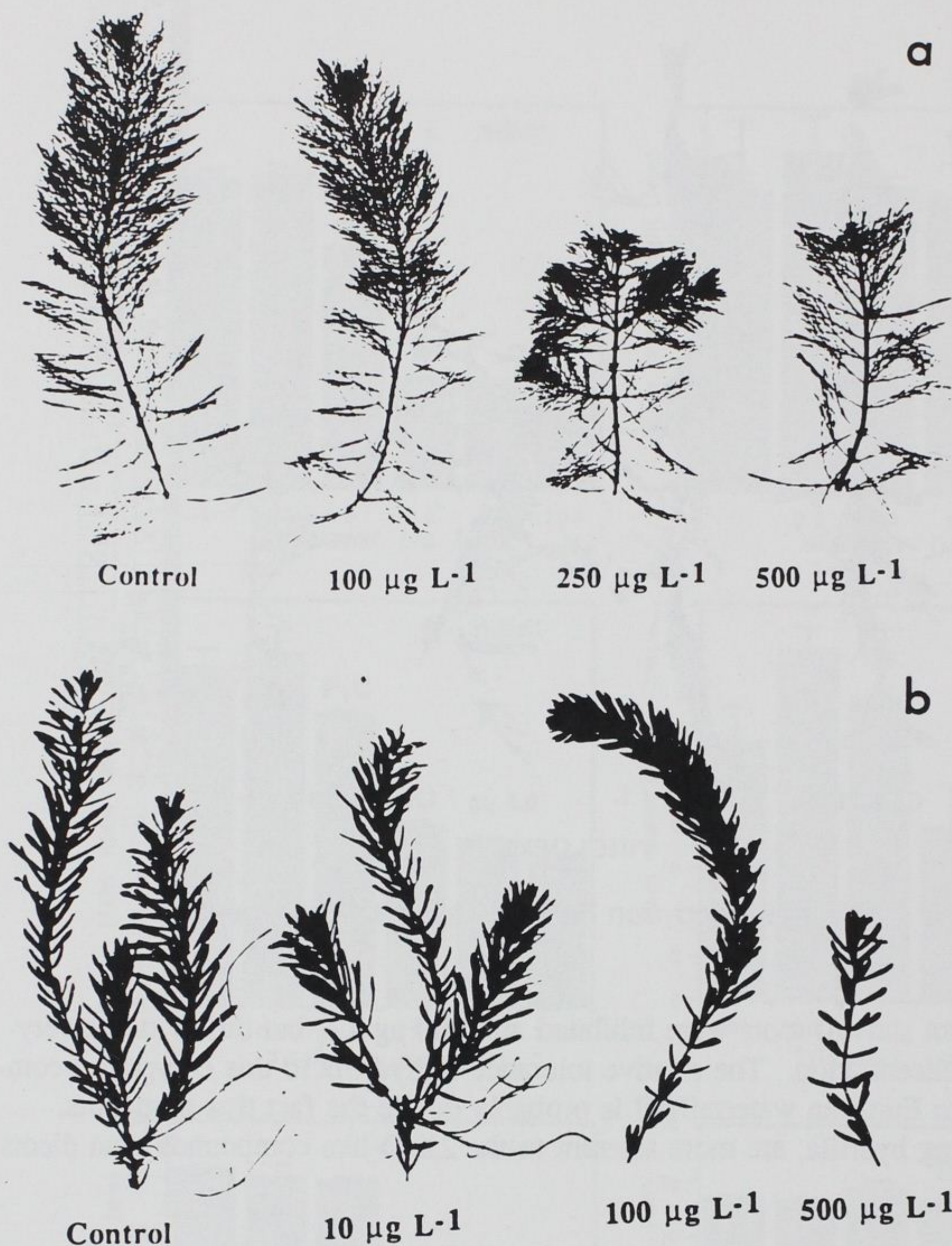


Figure 5. Effect of amidochlor (Limit) at 4 weeks  
 a. Eurasian watermilfoil  
 b. Hydrilla

less than 1 cm over the initial segment (Figure 5b) and turned red from excess anthocyanin production.

## Discussion

In general, imazapyr and triclopyr appeared to be herbicidal to Eurasian watermilfoil and hydrilla. Although main stem length reductions were observed, they occurred at concentrations that adversely affected photosynthetic rate, chlorophyll content, and root initiation. The effect of these



compounds was "all or nothing." In other words, at low concentrations they produced little stem length reduction (although they might be affecting other parameters), but at the next highest concentration, often within less than  $10 \mu\text{g L}^{-1}$  of a noninhibitory concentration, growth essentially ceased and additional parameters were affected.

In agreement with work by Anderson (1988) main stem length in Eurasian watermilfoil does appear to be reduced at low concentrations ( $6$  to  $100 \mu\text{g L}^{-1}$ ) of bensulfuron methyl. In addition, a short-term exposure of only  $12$  hr at  $60 \mu\text{g L}^{-1}$  was required to maintain height-reduced plants after a 4-week recovery period. However, our bioassay medium has limitations. The system is a purely liquid one. The plants are short segments maintained under ideal growing conditions. They are not rooted in soil, nor do they have roots at the time of chemical application. It is conceivable that our bioassay plants would take up compounds much more quickly than in a "natural" system where the chemical might be inactivated by soil or where the plants might physiologically be less able to take up the chemical rapidly. We have seen evidence of this in work with the gibberellin synthesis inhibitors where Eurasian watermilfoil is susceptible to concentrations as low as  $7.5 \mu\text{g L}^{-1}$  in the laboratory bioassay (Netherland and Lembi 1992) but requires more than  $75 \mu\text{g L}^{-1}$  for significant main stem length reduction in small-scale field tests (Lembi and Chand 1992). Nelson and Van (1991) found that a 21-day exposure at  $25$  and  $50 \mu\text{g L}^{-1}$  was required to prevent milfoil from topping out in a controlled-environment aquarium system.

Our data suggest that at rates of  $6$  to  $100 \mu\text{g L}^{-1}$  bensulfuron methyl can have adverse impacts on physiological parameters such as photosynthetic rate and root initiation. This may be one of the reasons why, in the field, bensulfuron methyl has not produced consistent growth regulating effects (Netherland, pers. commun.). Under certain environmental and physiological conditions the compound may act more as a herbicide than as a growth regulator. In addition, some observations of plant growth regulation in greenhouse experiments may be based on limited time frames. Overt necrosis may not be observed because chlorophyll content seemed to remain relatively stable throughout the concentration range tested here. Therefore, plants may be green and appear healthy and growth regulated for a period of time before they actually begin to undergo senescence and death. In a 6-week exposure to  $60 \mu\text{g L}^{-1}$ , our test plants became brittle with many morphological abnormalities (although photosynthetic rates remained normal), suggesting that long-term exposures at these relatively low concentrations may be herbicidal.

The compound with the greatest potential for growth regulation in this study was amidochlor. The compound was effective as a growth regulator on Eurasian watermilfoil over a concentration range of at least  $250$  to  $1,000 \mu\text{g L}^{-1}$ . Amidochlor appeared to be herbicidal to hydrilla. A major problem with ever introducing this compound to market may be the high dosages required for effective growth regulation on Eurasian watermilfoil, particularly if it turns out that field rates are higher than those predicted here.



## Summary

Triclopyr and imazpyr appear to be herbicidal rather than growth regulatory on hydrilla and Eurasian watermilfoil. Bensulfuron methyl may have potential as a growth regulator on Eurasian watermilfoil, but clearly more work is needed to identify herbicidal versus growth regulating properties. Amidochlor is an effective growth regulator on Eurasian watermilfoil, although it appears that "high" concentrations of the compound are required (at least  $250 \mu\text{g L}^{-1}$ ) for effective main stem length reduction.



### **3 Effect of Light on Response of Hydrilla and Eurasian Watermilfoil to Gibberellin Synthesis Inhibitors**

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#### **Introduction**

It is well known that the addition of gibberellic acid to plants results in stem elongation (e.g. see Devlin and Witham 1983). Since plant stems growing under low light conditions are longer (etiolated) than those grown under high light, the conclusion is that internal gibberellic acid (or its biosynthesis) has either been activated under low light or destroyed under high light. Which of these two hypotheses is correct is unknown (Devlin and Witham 1983, Moore 1989), but it is generally acknowledged that the formation and/or activity of gibberellin under low light results in greater rates of stem elongation than under high light. In aquatic situations, submersed plants are often subjected to low light conditions due to turbidity or to light attenuation with increasing depth. The question that must be asked in regard to gibberellin synthesis inhibitor activity is whether these compounds can overcome the increase in gibberellin activity under low light conditions.

We therefore designed an experiment in which flurprimidol-treated and untreated hydrilla and Eurasian watermilfoil were grown under low light ( $4\text{--}18 \mu\text{E m}^{-2} \text{ sec}^{-1}$ ) and high light ( $800\text{--}1,000 \mu\text{E m}^{-2} \text{ sec}^{-1}$ ) conditions.

#### **Materials and Methods**

Stem tip sections 6 cm long from hydrilla and Eurasian watermilfoil cultures grown as described above were planted into a loam soil in styrofoam cups (one stem tip per cup). A small amount of a controlled release fertilizer was added to the soil prior to planting. A thin layer of sand was placed over the soil to prevent sediment dispersion when placed in water. Glass jars



(3.5 L) were filled with Smart and Barko medium (1985), and the cups with plants were placed in the jars (one cup with hydrilla and one cup with milfoil per jar). A layer of plastic wrap was placed over the mouth of the jar to prevent excessive water loss. The jars were placed on a greenhouse bench. Experiments were conducted in July 1992. Ambient light at midday on bright sunny days was  $800\text{--}1,000\ \mu\text{E m}^{-2}\text{ sec}^{-1}$ . One set of jars was placed under ambient light conditions; a second set of jars was placed under nylon shade cloth and cheesecloth to obtain an irradiance of  $4\text{--}18\ \mu\text{E m}^{-2}\text{ sec}^{-1}$  at midday. Light measurements were made with a LI-COR meter with a spherical bulb. Two fans were directed toward the jars to prevent excessively high temperatures. Water temperatures during the course of the experiment averaged  $25\ ^\circ\text{C}$  and never rose above  $30\ ^\circ\text{C}$ .

Since the plants had been growing in culture chambers under relatively low light conditions ( $400\ \mu\text{E m}^{-2}\text{ sec}^{-1}$ ), they were allowed to acclimate to the higher light in the greenhouse in the following way: four layers of cheesecloth were placed over all jars for 2 days; this was followed by one layer of cheesecloth for 1 day. The next day, all plants were treated and placed under the high or low light conditions described above. Treatments were 0, 7.5, 75, and  $750\ \mu\text{g L}^{-1}$  (active ingredient, a.i.) of flurprimidol (50 percent WP, DowElanco Products Company, Indianapolis). Each light and concentration treatment consisted of three replicates. The experiment was repeated. Both experiments showed the same trends, but only the data from one experiment are reported here. Main stem lengths were measured 7 days after treatment on high light plants and 10 days after treatment on low light plants.

## Results

Although the number of internodes of low light and high light control ( $0\ \mu\text{g L}^{-1}$ ) plants was similar (17-19 in milfoil; 30-35 in hydrilla), the length of the internodes differed. At high light mean internode length was 0.53 cm in milfoil and 0.39 cm in hydrilla. At low light mean internode length was at least twice as long: 1.2 cm in milfoil and 1.6 cm in hydrilla. The increase in internode length resulted in control main stem lengths that were 55 percent and 73 percent longer on low light milfoil and hydrilla plants, respectively, compared to the high light plants (Figure 6).

Flurprimidol treatment of high light plants did not result in statistically significant differences in main stem length (Figure 6). Although the control plants were slightly longer than the treated plants, the stem length inhibition normally observed in treated plants probably did not have a chance to develop because of the short 7-day exposure times.

Main stem lengths of all treated low light plants of both milfoil and hydrilla were statistically reduced ( $P < 0.05$ ) compared to the low light control plants (Figure 6). In milfoil, main stem lengths of treated low light and high light plants did not differ. In hydrilla, treated low light plants were longer than the



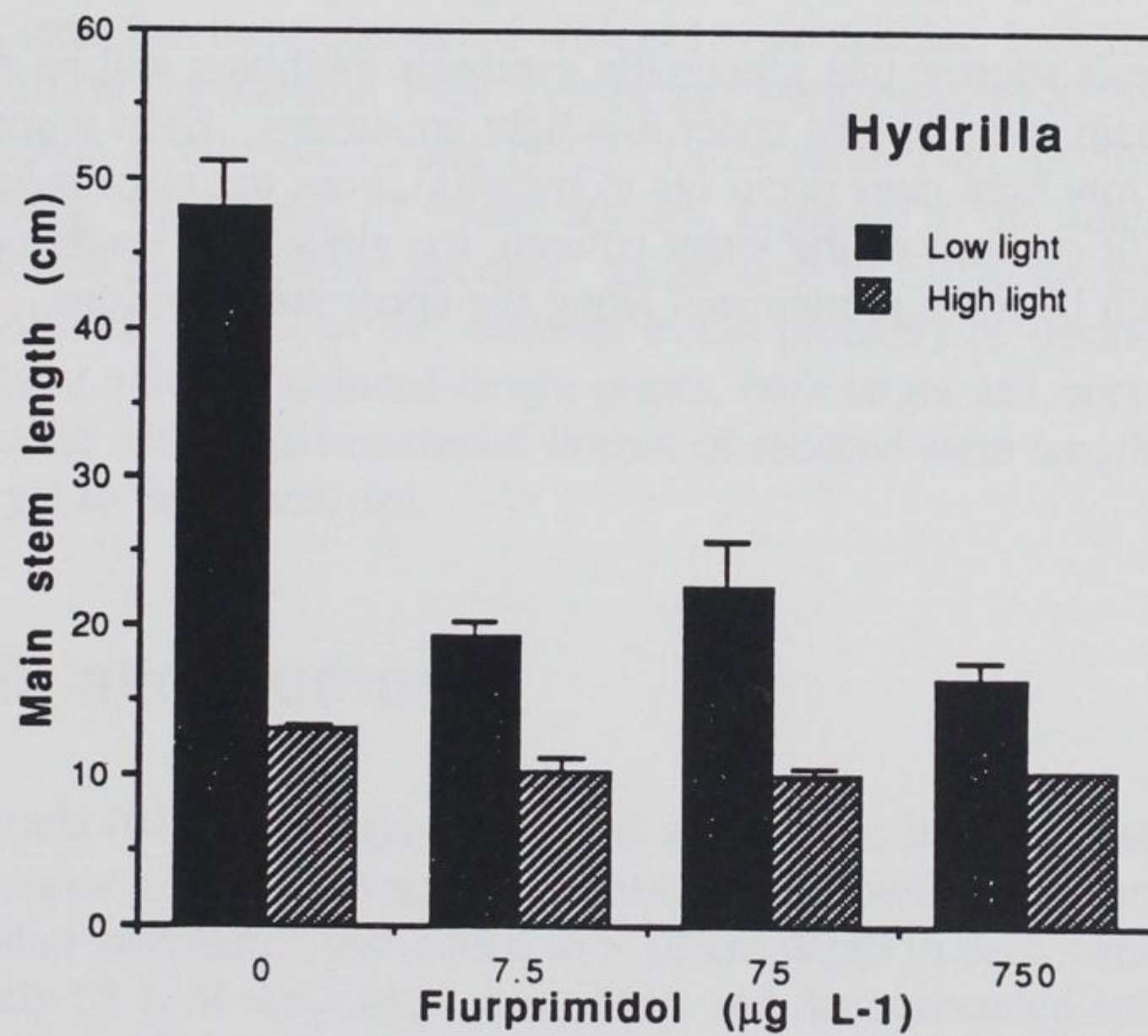
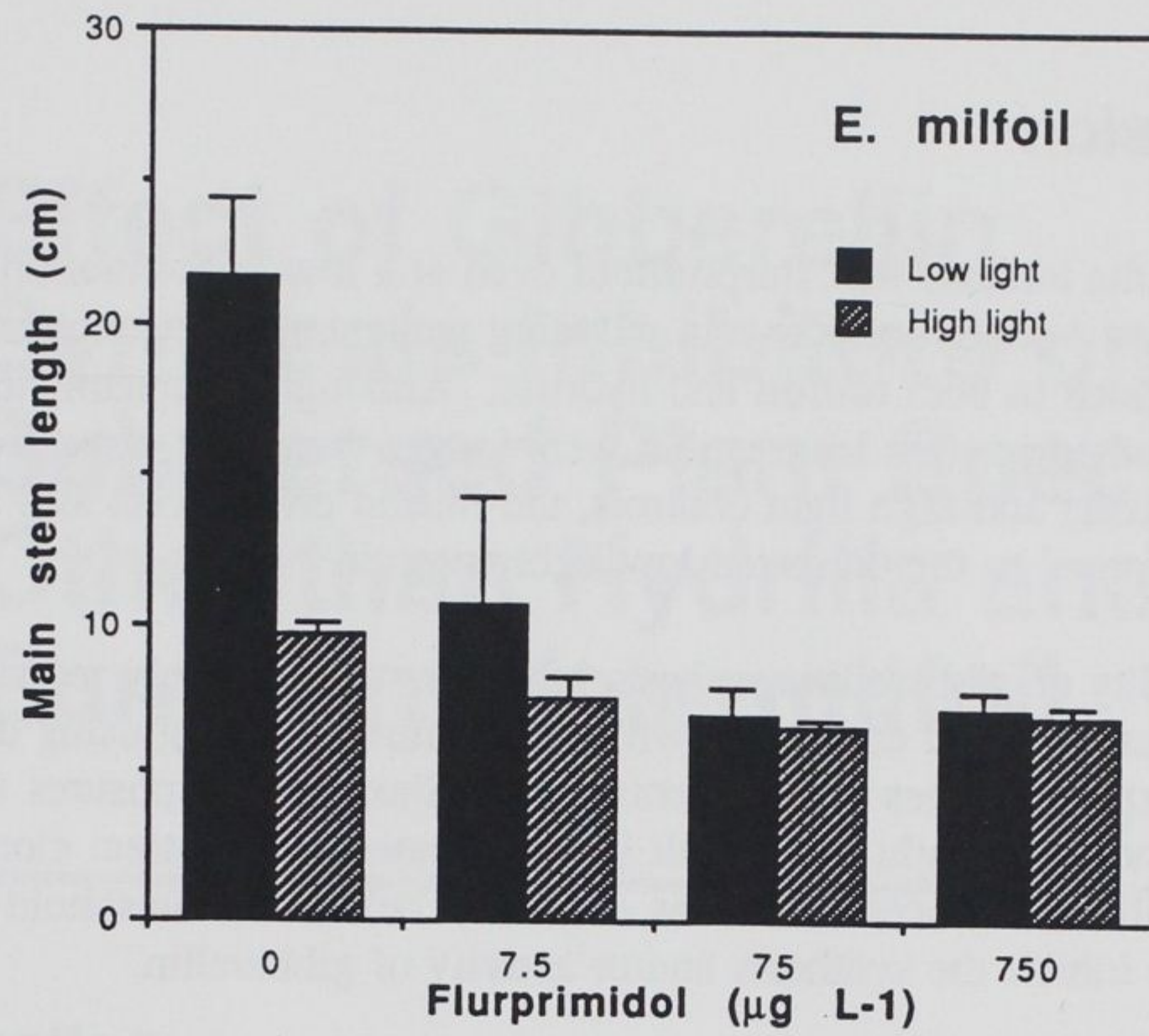


Figure 6. Effect of flurprimidol on main stem lengths of Eurasian watermilfoil and hydrilla grown under high light ( $800\text{--}1,000 \mu\text{E m}^{-2} \text{sec}^{-1}$ ) and low light ( $8\text{--}14 \mu\text{E m}^{-2} \text{sec}^{-1}$ ) conditions



high light plants treated with the same flurprimidol concentrations but were, as noted above, reduced when compared to the untreated controls.

## Discussion

The results indicate that flurprimidol even at a low concentration of  $7.5 \mu\text{g L}^{-1}$  is, at least initially, effective in reducing main stem elongation under low light conditions in both milfoil and hydrilla. Although all treatments of hydrilla resulted in stem lengths that were longer than their respective high light treatments and high light controls, the treated plants were still reduced in height compared to the untreated low light controls.

The ability of gibberellin synthesis inhibitors to effectively reduce plant height under low light conditions will require further testing using different inhibitor exposure times and concentrations. Short-term exposures to the inhibitor under low light may result in the release of main stem elongation once the inhibitor concentration has decreased below the threshold level required to inhibit the synthesis and/or activity of gibberellin.

## Summary

Initial tests suggest that gibberellin synthesis inhibitors will be effective in reducing main stem lengths under low light conditions. Even if some elongation under low light does occur (as in hydrilla), once the plant reaches the more well-lit portions of the water column, the gibberellin synthesis inhibitor effect should be more pronounced along the upper stem portions.



## **4 Effect of Gibberellin Synthesis Inhibitors on Submersed Plant Species Other than Hydrilla and Eurasian Watermilfoil**

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### **Introduction**

Most of the research to date on gibberellin synthesis inhibitor effects on submersed plants has been conducted with only two species, hydrilla and Eurasian watermilfoil (Netherland 1989, Lembi and Netherland 1990, Netherland and Lembi 1992, Lembi and Chand 1992). Other submersed species could also be affected. In the case of weedy species, exposure to the inhibitors would be viewed as a positive outcome. In the case of native or nontarget species, a minimal impact of the inhibitor would probably be desired. However, the habitat value of reduced-height plants, both target and nontarget, is unknown, so the actual environmental impact of reduced stem length in native species has yet to be determined.

### **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. Submersed plant species were collected from Lakes Wawasee, Shook, and Backwater in Kosciusko Co., Indiana. Three stem apices (approx. 7 cm in length) of a species, without roots, were planted per barrel. Up to three species were planted per barrel. The plants were allowed to acclimate for at least 10 days prior to flurprimidol treatment. Flurprimidol (50 percent 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 ensure even dispersal.



Four experiments were initiated during the summer 1992. In the first two (treatment dates 23 May and 6 June), the barrels were treated with 0, 75, and 200  $\mu\text{g L}^{-1}$  a.i. flurprimidol. Three days after treatment, the water in both control and treated barrels was siphoned off and replaced with fresh, untreated water. The plants were harvested 4 weeks after the initial treatment. In the third and fourth experiments (treatment dates 26 July and 1 August), the barrels were also treated with 0, 75, and 200  $\mu\text{g L}^{-1}$  a.i. flurprimidol, but the water was not removed. The plants were left in treated water over the 4-week period prior to harvest.

Length measurements were taken using a centimeter ruler. Fresh weights were also taken. Each treatment consisted of three replicate barrels. Statistical analysis consisted of ANOVA and separation of means using the Student-Newman-Keuls test. Significance was set at  $P < 0.05$ .

## Results

The species that were tested using the 3-day exposures were *Elodea canadensis*, *Sagittaria graminea* (23 May treatment), *Potamogeton nodosus*, and *Ceratophyllum demersum* (6 June treatment). The plants did not grow very well. In part this was due to the unusually cold, overcast conditions during the early portion of the summer. Untreated control main stem lengths were (mean  $\pm$  SE)  $12.6 \pm 1.2$  cm for *S. graminea* (leaf length),  $11.0 \pm 3.0$  cm for *P. nodosus*, and  $19.1 \pm 3.5$  cm for *Ceratophyllum*. *Elodea* initially grew as horizontal runners. Lateral vertical stems on this plant measured only  $4.2 \pm 0.4$  cm.

The plant which showed the most marked response to flurprimidol was *P. nodosus* with a stem length reduction of 43 percent at 200  $\mu\text{g L}^{-1}$ . Even though main stem length was reduced, the plants were "larger" than untreated plants. Individual treated plants had significantly more submersed leaves at 200  $\mu\text{g L}^{-1}$  (25 versus 11) and more upright stems (8 versus 4). Although not statistically significant, these treated plants had longer rhizomes (70 cm versus 41 cm) and greater fresh weights ( $5.7 \pm 2.1$  g versus  $2.7 \pm 0.9$  g) than untreated plants.

Although *Elodea* and *Sagittaria* plants showed a trend toward length reduction at 75 and 750  $\mu\text{g L}^{-1}$ , there were no significant differences between treated and untreated plants (data not shown). There was no tendency toward main stem length reduction in *Ceratophyllum*. In fact, the treated plants were slightly longer (21 cm) than control plants (19 cm). However, the number of lateral branches and fresh weights were lower in treated (7.3, 2.5 g, respectively at 200  $\mu\text{g L}^{-1}$ ) than untreated (22, 6.1 g, respectively) plants.

The species that were tested with 4-week exposures were *Vallisneria spiralis*, *Heteranthera dubia*, *Najas flexilis* (26 July treatment), *Elodea canadensis*, *Ceratophyllum demersum*, and *Potamogeton foliosus* (1 August



treatment). All control plants grew somewhat better than did those earlier in the summer.

The main stem lengths of *Heteranthera dubia*, *Najas flexilis*, *Elodea canadensis*, and *Potamogeton foliosus* were significantly reduced at 75 and 200  $\mu\text{g L}^{-1}$  (Table 1). Results on main stem lengths of *Ceratophyllum* were similar to those of the short-term exposure tests. Although there was a tendency toward stem length reduction, the differences between treated and untreated plants were not significant. Unfortunately, we did not measure number of lateral stems or fresh weight of *Ceratophyllum* in this test. The leaf length of *Vallisneria* also was not reduced at any concentration (Table 1).

<b>Table 1</b> <b>Main Stem Lengths (cm) of Submersed Plants Treated with Flurprimidol in Outdoor Barrels for 4 Weeks. (Means (<math>\pm</math>SE) within a row with same letters are not significantly different at the 0.05 level using the Student-Newman-Keuls test)</b>			
Plant Species	Flurprimidol ( $\mu\text{g L}^{-1}$ )		
	0	75	200
<i>Vallisneria americana</i> <sup>1</sup>	40.2 (11.4)a	36.5 (0.9)a	33.9 (3.4)a
<i>Heteranthera dubia</i>	34.6 (4.1)a	18.7 (1.1)b	10.6 (4.5)b
<i>Najas flexilis</i>	26.6 (1.7)a	12.2 (2.1)b	13.8 (1.2)b
<i>Elodea canadensis</i>	16.6 (3.4)a	7.8 (0.8)b	7.3 (1.6)a
<i>Ceratophyllum demersum</i>	28.9 (2.6)a	20.1 (4.5)a	20.1 (4.5)a
<i>Potamogeton foliosus</i>	15.6 (0.9)a	5.7 (1.1)b	4.5 (0.4)b
<sup>1</sup> Leaf length.			

## Discussion and Summary

Better growing conditions are needed to determine whether short-term exposures will reduce main stem lengths in the species tested as they do in hydrilla and Eurasian watermilfoil (Lembi and Chand 1992). Although the results of these tests, including the 4-week exposure experiments, are preliminary, taken in total, they do suggest that a broad spectrum of aquatic plant species are sensitive to flurprimidol. The only plants that did not seem to be affected were the *Vallisneria americana* (leaf length) and *Ceratophyllum demersum* (main stem length), although the overall growth of *Ceratophyllum* plants may have been reduced. *Vallisneria americana* (and *S. graminea*) has a rosette growth form, i.e. the internodes are already compressed and the length of the plant is mainly due to elongate leaf blades. Therefore, the lack of effect of flurprimidol on overall length (assuming leaf blade elongation is not enhanced by gibberellic acid) of this plant is not surprising. It would be



interesting to know if gibberellic acid has an effect on stem elongation in *Vallisneria*. In fact, additional studies on the effect of gibberellic acid on submersed plants with different growth habits might allow us to better predict the inhibitory effects of gibberellin synthesis inhibitors.



# 5 Response of Hydrilla and Eurasian Watermilfoil to Flurprimidol Dosage and Exposure Times

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## Introduction

As noted above, laboratory bioassays (Netherland 1989, Lembi and Netherland 1990, Netherland and Lembi 1992) have shown that inhibitors of gibberellin synthesis such as flurprimidol, paclobutrazol, and uniconazole can reduce plant height in hydrilla and Eurasian watermilfoil. However, these studies were conducted under "ideal" conditions, i.e. totally liquid medium, optimal growing conditions, and constant exposure to the inhibitor. Under field conditions, responses to gibberellin inhibitors might differ from those under laboratory conditions. For example, rooted plants may have different uptake capacities than plants floating in a medium, the presence of sediments could affect root uptake of the inhibitor, and inhibitor concentrations may be diluted over time. In order to begin to test these compounds under field conditions, we exposed hydrilla and Eurasian watermilfoil grown out-of-doors in 67-L barrels with bottom sediment to several flurprimidol concentrations. 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 in length) without roots of healthy Eurasian watermilfoil (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 percent WP, DowElanco 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 ensure even dispersal. Flurprimidol concentrations were 0, 75, and 750  $\mu\text{g L}^{-1}$  for hydrilla and 0, 7.5, 75, and 200  $\mu\text{g L}^{-1}$  for milfoil.

Treatment dates in 1989 were 5 June for Eurasian watermilfoil and 31 July for hydrilla. Treatment dates in 1990 were 1 June for Eurasian watermilfoil and 31 July 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 hr and 1, 3, 7, 14, and 28 days, although the hydrilla exposure times in 1990 were limited to 1 and 2 hr and 1 and 3 days. Flurprimidol concentrations in the 1990 milfoil test were monitored by gas chromatography (GC) and decreased by 85 percent over the 28-day period. In a separate study (Chand and Lembi 1991) approximately 88 percent 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 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 4 weeks in untreated water, the plants were harvested. Therefore, plants that had been exposed to flurprimidol (including the 0  $\mu\text{g L}^{-1}$  concentration) for 2 hr and 1 and 3 days were harvested at approximately 4 weeks after treatment. The plants that had been exposed to flurprimidol (including the 0  $\mu\text{g L}^{-1}$  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 centimeter 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 hr and weighed.

Each exposure time/concentration combination consisted of two replicates. Plant lengths and weights in the untreated barrel replicates from the 2-hr and 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 ANOVA, and the means of each parameter measured at each date were separated using the Student-Newman-Keuls multiple range test (Zar 1974). Significance was set at  $P < 0.05$ .



## Results and Discussion

Untreated hydrilla grew best in 1989. Main stem lengths ranged from 42 to 45 cm in all control barrels. This compared with a stem length of only  $20 \pm 1$  (SE) cm in the 4-week-old controls in 1990. In contrast, the untreated Eurasian watermilfoil plants grew best in 1990, with main stem lengths averaging  $59 \pm 8$  cm in 1990 versus  $35 \pm 7$  cm in 1989 in the 4-week-old controls and  $86 \pm 2$  cm in 1990 versus  $53 \pm 3$  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 Eurasian watermilfoil growth and perhaps somewhat detrimental to hydrilla growth, even though we always initiated our Eurasian watermilfoil 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 Eurasian watermilfoil 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 Eurasian watermilfoil, 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  $750 \mu\text{g L}^{-1}$ ) (Figure 7A). After 2 hr exposure and 4 weeks recovery, main stem length at  $75 \mu\text{g L}^{-1}$  was 64 percent of the main stem length of the 4-week-old control; at  $750 \mu\text{g L}^{-1}$  main stem length was 43 percent of that of the 4-week-old control. At exposure periods of 3 days and longer, main stem lengths at  $75$  and  $750 \mu\text{g L}^{-1}$  were approximately 40–47 percent and 30–42 percent, respectively, of the main stem lengths of the controls. In 1990, flurprimidol exposure for only 1 hr resulted in significantly reduced stem lengths compared to the controls (75 and 60 percent of the control length at  $75$  and  $750 \mu\text{g L}^{-1}$ , 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 1992).

Flurprimidol appeared to cause a decrease in hydrilla dry weights when compared to the untreated controls (Figure 7B). The effects were greatest at the  $750\text{-}\mu\text{g L}^{-1}$  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  $75 \mu\text{g L}^{-1}$  was reduced by 54 percent but dry weight was reduced by only 34 percent. The reason for this was probably due to the proliferation of stolons on the treated plants. In 1990, treated plants had an average of  $2 \pm 0.5$  and  $4 \pm 0.75$  stolons at  $75$  and  $750 \mu\text{g L}^{-1}$ , respectively, compared to only  $1 \pm 0$  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 8). Although we did not count the number of main stems in this



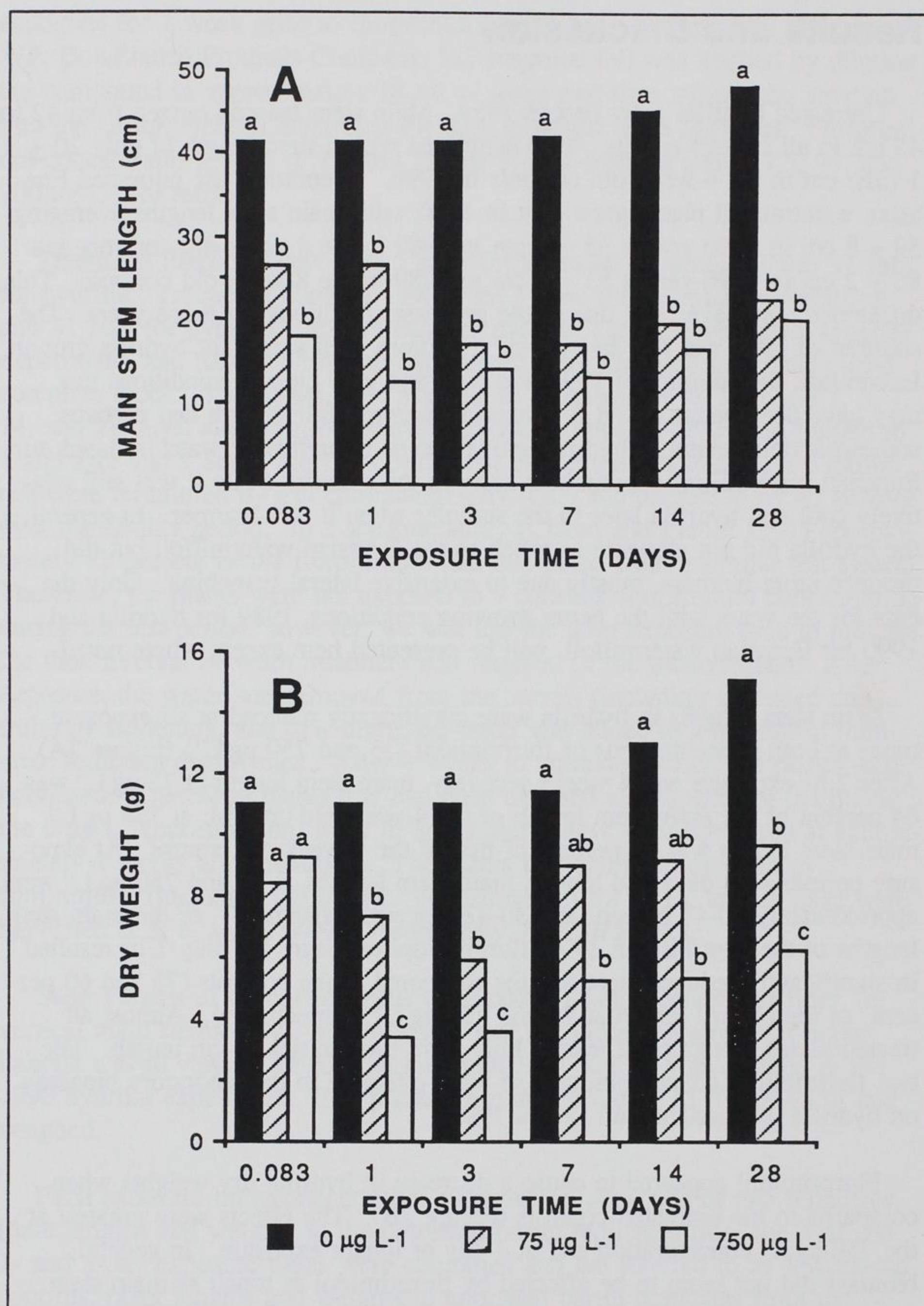


Figure 7. Effect of exposure time of flurprimidol on (A) mean main stem length and (B) mean dry weight in hydrilla in 1989. The plants were allowed to recover for 4 weeks following exposure. 0.083 days = 2 hr

study, Netherland (1989) found significantly more main stems produced by hydrilla grown in barrels and exposed to uniconazole, another gibberellin synthesis inhibitor, than by untreated plants.



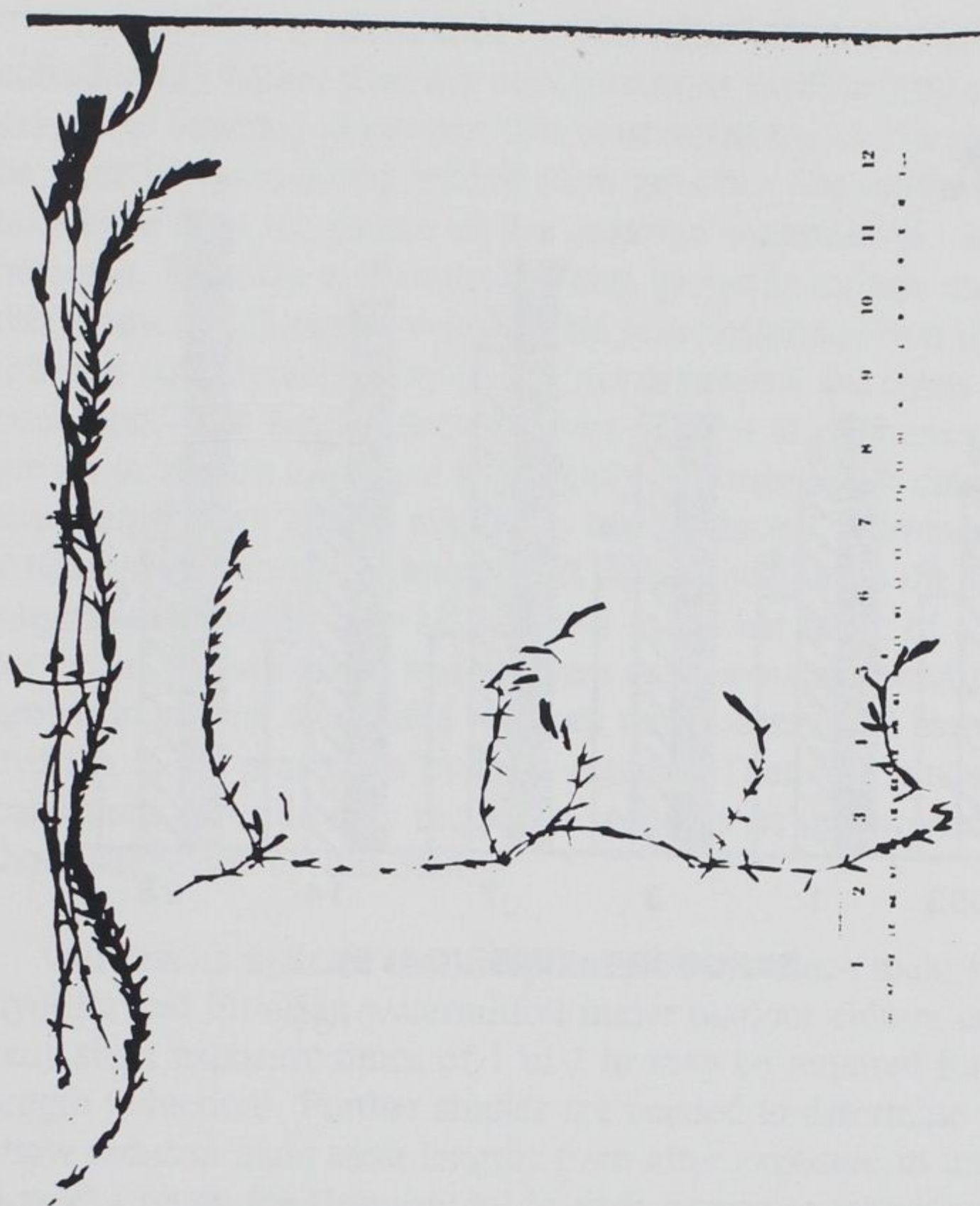


Figure 8. Hydrilla plant treated with  $75 \mu\text{g L}^{-1}$  flurprimidol on the right; untreated hydrilla plant on the left. The plants are oriented similarly with respect to the substratum

In 1989, a poor growth year for Eurasian watermilfoil 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  $75 \mu\text{g L}^{-1}$ . In 1990, we used flurprimidol concentrations of  $75$  and  $200 \mu\text{g L}^{-1}$ . At all exposure times, Eurasian watermilfoil showed reduced stem lengths at  $200 \mu\text{g L}^{-1}$  (Figure 9A). Only after the 28-day exposure did flurprimidol at  $75 \mu\text{g L}^{-1}$  begin to produce a significant reduction in stem length. Main stem lengths at  $200 \mu\text{g L}^{-1}$  were 37-65 percent 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  $200\text{-}\mu\text{g L}^{-1}$  treatments and at 14- and 28-day exposures for  $75\text{-}\mu\text{g L}^{-1}$  treatments (Figure 9B). Stolons were seldom produced by Eurasian watermilfoil 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.



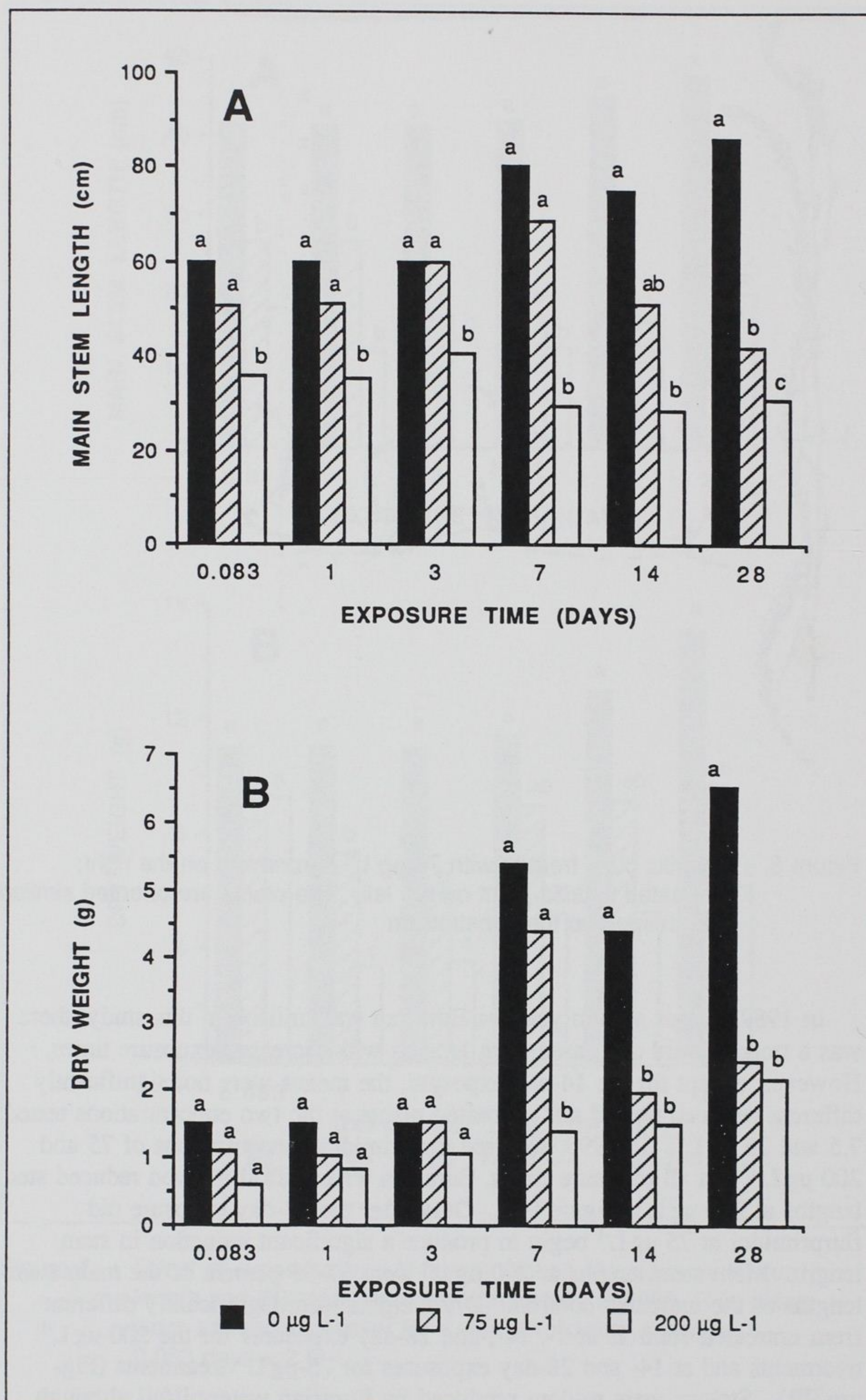


Figure 9. Effect of exposure time of flurprimidol on (A) mean main stem length and (B) mean dry weight in Eurasian watermilfoil in 1990. The plants were allowed to recover for 4 weeks following exposure. 0.083 days = 2 hr



The effective concentration of  $200 \mu\text{g L}^{-1}$  for Eurasian watermilfoil was considerably higher than the concentrations predicted by the laboratory bioassay. The bioassay suggested that concentrations as low as  $0.75 \mu\text{g L}^{-1}$  would be effective in reducing milfoil stem growth. The reason for this difference may have been the source of the Eurasian watermilfoil. In the laboratory bioassay, Eurasian watermilfoil plants grown in culture medium were used. In this study, the Eurasian watermilfoil 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  $200 \mu\text{g L}^{-1}$  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 present in Indiana waters). Therefore, under natural growing conditions, hydrilla may require concentrations of flurprimidol higher 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 hr 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 percent 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.

## Summary

The 2-hr exposures to flurprimidol significantly reduced vertical main stem length at concentrations of  $750 \mu\text{g L}^{-1}$  for hydrilla and  $200 \mu\text{g L}^{-1}$  for Eurasian watermilfoil for at least 28 days posttreatment. Treated hydrilla plants produced more stolons per plant than untreated plants resulting in a "rug-like" carpet on the bottom of the barrel. Our results suggest that further field testing of short-term exposures to gibberellin synthesis inhibitors is warranted.



## **6 Procedures for Detecting Flurprimidol Residues in Water, Plant Tissues, and Soil**

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### **Introduction**

An ideal plant growth regulator or herbicide, whether applied directly to aquatic weeds or to terrestrial sites with potential for residue runoff into an aquatic environment, should have low persistence. However, no information is available on persistence of any of the gibberellin synthesis inhibitors in the aquatic environment. The purpose of this portion of the study was to develop methods of extracting flurprimidol residues from Eurasian watermilfoil (roots, shoots, and buds), and to determine residues in water, soil, and plant parts.

### **Materials and Methods**

#### **Extraction from plant tissues**

Eurasian watermilfoil tissue was freeze-dried, macerated into a fine powder, and stored. All solvents used for extractions were high-performance liquid chromatographic (HPLC) grade. Two different methods of extraction (Reed 1988, Stahly and Buchanan 1986) were evaluated. The most satisfactory method, resulting in less contamination from plant pigments, was the extraction method of Stahly and Buchanan (1986) but with the following modifications. (1) Plant samples were extracted with a blender in 80 percent methanol at 55 °C for 30 min rather than at room temperature for 5 min. (2) Samples were first purified through 1 g LC-florisil SPE (Suppelco, Inc.) tubes; the flurprimidol was eluted with a 5-ml mixture of anhydrous ether and methanol (97:3 vol/vol) and evaporated in vacuo. Residues were dissolved in 1 ml of 100 percent methanol, and 24 ml of water was added to dilute the solution to 4 percent methanol. In the final step of purification, 0.5-g Sep-Pak C<sub>18</sub> cartridges (Water Associates, MA) were conditioned with 5 ml of 100 percent



methanol and then 10 ml of 4 percent methanol. The samples were loaded on the cartridges with a 10-ml rinse of 4 percent methanol. Flurprimidol was eluted with 5 ml of 80 percent methanol which was collected, vacuum evaporated, and dissolved in 100 percent anhydrous methanol for gas chromatography (GC) and/or GC-mass spectrometry (GC-MS) analysis.

### **Extraction from soil**

Free water was removed from each soil sample by vacuum-filtering the soil through Whatman No. 1 filter paper in a Buchner funnel. The extraction method for the wet soil was the same as that used for plant tissue.

### **Extraction from water**

Flurprimidol was extracted from water samples by the method described by West (unpublished, available from Eli Lilly Research Laboratories, Indianapolis, IN). The major modification was eliminating the hexane addition prior to eluting the sample through the Sep-Pak C<sub>18</sub>. Elution was conducted with 80 percent instead of 100 percent methanol. Fewer impurities were obtained when this sequence was used.

### **Recovery of known amounts of flurprimidol**

Watermilfoil plants were grown under controlled environmental conditions ( $25 \pm 1$  °C,  $400 \mu\text{E m}^{-2}\text{s}^{-1}$ , 16:8 hr light:dark) in 3-L flasks (Selim et al. 1989, Smith et al. 1989). The plants were harvested after 6-8 weeks of growth and were washed twice with distilled water prior to freeze-drying. Flurprimidol (99.8 percent technical grade, Eli Lilly) dissolved in 100 percent methanol was added to 1-2 g of the freeze-dried, macerated plant tissue, 25-200 ml of well water, and 20 g wet weight of soil.

### **Small-scale field experiments**

Analysis of flurprimidol residues in plant parts, soil, and water in a small-scale outdoor experiment was conducted during June/July 1989. Metal barrels (67-L capacity) were lined with plastic liners. 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. Ten healthy milfoil stem apices (10 cm in length) without roots were planted in each barrel and allowed to acclimate for 1 week prior to flurprimidol treatment on 4 June 1989. Flurprimidol (50 percent WP, Elanco Products Company, Indianapolis, IN) was applied at a concentration of 0.0 (control) and  $500 \mu\text{g a.i. L}^{-1}$  with three replicates per treatment. Water samples were taken immediately after treatment and 28 days after treatment when plants were harvested and soil was sampled for



flurprimidol analysis. The plants were washed twice with distilled water and segregated into shoots, lateral buds, and roots. The plant parts were blot-dried, and their wet weight was recorded before freeze-drying within 24 hr of collection. Soil cores were taken using a hollow plastic cylinder (5 cm inner diameter by 15 cm in length). Water and soil samples were frozen for storage. For analysis, thawed soil samples were divided into upper and lower 5-cm layers before removing the free water.

To study the dissipation of flurprimidol in water over time, another small-scale barrel experiment, similar to the experiment described above, was conducted outdoors. In this experiment only two milfoil apices were planted in each barrel. On 4 June 1989 flurprimidol was applied at concentrations of 0.0 (control), 7.5, and 75.0  $\mu\text{g a.i. L}^{-1}$ . There were two replicates per treatment. Water samples of 1 L were taken from each barrel prior to treatment, immediately after treatment, and 2 hr and 1, 3, 7, 14, and 28 days after treatment.

## GC and GC-MS

GC was conducted using a Varian 3400 GC equipped with a model 8035 autosampler, 1075 split/splitless capillary injector set at a split ratio of 1:6 with a 2- $\mu\text{l}$  injection volume; thermionic specific detector (TSD) (Varian Associates, Inc., Walnut Creek, CA); and a DB-17 (30 m x 0.32 mm) fused silica capillary column (J & W Scientific, Folsom, CA). Gas flow velocity for the hydrogen carrier gas was 45  $\text{cm min}^{-1}$ ; for make-up gas  $\text{N}_2$ , 30  $\text{ml min}^{-1}$ ;  $\text{H}_2$ , 4.95  $\text{ml min}^{-1}$ ; and air, 175  $\text{ml min}^{-1}$ . The TSD bead current was 2.950 A, and bias voltage was -4.0 V. The temperature for chromatography was 250  $^{\circ}\text{C}$  for the injector and detector. The initial column temperature was 150  $^{\circ}\text{C}$  for 1 min followed by a 3  $^{\circ}\text{C min}^{-1}$  increase to a final temperature of 230  $^{\circ}\text{C}$  with a 5-min hold time. Under these conditions, flurprimidol retention time was 11.74 min.

GC-MS was conducted using a Hewlett-Packard GC 5890A with a HP mass selective detector (MSD) 5970 and a HP7673A autosampler. The same column, injection volume (splitless mode), and temperature program as in the GC analysis were used, except that the initial column temperature was 170  $^{\circ}\text{C}$ . Helium gas flow was 32  $\text{ml min}^{-1}$ . Electron ionization was at 70 eV with a scan range of  $m/e$  40-320. The retention time of flurprimidol was 11.89 min.

Standard curves for GC were developed by injecting 2- $\mu\text{l}$  volumes of standard solutions (0.1, 0.25, 0.5, 1.0, 2.5, 5.0, 10.0, 25.0, 50.0, and 100  $\mu\text{g ml}^{-1}$ ) of technical grade flurprimidol (99.8 percent pure) in 100 percent methanol. While running samples on the GC or GC-MS every third or fourth sample was a flurprimidol standard to detect variability in the sensitivity of the instruments.



## Results and Discussion

The peak area response of the TSD was linear over a concentration range of 0.1-100  $\mu\text{g ml}^{-1}$ . The average correlation coefficient for peak area linearity of the standard solutions was 0.995-0.998 on 7 different days. As noted by West and Rutherford (1986) we also found that the peak area response of the GC-MS was highly variable on different days and was dependent on the autotune of the instrument. Consequently, GC-MS was only used for the confirmation of flurprimidol residues. The mass spectrum of technical grade flurprimidol at 5  $\mu\text{g ml}^{-1}$  and an injection volume of 1  $\mu\text{l}$  consists of four major ions at  $m/e$  values of 79, 107, 189, and 269 (Figure 10) with relative intensities (percent) of 20, 100, 7, and 69, respectively; retention time was 11.89 min. The molecular ion (MW 312.3) was not recorded until the flurprimidol concentration was at least 25  $\mu\text{g ml}^{-1}$ . Even at this concentration, the relative intensity of the molecular ion was less than 1 percent. In all treated samples (fortified and field) the retention time of flurprimidol was the same. The relative intensities (percent) of ions 79, 107, 189, and 269 were 17-23, 100, 4-8, and 59-65, respectively, which confirms the identity of flurprimidol.

The extraction efficiency at different levels of fortification was tested by spiking plant, water, and soil samples with known concentrations of flurprimidol (Table 2). Mean recoveries were 86.8 percent from watermilfoil shoots, 85.2 percent from roots, 79.3 percent from loam soil, and 93.3 percent from water. These recoveries were similar to those obtained for a soil-grass mixture (78 percent), soil (80 percent) (West and Rutherford 1986), and peach leaves (83.6 percent) (Reed 1988), which were also analyzed by GC, and considerably better than those obtained from plant tissue (40 percent) which was analyzed with HPLC (Booth et al. 1989).

Flurprimidol concentrations in field-grown plants after 28-day exposures were highest in the buds (Table 3). Flurprimidol was also found in the stems and roots. The milfoil plants at the time of treatment did not have roots but produced them during the exposure period. This suggests that the flurprimidol either moved basipetally in the plant or entered newly forming roots via the water-soil solution. The former seems unlikely since most of the literature on terrestrial plants suggests that flurprimidol and other gibberellin synthesis inhibitors are translocated primarily in the xylem (e.g. Sterrett 1988). The compound probably entered all of the plant parts through the aqueous medium; however, a slightly higher accumulation of flurprimidol in buds may indicate some upward movement in the plant. Sterrett and Tworkoski (1987) found that 10 percent of the flurprimidol applied to woody terrestrial plants by stem injection had moved into new shoots by 35 days after treatment. The majority of the compound remained near the application site, and none was detected in the roots.

The amount of flurprimidol applied to each barrel at a dose of 500  $\mu\text{g a.i. L}^{-1}$  was approximately  $3.49 \times 10^7$  ng. At the end of 28 days, a total of approximately  $4.1 \times 10^6$  ng of flurprimidol was recovered in the plant,



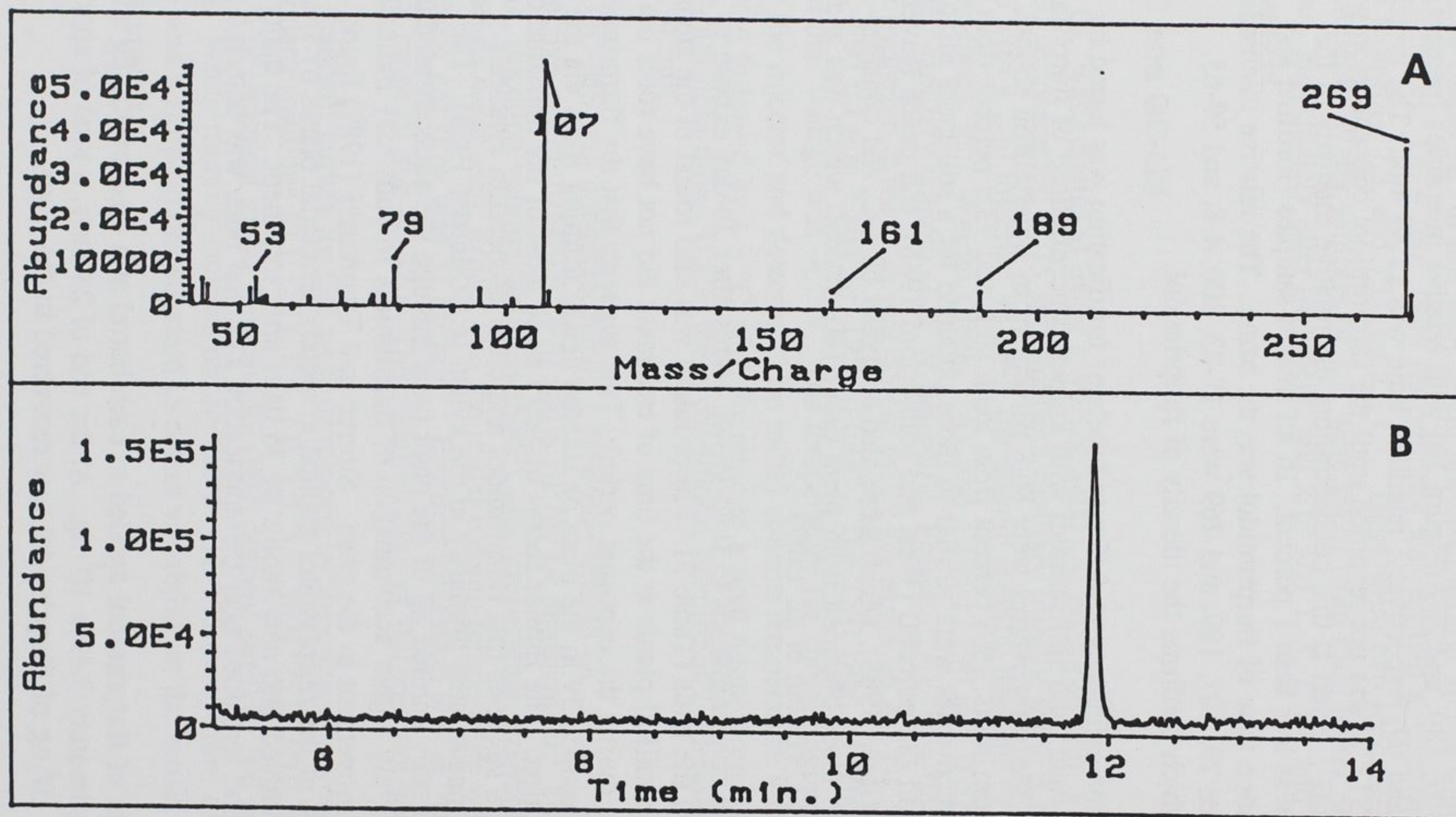


Figure 10. GC-MS characteristics of flurprimidol at 5-ng injection. (A) Mass spectrum; the major ions of flurprimidol are 79, 107, 189, and 269. (B) Total ion chromatogram



**Table 2**  
**Recovery of Flurprimidol from Eurasian Watermilfoil Shoots, Roots, Soil, and Water Samples Spiked with Different Flurprimidol Concentrations**

Sample Type	Added Flurprimidol <sup>a</sup>	Recovery Level <sup>a,b</sup>	% Recovery
Plant shoots	100	83 ± 2	82.9
	400	360 ± 52	89.6
	2,000	1,847 ± 91	90.6
	5,000	4,225 ± 24	83.9
	10,000	8,725 ± 34	87.2
Plant roots	100	78 ± 4	78.5
	400	332 ± 23	83.0
	2,000	1,744 ± 35	87.2
	5,000	4,505 ± 40	90.1
	10,000	8,710 ± 38	87.1
Loam soil	25	17 ± 4	68.4
	100	79 ± 3	79.3
	200	182 ± 6	91.0
	500	388 ± 17	77.6
	1,000	801 ± 49	80.1
Water	10	9 ± 2	88.5
	25	23 ± 2	91.0
	125	117 ± 19	93.4
	250	236 ± 18	94.3
	500	476 ± 15	95.3
	1,000	972 ± 11	97.2

<sup>a</sup> ng g<sup>-1</sup> dry weight basis in plant shoots and roots, ng g<sup>-1</sup> fresh weight basis in soil, ng ml<sup>-1</sup> in water.

<sup>b</sup> Each value is the mean ± SD of two experiments with two replicates each.

soil, and water components (Table 3). Residues in the combined plant parts accounted for only 0.039 percent of the total flurprimidol recovered (Table 3). Soil accounted for 39.6 percent of the total recovered, although the highest concentration and recovery was found in the upper 5-cm soil layer. Of the total recovered in the wet soil, approximately 4.2 percent had moved into the lower 5 cm of soil. The flurprimidol concentration in the free water from the upper layer was approximately the same as the concentration detected in the water from the barrel; however, no flurprimidol was detected in the free water from the lower 5 cm of soil. Flurprimidol is weakly adsorbed and easily desorbed from soils (Lilly Research Laboratories 1983) and therefore appears to be readily available for plant uptake and leaching. In leaching columns, 7.3 percent of applied flurprimidol has moved through 30 cm of terrestrial soils after 45 days (Lilly Research Laboratories 1983).

Approximately 60 percent of the recovered flurprimidol was present in the water fraction (Table 3). However, this only represented approximately 7 percent of that initially applied ( $3.49 \times 10^7$  ng); the estimated half-life was 8 days. The actual analysis of flurprimidol residues with time showed that its half-life in water at 7.5 and 75.0 µg a.i. L<sup>-1</sup> was 6.8 days (Figure 11). In addition to



**Table 3**

**Recovery of Flurprimidol from Eurasian Watermilfoil Plant Parts, Water, and Soil Collected from Barrels 28 Days After Treatment with 500  $\mu\text{g L}^{-1}$  Flurprimidol. Means  $\pm$  SD**

Sample <sup>a</sup>		Flurprimidol		Distribution (%) of Recovered Flurprimidol
Type	Wet Wt or Vol Barrel <sup>1</sup>	Concentration <sup>b</sup>	Total (ng) Barrel <sup>1</sup>	
Stems	12.8 $\pm$ 0.8	67 $\pm$ 11	870 $\pm$ 20	0.021
Buds	3.6 $\pm$ 1.8	93 $\pm$ 21	332 $\pm$ 20	0.008
Roots	8.1 $\pm$ 1.8	51 $\pm$ 1	410 $\pm$ 10	0.010
Soil				
Upper 5 cm	7,065 $\pm$ 432	220 $\pm$ 51	1.56 $\times 10^6 \pm 3.94 \times 10^5$	37.9
Lower 5 cm	6,664 $\pm$ 368	10 $\pm$ 2	6.80 $\times 10^4 \pm 1.10 \times 10^4$	1.7
Water	55,000	45 $\pm$ 12	2.47 $\times 10^6 \pm 2.12 \times 10^5$	60.4
Total recovery (ng) after 4 weeks			4.10 $\times 10^6$	
Total applied (ng)			3.49 $\times 10^7$	
Dissipation (%) in 4 weeks			88.3	

<sup>a</sup> Wet weight (g) of Eurasian watermilfoil and soil; volume (ml) of water.  
<sup>b</sup> ng g<sup>-1</sup> fresh weight basis in Eurasian watermilfoil and soil; ng ml<sup>-1</sup> in water.



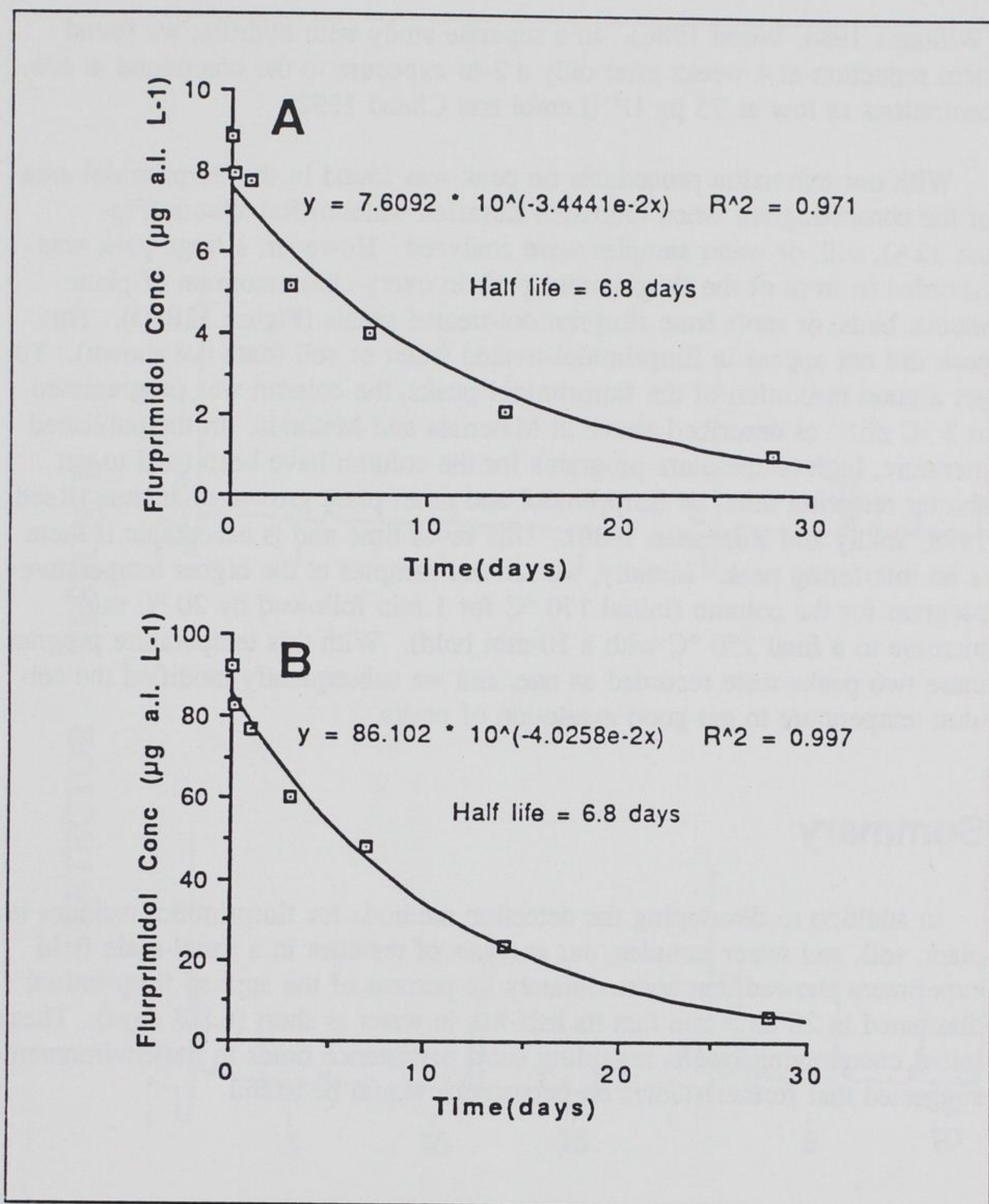


Figure 11. Recovery of flurprimidol in barrel water. (A)  $7.5 \mu\text{g L}^{-1}$  treatment and (B)  $75 \mu\text{g L}^{-1}$  treatment. Half-life of flurprimidol at both concentrations was 6.8 days

loss to the soil and plant components, flurprimidol is highly susceptible to photolysis with a half-life of 3 hr in pure water under high light intensities (Lilly Research Laboratories 1983).

Approximately 88.3 percent of the flurprimidol initially applied had disappeared within the 28-day period (Table 3). However, even at low concentrations, flurprimidol may retain its activity in reducing plant elongation. At least 2 or 3 years of stem elongation reduction have been monitored on woody species using foliar or soil drench applications of flurprimidol and paclobutrazol



(Williams 1984, Wood 1986). In a separate study with hydrilla, we found stem reduction at 4 weeks after only a 2-hr exposure to the compound at concentrations as low as  $75 \mu\text{g L}^{-1}$  (Lembi and Chand 1992).

With our extraction procedures no peak was found in the flurprimidol area of the chromatogram when untreated Eurasian watermilfoil shoots (Figure 12A), soil, or water samples were analyzed. However, a large peak was recorded in front of the flurprimidol peak in every chromatogram of plant shoots, buds, or roots from flurprimidol-treated plants (Figure 12B-D). This peak did not appear in flurprimidol-treated water or soil (data not shown). To get a good resolution of the flurprimidol peaks, the column was programmed at  $3^\circ\text{C min}^{-1}$  as described above in Materials and Methods. In the published literature, high temperature programs for the column have been used to get shorter retention times of flurprimidol and other plant growth retardants (Reed 1988, Stahly and Buchanan 1986). This saves time and is acceptable if there is no interfering peak. Initially, we ran our samples at the higher temperature program for the column (initial  $170^\circ\text{C}$  for 1 min followed by  $20^\circ\text{C min}^{-1}$  increase to a final  $250^\circ\text{C}$  with a 10-min hold). With this temperature program these two peaks were recorded as one, and we subsequently modified the column temperature to get good resolution of peaks.

## Summary

In addition to developing the detection methods for flurprimidol residues in plant, soil, and water samples, our analysis of residues in a small-scale field experiment showed that approximately 88 percent of the applied flurprimidol dissipated in 28 days and that its half-life in water is short (6.8-8 days). These initial encouraging results regarding short persistence times in the environment suggested that further studies on persistence would be useful.



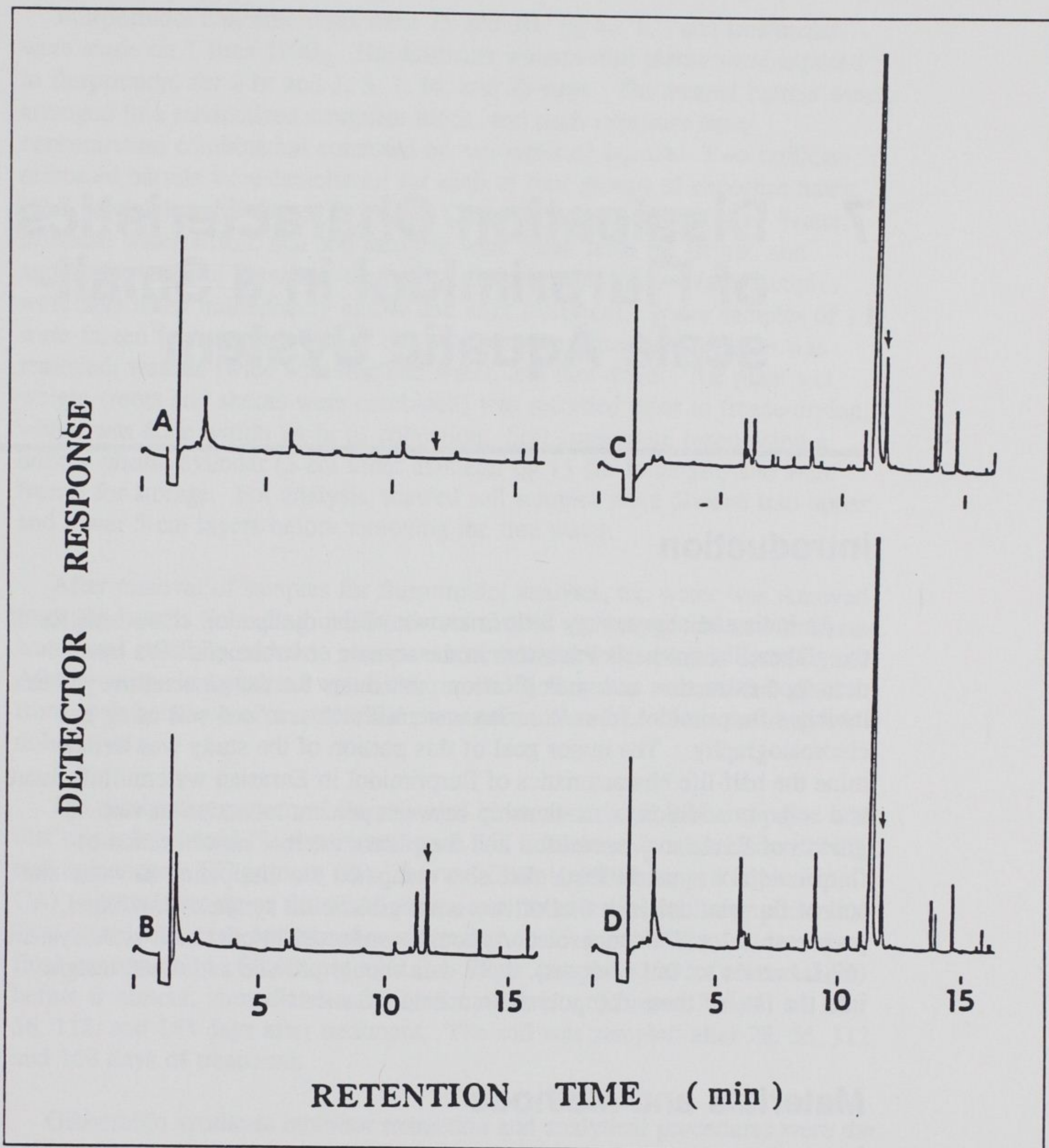


Figure 12. Capillary GC analysis of flurprimidol. (A) Shoots of untreated Eurasian watermilfoil (no flurprimidol detected), (B) roots, (C) shoots, and (D) buds of flurprimidol-treated Eurasian watermilfoil after preparation through florisil and  $C_{18}$ . Peak identification, RT = 11.74 min (arrows)



# **7 Dissipation Characteristics of Flurprimidol in a Small-scale Aquatic System**

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## **Introduction**

As indicated above, very little is known of the dissipation characteristics of the gibberellin synthesis inhibitors in the aquatic environment. We have described extraction and quantification procedures for the gibberellin synthesis inhibitor flurprimidol from Eurasian watermilfoil tissue and soil using gas chromatography. The major goal of this portion of the study was to determine the half-life characteristics of flurprimidol in Eurasian watermilfoil tissue and soil. In addition, a relationship between percent reduction in vertical growth of Eurasian watermilfoil and the plant's internal concentration of flurprimidol was established. We also compared the dissipation in water and soil of flurprimidol with that of two other gibberellin synthesis inhibitors, paclobutrazol and uniconazole. Although conducted in our small-scale system (67-L barrels set out-of-doors), these data should provide additional insight into the fate of these compounds in a field situation.

## **Materials and Methods**

Metal barrels (67-L capacity) with plastic liners were set in an unshaded outdoor area. Loam soil (46.5 percent sand, 41.0 percent silt, 12.4 percent clay; 1.6 percent organic matter, pH 6.2; 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 soil was allowed to settle for 2 to 3 days. Two stem apices (10 cm in length) of healthy Eurasian watermilfoil from Martel pond (Tippecanoe Co; Indiana) were planted per barrel and allowed to acclimate for 7 days prior to flurprimidol treatment. Flurprimidol (50 percent WP, DowElanco Products Company, Indianapolis, IN) was applied by diluting the compound in 10 ml of water, then gently stirring the solution into the barrels, without disturbing the soil, to ensure even dispersal.



Flurprimidol concentrations were 75 and 200  $\mu\text{g a.i. L}^{-1}$ , and treatments were made on 1 June 1990. The Eurasian watermilfoil plants were exposed to flurprimidol for 2 hr and 1, 3, 7, 14, and 28 days. The treated barrels were arranged in a randomized complete block, and each exposure time/concentration combination consisted of two replicate barrels. Two replicate untreated barrels were established for each of four groups of exposure times: (1) 2 hr and 1 and 3 days, (2) 7 days, (3) 14 days, and (4) 28 days. Water, Eurasian watermilfoil, and soil samples were taken from the treated and appropriate control barrels at the end of the exposure time. Water samples were also taken immediately before and after treatment. Water samples of 1 L were frozen for storage. One of the two Eurasian watermilfoil plants was removed, washed twice with distilled water, and blot-dried. The plant wet weight (roots and shoots were combined) was recorded prior to freeze-drying, which was done within 24 hr of collection. Soil cores were taken using a hollow plastic cylinder (5 cm inner diameter by 15 cm in length) and were frozen for storage. For analysis, thawed soil samples were divided into upper and lower 5-cm layers before removing the free water.

After removal of samples for flurprimidol analysis, the water was removed from the barrels (including untreated controls) by siphoning, and new untreated water was carefully added in a manner to minimize soil sediment disturbance. After 4 weeks in untreated water, the remaining Eurasian watermilfoil plant from each barrel was harvested and prepared as described above for flurprimidol analysis.

The dissipation pattern of flurprimidol in water and soil was compared with that of paclobutrazol (21.8 percent liquid, ICI Americas, Inc., Goldsboro, NC) and uniconazole (50 percent WP, Chevron Chemical Company, Richmond, CA) in barrels set up as described above except that no plants were present. Two barrels for each compound were treated on 19 June 1990 to achieve a final concentration of 1,000  $\mu\text{g a.i. L}^{-1}$ . Water samples (100 ml) were taken before treatment, immediately after treatment, and at 2 hr and 1, 3, 7, 14, 28, 56, 112, and 168 days after treatment. The soil was sampled after 28, 56, 112, and 168 days of treatment.

Gibberellin synthesis inhibitor extraction and analytical procedures were the same as those described above for water, plant tissue, and soil and published in Chand and Lembi (1991). Standards were technical grade flurprimidol (99.85 percent, Eli Lilly and Company, Indianapolis, IN), paclobutrazol (97.5 percent, ICI Americas, Richmond, CA) and uniconazole (78.5 percent, Valent U.S.A. Corporation, Walnut Creek, CA). Since all three gibberellin synthesis inhibitors behave similarly during extraction (Reed 1988) we used flurprimidol as an internal standard to quantify the residues of uniconazole and paclobutrazol and used paclobutrazol for the quantification of flurprimidol. Residues were quantified using a Varian 3400 GC equipped with a model 8035 autosampler, thermionic specific detector (TSD), and a DB-17 (30 m X 0.32 mm) fused silica capillary column. To confirm the identity of the compounds, mass spectra of technical grade flurprimidol, paclobutrazol, and uniconazole (dissolved in 100 percent methanol) were obtained using a Hewlett



Packard GC 5890A gas chromatography-mass spectrometer with HP mass selective detector (MSD) 5970 and a HP7673A autosampler.

## Results

Flurprimidol was not detected in untreated barrels or in barrels sampled before treatment. Water samples collected immediately after treatment showed flurprimidol residues were slightly less, but within 10 percent, of the target amounts of 75 and 200  $\mu\text{g L}^{-1}$ .

Flurprimidol was present in water, plant tissue, and soil in the treated barrels throughout the 28-day sampling period (Table 4). The amounts in water and plant tissue decreased over the 28-day period at both treatment concentrations but generally increased in the upper and lower soil layers. At 28 days, the amounts of flurprimidol remaining in the barrels were 14.6 and 14.4 percent of that initially applied at 75 and 200  $\mu\text{g L}^{-1}$ , respectively. These percent recoveries match reasonably well with an 11.7 percent recovery reported in Chand and Lembi (1991) conducted in 1989 in which barrels were treated with 500  $\mu\text{g L}^{-1}$  of flurprimidol.

Residue data, when calculated as  $\text{ng ml}^{-1}$  of water and  $\text{ng g}^{-1}$  fresh weight of plants and soil, were used to determine dissipation curves and half-lives of flurprimidol in water and plant tissue. Best fit regression equations (Figures 13,14) showed that the half-life of flurprimidol in water was 8.4 and 9.8 days at 75 and 200  $\mu\text{g L}^{-1}$ , respectively. The compound was present at its maximum value in water within 1 day of treatment and then decreased through the 28-day period. The half-life of the compound in milfoil tissue was similar to that in water: 9.1 and 8.8 days at 75 and 200  $\mu\text{g L}^{-1}$ , respectively. Maximal concentrations of flurprimidol in milfoil tissue appeared within 1 to 3 days after treatment and then decreased. In contrast to water and plant tissue, flurprimidol concentration in the upper 5 cm of soil increased over the first 7 days after treatment; after that point, the rate of increase appeared to level off.

Of the flurprimidol still detectable in the barrels at the end of the 28-day period (14.6 and 14.4 percent of the initial amount applied), the highest percentage (64.1 and 78.1 percent of the total at 75 and 200  $\mu\text{g L}^{-1}$ , respectively) was found in the water (Table 5). The upper 5-cm soil layer contained the next highest percentage (34.8 and 21.3 percent of the total at 75 and 200  $\mu\text{g L}^{-1}$ , respectively). Very little of the compound had moved into the lower soil layers. Plant tissue contained less than 0.1 percent of the remaining flurprimidol, a result of the small amount of plant tissue present (0.06 percent of the total weight) in relation to the other components. However, when the residues in plant, soil, and water were compared on a per unit wet weight basis during the treatment period, the plant tissue contained as much as 87 times the amount of flurprimidol present in the soil and between 2.93 and 6.39 times the



**Table 4**  
**Mass Balance of Total Flurprimidol Residues in Water, Soil, and Plant Tissues [% = percent of applied**  
**(4072.8  $\pm$  16  $\mu\text{g}$  per barrel for 75  $\mu\text{g L}^{-1}$  and 11584.2  $\pm$  470  $\mu\text{g}$  per barrel for 200  $\mu\text{g L}^{-1}$  Flurprimidol)]. Means  $\pm$  SD**

Days After Application	Flurprimidol Residues (µg)									
	Water	%	Soil(U) <sup>a</sup>	%	Soil(L) <sup>b</sup>	%	Plant	%	Total	%
75 µg L <sup>-1</sup> Treatment										
0.08	3784±184	92.9	20.7±1.9	0.51	1.85±0.11	0.04	0.32±0.13	0.01	3806.8	93.5
1.0	3624±95	88.9	72.4±2.6	1.78	2.17±0.46	0.05	0.53±0.04	0.01	3699.1	90.8
3.0	3175±19	77.9	80.5±3.0	1.98	2.39±0.27	0.06	0.66±0.08	0.02	3258.0	79.9
7.0	2469±239	60.6	118.1±0.4	2.90	3.03±0.15	0.07	0.73±0.19	0.02	2590.7	63.6
14.0	1402±363	34.4	151.6±7.5	3.72	5.24±0.93	0.13	0.48±0.28	0.01	1559.3	38.3
28.0	380±3	9.3	206.3±87.7	5.06	6.37±0.10	0.16	0.27±0.09	0.01	592.9	14.6
200 µg L <sup>-1</sup> Treatment										
0.08	11090±295	95.7	58.4±0.2	0.51	2.04±0.04	0.02	0.98±0.23	0.01	11151.2	96.3
1.0	11351±340	97.8	292.2±26.1	2.52	3.57±0.44	0.03	1.13±0.27	0.01	11647.7	100.4
3.0	9545±261	82.4	257.7±35.2	2.22	3.78±0.57	0.03	2.01±0.07	0.02	9808.9	84.7
7.0	7080±188	61.2	366.8±14.5	3.17	3.71±0.24	0.03	1.26±0.05	0.01	7451.7	64.4
14.0	3900±45	33.7	329.5±89.5	2.84	8.78±0.88	0.08	0.65±0.12	0.01	4238.9	36.6
28.0	1302±139	11.2	355.8±23.9	3.07	9.92±0.42	0.08	0.31±0.01	0.01	1668.0	14.4
<sup>a</sup> U = upper 5 cm. <sup>b</sup> L = lower 5 cm.										



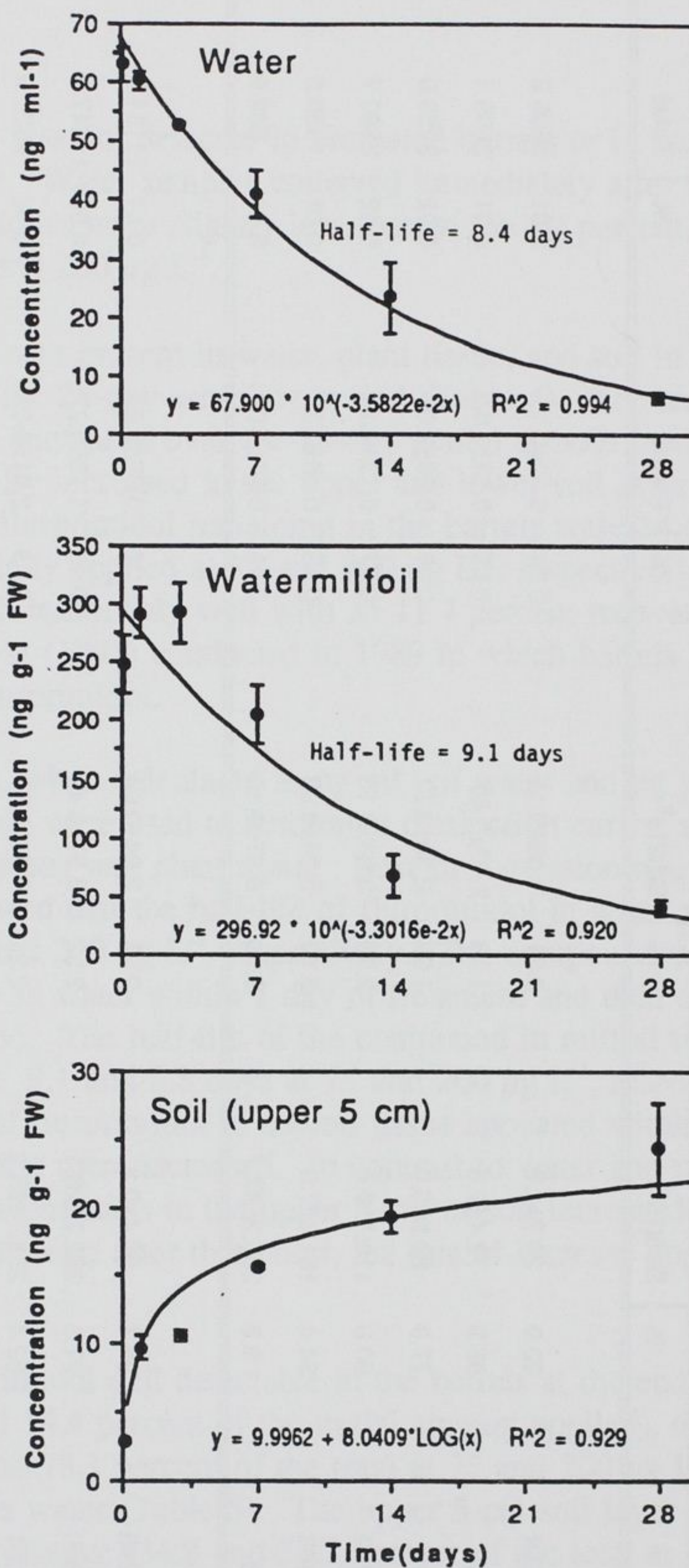


Figure 13. Dissipation curves of flurprimidol in water, watermilfoil tissue, and the upper 5-cm soil layer. Treatment was at 75  $\mu\text{g L}^{-1}$ . Bars =  $\pm 1$  S.D.



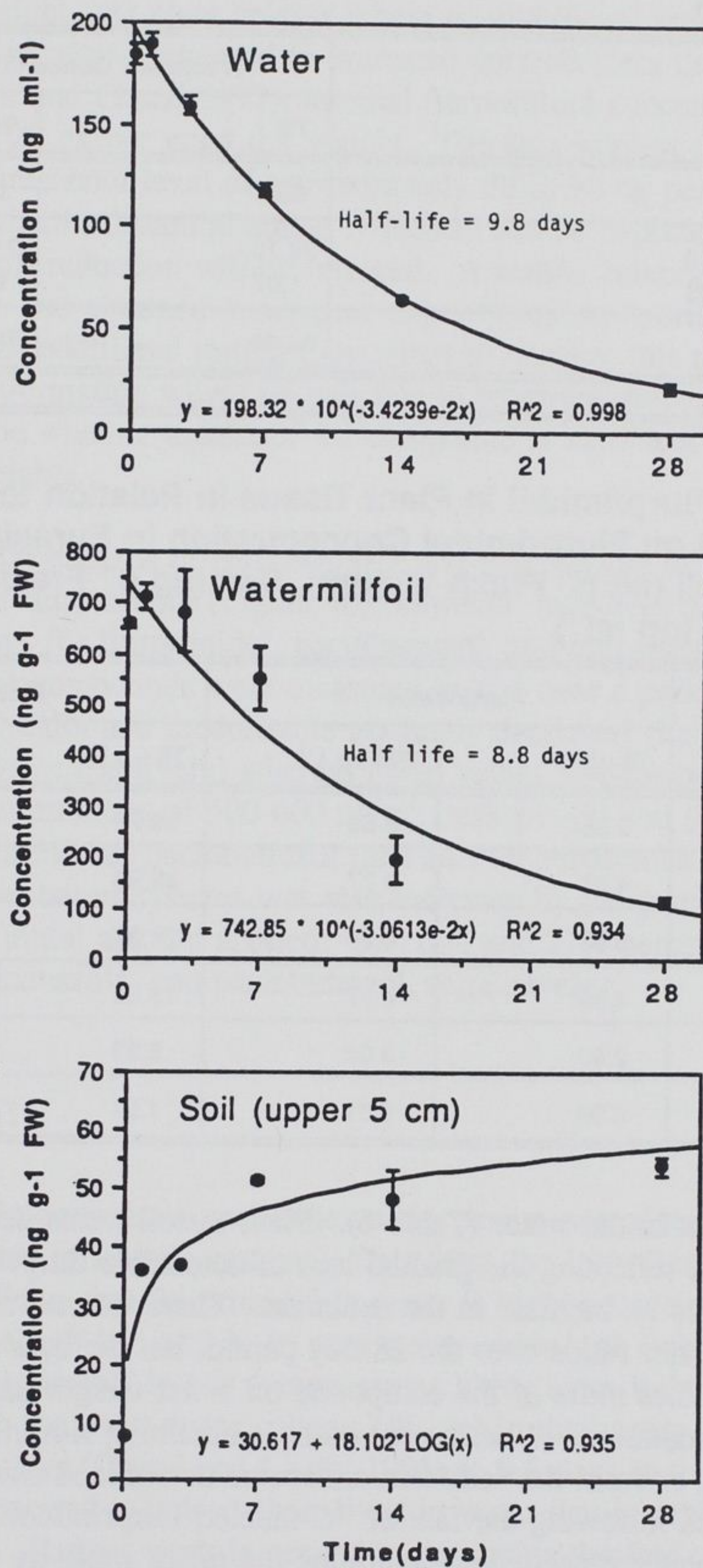


Figure 14. Dissipation curves of flurprimidol in water, watermilfoil tissue, and the upper 5-cm soil layer. Treatment was at 200  $\mu\text{g L}^{-1}$ . Bars =  $\pm$  S.D.



<b>Table 5</b> <b>Distribution (%) of Recovered Flurprimidol 28 Days After Application</b>		
Sample	Treatment Concentration ( $\mu\text{g L}^{-1}$ )	
	75	200
Plant	0.05	0.02
Soil (upper 5 cm)	34.79	21.33
(lower 5 cm)	1.07	0.59
Water	64.09	78.06

<b>Table 6</b> <b>Ratios of Flurprimidol in Plant Tissue in Relation to Water and Soil Based on Flurprimidol Concentration in Eurasian Watermilfoil (<math>\text{ng g}^{-1}</math> Fresh Weight), Soil (<math>\text{ng g}^{-1}</math> Fresh Weight), and Water (<math>\text{ng ml}^{-1}</math>)</b>				
Days After Treatment	Plant/Water		Plant/Soil	
	75 $\mu\text{g L}^{-1}$	200 $\mu\text{g L}^{-1}$	75 $\mu\text{g L}^{-1}$	200 $\mu\text{g L}^{-1}$
0.08	3.95	3.56	86.79	85.81
1.0	4.83	3.77	29.90	19.62
3.0	5.53	4.29	27.37	18.43
7.0	4.99	4.67	13.03	10.71
14.0	2.93	3.02	3.53	4.05
28.0	6.39	5.31	1.67	2.13

amount present in the water (Table 6). Plant-to-soil ratios decreased over the 28-day period, reflecting the gradual loss of detectable flurprimidol from the plant tissue and its increase in the sediment. There was no consistent trend in the plant-to-water ratios over the 28-day period, but the data suggest that the plant accumulates more of the compound on a wet weight basis than either the water or the sediment. Whether the plant is obtaining flurprimidol from the water column or from the sediment cannot be determined using these data. Further studies following the fate of  $^{14}\text{C}$ -labeled flurprimidol applied to the soil versus the water are needed to determine the major route by which the plants take up the compound.

Plants that had been exposed to flurprimidol for varying periods and then allowed to grow in untreated water for 28 days were harvested, measured for main stem length (data above and in Lembi and Chand 1992), and analyzed for flurprimidol residues. In this way we obtained plants with different percent reductions in main stem lengths and internal flurprimidol concentrations. The relationship between percent length reduction and internal concentration was



linear and positive: percent main stem reduction increased with increasing internal flurprimidol concentration (Figure 15). Plants that were reduced in length by at least 60 percent contained approximately 55 to 85 ng flurprimidol per gram dry weight. At plant heights where no significant reduction in length had been obtained when compared to untreated controls (less than 20 percent reduction; Lembi and Chand 1992), internal flurprimidol concentrations were between 13 and 22 ng per gram dry weight. The data suggest that there may be an internal threshold level of approximately 20 to 30 ng per gram dry weight, above which substantial length reduction can be expected and below which stem length reduction will be minimal. A similar relationship and threshold value was obtained in separate experiments on hydrilla (data not shown). Although additional testing is required to confirm this particular relationship, such information would be valuable in screening treated plants in the field to determine whether uptake of the compound is sufficient to give reduced plant heights.

The dissipation patterns of paclobutrazol and uniconazole in water were similar to that of flurprimidol (Figure 16); however, half-lives differed: 9.3, 24.4, and 5.2 days for flurprimidol, paclobutrazol, and uniconazole, respectively. When the compounds were measured in soil over a period of 28 to 168 days, flurprimidol and uniconazole gradually decreased (half-lives of 178 and 102 days, respectively) whereas mean values of paclobutrazol remained at concentrations of 500-600 ng g<sup>-1</sup> fresh weight soil (Figure 17). The longer persistence of paclobutrazol (and shorter persistence of uniconazole) in these barrel systems was also indicated by the percent recovery at 168 days of the initial amount applied: 3.9, 0.9, and 27.4 percent of the flurprimidol, uniconazole, and paclobutrazol, respectively.

## Discussion

Virtually no information is available on the persistence of the gibberellin synthesis inhibitors in aquatic systems. Data from the Lilly Research Laboratories (1983) indicate that flurprimidol in water is highly susceptible to photolysis, with a half-life of 3 hr in pure water under high light intensities. Photolysis of flurprimidol in our system seems likely since light could readily penetrate through the short water column (46 cm) in the barrels. Flurprimidol half-lives of 6.8 days (Chand and Lembi 1991) to 9.8 days in this work indicate that the compound is indeed short-lived in water that is subject to good light penetration. Further work is needed to determine the fate of flurprimidol in water under lower light conditions.

Clearly, the persistence of different gibberellin synthesis inhibitors in water varies. The only obvious structural difference between the shortest-lived compound, uniconazole, and the longest-lived compound, paclobutrazol, is the presence of a covalent double bond in uniconazole. In paclobutrazol, the carbons are saturated with hydrogen. However, the stereoisomers of the



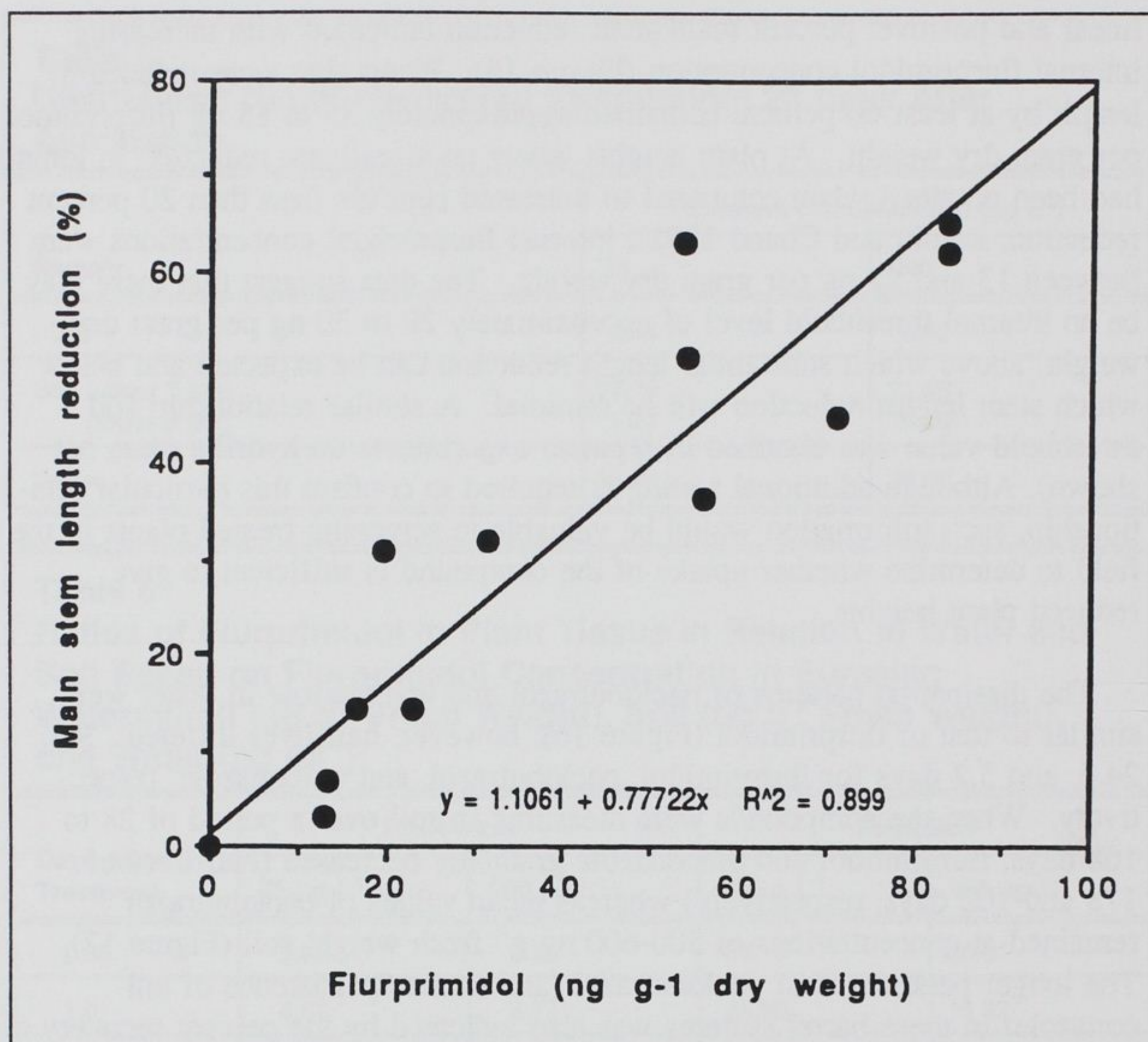


Figure 15. Relationship of internal flurprimidol content in milfoil tissue and percent main stem length reduction. Main stem length reductions of less than 20 percent were not statistically different from untreated controls (data in Lembi and Chand 1992)

biologically active forms of these two compounds also differ, a fact that Steffens (1988) suggested might partially account for the fact that uniconazole was the more effective of the two compounds in reducing stem length in greenhouse-grown apple trees, even at 115 days after treatment. Whether uniconazole in apple stem tissue is simply more biologically active than paclobutrazol or whether it persists longer, thereby providing long-term stem reduction, is unknown. In laboratory studies, uniconazole also was more effective than either flurprimidol or paclobutrazol in reducing main stem length in hydrilla (Netherland and Lembi 1992). This finding along with our data showing a shorter persistence time in water and soil suggests that the effectiveness of uniconazole may be due to biological activity rather than to persistence.

The majority of data on the persistence of the gibberellin synthesis inhibitors in the environment is from terrestrial systems. The compounds are relatively persistent in plant tissues and soil and are effective in reducing stem length in tree species for at least 3 years (Williams 1984, Tukey 1986, Mauk et al. 1990) and in herbaceous species for several months (Dernoeiden 1984).



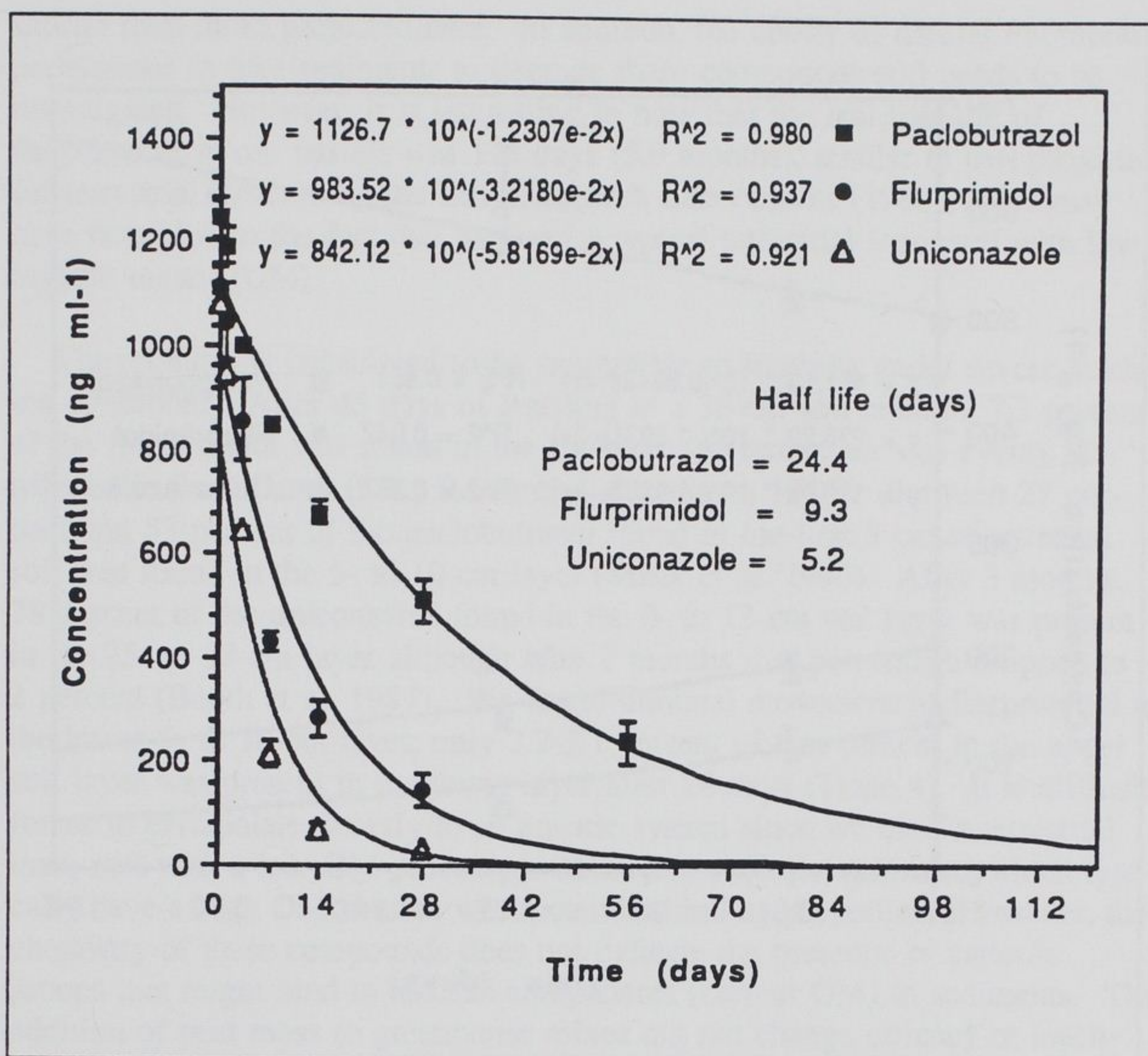


Figure 16. Dissipation of paclobutrazol, flurprimidol, and uniconazole in water. Treatment was at 1,000  $\mu\text{g L}^{-1}$ . Bars =  $\pm 1$  S.D.

As much as 22 percent of the paclobutrazol detected at one week was still present in apple tissues 13 weeks after a soil drench application (Reed, Curry, and Williams 1989), and as much as 81 percent of <sup>14</sup>C-flurprimidol was recovered in apple tissue 35 days after stem injection (Sterrett and Tworkoski 1987). The compounds can be applied effectively to the foliage (Lehman and Unrath 1988), suggesting that terrestrial plants can take up these compounds through their leaves as well as from the roots and via stem injection. The detection of nearly maximum amounts of flurprimidol in milfoil tissues 2 hr after treatment suggests that submersed plants may, at least initially, take up the compound from the water, presumably through the shoots. Although the compound dissipates from the milfoil tissue over the 28-day period with a half-life similar to that in water, some accumulation of flurprimidol in the tissue does appear to occur (Table 3). The plant/water ratios seem relatively stable over the 28-day period, suggesting that as the compound dissipates from the water, it is also dissipating from the plant tissue. Thus, there may be an equilibrium between tissue-held flurprimidol and that present in the water, so that flurprimidol concentration in the water may be an important factor in dictating internal tissue concentration.



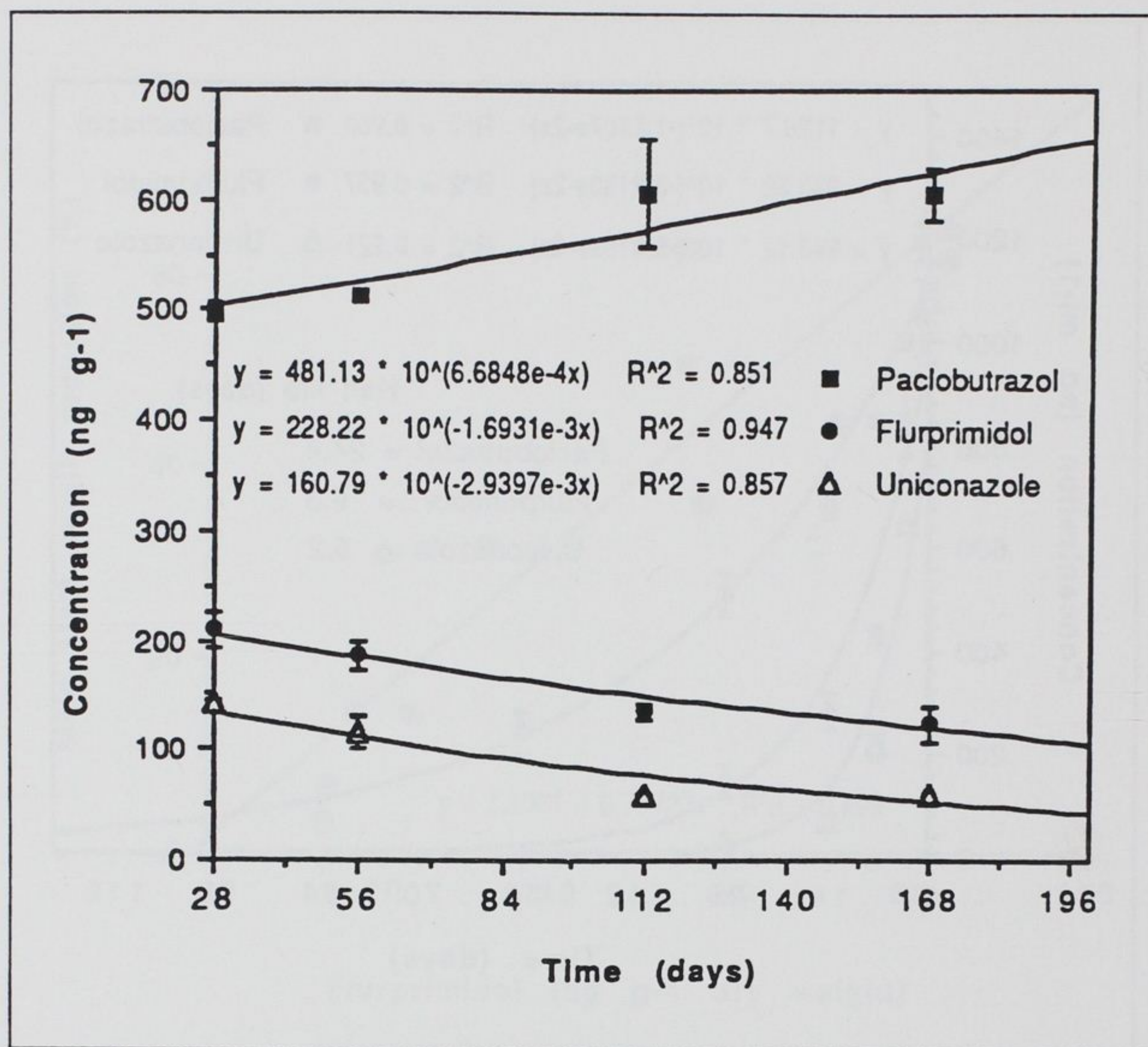


Figure 17. Dissipation of paclobutrazol, flurprimidol, and uniconazole in soil. Treatment was at  $1,000 \mu\text{g L}^{-1}$ . Bars =  $\pm$  S.D.

Another potential route of submersed plant uptake is through the sediment. The fact that the plant/soil ratios decreased over the 28-day period does not negate the possibility of sediment uptake. Even though flurprimidol concentration in the plant does not seem to respond in a positive way to changes in flurprimidol concentration in the soil, the concentrations in both the plant and the soil may be so high that they mask potential uptake and/or equilibrium relationships.

In terrestrial systems, the half-life of flurprimidol is estimated to be less than 6 months under conditions of adequate rainfall or irrigation (Lilly Research Laboratories 1983). However, paclobutrazol only decreased at a rate of 50 percent per year over a 3-year period in an apple orchard in North Carolina (Mauk et al. 1990). Thus, there is some indication from terrestrial systems that paclobutrazol may be more persistent in soil than flurprimidol, as it also appeared to be in our system. Since our long-term soil persistence study was initiated in mid-June and the last sampling occurred in early December, when lake water temperatures in central Indiana are typically between 5 and 10 °C (unpubl. data), it is possible that under warmer climatic conditions in other parts of the country the half-lives of all three compounds will be



shorter than those projected here. In addition, the ability of natural microbial populations in lake sediments to degrade these compounds still needs to be investigated. However, it is interesting to note that the soil half-life of flurprimidol in our barrels was 178 days (5.9 months), similar to that projected for terrestrial systems by the Lilly Research Laboratories (1983). This may have been due to the fact that we used a typical terrestrial loam soil with low organic matter (OM).

Flurprimidol is considered to be susceptible to leaching under severe leaching conditions. After 45 days of leaching in a 30-cm soil column, 7.3 percent of the flurprimidol was found in the leachate; the remainder was evenly distributed in the column (Lilly Research Laboratories 1983). Between 27 percent and 53 percent of the paclobutrazol found in the first 5 cm of a treated soil was found in the 5- to 10-cm layer (Mauk et al. 1990). After 3 months, 28 percent of the uniconazole found in the 0- to 13-cm soil layer was present in the 25- to 38-cm layer although after 7 months that percentage dropped to 2 percent (Booth et al. 1989). We found minimal movement of flurprimidol to the lower 5- to 10-cm layer; only 2.7-3.1 percent of that present in the upper soil layer was present in the lower layer after 28 days (Table 4). It is difficult for us to extrapolate directly to an aquatic system since we used a terrestrial loam soil with a low OM percentage rather than aquatic sediments which typically have a high OM content with potential binding properties. However, the chemistry of these compounds does not indicate the presence of cationic groups that might bind to anionic components (clay or OM) in sediments. The addition of peat moss to greenhouse mixes did not change efficacy or leachability of the compounds when compared to their performance in mineral soils (Larson, Long, and Bonamino 1974, Bonamino and Larson 1978, Barrett 1982). This suggests that the compounds are not tightly bound to organic matter, a factor that could influence both leachability and persistence. Interestingly, the addition of pine bark to greenhouse mixes did decrease efficacy and leachability; Barrett (1982) suggested that a hydrophobic attraction between nonpolar portions of the compounds and the bark accounted for increased binding.

## Summary

Flurprimidol shows rapid dissipation in water (8.4- to 9.8-day half-life) that is well penetrated by light. Although flurprimidol dissipates rapidly from plant tissue (8.8- to 9.1-day half-life), apparently low dosages (25-30 ng g<sup>-1</sup> dry weight of plant tissue) are sufficient to achieve significant main stem length reduction. The compound dissipates slowly from the soil (178-day half-life), but further studies using lake sediments are required before a complete picture of sediment persistence is obtained. A half-life of 6 months in sediment may be advantageous in providing a source of compound for long-term plant uptake. The dissipation characteristics of the three gibberellin synthesis inhibitors in water and soil appear to differ; these differences may turn out to be important factors in determining which of these compounds is eventually developed for the aquatic market. Further information will also be needed on



the breakdown products of these compounds and their dissipation characteristics.



## 8 Final Conclusions and Recommendations

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Results of these studies lead to the following conclusions and recommendations:

- a. Compounds other than the gibberellin synthesis inhibitors that show a potential to reduce main stem length in submersed plants are bensulfuron methyl and amidochlor. Bensulfuron methyl may be inconsistent at providing growth regulation, and further study is required to determine the exact conditions under which the compound is herbicidal or regulatory. Amidochlor should be tested in small-scale outdoor systems to verify effective dosages and length of control.
- b. The gibberellin synthesis inhibitors may be somewhat less effective at reducing main stem lengths at low light intensities (particularly in hydrilla), but they still appear to be active under low light. As the plants reach the upper portions of the water column, the combination of inhibitor and high light should cause stem reduction (assuming internal plant concentrations of the inhibitor are still optimal). No further study is recommended, but this is a parameter that should be monitored carefully if these compounds are tested in the field.
- c. Flurprimidol appears to affect a broad spectrum of submersed plants, with the possible exceptions of *Ceratophyllum* and *Vallisneria*. In addition to continuing testing on a number of plant species under better growing conditions, the effects of height-reduced plants on species competition and habitat value should be studied. A study of the effect of gibberellic acid and the gibberellin synthesis inhibitors on submersed species with different growth habits could provide additional predictive information on selectivity.
- d. Short-term exposures (as low as 2 hr) of flurprimidol appear to effectively reduce main stem lengths in hydrilla and Eurasian watermilfoil for up to 4 weeks. Further studies in more controlled systems such as the WES aquaria are needed to determine optimal exposure time/dosage conditions.



- e. In addition to developing the procedures for detecting the gibberellin synthesis inhibitors in plant, water, and sediment samples, our studies suggest relatively rapid dissipation of flurprimidol and uniconazole from the water and plant tissues. Sediment persistence is longer (half-life of 6 months), and paclobutrazol appears to be considerably more persistent in water and sediment than the other two compounds. The actual role of sediments in storing and making these compounds available for long-term plant uptake is unknown and should be investigated using  $^{14}\text{C}$ -flurprimidol labeling experiments.
- f. Other characteristics of the gibberellin synthesis inhibitors in aquatic systems should be studied, such as metabolite structure and persistence and toxicology. However, it may not be feasible to study these aspects unless a governmental or industrial entity plans to develop these compounds for EPA registration for aquatic systems.



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1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE January 1994	3. REPORT TYPE AND DATES COVERED Final report
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4. TITLE AND SUBTITLE Plant Growth Regulators as Potential Tools in Aquatic Plant Management: Efficacy and Persistence in Small-Scale Tests	5. FUNDING NUMBERS
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6. AUTHOR(S) Carole A. Lembi Tara Chand-Goyal	
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7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Department of Botany and Plant Pathology Purdue University West Lafayette, IN 47907-1155	8. PERFORMING ORGANIZATION REPORT NUMBER
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9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Engineer Waterways Experiment Station Environmental Laboratory 3909 Halls Ferry Road Vicksburg, MS 39180-6199	10. SPONSORING / MONITORING AGENCY REPORT NUMBER  Contract Report A-94-1
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11. SUPPLEMENTARY NOTES  Available from National Technical Information Service, 5285 Port Royal Road, Springfield, VA 22161.
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12a. DISTRIBUTION / AVAILABILITY STATEMENT  Approved for public release; distribution is unlimited.	12b. DISTRIBUTION CODE
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13. ABSTRACT (Maximum 200 words) Bioassay and small-scale test systems were used to determine the efficacy and persistence of plant growth regulators with potential for aquatic plant management. The goal of the project was to identify compounds that reduce plant height and thus weediness of the submersed flowering plants hydrilla ( <i>Hydrilla verticillata</i> Royle) and Eurasian watermilfoil ( <i>Myriophyllum spicatum</i> L.). Laboratory bioassays indicated that triclopyr and imazapyr were herbicidal rather than growth regulatory on these species. Bensulfuron-methyl and amidochlor showed growth regulatory properties on Eurasian watermilfoil, and further research may be warranted on these compounds. A number of studies were conducted on flurprimidol, a gibberellin synthesis inhibitor which reduces stem internode length, thereby effectively reducing vertical stem length. Two-hour exposures to flurprimidol significantly reduced vertical stem length at concentrations of 750 µg L <sup>-1</sup> for hydrilla and 200 µg L <sup>-1</sup> for Eurasian watermilfoil for at least 28 days post-treatment, suggesting that short-term exposures may be efficacious under certain circumstances. Flurprimidol was less effective at reducing stem lengths under low light intensities (4-18 µE m <sup>-2</sup> sec <sup>-1</sup> ) than under high light (800-1000 µE m <sup>-2</sup> sec <sup>-1</sup> ), but stem lengths were shorter under low light than in the untreated controls. As the plants reach the more well-lit portions of the water column, vertical growth should be reduced if flurprimidol is still present at (Continued)
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14. SUBJECT TERMS  (See reverse)	15. NUMBER OF PAGES 66
	16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT UNCLASSIFIED	18. SECURITY CLASSIFICATION OF THIS PAGE UNCLASSIFIED	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
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physiologically active concentrations. Additional plants that were affected by flurprimidol included waterstar-grass (*Heteranthera dubia*), slender naiad (*Najas flexilis*), elodea (*Elodea canadensis*), and leafy pondweed (*Potamogeton foliosus*). Total plant lengths in *Vallisneria americana* and coontail (*Ceratophyllum demersum*) were not significantly affected by flurprimidol. Gas chromatographic procedures for detecting flurprimidol residues in water, plant tissues, and soil were developed. Flurprimidol dissipated rapidly from water (8.4 to 9.8 day half-life) and plant tissue (8.8 to 9.1 day half-life) and slowly from the soil (178 day half-life). Further studies are needed to determine the potential for long-term plant control, with emphasis placed on determining whether uptake of flurprimidol is from the water or hydrosol. Our studies to date suggest that the gibberellin synthesis inhibitors provide a viable alternative as an aquatic plant management strategy.

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Amidochlor  
Aquatic plants  
Bensulfuron methyl  
Bioassay  
Environmental persistence  
Eurasian watermilfoil  
Flurprimidol  
Gas chromatography  
Imazapyr  
Paclobutrazol  
Plant growth regulator  
Residues  
Triclopyr  
Uniconazole