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AQUATIC PLANT CONTROL
RESEARCH PROGRAM

TECHNICAL REPORT A-90-7

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AD-A226 125 BIOASSAY OF PLANT GROWTH REGULATOR ACTIVITY ON AQUATIC PLANTS

by

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July 1990

Final Report

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Prepared for DEPARTMENT OF THE ARMY
US Army Corps of Engineers
Washington, DC 20314-1000

Monitored by Environmental Laboratory
US Army Engineer Waterways Experiment Station
3909 Halls Ferry Road, Vicksburg, Mississippi 39180-6199



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SECURITY CLASSIFICATION OF THIS PAGE

REPORT DOCUMENTATION PAGE				Form Approved OMB No. 0704-0188	
1a. REPORT SECURITY CLASSIFICATION Unclassified			1b. RESTRICTIVE MARKINGS		
2a. SECURITY CLASSIFICATION AUTHORITY			3. DISTRIBUTION/AVAILABILITY OF REPORT		
2b. DECLASSIFICATION/DOWNGRADING SCHEDULE			Approved for public release; distribution unlimited.		
4. PERFORMING ORGANIZATION REPORT NUMBER(S)			5. MONITORING ORGANIZATION REPORT NUMBER(S)		
			Technical Report A-90-7		
6a. NAME OF PERFORMING ORGANIZATION Purdue University, Department of Botany and Plant Pathology		6b. OFFICE SYMBOL (if applicable)	7a. NAME OF MONITORING ORGANIZATION USAEWES Environmental Laboratory		
6c. ADDRESS (City, State, and ZIP Code) West Lafayette, IN 47907			7b. ADDRESS (City, State, and ZIP Code) 3909 Halls Ferry Road Vicksburg, MS 39180-6199		
8a. NAME OF FUNDING/SPONSORING ORGANIZATION US Army Corps of Engineers		8b. OFFICE SYMBOL (if applicable)	9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER		
8c. ADDRESS (City, State, and ZIP Code) Washington, DC 20314-1000			10. SOURCE OF FUNDING NUMBERS		
			PROGRAM ELEMENT NO.	PROJECT NO.	TASK NO.
					WORK UNIT ACCESSION NO.
11. TITLE (Include Security Classification) Bioassay of Plant Growth Regulator Activity on Aquatic Plants					
12. PERSONAL AUTHOR(S) Lembi, Carole A.; Netherland, Michael D.					
13a. TYPE OF REPORT Final report		13b. TIME COVERED FROM _____ TO _____		14. DATE OF REPORT (Year, Month, Day) July 1990	
				15. PAGE COUNT 36	
16. SUPPLEMENTARY NOTATION Available from National Technical Information Service, 5285 Port Royal Road, Springfield, VA 22161.					
17. COSATI CODES			18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)		
FIELD	GROUP	SUB-GROUP			
			See reverse.		
19. ABSTRACT (Continue on reverse if necessary and identify by block number)					
→ A laboratory bioassay system was developed to determine whether inhibitors of gibberellin synthesis could reduce plant height but maintain physiological competence in two weedy submersed aquatic plants, hydrilla (<i>Hydrilla verticillata</i> Royle) and Eurasian watermilfoil (<i>Myriophyllum spicatum</i> L.). The gibberellin synthesis inhibitors tested were uniconazol, flurprimidol, and paclobutrazol. Milfoil was more sensitive to the inhibitors than hydrilla. All three compounds were effective in reducing both main and lateral stem lengths in hydrilla, although the number of lateral stems was greatly increased over untreated controls. Hydrilla produced a stoloniferous growth habit, in contrast to milfoil in which the dominant growth form was a single reduced stem with numerous compressed buds. Photosynthesis, measured as oxygen evolution, was not significantly affected in either plant at dosages in which stem reduction was obtained. Hydrilla had to be in constant contact with the compound in order to be affected, whereas milfoil required only 1 day of exposure to produce (Continued)					
20. DISTRIBUTION/AVAILABILITY OF ABSTRACT ☒ UNCLASSIFIED/UNLIMITED ☐ SAME AS RPT ☐ DTIC USERS			21. ABSTRACT SECURITY CLASSIFICATION Unclassified		
22a. NAME OF RESPONSIBLE INDIVIDUAL			22b. TELEPHONE (Include Area Code)		22c. OFFICE SYMBOL

Unclassified

SECURITY CLASSIFICATION OF THIS PAGE

18. SUBJECT TERMS (Continued).

Aquatic plants	Hydrilla
Bensulfuron methyl	Paclobutrazol
Bioassay	Plant growth regulator
Eurasian watermilfoil	Uniconazol
Flurprimidol	

19. ABSTRACT (Continued).

long-term effects. The bioassay suggests that these gibberellin synthesis inhibitors would have minimal adverse impacts on plant health but would be effective at reducing plant height in aquatic systems.

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Unclassified

SECURITY CLASSIFICATION OF THIS PAGE

Preface

This study was conducted as a part of the US Army Corps of Engineers Aquatic Plant Control Research Program (APCRP). Funds for the study were provided by the Headquarters, US Army Corps of Engineers (HQUSACE), under Department of the Army Appropriation No. 96X3122, Construction General. The APCRP is managed by the US Army Engineer Waterways Experiment Station (WES), under the Environmental Resources Research and Assistance Programs, Mr. J. L. Decell, Manager. The HQUSACE Technical Monitor for the APCRP was Mr. James W. Wolcott.

The principal investigator for this work was Dr. Carole A. Lembi, Department of Botany and Plant Pathology, Purdue University, West Lafayette, IN, who, along with Mr. Michael D. Netherland, Graduate Assistant, prepared the report.

The research was monitored at the WES by Ms. Linda S. Nelson and Dr. Howard E. Westerdahl of the Aquatic Processes and Effects Group (APEG), Ecosystem Research and Simulation Division (ERSD), Environmental Laboratory (EL). The study was conducted under the general supervision of Dr. John Harrison, Chief, EL, and Mr. Donald L. Robey, Chief, ERSD, and under the direct supervision of Dr. Thomas L. Hart, Chief, APEG. The report was edited by Ms. Jessica S. Ruff of the WES Information Technology Laboratory.

Commander and Director of WES was COL Larry B. Fulton, EN. Technical Director was Dr. Robert W. Whalin.

This report should be cited as follows:

Lembi, Carole A., and Netherland, Michael D. 1990. "Bioassay of Plant Growth Regulator Activity on Aquatic Plants," Technical Report A-90-7, US Army Engineer Waterways Experiment Station, Vicksburg, MS.

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BIOASSAY OF PLANT GROWTH REGULATOR ACTIVITY ON AQUATIC PLANTS

Introduction

1. Although nuisance aquatic plants can have detrimental effects on a body of water, some aquatic plant growth is considered desirable (Wiley et al. 1984, Engel 1985). Submersed aquatic plants provide oxygen through photosynthesis, habitat for fish and fish food organisms, and bottom sediment stabilization. Unfortunately, aquatic plant management strategies often result in severe reduction or elimination of most plants in the area of treatment, since the primary technique is to use aquatic herbicides, most of which are non-selective. Rapid plant decomposition may also result in adverse effects on other components of the aquatic system.

2. Another potential approach to managing aquatic vegetation may be through the manipulation of natural plant hormonal processes. Certain substituted pyrimidine and triazole compounds have been found to inhibit the synthesis of gibberellin in plants and plant homogenates (Lever, Shearing, and Batch 1982; Rademacher et al. 1984; Hedden and Graebe 1985). These gibberellin synthesis inhibitors (GSIs) reduce stem length of terrestrial plants in species ranging from grasses to trees without altering viability or morphological differentiation such as seedhead development.

3. The primary goal of this study was to develop a simple bioassay system from which it could be determined if GSIs can reduce the rate of stem elongation in submersed aquatic plants without killing the plants. This presumably would lead to a lawn or "turf" at the bottom of the body of water that would not be weedy because the plants are short. This turf, however, would be composed of functional plants able to provide oxygen, habitat, and bottom stabilization.

4. Once the bioassay system was perfected, the specific goals of the project were to determine the following:

- a. The effects of the GSIs (and other plant growth regulating compounds as time permitted) on stem length and other associated length and biomass parameters (growth parameters).
- b. The effects of GSIs on the physiological competence of the plants, with emphasis on photosynthesis and respiration (physiological parameters).

- c. The length of time in which GSI effects persist.
- d. Effective exposure times for GSI effects to be expressed.

Materials and Methods

Plant cultures

5. Algal-free cultures of Eurasian watermilfoil (*Myriophyllum spicatum* L.) and the dioecious strain of hydrilla (*Hydrilla verticillata* Royle) were obtained from Drs. John Andrews of the University of Wisconsin and Steve Klaine of Memphis State University, respectively. Hydrilla was grown in 10-percent Hoagland's solution, and watermilfoil, in a modified Gerloff's solution (Andrews 1980) in 3-l round-bottomed flasks. Stock cultures of both plants were maintained in controlled environment chambers at $25^{\circ} \pm 1^{\circ} \text{C}$, $400 \mu\text{E m}^{-2} \text{sec}^{-1}$ and a 16:8 hr light-dark cycle. Initially, watermilfoil cultures were bubbled continuously with a 5-percent CO_2 -enriched air mixture to provide an inorganic carbon source. Later, both hydrilla and watermilfoil media were buffered after autoclaving with 10 ml ℓ^{-1} of a 2-g/100-ml stock solution of NaHCO_3 , and the CO_2 bubbling was discontinued. The plant cultures were routinely checked for algal contamination, and only those cultures not contaminated were used for experiments.

Bioassay conditions

6. Four-centimeter-long apical shoot segments were excised from parent plants and transferred to 250-ml flasks (one shoot per flask) with 150 ml of the appropriate culture medium and GSI. Inhibitors used were uniconazole, flurprimidol, and paclobutrazol (50-percent wettable powders). A later experiment involved exposure of plants to bensulfuron methyl. Experimental flasks were placed under the same growing conditions as stock cultures but were not provided with CO_2 . Early in the experiments, it was learned that bubbling such small volumes of culture medium with CO_2 drove down the pH to levels that were injurious to the plants. For this reason, the bicarbonate buffer was added to both stock and experimental media. Sufficient levels of CO_2 or bicarbonate were available to sustain good growth of milfoil plants during the experimental period.

7. All dose response experiments were conducted for a 4-week period with measurements taken at 0, 1, 2, and 4 weeks. The majority of data presented in this report consist of 4-week measurements.

Growth parameters

8. Growth parameters 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

9. Chlorophyll analyses were conducted on fresh tissue using a dimethylsulfoxide extraction according to the method of Hiscox and Israelstam (1979) and are expressed 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 in an environmental growth chamber under the same growth conditions as during treatment but on a shaker table. Bottles were allowed to shake gently for 60 to 90 min and were then removed from the chamber and measured for DO. Respiration rates were measured in the same way but in a dark BOD bottle with lights turned off in the chamber during the 60- to 90-min incubation period. Dissolved oxygen evolution is expressed per unit fresh weight per unit time.

Exposure time/duration of effect

10. In separate experiments, milfoil was exposed to 75 and 150 $\mu\text{g } \ell^{-1}$ and hydrilla to 750 $\mu\text{g } \ell^{-1}$ of the gibberellin synthesis inhibitor for periods of 1, 3, 7, and 14 days. At each of these times, shoots were removed from the treatment; rinsed thoroughly with distilled, autoclaved water; placed in fresh, untreated medium; and returned to the environmental control chamber. Plants were measured for regrowth at 2, 4, and 6 weeks. This protocol provided information on both the required exposure time and the duration of effect.

Experimental design and statistical analysis

11. The dose response experiments were arranged according to the following protocol: 4 GSI dosages plus untreated controls $\times$ 3 replicates $\times$ 3 measurement dates. Because of slight light irradiance variations within the growth chamber, the flasks within a date were arranged in a randomized block design by replicate. Measurements were taken in the following sequence: plants were first monitored for photosynthesis and respiration. Growth

parameters were then measured, and fresh weights were taken. 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.

12. The exposure time/duration of effect experiments were arranged according to the following protocol: 1 GSI dosage $\times$ 3 replicates $\times$ 5 exposure times $\times$ 3 measurement dates. The flasks within an exposure time were arranged in a randomized block design by replicate.

13. Each experiment was repeated once, and the data were subjected to analysis of variance. Means of the dosage responses of each parameter measured at each date and among dates were separated using the Student-Newman-Keul's multiple range test at a 95-percent confidence interval. The data shown here are from one set of experiments. However, the data from both experiments were also pooled and analyzed, and the statistical results were identical to those from the individual experiments.

Small-scale outdoor testing

14. A separate set of experiments was conducted in a small-scale experiment to verify the results from the laboratory bioassay. Civil defense barrels (67-l capacity) were lined with plastic liners, and garden soil was added to a 6-in. (15-cm) depth. Well water was added, and the suspended soil was allowed to settle. Six-centimeter hydrilla segments (one or two per barrel) were planted and allowed to become established for 1 week prior to treatment. Treatment was with uniconazole, either 50-percent wettable powder or 0.15-percent granular. Concentrations tested during the summer of 1988 were 0, 7.5, 75, 750, and 1,500 $\mu\text{g l}^{-1}$. The exposure period was from May 25 to June 29 (5 weeks). Plants were removed from the treatment, measured, and weighed for growth parameters. A second experiment was conducted from August 20 to October 15 (8 weeks). The only data reported from this second experiment are tuber numbers.

Results

Bioassay

15. The use of algal-free cultures of hydrilla and Eurasian watermilfoil resulted in good growth of untreated plants over the normal 4-week test period. Main stem lengths increased at mean rates of 0.53 cm/day in hydrilla and 0.41 cm/day in milfoil (doubling times of 7.4 and 10 days, respectively). Untreated plants produced lateral shoots and roots from nodal tissues but did

not flower or produce tubers. Percent dry weight of these plants decreased during the 4-week period, from an initial value of 28.1 to 15.8 percent at 4 weeks for hydrilla and an initial value of 14.7 to 8.7 percent at 4 weeks for milfoil, indicating active utilization of stored materials and active growth of the plants.

Growth parameters

16. Milfoil was considerably more sensitive to the gibberellin synthesis inhibitors than hydrilla (Figure 1). After 4 weeks of exposure, main stem elongation of milfoil was significantly inhibited (approximately 60-percent decrease in main stem length compared with untreated main stem length) at uniconazol, flurprimidol, and paclobutrazol concentrations as low as $7.5 \mu\text{g } \ell^{-1}$. In an attempt to determine the lowest effective concentration, milfoil was exposed to GSIs at concentrations between 0.1 and $7.5 \mu\text{g } \ell^{-1}$. Main stem length was not affected at 0.1 and only variably at $0.2 \mu\text{g } \ell^{-1}$ (data not shown). Exposure to $0.75 \mu\text{g } \ell^{-1}$ did show consistent results and produced an approximately 45-percent decrease in main stem length. It was not possible to establish the upper dosage limit. Dosages at $750 \mu\text{g } \ell^{-1}$ and higher resulted in knotted and abnormal-looking plants. However, these plants were still photosynthesizing, although to a much lesser degree than untreated plants (39 percent of untreated controls) or plants exposed to lower GSI concentrations.

17. Hydrilla, although sensitive to all inhibitors and most of the concentrations tested (Figure 1a), showed only a 40-percent (flurprimidol) to 58-percent (uniconazol) reduction in main stem length over untreated controls at concentrations as high as $750 \mu\text{g } \ell^{-1}$. Concentrations of $75 \mu\text{g } \ell^{-1}$ resulted in a 35-percent (flurprimidol) to 48-percent (uniconazol) decrease in main stem length compared with untreated main stem length. No effect on main stem length was found at $7.5 \mu\text{g } \ell^{-1}$ (data not shown). Presumed toxic effects such as brittleness and an increased red pigmentation (presumably anthocyanins) were noted at GSI levels of $1,500 \mu\text{g } \ell^{-1}$ and above (data not shown). These symptoms appeared after a 2-week exposure with the $1,500\text{-}\mu\text{g } \ell^{-1}$ concentration and by 1 week with a $3,000\text{-}\mu\text{g } \ell^{-1}$ concentration.

18. The effects of GSIs on treated plants were visible as soon as untreated shoots began elongating, i.e., within at least 1 week of treatment (Figure 2). Main stem lengths at 1, 2, and 4 weeks for both milfoil and hydrilla were significantly reduced at all treatment concentrations compared with the untreated controls.

19. At a GSI concentration of $75 \mu\text{g l}^{-1}$ or less after 4 weeks of exposure, hydrilla lateral stem production was stimulated, resulting in a mean of four lateral stems per main stem, in contrast to two lateral stems produced per untreated main stem (Figure 3a). However, the length per lateral (3 cm) at this concentration was lower than that in the untreated controls (7 cm). If all stem lengths are added (main plus laterals), the overall length of the paclobutrazol- and flurprimidol-treated shoots was approximately the same as in the untreated shoots (Figure 4). On the other hand, uniconazol treatment at $75 \mu\text{g l}^{-1}$ produced total stem lengths significantly less than those in untreated shoots. These results were also reflected in slightly higher (and significantly different at the 0.05 level) fresh weights in the paclobutrazol- and flurprimidol-treated versus untreated shoots (Figure 5), whereas uniconazol-treated plants showed a significant decrease in fresh weight. Root growth was stimulated in much the same way, although all three GSIs produced basically the same effect. Root numbers per plant (Figure 6) were higher at the $75 \mu\text{g l}^{-1}$ concentration than in the untreated controls (an average of eight versus four), but the length per root was considerably shorter (8 versus 14 cm).

20. Gibberellin synthesis inhibitor concentrations higher than $75 \mu\text{g l}^{-1}$ resulted in hydrilla lateral stem numbers, lateral stem lengths, total stem lengths, and fresh weights that were significantly lower than those of the untreated controls. Of the three GSIs tested on hydrilla, uniconazol appeared to be the most effective at the same concentrations in terms of suppressing main stem length, number of lateral stems, total stem lengths, and fresh weights.

21. The GSI effect on lateral stem production in milfoil was markedly different than in hydrilla. In hydrilla, the number of lateral stems decreased with increasing GSI concentration (from 75 to $750 \mu\text{g l}^{-1}$); however, in milfoil, the number of lateral stems increased with increasing concentration (0.75 to $75 \mu\text{g l}^{-1}$) (Figure 3). The number of lateral stems produced in milfoil was also higher than in hydrilla. As many as 13 lateral stems per main stem were produced on uniconazol-treated milfoil plants ($75 \mu\text{g l}^{-1}$), in contrast to only one per main stem on untreated plants and four per main stem on treated hydrilla plants. Where lateral stems on treated hydrilla did undergo some elongation, the lateral stems produced on treated milfoil remained dense and compacted at the base of the leaf axils, usually measuring no more than 0.5 cm in length. The end result of many extremely short

laterals was that both total stem lengths and fresh weights of treated milfoil plants were always significantly lower than those of untreated plants (Figures 4 and 5). No laterals were produced on plants exposed to an extremely low GSI dose of $0.75 \mu\text{g } \ell^{-1}$.

22. The effects of GSIs on milfoil root numbers was variable (Figure 6). Paclobutrazol appeared to have no effect on root numbers, whereas flurprimidol reduced root numbers at the higher concentrations. Root lengths were somewhat suppressed in GSI-treated plants (6-cm average compared with 8-cm average in untreated plants).

23. The differences in GSI effects on growth parameters of hydrilla and milfoil resulted in different morphologies. Hydrilla plants at "low" concentrations tended to be shortened but bushy, with many laterals and roots (Figure 7a). As GSI concentrations increased, the numbers of laterals and roots decreased, leaving only single shortened main stems. The number of internodes on these shortened plants was the same as the number in untreated plants. In milfoil, increasing dosages resulted in greater numbers of compacted lateral buds (Figure 7b). Since these plants had not grown in relation to initial plant segments, the number of internodes was the same as the initial number.

24. An attempt was made to determine whether the compacted buds on treated milfoil plants would sprout into new plants. Whether left on the parent plant or excised from it, and even after rinsing and exposure to fresh medium for 12 weeks, these "shoot buds" did not sprout or elongate. Also, to stimulate elongation, plants with buds were treated with 10^{-5} M gibberellic acid (GA). The GA was applied to untreated controls, to treated plants at the time of GSI treatment, and to treated plants that had been removed from a 4-week GSI exposure. Untreated controls and plants treated at the same time grew into long shoots with extremely long internodes (Figure 8). In plants that had been removed from GSI treatment and then exposed to GA, all compacted buds elongated and grew into long lateral shoots (Figure 8). Gibberellic acid also had a stimulatory effect on hydrilla stem elongation when applied at the same time as the GSI (Figure 9).

Physiological parameters

25. Net photosynthesis tended to decrease with increasing GSI concentration in both hydrilla and milfoil (Figure 10). However, the effect was not statistically significant at those concentrations that produced shortened but healthy-looking plants (as described above). It was not until concentrations of $1,500 \mu\text{g } \ell^{-1}$ for hydrilla and $375 \mu\text{g } \ell^{-1}$ for milfoil were achieved that net

photosynthesis was affected in an adverse way. Although net photosynthesis appeared to decrease with increasing GSI concentration, respiration rates remained generally similar at all concentrations and compounds illustrated in Figure 11 (with the unusual exception of uniconazol on hydrilla, possibly due to faulty readings). Thus, the tendency toward a decrease in net photosynthesis was not due to increased respiration but to a decrease in gross photosynthesis.

26. Chlorophyll content per gram fresh weight also was not significantly affected at any concentration or by any compound at the range in which plants appeared to be healthy (Figure 12). Again, at very high concentrations (e.g., $1,500 \mu\text{g l}^{-1}$ paclobutrazol) on hydrilla, chlorophyll content on a fresh weight basis decreased significantly, by more than 50 percent over untreated controls (Table 1). As described above, these hydrilla segments were red in color, and it was apparent that they had lost considerable chlorophyll. In milfoil, chlorophyll per gram fresh weight tended to increase with increasing concentration.

27. It is interesting that chlorophyll per gram dry weight in hydrilla and milfoil was significantly lower than untreated controls, particularly at the higher but nontoxic concentrations (Table 1). This was probably due not to an actual loss in chlorophyll but to a concomitant increase in percent dry weight of the plants, which appeared to be due to a higher production of starch at these concentrations. Toward the end of the 4-week test period, treated plants appeared to be converting photosynthate into starch rather than utilizing photosynthate for growth. This was indicated by an increase in percent dry weight of untreated plants. Percent dry weight at 1 week was 15 for hydrilla and 24 for milfoil; at 4 weeks, percent dry weight was 28.9 and 44.5 for hydrilla ($750 \mu\text{g l}^{-1}$) and milfoil ($75 \mu\text{g l}^{-1}$). Starch content of hydrilla at 4 weeks was statistically different between untreated and treated plants, e.g., 89.2 and $151.3 \mu\text{g/g}$ dry weight at 0 and $750 \mu\text{g l}^{-1}$ paclobutrazol (data not shown).

Exposure time/duration of effect

28. Hydrilla plants recovered to untreated control total stem lengths after a 6-week recovery period no matter how long the plants had been initially exposed to the GSI (Figure 13). It appeared that a 3-day exposure resulted in more lateral stem production than the other treatments. Milfoil, on the other hand, remained suppressed, even after only a 1-day exposure to

Table 1
Effects of Paclobutrazol at 4 Weeks on Chlorophyll (Chl) and
Dry Weight in Hydrilla and Milfoil

<u>Paclobutrazol</u> <u>Concentration</u> $\mu\text{g } \ell^{-1}$	<u>Milligrams Chl/</u> <u>Gram Fresh Weight</u>	<u>Percent</u> <u>Dry</u> <u>Weight</u>	<u>Milligrams Chl/</u> <u>Gram Dry Weight</u>
Hydrilla			
0	1.24	14.7	8.4
75	1.48	13.2	11.2
375	1.32	23.6	5.6
750	1.34	28.9	4.6
1,500	0.58	39.6	1.5
Milfoil			
0	1.03	16.4	6.3
7	1.16	27.8	4.2
20	1.22	33.0	3.7
75	1.06	44.5	2.4

the GSIs (Figure 13). Only data from the $150 \mu\text{g } \ell^{-1}$ dosage are shown, but results on milfoil were similar for concentrations of $75 \mu\text{g } \ell^{-1}$. Uniconazol appeared to be more effective than paclobutrazol and flurprimidol at maintaining growth at the 4-cm initial main stem length.

Small-scale outdoor testing

29. Untreated hydrilla plants grew extremely well in the test barrels. Two 6-cm-long segments produced over 4,800 cm of total stem length by 5 weeks. Both wettable powder and granular formulations of uniconazol at $1,500 \mu\text{g } \ell^{-1}$ and the granular formulation at $750 \mu\text{g } \ell^{-1}$ caused severe adverse effects; i.e., the plants turned red, and total stem length at the end of 5 weeks was only 0.002 percent that of untreated plants. At $75 \mu\text{g } \ell^{-1}$, the wettable powder reduced stem total length to 59 percent that of untreated controls; treatment at the higher concentration ($750 \mu\text{g } \ell^{-1}$) produced plants that were 47 percent of untreated total stem length (Figure 14). The effect of the granular formulation at $75 \mu\text{g } \ell^{-1}$ was similar to that of the $75 \mu\text{g } \ell^{-1}$ concentration of wettable powder. Plants treated at $75 \mu\text{g } \ell^{-1}$ averaged

469 lateral stems (compared with 181 for untreated plants), but mean lateral stem length of treated plants was only 6 cm compared with 25 cm for untreated plants. The number of roots was greater among treated plants than among untreated plants. The fresh weight of treated plants, even though composed of numerous lateral stems and roots, was only 72 percent that of untreated plants at $75 \mu\text{g } \ell^{-1}$ and 48 percent that of untreated plants at $750 \mu\text{g } \ell^{-1}$. It is interesting to note that $750 \mu\text{g } \ell^{-1}$ uniconazol resulted in the production of fewer lateral shoots and roots than the lower concentration. The lowest concentration used ($7.5 \mu\text{g } \ell^{-1}$) was somewhat less efficacious than the $75\text{-}\mu\text{g } \ell^{-1}$ concentration, but reductions in stem length were still observed, i.e., stem length was 79 percent and fresh weight was 81 percent of the untreated plants.

30. Higher concentrations produced plants that never reached a vertical height greater than 10 cm, even with some lateral branch production (Figure 15). At lower concentrations, the vertical height was about 20 cm, the additional height caused primarily by lateral shoot production. Untreated plants reached vertical heights of approximately 55 cm (Figure 15). When the plants were pulled from the barrel, the treated plants showed a definite stoloniferous growth habit in contrast to the elongated stems of the untreated plants (Figure 16).

31. Some tubers were formed, and these were also counted. Untreated plants averaged 10.6 tubers per barrel. Treated plants produced 4.3, 7.3, and 2.6 tubers per barrel at 7.5, 75 and $750 \mu\text{g } \ell^{-1}$, respectively.

Bensulfuron methyl testing

32. This compound was tested only on hydrilla. Concentrations below $0.1 \mu\text{g } \ell^{-1}$ started to turn shoot tips brown but had no effect on plant growth, whereas concentrations at or above $0.4 \mu\text{g } \ell^{-1}$ were herbicidal. The time required to obtain a herbicidal effect decreased with increasing concentration; for example, at $50 \mu\text{g } \ell^{-1}$, toxic effects were observed within 2 days of treatment whereas at $0.5 \mu\text{g } \ell^{-1}$ toxic effects were not observed until 1 week after treatment. Toxic effects included stunted growth and reddening of the plants and a cessation of photosynthesis. At nontoxic concentrations (between 0.1 and $0.4 \mu\text{g } \ell^{-1}$, the compound caused the plants to stop growing. At 4 weeks, the plants still appeared green and healthy, but shoot tips looked abnormal (either spindly or with reduced leaves), and photosynthetic rates were approximately half those of the untreated plants.

Discussion

33. All three gibberellin synthesis inhibitors were effective in reducing stem length and other growth parameters in hydrilla and milfoil. Differences were noted, however, in the response of the two species to the compounds. The effective dosage range for both plants appears to be relatively broad: 0.75 to 75 $\mu\text{g l}^{-1}$ and possibly higher for milfoil, and 75 to 750 $\mu\text{g l}^{-1}$ for hydrilla. This is in contrast to bensulfuron methyl, in which growth-regulating activity appeared only at a very narrow range (0.1 to 0.4 $\mu\text{g l}^{-1}$). Toxic effects of the GSIs on hydrilla appeared at 1,500 $\mu\text{g l}^{-1}$. High GSI concentration effects on milfoil were more subtle; although severely stunted in growth and with many lateral buds, the plants appeared to be alive. On the other hand, milfoil was considerably more sensitive and responded to much lower GSI concentrations than hydrilla. Not only was this evident from initial stem length reductions but from the recovery experiments as well. Hydrilla required constant contact with the compound in order to be affected, whereas milfoil required only a day of exposure for long-term effects.

34. The plants also differed in their morphological responses to the compounds. Milfoil remained as a single main stem segment with numerous compressed buds in the leaf axils. Only at the lowest effective GSI concentration (0.75 $\mu\text{g l}^{-1}$) were main stems still shortened but without the production of lateral buds. The possibility exists that production of numerous lateral buds (possibly analogous to winter buds or turions) might result in an infestation of milfoil if these buds were to become detached from the parent plant and sprout at a nontreated site. However, it was not possible to induce sprouting in these buds without the application of gibberellic acid. Thus, it seems unlikely that they will sprout in the field. One obvious means of estimating bud production would be to lower the GSI concentration to at least 0.75 $\mu\text{g l}^{-1}$.

35. In contrast, lateral buds in hydrilla sprouted and elongated (although not at the same rate as untreated plants) to produce a plant with numerous lateral stems and roots. Roots were produced at the nodal areas of the stems, usually where a lateral stem was initiated. The overall appearance of a treated hydrilla plant, even in the bioassay system, was one of stoloniferous growth. The stimulation of rooting in plants has been noted on terrestrial plants (Sankla et al. 1985, Fletcher et al. 1988). In contrast to milfoil, increasing the GSI concentration may reduce lateral stem production.

Although a stoloniferous, carpet-like growth is desirable, it is also desirable with a very aggressive weed such as hydrilla to reduce overall plant biomass. That this can be accomplished was suggested by the small-scale field test in which a $750\text{-}\mu\text{g l}^{-1}$ concentration resulted in carpet-like growth consisting of only 48 percent of the untreated fresh weight and with numbers of shortened lateral shoots and roots that were not too much higher than those of the untreated plants. These field results were generally predicted by the bioassay.

36. Among terrestrial plants, dicots appear to be more susceptible to GSIs than monocots (personal observation, A. Hammer, Purdue University). Similar results were observed in this study in which milfoil, a dicot, was more sensitive to the GSIs than hydrilla, a monocot. A single application on terrestrial dicots often results in season-long control whereas several applications may be required to produce the same effect on monocots. In addition, terrestrial dicots appear to be more tolerant to a broader range of concentrations than monocots (Hammer, personal observation). This was also found to be true with milfoil and hydrilla in this study. Further studies will be required to determine if this generalization can be applied to other submersed aquatic species, such as pondweeds and naiads which are monocots and coontail which is a dicot.

37. In general, all three GSIs produced similar growth-regulating effects; however, at the same dosages uniconazol appeared to be slightly more effective at suppressing elongation than the other compounds. The molecular weight of uniconazol (291.5) is slightly lower than that for flurprimidol (312.3) and paclobutrazol (293.5). Therefore, even on the basis of moles of active ingredient, uniconazol appears to be the most active of the three compounds.

38. Physiological competence of the plants did not appear to be affected at the concentrations required to reduce stem length. Although the plants in the bioassay tended to increase in dry weight and starch content over the 4-week test period, this is to be expected as nutrients become limiting and photosynthesis slows. This sort of effect was not noted in the small-scale field test, where 4 weeks after treatment starch content and dry weight were not significantly different from those of the plants used to start the experiment. This suggests that the plants in the field were not limited for photosynthesis and were using photosynthate for growth throughout the experiment rather than sequestering it into starch.

39. Some concern has been expressed about the possibility that GSI treatment may stimulate the production of tubers in hydrilla. Studies indicate that gibberellic acid treatments decrease the number of tubers (Klaine and Ward 1984, Ewing 1987), suggesting that as long as the plant is undergoing growth or elongation, tuber formation will be suppressed. It would then follow that the inhibition of growth by a GSI might stimulate tuber production. Tubers were never produced in our bioassay; however, they were produced in our small scale-field tests. No significant difference in tuber number was found between treated and control barrels. Since excellent shoot growth was occurring in both treated and control barrels, it may be that the shunting of photosynthate into starch and tuber production had not yet been triggered by limiting growth conditions. It should be noted that tuber production in these barrels was low and was cut short by the rapid onset of cold weather. Analogous structures (lateral buds/winter buds or turions) in milfoil were produced, as noted above.

40. Our results to date, from bioassay and preliminary field tests, strongly suggest that the gibberellin synthesis inhibitors can play a role in aquatic plant management by suppressing weedy growth without disrupting the physiological competence of the plants.

Conclusions

41. Results of this study lead to the following conclusions:

- a. The bioassay used in this study is appropriate for rapid screening of compounds at a variety of concentrations and exposure times and for predicting GSI growth responses in the field.
- b. Uniconazol, paclobutrazol, and flurprimidol are effective at reducing stem length and other growth parameters in hydrilla and Eurasian watermilfoil.
- c. Eurasian watermilfoil is more sensitive to these compounds and exhibits different morphological effects than hydrilla.
- d. Concentrations at the low end of the dosage range for milfoil and at the high end of the dosage range for hydrilla may be required to reduce the number of lateral buds produced and the risk of increased infestation.
- e. GSI-treated plants remain physiologically competent at a broad dosage range. This is in contrast to a herbicide such as bensulfuron methyl, in which the plant growth-regulating dosage range appears to be very narrow.

- f. It appears that hydrilla must be kept in contact with the GSI for growth to remain suppressed, whereas milfoil requires only a short exposure to the compound for continued suppression of growth. This particularly needs verification in the field.
- g. Outdoor barrel treatments indicate that uniconazole does alter the regular growth form of hydrilla by keeping the plant in a low, rug- or carpet-like form. Both wettable powder and granular formulations were effective, but the granular formulation produced more variable results.

References

- Andrews, J. H. 1980. "Plant Pathogens As Agents for Biological and Integrated Control of Aquatic Plants," Wisconsin Technical Completion Report, Project No. OWRT A-0760-WIS, Water Resources Center, University of Wisconsin, Madison, WI.
- Engel, S. 1985. "Aquatic Community Interactions of Submerged Macrophytes," Technical Bulletin 156, Department of Natural Resources, Madison, WI.
- Ewing, E. E. 1987. "The Role of Hormones in Potato (*Solanum tuberosum* L.) Tuberization," Plant Hormones and Their Role in Plant Growth and Development, P. J. Davies, ed., Martinus Nijhoff Publications, pp 515-538.
- Fletcher, R. A., Asare-Boamah, N. K., Krieg, L. C., Hofstra, G., and Dumbroff, E. B. 1988. "Triadimefon Stimulates Rooting in Bean Hypocotyls," Physiologia Plantarum, Vol 73, pp 401-405.
- Hedden, P., and Graebe, J. E. 1985. "Inhibition of Gibberellin Biosynthesis by Paclobutrazol in Cell-Free Homogenates of *Cucurbita maxima* Endosperm and *Malus pumila* Embryos," Journal of Plant Growth Regulation, Vol 4, pp 111-122.
- Hiscox, J. D., and Israelstam, G. F. 1979. "A Method for the Extraction of Chlorophyll from Leaf Tissue Without Maceration," Canadian Journal of Botany, Vol 57, pp 1332-1334.
- Klaine, S. J., and Ward, C. H. 1984. "Environmental and Chemical Control of Vegetative Dormant Bud Production in *Hydrilla verticillata*," Annals of Botany, Vol 53, pp 503-514.
- Lever, B. G., Shearing, S. J., and Batch, J. J. 1982. "PP333 - A New Broad Spectrum Growth Retardant," Proceedings, 1982 British Crop Protection Conference on Weeds, pp 3-10.
- Rademacher, W., Jung, J., Graebe, J. E., and Schwenen, L. 1984. "On the Mode of Action of Tetcyclacis and Triazole Growth Retardants," Biochemical Aspects of Synthetic and Naturally Occurring Plant Growth Regulators, Monograph 11, British Plant Growth Regulation Group Publications, pp 1-11.
- Sankla, N., Davis, T. D., Upadhyaya, A., Sankla, D., Walser, R. H., and Smith, B. N. 1985. "Growth and Metabolism of Soybean As Affected by Paclobutrazol," Plant Cell Physiology, Vol 26, pp 913-921.
- Wiley, M. J., Gordon, R. W., Waite, S. W., and Powless, T. 1984. "The Relationship Between Aquatic Macrophytes and Sport Fish Production in Illinois Ponds: A Simple Model," North American Journal of Fisheries Management, Vol 4, pp 111-119.

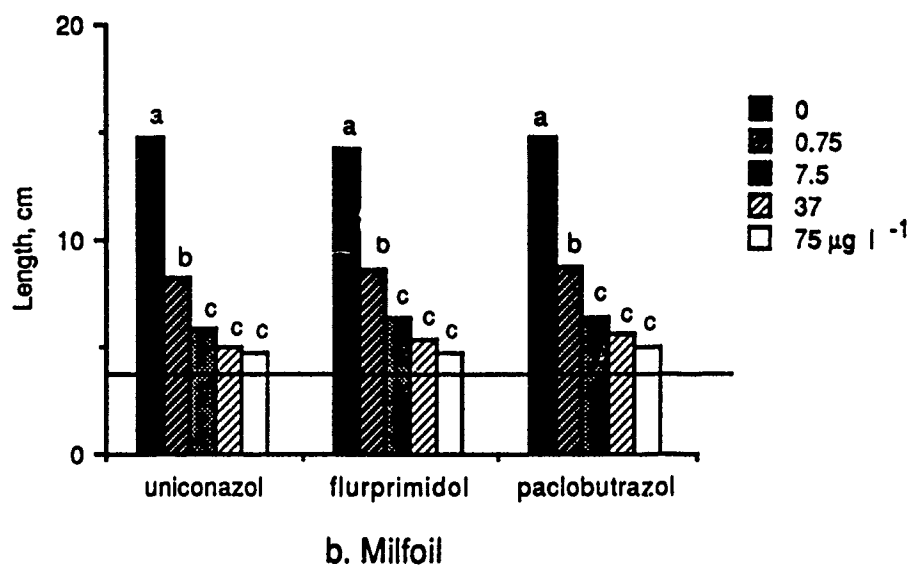
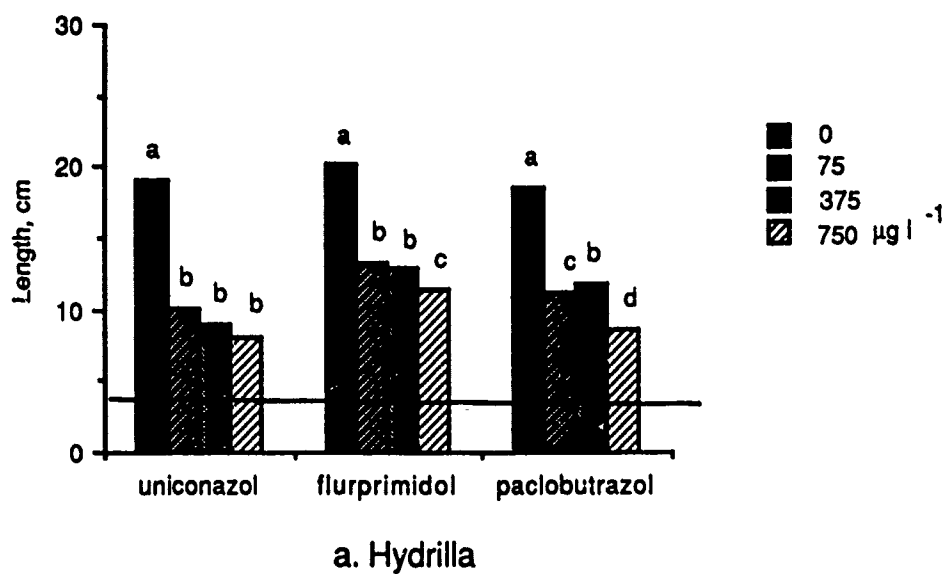


Figure 1. Effect of GSIs on main stem length in hydrilla and milfoil at 4 weeks. Horizontal line represents initial 4-cm length. Note differences in concentration ranges and in y-axis scales between the two plants

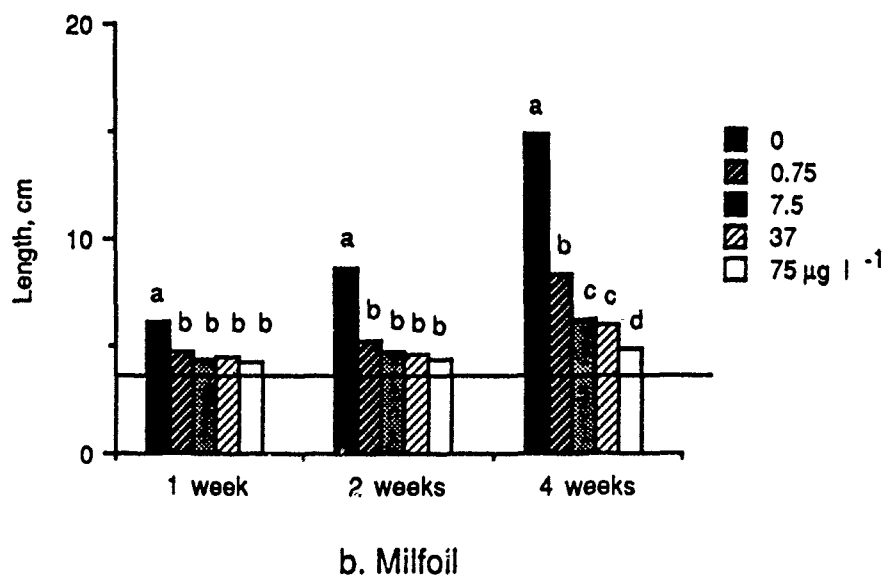
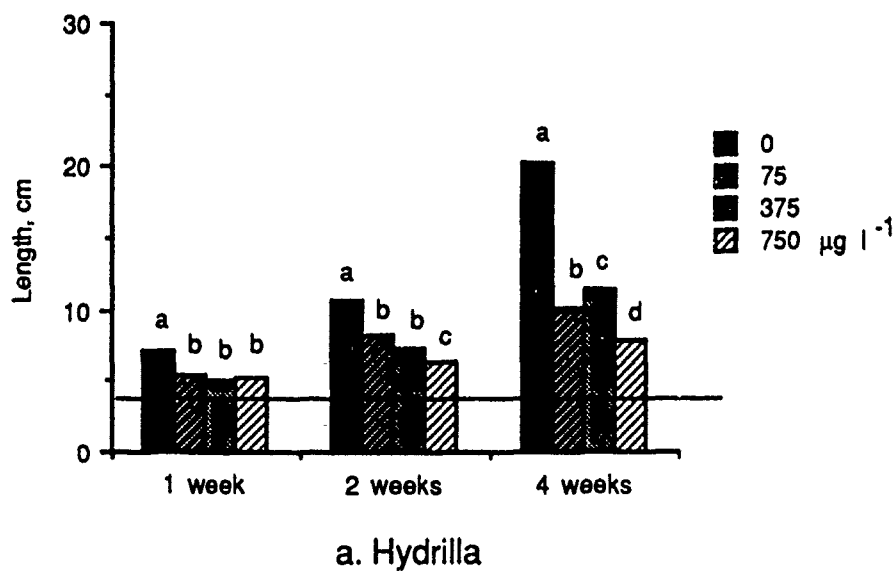
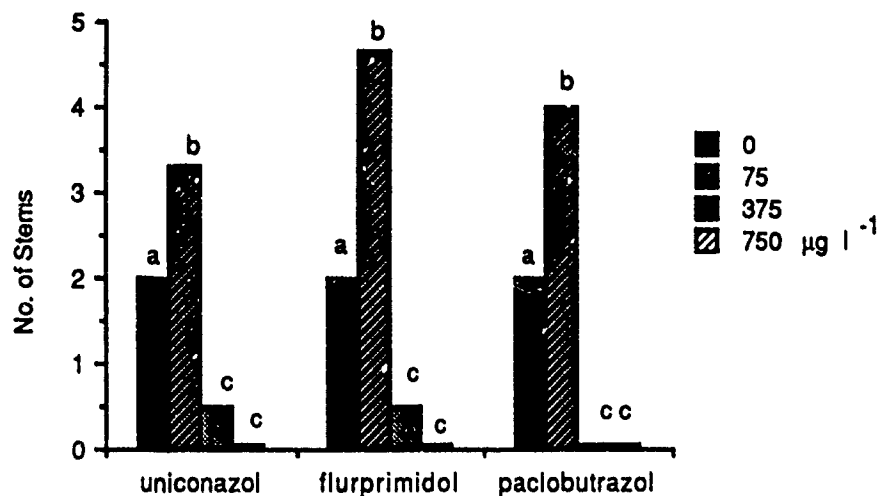
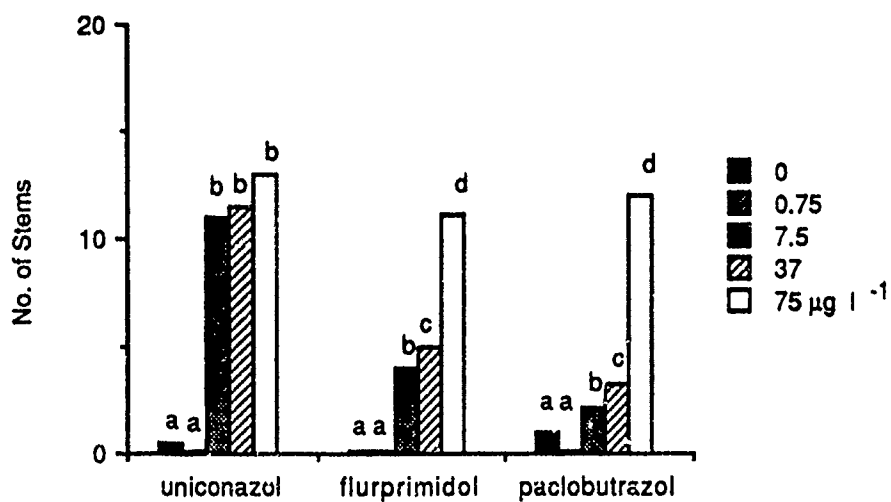


Figure 2. Effect of paclobutrazol on main stem length in hydrilla and milfoil at 1, 2, and 4 weeks. Horizontal line represents the initial 4-cm length. Note differences in concentration ranges and in y-axis scales between the two plants

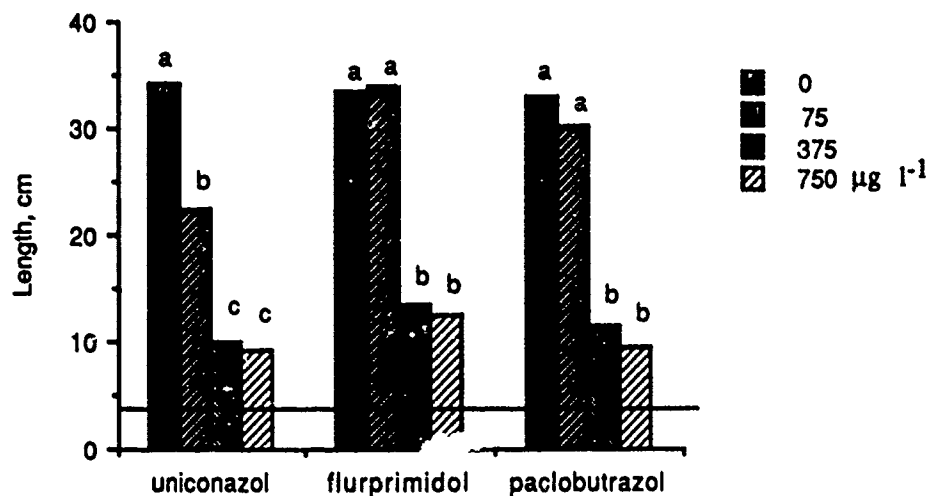


a. Hydrilla

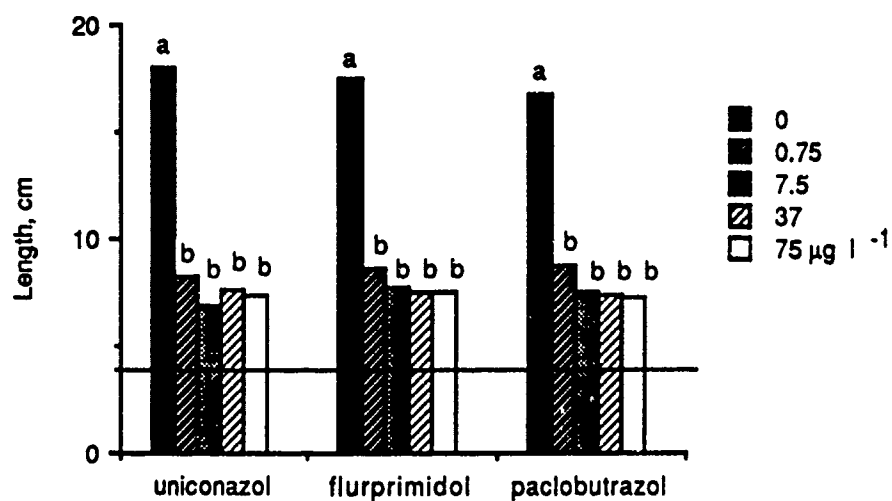


b. Milfoil

Figure 3. Effect of GSIs on lateral stem number in hydrilla and milfoil at 4 weeks. Note differences in concentration ranges and in y-axis scales between the two plants

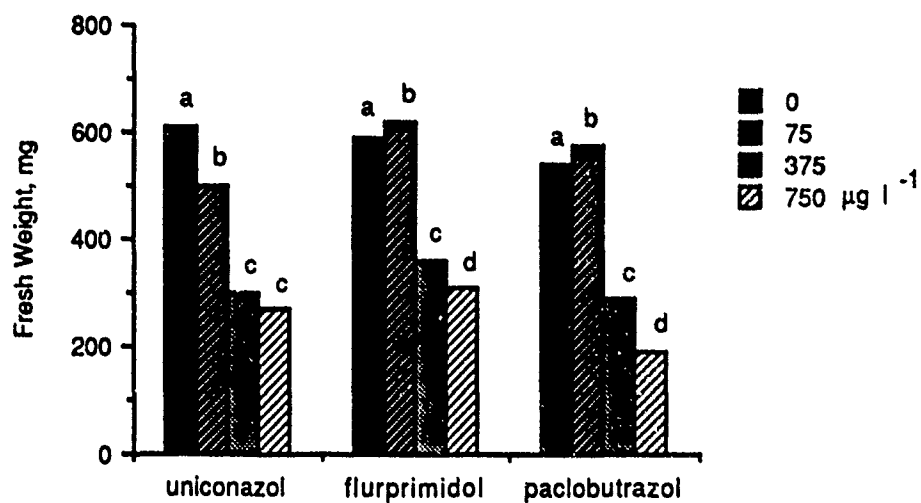


a. Hydrilla

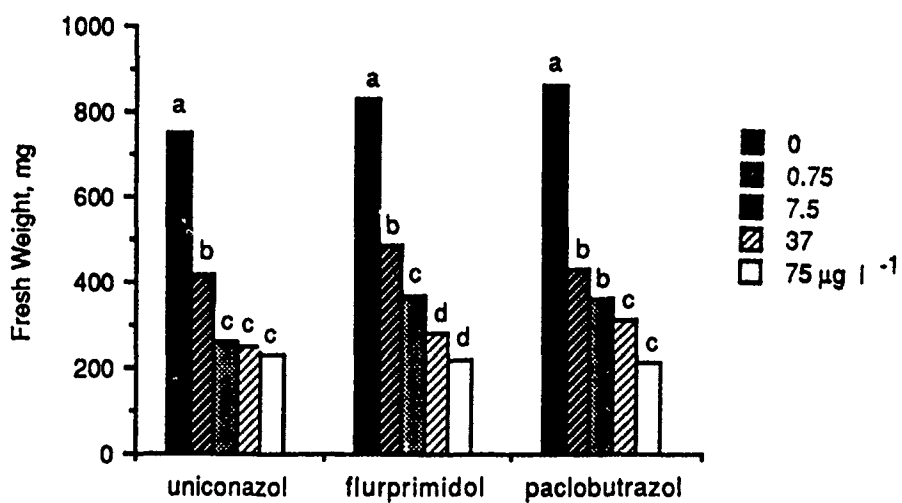


b. Milfoil

Figure 4. Effect of GSIs on total stem length (main + laterals) in hydrilla and milfoil at 4 weeks. Note differences in concentration ranges and in y-axis scales between the two plants

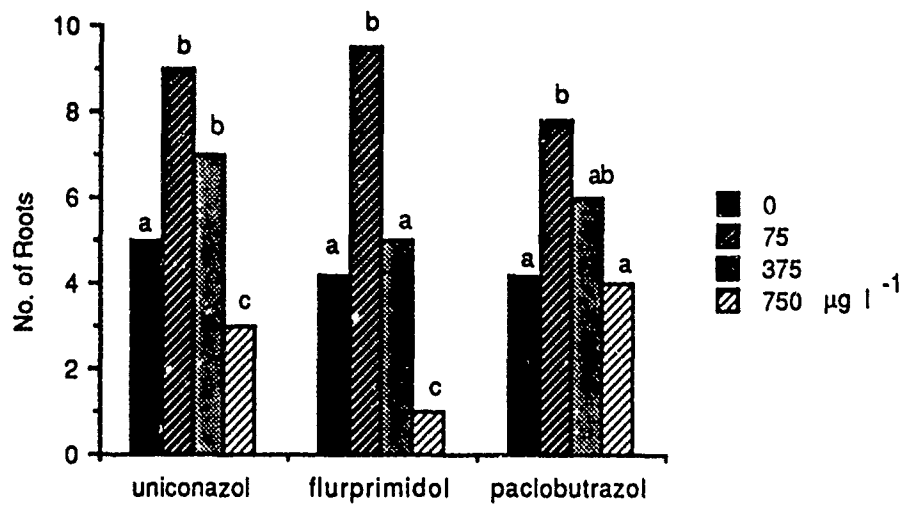


a. Hydrilla

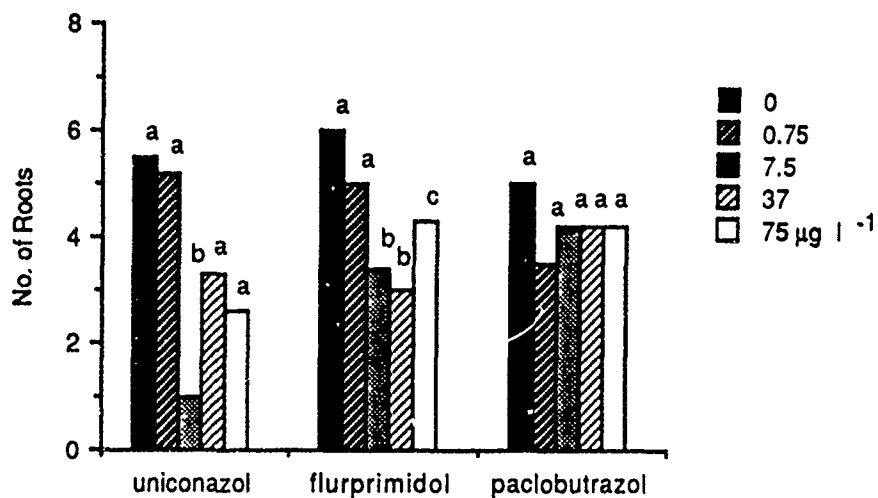


b. Milfoil

Figure 5. Effect of GSIs on fresh weights of hydrilla and milfoil segments at 4 weeks. Note differences in concentration ranges and in y-axis scales between the two plants



a. Hydrilla



b. Milfoil

Figure 6. Effect of GSIs on root numbers in hydrilla and milfoil at 4 weeks. Note differences in concentration ranges and in y-axis scales between the two plants

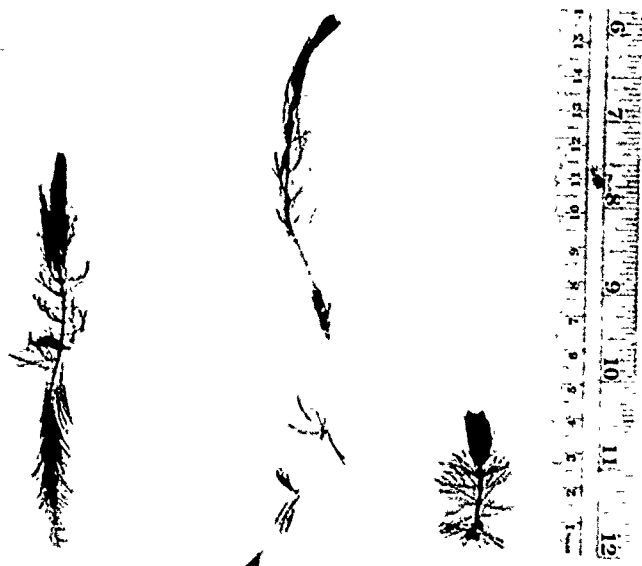


a. Hydrilla (treatment concentrations (left to right): 1,500, 375, 75, and 0 $\mu\text{g l}^{-1}$)



b. Milfoil (treatment concentrations (left to right): 0, 0.75, and 75 $\mu\text{g l}^{-1}$)

Figure 7. Effect of paclobutrazol treatments at 4 weeks



a. Untreated (left), treated with uniconazole ($7.5 \mu\text{g l}^{-1}$) and gibberellic acid (10^{-5} M) (center), and treated with uniconazole ($7.5 \mu\text{g l}^{-1}$) only (right), after 6 weeks

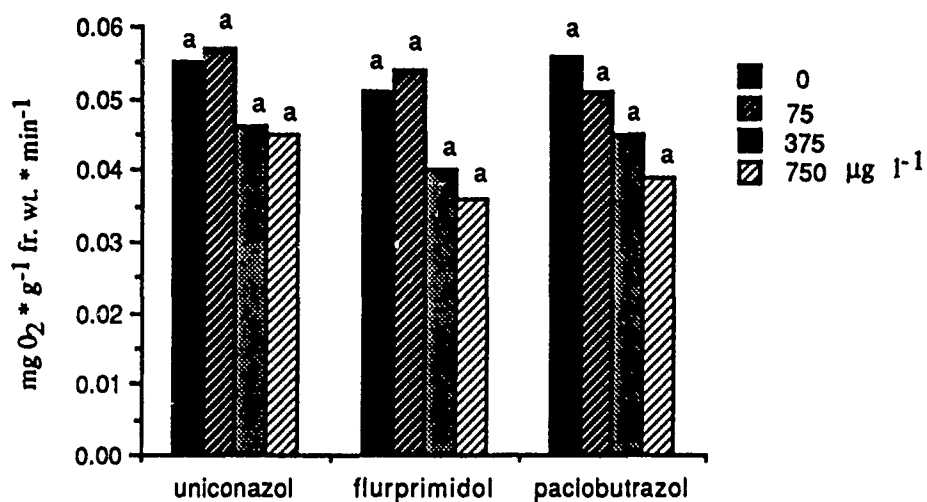


b. Untreated (left), treated with paclobutrazol ($7.5 \mu\text{g l}^{-1}$) (center), and treated with paclobutrazol ($375 \mu\text{g l}^{-1}$) for 4 weeks followed by 2-week exposure to gibberellic acid (10^{-5} M) (right)

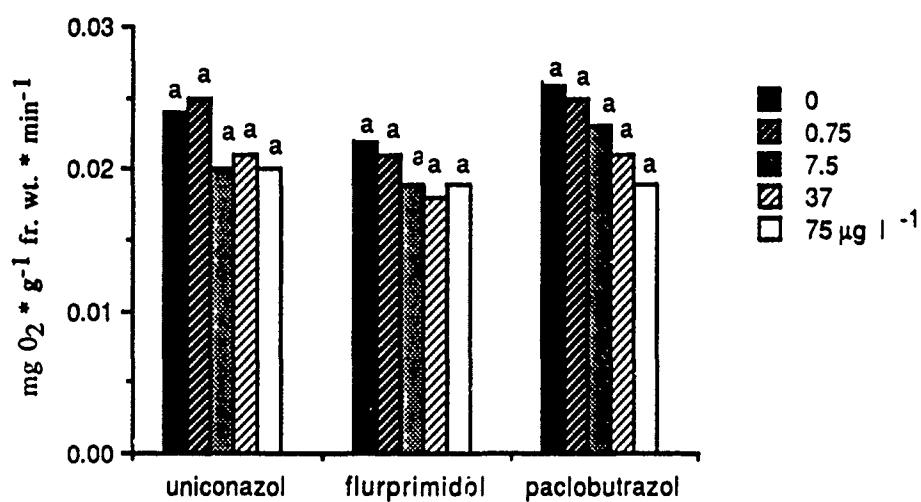
Figure 8. Response of GSI-treated milfoil to gibberellic acid



Figure 9. Response of GSI-treated hydrilla to gibberellic acid. Untreated (left), treated with uniconazole ($375 \mu\text{g l}^{-1}$) and gibberellic acid (10^{-5}) (left center), treated with uniconazole ($375 \mu\text{g l}^{-1}$) (right center), and treated with paclobutrazol ($375 \mu\text{g l}^{-1}$) (right), after 6 weeks

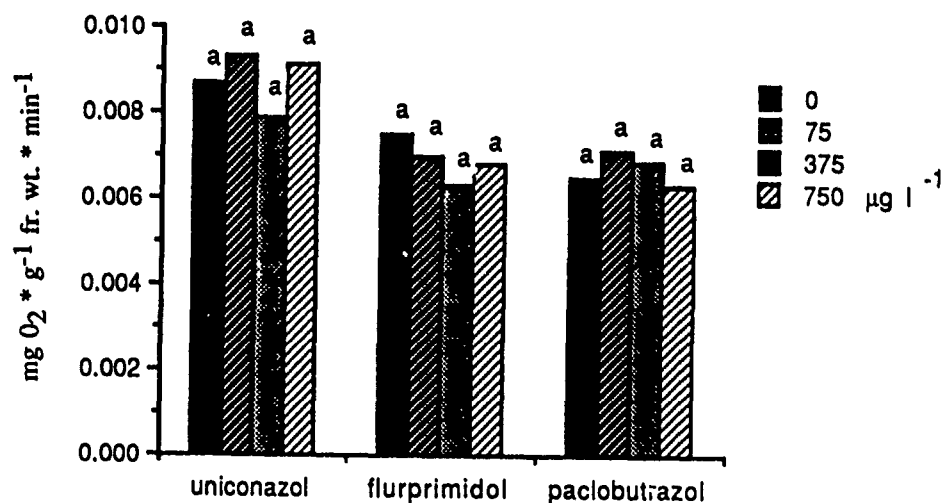


a. Hydrilla

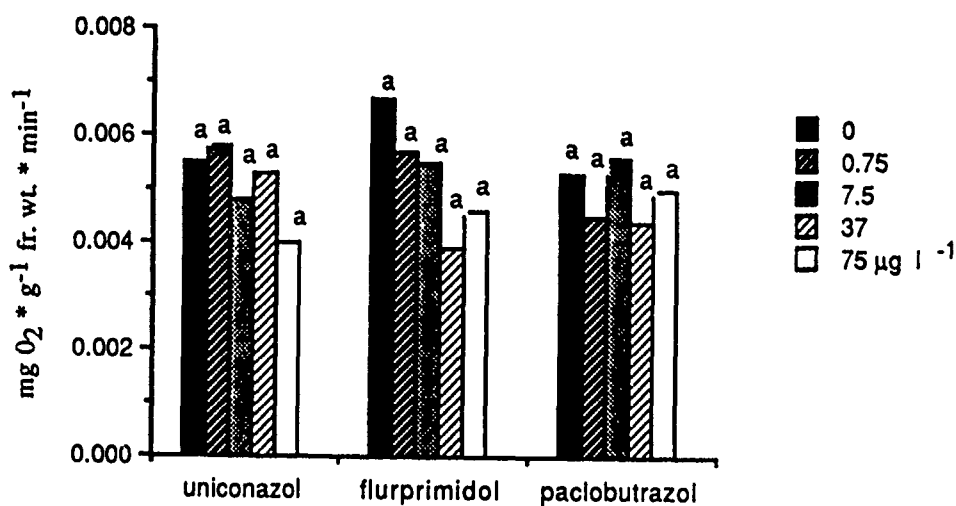


b. Milfoil

Figure 10. Effect of GSIs on net photosynthesis in hydrilla and milfoil at 4 weeks. Note differences in concentration ranges and in y-axis scales between the two plants

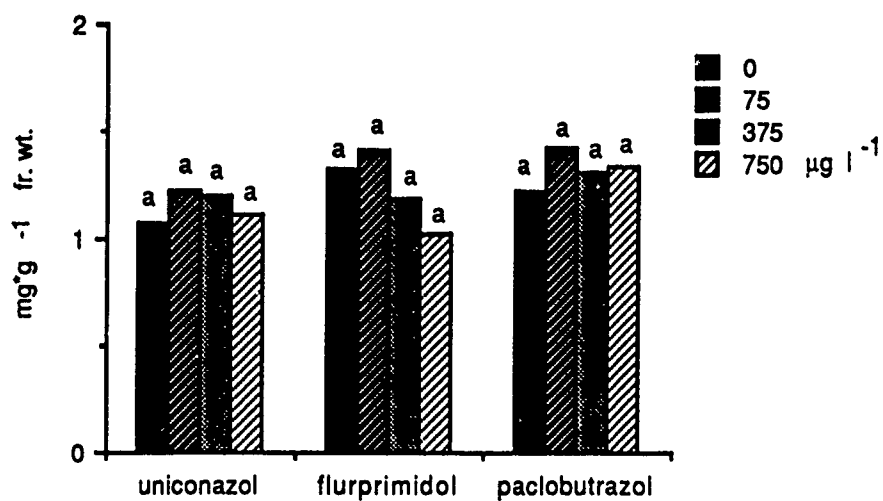


a. Hydrilla

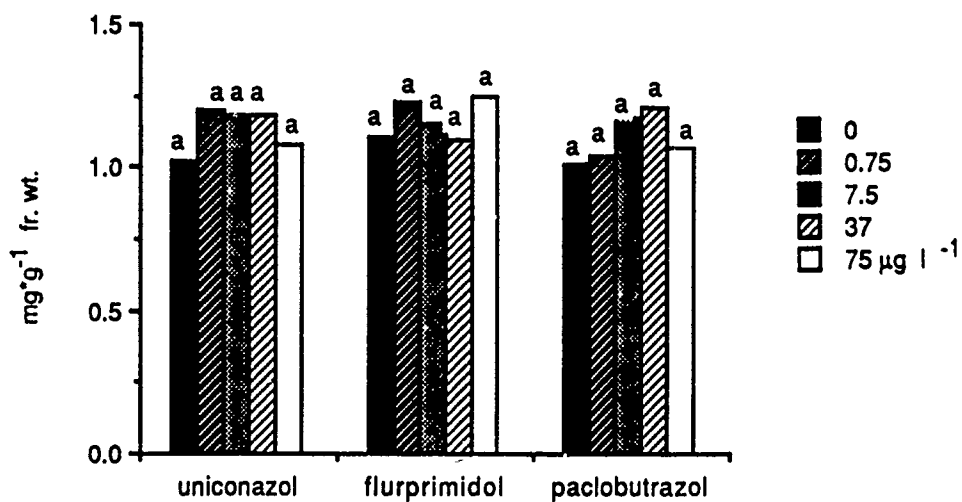


b. Milfoil

Figure 11. Effect of GSIs on respiration in hydrilla and milfoil at 4 weeks. Note differences in concentration ranges and in y-axis scales between the two plants

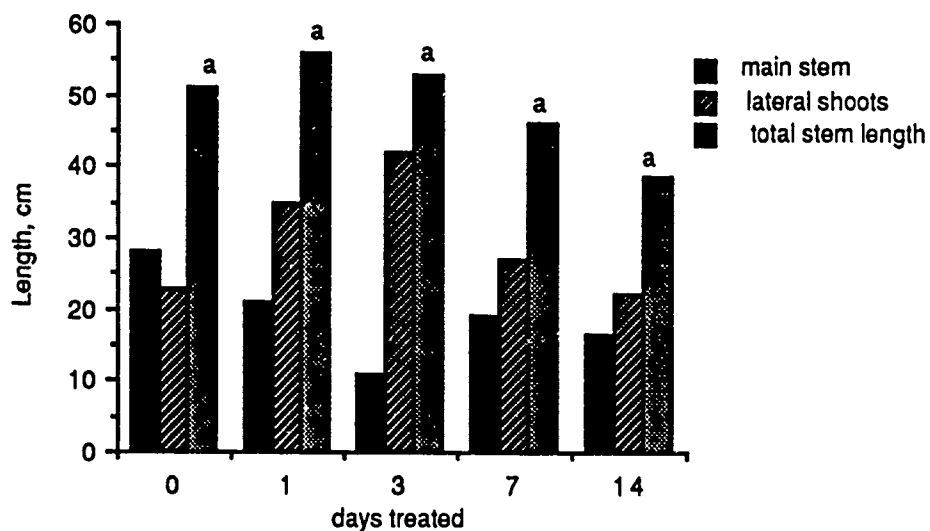


a. Hydrilla

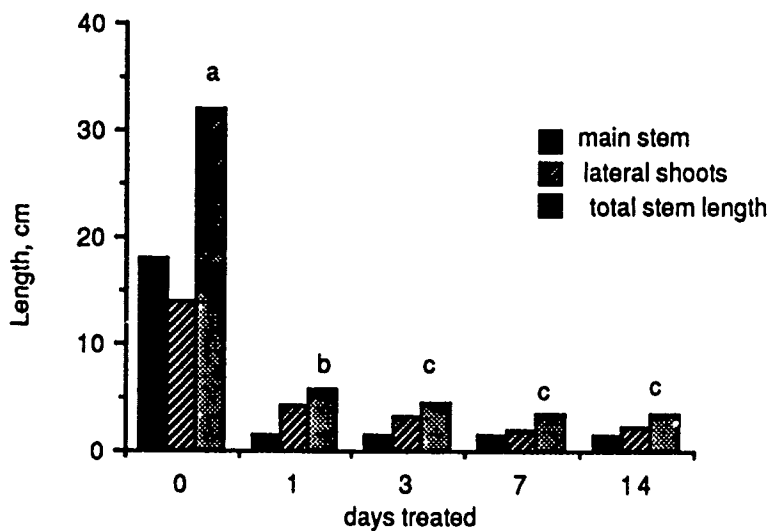


b. Milfoil

Figure 12. Effect of GSIs on total chlorophyll in hydrilla and milfoil segments at 4 weeks. Note differences in concentration ranges and in y-axis scales between the two plants

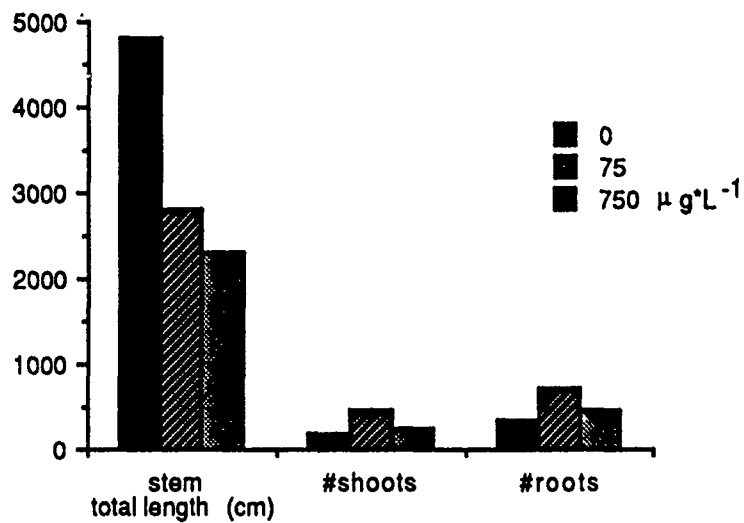


a. Hydrilla (uniconazole, $750 \mu\text{g l}^{-1}$)

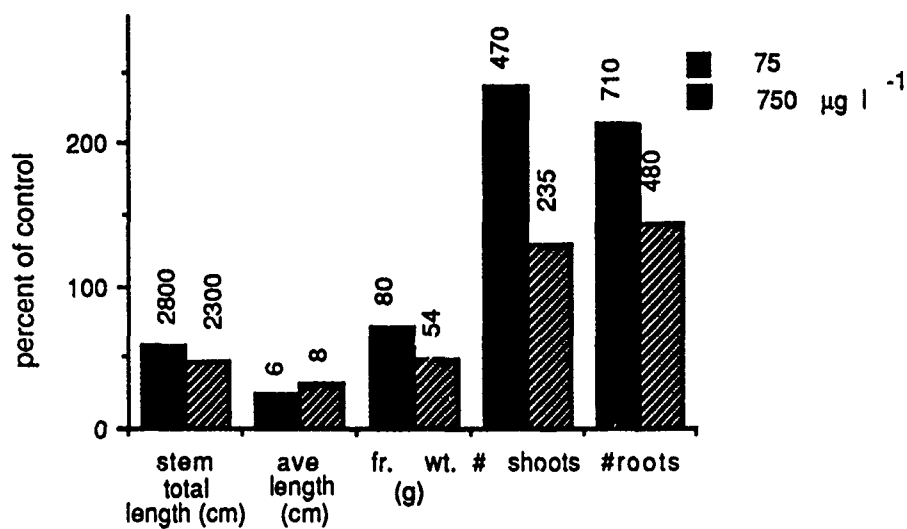


b. Milfoil (uniconazole, $15 \mu\text{g l}^{-1}$)

Figure 13. Six-week recovery of hydrilla and milfoil after exposure to uniconazole for various time periods. Separation of means shown only for total stem length data. Note differences in concentration and in y-axis scales between the two plants

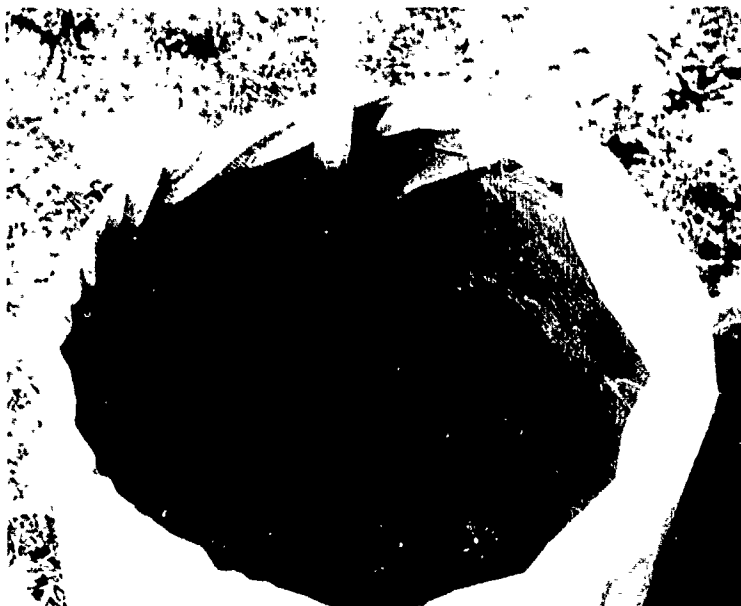


a. Growth data (per barrel)

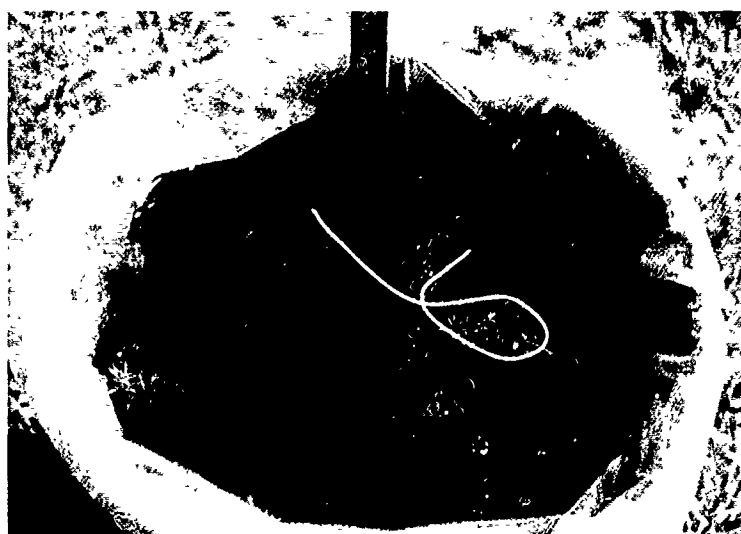


b. Growth data as percentage of untreated controls.
(Numbers above bars indicate actual measurements)

Figure 14. Effect of uniconazol on hydrilla in small-scale barrel tests at 5 weeks



a. Treated plants ($75 \mu\text{g l}^{-1}$)



b. Untreated plants

Figure 15. Comparison of effect on hydrilla of
uniconazol treatment in small-scale barrel
tests



Figure 16. Comparison of treated (left) and untreated (right) hydrilla plants pulled from small-scale barrel tests. Note stoloniferous growth of treated plant