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Plant Growth Regulation: A Viable Concept In Aquatic Plant Management?

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TECHNICAL REPORT NUMBER 194

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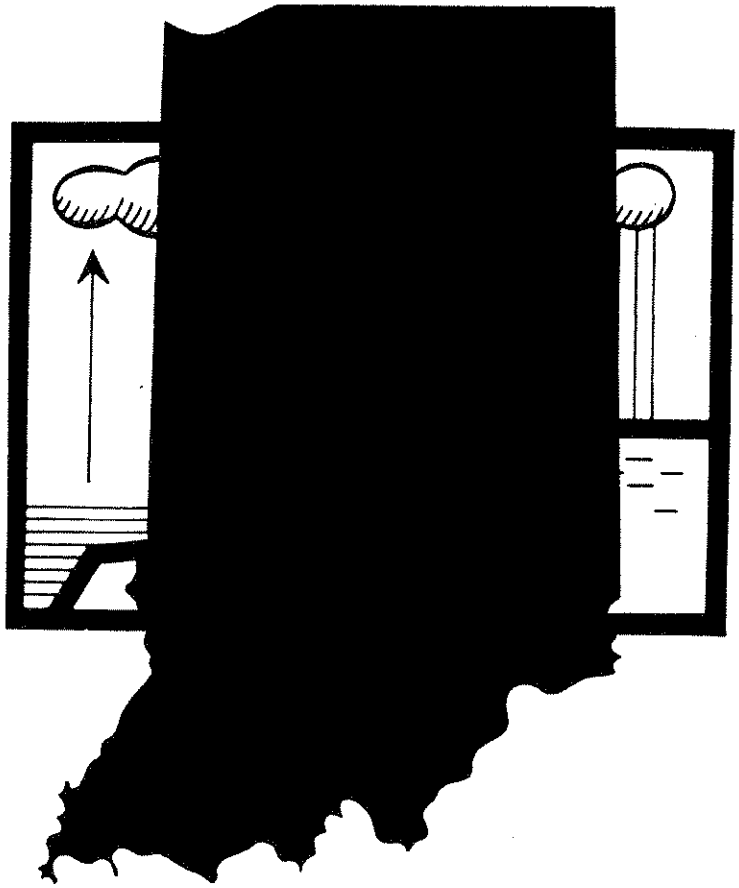

by

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October 1992

PURDUE UNIVERSITY
WATER RESOURCES RESEARCH CENTER
WEST LAFAYETTE, INDIANA

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PLANT GROWTH REGULATION: A VIABLE CONCEPT IN AQUATIC PLANT MANAGEMENT?

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Project personnel were Carole A. Lembi (Professor, Purdue University) and Michael D. Netherland, who received an M.S. degree based on this research and is currently employed as a research biologist with the Chemical Control Team, Aquatic Plant Control Research Program, Waterways Experiment Station, U. S. Army Corps of Engineers, Vicksburg, Mississippi. Cathy Malast is employed as a microbiologist by a city of Indianapolis sewage waste treatment facility. Laurie Buck is attending IUPUI in the medical technician program.

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ABSTRACT

A laboratory bioassay showed that inhibitors of gibberellin synthesis (flurprimidol, paclobutrazol, and uniconazole) reduced plant height but did not affect physiological parameters such as photosynthesis, respiration and chlorophyll content in two weedy submersed aquatic plants, hydrilla and Eurasian watermilfoil. Eurasian watermilfoil was sensitive to these compounds at concentrations as low as 0.75 µg/L. Hydrilla sensitivity was in the range of 75 to 750 µg/L. The three compounds reduced main and lateral stem lengths in hydrilla; however, at 75 µg/L the number of lateral stems and roots was greatly increased over untreated controls resulting in a stoloniferous growth habit. This carpet-like growth was also obtained in small-scale field tests (conducted in 67-L barrels set out-of-doors) on hydrilla using 75 µg/L uniconazole. The dominant growth form of treated Eurasian watermilfoil was a single shortened stem with numerous compacted buds. Photosynthesis, respiration, and chlorophyll content were not affected in either plant at non-toxic dosages in which stem reduction was obtained. Both plants required only a 24 hour exposure to maintain stem length reduction for 6 weeks after transfer to untreated medium. Our results indicate that the gibberellin synthesis inhibitors would be effective at reducing aquatic plant height and thereby providing non-weedy but functional plant stands in aquatic systems.

INTRODUCTION

The growth of excessive amounts of aquatic vegetation in Indiana's lakes and ponds is a major management concern. Aquatic weeds prevent the use of a body of water for recreation, irrigation, and as a source of potable water. Some of the most severe effects are on fish, both in natural and hatchery/aquaculture systems, in which weeds compete for space, cause imbalances in fish populations by providing excessive cover for forage species, and produce stress conditions by lowering the oxygen content of water at night. Extremely weed-infested areas are subject to fish kills, particularly during the winter as the large amounts of plant biomass decompose producing anoxic conditions. A primary management problem, both in terms of concern and amount of money spent, for a typical lake association in Indiana and elsewhere in the United States is aquatic weed control (Indiana Stream Pollution Control Board 1976, Trudeau 1982).

Some aquatic plant growth, however, is desirable (Wiley et al. 1984, Engel 1985). Rooted underwater (submersed) plants provide oxygen through photosynthesis, habitat for fish and fish-food organisms, and bottom sediment stabilization. Unfortunately, aquatic weed control 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 following herbicide use may also result in adverse effects on non-target components of the aquatic system.

Another potential approach to managing aquatic vegetation may be through the manipulation of natural plant hormonal processes. One such plant hormone, gibberellin, stimulates the elongation of intact plant stems (Salisbury and Ross 1985). Almost all terrestrial flowering plants that have been exposed to exogenously applied gibberellin show an elongation response. Although research on aquatic flowering plants is limited, Sastroutomo (1981) showed an elongation response in curlyleaf pondweed (*Potamogeton crispus*) upon exposure to gibberellic acid. Endogenous gibberellins have been detected in other aquatic plants such as *Callitriche* (Sculthorpe 1971) and

Wolfiella (Pieterse et al. 1971), and it is generally accepted that elongation in both terrestrial and aquatic flowering plants is regulated by gibberellins (Sculthorpe 1971).

Certain substituted pyrimidine and triazole compounds have been found to inhibit the synthesis of gibberellin in terrestrial plants and plant homogenates (Lever et al. 1982, Rademacher et al. 1984, Hedden and Graebe 1985). These gibberellin synthesis inhibitors reduce stem length in species ranging from grasses to trees without altering viability or morphological differentiation such as flowering and seedhead development.

The primary goal of our study was to determine, using a simple laboratory bioassay system, if gibberellin synthesis inhibitors could reduce the rate of stem elongation in submersed aquatic plants without killing the plants. This presumably would lead to a lawn or "turf" of healthy plants at the bottom of a 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.

The specific goals of the project were to determine the following:

1. The effects of gibberellin synthesis inhibitors on stem length and other associated length and biomass parameters (growth parameters).
2. The effects of gibberellin synthesis inhibitors on the physiological competence of the plants, with emphasis on photosynthesis and respiration (physiological parameters).
3. Effective exposure times in order for gibberellin synthesis inhibitor effects to be expressed.

The plants chosen as test organisms were Eurasian watermilfoil (*Myriophyllum spicatum* L.) and hydrilla (*Hydrilla verticillata* Royle). These two species are considered to be the most economically and ecologically devastating submersed weeds in the United States. Eurasian watermilfoil is the major aquatic weed species in lakes and reservoirs in Indiana and the midwest as well as other parts of the United States and Canada. Its invasion of the Okanagan and Columbia River Basins in British Columbia and Washington has prompted massive research and control programs in those areas (Newroth 1979) that continue to this day. Hydrilla is even more

competitive than Eurasian watermilfoil (Van et al. 1976, 1978) and, because of its tolerance to herbicides, is considered the most noxious aquatic weed in the U.S. Although not currently found in Indiana or other parts of the midwest, hydrilla is spreading rapidly from its initial introduction in Florida in 1960. It is a major weed throughout the southeast and has the potential to grow in northern waters as evidenced by current infestations in the Potomac River, in the Tennessee River Valley, and in northern California.

MATERIALS AND METHODS

Plant materials and culture

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. The cultures were grown in a growth chamber under constant environmental conditions of temperature (25 $\pm$ 1 C), photosynthetic photon fluence rate (400 $\mu E/m^2/s \pm 10\%$) and photoperiod (16:8 h light:dark). Apical tips of Eurasian watermilfoil were grown in modified Gerloff's medium (initial pH 7.6) (Selim et al. 1989), and apical tips of hydrilla were grown in 10% Hoagland's medium (initial pH 7.2) (Hoagland and Arnon 1960). Both media were autoclaved (20 min at 250 C) and then buffered with a 0.24 M stock solution of $NaHCO_3$ (passed through a 0.45 μm membrane filter to prevent contamination) to achieve a final $NaHCO_3$ concentration of 2.3×10^{-3} M. Eurasian watermilfoil cultures were aerated continuously with air enriched with 0.5% CO_2 (Selim et al. 1989). Stock culture plants were transferred to fresh media every 20-25 days to maintain optimal growth. Stem sections (at least two internodes) inoculated into fresh medium produced lateral shoots which were excised at approximately 3 weeks of growth, transferred to fresh medium, and grown for an additional week before use in experiments. The plants were free of algae but were not axenic.

Bioassay conditions

Four-cm apical segments were excised from the shoots (using sterile techniques) and transferred to 250 mL Erlenmeyer flasks (one apical shoot per flask) containing 150 mL of the appropriate culture medium and inhibitor. The inhibitors used were 50% wettable powders of flurprimidol ([α-(1-methylethyl)-α-(4-trifluoromethoxy)phenyl]-5 pyrimidinemethanol), 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]). Uniconazole and paclobutrazol are substituted triazoles; flurprimidol is a substituted pyrimidine. The chemical structures of these compounds are illustrated in Figure 1. The experimental flasks were then placed in growth chambers under the same conditions described for the stock cultures.

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. Each measurement period included its own set of replicates. Inhibitor concentrations were 0, 7.5, 75, 150, 375, 750 and 1000 µg/L for hydrilla and 0, 0.75, 7.5, 37.5, 75, 100 and 750 µg/L for Eurasian watermilfoil. Inhibitors were not replenished during the course of the experiment. An initial screen was conducted to determine the appropriate concentration range for each plant.

Growth parameters

Growth parameters included main stem length, lateral stem length and number, total stem length, root length and number, and fresh and dry weights. All length measurements were taken with a cm ruler. Dry weights were taken on plants dried at 70 C for 48 hours.

Physiological parameters

Physiological parameters tested included net photosynthesis, respiration, and chlorophyll content. Photosynthesis and respiration, measured as dissolved oxygen evolution and uptake, respectively, were monitored using a digital pH meter equipped with a dissolved oxygen electrode (Selim et al. 1989). For photosynthesis, single plant segments were placed in a 300-mL Biological Oxygen Demand (BOD) bottle with fresh medium at a known dissolved oxygen concentration.

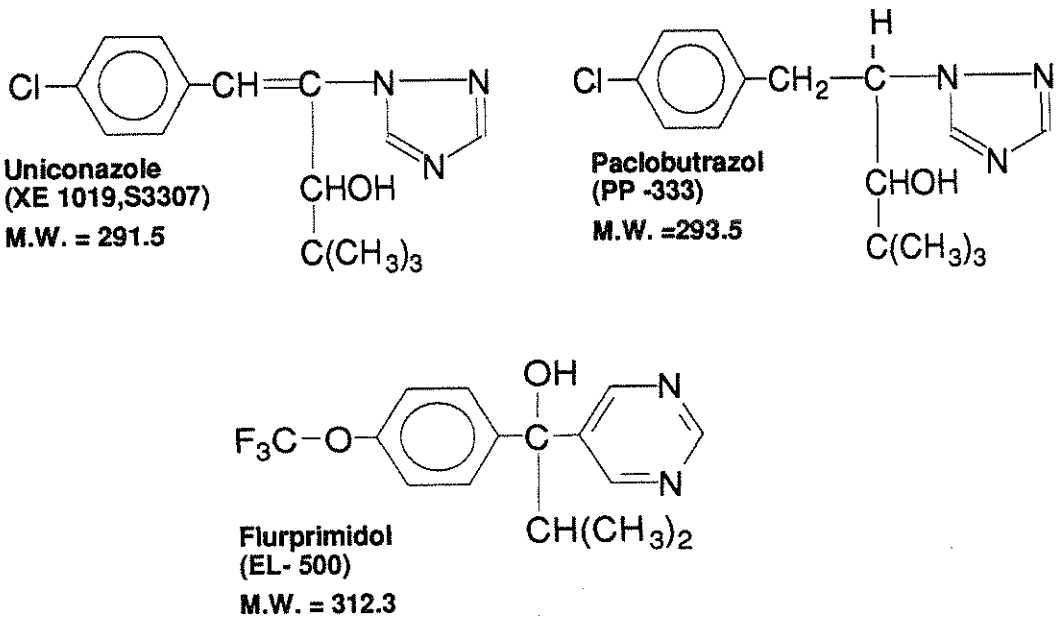

Figure 1. Chemical structure of three gibberellin synthesis inhibitors.

The bottles were placed on a gyratory shaker table in the growth chamber under the same conditions described above. Bottles were allowed to shake gently (80-90 rpm) for 60-90 minutes and were then removed from the chamber and measured for dissolved oxygen. Medium in BOD bottles without apices also was measured at the end of the period and never varied more than .01 mg/L from the initial measurement of dissolved oxygen. For respiration, the same plants were removed from the BOD bottles and placed in dark BOD bottles with a known oxygen concentration. The dark BOD bottles were placed in the growth chamber (lights were turned off as a precaution) on the shaker table and incubated for 60-90 minutes. Final dissolved oxygen concentration is expressed as mg O_2/g fresh weight/min. Total chlorophyll (chlorophyll a and b) was measured on fresh tissue (the apical 4-6 cm) using a DMSO extraction method (Hiscox and Israelstam 1979). Total chlorophyll is expressed as mg chlorophyll/g fresh wt.

Exposure time

Eurasian watermilfoil was exposed to 75 µg/L and hydrilla to 750 µg/L of each gibberellin synthesis inhibitor for periods of 1,3, 7, and 14 days. After exposure, the plants were removed from the treatment solution, rinsed thoroughly with distilled water, placed in fresh untreated medium and returned to the growth chamber. Regrowth was monitored at 2,4 and 6 weeks after transfer. This protocol provided information on both the required exposure time and the duration of effect.

Experimental design and statistical analysis

Each treatment consisted of three replicates 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 4-6 cm of the plant was removed and used for chlorophyll analysis. Dry weight was taken on the remaining portion of the stem. Data from each sampling time (0, 1, 2 and 4 weeks) were analyzed separately. Studies were repeated, and data from the two experiments were combined for analysis of variance (ANOVA). The effect of the inhibitors on growth and

physiological parameters was examined by regression analysis and testing for linear response of a particular measurement to the added inhibitor rates at each sampling time.

Small-scale outdoor testing

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 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% wettable powder or 0.15% granular. Concentrations tested during the summer of 1988 were 0, 7.5, 75, 750, and 1500 µg/L. The exposure period was from 25 May to 29 June (5 weeks). Plants were removed from the treatment, measured and weighed for growth parameters.

RESULTS AND DISCUSSION

Bioassay

The bioassay conditions resulted in good growth of untreated plants over the four week test period. Main stem lengths increased at mean rates of 0.53 ± 0.003 cm/day in hydrilla and 0.46 ± 0.002 cm/day in Eurasian watermilfoil (doubling times of 7.4 and 8 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 experimental period, from an initial value of 28% to 15% at four weeks for hydrilla and an inital value of 20% to 15% at four weeks for Eurasian watermilfoil, indicating active growth and utilization of stored materials.

Growth parameters

Hydrilla was sensitive to the gibberellin synthesis inhibitors at a range of 75 µg/L to 750 µg/L (Fig. 2) with main stem lengths of 41% (paclobutrazol) to 33% (uniconazole) that of untreated controls after 4 weeks at 750 µg/L. Inhibitor dosages of 75 µg/L resulted in a 63% (paclobutrazol) to 50% (uniconazole) decrease in main stem length compared to untreated stem length. At the lowest dose tested, 7.5 µg/L, no change occurred in plant growth, physiology or morphology.

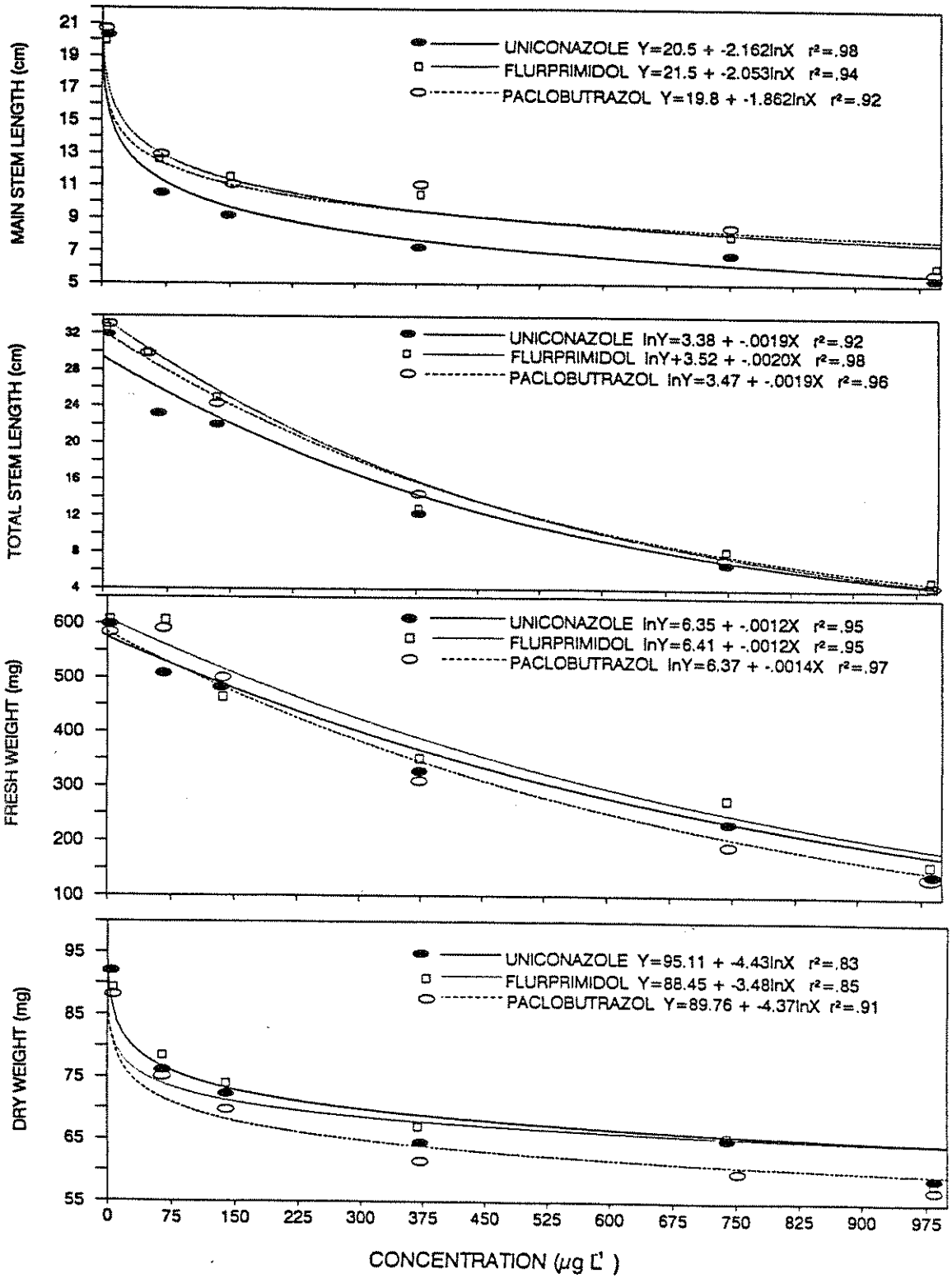

Figure 2. Hydrilla main stem length, total stem length, fresh weight, and dry weight at four weeks after treatment. Initial main stem length = 4 cm. Data were transformed to achieve the best curve fit following regression analysis.

Hydrilla growth at concentrations of 1000 and 1500 µg/L was minimal, no more than 1-2 cm over the initial 4 cm length; the plants became very brittle at these dosages with an increase in red pigmentation (probably anthocyanins). Photosynthesis was completely inhibited at these concentrations, and the plants were presumed to be dead.

Although not statistically comparable, Eurasian watermilfoil was approximately 100X more sensitive to the inhibitors than hydrilla. Main stem length of Eurasian watermilfoil was reduced at dosages of 0.75 to 100 µg/L (Fig. 3). Main stem lengths of plants treated with 0.75 µg/Lwere approximately 67% those of controls after 4 week exposures. Dosages below 0.75 µg/L (0.1 and 0.2 µg/L) were inconsistent in reducing main stem length in Eurasian watermilfoil (data not shown). Eurasian watermilfoil plants treated at 750 µg/L remained at the initial 4 cm length (data not shown) and were abnormal in appearance with many compacted lateral buds. However, they remained green in color and still photosynthesized (at about half the rate of untreated controls).

The effects of the gibberellin synthesis inhibitors on lateral stem production differed between the two species. Hydrilla treated at 75 µg/L with any of the three compounds produced more lateral stems per plant (4.2 ± 0.7) compared to untreated plants (2.0 ± 0). However, the length per lateral (3 ± 1.5 cm for all three compounds) at 75 µg/L was lower than that of the untreated controls (7 ± 2 cm), indicating that the inhibitors were affecting lateral stem elongation as well as main stem elongation. The increased lateral branching obtained at flurprimidol and paclobutrazol dosages of 75 µg/L resulted in total (main plus lateral) stem lengths and fresh weights that were not different from those of the controls (Fig. 2). Uniconazole at 75 µg/L was more effective at reducing total stem length because this treatment produced the lowest number of lateral stems (3.3) as well as the shortest main stem lengths (Fig. 2). All three compounds at 75 µg/L produced plants with a branched, stoloniferous growth form (Fig. 4).

As inhibitor dosage increased, lateral branching was inhibited, and a main stem with very compact internodes was produced (Fig. 4). The lack of lateral stem production at higher dosages (150, 375, and 750 µg/L) resulted in greatly reduced total stem lengths and fresh and dry weights (Fig. 2) compared to untreated controls.

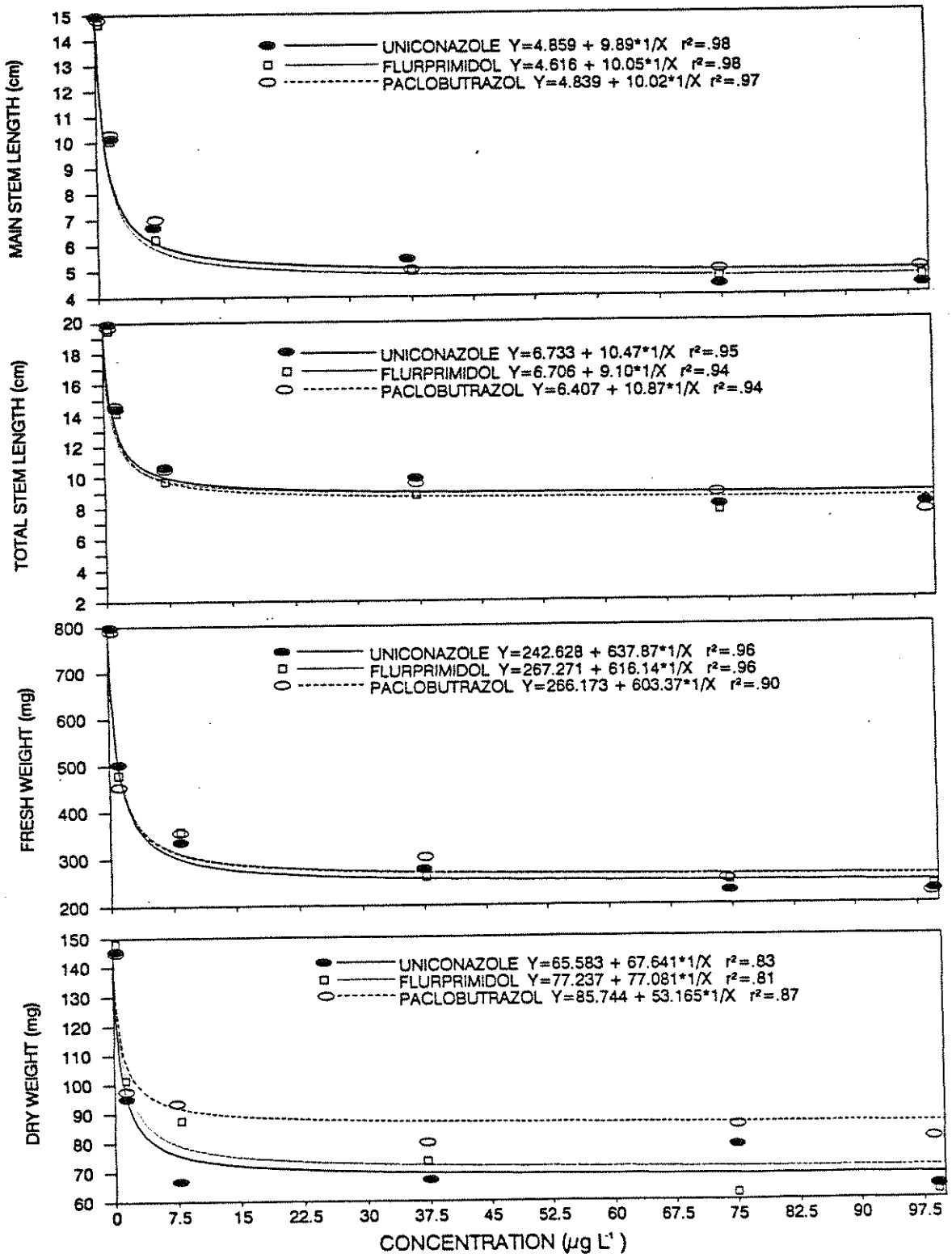

Figure 3. Eurasian watermilfoil main stem length, total stem length, fresh weight and dry weight at four weeks after treatment. Initial main stem length = 4 cm. Data were transformed to achieve the best curve fit following regression analysis.

Figure 4. Hydrilla after four weeks treatment with paclobutrazol. Concentrations (from left to right) are 0, 75, 375 and 1500 µg/L. Initial plant height = 4 cm.

At inhibitor dosages of 0.75 μg/L, Eurasian watermilfoil internodes became very compact resulting in a single shortened main stem (Fig. 5). No lateral buds or stems were observed on these plants. Control plants consistently produced one lateral shoot per plant whereas flurprimidol and paclobutrazol treatments of 7.5 μg/L increased lateral stem production (3.5 ± 0.5). Uniconazole at this dosage resulted in a much greater proliferation of lateral stems (11 ± 1.3). In contrast to hydrilla, however, Eurasian watermilfoil lateral stems remained as buds, i. e. they never elongated and rarely measured more than 0.5 cm in length (Fig. 5). The lack of main stem growth and lateral stem elongation in Eurasian watermilfoil resulted in total stem lengths and fresh and dry weights (Fig. 3) that were always significantly lower than untreated controls.

Increased lateral bud production by Eurasian watermilfoil (and hydrilla at 75 mg/L) in response to treatment may be explained by the effects of these inhibitors on metabolic pathways other than gibberellin biosynthesis. Gibberellin synthesis inhibitors have been shown to increase cytokinin-like activity in rice (Izumi et al. 1988). An increase in cytokinin production enhances lateral shoot initiation and elongation in plants; however, if this was the case in Eurasian watermilfoil the elongation of these buds was apparently reduced by the gibberellin synthesis inhibitors.

Following inhibitor treatment, many attempts were made to allow the compacted lateral buds of Eurasian watermilfoil to elongate during prolonged recovery periods in fresh, untreated medium, but none of these attempts was successful, even after 6 months exposure to untreated medium (data not shown). Exogenous applications of gibberellic acid (GA_3) are known to overcome the effects of these inhibitors (Izumi et al. 1984, Rademacher and Jung 1981). GA_3 was added to previously treated Eurasian watermilfoil stems with compacted lateral buds to determine whether it could overcome the effects of the gibberellin synthesis inhibitors. GA_3 (10^{-5} and 10^{-8} M) alone and GA_3 plus inhibitors (at concentrations of 75, 375 and 750mg/L) were added to experimental flasks containing stems with intact lateral buds and flasks containing excised lateral buds. All attached and excised buds began rapidly elongating within 2 days (Figs. 6, 7). Shoots with lateral buds and a total stem length averaging 6 cm grew to produce plants with total stem lengths of 84

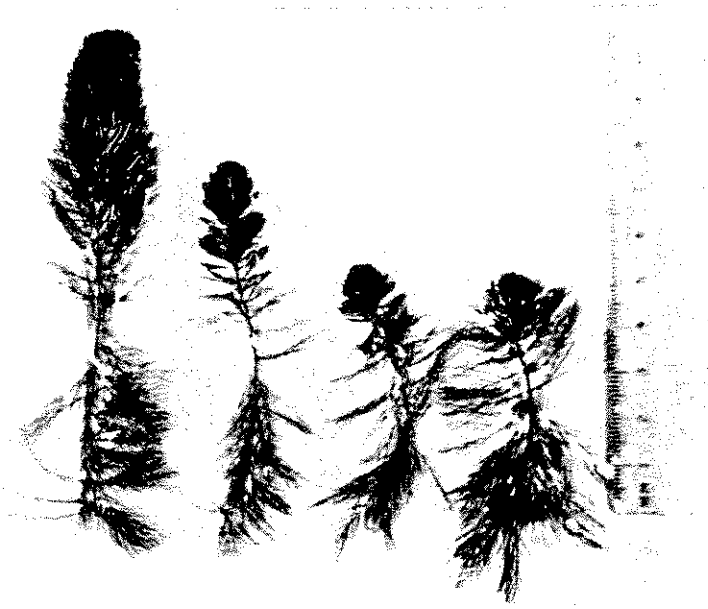

Figure 5. Eurasian watermilfoil after four weeks treatment with paclobutrazol. Concentrations (from left to right) are 0, 7.5, 37.5 and 75 µg/L. Note lateral buds at the 75 µg/L concentration. Initial plant height = 4 cm.

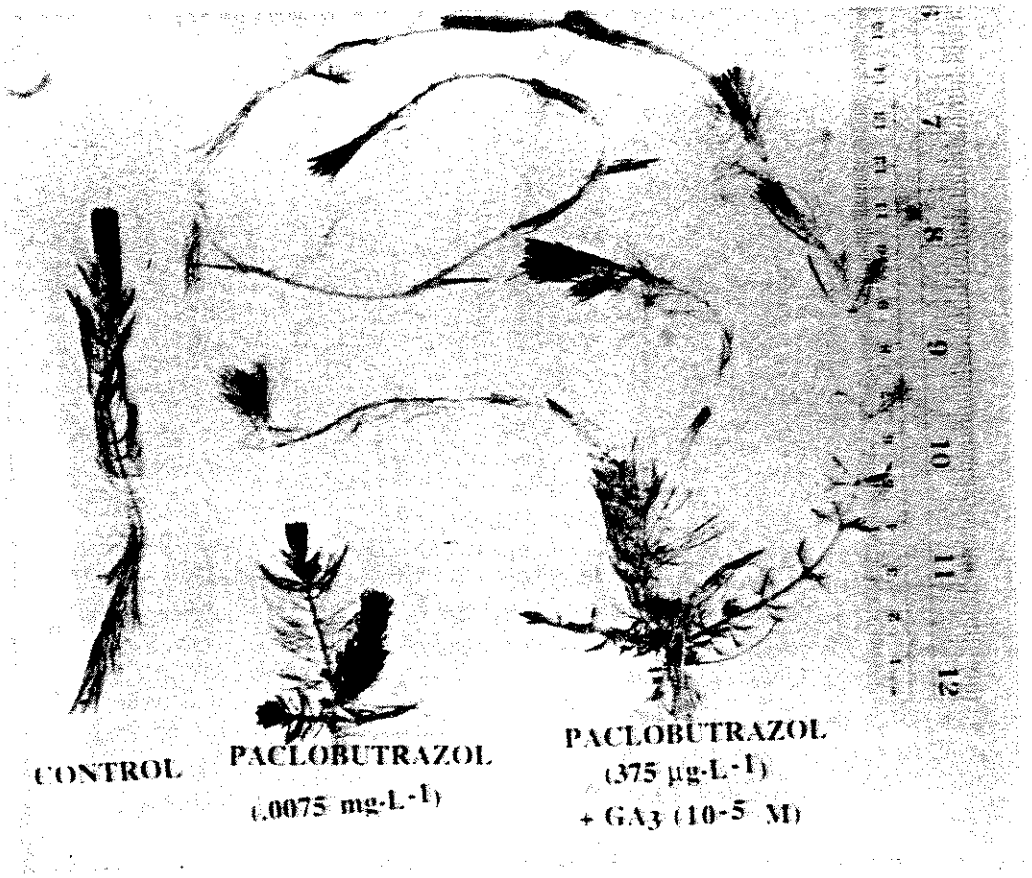

Figure 6. Response of gibberellin synthesis-inhibited Eurasian watermilfoil to gibberellic acid (GA_3). Left, untreated; center, treated with paclobutrazol at 7.5 µg/L for 4 weeks; right, treated with paclobutrazol at 375 µg/L for 4 weeks followed by 2-week exposure to GA_3 at 10^{-5} M. Notice that lateral buds on GA_3-treated plants have sprouted and elongated.

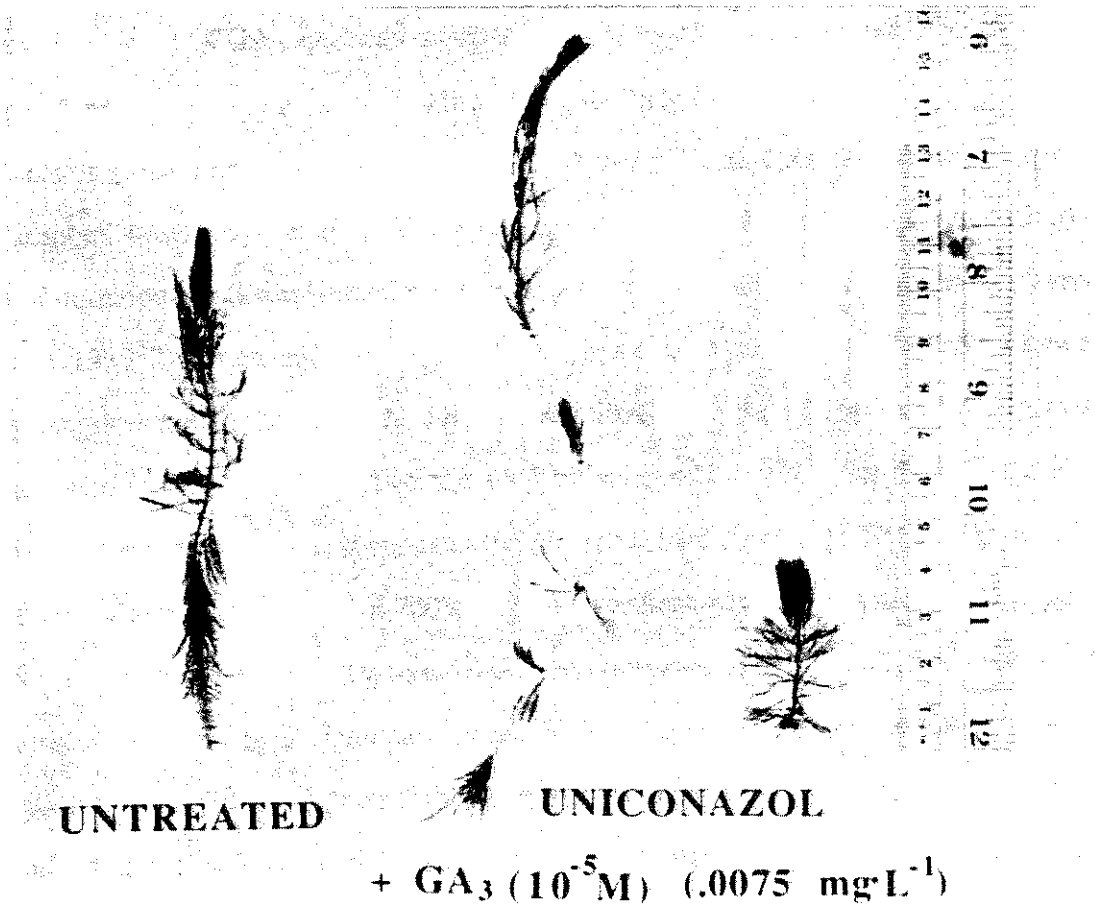

Figure 7. Response of gibberellin synthesis-inhibited Eurasian watermilfoil to gibberellic acid (GA_3). Left, untreated; center, treated with uniconazole at 7.5 µg/L and GA_3 at 10^{-5} M at the same time; right, treated with uniconazole at 7.5 µg/L only. After 6 weeks.

cm in 10 days. Buds that had remained dormant for 6 months posttreatment also began elongating within 2 days of GA_3 application. Exogenous GA_3 also had a stimulatory effect on hydrilla stem elongation when applied at the same time as the inhibitor (Fig. 8).

The effects of gibberellin synthesis inhibitors on roots of terrestrial plants include increases in number (Campbell 1986, Davis et al. 1985), no change in number (Wample and Culver 1983), production of numerous thickened lateral roots (Sankhla et al. 1985) and decreases in root number (Steffens et al. 1983, Temman and Elkins 1984). In our study, the inhibitors affected root number and average length in both hydrilla and Eurasian watermilfoil. At 75 mg/L or less, hydrilla root numbers increased (an average of 8 ± 2 versus 4 ± 1 in untreated controls), but the average length per root was shorter than in untreated controls (8 ± 2 versus 14 ± 3 cm). At 375 mg/L, root numbers generally increased whereas at 750 mg/L root numbers generally decreased (data not shown); however, average root lengths remained short compared to controls (1.5 cm versus 14 cm). The results of treatments on Eurasian watermilfoil roots were somewhat variable. The only treatment to have a significant effect on Eurasian watermilfoil average root length (1 cm versus 8 cm in controls) was at 75 mg/L. In both plant species the shortened roots were 2-3 times thicker compared to control roots. It should be noted that the use of apical tips (cuttings) grown in liquid medium could have affected the responses of the roots to the inhibitor. Paclobutrazol has been reported to stimulate adventitious root formation on cuttings of terrestrial plants (Davis et al. 1985).

Inhibitor effects on treated plants were evident as soon as untreated shoots began elongating. For example, total (main plus lateral) stem lengths of uniconazole-treated hydrilla and Eurasian watermilfoil at 1 and 2 weeks were reduced at most treatment concentrations compared to untreated controls (Fig. 9). All growth measurements taken at 1 and 2 weeks showed a similar trend and therefore are not included here.

Treated plants exhibited the same type of response to all three inhibitors. Uniconazole appeared to be the most effective compound of the three at reducing main and total stem lengths in hydrilla (Fig. 2). The molecular weights of the three compounds are similar: uniconazole = 291.5, paclobutrazol = 293.5, and flurprimidol = 312.3. Therefore, the apparent effectiveness of

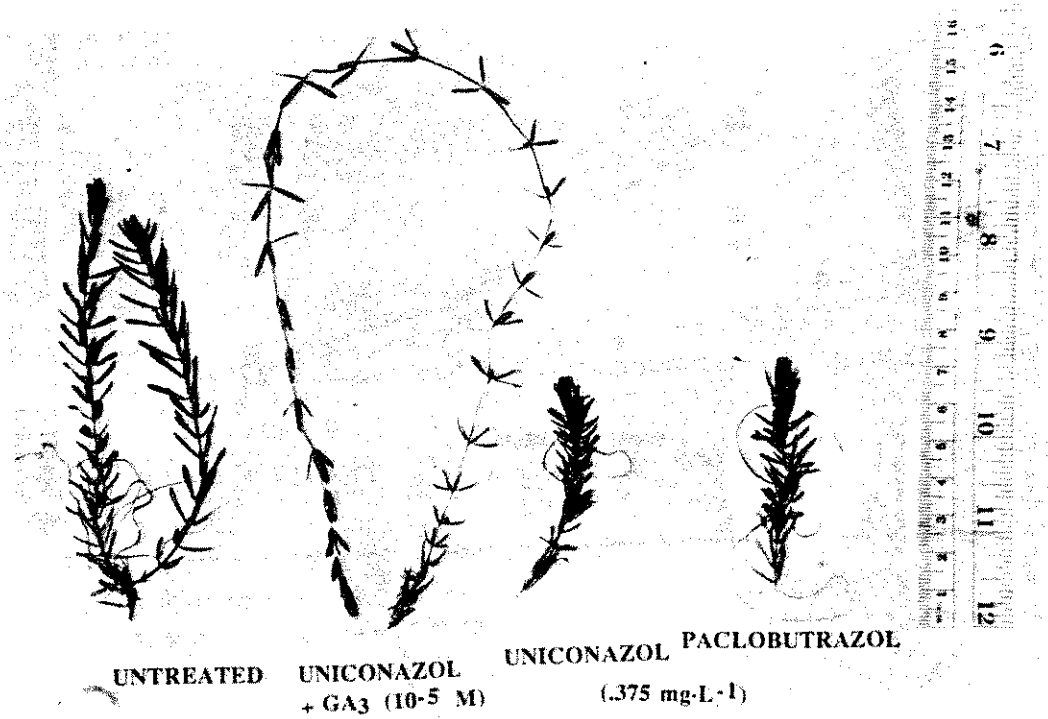

Figure 8. Response of gibberellin synthesis-inhibited hydrilla to gibberellic acid (GA_3). Left, untreated; left center, treated with uniconazole at 375 µg/L and GA_3 at 10^{-5}M at the same time; right center, treated with uniconazole at 375 µg/L; right, treated with paclobutrazol at 375 µg/L. After 6 weeks.

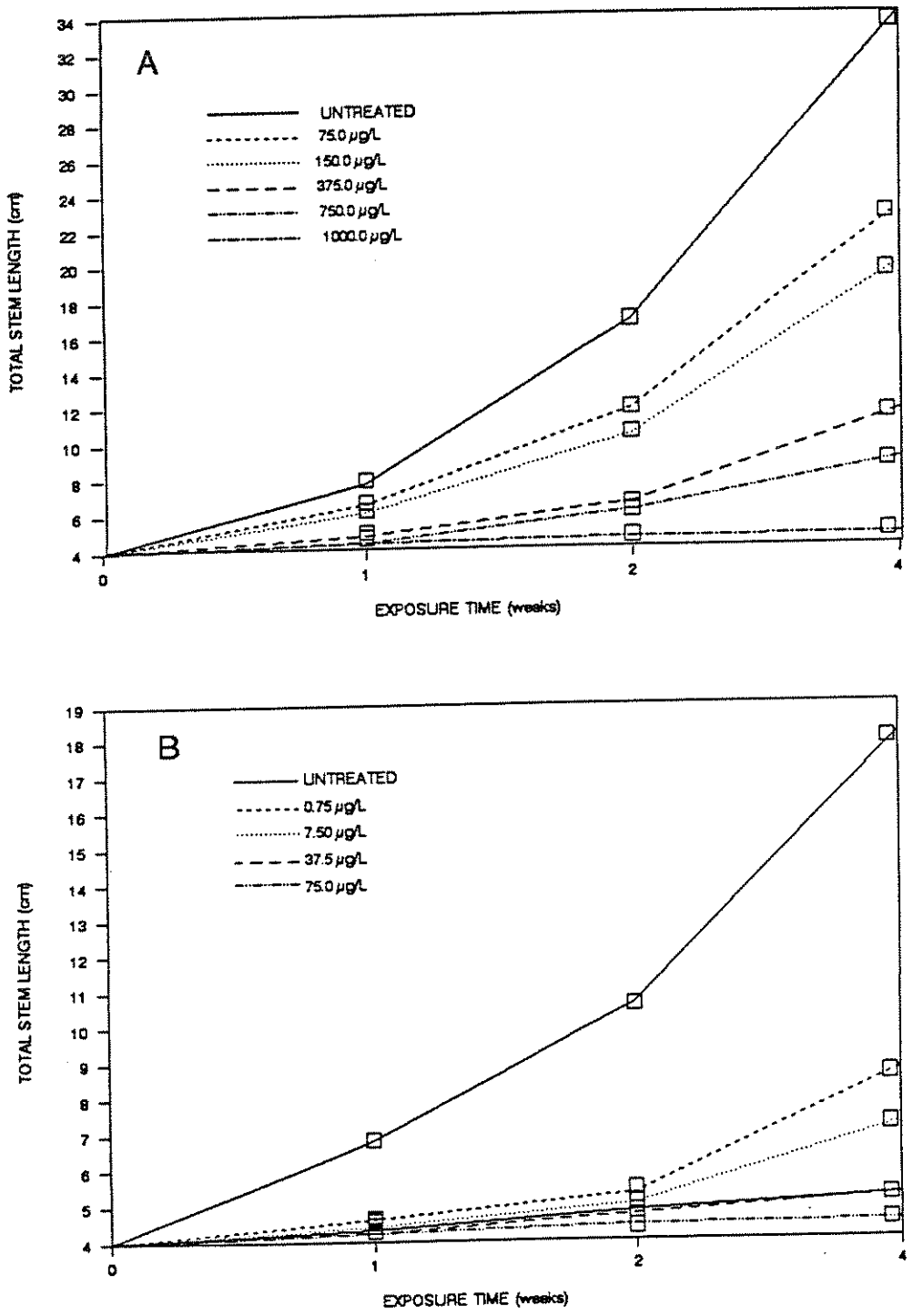

Figure 9. Total stem lengths of hydrilla (A) and Eurasian watermilfoil (B) after 1, 2, and 4 weeks treatment with uniconazole. 1,2, and 4 week data were subjected to ANOVA and significant differences ($P<.05$) existed within each treatment period.

uniconazole cannot be attributed to a higher molar concentration when the three compounds were compared. Studies comparing these three inhibitors on terrestrial plants have shown uniconazole to be most effective (Steffens 1988, Sterret 1988).

Physiological parameters

Net photosynthetic rates, respiration rates, and chlorophyll content per gram fresh weight of treated hydrilla and Eurasian watermilfoil at any concentration or for any compound were not significantly different from those of untreated controls except, as previously noted, at the highest concentrations tested (1000 µg/L for hydrilla; 750 µg/L for Eurasian watermilfoil). At 75 µg/L paclobutrazol, hydrilla photosynthesis rate, respiration rate and chlorophyll content was 50.2 ± 4.5 µg O_2/g fr wt/ min, 7.4 ± .9 µg O_2/g fr wt/min, and 1.30 ± .07 mg chl/g fr wt, respectively, and for Eurasian watermilfoil at 7.5 µg/L paclobutrazol these values were 21.6 ± 1.5 µg O_2/g fr wt/min, 5.2 ± .8 µg O_2/g fr wt/min, and 1.23 + .05 mg chl/g fr wt, respectively. The ability of the treated plants to retain these aspects of physiological competence at dosages which are non-toxic but provide significant main stem length reduction indicates that the plants would remain functional in an aquatic system.

Effects of these inhibitors on photosynthesis of terrestrial plants have varied with studies and species used and include inhibition (Wample and Culver 1983), stimulation (Jaggard et al. 1982) and no effect (Dejong and Doyle 1984, Sankhla et al. 1985). A characteristic response of terrestrial plants to gibberellin synthesis inhibitor treatment is intensified leaf greening. This has been attributed to a decreased leaf surface area containing an amount of chlorophyll nearly equivalent to that in untreated leaves (Wample and Culver 1983). The new growth of both Eurasian watermilfoil and hydrilla exhibited a darker green color after treatment. Visually, the treated plants appeared to have smaller leaves than the controls; if true, a decrease in leaf area may have resulted in the appearance of increased leaf chlorophyll.

When expressed on a gram per dry weight basis, chlorophyll content of hydrilla was lower in treated plants (375 and 750 µg/L) than in untreated controls (Table 1). This probably was not due to a decrease in chlorophyll content but to the increase in percent dry weight which occurred with

Table 1. Chlorophyll content on a fresh and dry weight basis and percent dry weight of hydrilla at four weeks after treatment. Data are means of three replicates from one experiment.

Inhibitor	Conc.	Chlorophyll		Dry wt.
	(µg/L)	(µg/g fr. wt.)	(µ/g dry wt.)	(%)
Uniconazole	0	1.23	7.7	15.8
	75	1.30	8.6	15.0
	150	1.26	7.2	16.8
	375	1.25	5.5	22.6
	750	1.20	4.7	25.6
Linear response		--[a]	0.05[b]	0.05[b]
Flurprimidol	0	1.32	8.7	15.1
	75	1.40	9.0	15.5
	150	1.27	7.8	17.4
	375	1.24	5.2	23.4
	750	1.26	4.5	27.8
Linear response		--[a]	0.05[b]	0.05[b]
Paclobutrazol	0	1.28	8.6	14.8
	75	1.44	9.8	14.6
	150	1.38	7.6	18.3
	375	1.40	5.9	23.6
	750	1.35	4.6	28.9
Linear response		--[a]	0.05[b]	0.05[b]

[a] ANOVA indicated no significant difference at the 0.05 level.

[b] Test for linear response of chlorophyll and dry weight over treatment concentrations within compounds and within column.

increasing concentrations of inhibitor (Table 1). A similar trend was observed in Eurasian watermilfoil (data not shown). The increased dry weight probably was due, at least in part, to an increase in carbohydrate content since an analysis of paclobutrazol-treated hydrilla showed an increase in starch content over untreated controls (data not shown). Accumulations of nonstructural carbohydrates in all parts of triazole-inhibited plants have been reported (Steffens, 1980, Wang et al. 1986).

Exposure time/duration of effect

The main stem lengths of hydrilla and Eurasian watermilfoil remained reduced six weeks after transfer from 24 hour or longer exposures to 750 µg/L and 75 µg/L, respectively (Tables 2 and 3). This suggests that very short exposure periods will result in relatively long-lasting effects. Lateral stems of Eurasian watermilfoil remained suppressed throughout the six-week period resulting in significantly reduced total stem lengths (Table 3). Lateral stems in hydrilla, however, continued to grow following exposure times ranging from 24 hours to 14 days (Table 2). This resulted in total stem lengths that were not different from control plants six weeks after transfer to fresh medium. Thus, although reduced in vertical height, hydrilla treated with 750 µg/L and rinsed after 24 hours produced a bushy, stoloniferous growth form similar to that achieved with a four week exposure to 75 µg/L.

Small-scale outdoor testing

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 uniconazole at 1500 µg/L and the granular formulation at 750 µg/L caused severe adverse effects; i.e. the plants turned red, and total stem length at the end of 5 weeks was only 0.002% that of untreated plants. At 75 µg/L, the wettable powder reduced total (main plus lateral) stem length to 59% that of untreated controls; treatment at the higher concentration (750 µg/L) produced plants that were 47% of untreated total stem length (Fig. 10). The effect of the granular formulation at 75 µg/Lwas similar to that of the 75 µg/L concentration of wettable powder (data not shown). Plants treated at 75 µg/L averaged 470 lateral shoots (compared with 181 for untreated

Table 2. Hydrilla stem lengths after recovery following treatment with 750 µg/L at different exposure times. Total stem length = main stem length + total lateral stem length.

		6 week recovery		
Inhibitor	Exposure time	Main stem length	Total lateral stem length	Total stem length
	(days)	(cm)	(cm)	(cm)
Uniconazole	0	29	23	52
	1	11	40	51
	3	9	41	50
	7	15	32	47
	14	16	28	44
Linear response		NS[b]	NS[b]	--[a]
Flurprimidol	0	28	22	50
	1	10	39	49
	3	10	41	51
	7	13	33	46
	14	15	28	43
Linear response		NS[b]	NS[b]	--[a]
Paclobutrazol	0	30	21	51
	1	11	40	51
	3	11	38	49
	7	10	35	45
	14	12	36	48
Linear response		NS[b]	NS[b]	--[a]

[a] ANOVA indicated no significant difference at the 0.05 level

[b] Test for linear response of stem length over exposure time within compounds and within column.

Table 3. Eurasian watermilfoil stem lengths after recovery following treatment with 75 $\mu g \cdot L^{-1}$ at different exposure times. Total stem length = main stem length + total lateral stem length.

		6 week recovery		
Inhibitor	Exposure time	Main stem length	Total lateral stem length	Total stem length
	(days)	(cm)	(cm)	(cm)
Uniconazole	0	19	15	34
	1	5	5	10
	3	5	6	11
	7	5	5	10
	14	5	3	8
Linear response[a]		NS	0.05	0.05
Flurprimidol	0	21	15	36
	1	7	7	14
	3	6	8	14
	7	5	7	12
	14	5	6	11
Linear response[a]		0.05	0.05	0.05
Paclobutrazol	0	20	17	37
	1	9	6	15
	3	7	5	12
	7	7	6	13
	14	6	5	11
Linear response[a]		0.05	0.05	0.05

[a] Test for linear response of stem length over exposure time within compounds and within column.

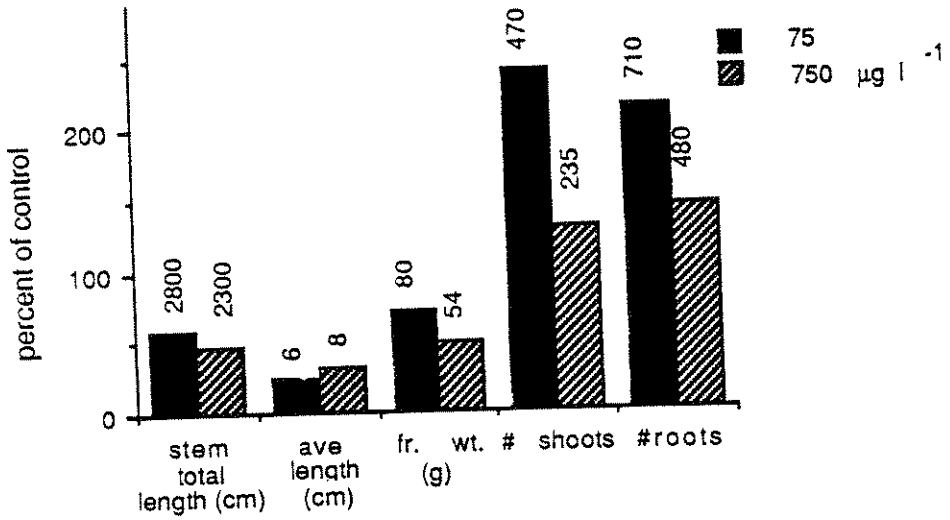

Figure 10. Effect of uniconazole on hydrilla in small-scale barrel tests at 5 weeks. Growth data as % of untreated controls. Numbers above bars indicate actual measurements.

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% that of untreated plants at 75 µg/L and 48% that of untreated plants at 750 µg/L. It is interesting to note that 750 µg/L uniconazole resulted in the production of fewer lateral shoots and roots than the lower concentration. The lowest concentration used (7.5 µg/L, data not shown) was somewhat less efficacious than the 75 µg/L concentration, but reductions in stem length were still observed, i.e. stem length was 79% and fresh weight was 81% of the untreated plants.

Uniconazole concentrations of 75 and 750 µg/L produced plants that never reached a vertical height greater than 10 cm, even with some lateral branch production (Fig. 11). At 7.5 µg/L, 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 (Fig. 11). 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 (Fig. 12).

CONCLUSIONS

Both hydrilla and Eurasian watermilfoil were susceptible to main stem length reduction upon exposure to the gibberellin synthesis inhibitors without effects on photosynthesis or respiration. However, sensitivity and morphological responses differed between the two species. Effective dosages were low for Eurasian watermilfoil, ranging between 0.75 and 75 µg/L whereas hydrilla was affected at a concentration range between 75 and 750 µg/L. Toxic effects of the gibberellin synthesis inhibitors appeared at 1000-1500 µg/L on hydrilla. High inhibitor concentration effects on Eurasian watermilfoil were more subtle; although severely stunted in growth and with many lateral buds, the plants appeared to be alive at concentrations as high as 750 µg/L.

Eurasian watermilfoil responded to increasing concentrations by producing an increased number of compact lateral buds along the length of the stem but with very little main stem growth.

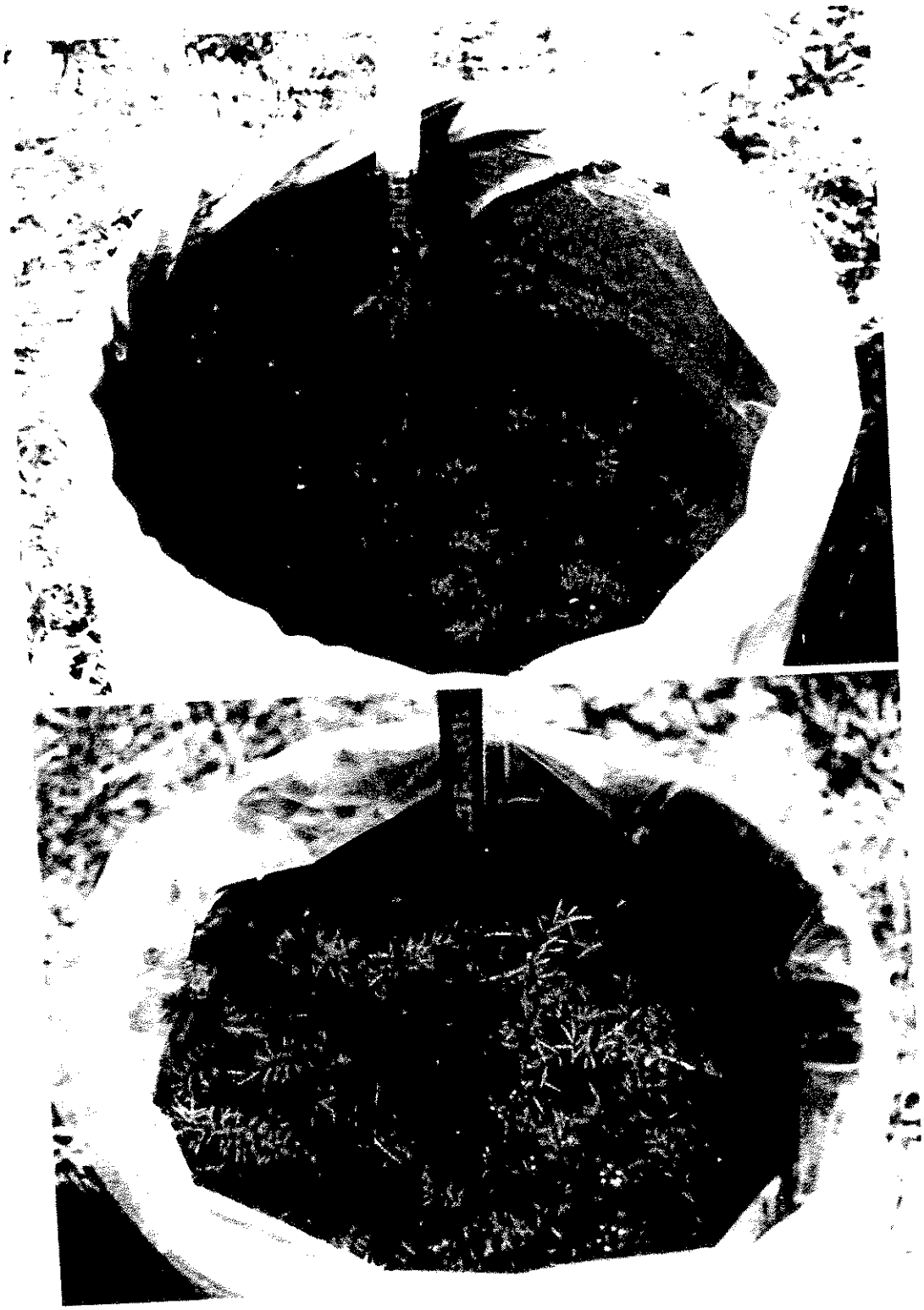

Figure 11. Comparison of effect of uniconazole treatment on hydrilla in small-scale barrel tests. Top, plants treated with 75 µg/L. Bottom, untreated plants.

Figure 12. Comparison of treated (left) and untreated (right) hydrilla plants pulled from small-scale barrel tests. Note stoloniferous growth of treated plant.

The potential of these lateral buds to serve as a source of new infestation (like turions) if they were to become detached from the parent plant is a concern. However, our inability to induce these buds to elongate (given favorable laboratory growing conditions over a 6 month period) without GA_3 application may not make them good candidates for regrowth in the field. An obvious way to eliminate bud production in milfoil would be to use lower concentrations of inhibitor so that only a shortened main stem with compact internodes would form.

In hydrilla, increased lateral shoot and root production at 75 $\mu g \cdot L^{-1}$ resulted in plants with reduced vertical height and a stoloniferous appearance. However, in contrast to Eurasian watermilfoil, lateral stem production in hydrilla appeared to decrease with increasing gibberellin synthesis inhibitor concentrations. Higher concentrations resulted in a single main stem with very compact internodes and no lateral branches. Although buds were not visible, nodal areas obviously had the potential to produce lateral branches, as suggested by the rapid regrowth of lateral branches during recovery in untreated medium.

Both hydrilla and Eurasian watermilfoil appear to be able to take up the inhibitors at concentrations that reduce main stem length within 24 hours of treatment and to retain the inhibitors (or at least their effect) for up to 6 weeks. If these characteristics persist in the field, the potential for long-term stem length reduction at parts per billion concentrations would make this strategy very appealing.

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 µg/L concentration resulted in carpet-like growth consisting of only 48% of the untreated 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.

Although our results must now be followed by large-scale field testing, they suggest that gibberellin synthesis inhibitors could provide a new strategy in aquatic plant management by maintaining shortened but healthy plants in the aquatic ecosystem.

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