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THE 2,4-D THRESHOLD CONCENTRATIONS FOR CONTROL OF EURASIAN WATERMILFOIL AND SAGO PONDWEED

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The objective of this study was to use a modified diluter system to determine the minimum sustained (threshold) concentrations of 2,4-dichlorophenoxy acetic acid (2,4-D) required to control the growth of Eurasian watermilfoil (*Myriophyllum spicatum* L.) and Sago pondweed (*Potamogeton pectinatus* L.). This study was designed to incorporate recommended improvements and to verify results from a previous pilot study. The diluter system delivered five different concentrations of 2,4-D to five sets of four test aquaria. Another

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set of four reference aquaria received only tapwater. Each aquarium contained meristematic cuttings of *M. spicatum* and germinated tubers of *P. pectinatus* planted in beakers containing a standard hydrosoil. Plant injury was assessed after 11 weeks of continuous exposure to the various 2,4-D concentrations. Based on the results of this study, the 2,4-D threshold concentrations required to control *M. spicatum* and *P. pectinatus* were determined to be 0.05 to 0.10 mg/ℓ and 0.10 to 0.25 mg/ℓ, respectively. This coincided with previous results obtained from a 6-week pilot study.

PREFACE

This study was conducted as part of the U. S. Army Corps of Engineers Aquatic Plant Control Research Program (APCRP) under Chemical Control Technology Development. Funds for the effort were provided by the Office, Chief of Engineers, under Department of the Army Appropriation No. 96X3122, Construction General, 902740, through the APCRP at the U. S. Army Engineer Waterways Experiment Station (WES), Vicksburg, Miss.

The work was initiated in June 1981 under the direct supervision of Mr. Donald L. Robey, Chief, Ecosystem Research and Simulation Division (ERSD), Environmental Laboratory (EL), and Mr. Jim Brannon, Acting Chief, Aquatic Processes and Effects Group (APEG), ERSD. Mr. J. Lewis Decell was Program Manager, APCRP; Dr. John Harrison was Chief, EL.

The principal investigators for this work were Mr. Jerry F. Hall and Dr. Howard E. Westerdahl of APEG. Mr. Ronald E. Hoeppel and Ms. Lena Williams assisted in the study. The Tennessee Valley Authority, Laboratory Branch, in Chattanooga, Tenn., provided analysis of 2,4-D residues in water samples.

The Commander and Director of WES during conduct of the study and preparation of the report was COL Tilford C. Creel, CE. Technical Director was Mr. F. R. Brown.

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PART I: INTRODUCTION

Background

1. Species of exotic and indigenous aquatic plants can proliferate in waterways, recreational lakes, and other aquatic environments to such an extent as to impair transportation, recreation, water supply, and irrigation. In many cases, it has become necessary to control these aquatic plants. A number of methods for controlling nuisance aquatic plant species are currently being used, including chemical, biological, mechanical, environmental management, and integrated techniques. Chemical control techniques are the most commonly used by Corps of Engineers (CE) field offices with active aquatic plant control programs (Dardeau and Hogg 1982).

2. Two aquatic plants presently considered to be nuisance species are Eurasian watermilfoil (*Myriophyllum spicatum* L.) and Sago pondweed (*Potamogeton pectinatus* L.). *Myriophyllum spicatum*, an extremely successful competitor, is a major problem in CE waterways. *Potamogeton pectinatus* is of equal concern to the Bureau of Reclamation because of its ability to impede water flow in irrigation systems in the western United States.

Purpose

3. The purpose of this study was to verify earlier determined threshold concentrations of the phenoxy herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) (Westerdahl et al. 1982) by continuously exposing plants of *M. spicatium* and *P. pectinatus* to various low dosages of this compound. The threshold 2,4-D concentration is here defined as the sustained minimal concentration required to inhibit plant growth and produce control over an extended time period.

PART II: MATERIALS AND METHODS

Diluter System

4. A diluter system was used to deliver different concentrations of 2,4-D to 20 aquaria (Figure 1). This system was modified according to recommendations made following a 6-week pilot study using 2,4-D (Westerdahl et al. 1982). The multicompartmented acrylic chamber (Figure 1) was wrapped with aluminum foil during the course of the experiment to prevent algal growth such as occurred during the pilot study. Excessive fungal growth in the stainless steel distribution cannisters also created a problem during the pilot study. Periodic cleaning of the cannisters eliminated this problem during the course of the study. Tubing leading from the distribution cannisters to the test aquaria was of larger inside diameter than that used during the aforementioned pilot study to permit rapid drainage and prevent air entrapment. All of the tubing was held in a downward direction sloping toward the aquaria.

5. The modified diluter system and aquaria were located in a controlled-environment greenhouse covered with 55 percent shade fabric. Supplemental lighting was provided by a light bank suspended above the aquaria. Photosynthetically active radiation (PAR), representing the 400- to 700-nm wavelength, was measured above several aquaria using a Lambda, Inc., PAR meter. The mean PAR received by the aquaria was approximately 1600 $\mu E \cdot m^{-2} \cdot sec^{-1}$ (Figure 2), which corresponds to 75 percent of solar noon sunlight received at this latitude.

Experimental Design

Aquaria

6. Based on previous laboratory research (Westerdahl et al. 1982), the 2,4-D concentrations selected were 0.00, 0.03, 0.05, 0.10, and 0.25 mg/ℓ. A simple randomized experimental design was used to assign each 2,4-D concentration to each of four replicate aquaria. To offset potential bias resulting from spatial differences in insolation, each

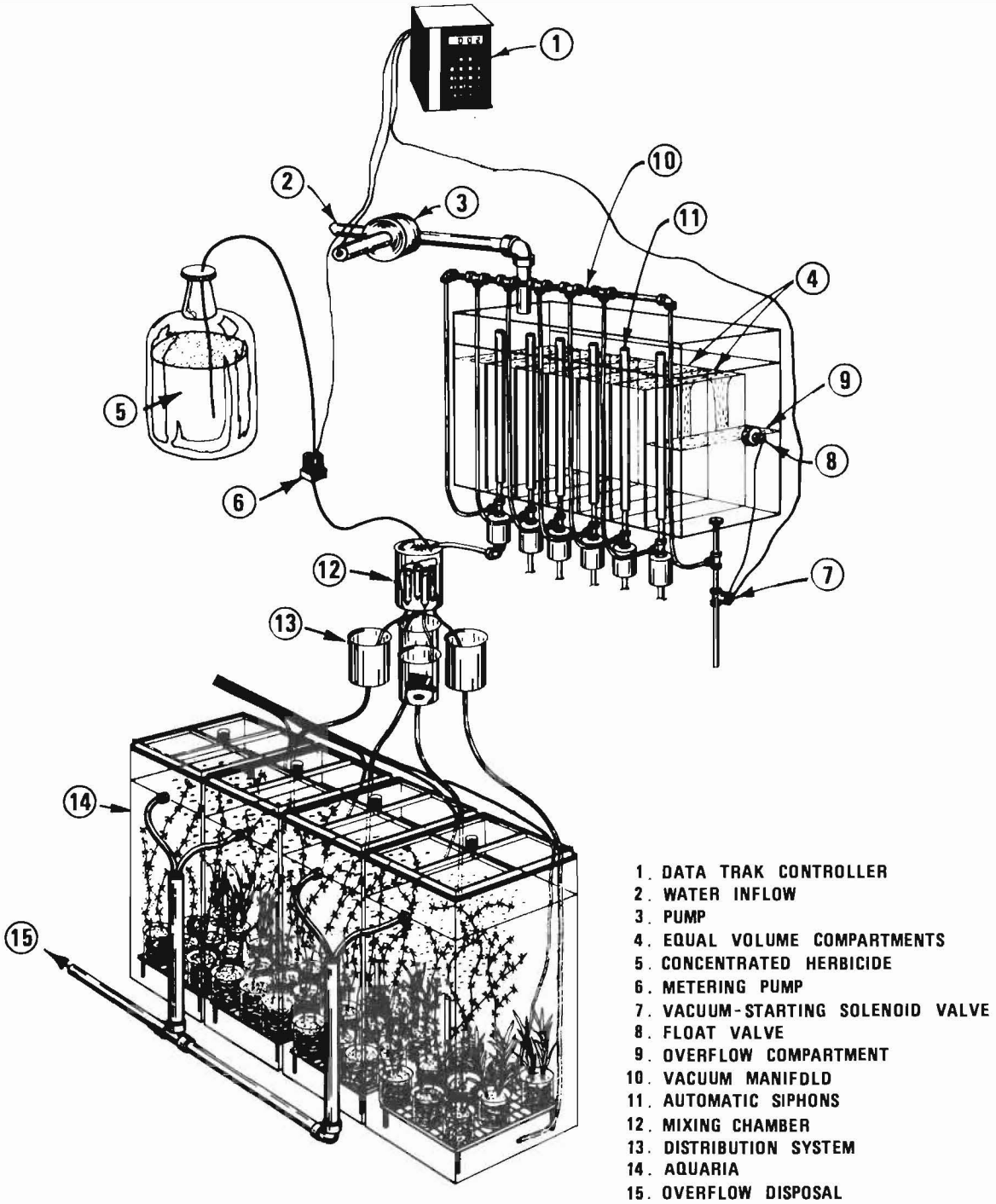

Figure 1. Schematic of the modified diluter system

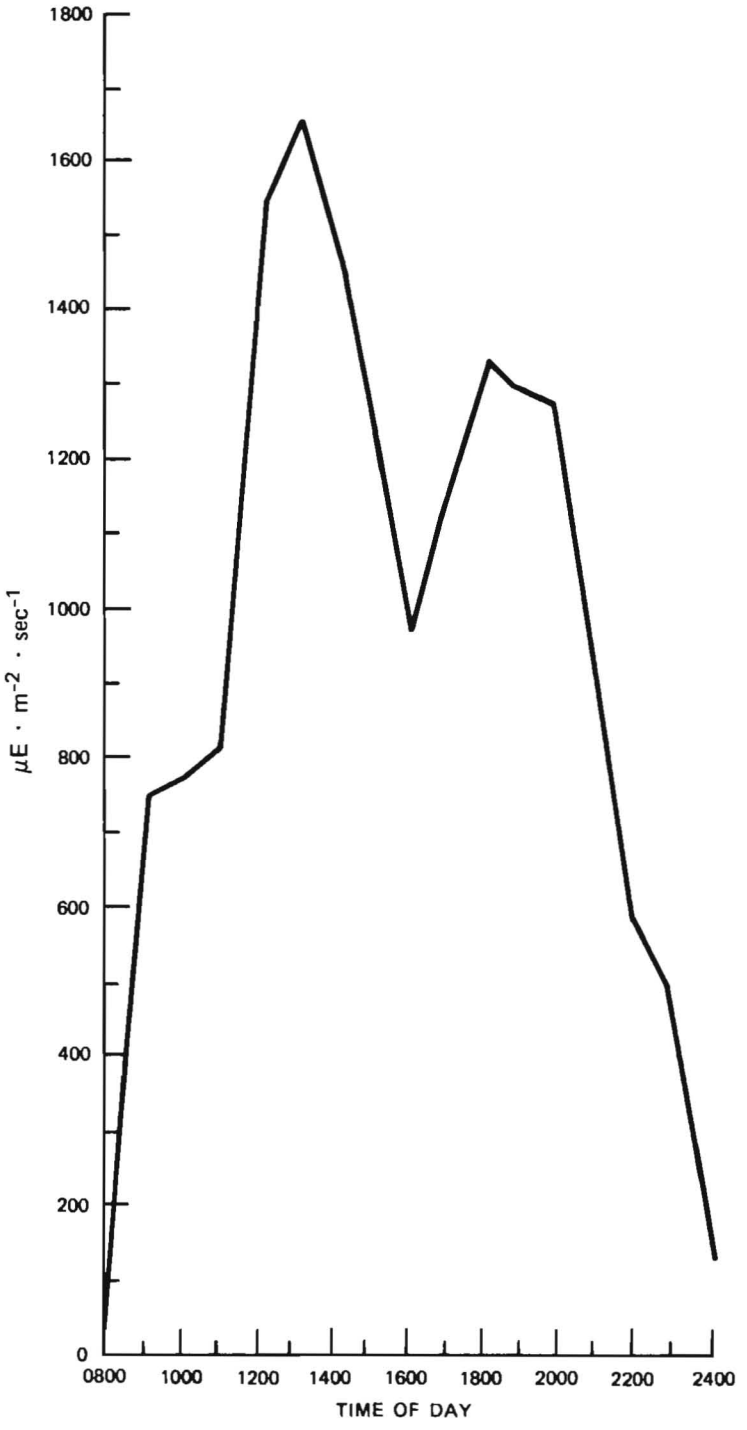

Figure 2. Photosynthetically active radiation received by test aquaria during July-August 1981 (aquaria were located in a greenhouse covered with 55 percent shade fabric)

aquarium was numbered and randomly assigned to one of two east-west oriented rows. The test aquaria were wrapped in black plastic so that only light from the overhead light bank was allowed to enter from the top of the aquaria. This prevented the development of an algal mat on the side walls of each aquarium and on the plants such as occurred in the pilot study.

7. The water used for operating the diluter system was uncontaminated tapwater originating from a deep well supply used by Vicksburg, Miss., that had passed through activated charcoal and a 0.45-μ cartridge filter. Water temperature was maintained at $25^{\circ} \pm 2^{\circ}C$ throughout the study.

8. Mature plants of *M. spicatum* were obtained from Lake Seminole near Chattahoochee, Fla. Germinated tubers of *P. pectinatus* were obtained from Wildlife Nurseries, Inc., in Oshkosh, Wis.

9. A stock culture of *M. spicatum* was maintained in reconstituted hard water (U. S. Environmental Protection Agency (EPA) 1975) using a standard hydrosoil containing by volume 70 percent washed sand and 10 percent Michigan peat as a planting medium. Approximately 4 weeks prior to testing, four 15-cm meristematic cuttings of *M. spicatum* were planted in each 250-ml glass beaker by burying the cut end of the plants approximately 5 cm in the hydrosoil. A finely sieved, washed sand was placed over the hydrosoil to an approximate depth of 2 cm to prevent peat fragments from floating into the overlying water. Four germinated tubers of *P. pectinatus* were planted in each beaker and covered with 3 cm of hydrosoil and 2 cm of fine sand. Five beakers each of *M. spicatum* and *P. pectinatus* were placed in the designated aquaria. During these 4 weeks only water flowed through the aquaria to permit root development and acclimation of the plants prior to exposure to 2,4-D.

Laboratory Analysis

10. Herbicide residue analyses were performed using approved, standard procedures (American Public Health Association (APHA) 1976)

by the Tennessee Valley Authority Laboratory Branch in Chattanooga, Tenn. Water samples for 2,4-D analysis were obtained at day 4, 14, 31, 45, 56, and 77 following initiation of the experiment. Inflow and outflow samples were collected from each aquarium. At the end of 11 weeks, the aquaria were dismantled and the remaining plants were removed from each beaker by washing the hydrosoil with deionized water. Shoots and roots of each *M. spicatum* plant within a beaker were separated and dried at 70°C for 36 hr, weighed, and recorded (accuracy: ±0.5 mg). Plants of *P. pectinatus* were not divided into shoots and roots because additional rhizomes developed within the same and adjacent beakers making it difficult to identify the original plants (Westerdahl et al. 1982).

Data Analysis

11. The effects of various 2,4-D concentrations on the growth of *M. spicatum* and *P. pectinatus* were determined using percent plant injury (0 = no control, 100 = total kill) and a set of qualitative factors currently in use at the Aquatic Plant Management Laboratory in Fort Lauderdale, Fla. (Hoeppel and Westerdahl 1981). The qualitative factors include heavy algal cover, roots evident, absence of meristems on stems and branches, leaf loss, evidence of solarization, stem flaccidity, degree of node or internode decomposition, stem and branch tip decomposition, general decomposition of plants, advanced decomposition (only a few stems remaining intact), complete disintegration of plant material, and subsequent regrowth. The mean shoot and root biomass, expressed as dry weight and representing the four replicate aquaria, were computed for each 2,4-D concentration and compared to the reference aquaria. The results assisted in determining the estimated threshold 2,4-D concentration when considered with the plant injury data. Height measurements of the tallest *M. spicatum* per beaker in each aquarium were recorded initially and at weekly intervals thereafter. The purpose was to measure growth effects on the plants resulting from constant exposure to various 2,4-D concentrations.

12. Data were statistically evaluated by analysis of variance as

a test of differences between means. Duncan's multiple-range test (Duncan 1955) was used to make comparisons among the shoot and root biomass means for each of the various 2,4-D concentrations. Dunnett's test (Dunnett 1955) was used to compare all experimental means with the reference means. Mean plant height data for each 2,4-D concentration were compared to the plant height means from the reference aquaria. Mean shoot and root biomass data were also compared to the biomass means from the reference aquaria.

PART III: RESULTS AND DISCUSSION

Diluter System Operation

13. The modified diluter system proved reliable and accurate in delivering water volumes with specific 2,4-D concentrations to each of the test aquaria. As the study progressed, more frequent calibration of the metering pumps was necessary to ensure proper dilution of the 2,4-D stock solution. Intermittent readjustment of the automatic siphons (Figure 1) was also necessary to ensure delivery of equal water volumes to each of the aquaria.

14. Over extended periods of operation, increased maintenance was required to control attached filamentous algal growth in tubing connecting the distribution cannisters with the test aquaria. Also, excessive fungal growth in the distribution cannisters receiving the 2,4-D required routine removal from the cannisters.

2,4-D Residues in Water

15. Periodic analysis of the inflow and outflow water of each aquarium for 2,4-D concentrations permitted evaluation of the 2,4-D passing through different aquaria. Figure 3 summarizes these data for each 2,4-D treatment rate. Discrepancies in inflow data were the result of difficulty in maintaining the proper calibration of the metering pumps at the lower sustained 2,4-D concentrations. Air leakage into the teflon tubing that connected the 2,4-D stock solution with the metering pump and the metering pump with the mixing cannisters was observed. This accounted for some of the variability seen in the inflow 2,4-D concentrations through day 15 (Figure 3).

16. Variability in outflow 2,4-D concentrations suggested that the macrophytes, algae, fungi, and organic debris probably act as sinks for the 2,4-D in the aquaria (Westerdahl et al. 1982). Therefore, collection of outflow samples would not provide accurate determinations of the 2,4-D concentration delivered to each aquarium.

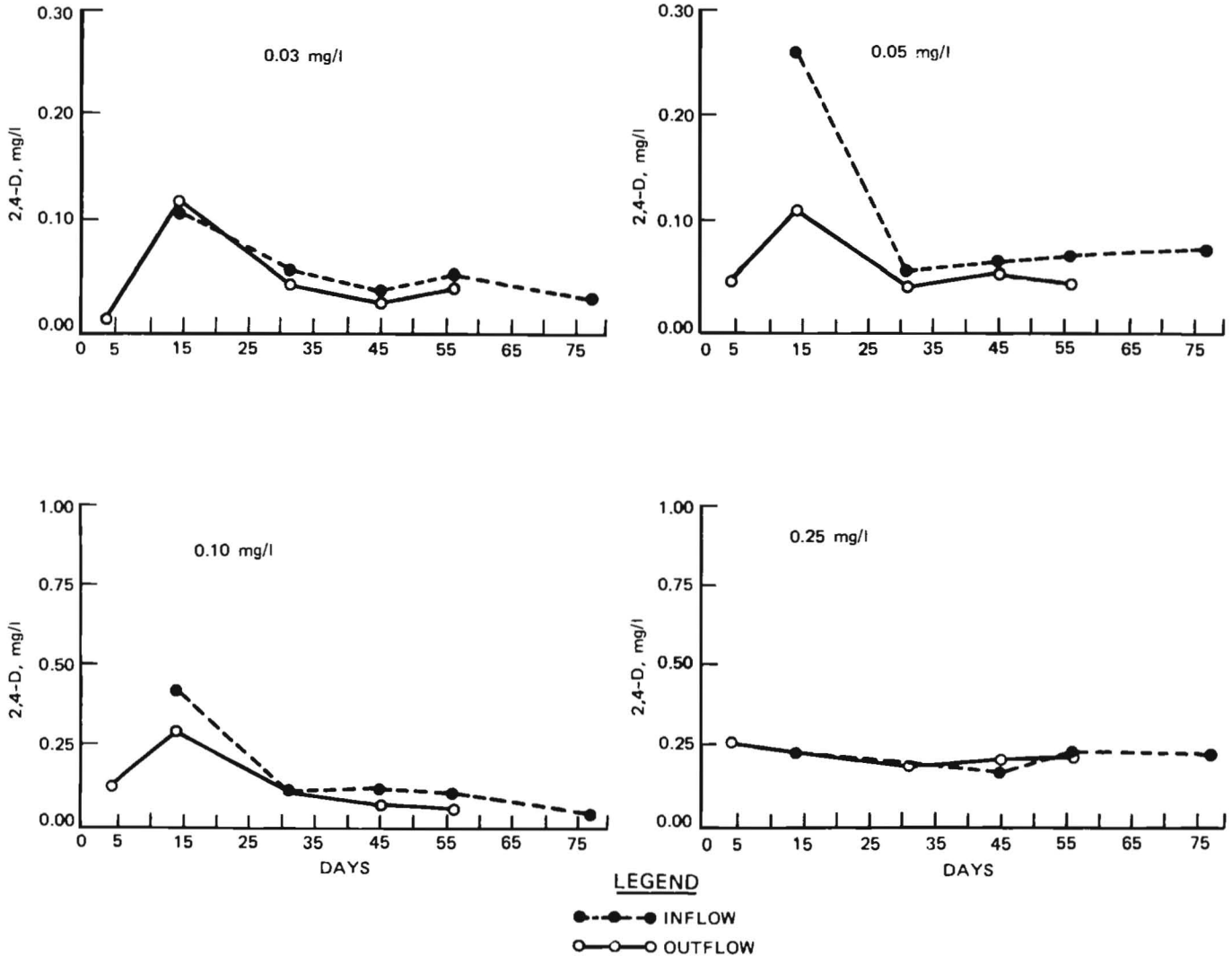

Figure 3. Mean 2,4-D residue concentrations in inflow and outflow water of aquaria treated continuously at 0.03, 0.05, 0.10, and 0.25 mg/ℓ

Growth Response of the Select Plants

17. Weekly measurements of the maximum *M. spicatum* shoot length per beaker in each aquarium provided an estimate of growth inhibition resulting from exposure to different 2,4-D concentrations (Table 1). When compared to the reference, inhibition of growth as evident by plant height was statistically significant only at the 2,4-D concentration of 0.10 mg/ℓ. This inhibition first became evident on day 35 of the experiment (Figure 4).

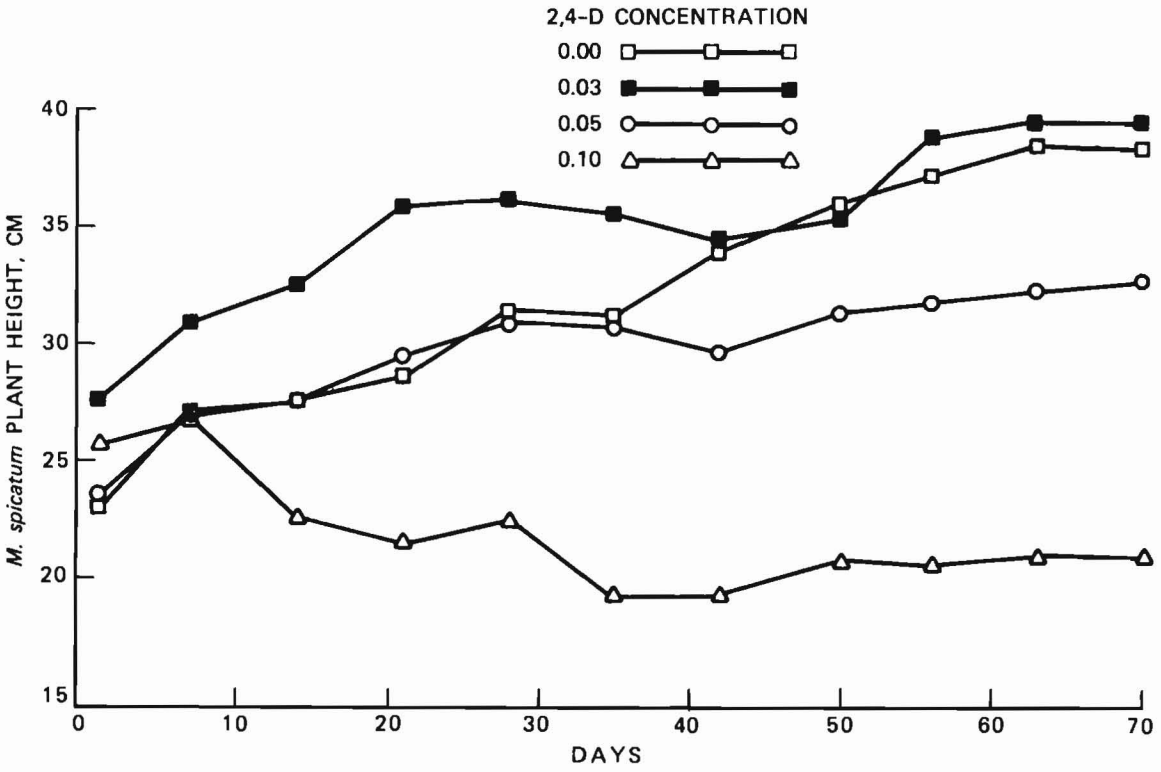

Figure 4. *Myriophyllum spicatum* L. shoot height response to three sustained 2,4-D concentrations over an 11-week study period

18. Table 2 illustrates the effects of various 2,4-D concentrations on shoot and root biomass of *M. spicatum* following an 11-week continuous exposure to 2,4-D. At a 2,4-D concentration of 0.03 mg/ℓ, there was a 28-percent reduction in shoot biomass and a 23-percent reduction in root biomass compared to the reference. At a 2,4-D concentration of

0.05 mg/ℓ, there was a 44- and a 54-percent reduction in shoot and root biomass, respectively. At a 2.4-D concentration of 0.10 mg/ℓ, there was a 71-percent reduction in shoot biomass and a 77-percent reduction in root biomass as compared to the reference. Shoot biomass values at all three treatment concentrations were statistically different from the shoot biomass value of the reference. The 2,4-D concentration of 0.10 mg/ℓ was the only treatment level having a root biomass value significantly different than that of the reference.

19. Several reviews (Robertson and Kirkwood 1970; Crafts and Crisp 1971) have concluded that foliar-applied phenoxy herbicides generally move with photosynthates in the phloem. The pattern observed was translocation from photosynthetically active leaves to meristematic tissue where the photosynthates are metabolized. Although phenoxy herbicides may be absorbed by roots (Scott and Norris 1970), certain factors, e.g. diffusion and reduced sediment conditions, impair 2,4-D movement and plant uptake (Donaldson, Bayer, and Leonard 1973). In this study, it was assumed that the primary absorption of 2,4-D was through the leaves since 2,4-D was added to the water and not the hydrosoil. Moreover, 2,4-D diffusion through the reduced hydrosoil would probably be slower than translocation. The plants of *M. spicatum* continuously exposed to 0.10 mg/ℓ of 2,4-D showed shoot and root biomass values significantly different from those of the reference. Translocation of 2,4-D from leaves to the roots apparently resulted in less root growth relative to the reference or possibly death of the root system. This view is supported by Ashton and Crafts (1981) who stated that extended exposure at relatively low dosage brings about growth responses in regions distant from the point of application.

20. Table 3 illustrates the effects of various 2,4-D concentrations on the total biomass of *P. pectinatus* following an 11-week continuous exposure to 2,4-D. At a 2,4-D concentration of 0.03 mg/ℓ, there was a 19-percent reduction in total biomass. At a 2,4-D concentration of 0.05 mg/ℓ, there was a reduction in total biomass of 36 percent. At a 2,4-D concentration of 0.10 mg/ℓ, there was a 51-percent reduction in total biomass. At a 2,4-D concentration of 0.25 mg/ℓ, there was a

60-percent reduction in total biomass compared to the reference. Total biomass was significantly different from the reference at all 2,4-D concentrations except for the 0.03-mg/ℓ treatment.

Assessment of Plant Injury

21. Regression analysis was used to compare the percent injury incurred by *M. spicatum* and *P. pectinatus* when exposed continuously to the selected 2,4-D concentrations (Figures 5 and 6). The best-fit regression equation ($y = bx$) was used to estimate the time required to produce 50-percent injury for each 2,4-D concentration. The minimum length of time required to produce 50-percent injury to *M. spicatum* was approximately 3.5 weeks with a continuous exposure to 0.03 to 0.05 mg/ℓ of 2,4-D and 1 week with 0.10 mg/ℓ of 2,4-D. For *P. pectinatus*, this time period was approximately 10 weeks with 0.05 mg/ℓ, 3 weeks with 0.10 mg/ℓ, and slightly less than 3 weeks with 0.25 mg/ℓ of 2,4-D. The 2,4-D threshold concentrations required to control *M. spicatum* and *P. pectinatus* appear to be between 0.05-0.10 mg/ℓ and 0.10-0.25 mg/ℓ, respectively. These results are similar to those observed in the pilot study (Westerdahl et al. 1982).

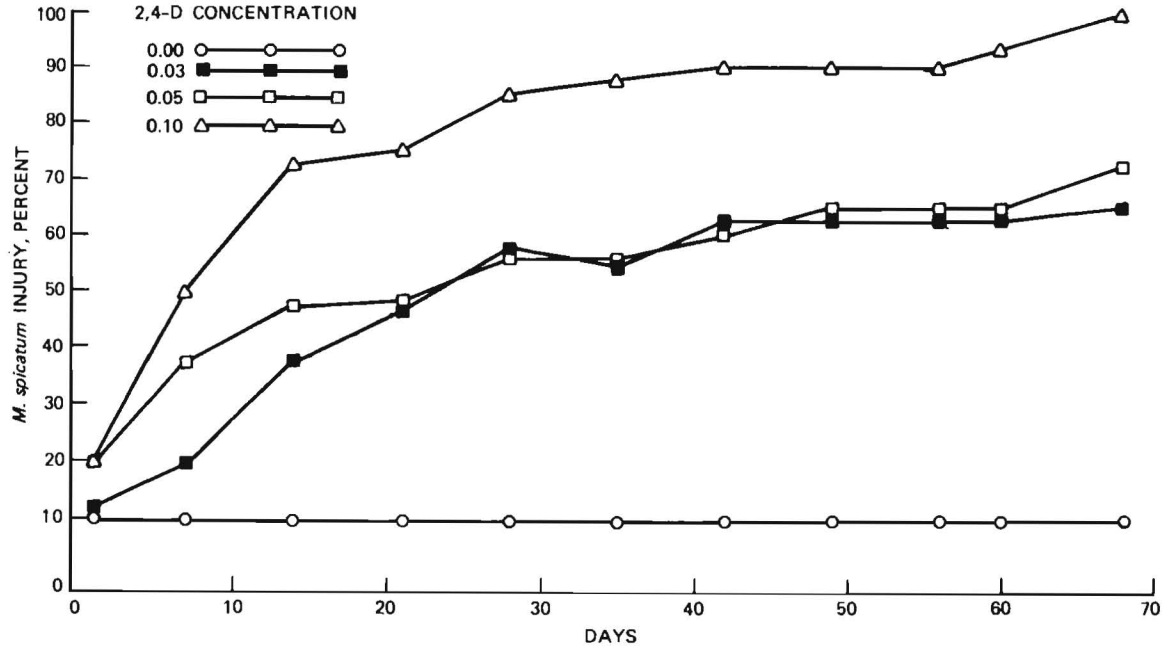

Figure 5. *Myriophyllum spicatum* L. response to three treatment concentrations of 2,4-D over an 11-week study period

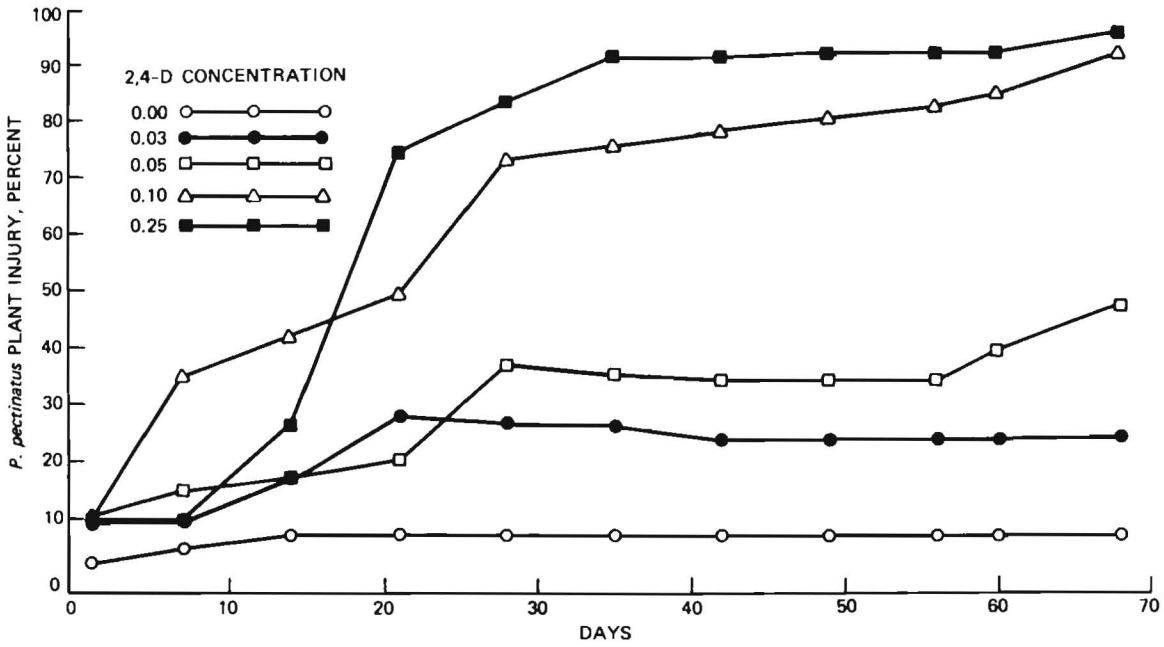

Figure 6. *Potamogeton pectinatus* L. response to four treatment concentrations of 2,4-D over an 11-week study period

PART IV: CONCLUSIONS AND RECOMMENDATIONS

Conclusions

22. The following conclusions can be drawn concerning the operation of the modified diluter system and estimates of the threshold 2,4-D concentrations required to control growth of *M. spicatum* and *P. pectinatus*:

a. The modified diluter system was reliable and accurate in dispersing preprogrammed water volumes and 2,4-D concentrations to each of the test aquaria.

b. The excessive algal growth observed in the aquaria during the pilot study was eliminated by wrapping the aquaria in black plastic.

c. The threshold 2,4-D concentrations required to control *M. spicatum* and *P. pectinatus* were determined to be 0.05-0.10 mg/ℓ and 0.10-0.25 mg/ℓ, respectively.

Recommendations

23. The following is a list of recommendations:

a. Tubing of the diluter system should be wrapped in aluminum foil to reduce algal growth during the course of the study.

b. All teflon tubing connected to the metering pumps should be replaced with Bev-a-Line-type tubing of an appropriate diameter to prevent air leakage into the system.

c. Periodic monitoring of 2,4-D residue in water should be confined to collection of inflow rather than inflow and outflow samples. Approximately 4000 ℓ of water pass through each aquaria over a 12-week study period. Since there is an inherent flow variability of ± 10 percent with this diluter system, herbicide analysis of discrete 1-ℓ samples taken infrequently would be less representative than composite 1-ℓ samples taken over a defined period of time throughout the study.

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Table 1

Mean Maximum Shoot Height (centimetres) of *M. spicatum*

Exposed to Various 2,4-D Concentrations

2,4-D Concentrations mg/ℓ	Day										
	1	7	14	21	28	35	42	50	56	63	70
Reference	22.0 ±1.5*	27.1 ±1.0	27.5 ±1.1	28.6 ±0.9	32.7 ±1.3	31.2 ±1.6	33.9 ±1.7	35.9 ±1.7	37.2 ±2.0	38.7 ±2.3	38.3 ±2.2
0.03	27.7 ±1.9	30.9 ±2.0	31.1 ±2.7	35.9 ±2.2	36.2 ±2.5	35.6 ±2.3	34.5 ±2.2	35.4 ±2.4	38.8 ±2.2	39.5 ±2.8	39.5 ±2.8
0.05	23.5 ±1.8	26.9 ±2.1	27.6 ±2.3	29.4 ±2.6	30.9 ±2.5	30.6 ±2.7	29.6 ±2.8	31.3 ±2.7	31.7 ±2.8	32.2 ±3.0	31.6 ±3.3
0.10	22.8 ±2.4	26.7 ±1.6	22.6 ±1.2	21.5 ±1.7	22.5 ±1.9	19.1 ±1.8	20.7** ±2.0	20.7** ±2.0	20.5** ±2.1	20.6** ±2.3	20.2** ±2.2

* ± Standard error of mean, n = 20.

** Significant at 0.05 level.

Table 2

Root and Shoot Biomass of *M. spicatum* Following 11-Week Continuous Exposure to Three 2,4-D Concentrations

2,4-D Acid mg/ℓ	Biomass, g*	
	Shoots	Roots
Reference	1.04 ± 0.07**	1.17 ± 0.13
0.03	0.75 ± 0.05†	0.90 ± 0.19
0.05	0.58 ± 0.06†	0.54 ± 0.06
0.10	0.30 ± 0.06†	0.27 ± 0.05†

* Expressed as dry weight.
** Mean ± standard error, n = 20.
† Significant at 0.05 level.

Table 3

Total Biomass of *P. pectinatus* Following 11-Week Continuous Exposure to Four 2,4-D Concentrations

2,4-D Acid mg/ℓ	Total Biomass, g*
Reference	0.86 ± 0.02**
0.03	0.70 ± 0.01
0.05	0.55 ± 0.02†
0.10	0.42 ± 0.009†
0.25	0.34 ± 0.008†

* Expressed as dry weight.
** Mean ± standard error, n = 20.
† Significant at 0.05 level.

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