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Endothall Concentration and Exposure Time Relationships for the Control of Eurasian Watermilfoil and Hydrilla[1]

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ABSTRACT

Herbicide concentration and exposure time relationships were determined for endothall (the dipotassium salt of 7-oxabicyclo [2,2,1] heptane-2,3- dicarboxylic acid) and control of Eurasian watermilfoil (*Myriopyllum spicatum* L.) and dioecious hydrilla (*Hydrilla verticillata* L. F. Royle) under controlled-environment conditions. Sixteen endothall concentration and exposure time combinations were tested for Eurasian watermilfoil: concentrations ranged from 0.5 to 5.0 mg acid equivalent (ae)/l; exposure times ranged from 2 to 72 hr. Twenty-seven endothall concentration and exposure time combinations were tested for hydrilla: concentrations ranged from 1.0 to 5.0 mg ae/l; exposure times ranged from 6 to 72 hr. Plant control was based on shoot and root biomass harvested at the end of the experiments. Weekly visual injury ratings were used to characterize efficacy during the course of the experiments. Plant control increased (biomass decreased), as either concentration or exposure time increased. A threshold level was reached in which a concentration/exposure time combination provided satisfactory control. Severe Eurasian watermilfoil injury (> 85% biomass reduction) occurred when exposed to 0.5 mg ae/l for 48 hr, 1.0 mg ae/l for 36 hr, 3.0 mg ae/l for 18 hr, and 5.0 mg ae/l for 12 hr. Severe hydrilla injury (> 85% biomass reduction) occurred when exposed to 2.0 mg ae/l for 48 hr, and 3.0, 4.0 and 5.0 mg ae/l for 24 hr. The 1.0 mg ae/l treatment failed to produce severe hydrilla injury at the maximum exposure time tested of 72 hr. Increased control of Eurasian watermilfoil and hydrilla is likely for treatments in systems where plants remain in contact with endothall concentrations and exposure times greater than the developed threshold levels.

Key words: Herbicide, chemical control, endothall, biomass, exposure time, Eurasian watermilfoil, hydrilla.

INTRODUCTION

Chemical treatment of submersed plants often is necessary in flowing-water systems, and in unprotected areas of lakes and reservoirs. The dissipation of herbicides in these plant-infested systems can be influenced by flow-generated, thermal and wind-induced water circulation patterns (Fox *et al.* 1990; Getsinger *et al.* 1990). Herbicide dissipation can reduce exposure time in the target area resulting in insufficient plant control. Since the efficacy of a submersed herbicide application is related to the length of time a target species is exposed to dissipating concentrations of herbicide, the determination of herbicide concentration/exposure time relationships should improve the ability to predict plant control in hydrodynamic systems. The unique properties of a herbicide with respect to the target plant (e.g. mode of action, rate of application, environmental half-life, plant uptake rate, plant biomass and growth stage, and plant susceptibility) require that concentration/exposure time relationships be developed for each major weed species. These relationships have been partially developed for some aquatic herbicides and target weeds (Hall and Westerdahl 1984; Van and Conant 1988; Green and Westerdahl 1990).

The herbicide endothall is widely used for large-scale and spot treatments of hydrilla and Eurasian watermilfoil in hydrodynamic systems. Endothall is described as a contact-type, membrane-active herbicide (Ashton and Crafts 1981), which implies an initial rapid uptake by the plant. However, several studies have shown slow initial uptake rates of endothall by submersed weeds (Haller and Sutton 1973; Reinert and Rogers 1986; Van and Conant 1988). Haller and Sutton suggested that this slow initial uptake rate may present problems in controlling these plants in flowing water. Furthermore, applications of endothall have resulted in variable plant control in situations where herbicide concentration dissipated to low levels approximately 24 hr after treatment (Price 1969; Westerdahl 1983; Killgore 1984; Reinert and Rodgers 1986). This variability in efficacy illustrates the need for establishing functional relationships between endothall concentration, exposure time, and plant control. The determination of these relationships will aid in the development of improved formulations and application techniques for situations where reduced contact time presents a problem.

The objectives of this study were to examine, under laboratory conditions, the relationship between endothall concentrations and exposure times for controlling Eurasian watermilfoil (hereafter called milfoil) and dioecious hydrilla. These relationships can be used to provide guidance for the application of endothall in hydrodynamic systems.

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MATERIALS AND METHODS

Experiments were conducted in two separate, but similar, laboratory systems. The endothall/milfoil experiments were conducted in a system developed at the US Army Engineer Waterways Experiment Station (WES), Vicksburg, MS, to study 2,4-D/milfoil concentration and exposure time relationships (Green and Westerdahl 1990). This system consisted of 24, 55 l (15 gal) aquaria (0.90 m tall x 0.09 m^2:) located in a controlled-environment room. Overhead lighting was provided by a combination of 400 watt mercury vapor lamps and 250 watt high-pressure sodium lamps. Mean photosynthetically active radiation received by the aquaria at the water surface was 490 ± 72 uE/m^2/sec, with a photoperiod of 13L:11D. Water temperature was maintained at 21 ± 2 C throughout the experiment. Milfoil apical tips used as planting stock in this study were collected from the Suwannee River, FL.

The endothall/hydrilla experiments were conducted in a similar system consisting of 48, 55 l aquaria located in a controlled-environment chamber. Overhead lighting was provided by lamps as described above. Mean PAR measured at the water surface was 580 ± 58 μE/m^2/sec, with a photoperiod of 14L:10D. Water temperature was maintained at 25 ± 2 C. Hydrilla apical tips used for planting stock in this study were collected from Lake Seminole, GA, and the Suwannee River, FL.

Sediment for both systems was obtained from Brown's Lake at the WES and enriched with macro- and micro-nutrients (Ra·pid·gro with Forti·5^{tm}, Ra·pid·gro Corp.) to eliminate possible nutrient deficiencies or limitations during the course of the studies. Containers (300 ml polyvinyl chloride (PVC) beakers for the milfoil studies and 300 ml glass pyrex for the hydrilla studies) were filled with sediment and four, 15-cm apical stem sections of the selected target species were planted (5 cm deep) in each beaker. A thin layer of silica sand was placed on top of the sediment to prevent resuspension of sediment during water exchange periods. Eleven beakers containing apical tips of the target species were placed in each aquarium. Each aquarium was independently supplied with a continuous flow of simulated hard water solution (Smart and Barko 1984), except when herbicide exposures were being conducted. Peristaltic pumps (Cole-Parmer Model No. 7568-00) were calibrated to exchange the water volume (50 l) of each aquarium every 24 hours. Air was bubbled through each aquarium to provide a source of carbon dioxide and to circulate the water. Each aquarium was outfitted with separate PVC drain and fill lines to expedite the removal and refill of water during the herbicide flushing procedure.

Plants were allowed to grow 2.5 to 3 weeks prior to herbicide treatment. This pretreatment growth period ensured the development of a healthy, viable root mass. One randomly selected beaker of plant material was removed from each aquarium (10 beakers per aquarium remained) prior to chemical treatment. This harvested material was separated into roots and shoots, and dried to a constant weight. An overall average weight (± 1 SD) was obtained and this weight was multiplied by 10 to estimate biomass of the remaining 10 beakers of plants within each aquarium. Estimated dry weight (DW) of shoot mass treated in the milfoil test runs was 13.1 ± 1.9 g. The dry weight estimate of milfoil root mass was 2.4 ± 0.26 g. Estimated hydrilla shoot mass was 11.1 ± 1.5 g DW. Hydrilla root mass was estimated at 2.2 ± 0.34 g DW. This would represent equivalent field biomass levels of approximately 145 g DW/m^2 for milfoil shoots and 26 g DW/m^2 for milfoil roots and 123 g DW/m^2 for hydrilla shoots and 24 g DW/m^2 for hydrilla roots. Grace and Wetzel (1978) reported that seasonal maximal biomass of milfoil measured in various field locations ranged from 32 to 360 g DW/m^2, while Harlan *et al.* (1985) reported that peak biomass for hydrilla in eight southeastern waterbodies ranged from 52 to 890 g DW/m^2. Pretreatment shoot biomass in our studies most closely approximates spring to early summer biomass reported for milfoil and hydrilla shoots (Bowes *et al.* 1979; Perkins *et al.* 1980; Painter 1988).

The endothall/milfoil study consisted of 16 concentration/exposure time treatments (including untreated references), conducted in three independent runs (Table 1). Each treatment was replicated three times and randomly assigned to a test aquarium. All treatment concentrations are reported as the acid equivalent of the endothall formulation. Treatments of 1.0 mg/l for 12 and 24 hr, 3.0 mg/l for 12 and 24 hr, and 5.0 mg/l for 12 hr were run twice to statistically compare results between different runs. Reference treatments were statistically compared among the three independent runs.

The endothall/hydrilla study consisted of 27 concentration/exposure time treatments (including untreated references) conducted in two indpendent test runs (Table 1). Each treatment was replicated three times and randomly assigned to a test aquarium. All treatment concentrations are reported as the acid equivalent of the endothall formulation. The 1.0 mg/l for 36 hr, 3.0 mg/l for 24 hr, 5.0 mg/l for 12 hr, and the reference treatment were used in both test runs for statistical comparison.

Endothall stock solutions used for treatment were prepared from the commercial formulation Aquathol K^{tm} (Atochem North America, Inc.). At the time of treatment, the flow-through water system was deactivated. Calculated volumes of the endothall stock solution were added to the aquaria to provide the desired treatment concentrations. At the end of the assigned exposure time, each aquarium was emptied and refilled with fresh water 3 times to remove endothall residues. After rinsing, the flow-through water system was activated and continued to operate until termination of the experiment. Water samples were collected and analyzed for endothall residues within 5 min after application to verify treatment concentrations, just prior to rinsing of aquaria to determine loss of herbicide (via chemical and boilogical processes), after rinsing over a range of 5 min to 8 hr to verify residue removal. Residue samples were analyzed by A&L Mid West Laboratories, Inc., Omaha, NE, and Columbia Laboratories, Inc., Corbett, OR. Results of residue analyses indicated that endothall loss during the exposure period was negligible for all exposure times in all milfoil and hydrilla test runs. All samples taken after the rinsing procedure displayed residue levels below the detection limit (10 μg/l).

TABLE 1. ENDOTHALL CONCENTRATIONS AND EXPOSURE TIMES AGAINST EURASIAN WATERMILFOIL AND HYDRILLA.

Eurasian watermilfoil			Hydrilla		
Concentration (mg/l)	Exposure Time (hr)	Run	Concentration (mg/l)	Exposure Time (hr)	Run
Ref	0	1,2,3	Ref	0	1,2
0.5	36	3	1.0	6	1
0.5	48	3	1.0	12	1
0.5	72	3	1.0	24	1
			1.0	36	1,2
1.0	2	1	1.0	48	1
1.0	12	1,2	1.0	60	2
1.0	18	1	1.0	72	2
1.0	24	2,3			
1.0	36	3	2.0	18	2
			2.0	48	2
3.0	2	1	2.0	48	2
3.0	12	1,2			
3.0	18	2	3.0	6	1
3.0	24	2,3	3.0	12	1
			3.0	18	2
5.0	2	1	3.0	24	1,2
5.0	12	1,2	3.0	30	2
5.0	18	2	3.0	36	1
			3.0	48	1
			4.0	12	2
			4.0	18	2
			4.0	24	2
			4.0	30	2
			5.0	6	1
			5.0	12	1,2
			5.0	24	1
			5.0	36	1
			5.0	48	1

Milfoil was allowed to respond to the herbicide application for a posttreatment period of four weeks. Based on previous studies (Green and Westerdahl 1990), 28 days posttreatment provided ample time for initial knockdown of standing mass and for plants to recover from herbicide injury. The monitoring period for hydrilla regrowth was extended to 42 days posttreatment, due to problems in determining the degree of injury and eventual fate of many of the treated plants. During the posttreatment periods, weekly visual evaluations of hydrilla and milfoil injury were conducted. At the conclusion of the posttreatment period, a final visual evaluation was made and plants from each aquarium were harvested, separated into viable roots and foliage, and oven-dried (70 C for 48 hr) to a constant weight.

For our purposes, control is defined as the percent reduction in shoot and root biomass of treated plants versus untreated references. Likewise, the term injury refers to the visual assessment of shoot damage of treated plants versus untreated references. Visual evaluations were used to characterize initial plant response to herbicide treatment, weekly progression of injury symptoms, and initiation of healthy regrowth of the target plants.

Biomass data were statistically evaluated by analysis of variance (ANOVA). Duncan's Multiple Range Test at the 95% confidence level was used to separate shoot and root biomass means within a treatment run for each of the concentration/exposure times (including reference treatments) tested. A t-test performed on dry weight data at concentration/exposure time combinations (including reference treatments) that were repeated, revealed that no significant difference ($p < .05$) existed between similar treatments in independent experimental runs.

RESULTS AND DISCUSSION

Milfoil Efficacy. Milfoil control increased with increasing endothall concentrations and/or exposure times. All treatments resulted in a significant reduction of shoot and root biomass levels compared to the untreated reference aquaria at 4 weeks posttreatment, except 1 mg/l for 2 hr (Figures 1 and 2). Visual estimates suggested that the initial knockdown or injury of milfoil at 1 week posttreatment was considered good to excellent for most treatments. The only exceptions occurred in treatments of 1.0 mg/l for 2 and 12 hr, and 3.0 and 5.0 mg/l at 2 hr exposure time in which shoot biomass was reduced 9 to 45% compared to reference aquaria (Figure 1). These treatments were considered ineffective, as shoots rapidly recovered and began producing new, healthy biomass at 1 week posttreatment.

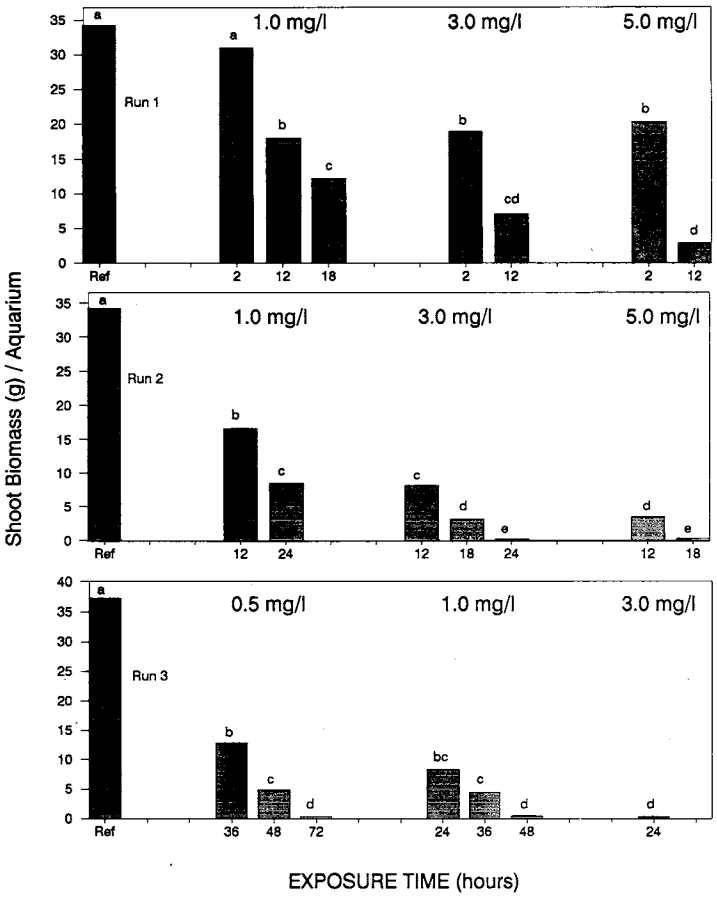

Figure 1. Eurasian watermilfoil shoot biomass harvested at 28 days posttreatment. Within a treatment run, different letters among concentration/exposure time combinations indicate significant differences at the 5% level according to Duncan's Multiple Range Test.

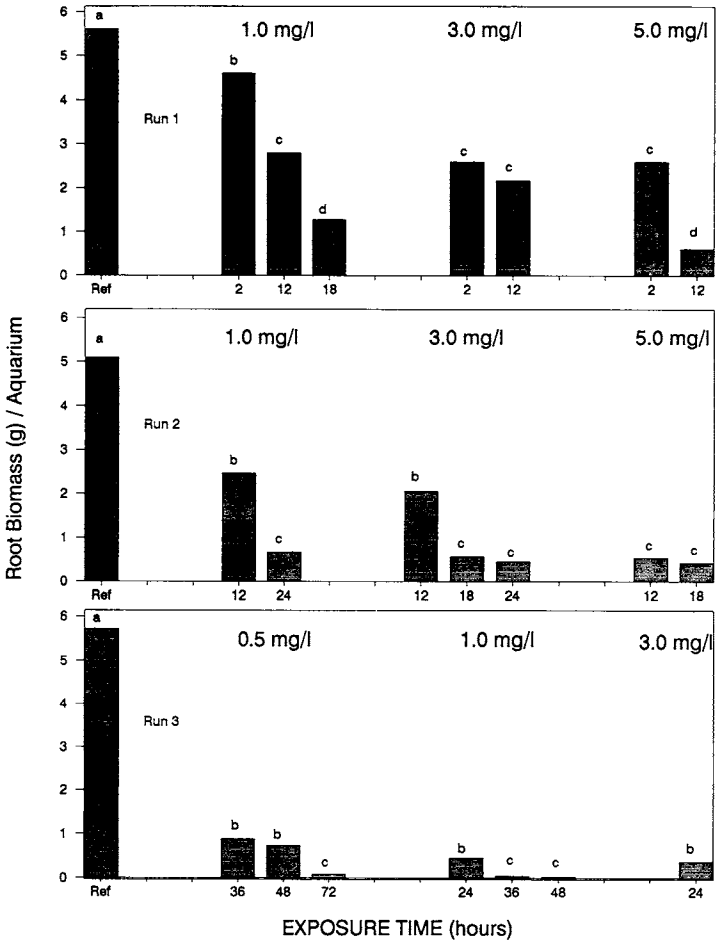

Figure 2. Eurasian watermilfoil root biomass harvested at 28 days posttreatment. Within a treatment run, different letters among concentration/exposure time combinations indicate significant differences at the 5% level according to Duncan's Multiple Range Test.

Treatments of 0.5 mg/l for 36 hr, 1.0 mg/l for 18 and 24 hr, and 3.0 mg/l for 12 hr resulted in an excellent knockdown of the existing biomass. Although biomass was reduced 60 to 77% in these treatments (Figure 1), production of a large number of viable shoots (15 to 25 per aquarium) from root crowns suggested the potential for complete regrowth. This regrowth began to appear at 2 weeks posttreatment and shoots began reaching the water surface by the third week posttreatment.

Treatments of 5.0 mg/l for 12 hr, 3.0 mg/l for 18 hr, 1.0 mg/l for 36 hr, and 0.5 mg/l for 48 hr resulted in complete initial knockdown of milfoil and production of very few viable shoots (5 to 6 per aquarium), which reached heights of 1 to 3 cm a few days prior to harvest. Harvested shoot biomass was reduced 92 to 95% compared to reference aquaria (Figure 1).

Treatments of 5.0 mg/l for 18 hr, 3.0 mg/l for 24 hr, 1.0 mg/l for 48 hr and 0.5 mg/l for 72 hr, also resulted in complete milfoil knockdown and a shoot biomass reduction of > 98% compared to references (Figure 1). Some replicates produced one or two short, rooted shoots (0.5 to 1 cm); however, many replicates failed to produce any living tissue. Most milfoil regrowth came from previously-formed, small rootcrowns.

The amount of root biomass harvested following treatment was related to shoot recovery, i.e. treatments which resulted in rapid shoot recovery had the highest levels of root biomass (Figure 2). At higher concentrations and exposure times the destruction of milfoil root systems was nearly complete. Root destruction was a good indicator of the potential for damaged plants to recover. Root destruction was probably due to the rapid loss of photosynthetic tissues, which prevented transport of photosynthate to the root system. Translocation of endothall is also known to occur in some aquatic plants (Thomas and Seaman 1968) and should not be ruled out as a possible source of root injury.

The ability of endothall to initially damage milfoil shoots at most concentrations and exposure times tested was evident from this study. Increased milfoil injury was directly proportional to the length of time plants were in contact with a given concentration of endothall. Results indicate that endothall at concentrations of 0.5, 1.0, 3.0 and 5.0 mg/l should be maintained for at last 48, 36, 18 and 12 hr respectively, to achieve > 85% reduction of milfoil biomass.

Hydrilla Efficacy. Hydrilla injury also increased with increasing endothall concentrations and/or exposure times. As expected, hydrilla required higher concentrations and longer exposure times than did milfoil to achieve acceptable levels of control. Treatments of 1.0 mg/l for 6 and 12 hr, and 3.0 mg/l for 6 hr produced minimal visual evidence of injury, and hydrilla shoot growth and vigor appeared equal to reference aquaria throughout the experiment. Shoot biomass in these treatments was not significantly different from reference aquaria (Figure 3). Root mass was reduced only 10 to 25% by these treatments (Figure 4).

Treatments of 2.0, 3.0 and 4.0 mg/l for 12 hr, 2.0, 3.0 and 4.0 mg/l for 18 hr, 1.0 and 2.0 mg/l for 24 hr, and 5.0 mg/l for 6 hr reduced shoot biomass 27 to 52% (Figure 3). Initial shoot injury symptoms were pronounced in these treatments, but recovery from both injured shoots and rootcrowns began within one week of treatment. New growth from rootcrowns began reaching the water surface by the third week posttreatment. Root biomass reductions varied from 21 to 72% of reference treatments (Figure 4). Although root biomass was greatly reduced by some of these treatments (especially at concentrations of 4.0 and 5.0 mg/l), the presence of apparently healthy regrowth suggested that complete recovery could occur.

Treatments of 1.0 mg/l for 36, 48, 60 and 72 hr, and 5.0 mg/l for 12 hr all significantly reduced hydrilla shoot mass (Figure 3). Existing shoot mass was severely injured and little regrowth from lateral buds was observed. Most regrowth came from injured rootcrowns which had recovered by the third week posttreatment, and had produced 15 to 25 healthy shoots per aquarium. Root biomass reductions (56 to 81%) paralleled reductions in shoot mass in these treatments. Although shoot and root biomass were significantly reduced, healthy regrowth signaled a potential for quick recovery.

Treatments of 2.0 mg/l for 48 hr, 3.0 mg/l for 24, 30, 36 and 48 hr, 4.0 mg/l for 24 and 30 hr, and 5.0 mg/l for 24, 36 and 48 hr all severely injured shoot and rootcrown

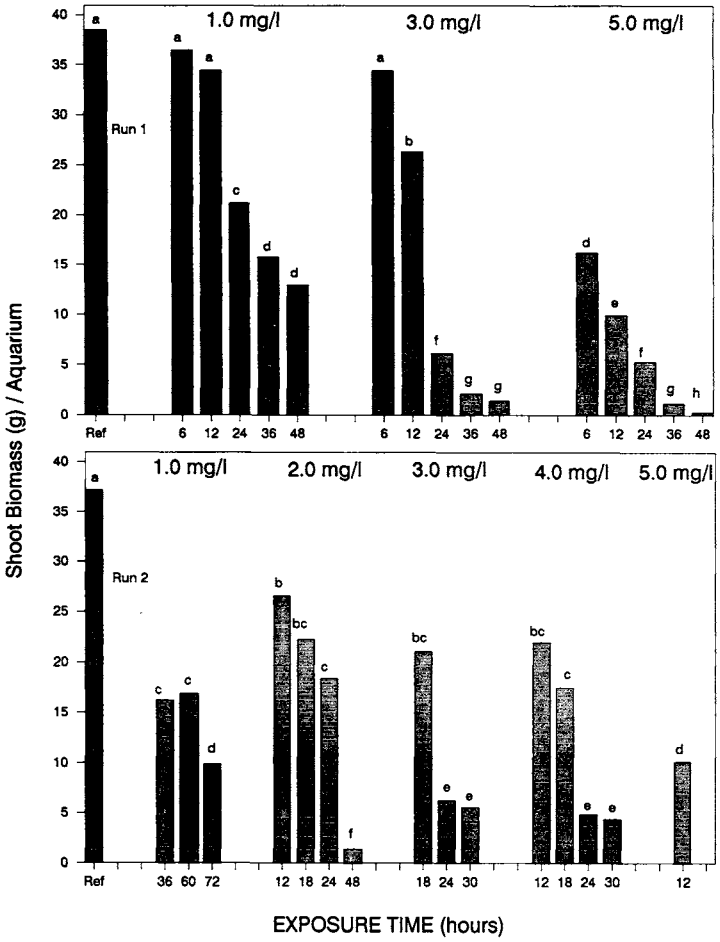

Figure 3. Hydrilla shoot biomass harvested at 42 days posttreatment. Within a treatment run, different letters among concentration/exposure time combinations indicate significant differences at the 5% level according to Duncan's Multiple Range Test.

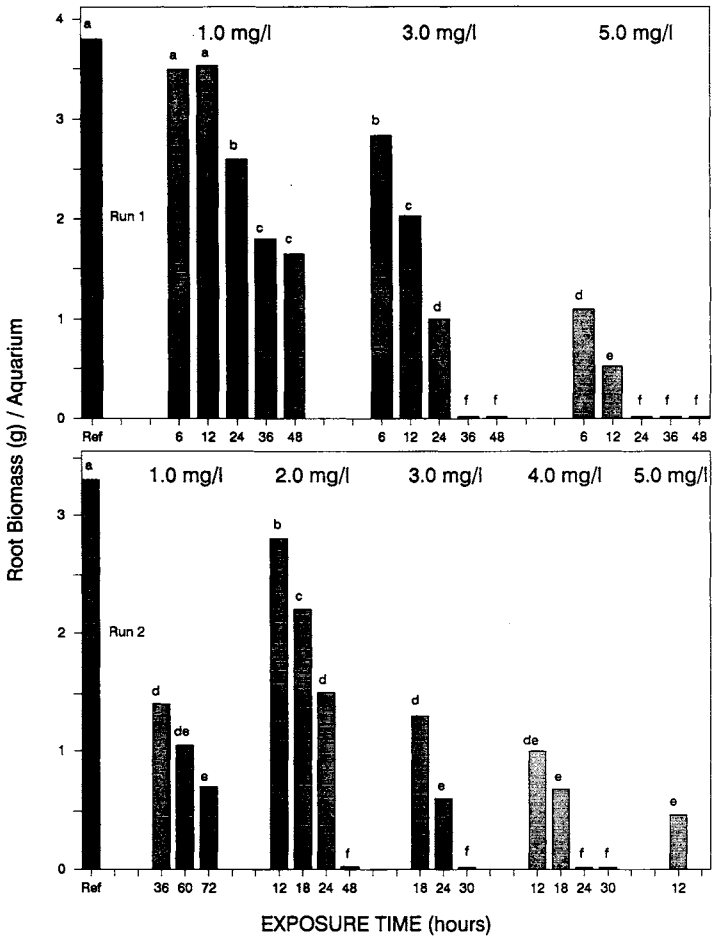

Figure 4. Hydrilla root biomass harvested at 42 days posttreatment. Within a treatment run, different letters among concentration/exposure time combinations indicate significant differences at the 5% level according to Duncan's Multiple Range Test.

tissue, and reduced shoot biomass 88 to 98% (Figure 3). These treatments were also characterized by a lack of regrowth from rootcrowns and destruction of existing root tissue (Figure 4.).

Hydrilla exposed to high concentrations (3.0, 4.0 and 5.0 mg/l) and long contact times (>24 hr) was severely damaged and stems often became detached from the root system. Some of this tissue did not readily decompose, therefore its viability remained questionable. During harvest, all potentially viable tissue was included for the determination of final biomass. In most cases, the presence of healthy roots attached to vigorously growing shoots was evidence of the potential for rapid regrowth. The ability to destroy the hydrilla rootcrowns and root system seemed to be the key to preventing successful regrowth after treatment.

Our results compare favorably with the work of Van and Conant (1988). For example, they showed that at 1.0 mg/l, a 72 hr contact time of endothall was required to achieve 80% hydrilla control, while in our study this contact time resulted in a 76% reduction of hydrilla biomass. Results from the high concentration/short exposure times were also similar, as approximately 80% hydrilla control was obtained at 5 mg/l for 12 hr in both studies. Results from this study indicate that endothall at 2.0 mg/l for 48 hr, and 3.0, 4.0, and 5.0 mg/l for 24 hr will be required to achieve >85% reduction of hydrilla.

Field Applications Of Laboratory Results. One of the objectives in determining the effects of herbicide concentration and exposure time on submersed plants is to provide guidance for improved use of herbicides in the field. Using results from this study, relationships have been developed to help predict the efficacy of endothall under varying concentrations and exposure times (Figures 5 and 6). Based on these relationships, increasing levels of plant control would be expected as herbicide concentrations and exposure times increase. As presented in Figures 5 and 6, endothall dissipation curves that fall within Zone A would provide <70% plant control; within Zone B, from 70 to 85% control; and within Zone C, from 85 to 100% control.

It should be noted that exposure times in the field will differ from the static exposures conducted in the laboratory; that is, plants in the field will be exposed to a dissipating concentration of herbicide over time. This dissipation would shorten herbicide contact times and could adversely affect the level of plant control desired. Treatments in large, open-water or flowing systems have resulted in nondetectable or very low levels of endothall in the water

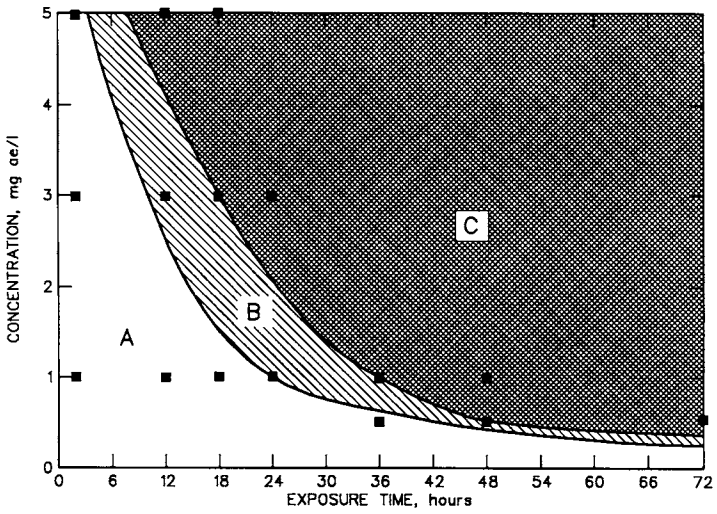

Figure 5. Endothall concentration and exposure time relationships for control of Eurasian watermilfoil. Solid squares represent actual endothall concentration/exposure time (CET) test coordinates. Zones A, B, and C were estimated using these test coordinates. Zone A represents CET combinations that should provide < 70% milfoil control along with a high probability of rapid regrowth within 1 week posttreatment; Zone B represents CET combinations that should provide between 70 and 85% milfoil control with regrowth beginning approximately 3 to 4 wks posttreatment; and Zone C represents CET combinations that should provide 85 to 100% milfoil control with very limited regrowth up to 4 weeks posttreatment.

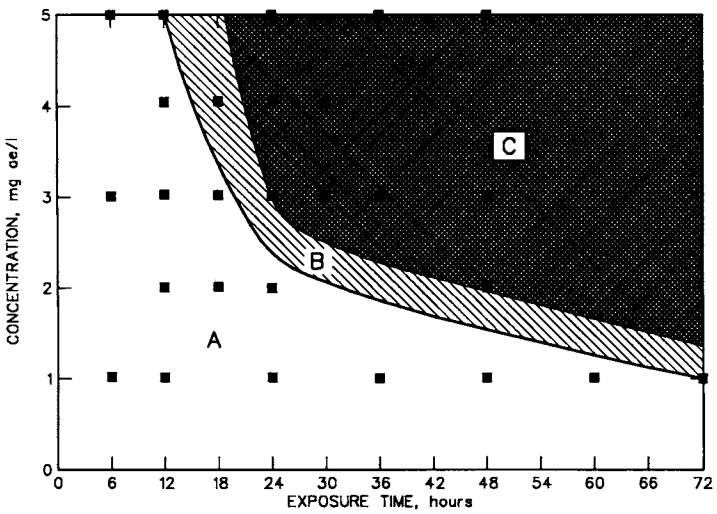

Figure 6. Endothall concentration and exposure time relationships for control of hydrilla. Solid squares represent actual endothall concentration/exposure (CET) time test coordinates. Zones A, B, and C were estimated using these test coordinates. Zone A represents these CET combinations that should provide < 70% hydrilla control and a high possibility of rapid regrowth within 1 to 2 weeks; Zone B represents CET combinations that should provide between 70 and 85% hydrilla control with regrowth beginning at 4 to 6 weeks posttreatment; and Zone C represents CET combinations that should provide 85 to 100% hydrilla control with very limited or no regrowth up to 6 weeks posttreatment.

within 24 hr post-treatment, as well as inconsistent efficacy (Westerdahl 1983; Killgore 1984; Reinert and Rodgers 1986). The rate of endothall dissipation can be affected by many variables including water- exchange characteristics, thermal stratification which can prevent herbicide mixing, dispersion, plant uptake, adsorption to suspended particulates, and microbial degradation. In addition, the ability of field plants to produce greater biomass and larger root-crowns may make them more tolerant to herbicide treatments compared to laboratory-grown plants. Therefore, the direct application of laboratory results to the field should be viewed with some degree of caution, and these results should be verified under field conditions.

While difficulty remains in precisely predicting field efficacy based upon laboratory results, the relationship between increased endothall concentration and exposure time, and increased efficacy, has been clearly established. The development of concentration/exposure time relationships should help manufacturers design improved endothall formulations, and provide guidance for the most effective use of endothall.

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