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Response of Eurasian Watermilfoil to 2,4-D Concentrations and Exposure Times

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ABSTRACT

Herbicide concentration and exposure time relationships were determined for 2,4-D (2,4-dichlorophenoxy acetic acid) and control of Eurasian watermilfoil (*Myriophyllum spicatum* L.) under laboratory conditions. Fourteen 2,4-D concentration and exposure time combinations were tested: 0.5 mg acid equivalent (ae)/1 for 12, 24 36, 48 60, and 72 hours; and 1.0 and 2.0 mg ae/1 for 12, 24, 36 and 48 hours. Eurasian watermilfoil control was based on visual plant injury and harvested biomass (4 weeks after treatment. Plant injury increased and harvested biomass decreased with increasing 2,4-D concentrations and exposure times, to a threshold above which satisfactory plant control was achieved. Severe Eurasian watermilfoil injury occurred when exposed to 0.5 mg ae/1 for 72-hr, 1.0 mg ae/1 for 36-hr, and the 2.0 mg ae/1 for 24-hr. The threshold levels for control of Eurasian watermilfoil was established in the 1.0 mg ae/1 for 48-hr, and 2.0 mg ae/1 for 36 and 48-hr

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exposures. Effective control of Eurasian watermilfoil is suggested for treatments in both dynamic and static aquatic environments, when plants remain in contact with 2,4-D concentrations for exposures times greater than the developed threshold levels.

Key words: Herbicide, submersed plants, time: dose relationships, biomass.

INTRODUCTION

Hydrodynamic processes in rivers and reservoirs with relatively short hydraulic retention times or significant wind-induced or tidally-influenced circulation patterns can dilute and displace herbicides within treated areas. As a result, submersed aquatic plant exposure may be less than that required to provide complete control. Herbicide efficacy in these environments is influenced by the herbicide concentration in the water or sediment (depending on primary mode of action), the length of time the targeted plant species remains exposed to herbicide concentrations, and the growth stage of the target plant at the time of treatment.

Previous studies using the herbicide endothall (7-oxabicyclo [2,2,1]heptane-2,3-dicarboxylic acid) to control hydrilla (*Hydrilla verticillata* Royle.) and 2,4-D to control Eurasian watermilfoil have demonstrated the variability in plant control in dynamic aquatic environments. Fox *et al.* (1988) suggested that proper timing of endothall applications in a spring and tidally-influenced area of the Crystal River, Florida will maximize herbicide exposure, and may improve hydrilla control. Lim and Lozoway (1976), the British Columbia Water Investigations Branch (1980), Hoeppel and Westerdahl (1983) and Getsinger and Westerdahl (1984) observed variability in Eurasian watermilfoil control following applications of liquid and granular 2,4-D to areas of large lakes and reservoirs. Many of these 2,4-D applications resulted in low herbicide concentrations within the water and short periods of plant exposure.

The variability in herbicide efficacy resulting from operational treatments demonstrate the need for establishing the functional relationships between herbicide concentration, exposure time and plant control. These relationships should be helpful to both herbicide manufacturers and applicators, in the design of herbicide formulations and the improvement of application techniques. Ultimately, better plant control practices will be achieved.

The objectives of this study were to examine the relationships between 2,4-D concentration and exposure time for the control of Eurasian watermilfoil under laboratory conditions, using various concentrations and exposure times, and to compare these findings with previous 2,4-D / Eurasian watermilfoil exposures.

MATERIALS AND METHODS

The laboratory system used for this study was a modification of the system used by Hall *et al.* (1982) and Westerdahl *et al.* (1983). The system consisted of 24, 55 l (15 gal) vertical aquaria (0.75 m x 0.3 m^2) located in a controlled environment greenhouse. Supplemental lighting was provided at a light:dark cycle of 13:11 hours. The mean photosynthetically active radiation received by the aquaria was approximately 1600 $\mu E \cdot m^{-2} \cdot sec^{-1}$ which corresponds to 75 percent of solar noon sunlight received at the latitude where the laboratory was located (Hall *et al.*, 1982). Four apical shoots (15 cm long) of Eurasian watermilfoil collected from the field were planted 5 cm deep in sediment contained in 300 ml glass beakers. Sediment was collected from Brown's Lake, Waterways Experiment Station, Vicksburg, Mississippi and enriched with macro- and micronutrients (Ra·pid·gro with Forti·5^{tm}, Ra·pid·gro Corp.) to eliminate nutrient deficiency. Eleven beakers containing four propagules were placed in each aquarium. Each aquarium was independently supplied with a continuous flow of simulated hard water solution (Hall *et al.*, 1982; EPA, 1975) except when herbicide exposures were being conducted. The water volume (50 l) of each aquarium was displaced with fresh, simulated hard water every 24 hours. Air was bubbled through each aquarium to provide a source of carbon dioxide and to circulate the water. Water temperature was maintained at 21 ± 2 C.

The study consisted of 16 treatments, including 14 2,4-D exposure combinations and 2 untreated references. Eurasian watermilfoil was exposed to 0.5 mg ae/l for 12, 24, 36, 48, 60 and 72 hours; and, 1.0 and 2.0 mg ae/l for 12, 24, 36 and 48 hours. All herbicide exposures were conducted in randomly selected triplicate aquaria. The herbicide exposures were separated into two independent test runs.

Plants were treated when the shoot apices reached to within 5 to 10 cm of the water surface (two weeks). One randomly selected beaker of plant material was removed from each of the 24 aquaria, immediately proceeding 2,4-D application to provide an estimate of treated biomass. The plant material of each beaker was placed in a single container and dried to a consistent weight. This weight was then divided by 24 and multiplied by 10 to estimate the biomass remaining in each aquarium. The estimated dry weight biomass treated in the two test runs was 11.2 and 11.1 g/aquarium, respectively. These concentrations would be equivalent to 37 g/m^2 dry weight at a water depth of 0.5 m.

The 2,4-D stock solutions used for treatment were prepared from analytical grade 2,4-D acid (>97% acid). The 2,4-D acid was dissolved in 50 ml ethyl alcohol and diluted with distilled water, to make one-liter stock solutions. At the time of treatment, the continuous water flow system was turned off. Calculated volumes of the 2,4-D stock solution were then added to the aquaria to provide the treatment concentrations. At the required exposure time, each aquarium was emptied and refilled with fresh water at least 3 times to remove 2,4-D residues. After rinsing, the continuous water flow system was turned on and continued to run until termination of the test run.

Three water samples were taken from each aquarium for 2,4-D residue analysis: 1) immediately after treatment to verify treatment concentrations; 2) just prior to the first rinse to determine residue level decline over the exposure time; and 3) after the final rinse to verify the removal of 2,4-D residues. Residue samples were analyzed by the Analytical Laboratory Branch, Tennessee Valley Authority, Chattanooga Tennessee. The mean 2,4-D residue con-

centration at the time of treatment was: 0.51 mg ae/l (0.01 standard error (SE)) for the 0.5 mg ae/l treatments; 1.02 mg ae/l (0.06 SE) for the 1.0 mg ae/l treatments; and, 2.03 mg ae/l (0.06 SE) for the 2.0 mg ae/l treatments. Residue dissipatlon over the exposure time was negligible. All residue levels following the final rinse were at, or below the detection limit (0.01 mg ae/l).

Since results of other 2,4-D / Eurasian watermilfoil studies (Elliston and Steward, 1972; Hall *et al.*, 1982; and, Westerdahl *et al.*, 1984) indicated that plant injury occurred by 4 weeks posttreatment, the posttreatment duration of this study lasted 4 weeks.

Eurasian watermilfoil control in this study was determined by comparing the results of two efficacy evaluations at 4 weeks posttreatment: 1) visual estimates of plant injury; and, 2) harvested biomass. Percent injury was assessed by rating apparent injury incurred for each replicate treatment relative to the appearance of the reference aquaria. A value of 100 percent would equal complete control, no apparent living tissue surviving treatment. Total harvested biomass (dry weight) was determined by collecting all non.decomposed plant material within each replicate and separating it into roots and shoots. The dry weight biomass for roots and shoots were combined for each replicate to provide total biomass.

RESULTS AND DISCUSSION

Results from this study support the generalized relationships between 2,4-D exposure and Eurasian watermilfoil control: plant injury increases with increasing concentrations and exposure times. The 2,4-D treatments that produced little or no injury to Eurasian watermilfoil, 4 weeks after treatment, were the 0.5 mg ae/l for 12 and 24 hr exposures and the 1.0 mg ae/l for 12 hr exposure. The visual plant injury in these aquaria was less than 20 percent (Figures 1 and 2). Epinasty (shoot and leaf curling) and epidermal rupture of the young tissue around the nodes, occurred over the first few days after treatment, but all replicates at these exposures contained healthy vegetation at the time of harvest. The harvested biomass from these

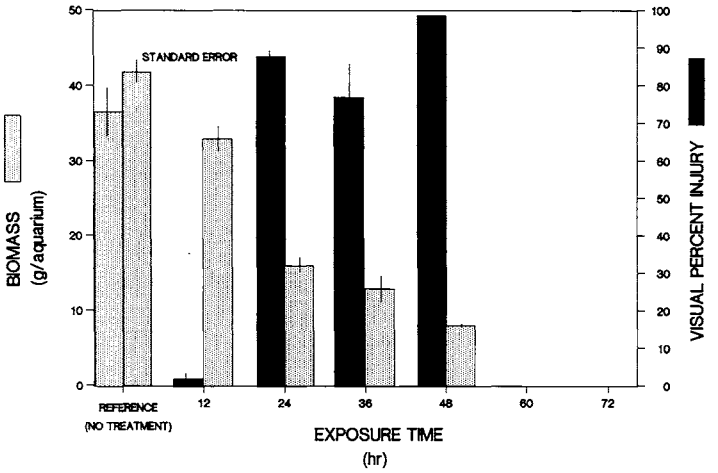

Figure 2. Harvested biomass and visual percent injury to Eurasian watermilfoil at 1.0 mg ae/l 2,4-D exposures.

treatments was less than that harvested from the references (between 69 and 94 percent dry weight), but was considerably greater than the biomass harvested from the remaining treatments.

Eurasian watermilfoil injury, ranging from 22 to 88 percent was observed in the 0.5 mg ae/l for 36, 48 and 60 hr, the 1.0 mg ae/l for 24 hr, and the 2.0 mg ae/l for 12 hr exposures (Figures 1, 2 and 3). The initial visible injury from these exposures suggested that control might be achieved but regrowth of the Eurasian watermilfoil occurred within the four week evaluation period. All replicates within each treatment contained viable roots and shoots at harvest, with the exception of one replicate of the 0.5 mg ae/l for 60 hr exposure. which did not contain visually evident viable root tissue and contained only one living shoot fragment. The harvested biomass from these treatments was considerably less than that in the references (between 40 and 65 percent).

The 2.0 mg ae/l for 24 hr, 1.0 mg ae/l for 36 hr, and 0.5 mg ae/l for 72 hr exposures produced severe plant injury. Plant injury was estimated at 96, 77 and 95 percent

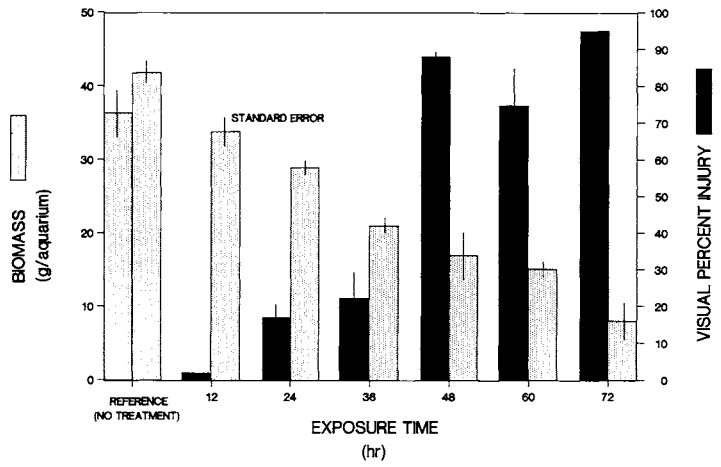

Figure 1. Harvested biomass and visual percent injury to Eurasian watermilfoil at 0.5 mg ae/l 2,4-D exposures.

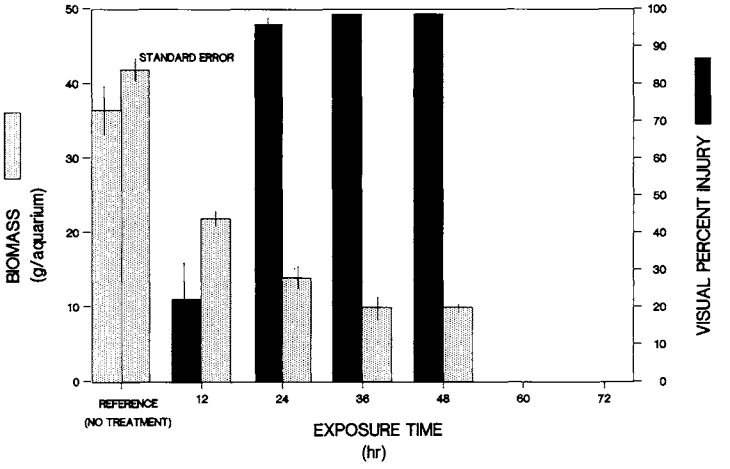

Figure 3. Harvested biomass and visual percent injury to Eurasian watermilfoil at 2.0 mg ae/l 2,4-D exposures.

for these three treatments, respectively (Figures 1, 2 and 3). Harvested biomass in these treatments ranged from 20 to 35 percent of that harvested in the references. Viable shoot tissue was harvested from all replicates of the 0.5 mg ae/l for 72 hr, and 1.0 mg ae/l for 36 hr exposures, and from two of the three replicates of the 2.0 mg ae/l for 24 hr exposure. Only two replicates contained viable root material in the 0.5 mg ae/l for 72 hr and 1.0 mg ae/l for 36 hr exposure. No viable root material was harvested from the 2.0 mg ae/l for 24 hr exposure.

Complete Eurasian watermilfoil control occurred in the 1.0 mg ae/l for 48 hr exposure, and 2.0 mg ae/l for 36 and 48 hr exposures. Visual estimates of plant injury approached 100 percent in all three treatments (Figures 2 and 3). Plant injury and death was severe enough, that the biomass harvested four weeks after treatment was less than that estimated when treated, suggesting that some plant decomposition had occurred. The 2.0 mg ae/l for 36 hr exposure contained no viable shoots at the time of harvest, and the 1.0 and 2.0 mg ae/l for 48 hr exposures contained one replicate each, with one visually evident viable shoot fragment. None of the 1.0 mg ae/l for 48 hr replicates contained viable root tissue and the 2.0 mg ae/l for 36 and 48 hr exposures contained one replicate each, with measurable root biomass.

Eurasian watermilfoil control in the field can be predicted based on the results obtained (Figure 4, shaded area). For example, Eurasian watermilfoil control should be achieved when exposed to a minimum 2,4-D concentration of 0.5 mg ae/l for a period of time greater than 72 hours, 1.0 mg ae/l for greater than 36-48 hours, and 2.0 mg ae/l for greater than 24-36 hours.

These results conform well with previous laboratory studies conducted by Elliston and Steward (1972), Hall *et al.* (1982), and Westerdahl *et al.* (1983), if the results of Figure 4 were extended to include their data. Elliston and Steward (1972) conducted very similar tests and determined that Eurasian watermilfoil control was achieved when plants were exposed to 2,4-D at a concentration of 1.0 mg ae/l for 48 hours, and 2.5 mg ae/l for 8 hours. However, total control was not achieved at 0.5 mg ae/l, even after 96 hours of exposure. It was determined by Hall *et al.* (1982) that Eurasian watermilfoil may be controlled with 0.25 mg ae/l 2,4-D after 35 days continuous exposure. The laboratory study of Westerdahl *et al.* (1983) found that Eurasian watermilfoil was controlled after 21 days continuous exposure to 2.4-D, ranging from an initial concentration of 1.0 mg ae/l and dissipating to 0.1 mg ae/l when total control was established.

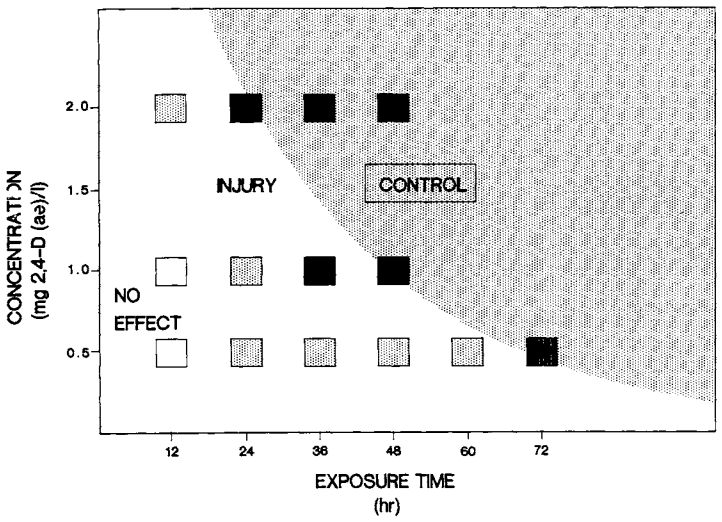

Figure 4. The 2,4-D concentration and exposure time relationships for control of Eurasian watermilfoil. Rectangles represent actual 2,4-D concentration, exposure time test coordinates. The open rectangles represent treatments providing no control. The less dense stippled rectangles represent treatments providing plant injury. The more dense stippled rectangles represent treatments providing severe plant injury. The completely filled rectangles represent treatments providing control. The shaded area of the graph represents the 2,4-D concentration exposure time coordinates that should provide plant control.

Eurasian watermilfoil exposures in the field differ from that of the static exposures conducted in the laboratory. Plants in the field, following treatment, will be exposed to a dissipating concentration of herbicide over time. Herbicide concentration will be dependent upon the rate of application and the formulation applied, whether it be liquid solid, or controlled release. The rate of herbicide dissipation and therefore, the magnitude of plant exposure, will be dependent upon water exchange characteristics and herbicide diffusion, uptake, adsorption and decomposition.

Eurasian watermilfoil control in the field would be expected if 2,4-D residue dissipation provided concentration and exposure time curves that lie within the region of control presented in Figure 4 or that intersect and enter the region of control. Residue dissipations which fall short of entering the coordinates of control would only injure Eurasian watermilfoil with the degree of injury increasing as the dissipation curves approach the threshold coordinates.

Variable Eurasian watermilfoil control would be expected from field applications that provided 2,4-D exposures approaching or intersecting the threshold of control at the lower concentrations (<1.0 mg ae/l). A 2,4-D dissipation curve that resulted in 0.75 mg ae/l at 72 hours would be expected to provide Eurasian watermilfoil control. Whereas, a 2,4-D dissipation curve that resulted in 0.25 or 0.50 mg ae/l at 72 hours would only provide marginal to severe injury. The difference in 2,4-D concentrations (0.5 mg ae/l) at this point in time of exposure (72 hours) might mean the difference between total Eurasian watermilfoil control and variable plant injury. The low concentration exposures for the longer time periods might also explain the spatial variability which sometimes occurs in field treatments where patches of good control are surrounded by areas of plants that are variably injured. The patches of controlled plants might have been exposed to slightly higher concentrations for the same or greater amount of time than the surrounding plants.

Calculated dissipation rates resulting from the aqueous concentrations of 2,4-D residues reported from the field studies of Hoeppel and Westerdahl (1983) and Getsinger and Westerdahl (1984) conducted in Lake Seminole, Georgia support the findings presented in this study. Hoeppel and Westerdahl (1983) applied two 2,4-D formulations (dimethylamine (DMA) and butoxethyl ester (BEE)) at two

different rates (45 kg aeba and 22.5 kg aeha) to 10 ha plots containing Eurasian watermilfoil. The aqueous 2,4-D dissipation curves resulting from these applications are presented in Figure 5. Three of four field applications (DMA 22.5 kg ae/ha, BEE 45 kg ae/ha and BEE 22.5 kg ae/ha) were documented as producing Eurasian watermilfoil injury, but the plants recovered and reestablished standing crop within 70 days after treatment. The initial 2,4-D concentrations were low and the dissipating exposures never entered the concentration and exposure time coordinates of control presented in this study (Figure 5, shaded area). The one effective field treatment (DMA 45 kg ae/ha) in which the treated Eurasian watermilfoil was controlled the entire growing season, maintained 2,4-D exposure well within the area of control presented in Figure 5.

The initial aqueous 2,4-D concentrations f Gtsinger and Westerdahl (1984) were low (0.07 - 0.13 mg ae/1), barely above the minimum effective levels determined by Hall *et al.* (1982) and Westerdahl *et al.* (1983). The estimated concentration and exposure times calculated from these field applications would be expected, based on the results of the laboratory research, to produce Eurasian watermilfoil injury, but not complete control. The Eurasian watermilfoil treated in the field tests of Getsinger and Westerdahl (1984) exhibited approximately 60 to 85 percent control four weeks after treatment followed by rapid regrowth and reestablishment of the Eurasian watermilfoil standing crop (8 weeks after treatment).

Results from field studies conducted by Lim and Lozoway (1976) and the British Columbia Water Investigations Branch (1980) with 2,4-D and Eurasian watermilfoil in lakes of the Okanagan Valley, British Columbia, Canada follow the same trends and relate well with relationships developed in the laboratory. Maximum aqueous 2,4-D residues collected by Lim and Lozoway (1976) were 0.14 and 0.06 mg ae/1 within the two treated plots on the second day after treatment. Residues were below detection 72 hours after treatment. Both 2,4-D treatments (Lim and Lozoway, 1976) caused injury to the Eurasian watermilfoil and reduced plant growth in comparison to the untreated reference plot. However, vegetative control was not achieved. These authors concluded that the ineffectiveness of control was the result of low residue concentrations combined with the short exposure times.

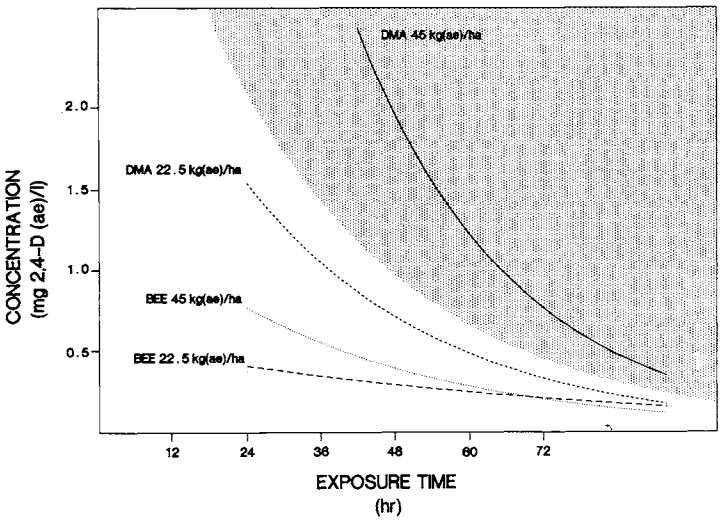

Figure 5. Dissipation of 2,4-D residues in the field in relation to laboratory results. Residue dissipation data obtained from Hoeppel and Westerdahl (1983).

Similar efficacy results occurred in field tests conducted by the British Columbia Water Investigations Branch (1980) where different aquatic systems containing Eurasian watermilfoil were treated with 2,4-D. Again, aqueous 2,4-D residue concentrations were low and exposure time varied among the systems treated. An entire lagoon, with no input of water from flowing streams or rivers, was treated with a combination of different application rates in different areas. The overall treatment provided a maximum 2,4-D residue concentration of 1.26 mg ae/1 near the surface on the day of treatment and 4.0 mg ae/1 near the bottom of the water column 6 days after treatment. Residues near the bottom averaged 0.68 mg ae/1 for the first ten days and were still detected 22 days after treatment. These concentrations and exposure times (dissipation curve) would fall well within the area of Eurasian watermilfoil control (Figure 4) developed in this study. Eurasian watermilfoil control in this lagoon was achieved for the entire growing season and continued into the next growing season (British Columbia Water Investigations Branch, 1980). The other sites treated by the British Columbia Water Investigations Branch (1980) were conducted in sections of large lakes, and Eurasian watermilfoil control was highly variable. No relationship seemed to exist between the different treatment rates and the resulting 2,4-D concentrations. The variability in efficacy was presumed to be influenced by water movement and the physiological condition of the plants at the time of treatment.

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