Technical Report A-93-2
January 1993

US Army Corps of Engineers
Waterways Experiment Station

Aquatic Plant Control Research Program

Efficacy of Bensulfuron Methyl on Eurasian Watermilfoil

by Linda S. Nelson, Michael D. Netherland
Environmental Laboratory

Prepared for Headquarters, U.S. Army Corps of Engineers

The contents of this report are not to be used for advertising, publication, or promotional purposes. Citation of trade names does not constitute an official endorsement or approval of the use of such commercial products.

Aquatic Plant Control Research Program

Technical Report A-93-2
January 1993

Efficacy of Bensulfuron Methyl on Eurasian Watermilfoil

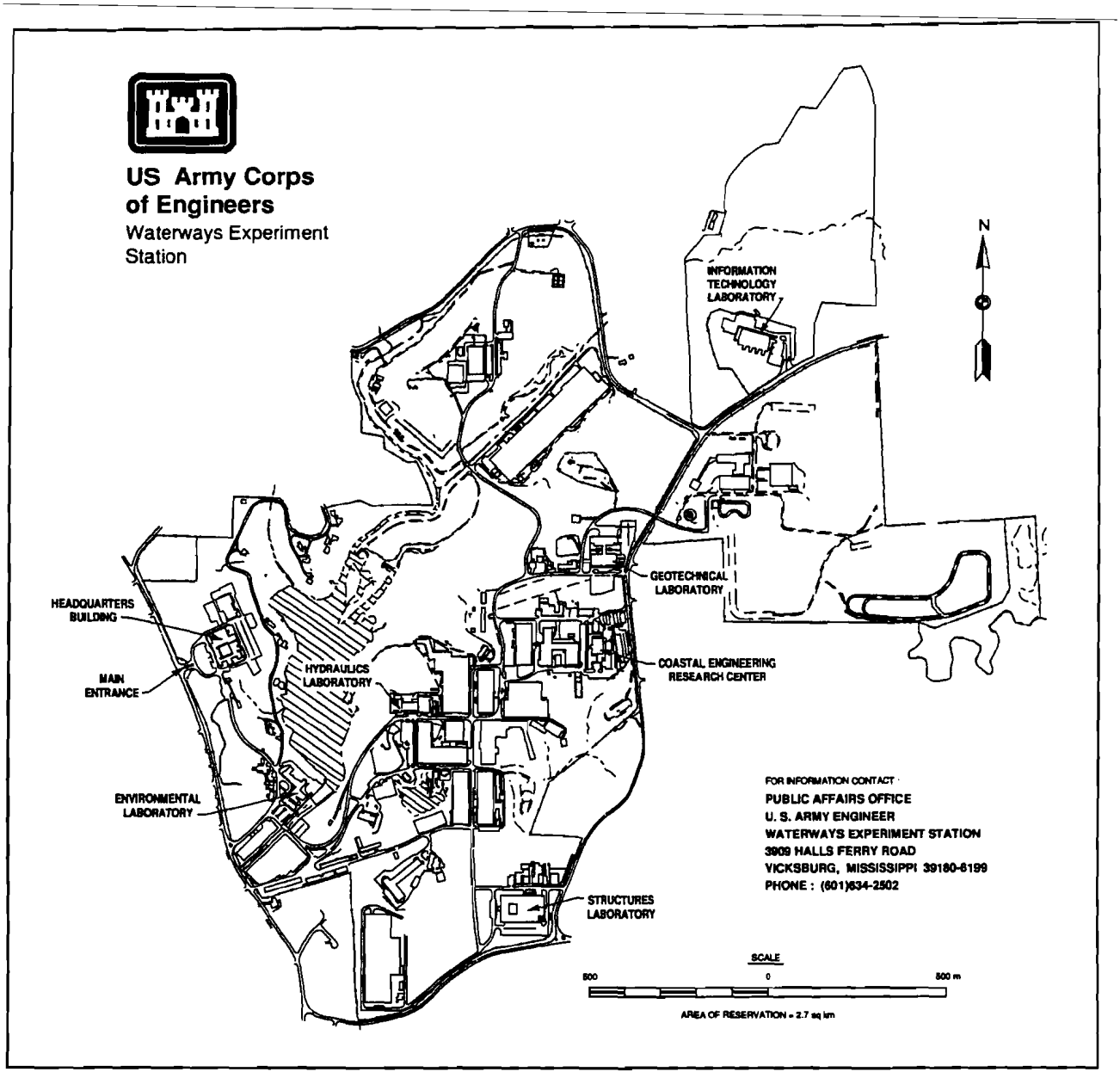
by Linda S. Nelson, Michael D. Netherland
Environmental Laboratory

U.S. Army Corps of Engineers
Waterways Experiment Station
3909 Halls Ferry Road
Vicksburg, MS 39180-6199

Final report

Approved for public release; distribution is unlimited

Prepared for U.S. Army Corps of Engineers
Washington, DC 20314-1000



Waterways Experiment Station Cataloging-in-Publication Data

Nelson, Linda S.

Efficacy of bensulfuron methyl on Eurasian watermilfoil / by Linda S. Nelson, Michael D. Netherland ; prepared for U.S. Army Corps of Engineers.

25 p. : ill. ; 28 cm. — (Technical report ; A-93-2)

Includes bibliographical references.

1. Eurasian watermilfoil — Control. 2. Aquatic herbicides — Evaluation. 3. Aquatic weeds — Control. 4. Herbicides — Testing. I. Netherland, Michael D. II. United States. Army. Corps of Engineers. III. U.S. Army Engineer Waterways Experiment Station. IV. Aquatic Plant Control Research Program (U.S. Army Engineer Waterways Experiment Station) V. Title. VI. Series: Technical report (U.S. Army Engineer Waterways Experiment Station) ; A-93-2.

TA7 W34 no.A-93-2

Preface

The work reported herein was conducted as part of the Aquatic Plant Control Research Program (APCRP), Work Unit 32578. The APCRP is sponsored by the Headquarters, US Army Corps of Engineers (HQUSACE), and is assigned to the US Army Engineer Waterways Experiment Station (WES) under the purview of the Environmental Laboratory (EL). Funding was provided under Department of the Army Appropriation No. 96X3122, Construction General. The APCRP is managed under the Environmental Resources Research and Assistance Programs (ERRAP), Mr. J. L. Decell, Manager. Mr. Robert C. Gunkel was Assistant Manager, ERRAP, for the APCRP. Technical Monitor during this study was Ms. Denise White, HQUSACE.

The Principal Investigator for the study was Dr. Kurt D. Getsinger, Ecosystem Processes and Effects Branch (EPEB), Environmental Processes and Effects Division (EPED), EL, WES. The study was conducted and the report prepared by Ms. Linda S. Nelson and Mr. Michael D. Netherland, EPEB.

Reviews of the report were provided by Dr. Getsinger and Dr. Susan L. Sprecher, AScI Corporation. Technical assistance was provided by Dr. Sprecher, Mr. Glenn Turner, and Ms. Anne Stewart, AScI Corporation, and Mr. Brian York, Ms. Kim Deevers, and Ms. Sheron Burt, EPED. The cooperation of E.I. du Pont de Nemours & Co. for providing the bensulfuron methyl formulation and residue analysis for this study is greatly appreciated.

This investigation was performed under the general supervision of Dr. John Harrison, Director, EL; Mr. Donald L Robey, Chief, EPED; and Dr. Richard E. Price, Acting Chief, EPEB.

At the time of publication of this report, Director of WES was Dr. Robert W. Whalin. Commander was COL Leonard G. Hassell, EN.

This report should be cited as follows:

Nelson, Linda S., and Netherland, Michael D. 1993. "Efficacy of Bensulfuron Methyl on Eurasian Watermilfoil," Technical Report A-93-2, US Army Engineer Waterways Experiment Station, Vicksburg, MS.

Contents

EFFICACY OF BENSULFURON METHYL ON EURASIAN WATERMILFOIL

Introduction

1. As nuisance aquatic plant infestations continue to increase throughout the United States, so does the need for developing additional management tools, such as new herbicides and plant growth regulators. One such compound being considered for aquatic registration is bensulfuron methyl (methyl 2-[[[[[(4,6-dimethoxy-2-pyrimidinyl)amino]carbonyl]amino]sulfonyl]methyl]benzoate). Bensulfuron methyl is currently registered as a herbicide (Londax^{R}) for use in rice production; however, recent studies have demonstrated its effectiveness on several aquatic plant species including hydrilla (*Hydrilla verticillata* Royle), Eurasian watermilfoil (*Myriophyllum spicatum* L.), and several species of *Potamogeton* (Haller, Fox, and Hanlon 1992; Van and Vandiver 1992; Anderson 1988). Large-scale evaluations for use in aquatic systems have been conducted under an Experimental Use Permit issued by the US Environmental Protection Agency (Getsinger et al. 1992, Langeland 1992, Pringle and Sisneros 1992).

2. Bensulfuron methyl is a member of the sulfonylurea herbicide group developed by E.I. du Pont de Nemours & Co., Wilmington, DE. Sulfonylureas are characterized by their high levels of activity at application rates as low as 0.002 kg/ha (0.03 oz per acre). Following application, plant uptake of bensulfuron methyl occurs readily through roots and foliage and, once inside the plant, is translocated via xylem and phloem. The mode of action of bensulfuron methyl is inhibition of the plant enzyme acetolactate synthase, which is necessary for the synthesis of two amino acids, valine and isoleucine (Beyer et al. 1988). Without these essential amino acids, plant growth ceases, often within 4 to 6 hr after application. Following growth cessation, visual symptoms of plant injury (chlorosis, leaf bending and curling, leaf discoloration because of enhanced anthocyanin production, and necrosis) usually appear within 1 to 2 days (E.I. du Pont de Nemours & Co. 1988). Although growth cessation immediately follows treatment with bensulfuron methyl, actual plant death occurs gradually as plants utilize and eventually deplete internal carbohydrate reserves.

3. In addition to very low application rates, sulfonylurea herbicides have relatively rapid dissipation characteristics and good toxicity profiles, indicating a high margin of safety with respect to the environment. Under

field conditions (in rice), the reported half-life of bensulfuron methyl is 5 to 10 days in water and 4 to 8 weeks in soil (Weed Science Society of America 1989). Degradation occurs via microbial breakdown and chemical hydrolysis, and is related to soil and water pH and temperature. Results of dissipation studies have shown that the rate of degradation is enhanced at warmer temperatures and as soil and water pH decrease below neutrality (Beyer et al. 1988). Animal toxicology studies have demonstrated that bensulfuron methyl is neither mutagenic nor teratogenic and exhibits low toxicity to fish, wildlife, and other organisms (Beyer et al. 1988). Table 1 provides a summary of the toxicological data for bensulfuron methyl. The fact that bensulfuron methyl affects a plant enzyme system (acetolactate synthase) that is nonexistent in animals helps explain its low toxicity to nontarget organisms.

Table 1*

Toxicological Properties of Bensulfuron Methyl

Study Conducted	Species Exposure		BSM Concentration
Acute oral	LD_{50}** rat		>5,000 mg/kg
Chronic feeding	Rat (2-year)		NOEL† 750 ppm
Mutagenicity	Negative in five assays††		
Teratogenicity	Rat		Negative
Wildlife	Oral LD_{50}	Mallard duck	>2,510 mg/kg
	Dietary LC_{50}‡ (8 day)	Mallard duck	>5,620 ppm
		Bobwhite quail	>5,620 ppm
	Honey bee	5% mortality	>12.5 µg/bee
Aquatics	LC_{50} (48 hr)	Carp	>1,000 ppm
		Daphina	>100 ppm
	LC_{50} (96 hr)	Bluegill sunfish	>150 ppm
		Rainbow trout	>150 ppm

* After Beyer et al. (1988).

** LD_{50} = lethal dose, given as milligram per kilogram of body weight, which kills 50 percent of a group of test organisms.

† NOEL = no observable effect level.

†† Assays included: Ames, unscheduled DNA synthesis, CHO/HGPRT gene mutation, in vivo bone marrow studies, and in vivo chromosome studies.

‡ LC_{50} = lethal concentration which kills 50 percent of the individuals, plant or animal.

4. Although the acetolactate synthase enzyme is present in all plants, not all species are susceptible to bensulfuron methyl, indicating some degree

of selectivity. Tolerant plants (e.g., *Leptochloa* spp. and most varieties of *Indica* rice) can quickly metabolize the active ingredient to herbicidally inactive compounds, whereas susceptible species cannot. In addition, the range in sensitivity to bensulfuron methyl among plants is wide. Beyer et al. (1988) reported that the differential sensitivity of plants to sulfonylurea herbicides can be over 1,000-fold. Greenhouse studies on crop tolerance showed that the growth of onion and wheat was not affected by bensulfuron methyl until concentrations reached 1,000 g active ingredient (ai)/ha, whereas the growth of mustard and spinach was suppressed at rates of 0.4 and 2.0 g ai/ha, respectively (E.I. du Pont de Nemours & Co. 1988). Furthermore, many herbicides, including the sulfonylureas, exhibit growth-regulating effects on plants when applied at sublethal concentrations. In other words, by adjusting application rates, a desired degree of vegetation control can be achieved. The benefits of growth regulation versus the complete removal of plant biomass (as with a herbicide) in an aquatic system have been identified by several researchers and include: oxygen production through photosynthesis, sediment stabilization, and habitat maintenance (Anderson 1988; Klaine and Knowles 1988; Lembi and Netherland 1990). As a selective herbicide with growth-regulating properties, bensulfuron methyl would benefit management strategies in which the objective of chemical treatment is to maximize control of a target plant species while minimizing effects on nontarget, desirable species.

5. Understanding the relationship between rate of application and the length of time a chemical is in contact with a target plant species (contact or exposure time) is also important to achieve desired plant control. This is especially critical in aquatic systems where water flow and thermal- and wind-induced circulation patterns influence herbicide dispersion and, consequently, treatment performance (Getsinger, Green, and Westerdahl 1990; Fox, Haller, and Getsinger 1991). Concentration/exposure time relationships have been described for several aquatic herbicides and can be helpful in predicting treatment success under field conditions (Hall, Westerdahl, and Stewart 1984; Van and Conant 1988; Green and Westerdahl 1990; Netherland, Green, and Getsinger 1991; Netherland and Getsinger 1992).

6. To date, most of the bensulfuron methyl research conducted on aquatic plants has focused on hydrilla. Several investigators have observed reduced shoot growth and tuber formation of hydrilla following treatment with bensulfuron methyl (Anderson 1988; Haller, Fox, and Hanlon 1992; Van and

Vandiver 1992). Reduced hydrilla reproduction by germinating tubers and turions was also observed under field conditions (Haller, Fox, and Hanlon 1992).

7. Investigations concerning the effectiveness of bensulfuron methyl on another troublesome, submersed aquatic species, Eurasian watermilfoil (hereafter referred to as milfoil), are limited. Therefore, the objective of the following studies was to determine the effects of selected concentrations and exposure times of bensulfuron methyl on the growth of milfoil.

Materials and Methods

8. Experiments were conducted in two similar laboratory systems developed at the US Army Engineer Waterways Experiment Station, Vicksburg, MS. The system used for Studies 1 and 2 consisted of 24, 55-ℓ aquaria (0.75 m tall by 0.09 m^2) located in a controlled-environment room. Overhead lighting was provided by a combination of 400-w, mercury vapor lamps and 250-w, high-pressure sodium lamps. The mean photosynthetically active radiation (PAR) measured at the water surface was 450 ± 50 $\mu E/m^2/sec$, with a photoperiod (light:dark cycle) of 13:11 hr. Water temperature was maintained at 25 ± 3 °C throughout both experiments.

9. Studies 3 and 4 were conducted in a controlled-environment growth chamber equipped with 36, 55-ℓ aquaria. Overhead lighting was provided by lamps as previously described, with a mean PAR measured at the water surface of 510 ± 45 $\mu E/m^2/sec$ and a light:dark cycle of 13:11 hr. Water temperature was maintained at 24 ± 2 °C.

10. Sediment for all studies was collected from Brown's Lake, Vicksburg, MS, and was amended with commercially available fertilizers (Rapid-gro, 20-15-15, and Osmocote, 14-14-14) to avoid possible nutrient deficiencies or limitations during the course of each study. Milfoil was supplied by the Lewisville Aquatic Ecosystem Research Facility, Lewisville, TX. Containers (300-ml glass or polyvinyl chloride beakers) were filled with sediment, and four, 10- to 15-cm apical shoots of milfoil were planted (5 cm deep) into each beaker. A thin layer of silica sand was added to the sediment surface of each beaker to prevent suspension of sediment during water exchange periods. Aquaria were independently supplied with a simulated hard water solution (Smart and Barko 1984) via peristaltic pumps that were calibrated to provide a complete water volume exchange every 72 hr. Air was bubbled through each

aquarium to provide a source of carbon dioxide and thorough mixing of the water column.

11. Bensulfuron methyl stock solutions used for all treatments were prepared from the commercial formulation Londax^{R} (dry flowable, 60 percent ai). All treatment concentrations are reported as micrograms per liter (parts per billion) of the active ingredient. At the time of treatment, the flow-through water system was deactivated (peristaltic pumps turned off), and calculated volumes of the bensulfuron methyl stock solution were added to the aquaria to provide the desired treatment concentrations. At the end of the assigned exposure times, each aquarium was drained and refilled with fresh water three times to remove chemical residues, after which the peristaltic pumps were reactivated, providing water exchange for the duration of the experiment. Water samples were collected and analyzed for chemical residues following the rinse cycle. Results from these analyses indicated that >99 percent of bensulfuron methyl residues were removed following the drain procedure.

12. The treatments (concentration × exposure time) evaluated in these studies are summarized in Table 2. The variation in experimentation among studies is indicated in the following paragraphs.

Studies 1 and 2

13. Studies 1 and 2 each consisted of eight bensulfuron methyl concentration/exposure time treatments ranging from very low to extremely high treatment rates. Each treatment was replicated three times and randomly assigned to a test aquarium. Beakers planted with milfoil (11 beakers per aquarium in Study 1 and 9 beakers per aquarium in Study 2) were placed in each aquarium and allowed to grow for 2 weeks to establish new shoot and root growth. After 2 weeks of growth, rapidly elongating shoots were trimmed back to a height of 20 cm; 1 week thereafter, chemical treatments were applied. Shoots were trimmed back to a uniform height to facilitate evaluation of the growth-regulating potential of bensulfuron methyl on small shoots supported by a healthy root system.

14. Immediately prior to treatment, one randomly selected beaker of plant material was removed from each aquarium. Mean shoot and root dry weights (DW) ± one standard deviation were measured; these values were multiplied by the number of beakers remaining in each aquarium to provide an estimate of pretreatment biomass. The estimated shoot and root biomass treated in

Table 2

Bensulfuron Methyl Treatment Rates and Exposure Time Periods

Rate µg/ℓ or ppb	Exposure Time days
Study 1	
0 (Untreated)	0
50	14
75	14
5	21
10	21
25	21
50	21
5	28
Study 2	
0	0
230	7
1150	7
1730	7
2300	7
4600	7
1150	14
2300	14
Study 3	
0	7, 14, 21, 28, 35, 42
50	(same for all concentrations)
75	
100	
125	
150	
Study 4	
0	0
50	28
50	49

Study 1 was 3.4 ± 1.5 g DW and 0.8 ± 0.3 g DW, respectively. Estimated pretreatment biomass for Study 2 was 6.1 ± 1.8 g DW for shoots and 3.7 ± 1.3 g DW for roots. The same procedure for estimating pretreatment biomass was used in all studies.

15. Milfoil was harvested at 5 weeks posttreatment in Study 1 and at 6 weeks in Study 2. Harvested plants were separated into viable roots and shoots, and oven-dried (70 °C for 48 hr) to a constant weight. Shoot and root biomass data were subjected to analysis of variance (ANOVA) and treatment effects separated using Duncan's Multiple Range Test. Weekly visual

observations were also recorded to characterize the initial plant response to bensulfuron methyl treatment, the progression of injury symptoms, and the initiation of regrowth.

Study 3

16. Study 3 consisted of six bensulfuron methyl concentrations ranging from 0 to 150 $\mu g/\ell$, subjected to a series of exposure times ranging from 7 to 42 days. Treatments were not replicated; however, 36 different concentration/exposure time combinations were evaluated. Eight beakers containing milfoil were placed in each aquarium and given a 3-week pretreatment growth period. Plant growth was vigorous, and many shoots had reached the water surface by the time of treatment. Estimated pretreatment shoot and root biomass was 4.4 ± 0.5 g DW and 1.1 ± 0.3 g DW, respectively. Three weeks following treatment, a beetle (unidentified taxonomically) began feeding on stems at the water surface, causing apical shoots to detach from the parent plants. The insecticide malathion was applied at 0.25 mg/ℓ to all aquaria at 3 and 6 weeks posttreatment to control these insect infestations.

17. At the conclusion of the study (8 weeks posttreatment), plants were harvested, and roots and shoots were separated and dried using techniques described in Studies 1 and 2. Linear regression procedures were used to relate plant biomass to increased exposure times at each bensulfuron methyl treatment rate tested. Visual ratings of plant injury were recorded weekly.

Study 4

18. Study 4 consisted of three bensulfuron methyl concentration/exposure time treatments applied to milfoil grown from 3-month-old rootcrowns or 10-cm apical cuttings. Rootcrowns were trimmed of above sediment biomass prior to planting. Six beakers containing either apical cuttings or rootcrowns were placed in each aquarium and were allowed to establish new growth for 1 week prior to chemical treatment. At the time of treatment, several small shoots had emerged from the trimmed rootcrowns, whereas growth of shoots from apical cuttings were negligible. The estimated shoot and root biomass treated was 0.76 ± 0.14 g DW and 0.04 ± 0.02 g DW, respectively, for plants grown from apical cuttings and 1.10 ± 0.21 g DW and 4.40 ± 0.87 g DW, respectively, for plants grown from rootcrowns.

19. Treatments were arranged in a completely randomized design with three replicates. Plant biomass was harvested 11 weeks after treatment, and shoots and roots were separated and dried as previously described. Biomass

data were analyzed using ANOVA, and treatment effects were separated using the Duncan's Multiple Range Test.

Results and Discussion

Study 1

20. Five treatments significantly reduced milfoil shoot biomass in Study 1 (Figure 1). Reductions ranged from 26 to 69 percent when compared with untreated plants, with the most effective treatment being a 21-day exposure to 50 $\mu g/\ell$ of bensulfuron methyl. Higher concentrations at shorter exposure periods were less effective, suggesting that contact time is an important factor in determining treatment success.

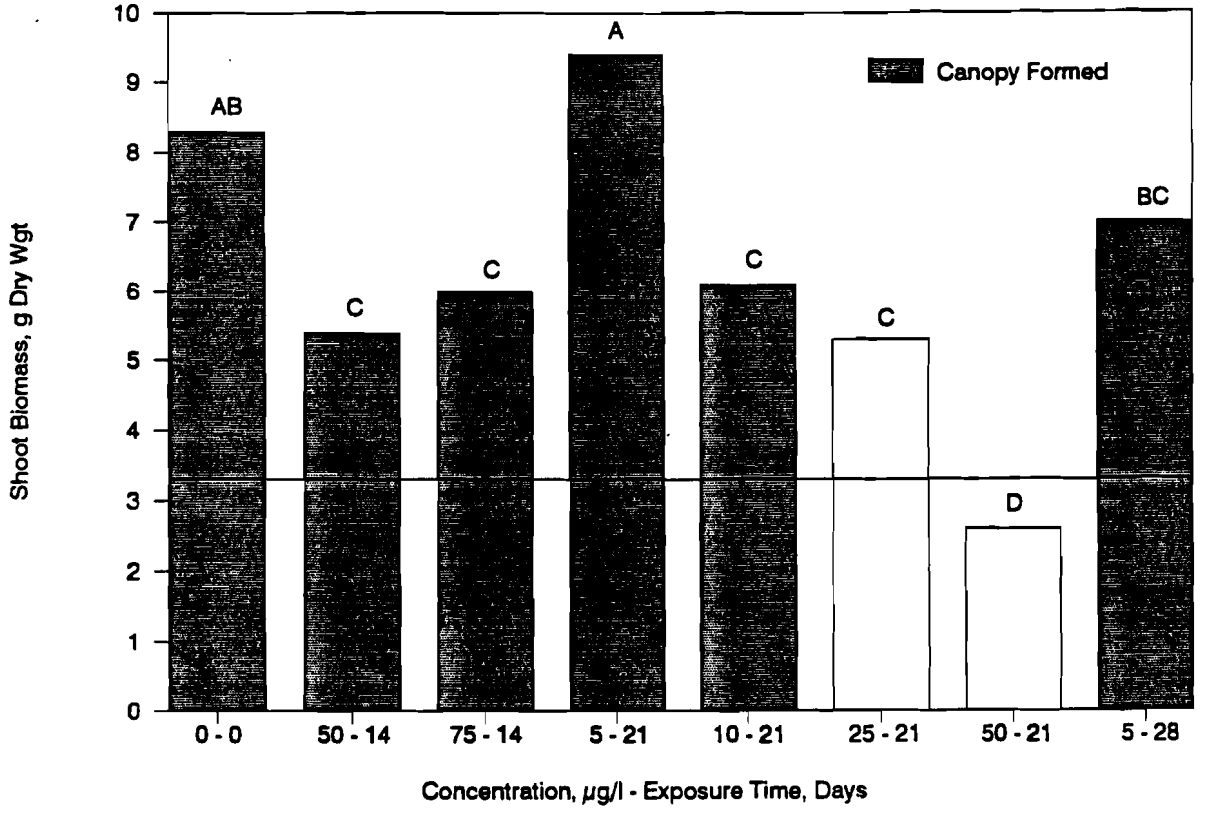


Figure 1. Effects of bensulfuron methyl on shoot biomass of Eurasian watermilfoil harvested at 5 weeks posttreatment. Pretreatment shoot biomass = 3.4 ± 1.5 g dry weight. Different letters among treatments indicate significant differences at the 5-percent level according to Duncan's Multiple Range Test

21. Two treatments, 21- and 28-day exposures to 5 $\mu g/\ell$, showed no significant difference in biomass production from that of untreated plants.

Plants subjected to these treatments showed initial injury symptoms (leaves of shoot apices appeared compressed and slightly chlorotic) but continued to grow during the exposure period, suggesting that under these experimental conditions, milfoil was tolerant to low doses of bensulfuron methyl. Similarly, milfoil treated with 10 $\mu g/\ell$ and exposed for 21 days exhibited active growth while in contact with bensulfuron methyl; however, final biomass was significantly reduced by 26 percent. Active growth during treatment further suggests that at low concentrations (<10 $\mu g/\ell$), milfoil can metabolize bensulfuron methyl quickly enough to prevent complete inhibition of the acetolactate synthase enzyme system. In all other treatments, substantial regrowth of milfoil was evident only after the chemically treated water was removed following the designated exposure period. Regrowth emerged from rootcrowns, lateral buds along stem nodes, and injured apical shoots, and was evident 1 to 2 weeks following removal of bensulfuron methyl from the water column.

22. Initial injury symptoms observed on milfoil treated with bensulfuron methyl concentrations of 25 $\mu g/\ell$ and higher were described as a chlorosis and/or browning of apical shoots, with some upper leaf drop and/or downward bending of foliage. These symptoms were evident 1 week following treatment. The appearance of injury symptoms at active growing points (shoot tips) was expected, given the mode of action of bensulfuron methyl. The development or progression of injury was also characteristic of a systemic (translocated) herbicide.

23. Formation of small, axillary buds along the nodes of most stems was also noted at these higher concentrations, but buds did not further develop during the chemical exposure period. Plant stems were also affected with necrotic lesions visible on lower stems 2 weeks following chemical application. In some instances new foliage showed morphological differences, such as reduced leaf area and/or a lobed leaf shape. Despite a three-fold difference in chemical concentration, the degree of injury varied little between plants treated with 25 $\mu g/\ell$ and those treated with 75 $\mu g/\ell$.

24. By the end of the 5-week experiment, regrowth was observed in all treatments, and plants had "canopied" or grown to the water surface in all but two treatments (25 and 50 $\mu g/\ell$ at 21-day exposures). Although the final data showed significant reductions in biomass production, canopy formation indicated strong regrowth potential and was considered an inadequate treatment. Despite slight increases in root biomass with several treatments, no

significant differences in root growth were observed compared with untreated plants (Figure 2).

25. Results of this study differ from studies by Anderson (1988) in which milfoil shoot and root DW were reduced by 50 to 70 percent and 40 to 77 percent, respectively, after a 4-week exposure to bensulfuron methyl concentrations of 1 to 20 $\mu g/\ell$. Variation in response may be due, in part, to the difference in age of plant material used in experimentation (1-week-old plants versus the 3-week-old plants used here). Other studies have reported an increased efficacy with bensulfuron methyl on younger plants. Haller, Fox, and Hanlon (1992) observed that in the field, hydrilla sprouting from tubers and turions was more susceptible to bensulfuron methyl than mature plants. Studies conducted on the emergent species *Cyperus difformis* and *Scirpus mucronatus* also revealed that less bensulfuron methyl was required to kill younger

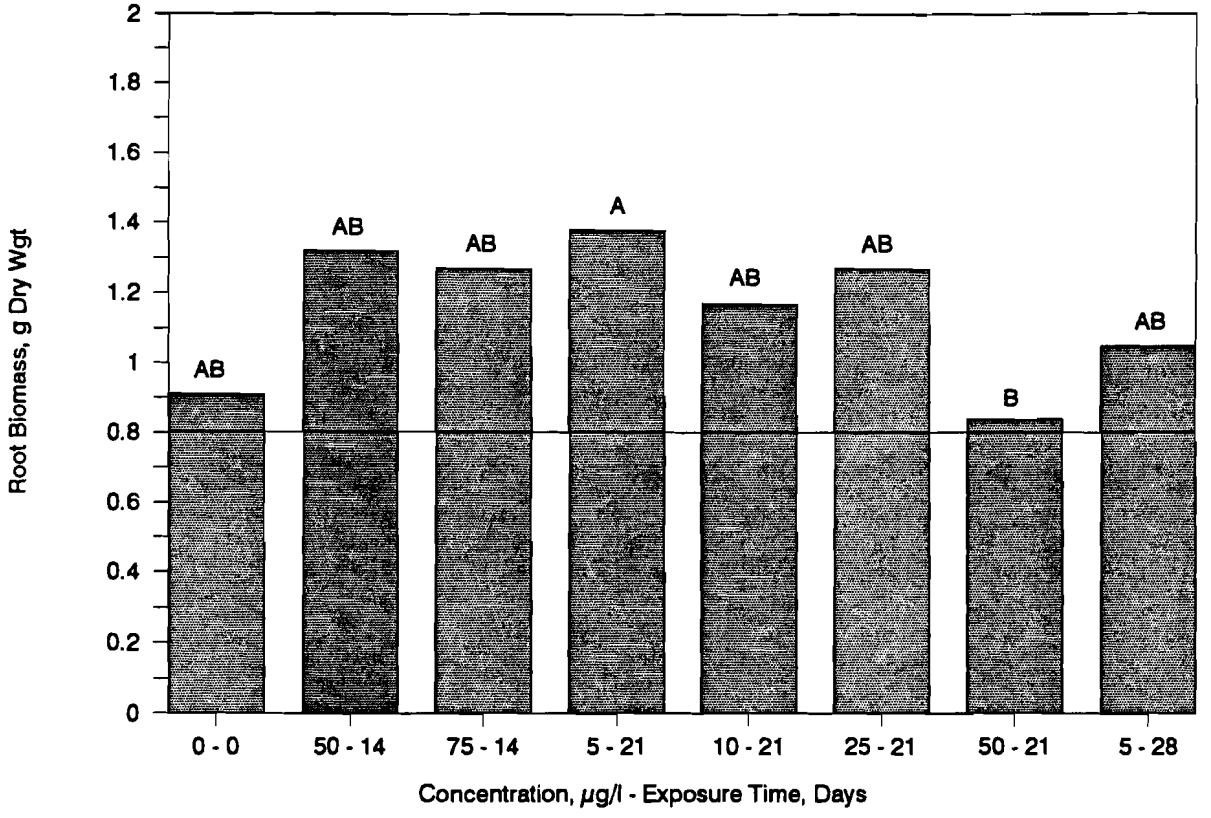


Figure 2. Effects of bensulfuron methyl on root biomass of Eurasian watermilfoil harvested at 5 weeks posttreatment. Pretreatment root biomass = 0.8 ± 0.3 g dry weight. Different letters among treatments indicate significant differences at the 5-percent level according to Duncan's Multiple Range Test

plants (seedling stage) than plants at the two- to three-leaf stage (E.I. du Pont de Nemours & Co. 1988).

26. Under the experimental conditions reported herein, an exposure period of 21 days to concentrations of 25 to 50 $\mu g/\ell$ was necessary to maintain acceptable growth suppression for the duration of the experiment (5 weeks following treatment). Although plants of these treatments had not yet formed a canopy, regrowth was apparent. The authors speculate that given more time (1 to 2 weeks), new growth also would have reached the water surface on these treatments.

Study 2

27. Initial response of milfoil to all bensulfuron methyl treatments was evident 1 week after application and included the following symptoms: reddening of shoot tips, downward bending of upper leaves, and bunched or compacted leaves at shoot apices. Effects were more pronounced with increasing concentration.

28. At 2 weeks posttreatment, untreated plants had formed a dense canopy at the water surface, and new growth was visible on plants that had been subjected to a 7-day exposure of 230, 1,150, and 1,730 $\mu g/\ell$ of bensulfuron methyl. New growth emerged from rootcrowns, injured shoot tips, and along lateral stem nodes, and was green but not robust. Leaf area was reduced and new stem growth appeared spindly. Very little growth was evident on plants treated with a 7-day exposure to concentrations of 2,300 and 4,600 $\mu g/\ell$. In fact, injury symptoms were still prevalent; stem necrosis was visible causing some stem breakage, and new growth that was present showed signs of chemical injury (as previously described). Plants exposed for 14 days to 1,150 and 2,300 $\mu g/\ell$ were unhealthy, with severe stem and leaf necrosis, stem breakage, and no sign of new shoot development.

29. Visual observations recorded 4 weeks following chemical application revealed that all treatments showed signs of recovery; new, normal-looking growth emerged from rootcrowns, injured shoot tips, floating plant segments (detached from decaying stems), and lateral nodes along stems. Results were similar to those observed in Study 1, in that regrowth occurred 1 to 2 weeks following removal of the chemically treated water.

30. At the conclusion of the experiment (6 weeks posttreatment), final biomass data showed that all treatments significantly reduced shoot and root growth (Figures 3 and 4). Compared with untreated plants, biomass reductions

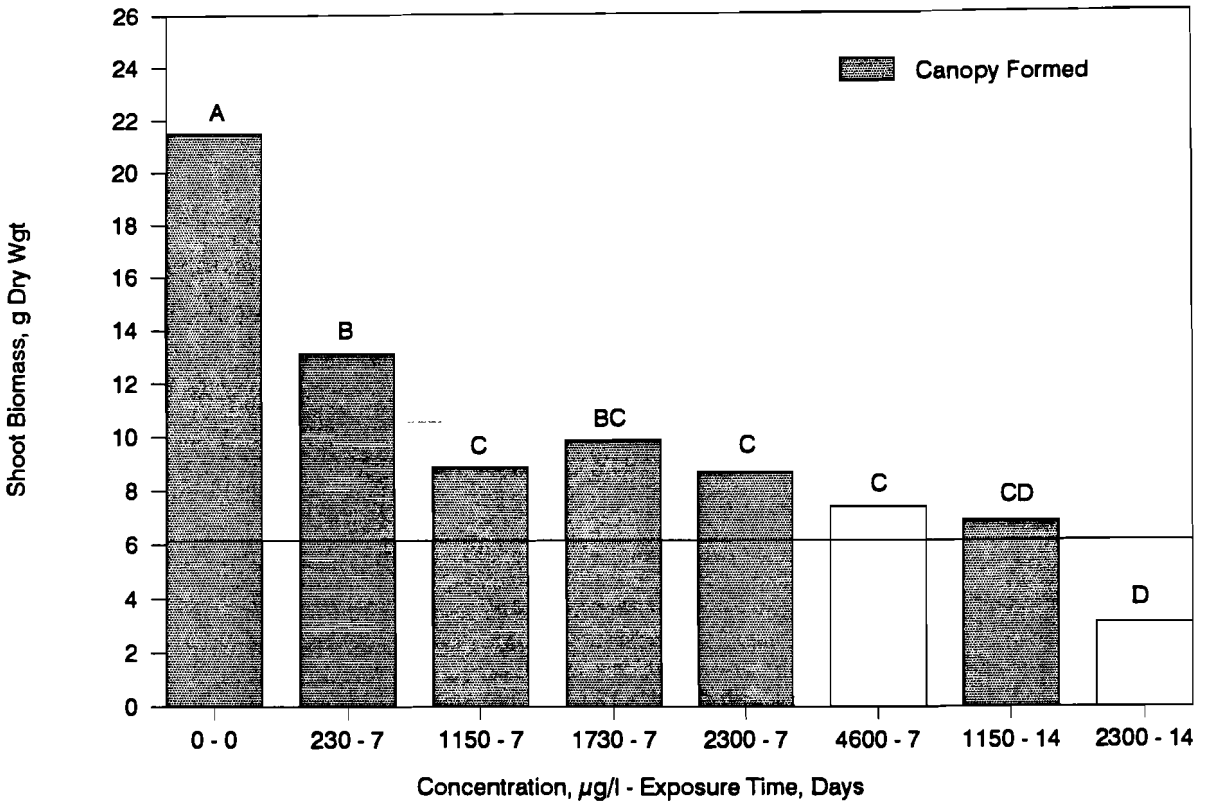


Figure 3. Effects of bensulfuron methyl on shoot biomass of Eurasian watermilfoil harvested at 6 weeks posttreatment. Pretreatment shoot biomass = 6.1 ± 1.8 g dry weight. Different letters among treatments indicate significant differences at the 5-percent level according to Duncan's Multiple Range Test

ranged from 39 to 86 percent for shoots and 43 to 73 percent for roots, with the most effective treatment being a 14-day exposure of 2,300 µg/ℓ of bensulfuron methyl. Root biomass decreased below pretreatment levels with several treatments, indicating root tissues were decaying. It should be noted that the degree of chemical activity or effectiveness was not proportional to increasing bensulfuron methyl concentrations, as most treatments were not significantly different from each other (e.g., 7-day exposure to 1,150 µg/ℓ versus 4,600 µg/ℓ). However, plants treated with the same chemical concentration (2,300 µg/ℓ), but exposed for different lengths of time (7 to 14 days), were significantly different. Thus, a longer exposure period was more efficacious than increasing bensulfuron methyl concentration. A flat response to increasing chemical concentration similar to that observed in this study has been observed with other sulfonylureas. Brewster and Appleby (1983) found

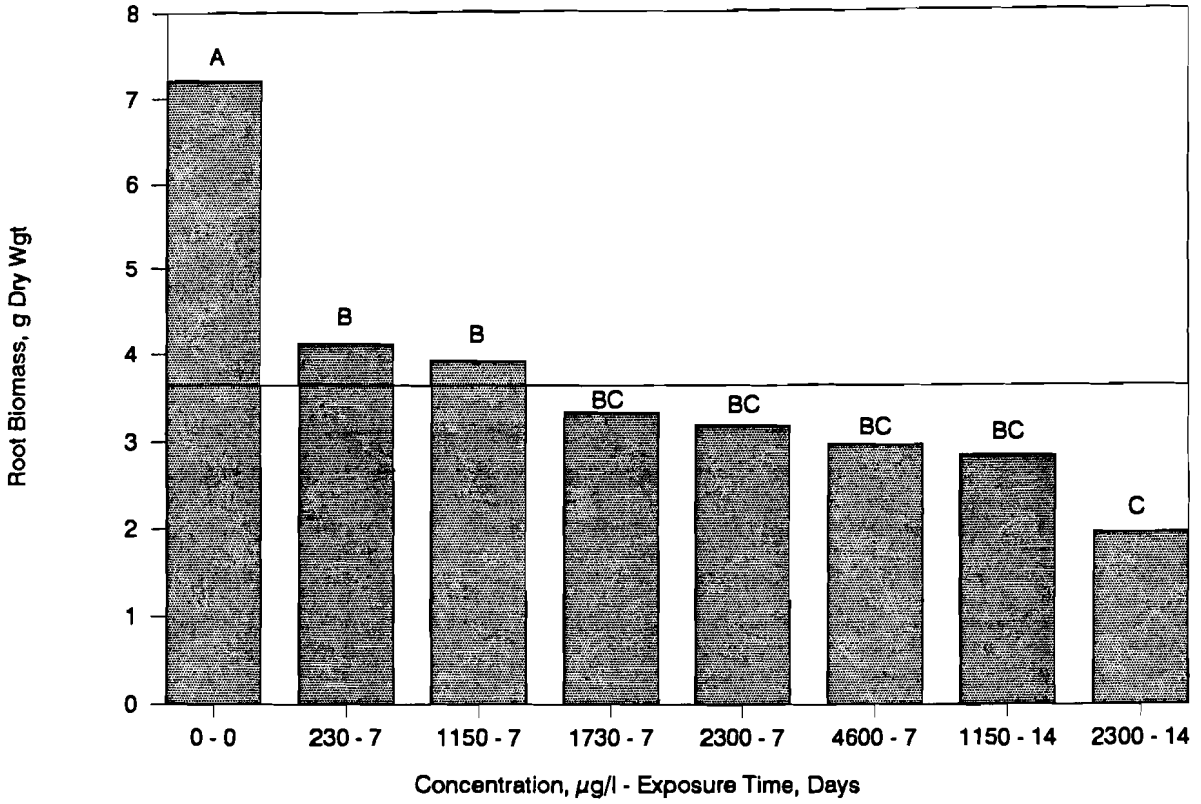


Figure 4. Effects of bensulfuron methyl on root biomass of Eurasian watermilfoil harvested at 6 weeks posttreatment. Pretreatment root biomass = 3.7 ± 1.3 g dry weight. Different letters among treatments indicate significant differences at the 5-percent level according to Duncan's Multiple Range Test

that increasing the concentration of chlorsulfuron (2-chloro-N-[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)amino]carbonyl]benzenesulfonamide) 16-fold (from 0.04 kg ai/ha to 0.56 kg ai/ha) did not further reduce wheat yields.

31. Despite significant differences in biomass production, plants had grown to the water surface (canopied) in all but two treatments (4,600 µg/ℓ at 7 days and 2,300 µg/ℓ at 14 days) by the end of the study. Extensive regrowth of milfoil to the water surface is neither desirable nor acceptable in field situations. Moreover, total plant control was not achieved even though the application rates ranged as high as 46 times the recommended label rate of 100 µg/ℓ. The ability of plants to recover from such high concentrations further suggests that milfoil may be capable of metabolizing bensulfuron methyl.

32. Results of Studies 1 and 2 show that bensulfuron methyl acts similarly to another aquatic herbicide, fluridone (1-methyl-3-phenyl-5-[3-(trifluoromethyl)phenyl]-4(1H)-pyridinone), in that both require long exposure or contact times to achieve efficacy. Hall, Westerdahl, and Stewart (1984) and Van and Conant (1988) found that a long exposure (several days) to low and high fluridone concentrations was necessary for control of hydrilla and milfoil. Field treatments designed to maintain low doses of fluridone over long periods of time have also shown excellent control of hydrilla and milfoil in river and lake systems in Florida and Washington (Getsinger, Fox, and Haller 1992). Van and Conant (1988) further state that systemic or translocated herbicides, such as fluridone, have much slower uptake rates than contact herbicides and thus require longer exposure times to be effective. Since bensulfuron methyl is also characterized as a systemic herbicide, the long contact times required for growth suppression in these studies was not surprising.

Study 3

33. Observations recorded 7 days after chemical treatment indicated that milfoil growth had slowed and plant injury was apparent. Injury symptoms were similar to those observed in Studies 1 and 2 and included deformed leaves on shoot tips, downward bending of leaves at upper nodes, some stem necrosis, and formation of lateral buds along stem lengths. There was no visual difference in the degree of injury between plants treated with 50 $\mu g/\ell$ and 150 $\mu g/\ell$. One week later, the number of stems with necrotic lesions and lateral buds had increased. By the next evaluation period (21 days posttreatment), insect damage was evident on plants in all treatments. Damage (stem boring) was confined to shoots lying on the water surface and only impacted upper stem integrity. An unidentified, small, black beetle was found, and malathion was applied at this time. Malathion controlled beetle populations without causing additional injury to the milfoil.

34. The first sign of recovery from bensulfuron methyl treatment was noted 21 days posttreatment on plants exposed for 7 and 14 days to all chemical concentrations. Lateral shoots developed and new growth appeared normal. One week later, regrowth from lateral buds was so extensive in these treatments that, visually, they could not be distinguished from untreated plants. As in previous studies recovery of other treatments occurred 1 to 2 weeks following completion of the exposure period and removal of chemically treated

water from the system. Even plants exposed to bensulfuron methyl concentration of 150 $\mu g/\ell$ for 42 days supported new growth by 14 days posttreatment.

35. Biomass data collected at the conclusion of this study revealed that changes in shoot biomass were highly correlated with exposure time (Figure 5). Statistical comparison of regression coefficients (t-test)

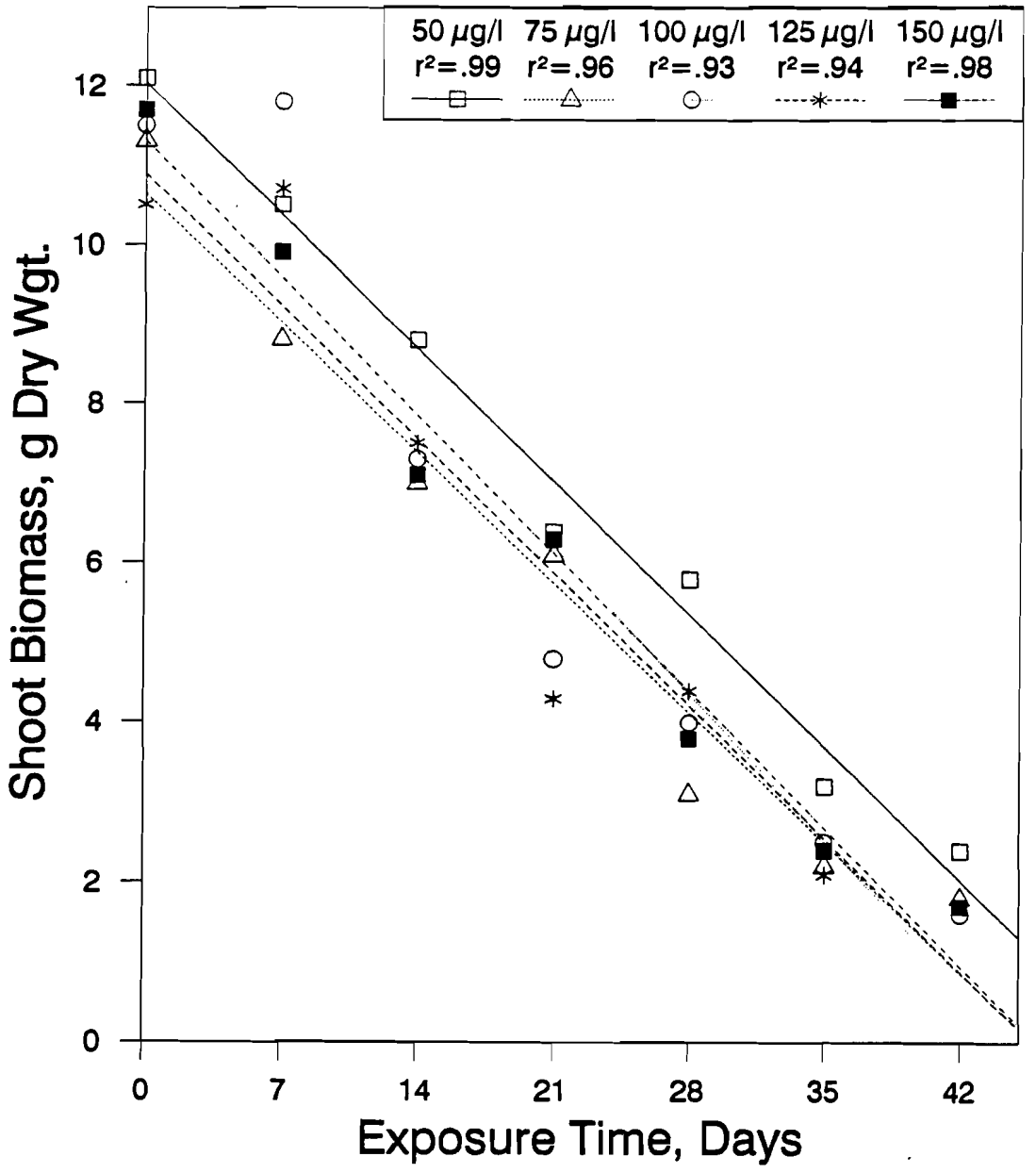


Figure 5. Effects of bensulfuron methyl on shoot biomass of Eurasian watermilfoil harvested at 8 weeks posttreatment. Pretreatment shoot biomass = 4.4 ± 0.5 g dry weight. Equations for regression lines are as follows: 50 $\mu g/\ell$, y = 12.2 - 0.238x; 75 $\mu g/\ell$, y = 10.93 - 0.239x; 100 $\mu g/\ell$, y = 11.96 - 0.271x; 125 $\mu g/\ell$, y = 11.0 - 0.239x; 150 $\mu g/\ell$, y = 11.4 - 0.251x

indicated that linear relationships between biomass and exposure time were the same at all chemical concentrations. Compared with untreated plants, shoot biomass production decreased by an average of 10 to 86 percent as exposure time to bensulfuron methyl concentrations increased from 7 to 42 days. Results agree with data from Studies 1 and 2 and add support to the finding that longer contact time is more important than increasing bensulfuron methyl concentration in suppressing milfoil growth. Root growth showed no linear relationship to exposure period or chemical concentration (Figure 6).

Study 4

36. Milfoil grown from 7-day-old apical cuttings showed signs of growth inhibition 1 week following treatment with 50 $\mu g/\ell$ of bensulfuron methyl. Injury symptoms persisted through 42 days posttreatment with several stems showing severe necrosis and/or complete plant death. Regrowth did not begin until 49 and 63 days after treatment for plants exposed to bensulfuron methyl for 28 and 49 days, respectively. Regrowth was not as vigorous as in previous studies, indicating that younger plants (7-day-old apical cuttings) may be more sensitive to bensulfuron methyl treatment. Established milfoil (3 weeks old) used in Studies 1 through 3 also had expressed injury symptoms and biomass reductions as a result of chemical treatment, but regrowth was so extensive (even at rates as high as 2,300 $\mu g/\ell$), that many treatments were considered inadequate.

37. Effects on biomass production were also greater than in previous studies. Final biomass data measured 11 weeks posttreatment revealed that both treatments significantly reduced shoot and root biomass production by an average of 90 and 75 percent, respectively, compared with untreated plants (Figure 7). Anderson (1988) also showed large decreases (up to 70 percent) in biomass production when treating 7-day-old milfoil shoots grown from apical cuttings. Contrary to results gathered in Studies 1 through 3 was the fact that, in this study, no statistical differences were observed between the 28- and 49-day exposure periods, again suggesting that younger plants were extremely sensitive to prolonged contact with bensulfuron methyl.

38. Results were similar among 7-day-old plants initiated from 3-month-old rootcrowns (Figure 8). Untreated plants had developed a canopy of vegetation 28 days after study initiation, whereas growth of treated plants was suppressed shortly after chemical treatment and did not generate any new growth until 63 days posttreatment. Biomass data collected at the conclusion of the study (11 weeks posttreatment) showed that shoot and root growth of

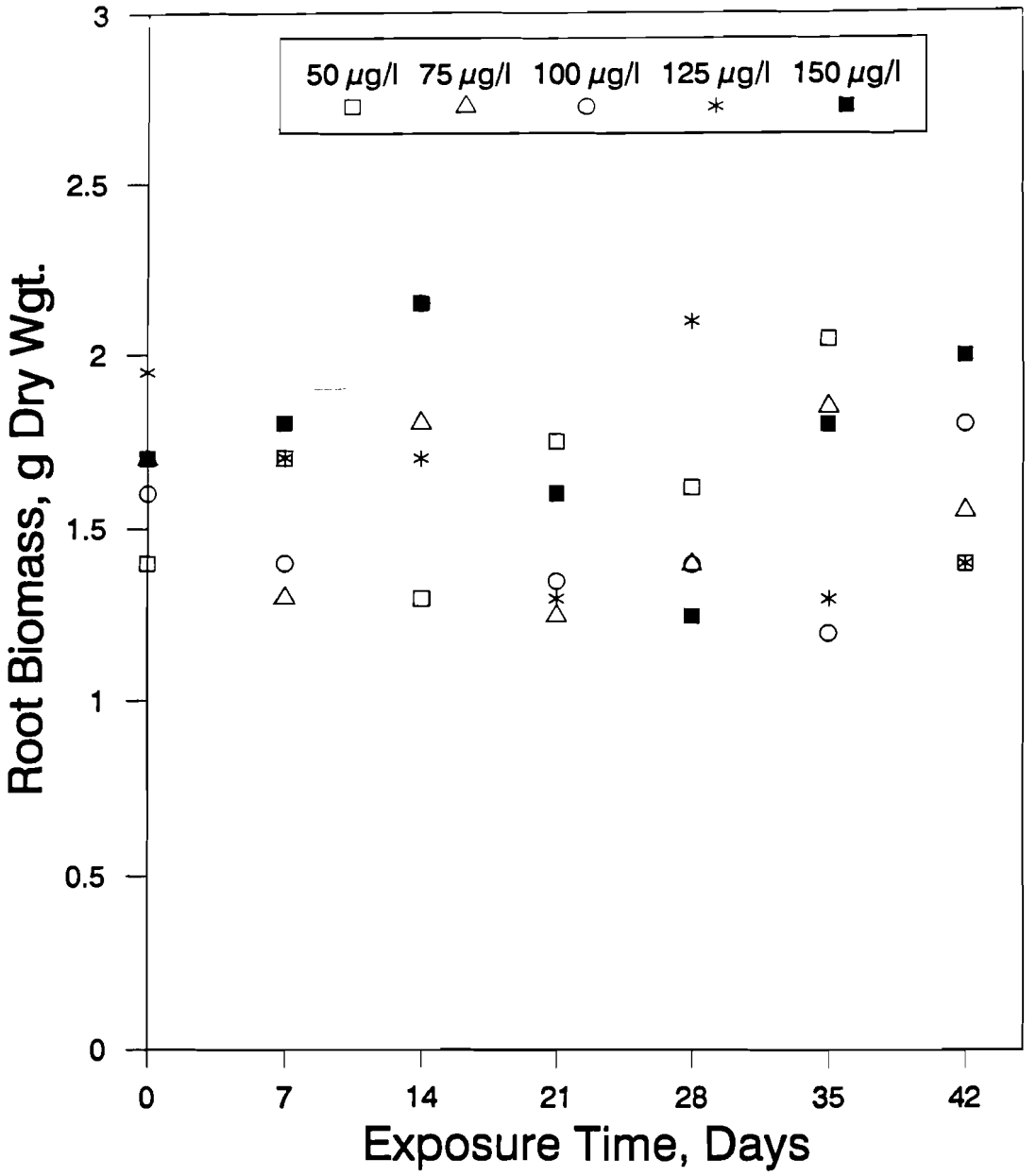


Figure 6. Effects of bensulfuron methyl on root biomass of Eurasian watermilfoil harvested at 8 weeks posttreatment. Pretreatment root biomass = 1.1 ± 0.3 g dry weight. Regression analysis showed no linear relationship between root biomass and exposure time

bensulfuron methyl-treated plants was significantly reduced compared with untreated plants. The average decrease in shoot and root growth was 90 and 97 percent, respectively. There was no significant difference between the two bensulfuron methyl treatments. Observation of root biomass at the time of harvest revealed that the root system of chemically treated plants had been

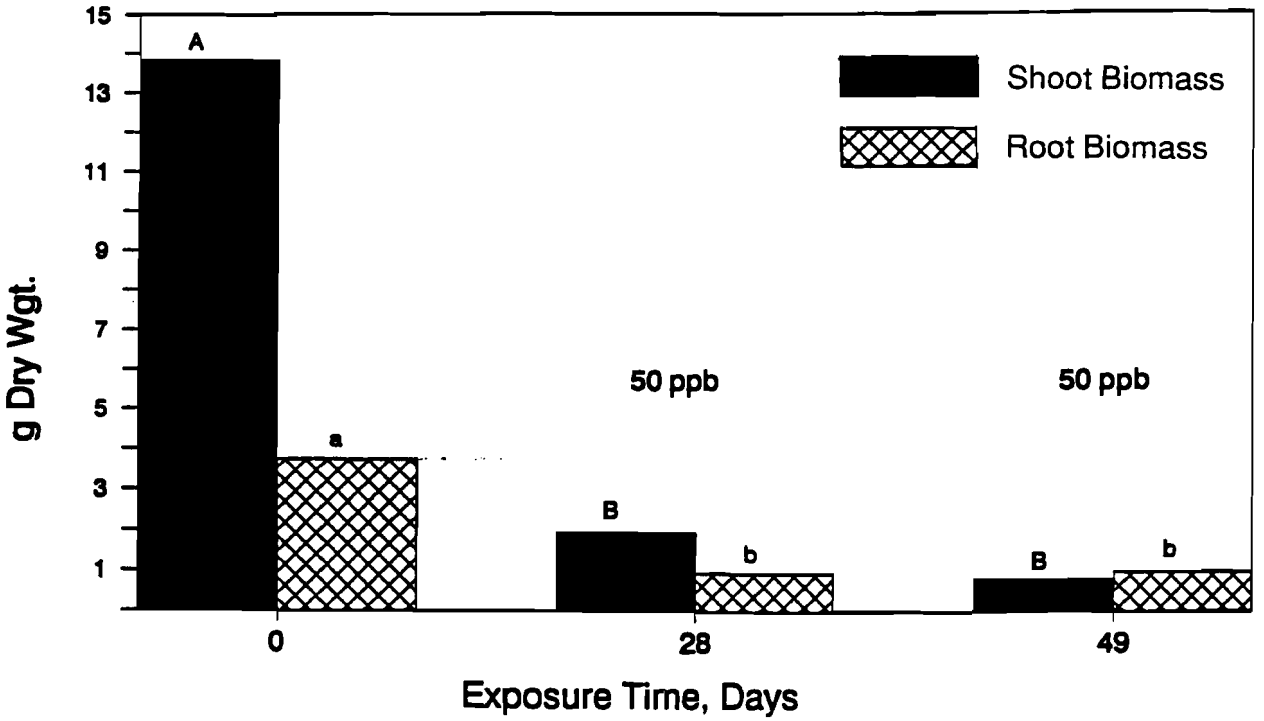


Figure 7. Effects of bensulfuron methyl on shoot and root biomass of Eurasian watermilfoil harvested at 11 weeks posttreatment. Plants were grown from apical cuttings and allowed a 7-day pretreatment growth period. Pretreatment shoot and root biomass was 0.76 ± 0.14 g dry weight and 0.04 ± 0.02 g dry weight, respectively. Different letters among treatments indicate significant differences at the 5-percent level according to Duncan's Multiple Range Test

severely depleted during the course of the experiment. Root biomass had decreased from 4.4 g DW (measured at pretreatment) to less than 1 g DW at the conclusion of the study. It is possible that there was not enough aboveground biomass on plants grown for only 7 days prior to treatment to support such a large root system, and, consequently, roots decayed. Again, results suggest that bensulfuron methyl is more effective on milfoil when applied to young shoot tissue.

Conclusions

39. Results of these studies show that the bensulfuron methyl is effective at reducing the growth of Eurasian watermilfoil; however, complete plant control (total plant death) was not achieved at the rates and exposure times

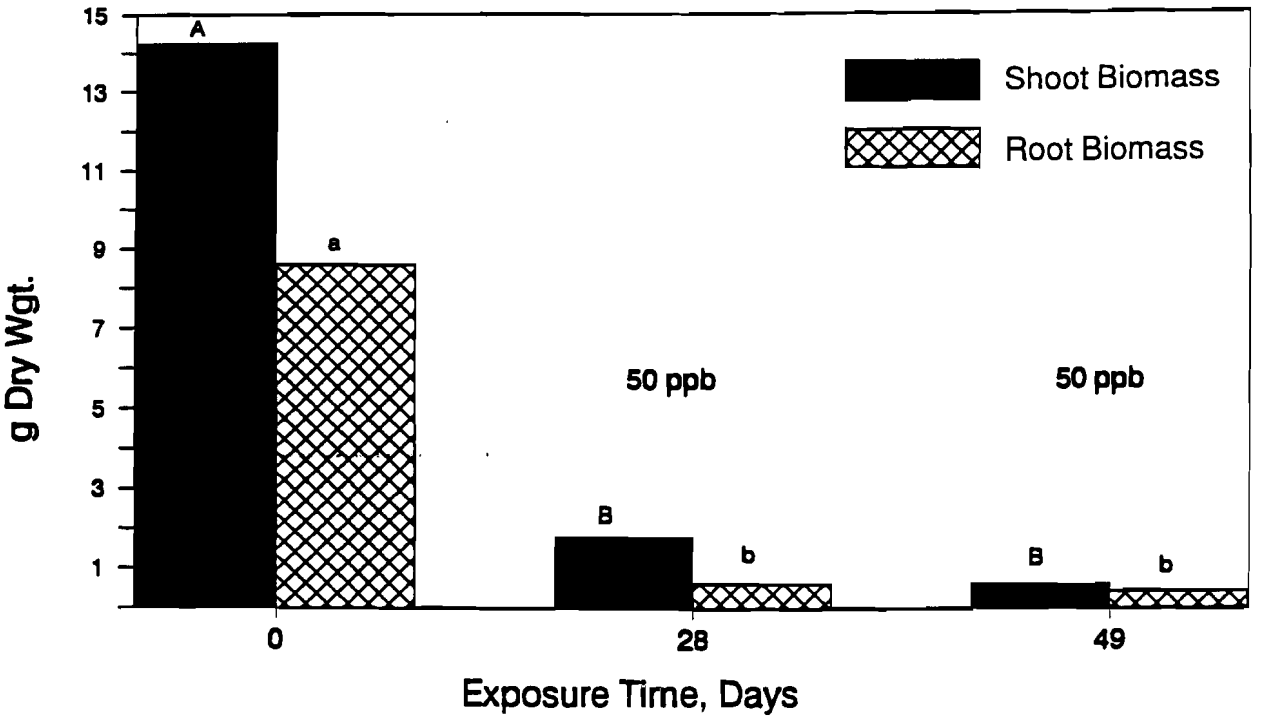


Figure 8. Effects of bensulfuron methyl on shoot and root biomass of Eurasian watermilfoil harvested at 11 weeks posttreatment. Plants were grown from 3-month-old root-crowns and allowed a 7-day pretreatment growth period. Pretreatment shoot and root biomass was 1.10 ± 0.21 g dry weight and 4.40 ± 0.87 g dry weight, respectively. Different letters among treatments indicate significant differences at the 5-percent level according to Duncan's Multiple Range Test.

tested. Increasing exposure time was more efficacious than increasing bensulfuron methyl concentration. Contact time was also critical for maintaining growth suppression, as plants showed strong regrowth 1 to 2 weeks after exposure to bensulfuron methyl was terminated. Results also indicate that bensulfuron methyl applications to mature plant stands will not be as effective as applications to young, germinating plants.

Recommendations

40. Based on the results of these studies, the following recommendations are made:

a. Further studies evaluating bensulfuron methyl on Eurasian watermilfoil should be conducted in outdoor, mesocosm systems

to simulate fieldlike conditions. Test protocols should investigate the effectiveness of long exposure periods (21 days and longer) to bensulfuron methyl rates of 25 $\mu g/\ell$ and higher. Studies should also evaluate the effectiveness and feasibility of sequential applications of bensulfuron methyl.

b. Additional studies to evaluate the selectivity of bensulfuron methyl on desirable, native aquatic species should be conducted.

c. Further field evaluations of bensulfuron methyl efficacy should be conducted to validate laboratory and mesocosm results.

d. Studies using bensulfuron methyl in combination with a biocontrol agent as an integrated management approach for controlling nuisance aquatic plant species should be initiated. The difficulty with the use of some biological control agents is that growth rate of the target plant species often exceeds the growth and reproductive rate of the biological control agent (e.g., insects). Using bensulfuron methyl to slow plant growth may allow the necessary time needed for insect populations to establish, as well as make target plants more susceptible to predation.

References

Anderson, L. 1988. "Growth Regulator Activity of Bensulfuron Methyl in Aquatic Plants," Chemical Vegetation Management, J. E. Kaufman and H. E. Westerdahl, eds., Plant Growth Regulator Society of America, San Antonio, TX, pp 127-145.

Beyer, E. M., Duffy, M. J., Hay, J. V., and Schlueter, D. D. 1988. "Sulfonylurea Herbicides," Herbicides: Chemistry, Degradation, and Mode of Action, Vol 3, P. C. Kearney, ed., Marcel Dekker, Inc., pp 117-189.

Brewster, B. D., and Appleby, A. P. 1983. "Response of Wheat (*Triticum aestivum*) and Rotation Crops to Chlorsulfuron," Weed Science, Vol 31, pp 861-865.

E.I. du Pont de Nemours & Co. 1988. "Londax for Rice," Du Pont Technical Bulletin, Wilmington, DE.

Fox, A. M., Haller, W. T., and Getsinger, K. D. 1991. "Factors that Influence Water Exchange in Spring-fed Tidal Canals," Estuaries, Vol 14, pp 404-413.

Getsinger, Kurt D., Fox, Allison M., and Haller, William T. 1992. "Controlling Submersed Plants With Herbicides in Flowing Water Systems," Proceedings, 26th Annual Meeting, Aquatic Plant Control Research Program, Miscellaneous Paper A-92-2, US Army Engineer Waterways Experiment Station, Vicksburg, MS, pp 103-105.

Getsinger, Kurt D., Green, W. Reed, and Westerdahl, Howard E. 1990. "Characterization of Water Movement in Submersed Plant Stands," Miscellaneous Paper A-90-5, US Army Engineer Waterways Experiment Station, Vicksburg, MS.

Getsinger, K. D., Sprecher, S. L., Langeland, K. A., Haller, W. T., Fox, A. M., and Joyce, J. C. 1992. "Field Dissipation Studies of Bensulfuron Methyl for Aquatic Uses and Aquatic Impact Uses and Field Accumulation Studies of Bensulfuron Methyl for Aquatic Non-Target Organisms," Du Pont Report No. AMR-1168-88, Wilmington, DE.

Green, W. R., and Westerdahl, H. E. 1990. "Response of Eurasian Watermilfoil to 2,4-D Concentrations and Exposure Times," Journal of Aquatic Plant Management, Vol 28, pp 27-32.

Hall, Jerry F., Westerdahl, Howard E., and Stewart, Troy J. 1984. "Growth Response of *Myriophyllum spicatum* and *Hydrilla verticillata* When Exposed to Continuous, Low Concentrations of Fluridone," Technical Report A-84-1, US Army Engineer Waterways Experiment Station, Vicksburg, MS.

Haller, W. T., Fox, A. M., and Hanlon, C. A. 1992. "Inhibition of Hydrilla Tuber Formation by Bensulfuron Methyl," Journal of Aquatic Plant Management, Vol 30, pp 48-49.

Klaine, S. J., and Knowles, B. J. 1988. "Plant Growth Regulators: Strategy for Use in Aquatic Plant Management Schemes," Chemical Vegetation Management, J. E. Kaufman and H. E. Westerdahl, eds., Plant Growth Regulator Society of America, San Antonio, TX, pp 102-115.

Langeland, K. A. 1992. "Field Dissipation (Closed Pond) Studies of Bensulfuron Methyl for Aquatic Uses and Aquatic Impact Uses and Field Accumulation (Closed Pond) Studies of Bensulfuron Methyl for Aquatic Non-Target Organisms," Du Pont Report No. AMR-1350-88, Wilmington, DE.

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

Netherland, M. D., and Getsinger, K. D. 1992. "Efficacy of Triclopyr on Eurasian Watermilfoil: Concentration and Exposure Time Effects," Journal of Aquatic Plant Management, Vol 30, pp 1-5.

Netherland, M. D., Green, W. R., and Getsinger, K. D. 1991. "Endothall Concentration and Exposure Time Relationships for the Control of Eurasian Watermilfoil and Hydrilla," Journal of Aquatic Plant Management, Vol 29, pp 61-67.

Pringle, J. C., and Sisneros, D. 1992. "Field Dissipation Studies of Bensulfuron Methyl for Aquatic Uses and Aquatic Impact Uses and Field Accumulation Studies of Bensulfuron Methyl for Aquatic Non-Target Organisms," Du Pont Report No. AMR-1169-88, Wilmington, DE.

Smart, R. M., and Barko, J. W. 1984. "Culture Methodology for Experimental Investigations Involving Rooted Submersed Aquatic Plants," Miscellaneous Paper A-84-6, US Army Engineer Waterways Experiment Station, Vicksburg, MS.

Van Thai, K., and Conant, Richard D., Jr. 1988. "Chemical Control of Hydrilla in Flowing Water: Herbicide Uptake Characteristics and Concentrations versus Exposure," Technical Report A-88-2, US Army Engineer Waterways Experiment Station, Vicksburg, MS.

Van, T. K., and Vandiver, V. V. 1992. "Response of Monoecious and Dioecious Hydrilla to Bensulfuron Methyl," Journal of Aquatic Plant Management, Vol 30, pp 41-44.

Weed Science Society of America. 1989. Herbicide Handbook, 6th ed., pp 31-32.

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	January 1993	Final report

4. TITLE AND SUBTITLE

Efficacy of Bensulfuron Methyl on Eurasian Watermilfoil

5. FUNDING NUMBERS

6. AUTHOR(S)

Linda S. Nelson
Michael D. Netherland

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

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

8. PERFORMING ORGANIZATION REPORT NUMBER

Technical Report
A-93-2

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)

US Army Corps of Engineers, Washington, DC 20314-1000

10. SPONSORING/MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

Available from National Technical Information Service, 5285 Port Royal Road, Springfield, VA 22161

12a. DISTRIBUTION/AVAILABILITY STATEMENT

Approved for public release; distribution is unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200 words)*

Studies to evaluate the efficacy of bensulfuron methyl on Eurasian watermilfoil (*Myriophyllum spicatum* L.) were conducted in a 55-ℓ aquaria system under controlled-environment conditions. Forty-two bensulfuron methyl concentration and exposure time combinations were tested: concentrations ranged from 0 to 4,600 µg/ℓ; exposure times ranged from 7 to 49 days. Chemical effectiveness was based on shoot and root biomass harvested at the conclusion of each experiment. Visual injury ratings were recorded weekly to characterize chemical activity. Herbicide injury was evident 1 week after application on all treatments and included one or more of the following symptoms: leaf chlorosis, deformed leaves on shoot tips, downward bending of leaves at upper nodes, stem necrosis, and formation of axillary buds. Following a 7-, 14-, 21-, 28-, 35-, 42-, and 49-day exposure period at concentrations ranging from 10 to 4,600 µg/ℓ, plant biomass was reduced 10 to 90 percent compared with untreated plants. Increasing

(Continued)

14. SUBJECT TERMS		15. NUMBER OF PAGES
Bensulfuron methyl	Exposure time	25
Biomass	Herbicide	16. PRICE CODE
Eurasian watermilfoil	Sulfonylurea	

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
UNCLASSIFIED	UNCLASSIFIED		

13. (Concluded)

exposure time was more efficacious than increasing bensulfuron methyl concentration. Younger milfoil plants (7 days old) were also susceptible to bensulfuron methyl application than plants that were allowed a 3-week pretreatment growth period. Bensulfuron methyl concentrations <10 $\mu g/\ell$ did not significantly reduce milfoil growth. Effects on roots were variable depending on concentration and plant age. Only treatments of 230 $\mu g/\ell$ and higher inhibited root growth on plants grown for 3 weeks prior to treatment, whereas 50 $\mu g/\ell$ was sufficient to reduce root growth of younger plants. Regrowth was observed on all treatments, indicating the potential for plant recovery. Regrowth emerged from rootcrowns, axillary buds, and injured shoot apices and was evident 1 to 2 weeks following completion of the exposure period and removal of the bensulfuron-methyl-treated water. Complete plant control (plant death) was not achieved at the concentrations and exposure times tested in these studies.