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Bensulfuron Methyl Activity on Eurasian Watermilfoil[1]

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ABSTRACT

The efficacy of bensulfuron methyl (BSM) at 40 concentration and exposure time combinations was evaluated on Eurasian watermilfoil (*Myriophyllum spicatum* L.) under controlled-environment conditions. BSM concentrations ranged from 0 to 4600 µg/l; exposure times ranged from 7 to 42 days. Efficacy was based on shoot and root biomass harvested at the conclusion of each experiment. Herbicide injury was evident 1 week after application on all treatments, and symptoms included leaf chlorosis, deformed leaves on shoot tips, downward bending of leaves at upper nodes, stem necrosis, and formation of axillary buds. Following 7-, 14-, 21-, 28-, 35-, and 42-day exposure periods at concentrations ranging from 10 to 4600 µg/l, biomass was reduced 10 to 90% compared to untreated plants. Increasing exposure time was more efficacious than increasing concentration. BSM concentrations <10 µg/l did not significantly reduce growth. Effects on roots were variable depending on concentration and plant age. Only treatments of 230 µg/l and higher inhibited root growth on plants grown for 3 weeks prior to treatment. 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 BSM-treated water. Plant death was not achieved at the concentrations and exposure times tested in these studies.

Key words: herbicide, sulfonylurea, exposure time, *Myriophyllum spicatum* L.

INTRODUCTION

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. Bensulfuron methyl (methyl 2-[[[[[(4,6-dimethoxy-2-pyrimidinyl)amino]carbonyl]amino]sulfonyl] methyl]benzoate) is currently registered as Londax® herbicide for use in rice production. However, recent studies have demonstrated efficacy on several aquatic plant species including hydrilla (*Hydrilla verticillata* Royle), Eurasian watermilfoil, and several species of *Potamogeton* (Anderson 1988, Haller *et al.* 1992, Van and Vandiver 1992). Large-scale evaluations for use in aquatic systems have been conducted under an Experimental Use Permit issued by the U.S. Environmental Protection Agency (Getsinger *et al.* 1992b, Langeland 1992, Pringle and Sisneros 1992).

Bensulfuron methyl (BSM) is a member of the sulfonylurea herbicide group which is characterized by high levels of activity at application rates as low as 0.002 kg/ha (0.03 oz/acre). Plant uptake of BSM occurs readily through roots and foliage, and once inside the plant, is translocated via the xylem and phloem. The mode of action of BSM is inhibition of the plant enzyme acetolactate synthase (ALS), which is necessary for the synthesis of two essential amino acids, valine and isoleucine (Beyer *et al.* 1988). Visual symptoms of plant injury (chlorosis, leaf bending and curling, leaf discoloration due to enhanced anthocyanin production, and necrosis) usually appear within 1 to 2 days posttreatment (Du Pont 1988). Although growth cessation immediately follows treatment with BSM, plant death occurs gradually as plants utilize and eventually deplete internal carbohydrate reserves.

The fact that BSM affects a plant enzyme system that is nonexistent in animals helps explain its low toxicity to nontarget organisms. Although the ALS enzyme is present in all plants, not all species are highly susceptible indicating some degree of selectivity to BSM. Tolerant plants (*e.g.*, rice), can quickly metabolize the active ingredient to herbicidally inactive compounds, whereas susceptible species cannot. In addition, the range in sensitivity to BSM among plants is wide. Beyer *et al.* (1988) reported that the differential sensitivity of plants to sulfonylurea herbicides can be over 1000-fold. As a potentially selective herbicide, BSM could benefit management strategies in which the objective of the treatment is to control a target species with minimal harm to desirable native species. Furthermore, sulfonylureas exhibit growth-regulating effects on plants when applied at sublethal concentrations. The potential benefits of growth regulation versus the complete removal of plant biomass (as with a herbicide) in an aquatic system have been suggested by several researchers (Anderson 1988, Klaine 1988, Lembi and Netherland 1990).

Understanding the relationship between rate of application and length of time a chemical is in contact with a target plant species is also important to achieve desired plant control. This is especially critical in systems where water flow and thermal- and wind-induced circulation patterns influence

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herbicide dispersion and, consequently, treatment performance (Getsinger *et al.* 1990 and Fox *et al.* 1991). Concentration/exposure time (CET) relationships have been described for several aquatic herbicides and can be helpful in predicting treatment success under field conditions (Hall *et al.* 1984, Van and Conant 1988, Green and Westerdahl 1990, Netherland *et al.* 1991, Netherland and Getsinger 1992).

To date, most of the BSM research conducted on aquatic plants has focused on hydrilla. Several investigators have observed reduced shoot growth and tuber formation of hydrilla following treatment with BSM (Anderson 1988, Haller *et al.* 1992, Van and Vandiver 1992). Reduced hydrilla reproduction by germinating tubers and turions was also observed under field conditions (Haller *et al.* 1992).

Investigations concerning the effectiveness of BSM on 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 BSM on the growth of milfoil.

MATERIALS AND METHODS

Three separate experiments were conducted in two similar laboratory systems at the U.S. Army Engineer Waterways Experiment Station (WES), Vicksburg, MS. The system used for Studies 1 and 2 consisted of twenty-four, 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) was 450 ± 50 $\mu E/m^2/sec$, with a photoperiod of 13:11 hr. Water temperature was maintained at 25 ± 3C throughout both experiments.

Study 3 was conducted in a controlled-environment growth chamber equipped with thirty-six 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.

Sediment for all studies was collected from Brown's Lake at the WES, and was amended with commercially available fertilizers (Ra-pid-gro, 20:15:15, and Osmocote, 15:15:15) 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. Beakers (300 ml) 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.

BSM stock solutions used for all treatments were prepared from the commercial formulation Londax® (dry flowable, 60% active ingredient). All treatment concentrations are reported as µg/l (ppb) 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 BSM stock solution were added to aquaria to provide 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% of BSM residues were removed following the drain procedure (data not presented).

Treatments (concentration x exposure time) evaluated in these studies are summarized in Table 1. Studies 1 and 2 each

TABLE 1. BENSULFURON METHYL TREATMENT RATES (µg/l), EXPOSURE TIME (days), AND CONCENTRATIONS (µg/l) IN EXPERIMENTAL TANKS AT 7 DAYS POSTTREATMENT.

Study	Target rate concentration	Exposure	Concentration at 7 days posttreatment
1	0 (untreated)	0	0.0
	50	14	52.1
	75	14	78.4
	5	21	ND[1]
	10	21	ND[1]
	25	21	—[2]
	50	21	—[2]
	5	28	—[2]
2	0	0	0.0
	230	7	210.3
	1150	7	1090.1
	1730	7	1611.0
	2300	7	2401.7
	4600	7	4560.3
	1150	14	—[2]
	2300	14	—[2]
3	0	7, 14, 21, 28, 35, 42	0.0
	50	same as above	52.9
	75	same as above	79.3
	100	same as above	102.5
	125	same as above	118.3
	150	same as above	148.0

[1]ND = not detected, both the 5- and 10-µg/l treatment concentration were below analytical detection limits by 7 days after treatment.
[2]No sample.

consisted of eight BSM CET treatments, ranging from very low to extremely high application rates. Each treatment was replicated three times and randomly assigned to a test aquarium. Beakers planted with milfoil were placed in each aquarium (11 beakers/aquarium in Study 1 and 9 beakers/aquarium in Study 2) 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 BSM on small shoots supported by a healthy root system.

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

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 (70C for 48 hr) to a constant weight. Shoot and root biomass data were subjected to analysis of variance (ANOVA) and treatment effects separated using Waller-Duncan *k*-ratio *t* Test. Weekly visual observations were also recorded to characterize the initial plant response to BSM treatment, the progression of injury symptoms, and the initiation of regrowth.

Study 3 consisted of five BSM concentrations ranging from 50 to 150 µg/l, subjected to a series of exposure times ranging from 0 to 42 days. Treatments were not replicated; however, 36 different CET 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.

At 8 weeks posttreatment, plants were harvested, and roots and shoots were separated and dried. Linear regression procedures were used to relate plant biomass to increased exposure times at each BSM treatment rate tested. Visual ratings of plant injury were recorded weekly.

RESULTS AND DISCUSSION

Study 1. Five treatments significantly reduced milfoil shoot biomass in Study 1 (Figure 1). Reductions ranged from 26 to 69% when compared to untreated plants, with the most effective treatment being a 21-day exposure to 50 µg/l BSM. Higher concentrations at shorter exposure periods were less effective, suggesting that contact time is an important factor in determining treatment success.

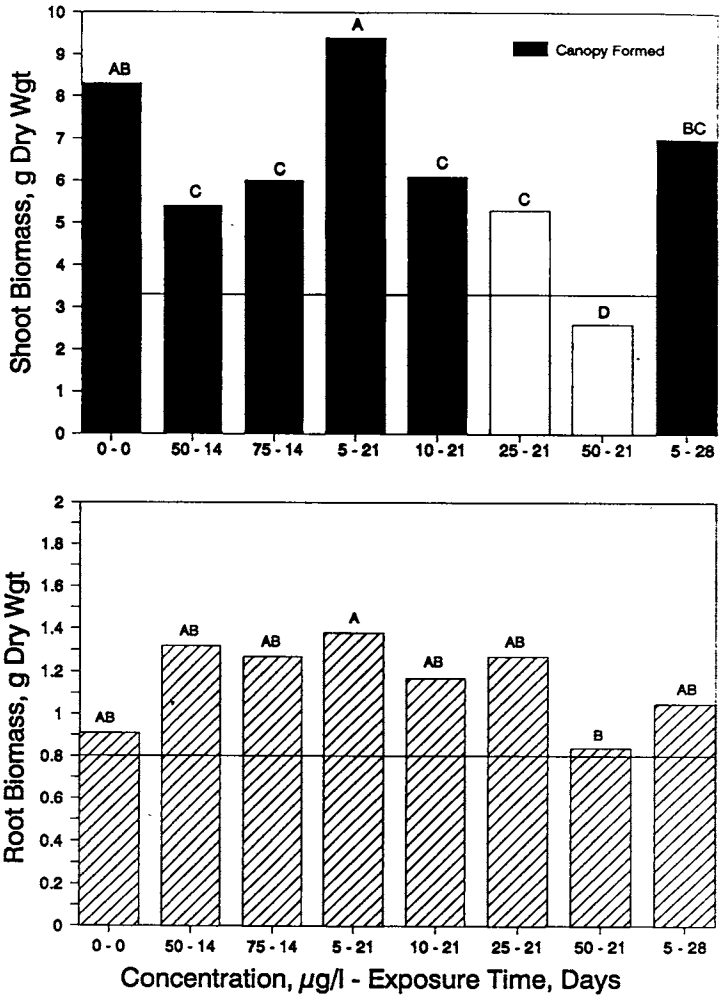

Figure 1. Effects of bensulfuron methyl on shoot and root biomass of Eurasian watermilfoil harvested at 5 weeks posttreatment. Horizontal lines represent pretreatment biomass; shoots = 3.4 ± 1.5 g DW, roots = 0.8 ± 0.3 g DW. Different letters among treatments indicate significant differences at the 5% level according to Waller-Duncan *k*-ratio *t* Test. For shoot biomass, unfilled bars represent those treatments in which a vegetative canopy did not form.

Two treatments, 21- and 28-day exposures to 5 µg/l, 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, indicating that under these experimental conditions, milfoil was tolerant to low doses of BSM. Similarly, milfoil treated with 10 µg/l and exposed for 21 days, exhibited active growth while in contact with BSM; however, final biomass was significantly reduced by 26%. Active growth during treatment further suggests that at low concentrations (<10 µg/l), milfoil can metabolize BSM quickly enough to prevent complete inhibition of the ALS

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 root crowns, lateral buds along stem nodes, and injured apical shoots, and was evident 1 to 2 weeks following removal of BSM from the water column.

Initial injury symptoms observed on milfoil treated with BSM concentrations of 25 µg/l 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 BSM.

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 µg/l and those treated with 75 µg/l.

By the end of 5 weeks, regrowth was observed in all treatments, and plants had grown to the water surface in all but two treatments (25 and 50 µg/l at 21-day exposures). Although the final data showed significant reductions in biomass, canopy formation showed strong regrowth potential indicating an inadequate treatment. Despite slight increases in root biomass with several treatments, no significant differences in root growth were observed compared to untreated plants (Figure 1).

Results of this study differ from studies by Anderson (1988), in which milfoil shoot and root DW were reduced by 50 to 70% and 40 to 77%, respectively, after a 4-week exposure to BSM concentrations of 1 to 20 µg/l. 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). Additional studies conducted in our laboratory indicate young milfoil plants (7 day-old apical cuttings) were much more sensitive to BSM than plants allowed to grow for 21 days pretreatment (data not presented). Other studies have also reported increased efficacy with BSM on younger plants. Haller *et al.* (1992) observed that in the field, hydrilla sprouting from tubers and turions was more susceptible to BSM than mature plants.

Under our experimental conditions, an exposure period of 21 days to concentrations of 25 to 50 µg/l 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 and that given more time (1 to 2 weeks), new growth would have probably reached the water surface on these treatments.

Study 2. Initial response of milfoil to all BSM treatments was evident 1 week after application and symptoms included reddening of shoot tips, downward bending of upper leaves, and bunched or compacted leaves at shoot apices. Effects were more pronounced with increasing chemical concentration.

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, 1150, and 1730 µg/l of BSM. New growth emerged from rootcrowns, injured shoot tips, and along lateral stem nodes, and was green but not robust. Leaves were visually smaller than those of untreated plants and new stem growth was spindly. Very little growth was evident on plants treated with a 7-day exposure to concentrations of 2300 and 4600 µg/l. In fact, injury symptoms were still prevalent and necrosis was visible causing some stem breakage. New growth that was present showed signs of chemical injury (as previously described). Plants exposed for 14 days to 1150 and 2300 µg/l were unhealthy, with severe stem and leaf necrosis, stem breakage, and no sign of new shoot development.

Visual observations recorded at 4 weeks posttreatment revealed that all treatments showed signs of recovery; new growth emerged from rootcrowns, injured shoot tips, floating plant segments (detached from decaying stems), and lateral nodes along stems. Similar to the first study regrowth occurred 1 to 2 weeks following removal of the chemically treated water.

At the conclusion of the experiment (6 weeks posttreatment), final biomass data showed that all treatments significantly reduced shoot and root growth (Figure 2). Compared to untreated plants, biomass reductions ranged from 39 to 86% for shoots and 43 to 73% for roots, with the most effective treatment being a 14-day exposure to 2300 µg/l of BSM. 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 BSM concentrations, as most treatments were not significantly different from each other (*e.g.*, 7-day exposure to 1150 µg/l versus 4600 µg/l). However, plants treated with the same chemical concentration (2300 µg/l) but exposed for different lengths of time (7 and 14 days) were significantly different. Thus, a longer exposure period was more efficacious than increasing BSM concentration. A flat response to increasing sulfonylurea concentration, similar to that observed in this study, has been observed by Brewster and Appleby (1983).

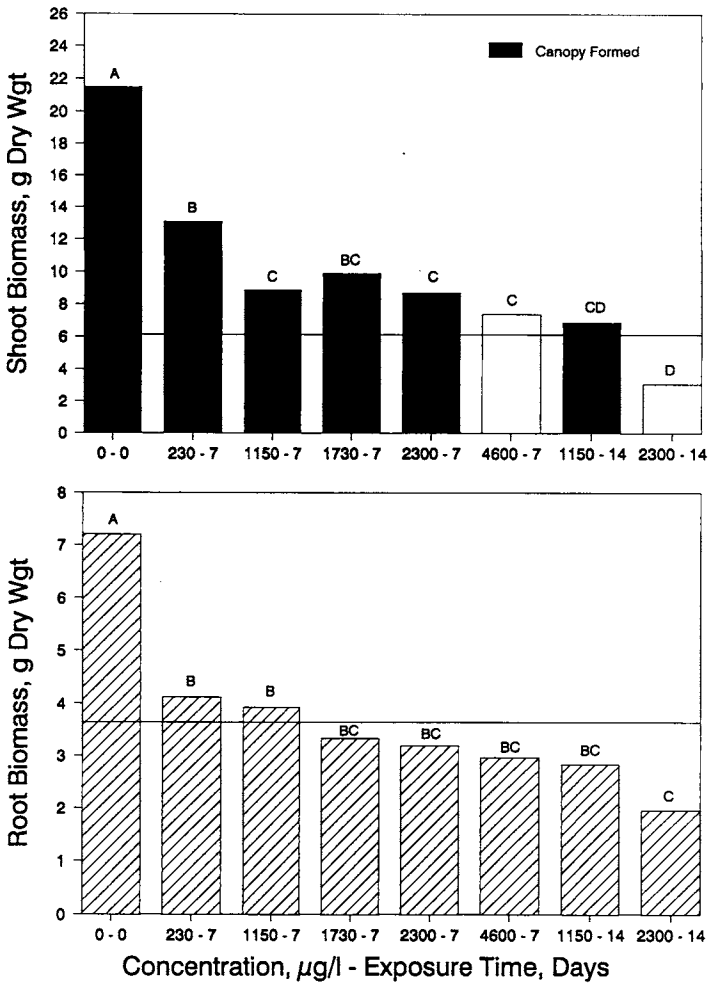

Figure 2. Effects of bensulfuron methyl on shoot and root biomass of Eurasian watermilfoil harvested at 6 weeks posttreatment. Horizontal lines represent pretreatment biomass; shoots = 6.1 ± 1.8 g DW, roots = 3.7 ± 1.3 g DW. Different letters among treatments indicate significant differences at the 5% level according to Waller-Duncan *k*-ratio *t* Test. For shoot biomass, unfilled bars represent those treatments in which a vegetative canopy did not form.

Despite significant differences in biomass production, plants had grown to the water surface (canopied) in all but two treatments (4600 µg/l at 7 days and 2300 µg/l at 14 days) by the end of the study. Extensive regrowth of milfoil to the 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 BSM.

Results of Studies 1 and 2 show that BSM acts similarly to another aquatic herbicide, fluridone (1-methyl-3-phenyl-5-[3-(trifluoromethyl) phenyl]-4(1*H*)-pyridinone), in that both require long exposure or contact times to achieve efficacy. Hall *et al.* (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 *et al.* 1992a). 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 BSM is also a systemic herbicide, the long contact times required for growth suppression in these studies was not surprising.

Study 3. Observations recorded 7 days after chemical treatment indicated that milfoil growth had slowed and plant injury (similar to that observed in Studies 1 and 2) was apparent. There was no visual difference in the degree of injury between plants treated with 50 µg/l and 150 µg/l. One week later, the number of stems with necrotic lesions and lateral buds had increased.

The first sign of recovery from BSM 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 they could not be visually distinguished from untreated plants. Recovery of other treatments occurred as in previous studies; 1 to 2 weeks following completion of the exposure period and removal of chemically treated water from the system. Even plants exposed to BSM concentrations of 150 µg/l for 42 days supported new growth by 56 days posttreatment.

Data collected at the conclusion of this study revealed that changes in shoot biomass were highly correlated with exposure time (Figure 3). Statistical comparison of regression coefficients (t-test) indicated that linear relationships between biomass and exposure time were the same at all concentrations. Compared to untreated plants, shoot biomass production decreased by an average of 10 to 86% as exposure time to BSM concentrations increased from 7 to 42 days. Results agree with data from Studies 1 and 2 and add support to the finding that longer BSM contact times are critical to maintaining suppression of milfoil growth. Root growth showed no linear relationship to exposure time or chemical concentration (data not presented).

Results of these studies showed that BSM was effective at reducing the growth of milfoil; however, complete plant control (total plant death) was not achieved at the rates and exposure times tested. Increasing exposure time was more efficacious than increasing BSM concentration. Contact time

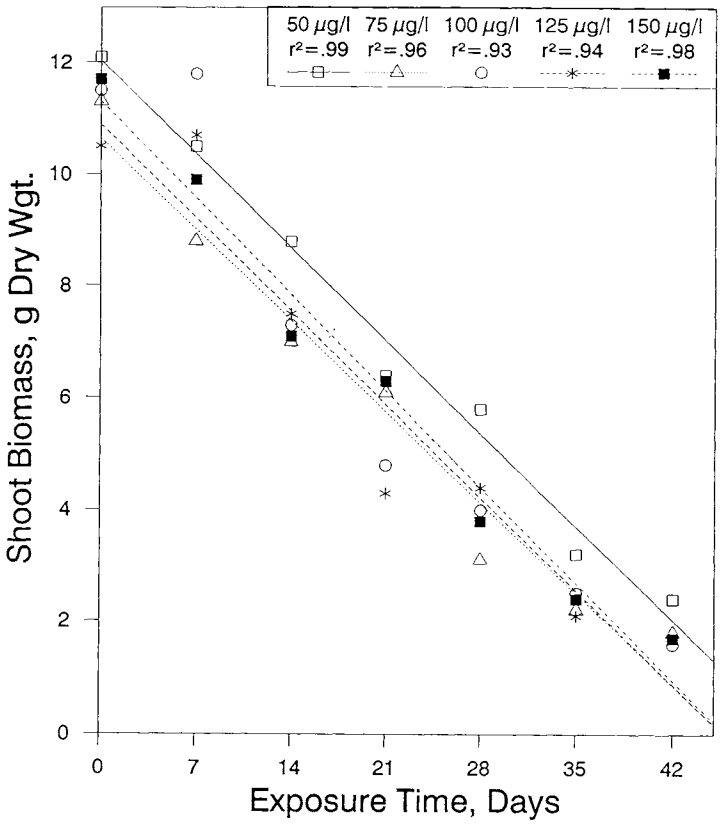

Figure 3. Effects of bensulfuron methyl on shoot biomass of Eurasian watermilfoil harvested at 8 weeks posttreatment. Pretreatment shoot biomass = 4.4 ± 0.5 g DW. Equations for regression lines are as follows: 50 µg/l, y = 12.2 - 0.238x; 75 µg/l, y = 10.93 - 0.239x; 100 µg/l, y = 11.96 - 0.271x; 125 µg/l, y = 11.0 - 0.239x; 150 µg/l, y = 11.4 - 0.251x.

was also critical for maintaining growth suppression, as plants showed strong regrowth 1 to 2 weeks after exposure to BSM was terminated.

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