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Mesocosm Evaluation of Bensulfuron Methyl Activity on Eurasian Watermilfoil, Vallisneria, and American Pondweed

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ABSTRACT

Concentration/exposure time relationships for the compound bensulfuron methyl (methyl 2-[[[[[(4,6-dimethoxy-2-pyrimidinyl)amino]-carbonyl]amino]sulfonyl] methyl]benzoate) against Eurasian watermilfoil (*Myriophyllum spicatum* L.), vallisneria (*Vallisneria americana* Michx.), and American pondweed (*Potamogeton nodosus* Poiret) were evaluated in a large outdoor mesocosm system. Initial treatment rates ranged from 25 to 100 μg/L, and plants were exposed for a 12-week period. Estimates of plant control were based on weekly measurements of shoot height, biomass harvested at 6 and 12 weeks posttreatment, and regrowth of root crowns following removal from herbicide-treated conditions. Biomass of Eurasian watermilfoil averaged approximately 50% less than that of untreated references after 6 weeks exposure to all treatments. At 12 weeks exposure, Eurasian watermilfoil biomass was reduced 96 to 98% compared to untreated references at all chemical rates tested. Shoot height of untreated Eurasian watermilfoil had reached the water surface (100 cm), but averaged less than 20 cm in height in all bensulfuron methyl treatments. Vallisneria and American pondweed exhibited greater than 95% reduction in biomass at all chemical rates tested compared to untreated references at 12 weeks posttreatment. Shoot heights of vallisneria and American pondweed were reduced greater than 90% at all bensulfuron methyl treatment rates compared to untreated references. When removed from herbicide-treated conditions, some root crowns from all species initiated regrowth. After 12 weeks exposure, results show that bensulfuron methyl contact time may be more important than rate of application for controlling the species tested.

Key words: Herbicide, chemical control, Mariner[R], Londax[R], *Myriophyllum spicatum, Potamogeton nodosus.*

INTRODUCTION

For many multiple-use water bodies the ideal aquatic plant management strategy is to remove nuisance vegetation while allowing desirable, non-weedy species to flourish. This approach can increase the biodiversity of the system and deter the reinvasion of nuisance plants (Nichols and Vennie 1991, Smart 1992). It is well known that herbicides and plant growth regulators can differentially control plant species; however, documentation for selectively managing submersed plants with chemicals is limited (Getsinger 1992). Factors that influence species-selectivity in aquatic systems include herbicide dose (concentration and length of exposure) and timing of application (Getsinger et al. 1992, Getsinger et al. 1993a, Fox et al. 1993). The activity spectrum of a herbicide has become increasingly important in the decision-making process when choosing chemicals to manage aquatic vegetation, particularly in public waters. If guidance can be provided on minimum effective dose and species selectivity, so that nuisance vegetation can be controlled while maintaining desirable plants, water resource managers and the public will be more likely to accept the use of herbicides as an ecologically sound management strategy.

In an effort to identify and develop new chemicals for selective control of nuisance submersed aquatic plants, the compound bensulfuron methyl has been evaluated in a variety of laboratory, mesocosm, pond, and field situations (Anderson and Dechoretz 1988, Langeland 1992, Pringle and Sisneros 1992, Getsinger et al. 1993b, Nelson and Netherland 1993). In addition to defining its herbicidal value, these studies have evaluated this sulfonylurea-class compound for its growth regulating and tuber inhibiting properties (Anderson 1988, Haller et al. 1992, Langeland and Laroche 1992, Van and Vandiver 1992, Nelson et al. 1993). Most of these studies have examined bensulfuron methyl efficacy on two major submersed weed species, Eurasian watermilfoil and hydrilla (*Hydrilla verticillata* (L.F.) Royle). Little is reported on the selective activity of bensulfuron methyl on submersed plants; however, a wide range of sensitivity among terrestrial plants has been observed (Beyer et al. 1988, E. I. du Pont de Nemours & Co. 1988). Although Du Pont Agricultural Products recently discontinued efforts to obtain aquatic registration for bensulfuron methyl (trade name, Mariner[R]) in the U.S., this herbicide is still widely used in rice production (as Londax[R] herbicide) and will continue to generate attention as a potential aquatic weed control agent in other parts of the world (Takeda et al. 1986, Bowmer et al. 1992).

This study was conducted to: a) verify laboratory results of bensulfuron methyl activity on the target species Eurasian watermilfoil; b) determine any selective properties of the compound on designated non-target plants; and c) demonstrate the effectiveness of a large, outdoor mesocosm system for determining herbicide concentration/ex-

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posure time relationships. Results from this and related studies will be used to recommend chemical strategies for selective control of submersed plants.

MATERIALS AND METHODS

The study was conducted over a 16-week period in an outdoor mesocosm system located at the US Army Engineer Waterways Experiment Station's (WES) Lewisville Aquatic Ecosystem Research Facility (LAERF) in Lewisville, Texas (Dick et al. 1993). Fifteen 7000-L aboveground fiberglass tanks (1.4 m tall x 2.6 m average diameter) were used as treatment basins. In September 1991, 4000 L of pond sediment was collected at the LAERF, sterilized, and amended with ammonium sulfate (24% N) at a rate of 20 g/m^3 to ensure adequate nutrient availability for the duration of the study. Prepared sediment was placed into 800, 4.4-L (20 cm h x 18 cm d) plastic containers and transported to an adjacent culture pond.

In late October 1991, sediment containers were saturated by filling the culture pond with Lewisville Lake water to a depth of 0.5 m. Apical tips of Eurasian watermilfoil (15 cm long) were collected from a LAERF culture pond and planted in 400 of the containers (3 per container). Water level in the culture pond was raised to a depth of 1.5 m (approximate water depth in the mesocosm tanks) and Eurasian watermilfoil was allowed to grow and overwinter until late March 1992. At that time, 200 of the remaining containers were planted with winter buds of vallisneria (obtained from Wildlife Nurseries, Inc., Oshkosh, WI) and 200 were planted with locally collected American pondweed tubers, both at a rate of 3 per container. After 4 weeks all species were breaking winter dormancy and containers with healthy plants were transferred into the mesocosm tanks. This planting and transfer scheme was devised to simulate the natural spring growth of these species.

Each of the fifteen mesocosm tanks were divided into quadrants using 0.6-cm mesh aquaculture netting. Groups of nine containers of each species were randomly assigned to quadrants in each tank. Once planted, each tank contained 2 quadrants of Eurasian watermilfoil, and 1 quadrant each of vallisneria and American pondweed. This arrangement kept species separated within each tank in order to reduce competitive interactions, but allowed for complete water exchange between quadrants. Tanks were filled with reservoir water with a measured pH of 7.8, DO of 8.5 mg/L, temperature of 22 C, and conductivity of 230 µmho/cm (Dick et al. 1993). Flowmeter valves on each tank were calibrated to provide a complete volume exchange (7000 L) every 24 hours. An airlift pipe was placed in the center of each tank to facilitate water column mixing.

After 5 days of acclimation, the flow-through system was deactivated and 1 container was removed from each tank quadrant. Shoots (stems, leaves, etc. above the sediment) and roots (roots, stolons, etc. below the sediment) from these containers were harvested and rinsed to remove epiphytes and sediment. Harvested materials were dried to a constant weight at 60 C in a convection drying oven. This material provided an estimate of pretreatment biomass.

Stock solutions of bensulfuron methyl were prepared from a 60% a.i. formulation (Du Pont DPX-F5384-200). Bensulfuron methyl treatment rates and dates of application were as follows: 25 µg/L, 50 µg/L, and 100 µg/L were applied on 14 May 1992; and a split treatment of 25 µg/L was applied on 14 May, 4 June, 25 June, and 16 July 1992. Untreated references were included as a treatment. All treatments were randomly assigned to tanks and were replicated 3 times. Previously conducted laboratory studies had shown these concentrations of bensulfuron methyl to be in the range of effective rates over extended periods of time (7 weeks) for Eurasian watermilfoil (Nelson and Netherland 1993).

Water temperatures were monitored using maximum-minimum thermometers throughout the study and were similar among all tanks, with daily means from 23.5 C (mid-May) to 29.5 C (early August). No temperature stratification was observed in the tanks, and diel fluctuations averaged 7.5 C (Dick et al. 1993). Other water quality parameters measured during the study included: pH=7.6-8.5; DO=8.5-10.0 mg/L; alkalinity=45-65 mg/L; conductivity=190-240 µmho/cm; turbidity=<1.0 NTU; ammonia-N=0.05-0.1 mg/L; nitrate-N=0.01-0.05 mg/L; potassium=1.5-2.5 mg/L; phosphorus (SRP)=0.0-0.005 mg/L (Dick et al. 1993).

Growth and standing biomass responses were monitored for 12 weeks following herbicide application. Observations of plant vigor and/or herbicide injury, and average shoot height (measured from surface of sediment to water surface) were made weekly. In addition, shoot biomass was harvested at 6 weeks (Eurasian watermilfoil only) and 12 weeks posttreatment and processed as described above.

At 6 weeks posttreatment, eight containers of Eurasian watermilfoil were removed from one quadrant of each tank. Shoots were harvested by cutting stems approximately 2 cm above the sediment, leaving root crowns and roots in place. After shoot harvest, these containers were placed in tanks filled with bensulfuron methyl-free water to monitor regrowth from root crowns for a period of 4 weeks. Regrowth from root crowns was used as an indicator of plant control.

Viable shoots of all species in the remaining containers were harvested in the same manner as above, 12 weeks after bensulfuron methyl application. Tanks were drained and refilled with reservoir water to monitor regrowth from these containers. The flow-through system was reactivated for a 5-day period (> 6 turnovers) to ensure removal of any remaining herbicide. Previously conducted fluorescent dye studies showed complete removal of dye-treated water using this rinse procedure (unpublished data). Posttreatment root crown viability for Eurasian watermilfoil, vallisneria, and American pondweed was assessed as described in the 6-week Eurasian watermilfoil harvest.

Biomass data were analyzed using SAS (SAS Institute, 1988). A one-way ANOVA was performed on mean biomass to test for significant differences between all treatments for the 6- and 12-week harvests. Dunnett's t-tests were then applied to compare mean biomass between each treatment and reference tanks. Additionally, final biomass for each tested species was analyzed using the Student-Newman-Keuls' procedure to ascertain differences between bensulfuron methyl concentrations within an exposure period. Shoot height estimates and posttreatment root crown viability were not statistically analyzed.

RESULTS AND DISCUSSION

All species were well-rooted and actively growing at the time of bensulfuron methyl application. Pretreatment shoot and root dry mass of each species (on a g/container ± SE and field-equivalent (g/m²) basis) are as follows: Eurasian watermilfoil, shoots = 7.2 ± 0.7 g/container (223 g/m²), roots = 0.4 ± 0.05 g/container (12 g/m²); vallisneria, shoots = 1.1 ± 0.1 g/container (34 g/m²), roots = 0.8 ± 0.1 g/container (24 g/m²); American pondweed, shoots = 4.0 ± 0.4 g/container (124 g/m²), roots = 0.5 ± 0.09 g/container (16 g/m²). These pretreatment field-equivalent biomass values are comparable to reports from a variety of locations (Grace and Wetzel 1978, Titus and Stephens 1983, Korschgen and Green 1988, J.D. Madsen, personal communication).

Standing biomass of Eurasian watermilfoil was significantly different (Dunnett's t-tests, $\alpha = 0.05$) between untreated references and bensulfuron methyl treatments 6 weeks following herbicide application (Figure 1). Also, there were no significant differences in biomass between bensulfuron methyl treatments (Student-Newman-Keul's, $\alpha = 0.05$). Biomass in reference tanks had increased nearly three-fold to reach a mean of 19.6 ± 1.3 g per container, with the 4 bensulfuron methyl treatments exhibiting less than half of the reference shoot biomass (range = 6.5 ± 0.9 to 9.5 ± 1.4 g per container). These results indicate that posttreatment biomass levels in the bensulfuron methyl-treated tanks remained essentially the same as pretreatment levels, demonstrating a plant growth regulator type effect. Shoot height of bensulfuron methyl-treated plants had decreased by approximately 50% (Figure 2), with most shoot tips and young leaves exhibiting necrosis. Although no viable apical tips were observed, mature shoots appeared healthy, suggesting a growth suppression effect following six weeks of exposure. In contrast, shoot height of reference plants had increased by 35%, and the viable apical tips were reaching the water surface by this time.

When placed in bensulfuron methyl-free water, root crowns from all treatments exhibited healthy regrowth within 4 weeks, indicating that complete control of Eurasian watermilfoil was not achieved. Other studies have shown that a bensulfuron methyl exposure period of ≤ 4 weeks resulted in growth suppression (rather than plant death) of Eurasian watermilfoil, particularly in older plants (Anderson 1988, Nelson and Netherland 1993, Nelson et al. 1993).

Standing biomass of Eurasian watermilfoil remained significantly higher (Dunnett's t-tests, $\alpha = 0.05$) in reference tanks compared with bensulfuron methyl treatments after 12 weeks of herbicide exposure (Figure 1). Biomass in reference tanks nearly doubled from 6 to 12 weeks posttreatment, reaching a mean of 35.6 ± 2.5 g per container. At this time, untreated plants had reached the water's surface, formed a canopy, and entered the flowering stage. In comparison, little if any viable shoot material was present in bensulfuron methyl-treated tanks, and most of that material consisted of severely stunted new growth from root crowns. There were no significant differences between

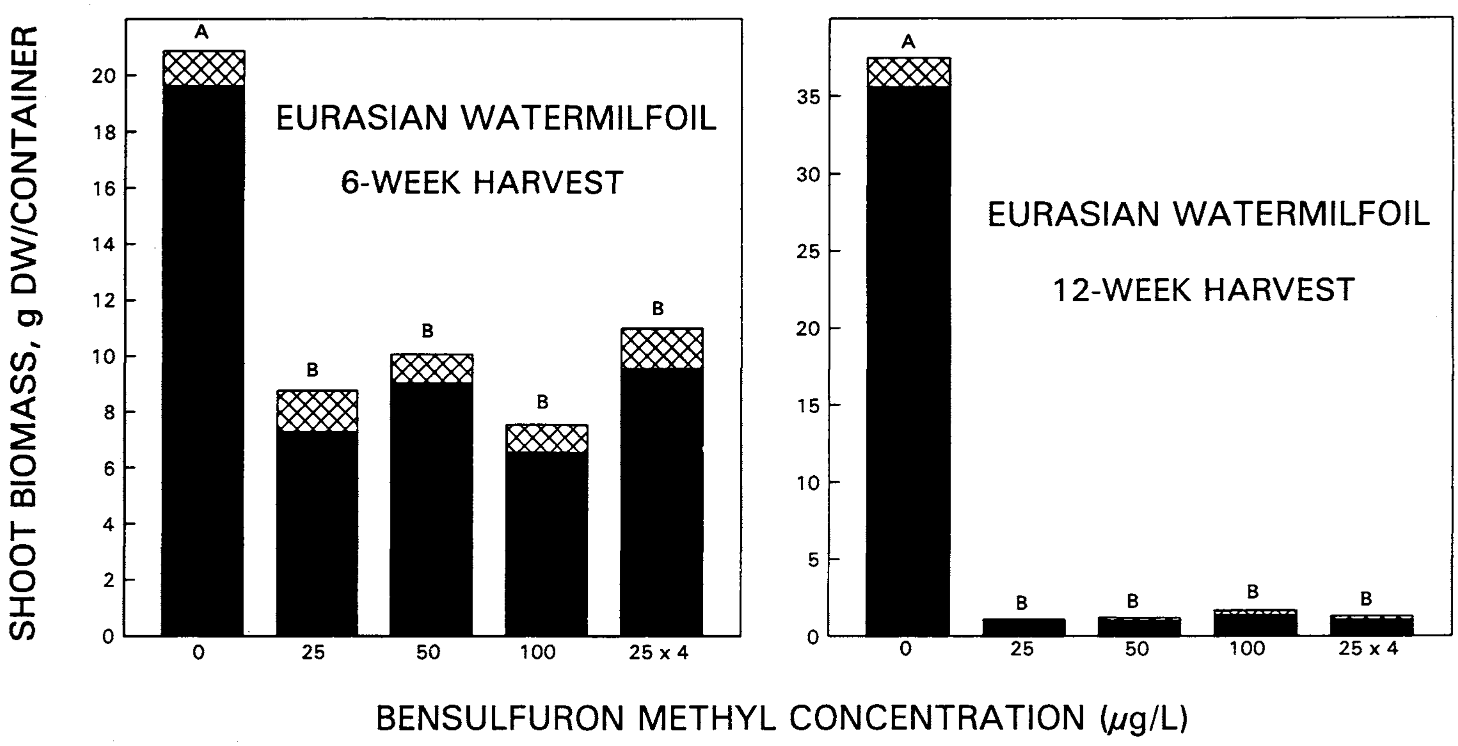

Figure 1. Eurasian watermilfoil (*Myriophyllum spicatum*) mean shoot biomass (± 1 SE) harvested 6 and 12 weeks following application of bensulfuron methyl. Mean biomass of each treatment was significantly different from the reference (Dunnett's t-tests, $\alpha = 0.05$).

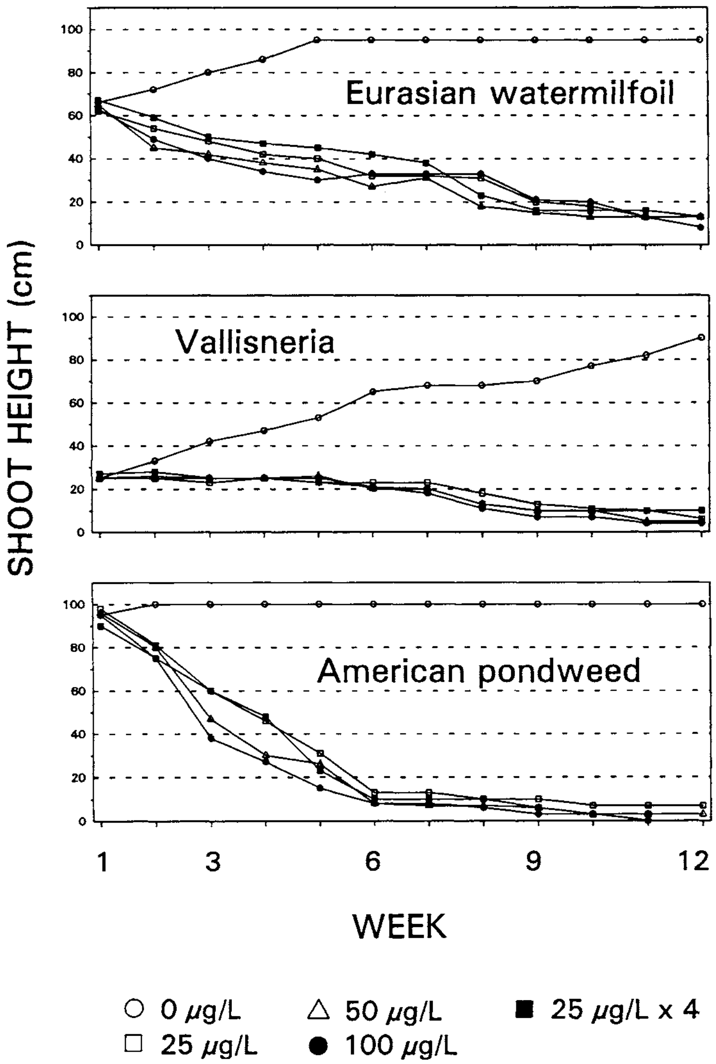

Figure 2. Eurasian watermilfoil (*Myriophyllum spicatum*), vallisneria (*Vallisneria americana*) and American pondweed (*Potamogeton nodosus*) average shoot heights during the bensulfuron methyl exposure period. Maximum water depth from sediment to surface was 1.0 meter.

biomass levels in the bensulfuron methyl-treated tanks. (Student-Newman-Keul's, α = 0.05). Shoot biomass in bensulfuron methyl-treated tanks was measured at a range of 0.9 ± 0.2 to 1.4 ± 0.3 g per container, which represented a 96 to 98% reduction in shoot mass compared to untreated plants. Shoot height of bensulfuron methyl-treated plants decreased to less than 20 cm by the 9th week and remained at that level through the 12th week (Figure 2). Despite the severe reduction in shoot mass with 12 weeks of herbicide exposure, root crowns exhibited healthy regrowth in all containers from all treatments following the 4-week culture period in bensulfuron methyl-free water. Incomplete Eurasian watermilfoil control has been found under a wide range of bensulfuron methyl rates and exposure periods in previously conducted laboratory studies. Nelson et al. (1993) showed that Eurasian watermilfoil recovered from bensulfuron methyl treatments that ranged from high doses and long exposure times (150 µg/L, 42 days) to extremely high doses and moderate exposure times (2300 µg/L, 14 days). Other studies by these investigators also suggested that bensulfuron methyl was more effective on Eurasian watermilfoil when applied to young tissue. Anderson (1988) reported shoot biomass reductions in Eurasian watermilfoil of up to 70%, but did not achieve complete plant control. Under field drawdown conditions, Bowmer et al. (1992) reported poor control of a related species, common watermilfoil (*M. papillosum* Orchard, sp. nov.), when exposed to initial bensulfuron methyl applications of 100, 200, and 300 g/ha.

Standing biomass of vallisneria was significantly different (Dunnett's t-tests, α = 0.05) between reference tanks and bensulfuron methyl treatments after 12 weeks (Figure 3). Shoot biomass increased in reference tanks to reach a posttreatment level of 19.0 ± 1.5 g per container. In addition, untreated shoots had reached heights of 90 cm and were flowering by the 12-week harvest (Figure 2). Although viable shoots had disappeared in all bensulfuron methyl-treated tanks by the 9th week, stunted regrowth comprised of small plants sprouting from stolons was observed by the end of the 11th week of herbicide exposure. At 12 weeks posttreatment, there were no significant differences in biomass between bensulfuron methyl-treated tanks (Student-Newman-Keul's, α = 0.05). Vallisneria shoot mass was measured at less than 0.03 g per container and shoot height was less than 10 cm in all bensulfuron methyl treatments. However, no individual plants grown from original propagules showed signs of recovery in any bensulfuron methyl-treated tanks.

Although no original plants seemed to survive the bensulfuron methyl exposure, vallisneria regrowth occurred in the majority of treated containers during the 4-week culture period in herbicide-free water: 100% at 0 µg/L; 96% at 25 µg/L; 92% at 50 µg/L; 62% at 100 µg/L; and 80% at 25 µg/L x 4. Bowmer et al. (1992) reported a 71 to 83% reduction in biomass in a related species, ribbonweed (*Vallisneria gigantea* Graebner), when stands of this plant growing in an irrigation channel were treated with bensulfuron methyl following a drawdown at 200 and 300 g/ha.

Standing biomass of American pondweed was significantly different (Dunnett's t-tests, α = 0.05) between reference and bensulfuron methyl treatments after 12 weeks (Figure 3). Shoot biomass in reference tanks increased to 21.3 ± 0.9 g per container by the 12-week harvest. Untreated shoots had formed a dense surface canopy by week 6 (Figure 2), and most plants had completed flowering by harvest time. In contrast, shoot mass in bensulfuron methyl-treated tanks was measured at less than 0.4 g per container, and shoot height was ≤10 cm. Viable shoots were absent by 9 weeks posttreatment, and no regrowth was evident during the remainder of herbicide exposure period. There were no significant differences in biomass between bensulfuron methyl-treated tanks (Student-Newman-Keul's, α = 0.05).

When transferred to herbicide-free water, regrowth was observed at 4 weeks in all of the untreated reference containers, but in only 4 to 17% of bensulfuron methyl-treated containers. Other investigators have shown that several members of the Potamogetonaceae are quite sensitive to bensulfuron methyl. Anderson and Dechoretz

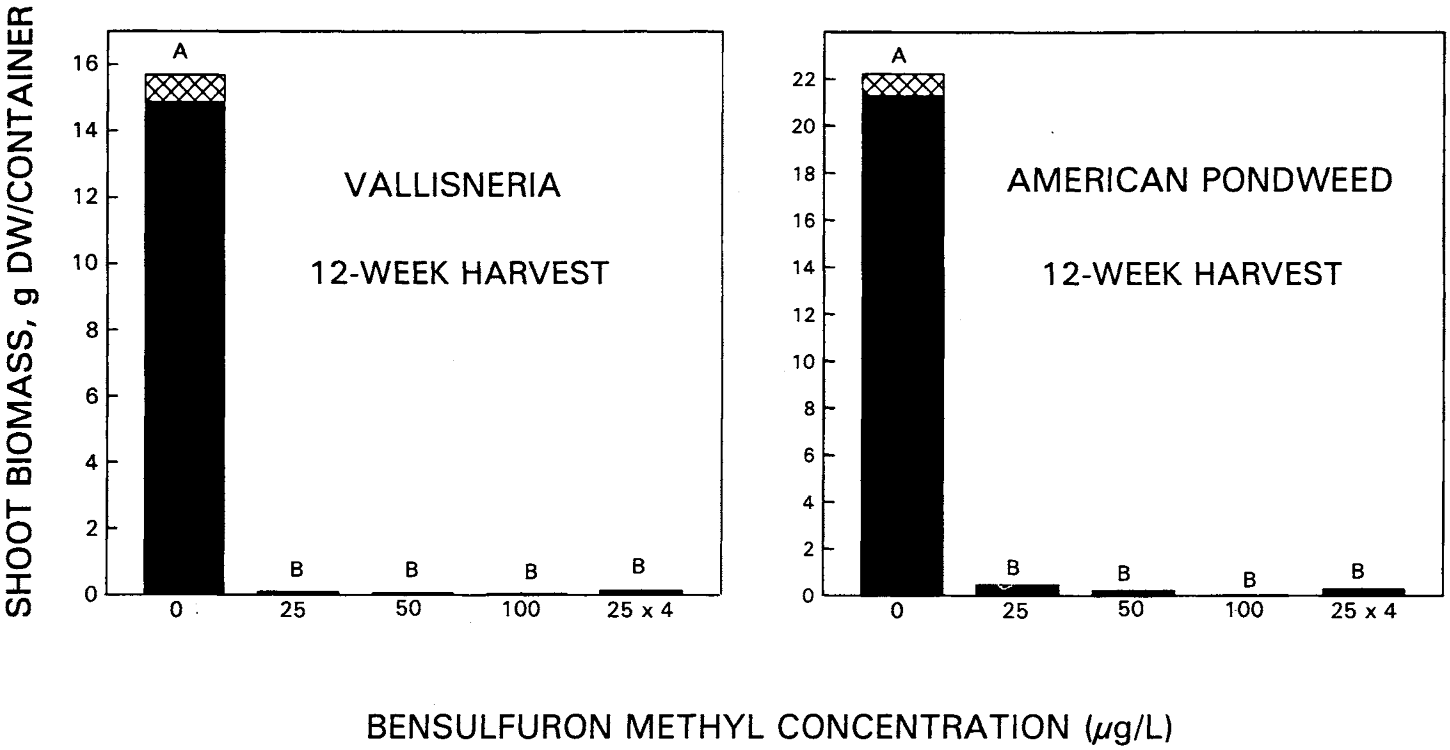

Figure 3. Vallisneria (*Vallisneria americana*) and American pondweed (*Potamogeton nodosus*) mean shoot biomass (± 1 SE) harvested 12 weeks following application of bensulfuron methyl. Mean biomass of each treatment was significantly different from the reference (Dunnett's t-tests, $\alpha = 0.05$).

(1988) reported a significant reduction in biomass and shoot length of American pondweed and sago pondweed (*P. pectinatus* L.) when exposed for 24 hours to ≥100 μg/L bensulfuron methyl. Bowmer et al. (1992) reported excellent control of floating pondweed (*P. tricarinatus* F. Muell. & A. Benn. ex A. Benn. (1892)) for up to 15 months, when treated with bensulfuron methyl at >100 g/ha following a drawdown.

Results of this mesocosm study have verified control of Eurasian watermilfoil using bensulfuron methyl concentration/exposure time relationships previously developed in the laboratory. Both mesocosm and laboratory evaluations have clearly shown that long exposures to bensulfuron methyl can suppress the growth of Eurasian watermilfoil, and that contact time is more important than application rate. Yet, evidence is mounting that this target plant can recover from a wide range of bensulfuron methyl doses, and that complete removal of Eurasian watermilfoil biomass using this herbicide may be difficult. It should be noted that growth conditions in laboratory and mesocosm systems may favor plant recovery following a herbicide treatment, and that stress factors which can augment the demise of herbicide-treated plants in the field (e.g., interspecific competition, light availability, nutrient fluctuations, microbial/algal interactions, and mechanical agitation from currents and/or waves) are limited. Therefore, field trials designed to confirm laboratory/mesocosm evaluations would be prudent prior to providing guidance for operational use of bensulfuron methyl.

In addition to suppressing the growth of Eurasian watermilfoil, bensulfuron methyl showed a high potential for controlling vallisneria and American pondweed. These findings confirmed results in the literature which indicated that the herbicide was efficacious against several pondweeds (including American pondweed) and a related vallisneria species. As with Eurasian watermilfoil, application rate seems to be much less important than exposure time. The effectiveness of bensulfuron methyl on several members of the Hydrocharitaceae and Potamogetonaceae implies that this chemical may not be suitable for selective control of Eurasian watermilfoil in typical, temperate zone submersed plant communities.

Some studies have suggested an age-related response to bensulfuron methyl (Anderson and Dechoretz 1988, Nelson and Netherland 1993), therefore evaluations of the compound's efficacy during various life cycle stages of Eurasian watermilfoil would be valuable. Perhaps an application of bensulfuron methyl during the early stages of Eurasian watermilfoil growth would avoid damage to desirable, non-target species. By using this approach, the selective control of Eurasian watermilfoil could be enhanced.

Since bensulfuron methyl seems to act primarily as a growth suppressant, the ideal application for this compound may be as a plant growth regulator (PGR), rather than as a herbicide. If used during the peak growth months (May-July), bensulfuron methyl could act as a broad spectrum PGR, yet allow for plant recovery later in the season. Also, it may prevent sufficient storage of carbohydrate reserves used for plant regrowth the following year. Other investigators have suggested a PGR-role for bensulfuron methyl as a component of chemical aquatic plant management strategies (Anderson 1988, Haller et al. 1992).

Finally, results from this study have demonstrated that the mesocosm system at LAERF can provide suitable conditions for supporting field-level populations of rooted submersed plants for up to 16 weeks, and can be reliably employed to develop herbicide concentration/exposure time relationships for use in controlling submersed plants.

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