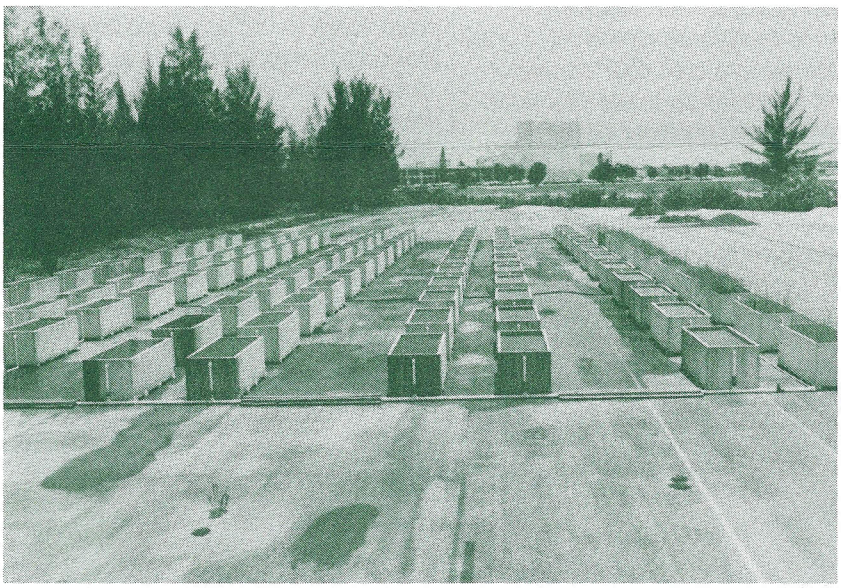
US Army Corps
of Engineers
Waterways Experiment
Station

Aquatic Plant Control Research Program

VOL A-91-1 September 1991

Growth regulation of Eurasian watermilfoil and hydrilla using bensulfuron methyl

by
Linda S. Nelson and Thai K. Van



Tank system used for evaluation of bensulfuron methyl on hydrilla at the USDA Aquatic Plant Management Laboratory

Classical strategies to manage aquatic vegetation often result in the severe reduction or elimination of plant biomass in the area of treatment. When this occurs, a valuable component of the aquatic ecosystem is removed. New technologies, such as plant growth regulators (PGRs), may provide an alternative management option in the near future, that of suppressing or inhibiting plant growth rather than complete removal of the plant. Plant growth regulators are synthetic compounds that, when applied to plants, alter or interfere with various growth processes, such as reduced stem elongation through inhibition of the plant hormone gibberellin. Several growth-regulating compounds are commercially available and for years have been used in many areas of plant science. For example, in agriculture, PGRs are used to reduce lodging (the leaning or falling over of plants) of many cereal and forage crops, thus facilitating an easier and more profitable harvest (Jung and Rademacher 1983; Wiersma, Oplinger, and Grey 1986; and Wiltshire and others 1989). Applied to fruit crops, growth regulators can promote fruit ripening, enhance fruit development and quality, and delay or promote fruit abscission (Wilson 1983 and Marini, Byers, and Sowers 1989). Plant growth regulators have also been used in the turfgrass industry to slow the growth rate of grasses, reducing mowing frequency and maintenance costs (Elkins 1983 and Nelson, Getsinger, and Luu, in preparation).

Recent studies have demonstrated that many PGRs which are active on terrestrial plants are also effective on aquatic plant species. Lembi and Netherland (1990) showed that paclobutrazol, uniconazol, and flurprimidol were effective in reducing stem length and other growth parameters in both hydrilla and milfoil. Furthermore, the physiological

competence (for example, photosynthesis and respiration) of these plants did not appear to be affected at the concentrations required to reduce stem length. Paclobutrazol also exhibits growth-retarding effects on waterhyacinth (Van 1988). Kane and Gilman (1991) found that shoot length of three species of *Myriophyllum* was significantly reduced when exposed to low concentrations of Cycocel[R] (chlormequat chloride), a growth regulator used on ornamental shrubs and flowers. Effects varied among species with *M. heterophyllum* exhibiting the greatest sensitivity. Although these and other studies indicate that PGRs are effective on aquatic plants, their use as a successful management tool requires further investigation.

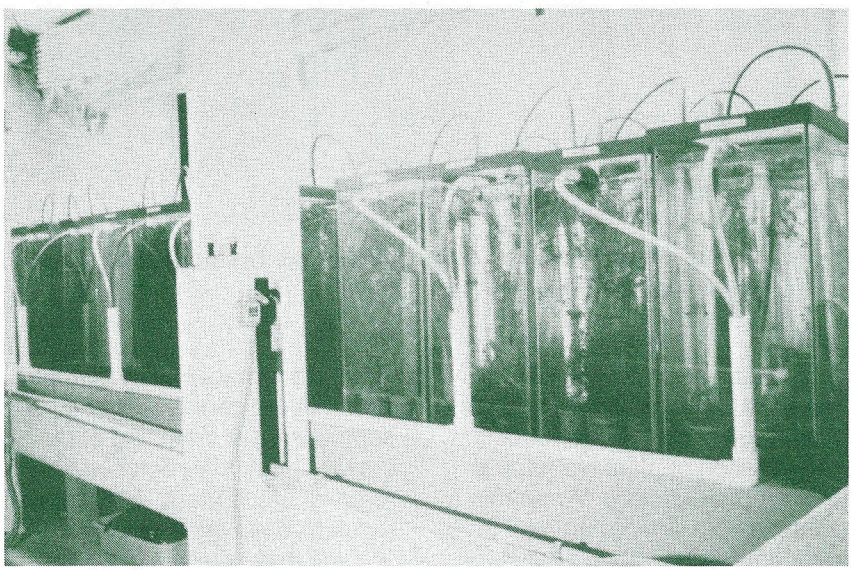
There are several advantages in evaluating the aquatic plant management potential of PGRs. First, using a growth regulator means applying lower concentrations of chemical to the waterbody. Plant growth regulators are generally applied at very low concentrations, that is, parts per billion. In fact, many herbicides, when used at low rates, exhibit growth-regulating effects rather than herbicide effects. Second, reducing the growth of submersed, aquatic vegetation rather than removing it from the waterbody allows a viable plant population to remain. Growth-regulated plants are short and therefore not a navigational or recreational nuisance, but still function as a part of the aquatic community; providing oxygen, sediment stabilization, and habitat for aquatic organisms. Finally, the concept of growth-regulating vegetation, rather than removing it from the system, is often viewed as being more environmentally compatible.

One promising PGR for use in aquatic systems is the compound bensulfuron methyl. This chemical is a member of the sulfonylurea herbicide group developed by E.I. DuPont de Nemours & Company. Sulfonylureas are active at extremely low rates (as low as 0.002 kilogram per hectare) and act to inhibit the plant enzyme acetolactate synthase (Beyer and others 1988). Inhibition of this enzyme, which is necessary for the production of essential amino acids, results in rapid cessation of growth. The absence of this enzyme in man and other animals helps to explain the low toxicity of these compounds to nontarget organisms. Bensulfuron methyl is currently registered as a herbicide (Londax[R]) for use in rice production and shows potential as a herbicide and growth regulator (when used at lower rates) for management of submersed aquatic plants (Lembi and Netherland 1990 and Anderson and Dechoretz 1988). Of all the PGRs evaluated for use on aquatic plant species thus far, bensulfuron methyl is nearest to receiving aquatic registration. DuPont will submit a petition to the US Environmental Protection Agency (EPA) for full aquatic registration of this product in the near future, and upon acceptance, will market the aquatic formulation under the trade name Mariner[R]. Bensulfuron methyl is currently under field investigation (through an EPA Experimental Use Permit) for its herbicide effectiveness against milfoil and hydrilla. In addition, studies are being conducted at the US Army Engineer Waterways Experiment Station (WES) and the USDA Aquatic Plant Management Laboratory, Ft. Lauderdale, to evaluate the potential of bensulfuron methyl as a PGR on Eurasian watermilfoil and hydrilla. This article is an update on the WES and USDA bensulfuron methyl research efforts.

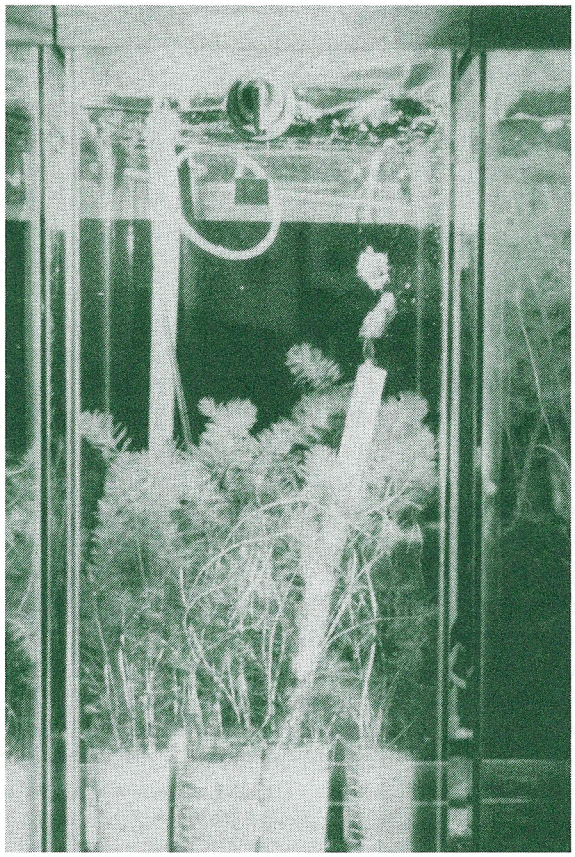
Bensulfuron methyl versus hydrilla

Materials and methods

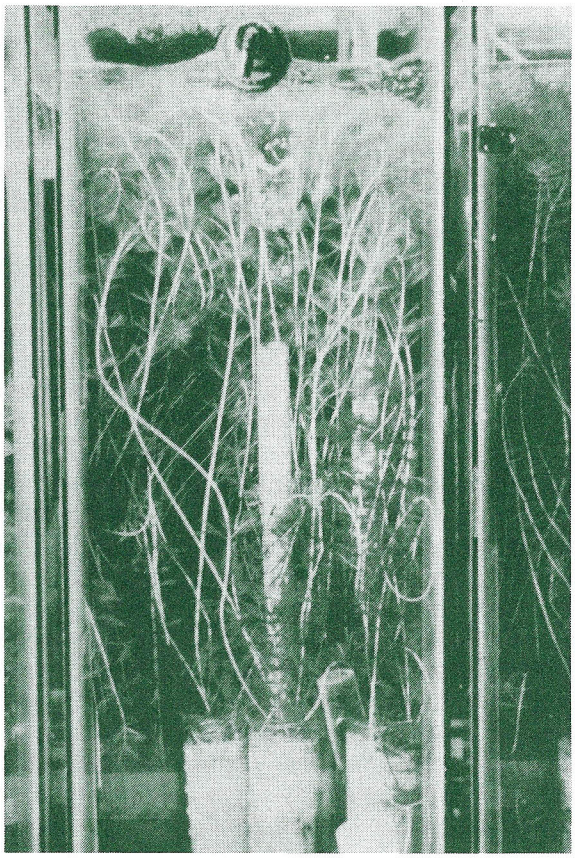
Monoecious and dioecious hydrilla [*Hydrilla verticillata* (L.f.) Royle] used in this study were obtained from stock cultures grown over a period of several months in outdoor aquaria at the USDA Aquatic Plant Management Laboratory. Monoecious hydrilla was established from tubers collected from the Potomac River, Virginia, while dioecious hydrilla was established from stem apices collected from Rodeo Lake, Florida.



A controlled-environment aquaria system used at WES to evaluate bensulfuron methyl effects on Eurasian watermilfoil



50 ppb bensulfuron methyl at 21 days' exposure



Untreated tank

Bensulfuron methyl effects on Eurasian watermilfoil 5 weeks following treatment (conducted at WES)

This investigation was conducted in outdoor tanks 0.8 metre wide by 2.2 metres long (1.7×10^{-4} hectare) filled with pond water to a maximum depth of 0.6 metre. Pond water was from the same source as previously described by Van and Steward (1986). Uniform low water pressure was maintained by constant overflow in a standpipe, and flow to individual tanks was regulated by small petcock valves to provide one water volume change every 24 hours. A system of 36 tanks arranged in three rows of 12 tanks each was used. Chemical treatments (concentration × exposure) were arranged as a 4 by 3 factorial with three replicates and were assigned to the tanks in a randomized block design.

Hydrilla tubers were allowed to germinate in pond water at 25 degrees Celsius under continuous light for 5 weeks before planting. Ten sprouted tubers 10 centimetres long were planted in plastic pots (25- centimeter diameter and 20 centimeters deep). Pots were filled with a rooting medium consisting of approximately 12 kilograms of sandy loam (60 percent sand, 26 percent silt, and 14 percent clay) enriched with 10 grams of a slow-release fertilizer, Sierra^{R}. Four pots of each hydrilla biotype were placed in each tank, and plants were allowed to grow for 2 weeks prior to chemical treatment. On August 1, 1990, bensulfuron methyl was applied to the tanks at concentrations of 0, 50, 100, and 200 micrograms per liter (µg/L). Plants were in contact with each of the four treatment concentrations for 3, 7, and 14 days. Water exchange was halted for the length of the designated exposure time, after which the tanks were flushed three times and the water exchange resumed. One pot of each plant biotype from each tank was harvested at 1, 2, 4, and 6 months after chemical treatment. Biomass was harvested, numbers of tubers counted, and dry weights determined. Data were subjected to analysis of variance using a split-split plot design with herbicide treatments as main plots, hydrilla biotypes as subplots, and harvest dates as sub-subplots. Only data of the 1- and 2-month harvests are reported in this article.

Results

Response of monoecious hydrilla 1 month after the bensulfuron methyl treatments is illustrated in Figure 1. (All values given in Figures 1-4 are means and standard deviations for three replicates.) An exposure for 3 days at 50 µg/L resulted in about 35 percent reduction of plant biomass. Increasing bensulfuron

methyl concentrations to 200 µg/L still provided only marginal control when chemical exposure was limited to 3 days. When exposure to the chemical was extended to 7 days, the 50 µg/L bensulfuron methyl treatment provided approximately 70 percent reduction of plant biomass. However, complete inhibition of both plant growth and tuber production in monoecious hydrilla required an exposure of 14 days to concentrations of 50 µg/L bensulfuron methyl or higher.

Regrowth began within 2 months in monoecious hydrilla, as evidenced by increases in plant weight in the second harvest (Figure 2). Plants recovered almost completely at all treatment concentrations when exposure to bensulfuron methyl was limited to 3 or 7 days. Inhibition of tuber production persisted after 2 months, even in treatments where plants had recovered from the initial herbicidal effects of bensulfuron methyl. After 2 months, untreated control plants produced an average of 281 tubers per pot, while no tubers were found with plants treated with 100 µg/L bensulfuron methyl and 14 days' exposure.

Similar results were obtained with dioecious hydrilla (Figures 3 and 4). The highest concentration of bensulfuron methyl (200 µg/L) and longest exposure time (14 days) were required to maintain adequate control of plant biomass after 2 months. Previous studies showed that dioecious hydrilla grown from tubers begins to produce new tubers after about 8 weeks (Van 1989). In this study, untreated control plants produced an average of 3 to 10 tubers per pot by the second harvest after 2 months (Figure 4), while tubers were still lacking in all plants that had been exposed to bensulfuron methyl for 14 days.

Bensulfuron methyl reduced plant growth in both monoecious and dioecious hydrilla at the lowest concentration tested, confirming an earlier report by Anderson and Dechoretz (1988). These authors also reported that effective control of monoecious hydrilla required 7 days of exposure to 25 to 50 µg/L bensulfuron methyl. Results reported herein indicate that a longer exposure time (minimum of 14 days) and higher rates of bensulfuron methyl (100 to 200 µg/L) are needed to achieve hydrilla control under Florida conditions. Heavy regrowth was observed after 2 months in both hydrilla biotypes when exposure to bensulfuron methyl was limited to less than 7 days.

Bensulfuron methyl also suppressed tuber formation in monoecious hydrilla at all concentrations tested. The suppression level of tuber formation was often much

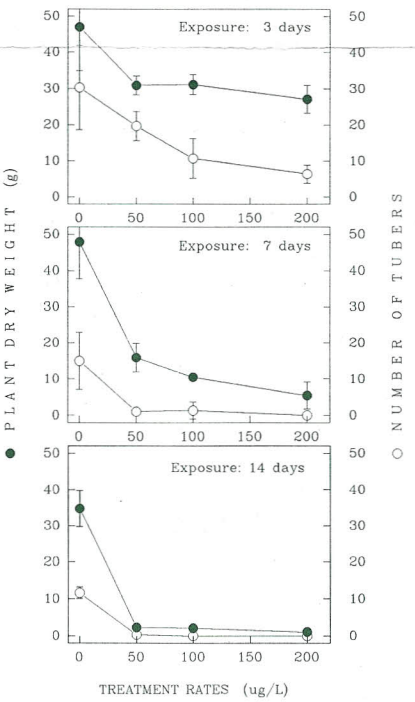


Figure 1. Effects of bensulfuron methyl on growth and tuber production in monoecious hydrilla 1 month after treatment

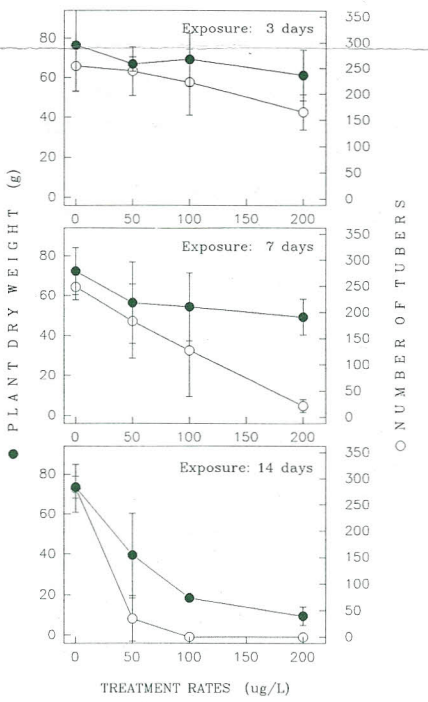


Figure 2. Effects of bensulfuron methyl on growth and tuber production in monoecious hydrilla 2 months after treatment

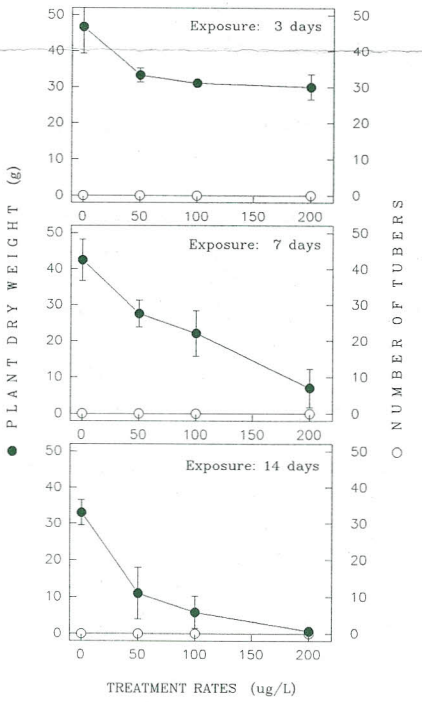


Figure 3. Effects of bensulfuron methyl on growth and tuber production in dioecious hydrilla 1 month after treatment

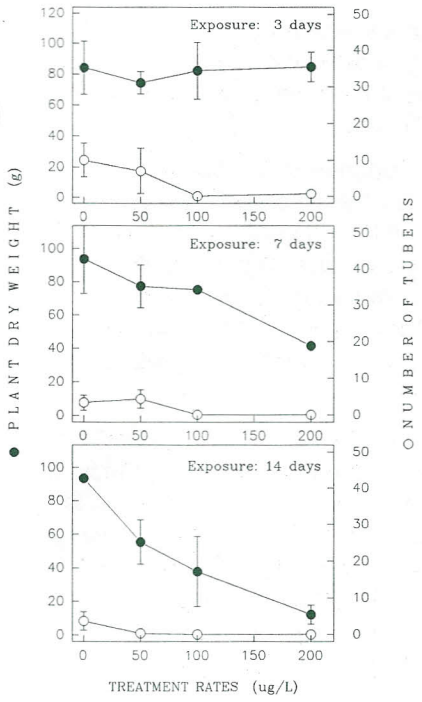


Figure 4. Effects of bensulfuron methyl on growth and tuber production in dioecious hydrilla 2 months after treatment

greater than the corresponding reduction of plant biomass exhibited by the same bensulfuron methyl treatment, suggesting that the inhibition of tuber formation was probably independent from a general retardation of plant growth. Furthermore, the growth-regulating effect of tuber inhibition persisted long after the plants had recovered from the initial herbicidal effects. Future harvests will be made after 4 and 6 months in an attempt to determine the length of time hydrilla tuber formation remains suppressed by the various bensulfuron methyl treatments.

Bensulfuron methyl versus Eurasian watermilfoil

Materials and methods

This experiment was conducted at WES in a controlled-environment aquaria system. The system consisted of twenty-four 55-litre aquaria (0.75 metre by 0.3 square metre) each independently supplied with a continuous flow of reconstituted hard water (Smart and Barko 1984). This allowed the total water volume (50 litres) of each aquarium to be exchanged every 24 hours. Air was bubbled through each aquarium as a source of carbon dioxide and for water circulation. Water temperature was maintained at 25 ± 2 degrees Celsius throughout the experiment. Overhead, supplemental lighting provided a light:dark cycle of 13:11 hours. The mean photosynthetically active radiation measured at the water surface was 450 microeinsteins per square metre per second.

Eurasian watermilfoil (*Myriophyllum spicatum* L.) used in this study was supplied by the Lewisville Aquatic Ecosystem Research Facility, Lewisville, Texas. Watermilfoil was separated into 10-centimetre apical segments and planted 5 centimetres deep into sediment-filled beakers. The sediment used was collected from Brown's Lake, Mississippi, and was amended with Ra-pid-gro^{R} to avoid any possible nutrient limitations. Nine beakers were placed in each aquarium, and plant segments were allowed to grow to establish new shoot and root growth. When adequate root growth was established (approximately 2 weeks), plants were trimmed back to a height of 20 centimetres; one week thereafter, chemical treatments were applied. One beaker of plants was randomly removed from each aquarium immediately prior to treatment to provide an estimate of treated biomass.

Established plants were exposed to static (flow-through water system turned off) treatments of varying bensulfuron methyl concentrations for 14-, 21- and 28-day periods (Table 1). Following the exposure period, aquaria were drained and rinsed three times to remove chemical-treated water, after which the continuous, flow-through water system was resumed for the duration of the experiment.

Table 1
Bensulfuron methyl treatment rates and exposure times

Rate µg/L or parts per billion	Exposure time days
0 (Control)	0
50	14
75	14
5	21
10	21
25	21
50	21
5	28

Treatments were arranged in a completely randomized design with three replicates. Visual ratings of plant injury were recorded weekly. At the conclusion of the experiment (5 weeks posttreatment), root and shoot biomass were measured for each treatment. Data were analyzed using analysis of variance, and treatment effects were separated using the Waller-Duncan Test.

Results

Five treatments significantly reduced Eurasian watermilfoil shoot biomass (Figure 5). Higher concentrations at longer exposure periods were most effective. Biomass reductions ranged from 26 to 69 percent when compared to the untreated controls, with the most effective treatment being a 21-day exposure to 50 µg/L bensulfuron methyl. Although not statistically significant, plants treated with a 21-day exposure to 5 µg/L bensulfuron methyl produced a slight increase in shoot biomass. Regrowth was observed on all treatments by the end of the experiment, and had "topped out" or grown to the water surface on all but two treatments (25 and 50 µg/L at 21-day exposures).

Regardless of significant reductions in biomass production, topped out vegetation indicates a strong regrowth potential and may be considered an inadequate treatment. Despite slight increases in root biomass with several treatments, no significant differences in root growth were observed compared to the untreated control (Figure 6).

Results from this experiment indicate that bensulfuron methyl is effective at reducing the growth of Eurasian watermilfoil and supports earlier conclusions by Anderson and Dechoretz (1988). As evidenced by the occurrence of regrowth, an exposure period of 21 days to concentrations of 25-50 µg/L was necessary to maintain acceptable growth suppression (not topped out) under the described experimental conditions. Higher concentrations at lower exposure periods were less effective, suggesting contact time is perhaps a critical factor in determining treatment success.

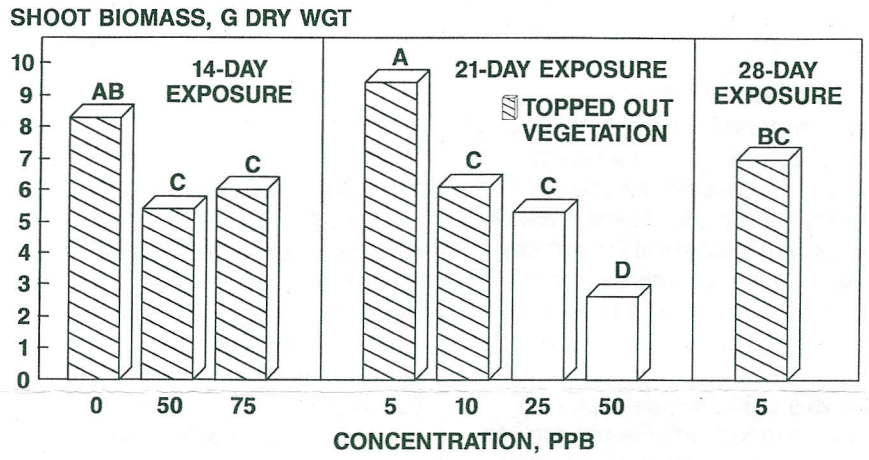


Figure 5. Effects of bensulfuron methyl on shoot biomass of Eurasian watermilfoil 5 weeks after treatment

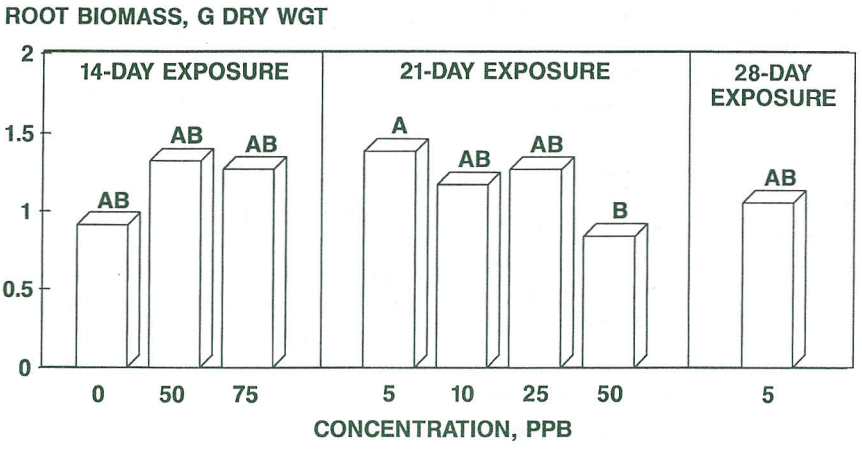


Figure 6. Effects of bensulfuron methyl on root biomass of Eurasian watermilfoil 5 weeks after treatment

Note: For Figures 5 and 6, data are means of three observations and letters denote significant differences at P = 0.05, according to the Waller-Duncan Test

Future research

Future research plans for evaluating bensulfuron methyl against target submersed plants include the following:

- Continue evaluation of bensulfuron methyl on both biotypes of hydrilla and Eurasian watermilfoil. Results thus far indicate that longer exposure periods or contact times are needed for adequate growth suppression of both plant species.
- Conduct additional studies to assess bensulfuron methyl effects on tuber production on both hydrilla biotypes. Evidence from initial studies shows that bensulfuron methyl is a potent inhibitor of tuber production in the monoecious biotype.
- Evaluate the effectiveness of bensulfuron methyl in mesocosms under field conditions. Field investigations are necessary for predicting the potential of bensulfuron methyl and other plant growth regulators as a management strategy.

References

Anderson, L. W. J., and Dechoretz, N. 1988. "Bensulfuron Methyl: A New Aquatic Herbicide," *Proceedings*, 22nd Annual Meeting, Aquatic Plant Control Research Program, Miscellaneous Paper A-88-5, US Army Engineer Waterways Experiment Station, Vicksburg, MS, pp 224-235.

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.

Elkins, D. M. 1983. "Growth Regulating Chemicals for Turf and Other Grasses," *Plant Growth Regulating Chemicals*,

Vol II, L. G. Nickell, ed., CRC Press, Boca Raton, FL, pp 113-130.

Jung, J., and Rademacher, W. 1983. "Plant Growth Regulating Chemicals--Cereal Grains," *Plant Growth Regulating Chemicals*, Vol I, L. G. Nickell, ed., CRC Press, Boca Raton, FL, pp 253-271.

Kane, Michael E., and Gilman, E. F. 1991. "*In Vitro* Propagation and Bioassay Systems for Evaluating Growth Regulator Effects on *Myriophyllum* Species," *Journal of Aquatic Plant Management*, Vol 29, pp 29-32.

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.

Marini, Richard P., Byers, Ross E., and Sowers, Donald L. 1989. "Growth Regulators and Herbicides for Delaying Apple Fruit Abscission," *HortScience* Vol 24, No. 6, pp 957-959.

Nelson, Linda S., Getsinger, Kurt D., and Luu, Kien T. "Evaluation of Plant Growth Regulators for Use in Grounds Maintenance at Military Installations," Technical Report in preparation, US Army Engineer Waterways Experiment Station, Vicksburg, MS.

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, T. K. 1988. "Integrated Control of Waterhyacinth with *Neochetina* and Paclobutrazol," *Journal of Aquatic Plant Management*, Vol 26, pp 59-61.

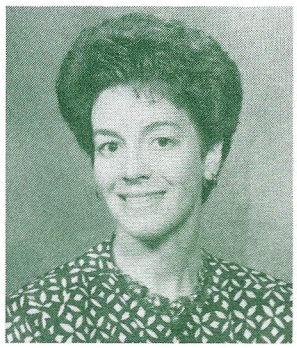
Van, T. K. 1989. "Differential Responses to Photoperiods in Monoecious and Dioecious *Hydrilla verticillata*," *Weed Science*, Vol 37, pp 552-556.

Van, T. K., and Steward, K. K. 1986. "The Use of Controlled-release Fluridone Fibers for Control of Hydrilla (Hydrilla verticillata)," *Weed Science*, Vol 34, pp 70-76.

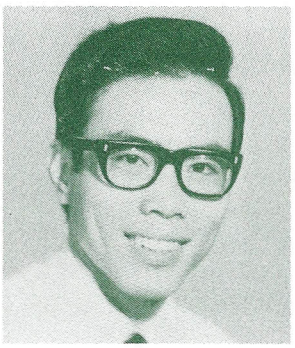
Wiersma, D. W., Oplinger, E. S., and Guy, S. O. 1986. "Environmental and Cultivar Effects on Winter Wheat Response to Ethephon Plant Growth Regulator," *Agronomy Journal*, Vol 78, No. 5, pp 761-764.

Wilson, W. C. 1983. "The Use of Exogenous Plant Growth Regulators on Citrus," *Plant Growth Regulating Chemicals*, Vol I, L. G. Nickell, ed., CRC Press, Boca Raton, FL, pp 207-232.

Wiltshire, J. J. J., Hebblethwaite, P. D., Esslemont, R. E., and McGilloway, D. A. 1989. "The Use of Growth Regulator RSW0411 in Seed Crops of *Lolium perenne* L.," *Grass and Forage Science*, Vol 44, pp 139-150.



Linda S. Nelson is a plant physiologist in the Environmental Laboratory, US Army Engineer Waterways Experiment Station. Her expertise within the Chemical Control Technology Team includes evaluating the use of plant growth regulators as a management tool for terrestrial (turfgrass) and aquatic environments. Ms. Nelson holds a Bachelor of Science degree in Biology from the University of South Dakota and a Master of Science degree in Agronomy from Iowa State University.



Dr. Thai K. Van is a research plant physiologist at the USDA Aquatic Plant Management Laboratory in Ft. Lauderdale, Florida. Dr. Van conducts research on the ecology, physiology, and management of aquatic plants. He holds a Bachelor of Science degree in Agronomy from the National Institute of Agriculture in Vietnam and a Doctor of Philosophy in Agronomy from the University of Florida.

In managing aquatic vegetation, new technologies, such as plant growth regulators (PGRs), may provide an alternative in the near future, that of suppressing or inhibiting plant growth rather than killing the plant itself. One promising PGR for use in aquatic systems is bensulfuron methyl. This issue features a report on testing done using bensulfuron methyl on monoecious and dioecious hydrilla and Eurasian watermilfoil.

AQUATIC PLANT CONTROL RESEARCH PROGRAM

This bulletin is published in accordance with AR 25-30 as one of the information dissemination functions of the Environmental Laboratory of the Waterways Experiment Station. It is principally intended to be a forum whereby information pertaining to and resulting from the Corps of Engineers' nationwide Aquatic Plant Control Research Program (APCRP) can be rapidly and widely disseminated to Corps District and Division offices and other Federal and State agencies, universities, research institutes, corporations, and individuals. Contributions are solicited, but should be relevant to the management of aquatic plants, as set forth in the objectives of the APCRP, which are generally to provide tools and techniques for the control of problem aquatic plant infestations in the Nation's waterways. These management methods must be effective, economical, and environmentally compatible. The contents of this bulletin 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 products. This bulletin will be issued on an irregular basis as dictated by the quantity and importance of information to be disseminated. Communications are welcomed and should be addressed to the Environmental Laboratory, ATTN: J.L. Decell, US Army Engineer Waterways Experiment Station, 3909 Halls Ferry Road, Vicksburg, MS 39180-6199, or call AC 601/634-3494.

LARRY B. FULTON
Colonel, Corps of Engineers
Commander and Director

DEPARTMENT OF THE ARMY
WATERWAYS EXPERIMENT STATION, CORPS OF ENGINEERS
3909 HALLS FERRY ROAD
VICKSBURG, MISSISSIPPI 39180-6199

OFFICIAL BUSINESS
CEWES-EP-I

BULK RATE
U.S. POSTAGE PAID
Vicksburg, MS
Permit No. 85