J. Aquat. Plant Manage. 31: 189-194

Fluridone Concentration and Exposure Time Requirements for Control of Eurasian Watermilfoil and Hydrilla

M. D. NETHERLAND,[1] K. D. GETSINGER[1] AND E. G. TURNER[2]

ABSTRACT

Fluridone concentration and exposure time requirements were evaluated for Eurasian watermilfoil (*Myriophyllum spicatum* L.) and hydrilla (*Hydrilla verticillata* (L.f.) Royle) under controlled-environment conditions. Results indicated that fluridone effectively inhibited growth and reduced biomass at rates of 12, 24 and 48 µg/l. Shoot and root biomass and total chlorophyll were reduced from 70 to 98% following 30-, 60- and 90-days exposures to fluridone. However, removal of fluridone at 30 and 60 days resulted in extensive regrowth following a 30-day recovery period. One exception was milfoil exposed to 48 µg/l for 60 days which was reduced by approximately 98% with no evidence of regrowth. The 48-µg/l treatment often resulted in greater biomass reduction in both species than the other rates; however, no significant differences were noted between the treatment rates of 12 and 24 µg/l. Results indicate that maintaining fluridone concentrations for >60 days at rates as low as 12 µg/l is critical for successful fluridone treatments.

Key words: aquatic weeds, herbicide, *Myriophyllum spicatum, Hydrilla verticillata.*

INTRODUCTION

Previous laboratory studies have shown that the efficacy of fluridone {1-methyl-3-phenyl-5-[3-(trifluoromethyl)phenyl]-4(1H)-pyridinone} against Eurasian watermilfoil and hydrilla is dependent upon the length of time these plants remain exposed to given concentrations of the herbicide (Hall *et al.* 1984, Van and Conant 1988, Netherland 1992). Studies with several other aquatic herbicides indicated concentration/exposure time (CET) requirements for these plants ranged from 6 hr to 4 days (Green and Westerdahl 1990,

[1]Environmental Laboratory, U.S. Army Engineer Waterways Experiment Station, 3909 Halls Ferry Rd., Vicksburg, MS 39180-6199.

[2]AScI Corporation, Vicksburg, MS 39180.

Netherland *et al.* 1991, Netherland and Getsinger 1992), whereas initial fluridone CET evaluations demonstrated that much longer exposures (21 to 84 days) were required to provide comparable reductions in biomass (Hall *et al.* 1984, Netherland 1992). In addition, these investigations showed that by maintaining low levels of fluridone (10 to 25 µg/l) in the water column over long periods of time plant control similar to that provided by much higher fluridone treatment rates could be achieved. In an effort to verify preliminary laboratory-based fluridone CET relationships, sequential applications of fluridone have been made to flowing-water systems in which low concentrations of the herbicide were maintained over long periods of time (Getsinger *et al.* 1992). These low-dose, long-exposure treatments have provided excellent plant control for at least one growing season.

Although successful plant control in previous laboratory and field treatments has been linked to maintaining low concentrations of fluridone for an extended period of time, these evaluations have only broadly quantified the relationship between concentration, exposure time, and efficacy. Therefore, this study was designed to more precisely determine fluridone CET relationships for controlling Eurasian watermilfoil and hydrilla. Further quantification of fluridone CET relationships will provide guidance for improving the management of Eurasian watermilfoil and hydrilla, particularly in flowing and other high water exchange environments.

MATERIALS AND METHODS

This study was conducted in a controlled-environment growth chamber with a photosynthetic photon flux density of 520 ± 50 µmoles/m/sec at the water surface, a 14L:10D photoperiod, and a water temperature of 24 ± 2C. Lighting was provided by 400-W high pressure sodium and GE multivapor lamps. Sediment was collected from Brown's Lake, Vicksburg, MS, and amended with fertilizer (Rapid-Gro® 20:15:15 (3 g/l) and slow-release Osmocote® 15:15:15 (5 g/l)). Glass beakers (300 ml) were filled with sediment and four 10- to 15-cm apical shoots were planted in each beaker. A thin layer (0.5 cm) of silica sand was added to the sediment surface of each beaker to prevent suspension of sediment during water exchange periods. Ten beakers containing four shoots of a single target species were placed in each 55-L aquarium (0.9 m tall by 0.09 m^2). Aquaria were independently supplied with a water culture solution (Smart and Barko 1984) via peristaltic pumps that were calibrated to provide a complete water volume exchange every 24 hr. Air was bubbled through each aquarium to provide a source of CO_2 and thorough mixing of the water column.

Eurasian watermilfoil (hereafter called milfoil) and hydrilla, collected from the Suwannee River, FL, were grown separately in 55-L aquaria. Milfoil was grown for 3 weeks prior to fluridone treatment while hydrilla was allowed to grow for 4 weeks. These periods allowed the actively growing plants to reach the water surface and encouraged the development of a healthy root mass. Immediately prior to treatment, one randomly selected beaker was removed from each aquarium. Mean shoot and root dry weights (DW ± SD) were measured and these values, multiplied by the number of beakers remaining in each aquarium, provided an estimate of pretreatment biomass. Pretreatment shoot weights (105 g DW/m^2 for milfoil and 90 g DW/m^2 for hydrilla) approximated spring to early summer field biomass reported for milfoil and hydrilla (Grace and Wetzel 1978, Bowes *et al.* 1979, Harlan *et al.* 1985). Following the initial growth period, plants were treated with fluridone at concentrations of 12 and 24 µg/l for a period of 30, 60, and 90 days, and 48 µg/l for 30 and 60 days. Each treatment (including untreated controls) was replicated three times and randomly assigned to a test aquarium.

Fluridone stock solutions were prepared from the commercial formulation Sonar® AS (4 lb active ingredient per gallon). All treatment concentrations are reported as µg/l (ppb) of the active ingredient fluridone. At the time of treatment, the flow-through water system was deactivated and fluridone was added to the aquaria. Following a 30-day exposure, all aquaria (including controls) were thoroughly drained. Rhodamine WT dye was added (10 µg/l) to each aquarium prior to draining and measured using a Turner Design® fluorometer. It was assumed that once dye concentrations reached zero, herbicide removal from the water column was complete. Residue analyses from previous studies conducted in this system showed that only 5 to 12% of the fluridone degraded over a 42-day period (data not shown). Minimal degradation in the chamber is attributed to the exclusion (due to 0.6-cm glass cover plates) of the ultraviolet light component (297 to 325 nm) primarily responsible for photolysis of fluridone (Mossler *et al.* 1989), and the fact that plant uptake accounts for a very small fraction of the fluridone removed from the water column over time (Marquis *et al.* 1981, Van and Steward 1986, Van and Conant 1988). Although degradation was not a major concern, due to the length of this study, aquaria were drained and re-treated at 30-day intervals to allow for an exchange of fresh water. Treatments designated as 60- and 90-day exposures were re-treated immediately following the drain procedure.

Plant response to fluridone treatment was monitored for a 90-day period, which allowed for potential plant recovery following the 30- and 60-day exposure periods. Visual assessments were used to characterize initial plant response to fluridone, progression of injury symptoms, and initiation of regrowth from shoots or rootcrowns. Two shoot apices (4 to 6 cm) per aquarium were sampled at 6, 30, 60, and 90 days

and analyzed for total chlorophyll (a and b) using a dimethyl sulfoxide (DMSO) extraction method (Hiscox and Israelstam 1979). Three beakers were removed from each aquarium at 30, 60 and 90 days, and shoots and roots were separated and oven-dried (70C for 48 hr) to a constant weight. Biomass and chlorophyll data were subjected to analysis of variance (ANOVA). Effects of the fluridone treatments on shoot biomass and chlorophyll content were examined by regression analysis to test for a linear response of each parameter over sampling time and between treatments at each sampling time.

RESULTS AND DISCUSSION

Growth of untreated milfoil and hydrilla was characterized by the formation of dense surface canopies that persisted throughout the study. Although biomass per harvest increased over time (Figures 1 and 2), the total biomass per

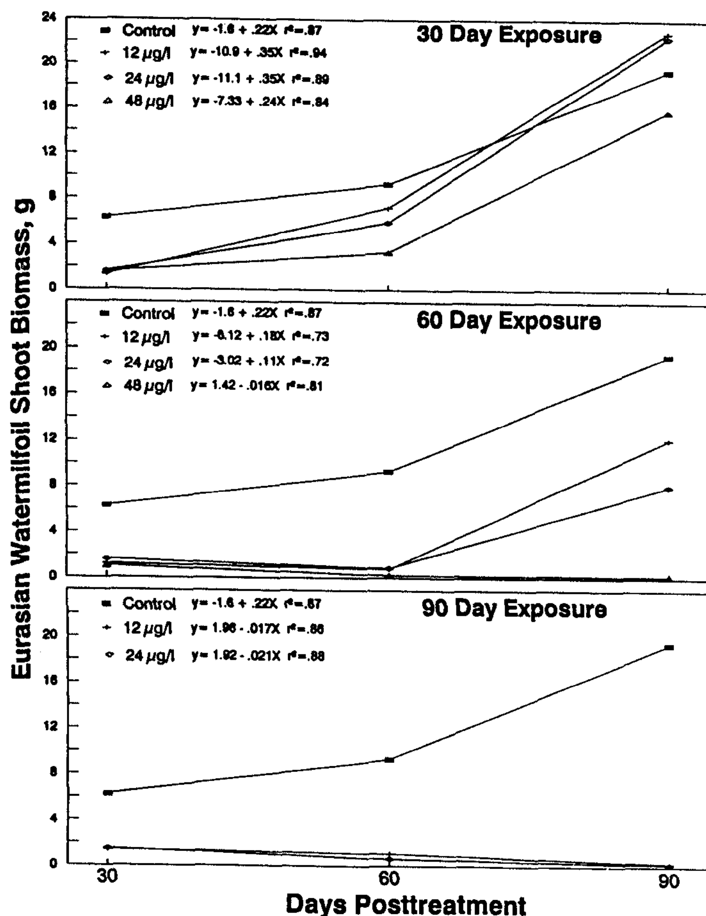

Figure 1. Effects of fluridone on shoot biomass of Eurasian watermilfoil harvested at 30, 60, and 90 days. Data points represent actual values. Regression equations (y = shoot biomass, x = days posttreatment) were calculated to determine if biomass showed a linear response to treatment over time.

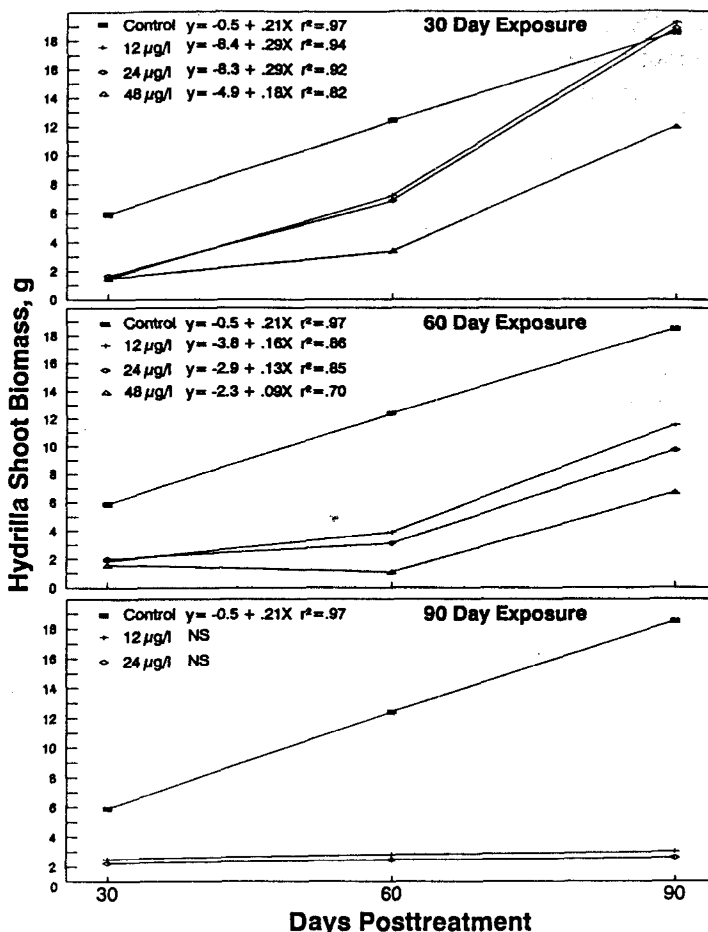

Figure 2. Effects of fluridone on shoot biomass of hydrilla harvested at 30, 60, and 90 days. Data points represent actual values. Regression equations (y = shoot biomass, x = days posttreatment) were calculated to determine if biomass showed a linear response to treatment over time. If the ANOVA procedure indicated that no significant differences existed between treatments, regression equations were labeled NS.

aquarium remained fairly constant from 30 through 90 days. By removing beakers over time, individual plants were able to increase biomass through utilization of open space.

Milfoil began to manifest fluridone symptoms by 6 days after treatment (DAT), as indicated by the 47 to 74% reduction of total chlorophyll in shoot tips (Table 1). Although actively growing apical shoots became albescent (bleached), elongation and growth of this tissue continued to occur for approximately 7 days. Growth had ceased by 10 DAT and albescent tissue became necrotic and detached from the stem. By 21 DAT all new growth from apical shoots had decayed (a canopy no longer existed) and lateral buds began to emerge from existing stem tissue or rootcrowns. All new shoot growth showed characteristic fluridone symptoms, yet stem tissue below the active growing points maintained a healthy green appearance. Results of the 30-day harvest indicated

that fluridone reduced milfoil biomass by 75% (Figure 1). Although treatment rate resulted in no significant differences in shoot biomass ($p = 0.97$), data indicated that the 24- and 48-µg/l treatments resulted in a 50 to 77% greater reduction of chlorophyll than the 12-µg/l treatment (Table 1).

TABLE 1. CHLOROPHYLL CONTENT OF EURASIAN WATERMILFOIL APICAL SHOOTS SAMPLED AT 6, 30, 60, AND 90 DAYS AFTER INITIAL FLURIDONE TREATMENT.

Treatment	Chlorophyll content (mg/g fresh weight)				Linear
(µg/l/day)	6 DAT	30 DAT	60 DAT	90 DAT	response[1]
Control	1.19	1.21	1.05	1.25	NS
12/30	0.64	0.45	0.86	1.31	0.05
24/30	0.43	0.23	0.85	1.20	0.05
48/30	0.31	0.16	0.72	1.13	0.05
12/60	0.62	0.42	0.39	0.96	NS
24/60	0.41	0.21	0.21	0.97	NS
48/60	0.28	0.13	0.04	0.02	0.05
12/90	0.59	0.47	0.28	0.09	0.05
24/90	0.37	0.19	0.11	0.02	0.05

[1]Test for linear response of chlorophyll content over sampling time within each treatment. NS = not significant at the 0.05 level.

Immediately following the 30-day treatment period, milfoil no longer exposed to fluridone began to recover. Regrowth from lateral buds and rootcrowns in the 12- and 24-µg/l treatments was rapid and plants reformed a canopy within 12 days. No residual response to fluridone was noted during the recovery period. Milfoil regrowth from the 48-µg/l treatments was delayed, and some of the early regrowth showed symptoms of residual fluridone. Following a 30-day recovery period (60-day harvest), it was difficult to discern fluridone-treated plants from untreated plants. Milfoil treated at 48 µg/l lagged behind the other treatments in biomass recovery (Figure 1); however, these plants were actively growing and forming a canopy. Shoot biomass recovery decreased linearly in response to increasing treatment concentrations (biomass = 8.44- 0.11*conc. $r^2 = 0.97$) following 30 days of recovery. Following 60 days of recovery the 12- and 24-µg/l treatments exceeded reference aquaria in biomass (Figure 1). The biomass and chlorophyll of the 48-µg/l treatment remained reduced, but the trend toward an increase in biomass over time indicated a complete recovery was likely following 30 days of fluridone exposure. In contrast, milfoil that remained exposed to fluridone (60- and 90-day exposures) continued to decline and new growth was limited to a few albescent shoots from lateral buds or rootcrowns. Stems were further defoliated and less vigorous, but, overall, the plants remained dormant. This was verified by the fact that shoot and root biomass levels of treated plants changed very little from 30 to 60 days. Shoot biomass at 60 days showed an 87% reduction in all treatments compared to the reference aquaria (Figure 1). Although no significant differences in shoot ($p = 0.68$) or root ($p = 0.51$) biomass existed between fluridone treatments, the 48-µg/l treatment was much less vigorous as stems were completely defoliated and brittle at harvest.

Milfoil recovery was slower following the 60-day fluridone exposure period. Although the 12-µg/l treatment began to recover immediately following the removal of fluridone, the 24- and 48-µg/l treatments remained inactive for 5 days following fluridone removal. By 14 days following removal of the fluridone, both the 12- and 24-µg/l treatments were actively growing and recovering. At the 90-day harvest, shoot biomass of these treatments, though 36 to 57% less than untreated plants, had recovered dramatically (8- to 12-fold increase in biomass) over the 30-day recovery period (Figure 1). The 30-day recovery, following the 60-day exposure, also showed that shoot biomass decreased linearly as treatment rates increased (biomass = 17.8 - 0.38*conc. $r^2 = 0.96$). The 48-µg/l treatments produced few new shoots from lateral buds; furthermore, these new tips were brittle and somewhat albescent. Biomass and chlorophyll continued to decrease over the 30-day recovery period (Figure 1), indicating the inability of milfoil to recover following this treatment.

Milfoil biomass and chlorophyll content continued to decrease during the 90-day exposure period. Following 90 days of exposure to fluridone, biomass and chlorophyll were reduced by approximately 93 to 99% compared to untreated controls (Figure 1, Table 1). The defoliated stems lacked shoot tips and were flaccid at harvest; however, some root tissue remained attached to rootcrowns. The fragile condition and extremely reduced biomass of the milfoil following the 90-day exposure indicated that recovery was unlikely, even in the optimal regrowth conditions experienced in the growth chamber.

Results indicated that fluridone exposure time was critical for the long-term control of milfoil. Growth ceased and biomass declined in the presence of all fluridone treatments tested. However, the 48-µg/l treatment was the only rate that prevented rapid regrowth following 60 days of exposure. Immediate regrowth following removal of fluridone from the water column indicates that the herbicide was not sequestered in plant tissue at phytotoxic levels. The minimum level of fluridone that must be maintained to produce phytotoxic symptoms has not been determined, and is likely dependent on the species and growth stage of the plant (Van and Conant 1988, Spencer and Ksander 1989, Spencer *et al.* 1989, Netherland 1992). However, Netherland (1992) reported that milfoil

exposed to 5 μg/l for 70 days was reduced by 40% compared to untreated plants, but treated plants continued to produce chlorophyll and significantly increased biomass over pretreatment levels.

Exposure of hydrilla to fluridone led to a 85 to 92% reduction in total chlorophyll by 6 DAT (Table 2). Albescent tissue continued to elongate and maintained its integrity during the 30-day exposure. This was in contrast to milfoil which ceased elongating at 7 days, as bleached tissue became necrotic and detached from the stem. Following the 30-day exposure period at concentrations of 12, 24 and 48 μg/l, hydrilla shoot mass was reduced 70% compared to untreated controls (Figure 2). Total chlorophyll at 30 DAT remained reduced by 85 to 92%, whereas root biomass was reduced by only 5 to 18% compared to untreated controls.

TABLE 2. CHLOROPHYLL CONTENT OF HYDRILLA APICAL SHOOTS SAMPLED AT 6, 30, 60, AND 90 DAYS AFTER INITIAL FLURIDONE TREATMENT.

Treatment	Chlorophyll content (mg/g fresh weight)				Linear
(μg/l/day)	6 DAT	30 DAT	60 DAT	90 DAT	response[1]
Control	1.05	1.01	1.12	1.04	NS
12/30	0.15	0.16	0.97	1.22	0.05
24/30	0.13	0.09	1.18	1.06	0.05
48/30	0.09	0.09	1.02	0.98	0.05
12/60	0.10	0.08	0.19	0.81	0.05
24/60	0.09	0.14	0.19	0.92	0.05
48/60	0.08	0.10	0.21	1.01	0.05
12/90	0.12	0.12	0.14	0.08	—[2]
24/90	0.07	0.08	0.06	0.04	—[2]

[1]Test for linear response of chlorophyll content over sampling time within each treatment. NS = not significant at the 0.05 level.
[2]ANOVA indicated no significant difference between treatments at the 0.05 level.

Removal of fluridone-treated water at 30 days resulted in an initial rapid growth of green shoot tips for a 4-day period, followed by a return of fluridone symptoms at 7 days. The reappearance of fluridone symptoms 1 week following the drain procedure indicates that fluridone was either not adequately removed from the system, or the compound remained sequestered within the plant tissue. This recurrence of symptoms indicates that the level of fluridone activity may be well below 12 μg/l. By 15 days recovery, hydrilla again produced healthy green shoots from stems and rootcrowns. The 60-day harvest (30 days of recovery) resulted in a 46% shoot biomass reduction in both the 12- and 24-μg/l treatments, and a 70% reduction in the 48-μg/l treatment (Figure 2). Although shoot biomass remained significantly decreased, total chlorophyll recovered to approach untreated control levels (Table 2), indicating active regrowth. This lag between the resumption of hydrilla regrowth and chlorophyll recovery following fluridone treatment also was noted by Spencer and Ksander (1989). Results of the 30-day exposure period indicated that biomass recovery was linear over time (30 and 60 days of recovery) and was reduced (35%) only by the 48-μg/l treatment (Figure 2).

Hydrilla exposed to fluridone for 60 days continued to produce albescent shoots from rootcrowns. Stems remained foliated and buoyant but were not actively growing. Shoot biomass was reduced from 70 to 85% by 60 DAT (Figure 2), whereas root biomass was only reduced 35 to 50%. Immediately following the 60-day drain procedure, all treated plants began to recover (no residual fluridone symptoms were apparent). During this 30-day recovery period hydrilla biomass nearly tripled (Figure 2). Although biomass was reduced by 35 to 60%, chlorophyll values and canopy formation by actively growing shoots indicated that recovery from all treatments was likely to occur.

Hydrilla exposed to fluridone for 90 days remained reduced by 88% at all harvest intervals (30, 60, and 90 days) compared to untreated controls (Figure 2). Although stems were flaccid and defoliated and chlorophyll was greatly reduced, no significant linear response in biomass reduction was noted over time. This was in contrast to milfoil biomass and chlorophyll which continued to decline over time.

Results indicated that fluridone exposure time was critical for the sustained control of hydrilla. Although significantly reduced following a 60-day exposure, hydrilla was able to recover from all fluridone rates tested. The ability of the plant to recover from 90-day exposures remained unclear.

Previous laboratory research has been conducted on fluridone CET effects on hydrilla (Van and Steward 1986, Van and Conant 1988, Spencer and Ksander 1989). These studies showed that increasing fluridone rates from 50 to 1,000 μg/l (150 μg/l is the maximum labeled rate) could reduce contact time requirements; however, a 10-fold increase in fluridone concentration often led to only marginal increases in efficacy. Hall *et al.* (1984) treated hydrilla and milfoil with fluridone at rates of 10 to 90 μg/l for a 12-week period and achieved a 75 to 90% reduction in shoot biomass; yet increasing the rate of fluridone did not result in a significant difference in shoot mass. These studies showed that over a long exposure period, low fluridone rates (~10 μg/l) were effective at inhibiting growth of submersed plants. Our results indicated that the shorter exposure periods (30 and 60 days) effectively reduced shoot mass, but were ineffective at preventing regrowth following removal of fluridone. One exception was milfoil treated at 48 μg/l for 60 days, which

significantly reduced biomass (98%) and prevented regrowth. Since the laboratory offers optimal conditions for plant regrowth following herbicide treatment (*e.g.* readily available light, stable water quality and temperature, low mechanical stress, etc.), perhaps an underestimation of efficacy can occur.

Based on information from the laboratory and the field, it is likely that the key to a successful fluridone treatment is in maintaining herbicidally active concentrations for periods exceeding 60 days. Moreover, recent success of sequential applications of fluridone to lotic systems can be explained by the ability to maintain low concentrations (<40 μg/l) over long periods of time (8 to 16 weeks).

ACKNOWLEDGMENTS

This research was conducted under the U.S. Army Corps of Engineers Aquatic Plant Control Research Program, Environmental Laboratory, U.S. Army Engineer Waterways Experiment Station. Permission was granted by the Chief of Engineers to publish this information. We thank Susan Sprecher, Ann Stewart, Jane Brazil, and Charles Mayfield for laboratory assistance in this study. We also thank DowElanco Inc. for providing fluridone for this study.

LITERATURE CITED

Bowes, G. A., A. S. Holaday and W. T. Haller. 1979. Seasonal variation in the biomass, tuber density, and photosynthetic metabolism of hydrilla in three Florida lakes. J. Aquat. Plant Manage. 17:61-65.

Getsinger, K. D., A. M. Fox and W. T. Haller. 1992. Controlling submersed plants with herbicides in flowing water systems. Proc. Aquat. Plant Control Research Program, Miscellaneous Paper A-92-2, U.S. Army Engineer Waterways Experiment Station, Vicksburg, MS. pp. 103-105.

Grace, J. B. and R. G. Wetzel. 1978. The production biology of Eurasian water-milfoil (*Myriophyllum spicatum* L.): A review. J. Aquat. Plant Manage. 16:1-11.

Green, W. R. and H. E. Westerdahl. 1990. Response of Eurasian watermilfoil to 2,4-D concentrations and exposure times. J. Aquat. Plant Manage. 28:27-32.

Hall, J. F., H. E. Westerdahl and T. J. Stewart. 1984. Growth response on *Myriophyllum spicatum* and *Hydrilla verticillata* when exposed to continuous, low concentrations of fluridone. Technical Report A-84-1, U.S. Army Engineer Waterways Experiment Station, Vicksburg, MS. 22 pp.

Harlan, S. M., G. J. Davis and G. J. Pesacreta. 1985. Hydrilla in three North Carolina lakes. J. Aquat. Plant Manage. 23:68-71.

Hiscox, J. D. and G. Israelstam. 1979. A method for the extraction of chlorophyll from leaf tissue without leaf maceration. Can. J. Bot.57:1332-1334.

Marquis, L. Y., R. D. Comes and C. P. Yang. 1981. Absorption and translocation of fluridone and glyphosate in submersed vascular plants. Weed Sci. 29:229-236.

Mossler, M. A., D. G. Shilling and W. T. Haller. 1989. Photolytic degradation of fluridone. J. Aquat. Plant Manage. 27:69-73.

Netherland, M. D. 1992. Herbicide concentration/exposure time relationships for Eurasian watermilfoil and hydrilla. Proc. Aquat. Plant Control Research Program, Miscellaneous Paper A-92-2, U.S. Army Engineer Waterways Experiment Station, Vicksburg, MS. pp. 79-85.

Netherland, M. D., and K. D. Getsinger. 1992. Efficacy of triclopyr on Eurasian watermilfoil: Concentration and exposure time effects. J. Aquat. Plant Manage. 30:1-5.

Netherland, M. D., W. R. Green and K. D. Getsinger. 1991. Endothall concentration and exposure time relationships for the control of Eurasian watermilfoil and hydrilla. J. Aquat. Plant Manage. 29:61-67.

Smart, R. M. and J. W. Barko. 1984. Culture methodology for experimental investigations involving rooted submersed aquatic plants. Miscellaneous Paper A-89-2, U.S. Army Engineer Waterways Experiment Station, Vicksburg, MS. 18 pp.

Spencer, D. F. and G. G. Ksander. 1989. Influence of iron on hydrilla's response to fluridone. J. Aquat. Plant Manage. 27:57-65.

Spencer, D. F., G. G. Ksander and L. C. Whiteand. 1989. Sago pondweed (*Potamogeton pectinatus*) tuber size influences its response to fluridone treatment. Weed Sci. 37:250-253.

Van, T. K., and K. K. Steward. 1986. The use of controlled-release fluridone fibers for the control of hydrilla (*Hydrilla verticillata*). Weed Sci. 34:70-76.

Van, T. K., and R. D. Conant. 1988. Chemical control of hydrilla in flowing water: Herbicide uptake characteristics and concentration versus exposure. Technical Report A-88-2, U.S. Army Engineer Waterways Experiment Station, Vicksburg, MS. 33 pp.