J. Aquat. Plant Manage. 30: 1-5

Efficacy of Triclopyr on Eurasian Watermilfoil: Concentration and Exposure Time Effects[1]

M. D. NETHERLAND[2] AND K. D. GETSINGER[2]

ABSTRACT

Herbicide concentration and exposure time relationships were determined for the triethylamine salt formulation of triclopyr (3,5,6-trichloro-2-pyridinyloxyacetic acid) and control of Eurasian watermilfoil (*Myriophyllum spicatum* L.) under controlled-environment conditions. Thirty-four combinations of triclopyr concentration and exposure time were tested: concentrations ranged from 0.25 to 2.5 mg acid equivalent (ae)/l; exposure times ranged from 2 to 84 hr. Plant control was based on shoot and root biomass harvested at 5 weeks posttreatment. Weekly visual injury ratings were used to characterize initial injury response and recuperative ability of plants following treatment. Plant response was rapid and most treatments resulted in severe injury to existing tissue with the majority of regrowth coming from rootcrowns and lower stems. Plant control increased (biomass decreased) as concentration and/or exposure times were increased until a threshold level was reached which provided complete plant control. Excellent control (>85% biomass reduction) was achieved at concentration/exposure time combinations of 0.25 mg ae/l for 72 hr, 0.5 mg/l for 48 hr, 1.0 mg/l for 36 hr, 1.5 mg/l for 24 hr, and 2.0 and 2.5 mg/l for 18 hr. Treatments of 2.5 mg/l for 2 hr, 1.0 mg/l for 6 hr, and 0.25 and 0.5 mg/l for 12 hr were ineffective and produced only minor initial injury symptoms followed by rapid plant growth. Results indicate that increased Eurasian watermilfoil control is likely in systems where plants remain in contact with triclopyr concentrations greater than developed threshold levels.

Key words: Herbicide, chemical control, Garlon 3A, exposure time, *Myriophyllum spicatum.*

INTRODUCTION

Following a herbicide application for submersed weed control, gravity flow, tides, and thermal and wind-induced circulation patterns can rapidly dilute and disperse herbicide residues from the treatment area (Fox *et al.* 1991, Getsinger *et al.* 1990). This rapid residue dissipation is generally considered desirable in an aquatic system; however, residues that disperse too quickly may result in a lack of plant control due to insufficient herbicide contact time. To assess the effect of rapid residue dissipation on efficacy of submersed applications, laboratory studies of herbicide concentration and exposure time (CET) interactions have been conducted against Eurasian watermilfoil at the US Army Engineer Waterways Experiment Station (Hall *et al.* 1984, Green and Westerdahl 1990, Netherland *et al.* 1991). Results of these studies indicate that the duration of exposure to a given concentration of herbicide is critical to achieving plant control. Determination of CET relationships should improve the ability to predict plant control in high water exchange environments.

Currently, the triethylamine salt formulation of the herbicide triclopyr is registered under an experimental use permit (EUP) for managing Eurasian watermilfoil and other nuisance aquatic species. Triclopyr is an auxin-type systemic herbicide used for broadleaf weed control in a variety of industrial, forestry, and other non-crop sites. Triclopyr's mode of action, translocation, and spectrum of weed control is similar to that of phenoxy herbicides, such as 2,4-D (2,4-dichlorophenoxy acetic acid) (WSSA 1989). Upon application to an aquatic system, triclopyr degrades and dissipates via chemical, biological, and physical processes. Triclopyr is subject to photodegradation (313 nm wavelength), and is further metabolized to carbon dioxide, water, and various organic acids by aquatic microorganisms (McCall and Gavit 1986, Dow Chemical 1988). This herbicide shows a low order of toxicity to microbial communities and higher aquatic organisms, and residue accumulation in sediment, shellfish, and fish is negligible (Getsinger and Westerdahl 1984, Dow Chemical 1988, Green *et al.* 1989).

While extensive data exists on the environmental fate and toxicology of triclopyr in aquatic systems, data concerning effectiveness against Eurasian watermilfoil, particularly in high water-exchange environments, is limited. This lack of efficacy information dictates the need for the development of triclopyr CET relationships for Eurasian watermilfoil control. Therefore, the objective of this study was to examine the effect of triclopyr CET combinations for controlling Eurasian watermilfoil (hereafter called milfoil). Results from this study can be used to provide guidance for the use of triclopyr in hydrodynamic systems.

MATERIALS AND METHODS

This study consisted of 34 CET treatments tested in three independent runs in a controlled-environment chamber system. Each experimental run consisted of 48, 55 l aquaria (0.90 m tall × 0.09 m^2) with overhead lighting providing mean photosynthetically active radiation of 614 ± 64 $\mu E/m^2/sec$ at the water surface. The photoperiod was 14L:10D, and water temperature was maintained at 22 ± 1 C.

[1]Part of these data were previously published in the US Army Corps of Engineers Aquatic Plant Control Research Program Annual Proceedings, Misc. Papers A-90-3 and A-91-3.

[2]Environmental Laboratory, US Army Engineer Waterways Experiment Station, 3909 Halls Ferry Rd., Vicksburg, MS 39180-6199. Received for publication October 11, 1991 and in revised form December 2, 1991.

Milfoil apical tips were collected from the Suwannee River, FL, and from culture ponds in Lewisville, TX. Four apical shoots (10-15 cm) were planted (5 cm deep) in 300-ml pyrex beakers that contained sediment (amended with RapidGro) obtained from Brown's Lake, Vicksburg, MS. A thin layer of silica sand in each beaker prevented resuspension of sediment during water exchange periods described below. Eleven beakers containing milfoil apical tips were placed in each aquarium. Flow-through pumps provided complete exchange of simulated hard water (Smart and Barko 1984) within each aquarium every 24 hr.

Milfoil was grown for 20 days prior to treatment with triclopyr. Previous experiments indicated this pretreatment period allowed for the development of a healthy shoot and root mass (Green and Westerdahl 1990, Netherland *et al.* 1991). One randomly selected beaker containing milfoil was removed from each aquarium (10 beakers remained) prior to chemical treatment. This harvested material was separated into shoots and roots and dried to a constant weight. An overall average weight (± 1 SD) was obtained and this weight was multiplied by 10 to estimate biomass of the remaining 10 beakers within each aquarium. Estimated dry weight (DW) values of shoot biomass treated in the three independent milfoil runs was 14.2 ± 1.3 g, 13.5 ± 1.2 g, and 13.7 ± 1.5 g DW per aquarium; while root biomass was 2.6 ± 0.34 g, 1.0 ± 0.31 g, and 2.9 ± 0.21 g DW per aquarium. This represents equivalent milfoil field biomass levels that ranged from 150 to 158 g DW/m^2 for shoots and 11 to 31 g DW/m^2 for roots, and was comparable to reports of maximal biomass levels from various field locations that ranged from 32 to 463 g DW/m^2 (Lim and Lozoway 1976, Grace and Wetzel 1978, Painter 1988).

Within each run, treatments were replicated three times and randomly assigned to test aquaria. Twelve treatments were repeated in separate experimental runs to allow statistical comparison of results among runs.

Triclopyr stock solutions were prepared from the commercial formulation Garlon 3A (DowElanco, Inc.), dissolved in distilled water. Treatment concentrations, reported as the acid equivalent of the triclopyr formulation, did not exceed the maximum EUP label rate of 2.5 mg/l. At the time of treatment, the flow-through water system was deactivated. Calculated volumes of triclopyr stock solution were added to the aquaria to provide the desired treatment concentrations. Upon termination of designated exposure times, aquaria were drained and refilled with fresh water 3 times to remove triclopyr residues. After rinsing, the flow-through system was reactivated for the duration of the experiment.

Water samples were collected within two minutes after treatment (to verify initial triclopyr concentrations), immediately prior to the first rinsing procedure (to determine residue loss during the exposure time), and 5 minutes to 96 hours after rinsing (to verify residue removal). Samples were analyzed for triclopyr residues by A&L Mid West Laboratories, Inc., Fort Wayne, IN.

Milfoil response to triclopyr was monitored for a posttreatment period of 35 days. Weekly visual assessments were made to document initial plant response to triclopyr, progression of injury symptoms, and initiation of any healthy milfoil regrowth. Surface mats of filamentous algae (e.g. *Oedogonium spp., Spirogyra spp., Pithophora spp.*) were removed by dip nets to encourage any potential for milfoil regrowth from rootcrowns and to aid in visual evaluations. Plants were harvested 35-days posttreatment, separated into shoots and roots, and oven-dried at 70 C to a constant weight.

Biomass data were statistically evaluated using analysis of variance (ANOVA). Treatment means, within a treatment run, were separated using Duncan's Multiple Range Test at the 0.05 level of significance. In addition a t-test at the 0.05 level of significance was used to compare biomass data from treatments (including references) that were repeated across experimental runs.

RESULTS AND DISCUSSION

Results from this study indicate that milfoil control is directly related to increased triclopyr concentrations and/or exposure times. Milfoil initially responded to all triclopyr treatments with symptoms characteristic of auxin-like growth regulators. Epinasty occurred rapidly as apical leaves curled and bent downward within 6 to 12 hr post-

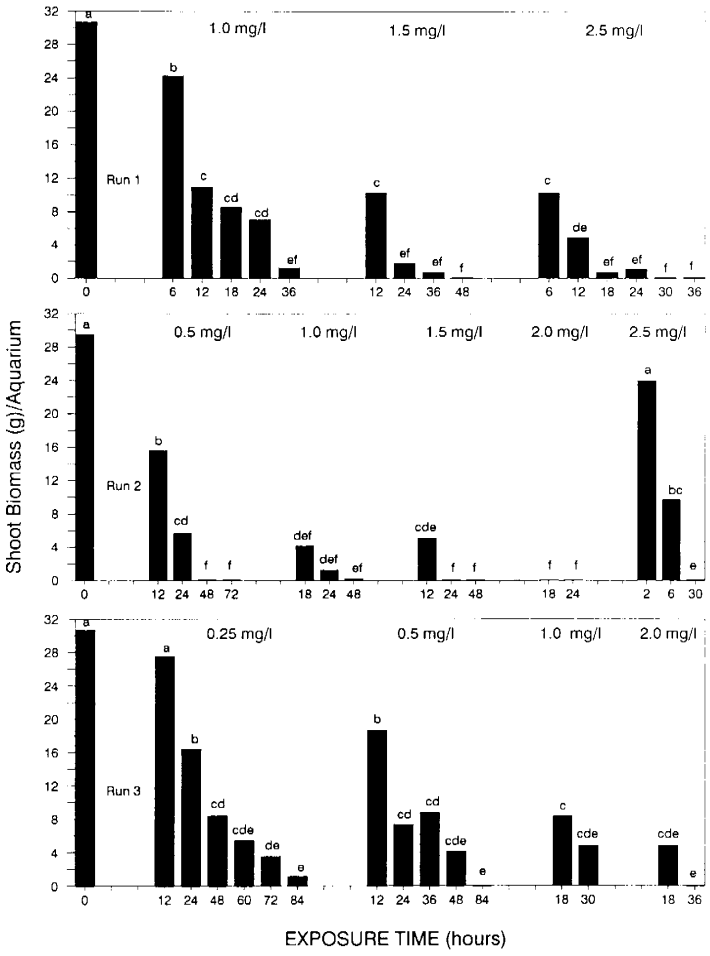

Figure 1. Eurasian watermilfoil shoot biomass harvested at 35 days posttreatment. Within a treatment run, different letters among concentration/exposure time combinations indicate significant differences at the 5% level according to Duncan's Multiple Range Test.

treatment; while shoot bending and twisting, and epidermal rupture, began within 36 hr posttreatment. Treatments of 2.5 mg/l for 2 hr, 1.0 mg/l for 6 hr, and 0.25 mg/l for 12 hr showed initial injury symptoms, although rapid regrowth (within 1 week) from injured apical tips and rootcrowns resulted in biomass reductions of only 11 to 19% compared to untreated reference aquaria (Figures 1 and 2). In these treatments, recovery of biomass surpassed pretreatment levels.

Treatments of 0.5 and 1.0 mg/l for 12 hr, and 0.25 mg/l for 24 hr resulted in severe injury with most shoot biomass at the canopy becoming epinastic and covered with filamentous algae (indicating possible release of nutrients from the injured plants) at 1 week posttreatment. While shoots at the canopy were senescing, uninjured lower stems and rootcrowns began producing healthy shoots within 2 weeks posttreatment. Regrowth occurred in all beakers and remained vigorous throughout the duration of the study. Shoot biomass was reduced 49 to 70%, whereas root biomass was reduced 37 to 55% compared to untreated references. The potential of milfoil to recover to pretreatment biomass levels in these treatments, was indicated by active shoot growth (from all beakers) which rapidly approached the water surface.

Treatments of 2.5 mg/l for 6 and 12 hr, 1.5 mg/l for 12 hr, 1.0 mg/l for 18, 24, and 30 hr, 0.5 mg/l for 24 and 36 hr, and 0.25 mg/l for 24, 48, and 60 hr severely impacted existing milfoil shoot tissue. Epinasty, followed by stem browning and leaf sloughing to the base of the stem, occurred in most beakers. Visual evaluations became difficult as filamentous algae covered senescing tissue up to 2 weeks posttreatment. By posttreatment week 3, many shoots (12-20) had emerged from surviving rootcrowns and/or stems. Although these shoots continued to grow for the remainder of the study, shoot biomass was reduced 70 to 85% and root biomass was reduced 58 to 87% compared to untreated references (Figures 1 and 2). These treatments completely eliminated shoot and root biomass in 50 to 70% of the beakers present in each aquarium. However, regrowth of milfoil from injured tissue suggests that exposure times did not allow sufficient triclopyr uptake and translocation throughout the plant and milfoil recolonization could occur.

Treatments of 2.0 and 2.5 mg/l at 18 and 24 hr, 1.5 mg/l at 24 and 36 hr, 1.0 mg/l at 36 and 48 hr, 0.5 at 48 and 72 hr, and 0.25 at 72, and 84 hr resulted in severe initial injury with minimal biomass recovery. Epinasty and stem browning followed by filamentous algal attachment to senescing stems occurred up to 3 weeks posttreatment. Algal biomass peaked at the height of milfoil injury (1 to 3 weeks posttreatment) and rapidly decreased as senescing tissue sank to the bottom of the aquaria. Visual evaluations indicated that 2 to 10 viable shoots (1-4 cm in length) had begun to grow from stem fragments and rootcrowns at 5 weeks posttreatment. Viable milfoil tissue was recovered from only 10 to 30% of beakers, resulting in shoot and root biomass reductions of 85 to 99% compared to untreated references (Figures 1 and 2). It remains uncertain if the milfoil regrowth seen in the sheltered, optimal growing conditions of the aquaria would survive in posttreatment conditions in the field.

Complete milfoil control was obtained with treatment combinations of 2.5 mg/l at 30 and 36 hr, 2.0 mg/l at 36 hr, 1.5 mg/l at 48 hr, and 0.5 mg/l at 84 hr. No viable shoot or root tissue remained following these treatments. This indicates that sufficient triclopyr uptake occurred, and translocation throughout the plant resulted in the death of all tissue.

Statistical comparison of harvested biomass values indicated that no significant difference ($p > .05$) existed among the majority of treatments (including references) that were repeated in separate experimental runs (data not presented). However, treatments of 1.5 mg/l at 24 hr, 1.0 mg/l at 18 and 24 hr and 0.5 mg/l at 24 and 48 hr produced significantly lower biomass values in the second run than in the first and third runs. Pretreatment root biomass in Run 2 was only 34 to 38% of the pretreatment root mass of Runs 1 and 3, and this condition may have resulted in enhanced milfoil sensitivity to triclopyr. Regrowth from these treatments in Runs 1 and 3 originated mainly from rootcrowns, whereas this source of regrowth was much reduced in the second run. Although biomass values were significantly lower in Run 2, the level of milfoil

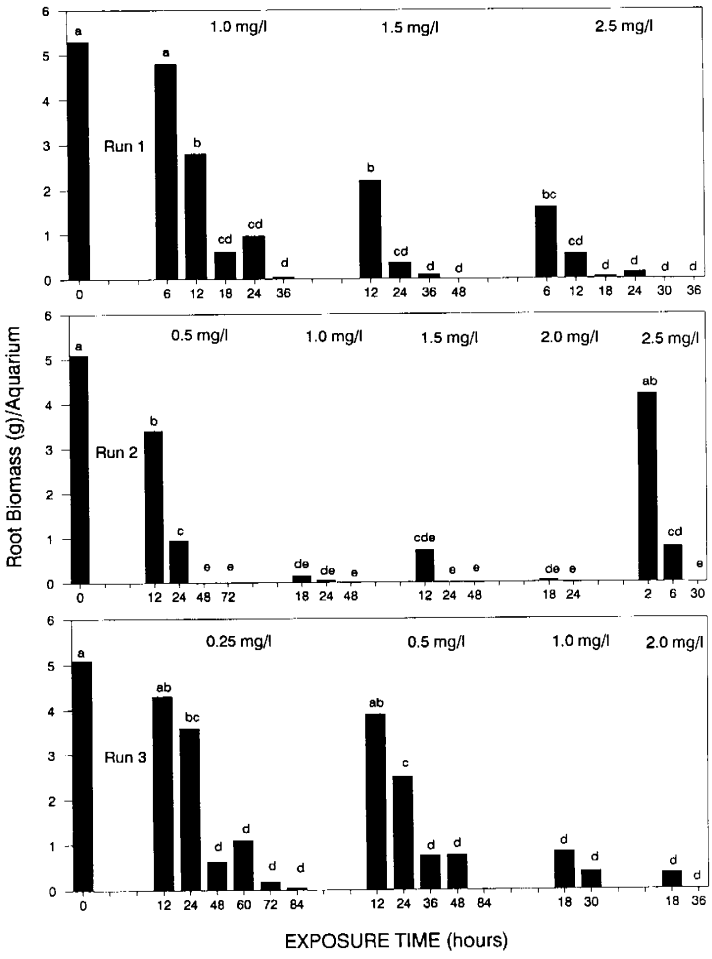

Figure 2. Eurasian watermilfoil root biomass harvested at 35 days posttreatment. Within a treatment run, different letters among concentration/exposure time combinations indicate significant differences at the 5% level according to Duncan's Multiple Range Test.

injury and control remained dependent on concentration and exposure time effects.

In summary, triclopyr effects on milfoil were rapid, and serious injury of existing shoot biomass occurred at most concentrations and exposure times tested. Although the majority of harvested biomass was the result of shoot regrowth from root crowns and injured stems, this recovery decreased as exposure times were increased within a concentration. Results indicate that triclopyr at concentrations of 2.5, 2.0, 1.5, 1.0, 0.5, and 0.25 mg/l should require a contact time of at least 18, 18, 24, 36, 48, and 72 hr respectively, to achieve >85% reduction of milfoil biomass.

Results of triclopyr residue analyses immediately after treatment, at the assigned exposure time prior to rinsing, and following the final rinse are summarized in Table 1. Results confirm that initial treatment concentrations were accurate, exposure time effects on residue loss were negligible, and that rinsing procedures provided complete removal of triclopyr residues.

The static nature of the treatments, and the absence of ultraviolet light, insured that any significant loss of triclopyr would depend on microbial/plant uptake and degradation. Although aquatic microorganisms have been reported to accumulate and metabolize triclopyr in the field (Dow Chemical 1988, WSSA 1989), the rate of microbial degradation remains uncertain and is likely dependent on temperature, application rate, and previous use of triclopyr with a treatment area. Likewise, field plants are reported to uptake and accumulate triclopyr (Green *et al.* 1989); however results of residue analyses from this, and similar laboratory studies, indicate plant uptake plays a minor role in herbicide residue loss from the water (Green and Westerdahl 1990, Netherland *et al.* 1991). Moreover, direct measurement of herbicide concentrations within plants indicates that tissue residues represent only 1 to 6% of the concentration in the ambient water (Van and Steward 1985, Reinert *et al.* 1985, Haller and Sutton 1973).

Photolysis has been reported to significantly influence triclopyr residue persistence, with predicted half-lives in water ranging from 2 to 10 hr, depending on water depth, time of year, and geographic location (McCall and Gavit 1986, WSSA 1989). From our CET results, predicted field half-lives (based solely on photolytic degradation) within this range would provide insufficient triclopyr concentration and exposure time to achieve acceptable milfoil control. It is likely that shading by target plants and the rapid extinction of ultraviolet radiation with water depth would reduce photolytic degradation of triclopyr in the field.

In many situations, triclopyr dissipation within the treatment site will be affected by dispersion due to gravity and wind-generated water flow. For instance, one field dissipation study resulted in triclopyr half-lives of 0.5 and 3.6 days in two treatment plots of similar depth and plant communities, leading the authors to conclude that water movement was a major factor influencing residue dissipation (Green *et al.* 1989).

The effect of variable field dissipation rates on triclopyr efficacy emphasizes the need to develop functional CET relationships. Using biomass data and visual observations from this study, a summary graph was developed for triclopyr efficacy against milfoil under varying concentrations and exposure times (Figure 3). Triclopyr dissipation curves that fall within the parameters of Zone A would provide < 70% milfoil control; within Zone B, from 70 to 85% control; and within Zone C, > 85% control. Multiple regression analysis of data combined from the three independent runs (% control = 16.1 + conc*19.4 + exptime* 1.33, $r^2 = .82$) indicates that this graph can be used as a predictive tool to estimate the level of milfoil control achieved following a triclopyr treatment.

It should be noted that the dynamic nature of triclopyr dissipation in the field differs from that of static laboratory exposures; that is, plants are exposed to a dissipating concentration of herbicide over time. It remains unclear if initial exposure to high concentrations of herbicide followed by dissipation, is more or less effective than static exposures. In addition, differences in recuperative capacity (particularly from rootcrowns), and differences in sen-

TABLE 1. TRICLOPYR CONCENTRATION (MG/L) IMMEDIATELY AFTER TREATMENT, PRIOR TO DRAINING AT THE END OF THE EXPOSURE PERIOD, AND FOLLOWING THE FINAL DRAIN.[a]

Calculated	Initial	Pre drain[b]	Post drain[c]
0.0	0.0	0.0	0.0
2.5	2.44 ± 0.10	2.36 ± 0.14	0.0
2.0	2.06 ± 0.15	2.02 ± 0.06	0.0
1.5	1.48 ± 0.11	1.51 ± 0.07	0.0
1.0	1.06 ± 0.06	0.98 ± 0.04	0.0
0.5	0.53 ± 0.07	0.48 ± 0.10	0.0
0.25	0.22 ± 0.04	0.19 ± 0.06	0.0

[a]Mean of 3 replicates ± 1 SD
[b]Sampled at longest exposure period for each concentration tested
[c]Sampled between 5 minutes and 96 hr after final drain

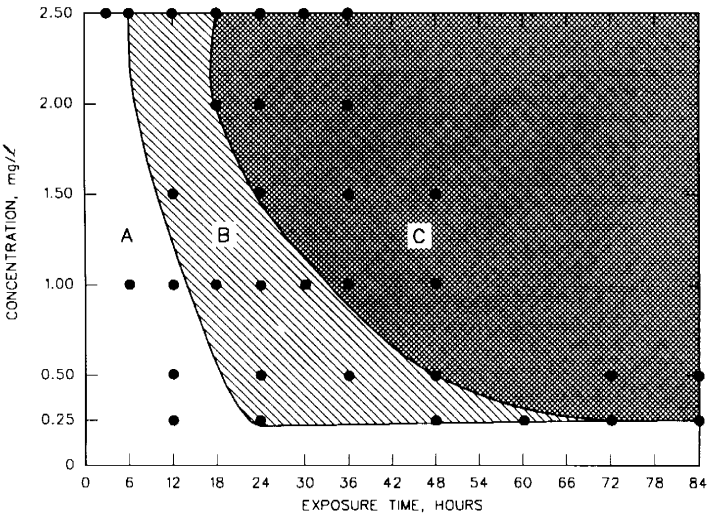

Figure 3. Summary of triclopyr concentration/exposure time (CET) relationships for control of Eurasian watermilfoil. Circles represent actual CET test coordinates. Zones A, B, and C were estimated using these coordinates. Zone A represents CET combinations that should provide < 70% milfoil control, regrowth from injured stems and rootcrowns is likely to occur within 2 weeks posttreatment; Zone B represents CET combinations that should provide 70 to 85% milfoil control with regrowth beginning 3 to 4 weeks posttreatment and; Zone C represents CET combinations that should provide > 85% milfoil control with very limited or no regrowth up to 5 weeks posttreatment.

sitivity between mature field plants and plants grown from cuttings, require caution when applying laboratory results to the field. Field studies are being conducted to determine triclopyr efficacy, dissipation rates and selectivity in various geographic locations. Preliminary observations indicate that tricylopyr, like 2,4-D, may have species-selective properties at rates used to control milfoil.

With similarities in mode of action, application rates and species selectivity, it is inevitable that comparisons of triclopyr and 2,4-D efficacy will be made. Results of this study and a comparable 2,4-D laboratory study (Green and Westerdahl 1990) indicate that CET requirements for milfoil control are very similar for these two herbicides. Efficacy of both compounds is directly related to the length of time milfoil is in contact with dissipating concentrations of each herbicide.

While difficulty remains in precisely predicting field efficacy from laboratory results, the relationship of increased triclopyr concentrations and exposure times to increased milfoil control has been established. The development of these CET relationships will help provide guidance for more effective use of triclopyr in aquatic systems.

ACKNOWLEDGMENTS

This research was conducted under the US Army Corps of Engineers Aquatic Plant Control Program, Environmental Laboratory, US Army Engineer Waterways Experiment Station. Permission was granted by the Chief of Engineers to publish this information. We thank Brian York, Kimberly Deevers, Sheron Burt, and Linda Nelson for laboratory assistance in this study. We also thank Dow-Elanco Inc. for providing triclopyr formulations for this study.

LITERATURE CITED

Dow Chemical Co., 1988. Triclopyr: Technical Information Guide. Midland, MI. 7 pp.

Fox, A. M., Haller, W. T., Getsinger, K. D. and Green, W. R. 1991. Characterization of water exchange in hydrilla-infested tidal canals of the Crystal River, Florida. Miscellaneous Paper A-9-2, US Army Engineer Waterways Experiment Station, Vicksburg, MS. 26 pp.

Getsinger, K. D., and Westerdahl, H. E. 1984. Field evaluation of Garlon 3A (triclopyr) and 14-ACE-B (2,4-D BEE) for the control of Eurasian watermilfoil. Miscellaneous Paper A-84-5, US Army Engineer Waterways Experiment Station, Vicksburg, MS. 12 pp.

Getsinger, K. D., Haller, W. T., and Fox, A. M. 1990. Herbicide application techniques for flowing water: Evaluation of submersed techniques using Rhodamine WT. In Proceedings, 24th Annual Meeting, Aquatic Plant Control Research Program, Miscellaneous Paper A-90-3, US Army Engineer Waterways Experiment Station, Vicksburg, MS. pp. 128-133.

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

Green, W. R., Westerdahl, H. E., Joyce, J. C., and Haller, W. T. 1989. Triclopyr (Garlon 3A) dissipation on Lake Seminole, Georgia. Miscellaneous Paper A-89-2, US Army Engineer Waterways Experiment Station, Vicksburg, MS. 19 pp.

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

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

Haller, W. T., and Sutton, D. L. 1973. Factors affecting the uptake of endothall ^{14}C by hydrilla. Weed Sci. 21:446-448.

Lim, P. G. and Lozoway, K. R. 1976. Studies of Aquatic Macrophytes Part X. A field experiment with granular 2,4-D for control of Eurasian watermilfoil. Water Investigations Branch Report No. 2613. Victoria, British Columbia.

McCall, P. J., and Gavit, P.D. 1986. Aqueous photolysis of triclopyr and its butoxyethyl ester and calculated environmental photodecomposition rates. Environmental Toxicol. and Chem. 5:879-885.

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

Painter, D. S. 1988. Long-term effects of mechanical harvesting on Eurasian watermilfoil. J. Aquat. Plant Manage. 26:25-29.

Reinert, K. H., Rodgers, J. H., Hinman, M. L., and Leslie, T. J. 1985. Compartmentalization and persistence of endothall in experimental pools. Ecotoxicol. and Environ. Safety 10:86-96.

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

Van, T. K. and Steward, K. K. 1985. The use of controlled-release fluridone fibers for the control of hydrilla (*Hydrilla verticillata*). Weed Sci. 34:70-76.

WSSA 1989. Herbicide Handbook of the Weed Science Society of America, 6th Edition, Champaign, IL. 301 pp.