

Subsurface Applications of Triclopyr and 2,4-D Amine for Control of Water Chestnut (*Trapa natans* L.)

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INTRODUCTION

The invasive floating-leaf plant water chestnut (*Trapa natans* L.) is a detriment to other aquatic organisms when it completely covers the water surface in lake coves and quiet stretches of rivers (Groth et al. 1996, Caraco and Cole 2002, Strayer et al. 2003). In the U.S., water chestnut is found in Vermont south to Virginia, with historic nuisance growth levels recorded in the Hudson-Mohawk River system, Lake Champlain, and the Potomac River (Countryman 1970 and 1978, Gangstad 1981, Kiviat 1993). While management activ-

ities controlled this plant in the 1960s and 1970s, it is once again becoming a widespread problem in northeastern waterways (Hummel and Kiviat 2004).

Water chestnut seeds germinate in early spring. Submerged stems that terminate in small leafy rosettes grow quickly to the water surface. Swollen air-filled petioles cause the rosettes to float and, by summer, there is a thick mat of rosette leaves and stems. Each rosette has 20 or more leaves that are 1 to 4.5 cm in length and rhomboid in shape. Flowering and seed ripening occur in mid-summer and continue into the fall, until a hard frost kills the floating leaves (Madsen 1993). The life cycle, distribution, and impacts of water chestnut has been reviewed by Hummel and Kiviat (2004).

As an annual bearing a relatively small number of seeds per plant, water chestnut is amenable to control, and possible eradication, with appropriate management methods timed to

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reduce plant numbers and prevent seed production (Smith 1955). Although control methods, such as mechanical harvesting (Elser 1966, Countryman 1978), cutting (Madsen 1993), and a combination of herbicide applications with hand-pulling (Greeley 1965) have been employed with varying degrees of success, the use of the aquatic herbicide 2,4-D (2,4-dichlorophenoxyacetic acid) has been the most effective in decreasing water chestnut populations (Smith 1955, Greeley and Steenis 1959, Greeley 1965). Currently, the 2,4-D ester formulated as a granular pellet is the only herbicide registered with a recommendation for control of the plant on the product label (see Aqua-Kleen®, Cerexagri, Philadelphia, PA and Navigate®, Applied Biochemists, Milwaukee, WI).

While 2,4-D formulations, rates and combinations have been previously described for water chestnut control, many recommendations need to be re-evaluated due to changes in formulations, the loss of certain aquatic herbicide registrations that were primary tank mixes (e.g., silvex: 2-(2,4,5-trichlorophenoxy propionic acid), and label modifications as a result of 2,4-D re-registration by the U.S. Environmental Protection Agency (USEPA) in 2005. With the availability of different formulations and newly-registered products (Netherland et al. 2005) as well as the development of chemical control strategies, such as low-dose treatments and early-season applications (Getsinger 1998, Poovey and Getsinger 2005), selective control of water chestnut could be achieved with current technology. It is well documented that 2,4-D can selectively remove dicots like water chestnut because most non-target native plants growing in northern lakes are monocots and are tolerant to 2,4-D at rates used in aquatic sites (Getsinger et al. 1982, Carpentier et al. 1988, Parsons et al. 2001).

Another auxin-like systemic herbicide, triclopyr (3,5,6-trichloro-2-pyridinyloxyacetic acid), may also be a candidate for selective control of water chestnut. Triclopyr received a Section 3 aquatic label from the USEPA in 2001. Dose-response characteristics of triclopyr against the weedy dicot, Eurasian watermilfoil (*Myriophyllum spicatum* L.), were determined through small-scale concentration and exposure time (CET) evaluations conducted in controlled-environment chambers (Netherland and Getsinger 1992), then verified in mesocosm and field evaluations (Turner et al. 1993, Rodgers et al. 1994, Smart et al. 1995, Getsinger et al. 1997, Petty et al. 1998, Poovey et al. 2004). This step-wise, multi-scale evaluation process has been the basis for the development of species-selective application techniques using both triclopyr and 2,4-D for controlling Eurasian watermilfoil.

A similar progression of small to large-scale evaluations for chemical control of water chestnut should be followed. Because there is no published information on the use of triclopyr for control of water chestnut, and the literature on 2,4-D is dated, the following study was a first step to investigate CET relationships using a subsurface application of these herbicides. Results will provide valuable information on the potential to selectively use both 2,4-D and triclopyr when managing water chestnut.

MATERIALS AND METHODS

This study was conducted at the U.S. Army Engineer Research and Development Center (ERDC), Vicksburg, MS

in a controlled-environmental growth chamber (58 m²). Ambient conditions were an air temperature of 20 ± 2°C, a mean light intensity (±1 SE) that ranged from 425 ± 56.2 to 572 ± 59.2 μmol m⁻² sec⁻¹, and a photoperiod of 14h:10h, light:dark cycle. Lighting was provided by 400 watt metal halide bulbs with glass plates situated underneath the light source.

Water chestnut seeds were field-collected from Lake Champlain, NY-VT and germinated in the growth chamber under ambient conditions over a 4-week period (mean weight, 4.98 ± 0.27 g, *n* = 18). One germinated seed with attached shoot (mean shoot length, 11.1 ± 0.69 cm, *n* = 18) was planted in a plastic container (1 L capacity, 11 cm W × 13 cm H) filled with natural lake sediment (Brown's Lake, Vicksburg, MS) and amended with 100 mg L⁻¹ ammonium chloride and 1 mg L⁻¹ Osmocote® fertilizer (18-6-12) to provide adequate nutrients for plant growth. After planting, the sediment in each container was capped with 1-cm layer of coarse-grit sand to prevent suspension of sediment particles in the water column. Fifty-six vertical aquaria (48 L capacity, 30.5 cm L × 30.5 cm W × 76 cm H) were filled with culture solution (Smart and Barko 1985) and two planted containers were placed in each aquarium. By 28 d, plants from each pot had formed 2 to 3 small rosettes at the water surface of each aquarium (50 to 75% surface coverage). At this time, before herbicide application, one container was harvested for a pre-treatment biomass estimate, while the remaining plants were treated with herbicide.

Stock solutions of 2,4-D as DMA™ 4 IVM (DowAgrosciences LLC, Indianapolis, IN) and triclopyr as Renovate™ 3 (SePRO Corporation, Carmel, IN) were prepared based on percent active ingredient (ai), then applied subsurface to aquaria using a pipette to achieve target concentrations in the water of 0.5, 1.0, and 2.0 mg ai L⁻¹. The highest rate evaluated was 2 mg ai L⁻¹ because it was the maximum label rate for 2,4-D when this study was conducted (February 2004), which was before 2,4-D re-registration. Two exposure times of 24 and 48 hours were used based on reported half-lives for these herbicides (Hoepfel and Westerdahl 1983, Woodburn et al. 1993, Petty et al. 2003). Following each exposure time, aquaria were completely emptied and refilled with fresh growth solution three times to remove all aqueous herbicide residues. The study continued for 8 weeks following herbicide applications.

Treatments were assigned to individual aquaria in a completely randomized manner and replicated four times. Herbicide efficacy was assessed through weekly visual observations and by harvesting above-sediment biomass at pretreatment and 56 days after treatment (DAT). For each harvest, biomass was collected from one container in each replicate, dried for 48 h at 70°C, and weighed. Biomass data in grams dry weight (g DW) for each herbicide were subjected to a two-way analysis of variance (ANOVA) to determine the herbicide concentration and exposure time effects. If statistical differences occurred between treatments, means were separated using the Holm-Sidak method (*p* ≤ 0.05).

Conductivity and pH were measured in each aquarium pretreatment and 56 DAT with a YSI 556 multi-parameter probe (Yellow Springs, OH). Water temperatures were monitored continuously in four reference aquaria with an Optic Stowaway Temperature Probe (Onset Computer Corp., Bourne, MA).

RESULTS AND DISCUSSION

Water temperatures in the aquaria ranged from 20 to 23°C. Mean conductivity increased slightly from 0.271 ± 0.49 at pretreatment to 0.295 ± 0.48 mS cm⁻¹ at 56 DAT, while pH remained 7.9 ± 0.1 throughout the study. These conditions were conducive to aquatic plant growth for small-scale experiments (Smart and Barko 1985).

Application rates of 2,4-D and triclopyr significantly reduced water chestnut shoot growth (Table 1, Figure 1); however, exposure times of 24 and 48 hours were not significant for either 2,4-D or triclopyr (Table 1), nor were there significant interactions between rate and exposure time for either 2,4-D or triclopyr (Table 1). That a doubling of exposure time did not affect water chestnut biomass levels was unexpected, since an increase in efficacy between a 24 and 48 hour exposure of 2,4-D and triclopyr can significantly boost effectiveness against other auxin-sensitive species (Green and Westerdahl 1990, Netherland and Getsinger 1992).

All herbicide treatments were significantly different from the untreated reference (Figure 1). Control of water chestnut with triclopyr significantly increased from 33% for plants treated with 0.5 mg ai L⁻¹ to 66% for plants treated with 2.0 mg ai L⁻¹. In contrast, all application rates of 2,4-D significantly controlled waterchestnut by 60 to 65% compared to the untreated reference. Plant stems were epinastic and chlorotic in all herbicide treatments by 7 DAT. Rosette disintegration and canopy collapse was evident in most 2,4-D treatments by 24 DAT; however, rosettes were again forming by the end of the study (56 DAT), indicating recovery from herbicide damage. There was no canopy collapse and new rosettes continued to form until 56 DAT in all triclopyr treatments.

In this study, herbicides were applied in the water column to plants with surface rosettes. Herbicide application at earlier growth stages, before rosettes reach the water surface when most of the biomass is comprised of submersed leaves and shoots, may increase efficacy of subsurface applications (Greeley and Steenis 1959). With this application approach, seed production would be eliminated, representing a long-term control strategy to lessen the potential re-infestation of water chestnut by seed bank depletion (Smith 1955). Early-season applications with endothall (7-oxabicyclo [2.2.1] heptane-2,3-dicarboxylic acid) have been used to reduce populations of curlyleaf pondweed (*Potamogeton crispus* L.) by suppressing plant growth and preventing turion production, thereby diminishing the turion bank over time (Woolf and Madsen 2004, Skogerboe et al. 2006).

TABLE 1. RESULTS OF A TWO-WAY ANALYSIS OF VARIANCE (ANOVA) FOR TRICLOPYR AND 2,4-D AMINE TREATMENTS (RATE = 0, 0.5, 1.0, AND 2.0 MG AI L⁻¹ AND EXPOSURE = 24 AND 48 H) ON WATER CHESTNUT SHOOT BIOMASS.

Source of Variation	DF	MS	F	P
Triclopyr				
Rate	3	81.336	9.269	<0.001
Exposure	1	6.994	0.797	0.318
Rate × Exposure	3	15.834	1.805	0.173
Error	24	8.775		
2,4-D amine				
Rate	3	94.976	12.578	<0.001
Exposure	1	3.264	0.432	0.517
Rate × Exposure	3	4.870	0.645	0.594
Error	24	7.551		

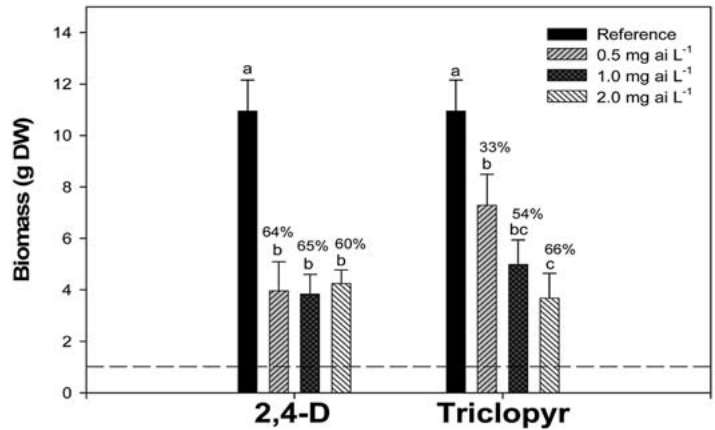


Figure 1. Mean shoot biomass (g DW \pm 1 SE, $n = 8$) of water chestnut 56 days after treatment with 0.5, 1.0, and 2.0 mg ai L⁻¹ 2,4-D amine and triclopyr. There were no significant differences between exposure times of 24 and 48 hours. Letters denote significant differences in biomass among herbicide concentrations (Holm-Sidak method, $p \leq 0.05$). Percentages above bars indicate % control relative to the reference. Dashed horizontal line indicates biomass at the time of treatment.

tane-2,3-dicarboxylic acid) have been used to reduce populations of curlyleaf pondweed (*Potamogeton crispus* L.) by suppressing plant growth and preventing turion production, thereby diminishing the turion bank over time (Woolf and Madsen 2004, Skogerboe et al. 2006).

Subsurface applications at current maximum treatment rates (2.5 mg ai L⁻¹ for triclopyr and 4 mg ai L⁻¹ for 2,4-D) and/or longer exposure times should also be evaluated—but this could increase injury to some non-target native plants where species selectivity is a concern. For example, subsurface field applications of triclopyr have been variable against elodea (*Elodea canadensis* Michx.; Getsinger et al. 1997, Poovey et al. 2004) and the desirable floating-leaf plants spatterdock (*Nuphar advena* (Ait.) Ait. f.) and fragrant water lily (*Nyphaea odorata* Ait.; Poovey et al. 2004).

An alternative to subsurface applications would be direct herbicide application to water chestnut apical meristems found in the floating rosettes above the water. This application technique using various 2,4-D amine and ester formulations successfully controlled water chestnut in the past (Smith 1955, Greeley 1965). Surface applications of both 2,4-D amine and triclopyr were effective, even at low rates, in reducing shoot biomass of another rosette-forming species, American frogbit (*Limnobium spongia* (Bosc) Steudel; Lange-land et al. 1995, Madsen et al. 1998).

Overall, the data demonstrated that at the floating rosette stage of growth, some control of water chestnut can be achieved using subsurface applications of both triclopyr and 2,4-D amine at rates (0.5 to 2.0 mg ai L⁻¹) and exposure times (24 to 48 h) that do not injure most monocots (Carpentier et al. 1988, Getsinger et al. 1997, Parsons et al. 2001, Poovey et al. 2004). Nonetheless, even the maximum control achieved in this evaluation (66%) would be considered less than optimal in most field situations. Different treatment strategies that might provide better water chestnut control require further investigation.

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LITERATURE CITED

- Caraco, N. F. and J. J. Cole. 2002. Contrasting impacts of a native and alien macrophyte on dissolved oxygen in a large river. *Ecol. Appl.* 12:1496-1509.
- Carpentier, A. G., D. L. Mackenzie and R. Frank. 1988. Residues and efficacy of two formulations of 2,4-D on aquatic macrophytes in Buckhorn Lake, Ontario. *J. Aquat. Plant Manage.* 26:29-37.
- Countryman, W. D. 1970. The history, spread and present distribution of some immigrant aquatic weeds in New England. *J. Aquat. Plant Manage.* 8:50-52.
- Countryman, W. D. 1978. "Nuisance aquatic plants in Lake Champlain." Lake Champlain Basin Study, LCBS-23. New England River Basins Commission, Burlington, VT.
- Elser, H. J. 1966. Control of water chestnut by machine in Maryland, 1964-1965. *Proceed. Northeast Weed Control Conf.* 23:682-687.
- Gangstad, E. O. 1981. Aquatic Plant Control Program of New York State. Final Rept. Office, Chief of Engineers, Washington, D.C.
- Getsinger, K. D., G. J. Davis, and M. M. Brinson. 1982. Changes in a *Myriophyllum spicatum* L. community following a 2,4-D treatment. *J. Aquat. Plant Manage.* 20:4-8.
- Getsinger, K.D. 1998. Chemical control research within the Corps of Engineers. *J. Aquat. Plant Manage.* 36:61-64.
- Getsinger, K. D., E. G. Turner, J. D. Madsen, and M. D. Netherland. 1997. Restoring native plant vegetation in a Eurasian watermilfoil-dominated plant community using the herbicide triclopyr. *Reg. Rivers Res. Manage.* 13:357-375.
- Greeley, F. R. 1965. Eradication of water chestnut. *The Conservationist* 19(4): 13-15.
- Greeley, F. R. and J. H. Steenis. 1959. Comparative tests of various herbicides on waterchestnut. *Proceed. Northeast Weed Control Conf.* 13:299-305.
- 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.
- Groth, A. T., L. Lovett-Doust and J. Lovett-Doust. 1996. Population density and module demography in *Trapa natans* (Trapaceae), an annual, clonal aquatic macrophyte. *Amer. J. Bot.* 83:1406-1415.
- Hoeppel, R. E. and H. E. Westerdahl. 1983. Dissipation of 2, 4-D DMA and BEE from water, mud, and fish in Lake Seminole, Georgia. *Water. Res. Bull.* 19:197-204.
- Hummel, M. and K. Kiviat. 2004. Review of world literature on water chestnut with implications for management in North America. *J. Aquat. Plant Manage.* 42:17-26.
- Kiviat, E. 1993. Under the spreading water-chestnut. *News for Hudsonia* 9:1-6.
- Langeland, K., B. Smith, N. Hill, S. Grace, M. Cole, J. Drew and E. Meadows. 1995. Evaluation of herbicides for control of American frogbit. *Aquatics* 17(3)16-19.
- Madsen, J. D. 1993. Waterchestnut seed production and management in Watervliet Reservoir, New York. *J. Aquat. Plant Manage.* 31:271-272.
- Madsen, J.D., C. S. Owens, and K. D. Getsinger. 1998. Evaluation of four herbicides for management of American frogbit (*Limnolobos spongiosa*). *J. Aquat. Plant Manage.* 36:148-150.
- 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., K. D. Getsinger, and D. R. Stubbs. 2005. Aquatic plant management: Invasive species and chemical control. *Outlooks Pest Manage.* 16(3):100-104.
- Parsons, J. K., K. S. Hamel, J. D. Madsen, and K. D. Getsinger. 2001. The use of 2,4-D to selectively control an early infestation of Eurasian watermilfoil in Loon Lake, Washington. *J. Aquat. Plant Manage.* 39:117-125.
- Petty, D. G., K. D. Getsinger, and K. B. Woodburn. 2003. A review of the aquatic environmental fate of triclopyr and its major metabolites. *J. Aquat. Plant Manage.* 41:1-8.
- Petty, D. G., K. D. Getsinger, J. D. Madsen, J. G. Skogerboe, W. T. Haller, A. M. Fox, and B. A. Houtman. 1998. Dissipation of the herbicide triclopyr in Lake Minnetonka, Minnesota. TR A-98-1, U.S. Army Engineer Waterways Experiment Station, Vicksburg, MS.
- Poovey, A. G. and K. D. Getsinger. 2005. Use of herbicides to control the spread of aquatic invasive plants. *J. ASTM Internat.* 2(10) online. Paper ID JA113254.
- Poovey, A. G., K. D. Getsinger, J. G. Skogerboe, T. J. Koschnick, J. D. Madsen, and R. M. Stewart. 2004. Small-plot, low-dose treatments of triclopyr for selective control of Eurasian watermilfoil. *Lake and Reservoir Manage.* 20:322-332.
- Rodgers, J. H., A. W. Dunn, and A. B. Jones. 1994. Triclopyr concentrations in Eurasian watermilfoil: Uptake under different exposure scenarios. *Proceedings, 28th Annual Meeting, Aquatic Plant Control Research Program. Miscellaneous Paper A-94-2, U.S. Army Engineer Waterways Experiment Station, Vicksburg, MS.* pp. 249-262.
- Skogerboe, J. G., A. G. Poovey, K. D. Getsinger, W. Crowell, and E. Macbeth. 2006. Early season, low dose applications of endothal to selectively control curlyleaf pondweed in Minnesota lakes. *APCRP Technical Notes Collection (TN APCRP-CC-00), U.S. Army Engineer Research and Development Center, Vicksburg, MS* (in press).
- Smart R. M. and J. W. Barko. 1985. Laboratory culture of submersed freshwater macrophytes on natural sediments. *Aquat. Bot.* 21:251-263.
- Smart, R. M., K. D. Getsinger, G. O. Dick, and J. G. Skogerboe. 1995. Preliminary report on species-selective control of Eurasian watermilfoil with triclopyr. *Proceedings, 29th Annual Meeting, Aquatic Plant Control Research Program. Miscellaneous Paper A-95-3, U.S. Army Engineer Waterways Experiment Station, Vicksburg, MS,* pp. 73-76.
- Smith, R. H. 1955. Experimental control of water chestnut (*Trapa natans*) in New York State. *N. Y. Fish Game J.* 2:173-193.
- Strayer, D. L., C. Lutz, H. M. Malcolm, K. Munger and W. H. Shaw. 2003. Invertebrate communities associated with a native (*Vallisneria spiralis*) and an alien (*Trapa natans*) macrophyte in a large river. *Freshwater Biol.* 48:1938-1949.
- Turner, E. G., M. D. Netherland, D. Sisneros, and E. R. Burns. 1993. Herbicide delivery systems. *Proceedings, 27th Annual Meeting, Aquatic Plant Control Research Program. Miscellaneous Paper A-93-2. U.S. Army Engineer Waterways Experiment Station, Vicksburg, MS,* pp. 165-175.
- Woodburn, K. B., W. R. Green, H. E. Westerdahl. 1993. Aquatic dissipation of triclopyr in Lake Seminole, Georgia. *J. Agric. Food Chem.* 41:2172-2177.
- Woolf, T. E. and J. D. Madsen. 2003. Seasonal biomass and carbohydrate allocation patterns in southern Minnesota curlyleaf pondweed populations. *J. Aquat. Plant Manage.* 41:113-118.