

## NOTES

# Submersed Plants and Algae as Factors in the Loss of Rhodamine WT Dye

E. G. TURNER<sup>1</sup>, M. D. NETHERLAND<sup>2</sup> AND K. D. GETSINGER<sup>2</sup>

### INTRODUCTION

Developed specifically for water tracing, the fluorescent dye, Rhodamine WT, has been utilized in numerous and varied hydrologic studies. Most often this dye has been used to determine flow rates and mixing dynamics in riverine environments (Hubbard et al 1982) or to characterize movement and dispersion of reservoir/lake influents (Johnson 1984). More recently, Rhodamine WT has been used to predict the potential rate of herbicide dissipation within submersed plant stands growing in lotic systems (Fox and Haller 1990; Fox et al 1991). Inherent to these studies is the assumption that the only significant process operating to reduce dye concentrations within the treatment area is the movement of dye-treated water out of the plant stand. Indeed, Rhodamine WT has been shown to be resistant to most processes that could lead to reduced dye concentrations. Smart and Smith (1976), and Smart and Laidlaw (1977) reported that loss of Rhodamine WT through photodegradation, biodegradation, and adsorption onto sediment would be negligible in most natural aquatic systems in studies of one week or less duration. Similarly, Fox et al (1991) reported that the loss of Rhodamine WT due to photodegradation/biodegradation in a Crystal River, FL, study was minimal over a 2-week period.

Another possible route for loss of dye from the water column is through plant/algal uptake. Although it is commonly assumed that plants and algae will not take up Rhodamine WT, hard data concerning this possibility is very limited. Donaldson and Robinson (1971) showed that certain terrestrial plants will readily take up Rhodamine WT through the transpiration stream when the dye is applied to the roots in aqueous solution. This suggests, albeit indirectly, that aquatic plants capable of transpiration (floating and emersed) could effect significant losses of dye from the water column, given the appropriate conditions. However, the possibility that submersed plants, which do not develop significant transpiration streams (Sculthorpe 1967), are capable of removing dye from the water column cannot be dismissed. One report is available which contains a vague reference to a study in Puerto Rico

in which a cloud of Rhodamine WT moving through a dense stand of hydrophytes was completely lost, presumably through plant uptake (Hubbard et al. 1982). In contrast, Smart and Laidlaw (1977) reported recovery rates of 100% and 98% of initial dye concentrations in a surface stream containing extensive weed beds after mean residence times of 3.5 and 11 hours, respectively. Unfortunately, plant species, density, and environmental conditions were not provided in either report.

It is clear that the ability of aquatic plants and associated algae to absorb Rhodamine WT has not been adequately investigated. To date, most, if not all dye/herbicide simulation studies of the type described above have been conducted in waters infested with the submersed plants hydrilla [*Hydrilla verticillata* (L.f.) Royle] or Eurasian watermilfoil (*Myriophyllum spicatum* L.) (Getsinger et al. 1990). Accordingly, data pertaining to dye uptake by these species, and algae, would be highly relevant to current and future dye studies by aquatic plant control researchers and managers. Therefore, this study was designed to determine the effects of hydrilla, Eurasian watermilfoil, and algae on the loss of Rhodamine WT from the water column.

### MATERIALS AND METHODS

Three different treatments plus two controls, each replicated three times, were utilized in this study. Each replicate consisted of one 55-liter aquarium (76 cm by 27 cm by 27 cm) containing material specific for each treatment and filled to a total volume of 48.6 liters with reconstituted hard water (pH 7.6). Treatments and controls were assigned to individual aquaria using a completely randomized design and were comprised of the following: 1) aquaria containing sediment, and either hydrilla, or Eurasian watermilfoil; 2) aquaria containing sediment and mixed algal communities; 3) aquaria containing sediment (control 1) or water only (control 2).

For treatments utilizing hydrilla and Eurasian watermilfoil (hereafter referred to as milfoil), four apical tips (15 cm in length) were planted in 300 ml beakers filled with nutrient-enriched lake sediment. Six of these beakers were placed in each aquarium. Inoculum for algal tanks consisted primarily of microplanktonic taxa (*Scenedesmus*, *Ankistrodesmus*, *Selenastrum*, *Closterium*, *Navicula*, *Pleurosigma*), with some larger, filamentous taxa included (*Spyrogyra* and *Oedogonium*). Algae tanks were amended with 10% Hoaglands solution and Rapid-Gro fertilizer.

<sup>1</sup>ASCI Corporation, Environmental Laboratory, US Army Engineer Waterways Experiment Station, 3909 Halls Ferry Rd., Vicksburg, MS 39180-6199

<sup>2</sup>Environmental Laboratory, US Army Engineer Waterways Experiment Station, 3909 Halls Ferry Rd., Vicksburg, MS 39180-6199. Received for publication April 26, 1991.

The study was conducted in a controlled-environment room with a water surface PPFD of  $452 \pm 63 \mu\text{E}/\text{m}^2/\text{s}$  (14L/10D). Water temperatures ranged from  $20 \text{ C} \pm 1.7$  (dark) to  $24.9 \text{ C} \pm 1.6$  (light). Air was bubbled continuously through each tank to ensure adequate water circulation and uniform dispersion of the dye throughout the study.

After 5 weeks of plant/algal growth, Rhodamine WT was injected into each aquarium resulting in an initial mean concentration of  $10.42 \pm 0.65 \mu\text{g}/\text{l}$ . Two 50-ml aliquots of water were collected from each tank at 5 minutes, 2, 4, 8, 16, and 24 hours after injection, and then at 12 hour intervals for an additional 72 hours. Fluorescence of each sample was measured using a Turner Designs Model 10-005 fluorometer modified for discreet sampling and corrected for temperature variation according to Smart and Laidlaw (1977). Upon termination of the experiment vascular plants were harvested and oven dried ( $70^\circ \text{C}$ ) to a constant weight. Three 150-ml aliquots of water were collected from each algal tank and filtered onto oven dried and preweighed glass fiber filters (Gelman Type A/E, 47 mm). Filters were oven dried for 4 days and reweighed. Dye concentrations (as percent dye remaining) were compared between treatments over time using ANOVA.

## RESULTS AND DISCUSSION

All treatments exhibited healthy, vigorous growth throughout the study. Biomass values for hydrilla ( $225.9 \pm 44.9 \text{ g DW}/\text{m}^2$ ) and milfoil ( $208.1 \pm 15.8 \text{ g DW}/\text{m}^2$ ) were generally similar to those reported for maximal standing crops in several US lakes (Bowes et al. 1979; Grace and Wetzel 1978). Algal biomass values ( $60.0 \pm 1.0 \text{ g DW}/\text{m}^3$  or approximately  $22 \text{ g C}/\text{m}^3$ ), as indicated by the extremely deep green color of the water, significantly exceeded reports of values for hypereutrophic lakes (Wetzel 1975).

Data are presented in Table 1 as percent dye remaining, relative to initial concentrations, in each treatment. There was a trend toward reduced dye concentrations after 72 hours, however, this was observed in all treatments and controls. More importantly, percent dye remaining at each hour was not significantly different between any treatments. Although root and foliar uptake provide possible routes of dye absorption, results of this study indicate that neither process removes significant amounts of dye from the surrounding water over a 4 day period. In addition, since the dye was applied only to the water column it is probable that very little, if any, penetrated the sediment to the root zone. This would also be true in most natural aquatic systems where dye studies have been conducted. With respect to algae, some dye may be taken up by algal cells in a manner similar to nutrient uptake. However, as with submersed plants, results from this study indicate that this apparently has a negligible effect on water column dye concentrations, even when phytoplankton biomass greatly exceeds that typically encountered in natural systems.

Whereas earlier studies provided evidence of Rhodamine WT's resistance to photodegradation, biodegradation, and adsorption, little data was offered relative to

TABLE 1. PERCENT RHODAMINE WT REMAINING WITHIN EACH EXPERIMENTAL TREATMENT OVER TIME.<sup>1</sup>

Hour	Hydrilla	Eurasian watermilfoil	Algae	Soil (Control 1)	Water Control 2)	SL <sup>2</sup>
0 <sup>3</sup>	100	100	100	100	---	
2	100(1.7)	96(2.8)	103(5.2)	101(0.6)	100(0.5)	0.08
4	104(1.8)	101(3.0)	106(4.7)	105(0.9)	104(1.6)	0.22
8	101(2.2)	98(2.6)	104(4.9)	103(0.9)	102(0.8)	0.12
16	102(1.7)	98(4.1)	105(4.8)	103(1.4)	102(1.4)	0.19
24	103(1.3)	98(3.8)	105(1.9)	104(1.9)	104(1.4)	0.18
36	106(1.4)	101(5.8)	107(5.4)	105(3.2)	107(2.5)	0.41
48	98(2.1)	93(3.7)	100(6.6)	98(1.3)	98(1.1)	0.19
60	100(0.7)	99(3.6)	103(9.9)	99(3.2)	101(3.2)	0.76
72	102(0.9)	97(4.3)	104(8.8)	101(1.2)	101(1.5)	0.42
84	95(0.7)	93(3.1)	98(10.5)	95(1.2)	96(1.8)	0.74
96	95(2.0)	91(3.1)	99(10.2)	95(0.4)	95(0.7)	0.38

<sup>1</sup>Values are means of 3 replicates ( $\pm 1 \text{ SD}$ )

<sup>2</sup>One way 95% ANOVA significant level

<sup>3</sup>Five minute postinjection measurements

plant uptake. Results of this work demonstrate that the loss of Rhodamine WT through submersed plant and algal uptake in the field should be insignificant over relatively short periods.

## ACKNOWLEDGEMENTS

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.

## LITERATURE CITED

- Bowes, G., Holaday, A. S., and Haller, W. T. 1979. Seasonal variation in the biomass, tuber density, and photosynthetic metabolism of hydrilla in three Florida lakes. *Journal of Aquatic Plant Management* 17:61-65.
- Donaldson, D. E., and Robinson, T. W. 1971. Fluorescent dyes, their uptake and translocation in plants. *Water Resources Research* 7(3):692-696.
- Fox, A. M., and Haller, W. T. 1990. "Use of Rhodamine WT to predict herbicide dissipation in moving water", Proceedings, EWRS 8th Symposium on Aquatic Weeds, Uppsala, Sweden, pp. 105-110.
- 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-91-2, US Army Engineer Waterways Experiment Station, Vicksburg, MS, 18 pp.
- Fox, A. M., Haller, W. T., and Shilling, D. G. 1991. Correlation of fluridone and dye concentrations in water following concurrent application. *Pesticide Science* 31:25-36.
- Getsinger, K. D., Fox, A. M., and Haller, W. T. 1990. "Understanding water exchange characteristics to improve the control of submersed plants", Information Exchange Bulletin A-90-2, US Army Engineer Waterways Experiment Station, Vicksburg, MS, 6 pp.
- Grace, J. B., and Wetzel, R. G. 1978. The production biology of watermilfoil (*Myriophyllum spicatum* L.): A review. *Journal of Aquatic Plant Management* 16:1-11.
- Hubbard, E. F., Kilpatrick, F. A., Martens, L. A., and Wilson, J. F., Jr. 1982. "Measurement of time of travel and dispersion in streams by dye tracing." In: Techniques of water resources investigations of the United States Geological Survey, US Geological Survey, Alexandria, VA, 44 pp.
- Johnson, M. C. 1984. "Fluorometric techniques for tracing reservoir inflows", Instruction Report E-84-1, US Army Engineer Waterways Experiment Station, Vicksburg, MS, 46 pp.

- Sculthorpe, C. D. 1967. The biology of aquatic vascular plants. Koeltz Scientific Books, Königstein/West Germany, 610 pp.
- Smart, P. L., and Laidlaw, I. M. S. 1977. An evaluation of some fluorescent dyes for water tracing. *Water Resources Research* 13 (1):15-3.
- Smart, P. L., and Smith, D. I. 1976. Water tracing in tropical regions, the use of fluorometric techniques in Jamaica. *Journal of Hydrology* 30:179-195.
- Wetzel, R. G. 1975. *Limnology*, W. B. Saunders Company, Philadelphia, PA, 743 pp.