# Influence of Dilute Acetic Acid Treatments on Survival of Monoecious Hydrilla Tubers in the Oregon House Canal, California

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### **ABSTRACT**

Hydrilla (Hydrilla verticillata (L.f.) Royle), a serious aquatic weed, reproduces through formation of underground tubers. To date, attacking this life-cycle stage has been problematic. The purpose of this study was to measure the impact of exposure to dilute acetic acid on monoecious hydrilla tubers under field conditions. In this field experiment, treatments were acetic acid concentration (0, 2.5, or 5%) and sediment condition (perforated or not perforated). Each of 60,  $1 \times 1$  m plots (in the Oregon House Canal) were randomly assigned to one treatment. Two weeks after treatment, we collected three samples from each plot. One was washed over 2 mm wire mesh screens to separate tubers from sediment. Relative electrolyte leakage was measured for one tuber from each plot. Five additional tubers from each plot were placed in a growth chamber and sprouting monitored for four weeks. A second sample from each plot was placed in a plastic tub and placed in an outdoor tank, filled with water. These samples were monitored for tuber sprouting. Relative electrolyte leakage increased significantly for tubers exposed to 2.5 or 5% acetic acid. Effects on tubers in perforated sediment were reduced. Exposure to acetic acid inhibited tuber sprouting by 80 to 100%, in both the growth chamber and outdoor tests. These results confirm findings from earlier laboratory/greenhouse experiments, and suggest that this approach may be useful in the management of hydrilla tuber banks in habitats where the water level can be lowered to expose the sediments.

Key words: aquatic plant management, tuber bank, vinegar, Hydrilla verticillata (L.f.) Royle, monoecious.

# INTRODUCTION

Hydrilla is an invasive aquatic weed in the United States. It reproduces by fragmentation, stolons, production of axillary turions, and subterranean turions (hereafter tubers). Like other weedy aquatic species (van Vierssen 1993), the underground vegetative propagules are particularly important to hydrilla's long-term survival in a given habitat. To date there have been few attempts to manage this important life cycle stage (Haller et al. 1976). Some have suggested that it may be possible to disrupt tuber formation or dormancy (Ogg et al.

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1969, Spencer and Ksander 1992, MacDonald et al. 1993, van Vierssen 1993) but, there have been few attempts to manipulate tuber survival in the sediment (Haller et al. 1976, Godfrey and Anderson 1994, Godfrey et al. 1994).

Previous work (Spencer and Ksander 1995) indicated that dilute solutions (0.1 to 5%) of the natural sediment component, acetic acid, (Ponnamperuma 1972, Miller et al. 1979, King and Klug 1982, Sansone and Martens 1982) greatly reduced sprouting and survival of hydrilla tubers in laboratory and greenhouse trials. The purpose of this work was to answer the following questions: 1) Does acetic acid affect survival of monoecious hydrilla tubers in an undisturbed canal setting? 2) Is there a difference between exposure to a 2.5 and 5% concentration? 3) Does perforating the sediment improve the treatment by enhancing penetration into the sediment? 4) Do important sediment properties change as a result of this treatment?

# **MATERIALS AND METHODS**

Part of this research was conducted in the Oregon House Canal which is located in Yuba County, California, about 90 km (56 miles) north of Sacramento, California. This is a small shallow canal (mean water depth of 75 cm, mean width at the bottom of the trapezoidal cross section was 1.04 m) which typically conveys water between April 15 and October 15. The following experiment was performed to evaluate the effect of acetic acid treatments on monoecious hydrilla tubers in undisturbed canal sediment. The experiment followed a two-way analysis of variance design with the two treatments being acetic acid (0, 2.5, and 5%) and sediment perforation (perforated vs. not perforated). The sediment perforation was accomplished by forming 15-cm deep holes into the sediment prior to treatment with acetic acid. This allowed evaluation of the effect of improved sediment penetration. There were 10 replications (plots) per treatment combination. Each plot (1 m × 1 m) was surrounded by sandbags on two sides. The sandbags were placed perpendicular to the long axis of the canal and thus the sloping canal walls formed natural barriers on the other sides. The sediment perforation treatment was applied with a potato fork (tines were 15 cm long) and, then the appropriate acetic acid treatment was added. Each plot was flooded to an average depth of 10 cm with the acetic acid treatment or well water as the control. Plots were randomly assigned to a particular treatment combination using the PLAN procedure (SAS Institute 1989).

TABLE 1. SEDIMENT CHARACTERISTICS FOR OREGON HOUSE CANAL PLOTS, VALUES ARE BASED ON 6 SAMPLES, SAMPLES FROM PERFORATED AND NON-PERFORATED PLOTS HAVE BEEN COMBINED. ANALYSIS OF VARIANCE DID NOT INDICATE SIGNIFICANT DIFFERENCES DUE TO ACETIC ACID TREATMENT.

Parameter	Acetic acid		Standard error
	(%)	Mean	
Bray Phosphorus (ppm)	0	5.60	1.14
	2.5	4.75	0.67
	5	3.55	0.31
NH <sub>4</sub> -N (ppm)	0	48.72	26.75
	2.5	47.65	23.88
	5	20.38	4.64
NO <sub>3</sub> -N (ppm)	0	3.27	0.92
	2.5	3.67	1.03
	5	2.05	0.39
K (ppm)	0	99.33	19.85
	2.5	86.33	6.48
	5	75.17	2.63
Organic Matter (%)	0	13.92	1.50
	2.5	14.20	1.40
	5	11.57	0.51

The initial treatments were made on October 29, 1997. Between November 1 and 10, 1997 we deployed a data logger (EasyLogger, Model 900 Series, Omnidata, Logan, Utah) with three temperature sensors attached at a site about 5 m upstream from the treatment area. The sensors were at 2.5, 7.5, or 15 cm deep in the sediment. The data logger was programmed to record temperature at 0.5 h intervals during this period.

Two weeks after the initial treatment, we removed three samples from each plot with a shovel. One sample was weighed (mean sample fresh weight was 2.5 kg; samples ranged from 0.5 to 4.2 kg) and processed to remove the tubers present by washing it over a 2-mm mesh size metal screen. The number of tubers present was counted and divided by the sample fresh weight to yield tubers per kg. One tuber from each plot was evaluated for relative electrolyte leakage (REL) using the procedure described by Hendry and Grime (1993). Previous work indicated that hydrilla tubers treated with acetic acid displayed increased REL and that increased REL was strongly correlated with the tuber subsequently not sprouting (Spencer and Ksander 1997). The square root of the number of tubers per kg of sediment (fresh weight) and REL were used as the response variables in the two-way analysis of variance (ANOVA). In addition, up to 5 tubers from each sample were placed in individual 250 ml flask containing 100 ml of well water. The top of the flask was covered loosely with foil and the flasks were placed in a growth chamber at 20 C. The tubers were examined weekly for the next 3 weeks and the number of tubers that had sprouted recorded. The proportion of tubers sprouting was calculated by dividing the number spouted by the total number of tubers from a particular treatment. The 95% confidence intervals for these proportions were calculated following Zar (1996). Final proportions (calculated after 21 days) were analyzed by logistic regression with acetic acid concentration and sediment perforation as explanatory variables (SAS Institute 1989).

A second sample was placed in a plastic container and then in an outside tank filled with water. At two to three day

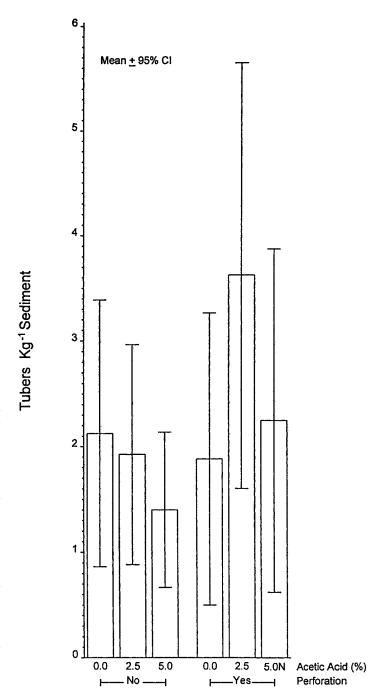


Figure 1. Mean number of tubers per kilogram fresh weight of sediment (and 95% confidence limits) for cores collected November 12, 1997, from experimental plots in the Oregon House Canal. N = 10.

intervals, we monitored tuber sprouting from these samples for six months. During this period the samples were exposed to ambient conditions at Davis, California.

The third sample was processed for sediment particle size distribution and organic content was estimated by loss on ignition at 550 C (Brower and Zar 1984). Additional sub-samples were sent to the University of California, Division of Agriculture and Natural Resources Analytical Laboratory for determination of extractable NH<sub>4</sub>-N, NO<sub>3</sub>-N, K, and available

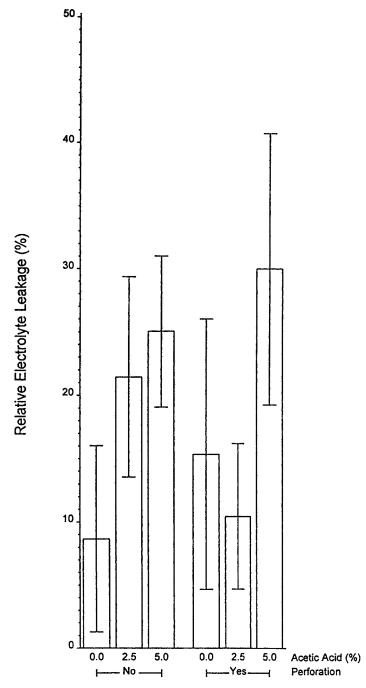


Figure 2. Relative electrolyte leakage (mean) for tubers collected from treated plots in the Oregon House Canal. Error bars represent 95% confidence limits, N=10.

 $PO_4$ -P (Bray P-1). Ammonium and  $NO_3$ -N were measured by the diffusion conductivity methods as described by Carlson et al. (1990). Available  $PO_4$ -P was determined using the procedures described by Bray and Kurtz (1945).

### **RESULTS AND DISCUSSION**

Sediment temperature varied from 10 to 20 C during the interval between initial treatment and retrieval of the cores.

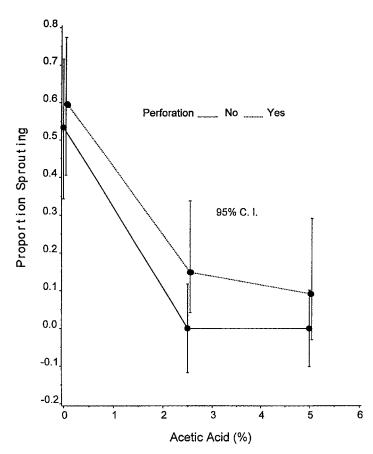


Figure 3. Proportion sprouting for tubers collected from treated plots in the Oregon House Canal.

Sediment temperature fluctuations were less pronounced with depth. The acetic acid treatments did not alter the measured sediment characteristics (Table 1). Sediment in the treated areas was 23% sand, 57% silt, and 20% clay on average (N = 18). Mean sediment moisture was 41% (N = 9) when the treatments were made.

The number of tubers per plot did not differ among treatment combinations (P > 0.05, ANOVA, Figure 1). This is not unexpected since the tubers were collected just 2 weeks after the initial treatment and sediment conditions (low temperatures, not flooded) may not have been conducive to rapid decay of damaged tubers that we have observed in similar greenhouse tests (Spencer and Ksander 1995).

Relative electrolyte leakage (REL) increased for tubers from plots treated with acetic acid (P < 0.001, Figure 2). Tubers from treated plots that were perforated did not display increased REL at 2.5% acetic acid, but did at 5% (Figure 2). This is reflected by the significant interaction term in the analysis of variance. It indicates that perforating the sediment may have reduced acetic acid effects by enhancing the rate at which the added acetic acid percolated through the soil thus reducing contact time with tubers. The proportion of tubers sprouting after 21 days in the growth chamber decreased with exposure to acetic acid (P < 0.0001, logistic regression, Figure 3). As with the REL results, a small proportion of tubers survived the 2.5% exposure if they were from plots with perforated sediments.

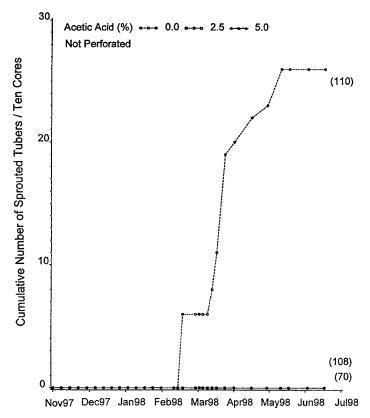


Figure 4. Cumulative number of sprouted tubers for cores collected from treated, non-perforated plots in the Oregon House Canal and submerged in an outdoor tank. The number in parenthesis represents the total number of tubers harvested from similar cores.

Results form cores monitored for sprouting over a 6-month period are shown in Figures 4 and 5. No tubers sprouted in the cores from the non-perforated plots that were treated with either 2.5 or 5% acetic acid, while the cores from control plots had 27 sprouted tubers. A similar response was observed for the cores from perforated plots, except that four tubers sprouted in the perforated plots treated with 2.5% acetic acid and 38 sprouted from control cores. This agrees with the other results that indicate that perforating the sediment actually lead to decreased affect of the acetic acid treatments.

Underground vegetative propagules are important to the long-term survival of some species of aquatic plants and, appear to be particularly important in the life cycles of weedy species (van Vierssen 1993). To date, attempts to manage this important life cycle stage have been aimed at disrupting their formation or dormancy (Ogg et al. 1969, Spencer and Ksander 1992) and we know of few attempts (Haller et al. 1976, Godfrey and Anderson 1994, Godfrey et al. 1994) to manipulate tuber survival in the sediment as has been suggested by Gunnison and Barko (1989) and Kremer (1993). Results of this study indicate that a solution of 2.5% acetic acid, equivalent to one-half the strength of vinegar, was effective in reducing survival of monoecious hydrilla tubers in this field test. These results imply that in habitats where the water level can be lowered to expose the sediments, it may be possible to develop a novel method for attacking this important life-cycle stage. Use of a naturally oc-

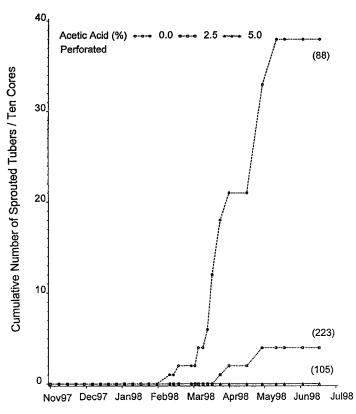


Figure 5. Cumulative number of sprouted tubers for cores collected from treated, perforated plots in the Oregon House Canal and submerged in an outdoor tank. The number in parenthesis represents the total number of tubers harvested from the first set of cores.

curring, short-lived, low molecular weight organic compound, such as acetic acid may be especially attractive. Continued evaluation of this approach in additional habitats with different sediment characteristics (clay content, organic content, porosity, etc.) may be illuminating and are essential to understanding the applicability of this approach to other aquatic sites that can be dewatered.

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