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Potential for Sediment-Applied Acetic Acid for Control of Invasive *Spartina alterniflora*

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ABSTRACT

Smooth cordgrass (*Spartina alterniflora*), a tall grass native to the east coast, has invaded Willapa Bay, Washington, and the San Francisco Bay, California. Management with glyphosate and imazapyr can be effective, but in the San Francisco populations, applications in several sites are confined to short periods in the fall in order to protect nesting habitats of Clapper rails (*Rallus longirostris*). Use of efficacious soil-active herbicides could mitigate this restriction. Acetic acid, a readily degraded natural product, has been shown to kill sediment-borne propagules of aquatic plants such as *Hydrilla verticillata* and *Stuckenia pectinatus*. Effects of acetic acid on sediment-free rhizomes of *S. alterniflora* were examined. Exposure of 0.1, 1.0, 1.5% vol/vol acetic acid for a few hours to several hours resulted in increased conductivity in distilled water compared to unexposed controls, indicating loss of cellular integrity and leakage of electrolytes. Regrowth from exposed rhizomes was significantly inhibited at higher (1.0% and 1.5%) concentrations applied for 2 or 4 hr. When rhizomes that had been directly exposed to 1.5% acetic acid were transferred to outdoor conditions in Albany, CA, both new shoot number and average plant height were reduced by over 90% at nine months post-treatment. The exposure to all concentrations of acetic acid for 4 hr also led to reduced frequency of inflorescence production, thus potentially diminishing the dispersal capacity of the treated plants. Field trials are needed to determine if judicious drenching of sediments with acetic acid (e.g., at low tide) may have utility as an alternative to foliar applied herbicides such as imazapyr and glyphosate.

Key words: soil-active herbicide, electrolyte, pore-water, HPLC, seawater, smooth cordgrass, vinegar.

INTRODUCTION

Control of rooted, invasive aquatic plants in tidal systems, whether marine or freshwater, presents several obstacles to managers. When herbicides are used as part of management approaches in these habitats, the periodic ebb and flood tidal cycles can remove foliar type herbicides and prevent adequate uptake of the active ingredient, or may simply dilute exposures to levels that are not efficacious. In addition, tidal flooding (i.e., high tides) may prohibit the use of standard ground-based spray equipment, and low tide phases may make the use of watercraft nearly impossible. This is the case with several attempts to control *Spartina alterniflora* in Washington State as well as invasive populations of *S. alterniflor*a and S. *altnerniflora* × *S. foliosa* hybrids in San Francisco Bay (Ayers et al. 2004a, b). Although imazapyr appears to be absorbed and translocated more quickly than glyphosate (Patten 2002, 2003), and is less prone to interference from salt and dust on foliage, tidal flooding is still a problem in lower elevations. In addition, seasonal restrictions on spray operations invoked by the U.S. Fish and Wildlife Service to protect nesting areas of the Clapper Rail (*Rallus longirostris*) in San Francisco Bay greatly limit the "window" of permissible applications to a few months in the fall. Although the winter period is available for such operations, the foliage of *S. alterniflora* and the hybrid plants usually has begun to senesce by late fall, and will not efficiently absorb or translocate herbicides such as glyphosate or imazapyr. Therefore, the ability to apply effective, sediment-active herbicides during the late fall to late winter may provide a useful tool in an integrated management program. The objective of this approach is to kill the over wintering rhizomes from which new vegetative growth will emerge, or to kill newly emerging culms. These types of herbicides may also prevent seeds from sprouting, or severely inhibit growth of seedlings. Examples of sediment-applied herbicides that have been used investigated for control of freshwater invasive weeds are provided in Table 1. Of these, only diuron is currently registered for drawdown use, but not for tidal areas.

Acetic acid, or natural vinegar, has been used as a contact weed control (and for control of other pests) for many years. However, more recently, it has been shown to be effective in killing the below-ground vegetative propagules of several freshwater plants (Spencer and Ksander 1997, 1999, 1995a, b). Exposures of these propagules to concentrations as low as 0.1% (vol/vol) for a few hours can dramatically retard sprouting from winter buds and tubers. Since typical food-grade vinegar (e.g., for use in salad dressing) is four to five

TABLE 1. HERBICIDES USED OR TESTED FOR USE ON/IN SEDIMENTS TO CONTROL AQUATIC WEEDS.

Herbicide:	Site/Use:
Diuron ("Karmex"/"Diurex")	De-watered canals (registered)
Metham sodium ("Vapam")	Exposed lake shores (State use only)
Fluridone ("Sonar")	De-watered canals (not currently registered for drawdown)
Bensulfuron methyl ("Londax")	De-watered canals (not registered)
Acetic acid (vinegar)	De-watered canals (not registered for that purpose)

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percent acetic acid, achieving effective concentrations in sediments seems feasible. Indeed, Spencer and Ksander showed that drenches into de-watered canals killed embedded tubers of the invasive plant hydrilla.

Others have reported on potential uses of acetic acid for control of terrestrial weeds (Mason 2005, Young 2004, Fausey 2003). Since acetic acid is also a natural product of soil microbes, and is easily degraded to CO_2, it would appear that this approach might be an acceptable fit given the constraints imposed by both the environment and by regulatory agencies. In fact, use of "immature" compost, which may generate very high levels of acetic acid (e.g., 1,700 to 2,400 ppm) via microbial action can suppress some weed growth and weed seed germination (Ozores-Hampton et al. 2002, 1999). This paper summarizes preliminary studies on the potential for use of acetic acid (vinegar) as a sediment-drench to kill rhizomes of *S. alterniflora*.

METHODS AND MATERIALS

Sources of Spartina alterniflora. In November 2003, clumps of rhizomes from healthy colonies of *S. alterniflora* were removed from San Francisco, South Bay area at Coyote Hills Regional Park (datum WGS: 122°5'41.61"W, 37°33'50.76"N), and transported to the USDA-ARS Exotic and Invasive Weed Research laboratory at UC Davis. These plants had been present for at least three years and therefore represented typical, well-established populations. Senescent shoots were removed and rhizomes were cleaned, and separated into sections, or clumps each of which contained from one to 3 shoot-buds (2 to 5 cm long) (Figure 1). Rhizomes were randomly assigned to treatments groups and the individual fresh weight of each rhizome clump was recorded. Each treatment was replicated 4 times, and each replicate consisted of four rhizome clumps contained in 1-liter glass beakers.

Treatments with acetic acid. Domestically available vinegar (ca. 4% vol/vol acetic acid) was used to make up dilutions to produce 0.1%, 0.05%, 1.50% acetic acid. The concentration of acetic acid in the vinegar was determined via HPLC (High Performance Liquid Chromatography) coupled to a UV detector. Groups of rhizomes were immersed in the acetic acid solutions for 2 or 4 hr at 18 to 22 C, removed, rinsed thoroughly with tap water, blotted and weighed. Two clumps of rhizomes were immediately planted in 500 ml (ca. 1/2 quart) plastic pots containing natural sediment removed from the original collection site at the Coyote Hills. These sediments had been passed through a 4 mm mesh sieve to remove any existing S. *alterniflora* rhizomes. Potted plants were transferred to a glass house at the USDA-ARS Davis facility and placed in shallow fiberglass pans (1.5 m by 2 m by 20 cm deep) containing 30 parts per thousand (ppt) artificial seawater (Instant Ocean™). The water level in the pans was maintained so that it reached approximately 5 cm from the lip of the pots. To assess the effect of the acetic acid treatments on the integrity of the rhizome tissues, the remaining two groups of rhizomes from each replicate were placed in 200 ml deionized water for 24 hr, after which the electrical conductivity of the bathing water was measured with a YSI Multiprobe datasonde. Elevated conductivity compared to untreated controls indicates cellular leakage and damage to tissues. Potted plants were kept in the glass house until mid-April, and then transferred to out door conditions at the USDA-ARS Western Regional Research Center (WRRC), Albany, CA. After having been re-potted into fresh, sieved sediments from Coyote Hills, plants were maintained in the same fiberglass shallow pans as in the glasshouse, but pans contained natural seawater from the Berkeley Marina, Berkeley, CA. Seawater was refreshed at two-week intervals thereafter. Growth was assessed by determining the number of new shoots (culms) formed, and total shoot lengths. At 10 months post-treatment with acetic acid, plants were transported to Davis where they were removed from the pots, sediments removed and the plants were separated into aboveground and belowground parts. Numbers of inflorescences were also recorded.

Acetic acid degradation. Since acetic acid is readily metabolized by bacteria, it is important to determine the duration of contact with rhizomes that might be expected in natural sediments. The effect of temperature and light on the breakdown of acetic acid in solutions were also determined. Acetic acid solutions were generated in artificial seawater (Instant Ocean) and in the pore water resulting from drenching replicated containers containing natural sediments from the Berkeley Marina. The pore water exposures were conducted at 20°C. Seawater solutions of acetic acid were kept at the following temperatures: 4°C, 15°C, 20°C, and 25°C and sampled for analysis at several time intervals. Half the solutions were kept in the dark by wrapping with aluminum foil; the other half was exposed to room lighting (ca. 200 µmols/m²/sec fluorescent lighting). Acetic acid concentrations were determined using HPLC methods modified from U.S. Customs Laboratory Methods (USCL Method 20-02), and Restek Corporation Applications Note #59177 (http://www.restekcorp.com). HPLC conditions were: 3.9 × 200 mm µBondapak C_{18} column with 0.1% phosphoric acid mobile phase pumped at 1 ml/min; detection wavelength: 210 nm. Sample injections were 100 µl. Quantification was determined by peak height at the retention time for standard, diluted glacial acetic acid. Detection level was 0.1 ppm.

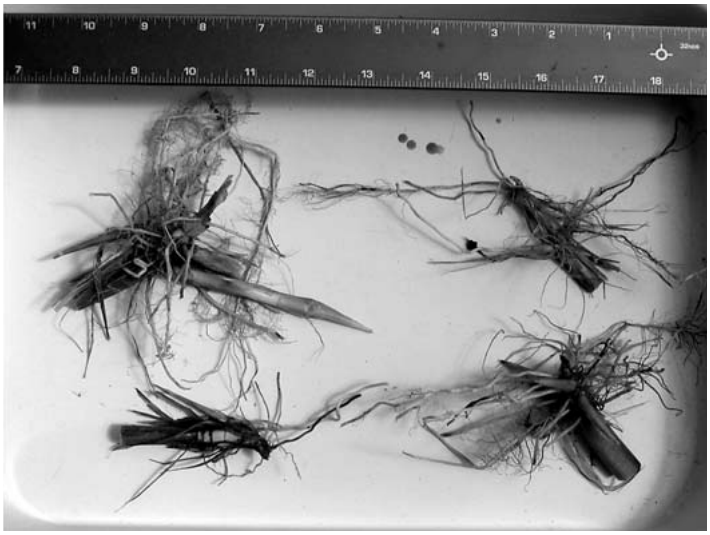

Figure 1. Examples of cleaned (sediment-free) *Spartina alterniflora* rhizomes.

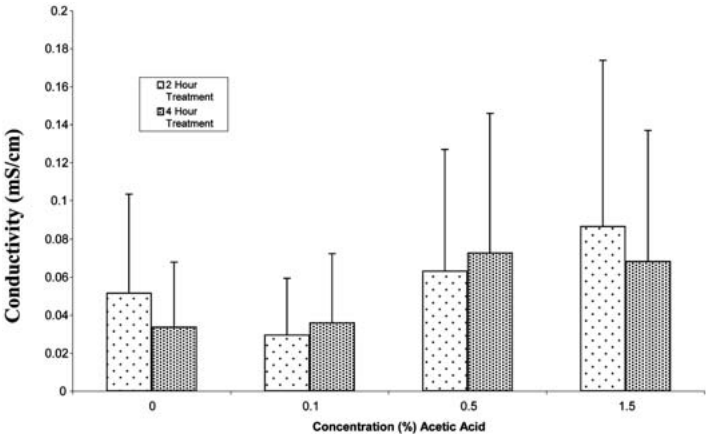

Figure 2. Effect of acetic acid exposure on electrolyte leakage in *Spartina alterniflora* rhizomes.

RESULTS AND DISCUSSION

Integrity of rhizome tissues. Both the 2 hr and 4 hr exposures at 1.5% acetic acid produced leakage of electrolytes in the cleaned rhizomes 24 hr after the end of the exposure period (Figure 2). Exposure at 0.1% did not produced apparent damage to the rhizomes. It should be noted, however, that other types of damage (e.g., to meristematic tissues) might not be reflected in overt symptoms such as leakage. Also, it is possible that continued measurement of conductivity (as the indication of electrolyte leakage) might indicate some effects at the 0.1% level.

Effect of acetic acid on shoot production and elongation. During the entire post-exposure grow-out period, 0.5 and 1.5% concentrations of acetic acid retarded shoot production in rhizomes exposed for 2 or 4 hr (Figures 3 and 4). However, it is clear that the 1.5% concentration with a 4-hr exposure produced the greatest inhibition of shoot production, particularly between the spring and fall period (Figure 4). The same trend was observed with shoot elongation (data not shown). The effects on shoot production were reflected in the final biomass produced ten months after the 4 hr exposures (Figures 5 and 6). Although aboveground (shoot) biomass was most dramatically reduced, there was also significant reduction in belowground (roots and rhizome) biomass with the 1.5% acetic acid exposure. There was no significant reduction in total plant biomass, however, in the plants whose rhizomes had been exposed to acetic acid for only 2 hours (data not shown).

Effects on inflorescence production. Control (untreated) plants and those exposed for only 2 hr at 0.1% or 0.50% acetic acid produced inflorescences between September and October, nine to ten months post-treatment (Figure 7). However, exposure to 0.1, 0.5 or 1.5% acetic acid for 4 hr reduced inflorescence production compared to controls.

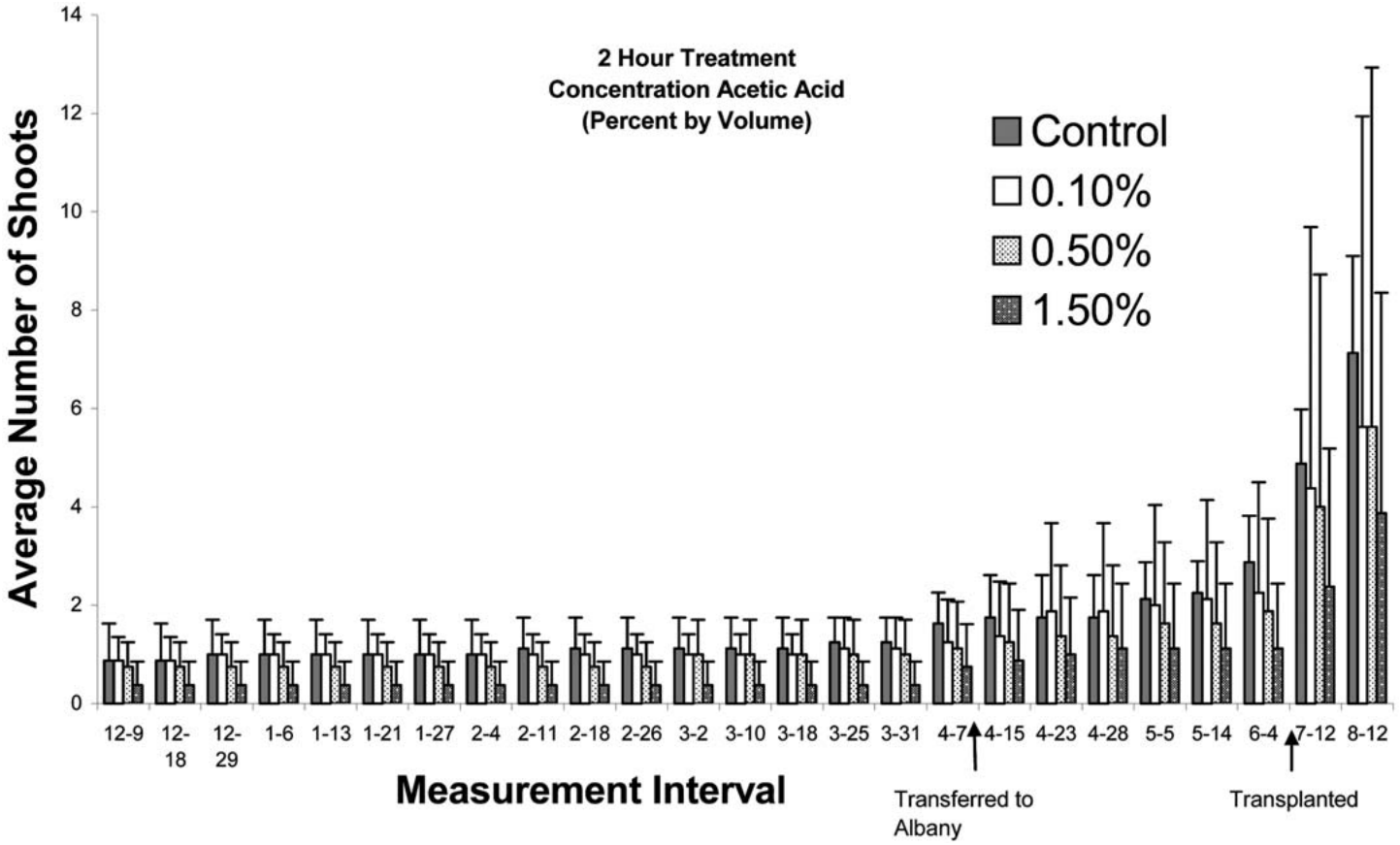

Figure 3. Shoot production from *Spartina alterniflora* rhizomes after exposure for 2 hr to acetic acid. Arrows indicate when exposed, potted plants were transferred from glass house conditions to outdoor conditions in Albany, CA, and when they were transplanted to new sediment.

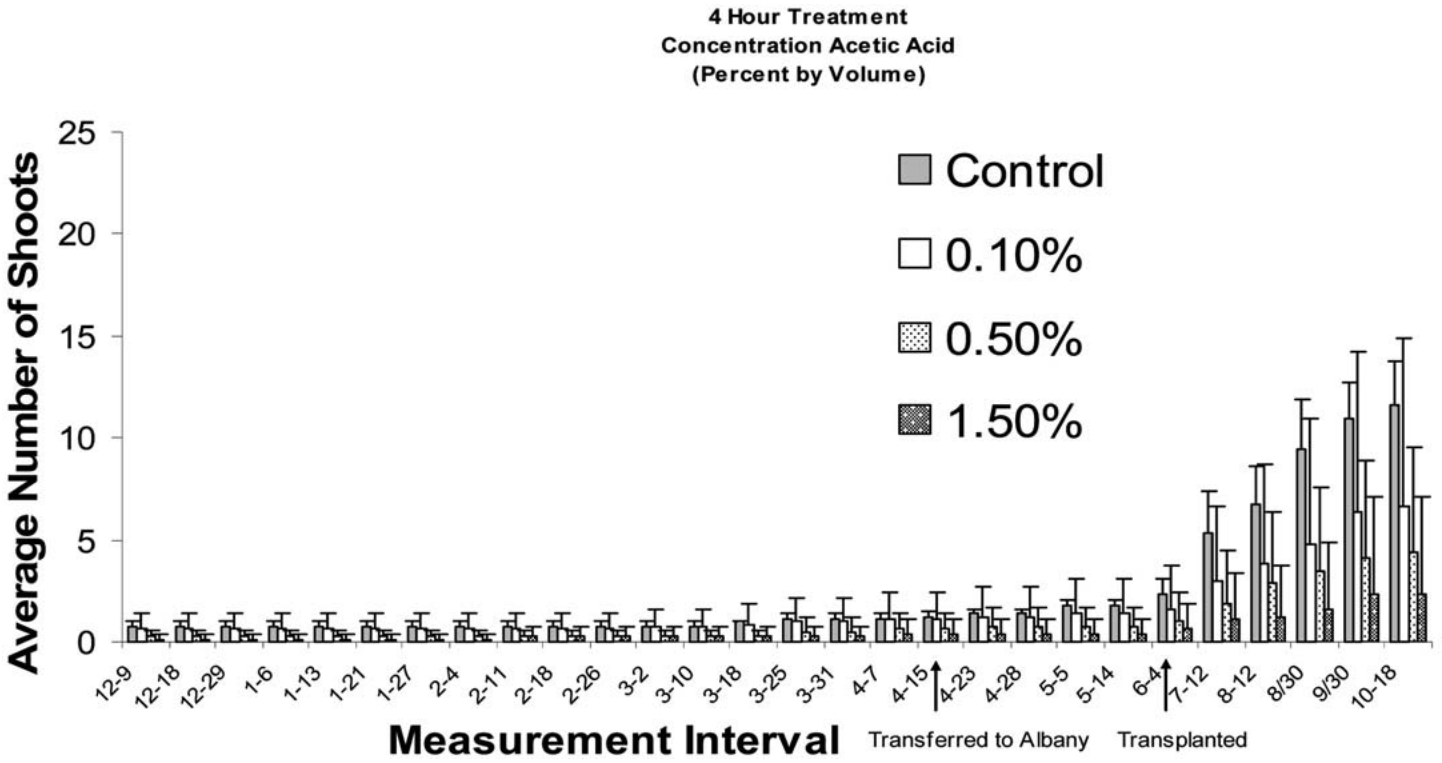

Figure 4. Shoot production from *Spartina alterniflora* rhizomes after exposure for 4 hr to acetic acid. Arrows indicate when exposed, potted plants were transferred from glass house conditions to outdoor conditions in Albany, CA, and when they were transplanted to new sediment.

Exposure to 1.5% acetic acid for 4 hr reduced inflorescence production by more that 90% (Figure 7).

Degradation of acetic acid. When clean seawater was used as the diluent, the half-life of acetic acid declined with increasing water temperature (Figure 8). In fact at 4°C, no detectable degradation was observed for up to 11 days. At 20°C, half-life was from 5-6 days, whereas at 15°C it was approximately 9 days. Presence or absence of low light levels had no apparent effect on half-life. When natural sediments were saturated with seawater containing acetic acid at 20°C, the half-life was approximately 24 hr (Figure 9). The more rapid breakdown of acetic acid in natural pore water may be due to the greater interactions with complex sediment constituents, or presence of bacteria or other microorganisms that can utilize it as a carbon source. These data suggest that field samples of pore water should be frozen immediately and may be held frozen for at least a week before analysis for acetic acid. The surface temperature of the sediments will probably have a significant effect on the duration of exposure to acetic acid when it is used as a drench to expose rhizomes in the field.

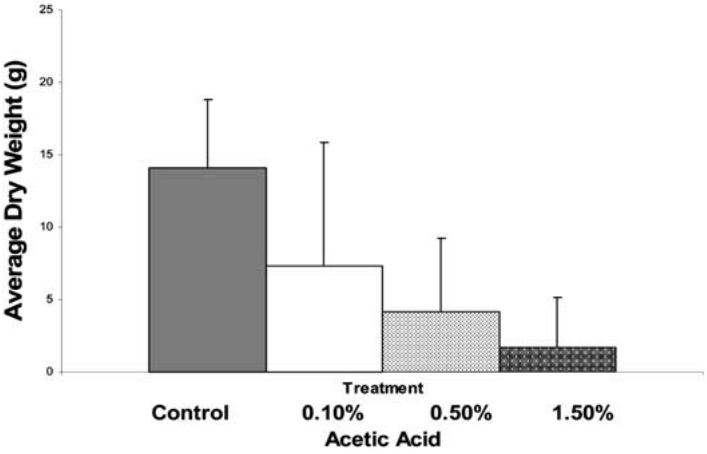

Figure 5. Effect of acetic acid on *Spartina alterniflora* shoot dry weight 10 months posttreatment.

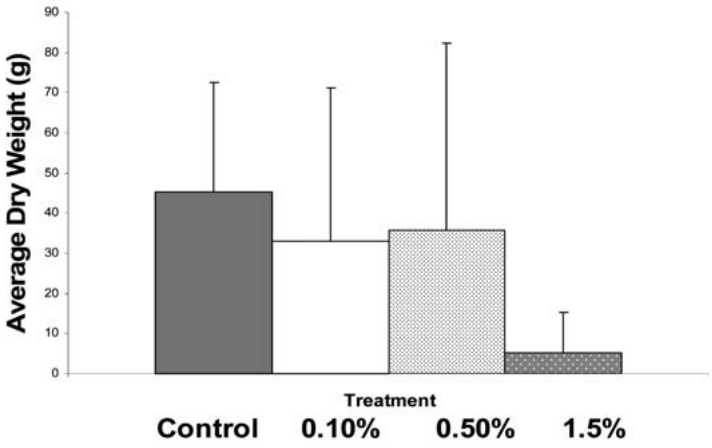

Figure 6. Effect of acetic acid on *Spartina alternflora* root dry weight 10 months posttreatment.

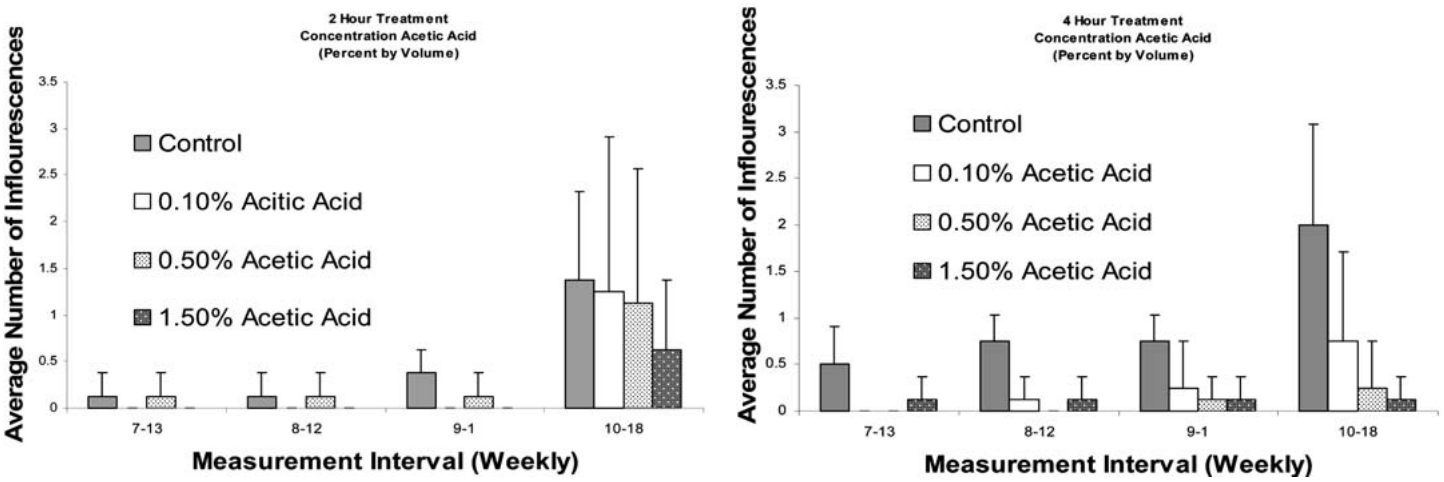

Figure 7. Effect of acetic acid exposures on inflorescence production in *Spartina alterniflora*: upper panel, 2 hr exposure to acetic acid; lower panel, 4 hr exposure to acetic acid.

Under typically cool temperatures of sediments during the winter (e.g., 8 to 10°C), acetic acid, applied at low tide, might remain long enough to provide efficacious exposures around *S. alterniflora* rhizomes. However, since the effects of ambient temperature on toxicity to acetic acid was not determined, cooler winter temperatures may necessitate longer exposures than four hours. Duration and degradation of acetic acid residues in field sediments supporting *Spartina alterniflora* growth will have to be determined since the presence of plant roots, rhizomes and rhizosphere microorganisms may affect degradation, as would the height of the "flood" tide.

The potential effect of tidal levels on dilution and diffusion of acetic acid is depicted as a hypothetical example in Figure 10. Variations in the slope of the tidal mudflats, and tidal durations would alter duration and dilution, and thus the contact time with rhizomes. The logistics of producing an effective, rapidly deployed acetic acid drench during a 3 to 4 hr ebb tide need to be determined. This could be achieved with temporarily deployed sprinkler systems or with commercially available "drench" ("soaker") hoses. Recent suggestions for using a "spike wheel" injection system to apply soil active herbicides might also be useful (Patten and

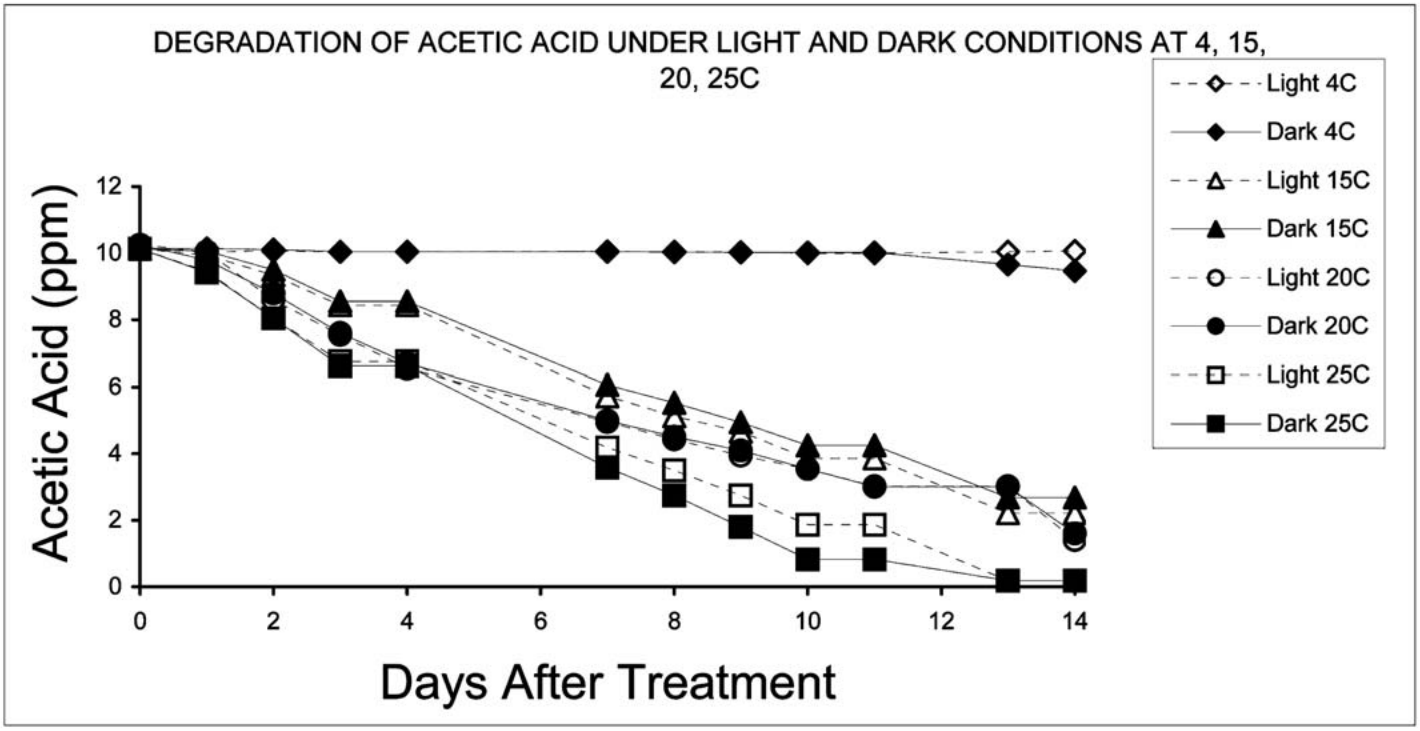

Figure 8. Degradation of acetic acid in seawater solutions maintained at 4°C to 25°C and in the dark or under 200 µmol/m²/sec fluorescent light.

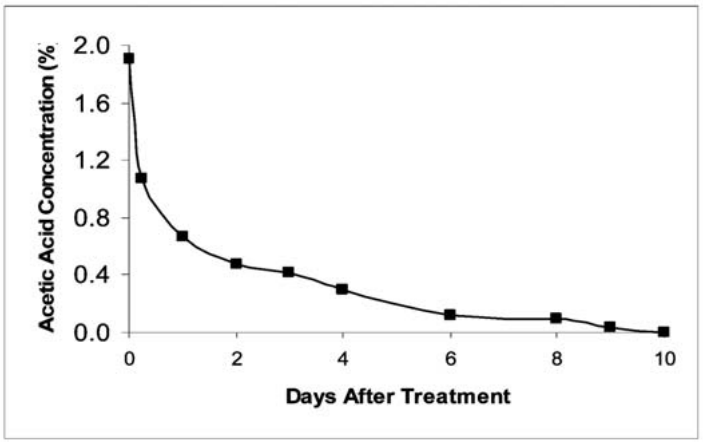

Figure 9. Degradation of acetic acid at 20°C in pore water derived from seawater solutions used to saturate natural sediments from San Francisco Bay.

Metzger 2006). Clearly the tidal cycles that result in large negative (very low) tides would produce the longest, and least diluted exposure to the rhizomes and roots. For higher elevation populations that are not flooded by high tides, it should be easier to sustain longer duration and higher concentrations of acetic acid. Further assessments are needed within naturally occurring populations of *S. alterniflora* and "hybrids". In addition, potential non-target impacts on infauna and desirable plants will have to be assessed. However, this approach, or one using other, effective sediment-active herbicides may provide an important adjunct to foliar herbicide applications, particularly since it targets the perennating structures during the winter when there is far less concern for impacts on nesting birds.

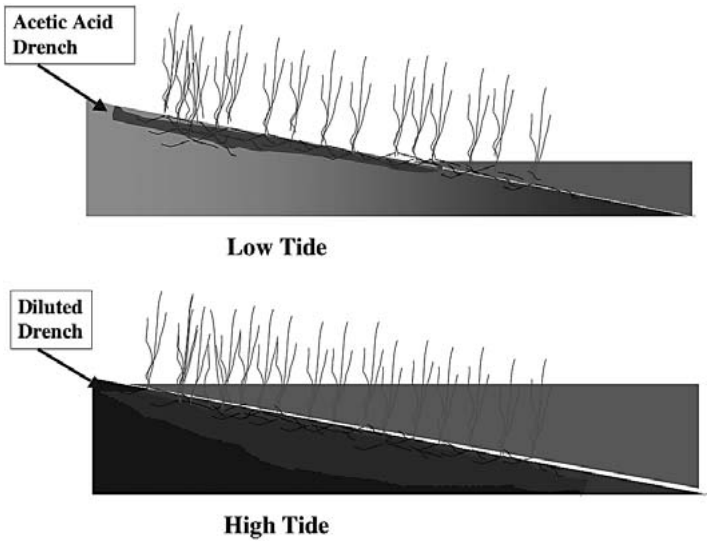

Figure 10. Depiction of potential acetic acid drench on *Spartina alterniflora* colony during low tide (top), and subsequent downward migration and dilution of the application during subsequent high tide (bottom).

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