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Curlyleaf pondweed (*Potamogeton crispus*) turion control with acetic acid and benthic barriers

THOMAS C. BARR III AND JOSEPH M. DITOMASO*

ABSTRACT

Aquatic weed propagules pose a serious, long-term management problem. Curlyleaf pondweed (*Potamogeton crispus* L.) produces numerous, asexual propagules that make traditional management difficult. In this study, we compared the effect of three benthic barrier materials (jute, polyethylene, and rubber) on the control of curlyleaf pondweed turions at the bench and mesocosm scales. In each replicated experiment, the bottom barriers covered the turions for 8 wk, and subsequently, turion viability was determined by percentage of sprouting. In the bench experiment, there was no significant inhibition in sprouting with any treatment, except the rubber material (52% reduction). Results in the mesocosm experiments showed slight effects on turion sprouting from jute and polyethylene, but far better control was achieved with rubber (70% reduction). In a second replicated study, also conducted at the bench and mesocosm scale, but only with the impermeable rubber barrier material, large- and small-class turions were exposed for 2 wk to dilute acetic acid ($C_2H_4O_2$, tapioca starch pearls, which facilitated slow release of the compound. Results of all experiments showed complete inhibition of sprouting turions at and above acetic acid concentrations of 83.3 mmol L^{-1} (0.5% v/v). The solid-starch formulation maintained continued, slow-release activity for at $\geq$ 3 d. Complete inhibition in sprouting occurred when relative electrolyte leakage was 31% in large-class turions and 49% in small-class turions. These findings demonstrate the potential of tapioca starch saturated with acetic acid and combined with impermeable benthic barriers as an effective method for the control of curlyleaf pondweed turion sprouting.

Key words: integrated pest management, invasive species, jute, polyethylene, *Potamogeton crispus*, rubber

INTRODUCTION

Curlyleaf pondweed (*Potamogeton crispus* L.; Potamogetonaceae) is a submersed, aquatic macrophyte found throughout the United States, the southern regions of Canada, and many other areas of the world (DiTomaso and Healy 2003). It is native to Eurasia and, within the United States, was first identified in 1859 in Delaware (Stuckey 1979). Large infestations of curlyleaf pondweed can impede water flow $\leq$ 90% in rivers and irrigation canals, clog and seriously damage water conveyance equipment, severely decrease recreational use, reduce land value adjacent to infested sites, and impede navigation (Bolduan et al. 1994, Nichols 1994). It can also causes serious ecological impacts by outcompeting native, aquatic, submersed species, which results in near-monotypic stands (Bratager et al. 1996).

Curlyleaf pondweed is a perennial that senesces as plants become dormant in summer, but the underground structures and young germinating plants are capable of surviving under cold winter conditions. Although plants can reproduce by seed and vegetatively from rhizomes and stem fragments, the primary method of reproduction and spread is through turions (Nichols and Shaw 1986). Turions are specialized stem buds that arise from the leaf axils or the tips of short, axillary branches. They detach from the main plant in midsummer and sprout in late summer to fall (DiTomaso and Healy 2003). The turions of curlyleaf pondweed are long lived (several years) and variable in their germination, which makes them extremely difficult to manage using chemical, cultural, or mechanical methods.

Benthic bottom barriers are a mechanical method of controlling aquatic organisms. They have been widely used in aquatic systems in the United States and Europe since the 1970s (Born et al. 1973, Nichols 1974, Mayer 1978, Murphy 1988, Eichler et al. 1995, Caffrey et al. 2010). For example, a bottom-barrier material was used to kill the Asian clam (*Corbicula fluminea* Müller) in Lake Tahoe, CA, by creating anoxic conditions (Wittman et al. 2012). In a marine environment in southern California, the invasive macroalgae killer alga [*Caulerpa taxifolia* (M. Vahl) C. Agardh], was eradicated using a combination of chemical control and benthic barrier material (Anderson 2007a). In another example, in Washington State, Eurasian watermilfoil (*Myriophyllum spicatum* L.) was successfully eradicated from a reservoir using bottom barriers (Zisette 2001). However, bottom barriers have not always proven successful in the management of weedy aquatic species. For example, in Clear Lake, CA, benthic bottom barriers were not successful in controlling hydrilla [*Hydrilla verticillata* (L. f.) Royle] (King 1999).

Benthic bottom barriers can be porous or nonporous materials that block light and/or gas exchange. Typical benthic barrier materials used for controlling aquatic weeds include jute, polyethylene [$(C_2H_4)nH_2$], fiberglass fabric, and rubber. Each of those products has specific advantages and

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*First and second authors, Graduate Student and Cooperative Extension Specialist, Department of Plant Sciences, MS4, University of California, One Shields Avenue, Davis, CA 95616. Corresponding author's E-mail: jmditomaso@ucdavis.edu. Received for publication January 16, 2014, and in revised form April 23, 2014.

disadvantages. Although benthic barriers are generally not considered cost effective for large areas (\$2.37 to \$13.45 m^{-2}), they may be a very effective management option for the control of new or small, aquatic-weed infestations, when managers are not authorized to use herbicides or other mechanical methods (Hofstra and Clayton 2012).

Jute is a natural plant product that sinks within a few minutes. It is less expensive than other barrier products and slowly decomposes and, thus, does not require removal the following season. It is highly porous and allows more light underneath than other materials, such as fiberglass-woven mats, polyethylene, or rubber. This reduces billowing and buoyancy and minimizes harm to the benthic fauna (Caffrey et al. 2010). In some systems, jute can kill weeds effectively by compacting existing growth, which subsequently decays after about 10 mo (Mayer 1978, Caffrey et al. 2010). However, although jute may be effective for some species, it does not target long-lived propagules (Caffrey et al. 2010).

Fiberglass and polyethylene fabrics are less porous than jute and have been widely used in terrestrial systems to suppress weeds by blocking light. Polyethylene fabric is also used for aquatic weed management, typically for smaller regions around boat marinas and docks; however, it is generally buoyant and subject to billowing. Like jute, a fiberglass benthic bottom is efficacious in some systems by compacting existing growth (Mayer 1978). Unfortunately, sedimentation on top of the barrier materials can contribute to regrowth unless the barriers are removed yearly and redeployed.

Rubber bottom barriers are nonporous and impermeable to both light and gas. Nonporous benthic materials can alter the physical and chemical habitat under the barriers by increasing ammonium and eliminating oxygen and light (Ussery et al. 1997). This can lead to anoxic conditions that can inhibit the sprouting and growth of aquatic weed propagules (Wu et al. 2009). One drawback is that aquatic sediments can produce gas bubbles that rise and collect under the barrier. Gas accumulation can dislodge barriers over time because of billowing and buoyancy.

The use of benthic barriers alone may not offer an effective tool for many management goals. Other than for the eradication of killer alga, there are few examples where benthic bottom barriers have been used in combination with other control methods, particularly chemicals. However, such integrated approaches have the potential for cost-effective eradication of new and isolated patchy sites of other invasive plant species in large lake and marine systems, where broadcast applications of herbicides are unfeasible or impractical. In addition, benthic barriers can provide a relatively fast, low-impact delivery method for chemical treatment; target a long-lived propagule "bank"; allow high concentrations of a compound confined below the barriers; and minimize downstream movement of chemicals to desirable, nontarget species by confining the compound to the region of application. Furthermore, confining a chemical to a specific region below the barrier can allow penetration of a chemical into the sediment, where it can target surface or subterranean propagules and root crowns. This is an important consideration because a few, small, persistent sediment tubers or other reproductive propagules generally avoid in-water herbicide applications and lead to reinfestation the following season.

Registered aquatic herbicides are effective but permits can be expensive and such herbicides may be prohibited in many aquatic systems. Alternatively, acetic acid ($C_2H_4O_2$) is a naturally occurring product of microorganisms in wetland soil and is degraded to carbon dioxide. Although it is not currently registered for use in aquatic systems, acetic at concentrations as low as 0.1% v/v, was shown to retard the growth and subsequent seed production of invasive smooth cordgrass (*Spartina alterniflora* Loisel.) in San Francisco Bay, CA (Anderson 2007b). Spencer and Ksander (1995, 1997) and Spencer et al. (2003) demonstrated that growth of hydrilla tubers, the winter buds of American pondweed (*Potamogeton nodosus* Poir.), and the tubers of sago pondweed [*Stuckenia pectinata* (L.) Börner] were inhibited with dilute acetic-acid treatments in dewatered irrigation systems. The inhibitory effect of acetic acid on plant tissues appears to be due to depolarization of the cell membranes, leading to metabolite leakage and cellular destruction (Spencer and Ksander 1999).

Acetic acid has the potential to be used on submersed, aquatic species in an integrated approach with nonporous, benthic bottom barriers. Such a system could allow for much smaller volumes of chemicals at high concentrations. This would not only reduce costs compared with less-targeted applications but also provide the opportunity to treat moving water without the need for drawdowns. Because acetic acid is water soluble, the development of a slow-release formulation, using starch [$(C_6H_{10}O_5)_n$] pellets, would provide a relatively simple method to increase exposure contact times for a variety of sediment propagules, including curlyleaf pondweed turions. Starch is a natural product that, like acetic acid, readily degrades into carbon dioxide in aquatic systems. In addition, we previously found starch to be relatively simple to incorporate with herbicides, even under nonlaboratory conditions. Like acetic acid, starch is widely available as a food source.

The objective of this study was, initially, to determine the effectiveness of porous and nonporous barrier materials on the inhibition of turion sprouting in curlyleaf pondweed. Subsequently, we evaluated the efficacy of a slow-release formulation of acetic acid in tapioca-starch "pearls," in combination with a nonporous barrier, on turion sprouting of curlyleaf pondweed and electrolyte leakage. Finally, we examined the depth of acetic acid penetration into the soil sediment as a way to estimate the potential for controlling surface or subterranean propagules.

MATERIALS AND METHODS

Plant collection and preparation

Turions of curlyleaf pondweed were collected from the north end of Fisherman's Cut near Brannon Island State Park, CA, in June 2012 (38°5′5″N; 121°38′48″W) and placed in cold water (4 C) in the dark until experimentation. Before the initiation of each experiment, a few turions were sprouted to ensure viability using the protocols from Sastroutomo (1981). Because Spencer and Ksander (1997) suggested that variation in propagule size may lead to differences in survivorship, we separated turions into two size classes by weight: small (50 to 100 mg) and large (150 to 250 mg). All experiments had four replicates of each size class for each treatment.

Benthic barrier comparisons

The experimental design for bench and mesocosm scale were both completely randomized designs with four replicates for each of the three treatments and an untreated control. Each experiment was repeated. In both bench and mesocosm experiments, the turions were untreated or covered with one of the three benthic bottom-barrier materials: 60-mm-pore mesh size jute fabric,[1] polyethylene fabric,[2] or 4-mm-thick rubber.[3] The jute allowed partial light penetration and full oxygen movement, polyethylene fabric allowed oxygen movement, and rubber barrier allowed neither light nor oxygen penetration. Untreated controls represented a treatment with no inhibition in light or oxygen penetration. In both studies, large and small turions (four replicates for each) were used separately to detect potential differences between the two size classes.

Bench-scale experiments were performed on the University of California, Davis, campus in June 2012. Deionized water (H_2O; 200 ml) was placed into foil-lined, 250-mL beakers with 10 mg of Osmocote slow-release fertilizer.[4] Lighting was supplied by fluorescent fixtures at 140 µmol m^{-2} s^{-1}. Wu et al. (2009) suggested that light at 150 to 200 µmol m^{-2} s^{-1} was most favorable for curlyleaf pondweed turion growth. Beakers were covered with jute, polyethylene fabric, or 4-mm rubber sheeting. Light meter[5] readings were measured under each barrier type (jute = 24 µmol m^{-2} s^{-1}, polyethylene = 3 µmol m^{-2} s^{-1}, and rubber = 0 µmol m^{-2} s^{-1}, respectively). Photoperiod was 12 h for the 8-wk treatment exposure. Rubber-barrier flask water was purged of oxygen (O_2) using nitrogen (N_2) gas and then quickly sealed with a rubber stopper to replicate anoxic conditions. The untreated control, jute, and polyethylene treatments were left unsealed. Temperature was stable at 26 C in the laboratory.

Mesocosm-scale experiments were performed in a greenhouse at the California Department of Food and Agriculture facility in Sacramento, CA, in June 2012. The greenhouse had temperatures ranging from 22 to 35 C and 1,210 µmol m^{-2} s^{-1} light, as measured on noon of June 21, 2012. Mesocosms were constructed from fiberglass and were 200 by 25 by 125 cm in size (516 L). Water depth was set at 25 cm and fresh carbon filtered tap water (alkalinity = 37 mg L^{-1}; pH 7.81) slowly and continuously dripping into the mesocosms. Water temperatures ranged from 24 to 29 C during the experiment. Each replicated experiment had four small turions and four large turions per treatment placed on the sediment in the middle of each pot. Pots were covered with jute, polyethylene fabric, or rubber sheeting. The rubber sheeting experiment was sealed by adding weights around the edges to minimize oxygen exchange. Light-meter readings were taken under each barrier type (jute = 68 µmol m^{-2} s^{-1}, polyethylene = 10 µmol m^{-2} s^{-1}, and rubber = 0 µmol m^{-2} s^{-1}, respectively). Sediments were collected from Owl Harbor (Twitchell Island, Isleton, CA), screened through 63-mm mesh to remove larger particles, and homogenized in a mixer. Pots were 15-cm-diam by 6-cm-deep with 3 cm of sediment added to each pot. Ten grams of Osmocote fertilizer (15–15–15 [N–P–K]) was added to each mesocosm.

Following an 8-wk treatment in both experiments, turions were removed and placed in a growth chamber with a 12-h photoperiod at 1,126 µmol m^{-2} s^{-1} light for 2 wk at a temperature of 5 C and for 1 additional wk at 30 C (Sastroutomo 1981). Posttreatment turion viability and survival were determined by visual inspection of new sprouts. For both the bench and mesocosm studies, the two replicated experiments were combined based on the similar means (chi-square test, $\chi^2 = 27.87$, df = 15). For large- and small-turions treatments, both data-set means were not significantly different and were normally distributed based on the chi-square test ($\chi^2 = 2.78$, df = 7); therefore, those data were also combined. Thus, for each treatment, there were 16 replicates.

Integration of acetic acid and nonporous benthic barrier

We initially developed a system for the slow-release of acetic acid under the rubber benthic barrier. To accomplish that, we used starch pellets made from tapioca, derived from the root of cassava (*Manihot esculenta* Crantz, Euphorbiaceae). Tapioca starch [$C_{27}H_{48}O_{20}$; 5-[3,4-dihydroxy-6-(hydroxymethyl)-5-methoxyoxan-2-yl]oxy-6-[[3,4-dihydroxy-6-(hydroxymethyl)-5-methoxyoxan-2-yl]oxymethyl]-2-[4,5-dihydroxy-2-(hydroxymethyl)-6-methyloxan-3-yl]oxyoxane-3,4-diol] was chosen because of its stability in water and its relatively rapid release rates for acetic acid compared with other starch types. Release rate was critical because the degradation time for acetic acid is relatively short (10 to 14 d) (Zhifeng et al. 2006, Anderson 2007b). Tapioca starch "pearls" (0.8 to 1.1 cm diam) were soaked in acetic acid. These acetic acid–loaded starch pearls remained intact for several days and were denser than water, which allowed them to sink.

Turions were covered with the 4-mm-thick rubber sheets. To correspond with the degradation rate of acetic acid (Anderson 2007b), we chose a 14-d turion exposure time. Untreated controls had the same conditions with tapioca pearls, except they lacked barrier material and acetic acid (starch loaded with deionized water). Treatments consisted of tapioca pearls each loaded with different rates of acetic acid, ranging from 20.8 mmol L^{-1} (0.125% v/v) to 332 mmol L^{-1} (2% v/v). Posttreatment turion viability was similar to that described for the benthic barrier comparison experiment.

The bench and mesocosm experiments were completely randomized designs with four replicates, and each experiment was repeated. Large and small turion size classes were separated to detect potential differences between size classes and efficacy of treatments.

Bench-scale experiments were performed in the laboratory on the University of California, Davis, campus in June 2012. Experiments were designed to test curlyleaf pondweed turion survival after exposure to both a liquid formulation of acetic acid and acetic acid loaded into tapioca pearls. The experiments had the same design, temperature, and lighting as described previously for 4-mm rubber sheeting treatment and untreated controls. As previously described, light-meter readings were 0 μmol m^{-2} s^{-1} under the rubber and 140 μmol m^{-2} s^{-1} in the untreated controls. Treatments consisted of 20 g of tapioca pearls, each loaded with 100 ml of different rates of acetic acid ranging from 0, 20.8, 41.5, 83, and 166 mmol L^{-1}, corresponding to 0, 0.125, 0.25, 0.5, and 1% v/v acetic acid. The tapioca pearls were allowed to absorb 100 ml of acetic acid for 30 min and was then placed into the beakers containing the turions, covered with the rubber barrier material or left uncovered for the control treatments.

Mesocosm-scale experiments were performed at the California Department of Food and Agriculture facility in Sacramento, CA, in June 2012, with similar design, temperature, water and soil conditions, and lighting as described previously for 4-mm rubber sheeting treatment and untreated controls. As with the benthic barrier experiment, each replicate had four small and four large turions placed on the sediment in the middle of each pot and then covered with 4-mm rubber sheeting. Light-meter readings were measured under each barrier to ensure no light dependency (rubber = 0 μmol m^{-2} s^{-1}). The volume in each pot in the mesocosm was 1 L. Starch loading was performed by using 200 g of tapioca pearls immersed in 1 L of liquid at 0, 20.8, 41.5, 83, 166, 332 mmol L^{-1}, corresponding to 0, 0.125, 0.25, 0.5, 1, and 2% v/v of acetic acid. The tapioca pearls were allowed to absorb the liquid for 30 min before use. Controls had the same dry weight of starch added to each replication without a rubber barrier or acetic acid.

The repeated experiments for both the bench and mesocosm experiments were combined based on the similar means for all treatments (chi-square test, $\chi^2 = 16.0$, df = 7). In these experiments, size classes were not combined for relative electrolyte leakage (REL) (chi-square test, $\chi^2 = 2.0$, df = 15); however, size classes were combined for the 14-d exposures for acetic acid at the bench and mesocosm scales (chi-square test, $\chi^2 = 28.7$, df = 15; and $\chi^2 = 32.5$, df = 15, respectively).

Release rates of acetic acid from starch

To assess acetic acid dilution rates in the water column using a liquid versus a starch-loaded formulation, we constructed a 20-L bucket chamber with an incoming tap-water flow rate of 3 L h^{-1} using a peristaltic pump.[6] Tap water entered at the bottom of the bucket with a pH of 7.86 (measured using a pH meter[7]) and overflowed out the top of the container. Incoming tap water was filtered with activated carbon to remove chlorine. The pH was measured 2 cm above the point of acetic acid application on the bottom center of the bucket. Measurements were made at 5-min intervals for the first 30 min, again at 60 min, and thereafter at 1-h intervals until 4 h, at which time, the dosing pumps were turned off. After 4 h, pH measurements were recorded 1 and 2 d after treatment (DAT). Initial dose for both liquid and starch-loaded (40 g of tapioca) treatments were 200 mL of 5% acetic acid to the 20-L bucket. The experiments were repeated and the results were combined across runs.

Electrolyte leakage

Experiments were performed on the University of California, Davis, campus in June 2012, with similar design, temperature, and lighting as described previously for the bench-scale experiments. Large and small turion size classes were separated based on weight with 40 to 80 mg for the small- and 120 to 200 mg for the large-size classes. Concentrations of acetic acid used for treatments were 0, 20.8, 41.5, 83, and 166 mmol L^{-1}, corresponding to a serial increase of 0, 0.125, 0.25, 0.5, and 1% v/v acetic acid. Following exposure for 7 d, turions were removed from the acetic acid treatments, rinsed with deionized water, and individually placed in a 15 mL glass test tube containing 5 mL of deionized water. Relative electrolyte leakage (REL) was measured using the Hendry and Grime (1993) procedure. The initial conductivity of the water was measured using a conductivity meter.[8] The conductivity was measured again 1 h later. Following that measurement, the test tubes containing the turions were placed in a boiling water bath for 10 min. Test tubes were then covered and allowed to stand at 26 C for 24 h. A final postboiling conductivity reading was taken. Total REL, as a percentage, was calculated from the following equation: *total electrolyte leaked* = (*conductivity at 1 h* − *initial conductivity*)/*conductivity 24 h after boiling* (Whitlow et al. 1992). Each experiment had four replications. The experiment was repeated with similar results (chi-square test, $\chi^2 = 16.0123$, df = 7); therefore, the two experiments were combined ($n = 8$).

Acetic acid leaching in soil column

Experimental conditions were similar to the mesocosm experiments, with similar design, temperature, water and soil conditions, and lighting as described previously. Fifty milliliters of 5% acetic acid was loaded into 30 g of tapioca starch pearls. The starch pearls were placed on top of the column filled with screened-clay Delta soil. Untreated controls also had 30 g of starch added to each replication, but the starch was loaded with only deionized water. The acetic acid soil-leaching profile was constructed using a 5-cm-diam polyvinyl chloride (PCV) pipe with plugged holes drilled at different depths for sampling the effluent. The pH of the soil solution was evaluated to determine acetic acid presence in the soil profile. Control pH was 7.86 measured using a pH meter. Approximately 2 ml of effluent was slowly withdrawn from each peeper depth and was then measured for pH in a clean, glass test tube. Data were recorded and then plotted as pH against time for each depth.

Analysis

To ensure homogeneity of variances, a Levene's test was performed before the ANOVA test. Computations of significant differences (Tukey-Kramer Honestly Significant Difference, $P < 0.05$) were based on ANOVA. Means are reported with their associated standard errors (SE). Statis-

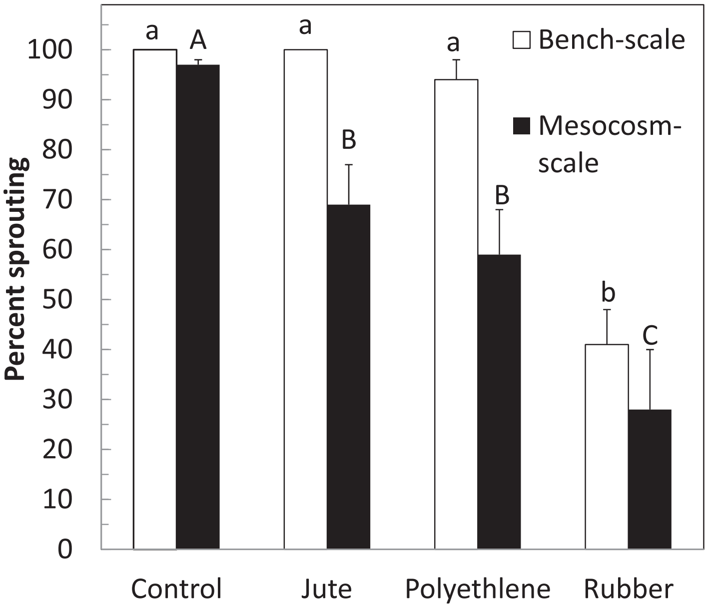

Figure 1. Percentage of sprouting in curlyleaf pondweed turions after an 8-wk exposure under benthic bottom-barrier treatments at the bench (open bars) and mesocosm (solid bars) scale. Data are combined from repeated experiments and size classes ($n = 16$ for all treatments). Means with the same letters within each study (lowercase for bench comparisons and uppercase for mesocosm comparisons) are not significantly different (Tukey's Honestly Significant Difference; $P \leq 0.05$).

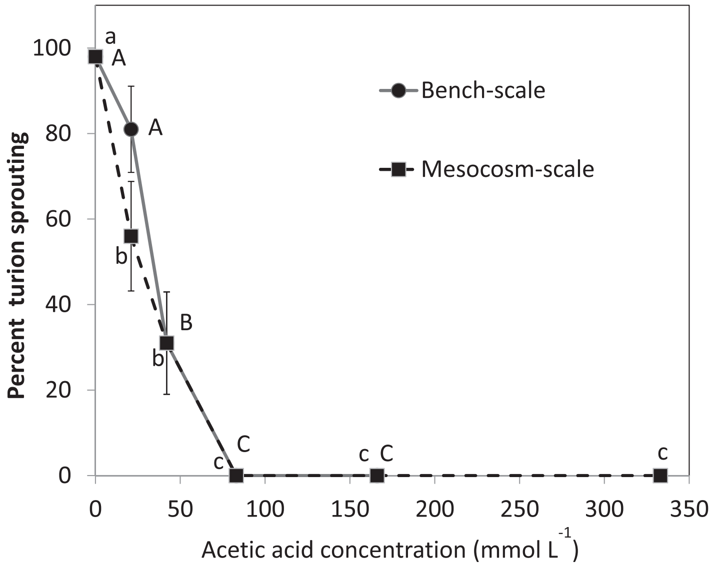

Figure 2. Sprouting percentage of curlyleaf pondweed turions after a 2 wk exposure to cassava starch (tapioca)–loaded acetic acid treatments under impermeable benthic bottom barriers in bench- and mesocosm-scale experiments ($n = 16$ for each treatment). Data are combined from repeated experiments and size classes. Means with same letters within each study (lowercase for mesocosm scale and uppercase for bench scale) are not significantly different for acetic acid concentration (Tukey's Honestly Significant Difference; $P \leq 0.05$).

tical analyses were performed using SAS JMP 8.0 statistical software.[9]

RESULTS AND DISCUSSION

Comparison of benthic barriers

Bench-scale treatments showed that the rubber barrier had a significant effect on curlyleaf pondweed turion sprouting ($P < 0.0001$) (Figure 1). Turion sprouting under the rubber (no light, no oxygen) benthic barrier treatment was 48% and significantly different from all other treatments. The untreated control (light and oxygen) and jute (reduced light, oxygen) treatments resulted in 100% turion sprouting, whereas the polyethylene benthic bottom-barrier treatments (no light, oxygen) failed to reduce turion sprouting (97%).

The mesocosm trial also demonstrated that the rubber barrier treatment had a significant effect on curlyleaf pondweed turion sprouting, with only 30% sprouting at the end of the trial ($P < 0.0001$) (Figure 1). Turion sprouting under the jute and polyethylene benthic bottom barriers was 72% and 70%, respectively. Although those rates are significantly reduced from the untreated controls (98% sprouting), even the rubber barrier would not be considered an effective long-term method of managing curlyleaf pondweed sprouting.

Although the results of the bench and mesocosm experiments differed somewhat in the response of turions to jute and polyethylene, both experiments clearly demonstrated that nonporous, benthic bottom-barrier materials were the most effective at inhibiting sprouting in curlyleaf pondweed turions. That inhibition was unlikely to be due to the elimination of light alone because other researchers have shown that aquatic weed propagules can survive on starch reserves without light for years in the hydrosoil (Kunii 1982, 1989, Netherland 1997, Madsen and Owens 1998). As has been suggested with other aquatic species (Wu et al. 2009, Wittman et al. 2012), the inhibitory effect of nonporous, benthic barriers on curlyleaf pondweed turions may be due to enhance anaerobic conditions in the sediments. Aquatic sediment anaerobiosis can produce phytotoxins, such as sulfide (S^{2-}), methane (CH_4), and acetic acid, which can cause tissue death (Van Wijck et al. 1992, Terrados et al. 1999). Despite its relative effectiveness, nonporous barriers cannot be used as the sole control technique to eradicate curlyleaf pondweed propagules; an integrated combination of acetic acid with anoxic stress may provide a far more-targeted and effective control method compared with each of these methods used alone.

Integration of acetic acid and nonporous benthic barrier

Both bench- and mesocosm-scale treatments showed that rubber-barrier treatments combined with starch-loaded acetic acid provided complete inhibition of sprouting curlyleaf pondweed turions for the 83, 166 and 332 mmol L^{-1} treatments (mesocosm) compared with the untreated control (Figure 2). The consistency among the results for the two turion size classes (data combined), two repeated experiments (data combined), and the two experimental designs (bench and mesocosm studies), strongly suggest that the effects of acetic acid under field conditions would also

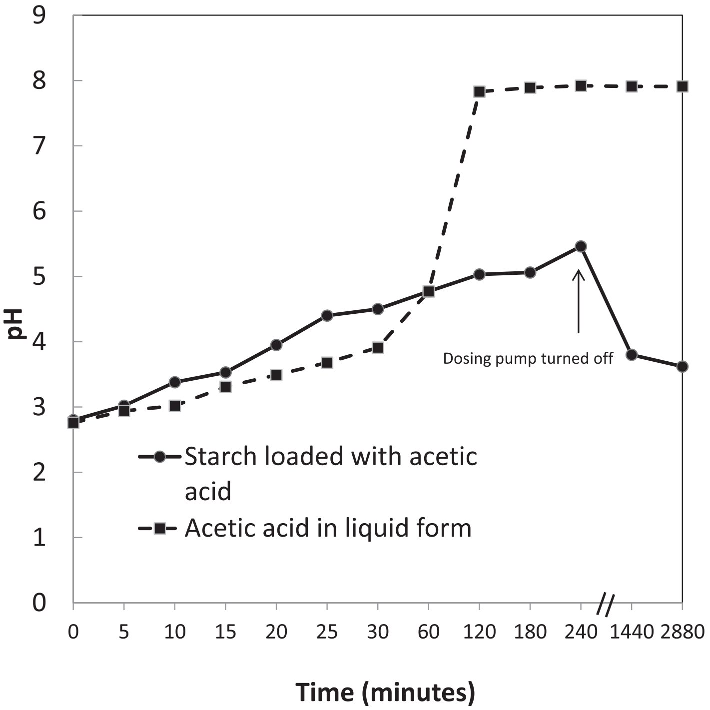

Figure 3. The acetic acid dilution rates into the water column by starch and by liquid formulations in a 10-L column container. The container was filled with tap water with an initial dose of 200 mL of 5% acetic acid and incoming tap-water flow rate of 3 L h^{-1}. After 240 min, the dosing pump was turned off. Data are combined from two experiments.

be efficacious. Thus, the combination of the rubber barrier and relatively low concentrations of acetic acid (equivalent to $> 0.5\%$) loaded into starch pearls indicate that this integrated approach may prove to be a valuable option for the control of curlyleaf pondweed turions. Although the use of acetic acid provided good control of aquatic weed propagules in other studies (Spencer and Ksander 1997, Anderson 2007b), its application was most effective in dewatered canals or under low-tide conditions. The combination of acetic acid under a nonporous benthic barrier may offer an effective tool in submersed, aquatic systems.

Release rates of acetic acid in starch

In the starch-loaded acetic acid formulation, the pH increased at a slightly faster rate compared with the liquid formulation until 60 min (Figure 3). After 60 min, the rate of dilution for the liquid formulation rapidly increased and equaled the tap water pH after 120 min. In contrast, the starch-loaded pH rose steadily until the 4 h (240 min) mark and, then, began to decline when the dosing pumps were turned off. Thus, long-term exposure to acidic conditions was greatly enhanced in the starch-loaded system. This would be expected to provide much better control of curlyleaf pondweed turions compared with direct transient exposure to a liquid formulation of acetic acid at equivalent rates.

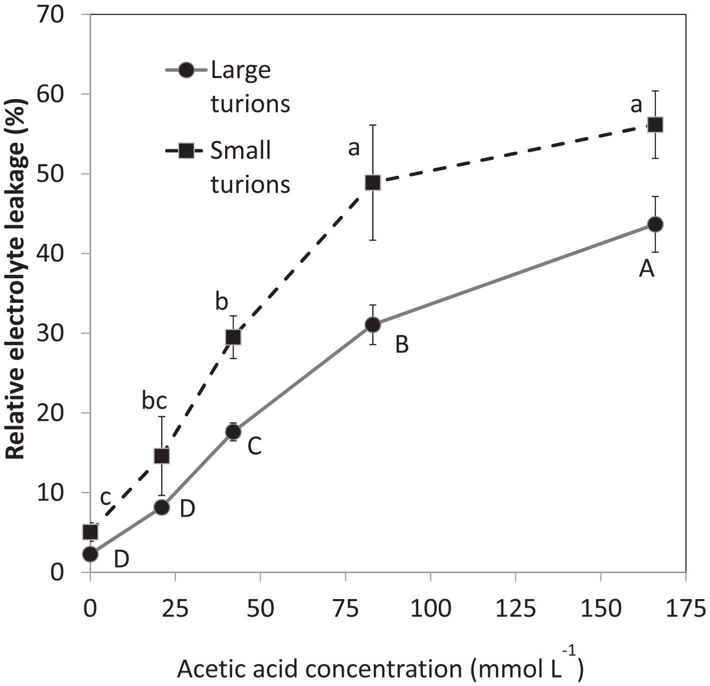

Figure 4. Percentage of relative electrolyte leakage at varying acetic-acid exposures for 7 d in large and small turion class sizes ($n = 8$ for each). Means with same letters within each turion study (lower case for small turions and upper case for large turions) are not significantly different for acetic-acid concentration (Tukey's Honestly Significant Difference; $P \leq 0.05$).

Electrolyte leakage

Large turions overall had a lower percentage of electrolyte leakage compared with small turions when exposed to acetic acid (Figure 4). That may be due to their increased volume, which prevented penetration of acetic acid to the center of the turions. In comparison to Figure 2, complete inhibition of sprouting occurred at concentrations 31% in the large turions and 49% in the small turions. Despite the complete inhibition in > 83.3 mmol L^{-1} for both the small and large turion sizes, at 83.3 mmol L^{-1} acetic acid, REL was sprouting at 83.3 mmol L^{-1} acetic acid and continued to increase with increasing acetic acid concentration. Differences between large and small turions indicate that, although there was a direct correlation between increased percentage of REL and turion sprouting, there was no specific threshold of REL that was associated with turion mortality.

Soil leaching of acetic acid

Acetic acid influenced the pH of the hydrosoil throughout the profile to a depth of 20 cm (Figure 5). Within the top 6 cm, there was no difference in soil pH at 240 min after exposure to 5% acetic acid loaded into 30 g of tapioca starch pearls. At hydrosoil depths below 6 cm, the influence of acetic acid became less pronounced, but even at 10 cm, the hydrosoil pH was 3.85 units more acidic than that of the soil at the start of the experiment. By 30 min after exposure, the pH at each depth reached equilibrium and remained about the same for the additional 210 min of the

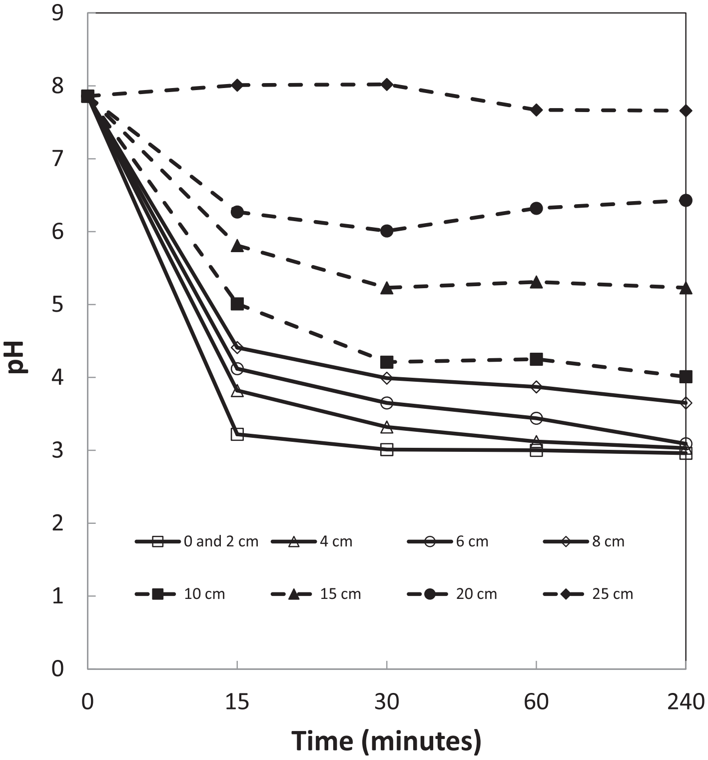

Figure 5. Acetic-acid leaching rates into sandy clay loam hydrosoil as a function of pH and sampling depth below the surface. Initial pH of pore water was 7.86. Each line represents water samples pH for each soil depth vs. time. Data are combined from two repeated experiments.

experiment. The results of this study are encouraging for the long-term management of curlyleaf pondweed vegetative propagules. Although it has been shown that 93 to 100% of monoecious hydrilla tubers are located in the top 12 cm of hydrosoil (Harlan et al. 1985), we observed that turions of curlyleaf pondweed are rarely buried and tend to occur primarily on top of the hydrosoil. This is expected because turions originate from vegetative tissues within the water column. Thus, the extended acidification of the hydrosoil in the top 10 cm may have a significant long-term effect on the survival of nearly all, if not all, of curlyleaf pondweed turions.

Implications for future management

Many of the negative economic and logistical trade-offs with the use of acetic acid were based on the method of delivery to the target weed. In this study, we have developed a more-effective method using a slow-release technology facilitated by loading dilute solutions of acetic acid into tapioca starch pearls. That technology, integrated with a nonporous, rubber benthic bottom barrier offers several advantages in the management of curlyleaf pondweed and, perhaps, other aquatic weeds. For example, deployment times can be reduced from months to 1 or 2 wk. This offers a cost-effective option for management of aquatic weeds compared with current benthic barrier practices. By using a thick-weighted, reusable, impermeable barrier with slow-release acetic-acid technology, shorter deployment times can allow more area to be treated over the course of a season compared with traditional benthic barriers used alone. For example, using four 6 by 6 m, weighted barriers for 1-wk deployment time could cover 2,304 m^2 of treatment area in 16 wk. With a traditional 16-wk treatment time, using only rubber benthic barriers, just 144 m^2 could be treated during the same period. In addition, such small, weighted barriers would be more economically practical for short treatment times compared with traditional long-deployment (months) benthic barriers.

Additional advantages over traditional use of bottom benthic barriers, the integrated approach used here could reduce the potential for gas build up and billowing that can compromise the utility of benthic bottom barriers, which can have a secondary benefit of reducing the potential for vandalism or boating and recreational hazards. Another advantage of using the combination of a benthic barrier and slow-release acetic acid is the possibility of avoiding patches of desirable native flora and fauna and minimizing leaching into the water column. Thus, management of an invasive or problematic species can be highly specific to smaller, patchy infestations.

Unlike broadcast herbicide applications in aquatic systems, benthic bottom barriers and slow-release acetic acid treatments can be used in unidirectional flow systems characteristic of rivers, irrigation canals, and smaller streams. In these areas, a systematic approach starting upstream and treating step-wise downstream could be used to eradicate weed propagules. Thus, the use of this integrated approach has the potential to be a highly precise, efficacious, and cost-effective method for the control of curlyleaf pondweed turions and, perhaps, other important aquatic weed species.

SOURCES OF MATERIALS

[1] Jute, EarthAid, 28447 Witherspoon Parkway, Valencia, CA 91355.

[2] Polyethylene weed block, Ben Meadows, P.O. Box 5277Janesville, WI 53547.

[3] Rubber, Rubber Sheet Roll, 9974 Molly Pitcher Highway, Shippensburg, PA 17257.

[4] Osmocote fertilizer, Scotts Company, 14111 Scottslawn Road, Marysville, OH 43041.

[5] Model MQ-200 Quantum with SQ-120 sensor, Apogee Instruments, 72 W. 1800 N., Logan, UT 84321.

[6] SP-3000 peristaltic pump, Aqua Medic, 5803 Byrd Drive, Loveland, CO 80538.

[7] Model PH370 pH meter, American Marine Inc., 54 Danbury Road, Suite 172, Ridgefield, CT 06877.

[8] CON 110 conductivity meter, Oakton Instruments, P.O. Box 5136, Oakton, Vernon Hills, IL 60061.

[9] SAS JMP 8.0 statistical software, SAS Institute, Inc., 100 SAS Campus Drive, Cary, NC 27513-2414.

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