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Integrating hot water under a benthic barrier for curlyleaf pondweed turion control

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ABSTRACT

The submersed aquatic macrophyte curlyleaf pondweed (*Potamogeton crispus* L.) is a widespread invasive plant of aquatic areas throughout the United States and much of the world. Its vegetative reproductive propagules, including turions, increase the difficulty of eradication or effective control. In this study, we evaluated the effect of heated water circulated under an insulated benthic bottom barrier as a potential nonchemical and rapid method to inhibit sprouting of turion propagules on or near the sediment surface. We exposed turions to water temperatures of 25, 40, 50, 60, 70 and 80 C for time periods ranging between 30 and 300 s in both bench- and mesocosm-scale experiments. Heated-water exposures significantly inhibited turions sprouting at 50 and 60 C in both experiments, but only gave complete inhibition in sprouting in the bench-scale experiment with exposure to 60 C for 300 s. In both bench- and mesocosm-scale experiment, 60-s or higher exposure times at 70 and 80 C gave complete inhibition in turion sprouting. The cost to maintain a 5-min exposure to 70 C water under a 1-m^2 insulated barrier is estimated at \$0.172 USD (~ \$1,720 ha^{-1}). This would be even more cost effective using wholesale energy cost estimates. Thus, this technique might be a practical and cost effective method of eradicating small incipient infestations of aquatic weed propagules, such as curlyleaf pondweed turions.

Key words: bottom barrier, heat, invasive species, *Potamogeton crispus*.

INTRODUCTION

Nearly all species of *Potamogeton* in the United States are widespread natives. The exception, however, is curlyleaf pondweed (*Potamogeton crispus* L.), a submergent aquatic species introduced from Eurasia and a widespread invasive plant throughout North America and many other regions of the world (DiTomaso and Healy 2003). In addition to its ecological effects on native habitats (Bratager et al. 1996), curlyleaf pondweed has significant economic impacts on many aquatic systems. For example, it has been shown to block the flow of water by up to 90% in rivers and irrigation canals, clog and damage water conveyance equipment, severely decrease recreational use, reduce land value adjacent to infested sites, and impede the movement of aquatic vessels (Bolduan et al. 1994, Nichols 1994).

The effective management of aquatic plants, including curlyleaf pondweed, often depends on understanding their reproductive biology. This is particularly important with submerged macrophytes, because most problematic species can reproduce through one or more vegetative propagules (DiTomaso and Healy 2003). although curlyleaf pondweed can reproduce sexually through seeds and vegetatively through rhizomes or stem fragments, it also produces copious amounts of asexual propagules known as turions. Turions are specialized stem buds that originate from leaf axils or the tips of short axillary branches. They can remain dormant for a few years and are capable of surviving unfavorable conditions (Nichols and Shaw 1986).

The turions of curlyleaf pondweed are formed in midsummer and can germinate in late summer to late fall (Nichols and Shaw 1986). Young dormant plants overwinter and grow quickly the following spring, providing an early advantage over competing vegetation.

There are few tools available that provide effective control of curlyleaf pondweed turions (Woolf and Madsen 2003, Johnson et al. 2012). Although herbicides can be effective in managing germinated propagules, they do not provide control of dormant reproductive structures, including turions. In addition, they can be difficult to use in flowing water systems, numerous patchy infestations, or areas where there is high concern for nontarget species. Thus, effective long-term control of germinating turions often requires several years of herbicide application (Johnson et al. 2012).

Previous studies showed that, when used alone, benthic bottom barriers can have variable results on management of aquatic macrophytes (Hofstra and Clayton 2012), but are typically ineffective for eradication of aquatic weeds due to limited-area coverage and rapid posttreatment regrowth (Ussery et al. 1997). We similarly found that the use of a nonporous rubber benthic barrier does not offer an effective long-term management tool for the control of curlyleaf pondweed (Barr 2013). However, in a marine environment in southern California, a combination of chemical control (chlorine tablets) and benthic barrier material was successful in eradicating the invasive macroalgae, *Caulerpa taxifolia* (M. Vahl) C. Agardh (Anderson 2005). Although not tested under field conditions, we have shown that the combination of rubber benthic bottom barrier and acetic acid in both bench- and mesocosm-scale experiments was an effective method of inhibiting curlyleaf pondweed turion sprouting (Barr 2013).

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The use of heat, as either direct flame, steam, or hot water, is a nonchemical weed control option occasionally used in terrestrial systems (Ascard et al. 2007). Few studies have examined the effects of heat on the management of aquatic weeds, with the exception of hot water to control Eurasian watermilfoil (*Myriophyllum spicatum* L.) fragments (Stanley 1975, Blumer et al. 2009). However, an integrated approach using appropriate heat intensity and dosing time has the potential to provide effective control of aquatic weed vegetative propagules, particularly those that remain close to the hydrosoil surface (e.g., turions). Furthermore, benthic barriers made from thicker silicone having weighted edges can give insulated containment of the heat, thus increasing the cost effectiveness and efficacy of the management method. In addition, the technology should be relatively easy to deploy at smaller scales and can be employed where no chemical usage is authorized.

One of the mechanistic challenges in developing a thermal-based management technique in aquatic submersed systems is how to physically apply heat efficiently to the precise location. In addition, little is known of the heat intensity and exposure period required to cause turion mortality, or if the integrated approach can be cost-effective compared to more traditional management strategies. To address these challenges, we examined the hot-water regimes required to kill curlyleaf pondweed turions deposited on the aquatic soil surface and also estimated the cost per area to successful prevent resprouting. We hypothesized that the proportion of sprouting turions would decrease with increasing temperature and time of exposure.

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, California, in June of 2012 (38°N, 122°W). Harvested turions were kept in cold water (4 C) in the dark. Some turions were sprouted to ensure viability using the protocols from Sastroutomo (1981). These were tested prior to the initiation of the experiment and repeated for each set of turions used in each experiment. Because Spencer and Ksander (1997) suggested that variation in propagule size might lead to differences in survivorship, we separated turions into two size classes: 50 to 100 mg and 150 to 250 mg.

Experimental design and analysis

The experimental design for both bench- and mesocosm-scale trials were complete randomized designs with four replicates for each of the turion sizes and heat treatments. In addition to the heat treatments, we also included an untreated control. The untreated controls (25 C) were exposed to the same conditions as the other treatments, except they lacked applied heat. Large and small turion size classes (four per size class per replicate) were split into groups to detect potential differences between size classes and efficacy of treatments. Each experiment was repeated a second time. The data from the repeated experiments were combined based on the nonstatistical differences in means for the bench-scale (chi square = 28.31, df = 15) and mesocosm-scale studies (chi square = 22.94, df = 16). Large and small class sizes were also combined, also based on the similar nonstatistically different means between each experiment (bench-scale, chi square = 21.18, df = 15; mesocosm-scale, chi square = 31.28, df = 15). Treatments in both bench- and mesocosm-scale experiments consisted of a combination of hot-water treatments at 25, 40, 50, 60, 70, or 80 C for exposure times of for 0, 30, 60, 120, or 300 s. Immediately after treatment, turions were rinsed with cool deionized water (15 C) and placed into a 16- by 25-mm test tube with 5 ml deionized water. This step ensured a return to similar field ambient water temperatures posttreatment and stopped the heat exposure effects on plant tissues. Posttreatment turion viability was based on visual inspection of new sprouts following a vernalization protocol as described by Sastroutomo (1981). In brief, turions were removed and placed in a growth chamber under 12-h photoperiod at 1,126 μmol m^{-2} s^{-1} light for 2 wk at a temperature of 5 C (maintained by fans) and an additional 1 wk at 30 C. Posttreatment turion viability and survival was based on visual inspection of new sprouts.

Bench-scale experiments were performed on the University of California, Davis campus in June 2012. A magnetic stirrer with a hotplate was used in conjunction with a temperature probe. Deionized water was used for the water bath treatments. Four turions (both small and large size classes) for each treatment were placed into a small wire basket and submersed into a hot-water bath for all treatment combinations.

Mesocosm-scale experiments were performed at the California Department of Food and Agriculture facility in Sacramento in June 2012. The greenhouse had temperatures ranging from 22 to 35 C and 1,210 μmol m^{-2} s^{-1} light, as measured at noon on 21 June 2012. Mesocosms were made from fiberglass and were 200 by 25 by 125 cm in volume (516 L). Water depth was set at 25 cm and fresh carbon-filtered tap water (alkalinity 37 mg L^{-1}; pH 7.81) was slowly and continuously dripped into the mesocosms. Water temperature for untreated controls ranged from 24 to 29 C during the experiment. Each replicated experiment had four small turions and four large turions placed on the sediment in the middle of each pot (four replicated pots per treatment). Sediments were collected from Owl Harbor (Twitchell Island, Isleton, CA), screened through a 63-mm mesh to remove larger particles, and homogenized in a mixer. Pots were 15 cm diameter by 6 cm deep with 3 cm of sediment added to each pot. Each pot was covered with 20-mm silicone foam-rubber sheeting.[1] The silicone sheet had a pair of PVC 1.27-cm-diam bulkheads attached to flexible 1.27-cm-diam PVC pipe to provide the heated water influent and effluent. An electric water heater[2] attached to a hot-water brass pump[3] with a 40-L steel reservoir was used to supply hot water to the treatments at different temperatures. A thermometer was used to measure the water temperature under the barrier. Replicate (pots) were subjected to a hot-water bath for all combinations of heat and exposure times.

To ensure homogeneity of variances, a Levene's test was performed prior to the ANOVA test. Computations of significant differences (Tukey-Kramer HSD, $P < 0.05$) were based on analyses of variance. Means reported carry their associated standard errors (SE). Statistical analyses were performed using SAS JMP 8.0 statistical software (SAS Institute Inc. 2009).

RESULTS AND DISCUSSION

Effect of heat on turion sprouting

In the bench-scale experiment, the effects of 25 C were not significantly different from 40 C and those of 80 C were not different from 70 C. Thus, the data for both the 25 and 80 C treatments are not presented. Results of the bench-scale heat treatments comparing exposure time versus percent sprouting for each temperatures showed that even a 30-s exposure to 70 and 80 C (data not shown) gave complete control of turion sprouting (Figure 1A). The only other combination that gave complete control of curlyleaf pondweed turions sprouting was a 300-s exposure at 60 C. In contrast, 40 C treatments did not significantly affect turion sprouting regardless of the exposure time, and 50 C gave between 69 and 75% suppression with exposures between 60 and 300 s. Plots of the data for temperature versus percent sprouting for each exposure time clearly show that all temperatures above 40 C had a significant effect on turion sprouting, but only 60 C at 300 s and all exposure times at 70 C gave complete control (Figure 1B).

In the mesocosm-scale experiment, again the 25 C treatment was not different from the 40 C treatment and, thus, the data are not presented. The data from the mesocosm experiment were very similar to that of the bench experiment, although curlyleaf pondweed turions in the mesocosm experiment were somewhat less sensitive to high temperature compared to the bench experiment. This could be the result of reduced efficiency under the barrier in the mesocosm experiment compared to the precision of maintaining temperatures in the bench experiment. For example, at 50 C and exposure times of 60 to 300 s, the inhibition in turion sprouting in the mesocosm experiment was between 25 and 44% (Figure 2A), compared to 69 and 75% in the bench experiment (Figure 2A). Additionally, although complete inhibition in turion sprouts occurred at a 60-s exposure to 70 and 80 C, only 80 C gave complete control of turions at a 30-s exposure time. Unlike the bench-scale experiment, even a 300-s exposure to 60 C did not completely suppress turion sprouting in the mesocosm experiment. When comparing temperature versus percent sprouting for each exposure time, it is again clear that all exposure times at 60 C gave significant reduction in turion sprouting, but only 80 C gave complete inhibition at all exposure times (Figure 2B).

The results of both the bench and mesocosm experiments are similar to those reported for Eurasian watermilfoil fragments. Stanley (1975) found that apical fragments exposed to 50 C for 300 s were completely killed. Using

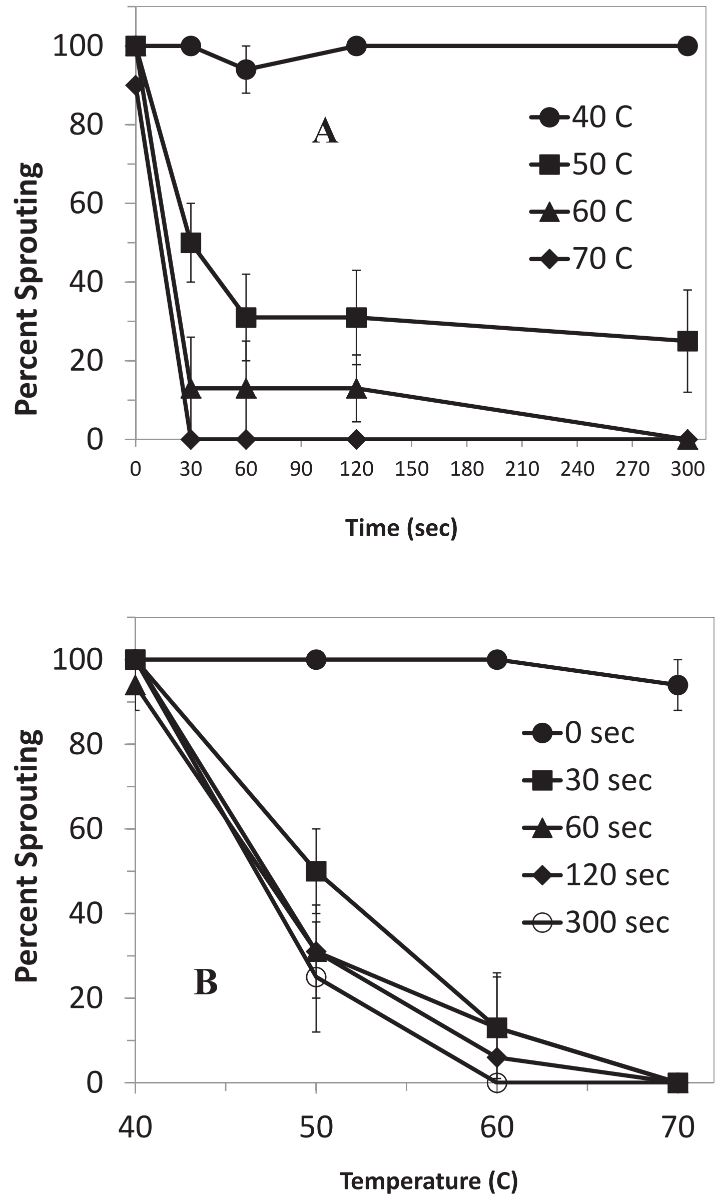

Figure 1. Sprouting percentage of curlyleaf pondweed (*Potamogeton crispus*) turions in bench-scale experiment after (A) time-based hot-water treatments at various temperature exposures and (B) temperature-based hot-water treatments at various time exposures. Data are combined from repeated experiments and size classes. Means $\pm$ 1 standard error (SE) ($n =$ 16) are presented.

20-cm Eurasian watermilfoil fragments with and without an apical meristem, Blumer et al. (2009) similarly showed that a 120-s exposure to temperatures $\geq$ 60 C gave complete kill of all fragments. Our studies showed slightly higher temperatures (70 C) to be required to achieve complete kill of curlyleaf pondweed turions. However, this is not surprising, considering the relative density of turions compared to Eurasian watermilfoil fragments (Nichols and Shaw 1986).

In terrestrial systems, the mechanism of action for heat treatments on plant tissues is thought to result from membrane destabilization, protein denaturation and coagulation, and an increase in reactive oxygen species that

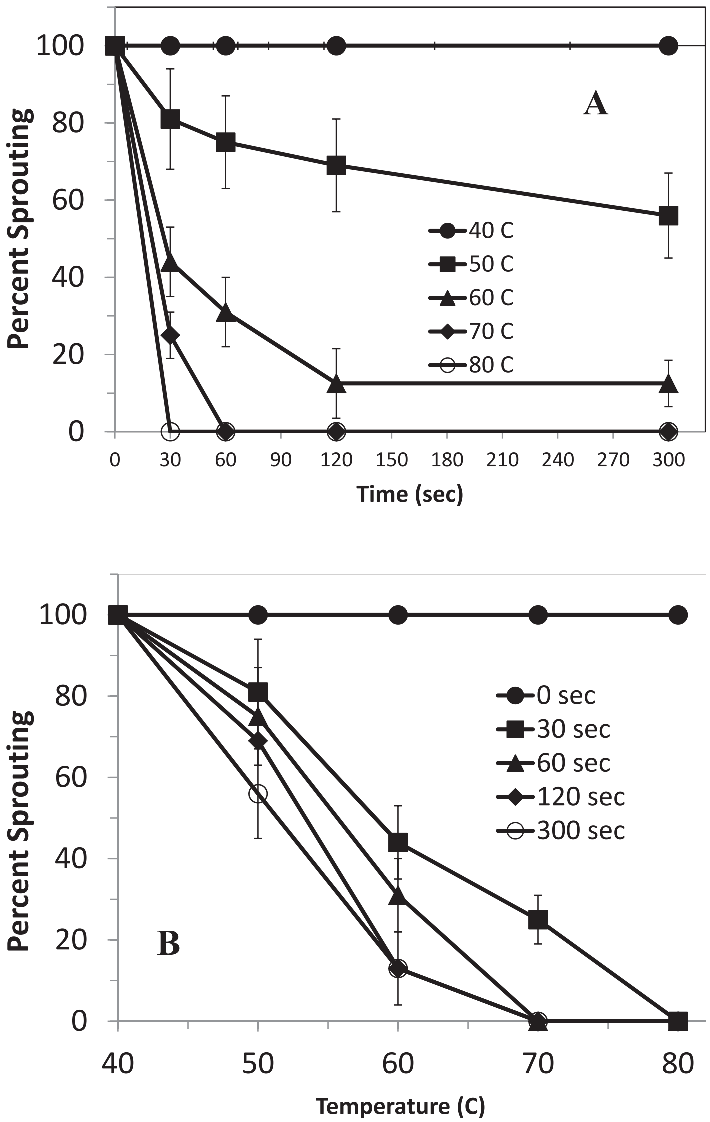

Figure 2. Sprouting percentage of curlyleaf pondweed (*Potamogeton crispus*) turions in mesocosm-scale experiment after (A) time-based hot-water treatments at various temperature exposures and (B) temperature-based hot-water treatments at various time exposures. Data are combined from repeated experiments and size classes. Means ± 1 standard error (SE) (n = 16) are presented.

subsequently damage cell proteins, membranes, and DNA (Leone et al. 2003). It is not clear which of these specific effects are responsible for cell death in curlyleaf pondweed turions, although all might contribute to mortality. Although we hypothesize that these same mechanisms apply similarly to aquatic systems, there is no direct evident to support our hypothesis. Nevertheless, the results of these experiments show that hot water, within a reasonable temperature range and exposure time, can effectively inhibit curlyleaf pondweed turion sprouting.

Economic analysis for the potential use of heat and benthic bottom barrier

Heating water involves significant energy and cost. Costs can be reduced if the infestation of curlyleaf pondweed is small or patchy and, thus, only a limited area is targeted for control. In addition, if the volume of hot water is small, the insulation good, and the required temperature is not exceedingly high, the practicality of this approach could be more realistic.

Natural gas, oil, propane, or diesel electric generators would typically provide field site energy supply for hot-water treatments. Hot-water electric- and oil-heated pressure washers (up to 95 C and flow rates up to 22 L min^{-1}) are commercially available for $1,500 to $5,000 USD. A typical insulated benthic bottom barrier hypothetically would be 3 by 3 m with raised channels, mesh, or blocks on the bottom surface. The channels would allow the water to flow evenly over the soil surface underneath the barrier. The required water volume for a treatment would be equal to the total area of barrier multiplied by the height of these channels. For each square meter of the barrier with 5-cm-high grooves, there would be approximately 50 L of water volume (10 dm length by 10 dm width by 0.5 dm height = 50 dm^3 = 50 L). The specific heat required for 50 L of water under a 1 m^2 barrier can be calculated by converting liters to kilogram (1 L = 1 kg), such that 50 L would be 50 kg of specific heat.

Typical ambient water temperatures vary but are often 10 C, to perhaps 20 C, for most aquatic systems. To achieve temperatures of 70 C for a 60-s exposure time would require a temperature rise of 60 C (assuming a starting point of 10 C) in a 50-L volume of water. To insure adequate kill of curlyleaf pondweed turions that could be just below the surface of the hydrosoil could require a 2- to 5-min exposure to 70 C. By using the following equation (Equation 1)

$$mC\Delta T = Q \qquad [1]$$

where Q is the heat added, m is the mass in joules kg^{-1} C^{-1}, C is the kg specific heat, and ΔT is the change in temperature, the amount of joules required to heat 50 L of water 60 C would be

(4,186 joules kg^{-1} C^{-1}) × (50 kg specific heat) × (70 C − 10 C) = 1.2558×10^7 joules.

Because 1 therm is equal to 1.0548×10^8 joules, 1.2558×10^7 joules would require 0.119 therms of natural gas to heat 50 L of water from 10 C to 70 C. Considering that the efficiency conversion for gas water heaters ranges between 0.6 to 0.85, as a result of some heat loss within the system, we used 0.725 as the middle range of conversion such that the required therms necessary to heat 50 L of water by 60 C would be 0.164 (0.119/0.725). The average residential cost of natural gas in the United States is $1.05 $therm^{-1}$, whereas the average industrial gas price, which includes agricultural and forestry usage, is $0.39 $therm^{-1}$ (U.S. Energy Information Administration 2012). At $1.05 $therm^{-1}$, the estimated cost of heating 50 L of water to 70 C under a 1-m^2 barrier would be $0.172. This would equate to $1,720 per hectare ($696 $acre^{-1}$). At a wholesale therm cost of $0.39, the cost would only be $639 ha^{-1}. It is important to note that this does not include the cost of labor and equipment, which could also be substantial.

This estimated cost is similar or even far less than current costs to control aquatic weeds, depending on the situation. However, although we built in an exposure time buffer for the control of turions, there is unlikely to be 100% efficiency of energy transfer and insulation even considering the efficiency conversion factor. The percent efficiency in energy transfer can greatly influence turion control. In addition, water would need to flow slowly through the bottom barrier to maintain effective thermal dose necessary to kill propagules. Higher flow rates would break the seal on the edges of the barrier and lead to a loss of recirculating hot water. The energy required to maintain this temperature over the 2- to 5-min treatment time would also vary. Transfer of heat through the pumping and piping system would reduce efficiency. In addition, pumps vary in their energy requirements and this is not factored into the cost analysis. This method might not be effective during peak midsummer biomass due to plants lifting the barrier well above the sediment and the lack of hot-water containment on the bottom. However, early- and late-season treatments might be particularly effective on young plants and sprouts.

Implications for future management

In this study, hot-water applications completely inhibited sprouting turions of curlyleaf pondweed at 70 and 80 C in both bench- and mesocosm-scale experiments. We speculate that the effects of hot water might be similar for young curlyleaf pondweed plants, as well as newly sprouted propagules, root crowns, and winter buds for other plant species. Such methods might also hold potential for treatment of invasive sessile invertebrates and marine algae.

There are several advantages to using hot water under an insulated benthic barrier. It is already possible to use a propane gas- or oil-powered water heater either on shore- or boat-mounted to supply circulated hot water under the benthic barrier via an insulated flexible hose. In addition, treatment times would be brief (5 min or less), thus allowing a crew to deploy and cover a large area with one small barrier in a single day. The proposed hot-water barrier method would be ideal for spot treatments, patchy smaller sites, flowing systems, rapid response treatments, and areas where no chemical usage is authorized or where permitting is cost-prohibitive.

Although Blumer et al. (2009) concluded that using hot water alone to kill fragments of Eurasian watermilfoil attached to watercraft was not feasible because of the high water temperatures required, they did not consider hot water as part of an integrated approach with the use of a benthic barrier. Given the relative straightforward engineering aspects of hot-water applications to the benthos in submersed aquatic habitats and the effectiveness demonstrated at both the bench- and mesocosm-scales, this integrated approach appears to have good potential for managing incipient populations of difficult-to-control aquatic weed propagules. However, because field applications are often more variable and less efficient than controlled bench- or mesocosm-scale studies, a higher temperature or longer exposure might be necessary to prevent sublethal field exposures. Further field research into the effects of hot water or the development of deployment technology could provide a method to reduce the energy requirements and improve the cost effectiveness of controlling aquatic weed propagules.

Should the technology prove to be practical, it is also possible that heat can be used in a more expanded integrated approach with both benthic bottom barriers and acetic acid. In a terrestrial system, for example, higher temperatures increased the control of Indian mustard [*Brassica juncea* (L.) Czern.] with acetic acid (Brainard et al. 2012). The combination of acetic acid, benthic barriers, and hot water might even give enhanced control at lower temperatures, and thus, reduced energy costs.

In summary, the engineering of small-scale hot-water systems would be relatively simple and safe for field use. Such a method would be best employed to treat young plants and the long-lived propagules in the early spring season or in the fall when foliar biomass is low.

SOURCES OF MATERIALS

[1]Silicon foam-rubber sheeting, RubberCal, Santa Ana, CA 92707.

[2]Electric water heater, Elkay model number LK498, Oak Brook, IL 60523.

[3]Hot-water brass pump, Grundfos model UPIS-10SU7P/TLC, Olathe, KS 66061.

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