Evaluating the Effects of Acetic Acid and d-Limonene on Four Aquatic Plants

Lyn A. Gettys[1], Kyle L. Thayer[1], and Joseph W. Sigmon[1]

ADDITIONAL INDEX WORDS. **broadleaf sagittaria, citric acid, *Eichhornia crassipes*, invasive species, natural herbicides, organic herbicides, pickerelweed, *Pistia stratiotes*, *Pontederia cordata*, *Sagittaria latifolia*, vinegar, waterhyacinth, waterlettuce**

SUMMARY. **The foundation of most aquatic weed management programs in Florida is synthetic herbicides because many of these U.S. Environmental Protection Agency (USEPA)-registered products are effective, selective, and inexpensive compared with other strategies such as mechanical harvesting. However, stakeholders have expressed concern regarding their use and managers are interested in exploring alternative methods for aquatic weed control. To that end, we evaluated the efficacy, selectivity, and costs of the "natural" products acetic acid and d-limonene (alone and in combination with each other and citric acid) on the invasive floating plants waterhyacinth (*Eichhornia crassipes*) and waterlettuce (*Pistia stratiotes*), and the native emergent plants broadleaf sagittaria (*Sagittaria latifolia*) and pickerelweed (*Pontederia cordata*). These products, plus an industry-standard synthetic herbicide (diquat dibromide), were applied once as foliar treatments to healthy plants, which were grown out for 8 weeks after treatment to allow development of phytotoxicity symptoms. A 0.22% concentration of diquat dibromide eliminated all vegetation, but neither "natural" product alone provided acceptable (>80%) control of floating weeds, even when applied at the maximum concentrations under evaluation (20% acetic acid, 30% d-limonene). Citric acid (5% or 10%) had no effect on the activity of acetic acid or d-limonene, but some combinations of acetic acid and d-limonene controlled floating weeds effectively without causing unacceptable damage to native plants. However, these treatments are much more expensive than the synthetic standard and managers would realize a 22- to 26-fold increase in product cost alone without factoring in other expenses such as additional labor and application time. Combinations of acetic acid and d-limonene may have utility in some areas where the use of synthetic herbicides is discouraged, but broad-scale deployment of this strategy would likely be prohibitively expensive.**

Florida's resource managers are charged with keeping aquatic vegetation at maintenance levels to facilitate navigation, flood control efforts, and other uses of state waters. This goal is most often achieved by using herbicides that have been approved by the U.S. Environmental Protection Agency (USEPA) for use in aquatic systems, with statewide oversight and coordination of treatments provided by the Florida Fish and Wildlife Conservation Commission (FWC, 2018, 2019a). For example, the FWC oversaw the expenditure of \$17.007 million and \$15.126 million in federal and state funds to control aquatic plants in Florida's public water bodies in fiscal year (FY) 2017–18 and FY 2018–19, respectively (FWC, 2018, 2019a). More than half of this funding (\$10.01 million and \$8.86 million in FY 2017–18 and FY 2018–19, respectively) was allocated for managing the submersed weed hydrilla (*Hydrilla verticillata*), whereas ≈25% of those monies (\$4.04 million in FY 2017–18 and \$4.19 million in FY 2018–19) was spent for floating plant control, which primarily comprise waterhyacinth (*Eichhornia crassipes*) and waterlettuce (*Pistia stratiotes*). Excessive growth of floating plants causes a number of problems in aquatic ecosystems, including reducing the penetration of oxygen and light into the water column by blocking the air–water interface, creating monocultures by outcompeting native plants, and interfering with flood control operations by creating large, dense mats that obstruct canals and water movement structures (Gettys, 2019). Waterhyacinth, a Brazilian species, was introduced intentionally to Florida during the late 1800s as a water garden ornamental and was released from cultivation soon thereafter (Gettys, 2020a). The native range of waterlettuce is cryptic and may include the southeastern United States, but the species exhibits aggressive growth and is considered invasive in Florida regardless of its true point of origin (Gettys, 2020b).

As with all pesticides registered by the USEPA, aquatic herbicides are only labeled for use if they "will not generally cause unreasonable adverse effects on the environment . . . taking into account the economic, social, and environmental costs and benefits of the use of any pesticide" (USEPA, 1996). However, public concerns regarding herbicide use in aquatic

Received for publication 3 Dec. 2020. Accepted for publication 17 Feb. 2021.

Published online 23 March 2021.

[1]University of Florida, Institute of Food and Agricultural Sciences, Fort Lauderdale Research and Education Center, 3205 College Avenue, Davie, FL 33314

This research was supported by the Florida Agricultural Experiment Station and by the U.S. Department of Agriculture, National Institute of Food and Agriculture (HATCH project FLA-FTL-005682). Funding was also provided by the Florida Fish and Wildlife Conservation Commission.

We thank Ian Markovich, Mohsen Tootoonchi, and Angie Diez for their invaluable contributions to this project.

Mention of a trademark, proprietary product, or vendor does not constitute a guarantee or warranty of the product and does not imply its approval to the exclusion of other products or vendors that also may be suitable.

This article is based on a presentation given at the 2020 Florida State Horticultural Society's annual meeting in Oct. 2020.

L.A.G. is the corresponding author. E-mail: lgettys@ufl.edu.

https://doi.org/10.21273/HORTTECH04769-20

Units

To convert U.S. to SI, multiply by	U.S. unit	SI unit	To convert SI to U.S., multiply by
3.7854	gal	L	0.2642
9.3540	gal/acre	$L \cdot ha^{-1}$	0.1069
2.54	inch(es)	cm	0.3937
25.4	inch(es)	mm	0.0394
0.4536	lb	kg	2.2046
28.3495	oz	g	0.0353
1	ppm	$mg \cdot L^{-1}$	1
(°F – 32) ÷ 1.8	°F	°C	(°C × 1.8) + 32

systems have added to the challenges faced by managers. The FWC "paused" chemical weed management activities in early 2019 in response to public outcry and to provide an opportunity for stakeholder voices to be heard. A primary message that arose from listening sessions during the pause was that the public believes "chemical" (herbicide) usage in aquatic systems should be reduced drastically (FWC, 2019b). Although other aquatic weed management options such as mechanical harvesting do exist, most have greatly increased costs and reduced efficacy compared with chemical control tools. The need for "softer" products that can be used for aquatic weed control is urgent, and exploration is needed to identify ways for managers to maintain water resources effectively without the use of synthetic herbicides.

"Natural" herbicides are used extensively by home gardeners, organic farmers, and others who wish to reduce their use of synthetic herbicides. Products used for natural weed control include acids [i.e., acetic acid (vinegar) and citric acid], oils [clove (eugenol) (*Syzygium aromaticum*), pine (*Pinus* sp.), peppermint (*Mentha* ×*piperita*), and citronella (*Cymbopogon* sp.)], soaps, iron- or salt-based herbicides, corn (*Zea mays*) gluten, and combinations of these products (Smith-Fiola and Gill, 2017). Acids and oils may destroy cell membranes, which can lead to cell leakage, plant desiccation, and plant death (Baker, 1970; Webber et al., 2018). When used in upland (terrestrial) areas, these are nonselective contact foliar sprays that kill most broad-leaved weeds.

There are a number of commercially available natural herbicides that list acetic acid as the active ingredient. The most common acetic acid concentration in single-ingredient products is 20% [e.g., Maestro-Gro Organic Vinegar (Maestro-Gro, Justin, TX), Vinagreen Natural Non Selective Herbicide (Fleischmann's Vinegar Co., Cerritos, CA), Weed Pharm Fast Acting Weed and Grass Killer (Pharm Solutions, Port Townsend, WA)]. Products containing 20% acetic acid are typically labeled as ready to use (although some can be diluted to a concentration of 10%), and label instructions regarding spot treatments state that target weeds should be sprayed to wet. Products that include broadcast instructions indicate that 15 to 30 gal/acre of product should be used.

There are many other acetic acid products on the market, but most are not specifically labeled for weed control. For example, a note on the website for Bradfield Natural Horticultural Vinegar (20% acetic acid) states, "Although many folks, especially in the organic culture, have historically used strong vinegars to abate vegetation growth, be advised that Acetic Acids of 8% or less when characterized as an inert ingredient, in a mixture, are exempt from registration by the USEPA as a pesticide under USEPA 'Minimum Risk Pesticide' FIFRA 25B, List 4A. Thus this product (at 20% acidity) is not to be labeled, marketed or characterized in any way as having any herbicidal virtues" (Bradfield Industries, 2019; USEPA, 2004b). Horticultural vinegar, with an acetic acid concentration of 30%, is available, but is not listed specifically as a herbicide. Acetic acid is corrosive and can damage application equipment when used in highly concentrated form, but Evans et al. (2009) stated that lower concentrations can be used if application volume is increased to deliver the same total amount of acetic acid. For example, 15% acetic acid applied at 68 gal/acre will reportedly yield similar results as 30% acetic acid at 34 gal/acre (Quarles, 2010).

Although citric acid is often a component in "natural" herbicide mixes, products that rely only on citric acid or citrus oil are less common. Avenger Weed Killer Concentrate (Cutting Edge Formulations, Buford, GA) contains 70% d-limonene. The label indicates the product should be diluted at 1:6 (10% d-limonene for small annual weeds) to 1:3 (17.5% d-limonene) for hard-to-control weeds, then sprayed to wet. GreenMatch Burndown Herbicide (Cutting Edge Formulations) is 55% d-limonene, and label instructions specify diluting at a 1:6 ratio (8% d-limonene) for broadcast treatments or a 1:4 ratio (11% d-limonene) for spot treatments.

Despite widespread interest in reducing synthetic herbicide use, there is a dearth of information available in the scientific literature regarding the efficacy of natural products such as acetic acid and d-limonene as weed control agents, either alone or in combination. Domenghini (2020) reported that acetic acid applied at a concentration of 20% or 30% could be a viable alternative to glyphosate, but that multiple applications would be necessary for prolonged weed control. Webber et al. (2018) evaluated 5% and 20% acetic acid solutions alone or with sweet orange (*Citrus sinensis*) or canola (*Brassica napus*) oil; they found that weed control increased as acetic acid concentration increased and that there was little or no advantage to adding either type of oil to the acetic acid solutions. Evans and Bellinder (2009) stated that broadcast applications of 15%, 20%, and 30% acetic acid mixed with 1.7% or 3.4% clove oil could be useful for weed suppression in sweet corn, onion (*Allium cepa*), and potato (*Solanum tuberosum*) cultivation. Shrestha et al. (2012) reported that a single application of 20% d-limonene provided up to 95% weed control in organic almond (*Prunus dulcis*) orchards 1 week after treatment, but that efficacy was reduced to 53% control 5 weeks after treatment, necessitating repeat applications every 5 to 6 weeks.

Even less information is available in the scientific literature regarding the use of natural products for weed control in aquatic ecosystems. These products are not labeled for use as herbicides in aquatic areas, so they have not been subjected to the many tests required before USEPA approval, which include environmental fate and ecological toxicity assessments (Stubbs and Layne, 2020), and their effects on aquatic fauna have not been well-characterized. However, Saha et al. (2006) reported that acetic acid had a 96-h 50% lethal concentration value of 273 $mg \cdot L^{-1}$ on tilapia (*Oreochromis mossambicus*) (i.e., tilapia populations exposed to this concentration for 96 h would be expected to experience death of half the population), and that dissolved oxygen and plankton populations were reduced after exposure to 17 $mg \cdot L^{-1}$ acetic acid. The USEPA (2004a) stated that technical-grade and formulated d-limonene is "practically nontoxic or slightly toxic to birds, fish and invertebrates," but Kim et al. (2013) reported that metabolites of d-limonene may cause skin irritation in humans and other animals. As stated earlier, acids and oils can destroy cell membranes (Baker, 1970; Webber et al., 2018) and thus should be considered nonselective and likely to cause damage to off-target flora that comes into contact with these products. Anderson (2007) reported that rhizomes of the emergent aquatic weed smooth cordgrass (*Spartina alterniflora*) had 90% reductions in shoot number and plant height 9 months after exposure to acetic acid. Acetic acid reduces hydrilla regrowth from root crowns (Spencer and Ksander, 1995), and inhibits viability and sprouting of hydrilla and sago pondweed (*Stuckenia pectinata*) tubers (Spencer and Ksander, 1997, 1999). However, using acetic acid to suppress submersed weed growth would only be practical when employed in conjunction with dewatering, because achieving an adequate concentration of acetic acid in the entire water column is virtually impossible and could cause significant off-target damage to other flora and fauna in the system. For these reasons, the scope of this research is limited to testing the effects of contact products on above-water vegetation (i.e., floating and emergent plant material).

The primary goals of this project were to evaluate the effects of acetic acid and d-limonene (alone and in combination with each other) on two floating invasive target species and two emergent desirable nontarget species, and to compare the costs of using these products vs. a synthetic USEPA-approved aquatic herbicide. According to the FWC National Pollution Discharge Elimination System report for calendar year 2018, diquat dibromide, which is nonselective, was the most commonly used herbicide for floating weed management, with a total of 7871.77 gal of formulation (37.3% diquat dibromide) applied (Clark and Dew, 2019), so this product will serve as the synthetic "standard practice" treatment in these experiments.

Materials and methods

Efficacy studies. Target (weed) species were waterhyacinth and waterlettuce, whereas nontarget (desirable) species were pickerelweed (*Pontederia cordata*) and broadleaf sagittaria (*Sagittaria latifolia*). Plants were treated in pairs of one invasive floating species and one native emergent species. Run 1 focused on waterhyacinth and broadleaf sagittaria, whereas run 2 focused on waterlettuce and pickerelweed.

Target species were field-collected or pulled from cultures maintained at the University of Florida Fort Lauderdale Research and Education Center (FLREC) in Davie, FL, and were moved to 18-gal plastic tubs filled with well water. Run 1 tubs (waterhyacinth) were amended with 10 g each of crushed 15N–3.9P–10K controlled-release fertilizer formulated for 6-month release in Florida (Osmocote Plus; ICL Specialty Fertilizers, Dublin, OH) and 7N–0P–0K iron chelate micronutrient (Sprint 330; BASF Corp., Research Triangle Park, NC). Run 2 tubs (waterlettuce) were amended with 3.4 g of 24N–3.5P–13.3K water-soluble fertilizer (Miracle-Gro Water Soluble All Purpose Plant Food; Scotts Miracle-Gro Products, Marysville, OH) and 1.2 g of 7N–0P–0K iron chelate micronutrient. Each tub was initially "seeded" with 10 plants of a target species, which were grown out for 4 to 6 weeks to allow development of more than 80% surface coverage.

Nontarget species were purchased from an aquatic nursery (Aquatic Plants of Florida, Myakka City, FL) and transported to a greenhouse at FLREC, where individual plants were transplanted into 2-L plastic pots without holes that were filled with sand [grain diameter 0.25–0.5 mm (Multi-Purpose Sand; Sakrete, Charlotte, NC)] amended with 4 g of the same controlled-release fertilizer used in run 1 tubs. Plants were grown out on greenhouse benches and irrigated twice per day (10:00 AM and 4:00 PM) with the equivalent of 0.5 inch of water per irrigation before being used in experiments. New shoots were cut back during this culture period to ensure that each 2-L pot contained a single nontarget plant. When target plant coverage reached more than 80% of the surface of the water, one potted nontarget plant was introduced to each tub (water depth, ≈20 cm above the surface of the pots) and all plants were then subjected to treatment.

Treatments were applied as single spot "spray to wet" foliar applications to above-water foliage, and all treatments included 1% v/v of a nonionic surfactant (Induce; Helena Agri-Enterprises, Collierville, TN) to aid in penetration and emulsification. Nine single-product treatments (5%, 7.5%, 10%, 15%, and 20% acetic acid; and 10%, 15%, 20%, and 30% d-limonene), 30 combination treatments (all combinations of single acetic acid and d-limonene treatments; all concentrations of acetic acid plus 5% and 10% citric acid), three synthetic standard-practice treatments (0.22%, 0.45%, and 0.89% diquat dibromide), and an untreated control were evaluated, with four replicates of each treatment. Base materials were 30% acetic acid (Green Gobbler 30% Vinegar Home and Garden; EcoClean Solutions, Copiague, NY), 100% d-limonene (100% Pure Technical Grade D-Limonene, EcoClean Solutions), 37.3% diquat dibromide (Tribune Herbicide; Syngenta Crop Protection, Greensboro, NC), and 100% citric acid (Milliard Citric Acid; Milliard Brands, Lakewood, NJ). Treatments were applied to run 1 and run 2 plants on 12 Nov. 2019 and 15 Jan. 2020, respectively. Plants were monitored weekly for 8 weeks after treatment and then the project lead assigned a numerical value of 0 through 10 to describe visual quality (0 = dead; 5 = fair quality, acceptable, somewhat desirable form and color, little to no chlorosis or necrosis; 10 = excellent quality, perfect condition, healthy and robust, excellent color and form). Although some authors (e.g., Cutelle et al., 2013; Koschnick et al., 2005; Mudge et al., 2007) report visual injury or damage resulting from herbicide treatments, we recorded visual quality, which has also been used to describe plant response to differing culture conditions (e.g., Gettys and Moore, 2018, 2019), herbicides (e.g., Gettys and Haller, 2009, 2010, 2012; Smith et al., 2014), salt stress (e.g., Tootoonchi et al., 2020), and other experimental factors. After visual scoring, a destructive harvest was conducted to collect all live biomass of floating species and all live aboveground shoots of emergent species. Harvested materials were placed

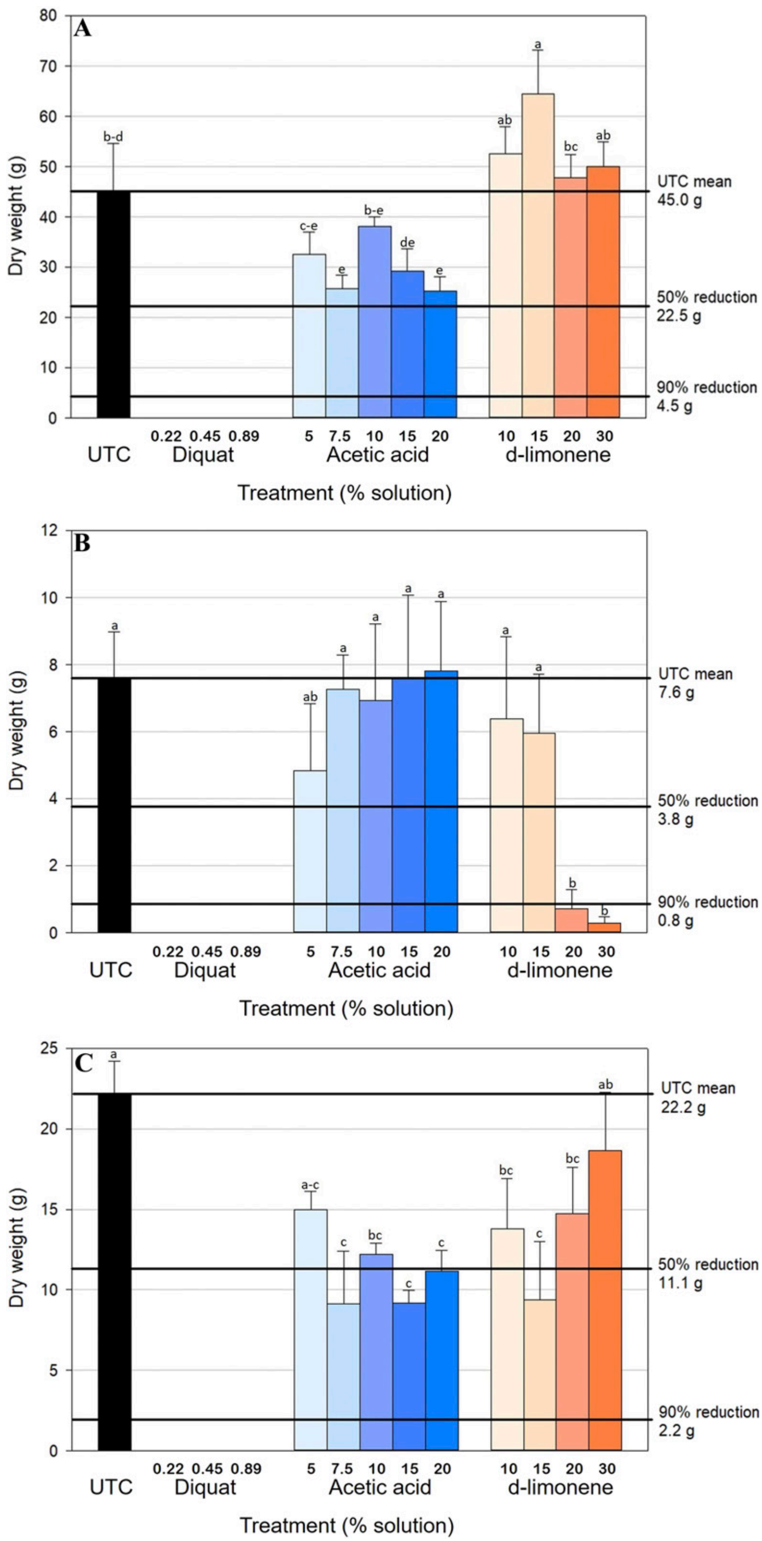

Fig. 1. Biomass of (A) waterhyacinth, (B) waterlettuce, and (C) pickerelweed 8 weeks after single-product treatment. Bars are the mean of four replicates and error bars represent 1 SD from the mean. Treatments coded with the same letter are not different at *P* = 0.05. The upper bold horizontal rule indicates the mean of untreated control (UTC) plants, whereas the central and lower bold horizontal rules indicate 50% and 90% reductions compared with UTC plants; 1 g = 0.0353 oz.

in paper bags and moved to a forced-air oven maintained at 65 °C for 2 weeks before being weighed. Visual evaluations and destructive harvests occurred on 7–9 Jan. 2020 (run 1) and 11–13 Mar. 2020 (run 2).

Visual data were arcsine-transformed before analysis to normalize distribution. Data within each species were evaluated using a generalized linear model (SAS version 9.4; SAS Institute, Cary, NC) to determine whether treatment means differed from those of untreated plants at *P* = 0.05. Treatment means of visual values and dried biomass were then compared with untreated controls. Haller and Gettys (2013) reported that an ideal herbicide treatment should cause a >90% reduction in these parameters in target weeds and a <50% reduction in nontarget native plants. Therefore, we used these values as benchmarks for efficacy on the floating weeds waterhyacinth and waterlettuce, and selectivity on the emergent native plants pickerelweed and broadleaf sagittaria.

Cost comparisons. A total of four diquat dibromide products, all formulated as 37.3% diquat dibromide, were used by the FWC in FY 2018–19 [67 gal Alligare Diquat (Alligare, Opelika, AL), 368 gal Diquat SPC 2L (Nufarm Americas, Burr Ridge, IL), 34 gal Reward (Syngenta Crop Protection), and 7407.77 gal Tribune (Syngenta Crop Protection)]. The majority (>94%) of diquat dibromide was applied as Tribune, which was purchased at the FWC's negotiated contract price of \$35.50/gal (vendor, Helena Agri-Enterprises; size, 2.5-gal jug) (Clark and Dew, 2019; Cleary and McNiel, 2019). As such, cost comparisons will assume a purchase price of \$35.50/gal for all diquat dibromide products.

Small volumes (e.g., 1 gal) of 30% acetic acid and technical grade d-limonene are \$24.99/gal and \$59.99/gal, respectively (Factory Direct Chemicals, 2019a, 2019b). When purchased in bulk, 30% acetic acid is \$8.00/gal (275-gal tote) and technical grade d-limonene is \$31.82/gal (4 × 55-gal drums) (Factory Direct Chemicals, 2019a, 2019b). If acetic acid and d-limonene were to be used for aquatic weed control in Florida, it is likely they would be purchased in bulk, so cost comparisons will assume a purchase price of \$8.00/gal for 30% acetic acid and \$31.82/gal for technical grade d-limonene.

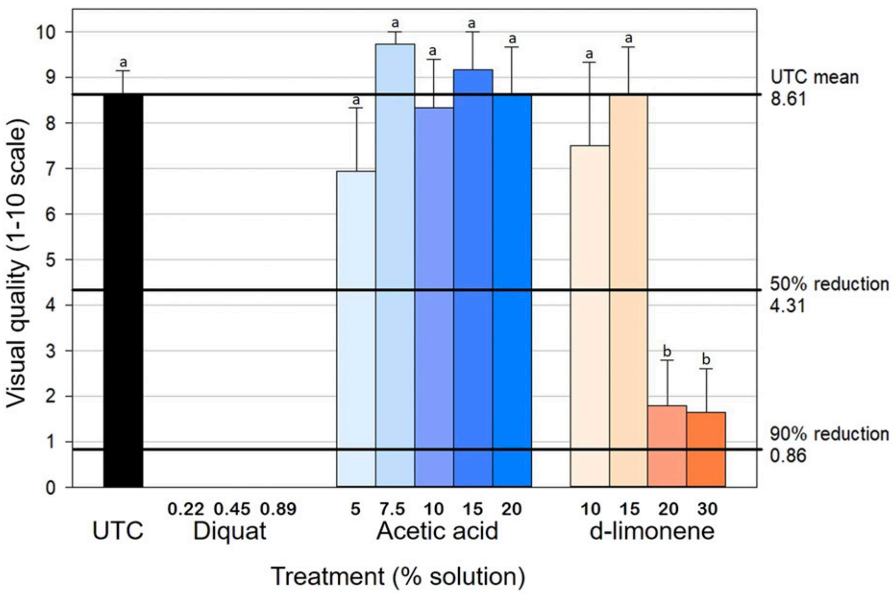

Fig. 2. Visual quality of waterlettuce 8 weeks after single-product treatment. A numerical scale of 0 through 10 is used to describe visual quality, where 0 is dead; 5 is fair quality, acceptable, somewhat desirable form and color, little to no chlorosis or necrosis; and 10 is excellent quality, perfect condition, healthy and robust, excellent color and form. Bars are the mean of four replicates and error bars represent 1 SD from the mean. Treatments coded with the same letter are not different at $P = 0.05$. The upper bold horizontal rule indicates the mean of untreated control (UTC) plants, whereas the central and lower bold horizontal rules indicate 50% and 90% reductions compared with UTC plants.

Results

Single products. The only single-product treatments that provided good control of both waterhyacinth and waterlettuce were diquat dibromide at 0.22%, 0.45%, and 0.89%, with all three concentrations completely eliminating both floating weeds. Unfortunately, all nontarget native plants were eliminated by these treatments as well. Although a goal of these experiments was to compare the efficacy of natural products to the synthetic herbicide diquat dibromide, it became clear that most natural treatments were much less effective than diquat dibromide, and comparisons between natural treatments and untreated controls would be more informative. Thus, diquat dibromide treatments were removed from data sets before further statistical analyses were conducted. Waterhyacinth biomass was affected by single-product natural treatments [$P < 0.01$ (Fig. 1A)], but no treatment reduced biomass by >50% or affected visual quality ($P = 0.66$). These treatments had an effect on waterlettuce biomass [$P = 0.03$ (Fig. 1B)] and visual quality [$P < 0.01$ (Fig. 2)], which were reduced by >90% and >75%, respectively, after treatment with 20% or 30% d-limonene, but no other single-product treatments were different from untreated control plants. Pickerelweed dry weight was reduced by most single-product treatments compared with untreated control plants [$P = 0.01$ (Fig. 1C)], but none reduced weight by >80%, and visual quality was unaffected ($P = 0.16$). Single natural products had no effect on broadleaf sagittaria dry weight ($P = 0.48$) or visual quality ($P = 0.68$).

Acetic acid and citric acid mixes. Combinations of 5% to 20% acetic acid and 5% or 10% citric acid did not reduce biomass or visual quality by at least 50% in any of the species evaluated in these experiments. Dry weight and visual quality of treated and untreated plants were not different ($P = 0.06$ to $P = 0.89$) in most cases. The sole exception was dry weight of waterlettuce ($P = 0.02$); treated plants were not different from untreated plants, but differences were detected among treatment combinations.

Acetic acid and d-limonene mixes. In contrast to single-product natural herbicide treatments and mixes of acetic and citric acids, some combinations of acetic acid and d-limonene had good efficacy on both floating weeds (waterhyacinth and waterlettuce biomass and visual quality, $P < 0.01$). The most promising combinations on waterhyacinth were 15% acetic acid plus 15%, 20%, or 30% d-limonene and 20% acetic acid with any concentration of d-limonene; these treatments reduced dry biomass by >80% (Fig. 3A) and visual quality by >60% (Fig. 4A). Most combinations of acetic acid and d-limonene had good efficacy on waterlettuce; dry weight (Fig. 3B) and visual quality ratings (Fig. 4B) were reduced compared with untreated control plants in all but a single treatment (5% acetic acid plus 10% d-limonene). Only 5 of the 20 treatment combinations failed to reduce biomass by >90% compared with untreated control plants, and two combinations failed to reduce visual quality by >50%. Pickerelweed biomass and visual quality were affected by combinations of acetic acid and d-limonene. Biomass was reduced in plants treated with any combination of acetic acid and d-limonene compared with untreated control plants [$P < 0.01$ (Fig. 3C)], and visual quality was reduced in 13 of the 20 treatments [$P = 0.03$ (Fig. 4C)]. Broadleaf sagittaria biomass was unaffected by mixes of acetic acid and d-limonene ($P = 0.34$). Visual quality of sagittaria was affected [$P = 0.04$ (Fig. 4D)], but treated plants were not different from untreated plants and all differences occurred among treatment combinations.

These results suggest that some combination treatments of acetic acid and d-limonene may be useful for managing populations of invasive waterhyacinth and waterlettuce while providing a level of selectivity with reduced damage to the native plants broadleaf sagittaria and pickerelweed.

Cost analysis of acetic acid and d-limonene mixes for floating weed management. The synthetic standard-practice treatments in these experiments used diquat dibromide. These experiments evaluated foliar treatments only and we envision that field treatments for floating weed management would be applied as spot treatments as opposed to broadcast treatments. Diquat dibromide can be applied at a concentration of up to 2% formulated product (equivalent to 0.89% diquat dibromide) for floating

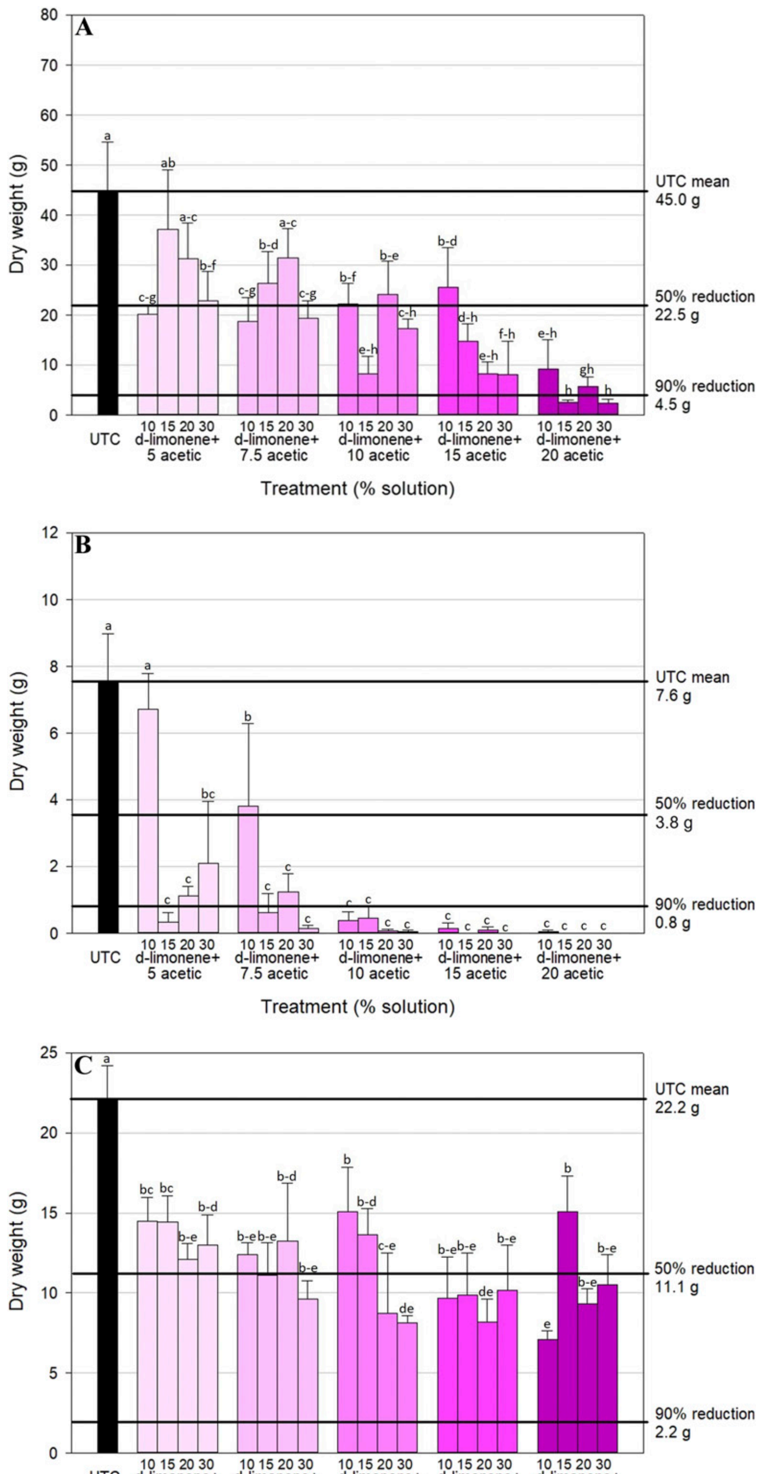

Fig. 3. Biomass of (A) waterhyacinth, (B) waterlettuce, and (C) pickerelweed 8 weeks after treatment with combinations of acetic acid and d-limonene. Bars are the mean of four replicates and error bars are 1 SD from the mean. Treatments coded with the same letter are not different at P = 0.05. The upper bold horizontal rule is the mean of untreated control (UTC) plants, whereas the central and lower bold horizontal rules indicate 50% and 90% reductions compared with UTC plants; 1 g = 0.0353 oz.

and marginal weeds, whereas spot treatments should use a concentration of 0.5% formulated product (equivalent to 0.22% diquat dibromide) (Syngenta Crop Protection, 2011). We evaluated three concentrations (0.22%, 0.45%, and 0.89%) of diquat dibromide, but the lowest concentration completely eliminated all plant material, so calculations are based on spot treatments using a 0.22% solution. As mentioned in the Materials and Methods, the FWC applied a total of 7871.77 gal of 37.3% diquat dibromide to floating plants in 2018 (Clark and Dew, 2019). Assuming all diquat field treatments were mixed to a concentration of 0.22% diquat dibromide, a total of 1,574,354 gal of ready-to-use (RTU) mix was made from the 7871.77 gal of concentrate purchased. The FWC's contract price for 37.3% diquat dibromide was \$35.50/gal, resulting in a total cost of \$283,383.72; after dilution to a 0.22% concentration, the final cost is \$0.1775/gal RTU, or ≈\$0.18/gal RTU.

As mentioned in the Materials and Methods, these calculations are based on purchase prices of \$8.00/gal for 30% acetic acid and \$31.82/gal for technical grade d-limonene. Neither acetic acid nor d-limonene resulted in acceptable control of waterhyacinth when applied alone, but some combinations of the two provided good control (>80% reduction in biomass) of waterhyacinth. These were 15% acetic acid plus 15%, 20%, or 30% d-limonene and 20% acetic acid with any concentration of d-limonene. The material costs to make RTU 15% or 20% acetic acid are \$1.20/gal or \$1.60/gal, respectively. The material costs for RTU d-limonene are \$3.18/gal (10%), \$4.77/gal (15%), \$6.36/gal (20%), and \$9.55/gal (30%). Thus, the least expensive efficacious natural treatment (20% acetic acid + 10% d-limonene; \$1.60 + \$3.18) for waterhyacinth is \$4.78/gal RTU, or nearly 26× more expensive than the synthetic standard (0.22% diquat dibromide) treatment (\$0.18/gal RTU). Assuming other application parameters (e.g., surfactant cost, labor) remain unchanged between the treatment types, and remembering that a total of 1,574,354 gal of RTU mix was used in 2018, switching completely from synthetic to natural products for waterhyacinth management would increase single-year product costs from \$283,383.72 (0.22% diquat dibromide) to \$7,525,412.12 (20% acetic acid + 10% d-limonene).

In contrast to waterhyacinth, there were many treatments that reduced waterlettuce biomass by at least 90% compared with untreated control

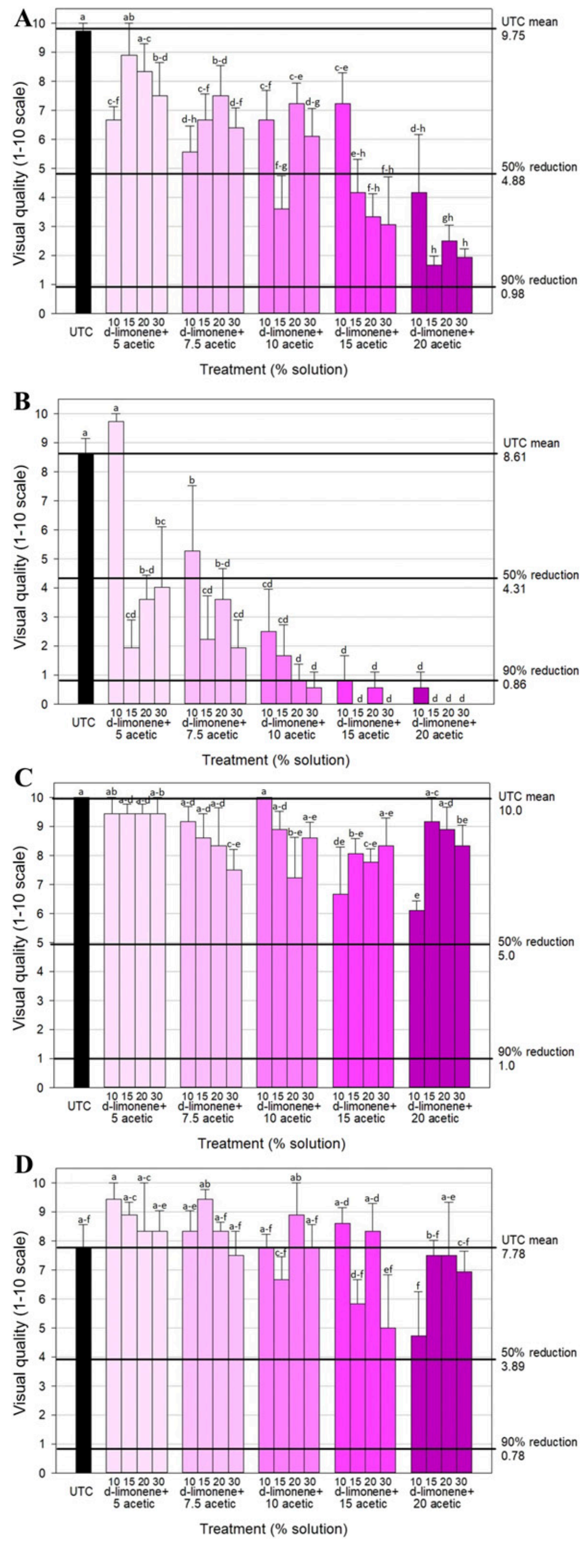

Fig. 4. Visual quality of (A) waterhyacinth, (B) waterlettuce, (C) pickerelweed, and (D) broadleaf sagittaria 8 weeks after treatment with combinations of acetic acid and d-limonene. A numerical scale of 0 through 10 is used to describe visual quality, where 0 is dead; 5 is fair quality, acceptable, somewhat desirable form and color, little to no chlorosis or necrosis; and 10 is excellent quality, perfect condition, healthy and robust, excellent color and form. Bars are the mean of four replicates and error bars represent 1 SD from the mean. Treatments coded with the same letter are not different at *P* = 0.05. The upper bold horizontal rule indicates the mean of untreated control (UTC) plants, whereas the central and lower bold horizontal rules indicate 50% and 90% reductions compared with UTC plants.

plants. Most efficacious treatments used combinations of acetic acid and d-limonene, but applications of 20% and 30% d-limonene alone also reduced waterlettuce biomass by 90%. The least expensive efficacious natural treatment (10% acetic acid + 10% d-limonene; \$0.80 + \$3.18) for waterlettuce is \$3.98/gal RTU, or ≈22× more expensive than the synthetic standard treatment (\$0.18/gal RTU). As with the caveats just described for waterhyacinth, switching completely from synthetic to natural products for waterlettuce management would increase single-year product costs from \$283,383.72 (0.22% diquat dibromide) to \$6,265,928.92 (10% acetic acid + 10% d-limonene).

The figures calculated here do not account for the likelihood that applications using natural products would take longer, resulting from the need to transport very large volumes of base material. For example, consider a spray boat with a 100-gal tank that uses diluent water drawn from the system being treated. Filling the tank once for a synthetic treatment would require the transport of 0.5 gal of 37.3% diquat dibromide, but filling the tank once with the least expensive efficacious natural treatment for waterhyacinth management would require the transport of 67 gal of 30% acetic acid and 10 gal of technical grade d-limonene, or ≈630 lb of materials (without factoring in the weight of the containers used to transport the materials). Rather than transporting base materials and adding them to the tank at the treatment site, applicators would likely add the natural treatment components to the tank at the ramp and would thus have to return to shore for reloading after applying 100 gal of RTU natural mix. In contrast, an applicator with a single 2.5-gal jug of 37.3% diquat dibromide would have enough base material to make 500 gal of RTU synthetic mix without returning to the ramp. As a result, replacing synthetic herbicides with natural products would not only greatly increase material costs but would also decrease productivity (as measured in acres treated per day).

Discussion

The natural products evaluated in these studies may have some utility for managing floating weeds such as waterhyacinth and waterlettuce selectively without causing unacceptable levels of damage to desirable native plants such as broadleaf sagittaria and pickerelweed. Acetic acid alone did not cause adequate (>50% reduction in biomass and visual quality) damage to either weed species, whereas d-limonene alone caused >90% reduction in biomass of waterlettuce only when applied at concentrations ≥20%. The addition of 5% or 10% citric acid to acetic acid had no effect on acetic acid efficacy, so further investigations with citric acid are not recommended. Some combinations of acetic acid and d-limonene provided acceptable control of both floating weeds, with biomass reductions of ≥80%. Waterlettuce was more sensitive to treatments than was waterhyacinth, which is likely a result of the structure of the plants. Both floating weeds have a rosette form, with leaves attaching to the base of the plant. However, the sessile leaves of waterlettuce create a "bowl" that can capture and hold liquids, which can result in longer exposure times, whereas the petiolate leaves of waterhyacinth facilitate drainage of liquids through the plant and into the water column.

Regardless of the target species and selected treatment chosen, replacement of current industry-standard aquatic herbicides with these natural products would result in profound increases in management costs. As mentioned, the material-only cost of treating floating weeds in Florida with 0.22% diquat dibromide in 2018 was $283,383.72. Treating a similar area with natural products would increase material-only costs to $7,525,412.12 for waterhyacinth and $6,265,928.92 for waterlettuce. Therefore, these natural products may have utility in select areas where the use of synthetic herbicides is discouraged, but broad-scale deployment of this management strategy would likely be prohibitively expensive.

Literature cited

Anderson, L. 2007. Potential for sediment-applied acetic acid for control of invasive *Spartina alterniflora*. J. Aquat. Plant Mgt. 45:100–105.

Baker, J.M. 1970. The effects of oils on plants. Environ. Pollut. 1(1):27–44, doi: 10.1016/0013-9327(70)90004-2.

Bradfield Industries. 2019. Horticultural vinegar supplemental info: 20% Acetic acid. 15 May 2019. <https://www.bradfieldind.com/vinegar.htm>.

Clark, R. and A. Dew. 2019. Florida Fish and Wildlife Conservation Commission annual report of pollutant discharges to the surface waters of the state from the application of pesticides 1 Jan. 2018 through 31 Dec. 2018. 20 Mar. 2019. <https://myfwc.com/media/19111/npdes-2018.pdf>.

Cleary, R. and D. McNiel. 2019. The herbicide bank handbook 2019–20. 15 Sept. 2019. <https://bugwoodcloud.org/CDN/floridainvasives/Herbicide_Bank_Handbook2020.pdf>.

Cutelle, M.A., G.R. Armel, J.T. Brosnan, D.A. Kopsell, W.E. Klingeman, P.C. Flanagan, G.K. Breeden, J.J. Vargas, R. Koepke-Hill, and M.A. Halcomb. 2013. Evaluation of container ornamental species tolerance to three p-hydroxyphenylpyruvate dioxygenase-inhibiting herbicides. HortTechnology 23:319–324, doi: 10.21273/HORTTECH.23.3.319.

Domenghini, J.C. 2020. Comparison of acetic acid to glyphosate for weed suppression in the garden. HortTechnology 30:82–87, doi: 10.21273/HORTTECH04453-19.

Evans, G.J. and R.R. Bellinder. 2009. The potential use of vinegar and a clove oil herbicide for weed control in sweet corn, potato, and onion. Weed Technol. 23(1):120–128, doi: 10.1614/WT-08-002.1.

Evans, G.J., R.R. Bellinder, and M.C. Goffinet. 2009. Herbicidal effects of vinegar and a clove oil product on redroot pigweed (*Amaranthus retroflexus*) and velvetleaf (*Abutilon theophrasti*). Weed Technol. 23(2):292–299, doi: 10.1614/WT-08-158.1.

Factory Direct Chemicals. 2019a. Citrus cleaners (d-limonene). 20 Mar. 2019. <https://www.factorydirectchemicals.com/collections/d-limonene>.

Factory Direct Chemicals. 2019b. Concentrated vinegar. 20 Mar. 2019. <https://www.factorydirectchemicals.com/collections/vinegars>.

Florida Fish and Wildlife Conservation Commission. 2018. Florida Fish and Wildlife Conservation Commission Invasive Plant Management Section annual report of activities conducted under the Cooperative Aquatic Plant Control Program in Florida public waters for fiscal year 2017–2018. 10 June 2020. <https://myfwc.com/media/19112/annualreport17-18.pdf>.

Florida Fish and Wildlife Conservation Commission. 2019a. Florida Fish and Wildlife Conservation Commission Invasive Plant Management Section annual report of activities conducted under the Cooperative Aquatic Plant Control Program in Florida public waters for fiscal year 2018–2019. 10 June 2020. <https://myfwc.com/media/22606/annualreport1819_ipm.pdf>.

Florida Fish and Wildlife Conservation Commission. 2019b. FWC implementing enhancements to aquatic plant management program. 10 Jan. 2021. <https://myfwc.com/news/all-news/aquatic-enhancements/>.

Gettys, L.A. 2019. Breaking bad: Native aquatic plants gone rogue and the invasive species that inspire them. HortTechnology 29:559–566, doi: 10.21273/HORTTECH04333-19.

Gettys, L.A. 2020a. Waterhyacinth, p. 71–74. In: L.A. Gettys, W.T. Haller, and D.G. Petty (eds.). Biology and control of aquatic plants: A best management practices handbook. 4th ed. Aquatic Ecosystem Restoration Foundation, Marietta, GA.

Gettys, L.A. 2020b. Waterlettuce, p. 75–78. In: L.A. Gettys, W.T. Haller, and D.G. Petty (eds.). Biology and control of aquatic plants: A best management practices handbook. 4th ed. Aquatic Ecosystem Restoration Foundation, Marietta, GA.

Gettys, L.A. and W.T. Haller. 2009. Tolerance of selected bedding plants to four herbicides in irrigation water. HortTechnology 19:546–552, doi: 10.21273/HORTSCI.19.3.546.

Gettys, L.A. and W.T. Haller. 2010. Response of selected foliage plants to four herbicides in irrigation water. HortTechnology 20:921–928, doi: 10.21273/HORTTECH.20.5.921.

Gettys, L.A. and W.T. Haller. 2012. Effect of herbicide-treated irrigation water on four vegetables. Weed Technol. 26(2):272–278, doi: 10.1614/WT-D-11-00120.1.

Gettys, L.A. and K.A. Moore. 2018. Greenhouse culture and production of four ornamental native wetland plants. HortTechnology 28:332–336, doi: 10.21273/HORTTECH03818-17.

Gettys, L.A. and K.A. Moore. 2019. Greenhouse production of native aquatic plants. HortTechnology 29:41–45, doi: 10.21273/HORTTECH04212-18.

Haller, W.T. and L.A. Gettys. 2013. Pond, selectivity and irrigation studies on potential new aquatic herbicides. Aquatics 35(2):6–10.

Kim, Y.W., M.J. Kim, B.Y. Chung, D.Y. Bang, S.K. Lim, S.M. Choi, D.S. Lim, M.C. Cho, K. Yoon, H.S. Kim, K.B. Kim, Y.S. Kim, S.J. Kwack, and B.-M. Lee. 2013. Safety evaluation and risk assessment of d-limonene. J. Toxicol. Environ.

Health B Crit. Rev. 16(1):17–38, doi: 10.1080/10937404.2013.769418.

Koschnick, T.J., W.T. Haller, and G.E. MacDonald. 2005. Turf and ornamental plant tolerances to endothall in irrigation water: I. Ornamental species. HortTechnology 15:318–323, doi: 10.21273/HORTTECH.15.2.0318.

Mudge, C.R., T.J. Koschnick, and W.T. Haller. 2007. Ornamental plant susceptibility to diquat in overhead irrigation water. J. Aquat. Plant Mgt. 45:40–43.

Quarles, W. 2010. Alternative herbicides in turfgrass and organic agriculture. IPM Pract. 32(5/6):1–8. <https://www.birc.org/MayJune2010.pdf>.

Saha, N.C., F. Bhunia, and A. Kaviraj. 2006. Comparative toxicity of three organic acids to freshwater organisms and their impact on aquatic ecosystems. Hum. Ecol. Risk Assess. 12(1):192–202, doi: 10.1080/10807030500430625.

Shrestha, A., M. Moretti, and N. Mourad. 2012. Evaluation of thermal implements and organic herbicides for weed control in a nonbearing almond (*Prunus dulcis*) orchard. Weed Technol. 26(1):110–116, doi: 10.1614/WT-D-11-00083.1.

Smith, H.C., J.A. Ferrell, and T.J. Koschnick. 2014. Flurprimidol performance on ornamental species in relation to trimming time and method of application. HortScience 49:1305–1308, doi: 10.21273/HORTSCI.49.10.1305.

Smith-Fiola, D. and S. Gill. 2017. Vinegar: An alternative to glyphosate? 10 June 2019. <https://extension.umd.edu//sites/extension.umd.edu/files/_docs/programs/ipmnet/Vinegar-AnAlternativeToGlyphosate-UMD-Smith-Fiola-and-Gill.pdf>.

Spencer, D.F. and G.G. Ksander. 1995. Influence of acetic acid on regrowth of dioecious hydrilla from root crowns. J. Aquat. Plant Mgt. 33:61–63.

Spencer, D.F. and G.G. Ksander. 1997. Dilute acetic acid exposure enhances electrolyte leakage by *Hydrilla verticillata* and *Potamogeton pectinatus* tubers. J. Aquat. Plant Mgt. 35:25–30.

Spencer, D.F. and G.G. Ksander. 1999. Influence of dilute acetic acid treatments on survival of monoecious hydrilla tubers in the Oregon House Canal, California. J. Aquat. Plant Mgt. 37:67–71.

Stubbs, D. and C.R. Layne. 2020. Requirements for registration of aquatic herbicides, p. 155–162. In: L.A. Gettys, W.T. Haller, and D.G. Petty (eds.). Biology and control of aquatic plants: A best management practices handbook. 4th ed. Aquatic Ecosystem Restoration Foundation, Marietta, GA.

Syngenta Crop Protection. 2011. Tribune herbicide label. 3 Jan. 2021. <https://www.greenbook.net/syngenta-llc/tribune>.

Tootoonchi, M., L.A. Gettys, K.L. Thayer, I.J. Markovich, J.W. Sigmon, and S. Sadeghibaniani. 2020. Ecotypes of aquatic plant *Vallisneria americana* tolerate different salinity concentrations. Diversity 12(2):65, doi: 103390/d12020065.

U.S. Environmental Protection Agency. 1996. Summary of the Federal Insecticide, Fungicide, and Rodenticide Act. 4 Jan. 2021. <https://www.epa.gov/laws-regulations/summary-federal-insecticide-fungicide-and-rodenticide-act>.

U.S. Environmental Protection Agency. 2004a. Exposure and risk assessment on lower risk pesticide chemicals: d-Limonene. 8 Jan. 2021. <https://archive.epa.gov/pesticides/reregistration/web/pdf/limonene_tred.pdf>.

U.S. Environmental Protection Agency. 2004b. List of inert pesticide ingredients, list 4A—minimal risk inert ingredients—by chemical name. 5 Jan. 2021. <https://www.epa.gov/sites/production/files/2015-10/documents/inerts_list4aname.pdf>.

Webber, C.L., III, P.M. White, Jr., J.W. Shrefler, and D.J. Spaunhorst. 2018. Impact of acetic acid concentration, application volume, and adjuvants on weed control efficacy. J. Agr. Sci. 10(8):1–6, doi: 10.5539/jas.v10n8p1.