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Effects of select herbicides for management of American lotus, white waterlily, and watershield

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ABSTRACT

Literature describing effective control measures for the floating-leaved plants American lotus (*Nelumbo lutea* Willd.), white waterlily (*Nymphaea odorata* Aiton), and watershield (*Brasenia schreberi* J.F. Gmel.) is minimal as these are usually considered as desirable species. However, floating-leaved plants can cause ecological, economic, and social problems when undergoing demographic expansions, usually following alterations of natural hydrologic cycles. Therefore, a mesocosm trial was conducted to determine the potential of foliar applications of seven aquatic herbicides to reduce abundance of the three target species at maximum and half-maximum label rates. Three of the herbicides (glyphosate, imazamox, and florpyrauxifen-benzyl) provided short- and long-term suppression (> 75% reduction) of white waterlily and watershield leaf density or biomass. As a follow-up trial, field work was conducted using glyphosate, imazamox, and florpyrauxifen-benzyl to determine plant response to these herbicides in a natural setting. All herbicides resulted in long-term (52 wk after treatment) leaf density reduction of white waterlily (64 to 100% reduction) and watershield (46 to 75% reduction; except 2.83 kg ae ha^{-1} glyphosate) in field sites while the abundance of American lotus increased. Reduction of white waterlily and watershield may have reduced competition thereby favoring higher abundance of lotus. Regardless, long-term (52-wk) reduction of white waterlily and watershield suggest the potential for these herbicides as operational management tools for nuisance populations of these species. Future work should evaluate chemical techniques for control of American lotus, where both timing of leaf emergence and potential interactions with other plant species must be considered in the design of those studies.

Key words: aquatic plant control, *Brasenia schreberi*, native nuisance species, *Nelumbo lutea*, *Nymphaea odorata*

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INTRODUCTION

Native aquatic plants are important components of lakes, rivers, and other aquatic ecosystems. Under natural hydrologic cycles, these plants typically exist in diverse mixtures of floating-leafed, submersed, and free-floating species that provide habitat for fish and other aquatic organisms (Gettys et al. 2021). These plants also provide invaluable ecosystem services such as oxygenation of the water column and mitigation of nutrient and sediment runoff, while adding aesthetic value to aquatic habitats and therefore are not often targeted for management.

Alteration of natural hydrologic cycles, however, through activities such as channelization or impoundment, can reduce aquatic habitat diversity and lead to dominance by one or a few plant species, even among native plants that typically are not considered a nuisance (Hall and Penfound 1944). Such changes in aquatic plant community composition can lead to ecological, economic, and social problems (Gettys et al. 2021). For example, the floating foliage of these plants can shade out submersed plants and algae and lead to decreased dissolved oxygen levels (Lawrence and Weldon 1965, Turner et al. 2010), which in turn can negatively affect aquatic fauna (Killgore and Hoover 2001). They can also restrict human recreational activities such as fishing and boating on waterbodies (Gettys et al. 2021). Additionally, allelopathy has been documented in some floating-leaved plants such as white waterlily (*Nymphaea odorata* Aiton) and watershield (*Brasenia schreberi* J.F. Gmel.), suggesting that these species could chemically suppress neighboring plant growth, in addition to the competitive effects mentioned above (Elakovich 1989). Successful control of troublesome populations of floating-leaved plants, even those representing native species, is a priority for wetland managers, especially in wetlands of high conservation and economic value.

Loakfoma Lake is a 184-ha (455-ac) manmade impoundment located in northeast Mississippi on the Sam D. Hamilton Noxubee National Wildlife Refuge (NNWR) that has become infested with white waterlily, watershield, and American lotus (*Nelumbo lutea* Willd.). Loakfoma Lake can be classified as a multipurpose waterbody as it provides recreational fishing and boating opportunities, serves as a migratory waterfowl refuge, is home to species of concern such as the American alligator (*Alligator mississippiensis*), and helps alleviate flooding along the Noxubee River (SDHNNWR 2014). In October 2018, the three plant species listed above covered approximately 87% of the surface of Loakfoma Lake (G. N. Ervin, unpubl. data), reducing its ability to perform these functions. Therefore, NNWR resource managers desired to reduce these nuisance plant species to restore multiple-use functionality to Loakfoma Lake.

Unfortunately, there is little literature describing effective control measures for American lotus, white waterlily, and watershield. Of the four management strategies used to control aquatic plants (mechanical, physical, biological, and chemical), mechanical (damage to the plant) and physical control (alteration of the physical environment) methodologies were unlikely to be effective on these species. Mechanical and physical control can be cost and/or labor prohibitive (Madsen et al. 2017) and may produce colonization of new habitat by dispersing propagules (mechanical) or stimulating growth of rhizomes (Haug et al. 2019). To date, there is no documented biological control agent for these species, although grass carp will occasionally feed on them (Theriot and Sanders 1975, Santha et al. 1994). Chemical control is the most common management strategy for floating-leaved plants due to its cost effectiveness and ease of implementation; however, there are minimal data regarding reduction of American lotus, white waterlily, or watershield by herbicide treatments (Riemer and Welker 1974, Robles et al. 2011, Turnage et al. 2015). Herbicides with different modes of action (MOA) may provide differing levels of control on nuisance vegetation. However, because there are multiple herbicide MOAs with documented control of plants in aquatic environments, resource managers can often times rotate herbicide use to achieve control of target vegetation (Shaner 2014, Gettys et al. 2021). Modes of action evaluated in the present work included aceto-lactate synthase (ALS) inhibition (imazapyr and imazamox), auxin mimicry (2,4-D, triclopyr, and florpyrauxifen-benzyl), protoporphyrinogen oxidase (PPO) inhibition (flumioxazin), and 5- enolpyruvylshikimate-3-phosphate (EPSP) inhibition (glyphosate).

The herbicides imazapyr and imazamox inhibit ALS, which produces the branched-chain amino acids valine, leucine, and isoleucine that are critical for plant growth (Shaner 2014). These herbicides have been used to control a broad suite of aquatic plants (Hanlon and Langeland 2000, Koschnick et al. 2007, Spencer et al. 2009, Emerine et al. 2010, Chaudhari et al. 2012, Wersal et al. 2014), but few data exist concerning these herbicides and the target species listed above.

The herbicides 2,4-D, triclopyr, and florpyrauxifen-benzyl mimic the plant enzyme auxin, which is used to regulate plant growth; however, target plant species are physiologically unable to regulate these chemicals, resulting in unregulated plant growth (i.e., epinasty) that eventually leads to plant death (Shaner 2014, Gettys et al. 2021). Triclopyr has activity on American lotus (G. Turnage, unpubl. data), but to our knowledge, information regarding control of white waterlily and watershield by triclopyr is lacking. Control of these three species by 2,4-D has been documented and recommended over the years (Durden and Blackburn 1972), but limited data exist describing selective control of these species with this herbicide (Couch and Nelson 1982, Klussman et al. 1983). A new aryl-picolinate herbicide (florpyrauxifen-benzyl) is also in the auxinic class of herbicides and has shown activity on multiple aquatic species, including some lily species (Richardson et al. 2016). The present work with this herbicide thus has the potential to document a novel tool for control of the target species.

Glyphosate is a broad-spectrum systemic herbicide that inhibits EPSP (). Inhibition of the EPSP enzyme blocks the production of the aromatic amino acids phenylalanine, tyrosine, and tryptophan that are needed for plant growth (Shaner 2014, Gettys et al. 2021). Glyphosate has seen wide use in aquatic environments and has documented activity on white waterlily and other species (Riemer and Welker 1974, Thayer and Haller 1985, Wersal et al. 2014). However, as described above, few data exist regarding the activity of glyphosate on American lotus and watershield.

Lastly, the contact herbicide flumioxazin inhibits the plant enzyme PPO (Shaner 2014, Gettys et al. 2021). PPO-inhibiting herbicides reduce pigment synthesis and membrane integrity, which leads to reduced photosynthesis and cellular destruction (Shaner 2014). Flumioxazin has been documented to control multiple species of aquatic vegetation (Richardson et al. 2008, Mudge et al. 2010, Mudge 2013, Poovey et al. 2013); however, few data exist evaluating its activity on the target species listed above.

In addition to differing response to various herbicide MOAs, application technique can affect the level of plant reduction attained when attempting to control nuisance vegetation (Langeland et al. 1995). In aquatic systems, herbicides are commonly applied to target vegetation from a boat. However, surface vessels are commonly limited in the amount of nuisance vegetation that can treated per day (approximately 8 ha d^{-1}) due to constraints on technology and manpower (D. Hill, pers. comm.). Consequently, herbicide applications from a boat may be cost ineffective on large infestations; in these situations, herbicide applications from aircraft may be more economical. In an effort to maximize the amount of target vegetation treated per flight (which is influenced by cargo weight limitations), aerial herbicide applicators typically reduce the amount of herbicide diluent applied per unit area (i.e., hectare) while increasing the herbicide concentration of the diluent, compared to surface-based herbicide applications. For example, some herbicide labels recommend that boat-based foliar herbicide applications utilize greater diluent volumes ($>$ 935 L ha^{-1}) while restricting aerial applications to lower volumes ($<$ 140 to 280 L ha^{-1}; Anonymous 2022a, b, c, d; Gettys et al. 2021). This application technique results in fewer diluent droplets per hectare but increases the herbicide concentration within each droplet such that the same amount of herbicide is being applied per hectare (Sperry et al. 2021a, b). As a result of this alternate application strategy, a drawback of aerial herbicide applications is that target vegetation may not receive uniform coverage compared to higher volume surface-based applications, thus reduceing density of droplets applied. This could potentially lead to reduced efficacy. To assess herbicide effectiveness against the target plant species, which are most often treated by aerial application, research methodology was modeled to assess the effectiveness of a real-world application scenario.

The purpose of this research was to 1) screen various herbicide MOAs in a mesocosm setting at high and low concentrations for the control of American lotus, white waterlily, and watershield using a common aerial application diluent rate and 2) validate successful treatments from the mesocosm scale on field populations at Loakfoma Lake.

MATERIALS AND METHODS

Mesocosm trial

A mesocosm trial was conducted in June 2018 at the Aquatic Plant Research Facility at Mississippi State University's R.R. Foil Plant Research Center in Starkville, MS. Target species (American lotus, white waterlily, and watershield) were collected from the Loakfoma Lake and planted in 3.8-L pots, which were placed in 1,135-L outdoor mesocosms filled with pond water (pH 7.8 to 8.2). Pots were filled with sand amended with 2 g L^{-1} fertilizer[1] (NPK = 15–9–12) to stimulate plant growth. American lotus seeds were scarified and placed in water for 5 d to stimulate sprouting, then two sprouted seedlings were placed in a pot. Watershield was planted by placing two 12.7-cm rhizome segments in each pot. Waterlily was planted by placing one 12.7-cm rhizome segment in each pot. Mesocosms were filled to a volume of 757 L (0.4 m depth). Six pots of each species were placed in each mesocosm, and plants were given 2 mo to establish prior to herbicide application. In total there were 15 treatments: an untreated reference and 14 herbicide treatments (high and low rates per herbicide). Additionally, three more mesocosms were established to harvest pretreatment specimens, to gather baseline data for a total of 48 mesocosms (three pretreatment and 45 treatment), containing 864 pots (288 per plant species).

Six systemic herbicides were evaluated: 2,4-D[2] (2.12 and 4.24 kg ae ha^{-1}), glyphosate[3] (2.83 and 5.67 kg ae ha^{-1}), triclopyr[4] (3.36 and 6.71 kg ae ha^{-1}), imazamox[5] (0.56 and 1.11 kg ai ha^{-1}), imazapyr[6] (0.42 and 0.84 kg ai ha^{-1}), and florpyrauxifen-benzyl[7] (0.02 and 0.05 kg ai ha^{-1}). The contact herbicide flumioxazin[8] (0.21 and 0.42 kg ai ha^{-1}) was also evaluated. Herbicides were applied to mature foliage of all three target species using a CO_2-pressurized backpack sprayer at a rate of 280 L ha^{-1} using a handgun with a Tee Jet 8002 fan nozzle[9] (application height was approximately 1 m). Each herbicide treatment was replicated three times and randomly assigned to mesocosms; each herbicide treatment included a 1% v/v nonionic surfactant[10]. Vegetation covered 70 to 80% of the water surface at the time of application, and water was not drained from tanks after application. This trial was not repeated due to the difficulty of replicating water quality in such a large number of mesocosms among trial runs.

Prior to herbicide application, leaf density (n m^{-2}) across each pretreatment mesocosm was recorded for each species; then plants were harvested, separated into aboveground (AG) and belowground (BG) biomass, placed in labeled paper bags, and dried in a forced air oven at 70 C for 5 d. After drying, biomass was measured and recorded for each sample, then plant metrics (leaf density and biomass) were converted to a per-square-meter basis (plant metric per m^2 = plant metric pot^{-1} × 54.95). After pretreatment harvest, herbicide treatments were randomly assigned to the remaining mesocosms such that each treatment was replicated three times. Symptomology and mortality of plants was recorded weekly for 8 wk and again at final harvest (52 wk). At 8 and 52 wk after treatment (WAT), leaf number was recorded and half of the pots for each species in each mesocosm were harvested and processed in the same manner as pretreatment specimens.

Field trial

The three most effective herbicide treatments from the mesocosm trial (glyphosate, imazamox, and florpyrauxifen-benzyl) were tested at the same herbicide, diluent, and surfactant rates in field plots in Loakfoma Lake in May 2019. In total, the field study comprised seven treatments (three herbicides × two rates, plus untreated reference plots), with five replicates each, for a total of 35 field plots. Each plot occupied 25 m^2. Plots were separated by a minimum of 20 m to reduce cross-contamination due to herbicide drift, and herbicide treatments were randomly assigned to treatment plots. Plots were selected that had similar coverage and density of all three species. The five reference plots were separated from the nearest treatment plot by approximately 40 m; additionally, reference plots were upstream of treatment plots based on the dominant direction of water flow through the reservoir. Mature foliage of three target species was present in each plot; however, density of American lotus leaves peaked after herbicide application (June).

Prior to herbicide applications, leaf and inflorescence density per species were recorded for each plot by randomly placing a 1-m^2 polyvinyl chloride frame in each plot five times and recording each metric within the frame. After pretreatment data collection, herbicides were applied from a boat using a backpack sprayer. After herbicide application, data was collected again 12 and 52 WAT.

Statistical analyses

Data were assessed for normality using a Shapiro-Wilks test; all data were nonparametric ($P < 0.05$ for all). Therefore, a Kruskal-Wallis one-way analysis of variance was used to assess each response variable. If differences were detected, a Fisher's Protected LSD test was used to further separate treatment means (R Core Team 2023). All statistical tests were conducted at the alpha = 0.05 significance level.

RESULTS AND DISCUSSION

Mesocosm trial

American lotus leaf density was reduced > 78% ($P <$ 0.0001) by every herbicide treatment except flumioxazin (0.21 and 0.42 kg ai ha^{-1}) when compared to reference plants (1,026 leaves m^{-2} sediment; Table 1). Lotus AG biomass (36.5 g dry weight [DW]) was reduced by every treatment except 2,4-D (2.12 kg ae ha^{-1}), imazapyr (0.84 kg ai ha^{-1}), and both rates of flumioxazin 8 WAT (Table 1). Lotus BG biomass (619.5 g DW) was not reduced ($P > 0.05$) 8 WAT compared to reference plants (Table 1). In addition, American lotus did not regrow during the next growing season (including reference plants); therefore, long-term analysis was not conducted for this species.

Table 1. Percentage of reduction in mean leaf number (n m^{-2}) and above- and belowground biomass (g dry weight m^{-2}) of American lotus, white waterlily, and watershield 8 wk after treatment with foliar applications of aquatic herbicides in a mesocosm setting.

Treatment[1]	American Lotus			White Waterlily			Watershield		
	Leaf No.	AG	BG	Leaf No.	AG	BG	Leaf No.	AG	BG
2,4-D 2.12 kg ai ha^{-1}	−78.6 cde	20.8 abc	−59.8 a	93.3 a	143.1 ab	42.1 a	19.0 a	−44.5 a	−4.1 abc
2,4-D 4.24 kg ai ha^{-1}	−100 e	−92.7 bc	−4.6 a	0 ab	4.7 abcd	−36.2 a	−34.5 a	−71.4 ab	13.9 a
Glyphosate 2.83 kg ai ha^{-1}	−100 e	−100 c	−64.4 a	−95.6 c	17.5 defg	−34.9 a	−94.0 b	−99.4 cd	−82.4 ef
Glyphosate 5.67 kg ai ha^{-1}	−100 e	−95.6 bc	−45.0 a	−91.1 c	−91.9 g	−49.1 a	−97.6 b	−98.0 cd	−95.5 f
Triclopyr 3.36 kg ai ha^{-1}	−100 e	−100 c	−72.1 a	−15.6 ab	−30.3 cdef	−46.3 a	0 a	−75.0 ab	−40.2 abcde
Triclopyr 6.71 kg ai ha^{-1}	−98.3 de	−100 c	−53.3 a	−20 ab	−29.4 cdef	5.1 a	−42.9 a	−73.9 ab	−18.2 abcd
Imazamox 0.56 kg ai ha^{-1}	−96.4 de	−98.6 bc	48.2 a	−93.3 c	−94.7 fg	10.9 a	−97.6 b	−99.2 cd	−40.9 abcde
Imazamox 1.11 kg ai ha^{-1}	−100 e	−100 c	12.0 a	−68.9 c	−34.4 bcdef	20.6 a	−98.8 b	−96.7 cd	−58.7 cdef
Imazapyr 0.42 kg ai ha^{-1}	−98.3 de	−100 c	−55.7 a	−13.3 b	3.1 abcd	−9.8 a	−96.4 b	−87.5 bc	65.8 a
Imazapyr 0.84 kg ai ha^{-1}	−87.5 bcd	−43.8 ab	−34.3 a	−77.8 c	−88.1 efg	−8.5 a	−97.6 b	−98.8 cd	−53.7 bcdef
Florpyrauxifen-benzyl 0.02 kg ai ha^{-1}	−100 e	−100 c	−24.1 a	−95.6 c	−96.9 g	−54.7 a	−100 b	−98.0 cd	−98.6 f
Florpyrauxifen-benzyl 0.05 kg ai ha^{-1}	−100 e	−100 c	−67.2 a	−77. 8 c	−61.9 defg	−45.4 a	−100 b	−100 d	−85.2 def
Flumioxazin 0.21 kg ai ha^{-1}	−66.1 ab	−3.6 ab	−19.6a	8.9 ab	62.5 abc	−13.5 a	−11.9 a	−56.4 a	48.7 abc
Flumioxazin 0.42 kg ai ha^{-1}	−78.6 abc	−40.0 a	−37.9a	66.7 ab	221.9 a	21.7 a	−21.4 a	−72.9 ab	44.9 ab

[1]Treatments sharing the same letters within a column are not different from one another at the $\alpha = 0.05$ significance level ($n = 3$); values in cells of each column are percentage of difference from the mean for each metric; a "−" represents reduction; shaded cells are those treatments that are different from references; AG biomass and BG biomass refer to above- and belowground biomass, respectively; reference means were 1025.7, 36.5, and 619.5 for American lotus Leaf No., AG, and BG (respectively), 247.7, 19.5, and 1018.4 for white waterlily leaf no., AG, and BG (respectively), and 1538.6, 115.9, and 260.5 for watershield leaf no., AG, and BG (respectively).

Glyphosate (2.83 and 5.67 kg ae ha^{-1}), imazamox (0.56 kg ai ha^{-1}), imazapyr (0.84 kg ai ha^{-1}), and florpyrauxifen-benzyl (0.02 and 0.05 kg ai ha^{-1}) reduced white waterlily leaf number by more than 68% at 8 WAT ($P < 0.0001$; Table 1), while no treatment affected leaf density 52 WAT ($P = 0.2763$; Table 2) compared to reference plants. Waterlily AG biomass was reduced 91% by glyphosate (5.67 kg ae ha^{-1}), 94% by imazamox (0.56 kg ai ha^{-1}), and 96% by florpyrauxifen-benzyl (0.02 kg ai ha^{-1}) 8 WAT; but was not reduced 52 WAT compared to reference plants for any treatment ($P > 0.05$; Tables 1 and 2). Waterlily BG biomass was not reduced by any herbicide treatments 8 WAT ($P = 0.0526$; Table 1). However, by 52 WAT, lily BG biomass was reduced 100% by glyphosate (5.67 kg ae ha^{-1}), 75% by imazamox (0.56 kg ai ha^{-1}), 78% by imazapyr (0.84 kg ai ha^{-1}), and 78 to 85% by florpyrauxifen-benzyl (0.02 and 0.05 kg ai ha^{-1}) compared to reference plants ($P = 0.0111$; Table 2).

Glyphosate (2.83 and 5.67 kg ae ha^{-1}), imazamox (0.56 and 1.11 kg ai ha^{-1}), imazapyr (0.42 and 0.84 kg ai ha^{-1}), and florpyrauxifen-benzyl (0.02 and 0.05 kg ai ha^{-1}) reduced watershield leaf density 94 to 100% ($P = 0.0008$) and AG biomass 87 to 100% ($P = 0.0018$) 8 WAT compared to reference plants (Table 1). At 52 WAT, leaf density was still reduced 80 to 100% with the aquatic herbicides glyphosate (2.83 and 5.67 kg ae ha^{-1}), imazamox (0.56 and 1.11 kg ai ha^{-1}), imazapyr (0.42 and 0.84 kg ai ha^{-1}), and florpyrauxifen-benzyl (0.02 and 0.05 kg ai ha^{-1}) compared to watershield references (Table 2). Watershield AG biomass was reduced 51 to 100% by glyphosate (2.83 and 5.67 kg ae ha^{-1}), imazamox (0.56 and 1.11 kg ai ha^{-1}), imazapyr

Table 2. Percentage of reduction of leaf number and above- and below-ground biomass of white waterlily and watershield 52 wk after exposure to foliar applications of aquatic herbicides in a mesocosm setting.

Treatment[1]	White Waterlily			Watershield		
	Leaf No.	AG	BG	Leaf No.	AG	BG
2,4-D 2.12 kg ai ha^{-1}	−8.1 a	−12.2 a	−29.3 a	53.9 a	101.4 a	−11.4 ab
2,4-D 4.24 kg ai ha^{-1}	−17.6 a	−59.9 a	−36.5 abcd	−7.0 ab	−26.1 ab	−21.8 ab
Glyphosate 2.83 kg ai ha^{-1}	−79.4 a	−60.2 a	−86.0 cde	−47.8 bc	−51.1 bc	−64.5 cd
Glyphosate 5.67 kg ai ha^{-1}	−100 a	−100 a	−100 e	−100 d	−100 d	−100 e
Triclopyr 3.36 kg ai ha^{-1}	−21.3 a	−49.9 a	−54.0 abcd	42.6 a	−7.1 ab	−43.6 abc
Triclopyr 6.71 kg ai ha^{-1}	−16.2 a	−42.6 a	−26.1 a	32.2 a	−6.5 a	−22.1 ab
Imazamox 0.56 kg ai ha^{-1}	1.5 a	−71.4 a	−75.9 bcde	−100 d	−100 d	−100 e
Imazamox 1.11 kg ai ha^{-1}	−27.9 a	188.6 a	−43.7 abc	−100 d	−100 d	−100 e
Imazapyr 0.42 kg ai ha^{-1}	10.3 a	1.8 a	−50.8 abc	−100 d	−100 d	−100 e
Imazapyr 0.84 kg ai ha^{-1}	5.1 a	−81.4 a	−78.0 cde	−80.0 cd	−83.7 cd	−86.5 de
Florpyrauxifen-benzyl 0.02 kg ai ha^{-1}	−59.6 a	−76.8 a	−85.1 de	−93.9 cd	−97.6 cd	−99.2 de
Florpyrauxifen-benzyl 0.05 kg ai ha^{-1}	−36.8 a	−10.5 a	−78.8 cde	−100 d	−100 d	−100 d
Flumioxazin 0.21 kg ai ha^{-1}	−17.6 a	−56.8 a	−40.9 ab	16.5 a	−22.6 ab	−45.1 bc
Flumioxazin 0.42 kg ai ha^{-1}	29.4 a	−16.8 a	−12.3 a	53.0 a	13.3 a	−36.6 abc

[1]Numbers in the first row are leaf no. or biomass of reference plants ($n = 3$; per m^{-2} sediment); values in cells of each column are percentage of reduction compared to reference plants; treatments sharing the same letters are not different from one another at the $\alpha = 0.05$ significance level within each column; shaded cells are those treatments that are different from references; a "−" represents reduction; AG biomass and BG biomass refer to above- and belowground biomass, respectively; reference means were 830.3, 477.0, and 4578.2 for white waterlily leaf no., AG, and BG (respectively), and 2106.4, 137.4, and 944.7 for watershield leaf no., AG, and BG (respectively).

Table 3. Percentage of reduction of inflorescence and leaf number for each target species 12 wk after treatment in field plots.

Treatment[1]	American Lotus		White Waterlily		Watershield	
	Infl. No.	Leaf No.	Infl. No.	Leaf No.	Infl. No.	Leaf No.
Glyphosate 2.83 kg ai ha^{-1}	−100 a	−78.1 d	−100 b	128.0 a	−100 a	−70.9 b
Glyphosate 5.67 kg ai ha^{-1}	−100 a	−70.3 d	−100 b	53.6 ab	−100 a	−74.2 bc
Imazamox 0.56 kg ai ha^{-1}	−100 a	2.3 bc	−100 b	82.9 b	−100 a	−86.7 bcd
Imazamox 1.11 kg ai ha^{-1}	0 a	4.7 ab	−100 b	35.4 bc	−100 a	−88.2 cd
Florpyrauxifen-benzyl 0.02 kg ai ha^{-1}	−100 a	−23.4 bc	−100 b	48.8 bc	−100 a	−83.2 cd
Florpyrauxifen-benzyl 0.05 kg ai ha^{-1}	−100 a	−67.2 cd	−100 b	132.9 a	−100 a	−91.1 d

[1]Numbers in the first row are values of reference plants (n = 5; per m^{-2} water surface); values in cells of each column are percentage of reduction compared to reference plants; treatments sharing the same letters are not different from one another at the $\alpha = 0.05$ significance level within each column; shaded cells are those treatments that are different from references; a "−" represents reduction; "Infl. No." refers to inflorescence number; reference means were 0.08 and 5.1 for American lotus infl. no. and leaf no. (respectively), 0.2 and 3.3 for white waterlily infl. no. and leaf no. (respectively), and 0.04 and 108.3 for watershield infl. no. and leaf no. (respectively).

(0.42 and 0.84 kg ai ha^{-1}), and florpyrauxifen-benzyl (0.02 and 0.05 kg ai ha^{-1}) 52 WAT ($P = 0.0010$); however, the low dose of glyphosate did not provide the same level of reduction (51% reduction) as the high doses of glyphosate or florpyrauxifen-benzyl or either dose of imazamox or imazapyr (100% reduction for all; Table 2). Watershield BG biomass was reduced 82 to 98% ($P = 0.0094$) by glyphosate (2.83 and 5.67 kg ae ha^{-1}) and florpyrauxifen-benzyl (0.21 and 0.42 kg ai ha^{-1}) 8 WAT compared to reference plants (Table 1). At 52 WAT, watershield BG biomass was reduced 45 to 100% by glyphosate (2.83 and 5.67 kg ae ha^{-1}), imazamox (0.56 and 1.11 kg ai ha^{-1}), imazapyr (0.42 and 0.84 kg ai ha^{-1}), florpyrauxifen-benzyl (0.02 and 0.05 kg ai ha^{-1}), and flumioxazin (0.21 kg ai ha^{-1}), but low rates of glyphosate and flumioxazin provided less reduction (64 and 45%, respectively) than the other treatments (86 to 100% reduction for all) compared to references ($P = 0.0007$; Table 2).

General symptomology of lotus, waterlily, and watershield in the mesocosms included leaf chlorosis in plants treated with flumioxazin, 2,4-D, florpyrauxifen-benzyl, glyphosate, and triclopyr at 2 WAT. Also at 2 WAT, epinasty development (e.g., downward leaf twisting) was evident in plants treated with 2,4-D, triclopyr, and florpyrauxifen-benzyl. Leaf chlorosis was evident in plants receiving imazapyr and imazamox treatments by 3 to 4 WAT. Due to the level of activity provided on white waterlily and watershield by glyphosate, imazamox, and florpyrauxifen-benzyl, these herbicides were selected for further evaluation in field plots on Loakfoma Lake.

Field trial

In field plots, none of the herbicides reduced American lotus inflorescence density 12 WAT ($P = 0.1174$; Table 3); however, lotus inflorescences were rarely documented throughout the duration of this trial. Glyphosate (2.83 and 5.67 kg ae ha^{-1}) and florpyrauxifen-benzyl (0.05 kg ai ha^{-1}) reduced American lotus leaf density 67 to 78% while low rates of florpyrauxifen-benzyl (0.02 kg ai ha^{-1}) reduced leaf density 23% compared to reference plants 12 WAT ($P < 0.0001$; Table 3). Imazamox treatments did not reduce lotus leaf density ($P < 0.0001$) compared to reference plants 12 WAT (Table 3). At 52 WAT, lotus leaf density had increased by over 300% in all treatment plots compared to reference sites ($P = 0.0012$; Table 4); likely due to competitive release from white waterlily and watershield.

All herbicide treatments reduced white waterlily inflorescence density 100% compared to references 12 WAT ($P = 0.0057$; Table 3) and maintained reduction (>84%) 52 WAT ($P = 0.0008$; Table 4). Lily leaf density increased 53 to 128% in plots treated with glyphosate (2.83 kg ae ha^{-1}), imazamox (0.56 kg ai ha^{-1}), and florpyrauxifen-benzyl (0.05 kg ai ha^{-1}) 12 WAT ($P = 0.0001$; Table 3) but was reduced 64 to 92% in all treatment plots compared to references 52 WAT ($P < 0.0001$; Table 4). Imazamox (0.56 kg ai ha^{-1}) provided greater waterlily leaf density reduction (92%) than florpyrauxifen-benzyl (64%; 0.02 kg ai ha^{-1}) 52 WAT; but neither treatment had a different leaf density reduction compared to all other herbicide treatments ($P < 0.0001$; Table 4).

Table 4. Percentage of reduction of inflorescence and leaf number for each target species 52 wk after treatment in field plots.

Treatment[1]	American Lotus		White Waterlily		Watershield	
	Infl. No.	Leaf No.	Infl. No.	Leaf No.	Infl. No.	Leaf No.
Glyphosate 2.83 kg ai ha^{-1}	a[2]	321.0 bc	−100 b	−89.4 bc	−85.3 b	−27.0 ab
Glyphosate 5.67 kg ai ha^{-1}	a[2]	1110.5 ab	−100 b	−78.1 bc	−96.1 b	−75.0 c
Imazamox 0.56 kg ai ha^{-1}	a[2]	610.5 ab	−100 b	−92.9 c	−83.3 b	−51.2 bc
Imazamox 1.11 kg ai ha^{-1}	a[2]	515.8 ab	−100 b	−88.4 bc	−84.6 b	−49.9 bc
Florpyrauxifen-benzyl 0.02 kg ai ha^{-1}	a[2]	442.1b	−100 b	−64.9 b	−63.1 b	−67.6 c
Florpyrauxifen-benzyl 0.05 kg ai ha^{-1}	a[2]	1336.8 a	−84.6 b	−70.1 bc	−90.5 b	−72.8 c

[1]Numbers in the first row are values of reference plants (n = 5; per m^{-2} water surface); values in cells of each column are percentage of reduction compared to reference plants; treatments sharing the same letters are not different from one another at the $\alpha = 0.05$ significance level within each column; shaded cells are those treatments that are different from references; a "−" represents reduction; "Infl. No." refers to inflorescence number; reference means were 0.0 and 0.8 for American lotus infl. no. and leaf no. (respectively), 0.5 and 12.4 for white waterlily infl. no. and leaf no. (respectively), and 12.2 and 157.1 for watershield infl. no. and leaf no. (respectively).

[2]Refers to error in the formula because the value in the reference was 0.

None of the herbicide treatments reduced watershield inflorescence density by 12 WAT compared to references ($P = 0.4231$; Table 3) but every treatment had suppressed inflorescence production 63 to 96% by 52 WAT ($P < 0.0001$; Table 4). Watershield leaf density was reduced 70 to 91% by all herbicide treatments compared to references 12 WAT ($P < 0.0001$; Table 3). However, high rates of florpyrauxifen-benzyl (0.05 kg ai ha^{-1}) provided greater watershield leaf reduction (91% reduction) than either glyphosate treatment (70 to 74% reduction) at 12 WAT ($P < 0.0001$; Table 3). Additionally, low rates of florpyrauxifen-benzyl (0.02 kg ai ha^{-1}) and high rates of imazamox (1.11 kg ai ha^{-1}) provided greater watershield leaf reduction (83 to 88% reduction) than low rates of glyphosate (2.83 kg ae ha^{-1}; 70% reduction) 12 WAT ($P < 0.0001$; Table 3). By 52 WAT, all treatments except low rates of glyphosate (2.83 kg ae ha^{-1}) reduced watershield leaf density 49 to 75% compared to reference plots ($P = 0.0003$; Table 4).

In field sites, glyphosate (both rates) resulted in extensive chlorosis, leaf margin curling, and leaf death 1 WAT. Most glyphosate-treated plots exhibited approximately 50% leaf mortality 2 WAT and at least 80% mortality by 3 WAT; thereafter, American lotus began to increase in abundance in these plots. Plots treated with florpyrauxifen-benzyl showed similar, but more severe, symptomology as glyphosate-treated plots, with plants showing extensive curling of leaf margins, epinasty, chlorosis, and leaf mortality 1 WAT. By 3 WAT, most florpyrauxifen-benzyl-treated plants exhibited 80 to 90% mortality, and American lotus was encroaching into vacant areas. In contrast to glyphosate and florpyrauxifen-benzyl, imazamox-treated plots exhibited low levels of leaf chlorosis 1 WAT, accompanied by low to moderate levels of leaf death in each species. By 8 WAT, many of the plots were dominated by white waterlily or newly produced American lotus and white waterlily foliage.

Most white waterlily and watershield foliage emerged at least 1 mo (March) earlier than American lotus, resulting in those two species having much greater coverage than American lotus in the plots at the time of treatment (mid-May). This resulted in substantially less direct treatment of American lotus and likely resulted in competitive release of this species from the other two species in treated plots, as evidenced by increased density of American lotus in treated plots 4 WAT (mid-June). Increased waterlily leaf density 12 WAT was likely due to production of new leaves following herbicide treatments and reduction of watershield leaf density. The increased dominance of American lotus in treatment plots persisted into the second growing season (52 WAT). Regardless of effects to American lotus, herbicide application to field plots resulted in long-term suppression of white waterlily and watershield (Table 4). Additionally, reduction of watershield inflorescences by all herbicide treatments suggests the treatments investigated here may be useful for long-term propagule reduction of watershield by reducing seed input to lake sediments. Propagule reduction is a major component of long-term control of nuisance aquatic plant populations because reduction of propagule banks reduces potential seedlings and thus nuisance plant growth in years following management activities (Skogerboe et al. 2008; Rohal et al. 2019; Turnage et al. 2019a, b).

Riemer and Welker (1974) reported 100% visual reduction of waterlily using glyphosate (3.36 and 6.72 kg ae ha^{-1}) 9 mo after herbicide application to infested ditches in Florida. The current work found 100% BG biomass reduction of mesocosm grown waterlily 52 WAT using glyphosate (5.67 kg ae ha^{-1}) and 78 to 89% reduction of leaf density 52 WAT in field plots using 2.83 and 5.67 kg ae ha^{-1} glyphosate, respectively (Tables 2 and 4). Riemer and Welker (1974) also used low diluent volume herbicide applications (205 L ha^{-1}), which, taken with our work, suggests that aerial applications of glyphosate could be used for large scale reduction of waterlily. Additionally, ultralow-volume herbicide applications to control aquatic vegetation are becoming more common and can be conducted with unoccupied aerial systems (UAS; Howell et al. 2023). Future research (field or mesocosm) should compare ultralow, low, and high carrier volume applications in the same trial to determine if UAS, aerial, or surface-based herbicide applications provide adequate control of target vegetation.

Glyphosate, imazamox, and florpyrauxifen-benzyl treatments were tested in field plots without repetition of the mesocosm trial because of the high levels of leaf density and biomass reduction (> 75%) exhibited by most treatment rates 52 WAT (Table 2). Furthermore, field trials found > 65% leaf density reduction 52 WAT (Table 4) for waterlily and watershield suggesting mesocosm replication was not needed as field data validated mesocosm results. Additionally, the inability to replicate water quality across 45 mesocosms for 1 yr negated the ability to have true repetition among trials at the mesocosm scale so field testing was conducted in lieu of a second round of mesocosm screening.

American lotus reduction 12 WAT by herbicide treatments (particularly glyphosate and florpyrauxifen-benzyl treatments) in field sites (Table 3) was not as great as that recorded in mesocosms 8 WAT (Table 1), which was likely due to a more extensive rhizome network in field sites providing greater regrowth potential. Asynchronous emergence of American lotus versus white waterlily and watershield resulted in much poorer long-term reduction of lotus in field sites, leading to a dominance of lotus in herbicide-treated plots 52 WAT. Lotus inflorescence production was low across field sites at both time points suggesting plant spread is mostly attained through rhizome elongation which is common for this species (Hall and Penfound 1944).

White waterlily, which was reduced 8 and 52 WAT in mesocosm trials, increased in abundance 12 WAT in field sites (Table 3) due to regrowth of numerous small leaves after older leaves had died from herbicide exposure. However, waterlily leaf and inflorescence reduction (Table 4) in field plots was evident 52 WAT suggesting glyphosate, imazamox, and florpyrauxifen-benzyl can provide resource managers long-term control of this species. Waterlily inflorescence reduction should be a goal of resource managers as this prevents seed production and recruitment to seed banks thereby reducing regrowth potential after management activities.

Watershield exhibited similar responses to glyphosate, imazamox, and florpyrauxifen-benzyl in both trials. In terms of the relative efficacy of the three chemicals, florpyrauxifen-benzyl usually had the highest levels of leaf density reduction in both trials, and glyphosate usually had the lowest levels, although these values weren't always significantly different. Field trials showed potential for added control of flowering in this species, which could enhance long-term management of nuisance populations of this species.

This is the first work documenting reduction of white waterlily or watershield in field sites using ALS inhibiting (imazamox) or auxin mimic (florpyrauxifen-benzyl) herbicides (Table 4). It was also one the few accounts of these typically nonnuisance native species being targeted for management under situations where growth had become weedy and control was required. Plant reduction by herbicides occurred faster for watershield (12 WAT) than waterlily in field plots (52 WAT; Tables 3 and 4) likely because waterlily has greater BG biomass (reference lily BG biomass = 1,018.4 g DW m^{-2}; reference watershield BG biomass = 260.5 g DW m^{-2}; Table 1) that may take longer for herbicides to reduce. Efficacy of multiple herbicide MOAs on target vegetation is beneficial for resource managers as this provides multiple control options for rotational practices to prevent the occurrence of herbicide-resistant plant populations while providing tank mix protocols for potential increased control. Future work should investigate rate reductions of the efficacious herbicides used in this project (i.e., glyphosate, imazamox, imazapyr, and florpyrauxifen-benzyl) as well as tank mixtures of these herbicides and others not investigated here.

SOURCES OF MATERIALS

[1]Osmocote® Plus, ICL Fertilizers, 4950 Blazer Memorial Parkway, Dublin, OH 43017.

[2]Weedar® 64 broadleaf herbicide, Nufarm Inc., 11901 S. Austin Ave, Alsip, IL 60803.

[3]Rodeo®, Dow AgroSciences LLC, 9330 Zionsville Road, Indianapolis, IN 46268.

[4]Navitrol® landscape and aquatic herbicide, Applied Biochemists, W175N11163 Stonewood Dr, Ste. 234, Germantown, WI 53022.

[5]Clearcast® herbicide, SePRO Corporation, 11550 North Meridian St, Suite 600, Carmel, IN 46032.

[6]Habitat® herbicide, SePRO Corporation, 11550 North Meridian St, Suite 600, Carmel, IN 46032.

[7]ProcellaCOR™ SC, SePRO Corporation, 11550 North Meridian St, Suite 600, Carmel, IN 46032.

[8]Clipper® SC aquatic herbicide, Nufarm Inc., 11901 S. Austin Ave, Alsip, IL 60803.

[9]TeeJet 8002 fan nozzle, TeeJet®Technologies, 1801 Business Park Dr., Springfield, MO, 62703.

[10]Top Surf®, Winfield Solutions, LLC, P.O. Box 64589 St. Paul, MN 55164.

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