

Evaluation of fluazifop-P-butyl and sethoxydim for *Hymenachne amplexicaulis* control in mixed and monotypic emergent plant communities

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ABSTRACT

West Indian marsh grass, *Hymenachne amplexicaulis*, is an invasive grass species that forms monotypic stands in Florida's freshwater marshes. It is typically managed using broad-spectrum herbicides that can have significant nontarget impacts. Grass-specific herbicides (graminicides), sethoxydim and fluazifop-P-butyl, represent an opportunity to control *H. amplexicaulis* and reduce nontarget impacts. Plots were established in a monotypic *H. amplexicaulis* stand in November 2017 by applying fluazifop-P-butyl at 0.42 or 1.12 kg ha⁻¹ or sethoxydim at 5.04 kg ha⁻¹, each with MSO at 1% v/v. Both graminicides significantly reduced *H. amplexicaulis* cover by 85% to 90% at 6 mo after initial treatment (MAT1), but by 9 MAT1, this control fell to 52 to 68% when compared to nontreated plots. Plots were retreated with the same herbicide treatments in August 2018 to assess longer-term efficacy. At 11 mo after second treatment (20 MAT1), graminicides reduced *H. amplexicaulis* cover by 75 to 88% and increased plant diversity, measured by Simpson's Diversity Index (*D*), compared to nontreated plots. At a second site with low *H. amplexicaulis* cover (3%), the same treatments maintained low *H. amplexicaulis* cover but did not eliminate the plant. *D* was not impacted by these graminicides, and there were few differences in *D* when treated and nontreated plots were compared by functional groups including monocotyledonous nongraminoid, dicotyledonous, and graminoid plants. Few differences emerged in functional groups between herbicide-treated and nontreated control plots. Functional groups were not affected by graminicide treatment over time beyond seasonality of examined species. These studies indicate both graminicides may be effective in controlling West Indian marsh grass while maintaining or improving plant diversity.

Key words: diversity, graminicide, West Indian marsh grass.

INTRODUCTION

Emergent invasive grasses are a threat to aquatic systems across Florida. Grass species such as torpedograss (*Panicum repens* L.), para grass [*Urochloa mutica* (Forssk.) T. Q. Nguyen], Tropical American watergrass (*Luziola subintegra* Swallen),

and West Indian marsh grass [*Hymenachne amplexicaulis* (Rudge) Nees] have been shown to displace native plant species and form dense, monotypic stands (Tarver 1979, Lambert et al. 2010, Enloe et al. 2018). Proliferation of invasive grasses can have negative effects on the greater wetland community by promoting secondary invasions and altering ecosystem processes including nutrient cycling, biomass accumulation, and water flow (Dudley 1998, Meyerson et al. 2000, Houston and Duivenvoorden 2002, Flory and Clay 2010, Flory and Bauer 2014). West Indian marsh grass (WIMG) is a relatively new threat and is becoming particularly problematic in the hydrologically fluctuating wetlands in the Kissimmee River and Kissimmee Chain of Lakes in Central Florida.

WIMG is one of the 81 highly invasive Category I species listed on the Florida Exotic Pest Plant Council's 2019 List of Invasive Plant Species (FLEPPC 2019). Native to the tropical and subtropical areas of South America, Central America, and the West Indies, WIMG was introduced into Florida as a potential forage species for cattle (David Hall, *personal communication*). Although the original date of introduction is unknown, the first herbarium record in Florida was collected in 1957 in Palm Beach County (Bair 1957). WIMG is now present in at least 26 contiguous counties from Miami-Dade County in the south to Lake County in central Florida, with an isolated population in Leon County in the northern part of the state (EDDMapS 2019). At first glance, WIMG may be confused for native maidencane (*Panicum hemitomon* Schult.) or American cupscale [*Sacciolepis striata* (L.) Nash]. Unlike maidencane or American cupscale, WIMG has characteristic stem-clasping leaves with prominent ear-shaped leaf bases, known as auricles. The leaf bases of American cupscale may also tend to clasp the stem, but they are not as prominent as those of WIMG. Another distinguishing characteristic, stems of WIMG are filled with spongy, white material known as aerenchyma, which helps stolons to float atop the surface of the water. Stems of American cupscale lack aerenchyma.

WIMG flowers and fruits in the fall, corresponding to September through December in Florida when day length decreases below 12 h (Tropical Weeds Research Center 2006, Jacono 2014). The panicles of WIMG can grow to half a meter in length and produce approximately 4,000 seeds per infructescence (Tropical Weeds Research Center 2006). Two-month-old seeds can have up to 85% viability, contributing to the invasive nature of WIMG (Campbell et al. 2009). Not only can WIMG reproduce from seed, but

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stem and stolon fragments as small as one node in length can regenerate and form entire plants (Jacono 2014).

WIMG grows quickly in disturbed habitats and responds well to flooding events by elongating its internode length, giving it a competitive advantage in areas where the water level fluctuates seasonally (Kibbler and Bahnisch 1999). Although there is little research on the mechanisms of WIMG invasion, it is inferred that the size of WIMG and its rapid response to flooding make it a better competitor for light than some smaller-statured native emergent species (Kibbler and Bahnisch 1999). Additionally, high seed viability may contribute to its ability to colonize susceptible habitats. WIMG tends to form dense monocultures that have been shown to decrease plant species richness, alter invertebrate family composition, and increase occurrence of introduced fish species in Australia (Houston and Duivenvoorden 2002).

In Florida, WIMG has been managed using the broad-spectrum herbicides glyphosate and imazapyr either alone or in combination (Sellers et al. 2008). However, the nonselective nature of these herbicides can have nontarget effects on the native plant community when treating mixed stands. Nonselective treatment can create an open space in which invasive species may colonize once again, especially if nearby populations or prominent seed banks exist (van der Valk 1981). Land managers need more selective methods when managing for emergent invasive grasses in mixed stands. In Australia, researchers have examined the use of a limited number of grass-specific herbicides for WIMG control (Vitelli et al. 2005).

Grass-specific herbicides, or graminicides, offer an alternative to management that uses broad-spectrum herbicides. Graminicides target a form of acetyl-coenzyme A carboxylase in members of Poaceae and do not negatively impact nongrass species (Burton et al. 1989, Kukorelli et al. 2013, Enloe and Netherland 2017). Graminicides have proven to be useful in agronomic systems and terrestrial habitat restoration (Burton et al. 1989, Clay et al. 2006, Barnes 2007). Research on torpedograss and para grass suggests the graminicides sethoxydim and fluzifop-P-butyl can be useful for invasive aquatic grass management (Enloe et al. 2018, Prince et al. 2019a). Sethoxydim has recently been granted a 24(c) registration for aquatic grass control in Florida (Anonymous 2017). Fluzifop-P-butyl is currently being assessed for aquatic use under a Florida Experimental Use Permit (Anonymous 2018).

Given the recent availability of these two graminicides for aquatic use and the growing issue of WIMG invasion, our objectives were 1) to assess sethoxydim and fluzifop-P-butyl for WIMG control in monotypic stands and mixed emergent plant communities on a central Florida lake margin and 2) to evaluate the plant community response to graminicide treatment.

MATERIALS AND METHODS

In November 2017, two studies were established in the northwest marsh of Cypress Lake near Kenansville, FL (28°05'00.8"N 81°20'24.4"W). Data from the United States Geological Survey indicate WIMG has sustained populations

in Cypress Lake since at least 1995 (EDDMapS 2019, USGS 2019). WIMG is present in both monotypic stands and mixed stands with native emergent vegetation including maidencane (*Panicum hemitomon* Schult.), smartweed (*Polygonum spp.* L.), Southern cutgrass (*Leersia hexandra* Swartz), Southern watergrass [*Luziola fluitans* (Michx.) Terrell & H. Rob.], pickerelweed (*Pontederia cordata* L.), arrowhead (*Sagittaria spp.* L.), American cupscale [*Sacciolepis striata* (L.) Nash], American lotus (*Nelumbo lutea* Willd.), lemon bacopa [*Bacopa caroliniana* (Walter) B.L. Rob.], and other species (Table 1). One study was set in a monotypic stand of WIMG and is hereafter referred to as the High WIMG Cover Study. The second study was set in a mixed stand of WIMG and native plant species and is hereafter referred to as the Low WIMG Cover Study. At the onset of these studies, essentially no other plant species were present in the high cover study; however, a total of 35 species representing 16 plant families were found in the Low WIMG Cover Study, including 14 dicots, 10 non-Poaceae monocots, 10 Poaceae monocots, and one fern (Table 1). All species were common wetland plants in Florida and included a mix of native and introduced species. Water depth in this area typically fluctuates from seasonally dry in late November through April to seasonally wet in May through mid-November, and both study sites experienced a mean wet season depth of 42 cm.

Twenty-eight plots, each 0.056 ha in size (9.1 m by 61 m), were established for the two studies. Sixteen plots were established in the Low WIMG Cover Study, and 12 were established in the High WIMG Cover Study. Plot corners were marked with permanent polyvinyl chloride (PVC) pipes 3 m in height. Treatments included fluzifop-P-butyl¹ at a broadcast rate of 0.42 kg ai ha⁻¹ or a spot treatment concentration equivalent to 1.12 kg ai ha⁻¹, sethoxydim² at a spot treatment concentration equivalent to 5.05 kg ai ha⁻¹, and a nontreated control. All herbicide treatments included a methylated seed oil adjuvant³ approved for use in aquatics at 1% v/v. Due to size constraints, plot replicates varied by study. The Low WIMG Cover Study contained four replicate plots per treatment, and the High WIMG Cover Study contained three replicate plots per treatment. Although it would have been very useful to include a broad-spectrum herbicide treatment, it was not feasible in this study due to study size constraints.

Initial herbicide treatments for the Low WIMG Cover and High WIMG Cover Studies occurred on 28 November and 1 December 2017, respectively. Under ideal conditions, treatments would occur in the late summer to early fall. However, water levels at ideal treatment times in 2017 were unexpectedly high due to a hurricane, and treatments were postponed until water levels receded and emergent vegetation had recovered. Treatments were applied using a handgun sprayer from an airboat at an application volume of 938 L ha⁻¹. The applicator calibrated the spray gun and made multiple practice passes before spraying plots to ensure spray volume accuracy. Applications were made by treating from the plot edge down the length of each plot so that treated areas were not run over by the airboat. This method prevented the formation of airboat trails in the plots, where herbicide efficacy on emergent plants has been

TABLE 1. SPECIES IDENTIFIED FROM BOTH CYPRESS LAKE WEST INDIAN MARSH GRASS STUDIES. FOUR SAMPLES COULD NOT BE IDENTIFIED TO THE SPECIES LEVEL BECAUSE THEY WERE SEEDLINGS AT THE TIME OF SAMPLING.

Scientific Name	Common Name	Family	Class
<i>Alternanthera philoxeroides</i>	Alligator weed	Amaranthaceae	Dicot
<i>Bacopa caroliniana</i>	Lemon bacopa, blue waterhyssop	Scrophulariaceae	Dicot
<i>Centella asiatica</i>	Spadeleaf	Apiaceae	Dicot
<i>Cirsium sp.</i>	Thistle	Asteraceae	Dicot
<i>Cyperus lecontei</i>	Le Conte's flatsedge	Cyperaceae	Monocot
<i>Cyperus odoratus</i>	Fragrant flatsedge	Cyperaceae	Monocot
<i>Diodia teres</i>	Poorjoe	Rubiaceae	Dicot
<i>Echinochloa walteri</i>	Coast cockspur grass	Poaceae	Grass
<i>Eclipta prostrata</i>	False daisy	Asteraceae	Dicot
<i>Eleocharis geniculata</i>	Canada spikesedge	Cyperaceae	Monocot
<i>Eleocharis interstincta</i>	Knotted spikerush	Cyperaceae	Monocot
<i>Eriocaulon sp.</i>	Pipewort	Eriocaulaceae	Monocot
<i>Eupatorium sp.</i>	Dogfennel	Asteraceae	Dicot
<i>Hydrocotyle umbellata</i>	Manyflower marshpennywort	Apiaceae	Dicot
<i>Hymenachne amplexicaulis</i>	West Indian marsh grass	Poaceae	Monocot
<i>Leersia hexandra</i>	Southern cutgrass	Poaceae	Grass
<i>Ludwigia grandiflora</i>	Large-flower primrose-willow	Onagraceae	Dicot
<i>Ludwigia leptocarpa</i>	Anglestem primrose-willow	Onagraceae	Dicot
<i>Luziola fluitans</i>	Southern watergrass	Poaceae	Grass
<i>Myriophyllum aquaticum</i>	Parrotfeather	Haloragaceae	Dicot
<i>Panicum hemitomon</i>	Maidencane	Poaceae	Grass
<i>Panicum repens</i>	Torpedograss	Poaceae	Grass
<i>Paspalidium geminatum</i>	Kissimmeegrass	Poaceae	Grass
<i>Paspalum acuminatum</i>	Brook crowngrass	Poaceae	Grass
<i>Paspalum distichum</i>	Knotgrass	Poaceae	Grass
<i>Phyla nodiflora</i>	Matchstick weed	Verbenaceae	Dicot
<i>Polygonum persicaria</i>	Spotted ladythumb	Polygonaceae	Dicot
<i>Pontederia cordata</i>	Pickerelweed	Pontederiaceae	Monocot
<i>Sacciolepis striata</i>	American cupscale	Poaceae	Grass
<i>Sagittaria lancifolia</i>	Bulltongue arrowhead	Alismataceae	Monocot
<i>Sagittaria latifolia</i>	Broadleaf arrowhead	Alismataceae	Monocot
<i>Salvinia minima</i>	Water spangles	Salviniaceae	Fern
<i>Scleria lacustris</i>	Lakeshore nutrush	Cyperaceae	Monocot
<i>Urochloa mutica</i>	Para grass	Poaceae	Grass
<i>Utricularia sp.</i>	Bladderwort	Lentibulariaceae	Dicot

shown to be poor (Enloe et al. 2018). Plots received a second treatment with the same herbicides in the same manner as the initial treatment on 28 August 2018 in both studies.

Baseline data were collected at five randomly placed points marked by permanent 1.5 m PVC poles along a single transect down the length of each plot on 20 November 2017. At each point, a 1 m² quadrat was centered on the permanently installed 1.5 m PVC pole. Plots were resampled at each subplot at 1, 3, 6, and 9 mo after initial treatment (MAT1). Visual estimates of percent cover were recorded by researchers from an airboat for each species present in the subplot for the Low WIMG Cover Study. At the time of initial treatment, plots in the High WIMG Cover Study only contained WIMG, and, therefore, only WIMG percent cover data were collected until the second treatment. Cover data were collected after the second treatment for all species in both studies at 1, 3, 6, and 11 mo after the second treatment (MAT2). Additionally, aerial photos were captured using an unmanned aerial vehicle⁴ 30–60 m above the water surface to observe herbicide treatments, but no numerical data were recorded from these images.

Statistical analysis

A completely randomized design was used for both studies. ANOVA was performed on all percent cover data

utilizing the emmeans package in RStudio[®] (Lenth 2019, RStudio Team 2015). In the Low WIMG Cover Study, one nontreated replicate was removed as an outlier because baseline percent WIMG cover was beyond two standard deviations of baseline mean WIMG cover in all plots, native plant coverage was low, and the plot was not representative of the rest of the study. Simpson's Diversity Index was calculated from the percent cover data for all sample dates in the Low WIMG Cover Study and at all sample dates after the second treatment for the High WIMG Cover Study using Equation 1:

$$D = \frac{1}{\sum_{i=1}^S p_i^2} \quad (1)$$

where D is the measure of the index, S is the total number of species in the community, and p_i is the proportion of S made up of the i th species (Beals et al. 1999). Simpson's Diversity Index has been used as a measure of diversity in many wetland studies, including to examine the effects of herbicide treatment for invasive grass control (Ailstock et al. 2001, Schooler et al. 2006, Chen et al. 2002). Results from diversity analyses were subjected to ANOVA to compare diversity between treatments and sample dates using the emmeans package in RStudio[®] (Lenth 2019, RStudio Team 2015). For the Low WIMG Cover Study, an additional

TABLE 2. WIMG MEAN PERCENT COVER RESPONSE OVER TIME TO INITIAL HERBICIDE TREATMENT USING SETHOXYDIM AND FLUAZIFOP-BUTYL IN THE HIGH WIMG COVER STUDY.

	Sample Date, % Cover ^{2,3}				
	0 MAT1 or 0 MAT2 ¹	1 MAT1 or 1 MAT2	3 MAT1 or 3 MAT2	6 MAT1 or 6 MAT2	9 MAT1 or 11 MAT2
First treatment					
Fluazifop-p-butyl (0.42 kg ha ⁻¹)	57 a ² X ³	4 b Y	1 b Y	11 b XY	40 ab XY
Fluazifop-p-butyl (1.12 kg ha ⁻¹)	77 a X	2 b Y	1 b Y	7 b Y	26 b Y
Sethoxydim (5.04 kg ha ⁻¹)	75 a X	0 b Y	1 b Y	7 b Y	33 b Y
Nontreated	64 a XY	42 a Y	64 a XY	75 a XY	82 a X
Second treatment					
Fluazifop-p-butyl (0.42 kg ha ⁻¹)	40 ab XY	2 b Y	2 b Y	1 b Y	19 b XY
Fluazifop-p-butyl (1.12 kg ha ⁻¹)	26 b Y	1 b Y	1 b Y	0 b Y	11 b Y
Sethoxydim (5.04 kg ha ⁻¹)	33 b Y	0 b X	2 b X	1 b X	23 b X
Nontreated	82 a X	94 a X	93 a X	85 a X	92 a X

¹MAT1 = months after first treatment, MAT2 = months after second treatment.

²Means followed by the same lowercase letter within a column and within the first or second treatment are not significantly different at the 5% level using Tukey's adjustment.

³Means followed by the same capital letter within a row are not significantly different at the 5% level using Tukey's adjustment.

ANOVA was conducted for percent cover of plant functional groups including grasses, nongrass monocots, and dicots. Herbicide treatment and sample date were considered fixed effects in all studies. For both studies, data met the assumptions for analysis of variance (ANOVA), and no transformation was necessary. Significance was determined at the 5% level using Tukey's HSD test for *post-hoc* analysis.

RESULTS AND DISCUSSION

High WIMG Cover Study

As soon as 1 MAT1, aerial images captured with an unmanned aerial vehicle indicated distinct herbicide injury symptoms in treated plots compared to the nontreated controls. Herbicide-treated WIMG showed characteristic graminicide injury symptoms, including bands of necrosis at the meristems and extensive leaf chlorosis and necrosis (Kukorelli et al. 2013). For WIMG cover, there was a significant interaction between herbicide treatment and sample date after the initial herbicide application ($P = 0.0011$). This interaction was largely driven by the strong difference in WIMG plant cover between the nontreated plots and the herbicide-treated plots. The nontreated plots had significantly higher average WIMG cover than nearly all herbicide-treated plots, and average WIMG cover was never lower than 42% at any sample date in nontreated plots (Table 2). Although there was some seasonal variation in average WIMG cover in the nontreated plots over time shown by a reduction in cover during the late fall compared to the following summer, the three herbicide treatments clearly provided control beyond the seasonality of this species. At 1 MAT1, all herbicide treatments reduced WIMG cover to near zero and were not different from each other. Control in the herbicide-treated plots was maintained through 6 MAT1; however, at 9 MAT1, average percent WIMG cover was only significantly lower than the nontreated plots in plots treated with the spot treatment rates of fluazifop-P-butyl (26%) and sethoxydim (33%). Average percent WIMG cover in plots treated with the broadcast rate of fluazifop-P-butyl was 40% at 9 MAT1, which was not

different from any other treatment or from the nontreated plots.

Perennial grasses often recover after only one herbicide treatment and require sequential herbicide applications to achieve control. In previous studies, one application of glyphosate on WIMG provided only 70% control 6 MAT and one application of sethoxydim provided 29% control of torpedograss at 6 MAT (Sellers et al. 2008, Enloe et al. 2018). The lower average percent cover values of WIMG treated with sethoxydim in this study at 6 MAT1 (7%) suggest that WIMG is more sensitive to sethoxydim than torpedograss. Further studies examining the sensitivity and within-season retreatment interval requirements of other invasive wetland grasses such as para grass and Tropical American watergrass are warranted.

After the second treatment, there was a significant interaction between sample date and herbicide treatment for WIMG cover ($P = 0.0046$). In this analysis, the interaction was driven by differences in cover over time between the plots treated with fluazifop-P-butyl, sethoxydim, and the nontreated plots. Both fluazifop-P-butyl treatments resulted in a significant change in cover over time. Average WIMG percent cover in plots treated with sethoxydim or the nontreated plots, however, did not change significantly by sample date (Table 2). Although cover in the sethoxydim treatment over time displayed a negative trend, there was considerable variation at the 0 MAT2 and 11 MAT2 sample dates, which may have masked a significant change over time. All herbicide treatments performed comparably within all sample dates and reduced WIMG cover to near zero at 1, 3, and 6 MAT2 and reduced cover to 11 to 23% at 11 MAT2 (Table 2). No herbicide treatment eliminated WIMG cover completely, suggesting that additional treatments would likely be needed to completely control WIMG. The source of WIMG recovery in the herbicide-treated plots was not clear, but high propagule pressure from surrounding stands in the marsh may have contributed to reinvasion of WIMG. Demographic studies that examine recruitment from seeds versus stolons following treatment would help to address this question.

In order to better understand the impact of graminicide treatment on the greater plant community, Simpson's Diversity Index was calculated for each plot and averaged

for each treatment. Simpson's Diversity Index provides a measure of both number and abundance of each species, providing an opportunity to describe a plant community's response to herbicide treatments beyond presence or absence of data. Analysis of Simpson's Diversity Index after the second treatment indicated species diversity responded to herbicide treatment ($P < 0.001$) and sample date ($P = 0.0191$), but not the interaction of the two factors. When data were pooled across sample dates, the nontreated plots had significantly lower species diversity ($D = 0.42$) than plots treated with graminicides ($D = 0.68$ to 0.73), and no herbicide treatments were statistically different from one another. When data were pooled across treatment, diversity at the final sample date, 11 MAT2, was lower than at 6 MAT2 ($D = 0.51$ and 0.73 , respectively), but neither 11 MAT2 nor 6 MAT2 was different from any other sample date. These results were likely due to the recovery of WIMG by the final sample date. An increase in species diversity following herbicide treatment is a highly desirable outcome of successful restoration efforts. Although analysis was performed only on data collected after the second treatment, the results provide evidence that graminicide treatments in the fall and subsequent late summer can provide short-term WIMG control and result in increased diversity when treating monotypic stands. In these studies, species recruitment into herbicide-treated plots may have occurred from both the seedbank and from the surrounding marsh.

Low WIMG Cover Study

For WIMG cover, there was no interaction between herbicide treatment and sample date after the first application ($P = 0.868$); however, both herbicide treatment and sample date were significant ($P = 0.0336$ and $P = 0.00393$, respectively). When pooled across sample dates after the first treatment, plots treated with sethoxydim had significantly lower WIMG cover (1%) than in the nontreated plots (4%). Both fluzifop-P-butyl treatments were not different from any other treatment (Table 3). WIMG cover was lowest at 1 and 3 MAT1 (0%) and highest at 9 MAT1 (5%), just before the second treatment. WIMG percent cover values at 0 and 6 MAT1 were not different from any other sample date.

After the second treatment, again both herbicide treatment ($P < 0.001$) and sample date ($P = 0.005$) were significant with no interaction between main effects ($P = 0.28049$). When pooled across sample dates after the second treatment, WIMG cover in plots treated with any of the three herbicide treatments was significantly lower than WIMG cover in the nontreated plots (Table 3). By 11 MAT2, WIMG began to recover and percent cover was significantly higher than that of 1, 3, and 6 MAT2. These data indicate that sethoxydim and both fluzifop-P-butyl treatments maintained low WIMG cover for nearly a year following a second treatment; however, no herbicide treatment completely eliminated WIMG, even though its initial cover was very low.

Simpson's Diversity Index was not affected by herbicide treatment in the Low WIMG Cover Study after the first or second herbicide treatments ($P = 0.232$ and $P = 0.156$,

TABLE 3. WIMG COVER RESPONSE TO INITIAL AND SECOND APPLICATIONS BY TREATMENT AND SAMPLE DATE FOR THE LOW WIMG COVER STUDY.

Main Effect	% Cover ¹	
	Response to Initial Treatment	Response to Second Treatment
Treatment		
Fluzifop-p-butyl (0.42 kg ha ⁻¹)	2 ab	1 b
Fluzifop-p-butyl (1.12 kg ha ⁻¹)	2 ab	2 b
Sethoxydim (5.04 kg ha ⁻¹)	1 b	1 b
Nontreated	4 a	10 a
Sample date		
0 MAT1 or 0 MAT2 ²	3 AB	5 AB
1 MAT1 or 1 MAT2	0 B	2 B
3 MAT1 or 3 MAT2	0 B	2 B
6 MAT1 or 6 MAT2	2 AB	2 B
9 MAT1 or 9 MAT2	5 A	—
11 MAT1 or 11 MAT2	—	7 A

¹Means followed by the same lowercase letter in a column are not significantly different from each other at the 5% level using Tukey's adjustment

²MAT1= months after initial treatment; MAT2 = months after second treatment.

respectively); however, sample date was significant after both first and second herbicide treatments ($P < 0.001$ and $P = 0.0183$, respectively). The variation in diversity with respect to sample date can be attributed to the seasonality of the study site. After the second treatment, Simpson's Diversity Index was impacted by sample date only ($P = 0.0183$). Simpson's diversity measure significantly increased at 3 MAT2 and 6 MAT2 ($D = 0.84$) compared to 1 MAT2 ($D = 0.76$), and these were not different from 0 MAT2 ($D = 0.80$). The variation in Simpson's measure of diversity is due to the seasonality of the species present in the study area and does not reflect the impact of the herbicides.

Other nontarget impacts

Plant species in the Low WIMG Cover Study were additionally separated into nongrass monocots, dicots, and grasses for an analysis by functional group, excluding WIMG and *Salvinia minima* (Table 1). Results following the first herbicide treatment indicated that the nongrass monocot group was sensitive to treatment and sample date, but not the interaction of the two factors (Table 4). Nongrass monocot cover was different between the two rates of fluzifop-P-butyl but was not different between any other treatment comparison. Nongrass monocot cover also decreased at 6 and 9 MAT1 compared to the earlier sample dates.

After the second treatment, nongrass monocot cover was higher in the spot-treatment rate of fluzifop-P-butyl than all other treatments, and all other treatments were not different from one another. After the second treatment, nongrass monocot mean percent cover was not different from at any sample date and ranged from 18 to 29%.

No herbicide treatment changed dicot cover compared to the nontreated plots. This lack of significance was as expected, given the selectivity of the graminicides. Dicot cover displayed a more seasonal pattern with the highest mean cover data collected at 6 MAT1 and 6 MAT2 (19%), and the lowest mean cover data collected in the early sample

TABLE 4. NONGRASS MONOCOT¹ RESPONSE TO INITIAL AND SECOND APPLICATIONS BY TREATMENT AND SAMPLE DATE FOR THE LOW WIMG COVER STUDY.

Main Effect	% Cover ²	
	Response to Initial Treatment	Response to Second Treatment
Treatment		
Fluazifop-p-butyl (0.42 kg ha ⁻¹)	20 b	21 b
Fluazifop-p-butyl (1.12 kg ha ⁻¹)	33 a	34 a
Sethoxydim (5.04 kg ha ⁻¹)	23 ab	23 b
Nontreated	26 ab	19 b
Sample date		
0 MAT1 or 0 MAT2 ³	33 A	18 A
1 MAT1 or 1 MAT2	33 A	27 A
3 MAT1 or 3 MAT2	27 AB	29 A
6 MAT1 or 6 MAT2	15 B	25 A
9 MAT1 or 9 MAT2	18 B	—
11 MAT1 or 11 MAT2	—	22 A

¹Reference Table 1 for nongrass monocot species included in this analysis.

²Means followed by the same lowercase letter within a column are not significantly different at the 5% level using Tukey's adjustment.

³MAT1 = months after initial treatment; MAT2 = months after second treatment.

date after each treatment with less than 20% difference between the highest and lowest values (data not shown). These data indicate that graminicide treatments did not significantly alter plant community composition, and the variability in cover was largely attributed to the seasonality of the dicot species present in the study.

Grass cover was impacted by both herbicide treatment and sample date main effects after the first and second treatments (Table 5). Following the first treatment, the fluazifop-P-butyl spot rate resulted in lower grass cover than the other herbicide treatments but was not different from the nontreated plots. Grass cover seasonally declined over the winter and early spring but recovered to pretreatment levels by 9 MAT1. Following the second treatment, grass cover was lower in the spot treatment rate than the broadcast rate of fluazifop-P-butyl but was not different between any other treatments. Grass cover declined at 1 MAT2 but quickly recovered over time and was significantly higher at 11 MAT2 than at the time of the second treatment. Although not explicitly clear, these data do suggest that the spot treatment rate of fluazifop-P-butyl may have some negative impact on total grass cover for the species present. Additional research to clarify this is ongoing.

At the conclusion of the study, both sethoxydim and the high rate of fluazifop-P-butyl provided significant control of WIMG compared to the baseline and nontreated plots in the High WIMG Cover Study. After the first treatment in the Low WIMG Cover Study, only sethoxydim provided significant control; however, all graminicides performed equally well after the second treatment in the Low WIMG Cover Study with little to no negative impact on the native plant community. These graminicides were able to maintain low WIMG cover in mixed stands with repeated treatments. This approach may provide an ideal option for land managers in areas of new invasion that still have favorable native plant cover to prevent further WIMG encroachment while preserving native species composition. Shifting management practices to include graminicides may require a

TABLE 5. GRASS¹ RESPONSE TO INITIAL AND SECOND APPLICATIONS BY TREATMENT AND SAMPLE DATE FOR THE LOW WIMG COVER STUDY.

Main Effect	% Cover ²	
	Response to Initial Treatment	Response to Second Treatment
Treatment		
Fluazifop-p-butyl (0.42 kg ha ⁻¹)	43 a	57 a
Fluazifop-p-butyl (1.12 kg ha ⁻¹)	26 b	38 b
Sethoxydim (5.04 kg ha ⁻¹)	41 a	50 ab
Nontreated	38 ab	52 ab
Sample date		
0 MAT1 or 0 MAT2 ³	37 BC	45 BC
1 MAT1 or 1 MAT2	20 D	31 C
3 MAT1 or 3 MAT2	28 CD	48 BC
6 MAT1 or 6 MAT2	56 A	54 AB
9 MAT1 or 9 MAT2	45 AB	—
11 MAT1 or 11 MAT2	—	69 A

¹Reference Table 1 for grass species included in this analysis.

²Means followed by the same lowercase letter within a column are not significantly different at the 5% level using Tukey's adjustment.

³MAT1 = months after initial treatment; MAT2 = months after second treatment.

paradigm shift in the effort required to achieve control while protecting native plant diversity.

Overall, these graminicides provided WIMG control, but control was limited to less than 1 yr. Graminicide applications reduced WIMG cover by over 70% compared to nontreated plots in the High Cover Study and reduced cover to as low as 1% in the Low Cover Study. Both graminicide options reduced, but did not eliminate, WIMG from either study. Results from the High WIMG Cover Study indicated that plant diversity increased following two treatments, and that diversity was maintained following treatments in the low cover study. Both outcomes are highly desirable and beneficial to aquatic systems where WIMG is present. Future research should examine variable treatment timing as a function of seedling versus stoloniferous recruitment posttreatment. Additionally, future studies should examine lower rates and concentrations of sethoxydim for WIMG control. Additional studies from more locations with varying native species composition and extent of WIMG invasion are needed to further support these conclusions, especially to detect any meaningful changes over seasons within years.

SOURCES OF MATERIALS

¹A12460 GRASS Herbicide, Syngenta Crop Protection, LLC, P.O. Box 18300, Greensboro, NC 27419.

²TIGR[®] herbicide, SePRO Corporation, 11550 N. Meridian St., Suite 600, Carmel, IN 46032.

³MSO Concentrate, Loveland Products, Inc., 14520 Co. Rd. 64, Greeley, CO 80631.

⁴Phantom 4 Pro, DJI, 14th Floor, West Wing, Skyworth Semiconductor Design Building, No. 18 Gaoxin South 4th Ave, Nanshan District, Shenzhen, 518057, China.

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