

Uruguay waterprimrose control with herbicides

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ABSTRACT

Uruguay waterprimrose [*Ludwigia hexapetala* (Hook. & Arn.) Zardini, Gu., & Raven] is an aggressive plant native to South America. Although it has been present in the United States for many years, it has rapidly increased over the last decade, especially in California and Florida. In many situations, the plant's extensive creeping stem biomass has generally resulted in limited control. Studies were conducted in mesocosms in Florida to examine both new active ingredients and commonly used tank mixes for control of both above- and below-water biomass. Aminopyralid, imazamox alone and in combination with flumioxazin, and glyphosate alone and in combination with flumioxazin or 2,4-D provided effective shoot control at 60 d after treatment (DAT) following winter and spring applications. florpyrauxifen-benzyl (benzyl 4-amino-3-chloro-6-(4-chloro-2-fluoro-3-methoxyphenyl)-5-fluoropyridine-2-carboxylate) provided good initial control at 35 DAT, but regrowth was similar to the nontreated control at 60 DAT. No herbicide treatment resulted in effective control of below-water biomass. These studies suggest Uruguay waterprimrose shoot growth may be controlled with multiple selective and nonselective options. However, below-water biomass control will likely be much more difficult. Based on results of this study, future work should focus on seasonality of treatment and sequential treatment intervals to address the below-water biomass issue.

Key words: chemical control, foliar application, invasive aquatic emergent.

INTRODUCTION

Uruguay waterprimrose [*Ludwigia hexapetala* (Hook. & Arn.) Zardini, Gu., & Raven] is a member of the creeping waterprimrose complex in the family Onagraceae. It and its closely related congener large-flower primrose-willow waterprimrose (*Ludwigia grandiflora* [Michx.] Greuter & Burdet), have become highly invasive in many lakes and rivers in Florida, California, and other areas of the western and southeastern United States. Uruguay waterprimrose is believed to be native to South America and was likely introduced as an ornamental plant (Kaufman and Kaufman 2012) in the mid-1800s. The species was also introduced to

Europe in the early 1800s and is now one of the most problematic aquatic weeds in France (EPPO 2011, Thouvenot et al. 2013a).

Uruguay waterprimrose occurs in the littoral areas of lakes, and along the shorelines of slow-moving rivers and canals. It primarily expands locally by creeping stems and long distance by water when stems are fragmented during storms or anthropogenic activities. Okada et al. (2009) found clonal fragmentation to be the primary means of dispersal in California, as very little genotypic variation was found in 27 populations sampled from three watersheds. This was also true for its congener, creeping waterprimrose. Although sexual reproduction has been documented for Uruguay waterprimrose, (Ruaux et al 2009), its importance in Florida is uncertain.

In France, Uruguay waterprimrose has been suggested to be an ecosystem transformer (sensu Richardson et al. 2000) due to its aggressive growth and high biomass production (Lambert et al. 2010). In a survey of 567 sites across France, Lambert et al. (2010) found *Ludwigia* spp. produced a maximum of 4,300 g dry matter m⁻² in a eutrophic river system. Such heavy, dense mats can restrict water flow and increase flood risks, increase sedimentation and organic matter accumulation, and create anoxic conditions during decomposition (Dandelot et al 2005). These matted conditions have also prompted concerns for increased costs and difficulty in mosquito control (Meisler 2008). Fishing, boating, navigation, and hunting have also been impacted in densely infested areas (Nehring and Kolthoff 2011).

In addition to its tremendous biomass potential, Uruguay waterprimrose also exhibits considerable morphological plasticity that is driven by season, light intensity, and water depth (Thouvenot et al. 2013b). The influence of these factors on plant morphology has also likely contributed to the taxonomic uncertainty of the *Ludwigia uruguayensis* complex (Zardini et al. 1991, Nesom and Kartesz 2000). However, the potential interaction of these factors with management strategies has not been studied.

Regarding management of Uruguay waterprimrose, physical and chemical control are the two primary methods used. In France and other areas where herbicide use is limited in aquatic systems, hand pulling and mechanical harvesting are used for control (Thouvenot et al. 2013a). Mechanical control has also been used in Florida at an approximate cost of \$24,000 ha⁻¹ (J. Schardt, pers. comm.). Given the high cost of mechanical harvesting, herbicide treatments are routinely applied to emergent stands of Uruguay waterprimrose and treatment may occur throughout most of the year in Florida, especially when new infestations are detected. However, there is little published

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data on effective herbicide treatments available for review. In greenhouse studies, Emerine et al. (2010), evaluated the performance of imazamox on *Ludwigia grandiflora* [Michx.] Greuter & Burdet and found it to be highly active, with a calculated EC₇₀ (the effective concentration required to reduce dry weight by 70%) of 116 g ha⁻¹ for shoot biomass at 5 wk after treatment (WAT). Richardson et al. (2008) found flumioxazin rates of 168 to 437 g ha⁻¹ provided 70 to 81% control of creeping waterprimrose [classified as *Ludwigia grandiflora* (M. Micheli) Greuter & Burdet subsp. *hexapetala* (Hook. & Arn.) Nesom & Kartesz] at 4 WAT and was generally more effective than carfentrazone. However, neither herbicide is a stand-alone tool for creeping waterprimrose but both carfentrazone and flumioxazin are widely used for floating-plant control. Additionally, both are tank mixed with imazamox for *Ludwigia* spp. control by aquatic managers in Florida. The interest in imazamox arises from its demonstrable selectivity (Rodgers and Black 2012) while the utility of the protoporphyrinogen oxidase (PPO) inhibitors arises from rapid activity, especially on free-floating weed species. The rapid growth rates and subsequent dense growth of many species result in the need for a follow-up treatment approximately 14 d after initial treatment. The herbicide 2,4-D amine has also been used as a selective treatment on other exotic *Ludwigia* spp. while glyphosate has been used in a nonselective manner (Chandrasena et al. 2002).

Given the increasing problems created by Uruguay waterprimrose, the objective of this study was to evaluate several new herbicide treatments and tank mixes that would be of interest to aquatic managers for Uruguay waterprimrose control. These included aminopyralid, florypyrauxifen-benzyl [4-amino-3-chloro-6-(4-chloro-2-fluoro-3-methoxyphenyl)-5-fluoro-pyridine-2-benzyl ester], imazamox, flumioxazin, glyphosate, and 2,4-D. Aminopyralid is a selective pyridine carboxylic acid that is effective on a wide range of terrestrial invasive plants. At the time of the study, it was under review for a proposed label expansion to include emergent plant control in aquatic environments. florypyrauxifen-benzyl is a new arylpicolinate herbicide in development for use in rice and aquatic weed control. florypyrauxifen-benzyl has been found to be highly effective on submersed plants including Eurasian watermilfoil (*Myriophyllum aquaticum*) and hydrilla [*Hydrilla verticillata* (L. f.) Royle], and emergent plants including alligator weed [*Alternanthera philoxeroides* (Mart.) Griseb.] (Richardson et al. 2016). Imazamox, flumioxazin, glyphosate, and 2,4-D are currently labeled for control of several aquatic weed species and are frequently tank mixed in certain combinations to increase the spectrum of aquatic weed control. However, their role as tank-mix partners for Uruguay waterprimrose control has not been well documented but is of great interest as the species continues to spread.

MATERIALS AND METHODS

A mesocosm study was conducted at the University of Florida, Center for Aquatic and Invasive Plants (CAIP) in Gainesville, FL, in 2015 and repeated in 2016. Creeping waterprimrose was collected from two populations from

TABLE 1. COMPARISON OF URUGUAY WATERPRIMROSE PERCENTAGE OF INJURY 10 AND 35 D AFTER TREATMENT (DAT) FOR EXPERIMENT-TREATMENT COMBINATIONS.

	Rate	Experiment 1		Experiment 2	
		10 DAT	35 DAT	10 DAT	35 DAT
Aminopyralid	0.11	47 ab	98 a	40 bcd	99 a
	0.22	58 a	98 a	68 ab	100 a
Glyphosate	4.2	28 b	78 b	18 de	62 b
Glyphosate + flumioxazin	4.2 + 0.14	48 ab	93 ab	77 a	95 a
Glyphosate + 2,4-D	4.2 + 4.3	53 ab	98 a	75 a	99 a
Imazamox	0.28	23 b	73 b	12 e	21 c
Imazamox + flumioxazin	0.28 + 0.14	40 ab	87 ab	82 a	93 a
florypyrauxifen-benzyl	1.35	48 ab	80 b	23 cd	100 a
	2.7	52 ab	77 b	22 cd	92 a

¹Means followed by the same letter within a column are not significantly different at $P = 0.05$ using Tukey's adjustment for multiplicity.

north and south Florida. The southern population was collected from the St. John's River at the southern end of Lake Harney (28°43'55.4"N, 81°02'33.2"W) near Geneva, FL, and the northern population was collected from Alligator Lake (30°10'06.6"N, 82°37'54.9"W) near Lake City, FL. For each population, creeping stems' sections were cut, placed in coolers, and transported to CAIP in Gainesville, FL. Collected stems were subsequently propagated in 900-L mesocosms, allowed to flower, and keyed to species as Uruguay waterprimrose (*Ludwigia hexapetala*). Voucher specimens from each collection were deposited at the University of Florida Herbarium, Florida Museum of Natural History. Collections were made in April and May 2015 for Lake Harney and Alligator Lake, respectively. Plants were grown until sufficient stem material was produced for the study. Two 15-cm stem cuttings were planted in 3.8-L pots filled with a commercial greenhouse potting soil¹ mixed with a complete slow-release fertilizer.² Three planted pots each were placed in 100-L tubs and a total of 30 tubs were planted. Tub served as experimental units for each study. Plants in the tubs were allowed to grow for 3 mo for the Alligator Lake population and 6 mo for the Lake Harney population until the creeping stem layer was well established in each pot. During this time, the water level was raised to 15 cm above the top of the 3.8 L-pots in each tub.

Treatments are provided in Table 1 and included two rates each of aminopyralid³ and florypyrauxifen-benzyl,⁴ imazamox⁵ alone and in combination with flumioxazin,⁶ and glyphosate⁷ alone and in combination with flumioxazin or 2,4-D.⁸ A methylated seed oil⁹ was added to the florypyrauxifen-benzyl, imazamox, and imazamox + flumioxazin treatments at 2.3 L ha⁻¹ and a nonionic surfactant¹⁰ was added to all other herbicide treatments at 0.25% v/v. Treatments were applied with a single-nozzle microsprayer equipped with a TeeJet¹¹ 800067 nozzle at an application volume of 467 L ha⁻¹. Treatments were applied to the Alligator Lake accession on 1 December 2015 and to the Lake Harney accession on 31 March 2016. In both studies, water in the florypyrauxifen-benzyl-treated tubs was exchanged three times over a 9-d period (every 3 d). All other tubs were maintained under static conditions.

For each study, visual assessment of injury was collected on a 0 to 100 scale, where zero was no injury and 100 was

complete desiccation. Data collection occurred at 10 and 35 d after treatment (DAT). The 10-d assessments were conducted because in Florida, many follow-up treatments are routinely done approximately 2 wk after initial treatment to address spray skips and dense growth in general. At 35 DAT, all live shoots were clipped at the water level, placed in paper bags, oven dried at 65 C for 72 h and weighed. Plants were then allowed to regrow for an additional 25 d. At this time, all shoot regrowth was harvested at the water level. Additionally, all below-water biomass, including creeping stems in the water column and all roots and pneumatophores in the substrate, were harvested, oven dried, and weighed as previously described.

Statistical analysis

This experiment was a completely randomized design with herbicide treatments randomly assigned to tubs with three replicate tubs per treatment within each experiment. A split-plot mixed-model analysis to account for repeated measurements of both visual percentage-of-control data at 10 and 35 DAT and above water biomass at 35 and 60 DAT were performed using SAS® PROC GLIMMIX¹² (Littell et al. 1996). Below-water biomass from the 60-DAT harvest was subjected to ANOVA in SAS. Experimental run (fall and spring), herbicide treatment (10 levels), and DAT were considered fixed effects. ANOVA was performed on the natural log of aboveground biomass and the arcsine transformation was used for visual percentage-of-control data. The use of these transformations (Snedecor and Cochran 1989) was based on graphical examination of normality and homogeneity of variance. No transformation was required for below-water biomass. A number of unplanned comparisons inherent in the study design were also used to test if there were significant biomass differences due to rate (aminopyralid, florpyrauxifen-benzyl) or due to tank mixes (flumioxazin with glyphosate or imazamox). The interaction of these differences with DAT was also tested for aboveground biomass. Mean comparisons were adjusted for multiplicity using Tukey's adjustment when making all pairwise comparisons and Dunnett's adjustment for direct comparison to the nontreated check. The nontreated check treatment was excluded from the analysis of percentage of control, as is commonly done, because it was always zero.

RESULTS AND DISCUSSION

Visual control

Although long-term data are more useful for understanding treatment effects on perennial species, short-term visual evaluations of control provide a rapid assessment that is useful to aquatic plant managers and can inform researchers regarding early trends in treatment efficacy. There was a significant herbicide treatment by experimental run by DAT interaction for control data collected at 10 and 35 DAT ($P < 0.001$). This was driven by several differences in the response to herbicide treatment between sample dates. For example, within experimental runs and sample dates, imazamox alone differed between experiments at 35

DAT, providing 73% control in Experimental Run 1, but only 21% control in Experimental Run 2. florypyrauxifen-benzyl also provided higher control in Experimental Run 1 at 10 DAT and lower control at 35 DAT. This was in contrast to aminopyralid, which provided similar control at both rates tested (Table 1). Glyphosate alone also resulted in a similar pattern of control across experimental runs and sample dates, with 28% control or less at 10 DAT and 62 to 78% control at 35 DAT. Additionally, all three tank mix treatments performed better at 10 DAT in Experimental Run 2 than in Run 1. However, these tank mix treatments were similar between runs at 35 DAT and provided 87 to 99% control.

In Experimental Run 1, the addition of either flumioxazin or 2,4-D to glyphosate did not improve Uruguay waterprimrose control at either 10 or 35 DAT compared to glyphosate alone. However, in Experimental Run 2, both tank-mix partners significantly increased control at both sample dates compared to glyphosate alone. A similar pattern emerged with imazamox and flumioxazin as the tank mix increased control to 93% compared to 21% for imazamox alone.

Biomass response

There was a significant herbicide treatment by DAT interaction ($P < 0.001$) for the aboveground Uruguay waterprimrose biomass response. This indicated differential performance of certain herbicide treatments between the two sampling times. For example, aminopyralid rapidly reduced above-water biomass at 35 DAT and maintained a similar reduction in regrowth at 60 DAT (Table 2). However, imazamox, which is an acetolactate synthase inhibitor, was very slow to work and did not significantly reduce biomass at 35 DAT compared to the nontreated control. At 60 DAT, imazamox almost completely eliminated above-water regrowth. florypyrauxifen-benzyl demonstrated the opposite trend as it reduced biomass at 35 DAT but was not different from the nontreated control at 60 DAT.

At 35 DAT, glyphosate + 2,4-D reduced biomass to a greater extent than glyphosate alone or glyphosate + flumioxazin. However, at 60 DAT, there were no differences between glyphosate and the glyphosate tank mixes with 2,4-D or flumioxazin (Table 2). The difference between biomass at 35 and 60 DAT for glyphosate and for glyphosate + flumioxazin supported the notion that although all three glyphosate treatments had similar final control, these two were slower to show activity than glyphosate + 2,4-D. For imazamox, the addition of flumioxazin did not reduce biomass at either 35 or 60 DAT compared to imazamox alone. While aquatic managers commonly tank mix a PPO-inhibiting herbicide with imazamox in many situations, these data do not indicate that it improves Uruguay waterprimrose control.

At 60 DAT, only the main effect of experimental run was significant for Uruguay waterprimrose below-water biomass. Experimental Run 1 had significantly higher biomass (23.9 g pot⁻¹) compared to Run 2 (13.6 g pot⁻¹). Herbicide treatment was not significant for below-water biomass and values ranged from 26.7 g in the nontreated control to 12.9

TABLE 2. URUGUAY WATERPRIMROSE BIOMASS RESPONSE TO HERBICIDES AT 35 AND 60 D AFTER TREATMENT (DAT).

Treatment	Rate (kg ha ⁻¹)	Above-water Biomass (g) ¹			Below-water Biomass (g) ¹
		35 DAT	60 DAT	Difference (35-60 DAT) ²	60 DAT
Aminopyralid	0.11	0.01 c ³	0.0 d	0.01	16.71 a
	0.22	0.01 c	0.0 d	0.01	12.94 a*
Glyphosate	4.2	1.16 ab	0.15 bc	1.01**	18.04 a
Glyphosate + flumioxazin	4.2 + 0.14	0.95 ab	0.06 cd	0.89**	15.82 a
Glyphosate + 2,4-D	4.2 + 4.3	0.01 c	0.01 cd	0.00	17.95 a
Imazamox	0.28	5.32 a	0.09 bcd	5.23**	23.16 a
Imazamox + flumioxazin	0.28 + 0.14	1.87 ab	0.11 bcd	1.76**	19.16 a
florpyrauxifen-benzyl	1.35	0.10 bc	1.24 ab	-1.14*	18.01 a
	2.7	0.28 b	1.14 ab	-0.86**	18.72 a
Nontreated	—	9.18 a	2.67 a	6.51*	26.67 a

¹Geometric means of above-ground biomass are reported here because the analysis was performed using log-transformed values.

²This tests if the difference between 35 and 65 DAT treatment biomass is significantly different from zero using an LSD of $P = 0.05$ (*) or $p = 0.01$ (**).

³Means followed by the same letter within a column are not significantly different at $P = 0.05$ using Tukey's adjustment for multiplicity.

*Means are significantly different from the nontreated control using Dunnett's test at $P = 0.05$.

**Means are significantly different from the nontreated control using Dunnett's test at $P = 0.01$.

g in the aminopyralid high rate and was not different between any treatments. However, Dunnett's test indicated that the high rate of aminopyralid significantly reduced below-water biomass when compared specifically with the nontreated control.

Preplanned contrasts between aminopyralid rates, florpyrauxifen-benzyl rates, imazamox with and without flumioxazin, and glyphosate with and without flumioxazin or 2,4-D supported the previous analyses (Table 3). Only glyphosate compared with glyphosate + 2,4-D for above-water biomass was found to be significantly different, along with the interaction with DAT. No preplanned comparisons resulted in significant differences for below-water biomass.

Despite the difference in experimental runs, these results provide a relatively clear understanding of Uruguay waterprimrose control with several herbicides and tank mixes. First, aminopyralid, which is not currently labeled for use in water, effectively controlled Uruguay waterprimrose, both short term (10 to 35 DAT) and longer term (60 DAT). Percentage of control data and shoot biomass were not different between the two aminopyralid rates, which correspond to the maximum broadcast rate and the maximum spot-treatment rate to treat less than 50% of a hectare (Anonymous 2016). The spot-treatment rate was also the only herbicide treatment that significantly reduced

underwater biomass when compared to the nontreated control with Dunnett's test. During the course of this research, the proposed label expansion for aminopyralid for aquatic use was not approved and its registration for aquatic use is uncertain.

florpyrauxifen-benzyl, which is highly effective as an in-water treatment for submersed species such as Eurasian watermilfoil and hydrilla, provided only short-term control of Uruguay waterprimrose. In both experiments, florpyrauxifen-benzyl provided 77 to 100% control at 35 DAT and was not different between the two rates tested. However, these were the only treatments that allowed significant regrowth between 35 and 60 DAT. Future research should examine florpyrauxifen-benzyl as an in-water treatment and evaluate its potential role as a tank-mix partner with other foliar-applied herbicides.

Imazamox worked very slowly and did not provide consistent control or reduce shoot biomass at 35 DAT across experimental runs. However, by 60 DAT, imazamox reduced shoot biomass to a similar level as most other herbicide treatments. The selectivity provided by this rate of imazamox warrants its use in mixed stands of many native species (Rodgers and Black 2012). The addition of flumioxazin increased control compared to imazamox alone in Experimental Run 2 but not Run 1. Additionally, this tank mix reduced shoot biomass in a similar manner to imazamox alone at 60 DAT. The utility of mixing PPO-inhibiting herbicides with amino acid inhibitors often includes increasing the spectrum of weed control or increasing efficacy on difficult-to-control species. Treating dense stands of Uruguay waterprimrose in Florida often requires an initial application and a follow-up treatment at approximately 2 wk. The lack of short-term visual effects has been cited by resource managers as a weakness of using imazamox alone or other slow-acting herbicides for *Ludwigia* spp. control.

For the closely related *Ludwigia grandiflora* [Michx.] Greuter & Burdet, Emerine et al. (2010) calculated the EC₇₀ for imazamox at 116 g ha⁻¹ for shoot dry weight reduction at 5 WAT. This was substantially more activity than we observed in our study at 5 WAT. This difference in results

TABLE 3. PREPLANNED COMPARISONS OF URUGUAY WATERPRIMROSE BIOMASS RESPONSE TO HERBICIDE RATE (AMINOPYRALID OR FLORPYRAUXIFEN-BENZYL) AND HERBICIDE TANK MIXES WITH EITHER GLYPHOSATE OR IMAZAMOX. SIGNIFICANT VALUES ARE GIVEN IN BOLD FOR CLARITY.

Preplanned Comparison	DF	Above Water		Below Water
		Rate or Tank Mix	Interaction with DAT ¹	Rate or Tank Mix
florpyrauxifen-benzyl rate	1	0.450	0.253	0.877
Aminopyralid rate	1	0.822	0.768	0.413
Imazamox ± flumioxazin	1	0.431	0.177	0.385
Glyphosate ± flumioxazin	1	0.399	0.512	0.628
Glyphosate ± 2,4-D	1	< 0.001	0.019	0.985

¹Interaction of herbicide rate or tank mix comparison with days after treatment (DAT) for above-ground biomass.

may be attributed to the age of the plants. In that study, plants were very young (~ 3 wk old) with limited shoot growth (15 to 20 cm), while we used well-established plants that were several months old. Other possible explanations may include differential sensitivity to imazamox between creeping waterprimrose and Uruguay waterprimrose. However, this has yet to be tested.

Overall, these results indicate that Uruguay waterprimrose shoot control can be achieved with several treatment options that vary from nonselective (glyphosate + 2,4-D) to selective (imazamox). However, it also points to the inherent difficulty in effective control of the below-water biomass accumulated by Uruguay waterprimrose, which is needed for long-term control and potential patch eradication. Further studies are also warranted in relation to potential seasonality of treatment effectiveness as these studies were conducted in the coolest part of the year. Specifically, studies that examine herbicide efficacy on well-established plants with spring and summer treatments are needed. Additionally, studies to examine retreatment intervals to reduce Uruguay waterprimrose below-water biomass over time should also be undertaken.

SOURCES OF MATERIALS

¹Professional top soil, Margo Garden Products, Inc., 134 Delia Nelson St. Folkston, GA 31537.

²Osmocote Plus, The Scotts Company, 14111 Scottslawn Road, Maryville, OH 43041.

³Milestone Specialty Herbicide, Dow AgroSciences LLC, 9330 Zionsville Road, Indianapolis, IN 46268.

⁴florpyrauxifen-benzyl, SePRO Corporation, 11550 North Meridian St., Suite 600, Carmel, IN 46032.

⁵Clearcast (imazamox 120 g ai L⁻¹). SePro Corporation, 11550 North Meridian St., Carmel, IN 46032.

⁶Clipper, Valent USA Corporation, P.O. Box 8025, Walnut Creek, CA 94596.

⁷Rodeo herbicide, Dow AgroSciences LLC, 9330 Zionsville Road, Indianapolis, IN 46268.

⁸Weedar 64, Nufarm Inc., 11901 S. Austin Ave., Alsip, IL 60803.

⁹MSO concentrate, Loveland Industries, P.O. Box 1286, Greeley, CO 80632.

¹⁰Induce, Helena Chemical Company, 225 Schilling Blvd., Suite 300, Collierville, TN 38017.

¹¹Teejet Technologies, 1801 Business Park Dr., Springfield, IL 62703.

¹²SAS® PROC GLIMMIX, SAS Institute, 100 SAS Campus Dr., Cary, NC 27513.

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