The influence of fluctuating water depth on herbicide efficacy for Uruguay water primrose (Ludwigia hexapetala) control

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ABSTRACT

Rapid fluctuations in water level are a common occurrence in many Florida waterbodies. While responses to fluctuating hydrology have been examined for many emergent invasive plant species, the influence these responses may have on herbicide efficacy is not well understood. Uruguay water primrose [Ludwigia hexapetala (Hook. & Arn.) Zardini, Gu, & Raven] is an aggressive, emergent species that is difficult to control due to its immense creeping stem biomass. Given the difficulty in management, our objective was to determine how changes in hydrology can influence its control using herbicides. Mesocosm studies were conducted in 2017 to examine efficacy of a single herbicide treatment (imazamox plus carfentrazone) in relation to varying initial and final water depth. Mesocosms were either maintained at 20 or 40 cm inundation throughout the experiment, or were subjected to a change in water level following herbicide treatment (mesocosms were either flooded from 20 to 40 cm, or underwent a drawdown from 20 to 40 cm). We found that herbicide effectiveness increased for plants that were subjected to a low initial water depth followed by an increase in water levels after herbicide treatment. Shoot biomass above the waterline regrew to levels that were similar to the nontreated control when water depths were consistently low, consistently high, or fluctuated from high to low following herbicide treatment. These results indicate that changing water depth influences Uruguay water primrose control and that the pattern of change contributes to this. Aquatic managers may optimize control with late spring treatments when water is low just before the onset of the rainy season.

Key words: carfentrazone, emergent invasive plant, hydroperiod, imazamox, Ludwigia hexapetala (Hook. & Arn.) Zardini, Gu, & Raven.

INTRODUCTION

Controlling emergent invasive plants in wetlands, marshes, and littoral zones is often challenging for aquatic managers. Emergent plants live in “two worlds” and are subject to both the benefits and stressors of the emerged and submersed environments. In Florida, emergent species are often subject to rapidly changing water levels. Sudden flooding occurs during the annual transition from dry to wet season in the early summer, or in late summer through fall following hurricanes (Kushlan 1986). Systems may also undergo rapid reductions in water level during flood prevention efforts prior to major storms. Water levels in coastal freshwater systems are also subject to tidal influence; for example, in the St. John’s River in eastern peninsular Florida, tidal influences can result in water depth fluctuations as far as 259 km upstream (Bourgerie 1999).

These hydrologic fluctuations can have a profound impact on plant physiology and morphology. For example, many emergent species exhibit high seasonal variation in leaf morphology and plant cuticle composition, strong adventitious rooting from the nodes of stem tissue, production of aerenchyma tissues for gas exchange, and the ability to tolerate rapid changes in water levels (Kibbler and Bahnisch 1999). For invasive plants, these responses to flooding may alter their competitive ability with native species (Conner et al. 2001, Zhang et al. 2014) and can potentially affect their response to herbicide applications (Prince et al. 2019). In particular, herbicide efficacy may be affected by the changes in leaf area, root:shoot ratios, or photosynthetic rates that result from flooding (Chen et al. 2002, Rood et al. 2010, Varanasi et al. 2016).

Plant response to changing water levels has been well studied for many invasive species, such as torpedograss (Panicum repens L.) (Hossain et al. 2002, Smith et al. 2004, Prince et al. 2019) and common reed (Phragmites australis (Cav.) Trin. ex Steud.) (Hellings and Gallagher 1992, Mauchamp et al. 2001, Vretare et al. 2001). However, few studies have examined the influence of flooding on herbicide efficacy for invasive emergent plants. Understanding the relationship between flooding and herbicide efficacy may be critical for successful management of species that have invaded Florida’s wetlands, such as Uruguay water primrose [Ludwigia hexapetala (Hook. & Arn.) Zardini, Gu, & Raven]. This emergent species, in the Onagraceae family, is native to South America and is believed to have been introduced to the United States in the mid 1800s (Kaufman and Kaufman 2012). The species is now present across the far western and southeastern United States, with Florida and California as hotspots of invasion. Uruguay water primrose primarily spreads by long creeping stems that float and are fragmented with disturbance. Stem fragmentation is strongly linked to its invasiveness, as Okada et al. (2009) found little genetic variation across a
wide range of populations in California. Creeping stems exhibit considerable tolerance to a range of environmental conditions, from complete inundation to full exposure when water levels are low. Uruguay water primrose is adapted to these types of fluctuations through morphological plasticity. Plants growing in deep water exhibited reduced root : shoot ratios and overall biomass compared to plants found in shallow water, as well as elongated stems and reduced lateral growth (Thouvenot et al. 2013).

Herbicide use and mechanical treatments on large lakes are the primary tools for controlling Uruguay water primrose. In a mesocosm study, Enloe and Lauer (2017) found several herbicide treatments provided effective control of shoot biomass above the waterline, including imazamox as a standalone treatment or in combination with the protoporphyrinogen oxidase inhibitor fluometuron. However, no herbicide treatment reduced shoot biomass below the waterline. This experiment was conducted at a constant water depth of 15 cm above the pot surface in each mesocosm. Given the limited control observed under constant flooded conditions and the importance of herbicide control for this species, it is crucial to understand herbicide efficacy on this species.

Our objective for this study was to evaluate the impact of fluctuating water levels pre- and post-herbicide treatment on herbicide efficacy for Uruguay water primrose. We hypothesized that 1) control (as measured by a decrease in biomass) would be better in consistently shallow versus consistently deep water, 2) raising the water level from shallow to deep after treatment would improve control, and 3) lowering the water level from deep to shallow after treatment would decrease control. A better understanding of these relationships would greatly assist managers in dealing with this difficult to control species.

MATERIALS AND METHODS

A greenhouse experiment was conducted in the spring and repeated in the summer of 2017 at the University of Florida’s Center for Aquatic and Invasive Plants in Gainesville, FL. Lateral stem fragments (approximately 15 cm in length) of Uruguay water primrose were planted in 90 pots (3.8 L) filled with a commercial potting mix1 and 15 g pot⁻¹ of a complete slow release fertilizer.2 Pots were maintained in shallow tubs filled with well water (7.4 pH) to a depth of 15 cm. Approximately 4 wk after planting, three pots each were placed in 100-L mesocosms (for a total of 30 mesocosms) and the water depth was raised to 30 cm above the soil level. These were grown for an additional 8 wk until plants were well established with submersed and emersed stem biomass.

Twelve weeks after planting, the mesocosms were assigned to one of two water depths (hereafter referred to as initial water depth): 20 or 40 cm. To achieve these initial depths, the water level was either raised to 40 cm or lowered to 20 cm and maintained for 7 d prior to herbicide treatment. Baseline data were then collected on three replicate tubs for each water depth. A digital image was captured of the shoot growth in each tub by placing a camera at 60 cm directly above the water surface. Each image was then subjected to analysis of green shoot cover using the CANOPEO app (Patrigiani and Ochsner 2015), which provided an accurate assessment of Uruguay water primrose shoot cover (leaves plus stems) above the waterline. Pots were then harvested and separated into submersed and emersed shoots as well as belowground components. These were oven dried at 65°C for 72 h and weighed.

Immediately following the baseline harvest, mesocosms were assigned to one of two treatments, including a herbicide treatment and a nontreated control. The herbicide treatment was a tank mix of imazamox3 (280 g ae ha⁻¹) plus carfentrazone4 (66 g ai ha⁻¹), which is commonly used in Florida for treatment of this species. A methylated seed oil5 was added to the herbicide treatment at 2.3 L ha⁻¹. The herbicide treatment was applied using a carbon dioxide–pressurized sprayer at an application volume of 935 L ha⁻¹ that was delivered through a single TeeJet® 800067 nozzle.6 Water was exchanged in all tubs 24 h after treatment and tubes were refilled to the same pretreatment levels of 20 and 40 cm. Care was taken during the exchange process to prevent washing the treated leaves, so they remained dry throughout the exchange.

At 72 h following herbicide treatment, water depth was again adjusted in each tub (hereafter referred to as final water depth) to simulate fluctuating or constant water depth. For mesocosms with an initial water depth of 20 cm, the water level in half of the replicates was raised to 40 cm to simulate a sudden flood event following herbicide application; the other half of these mesocosms was maintained at a constant 20 cm. For mesocosms with an initial water depth of 40 cm, half of the replicates were lowered to 20 cm to simulate a rapid drawdown, while the remaining half was maintained at 40 cm. This created a complete factorial arrangement of three factors: initial water depth (20 or 40 cm), herbicide treatment (herbicide or no herbicide), and final water depth (20 or 40 cm). This simulated constant conditions (high or low) and fluctuating water conditions (high to low, and low to high) associated with herbicide treatment. At 60 d after herbicide treatment, all pots were harvested, dried, and weighed as previously described for the baseline data.

Statistical analysis

Analysis of the baseline data compared means with respect to initial treatment depth only. The ANOVA was performed using log-transformed variables for all parameters measured. ANOVA for the main part of the experiment was performed using SAS® PROC GLIMMIX (Littell et al. 1996) with experimental run considered a random effect and initial depth, final depth, and herbicide treatment considered fixed effects for this completely randomized design within each run. Variance was not homogeneous across treatments and was grouped as determined by graphical examination of residuals and comparisons of Akaike’s Information Criteria for alternate error structures. Variance for pretreatment cover increased with cover but variance also varied by initial depth. This was resolved by
performing the analysis using the log-transform of cover with variance grouped by initial depth. The analysis of below-water shoot mass, total shoot mass, root mass, and total root plus shoot mass did not require transformation but variances were grouped for each combination of herbicide and final depth for total shoot, root, and total root plus shoot mass. The analysis of above-water shoot mass took into consideration that shoot mass could be zero and that variance was related to both mass and final water depth. This was resolved by considering above-water shoot biomass as counts that have a Poisson distribution with overdispersion parameters that differed by final depth. Post-hoc multiple comparisons were made at the 5% level using Tukey’s adjustment.

RESULTS AND DISCUSSION

Baseline Uruguay water primrose shoot cover strongly differed by initial depth. Shoot cover in the 20-cm depth mesocosms averaged 28% while cover in the 40-cm depth mesocosms was 14% (Table 1). This baseline difference was also significant in harvested above-water shoot biomass; plants grown in the 40-cm depth had 66% less cover compared to those in the 20-cm depth. No other baseline parameters differed between depths. This result was largely expected, as there was not enough time after initiation of flooding treatments for plants to respond in a manner that would result in a quantifiable reallocation of resources. Flooding may affect herbicide efficacy via the morphological and physiological responses of the plants themselves, or due to the high water level limiting the amount of plant tissue that is exposed to foliar applications (Prince et al. 2019). Our experimental design allowed us to isolate this difference in the amount of exposed shoot cover and biomass at the time of herbicide treatment on the experimental outcomes.

There was a significant three-way interaction between initial depth, herbicide treatment, and final depth for final above-water shoot biomass 60 d after treatment ($P = 0.002$). This interaction was driven by one treatment (treated with herbicide; initial depth = 20 cm, final depth = 40 cm). In this treatment, shoot biomass was nearly eliminated above the waterline and was significantly lower than all other treatments by 91 to 95% (Figure 1). There were no other differences in above-water shoot biomass among any treatments.

Sudden flooding, similar to what was imposed on plants in this experiment, can cause stress responses in plants; for example, gas exchange and light availability become severely limited for flooded leaves, thus reducing their photosynthetic capacity (Mommer and Visser 2005). However, *Ludwigia* spp. are well adapted to changes in water level (Thouvenot et al. 2013), and plants that were flooded, but not treated with herbicide, showed no reductions in shoot biomass compared to nonflooded plants. It is possible that emergent shoot loss due to the herbicide activity limited the ability of Uruguay water primrose plants to respond to flooding stress, resulting in increased control of shoot biomass. Observationally, herbicide treatment rapidly destroyed the abundant pneumatophores that were present just prior to treatment, and their recovery was slow to occur (S. F. Enloe, unpub. data). Additionally, sublethal herbicide concentrations present in surviving tissue may slow recovery through continued interference with plant metabolic processes. Herbicide application followed by flooding has been shown to increase control for other species, including torpedograss (*Enloe et al. 2018*) and paragrass [*Urochloa mutica* (Forssk.) T.Q. Nguyen] (Chaudhari et al. 2012).

The lack of any other significant differences in final above-water shoot biomass for this three-way interaction suggests some innate resiliency of Uruguay water primrose to both the herbicide treatment and the other water depth conditions imposed in this experiment. Shoot biomass above the waterline recovered from herbicide treatment in the consistently shallow (20-cm) or consistently deep (40-cm) water depth treatments, as well as in the fluctuation from deep to shallow (40- to 20-cm) treatment. Therefore, from an above-water shoot biomass perspective, we reject our hypothesis of better control in the constant shallow versus constant deep conditions. However, the data support our other two hypotheses, regarding better control in the shallow-to-deep herbicide treatment and poorer control in the deep-to-shallow herbicide treatment.

There were no interactions between the three factors for final below-water shoot biomass. However, it was influenced by herbicide treatment ($P < 0.001$) and final water depth ($P$

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**Table 1. Baseline parameter comparisons for Uruguay water primrose by initial water depth.**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>20 cm Mean ± SE</th>
<th>40 cm Mean ± SE</th>
<th>$P$ Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Above-water shoot cover (%)</td>
<td>28.3 ± 3.4</td>
<td>14.5 ± 1.7</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Above-water shoot biomass (g)</td>
<td>13.6 ± 3.8</td>
<td>5.1 ± 1.2</td>
<td>0.016</td>
</tr>
<tr>
<td>Below-water shoot biomass (g)</td>
<td>28.5 ± 4.7</td>
<td>36.6 ± 6.2</td>
<td>0.305</td>
</tr>
<tr>
<td>Total shoot biomass (g)</td>
<td>44.1 ± 7.4</td>
<td>41.7 ± 7.1</td>
<td>0.876</td>
</tr>
<tr>
<td>Root biomass (g)</td>
<td>3.6 ± 1.0</td>
<td>4.9 ± 0.9</td>
<td>0.516</td>
</tr>
<tr>
<td>Total biomass (g)</td>
<td>49.7 ± 8.2</td>
<td>46.6 ± 7.8</td>
<td>0.835</td>
</tr>
</tbody>
</table>

Figure 1. Uruguay water primrose above-water shoot biomass response to initial water depth, herbicide treatment of imazamox (280 g ha$^{-1}$) plus cafentrazone (66 g ha$^{-1}$), and final water depth. Bars represent means ± SE. Bars with the same letter are not significantly different at $P > 0.05$ using Tukey’s adjustment. Initial and final depth are expressed in centimeters.
Herbicide treatment reduced final below-water biomass (pooled across depths) from 45.2 ± 2.5 g tub⁻¹ to 31.1 ± 2.5 g tub⁻¹ (a 31% reduction from the nontreated control). This is somewhat in contrast to the findings of Enloe and Lauer (2017), who tested several herbicides and tank mixes for Uruguay water primrose control, but found none that significantly reduced underwater biomass. However, previous research (Enloe and Lauer 2017) did not evaluate the specific tank mix used in this study. In addition, water levels were lower in the prior study (15 cm) compared to the current study (20 to 40 cm).

Vegetative reproduction is the primary mechanism of Uruguay water primrose spread and regeneration, given that seed production is reported to be extremely limited (Okada et al. 2009). Shoot biomass below the waterline is primarily composed of the creeping stems that are responsible for vegetative reproduction; the observed reduction in underwater creeping stem biomass is therefore important, as it suggests that the imazamox plus carfentrazone tank mix may reduce the potential for regeneration. A 31% reduction with a single herbicide treatment, although limited, also suggests that repeated treatments may help to reduce the abundance of this aggressive species. Future research should examine this, as has been done for other species such as torpedograss (Enloe et al. 2018) and cogongrass [Imperata cylindrica (L.) P. Beauv.] (Aulakh et al. 2012).

Final water depth was also a significant main effect (P = 0.009) on below-water shoot biomass, which was 22% greater in mesocosms with a final water depth of 40 cm (42.9 g tub⁻¹) compared to a final depth of 20 cm (33.5 g tub⁻¹). This is indicative of Uruguay water primrose’s ability to proliferate creeping stems in deeper water and may contribute to the difficulty of controlling plants. Total root biomass was also influenced by herbicide treatment (P = 0.019), but no other factors or interactions were significant. When pooled across both depth factors, herbicide treatment reduced root biomass from 8.4 ± 1.0 g tub⁻¹ to 5.5 ± 0.6 g tub⁻¹ (34% reduction).

For total biomass (root plus above-water shoot plus below-water shoot), there was a significant two-way interaction between initial water depth and herbicide treatment (P = 0.049). In the 20-cm initial depth, herbicide treatment reduced final shoot biomass by 55% compared to the nontreated control (Figure 2). However, for the 40-cm initial depth, there was no difference in total biomass between the herbicide-treated and nontreated plants. This indicates that Uruguay water primrose growing at the initially greater depth was significantly more difficult to control than in the initially shallow depth.

This lack of control is consistent with previous findings by Prince et al. (2019), who found that torpedograss growing in flooded conditions was more difficult to control with the graminicides sethoxydim and fluazifop-P-butyl than in nonflooded conditions. Flooding may inhibit herbicide efficacy through a number of mechanisms. Prior research on the same Ludwigia species in France suggests that growth rates are decreased as water level increases (Thouvenet et al. 2013); this may reduce translocation of systemic herbicides such as imazamox. Flooding may also limit herbicide efficacy by reducing the number of leaves above the waterline, thus limiting foliar uptake of herbicide into the plant.

These results provide insight into the influence of fluctuating hydrology on herbicide efficacy for Uruguay water primrose management. Herbicide treatment was a key driver in influencing a change in most Uruguay water primrose biomass components (above water shoot, below water shoot, root, and total biomass), while initial and final water depths were somewhat less important. However, control of final above water shoot biomass increased when the water level was raised following herbicide treatment. This strongly indicates that flooding following herbicide treatment creates an added stressor that inhibits recovery of emergent plants. Applicators may be able to improve Uruguay water primrose control by treating in the late spring or early summer, just before the onset of the rainy season when water levels can rise rapidly. Unfortunately, it would be difficult to make that recommendation for prehurricane applications, as invasive plant treatment as a priority is rapidly diminished due to other pressing concerns. For fluctuating river systems such as the St. John’s River, strategies timed to treat Uruguay water primrose just prior to strong tidally driven increases in water level may also improve control. However, we acknowledge the difficulty for applicators to embrace such narrow windows of application timing due to the overwhelming need for continuous management operations throughout the year.

Further research should investigate seasonality of treatment, which is strongly linked to hydrologic conditions. Our studies were conducted over the spring and summer and do not account for shorter day length or cooler conditions in the fall, when many perennial plants increase allocation of photosynthates into storage organs for overwintering (Wersal et al. 2011, Wersal et al. 2013). It is unclear on the role that seasonality plays in Uruguay water primrose photosynthetic activity, especially in relation to storage.
Future work should carefully examine the physiology of carbohydrate storage in this species to better our understanding of possible weak links in its life cycle.

**SOURCES OF MATERIALS**

2. Osmocote Plus, The Scotts Company, 14111 Scottslawn Road, Maryville, OH 43041.
3. Clearcast (imazamox 120 g ai L⁻¹), SePro Corporation, 11550 North Meridian St., Carmel, IN 46032.
4. Stingray aquatic herbicide, SePro Corporation, 11550 North Meridian St., Carmel, IN 46032.
5. MSO concentrate, Loveland Products, Inc., 3005 Rocky Mountain Ave, Loveland, CO 80538.
6. TeeJet® Technologies, 1801 Business Park Dr., Springfield, IL 62703.

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**LITERATURE CITED**


