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In-water activity of glyphosate, 2,4-D, and diquat on waterhyacinth (*Eichhornia crassipes*)

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ABSTRACT

Waterhyacinth is an aggressive floating macrophyte that has been traditionally managed using foliar applications of 2,4-D and diquat. Recent research suggests that 20–25% of herbicide is lost to the water column. Here we evaluate the relative efficacy of subsurface applications of 2,4-D, diquat, and glyphosate to determine if spray loss from foliar applications provides additional efficacy through absorption from roots and submersed leaves. Plants were established in mesocosms and treated with diquat at rates of 100, 200, 400, 800, 1600, or 3200 μg L^{-1}. Both 2,4-D and glyphosate were applied at rates of 125, 250, 500, 1000, 2000, 4000, or 8000 μg L^{-1}. Total plant biomass was harvested after 28 days of static exposure. Results suggest that subsurface diquat applications are effective at waterhyacinth control, with total plant death observed at 3200 μg L^{-1} and biomass reductions of 92% at 1600 μg L^{-1}. Neither 2,4-D or glyphosate was effective at reducing waterhyacinth biomass regardless of application rate. Results suggest that spray loss from glyphosate and 2,4-D applications represents wasted product and cost, whereas spray loss from diquat may provide additional efficacy on waterhyacinth.

Key words: subsurface applications, chemical control, herbicides, invasive aquatic plants, floating aquatic plants.

INTRODUCTION

Waterhyacinth [*Eichhornia crassipes* (Mart.) Solms] is an aggressive aquatic macrophyte that forms dense floating mats. It was introduced to the United States in 1884 as an ornamental plant at the Cotton Centennial Exposition in New Orleans; by the early 1900s, it had spread throughout the southeastern United States as far north as Virginia (Penfound and Earle 1948). Waterhyacinth has since invaded over 57 countries and is widely accepted as the world's worst aquatic weed (Bhattacharya et al. 2015). Without management, waterhyacinth mats limit access to waterbodies, degrade water quality, and create ideal habitat for disease vectors such as mosquitoes and snails (Penfound and Earle 1948, Seabrook 1962, Schreiner 1980, Ofulla et al. 2010). To mitigate these effects, waterhyacinth populations in Florida are maintained at the lowest feasible level (i.e., maintenance control) through frequent monitoring and herbicide application (Mudge and Netherland 2014). Continued management is expensive; in Florida alone, the Florida Fish and Wildlife Conservation Commission (FWC) spent over $4 million managing floating plants from 2019 to 2020 (FWC 2021) and in the past has spent over $6 million a year on floating plant management (FWC 2010).

Waterhyacinth control has relied heavily on foliar applications of 2,4-D and diquat since the 1950s. These herbicides result in rapid symptom development followed by plant death. Visual markers allow applicators to distinguish between treated and nontreated plants preventing reapplication to those showing herbicide injury symptoms (Mudge and Netherland 2014). This is critical for managing free-floating plants in large bodies of water where multiple crews are working on the system simultaneously and plants move with wind and water currents. Foliar application of other products (such as glyphosate and penoxsulam) is also effective on waterhyacinth is are not as commonly used since herbicide symptoms take longer to become visual.

Foliar application techniques on aquatic plants typically consist of high carrier volume (935 L ha^{-1}) sprayed from a single-nozzle handgun (Haller 2020). This application technique has not changed for decades because of applicator familiarity, reliability, and frequency of success. While this foliar application technique is effective, high carrier volumes can appear excessive to some public stakeholders because of common misconceptions and chemophobia (Saleh et al. 2021, Evans and Rollins 2012). Exploring more discrete management techniques is important to improve public perception while maintaining effective control of invasive plants.

Recent research suggests that foliar applications using high carrier volumes can result in substantial herbicide spray loss into the water column (Mudge et al. 2021). When waterhyacinth plants were treated at 100 percent area covered (PAC), applications at 935 L ha^{-1} resulted in 20–25% spray loss. While this may result in wasted product and treatment cost, it is possible that herbicide entering the water could be absorbed by the roots and submersed leaves/stolons, thereby offering increased efficacy. Conversely, decreasing carrier volume resulted in greater spray retention and limited spray loss in applications to waterhyacinth, waterlettuce (*Pistia stratiotes* L.), and giant salvinia (*Salvinia molesta* D. S. Mitchel) (Sperry et al. 2022). Likewise, reduced carrier volume applications that result in lower spray coverage have been shown to provide equivalent or

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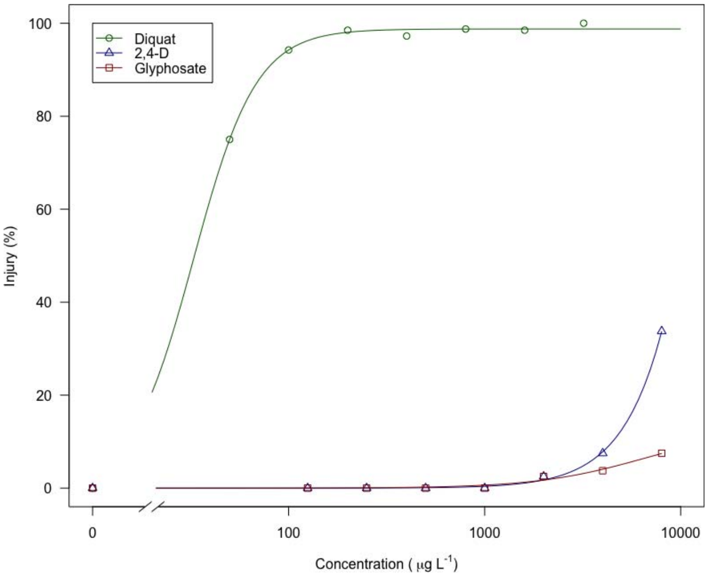

Figure 1. Dose response curves for injury (%) of *Eichhornia crassipes* plants in the second experimental run 28 days after subsurface applications of diquat (green line, open circles), 2,4-D (blue line, open triangles), or glyphosate (red line, open squares). All data series were fitted to a three-parameter log-logistic model, $Y = d/\{1 + \exp[b(\log x - \log e)]\}$. Symbols are means ($n = 4$) of observed injury.

enhanced waterhyacinth control compared to high carrier volume applications (Sperry and Ferrell 2021). Limited research has been conducted exploring the relationship between foliar spray deposition in the water column and efficacy of herbicide on floating plants. If herbicide deposition into the water column provides efficacy on waterhyacinth, application techniques could be adjusted to promote increased water column deposition. Therefore, the objective of this study was to evaluate the dose-response relationships of waterhyacinth to in-water exposures of 2,4-D, diquat, and glyphosate.

MATERIALS AND METHODS

A greenhouse experiment was established at the University of Florida's Center for Aquatic and Invasive Plants in Gainesville, FL. Waterhyacinth plants from local culture were established and maintained in 900 L stock mesocosms filled with well water amended with 100 mg L^{-1} fertilizer[1] and 20 mg L^{-1} chelated iron[2] prior to initiation of experiments. The first and second experimental runs were established in January and May 2021, respectively.

Waterhyacinth plants were selected for uniformity in size (approximately 15–30 cm in width) and established in 18.9 L mesocosms amended with the same fertilizers and rates described above for stock mesocosms. There were three plants per experimental unit. Mesocosms were maintained in a greenhouse for the duration of the experiment. After 14 days of acclimation, herbicide doses were administered subsurface using a syringe. Diquat[3] was applied at rates of 0, 100, 200, 400, 800, 1600, or 3200 μg L^{-1}. Both 2,4-D[4] and glyphosate[5] were applied at rates of 0, 125, 250, 500, 1000, 2000, 4000, or 8000 μg L^{-1}. We used rates above and below the maximum-labeled rates for each herbicide to evaluate dose-response relationships (see Seefeldt et al. 1995). Experiments were set up as a completely randomized design with four replications and herbicide exposures were static. At 28 days after treatment, plant biomass was harvested, dried in a forced-air drying oven at 60 C for four days, and weighed. Plant injury (%) was visually evaluated on a scale from 0 (no effect) to 100% (plant death) at 28 days after treatment in the second experimental run only.

Data were analyzed using nonlinear regression models in the *drc* package in R version 3.6.1 (Ritz et al. 2015). All data series were fitted to a three-parameter log-logistic model:

$$Y = \frac{d}{1 + \exp[b(\log x - \log e)]}$$

where Y is biomass (grams) or injury (percentage), d is the upper limit, x is the herbicide application rate, e is the value of x at the inflection point of the curve, and b is the slope of the curve at e (Ritz 2010). Lack-of-fit tests were performed to verify that each model was appropriate. Effective dose (ED_{50} and ED_{90}) values were also calculated for each herbicide to determine the application rate resulting in a 50% and 90% reduction in plant biomass. Biomass data were pooled across experimental runs for analysis.

RESULTS AND DISCUSSION

Injury was not recorded for the first experimental run. In the second experimental run, subsurface application of diquat resulted in the highest injury rating on waterhyacinth (Figure 1) when compared to applications of 2,4-D and glyphosate 28 DAT. A dose-response relationship was observed, where injury increased with increasing diquat concentration. Injury caused by 2,4-D was minimal except for the 8000 μg L^{-1} dose (Figure 1). Glyphosate resulted in minimal injury across the entire range of experimental concentrations. By 200 μg L^{-1}, plants treated with diquat reached almost 100% injury rating. At 8000 μg L^{-1}, plants treated with 2,4-D and glyphosate had injury ratings of approximately 34% and 7.5%, respectively.

Waterhyacinth biomass was reduced from in-water diquat exposure across all tested doses (Figure 2). Biomass was reduced 100 and 92% from diquat doses of 3200 and 1600 μg L^{-1}, respectively. This was supported by the low effective dose values, which indicated that concentrations (mean ± the 95% confidence interval) of 10.3 ± 21.5 and 669.8 ± 968.9 μg L^{-1} were required to achieve biomass reductions of 50 and 90%, respectively (Table 1). Glyphosate treatment resulted in no biomass reduction regardless of application rate, and we were unable to fit a dose-response curve or calculate effective dose values for this herbicide. 2,4-D applications resulted in biomass reductions only at the highest concentration of 8000 μg L^{-1} (where plants had on average 49 ± 9.5% less biomass than the nontreated controls) (Figure 2) and had higher effective dose values (ED_{50} and ED_{90} values of 8131.5 ± 908 μg L^{-1} and 10,069.7 ± 7969.6 μg L^{-1}, respectively). The maximum labeled rate of 2,4-D on waterhyacinth in the field is 4.3 kg ai ha^{-1}. Treating plants in 30 cm of water would result in 1400 μg L^{-1}, assuming all of the spray solution entered the water. This is

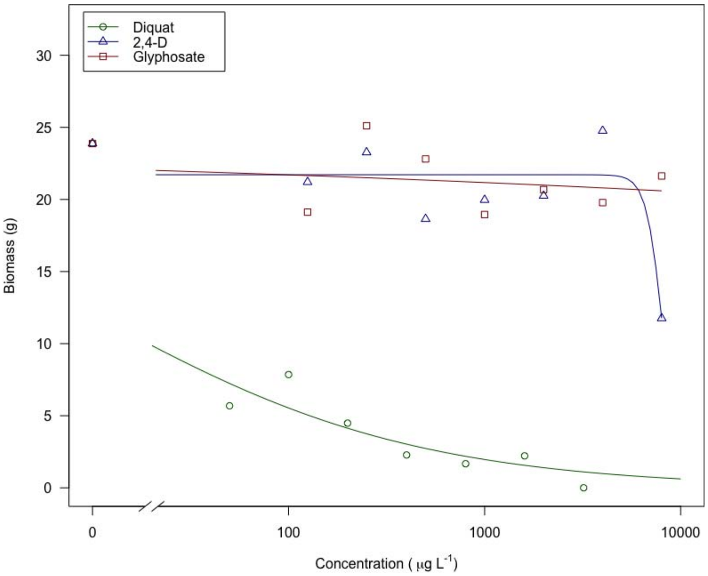

Figure 2. Dose response curves for biomass of *Eichhornia crassipes* plants 28 days after subsurface applications of diquat (green line, open circles), 2,4-D (blue line, open triangles), or glyphosate (red line, open squares). Diquat and 2,4-D were fitted to a three-parameter log-logistic model, $Y = d/\{1 + \exp[b(\log x - \log e)]\}$. Glyphosate data points are plotted with a trend line. Symbols are means ($n = 8$) of observed injury.

well below what is required to achieve 50% or 90% reductions in plant biomass based on our results (Table 1). In addition, the biomass reduction resulting from the highest 2,4-D application was still less than that observed at the lowest concentrations of diquat.

While 2,4-D is highly effective as a foliar treatment for waterhyacinth, it was not effective in subsurface applications in this experiment. Additionally, 2,4-D has an aqueous half-life of 13 days (Jote 2019) and is biodegraded by aquatic microorganisms (Hemmett and Faust 1969). It is unknown what effect dissipation had on efficacy in this study, but it is assumed that this limitation would be magnified under field conditions. Regardless, the maximum 2,4-D rate in the field for waterhyacinth is 0.69 kg ai ha^{-1}. If treating plants in 30 cm of water, this would result in 1407 μg L^{-1}, significantly below what is required to achieve 50 or 90% reductions in plant biomass (Table 1). These factors illustrate that 2,4-D will not produce acceptable results for subsurface applications on waterhyacinth.

Glyphosate also had minimal effects on waterhyacinth in this study. This was expected because glyphosate is inactive in water due to microbial degradation, interaction with salts, and its strong affinity to bind to suspended soil particles (Roberts et al. 1998). Under mesocosm conditions, glyphosate also has an aqueous half-life of 16 days (Souza et al. 2017), and we would expect even less activity in pond or lake water due to greater microbial activity. In addition, glyphosate is translocated in the phloem, so translocation from the roots is expected to be minimal (Roberts et al. 1998). Moreover, glyphosate is highly water soluble (15.7 mg/L), and it not likely to partition in plant tissues from an aqueous matrix (Shaner et al. 2014).

TABLE 1. EFFECTIVE DOSES REQUIRED TO CAUSE 50 AND 90% BIOMASS REDUCTION (ED_{50} AND ED_{90}) WITH 95% CONFIDENCE INTERVALS FOR *EICHHORNIA CRASSIPES* PLANTS 28 DAYS AFTER SUBSURFACE STATIC EXPOSURE OF 2,4-D, DIQUAT, AND GLYPHOSATE ($N = 8$).

	Concentration (μg L^{-1}), mean ± 95% C.I.	
Herbicide	ED_{50}	ED_{90}
2,4-D	8,131.5 ± 908	10,069.7 ± 7,969.6
Diquat	10.3 ± 21.5	669.8 ± 968.9
Glyphosate	ND[1]	ND

[1] ND = not determined because glyphosate exposure never resulted in 50% reduction in biomass.

We observed notable subsurface activity for diquat applications on waterhyacinth. Diquat is only minimally degraded by microorganisms (Simsiman 1976) and its aqueous half-life ranges from several hours to 2 days (Shaner et al. 2014). Diquat has been shown to move through the xylem in various plant species and competitively inhibits the reduction of NADP in chloroplasts (Thrower et al. 1965, Sheldrick 1967, Wong 2000). We observed that plants treated with diquat first showed phytotoxicity symptoms in the leaves, rather than petioles or root tissue. We hypothesize that subsurface applications of diquat translocated though the xylem in waterhyacinth roots and remained inactive until it reached the chloroplasts in the leaves. This is consistent with previous findings that indicated waterhyacinth has greater chlorophyll content in leaf tissues than in petioles (Dray et al. 2012). The maximum rate for diquat in the field is 0.36 kg ai ha^{-1}. Treating plants in 30 cm of water would result in 1481 μg L^{-1}, assuming all of the spray solution entered the water; this is well above what is required to achieve 50 or 90% reductions in plant biomass based on our results (Table 1).

These results suggest that diquat spray loss into the water column from foliar applications may provide supplemental control of waterhyacinth. However, this is a preliminary trial, and further research is needed to understand the utility of in-water diquat treatments for waterhyacinth control. In addition, our results suggest that spray loss of 2,4-D and glyphosate are not likely to provide additional phytotoxicity and represents wasted product and treatment cost. Research has shown that 20–25% of herbicide is lost to the water column from foliar applications at standard carrier volumes (935 L ha^{-1}), and that reducing carrier volume to 187 L ha^{-1} provides equivalent or enhanced waterhyacinth control (Mudge et al. 2021, Sperry and Ferrell 2021). Given this, and that spray loss to the water column is not likely to provide additional control, efforts are needed to reduce carrier volumes for glyphosate and 2,4-D applications. In addition, further research is needed to determine if subsurface applications of diquat can be useful under field conditions.

SOURCES OF MATERIALS

[1] 24-8-16, Miracle-Gro® All Purpose Plant Food, Scotts Company, Marysville, OH.

[2] Grow More Iron Chelate 10%, Grow More, Gardena, CA.

[3] Tribune, Syngenta Crop Protection LLC, Greensboro, NC.

[4] Alligare 2,4-D Amine, Alligare LLC, Opelika, AL.

[5] Roundup Custom, Bayer CropScience LLC, Research Triangle Park, NC.

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