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Absorption and translocation of florpyrauxifen-benzyl in ten aquatic plant species

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Abstract

Additional active ingredients are needed for use in aquatic systems to respond to new threats or treatment scenarios, enhance selectivity, reduce use rates, and mitigate the risk of herbicide resistance. Florpyrauxifen-benzyl is a new synthetic auxin developed for use as an aquatic herbicide. A study was conducted at North Carolina State University in which 10 μg L^{-1} of 25% radiolabeled florpyrauxifen-benzyl was applied to the isolated shoot tissue of 10 different aquatic plant species to elucidate absorption and translocation patterns in these species. Extremely high levels of shoot absorption were observed for all species, and uptake was rapid. Highest shoot absorptions were observed for crested floatingheart [*Nymphoides cristata* (Roxb.) Kuntze] ($A_{192} = 20$ μg g^{-1}), dioecious hydrilla [*Hydrilla verticillata* (L. f.) Royle] ($A_{192} = 25.3$ μg g^{-1}), variable watermilfoil (*Myriophyllum heterophyllum* Michx.) ($A_{192} = 40.1$ μg g^{-1}), and Eurasian watermilfoil (*Myriophyllum spicatum* L.) ($A_{192} = 25.3$ μg g^{-1}). Evidence of translocation was observed in all rooted species tested, with the greatest translocation observed in *N. cristata* (1.28 μg g^{-1} at 192 h after treatment). The results of this study add to the growing body of knowledge surrounding the behavior of this newly registered herbicide within aquatic plants.

Introduction

The dominance of invasive aquatic plant species can lead to numerous negative impacts on aquatic ecosystems. Through competition and displacement, invasive aquatic plants reduce native plant diversity and ultimately form dense monotypic stands of vegetation (Gause 1934; Hardin 1960; Schultz and Dibble 2012). These dense monotypic stands can reduce native fish and macroinvertebrate populations and diversity (Covich et al. 2004; Downing and Leibold 2002). They can also reduce overall ecosystem productivity and impede the natural flow of water through an ecosystem (Engelhardt and Ritchie 2002; Pitlo and Dawson 1993; Schultz and Dibble 2012). Additionally, these infestations negatively impact the recreational utility of bodies of water, reduce property values, and create habitat suitable for disease-carrying vectors (Gangstad and Cardarelli 1993; Halstead et al. 2003; Wilde et al. 2005; Zhang and Boyle 2010).

Herbicides are one of the most commonly used and cost-effective methodologies for the selective management of invasive aquatic weeds. Currently, there is a need for additional active ingredients in aquatic systems to respond to new threats or treatment scenarios, enhance selectivity, reduce use rates, and mitigate the risk of herbicide resistance (Cobb and Reade 2010; Getsinger et al. 2008).

Synthetic auxin herbicides have favorable properties for invasive plant control as they (1) pose limited risk to wildlife; (2) are easily absorbed and translocated throughout sensitive plants; and (3) are generally selective to control dicots, with minimal impact to monocots (Epp et al. 2016; Grossmann 2010; Netherland 2009). Only 17 herbicides and algaecides are currently registered for aquatic use, two of which, triclopyr and 2,4-D, can be considered auxin mimics (Netherland 2009). Recently, a new herbicide, florpyrauxifen-benzyl, was developed and registered for use in aquatics. Florpyrauxifen-benzyl is a synthetic auxin with a highly favorable toxicology profile (Miller and Norsworthy 2015; Wells and Taylor 2016). This herbicide has been shown to be highly effective in controlling some of the most invasive aquatic plant species in the United States, including hydrilla [*Hydrilla verticillata* (L. f.) Royle] and invasive watermilfoils (*Myriophyllum* spp.) (Netherland and Richardson 2016; Richardson et al. 2016).

To better understand the interaction between in-water herbicide concentrations and target species, it is important to understand herbicide absorption and translocation kinetics. Theoretically, the carboxylic acid functional group on florpyrauxifen-benzyl (lipid soluble) and other picolinate auxin herbicides allows for the herbicide to pass through the lipophilic phloem wall, and with a low pKa, the herbicide should ionize into the anionic form inside the phloem, effectively concentrating the herbicide in the phloem (Bromilow et al. 1990; Epp et al. 2016). This process is called "phloem trapping" and would allow for systemic movement of the ionized herbicide to growing shoot and root tissues (Epp et al. 2016). However, plant response to synthetic auxins is often species specific and may vary in an aquatic environment

compared with a terrestrial environment (Cobb and Reade 2010; Delbarre et al. 1996). Therefore, absorption and translocation of florpyrauxifen-benzyl need to be evaluated for species-specific responses to confirm theoretical hypotheses regarding the behavior of this herbicide within aquatic plants. In the last several years, protocols employing radiolabeled herbicides have been used to study the absorption and translocation of some herbicides in aquatic plants (Kniss et al. 2011; True-Meadows 2012; Vassios et al. 2014, 2017). The objective of this study was to elucidate absorption and translocation kinetics of florpyrauxifen-benzyl in 10 important aquatic plant species.

Materials and Methods

The methodology utilized in this study closely follows that of Vassios et al. (2014). Adaptations and specifics are discussed in the following sections.

Plant Propagation and Preparation

The 10 aquatic plant species utilized in this experiment were Eurasian watermilfoil (*Myriophyllum spicatum* L.), hybrid Eurasian watermilfoil (*Myriophyllum spicatum* L. × *Myriophyllum sibiricum* Kom.), variable watermilfoil (*Myriophyllum heterophyllum* Michx.), dioecious hydrilla, monoecious hydrilla, Brazilian egeria (*Egeria densa* Planch.), American eelgrass (*Vallisneria americana* Michx.), crested floatingheart [*Nymphoides cristata* (Roxb.) Kuntze], giant salvinia (*Salvinia molesta* Mitchell), and waterhyacinth [*Eichhornia crassipes* (Mart.) Solms]. Plant source locations are listed in Table 1. Submersed species and *N. cristata* were planted in flat-bottom 25-mm-diameter glass test tubes. The test tubes were filled with sterilized topsoil amended with slow-release fertilizer (Osmocote Smart Release 15-9-12®, Scotts, 14111 Scottslawn Road, Marysville, OH 43041) at a rate of 3 g L^{-1} of soil. Fifteen-centimeter apical segments were cut from stock plants and planted in the fertilizer-amended topsoil, and a thin layer of sand was placed on top. Once planted, submersed plants were established in dechlorinated tap water in a glass greenhouse with 30% shade cloth for 8 wk, with the exception of hybrid *M. spicatum* × *M. sibiricum*. Due to poor growth following initial planting, new 15-cm apical segments of hybrid *M. spicatum* × *M. sibiricum* were planted 2 wk before treatment. At the same time, submersed species that had been growing for 6 wk were trimmed to approximately 15 cm of shoot tissue. At 1 wk before treatment, plants were moved to the treatment room to acclimate. Treatment vessels were maintained under full-spectrum lights (21 µmol s^{-1} m^{-2}) with a 14-h light:10-h dark light regime for the duration of the experiment. Natural sunlight was blocked from entering the room just before and throughout the treatment. Air temperature was maintained at 23 ± 3 C, and air was circulated constantly throughout the experiment.

One to two days before treatment, plants were briefly removed from the growth chambers and placed in test tube racks with the majority of shoot tissue submerged in adjacent beakers of dechlorinated tap water. Water was dried from the surface of the sand and the walls of the test tube using paper towels to provide a tighter seal between the gel and the wall of the test tube. Once the surfaces were dried, a layer of agarose gel (1.5% v/v) (Phytagar, Invitrogen, Grand Island, NY 14075) was placed on the sand surface. The gel was maintained in a liquid state at a temperature of 30 to 38 C.

Two floating species, *S. molesta*, and *E. crassipes*, were also included in this experiment. Small (5- to 7-cm diameter) daughter plants of *E. crassipes* and small (3- to 5-node) *S. molesta* plants were selected from established stock plants. Because roots were already established on these plants, they were immediately transferred to the treatment room to acclimate without an establishment period. No agar separation was applied to floating species.

Table 1. A list of the plant species used in this experiment and the origins of the plant collections.

Scientific name	Origin
Myriophyllum spicatum	Roanoke Rapids Lake, NC
Myriophyllum spicatum × *Myriophyllum sibiricum*	Hayden Lake, ID
Myriophyllum heterophyllum	Private pond, Sanford, NC
Hydrilla verticillata	Gainesville, FL
Hydrilla verticillata	Shearon Harris Reservoir, NC
Egeria densa	Private pond, Raleigh, NC
Vallisneria americana	Lake Mattamuskeet, NC
Nymphoides cristata	Lake Marion, SC
Salvinia molesta	Stock plants from eradicated infestation near Wilmington, NC
Eichhornia crassipes	Private pond, Johnston County, NC

Shoot Absorption and Translocation

Treatments were carried out in a temperature- and light-controlled room. Glass treatment vessels (7.6 L) were filled with 7 L of tap water treated with water conditioner (API Tap Water Conditioner®, Mars Fishcare North America, 50 E. Hamilton Street, Chalfont, PA 18914). Each treatment vessel was equipped with a custom-built, 24-slot, stainless-steel test tube rack. To contain the 10 species slated for treatment, two tank types labeled "Tank A" and Tank B" were established, each with 4 submersed species and 1 floating species. Each tank type was replicated three times for a total of six treatment tanks. Six experimental units of five species were placed in Tank A and in Tank B for a total of 30 plants per tank. Ten different species were represented across the two tank types. Tank A species were *N. cristata*, dioecious *H. verticillata*, *M. spicatum*, *S. molesta*, and *M. heterophyllum*. Tank B species were *E. densa*, hybrid *M. spicatum* × *M. sibiricum*, monoecious *H. verticillata*, *V. americana*, and *E. crassipes*. Plants were placed in test tube racks within holding buckets before treatment, and all plants were lowered into the treatment vessels following application of the ^{14}C-labeled herbicide and formulation blank.

All six treatment vessels were treated at a rate of 10 µg ai L^{-1} florpyrauxifen-benzyl. A mixture of 75% nonradiolabeled technical material, 25% radiolabeled technical material, and sufficient formulation blank equivalent to the 300 g ai L^{-1} suspension concentrate commercial aquatic formulation (Procellacor SC, Dow AgroSciences, Specialty Synthesis Group, Indianapolis, IN 46268) was used to reach this target treatment rate. Radiolabeled and nonradiolabeled technical materials were dissolved in acetone separately. The radioactivity of the labeled solution was confirmed using a liquid scintillation counter (LSC), the concentration of the nonlabeled solution was confirmed via HPLC-MS, and then the two solutions were mixed with ratio of 25 labeled:75 nonlabeled. The formulation blank was diluted in water before application. Dissolved technical material was applied to the treatment vessels, followed immediately by the application of diluted formulation blank. The vessel was then stirred with a glass stirring rod to ensure uniform distribution of active ingredients and formulation. Following application, the test tube racks containing the plants to be treated were placed in each of the treatment vessels. This placement marked time zero for treatment, and three replicate plants of each species were harvested without exposure to treatment vessels.

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Plants were harvested at 0, 0.5, 1, 6, 12, 36, and 192 h following treatment. Preliminary studies had shown that florpyrauxifen-benzyl is absorbed very quickly (EJH, unpublished data, 2017). As such, these harvest times were selected to be more heavily weighted toward earlier times after treatment, compared with other studies, to capture the initial hyperbolic increase in absorption. At each designated harvest time, three replicate plants of each species were removed from the treatment vessels. Above- and belowground material was removed from the test tube and separated at the agar line. Each tissue type was triple rinsed with distilled water separately and then allowed to air dry. One milliliter of rinsate from the third rinse of each plant portion was analyzed by LSC to ensure that all surface radiation was removed and only absorbed radiolabeled material remained. Following 1 to 2 h of air-drying, plant tissue was placed in a paper bag. Paper bags with plant tissue were dried at 70 C for 48 h. Dry biomass data were collected for each plant tissue type using an analytical balance. Plant tissue was then rolled in ash-free filter paper, labeled, and stored at −4 C. Plant tissue and filter paper were combusted in a biological oxidizer (OX500®, RJ Harvey Instrument, 11 Jane Street, Tappan, NY 10983) for 2 min and collected in 15 ml of ^{14}C-trapping oxidizing cocktail (OX161®, RJ Harvey Instrument). The efficiency of the oxidizer was confirmed daily to meet EPA standards of 80% to 120% recovery. Following combustion, the radioactivity of samples was quantified by LSC (Tri-carb 2100TR Liquid Scintillation Counter®, Spectrofuge Corporation of North Carolina, 4915 Prospectus Drive, Suite A, Durham, NC 27713) for 4 min per sample. The trial was replicated twice separated in time.

Statistical Analysis

The statistical analyses followed those of Vassios et al. (2014, 2017). Herbicide concentration in micrograms of herbicide per gram of dry weight was calculated in Microsoft Excel (2010, Microsoft, Redmond, WA 98052) utilizing measured disintegrations per minute (dpm) of each sample, the measured dry biomass of each sample analyzed, and the specific activity of the radiolabeled florpyrauxifen-benzyl received. Levene's test for homogeneity of variance was run in MS Excel for each species before combining the two runs of concentration data for analysis. Following the Levene's test, means and standard errors were calculated using the JMP Pro (v. 13, SAS Institute, Cary, NC 27513) extension platform for SAS software. These tabulated values were then exported back to MS Excel and finally imported into SigmaPlot (v. 13, Systat Software, San Jose, CA 95110) for nonlinear regression analysis. The regression analysis fit the hyperbolic model adapted from Kniss et al. (2011) by Vassios et al. (2014, 2017) (Equation 1), where y is the predicted absorption at time x, and a and b are constants. Regression equations for belowground tissue for monoecious *H. verticillata*, dioecious *H. verticillata*, and hybrid *M. spicatum* × *M. sibiricum* could not be calculated in SigmaPlot using the nonhyperbolic regression equation (Equation 1) and were therefore excluded from the results.

$$y = \frac{ax}{1 + bx} \quad [1]$$

The plant concentration factor (PCF) metric is often used to compare absorption across different herbicides with varying properties and applied at varying rates. While this experiment focused on the absorption and translocation of only one herbicide at one rate, we felt the inclusion of the PCF metric would be beneficial for future comparisons across studies. PCF was calculated following the equation presented in Vassios et al. (2017) as adapted from de Carvalho et al. (2007) (Equation 2), where plant concentration is the concentration of the herbicide (ng) in plant tissues divided by the fresh biomass (g), and water concentration is the concentration of the herbicide in the treated water at the time of treatment (ng ml^{-1}).

$$\text{PCF} = \frac{\text{plant concentration}}{\text{water concentration}} \quad [2]$$

The nonlinear regression equations resulting from these analyses were used to calculate the predicted absorption at 192 h after treatment (HAT) (A_{192}) and the predicted time it would take to reach 90% of that absorption (t_{90}). These two metrics were recommended for comparisons of absorption in different species and plant portions in Vassios et al. (2017). The A_{192} value was used to compare the theoretical maximum absorption of different species, and t_{90} was used to compare the rate of absorption or how quickly the plant absorbed to its maximum.

It is important to note that fresh weights were only collected during run 2. Linear regression equations of second-run dry weight to fresh weight comparisons were conducted for each species with separate equations for shoot ($r^2 = 0.48$ to 0.97; $P < 0.001$) and root tissue ($r^2 = 0.49$ to 0.95; $P < 0.001$). The equations were used to estimate the fresh weights for run 1 samples.

Translocation of herbicide from aboveground to belowground tissue was calculated as the percent of total radioactivity in the plant that was observed in the belowground portion of the plant. The mean and standard error of this value for each time interval (hours after treatment) were plotted using JMP Pro (v. 13) graph builder.

Results and Discussion

The hyperbolic regression model fit *M. spicatum* aboveground florpyrauxifen-benzyl accumulation over time ($r^2 = 0.98$; Table 2; Figure 1). A rapid increase in product concentration in the plant was observed within the first 24 h, and this is reflected by the predicted t_{90} value of 24.21 h (Table 2). Results from PCF also show this maximum concentration occurring between the 12- and 36-h harvests (Table 3). The PCF_{192} (120) and A_{192} (18.14 µg g^{-1}) indicate very high levels of absorption for *M. spicatum* (Tables 2 and 3). The *M. spicatum* PCF_{192} (120 ± 34) for florpyrauxifen-benzyl was more than twice the *M. spicatum* PCF_{192} (35 ± 6) reported for triclopyr, another synthetic auxin (Vassios et al. 2017). This discrepancy indicates that florpyrauxifen-benzyl bioconcentrates in *M. spicatum* tissues to a greater extent than triclopyr. The results were not unexpected, given the high level of sensitivity of *M. spicatum* to florpyrauxifen-benzyl observed in other studies (Netherland and Richardson 2016).

Results of belowground tissue analysis indicate only limited translocation to root tissue with an A_{192} of 0.19 µg g^{-1} (Table 2), and an average translocation of 0.48 ± 0.28% at 192 HAT (data not shown). Vassios et al. (2014) found a high accumulation of triclopyr, an auxin-mimic herbicide, with both granular and liquid formulations; however, the authors indicate this may have been due in part to movement within the soil pore water, as the shoots and roots were not separated. In another study, Vassios et al. (2017) observed only 2.6 ± 0.3% of the absorbed triclopyr herbicide was translocated to belowground tissue. The high herbicidal activity of florpyrauxifen-benzyl compared with triclopyr may be relevant in interpreting translocation. Small-scale screening has documented that florpyrauxifen-benzyl is as much

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Table 2. Results of hyperbolic regression analysis and predicted values for the absorption (μg g^{-1}) at 192 h (A_{192}) and the time in hours to reach 90% of A_{192} (t_{90}) for aboveground (AG) and belowground (BG) tissue.

Plant		Predicted absorption values	
Species	Section	A_{192}	t_{90}
		—μg g^{-1}—	—h—
Myriophyllum spicatum	AG	18.14	24.21
	BG	0.19	0.37
Myriophyllum spicatum × *Myriophyllum sibiricum*	AG	10.43	5.91
Myriophyllum heterophyllum	AG	40.06	92.7
	BG	0.17	169.23
Hydrilla verticillata (dioecious)	AG	25.31	84.52
Hydrilla verticillata (monoecious)	AG	4.83	2.46
Egeria densa	AG	6.49	4.42
	BG	0.24	4.31
Vallisneria americana	AG	2.57	14.87
	BG	0.14	136.19
Nymphoides cristata	AG	20.06	71.32
	BG	1.28	181.15
Salvinia molesta	AG	7.76	12.85
Eichhornia crassipes	AG	8.66	28.41

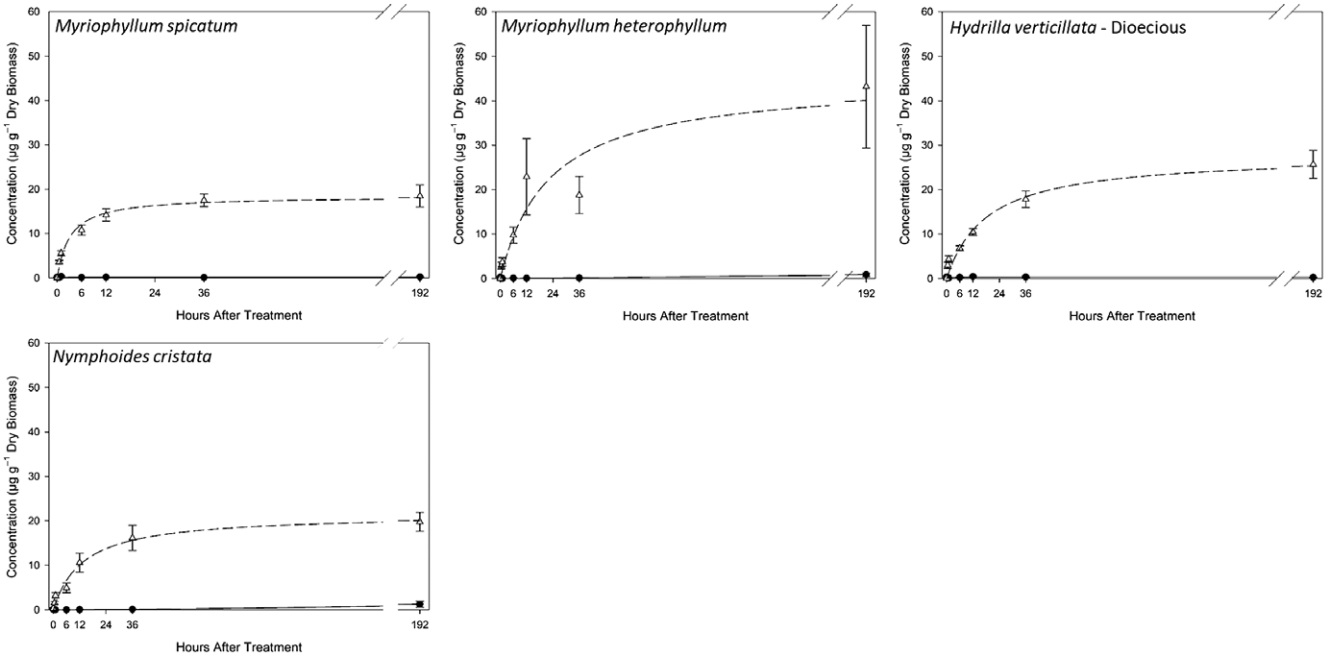

Figure 1. Hyperbolic nonlinear regression plots for *Myriophyllum spicatum*, *Myriophyllum heterophyllum*, dioecious *Hydrilla verticillata*, and *Nymphoides cristata*. Aboveground data are symbolized with a dashed line for the regression and open triangles for the mean concentration. Belowground data are symbolized with a solid line for the regression and closed circles for the mean concentration. Error bars represent the standard error of the mean. Please note the *y* axis scales in this figure display a maximum concentration of 60 μg g^{-1} dry biomass compared with only 20 μg g^{-1} dry biomass in Figure 2.

as 370 times more active on *M. spicatum* than triclopyr (Beets and Netherland 2018). Vassios et al. (2017) observed a belowground A_{192} of 71.42 μg ai g^{-1} with triclopyr in *M. spicatum*.

Absorption values for hybrid *M. spicatum* × *M. sibiricum* (A_{192} = 10.43 μg g^{-1}; PCF_{192} = 62 ± 11) were lower than that of *M. spicatum* (A_{192} = 18.14 μg g^{-1}; PCF_{192} = 120 ± 34) (Tables 2 and 3). However, hybrid *M. spicatum* × *M. sibiricum* reached 90% of maximum absorption faster (t_{90} = 5.91 h) than *M. spicatum* (t_{90} = 24.21 h) (Table 2). The lower absorption and lower PCF_{192} observed in this study explain the reported reduced sensitivity to florpyrauxifen-benzyl of the Hayden Lake hybrid *M. spicatum* × *M. sibiricum* compared with *M. spicatum* (Beets and Netherland 2018). Similar to *M. spicatum*, translocation in the Hayden Lake hybrid *M. spicatum* × *M. sibiricum* appears low as a fraction of total absorbed herbicide, with an average of 1.11 ± 0.28 % of the total amount of herbicide absorbed (data not shown). Regression equations for belowground tissue for hybrid *M. spicatum* × *M. sibiricum* could not be calculated in SigmaPlot using the nonhyperbolic regression equation. However, raw data indicated radioactivity in root tissue, thereby revealing some translocation occurred.

Myriophyllum heterophyllum florpyrauxifen-benzyl absorption (A_{192} = 40.06 μg g^{-1}) was more than twice that of pure *M. spicatum* and almost four times that of hybrid *M. spicatum* × *M. sibiricum*

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Table 3. Plant concentration factor (PCF) results for each species at each time interval in hours after treatment (HAT) expressed as the mean ± standard error.

	PCF					
	0.5 HAT	1 HAT	6 HAT	12 HAT	36 HAT	192 HAT
Myriophyllum spicatum	23 ± 3	37 ± 2	75 ± 11	82 ± 11	125 ± 31	120 ± 34
Myriophyllum spicatum × *Myriophyllum sibiricum*	30 ± 5	37 ± 6	53 ± 9	98 ± 30	50 ± 6	62 ± 11
Myriophyllum heterophyllum	18 ± 4	25 ± 5	63 ± 11	7 ± 10	145 ± 40	217 ± 64
Hydrilla verticillata (dioecious)	14 ± 4	18 ± 4	32 ± 7	47 ± 6	93 ± 22	90 ± 20
Hydrilla verticillata (monoecious)	14 ± 4	18 ± 5	26 ± 6	24 ± 4	26 ± 4	10 ± 2
Egeria densa	23 ± 3	35 ± 4	51 ± 8	48 ± 11	64 ± 14	33 ± 8
Vallisneria americana	2 ± 1	3 ± 1	4 ± 1	6 ± 1	7 ± 2	9 ± 3
Nymphoides cristata	5 ± 1	14 ± 7	13 ± 4	20 ± 4	34 ± 5	47 ± 11
Salvinia molesta	14 ± 2	27 ± 2	47 ± 7	63 ± 3	82 ± 8	62 ± 7
Eichhornia crassipes	10 ± 1	15 ± 2	34 ± 6	43 ± 5	49 ± 9	55 ± 9

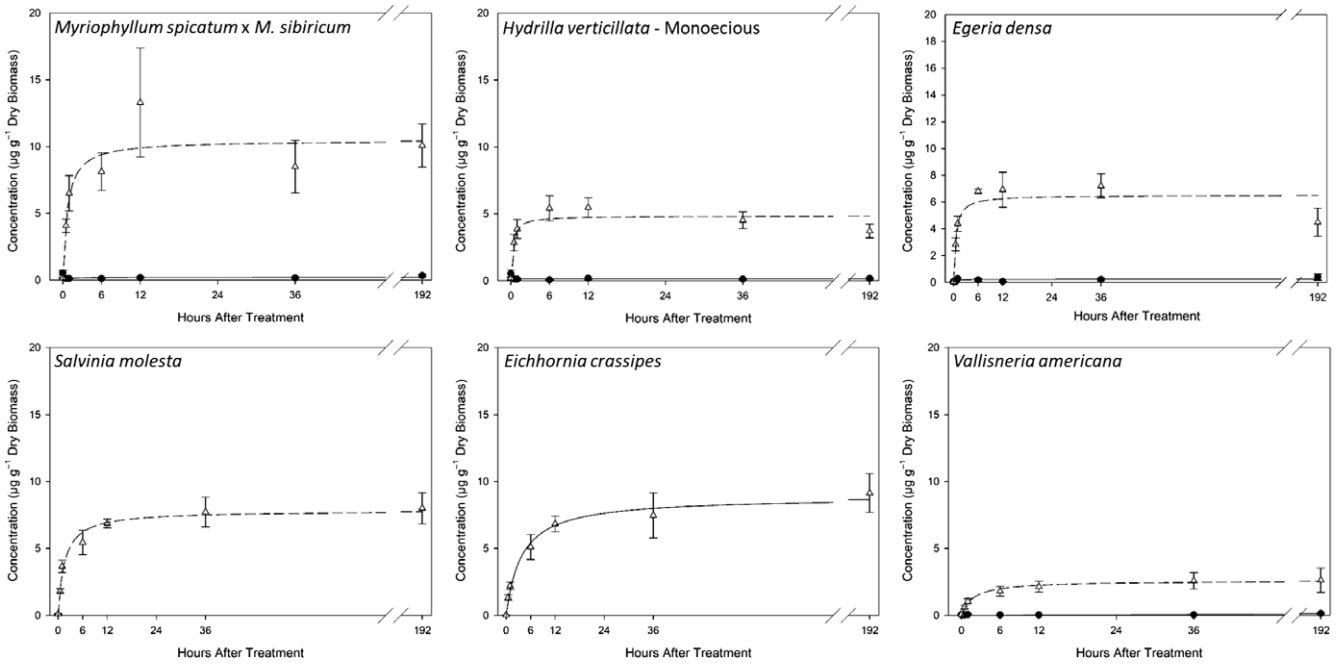

Figure 2. Hyperbolic nonlinear regression plots for *Myriophyllum spicatum* × *Myriophyllum sibiricum*, monoecious *Hydrilla verticillata*, *Egeria densa*, *Salvinia molesta*, *Eichhornia crassipes*, and *Vallisneria americana*. Aboveground data are symbolized with a dashed line for the regression and open triangles for the mean concentration. Belowground data are symbolized with a solid line for the regression and closed circles for the mean concentration. Error bars represent the standard error of the mean. Please note the *y* axis scales in this figure display a maximum concentration of 20 µg g^{-1} dry biomass compared with 60 µg g^{-1} dry biomass in Figure 1.

(Figure 1). However, the fit curve had a much slower rise to the maximum, as evidenced by the t_{90} of 92.7 h (Table 2). This high level of absorption might be an important factor favoring previously reported hypersensitivity of *M. heterophyllum* to florpyrauxifen-benzyl with an EC_{50} in small-scale, static exposure studies of less than 0.3 ppb (Richardson et al. 2016).

A hyperbolic nonlinear regression model fit dioecious *H. verticillata* shoot data very well ($r^2 = 0.97$). Vassios et al. (2017) observed an A_{192} for dioecious *H. verticillata* that was approximately 25% of that observed for *M. spicatum* when studying another auxin-mimic herbicide, triclopyr. In contrast, the results of this florpyrauxifen-benzyl study indicate that dioecious *H. verticillata* shoots ($A_{192} = 25.31$ µg g^{-1}) absorbed approximately 40% more herbicide than *M. spicatum* shoot tissue. Higher absorption in dioecious *H. verticillata* compared with *M. spicatum* is particularly interesting in light of the fact that efficacy studies found *M. spicatum* to be more sensitive ($EC_{50} = 0.11 \pm 0.11$ µg ai L^{-1}) than dioecious *H. verticillata* ($EC_{50} = 1.4 \pm 0.1$ µg ai L^{-1}), indicating higher sensitivity (Netherland and Richardson 2016). *Myriophyllum spicatum* is a dicot, and *H. verticillata* is a monocot. It is plausible that there is a difference in the way the herbicide is metabolized in these two plants, which could explain these seemingly contrasting results.

Several studies have indicated that experimental results utilizing dioecious *H. verticillata* cannot be universally applied to monoecious *H. verticillata* (McFarland and Barko 1987; Puri et al. 2007; Spencer and Anderson 1986; Steward and Van 1987; Sutton et al. 1992; True-Meadows et al. 2016). The aboveground absorption results of this study are yet another example of such. The predicted absorption in monoecious *H. verticillata* based on our results ($A_{192} = 4.83$ µg g^{-1}) is approximately 19% of the absorption predicted for dioecious *H. verticillata* ($A_{192} = 25.31$ µg g^{-1}) (Table 2; Figures 1 and 2). It should be noted that separate sensitivity studies have found florpyrauxifen-benzyl to be highly efficacious for both dioecious and monoecious *H. verticillata* (Netherland and Richardson 2016; Richardson et al. 2016). Beets and Netherland (2018) found that doubling the rate of florpyrauxifen-benzyl from 24 to 48 µg L^{-1} did not increase observed efficacy, nor did increasing the exposure from 24 to 72 h. In this

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same study, the authors observed a significant decrease in belowground biomass of dioecious *H. verticillata* (Beets and Netherland 2018).

Results for *E. densa* ($A_{192} = 6.49$ µg g^{-1}), another submersed monocot, indicate a shoot absorption pattern somewhere between that of monoecious *H. verticillata* and hybrid *M. spicatum* × *M. sibiricum* (Table 2). Whereas results for *V. americana*, a native submersed monocot, indicate a shoot absorption pattern that was lowest of all plant species evaluated in this experiment ($A_{192} = 2.57$ µg g^{-1}; $PCF_{192} = 8.89$) (Tables 2 and 3; Figure 2). This low level of absorption is consistent with the treatment results indicating no apparent response in relative abundance of *V. americana* to high levels (48 µg L^{-1}) of florpyrauxifen-benzyl (Sperry et al. 2021). Translocation was relatively low for both *V. americana* and *E. densa*.

The hyperbolic nonlinear regression model fit the shoot absorption data for *N. cristata*, the only floating-leaved dicot in this experiment, very well, with an r^2 of 0.97 (Table 2; Figure 1). Predicted shoot absorption for *N. cristata* at 192 HAT ($A_{192} = 20.06$ µg g^{-1}) was similar to that of *M. spicatum* and dioecious *H. verticillata* (Table 2). Again, this high level of absorption is congruent with the high level of sensitivity to florpyrauxifen-benzyl observed for *N. cristata* (Netherland and Richardson 2016). The highest level of belowground concentration of herbicide observed in this experiment was observed in *N. cristata*, with A_{192} of 1.28 µg g^{-1} (Table 2; Figure 1). Observations of high belowground concentrations of florpyrauxifen-benzyl are consistent with a mesocosm study in which treatment with 24 µg L^{-1} of the herbicide for 24 to 72 h resulted in significant reductions in belowground biomass (Beets and Netherland 2018).

Both *E. crassipes* and *S. molesta* showed moderate to high levels of absorption, with predicted absorption levels of 8.66 µg g^{-1} and 7.66 µg g^{-1}, respectively (Table 2; Figure 2). PCF values were also moderate to high at 61 for *S. molesta* and 55 for *E. crassipes* (Table 3). As this was an in-water treatment, shoots and roots were not separated to quantify translocation in these floating species.

While predicted shoot absorption values for monocots may appear to be on average lower than those of dicots in this experiment, it should be noted that this apparent difference was not significant in a one-way ANOVA ($P = 0.24$).

The percentage of herbicide translocated from shoot to root tissue has been low, less than 5% to 10%, in other experiments examining radiolabeled herbicide translocation in submersed aquatic plant species (Vassios et al. 2017). Evidence of florpyrauxifen-benzyl translocation was observed in all rooted plant species tested, as indicated by the radioactivity measured in isolated root tissues. However, more florpyrauxifen-benzyl translocation was expected than was observed based on the classification of this herbicide as an aryl-picolinate. Aryl-picolinates as a class of herbicides have been shown to translocate well in terrestrial plant tissues (Bromilow et al. 1990; Epp et al. 2016). One possible explanation for the reduced translocation observed could be due to the application of the formulation blank separate from the application of the active ingredient. This methodology was recommended by the manufacturer; however, it is hard to predict how the chemicals will behave when applied separately in a novel (aqueous) environment. Alternatively, it is possible that the herbicide impacted vascular tissue rapidly enough to reduce movement to belowground tissues, due in part to the small scale at which this experiment took place and the moderate florpyrauxifen-benzyl application rate used.

While synthetic auxin herbicides generally affect dicots more than monocots, sensitivity analyses for florpyrauxifen-benzyl have shown high activity on a select number of monocot invasive aquatic plants (Netherland and Richardson 2016; Richardson et al. 2016). Evidence of translocation was observed in all rooted species tested, and moderate to high translocation was observed in *N. cristata*. High levels of shoot absorption and high plant concentration factors were observed for susceptible species, and reduced absorption levels were absorbed for insensitive species. Finally, low absorption into native *V. americana* shoot tissues may help to explain florpyrauxifen-benzyl selectivity between this and other *Hydrocharitaceae* spp. such as *H. verticillata*. Overall, this study provides additional evidence that florpyrauxifen-benzyl is a good candidate for selective control of several aquatic plant species.

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