Endothall and florpyrauxifen-benzyl behavior in hydrilla (*Hydrilla verticillata*) when applied in combination

Author for correspondence:
Mirella F. Ortiz, Department of Plants, Soils, and Climate, Utah State University, 4820 Old Main Hill, Logan, UT 84322-4820.
(Email: Mirella.ortiz@usu.edu)

Mirella F. Ortiz[1] , Scott J. Nissen[2] and Franck E. Dayan[2]

[1]Graduate Student, Department of Agricultural Biology, Colorado State University, Fort Collins, CO, USA and [2]Professor, Department of Agricultural Biology, Colorado State University, Fort Collins, CO, USA

Abstract

Hydrilla [*Hydrilla verticillata* (L. f.) Royle] is often called the "perfect aquatic weed," as it has numerous physiological adaptations that make it highly aggressive and competitive. *Hydrilla verticillata* has historically been managed effectively using fluridone; however, the overreliance on this single mechanism of action (MOA) resulted in evolved fluridone resistance in the late 1990s. Where fluridone-resistant *H. verticillata* populations evolved, endothall became widely used for *H. verticillata* control. In 2018, florpyrauxifen-benzyl, a highly active auxin-mimic herbicide, was registered for *H. verticillata* control, and its use has increased since its introduction. Endothall and florpyrauxifen-benzyl provide two effective MOAs for *H. verticillata* management, and combining these two MOAs would be an effective strategy to delay further resistance evolution. The objective of this research was to determine whether combining endothall and florpyrauxifen-benzyl would significantly impact the behavior of either herbicide in dioecious (DHV) or monoecious (MHV) *H. verticillata* compared with their behavior when applied alone. Endothall and florpyrauxifen-benzyl absorption and accumulation alone and in combination were measured over a 192-h time course. Translocation patterns were also determined. Herbicide accumulation in MHV and DHV was not impacted when these herbicides were applied in combination. Endothall translocation from shoots to roots in DHV was not impacted (alone = 18.7 ± 1.4%; combination = 23.2 ± 2.2%); however, endothall shoot-to-root translocation in MHV was reduced from 16.2 ± 1.3% applied alone to 2.2 ± 0.1% when applied in combination with florpyrauxifen-benzyl. Florpyrauxifen-benzyl shoot-to-root translocation was reduced in both MHV and DHV when applied in combination with endothall. Florpyrauxifen-benzyl translocation was reduced by 16- and 6-fold in DHV and MHV, respectively. These data do not suggest that there would be operational impacts from endothall and florpyrauxifen-benzyl mixtures. Still, there appear to be changes in herbicide behavior, primarily shoot-to-root translocation, when these two herbicides are applied in combination.

Introduction

Hydrilla [*Hydrilla verticillata* (L. f.) Royle] is a submersed, invasive plant that has been described as the "perfect aquatic weed" due to numerous physiological adaptations that make it highly aggressive and competitive (Langeland 1996). Both monoecious and dioecious *H. verticillata* biotypes are present in the United States, and they have spread significantly from their initial introduction sites (Cook and Lüönd 1982). Dioecious *H. verticillata* (DHV) was first introduced in Florida as an aquarium plant in the 1950s and is commonly found in the southern United States, while monoecious *H. verticillata* (MHV) was first reported near Washington, DC, and Raleigh, NC, in 1980. Although MHV is still most commonly found from North Carolina northward, it has spread to northern and southern Alabama and northern Georgia (Gettys et al. 2020; Gettys and Leon 2021).

Before 1986, the serine/threonine protein phosphatase inhibitor endothall (HRAC Group 31; Bajsa et al. 2012) was frequently used for *H. verticillata* control at concentrations of several milligrams per liter (ppm) in the water column. Following its registration for aquatic use in 1986, fluridone (HRAC Group 12) became the primary tool for *H. verticillata* management because of its high efficacy, selectivity, and ability to control tuber production (Nawrocki et al. 2016). Fluridone is applied to the water column in micrograms per liter (ppb), and it was used intensively for decades (Dayan and Netherland 2005; Nawrocki et al. 2016). Fluridone's extensive use without any herbicide rotation or mixture, eventually selected for fluridone-resistant DHV in Florida in the late 1990s (Arias et al. 2005; Dayan and Netherland 2005; Michel et al. 2004; Netherland and Jones 2015; Puri et al. 2007). Only the female form of DHV is found in the United States, and its spread is limited to vegetative reproduction (Michel et al. 2004). Fluridone resistance in DHV has been the result of a somatic mutation, and this is the first case

of evolved herbicide resistance in a plant that does not rely on sexual reproduction (Ortiz et al. 2020).

The development of fluridone-resistant DHV reverted management strategies in Florida back to including endothall as the standard for the last two decades (Sperry et al. 2021). The overreliance on a single, effective mechanism of action (MOA) without rotations or mixtures, has led to suspected endothall resistance in some *H. verticillata* populations in central Florida as well (Giannotti et al. 2014).

The loss of fluridone for DHV management in Florida and the potential for new market opportunities were a driving force behind identifying and registering several new herbicide MOAs for aquatic plant management (Getsinger et al. 2008; Ortiz et al. 2020). In 2018, florpyrauxifen-benzyl (HRAC Group 4; Epp et al. 2016) was registered for aquatic use. Florpyrauxifen-benzyl is an auxin-mimic herbicide highly active in *H. verticillata*, even though this plant is a monocot species (Beets et al. 2019). Auxin-mimic herbicides are generally active on dicots, with minimal impact on monocots. Florpyrauxifen-benzyl is an excellent tool for *H. verticillata* control, as it is highly effective in both *H. verticillata* biotypes and is classified as a reduced-risk pesticide by the U.S. Environmental Protection Agency (Epp et al. 2016).

Herbicide rotations and mixtures are widely recommended to mitigate the evolution of herbicide resistance (Beckie and Reboud 2009). Terrestrial field research and computer simulation models suggest that combining herbicide MOAs as mixtures are the most effective measure at delaying resistance evolution (Beckie and Reboud 2009; Busi et al. 2020; Evans et al. 2018). Herbicide mixtures ensure that weeds, terrestrial or aquatic, are treated with two different MOAs, and weeds potentially resistant to one herbicide MOA are still controlled by another herbicide acting at a different site of action (Busi et al. 2020).

To date, only 15 herbicides and 9 different MOAs are registered for aquatic use (Ortiz et al. 2020). Consequently, when resistance develops in aquatic weeds, the options for alternative chemical controls are very limited. To reduce the opportunity for aquatic weeds to evolve resistance, there has been more interest in applying herbicide mixtures; however, there is very little information about how herbicide behavior might change when products are applied as mixtures. There are examples of both herbicide antagonism and synergism in terrestrial weed management, and the same could occur in aquatic systems (Kyser et al. 2021; Wersal and Madsen 2010, 2012). To better understand how herbicide behavior might change when used in combination, we investigated the behaviors of endothall and florpyrauxifen-benzyl applied alone and in combination. The objectives of this research were to determine herbicide absorption and translocation patterns when these herbicides were applied alone and in combination in MHV and DHV over a 192-h time course.

Materials and Methods

Plant Material

MHV and DHV tubers were collected from Shearon Harris Lake, NC (35.61°N, 78.95°W), and Orange Lake, FL (29.46°N, 82.17°W), respectively, in spring 2016 and cultured under greenhouse conditions for the last 5 yr. To produce uniform plant material for this research, 10-cm apical sections were cut from the previously propagated plants, and the distal ends were planted in 16 cm by 12 cm by 6 cm (1,152 cm^3) plastic pots filled with soil collected from Colorado State University's organic research farm.

Each pot was fertilized with 2 g of slow-release fertilizer (Osmocote Smart Release® Plant Food 15-9-12, Scotts Company, 14111 Scottslawn Road, Marysville, OH 43040), and six apical meristem shoots were planted in each pot for a total of 16 pots. Soil was covered by a 1-cm layer of washed sand. Plants were grown in dechlorinated tap water in 1.2 m by 1 m by 0.9 m (1,041 L) plastic tanks under greenhouse conditions until they produced roots. The photoperiod was 14:10-h light:dark, supplemental lighting was provided with 400-W sodium-halide light bulbs, and the greenhouse temperature was set at 24 C during the day and 18 C at night.

Approximately 3 wk after being transplanted, propagated apical shoots reached 13 to 15 cm in length. Shoots were removed from their original pots, and plants with well-developed roots were selected for absorption and translocation experiments. Roots were washed with tap water to remove soil residue and replanted into plastic test tubes (15-ml Conical Centrifuge Tubes, Thermo Fisher Scientific, 81 Wyman Street, Waltham, MA 02451). Test tubes were filled with unwashed silica sand. After plants were transferred into test tubes, a low melting point eicosane wax (Eicosane 99%, ACROS Organics, 81 Wyman Street, Waltham, MA 02451) was used to seal the top of each tube to isolate the root system from the water column (Frank and Hodgson 1964; Ortiz et al. 2019). Plants were moved to 4-L plastic tanks (22.7-cm tall by 17-cm diameter) filled with dechlorinated tap water and were allowed to acclimate for 24 h in the laboratory prior to herbicide treatment.

Herbicide Exposure

Twelve 4-L glass beakers (25-cm tall by 15-cm diameter) were filled with 3.5 L of dechlorinated tap water (pH 7.1). Six beakers were treated with [14C]endothall (56.6 mCi mmol^{-1} specific activity, Moravek Biochemicals, 577 Mercury Lane, Brea, CA 92821) combined with formulated dipotassium salt of endothall (Cascade®, United Phosphorus, 630 Freedom Business Center, Suite 402, King of Prussia, PA 19406) to achieve a final concentration of 2 mg L^{-1} in the water column. Three of these beakers were treated with endothall only, and the other three beakers were treated with endothall as described plus 3.8 µg L^{-1} of formulated, nonradiolabeled florpyrauxifen-benzyl (ProcellaCOR™ EC, SePRO, 11550 North Meridian Street, Suite 600, Carmel, IN 46032). The other six beakers were treated with [14C]florpyrauxifen-benzyl (57.1 mCi mmol^{-1} specific activity, Moravek Biochemicals) at 3.8 µg L^{-1}. Three of these beakers were treated with [14C]florpyrauxifen-benzyl only, and the other three beakers were treated with [14C]florpyrauxifen-benzyl plus 2 mg L^{-1} of formulated, nonradiolabeled dipotassium salt of endothall (Cascade®).

Each treatment was replicated three times for a total of 12 treatment beakers. Each [14C]endothall-treated tank contained 33.8 ± 1.0 kBq L^{-1}, while each [14C]florpyrauxifen-benzyl–treated tank contained 12.4 ± 0.5 kBq L^{-1}. The radioactivity in each treatment tank was confirmed using liquid scintillation spectroscopy (LSS) (Packard 2500R, PerkinElmer, 940 Winter Street, Waltham, MA 02451).

Each beaker contained 6 MHV, 6 DHV plants, and one empty tube waxed with a toothpick simulating a plant stem as a control to test the wax barrier efficacy. All plants were held by a round test tube rack (No-Wire Round Rack, Bel-Art Scienceware, 661 Route 23 South, Wayne, NJ 07470). During the experiment, plants were maintained in the laboratory at 22 C, with a 14:10-h light:dark period, supplemented with LED grow lights. Beakers were stirred

once a day, and treatment water volume was maintained by adding more water to the tanks daily. Plants were harvested at 6, 12, 24, 48, 96, and 192 h after treatment (HAT). Three replicates of each biotype were randomly harvested from a different tank at each time point, rinsed four times in clean tap water, and divided into shoots and roots. After separation, plant parts were dried at 60 C for at least 48 h, dry biomass was recorded for each plant part, and plant tissues were combusted in a biological oxidizer (OX500, R.J. Harvey Instrument, 11 Jane Street, Tappan, NY 10983) for 2 min, and absorbed ^{14}C was collected by a ^{14}C-trapping cocktail (OX161, R.J. Harvey Instrument). The efficiency of the oxidizer was tested before oxidizing plant parts, and it was always greater than 96%. After oxidation, radioactivity was quantified by LSS. The study was repeated twice.

Statistical Analysis

Before data from repeated experiments were combined for statistical analyses, Levene's test for homogeneity of variance ($\alpha = 0.05$ level of significance) was performed using R (v. 4.0.0, R Project) (Vassios et al. 2011). Means and standard errors for each experiment were back-calculated from dry weight, considering 90% of water content, determined based on 10 *H. verticillata* plants. Data collected from these experiments were analyzed using RStudio (v. 1.4.1717) and MS Excel and plotted with Prism 9 (GraphPad Software, 2365 Northside Drive, Suite 560, San Diego, CA 92108). Absorption and translocation over time were analyzed using a nonlinear regression analysis to fit a hyperbolic function (Kniss et al. 2011), where y is the predicted absorption at time x, and a and b are constants.

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

The plant concentration factor (PCF) was calculated to determine bioaccumulation, a metric often used in aquatic plant research to compare absorption across different herbicide concentrations and in different species. The equation used to calculate PCF was adapted from de Carvalho et al. (2007) and can be defined as:

$$\text{PCF} = \frac{\text{Herbicide concentration in plant (ng/g fresh biomass)}}{\text{Herbicide concentration in water (ng/ml)}} \quad [2]$$

The nonlinear regression equations resulting from these analyses were used to calculate two other values: predicted absorption at 192 HAT (A_{192}) based on the radiolabeled/nonradiolabeled herbicide ratio in the water column and the predicted time required for 90% of that absorption (t_{90}). The A_{192} value was used to compare the theoretical maximum absorption among different plant parts, plant species, and herbicides. The t_{90} was used to compare the absorption rate or how quickly the plant reached maximum absorption.

Results and Discussion

Plant roots were effectively isolated from the radiolabeled treatment solutions through the eicosane wax barrier. Only 0.041 ± 0.012 Bq ml^{-1} ($n = 6$) and 0.022 ± 0.007 Bq ml^{-1} ($n = 6$) of radioactivity were found in the non-plant toothpick test tubes 192 HAT for [^{14}C]endothall and [^{14}C]florpyrauxifen-benzyl treatments,
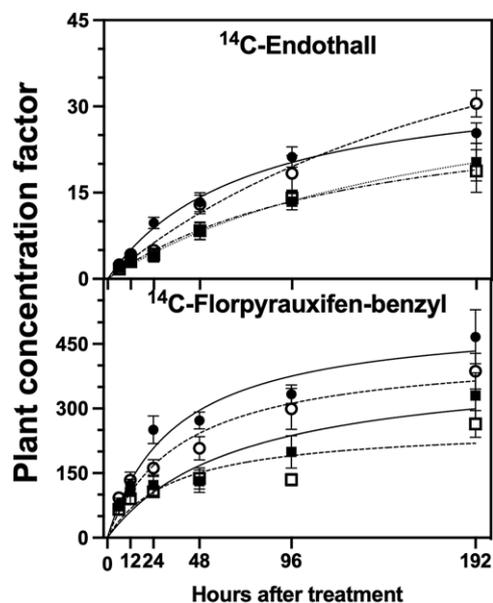
Figure 1. [^{14}C]endothall and [^{14}C]florpyrauxifen-benzyl bioaccumulation in *Hydrilla verticillata* biotypes (DHV, dioecious; MHV, monoecious) over a 192-h time period expressed as plant concentration factor (PCF). Filled circle, herbicide bioaccumulation in MHV alone; open circle, bioaccumulation in MHV in combination; filled square, herbicide bioaccumulation in DHV alone; open square, bioaccumulation in DHV in combination. Data presented are means and error bars are the standard error of the mean ($n = 6$).

respectively. There was no detected radioactivity in 5 of the 12 combined test tubes. Based on these data, this insignificant amount of radioactivity did not impact the results of the study.

Endothall absorption did not reach a maximum asymptote in either biotype when applied alone or in the presence of florpyrauxifen-benzyl (Figure 1). Although the asymptotic rise to maximum function is the most biologically relevant function to describe herbicide absorption (Kniss et al. 2011), previous research also demonstrated that endothall at 3 mg L^{-1} did not reach maximum asymptote for DHV and MHV (Ortiz et al. 2019).

The PCF$_{192}$ of [^{14}C]endothall applied alone was 20.2 ± 1.3 and 25.8 ± 0.7 in DHV and MHV, respectively. When applied in combination with florpyrauxifen-benzyl, [^{14}C]endothall PCF$_{192}$ in DHV and MHV was 19.0 ± 1.5 and 30.2 ± 0.9, respectively (Figure 1; Table 1), which was not statistically different than [^{14}C]endothall alone. The accumulation of this herbicide at 3 mg L^{-1} was 11.0 ± 0.9 and 6.6 ± 0.7 for DHV and MHV, respectively (Ortiz et al. 2019). The reason for greater herbicide accumulation in this study may be due to the difference in herbicide rate, as a lower concentration allows the plant to be more physiologically active and maintain a stronger concentration gradient. A similar endothall accumulation response was observed in curly-leaved pondweed (*Potamogeton crispus* L.) with PCF$_{192}$ of 4.8 ± 0.1 when exposed to 0.75 mg L^{-1} endothall and a lower PFC$_{192}$ of 3.9 ± 0.1 when exposed to a higher endothall concentration of 3 mg L^{-1} (Ortiz et al. 2022).

The PCF$_{192}$ for [^{14}C]florpyrauxifen-benzyl alone was 299.4 ± 21.3 and 433.5 ± 25.4 in DHV and MHV, respectively (Figure 1). Previous research reported that florpyrauxifen-benzyl PCF$_{192}$ at 10 μg L^{-1} was 90 ± 20 and 10 ± 2 for DHV and MHV, respectively (Haug et al. 2021). Greater herbicide accumulation in the current study is likely due to differences in the ratio of radiolabeled and nonradiolabeled herbicide, herbicide rate, and different herbicide formulations (Vassios et al. 2011). In the

Table 1. Predicted plant concentration factor at 192 h after treatment (HAT) (PCF$_{192}$), herbicide absorption (µg g^{-1}) at 192 HAT (A$_{192}$), and the time in hours required to reach 90% of A$_{192}$ (t_{90}).[a]

| Biotype[b]	Treatment	PCF$_{192}$	Plant part	A$_{192}$	t_{90}
DHV	[^{14}C]endothall	20.2 ± 1.3	Shoots	307.9 ± 20.2	159
			Roots	65.9 ± 3.7	150
	[^{14}C]endothall + florpyrauxifen-benzyl	19.0 ± 1.5	Shoots	260.9 ± 15.1	150
			Roots	72.4 ± 5.2	154
	[^{14}C]florpyrauxifen-benzyl	299.4 ± 21.3	Shoots	3.11 ± 0.39	112
			Roots	0.53 ± 0.08	147
	[^{14}C]florpyrauxifen-benzyl + endothall	219.4 ± 12.8	Shoots	2.94 ± 0.18	111
			Roots	0.018 ± 0.003	80
MHV	[^{14}C]endothall	25.8 ± 0.7	Shoots	382.3 ± 13.6	135
			Roots	87.5 ± 8.4	145
	[^{14}C]endothall + florpyrauxifen-benzyl	30.2 ± 0.9	Shoots	535.6 ± 25.6	160
			Roots	12.3 ± 0.8	135
	[^{14}C]florpyrauxifen-benzyl	433.5 ± 25.4	Shoots	4.91 ± 0.26	110
			Roots	0.097 ± 0.008	141
	[^{14}C]florpyrauxifen-benzyl + endothall	364.0 ± 17.0	Shoots	4.32 ± 0.23	115
			Roots	0.010 ± 0.002	114

[a]Values represent the mean, and error terms represent the standard error of the mean ($n = 6$).
[b]*Hydrilla verticillata* biotypes: DHV, dioecious; MHV, monoecious.

presence of 2 mg L^{-1} endothall, [^{14}C]florpyrauxifen-benzyl accumulation in DHV and MHV was 219 ± 12.8 and 364.0 ± 17.0, respectively (Table 1) and was not statistically different than [^{14}C]florpyrauxifen-benzyl alone.

Florpyrauxifen-benzyl high log K_{ow} (5.5) is very similar to 2,4-D butoxyethyl ester's (BEE) (log K_{ow} 5.3), another auxin-mimic herbicide used for aquatic plant management. The 2,4-D BEE PCF$_{192}$ in Eurasian watermilfoil (*Myriophyllum spicatum* L.) and hybrid watermilfoil (*Myriophyllum spicatum* × *Myriophyllum sibiricum* Kom.) was 35.11 ± 1.33 and 52.11 ± 1.09, respectively, substantially lower than florpyrauxifen-benzyl in DHV and MHV. Both florpyrauxifen-benzyl and 2,4-D BEE are pro-herbicides that are bioactivated into their acid forms once they are absorbed by the plant (Nandula et al. 2019). Depending on water chemistry and temperature, both herbicides can be hydrolyzed to the free acid in the water column and absorbed by plants. The acid form of florpyrauxifen-benzyl is significantly more lipophilic than 2,4-D acid, which could explain its higher accumulation.

Endothall absorption at 192 HAT (A$_{192}$) in DHV and MHV shoots was similar, 307.9 ± 20.2 µg g^{-1} and 382.3 ± 13.6 µg g^{-1}, respectively (Table 1); in combination with florpyrauxifen-benzyl, it was only impacted in MHV, with a 40% increase in herbicide absorption (DHV = 260.9 ± 15.1 µg g^{-1}; MHV = 535.6 ± 25.6 µg g^{-1}). Although endothall absorption increased by 40% in the presence of florpyrauxifen-benzyl, the time required to reach 90% of total herbicide absorbed (t_{90}) only increased by 18% (Table 1), meaning that endothall absorption by MHV was slower when in combination with florpyrauxifen-benzyl. In contrast, while florpyrauxifen-benzyl A$_{192}$ was not impacted in the presence of endothall, its absorption was significantly lower than endothall's, which was not unexpected, considering the rates at which each herbicide is applied (endothall = 2 mg L^{-1} and florpyrauxifen-benzyl = 3.8 µl^{-1}). Florpyrauxifen-benzyl absorption was very similar between the two biotypes, 3.1 ± 0.4 µg g^{-1} and 4.9 ± 0.3 µg g^{-1} in DHV and MHV, respectively (Table 1). A previous study using another herbicide formulation indicated similar florpyrauxifen-benzyl absorption in MHV, but four times greater absorption in DHV (Haug et al. 2021). The reason for the discrepancy between the two studies is unknown but may be attributable to the use of different herbicide formulations.
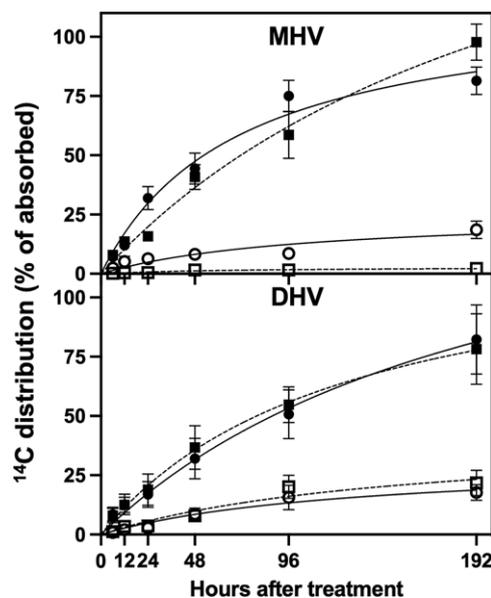
Figure 2. The ^{14}C distribution in *Hydrilla verticillata* biotypes (DHV, dioecious; MHV, monoecious) over 192 h following exposure to [^{14}C]endothall, expressed as percentage of total herbicide absorbed. Filled circle, percentage of [^{14}C]endothall alone in shoots; open circle, percentage of [^{14}C]endothall alone in roots; filled square, percentage of [^{14}C]endothall in combination with florpyrauxifen-benzyl in shoots; open square, percentage of [^{14}C]endothall in combination with florpyrauxifen-benzyl in roots. Data presented are means, and error bars are the standard error of the mean ($n = 6$).

Endothall shoot-to-root translocation was 18.7 ± 1.4% and 16.2 ± 1.5% when applied alone in DHV and MHV (Figure 2), respectively, supporting previously published data that endothall can translocate to the roots of both *H. verticillata* biotypes (Ortiz et al. 2019). In combination with florpyrauxifen-benzyl, endothall translocation in DHV was not impacted (23.2 ± 2.2%), but it was greatly reduced in MHV (2.20 ± 0.09%) (Figure 2). This reduced translocation in MHV could be due to the fast-acting properties of florpyrauxifen-benzyl affecting the plant's vascular tissue rapidly enough to reduce movement to belowground tissues. Florpyrauxifen-benzyl shoot-to-root translocation was 9.1 ± 1.1% and 1.3 ± 0.1% of total absorbed herbicide
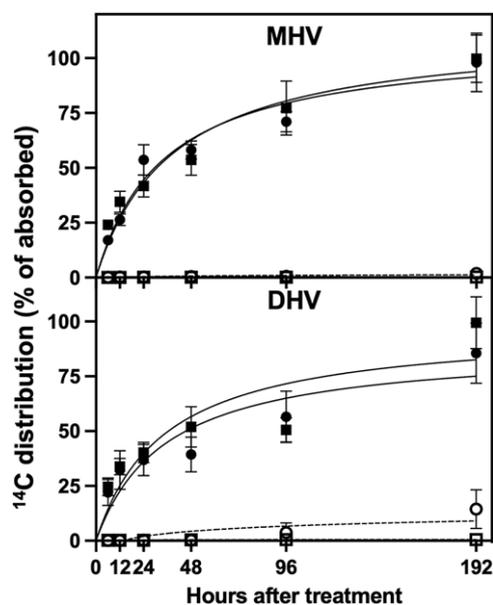
Figure 3. The ^{14}C distribution in *Hydrilla verticillata* biotypes (DHV, dioecious; MHV, monoecious) over 192 h following exposure to [^{14}C]florpyrauxifen-benzyl, expressed as percentage of total herbicide absorbed. Filled circle, percentage of [^{14}C]florpyrauxifen-benzyl alone in shoots; open circle, percentage of [^{14}C]florpyrauxifen-benzyl alone in roots; filled square, percentage of [^{14}C]florpyrauxifen-benzyl in combination with endothall in shoots; open square, percentage of [^{14}C]florpyrauxifen-benzyl in combination with endothall in roots. Data presented are means, and error bars are the standard error of the mean ($n = 6$).

found in the roots of DHV and MHV, respectively (Figure 3). In combination with endothall, florpyrauxifen-benzyl translocation was reduced in both *H. verticillata* biotypes (DHV = 0.5 ± 0.1%; MHV = 0.2 ± 0.03%) (Figure 3). Although florpyrauxifen-benzyl is highly active in both *H. verticillata* biotypes, the dose that corresponds to 50% inhibition of growth (GR$_{50}$) is lower in MHV than DHV (Netherland and Richardson 2016; Richardson et al. 2016). As both *H. verticillata* biotypes were exposed to the same dose of florpyrauxifen-benzyl (3.8 µg L^{-1}), it may be that MHV vascular tissue is affected more rapidly than DHV, impacting herbicide translocation to the roots.

In conclusion, although radioactive herbicide translocation to the roots was impaired when the herbicides were used in combination, this is the first study using radiolabeled herbicides to examine herbicide combination in aquatic plants; therefore, future research needs to be conducted to determine whether this reduced translocation negatively affects the overall effectiveness of this potential control strategy in the field. One major benefit of using endothall and florpyrauxifen-benzyl in combination is that both products require reduced exposure times relative to other systemic herbicides available for the aquatic system that are efficacious for *H. verticillata*. This study suggests that endothall and florpyrauxifen-benzyl have no negative impacts on each other's absorption and accumulation in *H. verticillata* and can be an excellent tool to delay the development of herbicide-resistant aquatic weeds when used in combination.

References

Arias RS, Netherland MD, Scheffler BE, Puri A, Dayan FE (2005) Molecular evolution of herbicide resistance to phytoene desaturase inhibitors in *Hydrilla verticillata* and its potential use to generate herbicide-resistant crops. Pest Manag Sci 61:258–268

Bajsa J, Pan Z, Dayan FE, Owens DK, Duke SO (2012) Validation of serine-threonine protein phosphatase as the herbicide target site of endothall. Pestic Biochem Physiol 102:38–44

Beckie HJ, Reboud X (2009) Selecting for weed resistance: herbicide rotation and mixture. Weed Technol 23:363–370

Beets J, Heilman M, Netherland MD (2019) Large-scale mesocosm evaluation of florpyrauxifen-benzyl, a novel arylpicolinate herbicide, on Eurasian and hybrid watermilfoil and seven native submersed plants. J Aquat Plant Manag 57:49–55

Busi R, Powles SB, Beckie HJ, Renton M (2020) Rotations and mixtures of soil-applied herbicides delay resistance. Pest Manag Sci 76:487–496

Cook CD, Lüönd R (1982) A revision of the genus *Hydrilla* (Hydrocharitaceae). Aquat Bot 13:485–504

Dayan FE, Netherland MD (2005) Hydrilla, the perfect aquatic weed, becomes more noxious than ever. Outlooks Pest Manag 16:277–282

de Carvalho RF, Bromilow RH, Greenwood R (2007) Uptake of pesticides from water by curly waterweed *Lagarosiphon major* and lesser duckweed *Lemna minor*. Pest Manag Sci 63:789–797

Epp JB, Alexander AL, Balko TW, Buysse AM, Brewster WK, Bryan K, Daeuble JF, Fields SC, Gast RE, Green RA, Irvine NM, Lo WC, Lowe CT, Renga JM, Richburg JS, *et al.* (2016) The discovery of Arylex (TM) active and Rinskor (TM) active: two novel auxin herbicides. Bioorgan Med Chem 24:362–371

Evans JA, Williams A, Hager AG, Mirsky SB, Tranel PJ, Davis AS (2018) Confronting herbicide resistance with cooperative management. Pest Manag Sci 74:2424–2431

Frank PA, Hodgson RH (1964) A technique for studying absorption and translocation in submersed plants. Weeds 12:80–82

Getsinger KD, Netherland MD, Grue CE, Koschnick TJ (2008) Improvements in the use of aquatic herbicides and establishment of future research directions. J Aquat Plant Manag 46:32

Gettys LA, Haller WT, Petty DG (2020) Biology and Control of Aquatic Plants: A Best Management Practices Handbook. 4th ed. Marietta, GA: Aquatic Ecosystem Restoration Foundation. 252 p

Gettys LA, Leon RG (2021) A population genetics approach for the study of fluridone resistance in hydrilla. Aquat Invasions 16:28–42

Giannotti AL, Egan TJ, Netherland MD, Williams ML, Knecht AK (2014) Hydrilla shows increased tolerance to fluridone and endothall in the Winter Park Chain of Lakes: considerations for resistance management and treatment options. Technical presentation to the Florida Aquatic Plant Management Society, Daytona Beach, FL. https://conference.ifas.ufl.edu/aw/archived/aw14/Presentations/Grand/Thursday/Session%209A/0850%20Giannotti.pdf. Accessed: February 9, 2022

Haug EJ, Ahmed KA, Gannon TW, Richardson RJ (2021) Absorption and translocation of florpyrauxifen-benzyl in ten aquatic plant species. Weed Sci 69:624–630

Kniss AR, Vassios JD, Nissen SJ, Ritz C (2011) Nonlinear regression analysis of herbicide absorption studies. Weed Sci 59:601–610

Kyser GB, Madsen JD, Miskella J, O'Brien J (2021) New herbicides and tank mixes for control of waterhyacinth in the Sacramento–San Joaquin Delta. J Aquat Plant Manag 59s:78–81

Langeland KA (1996) *Hydrilla verticillata* (LF) Royle (Hydrocharitaceae), "the perfect aquatic weed." Castanea 61:293–304

Michel A, Arias RS, Scheffler BE, Duke SO, Netherland M, Dayan FE (2004) Somatic mutation-mediated evolution of herbicide resistance in the nonindigenous invasive plant hydrilla (*Hydrilla verticillata*). Mol Ecol 13:3229–3237

Nandula VK, Riechers DE, Ferhatoglu Y, Barrett M, Duke SO, Dayan FE, Goldberg-Cavalleri A, Tétard-Jones C, Wortley DJ, Onkokesung N, Brazier-Hicks M, Edwards R, Gaines T, Iwakami S, Jugulam M, Ma R (2019) Herbicide metabolism: crop selectivity, bioactivation, weed resistance, and regulation. Weed Sci 67:149–175

Nawrocki JJ, Richardson RJ, Hoyle ST (2016) Monoecious hydrilla tuber dynamics following various management regimes on four North Carolina reservoirs. J Aquat Plant Manag 54:12–19

Netherland MD, Jones D (2015) Fluridone-resistant hydrilla (*Hydrilla verticillata*) is still dominant in the Kissimmee Chain of Lakes, FL. Invasive Plant Sci Manag 8:212–218

Netherland MD, Richardson RJ (2016) Evaluating sensitivity of five aquatic plants to a novel arylpicolinate herbicide utilizing an organization for economic cooperation and development protocol. Weed Sci 64:181–190

Ortiz MF, Nissen SJ, Gray CJ (2019) Endothall behavior in *Myriophyllum spicatum* and *Hydrilla verticillata*. Pest Manag Sci 75:2942–2947

Ortiz MF, Nissen SJ, Gray CJ (2022) Endothall absorption and translocation by curly-leaved and sango pondweed. J Aquat Plant Manag 60:34–38

Ortiz MF, Nissen SJ, Thum R, Heilman MA, Dayan FE (2020) Current status and future prospects of herbicide for aquatic weed management. Outlooks Pest Manag 31:270–275

Puri A, MacDonald GE, Altpeter F, Haller WT (2007) Mutations in phytoene desaturase gene in fluridone-resistant hydrilla (*Hydrilla verticillata*) biotypes in Florida. Weed Sci 55:412–420

Richardson RJ, Haug EJ, Netherland MD (2016) Response of seven aquatic plants to a new arylpicolinate herbicide. J Aquat Plant Manag 54:26–31

Sperry BP, Leary JK, Jones KD, Ferrell JA (2021) Observations of a submersed field application of florpyrauxifen-benzyl suppressing hydrilla in a small lake in central Florida. J Aquat Plant Manag 59:20–26

Vassios JD, Nissen SJ, Brunk GR (2011) Imazamox absorption, desorption, and metabolism by Eurasian watermilfoil. J Aquat Plant Manag 49:44–49

Wersal RM, Madsen J (2010) Combinations of penoxsulam and diquat as foliar applications for control of waterhyacinth and common salvinia: evidence of herbicide antagonism. J Aquat Plant Manag 48:21–25

Wersal RM, Madsen JD (2012) Combinations of diquat and carfentrazone-ethyl for control of floating aquatic plants. J Aquat Plant Manag 50:46–48