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Note

Mesocosm response of crested floating heart, hydrilla, and two native emergent plants to florpyrauxifen-benzyl: A new arylpicolinate herbicide

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INTRODUCTION

The development of new aquatic herbicides expands options available to resource managers for controlling invasive aquatic plants. Currently 14 herbicides are approved for aquatic use, and in many situations, controlling target invasive plants is balanced with the desire to enhance or conserve native aquatic vegetation (Netherland 2014). A new herbicide chemistry (4-amino-3-chloro-6-[4-chloro-2-fluoro-3-methoxyphenyl]-5-fluoro-pyridine-2-benzyl-ester), also identified as florpyrauxifen-benzyl, is currently being developed as an aquatic herbicide (Procellacor™) by SePRO Corporation (Carmel, IN) in partnership with Dow Agrosciences (Indianapolis, IN). This herbicide is also being developed for worldwide weed control in rice (Rinskor™) and other agricultural uses. The herbicide is part of a new class of synthetic auxins, the arylpicolinates, that differ in binding affinity compared to currently registered auxins such as 2,4-D and triclopyr (Lee et al. 2013, Bell et al. 2015). In small-scale laboratory screens, florpyrauxifen-benzyl was shown to be active on several aquatic weed species including crested floating heart (*Nymphoides cristata;* hereafter called CFH), hydrilla (*Hydrilla verticillata* L.f. Royle, both dioecious and monoecious biotypes), and Eurasian watermilfoil (*Myriophyllum spicatum* L.) (Netherland and Richardson 2016, Richardson et al. 2016). These studies suggested rapid activity under static conditions at concentrations from 1 to 27 μg L^{-1}. The testing of florpyrauxifen-benzyl in outdoor mesocosm experiments remains limited and requires further investigation to determine efficacy and selectivity under various exposure scenarios on more established plants.

Previous studies evaluating concentration and exposure time scenarios for registered aquatic herbicides on invasive and native plants have provided valuable information regarding potential herbicide use patterns (Green and Westerdahl 1988, Netherland et al. 1991, Netherland et al. 1993, Skogerboe et al. 2006, Glomski and Netherland 2007, Netherland 2011, Mudge et al. 2012, Glomski and Netherland 2013). Based on initial laboratory trials, florpyrauxifen-benzyl has the potential for a unique use pattern. The proposed low-use concentrations ($\sim$10 to 40 μg L^{-1}) are characteristic of slow-acting, low-dose products such as fluridone, penoxsulam, bispyribac, and topramezone; however, the rapid activity and potential for short exposure requirements (6 to 48 hr) is consistent with contact (e.g., endothall and diquat) and auxin-mimic herbicides (e.g., 2,4-D and triclopyr) that are used at concentrations in the range of 500 to 4000 μg L^{-1} (Netherland 2014). Florypyrauxifen-benzyl has characteristics that have potential to significantly reduce herbicide volumes associated with spot or partial-lake treatments of submersed invasive aquatic weeds.

CFH is an invasive aquatic plant that is continuing to spread in Florida and the southeastern United States, with its most notable invasion including establishment on several thousand acres in Lake Marion, SC. CFH forms dense surface mats of floating leaves that reduce light penetration and restrict water movement by reducing flow (Burks 2002). In addition to spread via fragmentation, CFH produces ramets, a vegetative propagule that will break away and float to a new location or will sink and remain dormant, evading foliar herbicide applications (Glomski et al. 2014). Herbicide efficacy depends on the age and life stage of the plant, and to date herbicide efficacy has been variable and unpredictable. Despite classification of CFH as a dicotyledon, evaluation of the auxin-mimic herbicides 2,4-D and triclopyr resulted in poor activity (Willey et al. 2014).

Hydrilla is another aggressive submersed aquatic invasive species that has been described as the "perfect aquatic weed" due to multiple traits that make the plant highly aggressive and competitive (Langeland 1996). Hydrilla can

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rapidly spread and occupy large expanses of lakes and reservoirs, and this ability for extensive growth can negatively impact recreation, flood control capacity, access, and aesthetics of both large and small water bodies. A number of registered herbicides can be used to control hydrilla, and the development of fluridone-tolerant hydrilla in Florida has been a key in registering alternate modes of action (five products since 2007) (Netherland 2014). Isolated populations of hydrilla in Florida have also shown tolerance to endothall (Giannotti et al. 2014). Both monoecious and dioecious hydrilla continue their spread into new regions, and so additional herbicides are needed to give resource managers increased flexibility (Richardson et al. 2016). A low use-rate systemic product with a short exposure requirement would provide managers with a novel strategy for targeting hydrilla.

A continued need for new herbicide modes of action exists for highly invasive plants such as hydrilla, CFH, and watermilfoils. The objectives of this study were to evaluate the activity of florpyrauxifen-benzyl against the invasive aquatic plants hydrilla and CFH and the native emergent plants sagittaria (*Sagittaria lancifolia*) and pickerelweed (*Pontederia cordata*) under a range of concentrations and exposures. This was done to determine initial activity and selectivity following short-term exposure scenarios on more established plants under outdoor mesocosm conditions. Previous laboratory studies have suggested rapid activity can be expected, and these mesocosm trials were conducted to confirm that use of florpyrauxifen-benzyl at low concentrations (12 to 48 μg L^{-1}) and comparatively short exposure periods (1 to 3 d) would impact the target plants.

MATERIALS AND METHODS

Two experiments were conducted at the University of Florida Center for Aquatic and Invasive Plants, Gainesville, FL. The first conducted from July 24, 2015, to August 21, 2015, and this study included hydrilla, CFH, and the native emergent plant sagittaria. The second experiment was conducted from September 8, 2015, to October 6, 2015, and included hydrilla, CFH, sagittaria, and pickerelweed. In the second experiment, the herbicide endothall, which is widely used for treatment of hydrilla and CFH, was added to provide a basis for comparison.

Experiment 1

Twenty-four 900 L (78 × 223 × 50 cm) concrete tanks were each planted with CFH, dioecious hydrilla, and sagittaria collected from culture tanks. All species were grown in 3.78-L plastic pots filled with Margo Professional Topsoil combined with Osmocote® (15-9-12) at 1.5 g kg^{-1} and capped with 5 cm of builder's sand. Plants were allowed to grow for 6 wk before treatment. All treatments were replicated six times and randomly assigned to each tank. Florpyrauxifen-benzyl (FPB) was applied to tanks (as SLF-9522, a 300 g a.i. L^{-1} suspension concentrate formulation). Tanks treated with 24 μg L^{-1} FPB were drained and filled with well water. A trickle flow was maintained for several days after the initial refill to remove any remaining herbicide concentrations. A 12 μg L^{-1} static treatment for 7 d was also evaluated in this trial. Hydrilla, CFH, and sagittaria were harvested from each tank at 28 d after treatment (DAT), and aboveground biomass was collected (above and belowground biomass was collected for sagittaria).

Water samples (~50 ml) were collected from treatment tanks at 2 hr after application and 24 hr postdrain to confirm initial treatment concentrations and removal of FPB following the drain procedure. Samples were analyzed via high performance liquid chromatography with tandem mass spectroscopy with limits of quantitation of 0.02 μg a.i. L^{-1} for FPB and 0.05 μg a.i. L^{-1} for a less-active acid metabolite.

Experiment 2

The tanks described above were utilized for the second experiment, and plant species included hydrilla, CFH, sagittaria, and pickerelweed. Hydrilla and CFH were grown in similar conditions to the first study, while young sagittaria and pickerelweed plants were purchased from a commercial grower, and plants were allowed to establish for 8 wk before study initiation. The second experiment included FPB applied at concentrations of 24 and 48 μg L^{-1} for 24 or 72 hr or a static exposure at 12 μg L^{-1}, and the dipotassium salt of endothall applied at 3000 μg a.i. L^{-1} for a 24 or 72 hr exposure. Because of the efficacy observed at 24 μg L^{-1}, CFH was not included in the 48 μg L^{-1} treatments. At the end of the exposure periods, water was exchanged as described above. All plants were harvested at 28 DAT and separated into above and belowground biomass.

Each treatment was replicated three times. Following all harvests, plant biomass was placed in a forced air-drying oven at 70 C and weighed to the nearest 0.1 g. Data analysis was conducted using the R statistical package (Version 3.3.1). Dry weight biomass data for each treatment were analyzed using ANOVA, and means were separated via a Tukey test ($\alpha = 0.05$), following testing of normal distribution and homogeneity of variance.

RESULTS AND DISCUSSION

Experiment 1

Analytical results for FPB confirmed that the initial measured concentrations were within ±15% of the target concentration of 24 μg L^{-1}. Water samples collected at 24 hr following the drain procedure resulted in no detection of the parent molecule FPB or its acid metabolite.

28-d harvest. FPB treatment resulted in rapid onset of symptoms by hydrilla. Within 3 to 6 d, plant tissue in the surface canopy was brittle and readily fragmented with slight agitation. Without some level of agitation, visual observations initially suggested limited impact of FPB. FPB exposure time had a significant effect on hydrilla biomass ($P = 0.002$; Figure 1A). Hydrilla biomass at 28 d was reduced by 68% following the 24-hr exposure and 80% following the 72-hr exposure to FPB. Surviving biomass from these treatments was rooted, but there was no evidence of

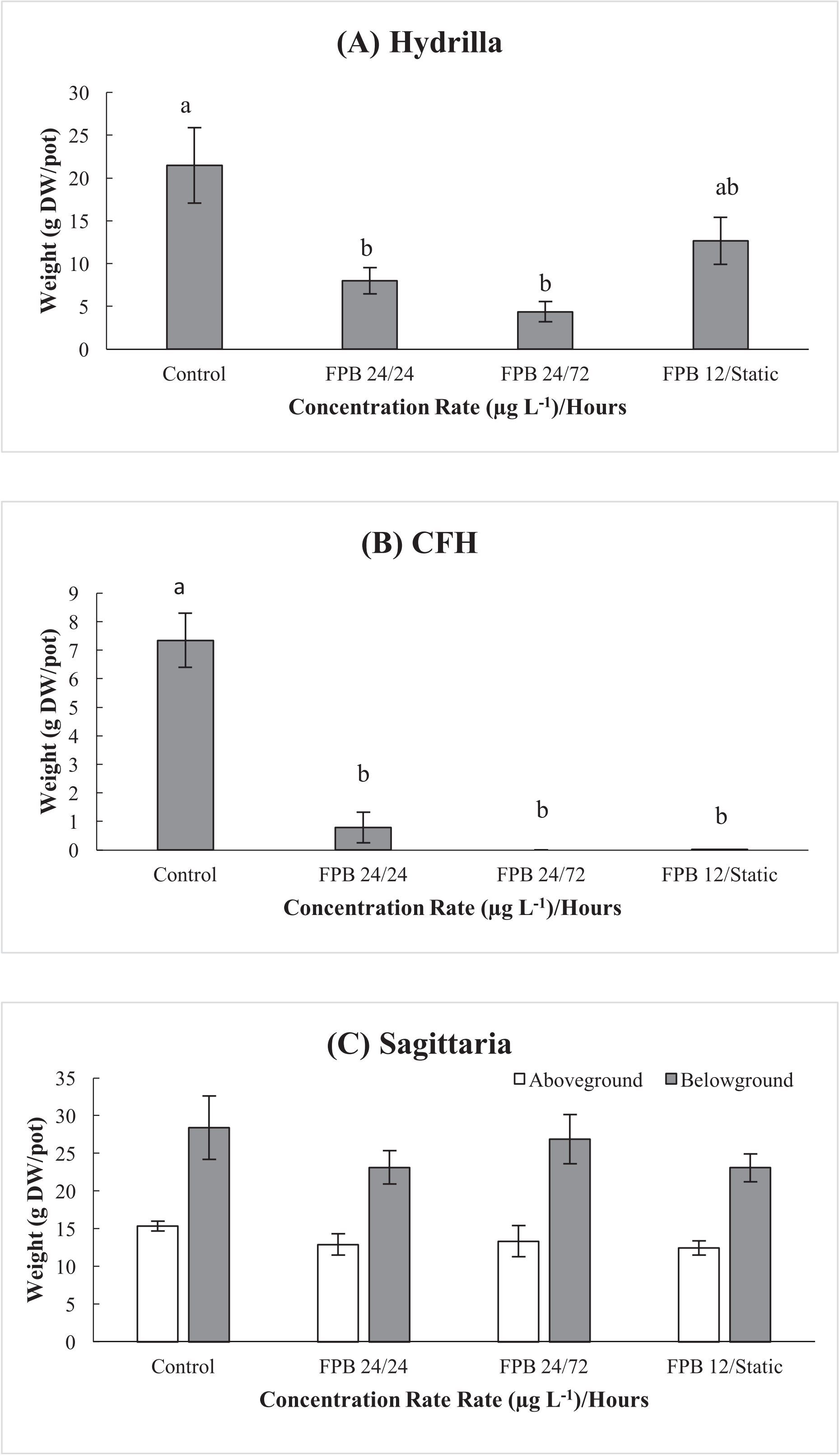

Figure 1. Dry weight biomass recorded at 28 d following treatment with florpyrauxifen-benzyl (FPB) at 24 µg L^{-1} for 24- and 72-hr exposures and a 12 µg L^{-1} static exposure on hydrilla (A), crested floating heart (B), and sagittaria (C). Bars represent mean values ($n = 6$) of dry weight ± SE. Letters above bars represent differences between treatment according to a Tukey's test ($\alpha = 0.05$).

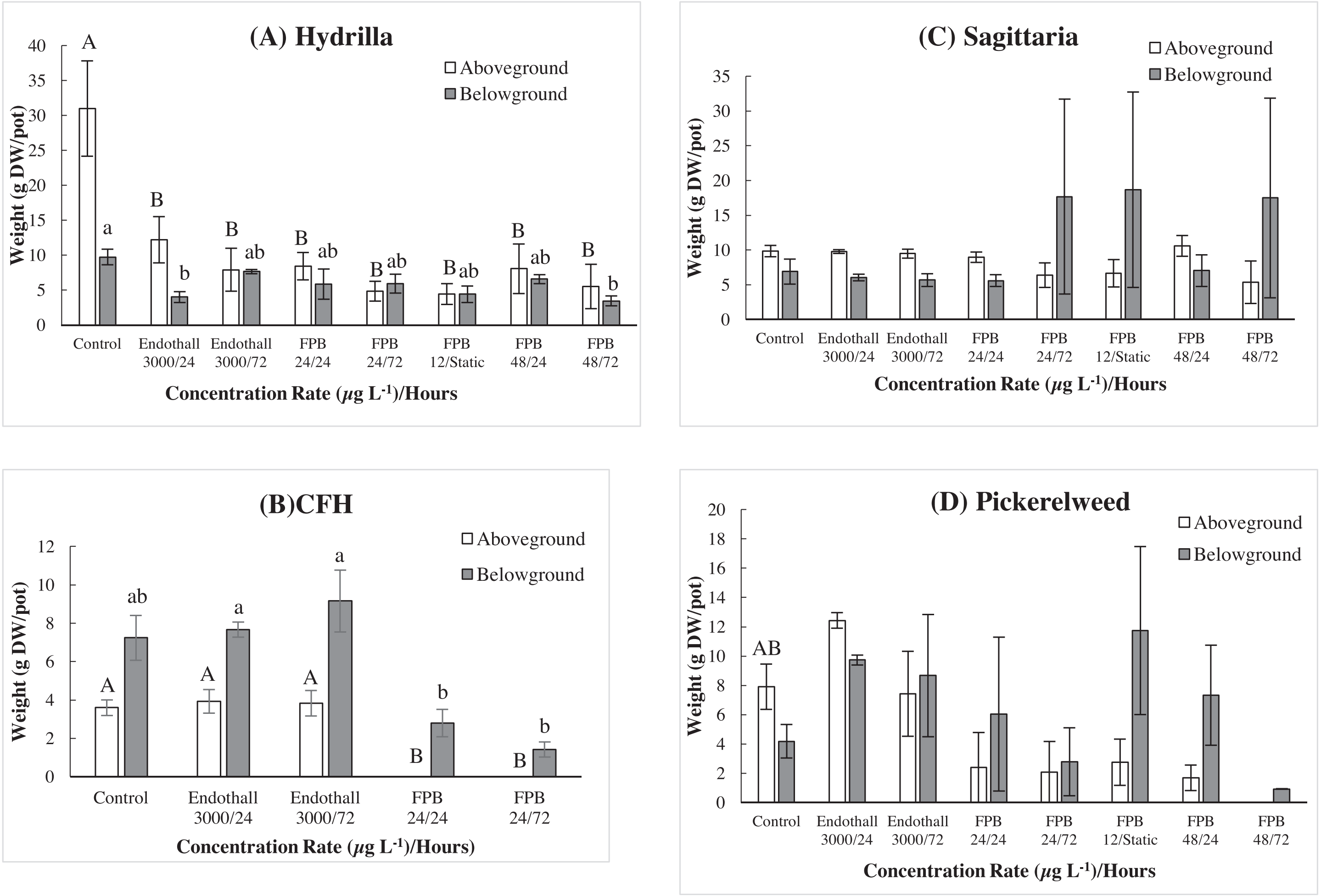

Figure 2. Dry weight biomass recorded at 28 d following treatment with florpyrauxifen-benzyl (FPB) at 24 and 48 μg L^{-1} for 24- and 72-hr exposures and a 12 μg L^{-1} static exposure on hydrilla (A), crested floating heart (B), sagittaria (C), and pickerelweed (D). Crested floating heart was not present in 48 μg L^{-1} treatments. Bars represent mean values ($n = 3$) of dry weight ± SE. Letters above bars represent differences between treatments for above and belowground biomass (compared separately) according to a Tukey's test ($\alpha = 0.05$).

recovery at 28 d. In contrast, extensive new aboveground biomass recovery was noted 21 DAT following the exposure to the static 12 μg L^{-1} treatment. Despite initial aboveground biomass fragmentation in the canopy, rapid regrowth from the rootcrown resulted in no biomass difference between this treatment and the untreated reference ($P = 0.14$).

FPB also resulted in rapid onset of symptoms by CFH within 3 d of application. These plants showed extensive visual symptoms with petiole elongation and bending and twisting associated with epinasty. Exposure time in all three treatments had a significant effect on CFH biomass relative to the untreated control ($P < 0.001$; Figure 1B). Mean biomass of CFH exposed for 24 hr was reduced 89%, while the 72-hr exposure resulted in 100% reduction (complete death). Additionally, the static exposure at 12 μg L^{-1} was highly effective and resulted in a 99% reduction compared to the control.

FPB resulted in limited initial visual symptoms associated with exposure of sagittaria. Some petiole bending was noted by 1 week after treatment; however, these symptoms were short-lived. There was no effect of treatment on mean sagittaria aboveground biomass ($P = 0.49$; Figure 1C) or belowground biomass ($P = 0.52$; Figure 1C).

Experiment 2

In the second experiment, FPB concentrations of 24 and 48 μg L^{-1} were evaluated on hydrilla, CFH, sagittaria, and pickerelweed. FPB treatment had a significant effect on mean hydrilla biomass at 28 DAT ($P = 0.001$; Figure 2A). The mean biomass of hydrilla was reduced by all treatments (61 to 86% reduction), yet there was no difference between any of the FPB treatments ($P > 0.76$). Increasing FPB concentration from 24 to 48 μg L^{-1} did not result in increased efficacy following both the 24- and 72-hr exposures. No differences in biomass were noted between FPB and endothall-treated hydrilla following similar exposure periods (Figure 2A).

A FPB treatment effect was detected for the mean biomass of CFH ($P < 0.001$; Figure 2B). The FPB treatments at 24 μg L^{-1} for 24- and 72-hr exposures resulted in complete control of aboveground biomass, while neither endothall treatment differed from the untreated reference. FPB treatments also affected mean belowground biomass ($P = 0.001$; Figure 2B) There was a 64% decrease in belowground biomass following FPB exposure for 24 hr and an 80% decrease following the 72-hr treatment ($P = 0.01$). The endothall treatments did not impact either above or belowground biomass of CFH. Neither FPB nor endothall treatments impacted mean sagittaria aboveground biomass ($P = 0.24$) or belowground biomass ($P = 0.83$) (Figure 2C).

There was a significant effect of FPB on the mean aboveground biomass of pickerelweed ($P = 0.002$; Figure 2D). While endothall did not impact pickerelweed, the FPB treatments resulted in 75 to 100% aboveground biomass reduction. FPB treatment did not have a significant effect on the mean belowground biomass of pickerelweed ($P = 0.40$) at 28 DAT.

Mesocosm results confirm that FPB was active on the target species CFH and hydrilla at concentrations ranging from 12 to 48 μg L^{-1}. Whereas previous laboratory studies were conducted on small-rooted plants under static conditions (Netherland and Richardson 2016, Richardson et al. 2016), these results confirm activity on larger, more robust plants following exposure times of 24 to 72 hr. We did not observe increased control of hydrilla when doubling FPB concentrations from 24 to 48 μg L^{-1} or increasing exposures from 24 to 72 hr. This result was not expected, and it suggests initial uptake of FPB by hydrilla is rapid and may quickly reach a plateau as evidenced by the observed tissue fragility or “shattering” and rapid growth cessation but delayed decay. Evaluation of exposure times ranging from 96 to 168 hr is recommended to determine if slightly longer exposure times at concentrations near 20 to 30 μg L^{-1} may result in greater control of hydrilla. These treatments were conducted in July and August, and the role of treatment timing still needs to be evaluated. Endothall did not perform as well as has been observed in spring and fall trials.

The activity of FPB on CFH suggests a novel herbicide use pattern may be possible for this plant. CFH has shown low susceptibility to several common aquatic herbicides including glyphosate, penoxsulam, endothall (dipotassium salt), and the auxin-mimics 2,4-D and triclopyr. The rapid auxin symptoms noted within days following a 24 μg L^{-1} treatment with FPB are in marked contrast to the lack of activity noted at concentrations of 2000 to 3000 μg L^{-1} with 2,4-D and triclopyr (Willey et al. 2014). Results following the 12 μg L^{-1} treatments with static exposures suggest a lower concentration threshold for CFH versus hydrilla. We did not evaluate a foliar spray pattern with FPB, but results suggest this should be evaluated.

There is some anecdotal evidence that initial water temperature and/or pH may impact the efficacy of FPB. Additional work in this area is suggested because products such as flumioxazin and 2,4-D ester formulations can be greatly influenced by factors such as pH and alkalinity (Glomski and Netherland 2008, Mudge et al. 2010). Given that FPB is an ester with hydrolysis as a secondary route of degradation at high pH (9+) (SePRO/Dow AgroSciences, unpublished EPA registration studies), the interaction between water quality, plant species, and herbicide activity should be further evaluated. Larger-scale mesocosm trials and/or early field development should also further assess longevity of CFH and hydrilla control at intervals beyond the 1-mo duration of these experiments. These trials were primarily designed to determine potential concentration and exposure time scenarios that should be further tested.

Sagittaria was not impacted under these treatment scenarios; however, we did observe significant aboveground biomass reduction of newly established pickerelweed. This result suggests that further evaluation of native emergent and submersed species is needed to document both efficacy and species selectivity of FPB. Additionally, understanding effects of FPB on a variety of phenological stages of native plant species would be beneficial.

The addition of endothall allowed for comparison against FPB activity on both invasive and native plants under similar exposure scenarios. Results suggest a similar late season response to both products by hydrilla, while CFH was much more sensitive to FPB when compared to endothall. Native emergent plants were not impacted by endothall, while FPB showed significant activity on the pickerelweed.

For future evaluations, we recommend addition of other invasive and native plants under a broader range of concentration and exposure scenarios and treatment timing. We would also encourage research on plants of different levels of maturity at different times of the year. Control and activity on a newly established submersed or emergent plant can be quite different when compared to a well-established plant of the same species.

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