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Large-scale mesocosm evaluation of florpyrauxifen-benzyl, a novel arylpicolinate herbicide, on Eurasian and hybrid watermilfoil and seven native submersed plants

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ABSTRACT

Eurasian watermilfoil (*Myriophyllum spicatum* L.; EWM) and hybrid Eurasian watermilfoil (*Myriophyllum spicatum* L. × *Myriophyllum sibiricum* Kom.; HWM) are problematic invasive submerged plants often managed with selective-use patterns of various aquatic herbicides. Since its confirmation HWM has been a concern due to reports of reduced herbicide efficacy across several modes of action, including the those of synthetic auxins. For the auxin-mimic herbicides, it is not clear whether the reduced efficacy is herbicide- or class-specific or whether it affects entire modes of action. The arylpicolinate herbicide florpyrauxifen-benzyl has shown promise for control of several invasive aquatic plant species, including watermilfoils, at lower use rates than currently used herbicides. A study was designed to evaluate concentration–exposure-time (CET) scenarios using florpyrauxifen-benzyl on well-established EWM and HWM, as well as on seven native species grown in 6,700-L tanks at the Lewisville Aquatic Ecosystem Research Facility in Lewisville, TX. The inclusion of native species allowed for insight on the selectivity of florpyrauxifen-benzyl. Florpyrauxifen-benzyl treatments were applied at three concentrations (3, 9, and 27 μg ai L^{-1}) for 6- and 24-h half-lives, as well as two concentrations (3 and 9 μg L^{-1}) as a static exposure. Eight CET scenarios were tested and biomass harvests were performed 30 and 60 d after treatment. Results indicated that all CET scenarios resulted in significant control of EWM and HWM, with HWM showing a lower sensitivity to florpyrauxifen-benzyl. Additionally, native species showed lower sensitivity to florpyrauxifen-benzyl and the new herbicide should provide selectivity when used for EWM or HWM control under the rate and exposure scenarios tested.

Key words: concentration–exposure time, *Myriophyllum spicatum*, *Myriophyllum spicatum x M. sibiricum*, selectivity.

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INTRODUCTION

Eurasian watermilfoil (*Myriophyllum spicatum* L.; EWM) and hybrid Eurasian watermilfoil (*Myriophyllum spicatum* L. × *Myriophyllum sibiricum* Kom.; HWM) are problematic submersed aquatic invasive plants in many North American waterways. Auxin-mimic herbicides, such as 2,4-D and triclopyr, are commonly used for selective of control of invasive populations of EWM, HWM, and other dicotyledonous species by stimulating auxin overdose (Netherland and Getsinger 1992, Poovey et al. 2007, Wersal et al. 2010). Differences in response to 2,4-D between EWM and HWM have led to discussion of whether this response is specific to 2,4-D or is a general response to auxin mimics. These synthetic auxins are more stable in their binding to auxin receptors than natural hormones, making the synthetic auxins more resistant to inactivation by the plant (Grossman 2010).

Moody and Les (2002) documented hybrid populations of watermilfoil, previously thought to be EWM, using nuclear ribosomal DNA analysis. Due to their highly similar morphology, DNA analysis is the most accurate method for discerning between EWM and HWM. The potential for inherited traits in HWM, such as increased invasiveness, hybrid vigor, or increased tolerance to herbicides, presents additional concerns for aquatic weed control programs (Thompson 1991, Ellstrand and Schierenbeck 2000, Moody and Les 2002). Chemical applications have the potential to create niche habitats for HWM if herbicides have reduced efficacy (LaRue et al. 2013). In this situation, EWM could be drastically reduced or eliminated by exposure to auxin herbicides, while HWM survives to spread and repopulate treated sites (Ellstrand and Schierenbeck 2000). However, it is important to consider that hybrid populations can arise independently, and herbicide response may vary greatly between hybrid populations due to inherited traits.

Development of a new class of synthetic auxins, the arylpicolinates, has resulted in production of the novel herbicide florpyrauxifen-benzyl, and it may provide a new tool to augment options for control of problematic aquatic weeds. The arylpicolinates 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 studies, florpyrauxifen-benzyl has been shown to be active on several aquatic weed species, including crested floating heart [*Nymphoides cristata* (Roxb.) Kuntze], hydrilla [*Hydrilla verticillata* (L.f) Royle—both dioecious and monoecious biotypes), and EWM (Netherland and Richardson 2016, Richardson et al. 2016). Results from these studies suggested that concentrations of florpyrauxifen-benzyl had activity on EWM well below typical use rates for 2,4-D and triclopyr.

Concentration and exposure-time (CET) requirements are key factors in evaluation of a new herbicide to determine use patterns. CET represents the amount of time that various herbicide concentrations are in contact with a plant and describes how an aquatic herbicide should affect a given plant species (Getsinger and Netherland 1997, 2018). Under operational herbicide use, a wide range of potential CET scenarios may occur due to various factors such as treatment scale, water flow or exchange, application rate, adsorption, degradation, and diffusion (Green and Westerdahl 1990; Skogerboe et al. 2006; Glomski et al. 2009; Glomski and Netherland 2010, 2014; Nault et al. 2014; Netherland and Glomski 2014; Netherland and Jones 2015). CET is species dependent and can play an important role in herbicide selectivity (Getsinger and Netherland 1997). There has been considerable research conducted to define the CET requirements for control of EWM using the herbicides 2,4-D (Green and Westerdahl 1990, Nault et al. 2014) and triclopyr (Netherland and Getsinger 1992, Netherland and Glomski 2014, Netherland and Jones 2015). Further investigation of CET requirements is needed to evaluate the efficacy and use patterns of the new compound, florpyrauxifen-benzyl.

The goal of this research was to evaluate a wide range of CET conditions to determine the effect of florpyrauxifen-benzyl on well-established EWM and HWM as well as on several native submersed species in large-scale mesocosms. Our objectives with this experiment were to determine the most effective CET combinations for EWM and HWM control and to observe the effect of these CET scenarios on several native species. Native submersed species from North America included American pondweed (*Potamogeton nodosus* Poir.), elodea (*Elodea canadensis* Michx.), water stargrass [*Heteranthera dubia* (Jacq.) MacMill.], Illinois pondweed (*Potamogeton illinoensis* Morong), and vallisneria (*Vallisneria americana* Michx.) from southern and northern locations. These species are considered desirable and less problematic than EWM and HWM.

MATERIALS AND METHODS

Plants were established on 15 September 2015 from apical stems or root nodes (*Vallisneria*) at the U.S. Army Engineer Research and Development Center's Lewisville Aquatic Ecosystem Research Facility in Lewisville, TX. Each 6,700-L mesocosm was planted with two 3-L pots each of American pondweed, Illinois pondweed, elodea, water stargrass, EWM, and HWM, and two populations of vallisneria from southern (Gainesville, FL) and northern (New York) locations. Specimens of HWM with reported tolerance to 2,4-D were used from a single population (Hayden Lake, ID) (Taylor et al. 2017, Beets and Netherland 2018). All plants were established in topsoil amended with Forestry Supply[1] 20–10–5 fertilizer tablets (4.5 g kg^{-1}). Plants were allowed to establish from 15 September 2015 to 16 April 2016, and then treated with herbicide as noted below. One treated and one control tank contained HOBO[2] data loggers to observe temperature fluctuations during the study period.

Florpyrauxifen-benzyl[3] treatments were applied at concentrations of 0, 3, 9 and 27 μg ai L^{-1} for 6- and 24-h water-exchange half-lives as well as at two concentrations (3 and 9 ai μg L^{-1}) as static treatments with no water exchange. Untreated water was circulated through the mesocosms at appropriate times to provide nominal target water-exchange half-lives (Netherland and Glomski 2014). Each of the nine treatments had three replications randomly assigned to mesocosms. Water samples were collected from representative treatments and analyzed via liquid chromatography and tandem mass spectroscopy to determine actual herbicide concentrations (EPA 2015). Harvests were conducted at 30 and 60 d after treatment, collecting aboveground biomass of plants. Samples were dried in a forced-air dryer at 70 C until desiccated and then weighed to the nearest 0.1 g. Results were analyzed using separate one-way ANOVAs and Tukey's HSD to determine statistical differences in aboveground biomass among treatments at each harvest period ($P = 0.05$). Heteroscedasticity (unequal variance in predicted vs. residual data) was an issue, and data for EWM and HWM were square root transformed to meet assumptions of normality and equal variance. Nontransformed data are presented.

RESULTS AND DISCUSSION

Temperature in the mesocosms ranged from 16.6 to 26.9 C with a mean temperature of 21.7 C during the study period. Herbicide analysis determined florpyrauxifen-benzyl degradation was in line with expectations based on dilution scenarios and the herbicide's physical chemistry and relatively fast photolytically driven breakdown (Table 1; Washington State Department of Ecology 2017). Sample concentration fluctuations are likely due to a combination of herbicide photolytic degradation (0.6 d half-life), plant uptake, and limitations in analysis due to herbicide solubility in water (10 to 15 μg L^{-1}).

Milfoil efficacy

Florpyrauxifen-benzyl provided near complete reduction of EWM and HWM biomass for up to 60 d following treatment even at the lowest concentrations and exposure times evaluated (Figures 1A and 1B). EWM biomass was significantly reduced by all CET scenarios, whereas untreated control biomass showed an increase between harvest periods (Figure 1A). All exposure scenarios resulted in large reductions in HWM biomass 30 and 60 d after treatment compared to the untreated control. However, 30 d after treatment, HWM biomass in the 3-μg L^{-1} 6-h treatment (the lowest scenario) was greater than HWM biomass in the other CET treatments

Table 1. Mean ± SE florpyrauxifen-benzyl concentration (μg L^{-1}) collected at hours after treatment (HAT) and days after treatment (DAT) intervals following treatment ($n = 3$). Dashes indicate time periods when no sample was collected.

CET scenario[1]	1 HAT	6 HAT	24 HAT	48 HAT	72 HAT	7 DAT	10 DAT	14 DAT
27 μg L^{-1} 6 h	16.2 ± 2.8	8.1 ± 0.66	3.9 ± 2.9	1.3 ± 0.21	—	—	—	—
27 μg L^{-1} 24 h	14.3 ± 2.1	8.9 ± 0.72	9.0 ± 0.31	8.6 ± 2.48	2.0 ± 0.42	0.84 ± 0.21	—	—
3 μg L^{-1} static	2.2 ± 0.2	—	—	—	1.6 ± 0.67	0.77 ± 0.43	0.10 ± 0.03	0.07 ± 0.03
9 μg L^{-1} static	6.9 ± 0.5	—	—	—	2.6 ± 0.04	1.2 ± 0.27	0.25 ± 0.04	0.08 ± 0.04

[1]CET: concentration–exposure-time.

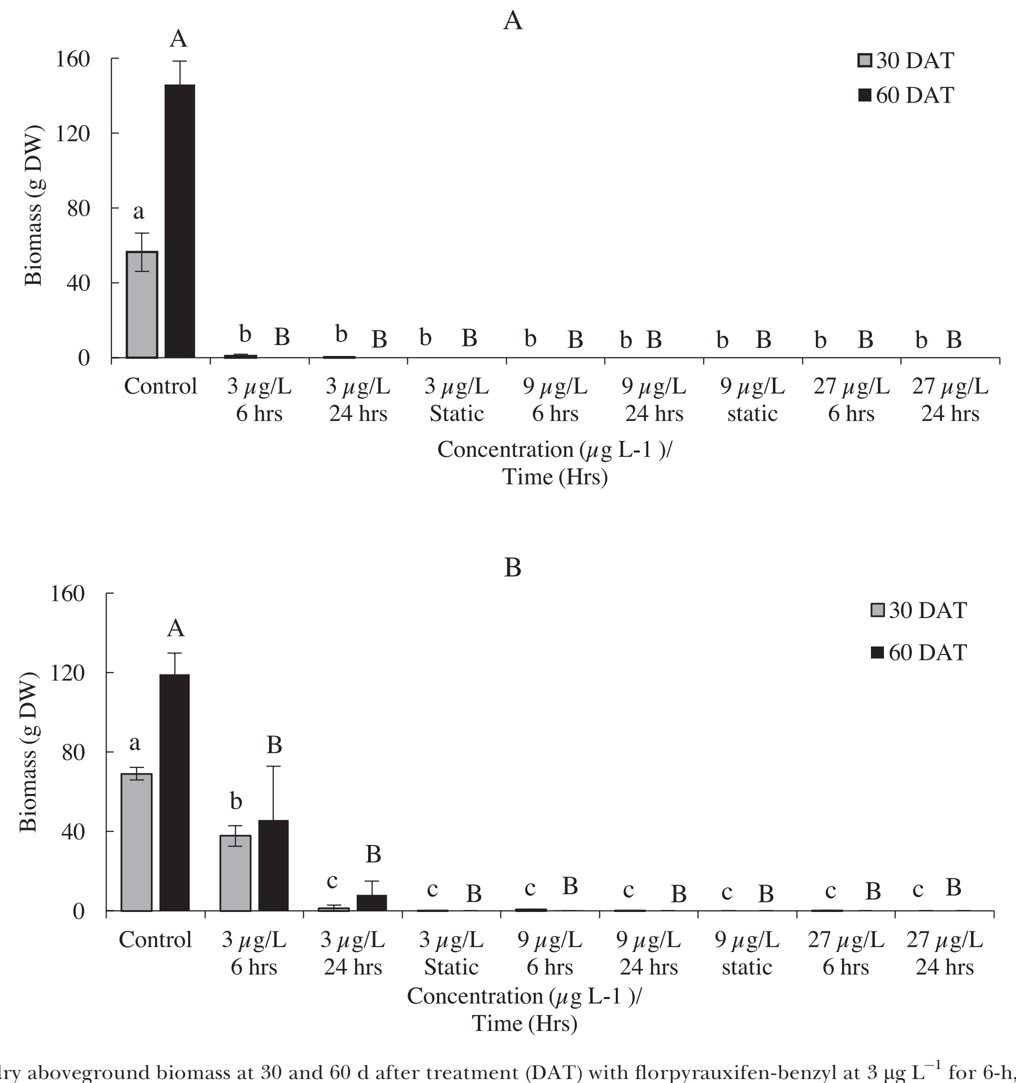

Figure 1. Mean ± SE dry aboveground biomass at 30 and 60 d after treatment (DAT) with florpyrauxifen-benzyl at 3 μg L^{-1} for 6-h, 24-h, and static water-exchange half-lives; 9 μg L^{-1} for 6-h, 24-h, and static water-exchange half-lives; and 27 μg L^{-1} for 6- and 24-h water-exchange half-lives on (A) EWM and (B) HWM ($n = 3$). Letters above bars represent differences between treatments according to Tukey's test ($\alpha = 0.05$). Uppercase letters indicate 60-d harvest dates that were analyzed separately.

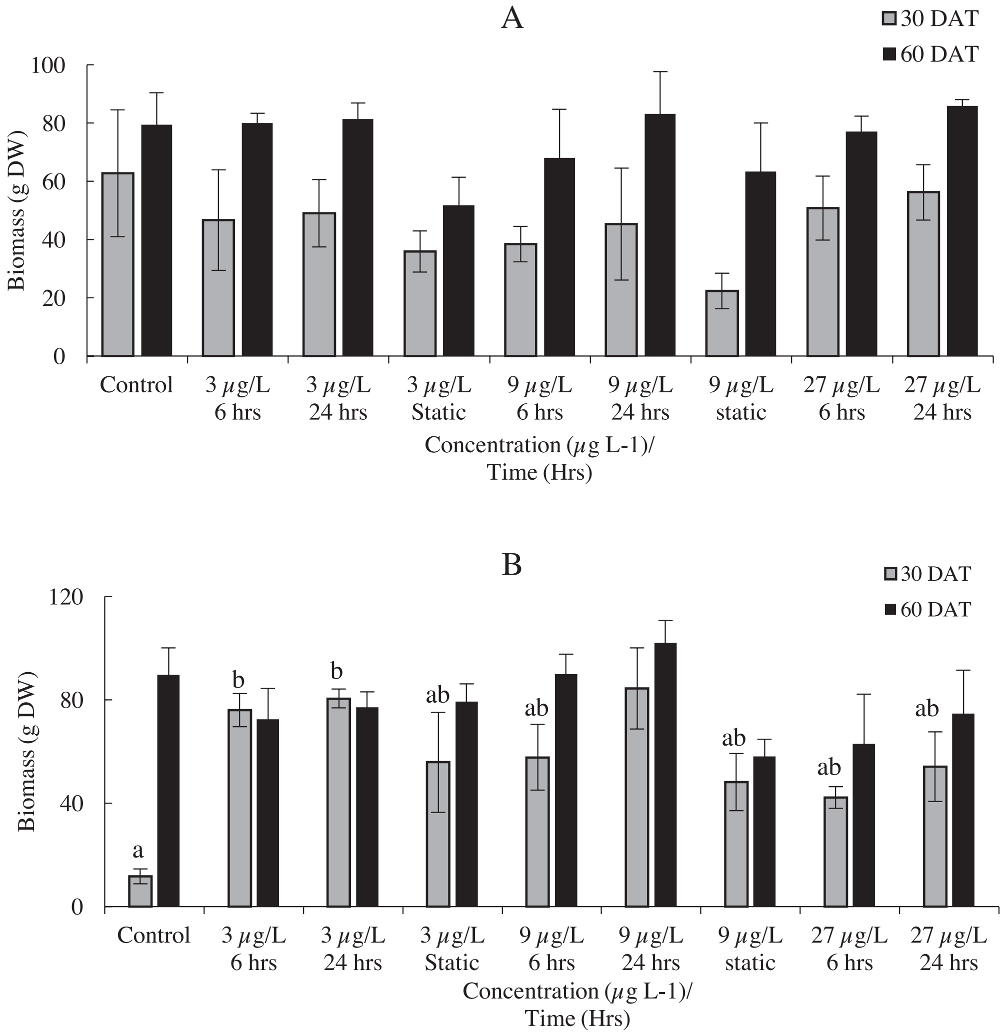

Figure 2. Mean ± SE dry aboveground biomass at 30 and 60 d after treatment (DAT) with florpyrauxifen-benzyl at 3 μg L^{-1} for 6-h, 24-h, and static water-exchange half-lives; 9 μg L^{-1} for 6-h, 24-h, and static water-exchange half-lives; and 27 μg L^{-1} for 6- and 24-h water-exchange half-lives on (A) American pondweed and (B) Illinois pondweed ($n = 3$). Letters above bars represent differences between treatments according to Tukey's test ($\alpha = 0.05$). Differences in mean biomass between 60-d treatments were not observed.

(Figure 1B). Differences in herbicide sensitivity between EWM and HWM have also been anecdotally observed in the field and seen in small-scale studies (Taylor et al. 2017, Beets and Netherland 2018). These use rates were also two orders of magnitude below the use rates for currently registered herbicides such as triclopyr and 2,4-D (Green and Westerdahl 1990, Netherland and Getsinger 1992, Nault et al. 2014) and suggest the potential use of florpyrauxifen-benzyl for watermilfoil control programs.

Native species efficacy

Overall, florpyrauxifen-benzyl had minimal effect on the native species evaluated in this study. It had no significant effect on American pondweed or Illinois pondweed biomass (Figures 2A and 2B) and some treatments of Illinois pondweed had greater biomass than the untreated control at 30 d. Increases in growth in treated mesocosms compared to untreated controls may be indicative of a lack of competition from the controlled milfoil. Elodea was not significantly affected by time or treatment (Figure 3A) and water stargrass showed the most treatment-related variability, with one treatment (3 μg L^{-1} for 6 h) showing a large increase in biomass and another (9 μg L^{-1} static) showing injury symptoms (Figure 3B). Given its sensitivity to 2,4-D, water stargrass may be a plant that requires further refinement of CET for selective milfoil treatments, and did not grow well in this study. No treatment scenario resulted in a significant reduction in southern vallisneria (Figure 4A). Northern vallisneria growth was minimal; however, northern vallisneria biomass in the 9-μg L^{-1} 24-h and 27 μg L^{-1} 24-h scenarios after 60 d was greater than the untreated control after 30 d ($P < 0.001$; Figure 4B).

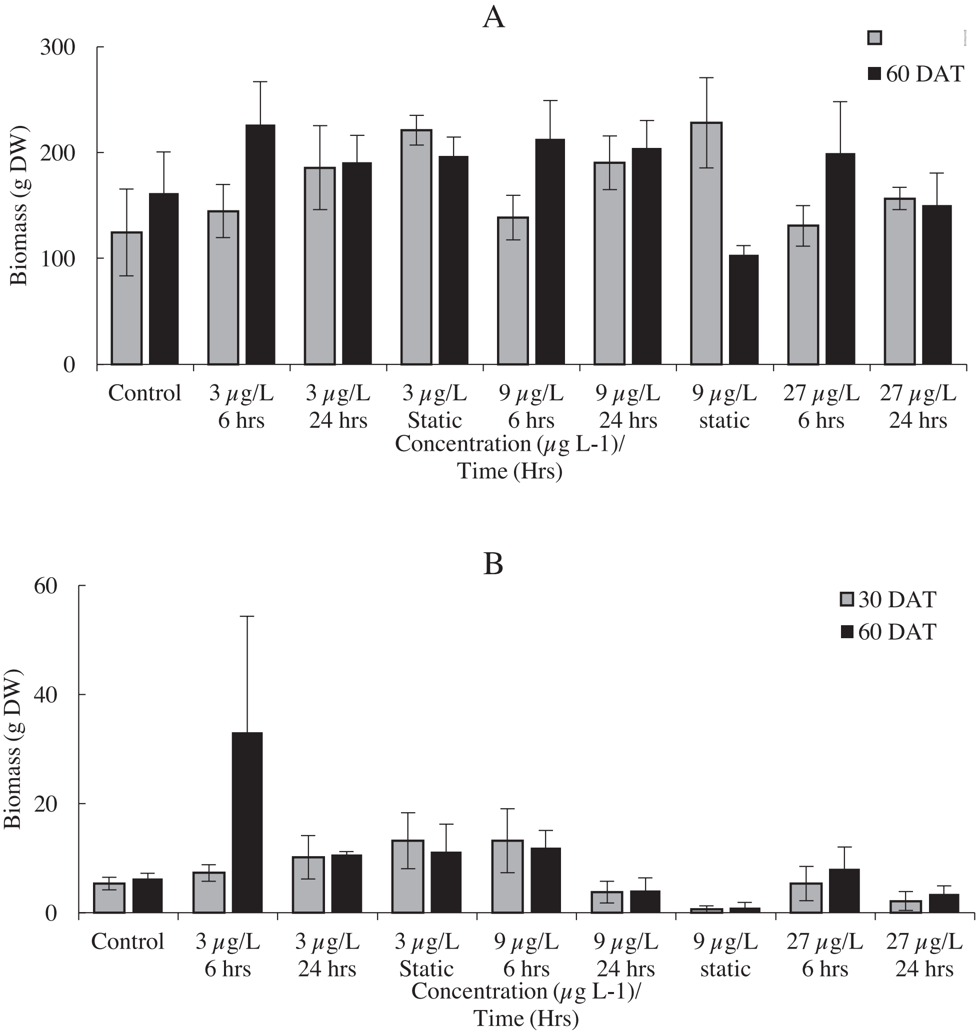

Figure 3. Mean ± SE dry aboveground biomass at 30 and 60 d after treatment (DAT) with florpyrauxifen-benzyl at 3 μg L^{-1} for 6-h, 24-h, and static water-exchange half-lives; 9 μg L^{-1} for 6-h, 24-h, and static water-exchange half-lives; and 27 μg L^{-1} for 6- and 24-h water-exchange half-lives on (A) elodea and (B) water stargrass ($n = 3$). Differences in mean biomass were not observed between treatments at 30 and 60 DAT.

Overall, this study confirms indications from preliminary studies of a high level of activity on EWM and HWM by florpyrauxifen-benzyl. In addition, exposure requirements were much shorter than expected, as evidenced by the strong control of EWM and HWM at the 3-μg L^{-1} 6-h water-exchange scenario. This information is promising for selective control of target milfoil populations when compared to the lack of response by native plants in the majority of CET scenarios. EWM and HWM were completely controlled in the 3-μg L^{-1} static treatments and also with higher herbicide concentrations whereas native species exhibited variable but largely insignificant responses to higher concentration as well as in both static treatments. While low-rate, static treatments are often used in targeting invasive aquatic species, hydrodynamic processes can greatly alter CET and therefore herbicide treatment efficacy. Static applications such as whole-lake treatments have the potential to lack selectivity, depending on the initial application rate. However, based on these results, florpyrauxifen-benzyl provides selective control of EWM and HWM under multiple CET scenarios.

In species-rich areas, the ability of low use rates to control milfoil invasions and allow the spread of native species via posttreatment regrowth and sustained control of EWM and HWM is vital to management. This study also indicated that prior small-scale trials were useful predictors of use patterns for larger-scale studies. Given the level of sensitivity of both EWM and HWM to the rates and exposures evaluated, the question of potential treatment-related differences between EWM and HWM was not adequately addressed. Although there is some evidence of increased tolerance by HWM, further trials (with this and additional strains of HWM) to determine if there are real differences in response to florpyrauxifen-benzyl are warranted.

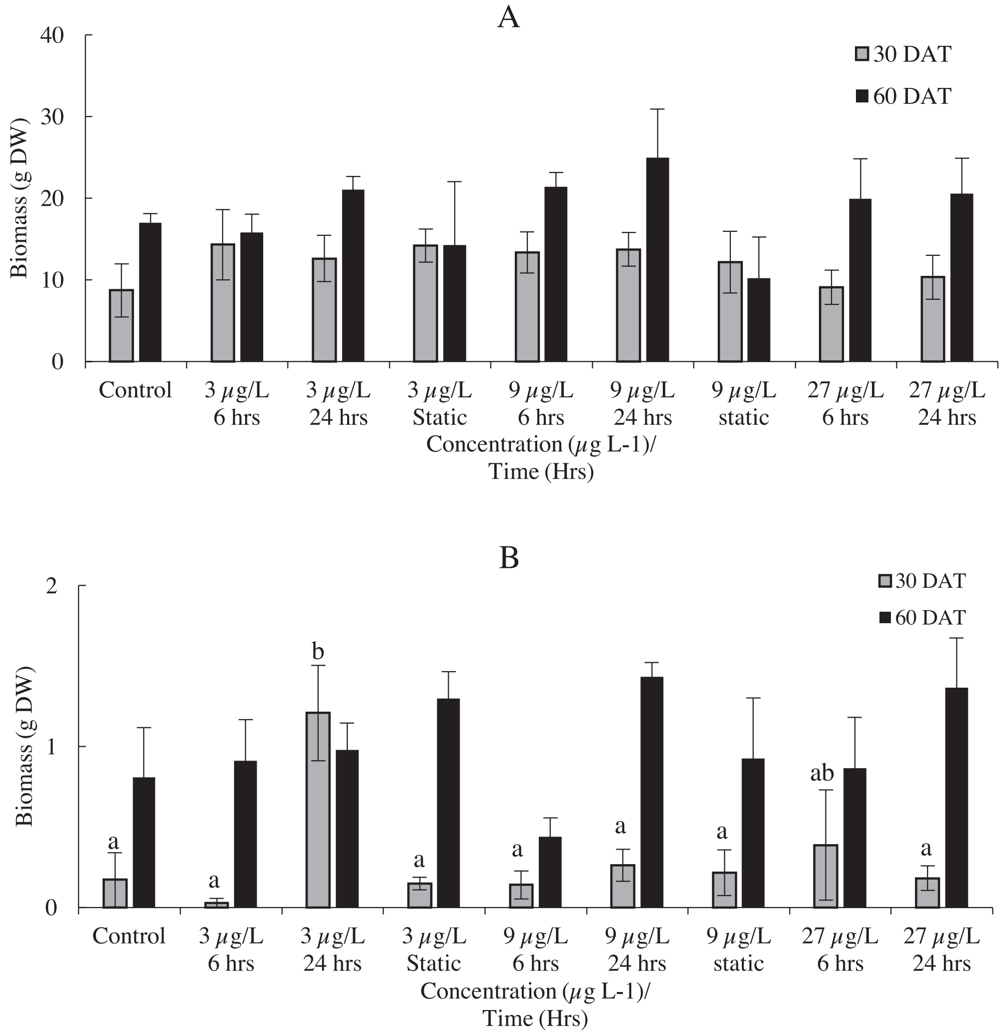

Figure 4. Mean ± SE dry aboveground biomass at 30 and 60 d after treatment (DAT) with florpyrauxifen-benzyl at 3 μg L^{-1} for 6-h, 24-h, and static water-exchange half-lives; 9 μg L^{-1} for 6-h, 24-h, and static water-exchange half-lives; and 27 μg L^{-1} for 6- and 24-h water-exchange half-lives on (A) southern vallisneria and (B) northern vallisneria ($n = 3$). Differences in mean biomass were not observed between treatments at 30 and 60 DAT for southern vallisneria or 60 DAT for northern vallisneria.

SOURCES OF MATERIALS

[1]Forestry Supply 20–10–5 fertilizer, The Scotts Company LLC, 14111 Scottslawn Road, Marysville, OH 43041.

[2]HOBO® Water Temperature Pro v2, U22-001, Onset® Computer Corporation, 470 MacArthur Blvd., Bourne, MA 02532.

[3]ProcellaCOR®, SePRO Corporation, 11550 North Meridian St., Suite 600 Carmel, IN 46032.

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