Selective Control of Invasive Watermilfoils with ProcellaCOR® Aquatic Herbicide and Response of Native Aquatic Plants.

January 28, 2019

Mark Heilman, Ph.D. – Senior Aquatic Technology Leader

Introduction

ProcellaCOR® (a.i. florpyrauxifen-benzyl) was registered as an aquatic herbicide by USEPA in February 2018 followed by successful treatments occurring throughout the US. Registration and permitted uses are currently in review with a handful of remaining states. ProcellaCOR is classified as reduced risk by USEPA based on its favorable environmental and human health profile. It has no EPA restrictions on use of treated water for direct human uses such as drinking, swimming, and fishing. Water use restrictions/precautions are currently limited to some forms of irrigation. ProcellaCOR use patterns reduce herbicide discharge rates by 100X or more versus the majority of older herbicide methods. The herbicide's physical properties support rapid uptake following in-water application and short exposure requirements for the control of target aquatic weeds. These characteristics allow for effective, systemic spot/partial treatments in areas of higher water exchange. ProcellaCOR's arylpicolinate classification represents a new category of WSSA Group 4 herbicides. Herbicides such as Renovate® (triclopyr) in Group 4 have shown favorable selectivity of control historically in the US for control of problem species such as Eurasian watermilfoil. ProcellaCOR improves upon these older herbicides with strong reductions in required use rates, similar or better selectivity, and better spot treatment performance.

The following overview highlights the ability to use ProcellaCOR selectively for the control of invasive and nuisance watermilfoils such as Eurasian watermilfoil (*Myriophyllum spicatum* or EWM), hybrid Eurasian watermilfoil (*M. spicatum* X *M. sibiricum* or HWM), parrotfeather (*M. aquaticum*), and variable watermilfoil (*M. heterophyllum*). Results of controlled research studies in partnership with university and federal research scientists will be highlighted along with field development and assessments of initial operational use. The summary should provide useful information on the invasive weed control properties of ProcellaCOR and set expectations on the responses of desirable non-target vegetation following application for invasive milfoil management. This review does not cover broader ecotoxicology of the herbicide, which can be found in other sources including several recent ecological risk assessments conducted by USEPA, the Washington Department of Ecology and other agencies.

ProcellaCOR EC Use Pattern for Invasive Watermilfoil Control

For invasive watermilfoil management, ProcellaCOR EC is applied typically per label guidance at rates of 1 – 5 Prescription Dose Units (PDU) per acre-foot (A-ft) of water in a management area of a lake, pond, or other aquatic site. A PDU represents ~3.2 fl oz of ProcellaCOR EC and translates to 0.00193 mg a.i. L^{-1} in 1 acre-foot of water. Therefore, common use rates will produce <0.01 mg a.i. L^{-1} in the water volume of a targeted management area in comparison to 1 – 4 mg a.i. L^{-1} rates of older WSSA Group 4 herbicides. Various scales of controlled studies in mesocosm trials by university and government partners show that the low rates of ProcellaCOR are effective under dilution conditions that simulate spot and partial applications targeted to invasive watermilfoils. Figure 1 is reproduced from Beets et al

2019 and shows ProcellaCOR controlling invasive watermilfoils (EWM and HWM) with short exposure scenarios realistically simulating spot/partial treatments under field conditions.

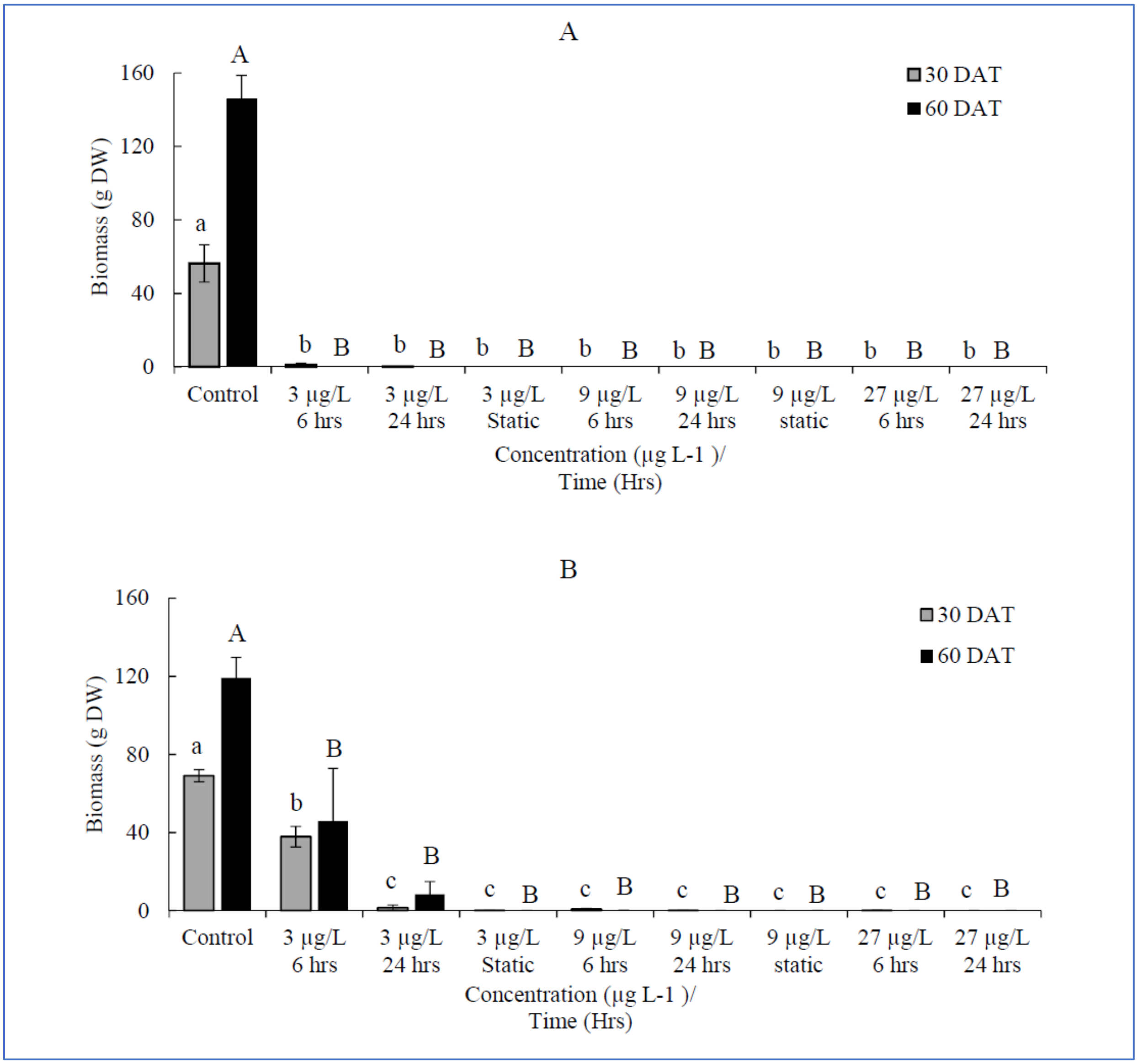


Figure 1. Mean (± SE) dry aboveground biomass at 30 and 60 days after treatment (DAT) with ProcellaCOR at 3 µg a.i. L^{-1} (equivalent to 1.55 PDU A-ft^{-1} ProcellaCOR EC) or 9 µg a.i. L^{-1} (4.66 PDU A-ft^{-1} ProcellaCOR EC) for 6 hr, 24 hr and static water exchange half-lives and 27 µg a.i. L^{-1} (14 PDU A-ft^{-1} ProcellaCOR EC) for 6 and 24 hr water-exchange half-lives on (A) EWM and (B) HWM. Letters above bars represent differences between treatments according to Tukey's test (α=0.05). Uppercase letters above bars indicate 60-day harvest dates that were analyzed separately. (reproduced from Beets et al 2019 – in press).

Figure 2. Representative photos of untreated control (left) and short-exposure treatment (right) with ProcellaCOR in ~1,800 gal mesocosm systems utilized in Beets et al. 2019. The treatment shown was the equivalent of 4.66 PDU A-ft^{-1} ProcellaCOR EC with 6 hr water exchange half-life simulating small spot treatments in lake systems. Both photos were taken at 4 weeks after ProcellaCOR treatments.

Responses of Non-target, desirable native aquatic vegetation to ProcellaCOR

The same Beets et al study referenced above included a number of common submersed US native aquatic plants (list below) and all of these species showed little or no impact at ProcellaCOR CET (concentration + exposure-time) scenarios sufficient to control the target invasive watermilfoils:

- American pondweed (*Potamogeton nodosus*)
- Illinois pondweed (*P. illinoensis*)
- elodea (*Elodea canadensis*)
- water stargrass (*Heteranthera dubia*)
- two populations of tapegrass (*Vallisneria americana*)
  from southern (Gainesville, FL) and northern (NY) locations

The responses of many common US native aquatic plant species to ProcellaCOR exposure have been documented in cooperative studies under controlled settings and also been validated by field experiences during the herbicide's development or early operational efforts since 2018 registration. A list of these species and their relative sensitivity to the herbicide when used for invasive watermilfoil control is presented in Table 1 along with citation of cooperating university or government group and relevant publication where available. Representative data from the majority of supporting studies are included in the Appendix of this document along with full versions of available relevant publications.

Table 1. Relative sensitivity of common native aquatic plant species to ProcellaCOR EC when utilizing typical PDU rates (1 – 5 PDU per acre-foot) for selective control of invasive watermilfoils.

Common name	Species name	Source/Publication	Relative Sensitivity
Northern watermilfoil	*Myriophyllum sibiricum*	Corps/UF – unpublished TX large mesocosm study	HIGH
Other watermilfoils	*Myriophyllum spp.*	*	HIGH*
Watershield	*Brasenia scherberi*	NCSU unpublished field trial; NHDES variable watermilfoil operations; labeled species for control	MODERATE - HIGH
American lotus	*Nelumbo lutea*	Corps/UF – unpublished	MODERATE - HIGH
White Water Lily	*Nymphaea odorata*	Corps/UF – unpublished; NHDES VWM operations; other US experiences	MODERATE
Yellow Pond Lily	*Nuphar spp.*	Corps/UF – unpublished; NHDES, MNDNR and other field outcomes	LOW-MODERATE
Stargrass	*Heteranthera dubia*	Beets et al. 2019	LOW-MODERATE**
Pickerelweed	*Pontedaria cordata*	Beets and Netherland 2018	LOW – MODERATE**
Coontail	*Ceratophyllum demersum*	Pend Oreille ID field demonstration; MNDNR evaluation; Corps-UF unpublished	LOW-MODERATE***
Sago Pondweed	*Stuckenia pectinata*	Northern US field demonstrations (minor reductions or expansion)	LOW
Arrowhead	*Sagittaria spp.*	Beets and Netherland 2018; Corps/UF - unpublished	LOW
Elodea	*Elodea spp.*	Beets et al. 2019; Netherland and Richardson 2016	LOW
Water marigold	*Bidens beckii (or Megalodonta beckii)*	Netherland and Richardson 2016	LOW
Naiads	*Najas spp.*	Corps/UF – unpublished; MN DNR 2018 field observations	LOW
Pondweeds	*Potamogeton spp.*	Beets et al. 2019; MNDNR, NHDES, and other field observations****	LOW
Bladderworts	*Utricularia spp.*	NHDES VWM operations	LOW
Vallisneria (tape grass)	*Vallisneria americana*	Beets et al. 2019; MNDNR and other field observations	LOW
Bulrush	*Schoenoplectus spp.*	Corps/UF and national 2018 field outcomes	LOW
Cattail	*Typha spp.*	Corps/UF and national 2018 field outcomes	LOW
Native grasses	*Panicum spp.*	Corps/UF and national 2018 field outcomes	LOW

Table 1 Notes: The following terminology capture descriptions of sensitivity in the table above.

- LOW – little or no response to standard rates for invasive watermilfoil control typically followed by expansion following milfoil control; in many cases, no symptoms will be observed but in some cases, light herbicide symptoms such as unusual growth or light chlorosis may be observed but they will be temporary and result in no control/reductions in the plant
- MODERATE – Initial symptoms will be more obvious within 1 – 2 weeks after treatment. Symptoms may be stronger with longer exposures in large-partial or full-site treatments. There may be some reductions in standing biomass immediately following treatment but generally

strong recovery. Competition with other more tolerant native plants may delay recovery under some conditions.

- HIGH – sensitivity less but close to invasive watermilfoil with intense symptoms. Notable reductions in density and coverage of the plant in question will often be observed.

* Projected responses for other milfoils are based on sensitivity of other species evaluated for the genus. Recovery potential compared to invasive watermilfoils is being assessed over time for initial operational treatments.

** visual symptoms but little or no impact when controlling EWM/HWM; may be controlled at high use rates with extended exposures (several days or more).

*** Coontail will show minor symptoms in most treatments but at CET scenarios with rates for spot/partial treatment of invasive watermilfoils, the effect is temporary and no notable reductions in species frequency have been documented.

**** not all species of pondweeds have been evaluated.

Cooperating Institutions: US Army Engineer Research and Development Center (ERDC – contact Dr. Kurt Getsinger representing the late Dr. Mike Netherland, also Dr. Chris Mudge), NC State University (NCSU – Dr. Rob Richardson), University of Florida (UF – contact Dr. Jason Ferrell)

APPENDIX

The following provides a brief review of additional detailed technical content included as supporting information for the above summary of selective invasive watermilfoil control with ProcellaCOR.

- **Beets, Heilman and Netherland 2019 (in press) - Journal of Aquatic Plant Management (JAPM)**
  - Large-Scale Mesocosm Evaluation of Florpyrauxifen-benzyl, a Novel Arylpicolinate Herbicide, on Eurasian and Hybrid Watermilfoil and Seven Native Submersed Plants
- **Beets and Netherland 2018 – JAPM**
  - Activity on hydrilla and crested floating heart but most relevant for northern US selectivity considerations is data on arrowhead and pickerel weed.
- **Beets and Netherland – part of Western APMS 2018 presentation on invasive watermilfoil control with ProcellaCOR.**
  - Highlights activity on multiple EWM and HWM accessions plus native northern watermilfoil (*M. sibiricum*)
- **Preliminary Report from Minnesota DNR on Summer 2018 treatment of Lake Jane, MN**
  - Selective control of HWM with partial treatment of ProcellaCOR EC
- **ProcellaCOR field evaluation on watershield in NC**
  - Field efficacy information to support sensitivity of the species to northern US patterns for milfoil control
- **Netherland and Richardson 2016 – Weed Science**
  - One of the first publications showing high activity on EWM with less sensitivity of a variety of common US native plants (ProcellaCOR coded as SX-1552)
- **Preliminary report on Pend Oreille ID demonstration treatment**
  - Collaborative field effort on EWM in spot treatment in Lake Pend Oreille system in northern Idaho…led by US Corps ERDC Aquatic Plant Control Research Program (Dr. Kurt Getsinger)
- **Draft manuscript for JAPM reviewing 2 year-post results of 2016 small-scale evaluation in Hopkinton NH by NHDES on variable leaf watermilfoil**
- **Pond demonstration treatment by SePRO at its NC research facility showing lack of ProcellaCOR activity on natives grasses and also a Michigan 2017 pond demo treatment showing lack of cattail activity while controlling EWM**
- **SePRO pond-scale evaluation of ProcellaCOR activity on spatterdock (*Nuphar advena*)**
- **Richardson et al 2016 – JAPM**
  - Another early screening study showing high activity on invasive watermilfoils and less activity on many common native plants.
- **Pond demonstration treatment on American lotus with US ERDC-APCRP in Texas**
- **Large mesocosm study by ERDC-APCRP in Texas**
  - Evaluation of sensitivity of white water lily and two species of bulrush to realistic exposure scenarios

Large-Scale Mesocosm Evaluation of Florpyrauxifen-benzyl, a Novel Arylpicolinate Herbicide, on Eurasian and Hybrid Watermilfoil and Seven Native Submersed Plants

Jens Beets, Mark Heilman, and Michael D. Netherland*

Abstract

Eurasian watermilfoil (EWM) and Hybrid Eurasian watermilfoil (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 synthetic auxins. For the auxin-mimic herbicides, it is not clear whether the reduced efficacy is herbicide or class specific or 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 scenarios using florpyrauxifen-benzyl on well-established EWM and HWM, as well as 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 a.i. L^{-1}) for 6 and 24-hour half-lives, as well as, two concentrations (3 and 9 μg L^{-1}) at a static exposure. Eight concentration exposure-time (CET) scenarios were tested and biomass harvests were performed 30 and 60 days after treatment. Results indicated that all concentration exposure scenarios resulted in significant control of EWM and HWM, with HWM showing a

* First author, Graduate student, Department of Agronomy, University of Florida, Institute of Food and Agricultural Sciences, Center for Aquatic and Invasive Plants, 7922 NW 71st St., Gainesville, FL 32653. Second author, SePRO Corporation, 11550 North Meridian Street, Suite 600, Carmel, IN 46032. Third author, Research Biologist, U.S. Army Engineer Research and Development Center, Center for Aquatic and Invasive Plants, 7922 NW 71st St., Gainesville, FL 32653. Corresponding author's E-mail: jbeets@ufl.edu.

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: Myriophyllum spicatum, *Myriophyllum spicatum x M. sibiricum*, Concentration Exposure Time, selectivity, ProcellaCOR

INTRODUCTION

Eurasian watermilfoil (*Myriophyllum spicatum* L.; EWM) and Hybrid Eurasian watermilfoil (*M. spicatum* L. *x M. 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 has led to discussion if this response is specific to 2,4-D or auxin mimics in general. 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 (Ellstrand and Schierenbeck 2000, Moody and Les 2002, Thompson 1991). 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 (tradename ProcellaCOR®), and it may provide a new tool to augment control options of problematic aquatic weeds. The arylpicolinates differ in binding affinity compared to currently registered auxins such as 2,4-D and triclopyr (Bell et al. 2015, Lee et al. 2013). 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, Getsinger and Netherland 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 (Nault et al. 2014, Netherland and Jones 2015, Green and Westerdahl 1990, Netherland and Glomski 2014, Glomski and Netherland 2010, Glomski and Netherland 2014, Glomski et al. 2009, Skogerboe et al. 2006). 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 and 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, HWM, and 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), as well as 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 9/15/2015 from apical stems or root nodes (*Vallisneria*) at the U.S. Army Engineer Research and Development Center's Lewisville Aquatic Ecosystem Research Facility (LAERF) in Lewisville, TX. Each 6,700L mesocosm was planted with two 3-L pots for each species of American pondweed, Illinois pondweed, elodea, water stargrass, EWM, HWM, and two populations of vallisneria from southern (Gainesville, FL) and northern (NY) locations.

Specimens of HWM with reported tolerance to 2,4-D were used from a single population (Hayden Lake, Idaho) (Beets and Netherland 2018, Taylor et al. 2017). All plants were established in topsoil amended with Forestry Supply[1] 20-10-5 fertilizer tablets (4.5 g/kg). Plants were allowed to establish from 9/15 to 4/16, 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 a.i. L^{-1}) for 6 and 24-hour water-exchange half-lives as well as two concentrations (3 and 9 a.i. 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 days 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 ANOVA and Tukey's HSD to determine statistical differences in aboveground biomass among treatments at each harvest period (p=0.05). Heteroscedascity (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; WA Dept. of Ecology 2017). Sample concentration fluctuations are likely due to a combination of herbicide photolytic degradation (0.6 day 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 days following treatment even at the lowest concentrations and exposure times evaluated (Figure 1a and b). 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 thirty and sixty days after treatment compared to the untreated control. However, 30 days after treatment HWM biomass in the 3 μg L^{-1} 6 hour treatment (the lowest scenario) was greater than HWM biomass in the other CET treatments (Figure 1b). Differences in herbicide sensitivity between EWM and HWM have also been anecdotally observed in the field and seen in small-scale studies (Beets and Netherland 2018, Taylor et al. 2017). 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, Nault et al. 2014, Netherland and Getsinger 1992) and suggest the potential use of florpyrauxifen-benzyl for milfoil control programs.

Native Species

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 (Figure 2a and b) and some treatments of Illinois pondweed had greater biomass than the untreated control at 30 days. 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 *Heteranthera* showed the most treatment related variability, with one treatment (3 μg L^{-1}/6 hr) 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, *Heteranthera* 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 hr and 27 μg L^{-1}/24 hr scenarios after 60 days was greater than the untreated control after 30 days ($p < 0.001$; Figure 4b).

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 hr 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 to use low use rates to control milfoil invasions and allow the spread of native species via post-treatment 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.

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® Aquatic Herbicide, SePRO Corporation. 11550 North Meridian Street, Suite 600 Carmel, IN 46032.

ACKNOWLEDGEMENTS

We would like to thank Chetta Owens, Carl Della Torre, Ben Willis, and Cody Hale with assistance in carrying out this study.

LITERATURE CITED

Beets J, Netherland MD. 2018. Laboratory and mesocosm evaluation of growth and herbicide response in Eurasian watermilfoil and four accessions of hybrid watermilfoil. [Abstract]. In: Proceedings of the Aquatic Plant Management Society Annual Meeting. APMS, Buffalo, NY. http://www.apms.org/wp/wp-content/uploads/Full-Program-and-Abstracts.pdf. Accessed August 1, 2018.

Bell JL, Schmitzer R, Weimer MR, Napier RM, Prusinska JM. 2015. Mode-of-action analysis of a new arylpicolinate herbicide. [Abstract]. In: Proceedings of the Weed Science Society of America Annual Meeting. WSSA, Lexington, KY: Weed Science Society of America. http://wssaabstracts.com/public/30/abstract-290.html. Accessed August 13, 2017.

Ellstrand NC, Schierenbeck KA. 2000. Hybridization as a stimulus for the evolution of invasiveness in plants?. Proc. Natl. Acad. Sci. 97(13):7043-7050.

EPA. 2015. Pesticide analytical methods: ECM for florpyrauxifen-benzyl & degradates in water – MRID 49677722. https://www.epa.gov/sites/production/files/2017-09/documents/ecm_-_florpyrauxifen-benzyl_degradates_in_water_-_mrid_49677722.pdf.

Getsinger KD, Netherland MD. 1997. Herbicide concentration/exposure time requirements for controlling submersed aquatic plants: summary of research accomplishments. US Army Corps of Engineers Aquatic Plant Control Program. Misc Paper A-97-2. 27 pp.

Getsinger KD, Netherland MD. 2018. Use of herbicides in areas of high water exchange: practical considerations. J. Aquat. Plant Manage. 56s: 39-43.

Glomski LM, Netherland MD, Nelson LS. 2009. Evaluation of 2,4-D and triclopyr against spatterdock and bulrush. APCRP Technical Notes Collection (ERDC/TN APCRP-CC-10). Vicksburg, MS. U.S. Army Engineer Research and Development Center, Vicksburg, MS.

Glomski LM, Netherland MD. 2010. Response of Eurasian and hybrid watermilfoil to low use rates and extended exposures of 2,4-D and triclopyr. J. Aquat. Plant Manage. 48:12-14.

Glomski LM, Netherland MD. 2014. Response of waterlily, spatterdock, and hardstem bulrush to liquid and granular triclopyr treatments. J. Aquat. Plant Manage. 52: 81-84.

Green WR, Westerdahl HE. 1990. Response of Eurasian watermilfoil to 2,4-D concentration and exposure times. J. Aquat. Plant Manage. 28:27-32.

Grossman K. 2010. Auxin herbicides: current status of mechanism and mode of action. Pest Manag. Sci. 66:113-120.

LaRue EA, Zuellig MP, Netherland MD, Heilman MA, Thum RA. 2013. Hybrid watermilfoil lineages are more invasive and less sensitive to a commonly used herbicide than their exotic parent (Eurasian watermilfoil). Evol. Appl. 6:462-471.

Lee S, Sundaram S, Armitage L, Evans JP, Hawkes T, Kepinski S, Ferro N, Napier RM. 2013. Defining binding efficiency and specificity of auxins for SCFTIR1/AFB-Aux/IAA co-receptor complex formation. ACS Chem Biol. 9:673–682.

Moody ML, Les MH. 2002. Evidence of hybridity in invasive watermilfoil (*Myriophyllum*) populations. Proc. Natl. Acad. Sci. USA 99:14867-14871.

Nault ME, Netherland MD, Mikulyuk A, Skogerboe JG, Asplund T, Hauxwell J, Toshner P. 2014. Efficacy, selectivity, and herbicide concentrations following a whole-lake 2,4-D application targeting Eurasian watermilfoil in two adjacent northern Wisconsin lakes. Lake Reserv. Manage. 30: 1–10.

Netherland MD, Getsinger KD. 1992. Efficacy of triclopyr on Eurasian watermilfoil: concentration and exposure time effects. J. Aquat. Plant Manage. 30:1-5.

Netherland MD, Glomski LM. 2014. Mesocosm evaluation of triclopyr on Eurasian watermilfoil and three native submersed species: the role of treatment timing and herbicide exposure. J. Aquat. Plant Manage. 52: 57-64.

Netherland MD, Jones KD. 2015. A three-year evaluation of triclopyr for selective whole-bay management of Eurasian watermilfoil on Lake Minnetonka, Minnesota. Lake Reserv. Manage. 31:4: 306-323.

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(1):181-190.

Poovey AG, Slade JG, Netherland MD. 2007. Susceptibility of Eurasian watermilfoil (*Myriophyllum spicatum*) and a milfoil hybrid (*M. spicatum* x *M. sibiricum*) to triclopyr and 2,4-D amine. J. Aquat. Plant Manage. 45:111-115.

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

Skogerboe JG, Getsinger KD, Glomski LM. 2006. Efficacy of diquat on submersed plants treated under simulated flowing water conditions. J. Aquat. Plant Manage. 44: 122-125.

Taylor LL, McNair JN, Guastello P, Pashnick J, Thum RA. 2017. Heritable variation for vegetative growth rate in ten distinct genotypes of hybrid watermilfoil. J. Aquat. Plant Manage. 55:51-57.

Thompson JD. 1991. The biology of an invasive plant. Biosci. 41:393-401.

Washington State Department of Ecology. 2017. Supplemental environmental impact statement for state of Washington aquatic plant and algae management. Washington Dept. of Eco. Pub. No. 17-10-020. 205 pp.

Wersal RM, Madsen JD, Woolf TE, Eckberg N. 2010. Assessment of herbicide efficacy on Eurasian watermilfoil and impacts to the native submersed plant community in Hayden Lake, Idaho, USA. J. Aquat. Plant Manage. 48:5-11.

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 where no sample was collected.

CET scenario	1 HAT	6 HAT	24 HAT	48 HAT	72 HAT	7 DAT	10 DAT	14 DAT
27 µg L^{-1} 6 hr	16.2 (2.8)	8.1 (0.66)	3.9 (2.9)	1.3 (0.21)	-	-	-	-
27 µg L^{-1} 24 hr	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)

LIST OF FIGURES

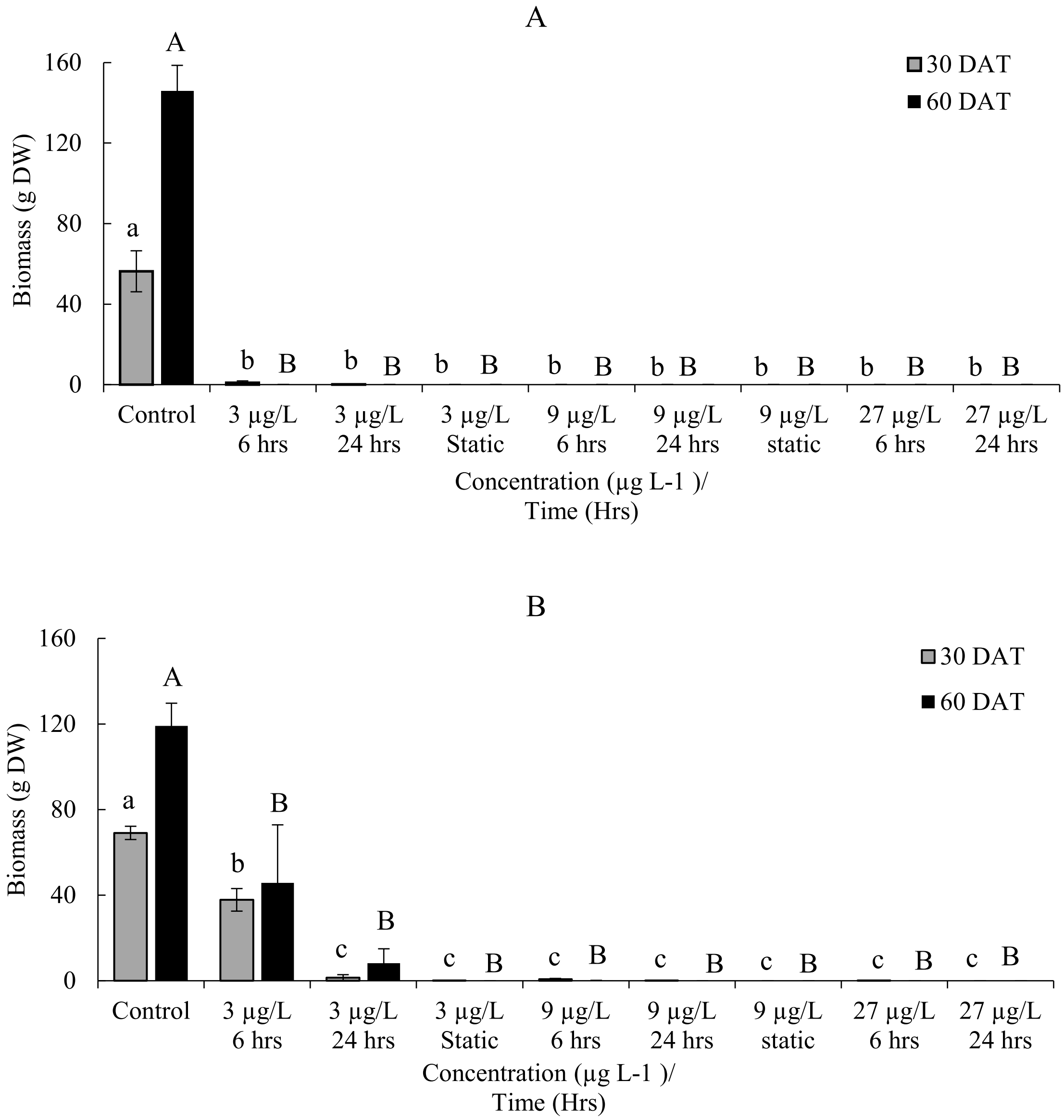


Figure 1. Mean (± SE) dry aboveground biomass at 30 and 60 days after treatment (DAT) with florpyrauxifen-benzyl at 3 µg L^{-1} for 6 hr, 24 hr and static water-exchange half-lives, 9 µg L^{-1} for 6 hr, 24 hr and static water-exchange half-lives, and 27 µg L^{-1} for 6 and 24 hr water-exchange half-lives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

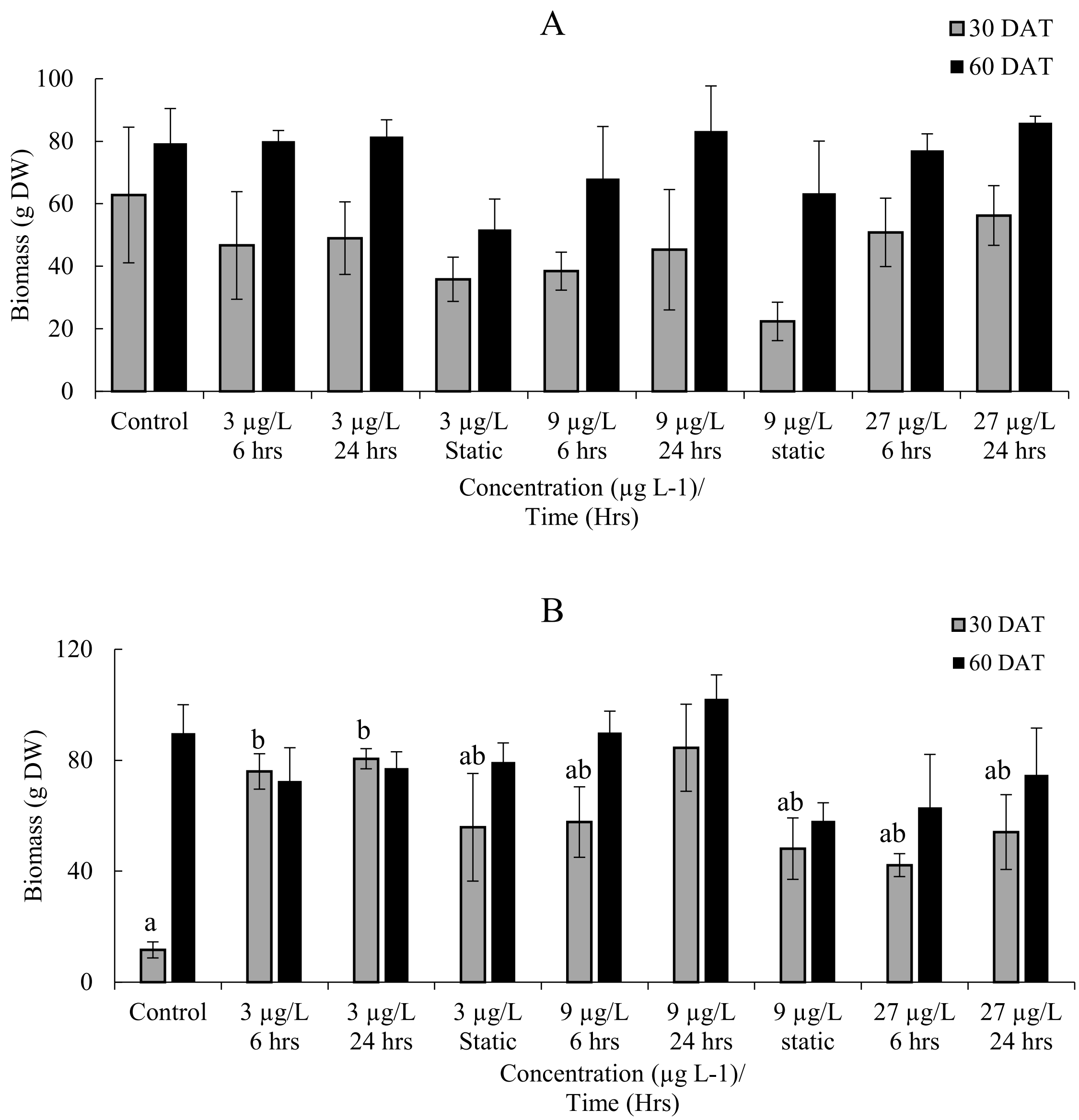
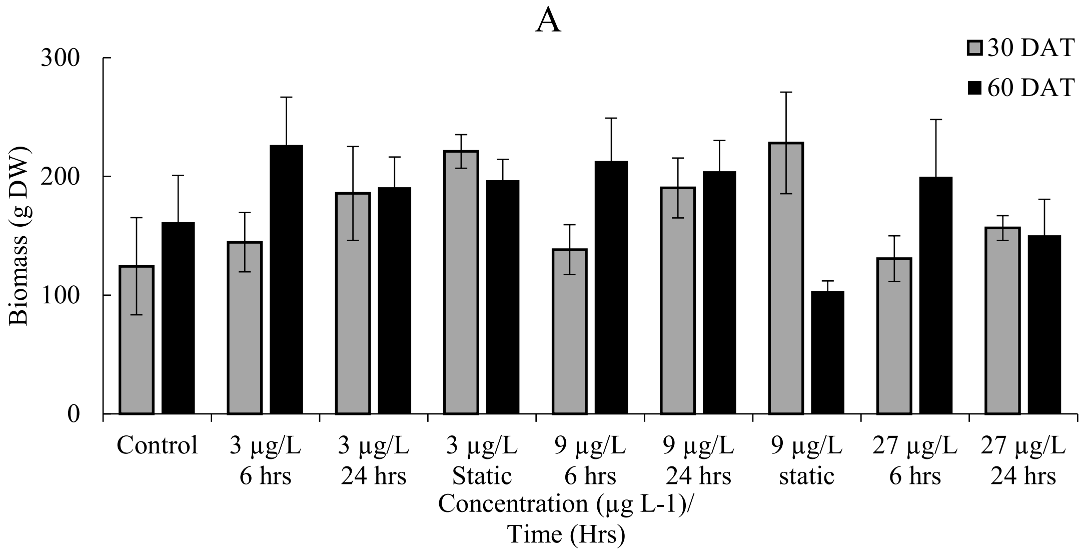


Figure 2. Mean (± SE) dry aboveground biomass at 30 and 60 days after treatment (DAT) with florpyrauxifen-benzyl at 3 µg L^{-1} for 6 hr, 24 hr and static water-exchange half-lives, 9 µg L^{-1} for 6 hr, 24 hr and static water-exchange half-lives, and 27 µg L^{-1} for 6 and 24 hr water-exchange half-lives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



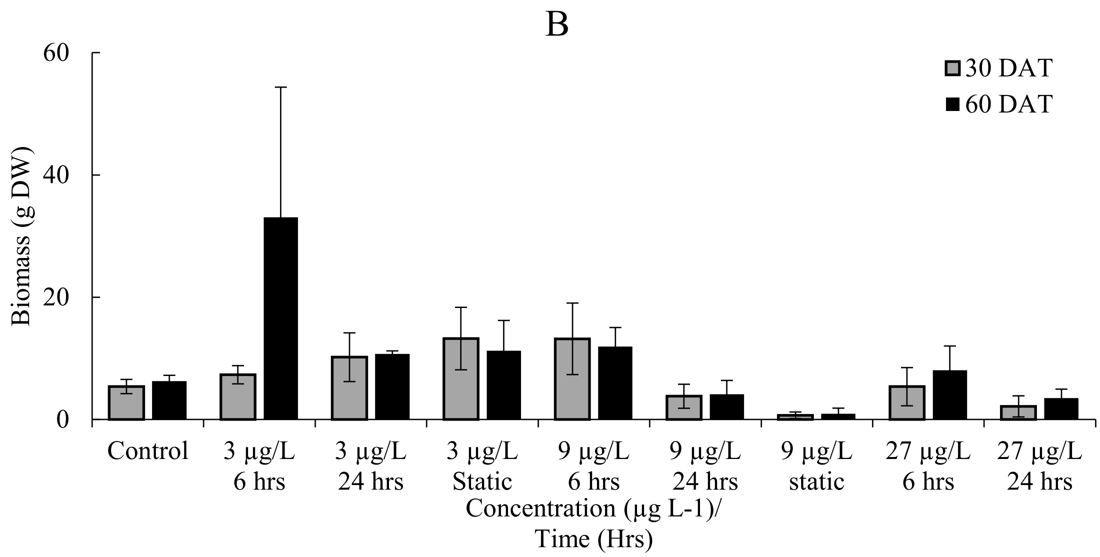
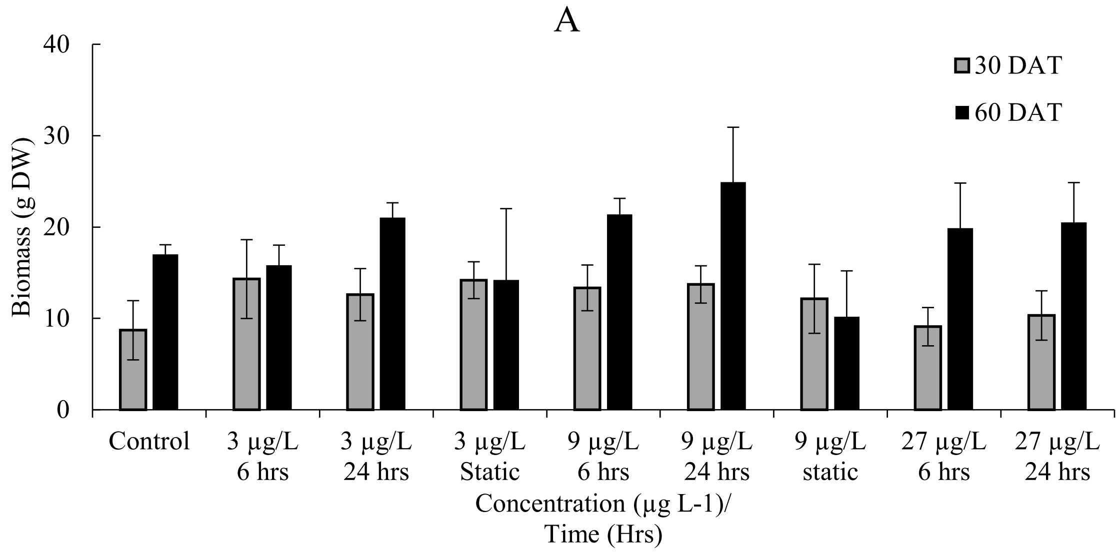


Figure 3. Mean (± SE) dry aboveground biomass at 30 and 60 days after treatment (DAT) with florpyrauxifen-benzyl at 3 µg L^{-1} for 6 hr, 24 hr and static water-exchange half-lives, 9 µg L^{-1} for 6 hr, 24 hr and static water-exchange half-lives, and 27 µg L^{-1} for 6 and 24 hr water-exchange half-lives on (a) elodea and (b) *Heteranthera* (n=3). Differences in mean biomass were not observed between treatments at 30 and 60 DAT.



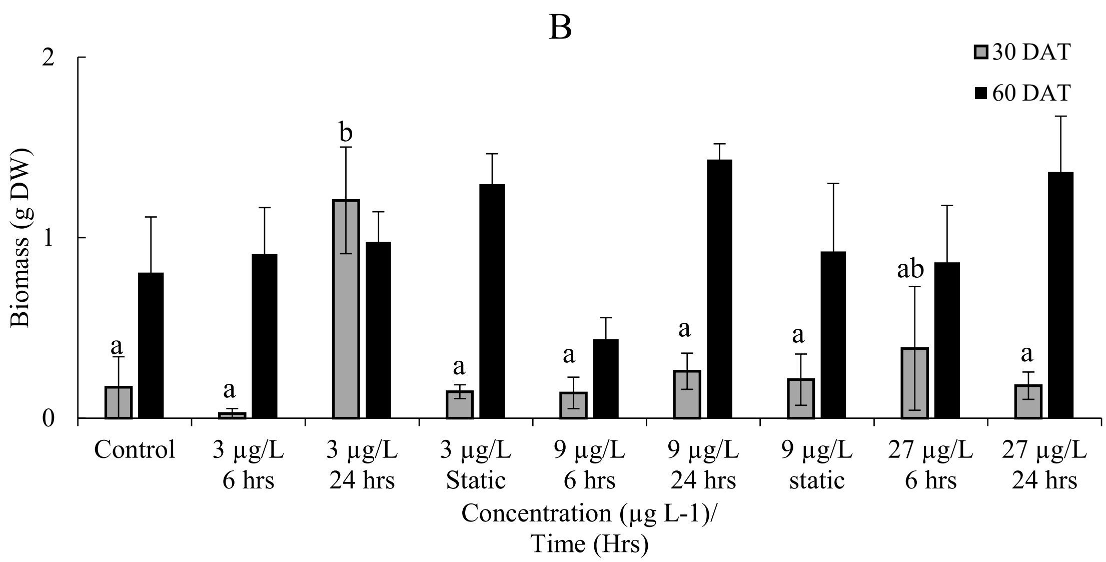


Figure 4. Mean (± SE) dry aboveground biomass at 30 and 60 days after treatment (DAT) with florpyrauxifen-benzyl at 3 µg L^{-1} for 6 hr, 24 hr and static water-exchange half-lives, 9 µg L^{-1} for 6 hr, 24 hr and static water-exchange half-lives, and 27 µg L^{-1} for 6 and 24 hr water-exchange half-lives 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 S. vallisneria or 60 DAT for N. vallisneria.

J. Aquat. Plant Manage. 56: 57–62

Note

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

JENS BEETS AND MICHAEL NETHERLAND*

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.

*First author, Graduate student, Department of Agronomy, University of Florida, Institute of Food and Agricultural Sciences, Center for Aquatic and Invasive Plants, 7922 NW 71st St., Gainesville, FL 32653. Second author, Research Biologist, U.S. Army Engineer Research and Development Center, Center for Aquatic and Invasive Plans, 7922 NW 71st St., Gainesville, FL 32653. Corresponding author's E-mail: jbeets@ufl.edu. Received for publication ____________ and in revised form ____________.

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 (~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 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

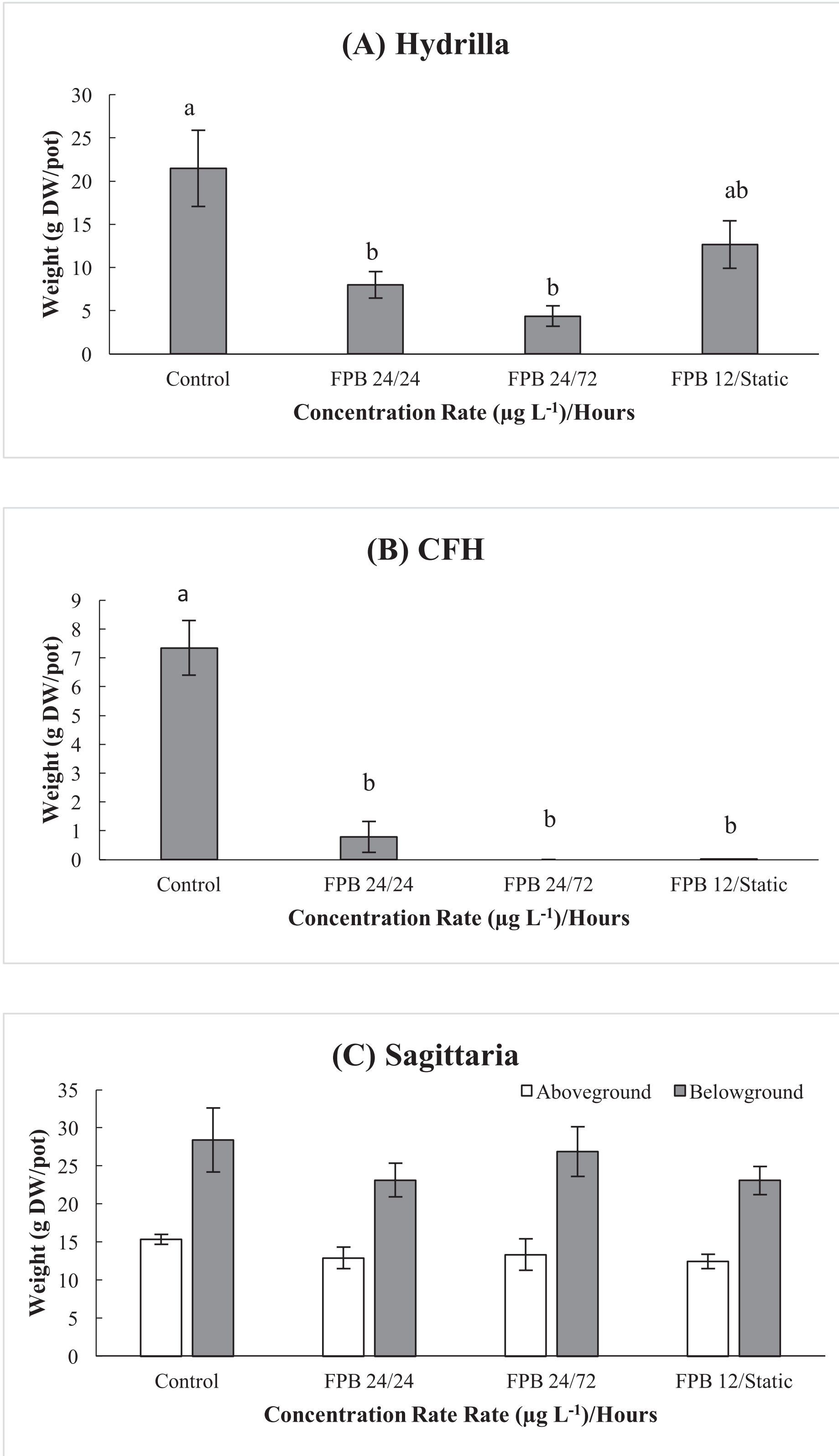


Figure 1. Dry weight biomass recorded at 28 d following treatment with florpyrauxifen-benzyl (FPB) at 24 µg L^{-1} for 24- and 72-hr exposures and a 12 µg L^{-1} static exposure on hydrilla (A), crested floating heart (B), and sagittaria (C). Bars represent mean values ($n = 6$) of dry weight ± SE. Letters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

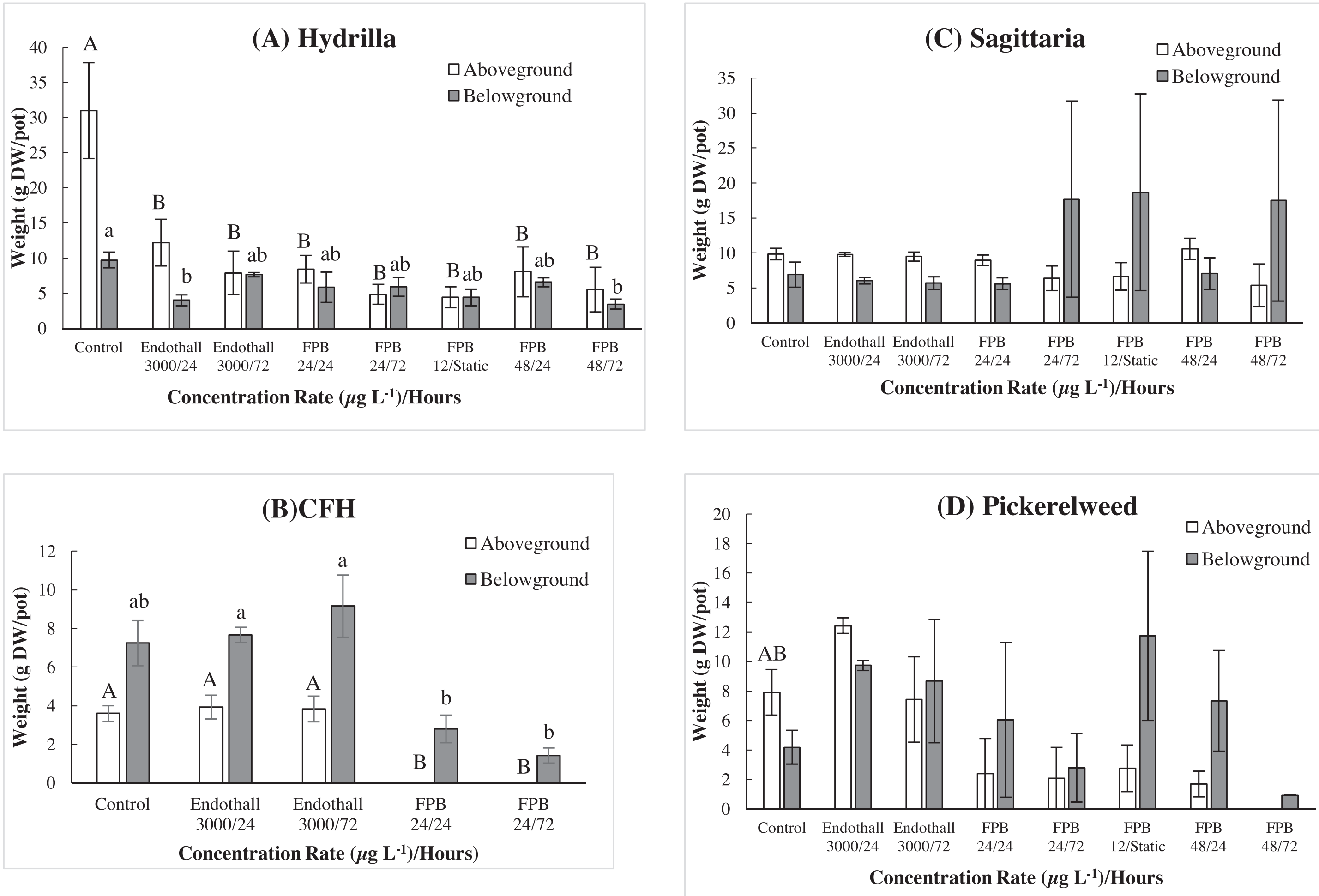


Figure 2. Dry weight biomass recorded at 28 d following treatment with florpyrauxifen-benzyl (FPB) at 24 and 48 μg L^{-1} for 24- and 72-hr exposures and a 12 μg L^{-1} static exposure on hydrilla (A), crested floating heart (B), sagittaria (C), and pickerelweed (D). Crested floating heart was not present 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.

ACKNOWLEDGEMENTS

The authors wish to thank the U.S. Army Corps of Engineers Aquatic Plant Control Research Program, the Florida Fish and Wildlife Commission Invasive Species Management Section, and the Aquatic Ecosystem Restoration Foundation for providing financial support to conduct this this research. Permission to publish was granted by the Chief of Engineers. SePRO Corporation provided florpyrauxifen-benzyl, technical guidance for these evaluations based on past internal testing of the herbicide, and analytical support.

LITERATURE CITED

Bell JL, Schmitzer R, Weimer MR, Napier RM, Prusinska JM. 2015. Mode of action analysis of a new arylpicolinate herbicide from Dow AgroSciences [Abstract]. In: Proceedings of the Weed Science Society of America Annual Meeting. WSSA, Lawrence, KS. 87 pp.

Burks KC. 2002. *Nymphoides cristata* (Roxb.) Kuntze, a recent adventive expanding as a pest plant in Florida. Castanea 67:206–211.

Giannotti AL, TJ Egan, MD Netherland, ML Williams, AK Knecht. 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/aw14/Presentations/Grand/Thursday/Session%209A/0850%20Giannotti.pdf. Accessed January 11, 2016

Glomski LM, Netherland MD. 2007. Efficacy of diquat and carfentrazone-ethyl on variable-leaf milfoil. J. Aquat. Plant Manage. 45:136–138.

Glomski LM, Netherland MD. 2008. Effect of temperature on 2, 4-D ester and carfentrazone-ethyl applications for control of variable-leaf milfoil. J. Aquat. Plant Manage. 46:119–121.

Glomski LM, Netherland MD. 2013. Use of a small-scale primary screening method to predict effects of flumioxazin and carfentrazone-ethyl on native and invasive, submersed plants. J. Aquat. Plant Manage. 51:45–48.

Glomski LM, Willey LN, Netherland MD. 2014. The efficacy of protox-inhibiting herbicides alone and in combination with glyphosate to control crested floating heart. J. Aquat. Plant Manage. 52:90–92.

Green YR, Westerdahl, HE. 1988. 2,4-D Concentration and Exposure Time Relationships for the Control of Eurasian Watermilfoil. Miscellaneous Paper A-88-8, US Army Engineer Waterways Experiment Station, Vicksburg, MS.

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

Lee S, Sundaram S, Armitage L, Evans JP, Hawkes T, Kepinski S, Ferro N, Napier RM. 2013. Defining binding efficiency and specificity of auxins for SCFTIR1/AFPB-Aux/IAA co-receptor complex formation. ACS (Am. Chem. Soc.) Chem. Biol. 9:673–682.

Mudge CR, Bultemeier BW, Haller WT. 2012. The Influence of pH and light on hydrilla (*Hydrilla verticillata*) photosynthesis and chlorophyll after exposure to flumioxazin. Weed Sci. 60:4–9.

Mudge CR, Haller WT, Netherland MD, Kowalsky JK. 2010. Evaluating the influence of pH-dependent hydrolysis on the efficacy of flumioxazin for hydrilla control. J. Aquat. Plant Manage. 48:25–30.

Netherland MD. 2011. Comparative susceptibility of fluridone resistant and susceptible hydrilla to four ALS inhibiting herbicides under laboratory and greenhouse conditions. J. Aquat. Plant Manage. 49:94–99.

Netherland MD. 2014. Chemical control of aquatic weeds. pp. 71–88 In: L. A. Gettys, W. T. Haller, and D. G. Petty (*eds.*). Biology and control of aquatic plants: A best management practices handbook, 3rd ed. Aquatic Ecosystem Restoration Foundation, Marietta, GA.

Netherland MD, Getsinger K, Turner E. 1993. Fluridone concentration and exposure time requirements for control of hydrilla and Eurasian watermilfoil. J. Aquat. Plant Manage. 31:189–194.

Netherland MD, Green W, Getsinger K. 1991. Endothall concentration and exposure time relationships for the control of Eurasian watermilfoil and hydrilla. J. Aquat. Plant Manage. 29:61–67.

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.

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

Skogerobe JG, Getsinger KD, Glomski LM. 2006. Efficacy of diquat on submersed plants treated under simulated flowing water conditions. J. Aquat. Manage. 44:122–125.

Willey LN, Netherland MD, Haller WT, Langeland KA. 2014. Evaluation of aquatic herbicide activity against crested floating heart. J. Aquat. Plant Manage. 52:47–56.

Laboratory and Mesocosm Evaluation of Growth and Herbicide Response in Eurasian and Four Accessions of Hybrid Watermilfoil

Jens Beets* and Michael D. Netherland
University of Florida- CAIP
Western Aquatic Plant Management Society

UF | IFAS
UNIVERSITY *of* FLORIDA

Subset of Presented Information at Western APMS – March 2018

Study 3- Growth and herbicide response of numerous HWM populations

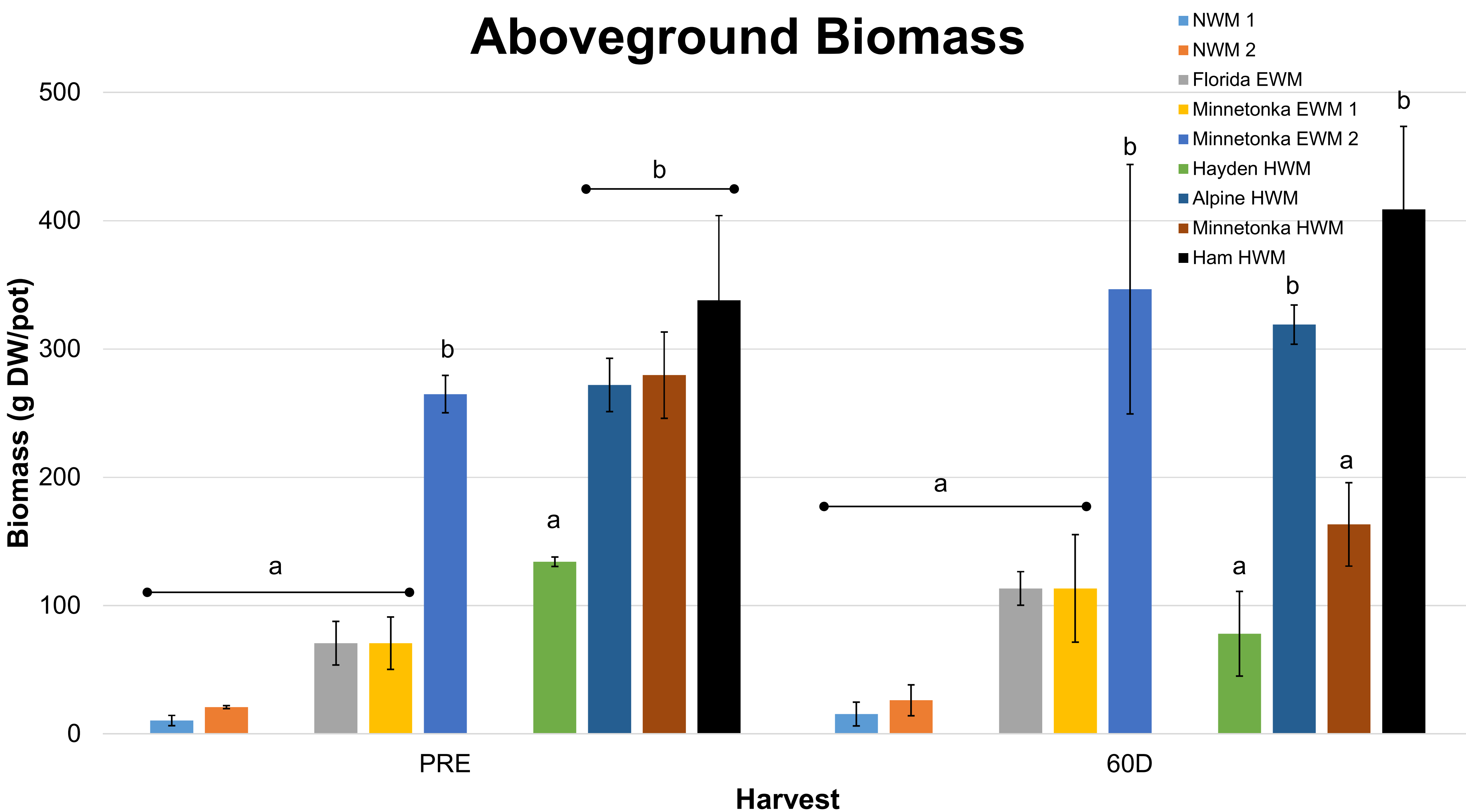
- Established 9/28/16
- Treated 4/12/17
- 6,700 L mesocosms at LAERF
- 3-L pots with 10cm apical stems
  - NWM
  - EWM: Crystal River, FL and Lake Minnetonka, MN
  - HWM: Hayden, ID; Ham Lake, MN; Minnetonka, MN; Alpine Lake, WI

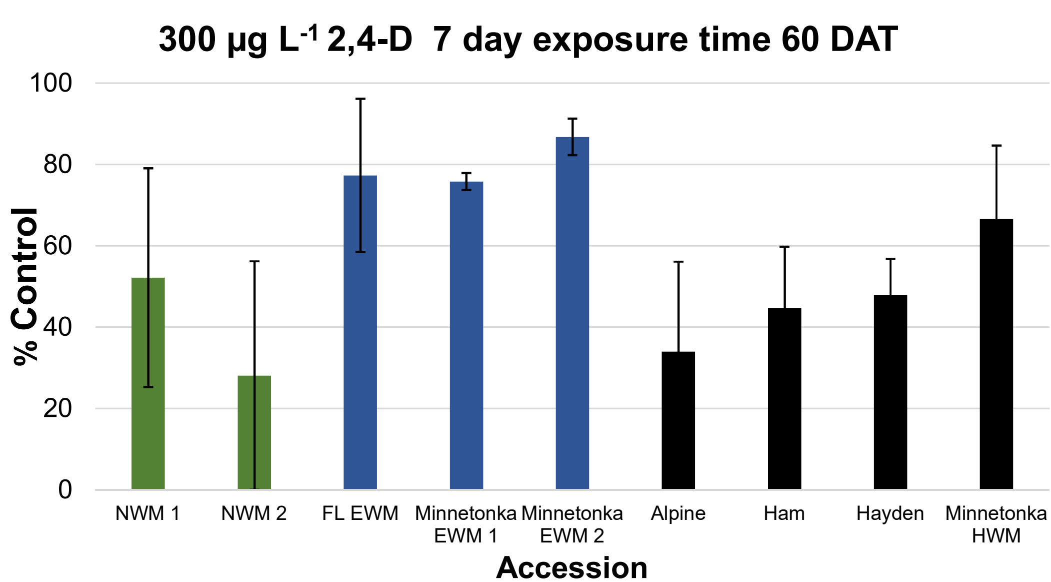
Treatment Rates

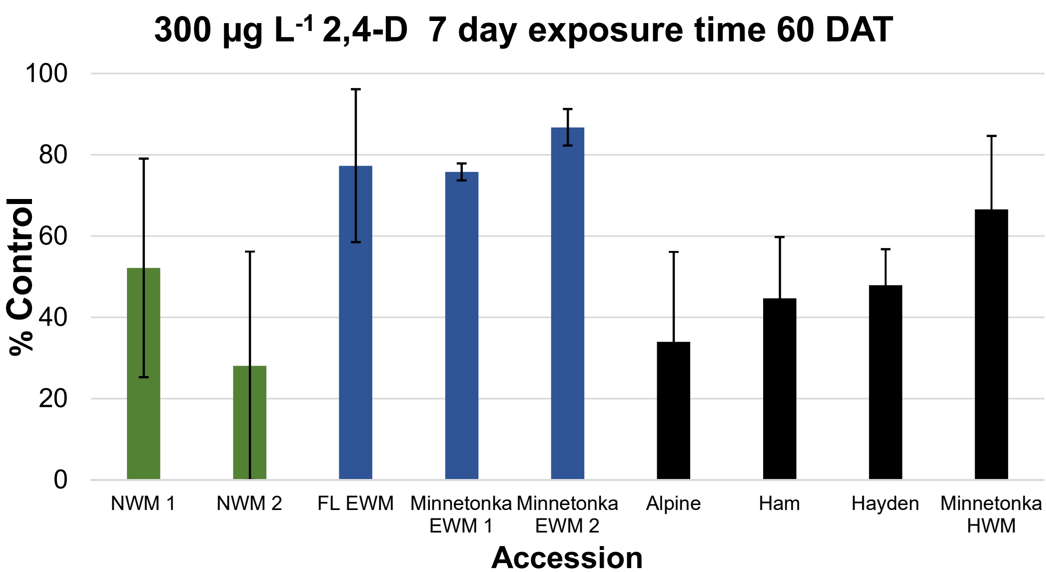
Treatment	Rate	Exposure Time
2,4-D	300 µg L^{-1}	7 days
2,4-D + Endothall	1200 µg L^{-1} + 300 µg L^{-1}	6 hours
2,4-D + Endothall	300 µg L^{-1} + 750 µg L^{-1}	7 days
Florpyrauxifen-benzyl	3 µg L^{-1} (1.6 PDU)	6 hours
Florpyrauxifen-benzyl	6 µg L^{-1} (3.1 PDU)	6 hours
Florpyrauxifen-benzyl	12 µg L^{-1} (6.2 PDU)	6 hours
Florpyrauxifen-benzyl	1.5 µg L^{-1} (0.8 PDU)	7 days

- 3 replications
- 30 and 60 day harvest of controls
- 60 day harvest of treated mesocosms

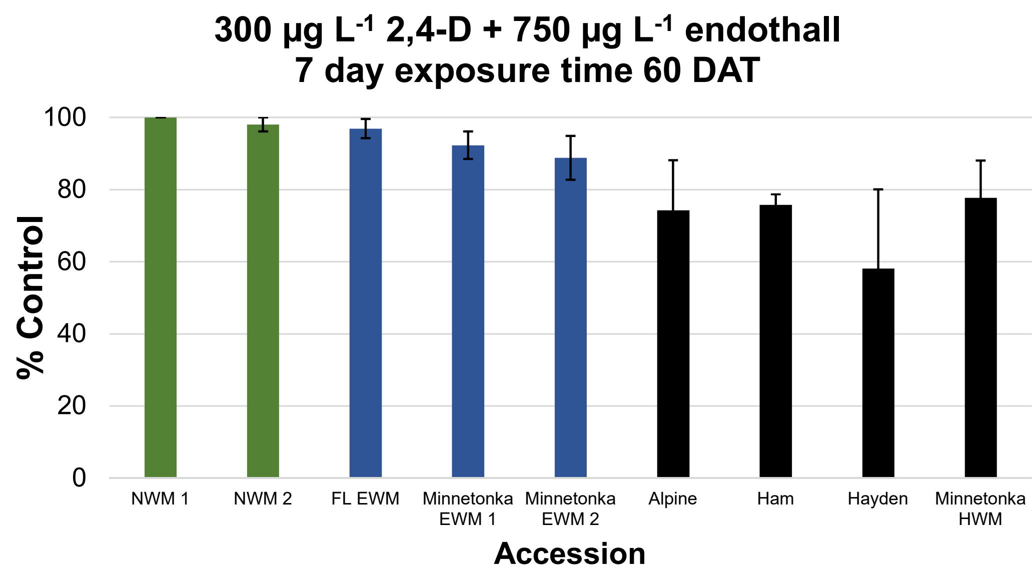
ProcellaCOR EC rate equivalents (in parentheses)


Aboveground Biomass
NWM 1
NWM 2
Florida EWM
Minnetonka EWM 1
Minnetonka EWM 2
Hayden HWM
Alpine HWM
Minnetonka HWM
Ham HWM
Biomass (g DW/pot)
500
400
300
200
100
0
a
b
a
b
a
b
a
b
a
b
PRE
60D
Harvest

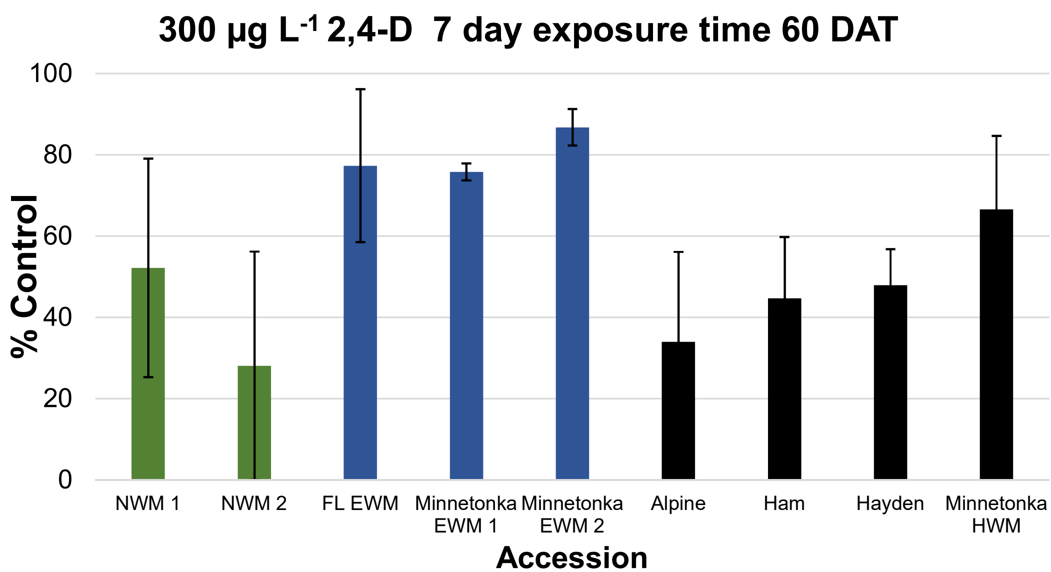

300 µg L-1 2,4-D 7 day exposure time 60 DAT
% Control
100
80
60
40
20
0
NWM 1
NWM 2
FL EWM
Minnetonka EWM 1
Minnetonka EWM 2
Alpine
Ham
Hayden
Minnetonka HWM
Accession

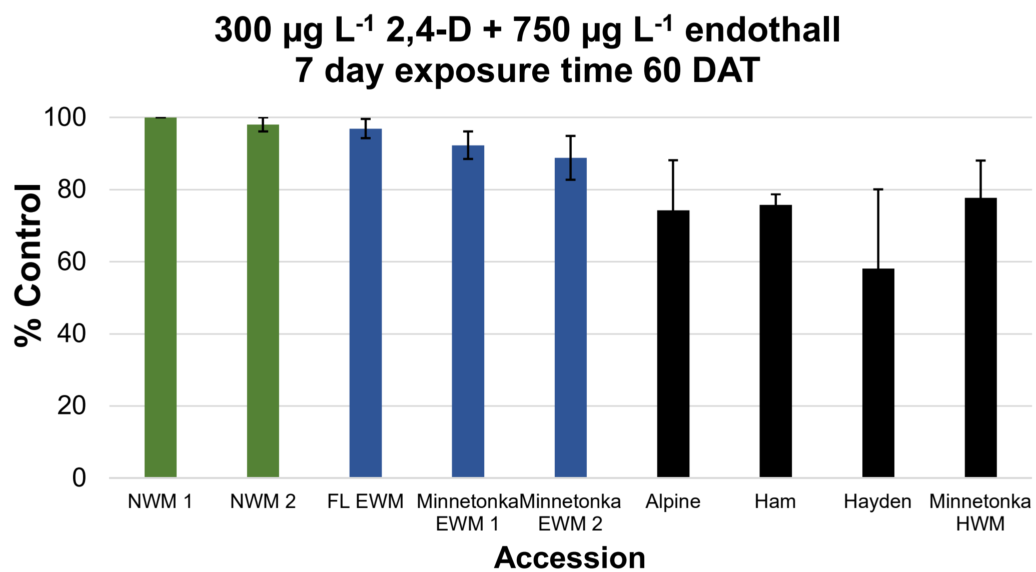


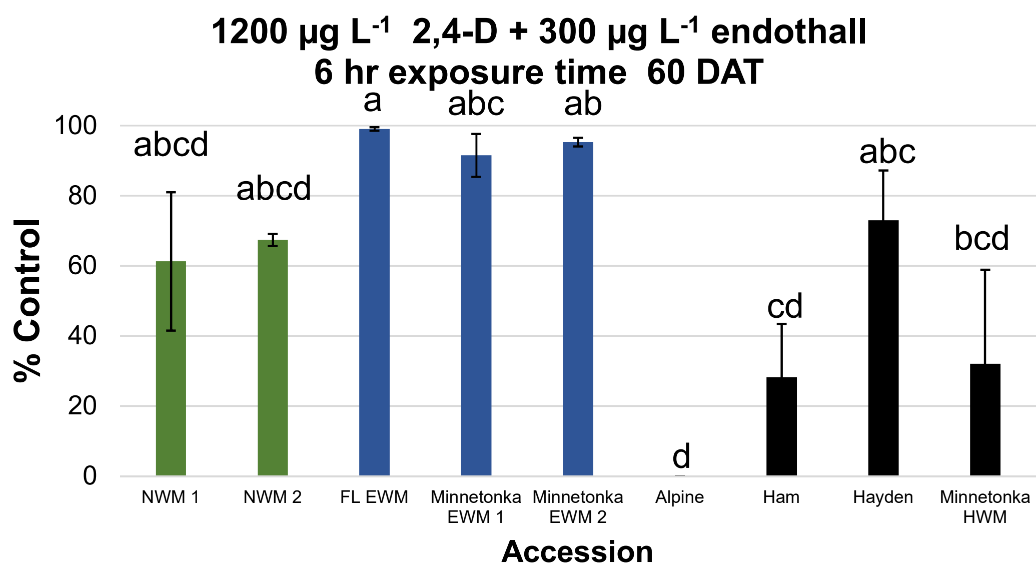
300 µg L-1 2,4-D 7 day exposure time 60 DAT
% Control
100
80
60
40
20
0
NWM 1
NWM 2
FL EWM
Minnetonka EWM 1
Minnetonka EWM 2
Alpine
Ham
Hayden
Minnetonka HWM
Accession

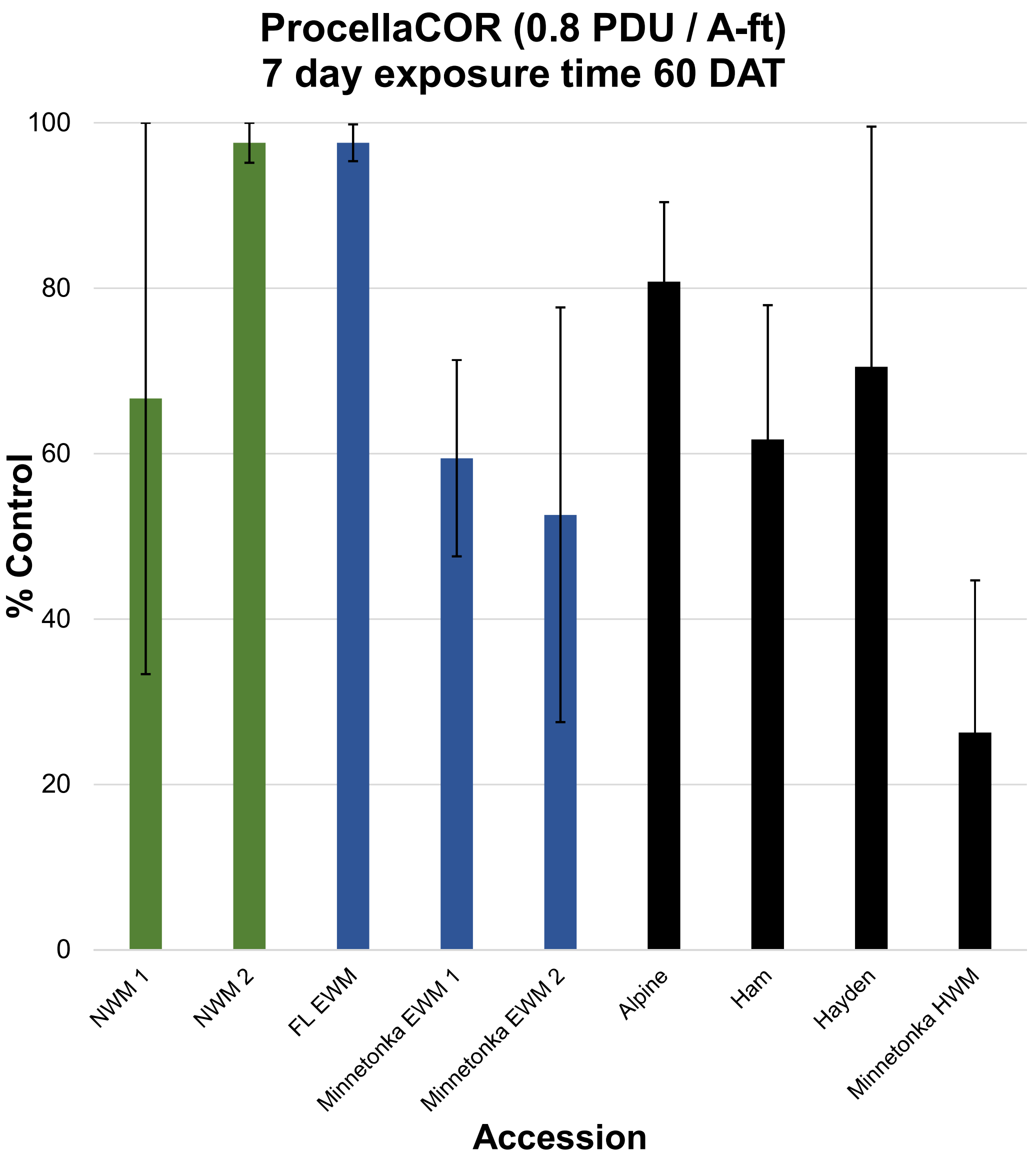


300 µg L-1 2,4-D + 750 µg L-1 endothall
7 day exposure time 60 DAT
% Control
100
80
60
40
20
0
NWM 1
NWM 2
FL EWM
Minnetonka EWM 1
Minnetonka EWM 2
Alpine
Ham
Hayden
Minnetonka HWM
Accession

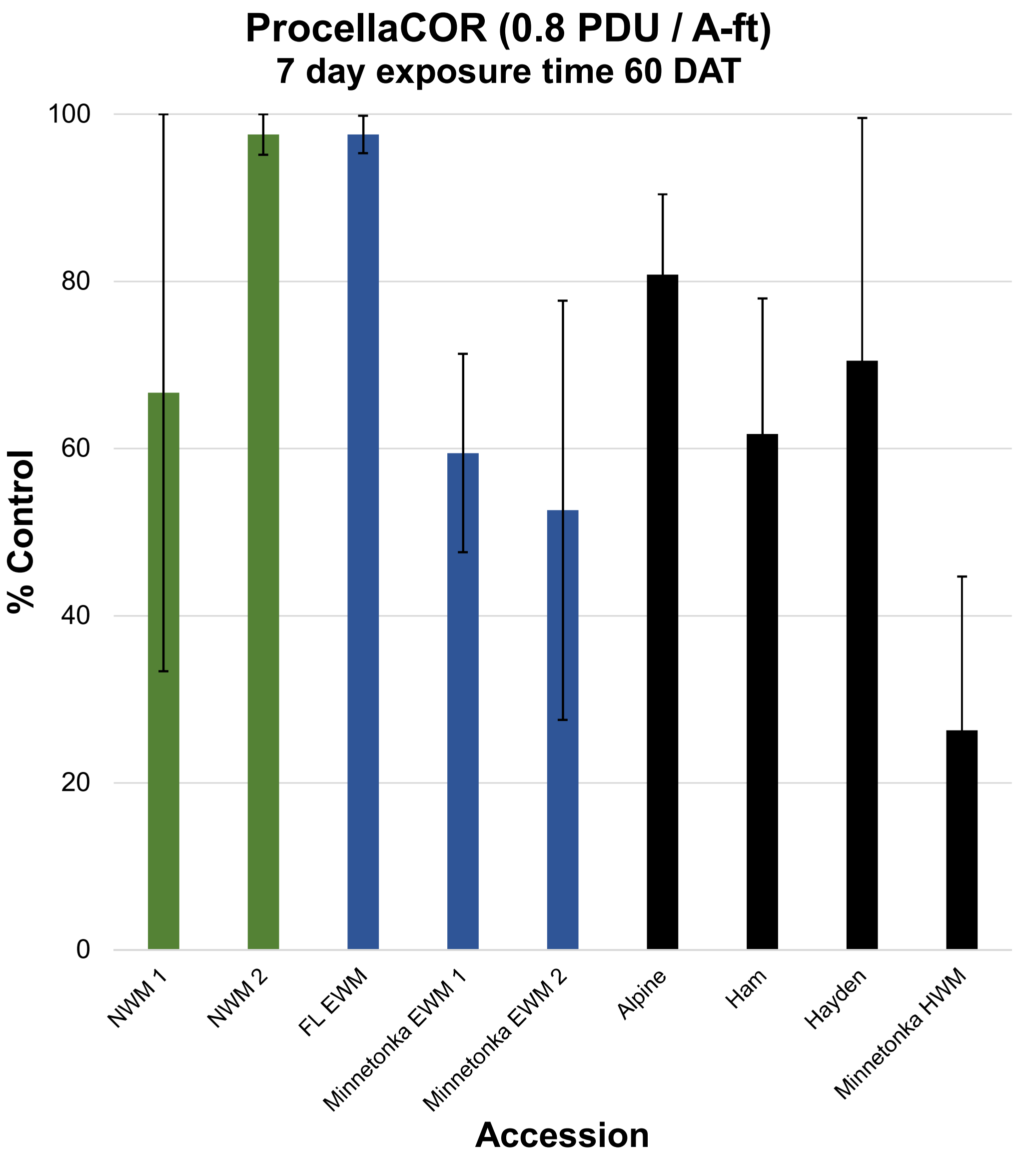

300 µg L-1 2,4-D 7 day exposure time 60 DAT
% Control
100
80
60
40
20
0
NWM 1
NWM 2
FL EWM
Minnetonka EWM 1
Minnetonka EWM 2
Alpine
Ham
Hayden
Minnetonka HWM
Accession


300 µg L-1 2,4-D + 750 µg L-1 endothall
7 day exposure time 60 DAT
% Control
100
80
60
40
20
0
NWM 1
NWM 2
FL EWM
Minnetonka EWM 1
Minnetonka EWM 2
Alpine
Ham
Hayden
Minnetonka HWM
Accession

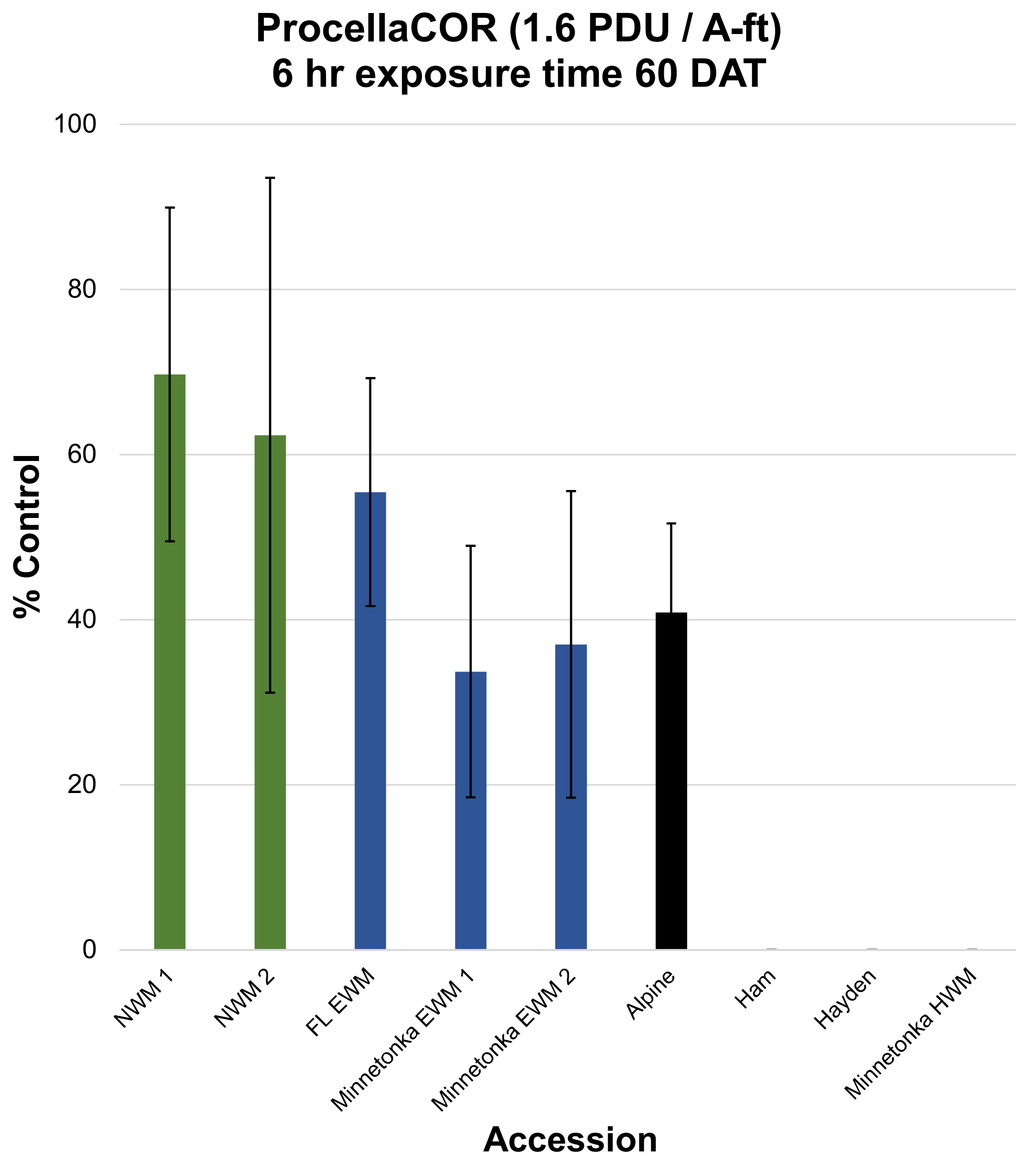

1200 µg L-1 2,4-D + 300 µg L-1 endothall
6 hr exposure time 60 DAT
% Control
100
80
60
40
20
0
abcd
abcd
a
abc
ab
d
cd
abc
bcd
NWM 1
NWM 2
FL EWM
Minnetonka EWM 1
Minnetonka EWM 2
Alpine
Ham
Hayden
Minnetonka HWM
Accession



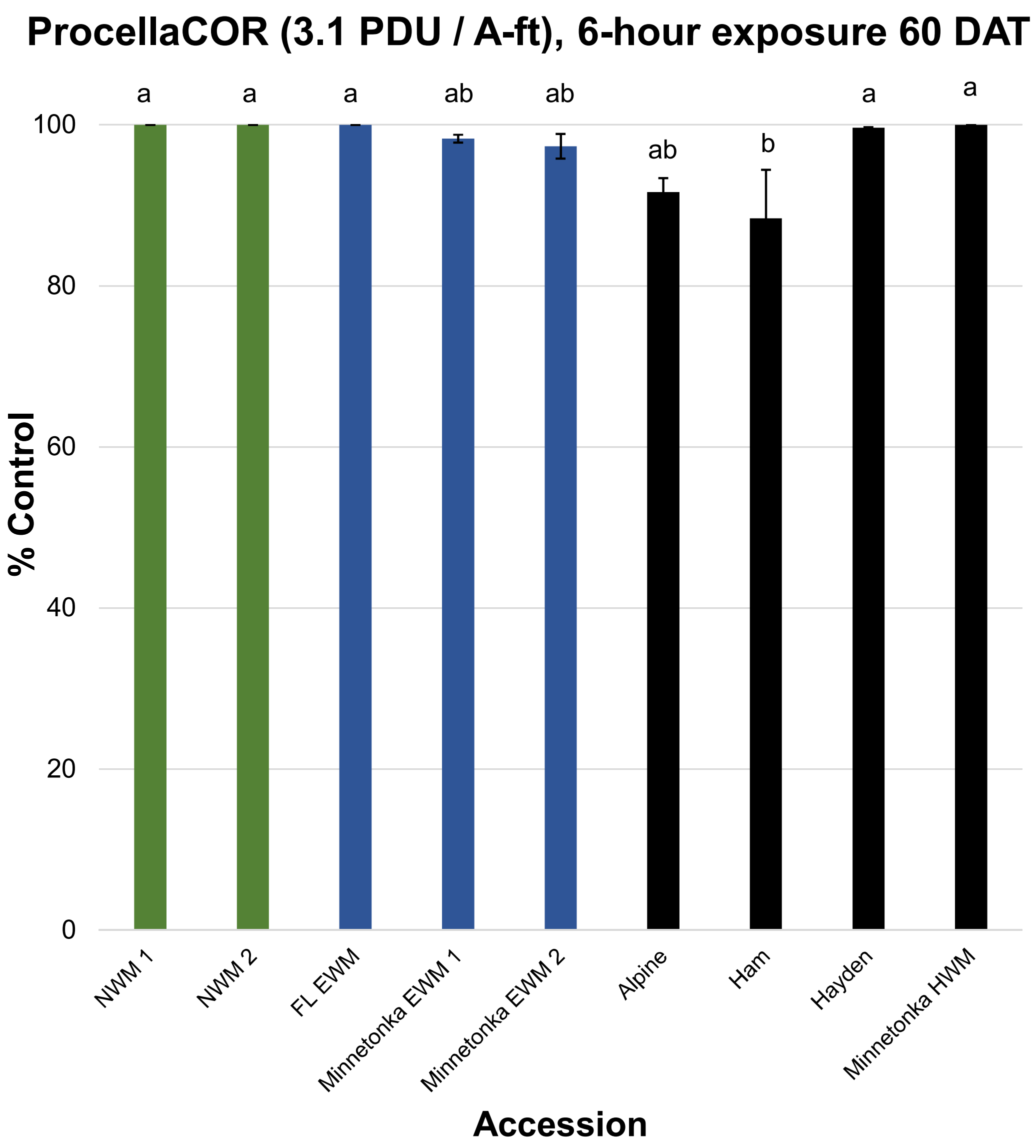
ProcellaCOR (0.8 PDU / A-ft)
7 day exposure time 60 DAT
% Control
100
80
60
40
20
0
NWM 1
NWM 2
FL EWM
Minnetonka EWM 1
Minnetonka EWM 2
Alpine
Ham
Hayden
Minnetonka HWM
Accession

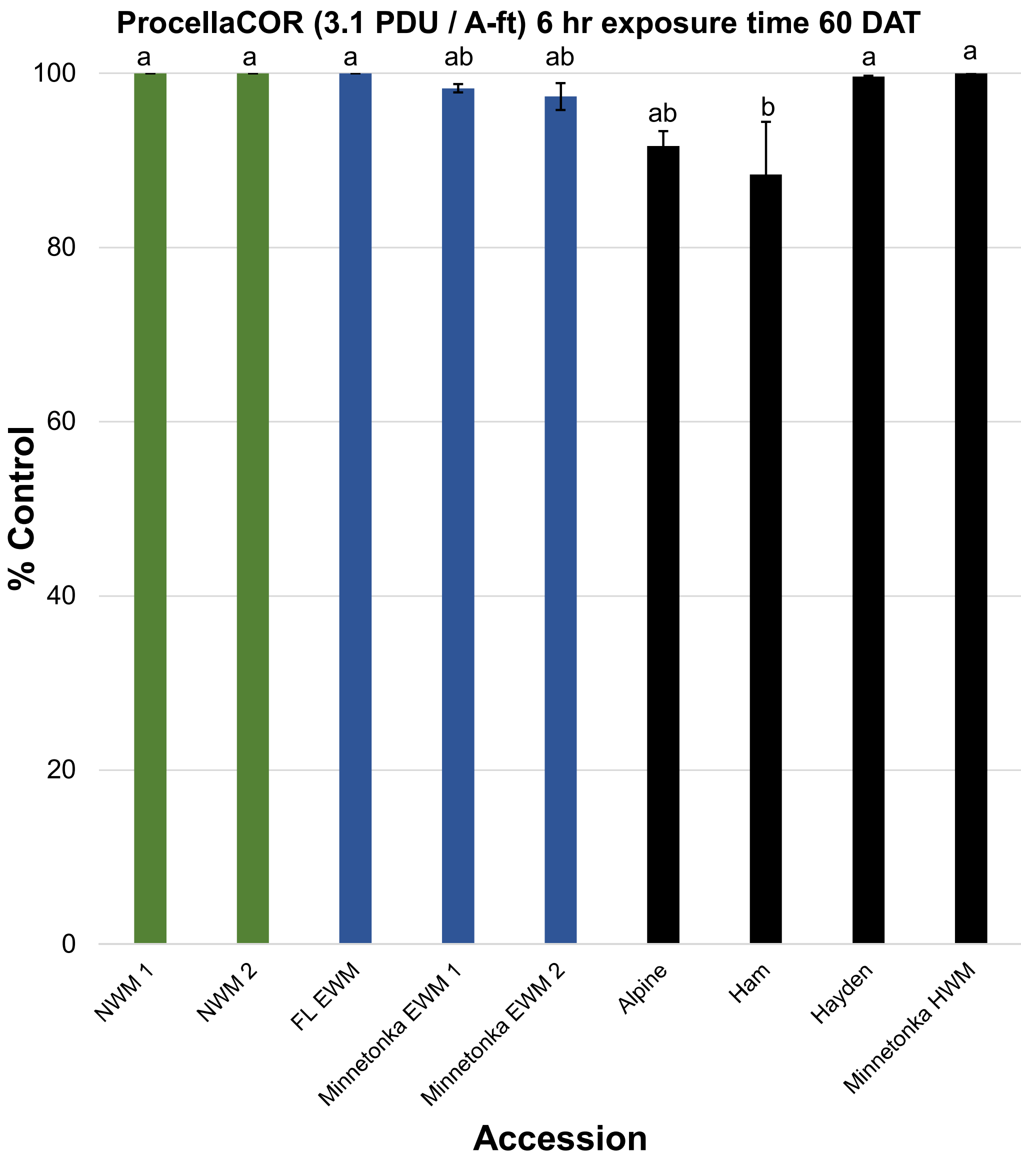


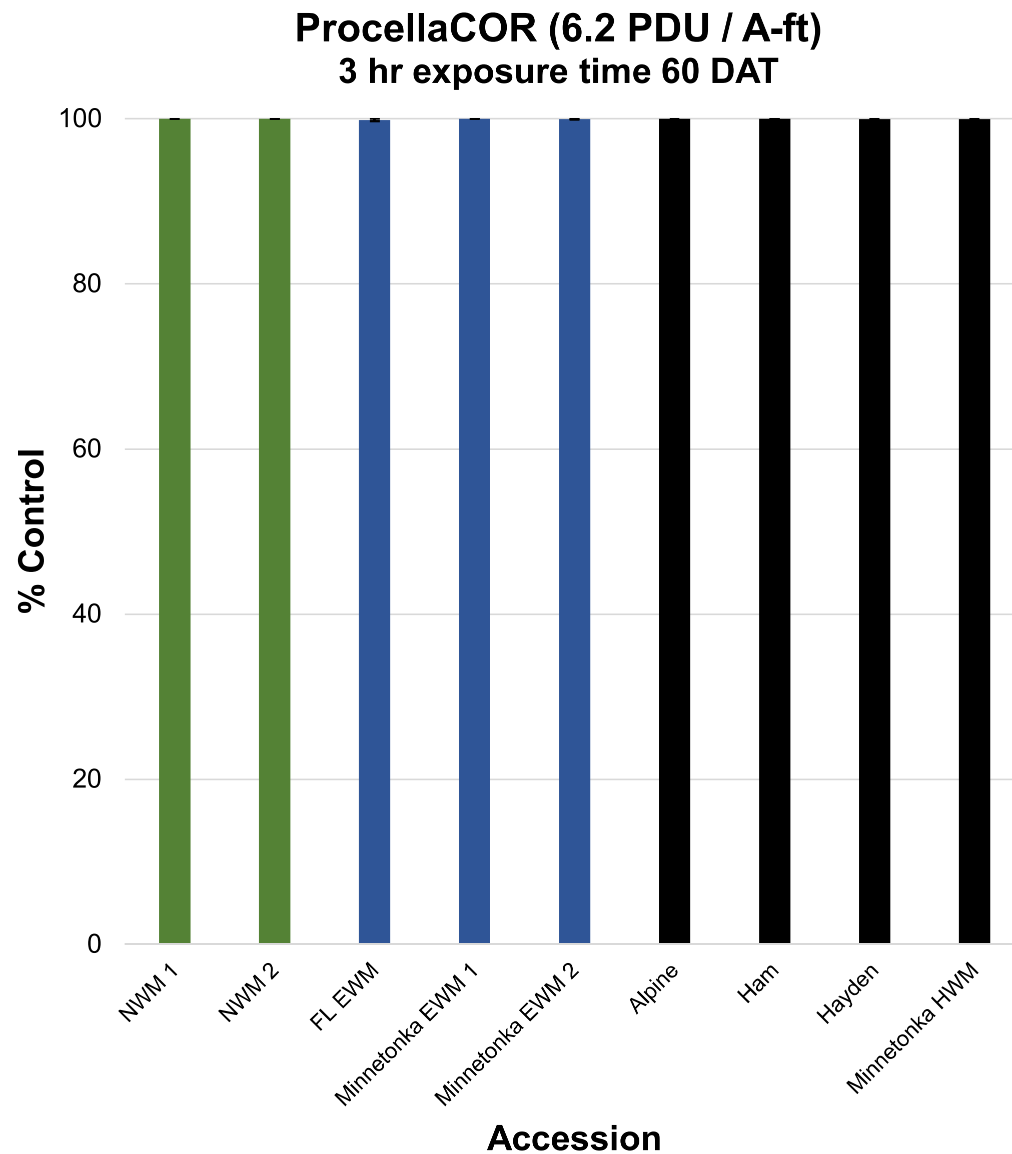
ProcellaCOR (0.8 PDU / A-ft)
7 day exposure time 60 DAT
% Control
Accession
NWM 1
NWM 2
FL EWM
Minnetonka EWM 1
Minnetonka EWM 2
Alpine
Ham
Hayden
Minnetonka HWM



ProcellaCOR (1.6 PDU / A-ft)
6 hr exposure time 60 DAT
% Control
Accession
NWM 1
NWM 2
FL EWM
Minnetonka EWM 1
Minnetonka EWM 2
Alpine
Ham
Hayden
Minnetonka HWM


ProcellaCOR (3.1 PDU / A-ft), 6-hour exposure 60 DAT
% Control
0
20
40
60
80
100
a
a
a
ab
ab
ab
b
a
a
NWM 1
NWM 2
FL EWM
Minnetonka EWM 1
Minnetonka EWM 2
Alpine
Ham
Hayden
Minnetonka HWM
Accession


ProcellaCOR (3.1 PDU / A-ft) 6 hr exposure time 60 DAT
% Control
Accession
0
20
40
60
80
100
a
a
a
ab
ab
ab
b
a
a
NWM 1
NWM 2
FL EWM
Minnetonka EWM 1
Minnetonka EWM 2
Alpine
Ham
Hayden
Minnetonka HWM


ProcellaCOR (6.2 PDU / A-ft)
3 hr exposure time 60 DAT
% Control
Accession
0
20
40
60
80
100
NWM 1
NWM 2
FL EWM
Minnetonka EWM 1
Minnetonka EWM 2
Alpine
Ham
Hayden
Minnetonka HWM

Summary – Study 3

- No significant growth in controls over time
- Differences in herbicide response and growth between accessions
  - Appear to be related to initial size
- 300 µg L^{-1} 2,4-D + 750 µg L^{-1} endothall treatment effective
- Florpyrauxifen-benzyl 6 µg L^{-1} (or 3.1 PDU EC / A-ft) 6hr and 12 µg L^{-1} (or 6.2 PDU EC / A-ft) 3 hr exposures effective

General Conclusions

- HWM showed greater tolerance to all auxin mimics
- ProcellaCOR (florpyrauxifen-benzyl) highly active on EWM and HWM
- Selected native species show florpyrauxifen-benzyl selectivity
- ProcellaCOR can effectively manage all watermilfoils with appropriate rate and CET scenarios

DEPARTMENT OF NATURAL RESOURCES

Hybrid Watermilfoil Management Report - DRAFT
Lake Jane, Washington County - ProcellaCOR® Treatment

Lake:	Jane, Washington County (DOW# 82010400)
Lake information:	153 surface acres (110 acres less than 15 feet deep), max depth 39 feet
Treatment date:	18 June 2018
Treatment type:	Spot treatment (ProcellaCOR®)
Pesticide applicator:	PLM Lake & Land Management Corps
Plant surveyor(s):	Keegan Lund, Kylie Cattoor, Wendy Crowell (MN DNR Invasive Species Program)
Author(s):	Kylie Cattoor, Keegan Lund, keegan.lund@state.mn.us, 651-259-5828
Report Date:	13 December 2018

Background:
Lake Jane is a smaller lake (153 acres) located near the city of St. Elmo in the greater Twin Cities metropolitan area. Lake Jane is a relatively deep lake (max depth 39 feet) that supports a diverse native aquatic plant community (25 native submersed aquatic plant species). The invasive watermilfoil (*Myriophyllum spicatum* x *Myriophyllum sibiricum* hybrid, hereafter HWM) was first reported in Lake Jane in 2012. The Minnesota Department of Natural Resources (DNR) confirmed the infestation and declared the lake as infested with Eurasian watermilfoil (for listing purposed both Eurasian watermilfoil and HWM are combined as one listing in Minnesota). DNR recognizes there are management implications (possible herbicide resistance) with certain strains of invasive watermilfoils and is not advocating one herbicide management approach versus another in this report.

Problem:
The Lake Jane Association began managing nuisance HWM through spot treatments first in 2015 (7.9 surface acres treated) and again in 2017 (11.1 surface acres treated) using the herbicide 2,4-D. According to DNR surveys and observations from the lake association, these spot treatments provided only seasonal control. Despite these spot treatments, milfoil continued to expand. By 2018, HWM dominated the plant community occupying more than 50% of the littoral zone (zone from 0-15 foot depth occupied by submersed aquatic plants).

Management Objective:
In early 2018, ProcellaCOR®Aquatic Herbicide (active ingredient, florpyrauxifen-benzyl) was approved by the Minnesota Department Agriculture as a registered pesticide for aquatic use in Minnesota. ProcellaCOR is marketed as a highly selective (notably milfoils, hydrilla, crested floating heart), fast acting, short term exposure herbicide in which the active ingredient requires 40x-100x lower use rates than other auxin-mimic herbicides such as 2,4-D or triclopyr. USEPA assessments presented no adverse risk to human health or non-target wildlife. This was the first application of this herbicide in Minnesota.

This treatment report is part of an evaluation of ProcellaCOR by the DNR to determine if: 1) ProcellaCOR effectively controls HWM through spot treatments for more than one season following treatment and 2) whether such control causes unacceptable harm to native plant species in lake. Four additional lakes in Minnesota: Minnetonka (Gray's Bay, Gideon's Bay and Carman's Bay; Hennepin Co), Orchard (Dakota Co), Ham and Crooked (Anoka Co) were treated in 2018 with ProcellaCOR. Additional data is available upon request.

ProcellaCOR Treatment:

On June 18, 2018, a 12-acre area (see **Map 1** below) on the north shore of Lake Jane was treated with ProcellaCOR EC. Average depth of the treatment area was approximately 6.5 feet and water temperatures were 25°C (77°F) during treatment.

A licensed pesticide applicator treated the area at 62.4 ounces per surface acre (19.5 Prescription Dose Units or PDUs – see ProcellaCOR EC product label or 3 PDU per acre foot) totaling 748.8 ounces (234 PDUs) of ProcellaCOR. The total cost of the treatment including product and labor was $15,000.00 or approximately $1,350.00 per acre and was guaranteed for three years by the manufacturer SePRO.

Herbicide concentration monitoring was not conducted during or following the treatment to measure dissipation. However, due to the rapid uptake of ProcellaCOR by target HWM based on past university research and the limited scale of management relative to untreated volume of the lake, extended contact time and off-site movement of effective concentrations to produce notable plant community effects outside of the management area were not anticipated.

Initial Results:

The effects of ProcellaCOR were determined by examining the distribution of individual aquatic plant species (including HWM) before and after treatment. The distribution of individual species was estimated by the species frequency, that is the percentage of sampling sites at which the plant is present over all sampled points within the littoral zone.

Submersed aquatic plants were collected using a two-sided sampling rake thrown at each sampling point. At each sampling point plant species and rake fullness was recorded. Plants were sampled across an evenly spaced grid (approximately 90 points, 35 meter spacing) within and adjacent to the treatment area via point-intercept survey methods. Surveys included a pre-treatment assessment followed by 2-post treatment assessments (19 days post treatment & 45 days post treatment).

Initial results indicate that the ProcellaCOR treatment reduced the frequency of Hybrid watermilfoil from 72% to 1%, 45 days post treatment. A single plant was found outside the treatment area but within sampled points during the final post-treatment assessment. There were overall no decreases to submersed native species following treatment of plants at a 10% or greater frequency (see **Table 1** below).

During the July 5 survey (19 days post treatment), HWM plants showed signs of herbicide damage such as brown stems with dead leaves attached. HWM plants were still present in large stands and had not dropped out of the water column (see **Photo 1** below). Additionally, native watershield (*Brasenia schreberi*), a floating-leaf plant, showed possible impacts from the treatment, notably leaves were folded or cupped downward but remained floating at the water's surface (see **Photo 2** below). Overall reductions in watershield were not observed 45 days post treatment.

After several weeks, the plant survey was repeated on July 31 (45 days post treatment). HWM plants appeared non-viable, were devoid of leaves, and had dark root crowns and limp stems showing no sign of regrowth (see **Photos 3 a & b below**). Among the more abundant native submersed plants observed at this time were Muskgrass, White-stem pondweed, Naiad, and Water celery. Native plants showed no signs of herbicide damage other than minor epinasty of Coontail as was expected from the treatment. Outside the treatment area, watershield had rebounded from previous observations on July 5 (see **Photo 4** below).

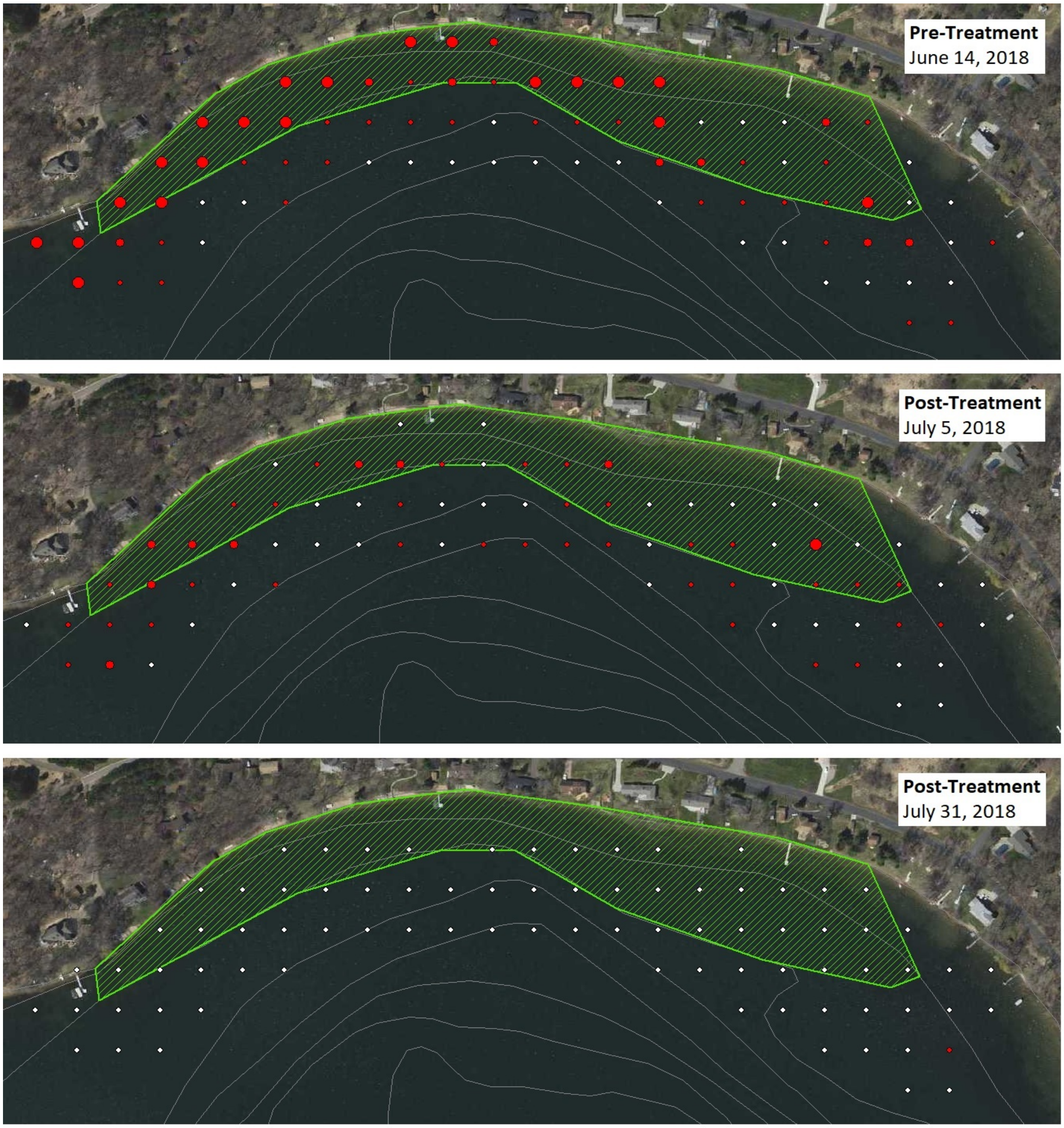
In general, the treatment of ProcellaCOR in Lake Jane provided a 100% reduction of HWM in the treatment area 45 days post treatment. Interestingly, northern watermilfoil remained present in the treatment area throughout the sampling efforts. Slight reductions in the frequency of occurrence of Small pondweed, Sago pondweed, and Canadian waterweed should be noted in future trials and evaluations of the herbicide. DNR will be conducting follow-up plant surveys in 2019 to determine whether the ProcellaCOR treatment provided any carry-over effect and relief from nuisance HWM in the 2018 treated areas.

Table 1- Point Intercept Metrics. Summary of point intercepts metrics for Lake Jane, Washington County (DOW# 82010400). Shaded values were calculated from littoral depth range.

Survey Metrics	JUNE 14 2018 (Pre-Treatment)	JULY 5 2018 (Post-Treatment)	JULY 31 2018 (Post-Treatment)
Treated (Y/N)	N	Y	Y
Surveyor	MN DNR	MN DNR	MN DNR
Total # Points Sampled	82	80	82
Max Depth of Growth (95%) in feet	13	13	14
# Point in Max Depth Range	71	76	78
# Points in Littoral (0-15 feet)	78	78	81
% Points w/ Submersed Native Taxa	91	97	99
Mean Submersed Native Taxa/ Point	2.4	2.5	2.6
# Submersed Native Taxa	12	11	13
# Submersed Non-Native Taxa	2	1	1

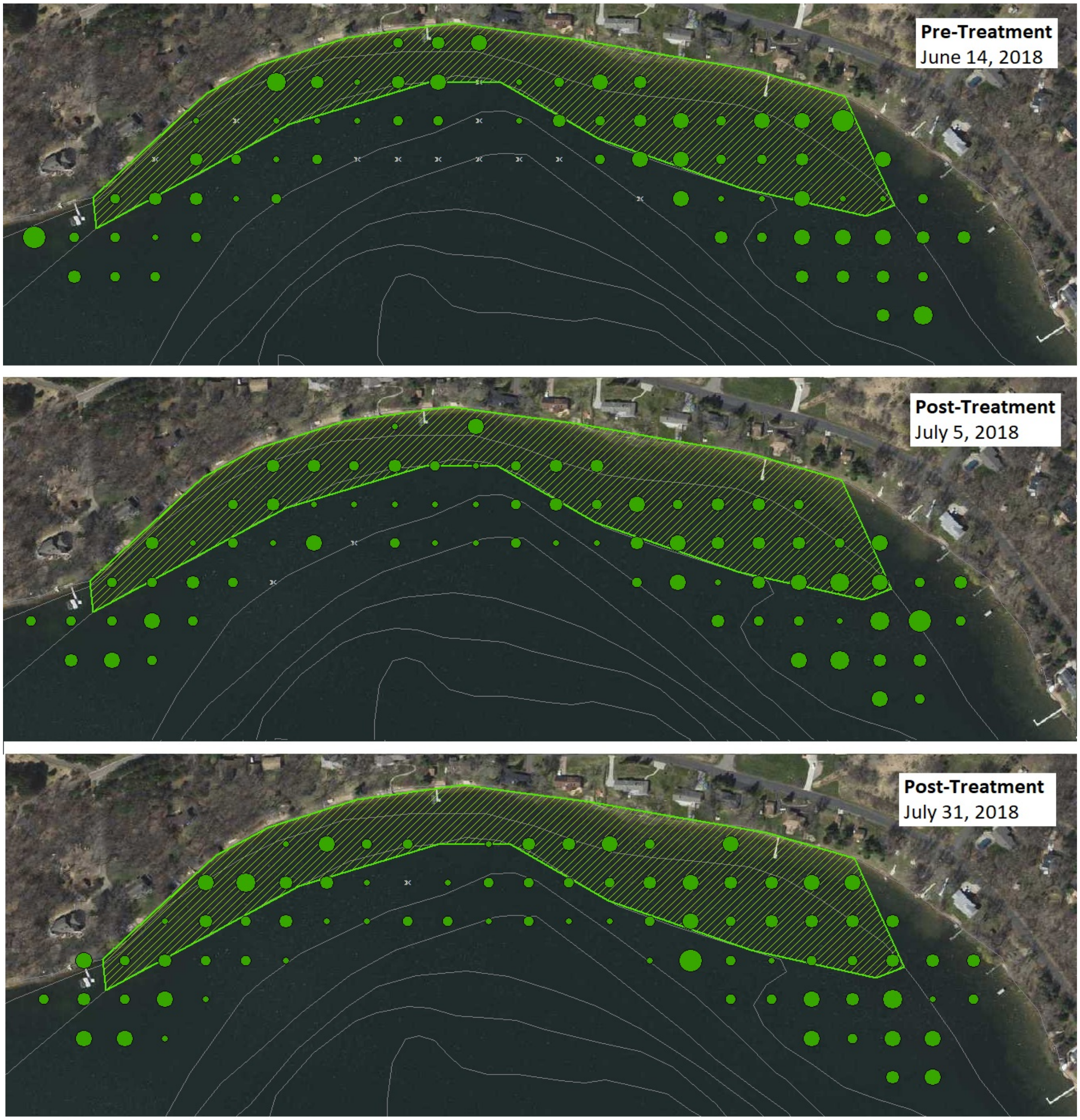
Table 2- Plant Frequency Occurrence. Historic percent frequency of occurrence for submersed vegetation within the littoral zone (0-15 feet) in Lake Jane, Washington County (DOW# 82010400). (*) denotes aquatic invasive plant. The χ-squared test showed significant shifts in plant species pre (June 14) and post (July 31) treatment with plants at 10% frequency of occurrence or greater. (-) denotes significant decrease, (+) denotes significant increase (*P < 0.05*), (*ns*) denotes no significant change.

Taxonomic Name SUBMERSED PLANTS	Common Name	JUNE 14 (Pre-Treatment)	JULY 5 (Post-Treatment)	JULY 31 (Post-Treatment)	χ – squared Test
*Myriophyllum spicatum x sibiricum**	Hybrid watermilfoil*	72	51	1	-
*Potamogeton crispus**	Curly-leaf pondweed*	27	0	0	-
Ceratophyllum demersum	Coontail	14	19	22	*ns*
Macroalgae	Muskgrass and Stonewort	29	31	36	*ns*
Eleocharis acicularis	Needle spikerush	0	3	2	
Elodea canadensis	Canadian waterweed	62	63	46	-
Myriophyllum sibiricum	Northern watermilfoil	3	0	2	
Najas spp.	Naiad	42	44	44	*ns*
Potamogeton amplifolius	Large-leaf pondweed	9	10	5	*ns*
Potamogeton gramineus	Variable-leaf pondweed	0	1	1	
Potamogeton nodosus	Long-leaf pondweed	3	0	7	
Potamogeton praelongus	White-stem pondweed	35	28	28	*ns*
Potamogeton pusillus	Small pondweed	5	0	0	
Potamogeton robbinsii	Fern pondweed	23	24	28	*ns*
Potamogeton zosteriformis	Flatstem pondweed	0	3	1	
Stuckenia pectinata	Sago pondweed	6	0	0	
Vallisneria americana	Water celery	10	26	32	+
FLOATING/EMERGENT PLANTS					
Brasenia schreberi	Watershield	1	1	1	
Nymphaea ordorata	White waterlily	0	0	4	
Sagittaria spp.	Arrowhead	9	1	0	



EWM Density Rating
- 0 - Not Found
- 1 - Sparse/Scattered
- 2 - Common
- 3 - Abundant
- 2018 Approved EWM Treatment Area= 12 ac

Map 1- Spatial distribution and rake density rating of Hybrid watermilfoil in Lake Jane, Washington County. Green shaded area represents treatment area (12-ac). Surveys conducted pre and post ProcellaCOR in 2018. Densities were based on a 0-3 scale.



Map 2- Spatial distribution and native species richness (# of native submersed taxa per sample point) in Lake Jane, Washington County. Green shaded area represents treatment area (12-ac). Surveys conducted pre and post ProcellaCOR treatment in 2018.

Photo 1- Hybrid watermilfoil plant observed inside the treatment area in Lake Jane, Washington County (DOW# 82010400). Survey conducted on July 5th, 2018 - 19 days post-treatment.

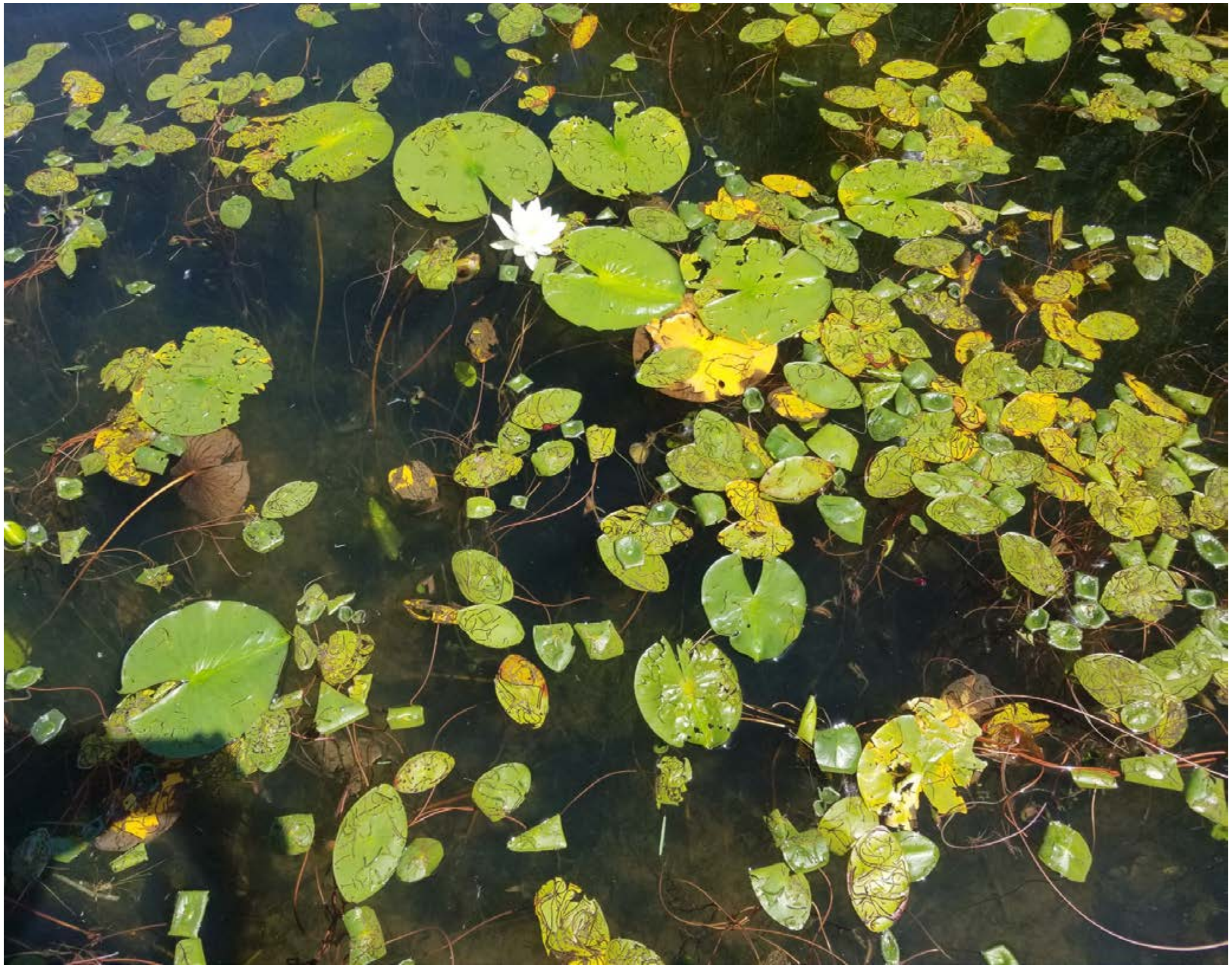


Photo 2- Native floating leaf plant, *Brasenia schreberi* observed near the treatment area in Lake Jane, Washington County (DOW# 82010400). Survey conducted on July 5th, 2018 - 19 days post-treatment.

Photo 3- Hybrid watermilfoil and native submersed plant samples from Lake Jane, Washington County (DOW# 82010400) inside the treatment area. Survey conducted on July 31st, 2018 - 45 days post-treatment.

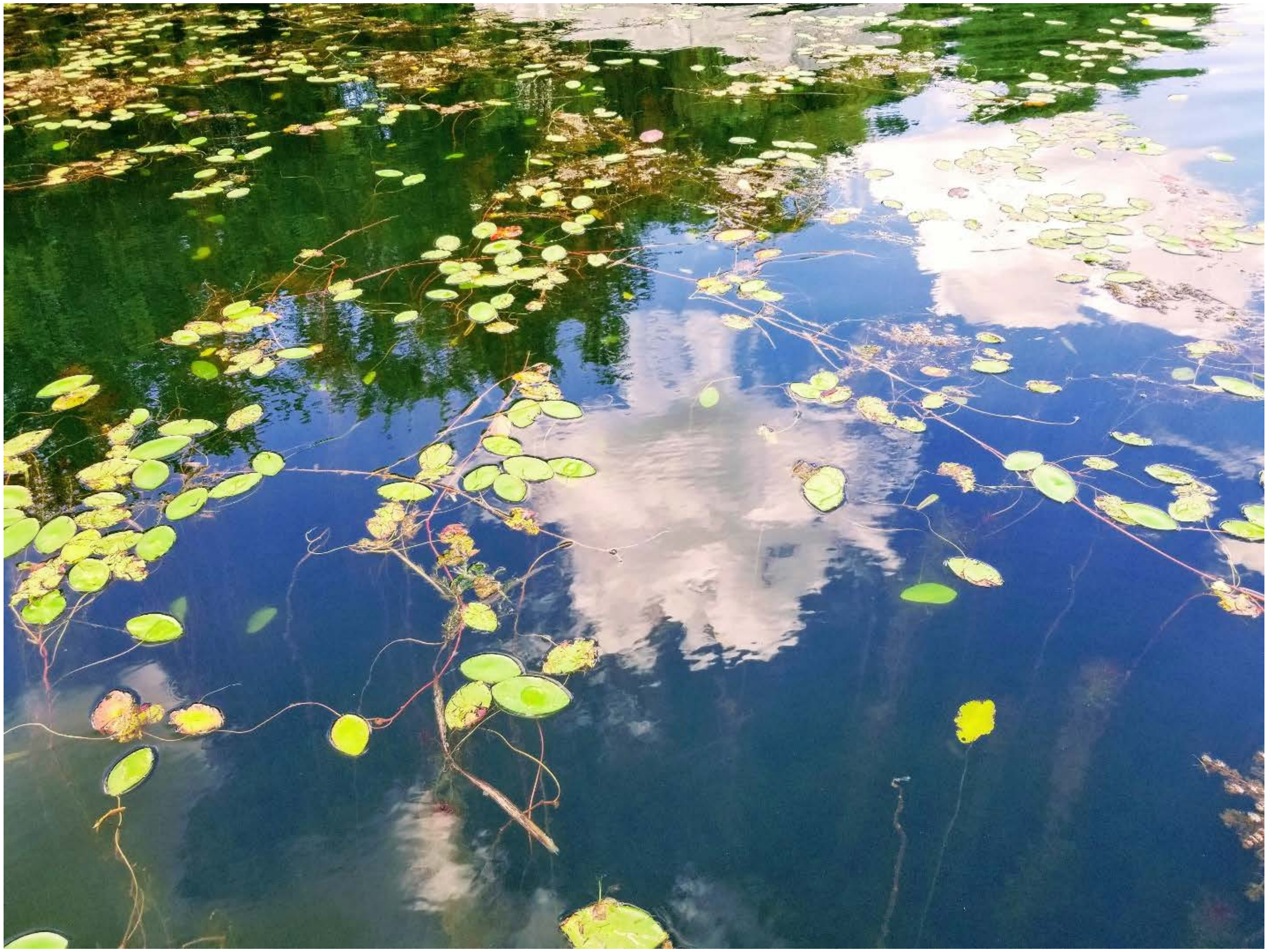


Photo 4- Hybrid watermilfoil (below surface) and native floating leaf, *Brasenia schreberi*, observed outside of the treatment areas in Lake Jane, Washington County (DOW# 82010400). Survey conducted on July 31st, 2018 - 45 days post-treatment.

ProcellaCOR Field Trial

NC State University

Watershield

An approximately 4-acre pond without water uses with a treatment area of 0.5 acre was treated with ProcellaCOR (equivalent to 7.8 PDU per acre-foot ProcellaCOR EC) on 7/24/2015. A surface application was performed and the weed of concern was watershield with moderate densities of creeping spike rush (*Eleocharis baldwinii*). The water temperature was 28.6 C, 7.33 pH, 2.23 mg/L DO and 0.036 g/L TDS at time of treatment. Biomass samples were taken at time of treatment, 1MAT and 2MAT. Two sample sites (1 & 2) were inside the treatment zone and two (3 & 4) were well outside the treatment area (Figure 1)

Figure 1. Biomass sample sites 1 & 2 inside the treatment area (red line) and 3 & 4 in the untreated area.

Results

By 2WAT a significant decrease in watershield abundance was visible and a significant decrease was seen in biomass in the treated area at 1MAT (Figure 2). Watermeal moved into the area making the post treatment results look deceiving however little to no watershield or spikerush is remaining at 1MAT. No regrowth was seen at 2MAT inside the treatment area. Very little bleed out from the treatment area was noted. Overall, ProcellaCOR was highly effective on watershield with good spot treatment characteristics.

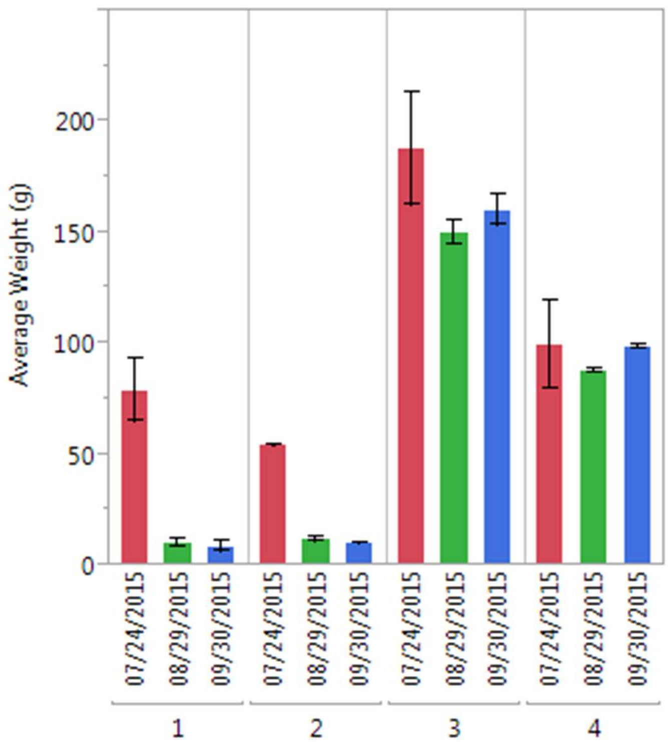


Figure 2. Average biomass weight (g) for the two treatment sites (1 &2) and two reference sites (3 & 4) at time of treatment, 1MAT and 2MAT.

Day of Treatment (highest watershield densities slightly off shore)

2WAT

1MAT

Weed Science 2016 64:181–190

Evaluating Sensitivity of Five Aquatic Plants to a Novel Arylpicolinate Herbicide Utilizing an Organization for Economic Cooperation and Development Protocol

Michael D. Netherland and Robert J. Richardson*

New arylpicolinate herbicide chemistry under development for rice, aquatic weed management, and other uses was evaluated using five aquatic plants. The herbicide 4-amino-3-chloro-6-(4-chloro-2-fluoro-3-methoxyphenyl)-5-fluoro-pyridine-2-benzyl ester—also identified as XDE-848 BE or SX-1552 (proposed International Organization for Standardization common name in review; active tradename Rinskor^{TM})—and its acid form (XDE-848 acid or SX-1552A) were evaluated on three dicots: (1) Eurasian watermilfoil (EWM), (2) megalodonta, and (3) crested floating heart (CFH), and two monocots: (1) hydrilla and (2) elodea. A small-scale Organization for Economic Cooperation and Development (OECD) protocol developed using EWM for registration studies was utilized. EWM and megalodonta were also evaluated in larger-scale mesocosms for comparison. In-water concentrations between 0.01 and 243 μg ai L^{-1} as SX-1552 or SX-1552A were applied under static conditions for 14 (growth chamber) or 28 d (mesocosm). EWM was susceptible to both SX-1552 and SX-1552A, with dry-weight 50% effective concentration (EC_{50}) values of 0.11 and 0.23 μg ai L^{-1} under growth chamber conditions. Megalodonta had EC_{50} values of 11.3 and 14.5 μg ai L^{-1} for the SX-1552 and SX-1552A. CFH was more sensitive to SX-1552 (EC_{50} = 5.6 μg ai L^{-1}) than to SX-1552A (EC_{50} = 23.9 μg ai L^{-1}). Hydrilla had EC_{50} values of 1.4 and 2.5 μg ai L^{-1}, whereas elodea was more tolerant, with EC_{50} values of 6.9 and 13.1 μg ai L^{-1} for SX-1552 and SX-1552A, respectively. For EWM mesocosm trials, EC_{50} values for SX-1552 and 1552A were 0.12 μg ai L^{-1} and 0.58 μg ai L^{-1}, whereas the megalodonta EC_{50} was 6.1 μg ai L^{-1}. Activity of SX-1552 on EWM, hydrilla, and CFH merits continued investigation for selective aquatic weed control properties. Results suggest that the OECD protocol can be used to screen activity of herbicides for multiple aquatic plant species.

Nomenclature: 4-Amino-3-chloro-6-(4-chloro-2-fluoro-3-methoxyphenyl)-5-fluoro-pyridine-2-benzyl ester; crested floating heart, *Nymphoides cristata* (Roxb.) Kuntze; elodea, *Elodea canadensis* Michx.; Eurasian watermilfoil, *Myriophyllum spicatum* L.; hydrilla, Hydrilla verticillata L.f. *Royle*; megalodonta, *Bidens beckii* Torr. Ex Spreng.

Key words: Aquatic herbicide, aquatic plant bioassay, aquatic plant toxicity, Beck's water-marigold, herbicide screening, invasive aquatic plants.

Aquatic weed control with herbicides is characterized by unique conditions and management objectives vs. agricultural or other terrestrial weed management (APMS 2014). Perhaps the two most significant differences in use of aquatic vs. terrestrial herbicides are (1) labeled use for direct application into water to achieve a target herbicide concentration and exposure and (2) high standards for targeting an invasive or nuisance plant with limited impact to multiple native or desirable plant species. In the typical agricultural setting direct application to water is prohibited and broad-spectrum weed control is provided for a single nontarget species. Aquatic herbicide registration by the U.S. Environmental Protection Agency and other international regulatory agencies requires demonstration of negligible risks to human health or the environment.

Risk assessments of aquatic herbicides consider human water uses and exposure (e.g., drinking, recreational use including swimming, and irrigation practices), other incidental exposure routes, and possible impact to nontarget biota: algae, fish, invertebrates, and nontarget aquatic vegetation. Stringent requirements for aquatic herbicide registration have limited the number of active ingredients approved for aquatic use. Although 244 herbicide active ingredients are currently registered in the United States, only 14 are registered as aquatic herbicides (NPIRS 2015). There is a technical need for additional

DOI: 10.1614/WS-D-15-00092.1

* Research Biologist, U.S. Army Engineer Research and Development Center, 7922 NW 71st Street, Gainesville, FL 32653; Associate Professor (ORCID: 0000-0002-3850-3709), North Carolina State University, Box 7620, Raleigh, NC 27695. Corresponding author's E-mail: Michael.D.Netherland@usace.army.mil

herbicides and alternative modes of action for aquatic weed management. New herbicides can improve response to new aquatic invaders, enhance selectivity to desirable native aquatic vegetation, reduce use rates, and mitigate risk of potential herbicide resistance development (APMS 2014; Getsinger et al. 2008).

To support the development of a potential new aquatic herbicide, a new chemistry was screened against several target and nontarget aquatic plants. The herbicide 4-amino-3-chloro-6-(4-chloro-2-fluoro-3-methoxyphenyl)-5-fluoro-pyridine-2-benzyl ester, is under development by Dow AgroSciences for rice (XDE-848 BE;proposed International Standardization Organization common name in review; active tradename Rinskor^{TM}) and other agricultural crops and is also under development in partnership with SePRO Corporation as an aquatic herbicide (SX1552; Procellacor^{TM}; Aquatic Herbicide Technology System). SX-1552 is a member of a new class of synthetic auxins in the arylpicolinate herbicide family. In preliminary screening, SX-1552 exhibited efficacy on several invasive U.S. aquatic weeds including the submersed plants hydrilla and EWM, and the floating-leaf plant CFH (SePRO Corporation, unpublished data). SX-1552 would represent a new chemical class for aquatic uses. Studies of *Arabidopsis thaliana* with mutations in select auxin-binding receptor proteins, along with direct molecule–protein interaction testing of these same receptor proteins, support that arylpicolinate chemistry including SX-1552 has a different binding affinity vs. 2,4-D and other synthetic auxins currently registered as herbicides (Bell et al. 2015; Lee et al. 2013; Villalobos et al. 2012; Walsh et al. 2006).

Laboratory studies and preliminary field dissipation studies indicate that SX-1552 in water is subject to rapid photolysis—a common mechanism of breakdown for several aquatic herbicides. SX-1552 can also convert partially via hydrolysis to an acid form (SX-1552A) with suspected reduced herbicidal activity.

Small-scale evaluation methods serve multiple purposes in aquatic herbicide development including characterization of relative activity for a particular mode of action and determination of weed spectrum including information on efficacy and selectivity. Several different small-scale methods have been utilized to characterize herbicidal activity on aquatic plants. Historically, baseline toxicity tests on duckweed (*Lemna* spp.) have driven regulatory assessment of pesticide risks to nontarget vascular aquatic plants (OECD 2006, USEPA 2012). Past small-scale laboratory testing to predict aquatic herbicide activity has included analysis of photosynthetic pigment concentrations after exposure to carotenoid biosynthesis inhibitors such as fluridone and topramezone (Berger et al. 2015; Glomski and Netherland 2011; Netherland et al. 1993). Contact aquatic herbicide activity for endothall (protein phosphate inhibitor), diquat (photosystem I inhibitor), flumioxazin, and carfentrazone (Protox inhibitors) have been quantified using conductivity testing of ion leakage (Glomski and Netherland 2013; Koschnick et al. 2006; MacDonald et al. 1993). For the auxin herbicides 2,4-D and triclopyr, controlled laboratory and greenhouse studies have defined concentration–exposure time relationships for EWM control (Green and Westerdahl 1990, Netherland and Getsinger 1992) and nontarget aquatic plant activity (Belgers et al. 2007; Hofstra and Clayton 2001; Netherland and Glomski 2014; Sprecher et al. 1998; Sprecher and Stewart 1995) that have been predictive of selective EWM control observed in the field (Nault et al. 2014; Parsons et al. 2001, Poovey et al. 2004, Wersal et al. 2010).

On the basis of the successful correlation of laboratory and mesocosm-scale studies and field evaluations with currently registered auxin-mimic aquatic herbicides, aquatic use pattern development for SX-1552 can be accelerated through initial data generation of laboratory-scale efficacy and selectivity. Realism of small-scale testing methodology for determinations of herbicidal efficacy, selectivity, and general ecological risk assessment is debated (Maltby et al. 2010). In 2014, a small-scale testing protocol using EWM was adopted by the OECD as a method to generate additional data for assessment of potential nontarget aquatic plant effects when *Lemna* spp. are not sensitive to the mode of action (OECD 2014). OECD method test results on EWM are now used in risk assessments supporting the registration of certain herbicidal modes of action in the European Union. There is minimal published data for aquatic herbicides that directly compare results of "microscale" laboratory screening with outcomes of larger-scale controlled studies using more established plants—typically at an aquarium or mesocosm scale under greenhouse or outdoor conditions. The OECD protocol (2014) describes the guidelines surrounding water and sediment testing for impacts of pesticides on rooted EWM. The results are used for registration purposes in Europe, and EWM was selected as the preferred species in cases where data are required for specific herbicidal modes of action or for a submerged, rooted dicotyledonous plant. The guidelines provide specifications for creating a sediment and water source used in the studies (OECD 2014; Smart and Barko 1985). Although the focus of the OECD protocol is on EWM sensitivity and risk assessment for registration, the potential for using this small-scale assay to test other submersed plant species or to test new herbicides for aquatic plant activity has not been evaluated. Potential benefits of using the OECD protocol as an initial screen for testing aquatic herbicides against multiple species of plants include: (1) small space requirements allow for significant replication; (2) use of rooted plants allows for increased confidence in efficacy testing; (3) protocol can be easily modified to fit research objectives; and (4) use of standard water and sediments will allow for improved comparison of results across laboratories.

The first objective of this study was to evaluate SX-1552 and SX-1552A against five submersed plant species (three dicots and two monocots) to confirm and compare activity and potential utility as an aquatic herbicide. The second objective was to determine if the growth chamber studies provided comparable results with larger-scale mesocosm trials. The third objective was to determine the potential utility of the OECD protocol for screening different herbicides or additional plant species.

Materials and Methods

EWM from the Crystal River, FL, dioecious hydrilla from Lake Cypress, FL, CFH from Lake Okeechobee, FL, and megalodonta (water marigold) and elodea from Lake Minnetonka, MN were utilized for growth chamber and greenhouse trials. Plants were grown in culture tanks at the University of Florida Center for Aquatic and Invasive Plants (Gainesville, FL) for use in studies. Stock cultures were maintained under ambient outdoor conditions, and robust growth was noted for all species through the evaluation period from September through April.

Growth Chamber Trials. In this study, the OECD protocol was utilized for evaluating the response of the dicots, EWM, megalodonta, and CFH, and the monocots, elodea and hydrilla, after SX-1552 applications to the water under controlled conditions.

Apical shoot tips of 6 cm in length were collected from culture tanks and thoroughly rinsed to remove epiphytes or carbonate crusts on the leaf tissue. Four apical shoots of a single species were each planted into 250-ml beakers containing 200 ml of sediment specified in the protocol (OECD 2014). At least 3 cm of the shoot were pushed into the sediment. The 250-ml beakers containing sediment and plants were then placed in 2-L beakers containing 1.75 L of culture water (Smart and Barko 1985). The 2-L beakers were then placed in Percival E-36L environmental growth chambers set to a temperature of 21 C, a photoperiod of 16 light (L) : 8 dark (D), and light intensity of 275 ± 27 μmol m^{-2} s^{-1}. For the hydrilla and CFH trials, the temperature was increased to 25 C to facilitate improved plant growth.

All plants were given a pretreatment establishment period ranging from 9 to 11 d. This allowed for an increase in shoot tissue and root formation at the nodes of tissue buried in the sediment before treatment. To determine if root formation was present, selected beakers were removed and checked for roots. Before initiating treatments, multiple root formation was observed for all species. The pretreatment pH of the water was within OECD specifications (7.5 to 8.0). Pretreatment measurements on shoot fresh weight, dry weight, and total stem length (including lateral shoots) were collected by removing one plant from each of the beakers (three apical shoots remained). As the expected response to SX-1552 was unknown for these species, nonreplicated range-finding studies were conducted to determine concentrations that would be evaluated for each species (data not shown).

Both the SX-1552 (herbicide formulation analytically validated 300 g ai L^{-1} suspension concentrate) and SX-1552A (analytical grade) were provided by the SePRO Corporation (Carmel, IN) and evaluated against EWM, megalodonta, CFH, elodea, and hydrilla. Stock solutions of both SX-1552 and SX-1552A were created for treatment of the 2-L beakers. Herbicide concentrations for growth chamber experiments are listed in Table 1. Once treated, static conditions were maintained over the 14-d incubation period. Deionized water was added to the beakers to replace water lost to evaporation. Entire plants were harvested at 14 d after treatment (DAT) and dried to a constant weight at 70 C for a minimum of 48 h.

Prior herbicide concentration monitoring and the lack of UV light in the growth chambers indicated limited potential for photolytic breakdown of SX-1552 in this test system. Water samples ($\sim$ 25 ml) were collected immediately after treatment and 1, 7, and 14 DAT in selected treatment beakers to determine initial and final exposure concentrations. Samples were analyzed via high-performance liquid chromatography with tandem mass spectroscopy with limits of quantitation of 0.02 μg ai L^{-1} for SX-1552 and 0.05 μg ai L^{-1} for SX-1552A. Each

Table 1. Overview of SX-1552 and SX-1552A concentrations used in growth chamber and mesocosm studies.

Plant species tested	Concentrations evaluated	Material tested
	$\mu g\ L^{-1}$	
Growth chamber studies		
Eurasian watermilfoil (dicot)	0, 0.01, 0.03, 0.1, 0.3, 1, 3, 9, 27, and 81	SX-1552 and SX-1552A
Water marigold (dicot)	0, 0.3, 1, 3, 9, 27, 81, and 243	SX-1552 and SX-1552A
Crested floating heart (dicot)	0, 1, 3, 9, 27, and 81	SX-1552 and SX-1552A
Hydrilla (monocot)	0, 0.3, 1, 9, 27, and 81	SX-1552 and SX-1552A
Elodea (monocot)	0, 0.1, 0.3, 1, 3, 9, 27, and 81	SX-1552 and SX-1552A
Greenhouse studies		
Eurasian watermilfoil	0, 0.01, 0.03, 0.1, 0.3, 1, 3, 9, and 27	SX-1552 and SX-1552A
Water marigold	0, 0.1, 0.3, 1, 3, 9, 27, and 81	SX-1552

treatment was replicated four times and each study was repeated.

Mesocosm Trials. Both EWM and megalodonta were evaluated under greenhouse conditions from October to December, 2015 to determine impact of SX-1552 on more established plants. For EWM, two studies using both the herbicide formulations of SX-1552 and SX-1552A were conducted, whereas only SX-1552 was tested for megalodonta. A series of 3.78-L pots was filled with Margo Professional topsoil (92% sand, 4% silt, 4% clay) amended with 1 g of fertilizer (Osmocote® 15–9–12) kg^{-1} of soil. Four apical shoots (10 cm) of each test species were planted in individual pots and placed in 95-L plastic tanks filled with well water. The plants were given a 28-d pretreatment establishment period under greenhouse conditions. Greenhouse lights were set to maintain a 16L : 8D photoperiod. Hobo water temperature loggers (Onset Computer Corp.) were placed in selected tanks to record temperature every 6 h.

Herbicide concentrations used for greenhouse evaluations are listed in Table 1. Treatments were static exposures, and the experiments were conducted for a period of 28 d. Supplemental water was added during the course of the study to replace water lost to evaporation. After the 28-d exposure period, shoot material was harvested and dried to a constant weight at 70 C for a minimum of 48 h.

Water samples were collected immediately after treatment, 7 DAT, and 28 DAT in selected tanks to determine exposure concentrations. Lack of potential for photolytic degradation has previously been demonstrated in studies conducted in these greenhouses (Netherland 2015). Each treatment was replicated three times, and each study was repeated.

Statistical Analysis. Equation 1 is the four-parameter log-logistic dose–response curve used to estimate EC_{50} for different measures of plant response. Estimation of this nonlinear regression model was performed using the drc package in R software (R 3.2.2, R Core Team 2015: https://www.R-project.org/). Methodology of this approach is described in detail by Knezevic et al. (2007) and Ritz and Streibig (2005):

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

The parameters b, c, d, and e estimate the relative slope at e, lower limit of Y, upper limit of Y, and midpoint of Y, respectively. The three-parameter form of Equation 1 ($c = 0$) was used when it was logical to restrict the lower limit to 0. The dependent variable Y consists of treatment averages ($n = 3$ or 4) within replicate studies ($n = 2$) for dry weight or for inhibition indices that relate response relative to the control calculated using dry weight, fresh weight, and plant length. The EC_{50} was estimated as the dose rate (x) corresponding to the midpoint (e) between the lower (c) and upper limit (d) for dry weight or the dose rate corresponding to 50% inhibition of specific growth rate or 50% inhibition in yield. Estimates of EC_{50} were compared for SX-1552 and SX1552A using the selectivity index (Ritz and Streibig 2005).

Final dry weight was estimated directly using model 1 as recommended by Knezevic et al. (2007). Graphical comparisons were performed by converting predicted values and sample means to percent dry weight reduction relative to the control. Model predictions were converted using the predicted upper limit (d) as the predicted control level and using the sample mean control (rate = 0) average for sample means.

Measures relative to the control were defined by specific study protocols as percent inhibition of specific growth rate (%Ir in Equation 2) and percent inhibition in yield (%Iy in Equation 3):

$$Ir = 100x(\mu_c - \mu_t)/\mu_c \qquad [2]$$

Specific growth rate in Equation 2 was calculated for control (μ_c) or treated (μ_t) as the natural log of the final divided by initial mean values divided by days (ln[final/initial]/days) for each replicate study. Equation 2 was modified when final size was less than initial size because this is when treatment-specific growth rates (μ_t) estimate necrosis/mortality on the basis of initial size rather than growth. Without modification, this results in no upper limit on %*Ir* and contradicts the log-logistic modeling approach used here. The focus on growth inhibition was maintained by restricting maximum %*Ir* to 100% (setting $\mu_t = 0$) when final size was less than initial size.

$$Ir = 100x(b_c - b_t)/b_c \qquad [3]$$

Mean growth (*b*) in Equation 3 was calculated for control (b_c) or treated (b_t) as the average final minus average initial for each replicate study. Inhibition of yield (%*Iy*) can exceed 100% when treatment growth is negative.

A Dunnett's test ($\alpha = 0.05$) comparing dry weight biomass of treated vs. nontreated plants was performed to determine a lowest observed effect concentration (LOEC) across the broad range of SX1552 concentrations tested.

Results and Discussion

Growth Chamber Trials. In 14-d assays, reference plant biomass increased by 2.8 to 5.1 times the initial biomass for the different test species. OECD guidelines require that doubling of biomass and mean coefficient of variation between reference plants be less than 35% (OECD 2014). Both of these requirements were met in all of our growth chamber studies. All nontreated control plants were robust and actively growing throughout the trials and at the time of harvest. Water sampling after treatments with the SX-1552 formulation at 1 DAT indicated that 41 to 56% of applied SX-1552 had remained in the parent form, whereas the rest had converted to SX-1552A. Results from water sampling at 7 and 14 d indicated that SX-1552 had fully converted to SX-1552A, with recoveries at 7 and 14 d ranging from 89 to 112% of nominal treatment concentrations. Samples collected at 1 and 14 DAT with SX-1552A resulted in recoveries ranging from 94 to 108% of nominal concentrations. Results of this water sampling confirmed that target concentrations were achieved.

EWM was sensitive to both SX-1552 and SX-1552A, with EC_{50} values of 0.11 and 0.23 μg ai L^{-1} (Table 2, Figure 1). For both formulations, the LOEC value was 0.1 μg ai L^{-1}. Symptom development was rapid with characteristic auxin-like epinasty of the apical shoots noticed within 1 d of treatment. Megalodonta sensitivity to SX-1552 and SX-1552A resulted in EC_{50} values of 11.3 and 14.5 μg ai L^{-1} respectively (Table 2, Figure 1). LOEC values of 3 and 9 μg ai L^{-1} were determined for SX1552 and SX1552-A, respectively, whereas a concentration of 81 μg ai L^{-1} reduced biomass by greater than 90%. The visual auxin symptoms were greatly reduced for megalodonta compared with EWM.

Elodea sensitivity to SX-1552 and SX-1552A yielded EC_{50} values of 6.9 and 13.1 μg ai L^{-1} respectively, with both forms yielding a LOEC value of 9 μg ai L^{-1} (Table 2, Figure 1) The EC_{50} values indicated a difference between SX-1552 and SX-1552-A, (Table 2). There was no viable biomass for harvest at the highest concentration evaluated in this trial (81 μg ai L^{-1}). Slight visual auxin-like symptoms were noted on this monocot at the higher concentrations; however, the primary symptom noted was necrosis along the length of the stems. Hydrilla was much more sensitive, with EC_{50} values of 1.4 μg ai L^{-1} (SX-1552) and 2.5 μg ai L^{-1} (SX-1552-A) and a LOEC of 1 μg ai L^{-1} (Table 2, Figure 1). A difference in the EC_{50} value for SX-1552 and SX-1552-A was also noted for hydrilla. There was very limited biomass for harvest at concentrations > 9 μg ai L^{-1}. In addition to auxin-like symptoms at the shoot tips, this monocot became brittle and shoots readily separated upon slight disturbance in the first day or two

Table 2. Final dry weight (g) 50% effective concentration (EC_{50}) comparisons (standard error) for Eurasian watermilfoil (EWM), megalodonta (MEG), elodea (ELO), Hydrilla (HYD), and crested floating heart (CFH) after exposure to SX-1552 and SX-1552A.

Study type	Formulation	EWM	MEG	ELO	HYD	CFH
				EC_{50} $(e)^a$		
Growth chamber	SX-1552	0.11 b (0.11)	11.3 a (2.0)	6.9 b (0.6)	1.4 b (0.1)	5.6 b (0.6)
	SX-1552A	0.23 ab (0.33)	14.5 a (2.8)	13.1 a (1.0)	2.5 a (0.3)	23.9 a (4.0)
Mesocosm	SX-1552	0.12 b (0.01)	6.1 b (0.2)	—	—	—
	SX-1552A	0.58 a (0.04)	—	—	—	—

[a] EC_{50} (μg ai L^{-1}) values with the same lowercase letter within a species are not significantly different at the 5% level.

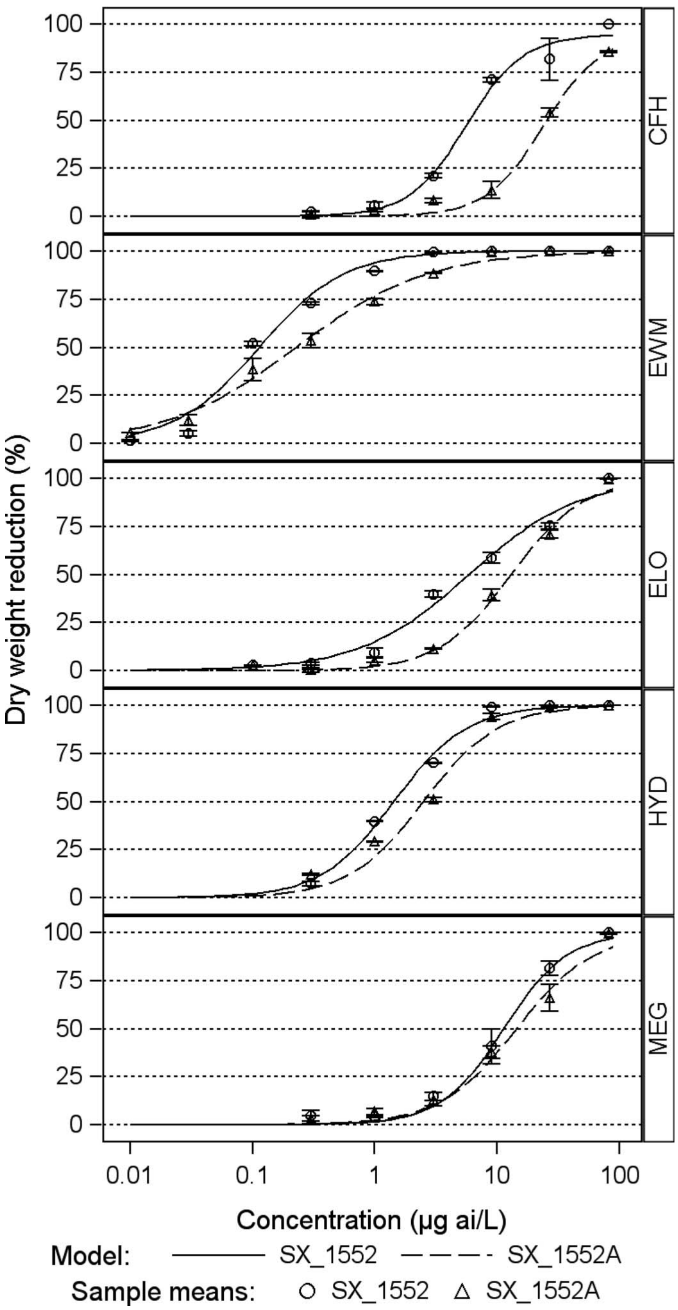


Figure 1. Logistic regression was used to plot dry-weight biomass reduction for five aquatic plant species after exposure to SX1552 (ester) and SX1552A (acid). Each symbol represents the mean value ($\pm$ standard error, $n = 4$). Abbreviations: CFH, crested floating heart; EWM, Eurasian watermilfoil; ELO, elodea; HYD, hydrilla; MEG, megalodonta.

posttreatment. At harvest, plants that had been treated at concentrations > 3 µg ai L^{-1} had waterlogged stems (aerenchyma tissue that is normally filled with air was full of water) and the limited amount of remaining tissue lacked integrity.

CFH also showed differential sensitivity to SX-1552 and SX-1552A, with EC_{50} values of 5.6 and 23.9 µg ai L^{-1} respectively (Table 2, Figure 1). The LOEC value for the formulation was 3 µg ai L^{-1}, whereas the SX-1552-A value was 9 µg ai L^{-1}. CFH displayed a rapid onset of visual symptoms with notable stem elongation within 1 d after exposure to concentrations from 1 to 3 µg ai L^{-1}. Although these initial symptoms were easy to distinguish, they did not translate to impacts on biomass at the lower treatment concentrations. There was some chlorosis noted on surface leaves within 5 to 10 DAT. A clear visual difference between the activity of SX-1552 and SX-1552-A was noted for this floating leaf plant.

Per the OECD protocol, EC_{50} values were also determined for several growth-based parameters. The three-parameter version ($c = 0$) of Equation 1 (parameter estimates not shown) was used to estimate percent inhibition of growth rate (*Ir*) and percent inhibition in yield (*Iy*). Estimates of EC_{50} are compared by formulation in terms of shoot length, fresh weight, and dry weight by species (Table 3). These data indicate some variation in predicted EC_{50} values for SX1552 against the different plant species. Specifically, higher EC_{50} values for the growth rate (*Ir*) data for elodea and CFH was noted. Nonetheless, most growth-based values were generally similar to the EC_{50} values determined on the basis of dry weights (Tables 2 and 3). Per the OECD guidelines, it is stated that "EC_{50} values calculated when using the % inhibition of yield (*Iy*) and average specific growth rate (*Ir*) are not comparable and this difference is recognized when using the results of the test." Overall, these analyses are being conducted on data that show consistent relationships within a species (e.g., dry weight vs. fresh-weight ratios or stem length vs. fresh weight). As such, the EC_{50} values were in general agreement regarding the sensitivity of each species to SX-1552 and SX-1552A.

Mesocosm Trials. Water temperatures ranged from 17.6 to 23.2 C during the course of mesocosm trials. During the 28-d pretreatment growth period, EWM biomass increased by a factor of 37.5 compared with initial shoot weights, and megalodonta increased by a factor of 18.4. During the 28-d study period, biomass of EWM increased by a factor 2.7 and megalodonta increased by a factor of 2.2. The combination of rapid growth rates and limited space eventually resulted in plants nearing or reaching carrying capacity and slowing growth rates in these tanks. All nontreated plants were robust and actively growing at the time of treatment and harvest. Results from water sampling at 7 and 28 DAT indicate that measured

Table 3. Estimation of 50% effective concentration (EC_{50}) (µg ai L^{-1}) as the dose that corresponds to 50% inhibition of growth rate (*Ir*) or inhibition in yield (*Iy*) in growth chamber (GC) and mesocosm (Meso) trials. EC_{50} (standard error) values within species followed by the same lowercase letter are not significantly different at the 5% level.

Study type	Form	Shoot length %*Ir*	Shoot length %*Iy*	Fresh weight %*Ir*	Fresh weight %*Iy*	Dry weight %*Ir*	Dry weight %*Iy*
				Eurasian watermilfoil			
GC	SX-1552	0.15b (0.01)	0.10b (0.01)	0.17b (0.01)	0.10b (0.01)	0.16c (0.01)	0.10c (0.01)
	SX-1552A	0.35a (0.03)	0.19a (0.02)	0.41a (0.04)	0.17a (0.02)	0.39b (0.04)	0.17b (0.02)
Meso	SX-1552	—	—	—	—	0.12d (0.01)	0.09c (0.01)
	SX-1552A	—	—	—	—	0.68a (0.06)	0.38a (0.03)
				Megalodonta			
GC	SX-1552	3.6b (0.4)	3.0b (0.5)	9.1 (0.9)	6.9a (0.7)	8.9a (1.0)	7.0a (0.8)
	SX-1552A	7.3a (0.6)	6.0a (0.8)	10.8a (1.0)	9.1a (1.0)	10.9a (1.8)	8.7a (2.7)
Meso	SX-1552	—	—	—	—	6.4b (0.7)	4.7a (1.0)
				Elodea			
GC	SX-1552	3.0b (0.2)	2.8b (0.5)	26.2a (18)	7.1a (2)	21.0a (12)	6.3a (1)
	SX-1552A	7.4a (0.7)	6.8a (1.2)	34.1a (47)	13.0a (3)	28.3a (11)	12.2a (2)
				Hydrilla			
GC	SX-1552	1.7b (0.2)	1.1b (0.1)	2.0b (0.2)	1.1b (0.1)	2.1b (0.2)	1.2b (0.1)
	SX-1552A	3.4a (0.4)	1.8a (0.2)	3.4a (0.2)	1.9a (0.2)	3.6a (0.3)	1.8a (0.2)
				Crested floating heart			
GC	SX-1552	5.9b (0.3)	5.4b (0.5)	7.0a (0.2)	4.9a (0.3)	7.2a (0.9)	5.0b (0.5)
	SX-1552A	26.6a (2.5)	17.6a (2.5)	41.1a (27)	26.1a (35)	33.2a (18)	21.0a (4)

concentrations of SX-1552 and SX-1552A were 87% ± 5% of the target concentrations.

EWM was sensitive to both SX-1552 and SX-1552A in larger-scale mesocosms under greenhouse conditions. Despite the larger initial size and more robust plants, EC_{50} values for SX-1552 and SX-1552A were 0.12 and 0.58 µg ai L^{-1} respectively. (Table 2). LOEC values were 0.1 and 0.3 µg ai L^{-1} for SX-1552 and SX-1552A. Within 1 to 2 d after exposure, plants became very brittle and stems fragmented into small pieces after slight disturbance. Comparison of growth chamber and mesocosm data suggests that despite different initial plant biomass and study conditions, EWM responded in a similar manner (Table 2, Figure 2). Megalodonta susceptibility in the mesocosm trials was generally similar to results observed in the growth chamber trials. The EC_{50} value for SX-1552 was 6.1 µg ai L^{-1}, whereas the LOEC was 9 (Table 2). Given the broad rate structure evaluated, there were minimal impacts on plant growth at 3 µg aiL^{-1}, whereas the 9 µg ai L^{-1} treatment resulted in > 65% biomass reduction. The EC_{50} value calculated for megalodonta was significantly lower for the greenhouse vs. the growth chamber trials (6.1 vs. 11.3 µg ai L^{-1}). It is possible that improved growth conditions in the mesocosms could explain the increased susceptibility of the megalodonta when compared with the space limitations observed in the 2-L beakers.

Results suggest that EWM is highly susceptible to both SX-1552 and SX-1552A. The EWM growth chamber and mesocosm trials were complementary and indicate that the EC_{50} values are well below

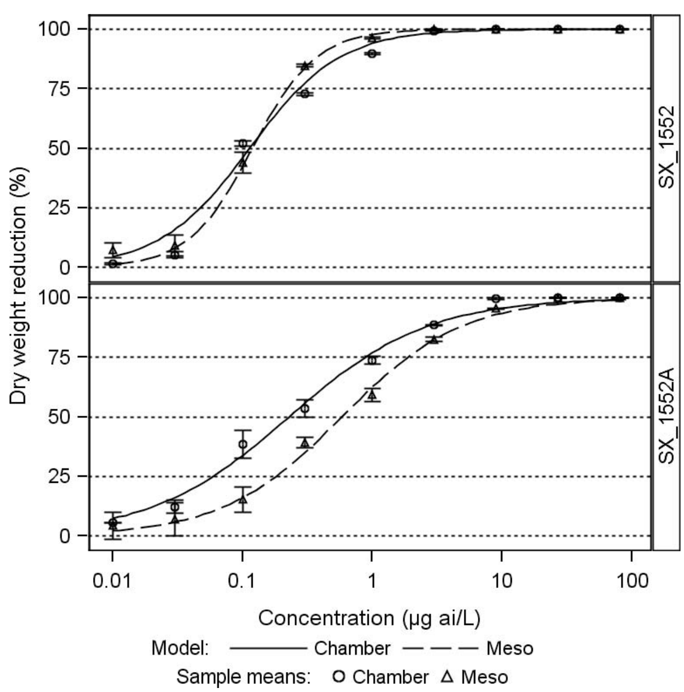


Figure 2. Logistic regression was used to plot dry-weight biomass reduction of Eurasian watermilfoil after exposure to SX1552 and SX1552A after growth chamber (chamber) and mesocosm (Meso) studies. Each symbol represents the mean value (± standard error, $n = 4$ for growth chamber trials and $n = 3$ for mesocosm trials).

1 μg L^{-1}. Across all species, SX-1552 resulted in lower EC_{50} values vs. SX-1552A; however, because of the rate structure evaluated the LOEC was often similar between the forms. The EC_{50} value for megalodonta was 63 to 102 times greater than for EWM. Interestingly, a dichotomy was also observed for the two monocotyledons. The EC_{50} values for the native elodea species were 4.9 to 5.4 times greater than that for the invasive species hydrilla. Given the invasive nature of both EWM and hydrilla in the United States, this level of SX-1552 activity warrants further investigation for potential use against these species.

These trials were based on extended static exposures to SX-1552, and therefore the results need to be viewed in context, as static exposures can result in enhanced activity against a given submersed species in small-scale systems (Mohr et al. 2013). For example, mesocosm evaluation of static exposures ($>$ 3 wk) of the auxin-mimic herbicides 2,4-D and triclopyr demonstrated high levels of activity for these herbicides on EWM at rates ranging from 25 to 75 μg ai L^{-1} (Glomski and Netherland 2010), yet typical use rates for these products range from 500 to 2,000 μg ai L^{-1}, as most treatments for submersed aquatic management are subject to rapid dispersion from the treatment site (Netherland 2015). The current results suggest that SX-1552 produces strong auxin-like symptoms, can result in rapid onset of injury and loss of EWM biomass, and is at least an order of magnitude more active on EWM when compared with products such as 2,4-D and triclopyr (Glomski and Netherland 2010; Green and Westerdahl 1990; Netherland and Getsinger 1992). Although 2,4-D and triclopyr can elicit symptoms on hydrilla at high concentrations, neither herbicide provides hydrilla control at maximum-labeled use rates in the range of 2,500 to 4,000 μg L^{-1}. In this study hydrilla lost tissue integrity at 3 μg ai L^{-1} and was completely controlled at a concentration of 9 μg ai L^{-1} after a 14-d static exposure period to SX1552.

In examining the potential utility for utilizing the OECD protocol to evaluate other herbicides or potential impacts on different plant species, there are several inherent strengths as well as a few caveats. The current results suggest that products like SX-1552 might be well suited to this screening method. However, slow-acting aquatic herbicides that target plant-specific enzymes such as fluridone (phytoene desaturase inhibitor [PDS]), penoxsulam (acetolactate synthase [ALS] inhibitor), and topramazone (hydroxyphenylpyruvate dioxygenase [HPPD] inhibitor) can require up to 2 to 4 mo to provide plant control (Netherland 2015). Use of a protocol that focuses on short-term changes in biomass and growth may not be optimal for predicting activity of slow-acting herbicides. Research using a water-only assay (e.g., recently sprouted tubers or apical shoot meristem growing in Hoagland's solution) has provided valuable data on short-term changes in pigments, growth inhibition, or impacts on root growth (Berger et al. 2015; Mohr et al. 2013; Netherland 2011, 2015). Additional testing using the OECD protocol on these slow-acting herbicides is recommended and extending the length of these trials to 28 d may provide additional data to separate between concentrations that are likely to provide growth regulation vs. those concentrations that are likely to kill the plant.

Fast-acting contact herbicides like diquat would demonstrate high levels of activity using this protocol, as EWM is very sensitive to this herbicide. Moreover, extended unrealistic exposures to diquat in these assays (due to lack of binding to suspended sediments or organic particulates in an assay) are not characteristic of field conditions. In this case, testing EWM would indicate that diquat is highly active for both regulatory and operational predictions; however, the impact of turbidity on diquat activity in the field would likely result in greatly reduced activity (Poovey and Getsinger 2002). Fast-acting products that require moderate exposure periods such as 2,4-D, triclopyr, endothall, and SX-1552 can be evaluated in a relatively short period of time and these products tend to perform in a similar manner under a broad range of environmental conditions (e.g., turbidity, pH, temperature, etc).

The growth chamber results with SX-1552 were validated at the mesocosm scale for the two dicot species tested. Such outcomes will likely vary for contact or systemic herbicides. Several submersed aquatic plants are highly susceptible to the rapid-acting protoporphyrinogen oxidase inhibitor flumioxazin under growth chamber conditions. Yet flumioxazin activity can be reduced under increasing pH as the molecule is rapidly hydrolyzed at a higher pH (Mudge and Haller 2006).

The OECD protocol offers a good model for screening inherent herbicide activity on submersed plants under relatively long-term exposures, but could easily overestimate risk when relying on a single species for risk assessment purposes. In this study, EWM was by far the most sensitive aquatic plant species to SX-1552. It could have also been the most tolerant, or shown no effect. Aquatic plant community interactions should be considered, involving multiple species of submersed or floating species. For example, in this study, the desirable native aquatic plants were more tolerant than the invasive species EWM and hydrilla. In addition, the exposure scenario should be kept in perspective after a terrestrial application of SX1552. Exposures significantly less than 14 or 28 d would generally be expected. Additional small-scale tests of other submersed native and invasive dicots and monocots at the chamber scale are recommended. The ability to utilize results from studies conducted at this scale provides an efficient and cost-effective method to screen plants under a variety of concentrations and exposure scenarios common to treatment of aquatic sites.

Overall these study results confirm a high level of SX-1552 activity on several aquatic species and the greater activity of SX-1552 and SX-1552-A. For SX 1552 the growth chamber studies were predictive of mesocosm results. Although the OECD protocol is currently specific to EWM for regulatory purposes in Europe, the current results suggest that this protocol (or modified versions of this protocol) could be used for multiple herbicides or aquatic plant species. Predicting herbicide activity on rare or threatened species or using this protocol to better refine knowledge of invasive plant response to a given herbicide are two areas where this small-scale assay could provide information that would improve study design for large-scale mesocosm testing.

Acknowledgments

The assistance of Jesse Stevens with study setup and data collection is greatly appreciated. Funding for this project was provided through the U.S. Army Engineer Research and Development Center, Aquatic Plant Control Research Program and through the Aquatic Ecosystem Restoration Foundation. Citation of trade names does not constitute endorsement or approval of the use of such commercial products. Permission to publish this paper was granted by the Chief of Engineers.

Literature Cited

[APMS] Aquatic Plant Management Society (2014) Herbicide Resistance Stewardship in Aquatic Plant Management. http://apms.org/wp/wp-content/uploads/2014/04/Herbicide-Resistance-Stewardship-in-Aquatic-Plant-Management.pdf. Accessed March 4, 2015

Belgers JDM, Van Lieverloo RJ, Van der Pas LJ, Van den Brink PJ (2007) Effects of the herbicide 2, 4-D on the growth of nine aquatic macrophytes. Aquat Bot 86:260–268

Bell JL, Schmitzer R, Weimer MR, Napier RM, Prusinska JM (2015) Mode-of-action analysis of a new arylpicolinate herbicide [Abstract]. 2015 Annual Meeting. Lexington, KY: Weed Science Society of America. http://wssaabstracts.com/public/30/abstract-290.html. Accessed May 15, 2015

Berger ST, Netherland MD, MacDonald GE (2015) Laboratory documentation of multiple-herbicide tolerance to fluridone, norflurazon, and topramazone in a hybrid watermilfoil (*Myriophyllum spicatum* × *M. sibiricum*) population. Weed Sci 63: 235–241

Getsinger KD, Netherland MD, Grue CE, Koschnick TJ (2008) Improvements in the use of herbicides and establishment of future research directions. J Aquat Plant Manage 46:32–41

Glomski LM, Netherland MD (2010) Response of Eurasian and hybrid watermilfoil to low use rates and extended exposures of 2,4-D and triclopyr. J Aquat Plant Manage 48:12–16

Glomski LM, Netherland MD (2011) Small-scale screening of submersed aquatic plants to the herbicide topramezone. APCRP Technical Notes Collection (ERDC/TN APCRP-CC-16). Vicksburg, MS: U.S. Army Engineer Research and Development Center. http://ed.erdc.usace.army.mil/aqua/. Accessed March 15, 2015

Glomski LM, Netherland MD (2013) Use of a small-scale primary screening method to predict effects of flumioxazin and carfentrazone-ethyl on native and invasive, submersed plants. J Aquat Plant Manage 51:45–48

Green WR, Westerdahl HE (1990) Response of Eurasian watermilfoil to 2,4-D concentrations and exposure times. J Aquat Plant Manage 28:27–30

Hofstra DE, Clayton JS (2001). Evaluation of selected herbicides for the control of exotic submerged weeds in New Zealand: I. The use of endothall, triclopyr and dichlobenil. J Aquat Plant Manage 39:20–24

Knezevic SZ, Streibig JC, Ritz C (2007) Utilizing R software package for dose–response studies: the concept and data analysis. Weed Technol 21:840–848

Koschnick TJ, Haller WT, Glasgow L (2006) Documentation of landoltia (*Landoltia punctata*) resistance to diquat. Weed Sci 54:615–619

Lee S, Sundaram S, Armitage L, Evans JP, Hawkes T, Kepinski S, Ferro N, Napier RM (2013) Defining binding efficiency and specificity of auxins for SCFTIR1/AFB-Aux/IAA co-receptor complex formation. ACS Chem Biol 9:673–682

MacDonald GE, Shilling DG, Bewick TA. 1993. Effects of endothall and other aquatic herbicides on chlorophyll fluorescence, respiration, and cellular integrity. J Aquat Plant Manage 31:50–54

Maltby L, Arnold D, Arts G, Davies J, Heimbach F, Pickl C, Poulsen, V, eds (2010) Aquatic Macrophyte Risk Assessment for Pesticides. New York, NY: CRC Press. 162 p

Mohr S, Schott J, Maletzki D, Hunken A (2013) Effects of toxicants with different modes of action on *Myriophyllum spicatum* in test sytems with varying complexity. Ecotox Environ Safe 97:32–39

Mudge CR, Haller WT (2006) Effect of pH on submersed aquatic plant response to flumioxazin. J Aquat Plant Manage 48:30–34

Nault ME, Netherland MD, Mikulyuk A, Skogerboe JG, Asplund T, Hauxwell J, Toshner P (2014) Efficacy, selectivity, and herbicide concentrations following a whole-lake 2,4-D application targeting Eurasian watermilfoil in two adjacent northern Wisconsin lakes. Lake Res Manage 30:1–10

Netherland MD (2011) Comparative susceptibility of fluridone resistant and susceptible hydrilla to four ALS inhibiting herbicides under laboratory and greenhouse conditions. J Aquat Plant Manage 49:100–106

Netherland MD (2015) Laboratory and greenhouse response of monoecious hydrilla to fluridone. J Aquat Plant Manage 53:178–184

Netherland MD, Getsinger KD (1992) Efficacy of triclopyr on Eurasian watermilfoil: concentration and exposure time effects. J Aquat Plant Manage 30:1–5

Netherland MD, Getsinger KD, Turner EG (1993) Fluridone concentration and exposure time requirements for control of hydrilla and Eurasian watermilfoil. J Aquat Plant Manage 32:189–194

Netherland MD, Glomski LM (2014) Mesocosm evaluation of triclopyr on Eurasian watermilfoil and three native submersed species: the role of treatment timing and herbicide exposure. J Aquat Plant Manage.52:57–64

[NPIRS] National Pesticide Information Retrieval System (2015) http://npirs.ceris.purdue.edu/. Accessed April 9, 2015

[OECD] Organization for Economic Cooperation and Development (2006) Test No. 221: Lemna sp. Growth Inhibition Test, OECD Guidelines for the Testing of Chemicals, Section 2. Paris: OECD Publishing. DOI: http://dx.doi.org/10.1787/9789264016194-en

OECD. (2014) Test No. 239: Water–Sediment *Myriophyllum spicatum Toxicity* Test, OECD Guidelines for the Testing of Chemicals,Section 2. Paris: OECD Publishing. DOI: http://dx.doi.org/10.1787/9789264224155-en

Parsons JK, Hamel KS, Madsen JD, Getsinger KD (2001) The use of 2, 4-D for selective control of an early infestation of Eurasian watermilfoil in Loon Lake, Washington. J Aquat Plant Manage 39:117–125

Poovey AG, Getsinger KD (2002) Impacts of inorganic turbidity on diquat efficacy against *Egeria densa*. J Aquat Plant Manage 40:6–10

Poovey AG, Getsinger KD, Skogerboe JG, Koschnick TJ, Madsen JD, Stewart RM (2004) Small-plot, low-dose treatments of triclopyr for selective control of Eurasian watermilfoil. Lake Res Manage 20:322–332

R Core Team (2015) R: A Language and Environment for Statistical Computing. Vienna, Austria: R Foundation for Statistical Computing. https://www.R-project.org/. Accessed August 1, 2015

Ritz C, Streibig JC (2005) Bioassay analysis using R. J Stat Softw 12:1–22

Sprecher SL, Getsinger KD, Stewart AB (1998) Selective effects of aquatic herbicides on sago pondweed. J Aquat Plant Manage 36: 64-68

Smart RM, Barko JW (1985) Laboratory of culture of submersed freshwater microphytes on natural sediments. Aquat Bot 21:251–263

Sprecher SL, Stewart AB (1995) Triclopyr effects on peroxidase activity in target and non-target aquatic plants. J Aquat Plant Manage 33:43–48

[USEPA] U.S. Environmental Protection Agency (2012) Aquatic Plant Toxicity Test using *Lemna* spp. Ecological Effects Test Guideline OCSPP 850.4400. Washington, DC: USEPA

Villalobos LI, Lee AC, De Oliveira S, Ivetac C, Brandt A, Armitage Sheard WL, Tan LB, Parry X, Mao G, Zheng H, Napier N, Kepinski RM, Estelle M (2012) A combinatorial TIR1/AFB–Aux/IAA co-receptor system for differential sensing of auxin. Nature Chem Biol 8:477–485

Walsh TA, Neal R, Merlo AO, Honma M, Hicks GR, Wolff K, Matsumura W, Davies JP (2006) Mutations in an auxin receptor homolog AFB5 and in SGT1b confer resistance to synthetic picolinate auxins and not to 2, 4-dichlorophenoxyacetic acid or indole-3-acetic acid in *Arabidopsis*. Plant Physiol 142:542–552

Wersal RM, Madsen JD, Woolf TE, Eckberg N (2010) Assessment of herbicide efficacy on Eurasian watermilfoil and impacts to the native submersed plant community in Hayden Lake, Idaho, USA. J Aquat Plant Manag 48:5–11

Received June 7, 2015, and approved September 26, 2015.

Associate Editor for this paper: Steven Seefeldt, University of Alaska at Fairbanks.

Preliminary report on the 2018 Cooperative Demonstration of Selective Control of Eurasian watermilfoil with ProcellaCOR® Aquatic Herbicide: Morton Slough – Pend Oreille, Idaho

Summary

In August 2018, a demonstration treatment of the new ProcellaCOR® Aquatic Herbicide (a.i. florpyrauxifen-benzyl) for selective control of invasive Eurasian watermilfoil (EWM) was cooperatively conducted in northern Idaho by the US Army Engineer Research and Development Center's Aquatic Plant Control Research Program (ERDC-APCRP or ERDC – P.I., Dr. Kurt Getsinger), the US Department of Agriculture (co-investigator Dr. John Madsen), SePRO (co-investigator Dr. Mark Heilman), and the Idaho State Department of Agriculture (ISDA assessment partner – Dr. Kim Hozler). Application support was provided by Aquatechnex LLC (Terry McNabb).

5 Prescription Dose Units (PDU) per acre-foot of ProcellaCOR EC were applied on August 13, 2018 to a 3.5-acre management area (average depth = 11 feet) located in the center of Morton Slough, a cove area within the Lake Pend Oreille/Pend Oreille River reservoir system. Extensive assessment and monitoring of the Morton Slough management area and an associated reference site (Riley Creek) were performed immediately prior to application and again 6 weeks after treatment. Water samples were collected at 12 locations (4 inside, 8 outside) at 1.5 feet below the water surface and at ~ 1 foot off of the bottom at 1, 5, 9, 24, 48 and 72 hours post application. Samples were kept frozen and overnight shipped for analysis via LC-MSMS (EPL Bioanalytical Services, Niantic, IL). The response of submersed aquatic plants was assessed by comparing pre-treatment and 6-week post treatment plant presence and density ratings (1 – 4 scale – 1-trace, 2-sparse, 3-moderate, 4-dense) using a throw rake method on a ~60-foot (18-meter) grid within the management area (42 locations). An untreated reference area (Riley Creek) located 5 miles away from the Morton Slough site was also assessed (20 locations) before and after treatment to detect potential seasonal changes or other non-treatment related conditions.

Analytical monitoring confirmed fast ProcellaCOR dissipation on the day of application with <1 µg a.i. L^{-1} measured at 9 hours following treatment (9.65 µg a.i. L^{-1} theoretical starting concentration following application). Prior to application, EWM in the Morton Slough management area was found at 95% frequency of occurrence with moderate to high densities of growth. At 6 weeks post treatment (September 24), EWM frequency decreased to 2% with just trace remaining plant biomass of questionable viability. Elodea and coontail were dominant native plants after treatment. As anticipated with the herbicide, northern watermilfoil (NWM) decreased in the management area following treatment. The ProcellaCOR application was highly selective in control of EWM with native species richness in the Morton Slough site increasing from 7 native species to 8 native species following treatment. There were some signs of normal seasonal senescence for species such as small pondweed (*Potamogeton pusillus*) that also occurred in the Riley untreated reference. EWM maintained high densities in the Riley untreated reference site at 6 weeks post application confirming the treatment effect associated with the ProcellaCOR application to the Morton Slough 3-acre management area.

A series of preliminary tables and figures are provided below to review outcome of this useful demonstration effort. Additional 1-year post assessment is planned for August 2019.

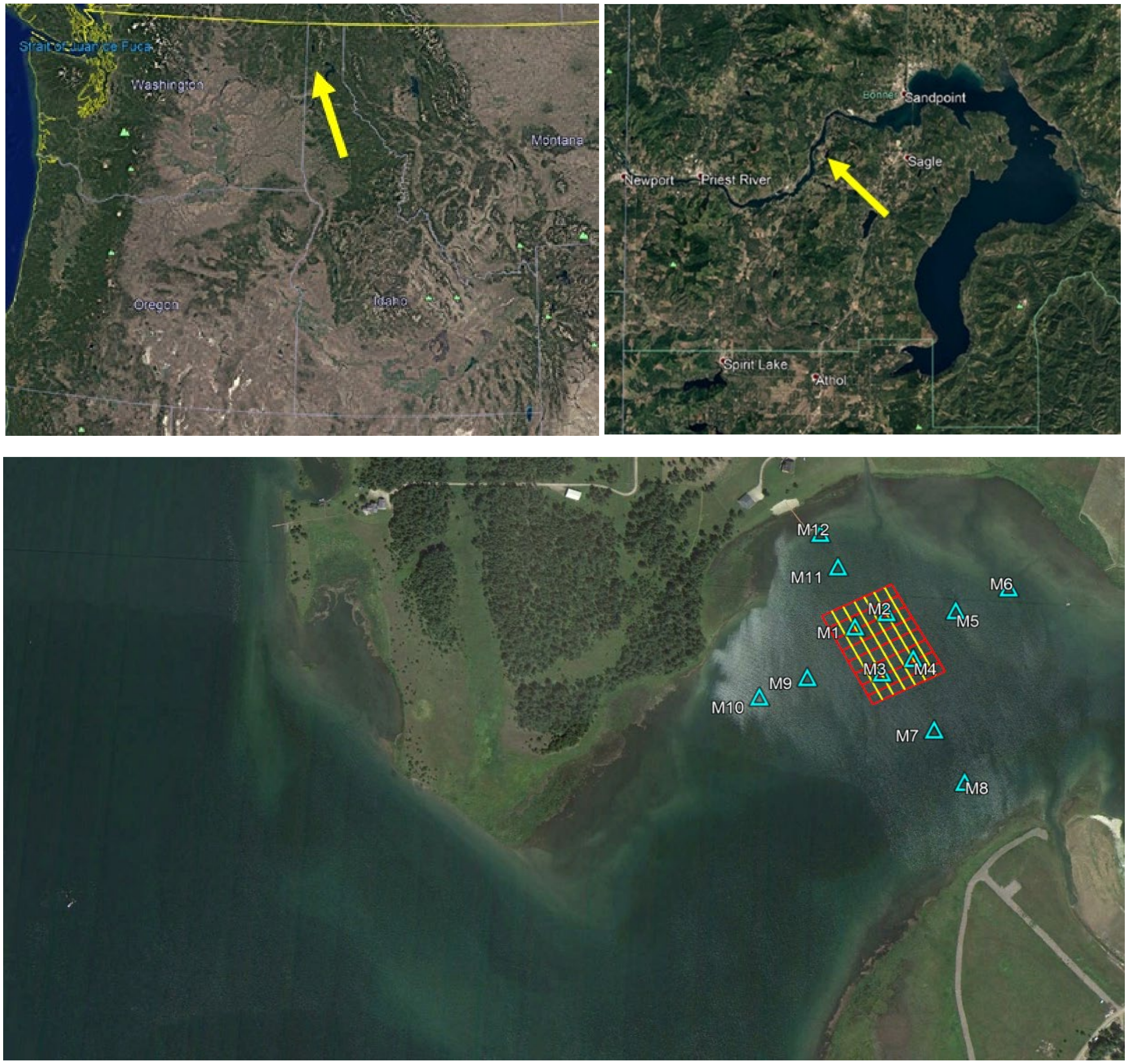
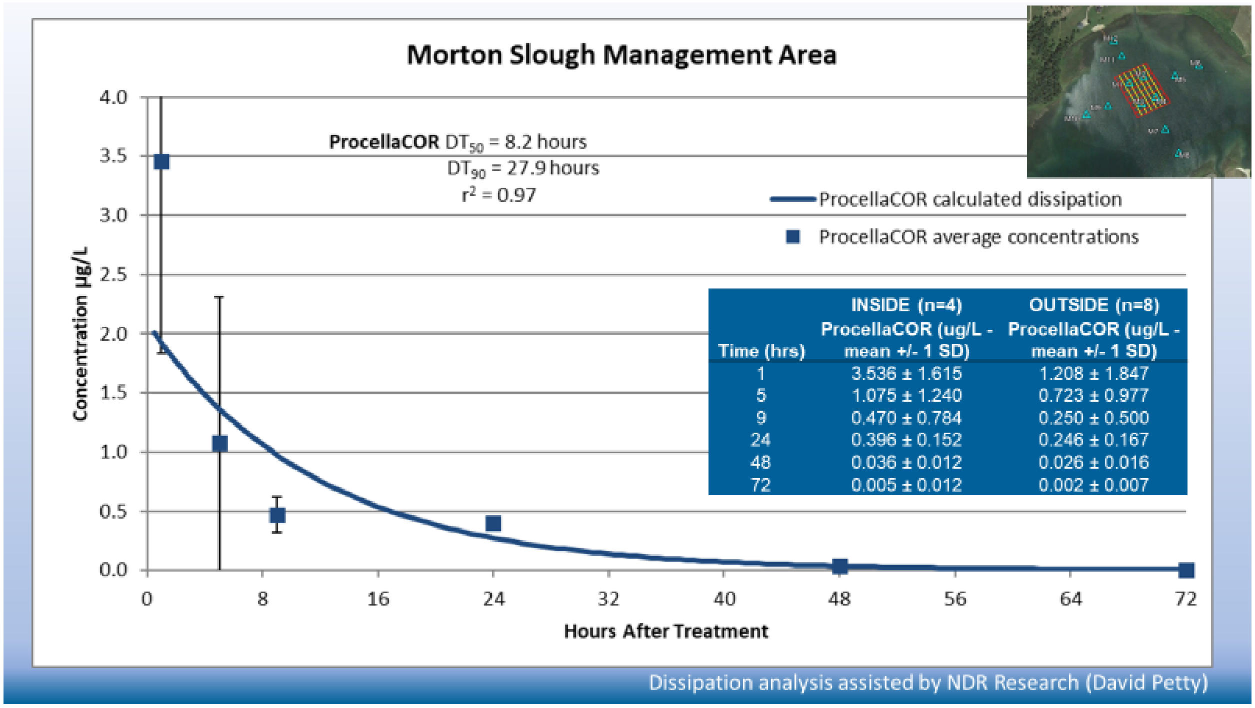


Figure 1. Location of Morton Slough site in the Pend Oreille system and layout of monitoring and assessment locations. Blue triangles are water sampling stations and the red/yellow grid intersection points were locations for rake sampling of vegetation. There was little or no water flow observed in the quiescent slough at time of application, which is seasonally normal hydrology for the site.



Time (hrs)	INSIDE (n=4) ProcellaCOR (ug/L - mean +/- 1 SD)	OUTSIDE (n=8) ProcellaCOR (ug/L - mean +/- 1 SD)
1	3.536 ± 1.615	1.208 ± 1.847
5	1.075 ± 1.240	0.723 ± 0.977
9	0.470 ± 0.784	0.250 ± 0.500
24	0.396 ± 0.152	0.246 ± 0.167
48	0.036 ± 0.012	0.026 ± 0.016
72	0.005 ± 0.012	0.002 ± 0.007

Figure 2. Dissipation of ProcellaCOR inside (4 sites – preliminary simple decay curve showing at most an 8-hour half life in management area) and immediately outside of the Morton Slough management area (8 sites). The inset table shows average concentrations of the herbicide inside and outside of the treatment area out to 72 hours after application. Herbicide analysis was performed independently by EPL Bioanalytical Services (Niantic, IL) using LC-MSMS.

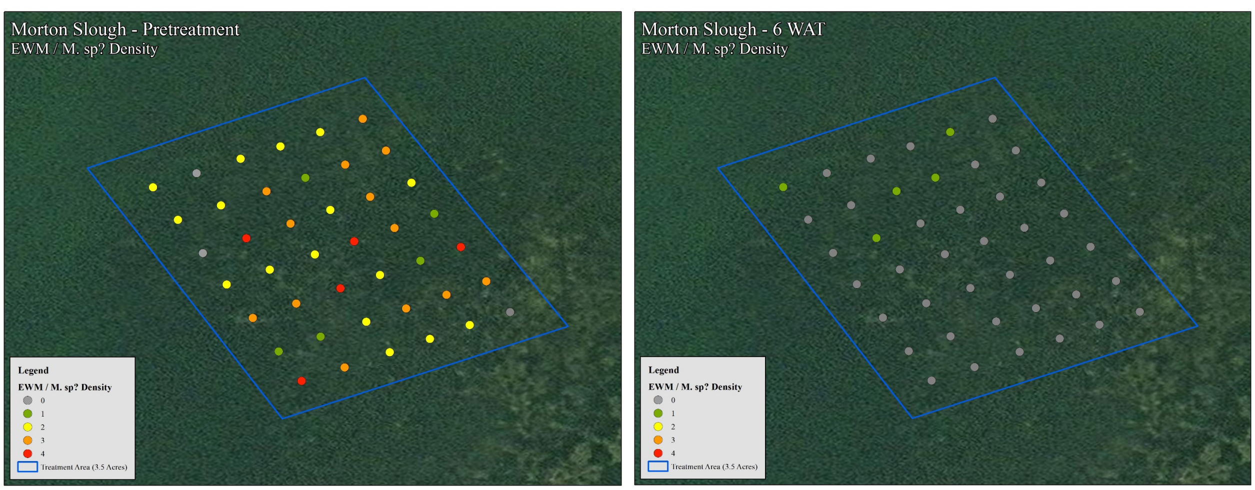


Figure 3. Maps of EWM / possible HWM combined frequency and densities in the Morton Slough management area before (left) and 6 weeks after (right) ProcellaCOR EC application.

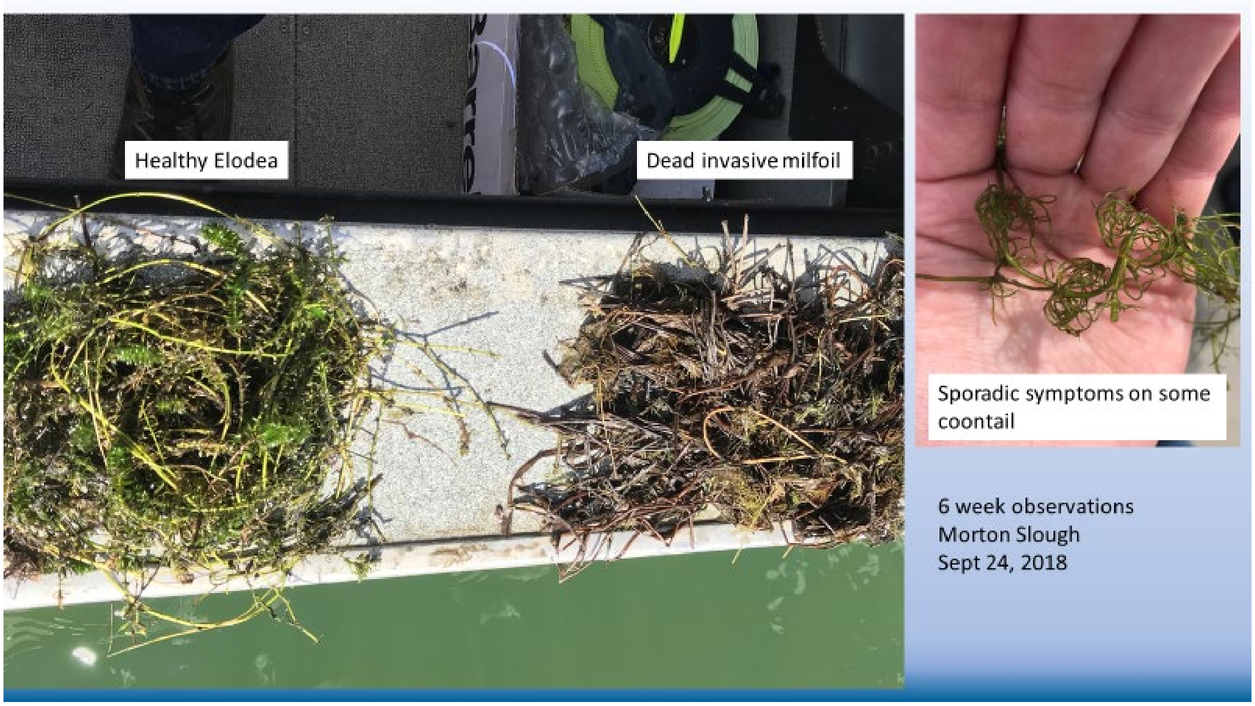


Figure 4. Photos of elodea, non-viable EWM, and coontail at the 6-week assessment of Morton Slough

Figure 5. Photos of healthy, topped-out EWM along adjacent untreated shoreline of Morton Slough in 6-7 feet of water confirming only localized EWM control associated with the mid-slough, 3.5-acre ProcellaCOR application

Table 1. Calculations of percent frequency of aquatic plant occurrence (%FOO) and average estimated rake densities (1 – 4 rating…trace, sparse, moderate, dense) before (PRE) and at 6 weeks after (6W Post) ProcellaCOR application in Morton Slough (treated – top table) and Riley Creek (untreated reference – bottom table).

PROCELLACOR TREATED					
	PRE			6W POST	
	MORTON SLOUGH			MORTON SLOUGH	
SPECIES	%FOO	AVG_DENS	SPECIES	%FOO	AVG_DENS
M_SPICATUM	95	2.5	M_SPICATUM	2	1.0
ELODEA_CAN	90	2.0	ELODEA_CAN	100	2.0
P_FOLIOSUS	88	1.2	P_FOLIOSUS	14	1.0
C_DEMERSUM	76	1.3	C_DEMERSUM	95	1.4
P_PUSILLUS	76	n/a	P_PUSILLUS		
P_CRISPUS	41	1.1	P_CRISPUS	26	1.0
M_SIBIRICU	39	1.3	M_SIBIRICU	10	1.0
S_PECTINAT	24	1.3	S_PECTINAT	5	1.0
P_ZOSTERIF	2	1.0	P_ZOSTERIF	14	1.0
P_RICHARDS			P_RICHARDS		
R_AQUATILI			R_AQUATILI		
CHARA			CHARA		
P_RICHARDS			P_RICHARDS		
P_FILAFORM			P_FILAFORM	19	1.0
UTRIC_SP			UTRIC_SP	2	1.0
			M_SP? (HWM?)	12	1.0
TOTAL NON-MILFOIL SPP			TOTAL NON-MILFOIL SPP		
	7			8	

UNTREATED REFERENCE					
	PRE			6W POST	
	RILEY CREEK			RILEY CREEK	
SPECIES	%FOO	AVG_DENS	SPECIES	%FOO	AVG_DENS
M_SPICATUM	95	1.5	M_SPICATUM	90	1.9
ELODEA_CAN	60	1.3	ELODEA_CAN	85	1.4
P_FOLIOSUS	5	1.0	P_FOLIOSUS		
C_DEMERSUM	15	1.0	C_DEMERSUM	50	1.3
P_PUSILLUS	95	3.5	P_PUSILLUS		
P_CRISPUS			P_CRISPUS	5	1.0
M_SIBIRICU	80	1.7	M_SIBIRICU	65	1.9
S_PECTINAT			S_PECTINAT	10	1.0
P_ZOSTERIF			P_ZOSTERIF	15	1.0
P_RICHARDS	5	1.0	P_RICHARDS	5	1.0
R_AQUATILI	25	0.6	R_AQUATILI	20	1.0
CHARA			CHARA	10	1.0
P_RICHARDS			P_RICHARDS		
P_FILAFORM			P_FILAFORM	5	1.0
UTRIC_SP			UTRIC_SP		
TOTAL NON-MILFOIL SPP			TOTAL NON-MILFOIL SPP		
	6			9	

Table 2. Morton Slough Pre and Post ProcellaCOR Treatment chi-square statistical comparison of frequency of occurrence changes in aquatic plant species (Wisconsin DNR standard protocol – Hauxwell et al. 2010)

Note: Possible HWM of questionable viability was noted at trace levels at 6 weeks. The identification was not confirmed genetically and should be considered conservatively. Pre-treatment EWM was also not genetically identified. HWM has been found in some Pend Oreille locations but invasive milfoil here did appear visually to be predominantly parental EWM prior to application.

Pre-treatment survey total points	41				
Post-treatment survey total points	42				
SPECIES	**Pre Present**	**Post Present**	**p**	**Significant Change**	**Increase/Decrease (proportional to # sampling points)**
Ceratophyllum demersum	31	40	0.01101	*	+
Chara	0	0			no change
Elodea canadensis	37	42	0.03800	*	+
Myriophyllum sibiricum	16	4	0.00168	**	-
Myriophyllum spicatum	**39**	**1**	**0.00000**	*******	**-**
Potamogeton crispus	17	11	0.14119	n.s.	-
Potamogeton filiformis	0	8	0.00328	**	+
Potamogeton foliosus	36	6	0.00000	***	-
Potamogeton pusillus	31	0	0.00000	***	-
Potamogeton zosteriformis	1	6	0.05217	n.s.	+
Stuckenia pectinata	10	2	0.01101	*	-
possible hybrid Eurasian watermilfoil	0	5	0.02267	*	+
Utricularia spp	0	1	0.32021	n.s.	+

Table 3. Riley Creek (untreated reference) Pre and Post Treatment chi-square statistical comparison of frequency of occurrence changes in aquatic plant species (WDNR protocol – Hauxwell et al. 2010)

Pre-treatment survey total points	20				
Post-treatment survey total points	20				
SPECIES	**Pre Present**	**Post Present**	**p**	**Significant Change**	**Increase/Decrease (proportional to # sampling points)**
Ceratophyllum demersum	3	10	0.01812	*	+
Chara	0	2	0.14679	n.s.	+
Elodea canadensis	12	17	0.07664	n.s.	+
Myriophyllum sibiricum	16	13	0.28809	n.s.	-
Myriophyllum spicatum	**19**	**18**	**0.54831**	**n.s.**	**-**
Potamogeton crispus	0	1	0.31118	n.s.	+
Potamogeton filiformis	0	1	0.31118	n.s.	+
Potamogeton foliosus	1	0	0.31118	n.s.	-
Potamogeton pusillus	19	0	0.00000	***	-
Potamogeton richardsonii	1	1	1.00000	n.s.	no change
Potamogeton zosteriformis	0	3	0.07172	n.s.	+
Ranunculus aquatilis	5	4	0.70495	n.s.	-
Stuckenia pectinata	0	2	0.14679	n.s.	+

Plant statistical analysis reference: Hauxwell, J., S. Knight, K. Wagner, A. Mikulyuk, M. Nault, M. Porzky and S. Chase. 2010. Recommended baseline monitoring of aquatic plants in Wisconsin: sampling design, field and laboratory procedures, data entry and analysis, and applications. Wisconsin Department of Natural Resources Bureau of Science Services, PUB-SS-1068 2010. Madison, Wisconsin, USA.

Field evaluation of spatially-targeted, selective control of variable-leaf watermilfoil in New Hampshire using florpyrauxifen-benzyl

Mark A. Heilman, Amy P. Smagula, and J.T. Gravelie*

*First and third authors: Senior Aquatics Technology Leader and GIS analyst for SePRO Corporation, Carmel, Indiana, USA

Second author: Limnologist for New Hampshire Department of Environmental Services, Concord, New Hampshire, USA

ABSTRACT:

Variable-leaf milfoil (*Myriophyllum heterophyllum*, Michx.) (VLM) is considered invasive in the Northeast US and has infested a variety of freshwater systems in New Hampshire. Selective spot application of herbicides to restore habitat impaired by this NH-exotic species while minimizing impact to water use are key objectives. The newly USEPA-registered, reduced-risk aquatic herbicide florpyrauxifen-benzyl (ProcellaCOR®) has shown excellent, selective, systemic activity on invasive watermilfoils including VLM. A cooperative field evaluation was conducted in 2016 to demonstrate the efficacy and monitor the dissipation of florpyrauxifen-benzyl in a spot treatment of VLM at a US Army Corps of Engineers facility in New Hampshire. A 0.4-ha spot area of VLM within a larger ~10-ha waterbody was treated in early August 2016. Water sampling and analytical testing documented <24 hours of contact time and excellent selective control within 3-6 weeks after application. VLM frequency of occurrence decreased from 83% cover of dense, mostly topped-out plants in the management area prior to application to 11% trace-densities in the summer after application. In July 2018, there was little recovery of VLM with continued expansion of natives plants (native species per site increased from 2.0 in 2016 to 5.0 at 2 years post in 2018) confirming extended, selective control potential with spot treatments.

KEY WORDS: ProcellaCOR®, *Myriophyllum heterophyllum, selectivity, dissipation*

INTRODUCTION

Variable-leaf milfoil (*Myriophyllum heterophyllum*, Michx.) is considered an invasive aquatic plant in parts of the northeast region of the United States. The invasive species is particularly widespread in waterbodies in New Hampshire with both economic (Halsted et al 2003) and ecological impacts (as summarized in Thum and Lennon 2010). Controlled testing in mesocosm-scale tanks systems (Glomski and Netherland, 2008a; Glomski and Netherland, 2008b; Getsinger et al 2003) and field evaluations (Haug and Bellaud 2013) have demonstrated the efficacy, use rates and selective potential of various herbicide technologies in controlling variable-leaf milfoil (VLM) as part of integrated management strategies. Recent advances in herbicide chemistries have led to products that provide good efficacy and longevity of control, a favorable toxicology profile, and decreasing use restrictions. A reduced risk profile is ultimately preferred by resource managers, who must balance the multitude of designated uses for surface water systems, including swimming, fishing, and other forms of recreation as well as drinking water consumption and irrigation.

Florpyrauxifen-benzyl (tradename ProcellaCOR®) is a newly USEPA-registered herbicide approved for aquatic use with a reduced-risk classification in early 2018. It has been documented to have high activity with short exposure requirements for the selective control of multiple invasive watermilfoils (Netherland Richardson 2016, Richardson et al. 2016, Beets et al 2019). Similar work in preparation for publication has documented favorable activity on VLM with short exposure (K. Foley and RJ Richardson, NC State University, in prep).

Prior to USEPA registration under conditions of no-water uses and limited scale for experimental field testing, a limited number of field evaluations were conducted in the US as part of the new aquatic herbicide's final development. One such study was conducted on VLM in New Hampshire with the objectives of evaluating the short-term and long-term efficacy and confirming dissipation of a small-scale, spot application of florpyrauxifen-benzyl in controlling VLM in a low pH (pH range of 5.0 to 5.5), soft-water aquatic site typical in New Hampshire, while evaluating impacts to non-target macrophyte species within and adjacent to the treatment area.

MATERIALS AND METHODS

Site Selection

Because florpyrauxifen-benzyl did not have USEPA registration at the time of this study, use of the product was limited to a maximum 0.4 ha treatment plot, in a relatively remote basin with restricted access. As such, the treatment plot was selected from a roughly 10 ha sub-basin at the Hopkinton-Everett Flood Control Area in Hopkinton, New Hampshire (Figure 1). The area is managed by the United States Army Corps of Engineers, New England District, as part of an extensive (>283 ha) flood control system associated with the Contoocook River system. The sub-basin is connected to the main flood control system by a shallow dredged canal which is marginally navigable only by small non-motorized craft, and it is also accessible by a gated dirt road over a flood control dike to the basin's shoreline. A treatment plot with high density VLM growth and a mixed native macrophyte community was selected for the study site (center of treatment block located at 71°42'36.787"W, 43°11'36.212"N). Water column depths throughout the basin range from 0.5-3 m, with a mean depth of about 1.2 m.

Herbicide Treatment

Under a special aquatic permit issued by the New Hampshire Department of Agriculture, the 0.4 ha study plot was treated with florpyrauxifen-benzyl at an application rate of 10 µg a.i. L^{-1} on August 8, 2016. The application was made by state licensed applicators with an airboat equipped with a calibrated pump connected to subsurface hoses. The boat was equipped with a GPS navigation system to ensure even distribution of the product . 141.9 mL of a 300 g a.i. L^{-1} test formulation (SLF-9522) was tank mixed with lake water and evenly applied throughout the treatment block (average water depth = 1.05 m or 3.5 feet) in under 30 minutes.

Herbicide Dissipation Monitoring

Herbicide dissipation was monitored at several intervals post-treatment: 6 hours after treatment (HAT), 1 day after treatment (DAT), 4 DAT and 7 DAT. At each sampling interval, grab samples were collected at six stations, including three within the treatment plot, and three around the sub-basin at distances ranging from 62 m to 102 m outside of the treatment block. Samples were collected into amber glass bottles with Teflon® lids and kept dark and refrigerated after collection to prevent photolysis in sunlight. Samples were also preserved in the field using 5% methanol and formic acid addition to keep sample pH low and prevent possible low-level hydrolysis. Water samples were shipped overnight and then analyzed by EPL Bio-Analytical Services (Niantic, IL, USA) using ultra-performance liquid chromatography with tandem mass spectrometry detection (UPLC/MS-MS).

Macrophyte Surveys

Macrophyte surveys were conducted pre-treatment (July 14, 2016), 3 weeks after treatment (WAT – VLM observations only – August 29, 2016), 6 WAT (September 19, 2016) and 1 year (1 YAT - July 10, 2017) and 2 years (2 YAT - July 13, 2018) after the July 2016 pre-treatment survey. A modified point-intercept sampling pattern implementing a rake toss method (single double-sided rake throw with approximately 3-meter bottom drag) was utilized to detect species of aquatic plants present and estimate their individual densities based on rake fullness at each of 30 points throughout the sub basin, including 9 points within the treatment plot (Figure 1). The rake fullness rating on a 1 – 5 scale utilized were based on the protocol commonly used by New Hampshire Department of Environmental Services: 1 – trace biomass, 2 – sparse biomass, 3 – moderate biomass, 4 – dense biomass, and 5 – dense, topped-out biomass.

RESULTS AND DISCUSSION

Herbicide concentrations in the 0.4-ha application area declined rapidly following treatment with average concentrations of 2.3 µg a.i. L^{-1} (23% of theoretical applied concentration) at 6 hours after application (HAT), 0.7 µg a.i. L^{-1} by 24 HAT, and 0.08 µg a.i. L^{-1} by 96 HAT. The maximum measured concentration of active ingredient detected post treatment was 3.2 µg a.i. L^{-1} at 6 HAT at site W3, which was located within the treatment block (Figures 1 & 3). By 1 DAT, all monitored sites had herbicide concentrations below 1 µg a.i. L^{-1}. Herbicide concentration decreased with distance from the treatment block with a maximum average concentration of 0.25 µg a.i. L^{-1} measured at 6 hours after application for the locations outside of the management area.

Pre-treatment vegetation assessment documented a mix of native macrophytes (13 taxa) and varying densities of VLM, which was the most common species in the the sub-basin at large (Table 1) and the 0.4 ha management area (Table 2). Prior to treatment, milfoil densities were high within the treatment block, and high to moderately high at 6 point-intercept sites around the sub-basin (maps in Figure 2). By three weeks post treatment milfoil exhibited signs of epinasty (browning, twisting of stems, defoliated stems) within the treatment plot, and in the periphery of the treatment zone. By six weeks post treatment (Figure 2B), milfoil biomass was absent within the treatment plot with the exception of a few dark/brittle defoliated stems detected at the bottom via rake toss. Reduced milfoil densities were also observed at point-intercept locations in the periphery of the treatment plot. Additionally, sites on the outer edges of the sub-basin showed slight declines in density, suggesting that low concentrations of florpyrauxifen-benzyl from dissipation outside of the plot resulted in some stress to VLM and likely also promoted competitive growth of tolerant native plants.

Native macrophytes persisted in apparent healthy condition through the treatment, with white water lily and watershield showing slight epinasty (petiole elongation and leaf margin curling) from 1-3 WAT; however, the leaves flattened out and appeared healthy by 6 WAT. By 6 WAT, while still detectable at most sites in the management area, VLM rake densities had dropped to trace levels from high densities prior to treatment. At 1 YAT, VLM was only present at 1 of 9 sites (11% FOO decreasing from 83% before treatment). The dominant native species found in the management area at 1 YAT were white water lily, Robbins pondweed, and large bladderwort. Several other bladderwort species and floating leaf pondweed were also common. The total number of native species detected in the management area increased from 8 in July 2016 to 11 in July 2017. The average number of native species found at each sampling location in the management area also increased from 2.3 in 2016 to 3.8 in 2017. At 2 YAT, there was little VLM recovery in the management area (increase to 2 of 9 sample sites with trace rake coverage) and a diverse native plant community similar in composition to 2017 1 YAT. Number of native plant species detected on average at each sampling point increased again from 3.8 per site to 5.0 per site in 2018.

In conclusion, a spot 0.4-ha application of florpyrauxifen-benzyl (ProcellaCOR) into a larger aquatic site (~10 ha) in New Hampshire quickly dissipated to <1 µg a.i. L^{-1} by 24 hours after application with minimal detectable concentrations outside of the management area. This short-low-level exposure provided favorable control of VLM out to two years post application. The treatment showed excellent selectivity with an increase in the abundance and diversity of native aquatic plants post treatment. Assessment of future VLM treatments with this new herbicide technology should focus on refining efficacy and selectivity under different rate, exposure, and seasonal timing. Methods of delivery (swath width, surface versus injection, etc) should also be explored under different levels of target/non-target plant biomass to identify techniques that most efficiently apply the herbicide to the target VLM.

ACKNOWLEDGEMENTS: The authors acknowledge R. Wolff of the NH Department of Agriculture for guidance and assistance with permitting, and M. Bellaud of SOLitude Lake Management, LLC in Massachusetts for permit application prep and application of the herbicide to the study site. We also thank J. Levesque, S. Dermody and others at the Army Corps of Engineers New England District Offices, and the Hopkinton Project for allowing access to study site.

LITERATURE CITED

Beets JP, Heilman MA, and 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. (in press)

Glomski LM, Netherland MD. 2008. Efficacy of Fluridone, Penoxsulam, and Bispyribac-sodium on Variable-leaf milfoil. J. Aquat. Plant Manag. 46: 193-196.

Halstead JM, Michaud J, Hallas-Burt S, Gibbs JP. Hedonic analysis of effects of a nonnative invader (*Myriophyllum heterophyllum*) on New Hampshire (USA) lakefront properties. Environmental Management. 2003 Sep 1;32(3):391-8.

Haug EJ, Bellaud MD. 2013. Efficacy of 2,4-D ester on variable-leaf milfoil control for partial lake treatments in New Hampshire waterbodies. J. Aquat. Plant Manag. 51: 49-52.

Glomski LM, Netherland MD. 2008. Effect of water temperature on 2,4-D ester and Carfentrazone-ethyl applications for control of variable-leaf milfoil. J. Aquat. Plant Manag. 46: 119-121.

Getsinger, KD, Sprecher SL, Smagula AP. 2003. Effects of Triclopyr on variable-leaf milfoil. J. Aquat. Plant Manag. 41: 124-126.

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(1):181-190.

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

Supplemental Environmental Impact Statement for State of Washington Aquatic Plant and Algae Management. 2017. Washington Department of Ecology – Water Quality Program. 205 pp.

Thum RA, Lennon JT. Comparative ecological niche models predict the invasive spread of variable-leaf milfoil (*Myriophyllum heterophyllum*) and its potential impact on closely related native species. Biological invasions. 2010 Jan 1;12(1):133.

TABLES

Table 1. Frequency of occurrence and average density rating (1-5 scale) of variable-leaf watermilfoil and New Hampshire native plants found in the entire ~10 ha Hopkinton sub-basin including the 0.4 ha area managed with florpyrauxifen-benzyl. <1 values for average density mean that a limited number of visual sightings of emergents present (0 rating) were part of the average. Surveys were performed Pre-treatment (July 14, 2016), 6 WAT (September 19, 2016), 1 YAT (July 10, 2017), and 2 YAT (July 13, 2018). (Chi-square statistical analyses in process for final manuscript)

TOTAL STUDY AREA (30 locations)									
Species		**Pre-treatment**		**6 WAT**		**1 YAT**		**2 YAT**	
Scientific Name	**Common Name**	**FOO**	**Avg. Density**	**FOO**	**Avg. Density**	**FOO**	**Avg. Density**	**FOO**	**Avg. Density**
Myriophyllum heterophyllum	**Variable-leaf watermilfoil**	**76%**	**3.0**	**41%**	**1.0**	**13%**	**1.5**	**27%**	**1.4**
Nymphaea odorata	White waterlily	72%	1.5	90%	1.4	73%	1.6	47%	1.6
Utricularia vulgaris	Large Bladderwort	64%	0.9	66%	1.2	77%	2.2	53%	1.5
Potamogeton robbinsii	Robbins Pondweed	48%	1.8	55%	2.1	67%	1.9	67%	2.6
Utricularia intermedia	Flat-leaved Bladderwort	40%	0.7	59%	1.4	30%	1.0	37%	1.0
Utricularia purpurea	Whorled bladderwort	40%	1.0	41%	1.0	3%	1.0	47%	1.1
Potamogeton natans	Floating Leaf Pondweed	20%	1.1	38%	1.5	30%	1.7	47%	1.1
Utricularia gibba	Humped Bladderwort	8%	0.8	14%	1.0	3%	1.0	37%	1.0
Najas guadalupensis	Southern Naiad	8%	1.8			3%	3.0	17%	1.2
Nuphar variegata	Yellow Waterlily	8%	1.3			7%	2.0	27%	1.1
Eleocharis robbinsii	Robbins spikesedge	4%	0.5			13%	1.0		
Utricularia radiata	Floating Bladderwort	4%	1.0	10%	1.0	17%	1.0	43%	1.0
Nymphoides cordata	Little Floatingheart	4%	1.0					7%	1.0
Potamogeton epihydrus	Ribbonleaf Pondweed	4%	1.0			3%	2.0		
Pontedaria cordata	Pickerelweed							3%	1.0
Potamogeton sp?	Thin Leaf Pondweed (sp?)			10%	1.0	10%	1.0	27%	1.4
Brasenia schreberi	Watershield			14%	1.0	20%	1.3	7%	1.5
Total Number of NH Native Species Detected		13		10		14		14	
Avg. Number of NH Native Species per site		3.2				3.7		4.8	

Table 2. Frequency of occurrence and average density rating (1-5 scale) of variable-leaf watermilfoil and New Hampshire native plants found within the 0.4-ha area in the Hopkinton sub-basin managed with florpyrauxifen-benzyl. <1 values for average density mean that a limited number of visual sightings of emergents present (0 rating) were part of the average. Surveys were performed Pre-treatment (July 14, 2016), 6 WAT (September 19, 2016), 1 YAT (July 10, 2017), and 2 YAT (July 13, 2018). (Chi-square statistical analyses in process for final manuscript)

MANAGEMENT AREA ONLY (9 locations)									
Species		**Pre-treatment**		**6 WAT**		**1 YAT**		**2 YAT**	
Scientific Name	**Common Name**	**FOO**	**Avg. Density**	**FOO**	**Avg. Density**	**FOO**	**Avg. Density**	**FOO**	**Avg. Density**
Myriophyllum heterophyllum	**Variable-leaf watermilfoil**	**83%**	**4.6**	**63%**	**1.0**	**11%**	**1.0**	**22%**	**1.0**
Nymphaea odorata	White waterlily	67%	1.3	88%	1.4	56%	1.6	78%	1.4
Utricularia vulgaris	Large Bladderwort	33%	1.0	63%	1.4	78%	2.3	78%	1.9
Potamogeton robbinsii	Robbins Pondweed	33%	1.0	75%	2.3	56%	1.6	44%	2.0
Utricularia purpurea	Whorled bladderwort	33%	1.0	38%	1.0			44%	1.0
Utricularia intermedia	Flat-leaved Bladderwort	17%	0.5	25%	2.0	44%	1.0	44%	1.0
Potamogeton natans	Floating Leaf Pondweed	17%	1.0	38%	2.0	22%	2.5	44%	1.0
Nymphoides cordata	Little Floatingheart	17%	1.0						
Potamogeton epihydrus	Ribbonleaf Pondweed	17%	1.0			11%	2.0		
Eleocharis robbinsii	Robbins spikesedge					11%	1.0		
Utricularia radiata	Floating Bladderwort			25%	1.0	33%	1.0	33%	1.0
Utricularia gibba	Humped Bladderwort			13%	1.0			44%	1.0
Pontedaria cordata	Pickerelweed							11%	1.0
Najas guadalupensis	Southern Naiad								
Potamogeton sp?	Thin Leaf Pondweed (sp?)					11%	1.0	33%	1.3
Brasenia schreberi	Watershield			13%	1.0	11%	1.0		
Nuphar variegata	Yellow Waterlily					22%	2.0	44%	1.3
Total Number of NH Native Species Detected		8		9		11		11	
Avg. Number of NH Native Species per site		2.3				3.8		5.0	

FIGURES

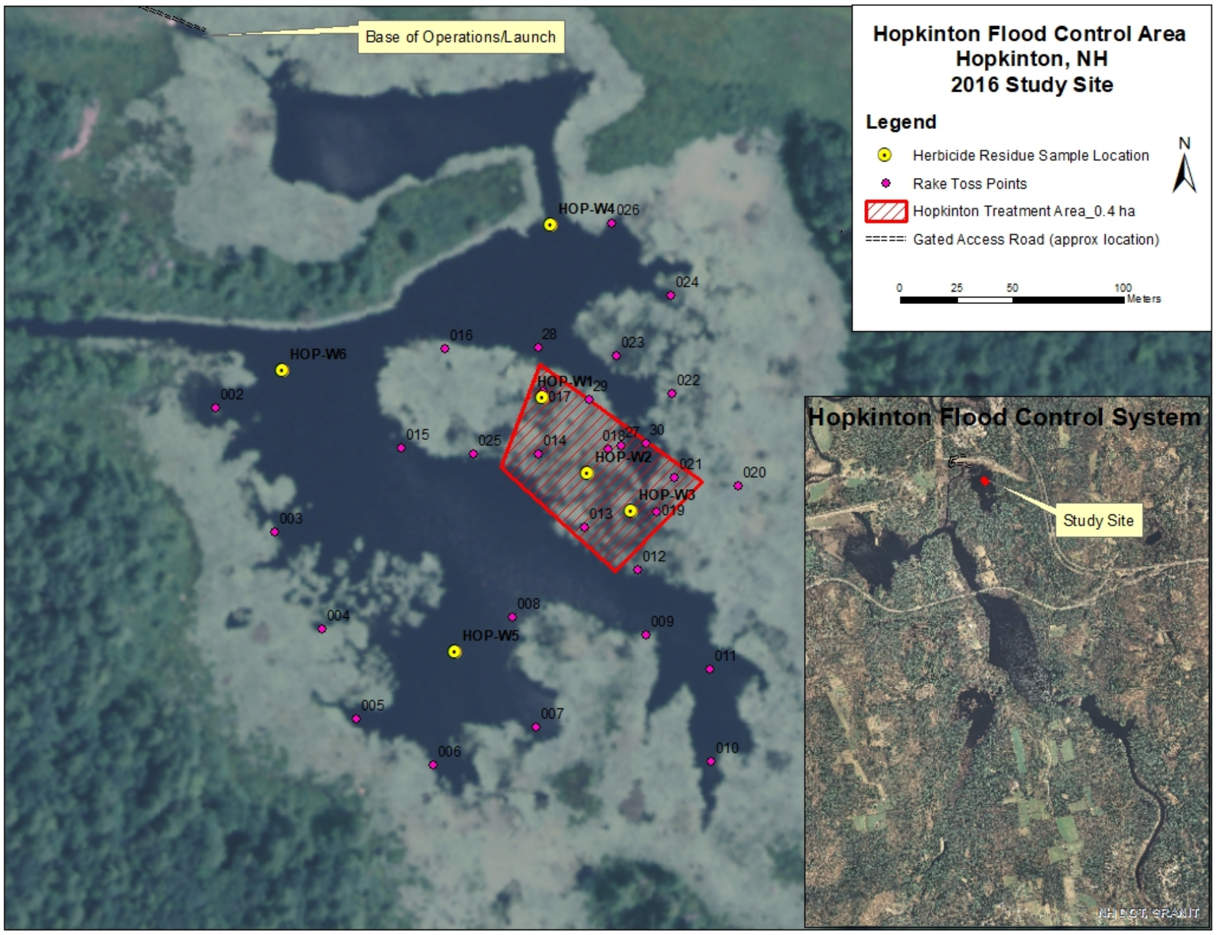


Figure 1 – Map of Hopkinton sub-basin site showing the 0.4 ha area of VLM treatment in early August 2016 plus vegetation and water sampling locations.

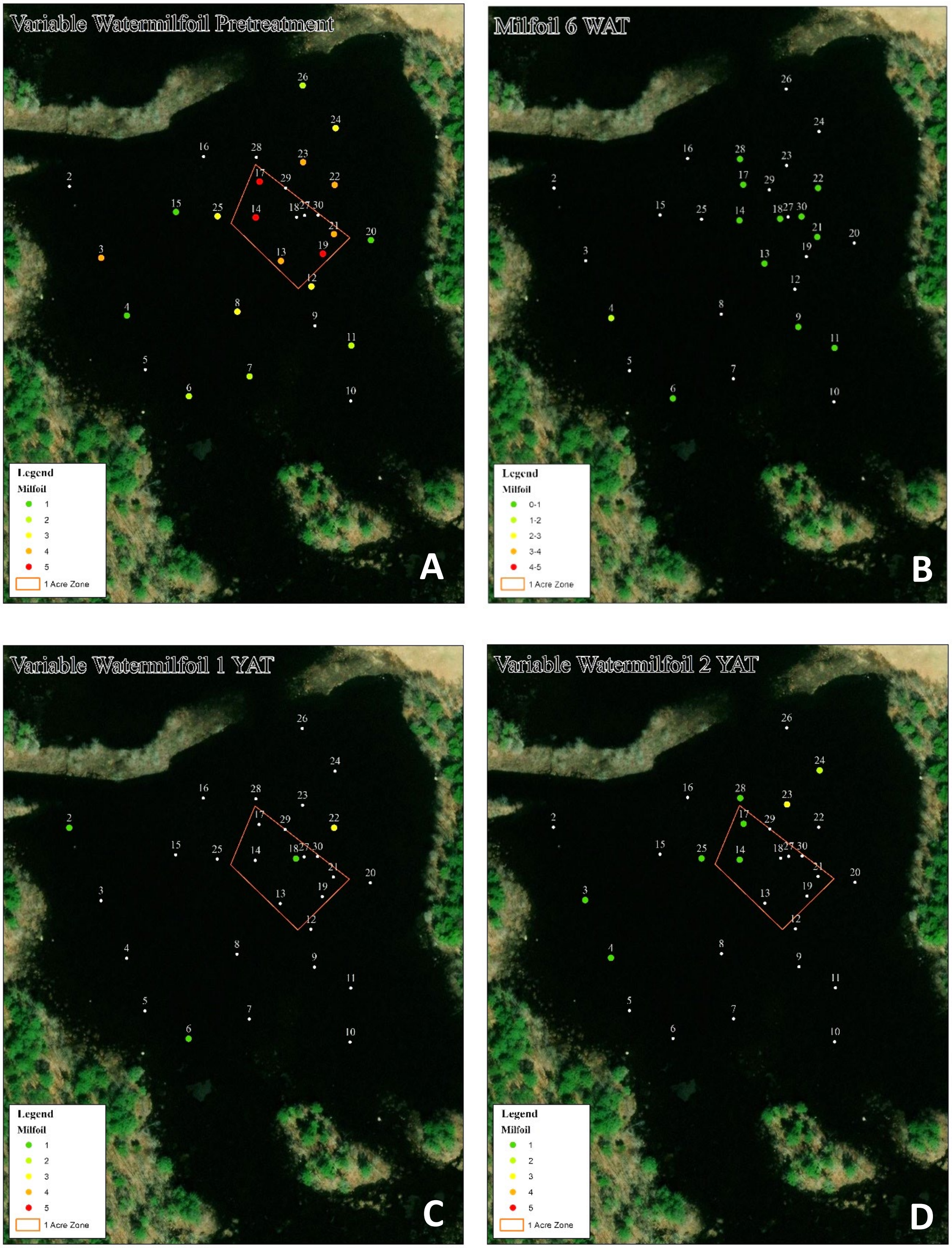


Figure 2 – Variable-leaf watermilfoil presence and estimated density in Hopkinton sub-basin (A) before August 8, 2016 treatment of 0.4 ha (1.0 acre) (Pre – July 14), (B) 6 weeks after application (September 19), 1 YAT (July 10, 2017), and 2 YAT (July 13, 2018).

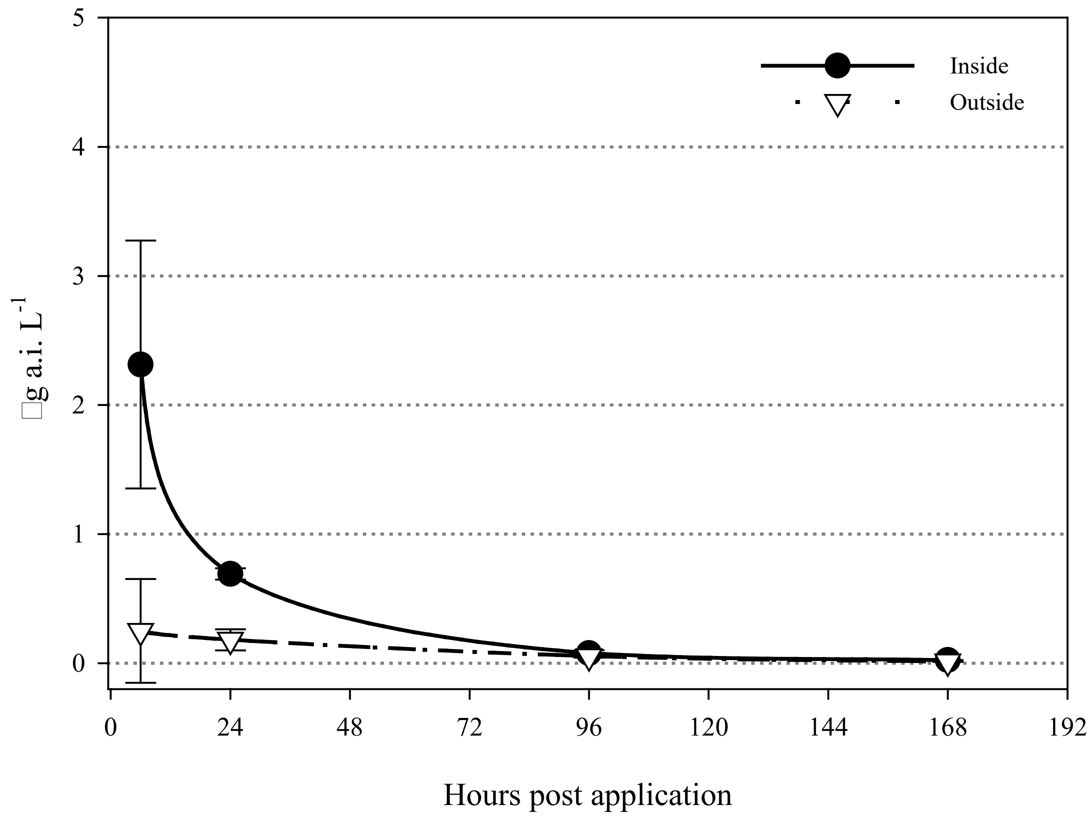


Figure 3. Dissipation of florpyrauxifen-benzyl in Hopkinton sub-basin after August 8, 2016 application. Targeted concentration of the application was equivalent to 10 µg a.i. L^{-1}. Error bars represent +/- 1 standard deviation for three stations inside the management area and three stations located 62 – 102 m outside of the management area.

ProcellaCOR effects on the native aquatic grasses Maidencane (*Panicum hemitomon*) and Egyptian paspalidium (*Paspalidium geminatum*) and native emergent cattail (*Typha* spp.)

Representative demonstration of tolerance in these common aquatic monocots.

ProcellaCOR has little activity on the vast majority of common aquatic monocots. There are select monocot responses such as the activity of the herbicide on hydrilla and members of the Hydrocharitaceae family. Native, perennial aquatic grasses such as those in the *Panicum* genus show no response to ProcellaCOR at up to maximum label rates.

Below is a photo of an early pond evaluation outcome at the SePRO Research and Technology Campus in eastern NC where a 0.1-acre experimental pond was treated at the equivalent of 15.5 PDU per A-ft EC (30 µg ai L^{-1}). No response was noted by the grass species maidencane and Egyptian paspalidium.

Fig 1. Healthy maidencane (back) and Egyptian paspalidium at one month following ProcellaCOR whole pond application at equivalent of 15.5 PDU EC.

Various field and controlled studies have shown tolerance of cattail to ProcellaCOR applications with either in-water and foliar treatments. A representative example of in-water treatment shows a small, shallow Michigan pond site (0.82 acres, average depth 2.1 feet) with shoreline cattail on day of application and no cattail impact post application (Fig 2). This site also contained dense Eurasian watermilfoil and several other common native submersed plants. Cattails were unaffected by treatment and selective control of Eurasian watermilfoil was also achieved with an equivalent of 2.6 PDU EC per A-ft of ProcellaCOR (Fig 3).

Fig 2. Shoreline cattail on day of application (June 5, 2017) (top) of ProcellaCOR at a whole pond rate equivalent to 2.6 PDU EC per A-ft and the same healthy cattail at approaching 6 weeks post application (July 14, 2017).

		EWM	Elodea	Sago	Illinois PW	CLP	Coontail	Naiad
10/5/2016	**%FOO**	100.0	22.2	11.1	11.1	0.0	11.1	0.0
	Avg Density (1 -4)	4.0	1.5	1.0	1.0	-	1.0	-
6/5/2017	**%FOO**	100.0	50.0	50.0	12.5	12.5	12.5	-
	Avg Density (1 -4)	2.9	1.0	2.3	3.0	1.0	1.0	-
TREATMENT JUNE 13								
6/29/2017	**%FOO**	**50.0**	50.0	75.0	25.0	25.0	37.5	25.0
	Avg Density (1 -4)	**0.5**	1.3	1.5	2.5	1.0	2.0	2.0
7/14/2017	**%FOO**	**25.0**	37.5	62.5	12.5	-	87.5	25.0
	Avg Density (1 -4)	**0.5**	2.0	2.0	4.0	-	1.3	2.5
8/16/2017	**%FOO**	**0.0**	62.5	37.5	25.0	-	62.5%	25.0
	Avg Density (1 -4)	**0**	1.2	1.0	3.0	-	1.2	2.0

Fig 3. Frequency and density ratings for submersed weeds within Michigan pond (8 sampling stations) treated with ProcellaCOR in early June 2017. An early fall 2016 survey documented Eurasian watermilfoil (EWM) dominance and then selective EWM control was achieved with competitive release of the native plants during the summer of 2017.

ProcellaCOR effects on spatterdock (*Nuphar advena*) – October 2016 Evaluation

SePRO Research and Technology Campus, Whitakers NC

ProcellaCOR was applied at three different target rates into water (not foliar spray) of three 0.1-acre experimental ponds at the SePRO research facility in eastern NC on August 24, 2016. Evaluation rates equivalent to 2.6, 5.2, and 10.4 PDU EC per A-foot (equivalent of 5, 10, 20 µg ai L^{-1}) were applied to target full water volume of separate ponds (static exposure scenario versus shorter, spot treatment scenario), and a 4^{th} pond was monitored as well as an untreated reference. The four study ponds each had well-established spatterdock growing in one corner. Spatterdock coverage in replicated 6.25 ft^2 (0.58 m^2) installed quadrats (3 per pond/spatterdock bed) was evaluated through photography and 'green surface cover' as determined using the *Canopeo* image analysis application (Patrignani et al 2015). Evaluations were performed at time of treatment, 7 days after application, and 28 days after application.

Spatterdock surface coverage was near 100% in all installed quadrats within the 3 ponds at the time of ProcellaCOR treatment. Spatterdock showed some visual symptoms (leaf curling and petiole extension) at all three ProcellaCOR rates. There was some senescence and replacement of older leaves with a rate-dependent response (less stress at low PDU, more at higher PDU), but only the 10.4 PDU rate (outside of common watermilfoil rates) evaluated showed surface coverage reductions in assessment quadrats greater than 50% at 4 weeks after application (Fig. 3). ProcellaCOR had higher activity on younger floating leaves compared to older emerged leaves. Overall, despite an atypical extended exposure scenario with the whole pond treatments versus more common partial treatment designs with shorter exposures, no ProcellaCOR treatment scenario produced major reductions in overall spatterdock coverage during the study. All three test ponds had healthy remaining spatterdock at the end of the evaluation. For invasive watermilfoil control, the results here along with early 2018 field observations support that spatterdock can be selectively maintained while controlling the exotic target species.

Fig 1. Untreated spatterdock on day of application and at one-month following application.

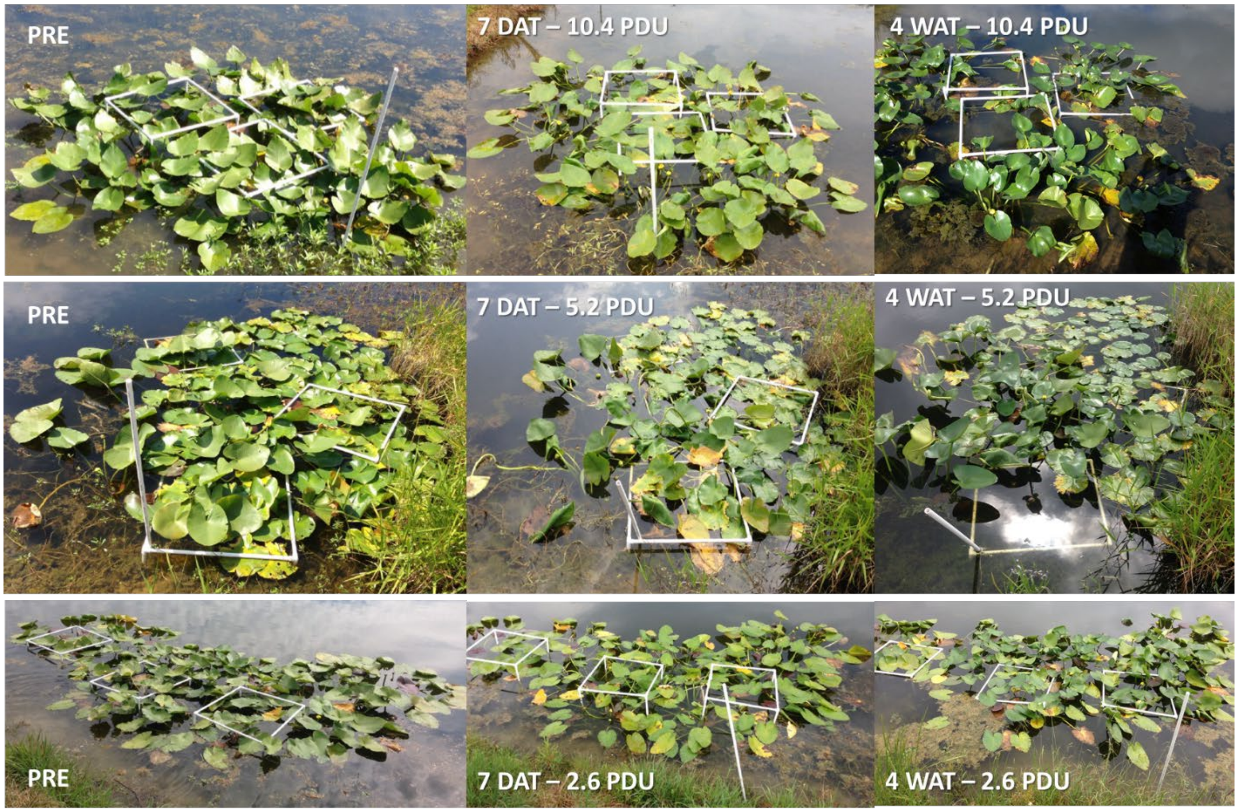


Fig 2. Spatterdock visual condition immediately prior to application (PRE - left) and 7 (center) and 28 days (right) following application of ProcellaCOR at 3 whole-pond, in-water rates: 2.6, 5.2, and 10.4 PDU per A-ft.

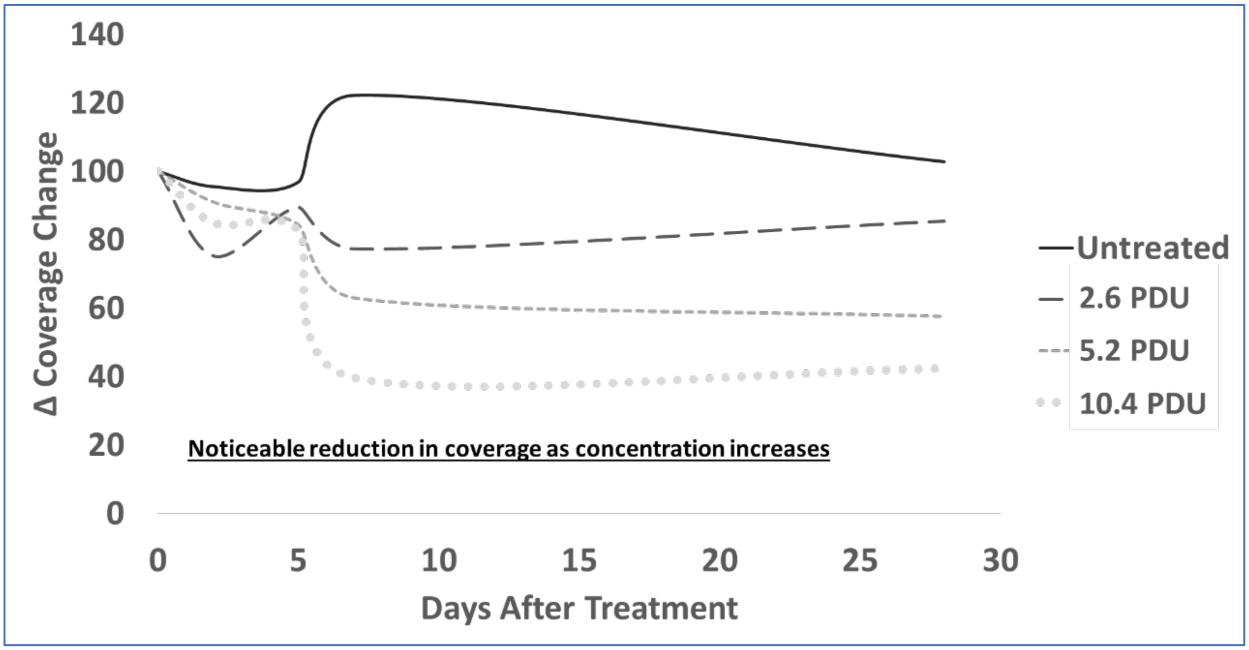


Fig 2. Trends in average water surface coverage by spatterdock in replicated quadrats deployed into mature plant beds within an untreated pond and 3 ponds treated at different ProcellaCOR whole-pond rates (2.6, 5.2, and 10.4 PDU per A-ft). Coverage changes were monitored through *Canopeo* image analysis.

Reference: Patrignani, A. and Ochsner, T.E., 2015. Canopeo: A powerful new tool for measuring fractional green canopy cover . Agronomy Journal, 107(6), pp.2312-2320.

J. Aquat. Plant Manage. 54: 26–31

Response of seven aquatic plants to a new arylpicolinate herbicide

ROBERT J. RICHARDSON, ERIKA J. HAUG, AND MICHAEL D. NETHERLAND*

ABSTRACT

The herbicide 4-amino-3-chloro-6-(4-chloro-2-fluoro-3-methoxyphenyl)-5-fluoro-pyridine-2-benzyl ester (SX-1552 or XDE-848 BE; proposed ISO common name in review) is a new arylpicolinate herbicide currently under development for weed management in rice (*Oryza sativa* L.) production, aquatic weed management, and other uses. Greenhouse research was conducted to evaluate the effect of SX-1552 and SX-1552A (an acid metabolite) on seven aquatic plants: alligatorweed [*Alternanthera philoxeroides* (Mart.) Griseb.], Carolina waterhyssop [*Bacopa monnieri* (L.) Pennell], fanwort (*Cabomba caroliniana* Gray), monoecious hydrilla [*Hydrilla verticillata* (L. f.) Royle], parrotfeather [*Myriophyllum aquaticum* (Vell.) Verdc.], variable watermilfoil (*Myriophyllum heterophyllum* Michx.), and American waterwillow [*Justicia americana* (L.) Vahl]. SX-1552 and SX-1552A were applied to these species as an in-water, 4-wk static exposure at rates of 0 to 81 $\mu g\ L^{-1}$. Fanwort was not controlled by SX-1552 at the rates evaluated, in contrast to the other species tested. Dry weight 50% effective concentration (EC_{50}) values were < 1 $\mu g\ L^{-1}$ SX-1552 for alligatorweed, monoecious hydrilla, parrotfeather, and variable watermilfoil. Carolina waterhyssop and American waterwillow SX-1552 EC_{50} values were 5.0 and 5.1 $\mu g\ L^{-1}$, respectively. These six species were less sensitive to SX-1552A with dry weight EC_{50} values of 1.6 to 77.1 $\mu g\ L^{-1}$. Plant control ratings also indicated that response of the six sensitive species increased from 2 to 4 wk after treatment. Further research is needed on additional species as well as concentration exposure-time determination for the species evaluated here.

Key words: herbicidal control, synthetic auxin.

INTRODUCTION

Despite an increased number of U.S. aquatic registrations in the past decade, additional technologies are still needed for successful management of aquatic weeds. Although 244 herbicide active ingredients are currently registered in the United States, only 14 are registered as aquatic herbicides (NPIRS 2015). Additional herbicides can improve control of weed species not optimally addressed by current product registrations, enhance selectivity to desirable native aquatic vegetation, reduce use rates, and mitigate risk of potential herbicide-resistance development (Getsinger et al. 2008, APMS 2014). Selectivity to native aquatic vegetation and longevity of control are key criteria in the management of invasive aquatic plants. Effects of a specific herbicide chemistry on a given target weed and co-occurring native plants, general characteristics of its mode of action, and herbicide concentration and exposure time (CET) achieved with in-water treatments dictate the selectivity and duration of control of aquatic herbicide treatments (Netherland and Getsinger 1992, Getsinger et al. 1993, Netherland et al 1997). Research and development of new aquatic herbicides is generally focused on finding new selective, systemic chemistries that have short exposure time requirements for in-water, partial-site treatment of major-target aquatic weeds, such as hydrilla [*Hydrilla verticillata* (L. f.) Royle] and Eurasian watermilfoil (EWM) (*Myriophyllum spicatum* L.).

Auxin-mimic herbicides (2,4-D and triclopyr) are well documented for their selective, systemic control of problem weeds, such as EWM and waterhyacinth [*Eichhornia crassipes* (Mart.) Solms. Auxins are a group of plant-growth hormones that affect many plant processes, such as root initiation, tropism, shoot growth, and development and apical dominance, among other essential plant-growth processes (Yamada 1954, Grossman 2010). In susceptible plants, synthetic auxins have the same impacts as would natural auxin overdose. However, synthetic auxins are more stable within plants and less susceptible to the plant's methods of inactivation as compared with the naturally produced auxins (Woodward and Bartel 2005). The prevailing theory until recently has suggested that synthetic auxins causes plants to essentially "grow themselves to death" (Gilbert 1946). The action of synthetic auxin overdosing can be summarized in three phases: the stimulation phase, during which, the plants metabolic activity is heightened, and abnormal growth occurs, such as stem curling and leaf epinasty; the inhibition phase, during which, growth is stunted, and several growth reducing physiological responses, such as stomatal closure and reduced carbon fixation, occur; and finally, the decay phase, characterized by cell and plant tissue death (Grossman 2010). The feedback mechanisms involved in this phased progression is much more complex than that proposed by Gilbert (1946), and it is because of these complexities that auxin mimics have differential action on monocots versus dicots and among different dicot species (Grossman 2010).

Synthetic indole-3-acetic acid (IAA) (auxin) derivatives were developed for use in plant management as early as 1940 (Cobb 1992). Synthetic auxins are translocated throughout the plant because of their similarity to natural auxins (Grossman 2010). Generally, dicotyledonous plants

*First and second authors, Department of Crop Science, North Carolina State University, Box 7620, Raleigh, NC 27695. Third author: U.S. Army Engineer Research and Development Center, 7922 NW 71st Street, Gainesville, FL 32653. Corresponding author's E-mail: rob__richardson@ncsu.edu. Received for publication July 30, 2015 and in revised form August 6, 2015.

are more susceptible to auxin mimics than monocots, whereas unicellular algae in the water column are not affected (Cedergreen and Streibig 2005). As such, synthetic auxins are often used to selectively control aquatic weeds to limit the impact to nontarget native plant and algal species (Madsen and Wersal 2009, Glomski and Netherland 2010, Wersal et al. 2010). Although currently registered auxin-mimic herbicides fit a number of needs for selective aquatic weed control, a systemic herbicide with this selective mode of action has not been previously identified with sufficient activity on hydrilla. Hydrilla may be considered the most problematic U.S. aquatic weed, and despite efforts to register several new herbicides for hydrilla control, the species continues to have the most urgent need for additional herbicide options (Hoyer et al 2005, Richardson 2008, APMS 2014). Several other aquatic weeds, such as crested floatingheart [*Nymphoides cristata* (Roxb.) Kuntze] and certain biotypes of hybrid watermilfoils (*Myriophyllum* spp. L.), show insufficient response to current auxin-mimic herbicides to be optimally controlled with typical use rates (LaRue et al 2013, Willey et al 2014).

The herbicide SX-1552,[1] 4-amino-3-chloro-6-(4-chloro-2-fluoro-3-methoxyphenyl)-5-fluoro-pyridine-2-benzyl ester is under development by Dow AgroSciences for rice production (XDE-848 BE; proposed ISO common name in review; active trade name Rinskor™) and other agricultural crops and is also in development in partnership with SePRO Corporation as an aquatic herbicide (SX-1552[2]; Procellacor™ Aquatic Herbicide Technology System). SX-1552 is a member of a new class of synthetic auxins in the arylpicolinate herbicide family. Studies of *Arabidopsis thaliana* with mutations in select auxin-binding receptor proteins, along with direct molecule-protein interaction testing of these same receptor proteins, support that arylpicolinate chemistry including SX-1552 has a different binding affinity versus 2,4-D and other currently registered synthetic auxin herbicides (Walsh et al. 2006, Villalobos et al. 2012, Lee et al. 2013, Bell et al. 2015). In preliminary screening, SX-1552 exhibited strong activity on several problematic U.S. aquatic plants, including the submersed weeds hydrilla and EWM, the free-floating weed waterhyacinth, and floating leaf weed crested floatingheart (M. D. Netherland and R. J. Richardson, unpub. data). SX-1552 would represent a new mode of action for hydrilla control and a number of other important aquatic weed management uses. The objective of this study was to evaluate the activity of SX-1552 and SX-1552A—a less-active acid metabolite—against seven aquatic plant species using a small-scale screening method under greenhouse conditions to confirm activity and potential utility of SX-1552 as an aquatic herbicide. SX-1552A was also evaluated because it is a major primary metabolite and has herbicidal activity.

MATERIALS AND METHODS

Propagation

Seven species were propagated for this evaluation: alligatorweed [*Alternanthera philoxeroides* (Mart.) Griseb.], fanwort (*Cabomba caroliniana* A. Gray), Carolina waterhyssop [*Bacopa caroliniana* (Walt.) B.L. Robins.], monoecious hydrilla, parrotfeather [*Myriophyllum aquaticum* (Vell.) Verdc.], variable watermilfoil (*Myriophyllum heterophyllum* Michx.), and American waterwillow [*Justicia americana* (L.) Vahl.]. Laboratory stock plants were used for the propagation of alligatorweed and parrotfeather. Variable watermilfoil shoot tissue, monoecious hydrilla subterranean turions, and American waterwillow stems were field-collected from local North Carolina sources. Carolina waterhyssop[3,4] and fanwort[5] were purchased from commercial sources. Alligatorweed, parrotfeather, and American waterwillow shoot tips were cut to approximately 15 cm long. These tips were first stored upright in dechlorinated tap water. Following the production of viable root tissue, tips were planted in soil and submersed in dechlorinated tap water for establishment. Approximately 10-cm sections of variable watermilfoil and fanwort shoot tissue were cut and immediately planted in soil and submersed in dechlorinated tap water for establishment. Carolina waterhyssop, purchased from an aquarium plant dealer, was first submersed in dechlorinated water with the roots in the nutrient gel provided by the dealer. The nutrient gel was removed after 1 wk, and shoots were then planted in soil and submersed in dechlorinated tap water for establishment. Monoecious hydrilla subterranean turions were collected at Lake Gaston, NC, and stored at 4 C before sprouting in dechlorinated tap water. Sprouted turions were planted in soil and submersed in dechlorinated tap water for establishment. All propagules were planted in 3 oz (89 ml) pots, filled with lake sediment collected from Roanoke Rapids Lake, NC. Collected soil was sifted to remove debris and propagules and homogenized before filling pots. After propagules of test species were planted, a thin layer of fine sand was placed over the lake sediment. Plants were allowed to establish for 1 wk after planting in soil. Experimental mesocosm size was 15 L, with plastic liner in each container. All mesocosms were maintained in a temperature-controlled, poly-covered greenhouse, with minimum temperature of 26 C.

Treatment

Each species underwent a 4-wk static exposure of 0, 0.3, 1, 3, 9, 27, or 81 $\mu g\ L^{-1}$ of SX-1552 or of SX-1552A,[6] the acid metabolite. Because of the limited maturity of tested plants, competition between plants did not appear to affect the growth of plants. Treatments were arranged into a randomized complete-block design with four replicates. The experiment was conducted twice, nonconcurrently, to confirm consistent results.

Data collection and analysis

Percentage of control of the treated plants was compared with untreated controls and was assessed visually at 2 and 4 wk after treatment (WAT). Plants were rated on a scale of 0 (no signs of impact) to 100% control (no living shoot tissue remaining). Intermediate symptomology of treatment varied by species and included evaluations of shoot swelling, stem twisting, leaflet curling, chlorosis, and tissue death. Visual observations are described, but data are not presented. The total length of all living shoot tissue was measured in millimeters before treatment and again after 4 wk of exposure. Because of tissue damage following herbicide treatment, intermediate measurements of living shoot tissue were determined to be too destructive to the remaining live tissue, and as such, only pretreatment and posttreatment measures were collected. Four weeks after treatment, above-sediment shoot biomass was harvested for both fresh-weight and dry-weight determination. The fresh biomass of all tissue harvested for each plant was measured within 2 h of harvesting, using a laboratory balance with 0.001-g accuracy. Shortly after harvest, excess moisture was allowed to drain from plant biomass. Following fresh-weight measurement, plant samples were placed in labeled paper bags for drying. Plant samples were dried to a constant mass at 60 C. The biomass of the dried plant tissue was again measured on a laboratory balance with 0.001-g accuracy.

Water samples were collected using glass instrumentation and stored in amber-color glass vials. Methanol (1.5 ml) was placed in each vial before collection of 29 ml sample water. Formic acid (1.2 ml) was titrated into the vial after collection to prevent potential hydrolytic degradation of SX-1552 by achieving approximate pH 3. After collection and acidification, samples were stored in a laboratory grade freezer at −5 C. Frozen samples were then shipped overnight on ice to EPL Bio Analytical Services (Ninantic, IL), for analysis via liquid chromatography with mass spectroscopy in a dedicated method developed for analysis of SX-1552 and its major metabolites in water in support of registration studies (EPL Method 477G696A-1, unpubl. data). Samples were collected from the first replicate of 3 $\mu g\ L^{-1}$, 9 $\mu g\ L^{-1}$, and 81 $\mu g\ L^{-1}$ concentrations for SX-1552 immediately after treatment to verify target concentrations. Mean starting concentrations were within 10% of target rates.

Water temperature and pH measurements were collected using a YSI field probe.[7] Measurements were made before treatment and weekly thereafter. Measurements were collected from all replicates of the untreated control, 9 $\mu g\ L^{-1}$, and 81 $\mu g\ L^{-1}$ treatment chambers before treatment and during the final percentage of control evaluation. Interim temperature and pH measurements were collected only from the replicates of the untreated control chambers.

All data were subjected to ANOVA in SAS software.[8] No significant treatment by experiment interactions were observed; therefore, data were pooled over experiments. Shoot length, fresh weight, and dry weight were converted to percentage of inhibition of the untreated control and then subjected to regression analysis along with visual control. The nonlinear equation $y = a(1 - \exp^{-bx})$ was used for all models in SigmaPlot software.[9] This model was used because it converged across all data sets, whereas the three- and four-parameter logistic equations evaluated did not. The 50% effective concentration (EC_{50}) concentrations were then determined for each regression model. In addition, a Dunnett's test ($\alpha = 0.05$), comparing biomass of treated plants to the nontreated control, was used to determine the lowest observed effect concentration (LOEC).

RESULTS AND DISCUSSION

Alligatorweed was sensitive to both SX-1552 and SX-1552A (Figure 1). Treatment symptomology on alligatorweed included increased stem growth, limited chlorosis, and stem swelling at and below the surface of the water, and progressed to tissue necrosis and plant death. Visual symptoms were observed at 2 WAT with SX-1552, whereas response to the acid form occurred more slowly (data not presented). At 4 wk after treatment, SX-1552 EC_{50} values ranged 0.96 to 1.8 $\mu g\ L^{-1}$, whereas SX-1552A EC_{50} values ranged 9.7 to 17.8 $\mu g\ L^{-1}$, indicating less sensitivity to the acid form (Table 1). Dry weight LOEC values were 1 and 9 for SX-1552 and SX-1552A, respectively.

Previous research has indicated that triclopyr may reduce the biomass of young alligatorweed plants (Hofstra and Clayton 2010) and that quinclorac may provide moderate control in a greenhouse setting (Kay 1992). Alligatorweed is generally not controlled by 2,4-D, which has been attributed to poor basipetal translocation (Earle et al. 1951). The control observed with SX-1552 was greater than would have been expected from either triclopyr or 2,4-D.

Carolina waterhyssop response was generally similar to alligatorweed with the plant being distinctly more sensitive to SX-1552 than SX-1552A (Figure 1). SX-1552A symptomology was minor at 2 wk after treatment but was more pronounced by 4 wk after treatment (data not presented). SX-1552 EC_{50} values ranged from 3.2 to 5.0 $\mu g\ L^{-1}$ (Table 1). SX-1552A EC_{50} values ranged from 9.7 to 17.8 $\mu g\ L^{-1}$. At SX-1552, rates of 9.7 $\mu g\ L^{-1}$ and greater, Carolina waterhyssop response progressed to eventual tissue and plant death. However, at rates lower than 3 $\mu g\ L^{-1}$. leaves were initially abscised, but some leaf tissue regrowth had occurred by trial conclusion. Conversely, Carolina waterhyssop plants exposed to low < 3 µg/L SX-1552A rates did not lose foliage. This plant response likely explains the disparity between shoot and weight inhibition EC_{50} values for SX-1552A. LOEC values were 9 µg/L for SX-1552 and 27 µg/L for SX-1552A again supporting better activity from the SX-1552 molecule (Table 1).

Unlike the other species evaluated, fanwort was not sensitive even with the static 4-wk exposure (Figure 1). Symptomology observed at the highest exposure rates included curling of young leaves and progressed to limited stem epinasty. Our evaluated rates were not sufficient to generate EC_{50} or LOEC values, and this is consistent with previous research on fanwort sensitivity to auxin mimics. Bultemeir et al. (2009) reported that 2,4-D, quinclorac, and triclopyr (maximum test rates of 4,400, 400, and 4,900 µg/L, respectively) did not reduce fanwort photosynthesis by 50%. Because of the relative tolerance of cabomba to synthetic auxins, there is no need to evaluate a broader rate range of SX-1552 to generate an EC_{50} value unless registered use rates will exceed 81 $\mu g\ L^{-1}$.

Monoecious hydrilla was sensitive to both SX-1552 and SX-1552A (Figure 1). EC_{50} values for all data at 4 WAT ranged from 0.71 to 1.6 $\mu g\ L^{-1}$, whereas the LOEC was 3 µg

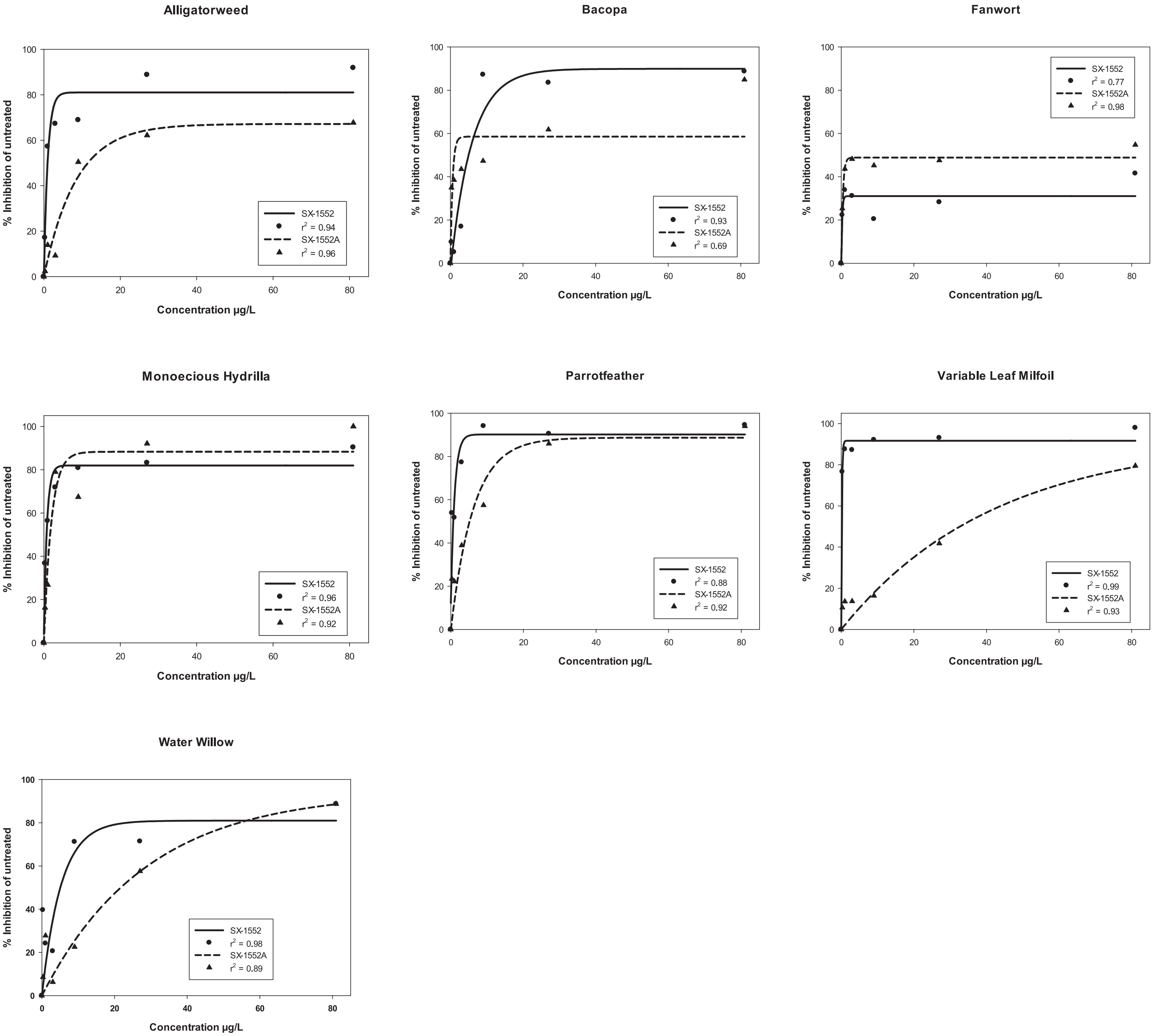


Figure 1. Plant dry weights at 4 wk after static exposure of SX1552 and SX-1552A at 0, 0.3, 1, 3, 9, 27, and 81 µg L^{-1} expressed as the percentage of inhibition of the untreated control. Regression analysis performed using the nonlinear equation $y = a[1 - \exp(^{-bx})]$.

L^{-1} (Table 1). Visual symptoms did progress from 2 to 4 WAT with both SX-1552 and SX-1552A (data not presented). Symptomology consisted of leaf pigmentation changes (purpling) and stunted growth, progressing to leaf curling, chlorotic/necrotic tissue, and eventual plant death. Hydrilla stem tissue also became fragile to touch and broke easily at nodes as symptomology progressed. Although hydrilla (like many other monocots) is commonly known to be tolerant of the synthetic auxins 2,4-D and triclopyr, quinclorac has been reported to provide significant control of hydrilla (Zawierucha et al. 2006). Our results are also consistent with those of (M. D. Netherland and R. J. Richardson, In Press), who found dioecious hydrilla EC_{50} values of 1.7 to 6.8 µg L^{-1} with both SX-1552 and SX-1552A. SX-1552 could provide a new mode of action for resistance management in control efforts for dioecious hydrilla (fluridone- and endothall-resistant dioecious biotypes have been detected in Florida (Michel et al 2004, APMS 2014, M. D. Netherland and R. J. Richardson, In Press) and also provide a new pattern of selectivity for removing hydrilla from mixed aquatic-plant communities. Future research should be conducted to determine this pattern of selectivity as well as the necessary concentration exposure time for both hydrilla biotypes.

TABLE 1. CALCULATED 50% EFFECTIVE CONCENTRATION (EC_{50}) VALUES FOR SEVEN AQUATIC PLANTS TREATED WITH SX-1552 AND SX-1552A AT CONCENTRATIONS RANGING FROM 0.3 TO 81 PARTS PER BILLION; VALUES WERE DERIVED FROM NONLINEAR REGRESSION ANALYSIS OF SHOOT LENGTH, FRESH WEIGHT, AND DRY WEIGHT CONVERTED TO PERCENTAGE OF INHIBITION OF THE UNTREATED PLANTS USING THE EQUATION $y = a[1 - \exp(-bx)]$, AND THE LOWEST OBSERVED EFFECT CONCENTRATION (LOEC) WAS DERIVED VIA DUNNETT'S TEST ($\alpha = 0.05$).

	EC_{50} Values ($\mu g\ L^{-1}$)–SX1552			SX-1552 ($\mu g\ L^{-1}$)	EC_{50} Values ($\mu g\ L^{-1}$) – SX1552A			SX-1552A ($\mu g\ L^{-1}$)
Species	Shoot Inhibition	Fresh wt Inhibition	Dry wt Inhibition	Dry wt LOEC	Shoot Inhibition	Fresh wt Inhibition	Dry wt Inhibition	Dry wt LOEC
Alligatorweed	1.37	1.8	0.96	1	15.8	17.8	9.7	9
Carolina waterhyssop	3.2	3.7	5.0	9	2.5	36.1	12.2	27
Carolina fanwort	> 81	> 81	> 81	> 81	> 81	> 81	> 81	> 81
Monoecious hydrilla	1.32	0.94	0.71	3	1.2	1.4	1.6	3
Parrotfeather	< 0.3	< 0.3	0.68	0.3	10.5	6.0	6.9	9
Variable watermilfoil	< 0.3	<0 .3	< 0.3	0.3	21.3	33.5	35.1	27
American waterwillow	1.4	9.3	5.1	9	74.8	59.1	77.7	81

The two milfoil species, parrotfeather and variable watermilfoil, were the most sensitive species evaluated to SX-1552 (Figure 1). Symptomology occurred within 1 WAT, particularly in plants treated with SX-1552, and rapidly increased. Increased stem growth and epinasty were the first observed symptoms, but this quickly progressed to tissue necrosis and plant death. Our rate range was generally not low enough to calculate SX-1552 EC_{50} values for most parameters, although dry weight inhibition of parrotfeather was 0.68 $\mu g\ L^{-1}$ (Table 1). Both plants were more tolerant to SX-1552A because parrotfeather had EC_{50} values of 6.0 to 10.5 $\mu g\ L^{-1}$ whereas variable watermilfoil had EC_{50} values of 21.3 to 35.1 $\mu g\ L^{-1}$ across plant-growth data. LOEC values for SX-1552 was 0.3 $\mu g\ L^{-1}$ on both species and 9 and 21 for SX-1552A on parrotfeather and variable milfoil, respectively. Progression of visual symptoms was also observed with both species from 2 to 4 WAT (data not presented).

The sensitivity of milfoil species to synthetic auxins is well documented. M. D. Netherland and R. J. Richardson (In Press) found Eurasian watermilfoil EC_{50} values of 0.17 to 1.4 $\mu g\ L^{-1}$ for SX-1552 and SX-1552A. Numerous other researchers have previously described sensitivity of Eurasian watermilfoil, parrotfeather, and variable watermilfoil to the synthetic auxins 2,4-D and triclopyr (Netherland and Getsinger 1992; Sutton and Bingham 1970; Parsons et al. 2001; Getsinger et al. 2003; Hofstra et al. 2006; Poovey et al. 2007; Haug and Bellaud 2013). Thus, *Myriophyllum* species are likely to be among the most sensitive to SX-1552, and these species may be significantly injured in SX-1552 treatment areas.

American waterwillow was more sensitive to SX-1552 than it was to SX-1552A (Figure 1). EC_{50} values ranged 1.4 to 9.3 $\mu g\ L^{-1}$ for SX-1552 and 59.1 to 77.7 $\mu g\ L^{-1}$ for SX-1552A, which was the largest difference in response among species evaluated (Table 1). Likewise, LOEC values were 9 and 81 $\mu g\ L^{-1}$ for SX-1552 and SX-1552A, respectively. In Piedmont Reservoirs, NC, American waterwillow is one of the most important native species, and hydrilla one of the most significant invaders. The difference in plant response between these species makes it likely that SX-1552 could selectively remove hydrilla from American waterwillow beds, a necessity for this use pattern.

Our results indicate that SX-1552 has the potential to control several important North American weed species. The strong activity of this new mode of action herbicide observed for monoecious hydrilla supports its development for selective hydrilla control. Additional high activity on invasive/nuisance milfoils, such as parrotfeather and variable watermilfoil, also support potential future fit in selective control of these species. The 4-wk static exposure used in these small-scale trials may overestimate control that could be obtained in field situations where plant establishment and degradation/dilution in typical partial treatment designs will reduce achieved exposure and can reduce efficacy. However, 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. In-Press. http://dx.doi.org/10.1614/WS-D-15-00092.1 showed that static greenhouse treatments of well-established Eurasian watermilfoil with SX-1552 provided control at similar $\leq$ 1 part per billion rates as observed in small-scale testing, similar to that presented here. Current results provide a good baseline for the establishment of CET protocols on more established plants necessary to fully develop field use patterns. Similar to use of currently registered auxin-mimic herbicides, focus should concentrate on partial treatment designs as these are expected to be the primary approach for potential use of SX-1552. The four week exposure also provided an important detail on the acid form; control of all species except cabomba increased from two to four weeks. In addition to CET trials, future research should also evaluate the sensitivity of additional target and nontarget, submersed plants so that a complete use pattern guidelines can be developed.

SOURCES OF MATERIALS

[1]SX-1552 SePRO Corporation, 11550 North Meridian Street, Suite 600, Carmel, IN 4603.

[2]SX-152A SePRO Corporation, 11550 North Meridian Street, Suite 600, Carmel, IN 46032.

[3]Carolina waterhyssop for run 1, The Fish Room, 1259 Kildaire Farm Road, Cary, NC 27511.

[4]Carolina waterhyssop for run 2, PetSmart, 2430 Walnut Street, Cary, NC 27518.

[5]Fanwort, LiveAquaria.com, 2253 Air Park Road, Rhinelander, WI 54501.

[6]SX-1552 and SX-1552A, SePRO Corporation, 11550 N. Meridian Street, Suite 600, Carmel, IN 46032.

[7]Field probe model 556, YSI, 1700/1725 Brannum Lane, Yellow Springs, OH 45387-1107.

[8]Statistical software, version 9.3, SAS Institute, 100 SAS Campus Drive, Cary, NC 27513-2414.

[9]SigmaPlot software, version 12.0, SigmaPlot Software, 225 W. Washington Street, Suite 425, Chicago, IL 60606.

ACKNOWLEDGEMENTS

The authors would like to acknowledge the following members of the NCSU Aquatic Weed Science Laboratory for their efforts with data collection: Shannon Auell, Evan Calloway, Tyler Harris, Andrew Howell, Steven Hoyle, Amy Miller, and Stephanie Nawrocki.

LITERATURE CITED

[APMS] Aquatic Plant Management Society. 2014 Herbicide Resistance Stewardship in Aquatic Plant Management. http://apms.org/wp/wp-content/uploads/2014/04/Herbicide-Resistance-Stewardship-in-Aquatic-Plant-Management.pdf. Accessed September 1, 2015.

Belgers JDM, Van Lieverloo RJ, Van der Pas LJ, Van den Brink PJ. 2007 Effects of the herbicide 2, 4-D on the growth of nine aquatic macrophytes. Aquat. Bot. 86(3):260–268.

Bell JL, Schmitzer R, Weimer MR, Napier RM, Prusinska JM. 2015 Mode-of-action analysis of a new arylpicolinate herbicide from Dow AgroSciences [Abstract]. In: Proceedings of the Weed Science Society of America Annual Meeting. WSSA, Lawrence, KS. http://wssaabstracts.com/public/30/abstract-290.html. Accessed August 1, 2015.

Berger ST, Netherland MD, MacDonald GE. 2015. Laboratory documentation of multiple-herbicide tolerance to fluridone, norflurazon, and topramezone in a hybrid watermilfoil (*Myriophyllum spicatum* × *M. sibiricum*) population. Weed Sci. 63(1):235–241.

Bultemeier BW, Netherland MD, Ferrell JA, Haller WT. 2009. Differential response among three phenotypes of *Cabomba caroliniana*. Inv. Plant Sci. Mgt. 2(4):352–359.

Cedergreen N, Streibig JC. 2005. The toxicity of herbicides to non-target aquatic plants and algae: Assessment of predictive factors and hazard. Pest Manag. Sci. 61:1152–1160.

Cobb AH. 1992. Auxin-type herbicides, pp. 82–106. In: Herbicides and Plant Physiology. Chapman and Hall, London, UK.

Earle TT, Riess K, Hidalgo J. 1951. Tracer studies with alligator weed using 2,4-D-C14. Science 28:695–696.

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 Manage. 46:32–41.

Getsinger KD, Netherland MD, Turner EG. 1993. Fluridone concentration and exposure time requirements for control of Eurasian watermilfoil and hydrilla. J. Aquat. Plant Manage. 31:189–194.

Gilbert FA. 1946. The status of plant growth substances and herbicides in 1945. Chem. Rev. 39:199–218.

Glomski LM, Netherland MD. 2010. Response of Eurasian and hybrid watermilfoil to low use rates and extended exposures of 2,4-D and triclopyr. J. Aquat. Plant Manage. 48:12–16.

Grossmann K. 2010. Auxin herbicides: Current status of mechanism and mode of action. Pest management science 66(2):113–120.

Hofstra DE, Clayton JS. 2001. Evaluation of selected herbicides for the control of exotic submerged weeds in New Zealand, I: The use of endothall, triclopyr and dichlobenil. J. Aquat. Plant Manage. 39:20–24.

Hoyer MV, Netherland MD, Allen MS, Canfield DE. 2005. Hydrilla Management in Florida: A Summary and Discussion of Issues Identified by Professionals with Future Management Recommendations. University of Florida LAKEWATCH. 68 pp. http://myfwc.com/media/1183545/InvasivePlants__HydMgtChlngs06.pdf. Accessed August 1, 2015.

Kay SH. 1992. Response of two alligatorweed biotypes to quinclorac. J. Aquat. Plant Manage. 30:35–40.

LaRue EA, Zuellig MP, Netherland MD, Heilman MA, Thum RA. 2013. Hybrid watermilfoil lineages are more invasive and less sensitive to a commonly used herbicide than their exotic parent (Eurasian watermilfoil). Evol. Appl. 6:462–471. doi: 10.1111/eva.12027

Lee S, Sundaram S, Armitage L, Evans JP, Hawkes T, Kepinski S, Ferro N, Napier RM. 2013. Defining binding efficiency and specificity of auxins for SCFTIR1/AFB-Aux/IAA co-receptor complex formation. ACS (Am. Chem. Soc.) Chem. Biol. 9(3):673–682.

Madsen JD, Wersal RM. 2009. Aquatic plant community and Eurasian watermilfoil (*Myriophyllum spicatum* L.) management assessment in Lake Pend Oreille, Idaho for 2008. Geosystems Research Institute Report 5032.

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.

Netherland MD, Getsinger KD. 1992. Efficacy of triclopyr on Eurasian watermilfoil: Concentration and exposure time effects. J. Aquat. Plant Manage. 30:1–5.

Netherland MD, Getsinger KD, Skogerboe JD. 1997. Mesocosm evaluation of the species-selective potential of fluridone. J. Aquat. Plant Manage. 35, 41–50.

[NPIRS] National Pesticide Information Retrieval System. 2015. Purdue University. http://ppis.ceris.purdue.edu/. Accessed Sept. 9. 2015.

Parsons JK, Hamel KS, Madsen JD, Getsinger KD. 2001. The use of 2,4-D for selective control of an early infestation of Eurasian watermilfoil in Loon Lake, Washington. J. Aquat. Plant Manage. 39:117–125.

Poovey AG, Slade JG, Netherland MD. 2007. Susceptibility of Eurasian watermilfoil (*Myriophyllum spicatum*) and a milfoil hybrid (*M. spicatum* × *M. sibiricum*) to triclopyr and 2, 4-D amine. J. Aquat. Plant Manage. 45:111–115.

Richardson RJ. 2008. Aquatic plant management and the impact of emerging herbicide resistance issues. Weed Technol. 22(1):8–15.

Sutton DL, Bingham SW. 1970. Uptake and translocation of 2,4-D-1-14C in parrot feather. Weed Sci. 18:193–196.

Villalobos LI, Lee AC, De Oliveira S, Ivetac C, Brandt A, Armitage Sheard WL, Tan LB, Parry X, Mao G, Zheng H, Napier N, Kepinski RM, Estelle M. 2012. A combinatorial TIR1/AFB–Aux/IAA co-receptor system for differential sensing of auxin. Nat. Chem. Biol. 8(5), 477–485.

Walsh TA, Neal R, Merlo AO, Honma M, Hicks GR, Wolff K, Matsumura W, Davies, JP. 2006. Mutations in an auxin receptor homolog AFB5 and in SGT1b confer resistance to synthetic picolinate auxins and not to 2, 4-dichlorophenoxyacetic acid or indole-3-acetic acid in *Arabidopsis*. Plant Physiol. 142(2):542–552.

Wersal RM, Madsen JD, Woolf TE, Eckberg N. 2010. Assessment of herbicide efficacy on Eurasian watermilfoil and impacts to the native submersed plant community in Hayden Lake, Idaho. J. Aquat. Plant Manage. 48:5–11.

Willey LN, Netherland MD, Haller WT, Langeland KA. 2014. Evaluation of aquatic herbicide activity against crested floating heart. J. Aquat. Plant Manage. 52:47–56.

Woodward AW, Bartel B. 2005. Auxin: Regulation action and interaction. Ann. Bot. 95:797–735.

Yamada SL. 1954. Auxin relationships of the rice coleoptile. Plant physiology 29(1):92.

Zawierucha J, Oliver GW, Evans RR, Horton T, Beran DD, Vollmer JG, Burns AJ, Birk JH, Miller DW, Knight TP. 2015. Method for controlling aquatic weeds. US patent number: 9,060,516. Assignee: BASF SE. Filed Dec. 20, 2006 and issued June 23, 2015.

ProcellaCOR on American lotus (*Nelumbo lutea*) – Lewisville TX – Summer 2018

Demonstration treatment with the US Army ERDC – Aquatic Plant Control Research Program

ProcellaCOR was applied into water (not foliar spray) of 0.65 acre pond (3.8 foot average depth) at 5 PDU EC per A-ft equivalent (9.7 µg ai per L) on June 12, 2018. Control outcome was rated on July 17 and again on August 20.

Lotus coverage

- June 12 (day of treatment) 95% healthy lotus coverage (photo not available)
- July 17 30% viable lotus coverage (photo – Fig 1 below)
- August 20 10% viable lotus coverage (photo – Fig 2 below)

Fig 1. TX lotus pond condition on July 17, 2018 at 5 weeks after ProcellaCOR application.

Fig 2. TX lotus pond condition on August 20, 2018 at 5 weeks after ProcellaCOR application.

ProcellaCOR Mesocosm Trial on White Water Lily (*Nymphaea odorata*) and two species of Bulrush (*Schenoplectus tabernaemontani* and *S. californicus*) – Lewisville TX – Summer 2016

Cooperative study with the US Army ERDC – Aquatic Plant Control Research Program

ProcellaCOR was applied as an in-water application to large outdoor mesocosm tanks (water depth 0.6m or 2 feet, volume ~3,400 liters) containing potted specimens of lily and the two bulrushes. Treatment scenarios were similar to Beets et al. 2019 with multiple concentration-exposure scenarios achieved through flow of untreated water through the tanks to provide a dilution half-life for most treatments.

PDU EC equivalent (μg a.i. L^{-1})	Exposure Time (flowthrough dilution half-life)
4.7 PDU (9)	6 hours
14 PDU (27)	6 hours
1.6 PDU (3)	24 hours
4.7 PDU (9)	24 hours
14 PDU (27)	24 hours
1.6 PDU (3)	Static
4.7 PDU (9)	Static
14 PDU (27)	Static

Shoot dry biomass was measured at 1 and 2 months after treatment. Two-month harvest results are provided here (Figures 1, 2) and similar results were observed at 1-month harvest. White water lily was sensitive to a 14 PDU rate under the 6h short exposure but not to the 4.7 PDU rate with short exposure. At intermediate to static exposures at 4.7 PDU, an upper end rate for invasive watermilfoil management, lily biomass was also reduced but not as strongly as higher CET scenarios and healthy re-growth was noted by the end of the study. The lower 1.6 PDU rate did not produce biomass reductions and only minor short-term symptoms were observed. The results show the potential for short-term lily injury and some reductions in surface coverage with higher rates and extended exposures to ProcellaCOR. These effects should be confined tightly to the direct area of management and field application designs and final rate selection should be able to mitigate potential lily stress under most conditions. Lily symptoms and short-term reductions are common for other WSSA Group 4 aquatic herbicides (e.g., triclopyr) so these responses are not surprising.

The two bulrushes were not impacted by any CET scenario in this study, and 2018 field responses confirm tolerance.

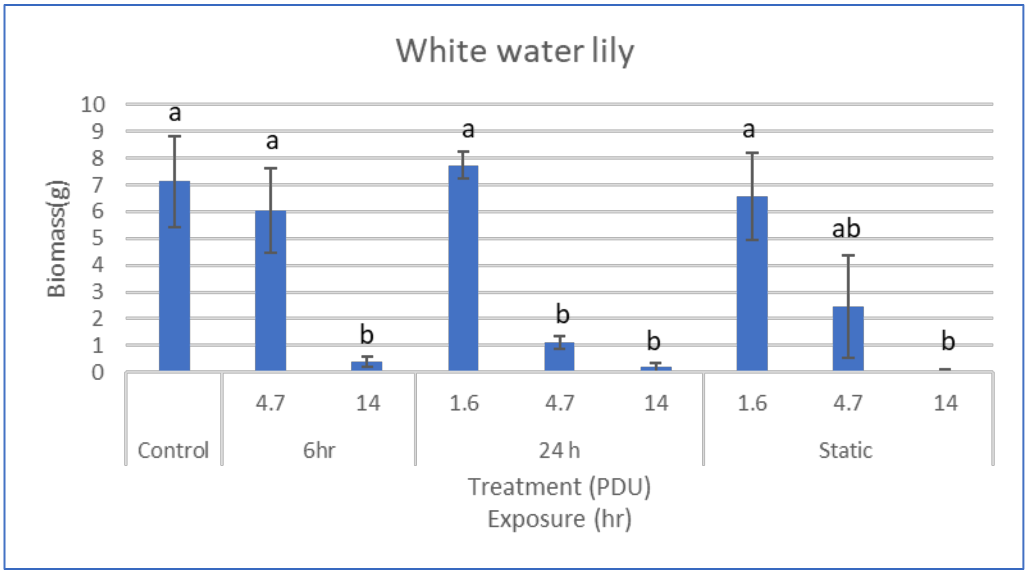


Figure 1. Dry shoot (aboveground) biomass of white water lily at 2 months after various mesocosm exposures to ProcellaCOR at rates equivalent to of 1.6 to 14 PDU EC per A-ft. Error bars are +/- 1SD (n = 3). Letters show significant difference between treatments (Tukey HSD; p = 0.05).

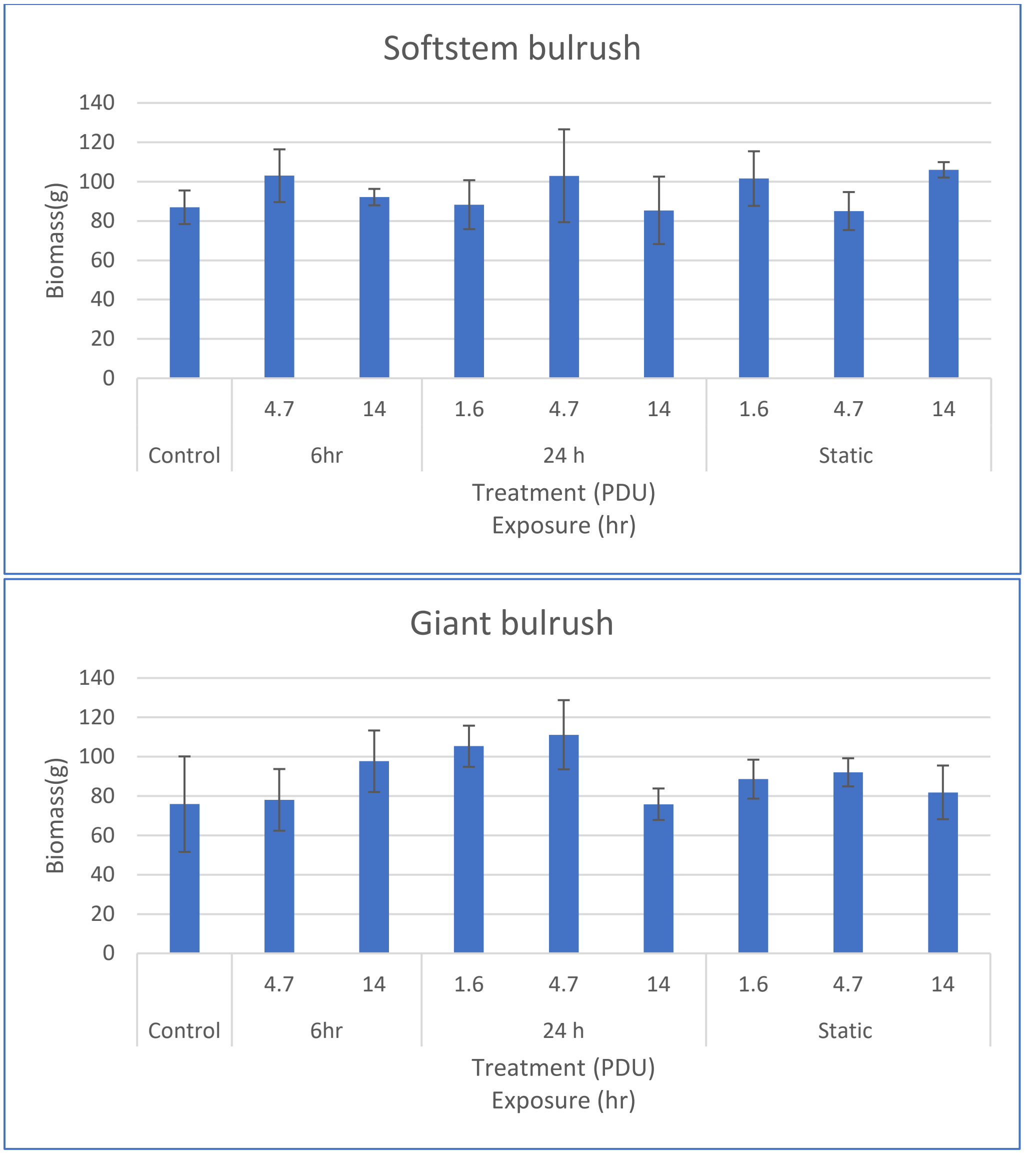


Figure 2. Dry shoot (aboveground) biomass of softstem bulrush (upper graph) and giant bulrush (low graph) at 2 months after various mesocosm exposures to ProcellaCOR at rates equivalent to of 1.6 to 14 PDU EC per A-ft. Error bars are +/- 1SD (n=3).