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Response of seven aquatic plants to a new arylpicolinate herbicide

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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 μg 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 μg 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 μg L^{-1}, respectively. These six species were less sensitive to SX-1552A with dry weight EC_{50} values of 1.6 to 77.1 μg 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

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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 μg 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 μg L^{-1}, 9 μg L^{-1}, and 81 μg 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 μg L^{-1}, and 81 μg 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 μg L^{-1}, whereas SX-1552A EC_{50} values ranged 9.7 to 17.8 μg 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 μg L^{-1} (Table 1). SX-1552A EC_{50} values ranged from 9.7 to 17.8 μg L^{-1}. At SX-1552, rates of 9.7 μg L^{-1} and greater, Carolina waterhyssop response progressed to eventual tissue and plant death. However, at rates lower than 3 μg 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 μg 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 μg L^{-1}, whereas the LOEC was 3 μg

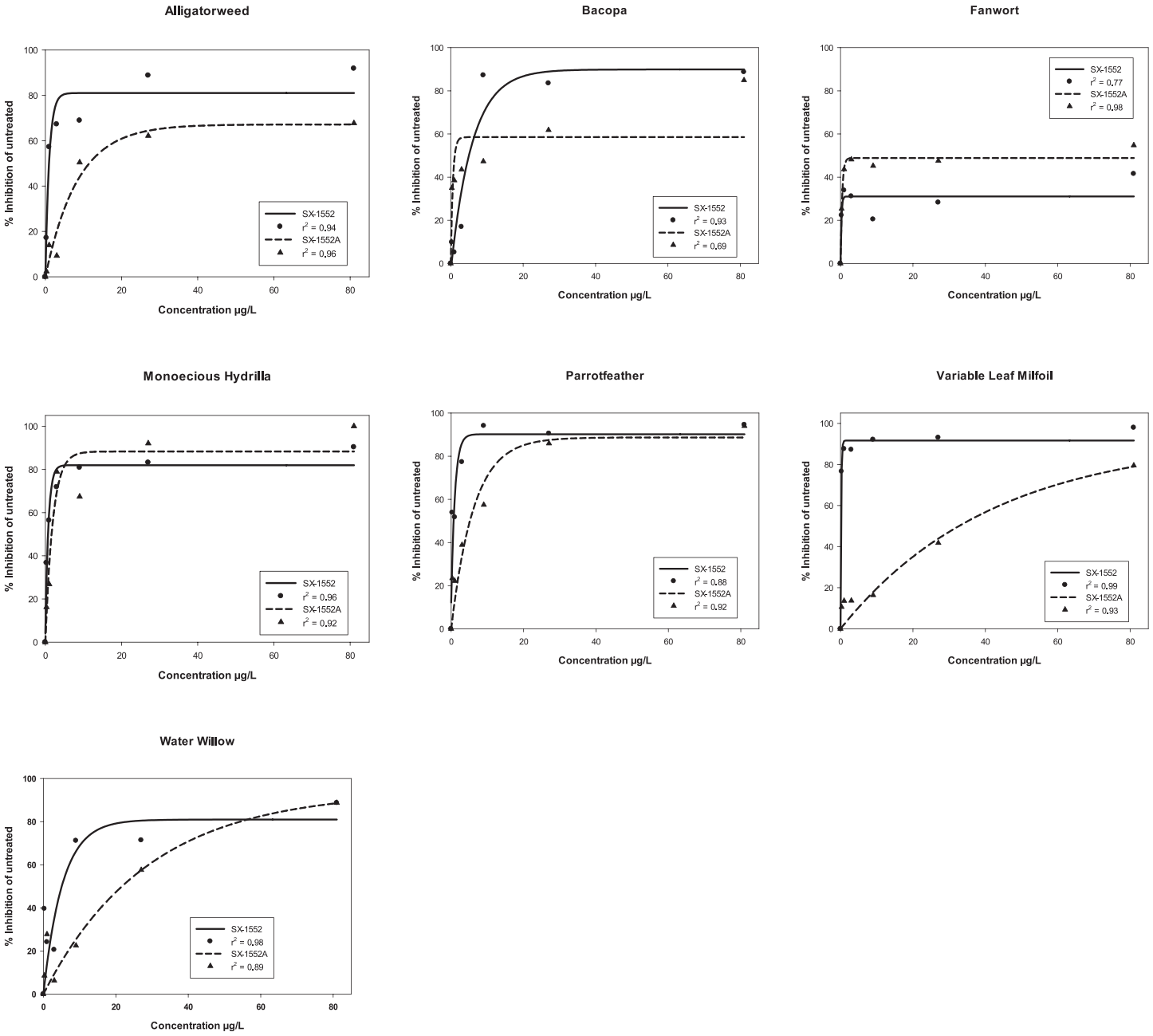

Figure 1. Plant dry weights at 4 wk after static exposure of SX1552 and SX-1552A at 0, 0.3, 1, 3, 9, 27, and 81 µg L^{-1} expressed as the percentage of inhibition of the untreated control. Regression analysis performed using the nonlinear equation $y = a[1 - \exp(^{-bx})]$.

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 (μg L^{-1})–SX1552			SX-1552 (μg L^{-1})	EC_{50} Values (μg L^{-1}) – SX1552A			SX-1552A (μg 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 μg L^{-1} (Table 1). Both plants were more tolerant to SX-1552A because parrotfeather had EC_{50} values of 6.0 to 10.5 μg L^{-1} whereas variable watermilfoil had EC_{50} values of 21.3 to 35.1 μg L^{-1} across plant-growth data. LOEC values for SX-1552 was 0.3 μg 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 μg 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 μg L^{-1} for SX-1552 and 59.1 to 77.7 μg L^{-1} for SX-1552A, which was the largest difference in response among species evaluated (Table 1). Likewise, LOEC values were 9 and 81 μg 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.

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