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Utilizing a Small-scale Primary Screening Method to Evaluate Activity of the Bleaching Herbicide Topramezone on Invasive and Native Submersed Plants

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PURPOSE: When evaluating potential use patterns of new aquatic herbicides, it is important to determine concentrations that impact target as well as non-target vegetation. Several new herbicide modes of action are currently being evaluated in the aquatic market, and efficacy and non-target plant selectivity are both important factors in developing a use pattern. Conventional indoor walk-in growth chamber and mesocosm studies require large spaces, significant time (2 to 4 months) and labor resources, and study designs are typically limited by the number of aquariums or mesocosm tanks in the study space. Given the large number of new herbicides being evaluated for aquatic use, a small-scale screening method was evaluated at the U.S. Army Engineer Research and Development Center (ERDC) to determine its potential pros and cons. The effect of a range of concentrations of the bleaching herbicide topramezone ([3-(4,5-dihydro-3-isoxazolyl)-2-methyl-4-(methylsulfonyl)phenyl](5-hydroxy-1-methyl-1*H*-pyrazol-4-yl)methanone) was evaluated on eight species of submersed aquatic plants over a 2- to 3-week period.

BACKGROUND: With the discovery of fluridone-resistant hydrilla (*Hydrilla verticillata* (L.f.) Royle) in Florida (Arias et al. 2005; Michel et al. 2004) several new herbicide modes of action have been evaluated for hydrilla control including acetolactate synthase (ALS), protoporphyrinogen oxidase (protox) and 4-hydroxyphenylpyruvate dioxygenase (4-HPPD) inhibitors. Given the 21-year lag between fluridone registration in 1986 and penoxsulam (ALS inhibitor) registration in 2007, the introduction of five new herbicide active ingredients with activity on hydrilla represents a significant challenge for research facilities to conduct the necessary efficacy and selectivity screening to aid in use pattern development.

The 4-HPPD inhibitor topramezone is currently being evaluated for aquatic use. Similar to fluridone, topramezone is a bleaching herbicide that targets a plant-specific enzyme; therefore, at proposed use rates it possesses low toxicity to mammals, fish, and invertebrates (Weed Science Society of America (WSSA) 2007). Topramezone was first registered for use in corn in 2006 and provides post-emergent control of many broadleaf and grass weeds (Grossman and Ehrhardt 2007). Topramezone inhibits the enzyme 4-HPPD thereby blocking the production of plastoquinone, an essential cofactor for phytoene desaturase (Grossmann and Ehrhardt 2007; Norris et al. 1995). Bleaching symptoms appear on new growth as an indirect result of carotenoid synthesis inhibition (WSSA 2007). Plants tolerant to topramezone have either a lower sensitivity of the 4-HPPD enzyme or selective metabolism (Grossmann and Ehrhardt 2007); however, topramezone is equally effective against fluridone-resistant and fluridone sensitive strains of hydrilla (Puri et al. 2009). To determine the

potential activity of topramezone on submersed aquatic plants, small-scale assays were conducted on three target and five non-target species.

MATERIALS AND METHODS: Small-scale screening was conducted to determine the activity of topramezone on native and non-native submersed plants. To determine the predictive potential of the small-scale screening, a follow-up large-scale greenhouse study was conducted on hydrilla. All trials were conducted at the ERDC Lewisville Aquatic Ecosystem Research Facility.

Small-scale screening. Methods similar to Netherland and Lembi (1992) were used to determine the effect of topramezone on both native and non-native submersed aquatic plants. Studies were conducted over a 4-month period using a Percival E-36L2 growth chamber set at 22 to 25 °C (depending on plant species) and a 16:8 light:dark photoperiod. Light intensity was 182 µmole photons m^{-2} s^{-1}. One 4-cm apical stem segment or one small daughter plant of wild celery was incubated in 150 mL of 10% Hoaglands growth media (Hoagland and Arnon 1950), with the addition of sodium bicarbonate, in each 250-mL flask. Flasks were treated at 0, 6, 12, 24, 48, 96, and 192 µg L^{-1} active ingredient (ai) topramezone. Native species tested were coontail (*Ceratophyllum demersum* L.), elodea (*Elodea canadensis* Michx.), Illinois pondweed (*Potamogeton illinoensis* Morong.), Southern naiad (*Najas quadalupensis* (Spreng.) Morong.), and wild celery (*Vallisneria americana* Michaux). Invasive species tested included curlyleaf pondweed (*P. crispus* L.), Eurasian watermilfoil (*Myriophyllum spicatum* L.), and hydrilla (*Hydrilla verticillata* (L.f.) Royle). Exposure periods varied from 2 to 3 weeks depending on plant growth. At the end of the exposure new growth beyond the original 4 cm as well as new lateral shoot growth were harvested and utilized for chlorophyll analysis via the method of Hiscox and Israelstam (1979). As noted above, bleaching herbicides have an indirect effect on chlorophyll production, and this parameter can be useful in determining active concentrations. Treatments were replicated three times and data were subjected to one-way analysis of variance (ANOVA) with means compared via the Student-Newman-Keuls method (SNK; $P \leq 0.001$).

Greenhouse validation. To validate the results of the small-scale screening, a traditional greenhouse study was conducted with hydrilla from January to March 2010. Plastic pots (750 mL) were filled with pond sediment amended with 3 g L^{-1} Osmocote (16-8-12). Each pot was planted with two 15-cm apical tips of hydrilla. Pots were topped with a 1-cm layer of sand and four pots were placed in each aquarium. Aquaria were filled with water from Lake Lewisville, TX. Aquaria were situated into 1000-L fiberglass tanks filled with water maintained at temperatures between 26 and 28 °C.

Six weeks after planting, aquaria were treated with a static exposure of 0, 10, 20, 30, 50 and 100 µg ai L^{-1} topramezone. Treatment rates evaluated were chosen based on current proposed use rates for Experimental Use Permit trials and therefore differ slightly from rates used in the small-scale screening. All treatments were replicated three times.

Twelve weeks after treatment (WAT), all viable shoot biomass was harvested, dried at 65 °C and weighed. Dry weight values were log transformed to meet the assumptions of normality and equal variance. Data were subjected to one-way analysis of variance (ANOVA) and means were compared via the Student-Newman-Keuls method (SNK; $P \leq 0.001$). Non-transformed data are presented.

RESULTS AND DISCUSSION:

Small-scale screening. Curlyleaf pondweed tips showed visual signs of bleaching 9 days after treatment (DAT). A decrease in chlorophyll content was observed at 6 µg L^{-1} and greater; however, the decrease in chlorophyll content leveled off at 24 µg L^{-1} (Figure 1B). The lack of a continued dose response by submersed plants following exposure to enzyme-specific herbicides has been previously described (Netherland et al. 1993; Netherland and Getsinger 1995). Regardless of application rate, elodea showed no signs of bleaching and chlorophyll content was not significantly

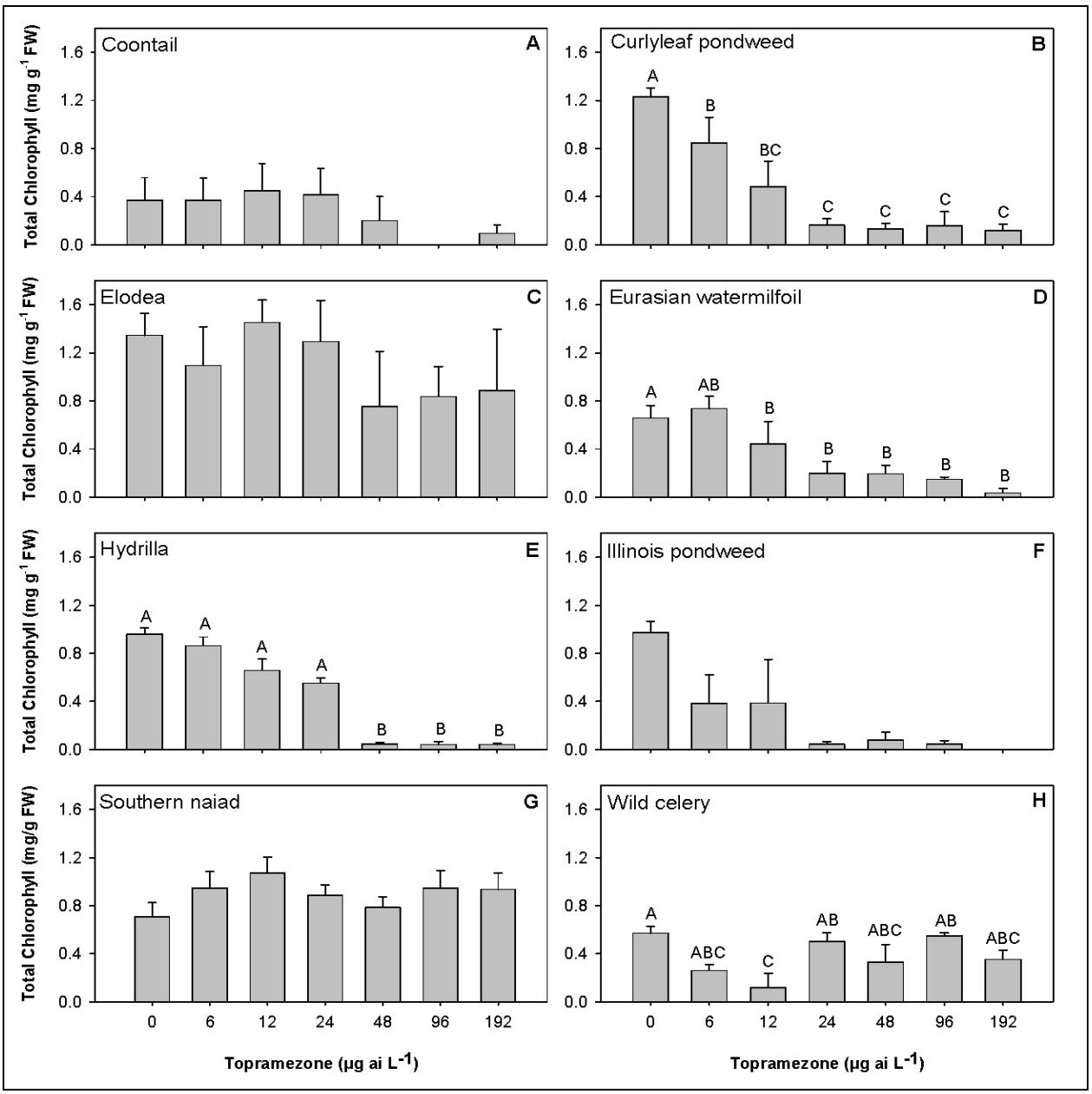

Figure 1. Mean (± SE) total chlorophyll (mg g^{-1} fresh weight (FW)) content of A) coontail, B) curlyleaf pondweed, C) elodea, D) Eurasian watermilfoil, E) hydrilla, F) Illinois pondweed, G) southern naiad, and H) wild celery 2 to 3 weeks after topramezone treatment. Bars sharing the same letter do not significantly differ from each other ($P \leq 0.001$).

different than the control (Figure 1C). Eurasian watermilfoil showed bleaching symptoms as early as 6 DAT and a decrease in chlorophyll content was noted at 12 µg L^{-1} and greater (Figure 1D). Hydrilla was also bleached at 6 DAT; however, only rates of 48 µg L^{-1} and higher were different than the control. There were no differences in chlorophyll content between the 48, 96, and 192 µg L^{-1} treatment rates (Figure 1E). Illinois pondweed showed signs of bleaching around 14 DAT; however, little to no chlorophyll content was recorded at 24 µg L^{-1} and greater. Due to variability in the data for Illinois pondweed, no statistical differences were detected (Figure 1F). Southern naiad showed no signs of bleaching and no differences were detected in chlorophyll content (Figure 1G). Wild celery showed no signs of bleaching and chlorophyll content was variable across the range of treatments (Figure 1H). Over the two-week exposure time, coontail had little to no new growth at rates of 6 µg L^{-1} and higher and red apical tips were observed at 96 and 192 µg L^{-1}. With little to no new growth, bleaching wasn't expected and chlorophyll content of treated plants was not different than the control for this species (Figure 1A).

While this method has been previously employed to evaluate gibberellins synthesis inhibitors, triazines, and bleaching herbicides against hydrilla and Eurasian watermilfoil (Netherland and Lembi 1992), results suggest that other invasive and native plants can be screened utilizing this methodology. Although most species used in this screening grew well in small flasks of culture solution, some such as coontail and cabomba (data not shown) did not grow well in these trials. Further research to determine the best culture methods and conditions, as well as the best plant sources (e.g. small plants, apical shoots, tubers) for each species can improve this method and allow for primary screening of a large number of species. Primary screens that provide data on the relative sensitivity of a species to a given herbicide will enhance the design of larger scale growth chamber and mesocosm studies. Given the cost, time, and limited number of replicates available in the larger systems, information that can improve study design is of significant value.

Greenhouse validation. Hydrilla stem apices were initially bleached 5 DAT at rates of 20 µg L^{-1} and higher. At 12 DAT, hydrilla treated at 20 and 30 µg L^{-1} had produced new green apical tips and were continuing to grow through the treatment; however, little to no new growth was observed with 50 and 100 µg topramezone L^{-1}. By 8 weeks after treatment (WAT) bleached tips became necrotic and detached from stems and no new tips were produced on hydrilla treated at 50 and 100 µg L^{-1}. Hydrilla biomass 12 WAT was reduced by 84 and 95 percent at rates of 50 and 100 µg L^{-1} topramezone, whereas rates of 10 to 30 µg L^{-1} were no different from untreated controls (Figure 2). These results are similar to those of the small-scale screening, which showed that rates of 48 µg L^{-1} and greater were needed to significantly reduce chlorophyll content (Figure 1E).

From the small-scale screening conducted, topramezone is effective against the invasive submersed plants curlyleaf pondweed, Eurasian watermilfoil and hydrilla; whereas the impact on native plants was variable with some of the native species exhibiting tolerance (elodea, Southern naiad).

FUTURE WORK: With new active ingredients being introduced into the aquatic herbicide market and a recent push for product combinations to improve efficacy or reduce resistance development, there is a need to conduct research on the impacts of these new products and combinations on both target and non-target vegetation. The current large-scale facilities are not adequate to handle such a large number of studies. Small-scale primary screening on submersed plants may provide both a cost-effective and timely alternative as well as a complementary approach to current mesocosm-scale and

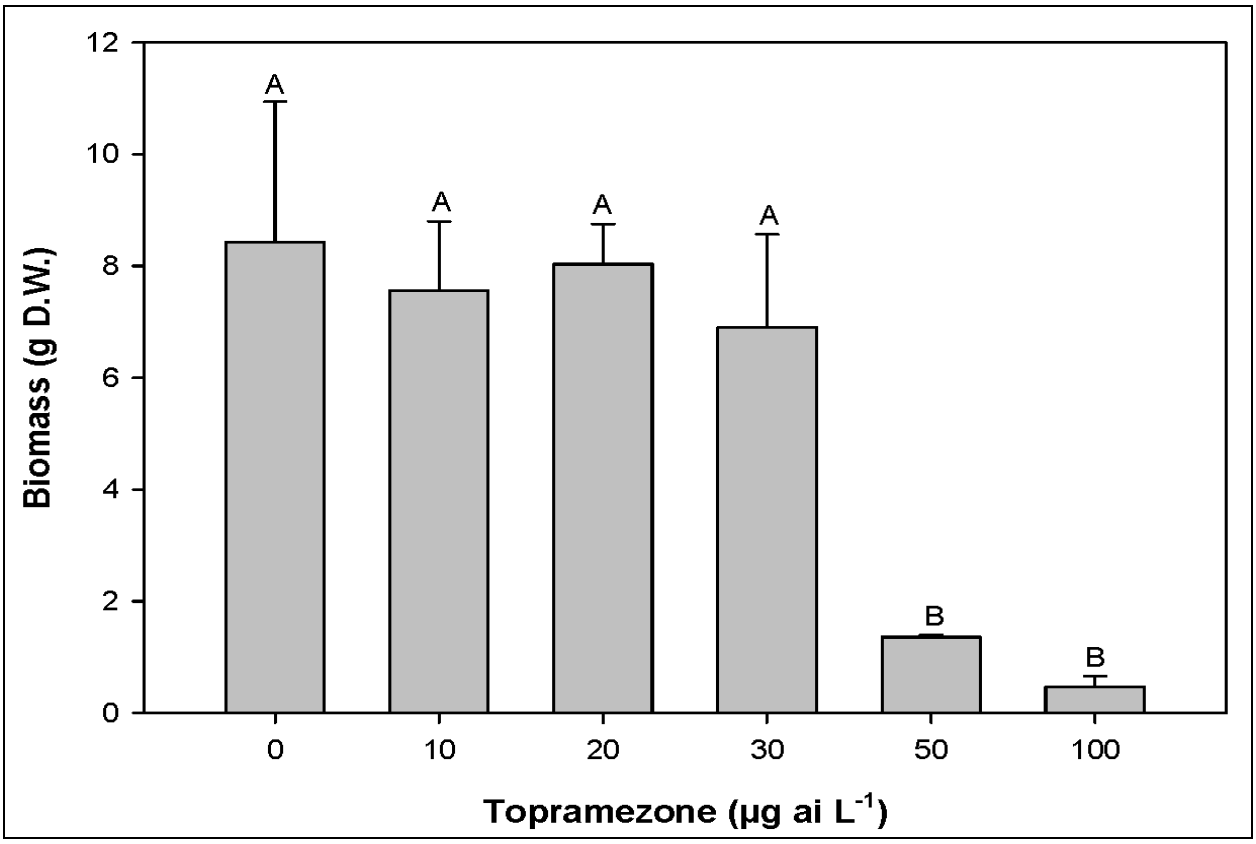

Figure 2. Mean (± SE) dry weight (DW) of hydrilla shoot biomass collected 12 weeks after topramezone treatment. Bars sharing the same letter do not significantly differ from each other (P ≤ 0.001).

field trials. As use patterns for target plants are further defined, future research will continue to focus on the effects of submersed topramezone applications on non-target emergent, floating-plant, and submersed species. Development of physiological end-points or growth measures will also be necessary to determine activity of herbicides with different modes of action.

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