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Notes

Laboratory response of monoecious *Hydrilla* to four slow-acting, enzyme-inhibiting herbicides

JOSHUA D. WOOD AND MICHAEL D. NETHERLAND

INTRODUCTION

The submersed, invasive plant hydrilla [*Hydrilla verticillata* (L. f.) Royle] has been described as "the perfect aquatic weed" (Langeland 1996), and it often requires management *via* use of registered aquatic herbicides and/or grass carp (Haller 2014). There are two genetically different biotypes of hydrilla found in the United States (dioecious and monoecious). Within the United States, the biology, phenology, and invasion range for these plants are distinct (McFarland and Barko 1987, Van 1989, Madeira et al. 1997, Madeira et al. 2004, and Benoit 2011). The monoecious biotype is found mainly in the northern-tier states (including North Carolina and Tennessee), spreading southward into Georgia and Alabama and northward toward Canada, whereas the dioecious biotype is found primarily in southern-tier states (U.S. Geological Survey 2016). The continued spread of monoecious hydrilla (M. hydrilla) into northern waters of the United States is of significant concern to resource managers (Netherland and Greer 2014). A recent literature review indicated that, of 1,246 articles on hydrilla, only 53 specifically mentioned the monoecious biotype (True-Meadows et al. 2016). The disparity in the published literature between the dioecious and monoecious hydrilla biotypes includes studies on ecology, plant biology, and management information, including the response to herbicides.

Prior herbicide research conducted on M. hydrilla has been limited to only 3 of the 15 registered aquatic herbicides: diquat, endothall (Van and Steward 1986, Van et al. 1987, Poovey and Getsinger 2010), and fluridone (Nawrocki 2011, Netherland 2015). Bensulfuron-methyl has also been studied (Van and Vandiver 1992), but that chemistry is not registered for aquatic use.

The need to register new herbicide modes of action was identified with the discovery of resistant populations of hydrilla to fluridone in dioecious hydrilla (D. hydrilla) in the early 2000s (Michel et al. 2004, Arias et al. 2005, Dayan and Netherland 2005, Puri et al. 2009). Six new herbicide ai have been registered for aquatic use since 2007: bispyribac, florpyrauxifen-benzyl, flumioxazin, imazamox, penoxsulam, and topramezone. Netherland (2011) conducted greenhouse trials that indicated that the acetolactate synthesis (ALS)–inhibiting herbicides bispyribac-sodium, imazamox, and penoxsulam were active on D. hydrilla at low concentrations (5 to 25 µg L^{-1}); however, no published data for those ALS herbicides exist for M. hydrilla. There are also no published data for M. hydrilla for the 4-hydroxyphenyl-pyruvatedioxegenase (4-HPPD) herbicide, topramezone.

The use of small-scale studies can be effective to determine efficacy and selectivity of an herbicide mode of action on an invasive, submersed species (Netherland and Richardson 2016). Small-scale studies allow for a broad range of concentrations to be tested in a small space over a short period. Results can suggest whether further testing is warranted and, if so, can give a range of the active concentrations. The Organisation for Economic Co-operation and Development (OECD) recently adopted a small-scale protocol to address herbicide activity, using rooted Eurasian watermilfoil (*Myriophyllum spicatum* L.) and provides specifications for sediment and water source used (OECD 2014). The results generated were used for registration purposes in Europe. Netherland and Richardson (2016) evaluated the potential of the OECD protocol as an initial screen for testing aquatic herbicides against other submersed plant species and recommended additional testing on slow-acting, enzyme-inhibiting herbicides. Potential advantages of this protocol were described and included 1) the high turnover and replication because of small space requirements, 2) the use of rooted plants in the place of floating fragments to allow for higher confidence in results, 3) the modifiable protocol to suit research objectives, and 4) standards for both water and sediment, which allow for comparison of results from different laboratories.

Given the recent spread of M. hydrilla and minimal publications on response to herbicides, additional research is needed. The objective of this study was to determine the activity of the slow-acting, enzyme-specific inhibitors bispyribac, fluridone, penoxsulam, and topramezone on M. hydrilla in environmentally controlled growth chambers.

*First and second authors: Former Graduate Research Assistant, Department of Agronomy, University of Florida, Center for Aquatic and Invasive Plants, Gainesville, FL 32611. Second author: U.S. Army Engineer Research and Development Center, Environmental Laboratory, Center for Aquatic and Invasive Plants, University of Florida, 7922 NW 71st St, Gainesville, FL 32653. Corresponding author's E-mail: joshuaw@sepro.com. Received for publication April 15, 2018 and in revised form October 5, 2018.

Figure 1. Image from trial 2 of M. hydrilla after 4-wk static exposure to penoxsulam. The three rows represent the three replications, and the columns are the treatment. The control (0 ppb) is on the left and the penoxsulam concentration increases from left to right (0–81 ppb).

MATERIALS AND METHODS

All studies were conducted at the University of Florida Center for Aquatic and Invasive Plants (UF CAIP, Gainesville, FL) in environmentally controlled growth chambers. M. hydrilla was originally collected from the Erie Canal, NY, and kept in culture in outdoor mesocosms at the UF CAIP for use in these studies.

Beakers (150 ml) were filled with a standardized sediment, as described in the OECD protocol (OECD 2014), and two newly sprouted tubers of M. hydrilla were planted. Newly sprouted tubers were approximately 7 d old, with shoot growth of 4 to 8 cm. The 150-ml beakers were placed into larger 2-L beakers filled with a culture solution (Smart and Barko 1985), also as prescribed by the OECD protocol. Beakers were placed into environmentally controlled growth chambers at 25 C with 14/10 h (day/night) photoperiods. The plants were allowed to acclimate for 3 d, and four beakers were removed to determine plant pretreatment weights. Then, the plants were treated with bispyribac[1] (80% soluble powder ai), fluridone[2] (60 g L^{-1} ai), topramezone[3] (336 g L^{-1} ai), and penoxsulam[4] (240 g L^{-1} ai) under static conditions for 4 wk at concentrations 0, 0.1, 0.3, 1, 3, 9, 27, and 81 µg L^{-1} (treatment dates: September 28, 2015, and March 23, 2016). Deionized water was added 1 time wk^{-1} to correct for water loss from evaporation, and treatments were replicated three times using a completely randomized design. At 4 wk after treatment (WAT; October 26, 2015, and April 20, 2016), plants were harvested (Figure 1) by removing both roots and shoots and were dried to a constant wt. The dry wt biomass was subjected to Weibull (type 1; Figure 2) nonlinear regression from which the 50% and 90% effective dose (EC_{50} and EC_{90}) values were calculated. There was no significant trial interaction; therefore, data from the repeated studies were pooled for analysis. Pretreatment weights (40 mg dry wt) were subtracted from data, and negative values were corrected for measurement error by giving a value of zero (35 of 192 data points) because biomass cannot have negative values.

RESULTS AND DISCUSSION

The untreated controls grew between 0.09 and 0.24 g across the four treatments. The EC_{50} values indicated high activity for bispyribac (19.4 µg L^{-1}), fluridone (1.7 µg L^{-1}), penoxsulam (1.0 µg L^{-1}), and topramezone (1.4 µg L^{-1}) on M. hydrilla. That was also reflected in the EC_{90} values of bispyribac (29.6 µg L^{-1}), fluridone (3.0 µg L^{-1}), penoxsulam (5.0 µg L^{-1}), and topramezone (22.3 µg L^{-1}) (Table 1). EC_{50} values for bispyribac were higher when compared with the other three herbicides, and EC_{90} values for bispyribac were higher when compared with fluridone and penoxsulam. Topramezone showed a large range for the 95% confidence interval (95% CI) for the EC_{90} valves, which may have contributed to the gradual slope of the generated curve and the lack of plant death at the highest concentrations tested.

Hydrilla has been documented as a polypoid plant having higher genetic variation compared with other aquatic

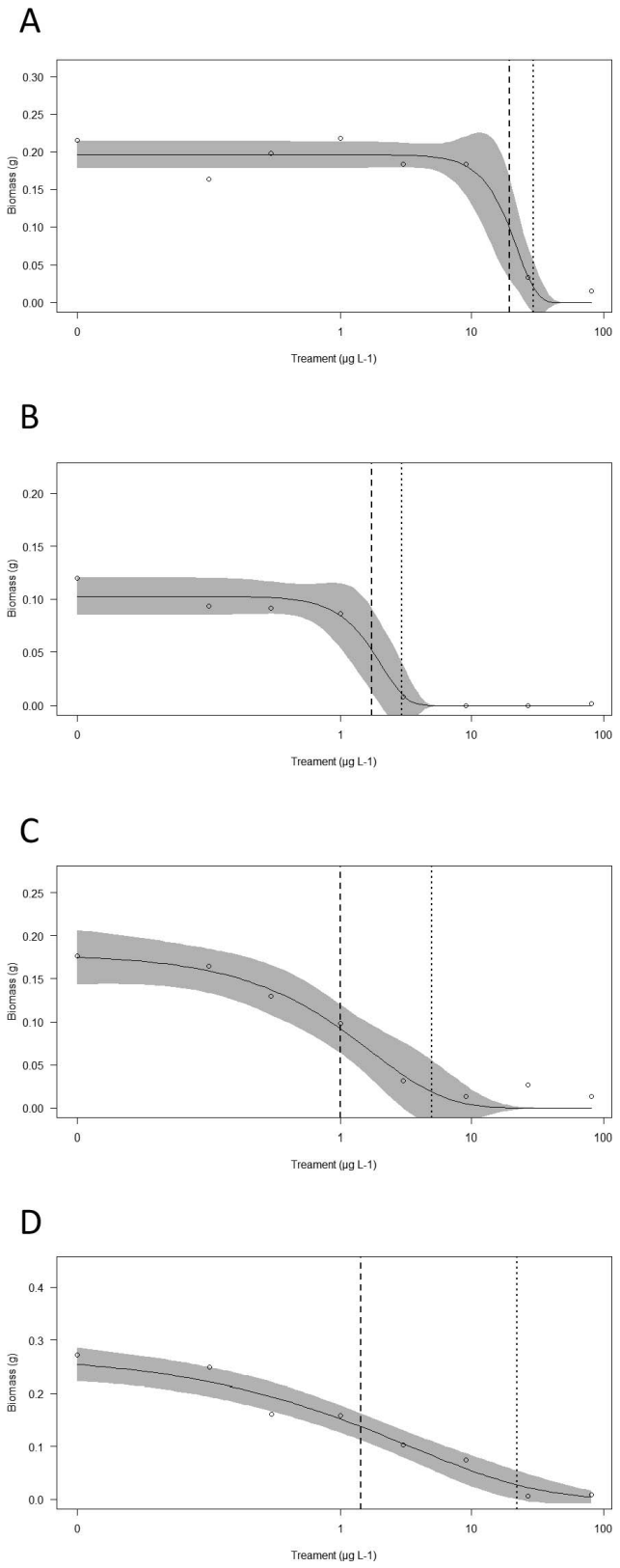

Figure 2. Nonlinear regressions for monoecious hydrilla growth following static exposure to eight concentrations (0 to 81 µg L^{-1}) of (A) bispyribac, (B) fluridone, (C) penoxsulam, and (D) topramezone over a 4-wk period. The dashed line represents the EC_{50} value, and the dotted line the EC_{90} value. The shaded area represents the 95% CI for the regression line.

Table 1. The 50% and 90% effective concentration (EC_{50} and EC_{90}) values with 95% confidence interval (95% CI) calculated from nonlinear curves based on dry wt biomass for the herbicides bispyribac, fluridone, penoxsulam, and topramezone.

	EC_{50}		EC_{90}	
Herbicide	µg L^{-1}	95% CI	µg L^{-1}	95% CI
Bispyribac	19.4	13.5–28	29.6	22.7–38.7
Fluridone	1.7	1.0–3.0	3.0	1.7–5.1
Penoxsulam	1.0	0.5–2.3	5.0	1.63–15.8
Topramezone	1.4	0.6–3.5	22.3	8.4–59.0

macrophytes (Barret et al. 1993, Arias et al. 2005). This means that different populations of hydrilla (both monoecious and dioecious) can be diploid, triploid, or tetraploid (Harlan et al. 1985, Nakamura and Kodono 1993, Arias et al. 2005). This could allow for individuals in the population to produce clones that could react differently to their environment (including exposures to herbicides), resulting in the rapid development of herbicide resistance. Even though fluridone resistance has yet to be documented in M. hydrilla, with the documented three populations of fluridone resistance (Michel et al. 2004) and two populations of endothall resistance (M. D. Netherland, unpubl. data) in D. hydrilla, it is only a matter of time before resistance is found in M. hydrilla populations because the high level of genetic variability is very similar between the two biotypes.

The activity of these herbicides on M. hydrilla is similar to that found for D. hydrilla. Under mesocosm conditions, Netherland (2011) found that D. hydrilla growth was inhibited at 5 µg L^{-1} for penoxsulam (which was the lowest rate tested) and 10 µg L^{-1} for bispyribac-sodium. Using walk-in growth chambers, Netherland and Getsinger (1995) reported that fluridone concentrations of 1 and 2 µg L^{-1} inhibited growth, but 3 µg L^{-1} was required to reduce biomass. Recent mesocosm research on newly emerged M. hydrilla exposed to fluridone yielded chlorophyll fluorescence and growth reduction of $>$ 85% at fluridone concentrations $>$ 3 µg L^{-1} (Netherland 2015).

Most operational treatments of M. hydrilla with fluridone target the plant when the biomass is still low and actively growing, soon after sprouted tubers have emerged from the soil (and before new tuber formation) (Netherland and Greer 2014, Nawrocki et al. 2016). A factor which can contribute to the rapid development of herbicide resistance in hydrilla is the high genetic variation in the same populations of hydrilla and even on the same plant (Arias et al. 2005). When the biomass is kept at low levels, the genetic variation of the population remains lower, thus reducing the likelihood of developing herbicide resistance. Similar treatment principles would likely apply to bispyribac, penoxsulam, and topramezone. Treatment of M. hydrilla when biomass remains low and is actively growing may also require reduced exposure times to achieve control. Additional mesocosm work is required to validate that hypothesis; however, that process could result in a significant reduction in the treatment life cycle and cost savings when planning treatments for M. hydrilla. Most operational treatments for monoecious hydrilla have been conducted

with fluridone. This work supports that use pattern but also suggests that other herbicides warrant consideration.

Recent findings of a significant lag period between the removal of fluridone exposure and recovery of photosynthetic pigments suggest that continuous exposure may not be necessary (Netherland 2015). Given the high level of activity of all herbicides tested, it is recommended that intermittent exposures be evaluated. The lag periods could be different for each different mechanism of action, which could result in less herbicide needing to be used to provide the same level of control. This information would provide managers greater flexibility when planning for multiple applications of these slow-acting, enzyme-inhibiting products to sustain an exposure period.

This work could offer a reliable alternative to mesocosm studies by reducing the amount of time it would take to screen select herbicides and allow for the finding of a narrower treatment range for larger-scale studies. Because M. hydrilla has shown sensitivity to the four herbicides tested, the next step would be to evaluate these herbicides in a mesocosm setting, using a narrower treatment window than that used in this study, and to test how well these results translate to a larger-scale setting. It might be worth comparing the tested herbicides to the different formulations of fluridone because most M. hydrilla treatments consist of the granular formulation.

SOURCES OF MATERIAL

[1]Tradewind, Valent USA Corporation, 1600 Riviera Ave, Walnut Creek, CA 94596.

[2]Sonar Genesis, SePRO Corporation, 11550 N Meridian St Suite 600, Carmel, IN 46032.

[3]Oasis, SePRO Corporation, 11550 N Meridian St Suite 600, Carmel, IN 46032.

[4]Galleon SC, SePRO Corporation. 11550 N Meridian St Suite 600, Carmel, IN 46032.

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