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Impact of Herbicide Combinations on Two Biotypes of the Submersed Plant *Vallisneria americana*

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PURPOSE: This technical note describes the results of a growth chamber study that evaluated the impact of combinations of the recently registered aquatic herbicides bispyribac-sodium, flumioxazin, imazamox, and penoxsulam with the previously registered aquatic herbicides diquat and endothall on two biotypes of vallisneria (*Vallisneria americana* Michaux).

BACKGROUND: One of the primary goals of aquatic weed management in public and private waters is to control the growth of invasive plant species while maintaining a diversity of native submersed and emergent species. Native aquatic plants can improve water clarity and quality, provide valuable fish and wildlife habitat, reduce sediment resuspension, and help prevent the spread of invasive plants (Savino and Stein 1982; Heitmeyer and Vohs 1984; Smart 1995; Dibble et al. 1996). Selective removal of invasive species is beneficial for continued existence and diversity of native vegetation.

Mixed populations of hydrilla (*Hydrilla verticillata* (L. f.) Royle) and non-target native plants such as vallisneria can often be found co-existing until the invasive plants ultimately take over the aquatic ecosystem. It is important to manage the weedy species before monotypic stands form. The dioecious biotype of hydrilla was present in more than 50,000 ha of Florida's public waters in 2007, with an approximate management cost of $16 million (Florida Department of Environmental Protection (FDEP) 2007). Of the 13 aquatic herbicides labeled (FIFRA-Section 3) by the U.S. Environmental Protection Agency (EPA), only bispyribac-sodium, copper, diquat, endothall (dipotassium and dimethylalkylamine salts), flumioxazin, fluridone, imazamox, and penoxsulam actively control this noxious weed (Vandiver 2002; Senseman 2007; Richardson 2008). While copper, diquat, and endothall have been registered since the 1960's, it was not until 1986 that fluridone was registered for aquatic use and penoxsulam and imazamox were not registered until 2007 and 2008, respectively. The spread of fluridone-resistant hydrilla to most of the large Florida water bodies has greatly reduced the utility of this herbicide. As a result, the contact herbicide endothall has been heavily utilized for the past several years in central Florida (MacDonald et al. 2001, Hoyer et al. 2005) and there is a concern that such heavy reliance on one mode of action may increase the chance for developing resistance to another class of herbicides. Several new herbicide modes of action have recently received federal registration or experimental use permits (EUP) in the United States for aquatic weed management. These products control hydrilla by inhibition of acetolactate synthase (ALS) (bispyribac-sodium, bensulfuron methyl, imazamox, and penoxsulam), inhibition of protoporphyrinogen oxidase (PPO) (flumioxazin), synthetic auxin (quinclorac), and inhibition of carotenoid biosynthesis (topramezone) (Mossler et al. 2006, 2007; Koschnick et al. 2007; Florida Department of Agricultural and Consumer Services (FDACS) 2008). Of the new herbicides listed above, bispyribac-sodium, flumioxazin, imazamox, and penoxsulam are the only products currently registered for aquatic use.

Optimal use patterns for efficacy and selectivity of the newly registered and EUP herbicides are still under investigation. The ALS inhibitors have resulted in varying levels of hydrilla control in operational field applications. These products are typically applied at low concentrations (5 to 100 µg active ingredient (a.i.) L^{-1}) and generally require long continuous exposures (>60 to 120+ d). Consequently, repeat applications are often necessary to maintain adequate exposure periods. In contrast to the long-term exposure requirements of the ALS herbicides, the contact herbicide flumioxazin is degraded by hydrolysis in ca. 4 d, 16 h, and 17 min at pH 5.0, 7.0, and 9.0, respectively (Katagi 2003). Consequently, field trials with flumioxazin have resulted in variable control of hydrilla with regrowth at or near the water surface in less than 2 months after treatment when applied to water with a pH >8.0 (Mudge 2007). The long exposure and higher concentration requirements of the ALS herbicides in addition to the rapid degradation of the PPO inhibitor flumioxazin result in disparate scenarios that have forced researchers and managers to find alternative ways to use these products.

Initial laboratory and small-scale field trials suggest that hydrilla control may be improved by applying some of these slow-acting ALS herbicides in combination with each other at low use rates or in combination with contact herbicides. Laboratory and field evaluations indicate combinations of penoxsulam and endothall decreased exposure requirements for penoxsulam, reduced endothall use rates for initial reduction of hydrilla biomass, and increased duration of control versus endothall alone (Heilman et al. 2009). These results suggest this use pattern may expand. Recent growth chamber research has demonstrated combinations of many of these products can effectively reduce hydrilla dry weight up to 97% 9 weeks after treatment (WAT).[1] Additional research is currently being conducted on the efficacy of these combinations on hydrilla; however, no research has been published and little is known about the impact of these combinations on non-target aquatic plants. Native submersed aquatic vegetation (SAV) such as vallisneria is ecologically important. If these herbicide combinations become an accepted practice for hydrilla management, non-target SAVs could be exposed to the treatment if vallisneria and hydrilla co-exist. Therefore, combinations of recently registered (bispyribac-sodium, flumioxazin, imazamox and penoxsulam) and previously registered (diquat and endothall) herbicides were applied alone and in combination at low use rates to determine selectivity. Two biotypes of vallisneria were chosen for this study as there have been anecdotal claims of differential herbicide susceptibility between the two biotypes. Vallisneria is an important food source for waterfowl and aquatic mammals (Fassett 1957), provides habitat for fish, sediment stabilization benefits, improved water quality and clarity, and can slow the invasion of nonindigenous aquatic plant species (Smart 1993, 1995; Smart et al. 1994; Smart and Dick 1999); differences in response between biotypes may require different management strategies.

MATERIALS AND METHODS: The study was conducted at the U.S. Army Engineer Research and Development Center (USAERDC) in Vicksburg, MS, in a large controlled-environment chamber equipped with sixty 55-L glass aquariums (0.75 m tall by 0.3 m^2) specifically designed and operated for growing submersed plants. The experiment was initiated on February 9, 2010. Conditions conducive to maintaining healthy submersed plant growth were utilized: temperature of 24.0 ± 1 C with a light intensity of 189.74 ± 74 µmol m^{-2} s^{-1} and a 14h:10 h (light:dark) photoperiod.

[1] Unpublished data. 2010. Dr. Christopher R. Mudge, Research Biologist, U.S. Army Engineer Research and Development Center, Vicksburg, MS and Dr. Linda Nelson, Program Manager, Aquatic Plant Control Research Program, U.S. Army Engineer Research and Development Center, Vicksburg, MS.

Narrow-leaf vallisneria was collected from Lake Tohopekaliga, FL and wide-leaf vallisneria was purchased from a commercial aquatic plant nursery (Suwannee Labs, Lake City, FL), and shipped overnight to USAERDC for use in this experiment. One healthy narrow-leaf (average 15 leaves per plant, 17.58±1.02 g fresh wt) and one healthy wide-leaf vallisneria (average six leaves per plant, 3.36±0.58 g fresh wt) were planted into 750-ml plastic beakers filled with 3:1 topsoil:sand sediment. The sediment was amended with Osmocote® 19-6-12 fertilizer (2 g L^{-1} sediment). A 1-cm layer of silica sand was added to the sediment surface to reduce sediment and nutrient suspension in the water column. Two beakers of narrow-leaf vallisneria were placed in each aquaria filled with growth culture solution (Smart and Barko 1985). Due to limited plant material, one beaker of wide-leaf vallisneria was placed in 33 of the 60 tanks. Plants were allowed to grow for 5 weeks before herbicide treatment. Herbicides including bispyribac-sodium (B), diquat (D), endothall (E), flumioxazin (F), imazamox (I), and penoxsulam (P) were applied alone and in combination at low use rates (Table 1). Herbicide combinations and concentrations were chosen to find potential synergy or additive effects between different herbicide modes of action. Previous growth chamber research has determined that many of these combinations were efficacious against hydrilla and warranted further evaluation against non-target

Table 1. Herbicides applied alone and in combination to narrow and wide-leaf vallisneria.[1]

Herbicide Treatments	Concentration (µg L^{-1})	Plants Treated[2]
Bispyribac-sodium (B10)	10	NL
Diquat (D100)	100	NL
Endothall (E500)	500	NL, WL
Flumioxazin (F50)	50	NL, WL
Imazamox (I50)	50	NL, WL
Penoxsulam (P5)	5	NL, WL
Bispyribac-sodium (B10) + Diquat 100	10 + 100	NL
Bispyribac-sodium (B10) + Endothall (E500)	10 + 500	NL
Bispyribac-sodium (B10) + Flumioxazin (F50)	10 + 50	NL
Bispyribac-sodium (B10) + Imazamox (I50)	10 + 50	NL
Bispyribac-sodium (B10) + Penoxsulam (P5)	10 + 5	NL
Diquat (D100) + Flumioxazin (F50)	100 + 50	NL
Diquat (D100) + Penoxsulam (P5)	100 + 5	NL
Endothall (E500) + Flumioxazin (F50)	500 + 50	NL, WL
Endothall (E500) + Imazamox (I50)	500 + 50	NL, WL
Endothall (E500) + Penoxsulam (P5)	500 + 5	NL, WL
Flumioxazin (F500) + Imazamox (I50)	50 + 50	NL, WL
Flumioxazin (F500) + Penoxsulam (P5)	50 + 5	NL, WL
Imazamox (I50) + Penoxsulam (P5)	50 + 5	NL, WL
Nontreated Control	0	NL, WL

[1] Herbicide combinations were applied in sequence to hydrilla 5 weeks after planting.
[2] Abbreviations: NL = narrow-leaf vallisneria; WL = wide-leaf vallisneria.

species.[1] Each herbicide, with regard to the combination treatments, was applied individually to the aquariums (not mixed together in a stock solution prior to treatment). Herbicide exposure times were as follows: bispyribac-sodium, static; diquat, 8 hr; endothall, 3 d; flumioxazin, 24 hr; imazamox, 14 d; and penoxsulam, static. At the appropriate time, aquaria were drained and filled to remove herbicide residue, and re-treated with the herbicide if additional exposure was required. The exposure times were chosen based on previous growth chamber research, product half-life under field conditions, and slower degradation in the growth chambers. The low-dose concentrations were selected to provide limited hydrilla control when applied alone, but the combination of the products would hopefully result in greater/increased control. Non-treated reference aquaria were also used to compare plant growth in the absence of herbicide. At treatment, water temperature was 22.6 ± 0.2 °C and pH was 7.62 to 8.62.

Three beakers of each plant species were used to measure pre-treatment shoot biomass. Narrow-leaf vallisneria pre-treatment shoot and root dry weights were 1.7 ± 0.2 and 0.5 ± 0.2 g, respectively, and wide-leaf vallisneria shoot and root dry weights were 0.6 ± 0.1 g and 0.5 ± 0.2 g, respectively. One beaker per aquaria was harvested 11 weeks after treatment (WAT) for shoot and root biomass. The study was randomized with three replications per treatment. All data were analyzed using analysis of variance and means separated using Fisher's Protected LSD ($p \leq 0.05$). Normality assumptions were assessed for all response variables. Wide-leaf vallisneria root biomass values did not meet normality assumptions and thus were transformed using a base-10 log transformation. Respective means were back-transformed for graphical depictions.

RESULTS AND DISCUSSION: All herbicides applied alone to narrow-leaf vallisneria, except the dipotassium salt of endothall (500 µg a.i. L^{-1}), did not reduce shoot biomass compared to the non-treated control 11 WAT (Figure 1). Only five of the thirteen combination treatments were injurious to narrow-leaf vallisneria. In particular, B10 + D100, B10 + E500, E500 + F50, E500 + I50, and E500 + P5 provided 47 to 83% shoot biomass reduction of this submersed aquatic plant species. Four of these injurious combinations included the fast-acting contact herbicide endothall. The combinations which included endothall were not significantly different from endothall alone; therefore, the injury is likely attributed to endothall rather than the herbicide combination. Skogerboe and Getsinger (2002) found endothall at 500 µg a.i. L^{-1} significantly reduced vallisneria dry weight 6 WAT. Combinations of the slow-acting systemic ALS herbicides bispyribac-sodium, imazamox, or penoxsulam as well as combinations with the PPO inhibitor flumioxazin were not injurious to narrow-leaf vallisneria shoots (Figure 1).

Stand-alone herbicide treatments provided similar results, with only E500 reducing narrow-leaf vallisneria root dry weight by 53% at the conclusion of the study (Figure 2). In addition, the combination treatment of B10 + D100, B10 + E500, E500 + F50, E500 + I50, and E500 + P5 decreased root biomass 43 to 74%. All other herbicides alone or in combination reduced narrow-leaf vallisneria root dry weight by ≤41%.

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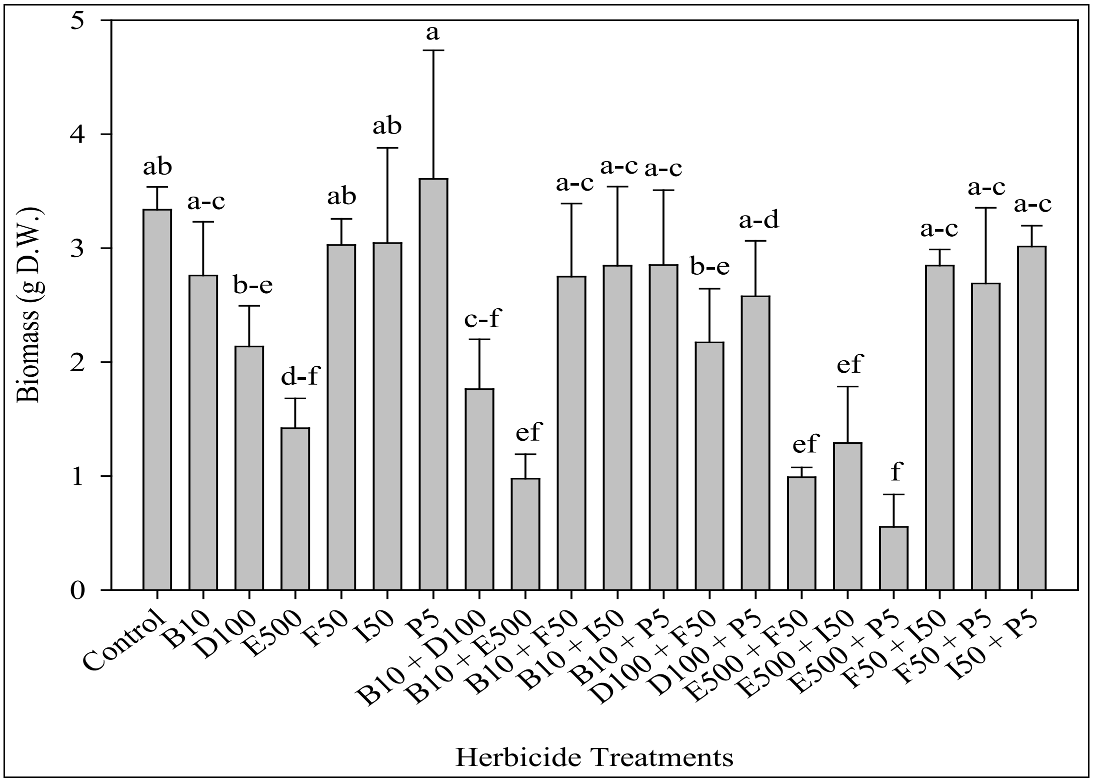

Figure 1. Effect of bispyribac-sodium (B), diquat (D), endothall (E), flumioxazin (F), imazamox (I), and penoxsulam (P) alone and in combination on mean (±S.E.) narrow-leaf vallisneria shoot dry weight 11 wk after treatment (WAT). Numbers behind treatment abbreviations represent herbicide concentrations (µg active ingredient (a.i.) L^{-1}). Means with the same letter are not significant according to Fisher's LSD at $p \leq 0.05$; n = 3.

Wide-leaf vallisneria shoots were affected by the herbicide treatments E500, E500 + F50, and E500 + I50 (Figure 3). This injury is most likely due to endothall alone, with minimal or no contribution from flumioxazin or imazamox. Research by Mudge and Haller (2010) demonstrated that a concentration of 1244 µg a.i. L^{-1} flumioxazin was required to reduce hydrilla dry weight biomass by 50% (EC_{50}) , when applied to water with pH 7.0. Wide-leaf vallisneria roots were affected more by stand-alone and combination herbicide treatments than any other plant response variable evaluated in this study (Figure 4). Flumioxazin, penoxsulam, and the combination of these two herbicides were the only treatments evaluated in this study that did not reduce root biomass 11 WAT. All other treatments decreased wide-leaf vallisneria root biomass 65 to 88% of the non-treated control with minimum differences between these treatments.

None of the individual or combination treatments completely eliminated biomass of these two non-target SAVs with only a few treatments reducing shoot or root biomass to below pre-treatment levels. Several of the treated plants were beginning to produce new leaves from older stems and rhizomes as well as new stolons (daughter plants) at 5 to 11 WAT. By reducing the use rates to ca. one-half or one-fourth of the current recommended rate, the herbicide exposure for non-target plants will be reduced, especially for those combinations containing contact + systemic or contact + contact. The reduced exposure period will likely result in less injury to non-target species. Many of the contact + systemic

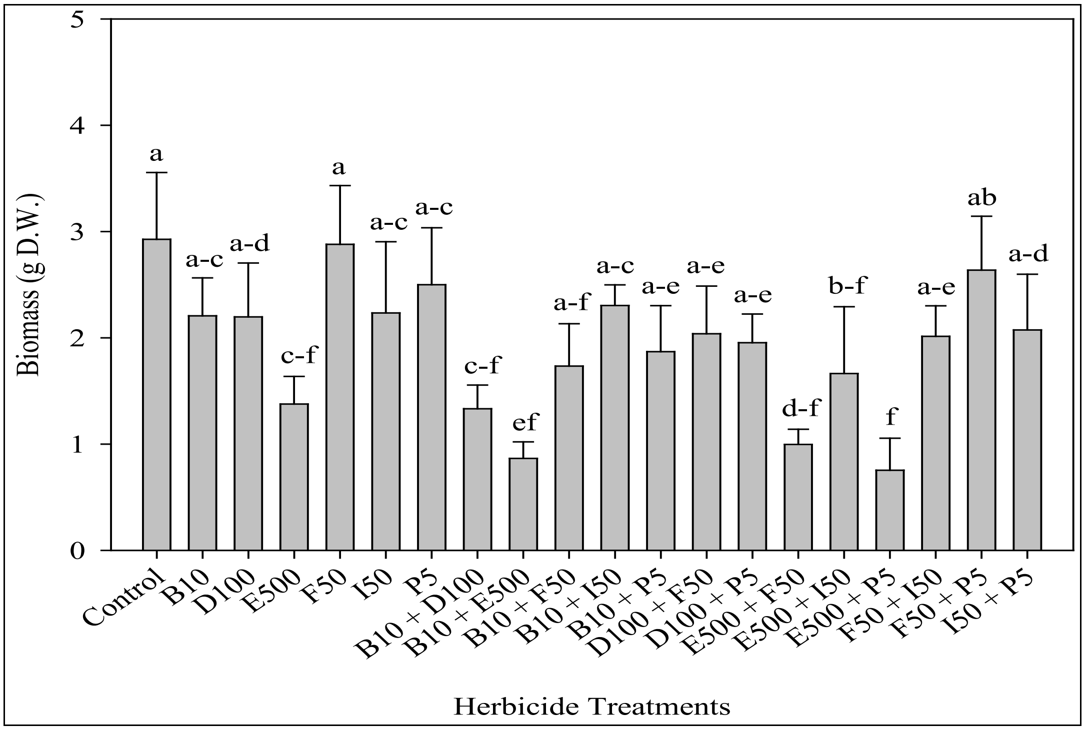

Figure 2. Effect of bispyribac-sodium (B), diquat (D), endothall (E), flumioxazin (F), imazamox (I), and penoxsulam (P) alone and in combination on mean (±S.E.) narrow-leaf vallisneria root dry weight 11 wk after treatment (WAT). Numbers behind treatment abbreviations represent herbicide concentrations (µg active ingredient (a.i.) L^{-1}). Means with the same letter are not significant according to Fisher's LSD at $p \leq 0.05$; n = 3.

combinations evaluated in this research also reduced hydrilla biomass 75 to 100% compared to the non-treated control in previous growth chamber experiments.[1]

Although no statistical comparison was made across plants, both narrow and wide-leaf vallisneria responded similarly to most herbicide treatments. The two plant biotypes evaluated in this study are just two of many vallisneria biotypes throughout the United States. Other narrow or wide-leaf accessions from various lakes may respond differently to these combinations. DNA marker research is currently being conducted at the University of Florida to determine the genetic relationship between vallisneria plants throughout Florida[2]. The submersed aquatic plant cabomba (*Cabomba caroliniana* Gray) has three distinct phenotypes and one recently became problematic in the northern regions of the United States (Bultemeier et al. 2009). All three cabomba phenotypes demonstrated distinct photosynthetic responses to a wide range of contact and systemic herbicides in growth chamber studies. In addition, two biotypes of alligatorweed [*Alternanthera philoxeroides* (Mart.) Griseb.] (slender stem and broad stem) responded differently to quinclorac, with the slender stem biotype more

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[2] Personal communication. 2010. L. A. Gettys, Research Assistant Scientist, University of Florida, Gainesville, FL 32653.

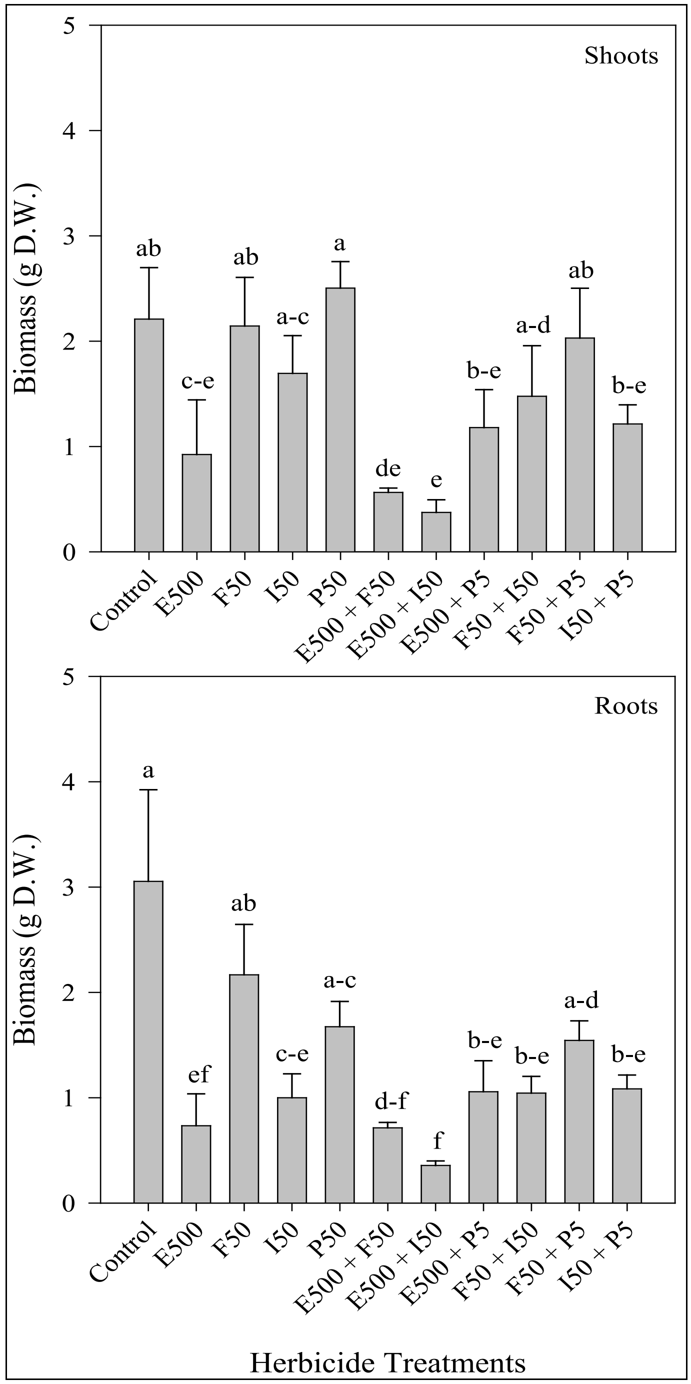

Figure 3. Effect of endothall (E), flumioxazin (F), imazamox (I), and penoxsulam (P) alone and in combination on mean (±S.E.) wide-leaf vallisneria shoot and root dry weight 11 wk after treatment (WAT). Numbers behind treatment abbreviations represent herbicide concentrations (µg active ingredient (a.i.) L^{-1}). Means with the same letter are not significant according to Fisher's LSD at p ≤ 0.05; n = 3.

susceptible than the broad stem biotype (Kay 1992). The aforementioned research and the current vallisneria research demonstrate the need for other plant species with different biotypes/ecotypes/ phenotypes that have shown differential herbicide response in the field to be further evaluated.

Systemic + systemic combinations (bispyribac-sodium, imazamox, or penoxsulam) provided no additional injury compared to these products applied alone. The ALS herbicides have shown promise for hydrilla control; however, these products need to be maintained in water for extended periods of time. Prolonged exposures will injure certain non-target SAV and emergent plant species, and there are concerns with development of ALS herbicide resistance in aquatic weed control. It is doubtful that systemic + systemic combinations, even at reduced concentrations, will reduce the exposure requirement or increase the level of hydrilla control. To date, no ALS herbicide combinations have been found to provide enhanced hydrilla control or to reduce exposure requirements.[1] Conversely, one of the benefits of combining fast and slow-acting herbicides such as penoxsulam and endothall has been the reduced exposure requirement to control hydrilla.

In conclusion, these data indicate that the Lake Tohopekaliga narrow-leaf and Suwannee wide-leaf vallisneria were minimally impacted by herbicide combinations. In addition, both taxa responded similarly to the herbicide treatments evaluated in this study. Other narrow and wide-leafed vallisneria biotypes in Florida or other regions of the United States may respond differently to these combinations, but vallisneria found in mixed SAV stands with hydrilla should survive herbicide treatments.

FUTURE WORK: This is the first round of trials to evaluate the impact of herbicide combinations on non-target plant species. This research will be repeated in outdoor mesocosms to verify the results of the chamber study. In the future, these combinations will be evaluated on other non-target submersed and emergent plant species. The results of these studies will be a useful tool for hydrilla management in water bodies throughout the United States where hydrilla and non-target plants co-exist. These data will provide lake managers with knowledge of which injurious combinations to avoid.

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