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Note

Evaluation of two ALS inhibiting herbicides on nontarget native aquatic and wetland plants

LYN A. GETTYS AND WILLIAM T. HALLER*

INTRODUCTION

The development of fluridone-resistant hydrilla populations (Michel et al. 2004, Arias 2005) in several Florida lakes in the late 1990s stimulated the screening and evaluation of additional herbicides for potential aquatic use, particularly for control of the submersed invasive species hydrilla [*Hydrilla verticillata* (L. f.) Royle]. A critical component of screening programs is evaluating potential herbicide damage to nontarget native aquatic and littoral/wetland plants, which provide numerous important ecosystem services. The acetolactate synthase (ALS)–inhibiting herbicides penoxsulam and bispyribac-sodium were evaluated first in controlled laboratory and greenhouse studies and subsequently in field studies under experimental use permits. These products were ultimately registered for aquatic use by the U.S. Environmental Protection Agency as Galleon SC (penoxsulam) in 2007 and Tradewind (bispyribac-sodium) in 2012 (Schardt and Netherland 2021). Although both herbicides are Weed Science Society of America Group 2 ALS inhibitors, they belong to different chemical families (penoxsulam: triazolopyrimidine; bispyribac-sodium: pyrimidinyl(thio)benzoate). Both were previously registered for grass, sedge, and broadleaf control in rice (application rates of 10 to 50 g ai ha^{-1} for penoxsulam and 20 to 40 g ai ha^{-1} for bispyribac-sodium) (Shaner 2014) and both are also labelled for use alone or in combination with other herbicides for weed control in certain turf grasses.

The aquatic labels for these two products list many of the same target aquatic plants and both can be applied in water to submersed weeds or as foliar treatments to floating and emergent species (SePRO 2013, Valent 2016). The recommended label rates for in-water concentrations for submersed weed control are 25 to 75 parts per billion (ppb) for penoxsulam and 20 to 45 ppb for bispyribac-sodium. However, concentrations at the lower end of recommended ranges are typically used to minimize damage to nontarget, native aquatic plants, which provide important ecosystem services that include energy reduction or calming, substrate stabilization, and food and habitat for fauna. Invasive plant management is quite different from agricultural weed control in that the intent in agricultural systems is to eliminate most or all weedy species to maximize crop production. In contrast, the goal of invasive weed control in more natural upland and aquatic sites is to control one or a few invasive species with little or no impact on native plants in order to minimize interference with ecosystem function.

Schardt and Netherland (2021) suggested that the contact time required for effective submersed weed control is > 45 d for both products; however, the penoxsulam label suggests 60 d of contact might be best for optimum control. Shaner (2014) reported that the half-lives in water of both products in flooded rice are variable, but are around 10 d. However, Schardt and Netherland (2021) suggested that half-lives may be longer (more than 30 d) in deeper systems. The combination of short half-lives and long exposure time requirements could result in reduced efficacy if single treatments are applied without additional applications to ensure that herbicide concentrations remain at effective levels.

As mentioned above, the objective of invasive plant management programs is to control one or more invasive species while minimizing damage to desirable native plants in order to maintain optimum ecosystem function. Nonselective herbicides have limited utility in invasive plant control programs in natural areas, aquatic systems, and upland habitats. Thus, the objective of these experiments was to evaluate the potential impacts of penoxsulam and bispyribac-sodium on 13 native aquatic and wetland plants commonly found in the southern United States.

MATERIALS AND METHODS

Experiments were conducted at the University of Florida (UF) Fort Lauderdale Research and Education Center (FLREC) in Davie and at the UF Center for Aquatic and Invasive Plants (CAIP) in Gainesville. Native plants evaluated at FLREC included submersed [eelgrass: *Vallisneria americana* Michx.; Illinois pondweed: *Potamogeton illinoensis* Morong; southern naiad: *Najas guadalupensis* (Spreng) Magnus; coontail: *Ceratophyllum demersum* L.] and select emergent (maidencane: *Panicum hemitomon* Schult.; Gulf Coast spikerush: *Eleocharis cellulosa* Torr.; cattail: *Typha latifolia* L.; soft rush: *Juncus effusus* L.) species. Native plants tested at CAIP were floating-leaved [fragrant white waterlily: *Nymphaea*

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* First author: Associate Professor, Fort Lauderdale Research and Education Center, Department of Agronomy, University of Florida IFAS, Davie, FL 33314. Second author: Professor Emeritus and former Director, Center for Aquatic and Invasive Plants, Department of Agronomy, University of Florida IFAS, Gainesville, FL 32653. Corresponding author's E-mail lgettys@ufl.edu. Received for publication March 10, 2021 and in revised form July 27, 2021.

odorata Aiton; spatterdock: *Nuphar advena* (Aiton) W.T. Aiton] and select emergent (pickerelweed: *Pontederia cordata* L.; broadleaf arrowhead: *Sagittaria latifolia* Willd.; lanceleaf arrowhead: *Sagittaria lancifolia* L.) macrophytes. Submersed plants were collected from field populations in Broward County (Florida), whereas emergent and floating-leaved species were purchased from commercial aquatic nurseries.[1,2] All plants were cultured in substrate composed of 50 : 50 (v/v) coarse builders' sand and commercially available topsoil consisting of a regionally formulated mix of organic and mineral components.[3] The topsoil was air-dried and processed through a 0.64-cm screen to remove stones, bark, and larger soil clumps prior to blending with the sand. Controlled-release fertilizer[4] was incorporated into the substrate at a rate of 2 g kg^{-1} of dry substrate (Mudge 2018).

A total of 90 mesocosms (95-L plastic nursery tubs) were prepared for each species. Five 10-cm-long apical cuttings of the submersed plants southern naiad, coontail, and Illinois pondweed and single plants of eelgrass were planted into separate 20-cm-diam 2-L plastic pots (180 pots per species). Two pots of a single species were then placed in a mesocosm that was filled with unchlorinated well water to a depth of 38 cm for a 3- to 4-wk initial grow-out period.

Single plants of the emergent species cattail, maidencane, Gulf Coast spikerush, soft rush, pickerelweed, lanceleaf arrowhead, and broadleaf arrowhead were planted in 2-L pots (180 pots per species) and two pots of a single species were placed in a mesocosm. The floating-leaved plants fragrant white waterlily and spatterdock were planted individually into 11.4-L plastic dishpans (90 dishpans per species) and a single planted dishpan was placed in a mesocosm. Water depth for emergent and floating-leaved species was initially set to a few centimeters over the substrate level in the pots or dishpans and gradually increased to a depth of 38 cm (24 to 25 cm over the substrate level) over a 3- to 5-wk grow-out period prior to herbicide treatment.

All plants were grown in a shadehouse covered with 30% shade cloth and water levels were maintained at 38 ± 2 cm throughout the study period. Plants were grown out during early spring and summer, then treated during late spring and summer. Average air temperature at CAIP ranged from 24 to 28 C (76 to 82 F), whereas FLREC temperatures were 28 to 31 C (83 to 87 F). Prior to herbicide treatments, 70 mesocosms of each species were selected based upon visual evaluation of uniform growth, then herbicide treatments (two herbicides by seven concentrations each) were randomly assigned. Five replicates (mesocosms) were prepared for each herbicide concentration treatment, resulting in 35 mesocosms per species per herbicide. Bispyribac-sodium was applied as Tradewind,[5] which is formulated as an 80% ai soluble powder, whereas penoxsulam was applied as Galleon SC,[6] which is formulated with 240 g ai L^{-1} (2 lb ai gal^{-1}). Water volume in the mesocosms was corrected to account for the volume displaced by the substrate-filled containers, then single herbicide treatments were applied and gently mixed into the water to achieve concentrations of 0, 10, 20, 40, 60, 160, and 240 µg L^{-1} (ppb). These experimental rates were based upon likely use rates for submersed weed control (penoxsulam: 10 to 30 ppb; bispyribac-sodium: 20 to 45 ppb) and were increased to include much higher concentrations. These ranges were selected with the hope that the maximum rate of each herbicide would cause near or total mortality of the plants being tested, which results in more accurate calculation of the effective concentration of herbicide expected to cause a 50% reduction in biometrics compared with untreated control plants (EC_{50}) derived from regression analysis. These experiments mimic a single application of these herbicides. However, the labels for both products indicate that the targeted area may be treated multiple times to maintain the desired herbicide concentration over time. For example, the label for Galleon SC specifies that no more than 150 ppb of penoxsulam should be applied during a single annual growth cycle. Single applications should be applied at a target concentration of 25 to 75 ppb (although treatments with up to 150 ppb are allowed), whereas multiple or split applications should have an initial target concentration of 10 to 30 ppb, with additional treatments applied as needed (up to the annual maximum of 150 ppb) to maintain the appropriate concentration. Although it is not likely that these high rates will be used operationally, it is possible that these concentrations could be used in some situations.

Plants were maintained for 65 to 75 d after herbicide treatment, then subjected to a destructive harvest. Live (green) leaves of the larger broadleaf species were counted at harvest before being combined with stems for obtaining dry shoot biomass values. Leaves are often reduced in size following treatment with ALS inhibitors, and a leaf was counted if it was completely unfurled and at or above the water line regardless of size. All live aboveground biomass in each mesocosm was collected (both pots of submersed and emergent species were combined), rinsed vigorously on a window screen, placed into paper bags, dried in a forced-air oven at 65 C until a constant weight was achieved, then weighed to determine shoot biomass per replicate (mesocosm). Dry shoot biomass and leaf count data were subjected to nonlinear regression (exponential decay) using SAS software.[7] Regression model components were used to calculate EC_{50} values and 95% confidence intervals for each herbicide. Responses were considered different if overlap occurred between 95% confidence intervals.

RESULTS AND DISCUSSION

Calculated EC_{50} values for shoot biomass of submersed plants treated with bispyribac-sodium or penoxsulam were not different within a species, but the four submersed species differed in their responses to bispyribac-sodium and penoxsulam (Table 1). Coontail was more tolerant of bispyribac-sodium than was eelgrass, but the EC_{50} for coontail biomass was not different from the EC_{50} values of Illinois pondweed or southern naiad. Similarly, coontail was more tolerant of penoxsulam than were eelgrass and Illinois pondweed, but was not different from southern naiad (Table 1).

Similar to the submersed species, there were differences in susceptibility to these ALS-inhibiting herbicides among the seven emergent species (Table 1). However, two

Table 1. Effective concentration of herbicide expected to cause a 50% reduction in biometrics compared with untreated control plants (EC$_{50}$ values; in parts per billion [ppb]) and 95% confidence intervals (95% CI) for dry shoot biomass (g) and number of leaves (selected species) for plants harvested 10 wk after a single in-water treatment with bispyribac-sodium or penoxsulam. With a few exceptions, species are listed from most susceptible to most tolerant within each growth type (submersed, emergent, floating-leaved). Bold type indicates significantly different within-species response (EC$_{50}$ values) to the two herbicides based on 95% confidence intervals.

	EC$_{50}$ (ppb) (95% CI)			
	Shoot biomass (g)		Number of leaves	
Species	Bispyribac-sodium (recommended use rate 20 to 45 ppb)	Penoxsulam (recommended use rate 10 to 30 ppb)	Bispyribac-sodium (recommended use rate 20 to 45 ppb)	Penoxsulam (recommended use rate 10 to 30 ppb)
Submersed				
Eelgrass	31 (17–141)[1] abcd[2]	26 (15–114)[1] bcd	n/a	n/a
Illinois pondweed	65 (35 to > 240)[1] bcde	40 (26–90)[1] bcd	n/a	n/a
Southern naiad	80 (47 to > 240) cde	119 (61 to > 240) cde	n/a	n/a
Coontail	> 240 (217 to > 240) e	> 240 (208 to > 240) e	n/a	n/a
Emergent				
Broadleaf arrowhead	27 (21–36)[1] ab	21 (17–27)[1] b	36 (24–69)[1]	19 (13–38)[1]
Maidencane	**28 (19–54)[1] abc**	**> 240 (210 to > 240) e**	n/a	n/a
Gulf Coast spikerush	93 (63–183) d	41 (27–87)[1] bcd	n/a	n/a
Cattail	116 (79–220) de	137 (81 to > 240) de	n/a	n/a
Pickerelweed	116 (76 to > 240) de	68 (46–131) cd	47 (34–73)[1]	28 (21–41)[1]
Soft rush	> 240 (215 to > 240) e	186 (73 to > 240) de	n/a	n/a
Lanceleaf arrowhead	**> 240 (> 240 to > 240) e**	**106 (70–213) d**	**> 240 (147 to > 240)**	**48 (37–67)**
Floating-leaved				
Fragrant white waterlily	**14 (11–21) a[1]**	**4 (3–5) a[1]**	14 (9–26)[1]	8 (5–22)[1]
Spatterdock	**122 (84–222) de**	**38 (27–68) bc[1]**	**110 (77–197)**	**27 (21–38)[1]**

[1] Indicates that an EC$_{50}$ value ± 95% confidence interval is below the recommended use rate for the specified product.

[2] Values within a herbicide column that are followed by the same letter are not different based on 95% confidence intervals.

emergent species differed in their responses to bispyribac-sodium and penoxsulam. Maidencane was considerably more susceptible to bispyribac-sodium (biomass EC$_{50}$ 28 ppb) than to penoxsulam (biomass EC$_{50}$ > 240 ppb). The reverse was true for lanceleaf arrowhead; based upon dry shoot biomass and leaf count, this species was more susceptible to penoxsulam (shoot biomass and leaf count EC$_{50}$ 106 and 48 ppb, respectively) than to bispyribac-sodium (both EC$_{50}$ values > 240 ppb). It is interesting to note that broadleaf arrowhead biomass is considerably more susceptible to both herbicides than is its congener lanceleaf arrowhead (Table 1).

Both floating-leaved plants were more susceptible to penoxsulam than to bispyribac-sodium (Table 1). Penoxsulam EC$_{50}$ values for both plants are below (fragrant white waterlily shoot biomass and leaves EC$_{50}$ 4 and 8, respectively) or near (spatterdock shoot biomass and leaves EC$_{50}$ 38 and 27, respectively) the recommended application rates of 10 to 30 ppb for this herbicide. Similarly, the EC$_{50}$ values for bispyribac-sodium on fragrant white waterlily (14 ppb for both shoot biomass and leaves) were lower than the recommended application rates (20 to 45 ppb), but spatterdock EC$_{50}$ values were considerably higher (shoot biomass and leaves 122 and 110, respectively). Shoot biomass and leaf count EC$_{50}$ values were very similar within each floating-leaved species.

We quantified number of live leaves when possible in this study to determine whether this parameter was related to or supported calculated EC$_{50}$ values for dry weight. Wersal and Madsen (2007) reported extensive lateral bud growth (witches' brooms) in *Myriophyllum aquaticum* (Vell.)Verdc. after treatment with the ALS-inhibiting herbicides imazapyr and imazamox, but they did not count leaves. We report live leaf counts for 10 species–herbicide combinations in our study, and found that the 95% confidence intervals for leaf count and dry weight EC$_{50}$ values overlapped in seven of these cases (both herbicides on broadleaf arrowhead, fragrant white waterlily, and spatterdock; bispyribac-sodium on lanceleaf arrowhead). In addition, 95% confidence values of the three remaining leaf counts were very close to those calculated for dry weight data. This suggests that live leaf number could be related to dry biomass and may be useful to increase confidence and provide additional support for calculated EC$_{50}$ values.

The high (> 240 ppb; above the maximum concentration tested) EC$_{50}$ values and 95% confidence intervals for both herbicides on coontail, bispyribac-sodium on lanceleaf arrowhead and soft rush, and penoxsulam on maidencane indicate that these herbicides had little or no impact on these species at the highest herbicide concentrations evaluated in this study. These values are well above the recommended label rates for submersed weed control, which suggests these nontarget species may experience some damage at the higher label rates, but are likely to survive and recover since $\geq 50\%$ of the live biomass remained at the conclusion of the study. In contrast, species with EC$_{50}$ values that are below or in the lower range of recommended use rates are likely to experience significant off-target damage after exposure to water treated at normal concentrations used for submersed weed control. For example, eelgrass, Illinois pondweed, broadleaf arrowhead, and fragrant white waterlily were sensitive to both ALS inhibitors, as evidenced by the fact that their EC$_{50}$ values ± 95% confidence interval were below the recommended use rate for the specified product (bispyribac-sodium: 20 to 45 ppb; penoxsulam: 10 to 30 ppb), and could be damaged by exposure to treated waters. Gulf Coast spikerush and spatterdock were sensitive to penoxsulam only, whereas pickerelweed leaf counts only were significantly reduced by bispyribac-sodium. Also, As mentioned before, these experiments mimic a single application of these herbicides, but both products may be used at higher concentrations or applied multiple times to maintain target concentrations over time. In the unlikely event that high concentrations are used operationally (e.g., a single application of penoxsulam at 150 ppb), it is possible that additional species evaluated in these experiments could be negatively affected because their EC_{50} values fall within this higher concentration range. Therefore, the EC_{50} values we present should be considered conservative.

Only 4 of the 13 species evaluated in this study differed in their susceptibility to these two herbicides. Koschnick et al. (2007) reported EC_{50} values for bispyribac-sodium and penoxsulam on softstem bulrush (*Schoenoplectus tabernaemontani* (C.C. Gmel.) Palla) and Egyptian panicgrass (*Paspalidium geminatum* (Forssk.) Stapf), but did not include 95% confidence intervals or other ways to compare these values. Their reported EC_{50} values for shoot weights in the 66-d study were 10 (softstem bulrush) and 134 ppb (Egyptian panicgrass) for penoxsulam, which is similar to the plants' responses to bispyribac-sodium (EC_{50} values 32 and 89 ppb for softstem bulrush and Egyptian panicgrass, respectively). Thus, it appears that these two herbicides, despite being in two different chemical families and having large molecular differences, have similar effects on a range of plants (Shaner 2014). Although this is not completely unexpected, it does conflict with Ren et al. (2000), who reported that minor differences in molecular structure may produce major differences in the weed spectrum controlled by ALS herbicides. For example, imazapyr and imazamox are both in the imidazolinone family of ALS inhibitors and the only molecular difference between the two is an extra oxygen and methyl group attached to the imazamox molecule. Imazapyr is considered a broad-spectrum herbicide and controls many species of plants, but imazamox has a limited spectrum of weeds controlled, which comprise mostly annual dicots and a few perennial grasses.

Bispyribac-sodium and penoxsulam are both registered and applied post-emergence for weed control in rice production, but there is little information published regarding the effect of foliar application of these products on aquatic plants. Because these two herbicides seemed to have similar activity within most of the species we evaluated in this study, it was of interest to compare the weed spectrums of these two products in rice fields. The "weeds controlled" sections of two rice herbicide labels (bispyribac-sodium: Regiment, Valent, 2012; penoxsulam: Grasp SC, Dow 2018) have significant overlap; they list around 80% of the same species and recommend that products be applied at 20 to 40 g ai ha^{-1} to young, actively growing weeds. In addition, the Louisiana State University Weed Management Guide lists 22 major rice weeds and their responses to several herbicides and herbicide combinations (Stephenson et al. 2020). Only three of the 22 weeds had differential responses (greater than ± 10%) to foliar applications of bispyribac-sodium or penoxsulam.

Although bispyribac-sodium and penoxsulam belong to different chemical families within the ALS inhibitors, there appears to be little difference in their effects on weeds in both aquatic and rice applications. Some of the native aquatic plants we evaluated in this study had EC_{50} values that fell within the recommended concentrations for submersed weed control (penoxsulam: 10 to 30 ppb; bispyribac-sodium: 20 to 45 ppb), which suggests that selectivity may be improved and off-target damage may be reduced by using rates on the low end of the recommended treatment range. However, a number of the native species in this study had calculated EC_{50} values that were significantly greater than the recommended use rates of these products; these plants may suffer off-target damage from submersed weed control treatments but are likely to recover from treatment effects over time. It is possible—and even likely—that different results might be observed under field conditions, especially if plants are subjected to longer exposure periods or split treatments. However, these data provide useful information regarding which plants are most likely to be impacted by operational treatments.

SOURCES OF MATERIALS

[1] Maidencane, Gulf Coast spikerush, cattail, and soft rush: Aquatic Plants of Florida, Myakka City, FL

[2] Fragrant white waterlily, spatterdock, pickerelweed, broadleaf arrowhead, and lanceleaf arrowhead: Suwannee Labs, Lake City, FL

[3] Professional Top Soil; Margo Garden Products, Folkston, GA 31537.

[4] Osmocote Plus 15-9-12; Everris – an ILC Fertilizers Company, Dublin, OH 43017.

[5] Tradewind, Valent USA Corporation, Walnut Creek, CA 94596.

[6] Galleon SC, SePRO Corporation, Carmel, IN 46032.

[7] SAS Software Version 9.4, SAS Institute, Cary, NC 27513.

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LITERATURE CITED

Arias RS, Netherland MD, Scheffler BE, Puri A, Dayan FE. 2005. Molecular evolution of herbicide resistance to phytoene desaturase inhibitors in *Hydrilla verticillata* and its potential use to generate herbicide-resistant crops. Pest. Manage. Sci. 61(3):258–268.

Dow. 2018. Grasp SC herbicide label. https://assets.greenbook.net/14-45-49-06-04-2018-D02-194-004__Grasp__SC__Specimen__Label.pdf. Accessed March 8, 2021.

Koschnick TJ, Netherland MD, Haller WT. 2007. Effects of three ALS-inhibitors on five emergent native plant species in Florida. J. Aquat. Plant Manage. 45:47–51.

Michel A, Arias RS, Scheffler BE, Duke SO Netherland MD. 2004. Somatic mutation-mediated evolution of herbicide resistance in the nonindigenous invasive plant hydrilla (*Hydrilla verticillata*). Mol. Ecol. 13:3229–3237.

Mudge CR. 2018. Propagation methods of submersed, emergent, and floating plants for research. J. Aquat. Plant Manage. 56s:2–9.

Ren TR, Yang HW, Gao X, Yang XL, Zhou JJ, Chang FH. 2000. Design, synthesis and structure-activity relationships of novel ALS inhibitors. Pest Manage. Sci. 56(3):218–226.

Schardt J, Netherland MD. 2021. Chemical control of aquatic weeds, pp. 163–178. In: L. A. Gettys, W. T. Haller, and D. G. Petty (eds). Biology and control of aquatic plants: A best management practices handbook. 4th ed. Aquatic Ecosystem Restoration Foundation, Marietta, GA.

SePRO. 2013. Galleon SC herbicide label. https://www.sepro.com/documents/Galleon__Label.pdf. Accessed March 8, 2021.

Shaner DL. 2014. Herbicide handbook. 10th ed. Weed Science Society of America, Lawrence KS. 513 pp.

Stephenson DO IV, Brown KP, Miller DK, Fontenot K, Lazaro LM, Mudge C, Orgeron A, Price R, Strahan RE, Sexton M, Webster EP (eds.). 2020. 2020 Louisiana suggested chemical weed management guide. Louisiana State University AgCenter Publication 1565, Baton Rouge, LA. https://www.lsuagcenter.com/portals/communications/publications/management__guides/louisiana%20suggested%20chemical%20weed%20control%20guide/louisiana%20chemical%20weed%20management%20guide%20complete%20book. Accessed August 9, 2020.

Valent. 2012. Regiment herbicide label. http://www.cdms.net/ldat/ld5HT001.pdf. Accessed March 8, 2021.

Valent. 2016. Tradewind herbicide label. http://www.cdms.net/ldat/ld9UO000.pdf. Accessed March 8, 2021.

Wersal RM, Madsen JD. 2007. Comparison of imazapyr and imazamox for control of parrotfeather (*Myriophyllum aquaticum* (Vell.)Verdc.). J. Aquat. Plant Manage. 45:132–136.