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Note

Effect of subsurface and foliar applications of bispyribac-sodium on water hyacinth, water lettuce, and giant salvinia

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INTRODUCTION

Bispyribac-sodium [2,6-bis(4,6-dimethoxypyrimidin-2-yloxy)benzoic acid] recently received a USEPA Section 3 aquatic registration for control of hydrilla and other nuisance aquatic plants. Similar to the herbicides penoxsulam [2-(2,2-difluoroethoxy)-N-(5,8-dimethoxy [1,2,4] triazolo[1,5-c]pyrimidin-2-yl)-6 (trifluoromethyl) benzenesulfonamide] and imazamox [2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1H-imidazol-2-yl]-5-(methoxymethyl)-3-pyridinecarboxylic acid], bispyribac-sodium inhibits the production of branched-chain amino acids by binding to the acetolactate synthase (ALS) enzyme (Tranel and Wright 2002). Without these essential amino acids, protein synthesis and growth are inhibited, ultimately resulting in plant death (WSSA 2007). While the ALS inhibitors target the same plant enzyme, the large number of ALS inhibitors registered for terrestrial use attests to significant differences in plant selectivity between these compounds; therefore, evaluation of two or three different ALS inhibitors on a suite of plant species may yield very different outcomes. For example, Koschnick et al. (2007) reported EC_{50} values for penoxsulam, bispyribac-sodium and imazamox on duck potato shoot biomass (*Sagittaria lancifolia* L.) to be 9, 105, 96 µg (9, 105, and 96 ppb) active ingredient (ai) L^{-1}, respectively. If the management goal was to control hydrilla without damaging stands of duck potato, then the use of penoxsulam may not be the product of choice since herbicide concentrations of 5 to 20 µg L^{-1} are required.

Systematic efficacy evaluations on both nontarget and target weeds are important for determining use patterns of the ALS herbicides. Previous work on water hyacinth (*Eichhornia crassipes* (Mart.) Solms) has shown that penoxsulam is effective as either a subsurface or foliar application (Richardson and Gardner 2007, Wersal and Madsen 2010). Also, imazamox is efficacious against water hyacinth and water lettuce (*Pistia stratiotes* L.) at higher subsurface application rates, but this product is ineffective against giant salvinia (*Salvinia molesta* D.S. Mitchell) (Emerine et al. 2010). Little to no information is available on the efficacy of bispyribac-sodium on water hyacinth, water lettuce or giant salvinia. Although other herbicides such as 2,4-D, carfentrazone-ethyl, chelated copper, diquat and glyphosate are effective on water hyacinth, water lettuce and/or giant salvinia (Westerdahl and Getsinger 1988, Lopez 1993, Nelson et al. 2001, Langeland et al. 2002, Glomski et al. 2003, Glomski and Getsinger 2006), the ALS inhibitors represent a new mode of action for controlling floating aquatic plants.

With the discovery of fluridone resistance in hydrilla (Michel et al. 2004, Arias et al. 2005) and diquat resistant dotted duckweed (Koschnick et al. 2006) it has become important to not rely on any one mode of action to control any invasive plant. While many aquatic herbicides including 2,4-D, diquat, and glyphosate have become cost effective mainstays of maintenance control programs, there are still issues with injury and selectivity when applied to native/non-native mixed communities containing bulrush (*Scirpus* spp.), spatter-dock (*Nuphar lutea* L.), cattail (*Typha* spp.), and other species (White 1965, Langeland et al. 2009, University of Florida 2011). New products need to be utilized in management programs that will not only minimize damage to nontarget species, but also provide acceptable control of target plants. Because of the limited amount of efficacy data available for bispyribac-sodium, studies were conducted to evaluate the activity of subsurface and foliar applications of bispyribac-sodium on the floating weeds water hyacinth, water lettuce and giant salvinia.

MATERIALS AND METHODS

The water hyacinth and water lettuce study was conducted outdoors June through August 2008 at the U.S. Army Corps of Engineers Lewisville Aquatic Ecosystem Research Facility (LAERF) in Lewisville, TX. Water hyacinth was obtained from cultures at LAERF and water lettuce was obtained from the University of Florida's Center for Aquatic and Invasive Plants (Gainesville, FL). To cover ca. 25% of the water surface for these trials, three to six plants of either water hyacinth or water lettuce were placed into

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each 76-L plastic container (ca. 49.5 cm (19.5 in) diam by 58.4 cm ht) filled with nutrient-amended Lake Lewisville water. Water was amended with Miracle-Gro® lawn fertilizer (36–6–6) at a rate that provided 10 mg N L^{-1} (10 ppm).

Plants were allowed to establish for one week prior to treatment. Bispyribac-sodium was applied as a subsurface treatment at concentrations of 5, 10, 20, 40, and 80 µg ai L^{-1} or as a foliar treatment at 5, 10, 20, 40, 59, and 119 g ai ha^{-1} (0.071, 0.14, 0.28, 0.56, 0.84, 1.7 oz ai A^{-1}). A nonionic aquatic surfactant (Cygnet Plus®, Flint, MI) at a rate of 0.25% v : v was added to foliar treatments for water hyacinth applications only. An untreated control was also included in the study. Foliar treatments were applied using a CO_2-pressurized sprayer (R&D Sprayers, Opelousas, LA) equipped with a hand-held, single-nozzle (TeeJet® solid cone spray tip) spray header calibrated to deliver a spray volume of 935 L ha^{-1}. Each tank was shielded during foliar application to prevent cross contamination of spray material between treatments. Subsurface applications were applied via pipette.

Treatments were randomly assigned and replicated four times. Eight weeks after treatment (WAT), all viable biomass was harvested and dried at 65 C to a constant weight. Data were transformed by taking the square root of the data to meet the assumptions of normality and equal variance. Data were then subjected to analyses of variance (ANOVA) procedures and means separated via the Student-Newman-Keuls method (SNK; $\alpha = 0.05$). Non-transformed data are presented.

Two giant salvinia studies were conducted in outdoor tanks from July through September 2009 at the U.S. Army Engineer Research and Development Center (USAERDC) in Vicksburg, MS. Giant salvinia plants were collected from outdoor cultures maintained at USAERDC. Equal amounts of fresh plant material (enough to cover 75% of the water surface; 11.8 ± 0.4 g dry weight) were placed into 76-L plastic containers. The containers were filled with tap water amended with Miracle-Gro lawn fertilizer (36–6–6) at a rate that provided 10 mg N L^{-1} and Aquashade™ (Applied Biochemists, Milwaukee, WI) at a rate of 1 mg L^{-1} to reduce light penetration and algal growth in the water column. The planted containers were placed inside 946 L tanks filled with water. The larger tanks served as a water bath to help maintain a consistent water temperature. Giant salvinia culture techniques were adapted from previous giant salvinia research (Nelson et al. 2001, 2007).

Plants were allowed to acclimate to container conditions for one week prior to herbicide treatment. At this time, a dense single layer of mature salvinia covering 100% of the water surface (23.1 ± 1.0 g dry weight) was formed. Bispyribac-sodium was applied as a subsurface treatment at concentrations of 5, 10, 20, 40, and 80 µg ai L^{-1} for study 1 and as a foliar treatment at 2.5, 5, 10, 20, 40 and 60 g ai ha^{-1} for study 2. Bispyribac-sodium was applied to giant salvinia using the same techniques and equipment as described above. The only difference was the nonionic surfactant Thoroughbred® was used instead of Cygnet Plus®. An untreated control was also included for both studies. Miracle-Gro was added at a rate that provided 10 mg N L^{-1} every 4 wk to each experimental unit to ensure adequate

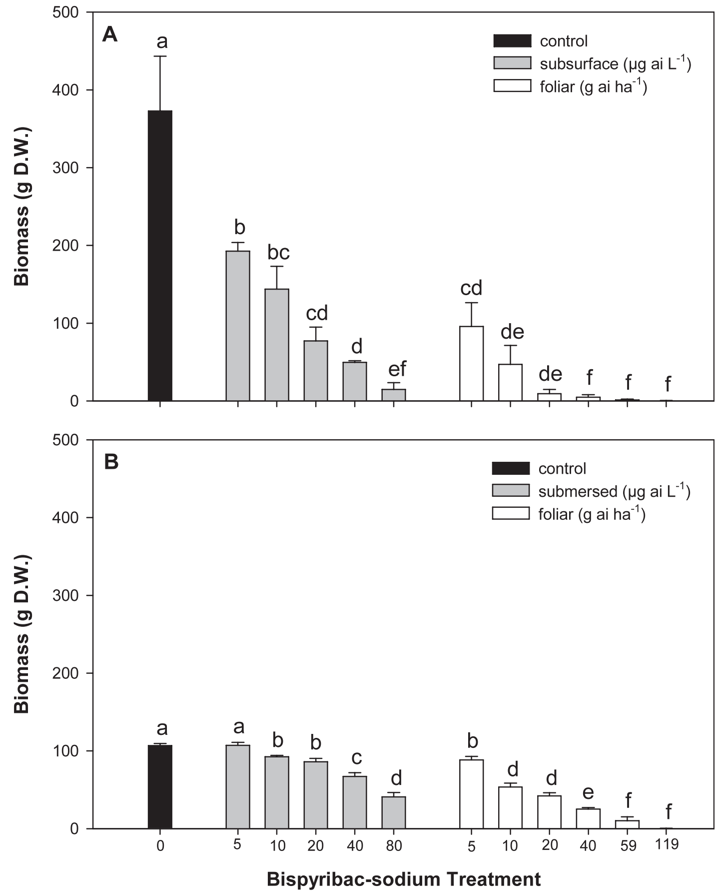

Figure 1. Mean (± SE) dry weight (D.W.) of (A) water hyacinth and (B) water lettuce biomass 8 weeks after treatment (WAT) with subsurface and foliar applications of bispyribac-sodium. Water hyacinth and water lettuce were harvested 8 wk after treatment (WAT). For each species, bars sharing the same letter do not significantly differ from each other. Data were subjected to ANOVA and means were separated using the Student-Newman-Keuls (SNK) method ($\alpha = 0.05$).

nutrients were available throughout the study. Both studies were concluded 12 WAT. All viable giant salvinia biomass was harvested at 12 WAT, dried to a constant weight (70 C for 1 wk), and recorded as mean dry weight. Treatments were randomly assigned and replicated four times for study 1 and three times for study 2. All data were subjected to ANOVA and means were separated using the SNK method ($\alpha = 0.05$).

RESULTS AND DISCUSSION

Two WAT all foliar treated water hyacinth was necrotic and/or chlorotic, whereas only plants treated at the highest subsurface concentration were severely injured. At 5 WAT, 95% or more of water hyacinth treated at foliar rates of $\geq$ 10 g ai ha^{-1} were visibly controlled; however, regrowth was observed in some plants at 10 and 20 g ai ha^{-1} throughout the remainder of the study. To be considered "controlled," plants were visibly brown and necrotic or had collapsed into the water column. At 8 WAT, water hyacinth biomass was reduced with all rates of bispyribac-sodium applied as either a subsurface injection or foliar spray (Figure 1A). Subsurface applications reduced biomass 48 to 96%, whereas foliar

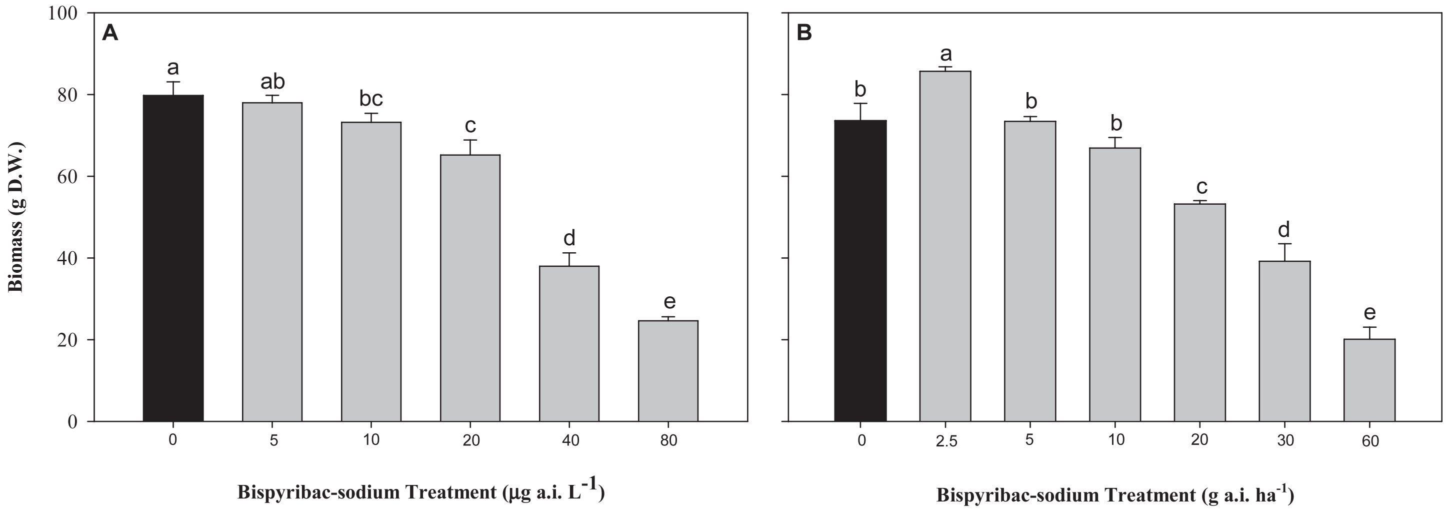

Figure 2. Mean (± SE) dry weight (D.W.) of giant salvinia biomass 12 wk after treatment (WAT) with (A) subsurface and (B) foliar applications of bispyribac-sodium. Bars sharing the same letter do not significantly differ from each other. Data were subjected to ANOVA and means were separated using the Student-Newman-Keuls (SNK) method ($\alpha = 0.05$).

applications reduced biomass 74 to 99%. The foliar applications of 40, 59, and 119 g ai ha^{-1} provided excellent water hyacinth control, measured as biomass reduction (98 to 99%). The maximum labeled foliar rate is 112 g ai ha^{-1} (2 oz A^{-1}). Although the 80 µg ai L^{-1} subsurface concentration reduced biomass by 96%, applications cannot exceed 45 µg ai L^{-1} per application and only four applications are allowed per year. All other subsurface concentrations and lower foliar rates evaluated would likely fail to provide acceptable water hyacinth control in the field if applied as a one time treatment. Overall, foliar applications of bispyribac-sodium are expected to provide excellent control of water hyacinth at rates of 40 to 112 g ai ha^{-1}.

At 2 WAT, subsurface applications of bispyribac-sodium at 40 and 80 µg ai L^{-1} caused minor chlorosis on water lettuce, and water lettuce treated with foliar applications of 40 g ai ha^{-1} and higher were chlorotic and/or necrotic. Water lettuce treated with subsurface bispyribac-sodium applications at concentrations $\geq$ 10 µg ai L^{-1}, as well as all foliar rates evaluated, resulted in significantly less biomass than the untreated control 8 WAT (Figure 1B). Percent control based on biomass reduction for subsurface applications ranged from 13 to 62%. This level of control would be considered unacceptable in the field, and remaining plants would serve as a source for re-infestation. In general, foliar applications were more effective than subsurface applications, with percent control based on biomass reduction ranging from 17 to 99%. Only the higher rates evaluated, 59 and 119 g ai ha^{-1}, could potentially provide acceptable control in the field, although re-treatment would probably be necessary to control re-growth. Future research needs to evaluate whether surfactants improve control of floating aquatic weeds with this product. For instance, previous research in creeping bentgrass (*Agrostis stolonifera* L.) fairways, demonstrated sequential bispyribac-sodium applications at 37 g ai ha^{-1} combined with spray adjuvants controlled annual bluegrass (*Poa annua* L.) similarly to 74 g ai ha^{-1} applied sequentially without adjuvants (McCullough and Hart 2008).

Visual injury symptoms of giant salvinia treated with either foliar or subsurface bispyribac-sodium applications 1 to 2 WAT included inhibition of new growth within the meristematic region and necrosis of older tissue. New fronds were tightly rolled almost in a bud, stunted, bright yellow, and similar to the classic ALS symptom "witches broom". The development of numerous small leaf material near the same growing point, or witches broom, is common in the imidazolinone family, another class of ALS herbicides, and it has been noted in trials on other aquatic species (Wersal and Madsen 2007). Leaf necrosis was noted for the first 2 WAT on all bispyribac-sodium foliar treated plants; however, these injury symptoms were nonexistent by 6 WAT for all foliar treatments except 60 g ai ha^{-1}. Plants treated with the highest foliar rate of bispyribac-sodium continued to show necrosis and growth regulation at the conclusion of the study.

At 2 to 6 WAT, healthy new fronds developed from older treated tissue on plants subjected to subsurface treatments of 5 to 20 µg ai L^{-1}. Approximately 50% of the older tissue for plants treated with concentrations $\geq$ 40 µg ai L^{-1} began to disintegrate and lose buoyancy by 6 WAT. Despite rapid injury, most treatments failed to growth regulate or continue plant desiccation after 6 WAT.

Subsurface applications of bispyribac-sodium at concentrations of 10 to 80 µg ai L^{-1} resulted in 8 to 69% giant salvinia control based on biomass reduction 12 WAT (Figure 2A). None of the treatments reduced biomass to less than pretreatment level (23.1 g) by the conclusion of the study. Although bispyribac-sodium concentrations $\geq$ 40 µg L^{-1} reduced dry weight $>$ 50%, plants began to recover from herbicide treatments before the conclusion of the study.

Foliar applications of bispyribac-sodium at 20 to 60 g ai ha^{-1} significantly reduced giant salvinia biomass by 28 to 73%, respectively (Figure 2B) when compared to untreated plants. Bispyribac-sodium applied at 5 and 10 g ai ha^{-1} did not reduce plant biomass 12 WAT, whereas the 2.5 g ai ha^{-1} treatment resulted in a 16% increase in biomass. The 2.5 g ai ha^{-1} treatment initially resulted in tissue desiccation, but plants quickly recovered and dry weight exceeded those of plants in the untreated control. Only the highest rate evaluated (60 g ai ha^{-1}) reduced giant salvinia biomass to below pretreatment level. The highest rate applied in this study, is below the maximum label rate of 112 g ai ha^{-1}; therefore future work should be done to determine if higher rates can improve control.

These data indicate bispyribac-sodium may temporarily growth regulate giant salvinia at lower concentrations (10 to 20 μg L^{-1}) and significantly reduce biomass at higher concentrations (40 to 80 μg L^{-1}) when applied as an in-water treatment; however, the plants may ultimately recover from a single subsurface treatment. Giant salvinia is difficult to control because of its prolific growth rate, mat-forming habit, and effective means of vegetative dispersal. This study was conducted under a best case scenario with only one layer of plants at herbicide treatment. Field infestations may achieve multiple layers of giant salvinia; up to one meter thick (Thomas and Room 1986). Consequently, repeat applications or tank mixing with other aquatic herbicides may be necessary to provide long-term control. However, the product label will restrict application rate and frequency (cannot exceed 45 μg L^{-1} day^{-1} or 4 applications yr^{-1}). Based on these data, bispyribac-sodium is not suitable to control giant salvinia as a one time subsurface application.

ALS herbicides, including bispyribac-sodium, can be more selective than 2,4-D, diquat, or glyphosate in areas where native and invasive plants co-exist. Previous research demonstrated the emergent native plants pickerelweed (*Pontederia cordata* L.) and duck potato (*Sagittaria lancifolia* L.) were marginally tolerant and tolerant, respectively, to bispyribac-sodium concentrations $\leq$ 40 μg L^{-1} (Glomski and Mudge 2009). Other research by Koschinick et al. (2007) showed EC_{50} values of 89 and 160 μg L^{-1} were required to reduce biomass of the grass species maidencane and panicgrass, respectively, further demonstrating the potential selectivity of bispyribac-sodium.

In these trials, bispyribac-sodium was efficacious against water hyacinth as a foliar and subsurface treatment, effective against water lettuce as a foliar treatment, and ineffective against giant salvinia regardless of application technique at rates tested. Future research should evaluate this herbicide at higher rates on giant salvinia and a range of rates on other floating, emergent, and submersed invasive species since limited information has been published.

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