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Short title: Metribuzin and *Amaranthus*

Optimizing metribuzin rates for herbicide-resistant Amaranthus weed control in soybean

Rishabh Singh¹, Aaron Hager², Sarah Lancaster³, Jason K. Norsworthy⁴, Karla Gage⁵, William Johnson⁶, Bryan Young⁶, Daniel Stephenson⁷, Jason Bond⁸, Kevin Bradley⁹, Amit Jhala¹⁰, Alyssa Essman¹¹, Lawrence E. Steckel¹², Thomas C. Mueller¹², Christy Sprague¹³, Travis Legleiter¹⁴, Rodrigo Werle¹⁵, Joseph Ikley¹⁶, Prashant Jha¹⁷ and Mithila Jugulam¹⁸

¹Graduate Research Assistant, Department of Agronomy, Kansas State University, Manhattan, KS, USA

²Professor, Department of Crop Sciences, University of Illinois, Urbana, IL, USA

³Assistant Professor, Department of Agronomy, Kansas State University, Manhattan, KS, USA

⁴Distinguished Professor, Department of Crop, Soil and Environmental Sciences, University of Arkansas, Fayetteville, AR, USA

⁵Associate Professor, School of Agricultural Sciences, Southern Illinois University, Carbondale, IL, USA

⁶Professor, Department of Botany and Plant Pathology, Purdue University, West Lafayette, IN, USA

⁷Professor, Dean Lee Research and Extension Center, Louisiana State University, Alexandria, LA, USA

⁸Professor, Delta Research and Extension Center, Mississippi State University, Stoneville, MS, USA

⁹Professor, Division of Plant Science and Technology, University of Missouri, Columbia, MO, USA

¹⁰Professor, Department of Agronomy and Horticulture, University of Nebraska-Lincoln, Lincoln, NE, USA

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¹¹Assistant Professor, Department of Horticulture and Crop Science, The Ohio State University, Columbus, OH, USA

¹²Professor, Department of Plant Science, University of Tennessee, Knoxville, TN, USA

¹³Professor, Department of Plant, Soil and Microbial Sciences, Michigan State University, East Lansing, MI, USA

¹⁴Associate Professor, Department of Plant and Soil Sciences, University of Kentucky, Lexington, KY, USA

¹⁵Associate Professor, Department of Plant and Agroecosystem Sciences, University of Wisconsin-Madison, Madison, WI, USA

¹⁶Associate Professor, Department of Plant Sciences, North Dakota State University, Fargo, ND, USA

¹⁷Professor, School of Plant, Environmental and Soil Sciences, Louisiana State University Agricultural Center, Baton Rouge, LA, USA

 $\textbf{Authors for correspondence:} \ \textbf{Mithila Jugulam, Email: m.jugulam@ag.tamu.edu; Sarah}$

Lancaster, Email: slancaster@ksu.edu

¹⁸Professor and Center Director, Texas A&M University, Beaumont, TX, USA

Abstract

Palmer amaranth and waterhemp are troublesome weeds in U.S. corn, soybean, and cotton production systems. Rapid evolution of resistance to herbicide from multiple sites of action in these species warrant alternate weed control options. Metribuzin applied preemergence can provide effective control of herbicide-resistant Amaranthus species. However, despite its decades of efficacy, many growers remain unaware of its weed control potential or are hesitant to use it due to concerns over crop injury. Field experiments were conducted in 2022 and 2023 in 15 states across the United States to investigate residual control of Palmer amaranth and waterhemp with metribuzin applied preemergence to soybean. Sites had either herbicide-resistant Palmer amaranth or waterhemp as the dominant weed species. Seventeen preemergence treatments were evaluated, including 13 doses of metribuzin (210 to 841 g ai ha⁻¹), a dose of sulfentrazone (420 g ai ha⁻¹), and a dose of S-metolachlor (1,790 g ai ha⁻¹), along with nontreated and a weed-free control plots. Weed control and soybean injury were visually assessed and recorded at 14, 28, and 42 d after application (DAA) of preemergence herbicides. Additionally, weed density, weed biomass, and soybean height were recorded 28 DAA followed by a measure of soybean yield at maturity. Weed control was analyzed as a function of metribuzin dose and environmental factors using a generalized additive model. Crop injury of not more than 5% was predicted even with 841 g ai ha⁻¹ of metribuzin. Metribuzin at 630 g ai ha⁻¹ was more effective than sulfentrazone in delaying weed emergence and reducing weed density, while 315 g ai ha⁻¹ of metribuzin outperformed S-metolachlor in both metrics. Metribuzin doses of 578 to 841 g ai ha⁻¹ provided greater than 95%, 90%, and 80% weed control, respectively, at 14, 28, and 42 DAA. Higher metribuzin doses of 578 to 841 g ai ha⁻¹ may be used safely to effectively control herbicideresistant Amaranthus weeds.

Nomenclature:

Metribuzin; S-metolachlor; sulfentrazone; Palmer amaranth, Amaranthus palmeri S. Watson; waterhemp, Amaranthus tuberculatus (Moq.) Sauer; corn, Zea mays L.; cotton, Gossypium L.; soybean, Glycine max (L.) Merr.

Keywords: Pigweeds, residual control, soybean injury

Introduction

The *Amaranthus* genus consists of approximately 50 species that are native to the Americas (Kigel 2018). Among these, the two dioecious species, Palmer amaranth and waterhemp, are consistently ranked among the most problematic weeds in U.S. cropping systems, especially soybean (Van Wychen 2022). Palmer amaranth densities of 0.33 to10 plants m⁻¹ of row at 8 to 12 wk after emergence have been reported to reduce soybean yield by 17% to 68%, respectively (Klingaman and Oliver 1994). Soybean yield losses of 43% were reported after 10 wk of waterhemp interference at densities of 89 to 362 plants m⁻² (Hager et al. 2002).

Herbicide resistance was first reported in Palmer amaranth in 1989 in South Carolina and in waterhemp in 1993 in Illinois (Heap 2024). The evolution of herbicide-resistant biotypes of Palmer amaranth and waterhemp has contributed to an increase in soybean production costs and the need to make complicated weed management decisions. Currently, Palmer amaranth populations in the United States have been confirmed with resistance to herbicides from nine sites of action (SOAs). These include inhibitors of acetolactate synthase (ALS; Group 2), microtubule assembly (Group 3), photosystem II (PS II; Group 5), enolpyruvylshikimate-3-phosphate synthase (EPSPS; Group 9), protoporphyrinogen oxidase (PPO; Group 14), very-long-chain fatty acids (VLCFAs; Group 15), 4-hydroxyphenylpyruvate dioxygenase (HPPD; Group 27), glutamine synthetase (GS; Group 10), and synthetic auxin herbicides (SAHs; Group 4) (Heap 2024). (Note: herbicide group numbers are assigned by the Herbicide Resistance Action Committee [HRAC] and the Weed Science Society of America [WSSA].) Waterhemp resistance in the United States has also been reported to herbicides from seven different SOAs including those that inhibit EPSPS, ALS, PS II, PPO, HPPD, VLCFAs, and SAHs (Heap 2024).

Introduction of glyphosate resistant crops in the late 1990s propelled weed management efforts to focus on using postemergence herbicides in place of combinations of preemergence plus postemergence options, thus accelerating the evolution of glyphosate resistance (Givens et al. 2009; Powles 2008). Oliveira et al. (2017) reported benefits of using preemergence herbicides to control annual broadleaf and herbicide-resistant weeds, including Palmer amaranth and waterhemp. Preemergence herbicides provide weed control during the first 3 to 4 wk after crop planting but they also reduce the selection pressure on postemergence herbicides (Butts et al. 2017; Knezevic et al. 2019; Tursun et al. 2016). Additionally, effective preemergence herbicides were found to be an important strategy to control other herbicide-resistant weeds (Norsworthy et

al. 2012). The current scenario of herbicide resistance by weeds has led to fewer postemergence weed control options, prompting growers to increase their use preemergence herbicides. Between 2006 and 2017, the hectarage of soybean fields treated with metribuzin, sulfentrazone, and *S*-metolachlor has increased by 16%, 21%, and 15%, respectively (USDA-NASS 2017).

Metribuzin is an asymmetric triazine herbicide. As a systemic herbicide metribuzin is readily absorbed by the roots and inhibits the flow of electrons through PS II, generating reactive oxygen species, which ultimately leads to plant death. Metribuzin was registered in the United States in 1973 and was soon adopted as a major preemergence herbicide for use in soybean production. However, use of metribuzin significantly dropped during the 1990s with total treated hectarage decreasing from 3.6 million ha in 1990 to 1.82 million ha in 1999 (US EPA 2003). Recent research has shown that metribuzin can provide good to excellent residual control of herbicide-resistant waterhemp and Palmer amaranth, including populations with metabolic resistance to atrazine (Meyer et al. 2015; Vennapusa et al. 2018; Vyn et al. 2007). Metribuzin is a common component of commercially available premixes for soybean because it can be naturally metabolized by soybean plants. However, when metribuzin is used in a premix combination, the dose (210 to 420 g ai ha⁻¹) is frequently too low to achieve the needed duration of residual control of Amaranthus species. For soybean producers who adopt metribuzin as a preemergence herbicide, there is often concern about early season crop injury, which may potentially lead to yield reductions. A typical symptom of metribuzin injury includes interveinal leaf chlorosis that progresses to necrosis, which is primarily evident on unifoliate and first trifoliate leaves. Risk of soybean injury increases with high soil pH (>7) and/or soils with low organic matter (OM) (<1%) due to greater availability of the herbicide (Hartzler 2017; Shaner 2014). Additionally, soybean injury also depends on variety and temperature, as cooler temperatures tends to reduce soybean emergence vigor and the plant's ability to metabolize the herbicide (Hartzler 2017).

Similar to the most soil-applied herbicides, the biologically effective dose of metribuzin is influenced by the interaction between the herbicide and other edaphic and environmental parameters that influence herbicide availability, retention, adsorption, and transport. Soybean tolerance to metribuzin is greatly influenced by metribuzin dose, soil OM, and amount of rainfall following application (Coble and Schrader 1973). Thus, we hypothesize that metribuzin rates greater than the current commercial premixes (210 to 420 g ai ha⁻¹) can effectively control

Palmer amaranth and waterhemp with no adverse effects on soybean growth and development. This study was designed to evaluate the entire use range of metribuzin across 13 treatments, with three major objectives: 1) determine the optimal metribuzin dose for residual control of herbicide-resistant Palmer amaranth and waterhemp under varied soil and environmental conditions; 2) evaluate early season growth and development of soybean following preemergence application of metribuzin at multiple doses; and 3) determine whether potential early season herbicide-induced injury could affect soybean yield.

Materials and Methods

Site Description

Field experiments were conducted during 2022 and 2023 in 15 states in the United States: Arkansas (Experiments AR'22 and AR'23); Illinois (IL'22, IL'23, SIL'22, and SIL'23); Indiana (IN'22 and IN'23); Iowa (IA'23); Kansas (KS'22 and KS'23); Kentucky (KY'22 and KY'23); Louisiana (LA'22 and LA'23); Michigan (MI'22 and MI'23); Mississippi (MS'22 and MS'23); Missouri (MO'22 and MO'23); Nebraska (NE'22 and NE'23); North Dakota (ND'22 and ND'23); Ohio (OH'22 and OH'23); Tennessee (TN'22 and TN'23); and Wisconsin (WI'22 and WI'23). Additional information on management practices for each site is summarized in Table 1. Soil characteristics and rainfall data for the duration of the experiment are summarized in Table 2. Site-years offered variability in soil texture, OM (1.5% to 6.6%), pH (5.5 to 8.0), interval between application and first precipitation (0 to 18 d after application of herbicide; DAA) and cumulative precipitation 42 DAA (46 to 343 mm). Naturally occurring infestations of herbicide-resistant Palmer amaranth or waterhemp at each site were evaluated for control (Table 1).

Experimental Design and Treatments

Experiments were conducted in a randomized complete block design with three or four replications. Plot size was approximately 3 m by 9.1 m and differed slightly among site-years primarily due to row spacing and management practice unique to each site. Soybean were planted at 300,000 to 390,000 seeds ha⁻¹ following preplant tillage to prepare a weed-free site at trial establishment. Tillage, planting, and herbicide application was completed within a 24- to 48-h window; therefore, all further assessments use herbicide application date as reference. A total of 17 single active—ingredient treatments were evaluated to determine the effective dose of

metribuzin to control Palmer amaranth and waterhemp. Treatment information is summarized in Table 3, which includes 13 treatments of metribuzin ranging from 210 to 841 g ai ha⁻¹ (subsequent treatments were in increments of 52 g ai ha⁻¹), along with two comparison herbicides, sulfentrazone (420 g ai ha⁻¹) and *S*-metolachlor (1,790 g ai ha⁻¹), and nontreated and weed-free controls. Herbicides were applied within 1 d after planting using a CO₂-pressurized backpack sprayer calibrated to deliver 140 L ha⁻¹ at most locations, except at the Illinois and Michigan sites in both years (Supplementary Table S1). The application rate and nozzle used at each site are listed in Supplementary Table S1. Weed-free plots were maintained that way either by mechanical or chemical weed control options unique to each site to reduce the effects of weed interference on soybean growth and development (Supplementary Table S1).

Data Collection

Weed parameters, including weed control, weed density, and weed biomass, were recorded only for Palmer amaranth or waterhemp at respective sites and will collectively be referred to as Amaranthus weeds considering their physiological, morphological, and biological similarities. Weed control was visually assessed and recorded at 14, 28, and 42 DAA of herbicide. Each experimental unit was visually evaluated on a scale of 0% to 100% relative to nontreated plots, with 0% representing no weed control and 100% representing complete weed control. Weed emergence was recorded as the number of days after planting until the first emergence of Amaranthus weeds. Additionally, weed density and aboveground weed biomass per square meter were collected using four 0.25-m² quadrants per plot at 28 DAA. Aboveground weed biomass was harvested separately from each plot in paper bags and dried in an oven at 65 C for 72 h. Soybean injury was visually assessed and recorded at 14, 28, and 42 DAA on a scale of 0% to 100% injury relative to nontreated plots, with 0% representing no injury and 100% representing complete mortality of soybean plants. Injury symptoms included physiological growth, emergence, interveinal chlorosis, and necrosis. The height of six randomly selected soybean plants per plot was measured to calculate the average soybean height. Additionally, soybean yield was recorded at maturity (Supplementary Table S2).

Statistical Analyses

Data from IL'22, IA'23, LA'23, MS'22, and MS'23 were excluded from further analysis due to technical errors or experimental failure, making the data insufficient for drawing any conclusions. All data were processed and analyzed using R Studio software with respective packages (R Core Team 2021). Soil moisture at the time of application was a categorical variable reported in seven levels (very dry, dry, fair, adequate, good, damp, and moist) and so were reduced to three levels: dry for very dry and dry site-years; fair for fair, adequate, and good siteyears; and *moist* for damp and moist site-years (Table 2). Soybean planting date was transformed to day of the year format to maintain consistency. Weather data collected from the respective weather stations were used to calculate precipitation values. Based on research by Meyer (2023), who identified 12.7 mm of precipitation within the first 2 wk after application as being critical for optimizing herbicide performance, this value was used as a threshold for further analysis. A principal component analysis (PCA) was conducted using the prcomp function to evaluate the differences across site-years (Venables and Ripley 2002). The following variables were included for calculating eigenvectors: soil texture (sand, silt, and clay %); soil OM; soil temperature at the time of herbicide application; soil pH; interval between application and first precipitation; amount of first precipitation; days between application and cumulative 12.7 mm precipitation; and cumulative precipitation at 42 DAA. Following PCA, a random forest approach was used to predict the importance score of covariates determining weed control using the RANDOMFOREST package (Liaw and Wiener 2002). Weed control was selected as the predicted variable for the random forest model and was evaluated as a linear function of all the variables used for PCA in addition to metribuzin dose, weed control days after application, seeding rate, row spacing, soil moisture at application, and planting date.

Weed control was analyzed using the generalized additive model (GAM) from the MGCV package specifying a beta regression family with a probit link to account for the bounded nature of percent control data (Wood 2011). Weed control was evaluated as a function of metribuzin dose, soil OM, soil pH, clay content, interval between application and first precipitation, amount of first precipitation, days between application, cumulative 12.7 mm precipitation, cumulative precipitation at 42 DAA, soil temperature, and soil moisture at application. A smooth term was included for metribuzin dose to capture the potential nonlinearity in the dose-response relationship. Weed density and weed biomass data were analyzed separately using GAM as a

function of metribuzin dose. Crop injury data were analyzed separately at 14, 28, and 42 DAA using a GAM with crop injury as a function of metribuzin dose. Predicted soybean response was then plotted against the metribuzin dose. The EMMEANS package was used to estimate marginal means for soybean height and yield data across treatments (Lenth 2023). The estimated means were then subjected to pairwise comparison using Tukey's honestly significant difference (HSD) test ($\alpha = 0.05$). Additionally, weed control with sulfentrazone and S-metolachlor was compared with that of 525 g ai ha⁻¹ of metribuzin by subjecting the estimated means to pairwise comparisons using Tukey's HSD ($\alpha = 0.05$). Crop injury and all weed parameters except weed control were evaluated separately for sulfentrazone and S-metolachlor treatments similar to soybean height and yield as outlined previously.

Results and Discussion

Principal Component and Random Forest Analyses

A PCA biplot was developed to summarize the geographical differences in terms of soil and precipitation parameters. Dimensions 1 and 2 combined explain 48.4% variability among siteyears. In the PCA biplot the IL'23 and MI'23 site-years were distantly separated from other sites along the principal components, primarily driven by interval between application and first precipitation and days between application and cumulative 12.7 mm precipitation (Figure 1). The IL'23 experiment received its first precipitation 18 DAA and required 23 d to accumulate 12.7 mm of precipitation, whereas the MI'23 experimental plots received their first precipitation 19 DAA and required 32 d to accumulate 12.7 mm of precipitation (Table 2). Data from IL'23 and MI'23 were analyzed separately from other site-years due to the delayed timing of precipitation following herbicide application. Precipitation within the first 2 wk of herbicide application is an important factor for herbicide incorporation and subsequent weed control (Landau et al. 2021; Meyer 2023). Because rain was substantially delayed at these two sites, they were categorized as representing a delayed precipitation condition. In contrast, the data from all site-years, except IL'23 and MI'23, were clustered together and labeled as having optimum precipitation conditions (Figure 1). This classification was based solely on the time required to accumulate 12.7 mm of precipitation, rather than total rainfall. The optimum precipitation condition subset was used for all further analyses. In contrast, the delayed precipitation condition subset was analyzed only for weed control due to its limited variability and small data set size.

In the context of random forest analysis, a variable importance plot quantifies the contribution of each variable to the overall predictive accuracy of the model. As expected, metribuzin dose was the largest factor in predicting weed control followed by weed control DAA (Figure 2). Weed control days after application is an indication of how long into the growing season weed control is achieved (i.e., 14, 28, or 42 DAA). Cumulative precipitation and amount of first precipitation were the two biggest co-variates among the precipitation parameters (Figure 2). Soil OM, soil texture, and soil pH were the major co-variates among the soil parameters. Seeding rate and row spacing had a minimal effect on predicting weed control. Therefore, these two variables were excluded from further analysis (Figure 2). Previous research suggests that seeding rate and row spacing have inconsistent effects on weed control in soybean, although some studies reported reduced weed density and increased weed control with higher seeding rates of soybean (Arce et al. 2009; Place et al. 2009). A meta-analysis revealed that narrow rows reduced weed biomass by 71%, yet 36% of soybean trials showed no significant weed suppression (Singh et al. 2023).

Weed Control

Optimum Precipitation Condition. Amaranthus weed control was assessed using a GAM, and all the co-variates had a significant effect in predicting weed control except soil pH and clay content (Table 4). This is likely due to a lack of variability in soil pH and clay content among site-years. The smooth term for dose was highly significant (effective degrees of freedom [edf] = 3.76, P < 0.001), indicating a flexible, nonlinear relationship. Derivatives of the smooth function revealed that the rate of increase in control was highest at low doses and plateaus at higher doses. Weed control was directly related to metribuzin dose. Effectiveness of metribuzin to control weeds decreased as time progresses in the growing season, with the overall greatest control achieved at 14 DAA and the least control at 42 DAA (Figure 3). At 14 DAA, 473 g ai ha⁻¹ of metribuzin provided 95% weed control with the maximum weed control of 98% achieved with 841 g ai ha⁻¹ of metribuzin (Figure 3). At 28 DAA, weed control was <95% for all metribuzin doses. However, 90% weed control was achieved using 630 g ai ha⁻¹ metribuzin with a marginal increase in weed control at greater doses of metribuzin at 28 DAA (Figure 3). For the lower doses of metribuzin (210 to 420 g ai ha⁻¹) weed control decreased at 42 DAA compared with control at 14 and 28 DAA. Eighty percent and 85% weed control was achieved with 578 and 683

g ai ha⁻¹, respectively, at 42 DAA. Weed control gradually increased with an increasing dose of metribuzin at 42 DAA with a maximum control of 89% achieved when 841 g ai ha⁻¹ was applied (Figure 3).

Metribuzin (525 g ai ha⁻¹) and sulfentrazone (420 g ai ha⁻¹) consistently provided similar weed control at 14, 28, and 42 DAA. In contrast, *S*-metolachlor (1,790 g ai ha⁻¹) resulted in significantly lower weed control at all evaluation times. At 14 DAA metribuzin and sulfentrazone provided >92% weed control compared with 88% control for *S*-metolachlor. Although metribuzin and sulfentrazone provided >78% weed control at 42 DAA compared with 59% control achieved with *S*-metolachlor (Table 5).

Delayed Precipitation Condition. Control of Amaranthus weed species was analyzed separately for IL'23 and MI'23 using a GAM. Metribuzin dose, weed control DAA, soil temperature, days between application, and cumulative 12.7 mm precipitation had a significant effect in predicting weed control. In previous research, soil temperatures were found to affect early season weed control only when rainfall was scant during the first 15 DAA (Landau et al. 2021). The smooth term for dose was highly significant (edf = 1.92, P < 0.001), indicating a flexible, nonlinear relationship. Weed control at 14 DAA was likely affected due to dry and fair soil moisture at the time of herbicide application followed by no precipitation up to 19 and 32 DAA. Weed control increased with an increasing dose of metribuzin. The effectiveness of metribuzin to control weeds decreased over time with overall greatest control occurring at 14 DAA with a metribuzin dose of 841 g ai ha⁻¹. At 14 DAA, 630 g ai ha⁻¹ of metribuzin provided 80% weed control with a maximum weed control of 91% achieved with 841 g ai ha⁻¹ of metribuzin. While 80% weed control was achieved at 28 DAA using 578 g ai ha⁻¹ metribuzin, this increased to 88% when 841 g ai ha⁻¹ metribuzin was applied. Weed control at 14 and 28 DAA was comparable with applications of 525 to 841 g ai ha⁻¹ metribuzin, while weed control decreased later into the growing season at 42 DAA. A maximum weed control of 76% was achieved at 841 g ai ha⁻¹ 42 DAA (Figure 4).

At the IL'23 and MI'23 site-years, metribuzin applied at 525 g ai ha⁻¹ provided significantly greater (82%) weed control than either sulfentrazone applied at 420 g ai ha⁻¹, which provided 30% control, or S-metolachlor at 1,790 g ai ha⁻¹, which provided 33% when assessed at 14 DAA (Table 6). However, by 28 and 42 DAA, no significant differences were observed among treatments. Weed control at 28 DAA ranged from 79% to 84%, and decreased slightly across treatments by 42 DAA, ranging from 64% to 74% (Table 6).

Amaranthus Weed Emergence. Weed emergence was recorded for 9 site-years (Supplementary Table S2). Weeds emerged as early as 6 DAA in some nontreated plots. An overall delayed weed emergence for the site-years in Missouri and Ohio was observed (data not shown). Sulfentrazone and S-metolachlor applications resulted in delayed weed emergence at 27 and 21 DAA, respectively (Figure 5). Metribuzin doses of 630 to 841 g ai ha⁻¹ delayed weed emergence longer than sulfentrazone and S-metolachlor, whereas 841 g ai ha⁻¹ metribuzin delayed emergence to an average of 33 DAA.

Amaranthus Weed Density and Biomass. Weed density was recorded for 20 site-years at 28 DAA (Supplementary Table S2). The lowest weed density was observed at ND'23, with an average of 9 plants m⁻², and the greatest density was observed at MO'23 where approximately 1,300 plants m⁻² were counted in the nontreated plot. We observed the lowest weed densities when 525 to 841 g ai ha⁻¹ of metribuzin were applied, which was similar when sulfentrazone was applied (Figure 6). Weed density after S-metolachlor treatment was similar to that when lower doses of metribuzin (210 to 315 g ai ha⁻¹) were applied (Figure 6).

Weed biomass was recorded for 16 site-years (Supplementary Table S2). Nontreated KS'23 and MO'23 plots accumulated the greatest weed biomass. We observed less weed biomass at 28 DAA when 525 to 841 g ai ha⁻¹ of metribuzin was applied (Figure 7), whereas similar mean weed biomass measurements of 6 and 9.4 g per m⁻² were recorded after applications of sulfentrazone and *S*-metolachlor, respectively, when assessed at 28 DAA.

In this research, effective control of *Amaranthus* weeds occurred when precipitation conditions were optimal, particularly when higher doses of metribuzin (578 to 841 g ai ha⁻¹) were used. A maximum control of 98% was achieved with applications of 841 g metribuzin ha⁻¹ when assessed at 14 DAA. The efficacy of metribuzin declined later in the growing season. At 28 DAA, 90% control was achieved with 630 g ai ha⁻¹ of metribuzin. Meyer et al. (2016) reported 69% control of Palmer amaranth 4 wk after treatment with 420 g ai ha⁻¹ of metribuzin, which was less than that when 1,068 g ai ha⁻¹ of *S*-metolachlor was applied, which provided 89% control in a coarse-textured soil with little OM. However, both *S*-metolachlor and metribuzin treatments provided similar reductions in Palmer amaranth and waterhemp density (Meyer et al. 2016). In our study, by 42 DAA the effectiveness of metribuzin decreased, especially at lower doses of 210 to 420 g ai ha⁻¹. However, the highest dose of 841 g ai ha⁻¹ still provided 89% control. In contrast, *S*-metolachlor exhibited a sharp decline in efficacy, dropping

to 59% control at 42 DAA. Additionally, results suggest that metribuzin (525 g ai ha⁻¹) and sulfentrazone (420 g ai ha⁻¹) offer superior residual *Amaranthus* control compared to *S*-metolachlor (1,790 g ai ha⁻¹). However, previous research suggests that sulfentrazone (280 g ai ha⁻¹) and *S*-metolachlor (1,787 g ai ha⁻¹) provided better residual control of Palmer amaranth than metribuzin (563 g ai ha⁻¹) (Ribeiro et al 2021b). That conclusion was based on the greenhouse bioassays conducted on silty loam soils at a depth of 0 to 10 cm with OM ranging from 2.6% to 3.1% and pH of 6.5 and 7 (Ribeiro et al 2021b).

When precipitation was delayed, we observed that metribuzin was more effective, potentially due to its higher solubility, whereas sulfentrazone and *S*-metolachlor exhibit reduced early season activity until adequate the soil was adequately moist. The solubility and soil half-life of these herbicides influence their water requirements for activity and persistence in the soil. Metribuzin, with its high solubility (1,100 mg L⁻¹ at 20 C) and moderate soil half-life (30 to 60 d) requires less initial moisture for activity and can quickly move into the weed seed zone (Shaner 2014). However, its mobility also increases the risk of leaching when rain is excessive. *S*-metolachlor, with lower solubility (488 mg L⁻¹ at 20 C) and a shorter soil half-life (15 to 50 d), requires more consistent and timely precipitation for optimal activity and is less prone to leaching (Shaner 2014). Sulfentrazone, with moderate solubility (780 mg L⁻¹ at pH 7) and a long soil half-life (121 to 302 d), is more persistent but may require higher moisture levels for activity, making its efficacy more dependent on sufficient and timely precipitation (Shaner 2014). Landau et al. (2021) also reported that the probability of effective control of three annual weed species increased as rainfall increased to a threshold of 10 mm. Additionally, herbicide combinations achieved maximum efficacy when rainfall was low (Landau et al. 2021).

Furthermore, this study highlights the efficacy of metribuzin to control herbicide-resistant *Amaranthus* weeds. Palmer amaranth and waterhemp populations at experimental sites were resistant to multiple herbicides (mostly to Group 2 and Group 9 herbicides) as listed in Table 1. Specifically, populations of Palmer amaranth and waterhemp in plots in Illinois, Indiana, and North Dakota were resistant to postemergence-applied Group 5 herbicides (potentially atrazine), yet weeds were effectively controlled with metribuzin. This suggests that metribuzin remains an effective herbicide for managing multiple herbicide–resistant populations of Palmer amaranth and waterhemp.

Soybean Response

Crop injury increased with an increasing dose of metribuzin with predicted injury of no more than 5% even at the highest dose of metribuzin (841 g ai ha⁻¹; Figure 3). Crop injury as high as 10% was recorded at LA'22, MI'22, OH'22, and TN'23 at 14 DAA, which recovered at 28 and 42 DAA (<5%; data not shown); whereas injury ranging up to 20% was reported at 42 DAA at the AR'22 and LA'22 plots (data not shown). There was no injury with applications of either sulfentrazone (420 g ai ha⁻¹) or *S*-metolachlor (1,790 g ai ha⁻¹; Table 7).

Soybean Height. Soybean height was recorded for 20 site-years at 28 DAA (Supplementary Table S2). Soybean growth varied across site-years depending on the relative weather conditions and soybean cultivar. Overall, there was no difference in soybean height among all treatments, especially comparing the nontreated and weed-free plots with those that received herbicide treatments, suggesting that herbicides had no effect on soybean height (Figure 8).

Soybean Yield. Soybean yield was recorded for 7 site-years at crop maturity (Supplementary Table S2). A minimum yield was recorded at KS'23, averaging 531 kg ha⁻¹ from nontreated plots. A maximum yield was recorded from weed-free plots in NE'23, averaging 4,300 kg ha⁻¹. Despite the absence of postemergence herbicide applications, preemergence herbicide treatments resulted in soybean yields that were similar to those observed in the weed-free control (Figure 9). This is likely due to reduced competitiveness of the weeds that survived preemergence herbicides. Previously, Adcock and Banks (2017) reported reduced weed competition and lowered water use after an application of 0.4 kg ha of metribuzin. Soybean yield following metribuzin application at 315 to 841 g ai ha⁻¹ and sulfentrazone applications were similar to yield from the weed-free plots. Lower yields were observed from plots that received *S*-metolachlor treatment and 210 g ai ha⁻¹ of metribuzin (Figure 9).

There was minimal soybean injury resulting from the application of metribuzin, even at the highest dose of 841 g ai ha⁻¹. The variability in injury at the OH'22, TN'23, AR'22, and LA'22 plots could be attributed to low OM and heavy rainfall following herbicide application, as herbicide adsorption is known to be strongly correlated with soil OM and texture (Blumhorst et al. 1990). Additionally, extended periods of cool, wet soil conditions during crop emergence are known to reduce soybean's ability to metabolize preemergence herbicides, potentially leading to soybean injury (Moomaw and Martin 1978; Osborne et al. 1995). Soybean plants, due to their

indeterminate growth habit, are known to compensate for herbicide injury occurring during early developmental stages (Cox and Cherney 2011, Ribeiro et al 2021a, Weidenhamer et al. 1989). This might be why we observed no negative effects on soybean height and yield in our study following metribuzin application. In previous studies, metribuzin (560 g ai ha⁻¹) and sulfentrazone (280 g ai ha⁻¹) applied preemergence did not reduce soybean yield on loam soil texture with soil OM ranging from 1.7% to 2.2% and pH from 6.7 to 7.5 (Arsenijevic et al 2021). However, sulfentrazone resulted in a 22% reduction in green canopy at the V2 growth stage and a 10% reduction in plant stand at maturity compared with a nontreated control. Despite these effects, overall soybean yield was increased by 3% with metribuzin and sulfentrazone treatments, and this can be attributed to a higher number of seeds produced per plant. Interestingly, in our study, sulfentrazone and S-metolachlor did not cause concerning crop injury levels. This contrasts with findings by Taylor-Lovell et al. (2001), who reported up to 61% soybean injury across 15 varieties following sulfentrazone (446 g ai ha⁻¹) application, with greater injury observed under prolonged wet and cool conditions after planting. Previous research has reported differential tolerance of soybean varieties to preemergence herbicides (Taylor-Lovell et al. 2001); however, this was beyond the scope of our study, and necessary comparisons were not included in the experimental design.

Practical Implications

Weed control remains a top priority for soybean producers throughout the United States. The challenge is accompanied by accelerating evolution of herbicide-resistant weeds. This multistate study demonstrates that metribuzin, a long-established soil-residual herbicide, remains a viable option for residual control of Palmer amaranth and waterhemp. Results suggest that metribuzin can be safely applied at higher rates than those commonly included in commercial premixes, particularly in optimum precipitation conditions. Doses ranging from 578 to 841 g ai ha⁻¹ can be judiciously incorporated into herbicide rotation strategies for effective control of *Amaranthus* weeds. Even under delayed precipitation conditions, metribuzin retained activity better than rates used for sulfentrazone (420g ai ha⁻¹) and *S*-metolachlor (1,790g ai ha⁻¹), likely due to metribuzin's higher solubility. The study also confirms effectiveness of metribuzin on weed populations that have become resistant to multiple herbicide SOAs, including PS II inhibitors such as atrazine.

Preemergence herbicides have regained importance for building effective weed management strategies. The weed control efficacy of preemergence herbicides likely outweighs the concerns associated with early season soybean injury. Incorporating higher rates of metribuzin into preemergence herbicide program can improve early season weed control, delay the critical period of weed control, and reduce the selection pressure on postemergence herbicides.

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Competing Interests

The authors declare they have no competing interests.

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Table 1. Management practices for 32 site-years included in this study.^a

Site-year	Location	Soybean	Seeding	Row	Plantin	Herbicide	Metribuzin	Plot	Wee	Resistance
		variety	rate	spacin	g date	applicatio	product	size	d	profile ^b
				g		n				
			seeds	m				m		
			ha^{-1}							
Arkansas	Fayetteville,	P48A14E	375,000	0.91	May 12	May 13	Tricor® 4F	3.7×7.	PA	2, 9
2023	AR							6		
Arkansas	Fayetteville,	AG48XF0	375,000	0.91	May 12	May 13	Tricor [®] 4F	3.7×7.	PA	2, 9
2022	AR							6		
Illinois 2022	Sidney, IL	AG33XF2	350,000	0.76	June 4	June 4	Metricor® DF	3×7.6	WH	2 ,4, 5, 9, 14, 15,
										27
Illinois 2023	Sidney, IL	AG33XF2	350,000	0.76	May 18	May 18	Metricor® DF	3×7.6	WH	2 ,4, 5, 9, 14, 15,
										27
Indiana	Francesville,	AG29XF1	367,500	0.76	May 11	May 11	Metricor® DF	2×9.1	WH	2, 4, 5, 9, 14, 27
2022	IN									
Indiana	Francesville,	AG30XF2	350,000	0.76	May 10	May 11	Metricor® DF	2×9.1	WH	2, 4, 5, 9, 14, 27
2023	IN									
Iowa 2023	_	_	_	_	_	_	_	_	_	_
Kansas 2022	Manhattan, KS	GH3982X	300,000	0.76	May 23	May 23	Metricor® DF	3×9.1	PA	2, 9, 14
Kansas 2023	Manhattan, KS	GH3982X	300,000	0.76	May 31	May 31	Metricor® DF	3×9.1	PA	2, 9, 14
Kentucky	Princeton, KY	Xitavo	350,000	0.76	May 16	May 16	Metricor® DF	3×9.1	PA	2, 9, 14
2022										
Kentucky	Princeton, KY	XO4522E	350,000	0.76	April	April 25	Metricor® DF	3×9.1	PA	2, 9, 14

2023					25					
Louisiana	Alexandria,	P49T62E	312,500	0.97	May 9	May 10	Glory® 4L	3.9×9.	PA	9
2022	LA							1		
Louisiana	_	_	_	_	_	_	_	_	_	_
2023										
Michigan	Lansing, MI	P24T35E	375,000	0.76	May 24	May 24	Metribuzin [®]	3×7.6	WH	2, 9
2022							75WG			
Michigan	Lansing, MI	AG26XF3	375,000	0.76	May 23	May 23	Metribuzin [®]	3×7.6	WH	2, 9
2023							75WG			
Mississippi	_	AG48XF2	325,000	1	April	_	Tricor® 4F	4×7.6	PA	_
2022					28					
Mississippi	_	AG48XF2	325,000	1	April	_	Tricor® 4F	4×7.6	PA	_
2023					17					
Missouri	Columbia, MO	MorSoy3861X	350,000	0.76	July 6	July 6	Metricor® DF	2×15.2	WH	9, 14
2022		E								
Missouri	Columbia, MO	XO3752E	375,000	0.76	May 3	May 3	Metricor® DF	2×15.2	WH	9, 14
2023										
Nebraska	Clay Centre,	AG 27XF1	335,000	0.76	May 18	May 18	Metricor® DF	3×9.1	PA	2, 9
2022	NE									
Nebraska	Clay Centre,	NK31-J9XF	337,500	0.76	April	April 25	Metricor® DF	3×9.1	PA	2, 9
2023	NE				25					
North	Spiritwood,	AG 09XF0	390,000	0.76	June 6	June 7	Tricor® 75DF	2×9.1	PA	2, 4, 5, 9, 27
Dakota 2022	ND									
North	Fargo, ND	AG 09XF0	390,000	0.76	May 24	May 24	Tricor® 75DF	2×9.1	WH	2, 9

Dakota 2022										
North	Fargo, ND	AG 07XF2	390,000	0.76	May 21	May 22	Tricor® 75DF	2×9.1	WH	2, 9
Dakota 2023										
Ohio 2022	Charleston,	P35T15E	375,000	0.76	May 24	May 24	Metricor® DF	3×9.1	WH	_
	ОН									
Ohio 2023	Charleston,	P35T15E	387,500	0.76	May 11	May 11	Metricor® DF	3×9.1	WH	_
	ОН									
S Illinois	Carbondale, IL	AG38XF1	350,000	0.76	May 31	June 1	Glory® 4L	2×10.7	WH	2, 9, 14
2022										
S Illinois	Carbondale, IL	AG38XF1	350,000	0.76	May 19	May 20	Glory® 4L	2×10.7	WH	2, 9
2023										
Tennessee	Holston, TN	AG45XF0	350,000	0.76	June 1	June 2	Tricor® DF	3×9.1	PA	2, 9
2022										
Tennessee	Holston, TN	AG48XF2	350,000	0.76	May 26	May 26	Tricor® 75DF	2×9.1	PA	2, 9
2023										
Wisconsin	Brooklyn, WI	NK22-C43E3	350,000	0.76	May 23	May 23	Metribuzin [®]	3×9.1	WH	2, 4, 5, 9, 14
2022							75DF			
Wisconsin	Brooklyn, WI	NK22-C43E3	350,000	0.76	May 17	May 17	Metribuzin [®]	3×7.6	WH	2, 4, 5, 9, 14
2023							75DF			

^aAbbreviations: PA, Palmer amaranth; WH, waterhemp.

^bResistance profile numbers represent herbicide Group numbers as designated by the Herbicide Resistance Action Committee and Weed Science Society of America.

Table 2. Soil characteristics and precipitation records across 27 site-years.^a

Site-year	Soil chara	acterist	ics						Precipitati	Precipitation records					
	Organic	pН	Textur	Sand	Silt	Clay	Moisture at	Temperature	Amount	Cumulative	Interval	Days	Irrigatio		
	matter		e				application	at	of first	precipitatio	between	between	n		
								application	precipitat	n 42 DAA	applicatio	application			
									ion		n and first	and			
											precipitati	cumulative			
											on	12.7 mm			
												precipitatio			
												n			
	%				%			С	mm		DAA				
Arkansas	1.55	6.3	Silt	19	73	7.5	Adequate	20	2	146	0	1	Rainfed		
2023			loam												
Arkansas	2	6.2	Silt	18	71	10	Adequate	24	1	133	2	4	Rainfed		
2022			loam												
Illinois	5	5.5	Silt	28	51	21	Dry	24	2	48	18	23	Rainfed		
2023			loam												
Indiana	2.4	7.5	Sandy	75	14	11	Normal	22	3	149	4	10	Rainfed		
2022			loam												
Indiana	3.2	6.7	Sandy	74	16	10	Normal	21	17	170	2	2	Rainfed		
2023			loam												
Kansas	3	6.1	Silt	10	76	14	Good	21	18	343	1	1	Rainfed		
2022			loam												
Kansas	2.6	6.2	Silt	10	76	14	Good	22	26	144	1	1	Rainfed		

2023			loam										
Kentucky	2.5	6.1	Silt	10	74	14.9	Dry	18	18	102	5	5	Rainfed
2022			loam										
Kentucky	2.5	6.1	Silt	10	74	14.9	Damp	17	11	128	2	4	Rainfed
2023			loam										
Louisiana	2.3	8	Silt	12	70	18	Fair	26	15	158	5	5	Rainfed
2022			loam										
Michigan	2.6	6.1	Sandy	52	23	25	Fair	21	7	88	1	3	Rainfed
2022			clay										
			loam										
Michigan	1.3	6.1	Loam	36	45	18	Fair	21	5	48	19	32	Rainfed
2023													
Missouri	1.9	6.8	Silt	12.5	67.	20	Very dry	33	2	108	0	5	Rainfed
2022			loam		5								
Missouri	1.8	5.9	Silt	12.5	67.	20	Dry	30	2	101	3	11	Rainfed
2023			loam		5								
Nebraska	2.5	6.5	Silty	17	58	25	Good	19	1	100	5	6	Irrigated
2022			clay										
			loam										
Nebraska	2.5	6.5	Silty	17	58	25	Good	19	4	99	2	13	Irrigated
2023			clay										
			loam										
North	6.6	7.4	Loam	33	46	20	Good	20	2	112	1	5	Rainfed
Dakota													

2022													
North	5.3	8	Silt	2	41	56	Good	20	3	122	1	6	Rainfed
Dakota			clay										
2022													
North	5.3	8	Silt	2	41	56	Dry	21	2	121	1	8	Rainfed
Dakota			clay										
2023													
Ohio 2022	2.3	6.6	Loam	35	40	25	Dry	23	18	150	1	1	Rainfed
Ohio 2023	3.2	7	Loam	40	35	25	Dry	22	22	94	1	1	Rainfed
S Illinois	2	6.9	Silt	8	72	20	Fair	24	10	91	6	6	Rainfed
2022			loam										
S Illinois	1.8	6.4	Silt	3	78	19	Fair	21	12	70	1	1	Rainfed
2023			loam										
Tennessee	2.5	6.3	Silt	24	54	22	Fair	28	13	46	2	2	Rainfed
2022			loam										
Tennessee	2.5	5.8	Loam	36	40	24	Dry	24	15	162	1	1	Rainfed
2023													
Wisconsin	2	7.9	Loam	40	41	19	Wet	21	39	165	2	2	Irrigated
2022													
Wisconsin	1.9	6.9	Loam	39	43	18	Damp	19	3	89	2	7	Irrigated
2023													

^aAbbreviation: DAA, days after application of herbicide.

Table 3. Herbicide treatments applied before soybean emergence.

Treatment number	Herbicide treatment ^a	Dose
		g ai ha ⁻¹
1	Nontreated	_
2	Weed-free	_
3	Metribuzin	210
4	Metribuzin	263
5	Metribuzin	315
6	Metribuzin	368
7	Metribuzin	420
8	Metribuzin	473
9	Metribuzin	525
10	Metribuzin	578
11	Metribuzin	631
12	Metribuzin	683
13	Metribuzin	736
14	Metribuzin	788
15	Metribuzin	841
16	Sulfentrazone	420
17	S-metolachlor	1,790

^aTrade names for metribuzin include Tricor 4F, Tricor 75DF, Metricor DF, Glory 4L, and Metribuzin 75WG, manufactured by UPL (Cary, NC), Syngenta Crop Protection (Greensboro, NC), Loveland Products (Loveland, CO). Sulfentrazone is sold under the trade name Spartan 4F by FMC (Philadelphia, PA). *S*-metolachlor is sold by Syngenta Crop Protection under the trade names Dual Magnum and Dual II Magnum.

Table 4. Estimated parameter values of the generalized additive model for weed control as a function of predicting variables for all site-years except Illinois 2023 and Michigan 2023.^a

Intercept	Predicting variable	Slope	P-value	
2.83	Weed control days after ap	plication	-0.031	< 0.01
	Soil organic matter, %	0.07	< 0.01	
	Soil pH	0.006	0.76	
	Clay, %	0.0003	0.81	
	Planting date (Julian days)	-0.016	< 0.01	
	Soil moisture	-0.36	< 0.01	
		Moist	-0.35	< 0.01
	Soil temperature at applica	tion in °C	0.08	< 0.01
	Days between application	on and first	0.065	< 0.01
	precipitation			
	Amount of first precipitation	on, in mm	0.04	< 0.01
	Days between appl	ication and	-0.078	< 0.01
	cumulative 12.7 mm precip	oitation		
	Cumulative precipitation	42 d after	0.004	0.048
	application, in mm			

^aAdjusted $R^2 = 0.41$; deviance explained = 59%.

Table 5. Percent weed control estimates as a function of herbicide treatment for all site-years except Illinois 2023 and Michigan 2023. a,b

Interval	Treatment ^c	Mean	percent	P-value
DAA		control		
14	Metribuzin	94.6	a	0.01
	Sulfentrazone	92.8	a	
	S-metolachlor	87.6	b	
28	Metribuzin	83.8	a	<0.01
	Sulfentrazone	85	a	
	S-metolachlor	73.2	b	
42	Metribuzin	78.3	a	<0.01
	Sulfentrazone	81.3	a	
	S-metolachlor	59	b	

^aAbbreviation: DAA, days after application of herbicide.

^bMeans within a column followed by lowercase letters represent significant differences identified by separation of means for each interval using Tukey's honest significant difference test ($\alpha = 0.05$).

^cWeed control estimates as a result of metribuzin treatment were evaluated when the herbicide was applied at 525 g ai ha⁻¹.

Table 6. Percent weed control estimates as a function of herbicide treatment for Illinois 2023 and Michigan 2023 site-years. ^{a,b}

Interval	Treatment ^c	Mean	percent	P-value
DAA		control		
14	Metribuzin	82.3	a	0.03
	Sulfentrazone	30	b	
	S-metolachlor	33.3	b	
28	Metribuzin	84.2	a	0.9
	Sulfentrazone	78.6	a	
	S-metolachlor	80.8	a	
42	Metribuzin	74	a	0.67
	Sulfentrazone	68	a	
	S-metolachlor	64.2	a	

^aAbbreviation: DAA, days after application of herbicide.

^bMeans within a column followed by lowercase letters represent significant differences identified by separation of means for each interval using Tukey's honest significant difference test ($\alpha = 0.05$).

^cWeed control estimates as a result of metribuzin treatment were evaluated when the herbicide was applied at 525 g ai ha⁻¹.

Table 7. Percent crop injury estimated as a function of herbicide treatment for all site-years except Illinois 2023 and Michigan 2023. a,b

Interval	Treatment	Mean	percent	P-value
DAA		control		
14	Sulfentrazone	3.7	a	0.32
	S-metolachlor	2.8	a	
28	Sulfentrazone	5.9	a	<0.01
	S-metolachlor	1.4	b	
42	Sulfentrazone	4.9	a	<0.01
	S-metolachlor	1	b	

^aAbbreviation: DAA, days after application of herbicide.

^bMeans within a column followed by lowercase letters represent significant differences identified by separation of means for each interval using Tukey's honest significant difference test ($\alpha = 0.05$).

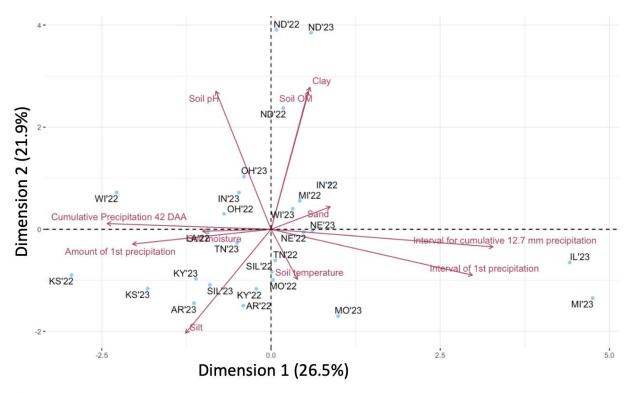


Figure 1. Principal component biplot for soil texture (sand, silt and clay), soil organic matter (OM), soil temperature at herbicide application, soil moisture, soil pH, precipitation interval after herbicide application (interval of first precipitation), amount of first precipitation, interval for cumulative 12.7 mm precipitation, and cumulative precipitation 42 d after application for all site-years. Site-years are represented by state location followed by experimental year: Arkansas (AR'22, AR'23), Illinois (IL'23, SIL'22, SIL'23), Indiana (IN'22, IN'23), Kansas (KS'22, KS'23), Kentucky (KY'22, KY'23), Louisiana (LA'22), Michigan (MI'22, MI'23), Missouri (MO'22, MO'23), Nebraska (NE'22, NE'23), North Dakota (ND'22, ND'23), Ohio (OH'22, OH'23), Tennessee (TN'22, TN'23) and Wisconsin (WI'22, WI'23).

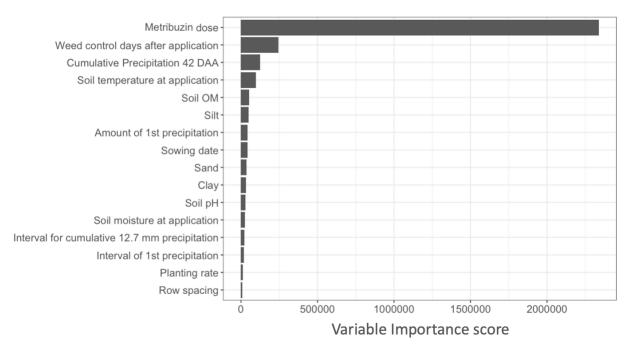


Figure 2. Variable importance plot for covariates determining weed control based on random forest model for all site-years except Illinois 2023 and Michigan 2023. Abbreviations: DAA, days after application of herbicide; OM, organic matter.

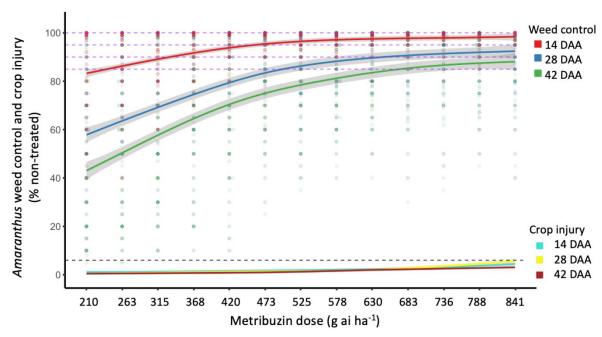


Figure 3. Soybean injury and *Amaranthus* weed control across metribuzin doses at 14, 28, and 42 d after application (DAA) for all site-years except Illinois 2023 and Michigan 2023. The shaded area around the regression line indicates the 95% confidence interval. The dotted black line represents 5% crop injury levels, and the dotted purple lines represent 100%, 95%, 90%, and 85% weed control, respectively. Each dot represents an individual weed control data point across treatments.

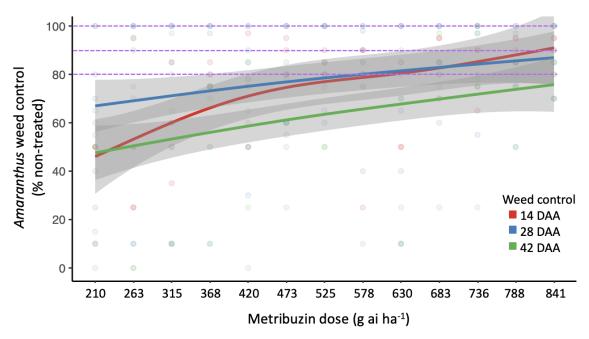


Figure 4. *Amaranthus* weed control across metribuzin doses at 14, 28, and 42 d after application (DAA) at the Illinois 2023 and Michigan 2023 site-years. The shaded area around the regression line indicates the 95% confidence interval. The dotted purple lines represent 100%, 90%, and 80% weed control, respectively. Each dot represents an individual weed control data point across treatments.

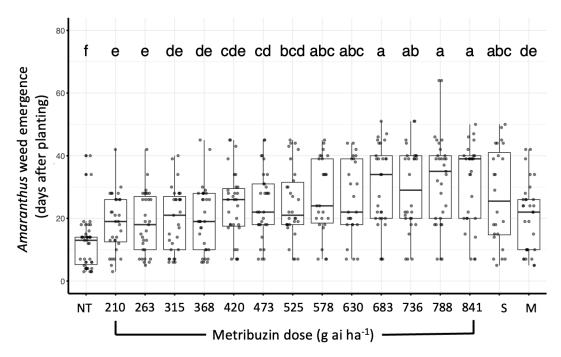


Figure 5. *Amaranthus* weed emergence from 9 site-years across metribuzin doses. The regression line represents weed emergence as a function of metribuzin dose. The shaded area around the line indicates the 95% confidence interval. The dashed lines represent mean weed emergence for sulfentrazone (S; 27 d after planting), and *S*-metolachlor (M; 20 d after planting). Each dot represents an individual weed emergence data collection point.

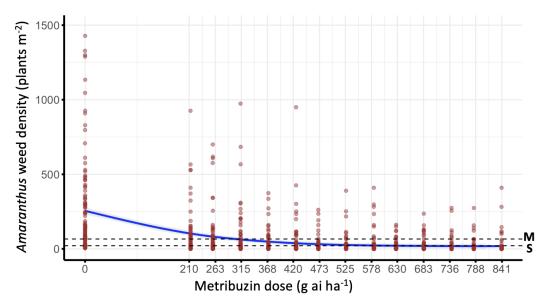


Figure 6. Amaranthus weed density from 20 site-years across metribuzin doses 28 d after application. The regression line represents weed density as a function of metribuzin dose. The shaded area around the line indicates the 95% confidence interval. The dashed lines represent mean weed density for sulfentrazone (S; 21 plants) and S-metolachlor (M; 66 plants). Each dot represents an individual weed density data collection point.

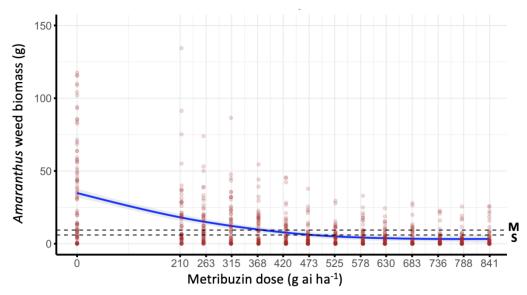


Figure 7. Amaranthus weed biomass from 16 site-years across metribuzin doses 28 d after application. The regression line represents weed biomass as a function of metribuzin dose. The shaded area around the line indicates the 95% confidence interval. The dashed lines represent mean weed biomass for sulfentrazone (S; 6 g) and S-metolachlor (M; 9 g). Each dot represents an individual weed biomass data collection point.

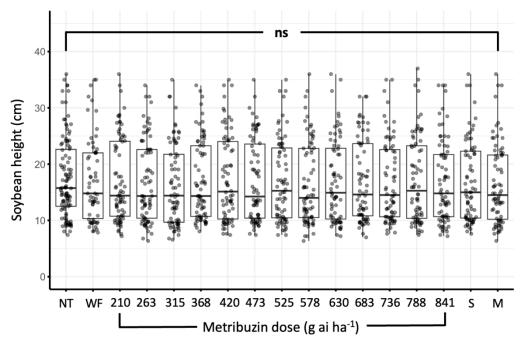


Figure 8. Soybean height from 20 site-years across herbicide treatment 28 d after application. Each dot represents an average soybean height data point. The boxes represent the 25th to 75th percentiles of interquartile ranges with the horizontal line inside each box indicating the median yield. The whiskers extend to the smallest and largest values within 1.5 times the interquartile range. Abbreviations: M, S-metolachlor; ns, no significant differences (identified by separation of means using Tukey's honestly significant difference test; $\alpha = 0.05$); NT, nontreated; S, sulfentrazone; WF, weed-free.

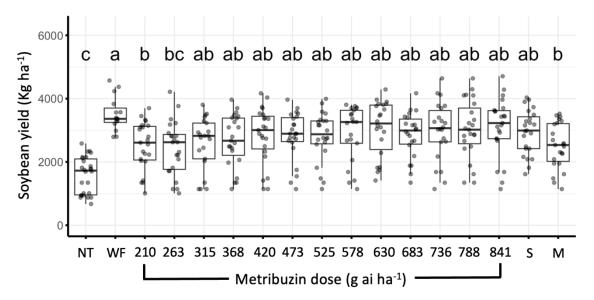


Figure 9. Soybean yield from 7 site-years plotted with herbicide treatment. Lowercase letters represent significant differences identified by separation of means using Tukey's honestly significant difference test ($\alpha = 0.05$). Each dot represents an individual soybean yield data point. The boxes represent 25th to 75th percentiles of interquartile ranges with the horizontal line inside each box indicating the median yield. The whiskers extend to the smallest and largest values within 1.5 times the interquartile range. Abbreviations: M, *S*-metolachlor; NT, nontreated; S, sulfentrazone; WF, weed-free.