Weed Technology

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Evaluating efficacy of preemergence soybean herbicides using field treated soil in greenhouse bioassays

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Research Article

Cite this article: Ribeiro VHV, Oliveira MC, Smith DH, Santos JB, Werle R (2021) Evaluating efficacy of preemergence soybean herbicides using field treated soil in greenhouse bioassays. Weed Technol. **35**: 830–837. doi: 10.1017/wet.2021.22

Received: 3 November 2020
Revised: 6 February 2021
Accepted: 7 March 2021
First published online: 6 April 2021

Associate Editor:
Mark VanGessel, University of Delaware

Nomenclature:
Acetochlor; chlorimuron-ethyl; cloransulam-methyl; dimethenamid-P; flumioxazin; imazethapyr; metribuzin; pyroxasulfone; saflufenacil; *S*-metolachlor; sulfentrazone; giant foxtail; *Setaria faberi* Herrm.; Palmer amaranth; *Amaranthus palmeri* S. Watson; cereal rye; *Secale cereale* L.; radish; *Raphanus sativus* L.; soybean; *Glycine max* (L.) Merrill

Keywords:
Bioindicator species; cover crops; herbicide persistence; integrated weed management; weed control

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Abstract

Amid widespread occurrence of herbicide-resistant weeds in the United States, the use of PRE herbicides and cover crops have resurged once again as important strategies for weed management in cropping systems. The objective of this experiment was to evaluate the length of soil residual weed control from PRE soybean herbicides and the detrimental impact of these herbicides on cover crop species using field treated soil in greenhouse bioassays. Greenhouse bioassays were conducted using soil from field experiments conducted in 2018 and 2019 in Arlington and Lancaster, WI. PRE herbicides consisted of imazethapyr, chlorimuron-ethyl, and cloransulam-methyl (acetolactate synthase [ALS] inhibitors); metribuzin (photosystem II [PS II] inhibitor); sulfentrazone, flumioxazin, and saflufenacil (protoporphyrinogen oxidase [PPO] inhibitors); acetochlor, *S*-metolachlor, dimethenamid-P, and pyroxasulfone (very long-chain fatty acid [VLCFA] inhibitors); and a nontreated control. Greenhouse bioassays were conducted using soil (depth, 0 to 10 cm) sampled at 0, 10, 20, 30, 40, and 50 d after treatment (DAT). Palmer amaranth and giant foxtail (weeds), and radish and cereal rye (cover crops) were used as bioindicators of herbicide levels in the soil. Bioassay results showed extended soil residual control of Palmer amaranth with sulfentrazone and pyroxasulfone; extended residual control of giant foxtail was observed with pyroxasulfone and *S*-metolachlor. Chlorimuron-ethyl and metribuzin were the most injurious herbicides to radish and cereal rye shortly after application, respectively, but minimal injury was observed from soil samples collected 50 DAT, indicating the use of PRE and fall-seeded cover crops in southern Wisconsin can be compatible. These results can support growers and practitioners with selection of effective PRE herbicides for Palmer amaranth and giant foxtail control and reduced impact on fall-seeded radish and cereal rye cover crops, altogether leading to more effective, diverse, and sustainable weed management programs.

Introduction

During the early 1990s, a significant percentage of the soybean production area in the United States was treated with PRE herbicides, particularly with chlorimuron-ethyl (20%), metribuzin (19%), and imazethapyr (11%; USDA 2020). Between 1996 and 2006, the soybean acreage in the United States planted with glyphosate-resistant (GR) varieties and the area treated with glyphosate increased by approximately 90% and 60%, respectively (Benbrook 2016; Duke and Powles 2009). The rapid adoption of the GR soybean technology shifted herbicide use patterns from PRE followed by POST herbicides from multiple sites of action (SOAs) to POST only application(s) of glyphosate (Duke 2015; Givens et al. 2009; Powles 2008). The wide adoption of GR soybean varieties and associated reliance on glyphosate use led to drastic reduction in herbicide diversity, induced weed species shifts, and accelerated the evolutionary rate of GR weeds in these production systems (Culpepper 2006; Green 2009; Johnson et al. 2009; Kniss 2018; Owen 2008; Owen and Zelaya 2005; Webster and Nichols 2012). Seventeen weed species evolved resistance to glyphosate between 1990 and 2020 in the United States (Heap 2020). Due to increased reports of GR weeds throughout the United States, the use of additional herbicide SOAs have become necessary for effective chemical weed management (Hager et al. 2003; Prince et al. 2012; Riggins and Tranel 2012; Werle et al. 2018). The soybean production area treated with PRE herbicides substantially increased from 2006 through 2017, particularly with sulfentrazone (21%), metribuzin (16%), *S*-metolachlor (15%), and flumioxazin (10%; USDA 2020), indicating higher herbicide SOA diversity for weed control in soybean cropping systems (Kniss 2018). The integration of PRE herbicides is an effective strategy for management of herbicide-resistant weeds

Table 1. Soil description, soybean cultivars, and planting and herbicide application dates for the field experiments in Wisconsin.

Site-year	Soil type	Organic matter	pH	Soybean cultivar	Planting	Herbicide application
		%				
Arlington 2018	Silty clay loam (8% sand, 56% silt, 37% clay)	2.6	6.5	AG21X8	June 12	June 12
Arlington 2019	Silt loam (10% sand, 65% silt, 25% clay)	4.1	6.6	AG21X8	May 13	May 13
Lancaster 2018	Silt loam (12% sand, 70% silt, 19% clay)	2.5	7.0	AG21X7	May 24	May 25
Lancaster 2019	Silt loam (10% sand, 73% silt, 17% clay)	3.1	7.0	AG24X7	May 23	May 26

(Norsworthy et al. 2012). PRE herbicides can reduce early season weed interference and give growers more flexibility for timely POST applications (Arneson et al. 2019; Butts et al. 2017; Knezevic et al. 2019; Tursun et al. 2016; Whitaker et al. 2011). Additionally, PRE herbicides can increase herbicide SOA diversity because there are limited options for effective POST herbicides (Beckie and Reboud 2009; Grey et al. 2014; Norsworthy et al. 2012).

Even though extended soil residual efficacy from PRE herbicides during the growing season is desirable for weed control, certain residual herbicides can persist (carryover) in the soil and negatively affect growth of subsequent crops, including cover crops (Curran 2016). The planting of cover crops after cash crop harvest for soil conservation and weed suppression purposes has increased in the United States, but successful cover crop establishment in corn-soybean rotations where PRE herbicides are used remains a concern (Cornelius and Bradley 2017; Oliveira et al. 2019; Whalen et al. 2019). For instance, metribuzin + chlorimuron-ethyl applied to soybean reduced the biomass of fall-planted alfalfa (*Medicago sativa* L.; >55%), indicating that alfalfa planting must be avoided where such herbicide combination has been applied within 4 mo (Walsh et al. 1993). Similarly, imazapyr + imazapic applied to corn reduced the fresh weight of subsequent pea (*Pisum sativum* L.), alfalfa, and annual ryegrass (*Lolium multiflorum* Lam.) by 23%, 75%, and 63%, respectively, 60 d after establishment (Alister and Kogan 2005). Generally, small-seeded cover crops, including clovers (*Trifolium* spp.), canola (*Brassica napus* L.), and annual ryegrass tend to be more sensitive to PRE herbicides than large-seeded species such as cereal rye and oats (*Avena sativa* L.; Curran 2016; Palhano et al. 2018).

While both the use of PRE herbicides in response to widespread occurrence of GR weeds and interest in adopting fall-seeded cover crops continue to rise throughout the United States (Heap 2020; Oliveira et al. 2019; USDA 2020), evaluations of soil residual efficacy of commonly used PRE herbicides in soybeans on broad spectrum weed control and subsequent cover crop establishment become imperative. The use of plants as bioindicator organisms of herbicide residue in soil (i.e., soil bioassays) has been widely adopted as an alternative technique to chemical extraction analytical methods (e.g., liquid chromatography, gas chromatography, mass spectrometry, capillary electrophoresis, and immunoassays; Geisel et al. 2008; Horowitz 1976; Mehdizadeh et al. 2017; Streibig 1988; Wang and Freemark 1995). Despite being time-consuming and labor-intensive, the adoption of bioassay techniques has advantages compared to analytical methods that include reduced cost, no need for expensive laboratory equipment, biological detection of low herbicide concentrations in soil, and reproducible results (Mehdizadeh et al. 2017; Riddle et al. 2013). Thus, the objective of this experiment was to evaluate the length of soil residual weed control from PRE soybean herbicides and the detrimental impact of these herbicides have on cover crop species using field-treated soil in greenhouse bioassays.

Table 2. Monthly mean air temperature and total rainfall from May through September.

	Arlington, WI			Lancaster, WI		
	2018[a]	2019	30-yr avg[b]	2018	2019	30-yr avg
Air temperature			C			
May	18	13	13	16	11	14
June	20	18	19	19	17	19
July	22	23	21	20	21	22
August	21	19	20	19	17	21
September	17	18	15	15	17	16
Average	20	18	18	18	17	18
Rainfall			mm			
May	180	172	94	163	143	105
June	122	141	119	162	119	134
July	52	118	106	137	161	109
August	222	153	99	231	81	107
September	118	146	90	308	471	80
Total	694	730	508	1001	975	535

[a]2018 and 2019 weather data obtained from in situ weather stations.
[b]30-yr avg data (1981–2010) were obtained from the Wisconsin State Climatology Office (~http://www.aos.wisc.edu/~sco/).

Materials and Methods

Field Experiments

Field experiments were conducted in 2018 and 2019 at the University of Wisconsin Arlington (43.30°N, 89.33°W) and Lancaster (42.83°N, 90.76°W) Agricultural Research Stations, near Arlington and Lancaster, WI, respectively, for a total of four experimental site-years. Soil characteristics for each site-year are described in Table 1. The experimental fields had been in a corn-soybean rotation and corn was grown the year before the experiment establishment at all site-years. Before soybean establishment, fields were tilled using a field cultivator. Soybean seeds were planted at a depth of 3 cm, with 76-cm row spacing, and 345,940 seeds ha^{-1}. Soybean cultivars and planting date information are detailed in Table 1. Experimental units were 3 m wide (four soybean rows) by 7.6 m long. Monthly mean air temperature and total rainfall during the soybean growing season were obtained from WatchDog 2700 weather stations (Spectrum Technologies®, Aurora, IL) installed at each site-year (Table 2). The experiments were conducted in a randomized complete block design with four replications. The treatments consisted of 11 PRE herbicides and a nontreated control (Table 3). The PRE herbicides investigated each had a single active ingredient to evaluate their soil residual efficacy independently (e.g., no mixtures or commercial premixes containing multiple active ingredients were evaluated in this research). Herbicides were applied within 3 d after soybean planting (Table 1). The herbicides were applied using a CO_2-pressurized backpack sprayer equipped with four Teejet XR11002 (Teejet, Springfield, IL) nozzles spaced 50.8 cm apart, at a height of 45 cm from the soil surface, 248 kPa operating pressure, at a speed

https://doi.org/10.1017/wet.2021.22 Published online by Cambridge University Press

Table 3. PRE herbicide, trade names, companies, site of action group, herbicide families, half-lives, and rates used in the field experiments.

Herbicide	Trade name®	Company	Location	Group (SOA#)[a]	Herbicide family	Half-life[b]	Rate
						—avg days—	—g ai ha^{-1}—
Chlorimuron-ethyl	Classic	Corteva	Indianapolis, IN	ALS (2)	Sulfonylurea	40	53
Cloransulam-methyl	FirstRate	Corteva	Indianapolis, IN	ALS (2)	Triazolopyrimidine	8–10	35
Imazethapyr	Pursuit	BASF	Durham, NC	ALS (2)	Imidazolinone	60–90	70
Metribuzin	Tricor DF	UPL	King of Prussia, PA	PSII (5)	Triazinone	30–60	563
Flumioxazin	Valor SX	Valent	Walnut Creek, CA	PPO (14)	N-phenylphthalimide	12–18	107
Saflufenacil	Sharpen	BASF	Durham, NC	PPO (14)	Pyrimidinedione	15–29	25
Sulfentrazone	Spartan	FMC	Philadelphia, PA	PPO (14)	Aryl triazinone	121–302	280
Acetochlor	Warrant	Bayer	St. Louis, MO	VLCFA (15)	Chloroacetamide	90	1260
Dimethenamid-P	Outlook	BASF	Durham, NC	VLCFA (15)	Chloroacetamide	35–42	945
Pyroxasulfone	Zidua	BASF	Durham, NC	VLCFA (15)	Pyrazole	16–26	179
S-metolachlor	Dual II Magnum	Syngenta	Greensboro, NC	VLCFA (15)	Chloroacetamide	112–124	1787

[a]Abbreviations: ALS, acetolactate synthase; PS II, photosystem II; PPO, protoporphyrinogen oxidase; SOA#, site of action; VLCFA, very long chain fatty acid.
[b]Half-life values were obtained from the WSSA Herbicide Handbook (10th ed.; Shaner 2014) other than saflufenacil and acetochlor, which were obtained from Camargo et al. (2013) and Jablonkai (2000), respectively.

of 4.8 km h^{-1}, calibrated to deliver 140 L ha^{-1} of spray solution. All sites received more than 20 mm of rainfall within 3 d of application. For reference, the half-lives of the PRE herbicides evaluated were obtained from the WSSA Herbicide Handbook (Shaner 2014), Camargo et al. (2013), and Jablonkai (2000) and are reported in Table 3.

To investigate the residual performance of the aforementioned PRE herbicides over time, soil samples from a depth of 0 to 10 cm were collected from the field experiments at 0, 10, 20, 30, 40, and 50 DAT using a 6-cm-diameter handheld soil sampler (Fiskars®, Middleton, WI). At each sampling time, five soil cores were collected adjacent to the two central soybean rows from each plot and combined into one composite sample inside a sealable plastic bag (~1,000 g). Soil samples were stored in a freezer (−20 C) until the onset of the greenhouse bioassays. Other than the PRE herbicides, no additional herbicides were applied to the field experiments.

Greenhouse Bioassays

Bioassays were conducted in the fall of 2018 (with the aforementioned soil samples collected in 2018) and in the fall of 2019 (with soil samples collected in 2019) in a greenhouse on the University of Wisconsin-Madison campus (43.07°N, 89.42°W). The bioassay experimental unit consisted of a 158 cm^3 seed tray cell (4.9 cm width × 5.7 cm length × 5.7 cm depth; 3601 Series T.O Plastics Inc., Clearwater, MN) filled with the soil samples from the field experiments. Composite soil samples within a site-year were thawed and combined across replications from the same PRE herbicide by sampling time, thoroughly mixed, and then split into the bioassay experimental units (seed tray cells). Four bioindicator species were used: two small-seeded weed species, Palmer amaranth (population Kei 3; Oliveira et al. 2020) and giant foxtail, which were collected in 2017 in Keith County, NE, and in 2018 in Columbia County, WI, respectively; and two cover crops, radish ('Tillage Radish'®; La Crosse Seed, La Crosse, WI) and cereal rye ('Guardian'® Winter Rye; La Crosse Seed). These species were selected given their commonality as either weeds or cover crops across cropping systems in the United States and to represent a range of seed sizes and plant families (Oliveira et al. 2019; WSSA 2019). To maintain consistent seeding rates for the weed species, the same volume of seeds was planted, and it averaged 60 and 20 seeds of Palmer amaranth and giant foxtail, respectively. Five seeds of each cover crop species were planted. Each species was grown in separate experimental units. The bioassays were conducted as a randomized complete block design with four replications. The bioassay was replicated twice in time with the soil collected per PRE herbicide over sampling time for each site-year. Temperature (14 C minimum, 27 C average, 34 C maximum) and relative humidity (57% average) were monitored throughout the greenhouse experiments using a WatchDog A150 Temp/RH logger (Spectrum Technologies®, Aurora, IL). Plant biomass was collected at 28 d after planting (DAP). Biomass samples were cut at the soil surface, placed in paper bags, and dried (70 C) until a constant weight was achieved. The biomass of plants grown in soil treated with herbicides from each sampling time were compared with that of the average nontreated control from each sampling time for each site-year and expressed as percent biomass compared to the nontreated control using the following equation:

$$Z = \left(\frac{B}{C}\right) \times 100 \quad [1]$$

where Z is percent biomass compared to that of the nontreated control (the closer to 100% the lower the herbicide impacted plant growth), *B* is the observed biomass for the respective experimental unit (g), and *C* is the average biomass of the nontreated control (g).

Accumulated growing degree day (GDD) units at the field soil sampling times were estimated and used as the explanatory variable to standardize the differences in planting dates and growing conditions across site-years (Tables 1 and 2). GDD was estimated based on recorded field soil temperature (0 to 2 cm) collected with a Watchdog 1650 Micro Station (Spectrum Technologies®, Aurora, IL). Daily soil GDD was calculated according to the equation described by McMaster and Wilhelm (1997):

$$GDD = \sum_{i=1}^{n}\left[\frac{(Tmax + Tmin)}{2}\right] - Tbase \quad [2]$$

where *Tmax* is the daily maximum soil temperature (C), *Tmin* is the daily minimum soil temperature, *Tbase* is the base temperature (5 C, which indicates the minimum temperature necessary for herbicide degradation in soil; Cupples et al. 2000), and *n* is the number of days after treatment. The first soil sampling at each site-year occurred immediately after PRE herbicide application thus representing the onset of GDD accumulation (0 DAT = 0 GDD).

https://doi.org/10.1017/wet.2021.22 Published online by Cambridge University Press

Statistical Analyses

Linear regression models were fitted to the percent biomass compared to the nontreated control (Z; response variable) and regressed against GDD (explanatory variable) using the *lm* function of the LM4 package (Bates et al. 2015) with R statistical software (version 4.0.2; R Core Team 2020). To enable stronger inferences, models were fitted to the data pooled across site-years for each PRE herbicide by bioindicator species combination. The intercept and slope of each model were used to assist with interpreting bioindicator species response to each herbicide, where the intercept indicates the injury expected at the highest herbicide concentration in soil (i.e., day of herbicide application [0 DAT = 0 GDD]), and the slope represents the biological response to herbicide dissipation over time for each species tested (Walker and Thompson 1977). The percent biomass at 100, 500, and 900 accumulated GDD (GDD accumulation range representative of the soil sampling interval across site-years; 0 to 50 DAT) was estimated for each bioindicator species from the linear regression models using the *predict* function of the LM4 package (Bates et al. 2015) to aid in the interpretation of the residual efficacy through the season.

The percent biomass of each bioindicator species across soil sampling time within each site-year were used to calculate the Area Under Biomass Production Curve (AUBPC). AUBPC used the *audpc* function of the AGRICOLAE package (Mendiburu 2019). The Shapiro-Wilk test was performed using the *shapiro.test* function of the STATS package to test for normality (Royston 1995), and the Levene test was performed using the *leveneTest* function of the CAR package to test the homogeneity of residual variance of the AUBPC data (Fox and Weisberg 2011) using R statistical software. AUBPC estimates were subjected to ANOVA using a mixed-effect model with the *lmer* function of the LM4 package (Bates et al. 2015). In the model, herbicide and bioindicator species were included as fixed effects and experimental runs nested within site-years were considered as random effects (assuming soil samples were collected from random sites in southern Wisconsin). If ANOVA indicated herbicide × bioindicator species interaction or main effects to be significant ($P < 0.05$), means were separated accordingly using Fisher's protected LSD test. AUDPC is a valuable tool commonly used in the field of plant pathology to estimate disease progress over time (Madden et al. 2007) and has been previously adopted by weed scientists to estimate crop injury from POST herbicides across distance or over time (Striegel et al. 2020; VanGessel et al. 2016). The AUBPC allowed estimation of a single response variable, and to thus rank the overall PRE herbicide impact on biomass of each bioindicator species over the period evaluated (0 to 50 DAT; 0 to 900 GDD), further supporting the linear regression results.

Results and Discussion

Field Experiments

Given the different planting times and environmental conditions, the accumulated GDD at each sampling time (0, 10, 20, 30, 40, and 50 DAT) varied at each site-year (Tables 1 and 2). Nonetheless, 100, 500, and 900 GDD were selected as a representative range of GDD accumulated during the soil sampling interval in this study to assist interpretations of PRE herbicide impact on bioindicator biomass (Tables 4 and 5). The days after PRE herbicide application that represent 100, 500, and 900 GDD were 5, 27, and 48 for the

Table 4. Estimated parameter values for the linear regression model for plant biomass of each bioindicator species by PRE herbicide combination evaluated in the greenhouse bioassays.

	Palmer amaranth		Giant foxtail		Radish		Cereal rye	
Herbicide (SOA#)[a]	Intercept (P-value)[b]	Slope (P-value)	Intercept (P-value)	Slope (P-value)	Intercept (P-value)	Slope (P-value)	Intercept (P-value)	Slope (P-value)
Chlorimuron-ethyl (2)	71.8 (<0.01)	0.021 (0.003)	50.6 (<0.01)	0.012 (0.12)	25.0 (<0.01)	0.057 (<0.01)	50.6 (<0.01)	0.034 (<0.01)
Cloransulam-methyl (2)	62.5 (<0.01)	0.031 (<0.01)	50.0 (<0.01)	0.040 (<0.01)	38.3 (<0.01)	0.067 (<0.01)	67.8 (<0.01)	0.016 (0.013)
Imazethapyr (2)	53.6 (<0.01)	0.039 (<0.01)	33.5 (<0.01)	0.058 (<0.01)	55.3 (<0.01)	0.045 (<0.01)	54.1 (<0.01)	0.037 (<0.01)
Metribuzin (5)	37.8 (<0.01)	0.067 (<0.01)	61.2 (<0.01)	0.034 (<0.01)	37.4 (<0.01)	0.079 (<0.01)	29.6 (<0.01)	0.071 (<0.01)
Flumioxazin (14)	23.9 (<0.01)	0.081 (<0.01)	51.9 (<0.01)	0.035 (<0.01)	63.3 (<0.01)	0.040 (<0.01)	38.9 (<0.01)	0.052 (<0.01)
Saflufenacil (14)	41.9 (<0.01)	0.061 (<0.01)	77.0 (<0.01)	0.003 (0.593)	84.6 (<0.01)	0.011 (0.033)	62.6 (<0.01)	0.022 (<0.001)
Sulfentrazone (14)	7.2 (0.097)	0.057 (<0.01)	21.8 (<0.01)	0.043 (<0.01)	73.0 (<0.01)	0.012 (0.114)	60.6 (<0.01)	0.021 (0.001)
Acetochlor (15)	55.8 (<0.01)	0.018 (0.03)	49.6 (<0.01)	0.021 (<0.01)	89.7 (<0.01)	-0.001 (0.86)	63.6 (<0.01)	0.015 (0.031)
Dimethenamid-P (15)	46.0 (<0.01)	0.051 (<0.01)	31.7 (<0.01)	0.063 (<0.01)	88.2 (<0.01)	0.004 (0.42)	51.9 (<0.01)	0.032 (<0.01)
Pyroxasulfone (15)	8.9 (0.040)	0.063 (<0.01)	3.3 (0.31)	0.035 (<0.01)	32.7 (<0.01)	0.072 (<0.01)	43.7 (<0.01)	0.044 (<0.01)
S-metolachlor (15)	27.3 (<0.01)	0.059 (<0.01)	3.7 (0.24)	0.055 (<0.01)	95.6 (<0.01)	0.001 (0.67)	38.6 (<0.01)	0.057 (<0.01)

[a]Abbreviations: DAT, days after treatment; GDD, growing degree days; SOA#, site of action group.
[b]The intercept indicates the injury expected at the highest herbicide concentration in soil (i.e., day of herbicide application [0 DAT = 0 GDD]) and the slope represents the biological response to herbicide dissipation over time for each species tested (0–900 GDD).

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Table 5. Area under biomass production curve estimated for the percent biomass compared to the nontreated control of each bioindicator species by PRE herbicide combination over time.

	Bioindicator species			
Herbicide (SOA#)[a]	Palmer amaranth[b]	Giant foxtail	Radish	Cereal rye
Nontreated control	71,619 a	65,974 a	73,374 a	60,638 a
Chlorimuron-ethyl (2)	66,067 ab	44,223 e	39,315 h	56,158 abc
Cloransulam-methyl (2)	61,550 bc	52,413 cd	53,591 fg	57,348 abc
Imazethapyr (2)	55,873 cde	47,749 cde	59,913 def	53,787 abc
Metribuzin (5)	55,277 cde	62,633 ab	58,839 defg	49,850 c
Flumioxazin (14)	49,062 ef	54,942 bc	65,217 bcd	50,908 bc
Saflufenacil (14)	57,844 cd	63,816 a	72,619 ab	60,272 ab
Sulfentrazone (14)	25,597 g	29,526 f	63,991 cde	57,836 abc
Acetochlor (15)	50,991 def	46,876 de	71,810 abc	57,782 abc
Dimethenamid-P (15)	55,437 cde	48,510 cde	71,386 abc	54,457 abc
Pyroxasulfone (15)	28,722 g	13,478 g	51,768 g	51,803 bc
S-metolachlor (15)	43,851 f	20,476 g	55,841 efg	52,322 bc
Herbicide × bioindicator species		<0.01		

[a]Abbreviations: SOA, site of action.
[b]Means within a column followed by the same letter are not significantly different at the 5% level according to Fisher's LSD test.

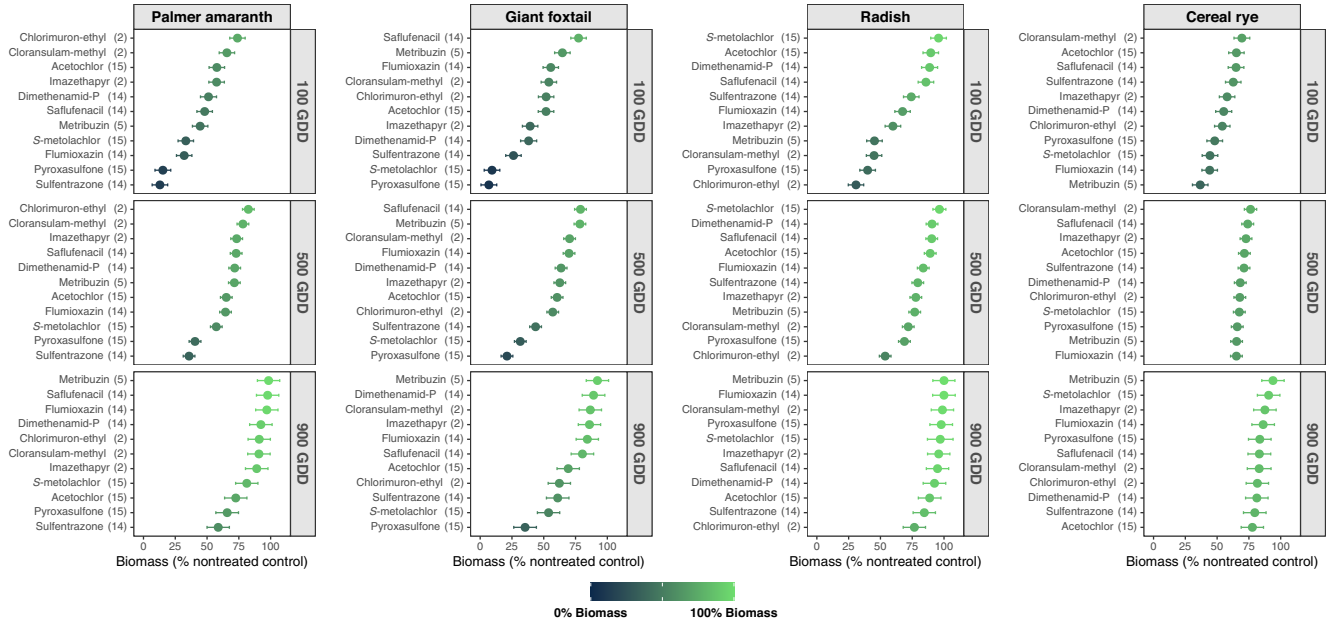

Figure 1. Estimated biomass (% biomass compared with the nontreated control) of each bioindicator species by PRE herbicide at 100, 500, and 900 growing degree days (GDD) across 4 site-years in southern Wisconsin. The days after PRE herbicide application that represent 100, 500, and 900 GDD were 5, 27, and 48 at Arlington 2018; 11, 38 and 59 at Arlington 2019; 5, 32, and 53 at Lancaster 2018; and 8, 36 and 55 at Lancaster 2019. Dots represent the means and dashes represent the 95% confidence intervals. PRE herbicides are ranked within each subfigure (bioindicator species by GDD combination) according to their impact on bioindicator biomass accumulation from least (100% biomass; light green) to highest (0% biomass; dark teal).

Arlington 2018 experiment; 11, 38, and 59 for Arlington 2019; 5, 32, and 53 for Lancaster 2018; and 8, 36, and 55 for Lancaster 2019.

Greenhouse Bioassays

The PRE herbicide × bioindicator species interaction was significant ($P < 0.01$), thus AUBPC was analyzed separately for each bioindicator species (Table 5), which further supported the decision to fit an individual linear regression model to each PRE herbicide by bioindicator species combination evaluated. Herein, the lower the intercept (% biomass at 0 GDD; Table 4), the lower the biomass estimated at 100, 500, and 900 GDD (Figure 1), and the lower the AUBPC values (Table 5), the more detrimental the impact of the PRE herbicide on the bioindicator species of interest.

Palmer Amaranth

Sulfentrazone (intercept = 7.2%, slope = 0.057) and pyroxasulfone (intercept = 8.9%, slope = 0.063) were the most detrimental PRE herbicides to Palmer amaranth through the soil sampling period (Table 4). Palmer amaranth grown in soil treated with sulfentrazone produced 13%, 36%, and 59% biomass compared with the nontreated control at 100, 500, and 900 GDD, respectively (Figure 1). In soil treated with pyroxasulfone, Palmer amaranth presented 15%, 41%, and 66% biomass compared with the

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nontreated control at 100, 500, and 900 GDD, respectively. The AUBPC analysis corroborates these results with the lowest AUBPC values with sulfentrazone (AUBPC = 25,597) and pyroxasulfone (AUBPC = 28,722) compared with the nontreated control (AUBPC = 71,619; Table 5). Sulfentrazone and pyroxasulfone are selective herbicides that provide effective residual weed control of small-seeded broadleaf species including Palmer amaranth (Gregory et al. 2005; Grey et al. 2014; Olson et al. 2011; Sweat et al. 1998). Moreover, flumioxazin and *S*-metolachlor also resulted in significant Palmer amaranth biomass reduction (≤33% biomass compared with the nontreated control) at 100 GDD (Figure 1). These results are validated by the high biomass reduction shortly after application of these herbicides (intercept = 23.9%, 27.3% for flumioxazin and *S*-metolachlor, respectively; Table 4) and supported by the lower AUBPC for Palmer amaranth with flumioxazin and *S*-metolachlor (AUBPC = 49,062 and 43,851, respectively; Table 5). The Palmer amaranth population used in this study was confirmed to be resistant to an ALS-inhibitor herbicide (imazethapyr at 70 g ai ha^{-1}; Oliveira et al. 2020), explaining why ALS-inhibitor herbicides were not as effective. The additional PRE herbicides evaluated were less effective in controlling Palmer amaranth, showing ≥48%, ≥57%, and ≥73% biomass compared with the nontreated control at 100, 500, and 900 GDD, respectively (Figure 1). Thus, according to our results, flumioxazin, pyroxasulfone, *S*-metolachlor, and/or sulfentrazone, can be effective PRE herbicide options to control ALS-inhibitor-resistant Palmer amaranth populations in soybeans.

Giant Foxtail

Pyroxasulfone (intercept = 3.3%, slope = 0.035) and *S*-metolachlor (intercept = 3.7%, slope = 0.055) had the greatest impact on giant foxtail biomass through the soil sampling period (Table 4). Giant foxtail produced 7%, 21%, and 35% biomass on the nontreated control at 100, 500, and 900 GDD in soil treated with pyroxasulfone (Figure 1). In soil treated with *S*-metolachlor, giant foxtail produced 9%, 32%, and 54% biomass at 100, 500, and 900 GDD, respectively. The AUBPC results corroborate the linear regression analysis showing lower biomass production over time by giant foxtail in the soil treated with pyroxasulfone (AUBPC = 13,478) and *S*-metolachlor (AUBPC = 20,476) compared with that of the nontreated control (AUBPC = 65,974; Table 5). The effectiveness of pyroxasulfone and *S*-metolachlor reducing giant foxtail growth supports the efficacy and selectivity of VLFCA-inhibitor herbicides on small-seeded annual grass species (Parker et al. 2005; Yamaji et al. 2014). Sulfentrazone (intercept = 21.8%; Table 4) also reduced giant foxtail growth by 26%, 44% and 61% of biomass at 100, 500, and 900 GDD, respectively (Figure 1) and AUBPC = 29,526 (Table 5). The remaining PRE herbicides investigated were less effective on giant foxtail, with ≥38%, ≥44%, and ≥61% biomass compared with the nontreated control 100, 500, and 900 GDD, respectively.

Radish

Chlorimuron-ethyl was the most detrimental herbicide to radish (intercept = 25%, slope = 0.057; Table 4). Radish biomass was lowest in soil treated with chlorimuron-ethyl throughout the sampling period, producing 31%, 54%, and 77% biomass compared with the nontreated control at 100, 500, and 900 GDD, respectively (Figure 1). Validating these findings, radish growing in the soil treated with chlorimuron-ethyl presented the lowest AUBPC (AUBPC = 39,315) compared with the nontreated control (AUBPC = 73,374; Table 5). Chlorimuron-ethyl soil residual efficacy has been shown to influence the establishment of subsequent broadleaf cover crop species (Bedmar et al. 2006; Brown et al. 2009; Ren et al. 2011). Previous research has also indicated that chlorimuron-ethyl reduced biomass and stand of radish seeded after soybean harvest by 19% and 40%, respectively (Cornelius and Bradley 2017). The persistence of chlorimuron-ethyl in soil varies according to the pH (Sharma et al. 2012). For instance, chlorimuron-ethyl persistence increased from 30 d at pH 5.9 to 69 d at pH 6.8 (Bedmar et al. 2006). Thus, it is likely that chlorimuron-ethyl was the most detrimental herbicide on radish due to the moderate soil pH in this study (6.5–7.0; Table 1). Conversely, cloransulam-methyl, metribuzin, and pyroxasulfone affected radish biomass (≤45% biomass compared with the nontreated control) at 100 GDD but not at 900 GDD (≥98% biomass compared with the nontreated control; Figure 1). The AUBPC findings support the linear regression results, showing lower AUBPC by cloransulam-methyl (AUBPC = 53,591), metribuzin (AUBPC = 58,839), and pyroxasulfone (AUBPC = 51,768) than the nontreated control (AUBPC = 73,374; Table 5). The lack of radish response at 900 GDD to cloransulam-methyl (slope = 0.067), metribuzin (slope = 0.079), and pyroxasulfone (slope = 0.072) likely occurred due to the higher dissipation of these herbicides over time (Figure 1; Table 4). On the other hand, radish showed constant biomass (79% biomass compared with the nontreated control) in soil treated with sulfentrazone at 500 and 900 GDD (Figure 1). The consistent reduction in radish biomass with sulfentrazone at 500 and 900 GDD is likely due to the extended half-life of this herbicide (121–302 d; Table 3; Shaner 2014). Despite sulfentrazone being less harmful to radish at 100 GDD (74% biomass compared with the nontreated control), this herbicide appeared to be as injurious as chlorimuron-ethyl to radish at 900 GDD (Figure 1). Cornelius and Bradley (2017) observed that radish showed 19% and 13% stand and biomass reduction, respectively, by sulfentrazone at 28 d after emergence. The residual efficacy of the remaining PRE herbicides evaluated in this bioassay were less detrimental to radish, presenting ≥60%, ≥78%, and ≥89% biomass compared with the nontreated control at 100, 500, and 900 GDD, respectively (Figure 1).

Cereal Rye

Metribuzin caused the highest injury to cereal rye growth at 100 and 500 GDD, producing 37% and 65% biomass compared with the nontreated control, respectively (intercept = 29.6%; Figure 1; Table 4). However, this herbicide presented rapid dissipation over time (slope = 0.071) resulting in minimal to no impact on cereal rye growth at 900 GDD (94% biomass compared with the nontreated control). The AUBPC analysis also indicates that metribuzin was the most detrimental herbicide to cereal rye growth (AUBPC = 49,850; Table 5) when compared with the nontreated control (AUBPC = 60,638). Metribuzin half-life is influenced by soil texture, organic matter content, temperature, and pH, and it tends to decrease as soil temperature and pH increase (Hyzak et al. 1974; Ladlie et al. 1976; Savage 1977; Sharom et al. 1976; Webster and Reimer 1976). Metribuzin degradation occurs primarily by soil microorganisms (Maqueda et al. 2009; Savage 1977); higher soil temperature and pH (>7) support higher microbial activity and consequently higher herbicide degradation (Maqueda et al. 2009; Singh et al. 2003). Thus, the increased soil temperature during the sampling period and the moderate soil pH in this study (6.5–7.0; Table 1) support the rapid degradation of metribuzin resulting in a low impact on cereal rye at 900 GDD (~50 DAT). Cornelius and Bradley (2017) observed that metribuzin reduced biomass (23%) and stand density (22%) of cereal rye

https://doi.org/10.1017/wet.2021.22 Published online by Cambridge University Press

seeded after soybean harvest. Cereal rye biomass was also reduced by pyroxasulfone, *S*-metolachlor, and flumioxazin at 100 and 500 GDD, with ≤48% and ≤67% biomass compared with the nontreated control, respectively (Figure 1). By 900 GDD, these herbicides had a minimal effect on cereal rye, with at least 84% biomass accumulation. According to the AUBPC analysis (Table 5), flumioxazin (AUBPC = 50,908), pyroxasulfone (AUBPC = 51,803), and *S*-metolachlor (AUBPC = 52,322) were ranked as detrimental herbicides to cereal rye. The other PRE herbicides assessed presented ≥54%, ≥68%, and ≥78% biomass compared with the nontreated control at 100, 500, and 900 GDD, respectively (Figure 1). Cornelius and Bradley (2017) reported that only 4 out of 27 herbicides tested adversely affected cereal rye establishment in terms of stand and biomass reduction. Furthermore, Smith et al. (2015) observed that cereal rye was not affected by commonly used soybean herbicides across 2 yr in Wisconsin and Indiana. Therefore, cereal rye is a resilient winter-hardy species and could fit well as a cover crop in systems where PRE herbicides are used for weed control purposes and the species is planted after soybean harvest (>50 DAT).

Sulfentrazone, pyroxasulfone, flumioxazin, and *S*-metolachlor were the most efficacious herbicides in the bioassay in terms of Palmer amaranth biomass production whereas pyroxasulfone, *S*-metolachlor, and sulfentrazone presented the highest residual impact on giant foxtail biomass. Thus, growers and practitioners should be able to use our results to support their selection of PRE herbicide(s) based on their weed infestations and benefit from a range of effective herbicide SOAs to include during multiyear crop rotations. Overall, our results showed that radish was less affected by PRE herbicides than cereal rye at 900 GDD (Figure 1). Most PRE herbicides evaluated herein would likely not affect radish and cereal rye established in the fall after soybean harvest under environmental conditions across southern Wisconsin.

Results of our bioassays can be of value to growers and practitioners considering herbicide options for enhanced control of small-seeded weed species such as Palmer amaranth and giant foxtail and reduced impact on establishment of subsequent cover crops such as radish and cereal rye. With caution, these results can also guide growers and practitioners with proper selection of herbicides to be used as part of a layered residual approach (i.e., inclusion of soil-residual herbicide with the POST program) in systems where a cover crop may be seeded after such applications. Additionally, our findings showcase the value of bioassays as a strategy to evaluate the biological residual efficacy of herbicides in soil using plant species of interest (e.g., weed and/or crops from a control and/or carryover perspective, respectively). The use of greenhouse bioassays can also reduce the impact of confounding environmental factors under field settings that may lead to uneven seedling establishment. Herein we also describe novel ways that bioassay results can be analyzed and displayed. Further research is needed to investigate the residual efficacy of PRE herbicide premixes containing multiple SOAs under different soil types and environments on different weed and cover crop species.

Acknowledgments. We thank staff members at the University of Wisconsin-Madison Arlington and Lancaster Agricultural Research Stations and personnel in the Wisconsin Cropping Systems Weed Science Laboratory for their technical assistance with the field and greenhouse projects. Thanks to Dr. Mark VanGessel and anonymous reviewers for their thoughtful edits and suggestions to this manuscript. No conflict of interest has been declared.

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