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

Victor H. V. Ribeiro1, Maxwel C. Oliveira2, Daniel H. Smith3, Jose B. Santos4 and Rodrigo Werle5

1Visiting Graduate Student, Department of Agronomy, University of Wisconsin, Madison, WI, USA; 2Research Associate, Department of Agronomy, University of Wisconsin, Madison, WI, USA; 3Southwest Regional Agronomist, Nutrient and Pest Management Program, University of Wisconsin, Madison, WI, USA; 4Professor, Universidade Federal dos Vales do Jequitinhonha e Mucuri, Diamantina, MG, Brazil and 5Assistant Professor, University of Wisconsin, Madison, WI, USA

Abstract

Amid widespread occurrence of herbicide-resistant weeds in the United States, the use of PRE herbicides and cover crops have resurfaced 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  | Silty clay loam (8% sand, 56% silt, 37% clay) | 2.6            | 6.5  | AG21X8          | June 12   |                      |
| Arlington  | Silt loam (10% sand, 65% silt, 25% clay)  | 4.1            | 6.6  | AG21X8          | May 13    |                      |
| Lancaster  | Silt loam (12% sand, 70% silt, 19% clay)  | 2.5            | 7.0  | AG21X7          | May 24    |                      |
| Lancaster  | Silt loam (10% sand, 73% silt, 17% clay)  | 3.1            | 7.0  | AG24X2          | May 23    |                      |

(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.

**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 CO₂-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 of...
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\textsuperscript{®}, 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\(^2\) seed tray cell (4.9 cm width \(\times\) 5.7 cm length \(\times\) 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 over sampling time for each site-year. GDD was estimated based on recorded field soil temperature (0 to 2 cm) collected with the Watchdog 1650 Micro Station (Spectrum Technologies\textsuperscript{®}, Aurora, IL). Daily soil GDD was calculated according to the equation described by McMaster and Wilhelm (1997):

\[
GDD = \sum_{i=1}^{n} \left( \frac{T_{\text{max}} + T_{\text{min}}}{2} - T_{\text{base}} \right) - T_{\text{base}}
\]

where \(T_{\text{max}}\) is the daily maximum soil temperature (C), \(T_{\text{min}}\) is the daily minimum soil temperature, \(T_{\text{base}}\) 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).

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

| Herbicide | Trade name\textsuperscript{a} | Company | Location | Group (SOA)\textsuperscript{a} | Herbicide family | Half-life\textsuperscript{b} | Rate | --- | --- |
|-----------|-------------------------------|---------|----------|-------------------------------|-----------------|-----------------|------|------|------|
| 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 | Tricolor | UPL | King of Prussia, PA | PSII (5) | Triazine | 30–60 | 563 |
| Flumioxazin | Valor SX | Valent | Walnut Creek, CA | Pyrazole 16 | N-phenylphthalimide | 12–18 | 107 |
| Sulfentrazone | Spartan | BASF | Durham, NC | Pyridiminedione | 15–29 | 25 |
| S-metolachlor | Dual II Magnum | Syngenta | Greensboro, NC | Chloroacetamide 112 | Chloroacetamide | 90 | 1260 |

\textsuperscript{a}Abbreviations: ALS, acetolactate synthase; PS II, photosystem II; PPO, protoporphyrinogen oxidase; SOA, site of action; VLCFA, very long chain fatty acid.

\textsuperscript{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.
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 lme4 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 according to Fisher’s protected LSD test. AUDPBC 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...
Estimated biomass (% biomass compared with the nontreated control) of each bioindicator species by PRE herbicide at 100, 500, and 900 growing degree days (GDD) (Figure 1). In soil treated with pyroxasulfone, Palmer amaranth produced 13%, 36%, and 59% biomass compared with the nontreated control at 100, 500, and 900 GDD, respectively. In the soil treated with saflufenacil, Palmer amaranth presented 15%, 41%, and 66% biomass compared with the nontreated control at 100, 500, and 900 GDD, respectively. The intercept (% biomass at 0 GDD; Table 4), the lower the biomass accumulation of Palmer amaranth (intercept 8.9%, slope 7.2%), was significantly different across PRE herbicides through the soil sampling period (Table 4).

**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.**

| Herbicide (SOA)‌<sup>a</sup> | Palmer amaranth<sup>b</sup> | 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 def| 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       | 33,991 cde| 57,836 abc|
| Acetochlor (15)                | 50,991 def                | 46,876 de      | 71,386 abc| 54,457 abc|
| Dimethenamid-P (15)            | 55,437 cde                | 48,510 cde     | 71,386 abc| 54,457 abc|
| Pyroxasulfone (15)             | 28,722 g                  | 13,476 g       | 51,768 g  | 51,803 bc |
| S-metolachlor (15)             | 43,851 f                  | 20,476 g       | 55,841 f  | 52,322 bc |

<sup>a</sup>Abbreviations: SOA, site of action.  
<sup>b</sup>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
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 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 (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.

**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 for 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.
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 seeding 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.

References
Alister C, Kogan M (2005) Efficacy of imidazolinone herbicides applied to imidazolinone-resistant maize and their carryover effect on rotational crops. Crop Prot 24:375–379
Arneson NJ, Smith DH, DeWerf R, Oliveira MC (2019) Residual control of waterhemp with pre-emergence herbicides in soybean. https://www.wwc
Heap I (2020) The international survey of herbicide resistant weeds. www.
Corns MS (2017) Determinancy and yield loss of waterhemp (Amaranthus rudis) control in soybean (Glycine max), Weed Technol 17:14–20
Heap I (2020) The international survey of herbicide resistant weeds. www.
Hyzak DL, Zimdahl RL (1974) Rate of degradation of metribuzin and two analogs in soil. Weed Sci 21:75–79
Jablonski I (2000) Microbial and photoytic degradation of the herbicide acetochlor. Int J Environ Anal Chem 78:1–8
Johnson WG, Davis VM, Kruger GR, Weller SC (2009) Influence of glyphosate-resistant cropping systems on weed species shifts and glyphosate-resistant weed populations. Eur J Agron 31:162–172

https://doi.org/10.1017/wet.2021.22 Published online by Cambridge University Press
Knezevic SZ, Pavlovic P, Osipitan OA, Barnes ER, Beiermann C, Oliveira MC, Lawrence N, Scott JE, Hala A (2019) Critical time for weed removal in glyphosate-resistant soybean as influenced by preemergence herbicides. Weed Technol 33:393–399

Knis AR (2018) Genetically engineered herbicide-resistant crops and herbicide resistant weed evolution in the United States. Weed Sci 66:260–273

Laddie S, Meggitt F, Penner D (1976) Effect of soil pH on microbial degradation, adsorption, and mobility of metribuzin. Weed Sci 24:477–481

Madden LV, Hughes G, Van Den Bosch F (2007) The study of plant disease epidemics. St. Paul, MN: APS Press. 421 p

Maqueda C, Villaverde J, Sopeña F, Undabeytia T, Morillo E (2009) Effects of soil characteristics on metribuzin dissipation using clay-gel-based formulations. J Agric Food Chem 57:3273–3278

Mehdizadeh M, Alembrahim MT, Roushani M (2017) Determination of two sulfonylurea herbicides residues in soil environment using HPLC and phytotoxicity of these herbicides by lentil bioassay. Bull Environ Contam Toxicol 99:93–99

McMaster GS, Wilhelrn WW (1997) Growing degree-days: one equation, two interpretations. Agr Forest Meteorol 87:291–300

Mendiburu F (2019) Agricolae: statistical procedures for agricultural research. R package version 1.3–1. https://CRAN.R-project.org/package=agricolae Accessed: January 20, 2020

Norsworthy JK, Ward SM, Shaw DR, Llewellyn RS, Nichols RL, Webster TM, Riddle RN, O’Mendiburu F (2019) Agricolae: statistical procedures for agricultural research. McAshton GS, Wilhelm WW (1997) Growing degree-days: one equation, two interpretations. Agr Forest Meteorol 87:291–300

Madden LV, Hughes G, Van Den Bosch F (2007) The study of plant disease epidemics. St. Paul, MN: APS Press. 421 p

Maqueda C, Villaverde J, Sopeña F, Undabeytia T, Morillo E (2009) Effects of soil characteristics on metribuzin dissipation using clay-gel-based formulations. J Agric Food Chem 57:3273–3278

Mehdizadeh M, Alembrahim MT, Roushani M (2017) Determination of two sulfonylurea herbicides residues in soil environment using HPLC and phytotoxicity of these herbicides by lentil bioassay. Bull Environ Contam Toxicol 99:93–99

McMaster GS, Wilhelrn WW (1997) Growing degree-days: one equation, two interpretations. Agr Forest Meteorol 87:291–300

Mendiburu F (2019) Agricolae: statistical procedures for agricultural research. R package version 1.3–1. https://CRAN.R-project.org/package=agricolae Accessed: January 20, 2020

Norsworthy JK, Ward SM, Shaw DR, Llewellyn RS, Nichols RL, Webster TM, Bradley KW, Friisvold G, Powles SB, Burgos NR, Witt WW, Barret M (2012) Reducing the risks of herbicide resistance: best management practices and recommendations. Weed Sci 60:31–62

Oliveira MC, Butts L, Werle R (2019) Assessment of cover crop management strategies in Nebraska, US. Agriculture 9:1–14

Oliveira MC, Giacomini DA, Arsenijevic N, Vieira G, Tranel PJ, Werle R (2020) Distribution and validation of genotypic and phenotypic glyphosate and PPO-inhibitor resistance in Palmer amaranth (Amaranthus palmeri) from southwestern Nebraska. Weed Technol 34:1–35

Olson BL, Zöllinger RK, Thompson CR, Peterson DE, Jenks B, Moechnig M, Stfhalmn PW (2011) Pyroxasulfone with and without sulflenzraone in sunflower (Helianthus annuus). Weed Technol 25:217–221

Owen MD, Zelaya IA (2005) Herbicide-resistant crops and weed resistance to herbicides. Pest Manag Sci 61:301–311

Owen MD (2008) Weed species shifts in glyphosate-resistant crops. Pest Manag Sci 64:377–387

Palhano MG, Norsworthy JK, Barber T (2018) Sensitivity and likelihood of residual herbicide carryover to cover crops. Weed Technol 32:236–243

Parker DC, Simons WF, Wax LM (2005) Fall and early preplant application timing effects on persistence and efficacy of acetamide herbicides. Weed Technol 19:6–13

Powles SB (2008) Evolved glyphosate-resistant weeds around the world: lessons to be learnt. Pest Manag Sci 64:360–365

Prince JM, Shaw DR, Givens WA, Owen MD, Weller SC, Young BG, Wilson RG, Jordan DL (2012) Benchmark study: IV. Survey of grower practices for managing glyphosate-resistant weed populations. Weed Technol 26:543–548

R Core Team (2020) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/. Accessed: November 15, 2020

Ren W, Wang M, Zhou Q (2011) Effect of soil pH and organic matter on desorption hysteresis of chlorimuron-ethyl in two typical Chinese soils. J Soil Sediment 11:552–561

Riddle RN, O’Sullivan J, Swanton CJ, Van Acker RC (2013) Field and greenhouse bioassays to determine mesotrione residues in soil. Weed Technol 27:565–572

Riggins CW, Tranel PJ (2012) Will the Amaranthus tuberculatus resistance mechanism to PPO-inhibiting herbicides evolve in other Amaranthus species? Int J Agron 2012:1–7

Royston P (1995) Remarks AS R94: A remark on algorithm AS 181: The W-test for normality. J R Stat Soc 44:547–551

Savage KE (1977) Metribuzin persistence in soil. Weed Sci 25:55–59

Shaner DL (2014) Herbicide Handbook. 10th ed. Lawrence, KS: Weed Science Society of America. 513 p

Sharma S, Banerjee K, Choudhury PP (2012) Degradation of chlorimuron-ethyl by Aspergillus nigerisolated from agricultural soil. FEMS Microbiol Lett 337:18–24

Sharam MS, Stephenson GR (1976) Behavior and fate of metribuzin in eight Ontario soils. Weed Sci 24:153–160

Singh BK, Walker A, Morgan JA, Wright D (2003) Role of soil pH in the development of enhanced biodegradation of fenamiphos. Appl Environ Microbiol 69:7035–7043

Smith DH, Legleiter TR, Bosak EJ, Johnson W, Davis VM (2015) Cover crop establishment issues following corn and soybean in the upper Midwest. Pages 52–53 in Proceedings of the 2015 Weed Science Society of America annual meeting. Lexington, KY: Weed Science Society of America.

Striegel S, Oliveira MC, Arneson N, Conley SP, Stoltenberg DE, Werle R (2020) Spray solution pH and soybean injury as influenced by synthetic auxin formulation and spray additives. Weed Technol 34:1–55

Striebig JC (1998) Herbicide bioassay. Weed Res 28:479–484

Sweat JK, Horak MJ, Peterson DE, Lloyd RW, Boyer JE (1998) Herbicide efficacy on four Amaranthus species in soybean (Glycine max). Weed Technol 12:315–321

Tursun N, Datta A, Sami M, Kantarci Z, Knezevic SZ, Singh, B (2016) The critical period for weed control in three corn (Zea mays L.) types. Crop Prot 90:59–65

[USDA] U.S. Department of Agriculture—National Agricultural Statistics Service (2020) Surveys: Agricultural Chemical Use Program. https://www.nass.usda.gov/Surveys/Guide_to_NASS_Surveys/Agricultural_Chemical_Use/index.php. Accessed: June 15, 2020

VanGessel MK, Johnson QR, Scott BA (2016) Evaluating postemergence herbicides, safener, and tolerant hybrids for corn response. Weed Technol 30:869–877

Walker A, Thompson JA (1977) The degradation of simazine, linuron and propyzamide in different soils. Weed Res 17:399–405

Walsh JD, DelPelico MS, Sims BD (1993) Influence of tillage on soybean (Glycine max) herbicide carryover to grass and legume forage crops in Missouri. Weed Technol 41:144–149

Wang W, Freemark K (1995) The use of plants for environmental monitoring and assessment. Ecotox Environ Safety 30:289–301

Webster GT, Reimer GJ (1976) Field degradation of the herbicide metribuzin and its degradation products in a Manitoba sandy loam soil. Weed Res 16:191–196

Webster TM, Nichols RL (2012) Changes in the prevalence of weed species in the major agronomic crops of the Southern United States: 1994/1995 to 2008/2009. Weed Sci 60:145–157

[WSSA] Weed Science Society of America (2019) Common Weed in Soybean Survey. http://wssas.net/wssa/weed/surveys/ Accessed: October 28, 2020

Werle R, Oliveira MC, Hala A, Proctor CA, Rees J, Klein R (2018) Survey of Nebraska farmers' adoption of dicamba-resistant soybean technology. Weed Technol 32:754–761

Whalen DM, Bish MD, Young BG, Hager AG, Conley SP, Reynolds DB, Steckel LE, Norsworthy JK, Bradley KW (2019) Evaluation of cover crop sensitivity to residual herbicides applied in the previous soybean (Glycine max (L.) Merr) crop. Weed Technol 33:312–320

Whitaker JR, York AC, Jordan DL, Culpepper AS, Soososke LM (2011) Residual herbicides for Palmer amaranth control. J Cotton Sci 15:89–99

Yamaji Y, Honda H, Kobayashi M, Hana R, Inoue J (2014) Weed control efficacy of a novel herbicide, pyroxasulfone. J Pestic Sci 39:165–169