Weed Technology

www.cambridge.org/wet

Evaluating cover crop tolerance to corn residual herbicides using field-treated soil in greenhouse bioassay

Tatiane Severo Silva[1], Nicholas John Arneson[2], Daniel Valadão Silva[3] and Rodrigo Werle[4]

[1]Postdoctoral Research Associate, Department of Department of Plant and Agroecosystem Sciences, University of Wisconsin–Madison, Madison, WI, USA; [2]Outreach Program Manager, University of Wisconsin–Madison, Madison, WI, USA; [3]Assistant Professor, Department of Agronomy, Federal Rural University of the Semi-Arid Region, Mossoro, Brazil and [4]Associate Professor, Department of Plant and Agroecosystem Sciences, University of Wisconsin–Madison, Madison, WI, USA

Research Article

Cite this article: Severo Silva T, Arneson NJ, Silva DV, Werle R (2023). Evaluating cover crop tolerance to corn residual herbicides using field-treated soil in greenhouse bioassay. Weed Technol. doi: 10.1017/wet.2023.72

Received: 12 May 2023
Revised: 17 August 2023
Accepted: 25 September 2023

Associate Editor:
David Johnson, Corteva Agriscience

Nomenclature:
Annual ryegrass, *Lolium multiflorum* Lam.; cereal rye, *Secale cereale* L.; radish, *Raphanus sativus* L.; red clover, *Trifolium pratense* L.; corn, *Zea mays* L.

Keywords:
Biomass production; carryover; cover crop interseeding; cover crop overseeding; herbicide injury; preemergence herbicides

Corresponding author:
Tatiane Severo Silva;
Email: severosilva@wisc.edu

Abstract

More growers across the U.S. Midwest are considering interseeding or overseeding cover crops into corn for soil health purposes. One challenge of this practice is the potential injury from soil residual herbicides applied preemergence (PRE) for weed control in corn to the interseeded and overseeded cover crop species. Field-treated soil was collected in 2021 and 2022 at Janesville, WI, and Lancaster, WI, to investigate the impact of PRE residual herbicides on establishment of interseeded and overseeded cover crops via greenhouse bioassay. Soil samples (0 to 5 cm depth) were collected from field experiments at 0, 10, 20, 30, 40, 50, 60, and 70 days after treatment (DAT). Treatments consisted of 14 single and multiple sites of action (SOAs) PRE herbicides plus a nontreated check (NTC). Four bioindicator cover crop species were used in the greenhouse bioassay: annual ryegrass, cereal rye, radish, and red clover. Cover crop biomass was collected 28 d after bioassay seeding. Cover crop species responded differently across herbicide treatments. Annual ryegrass and cereal rye were sensitive to treatments containing herbicide Group 15, whereas Groups 2, 4, 5, 14, and 27 had minimal impact on their establishment when field soil was collected at 30 DAT (interseeding scenario) and 70 DAT (overseeding scenario) compared to the NTC. Radish and red clover were sensitive to herbicide Groups 2, 4, and 27, whereas Groups 5, 14, and 15 had minimal impact on their establishment. Annual ryegrass, radish, and red clover were more sensitive to PRE herbicides containing two and three SOAs than to herbicides with a single SOA. On the basis of these greenhouse bioassay results, cover crop species should be carefully selected depending on the soil residual herbicide when interseeded and overseeded into corn. Field studies will be conducted to validate these results and support recommendations to growers interested in this system.

Introduction

The adoption of cover crops increases the crop diversity in continuous corn and corn–soybean [*Glycine max* (L.) Merr.] rotations across the U.S. Midwest (Brooker et al. 2020a). Cover crops can provide various benefits, including reduced soil erosion, improved water infiltration, enhanced nutrient cycling, and weed and insect pest suppression (Grint et al. 2022; Schipanski et al. 2014; Wallander et al. 2021). Although only 2% of agricultural hectares in the United States were sown with cover crops in 2017, the increase in cover crop adoption is promising, with a 50% increase in cover cropping from 2012 to 2019 (USDA-NASS 2019; Wallander et al. 2021). In Wisconsin, cover crops were established in 6% of the 3.7 million ha of cropland in 2017 (USDA-NASS 2019). One of the main challenges for successful cover crop establishment in corn cropping systems in the Upper Midwest is the short growing season (lack of degree days) for sowing and establishing cover crops following corn grain harvest (Kladivko et al. 2014; Smith et al. 2019).

In a continuous corn and corn–soybean rotation, cover crop species selection is typically limited to winter cereals, such as cereal rye. Legume species like crimson clover (*Trifolium incarnatum* L.) and field pea (*Pisum sativum* L.), as well as brassica species like radish and turnips (*Brassica rapa* L.), perform poorly when established late after corn grain harvest because of low temperatures (Curran et al. 2018; Noland et al. 2018; Rusch et al. 2020; Singer 2008). Interseeding or overseeding cover crops while the primary crop is still in the field increases growing season length and cover crop biomass potential relative to cover crop planted after harvest, enhancing the ecosystem benefits of cover cropping in corn production systems (Adler and Nelson 2020; Caswell et al. 2019). Herein, interseeding is defined as planting a cover crop early in the growing season when the corn is between the V3 and V8 vegetative growth stages

https://doi.org/10.1017/wet.2023.72 Published online by Cambridge University Press

(Smith et al. 2019; Youngerman et al. 2018). In contrast, cover crop overseeding is typically done by aerial seeding just before or at crop physiological maturity (Kladivko et al. 2014). These systems provide winter-sensitive legume cover crop species, such as crimson clover (Peterson et al. 2021; Youngerman et al. 2018) and red clover (Wallace et al. 2017), and brassica species, such as radish and turnips, a wider growing window before the winter (Brooker et al. 2020b).

A concern with interseeding and overseeding is whether soil residual herbicides applied for weed control will injure the cover crops (Adler and Nelson 2020; Brooker et al. 2020b). Researchers have investigated the impact of soil residual herbicides on interseeded cover crops into the V3 to V6 corn growth stages and reported injury depending on the active ingredient and cover crop species. In one interseeding study established at the V5 corn growth stage and conducted in Pennsylvania, annual ryegrass biomass was reduced >80% with pyroxasulfone and *S*-metolachlor applications, and red clover biomass was reduced >80% with mesotrione, compared to the nontreated control (Wallace et al. 2017). Brooker et al. (2020b) reported that Group 15 herbicides (acetochlor, dimethenamid-P, and pyroxasulfone) reduced annual ryegrass stand >60% at V3 and V6 interseeding timings; Group 2 herbicides (flumetsulam and rimsulfuron) reduced radish stand >70% compared to the nontreated control. The same authors described that cover crops can be interseeded into corn over the V3 and V6 stages but that species selection and herbicide label restrictions should be carefully considered. Thus additional studies are warranted to evaluate response of multiple cover crop species to soil residual herbicides under different soil types and environmental conditions, which are critical components influencing cover crop establishment, herbicide residual activity in the soil, and their interactions (Cornelius and Bradley 2017; Jursík et al. 2020).

Few studies have reported the potential herbicide residual injury to cover crops interseeded at the V3 to V5 corn growth stages and overseeded at the V10 to VT corn growth stages. More research is needed to support herbicide selection that provides effective weed control yet allows establishment of cover crops for growers adopting the interseeding and overseeding systems. Herein the tolerance of four common cover crop species (annual ryegrass, cereal rye, radish, and red clover) to a comprehensive list of labeled corn residual preemergence (PRE) herbicides is evaluated. The main purpose of this study was to investigate potential soil residual herbicide and cover crop combination options for interseeding (~V3 to V5 corn growth stages) and overseeding (~V10 to VT corn growth stages) scenarios via greenhouse bioassay.

Materials and Methods

Field Experiment Information

A field experiment was conducted in 2021 and 2022 at the Rock County Farm, Janesville, WI (42.43°N, 89.01°W), and the University of Wisconsin–Madison Lancaster Agricultural Research Station, Lancaster, WI (42.83°N, 90.76°W), to evaluate weed control in corn with multiple soil residual herbicides applied PRE. (For more information about the field experiment and weed control results, see Silva et al. [2023].) Briefly, soil properties, corn hybrids, and seeding rates for each location are summarized in Table 1. The Janesville-2021 and Lancaster-2021 fields had no history of residual herbicide application in the previous season. Authority® First (sulfentrazone [280 g ai ha^{-1}] + cloransulam-methyl [35 g ai ha^{-1}]) (FMC, Philadelphia, PA, USA) was applied PRE in the previous season for the Janesville-2022 field. Sequence® (glyphosate [800 g ai ha^{-1}] + *S*-metolachlor [1,100 g ai ha^{-1}]) (Sygenta, Greensboro, NC, USA) was applied at the V2 soybean growth stage in the previous season for the Lancaster-2022 field. Monthly average air temperature and accumulated precipitation during the data collection period were obtained from onsite weather stations (WatchDog® 2700, Spectrum Technologies, Aurora, IL, USA) and are summarized in Table 2.

The field experiment was conducted in a randomized complete-block design with four replications. The experimental units were 3 m wide (4 corn rows) × 9 m long. The treatments consisted of 14 soil residual herbicides applied PRE plus a nontreated check (NTC; Table 3). Herbicides were applied within a day after corn planting (Table 4) using a CO_2 pressurized backpack sprayer equipped with six TeeJet® TTI110015 flat-fan nozzles (TeeJet®, Springfield, IL, USA) spaced 50.8 cm apart at a boom height of 50 cm from the soil surface. The sprayer was calibrated to deliver 140 L ha^{-1} of spray solution at 241 kPa at a speed of 4.8 km h^{-1}.

Soil samples (0 to 5 cm depth) were collected from 14 residual PRE herbicide treatments (including herbicides with single and multiple sites of action [SOAs]) plus the NTC (Table 3) from field experiments conducted at the four site-years. Soil samples were collected at 0, 10, 20, 30, 40, 50, 60, and 70 d after treatment (DAT) to evaluate cover crops' response to herbicide residuals over time. A handheld 6-cm-diameter soil sampler (Fiskars, Middleton, WI, USA) was used to collect the soil samples. At each sampling time, six soil cores were collected from each plot adjacent to the two central corn rows, combined, and placed in a plastic bag (~1,000 g). Soil samples were stored in a freezer (−20 C) until the onset of the greenhouse bioassay experiment (approximately 4 mo after the first collection date). The corn growth stage at each soil sampling time was recorded according to Broeske and Lauer (2020; Table 4). No herbicides were applied to the field experiments other than the PRE herbicides evaluated.

Greenhouse Bioassays Using Cover Crops

In the fall of each year, the treated soil samples were used in greenhouse bioassays; that is, in fall 2021, greenhouse bioassays were conducted with the soil samples collected from the Janesville-2021 and Lancaster-2021 field experiments; in fall 2022, greenhouse bioassays were conducted with the soil samples collected from the Janesville-2022 and Lancaster-2022 field experiments. The bioassays were conducted in the Walnut Street Greenhouse at the University of Wisconsin–Madison. Each bioassay experimental unit consisted of a 210-cm^3 seed tray cell (6 cm length × 6 cm width × 5.9 cm depth; 804 Series, T.O. Plastics, Clearwater, MN, USA). The four field soil samples from each treatment within a site-year and sampling time were thawed and combined across replications (creating a composite sample). The composite sample was thoroughly mixed by hand to ensure uniform distribution of the herbicide among the eight resulting replicates (treated as four replications and two experimental runs). Experimental units (seed tray cells) were then filled with the respective mixed soil sample.

Annual ryegrass, cereal rye, radish, and red clover (La Crosse Seed, La Crosse, WI, USA) were used as bioindicator species. These species are among the most commonly adopted cover crops across cropping systems in the United States (USDA-SARE 2020) and have been successfully interseeded in Wisconsin corn systems (Smith and Ruark 2022). Germination tests were conducted before setting up the bioassay experiment to investigate seed viability.

Table 1. Soil properties, corn hybrids, and seeding rates for corn field experiments at Janesville, WI, and Lancaster, WI, 2021 and 2022.[a,b]

Site-year[c]	pH	OM	Sand	Silt	Clay	Soil type	Corn hybrid Name	Corn hybrid Manufacturer	Seeding rate
		%							seeds ha^{-1}
Janesville-2021	5.4	4.1	8	68	24	Plano silt-loam	NK 9653-5222EZ	Brevant, Indianapolis, IN	87,600
Janesville-2022	5.9	2.6	26	63	12	Plano silt-loam	NK 9653-5222EZ	Brevant, Indianapolis, IN	87,600
Lancaster-2021	6.6	2.5	10	76	14	Fayette silt-loam	B97T04SXE	Syngenta, Greensboro, NC	80,200
Lancaster-2022	5.3	4.1	18	65	18	Fayette silt-loam	P9998Q-N802	Pioneer, Johnston, IA	80,200

[a]The experimental areas were managed in a soybean–corn rotation; thus soybean was grown in the previous growing season before the experiment establishment at all experimental sites. Before corn planting, the experimental area was tilled using a field cultivator. Corn was planted 5 cm deep and in 76-cm row spacing at all experimental sites.
[b]Abbreviation: OM, organic matter.
[c]The Janesville-2021 experimental field was fertilized with 200 kg ha^{-1} of nitrogen (46-0-0); Lancaster-2021 with 128 kg ha^{-1} of nitrogen (46-0-0); Janesville-2022 with 112 kg ha^{-1} of nitrogen (32-0-0) and 32 kg ha^{-1} of sulfur in the form of ammonium thiosulfate (12-0-0-26S); Lancaster-2022 with 55 kg ha^{-1} of phosphorus + 112 kg ha^{-1} of potassium nitrate (4-19-38) applied early spring and 160 kg ha^{-1} of nitrogen (46-0-0).

Table 2. Monthly average air temperature and total precipitation from April through July at Rock County Farm, Janesville, WI, and Lancaster Agricultural Research Station, Lancaster, WI, in 2021 and 2022 and during the past 30 years.[a,b]

	Janesville			Lancaster		
	2021	2022	30-yr avg.	2021	2022	30-yr avg.
Air temperature						
			C			
April	8.8	—	8.2	9.0	5.4	7.9
May	14.8	17.8	14.9	14.4	15.5	14.3
June	22.8	20.7	20.6	22.2	20.2	19.8
July	22.2	22.1	22.5	22.1	22.2	21.8
Average	17.2	20.2	16.5	16.9	15.8	16.0
Rainfall						
			mm			
April	33.8	—	89.6	24.1	83.3	92.4
May	74.4	47.2	101.7	72.6	65.5	109.2
June	55.4	58.9	120.5	43.7	71.4	140.8
July	53.1	96.0	108.2	120.9	183.6	131.0
Total	216.7	202.2	420.1	261.3	403.8	473.4

[a]Weather data for 2021 and 2022 were obtained from onsite weather stations. The 30-yr average monthly weather data were obtained from the Wisconsin State Climatology Office (https://www.aos.wisc.edu/~sco/clim-history/acis_stn_meta_wi_index.htm).

Seeds were sown in pots filled with soil (four replicates of 25 seeds each), and at 10 d after sowing (DAS), the germinated seedlings were counted. The average percentage of germination for both years was 93%, 85%, 96%, and 94% for annual ryegrass, cereal rye, radish, and red clover, respectively. A preliminary experiment in additive series (Freckleton and Watkinson 2000; Galon et al. 2018) was also conducted in 2021 to determine the cover crop plant density for each species. The cover crop densities evaluated were 1, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, and 22 plants cell^{-1}, which corresponded to 17, 34, 67, 101, 134, 168, 201, 235, 268, 302, 335, and 369 plants m^{-2}. At 28 DAS, the aboveground biomass of the plants was harvested and dried at 60 C until constant dry biomass was obtained. The constant biomass production was obtained with a density of 8 plants cell^{-1} for cereal rye and radish (134 plants m^{-2}), 10 plants cell^{-1} for annual ryegrass (168 plants m^{-2}), and 18 plants cell^{-1} for red clover (302 plants m^{-2}) (data not shown). For the preliminary study and for the bioassay experiment, each cover crop species was grown in a separate experimental unit (Figure 1).

The greenhouse bioassay experiment was conducted as a completely randomized design with four replications. The experiment was repeated in time (two experimental runs) for each PRE herbicide treatment over sampling time and site-year. In 2021, the greenhouses were maintained at 28/25 C day/night temperature and 55% relative humidity. In 2022, the greenhouses were maintained at 24/21 C day/night temperature and 60% relative humidity. The slight differences in day/night temperature and relative humidity in the greenhouses between the two experimental years were because of external fall weather conditions (the greenhouse bioassay experiments were established on September 17, 2021, and September 8, 2022). Greenhouse conditions for both years were set to a 16/8-h day/night photoperiod using high-pressure sodium lightbulbs (400 W) to supplement the natural light. The greenhouse environmental conditions were monitored throughout the experiment using a WatchDog® A150 logger (Spectrum Technologies). Bioassays were watered twice a day and fertigated weekly using 20-10-20 water-soluble fertilizer (Peters® Professional; ICL Fertilizers, Dublin, OH, USA) providing 300 ppm of nitrogen and potassium and 150 ppm of phosphorus. At 28 DAS, bioassay cover crop injury was assessed using a scale of 0% to 100%, where 0% was no visible injury and 100% was complete plant necrosis. The aboveground biomass of indicator cover crop species growing in each tray cell (g pot^{-1}) was harvested, bagged, forced-air dried at 60 C for at least 7 d, and then weighed.

Statistical Analyses

All statistical analyses were performed using R Version 4.2.2 (R Development Core Team 2022). A linear correlation between

https://doi.org/10.1017/wet.2023.72 Published online by Cambridge University Press

Table 3. PRE herbicides, site of action groups, trade names, manufacturers, chemical families, half-lives, and rates used in the corn field experiments.[a]

Herbicide (SOA)	Trade name	Manufacturer	Chemical family	Half-life[b]	Rate
				d	g ai or ae ha^{-1}
Atrazine (5)	AAtrex®	Syngenta Crop Protection, Greensboro, NC	triazines	60	1,120
Simazine (5)	Princep® 4L	Syngenta Crop Protection	triazines	60	2,240
Acetochlor (15)	Harness®	Bayer Crop Science, St. Louis, MO	α-chloroacetamides	12	1,960
S-metolachlor (15)	Dual II Magnum®	Syngenta Crop Protection	α-chloroacetamides	112–124	1,791
Mesotrione (27)	Callisto®	Syngenta Crop Protection	triketones	5–15	175
Acetochlor (15) + mesotrione (27)	Harness® Max	Bayer Crop Science	α-chloroacetamides + triketones	—	1,971 + 185
Atrazine (5) + *S*-metolachlor (15)	Bicep Lite II Magnum®	Syngenta Crop Protection	triazines + α-chloroacetamides	—	1,310 + 1,634
Atrazine (5) + acetochlor (15)	Harness® Xtra	Bayer Crop Science	triazines + α-chloroacetamides	—	952 + 2,408
Saflufenacil (14) + dimethenamid-P (15)	Verdict®	BASF Corporation, Durham, NC	N-phenyl-imides + α-chloroacetamides	—	75 + 655
Flumetsulam (2) + clopyralid (4)	Hornet® WDG	Dow AgroSciences, Zionsville, IN	triazolopyrimidine + pyridine-carboxylates	—	52 + 168
S-metolachlor (15) + bicyclopyrone (27) + mesotrione (27)	Acuron® Flexi	Syngenta Crop Protection	α-chloroacetamides + triketone + triketone	—	1,602 + 45 + 179
Atrazine (5) + *S*-metolachlor (15) + bicyclopyrone (27) + mesotrione (27)	Acuron®	Syngenta Crop Protection	triazines + α-chloroacetamides + triketones + triketones	—	700 + 1,498 + 42 + 168
Flumetsulam (2) + clopyralid (4) + acetochlor (15)	SureStart® II	Corteva Agriscience	triazolopyrimidine + pyridine-carboxylates + α-chloroacetamides	—	42 + 133 + 1,315
Clopyralid (4) + acetochlor (15) + mesotrione (27)	Resicore®	Corteva Agriscience	pyridine-carboxylates + α-chloroacetamides + triketones	—	133 + 1,960 + 210

[a]Abbreviation: SOA, site of action.
[b]Average field half-life. Data are from Shaner (2014) and the Pesticide Properties Database (http://sitem.herts.ac.uk/aeru/ppdb/en/index.htm).

Table 4. Corn planting and herbicide application dates for each site-year and corn growth stage for each collection date.[a]

Site-year	Corn planted	Herbicide applied	Corn growth stage						
			10	20	30	40	50	60	70
			DAT						
Janesville-2021	26 Apr	28 Apr	—	V1	V3	V5	V7	V9	V10
Janesville-2022	10 May	11 May	V1	V3	V5	V7	V8	V10	VT
Lancaster-2021	28 Apr	29 Apr	—	V1	V3	V5	V7	V9	V10
Lancaster-2022	11 May	13 May	V1	V3	V5	V7	V8	V10	VT

[a]Abbreviation: DAT, days after treatment.

Figure 1. Cover crop species 14 d after sowing in each experimental unit (left). From bottom to top, the units represent days after treatment in the field from 0 to 70, and from left to right, the units represent the different treatments, starting with the nontreated check. Photos provide a closer view of the cover crops at 14 d (top right) and 28 d (bottom right) after sowing. The experimental units at the top left and bottom left represent radish and red clover, respectively, whereas the experimental units at the top right and bottom right represent cereal rye and annual ryegrass, respectively.

https://doi.org/10.1017/wet.2023.72 Published online by Cambridge University Press

bioassay injury and aboveground biomass production was performed using Pearson's analysis (*stat_cor* function, GGPUBR package; Kassambara 2022). Jitter violin plots combined with box plots were generated for annual ryegrass, cereal rye, radish, and red clover data to show the variance of the biomass values combining all treatments over site-years and sampling times.

Analysis of variance (ANOVA) was performed to compare different PRE herbicide treatments within each sampling time (0, 10, 20, 30, 40, 50, 60, and 70 DAT) for each bioindicator cover crop species instead of building multiple response curves over time. ANOVA provided more meaningful results when compared to regression models (data not shown). Bioassay aboveground biomass data for each cover crop species and sampling time were combined over site-years and over the two experimental runs in the greenhouse and analyzed with linear mixed-effect models using the function *lmer* from the LME4 package (Bates et al. 2015). Square root transformation models were used when fitting the bioassay biomass data to meet the assumptions of normality and homogeneity of variance of residuals for each cover crop species. Back-transformed means are reported in the results. PRE herbicide treatments were included as a fixed effect in the model; greenhouse bioassay experimental run and site-year and experimental run nested within site-year were considered random effects. Models were analyzed using ANOVA (*anova* function, CAR package; Fox and Weisberg 2019), and means were separated using Fisher's least significant difference (LSD) test (EMMEANS package; Lenth 2022) when a treatment effect was significant ($P \leq 0.05$).

The biomass response by PRE herbicide treatment on each cover crop species across soil sampling time and site-year was used to calculate the area under biomass stairs (AUBS). The AUBS was estimated using the *audps* function of the AGRICOLAE package (Mendiburu 2022). The AUBS referred to herein is an adaptation from the area under the disease progress stairs (AUDPS) commonly used in plant pathology to estimate disease progress over time (Simko and Piepho 2012). The AUDPS has also been adopted to estimate crop injury from postemergence (POST) herbicides over distance (Striegel et al. 2021) and herbicide impact on biomass bioindicator species (Ribeiro et al. 2021). The AUDPS (herein after called AUBS) concept applied to our bioassay data resulted in one value to estimate the impact of each residual herbicide applied PRE on the biomass of each cover crop species over sampling time. AUBS corresponds to the area under the step function considering adjusted weight for the first and last DAT. For instance, each biomass value was multiplied by 10 (interval between soil sampling), and the first and last assessment weights were extrapolated in the missing direction using half of the average interval duration between DAT observations (Simko and Piepho 2012). The higher the AUBS value that was obtained, the lower was the PRE herbicide injury on cover crops (Figure 2).

AUBS estimated values for each cover crop species by PRE herbicide treatment were submitted to ANOVA using a linear mixed-effect model following the previously described approaches for biomass data. AUBS values were also estimated for each cover crop species and combined across species by the number of herbicide sites of action of each PRE treatment (one, two, or three SOAs; Table 3). PRE herbicide SOAs were included as a fixed effect in the model. Experimental run and site-year and experimental run nested within site-year were fitted as random effects for each cover crop and all cover crops pooled together. Models were analyzed using ANOVA (*anova* function, CAR package; Fox and Weisberg 2019), and means were separated using Fisher's LSD test (EMMEANS package; Lenth 2022) when a treatment effect was significant ($P \leq 0.05$).

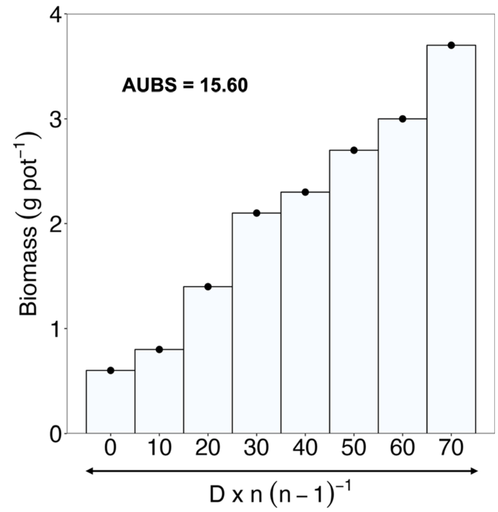

Figure 2. Graphical example of clopyralid + acetochlor + mesotrione herbicide effect on annual ryegrass aboveground biomass production (28 d after sowing the greenhouse bioassay) as a function of days after treatment in the field as calculated by the area under biomass stairs (AUBS). *D* is cover crop biomass, and *n* is the interval between days after treatment. The AUBS value was obtained from the simplified equation AUBS $= \bar{y} \times n$, where $\bar{y}$ is the arithmetic mean of all cover crop biomass assessments.

Results and Discussion

Cover Crop Response

The average relative cover crop growth at 28 DAS in the NTC was 0.40 g pot^{-1} for annual ryegrass, 0.68 g pot^{-1} for cereal rye, 1.71 g pot^{-1} for radish, and 0.23 g pot^{-1} for red clover. Soil residual herbicides applied PRE, measured via greenhouse bioassay 28 DAS, affected cover crop biomass for each field soil sampling time (0, 10, 20, 30, 40, 50, 60, and 70 DAT) ($P < 0.01$). Pearson's analysis showed negative correlations between visual injury rating values and biomass production (g pot^{-1}) for annual ryegrass, cereal rye, radish, and red clover at 28 DAS (Figure 3 A). The slope of the regression lines was $R = -0.73$, $R = -0.47$, $R = -0.72$, and $R = -0.71$ with $P < 0.001$ for annual ryegrass, cereal rye, radish, and red clover, respectively (Figure 3 A). These results indicate that visual injury rating is associated with cover crop biomass production and suggest that higher visual injury occurred when lower biomass was produced; therefore only the biomass data were considered for fitting the linear mixed-effect models and calculating AUBS values. Jitter violin plots combined with box plots showed the distribution and changes of the biomass values for each cover crop species, including all PRE herbicide treatments and the NTC at all sampling times (Figure 3 B). In general, high biomass values (more jitter points distributed above zero) were observed for cereal rye and radish. A similar shape of violin plots was observed for annual ryegrass and red clover, with a wider base and a high frequency of observation close to zero. This indicates that cereal rye and radish tended to be more tolerant than annual ryegrass and red clover to the residual herbicides applied PRE evaluated herein.

Herein we focus the discussion on the results from the field soil samples collected 30 and 70 DAT, but the complete results are also available in Tables 5 to 8. Using the cover crop greenhouse bioassay

Table 5. Effect of PRE herbicides on annual ryegrass biomass production at each sampling time and area under biomass stairs estimated for annual ryegrass biomass production by PRE herbicide over time in greenhouse bioassay using field-treated soil from Janesville, WI, and Lancaster, WI, in 2021 and 2022.[a,b]

Treatment (herbicide rate)	DAT in the field								
	0	10	20	30	40	50	60	70	AUBS
g ai or ae ha^{-1}	g pot^{-1} [c]								
Nontreated check	0.434 b	0.366 c	0.391 b	0.419 b	0.431 ab	0.360 b	0.365 bc	0.472 a	33.43 b
ATZ (1,120)	0.342 c	0.373 c	0.425 b	0.369 bc	0.350 bc	0.343 bc	0.444 ab	0.424 ab	32.48 b
SMZ (2,240)	0.152 d	0.162 d	0.205 c	0.369 bc	0.307 c	0.359 b	0.316 cd	0.401 ab	24.20 c
ACET (1,960)	0.020 gh	0.009 hj	0.060 fg	0.092 ef	0.156 d–f	0.160 e	0.317 cd	0.322 cd	12.19 e
S-MET (1,791)	0.002 i	0.002 i	0.008 ij	0.013 gh	0.028 h	0.023 f	0.028 h	0.057 f	2.07 h
MES (175)	0.465 b	0.628 a	0.633 a	0.525 a	0.505 a	0.505 a	0.433 ab	0.419 ab	42.08 a
ACET (1,971) + MES (185)	0.068 ef	0.035 fg	0.109 e	0.195 d	0.197 de	0.272 cd	0.254 d	0.366 bc	16.40 d
ATZ (1,310) + S-MET (1,634)	0.008 hj	0.002 i	0.01 h	0.005 h	0.025 h	0.041 f	0.033 gh	0.068 ef	2.22 h
ATZ (952) + ACET (2,408)	0.024 gh	0.009 hi	0.075 ef	0.061 f	0.147 ef	0.139 e	0.191 e	0.307 cd	10.68 e
SAFL (75) + DIM-P (655)	0.019 gh	0.017 gh	0.074 e–g	0.097 e	0.113 f	0.174 e	0.188 e	0.259 d	10.40 e
FLUM (52) + CLOP (168)	0.592 a	0.505 b	0.477 b	0.547 a	0.496 a	0.472 a	0.487 a	0.397 ab	40.99 a
S-MET (1,602) + BIP (45) + MES (179)	0.014 hi	0.007 hi	0.023 hi	0.019 g	0.033 h	0.035 f	0.059 g	0.059 f	3.23 g
ATZ (700) + S-MET (1,498) + BIP (42) + MES (168)	0.021 gh	0.014 h	0.043 gh	0.023 g	0.066 g	0.029 f	0.113 f	0.096 e	4.83 f
FLUM (42) + CLOP (133) + ACET (1,315)	0.106 de	0.093 e	0.162 cd	0.342 c	0.334 c	0.298 b–d	0.368 bc	0.365 bc	21.62 c
CLOP (133) + ACET (1,960) + MES (210)	0.041 fg	0.041 f	0.113 de	0.172 d	0.208 d	0.235 d	0.270 d	0.363 bc	15.60 d
LSD (0.05)	0.075	0.062	0.071	0.067	0.074	0.072	0.073	0.064	0.380
P-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

[a]Abbreviations: ACET, acetochlor; ATZ, atrazine; AUBS, area under biomass stairs; BIP, bicyclopyrone; CLOP, clopyralid; DAT, days after treatment; DIM-P, dimethenamid-P; FLUM, flumetsulam; IFT, isoxaflutole; LSD, least significant difference; MES, mesotrione; SAFL, saflufenacil; S-MET, *S*-metolachlor; SMZ, simazine.
[b]Means within a column with the same letter are not significantly different according to Fisher's LSD test at $P \leq 0.05$.
[c]Aboveground biomass 28 d after sowing the greenhouse bioassay.

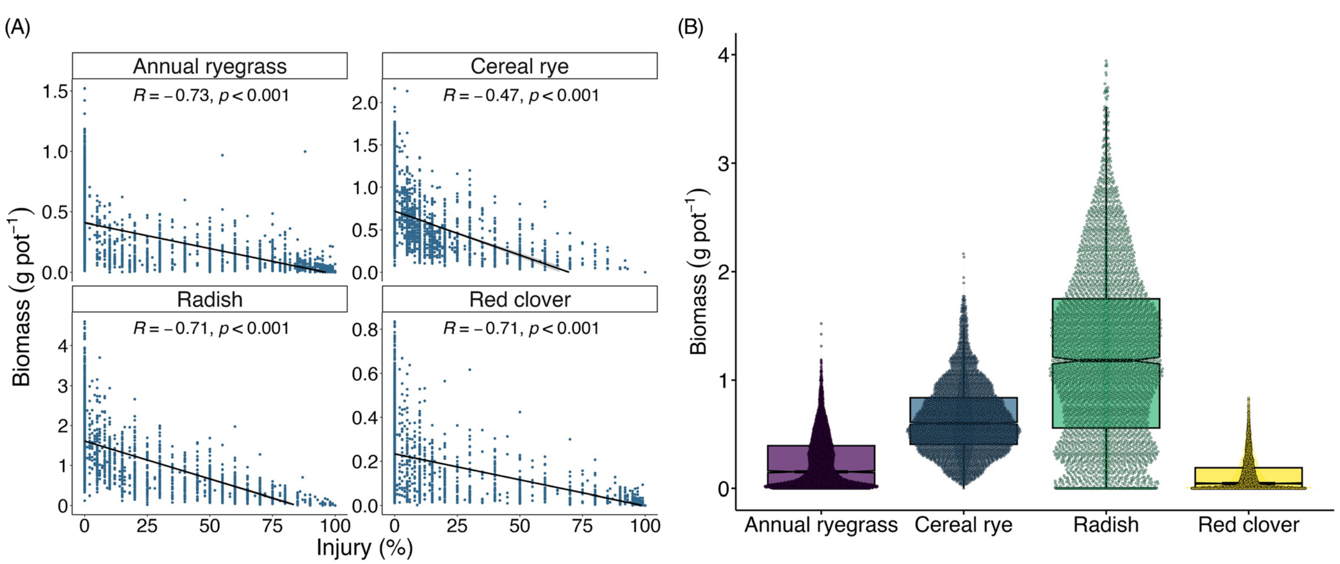

Figure 3. Pearson's linear correlation between herbicide injury (%) and aboveground biomass (g pot^{-1}) for annual ryegrass, cereal rye, radish, and red clover (A). The black solid lines show the linear trend, and the gray shaded areas represent 95% confidence intervals. Violin plots and box plots representing the aboveground biomass distribution (g pot^{-1}) combined for all treatments and sampling time of each cover crop species (B).

data (28 DAS), we assumed a situation of interseeding at 30 DAT (~V3 to V5 corn growth stages) and overseeding at 70 DAT (~V10 to VT corn growth stages; Table 4). This decision was taken considering that cover crop interseeding (planted during the vegetative corn growth stage) in Wisconsin can be successful between the V3 and V7 corn growth stages (Smith and Ruark 2022), and the overseeding is adopted just before or at corn maturity (Adler and Nelson 2020; Kladivko et al. 2014). The methodology adopted herein allowed us to evaluate the impact of the soil residual herbicide on the cover crop establishment in the absence of the crop canopy, which can also impact cover crop establishment (Ribeiro et al. 2021; Schmitt et al. 2021).

Interseeding and Overseeding Annual Ryegrass Scenario

Annual rye biomass 28 DAS in the interseeding scenario (field soil samples collected at 30 DAT; ~V3 to V5 corn growth stages; Table 4) was reduced by most soil residual herbicides applied PRE compared to the NTC (Table 5). Atrazine + *S*-metolachlor, *S*-metolachlor, *S*-metolachlor + bicyclopyrone + mesotrione, atrazine + *S*-metolachlor + bicyclopyrone + mesotrione, atrazine + acetochlor, acetochlor, and saflufenacil + dimethenamid-P had the most detrimental impact on annual ryegrass (0.005 to 0.097 g pot^{-1}) when compared to the NTC (0.419 g pot^{-1}) (Table 5). Clopyralid + acetochlor + mesotrione, acetochlor + mesotrione, and flumetsulam + clopyralid + acetochlor resulted an intermediate negative impact on annual ryegrass (0.172 to 0.342 g pot^{-1}). Only mesotrione, flumetsulam + clopyralid, atrazine, and simazine did not impact annual ryegrass biomass (0.369 to 0.525 g pot^{-1}) compared to the NTC (Table 5).

These results suggest that applying *S*-metolachlor, acetochlor, and premixes containing Group 15 herbicides is likely to impact annual ryegrass biomass at V3 to V5 corn. Group 15 herbicides are recommended for controlling grass weed species, and *S*-metolachlor also has an extended half-life, which might result in greater persistence (Shaner 2014) and consequently more risk for annual ryegrass injury. A previous field study reported stand reduction >60% of annual ryegrass interseeded at the V3 to V6 corn growth stages following PRE application of Group 15 herbicides (*S*-metolachlor, acetochlor, and dimethenamid-P) (Brooker et al. 2020b). Wallace et al. (2017) also observed unacceptable levels of annual ryegrass biomass reduction (>75%) for *S*-metolachlor (1,790 g ai ha^{-1}) when applied PRE at the V5 corn stage. However, unlike our results, Wallace et al. (2017), in a field study, found that dimethenamid-P (840 g ai ha^{-1}) and acetochlor (1,960 g ai ha^{-1}) applied PRE at standard label rates resulted in less than 20% of annual ryegrass biomass reduction at the V5 stage, which was suggested to be an acceptable level to farmers integrating weed control and soil conservation benefits. Stanton and Haramoto (2019), in a field experiment in Kentucky, reported that saflufenacil (70 g ai ha^{-1}) + dimethenamid-P (560 g ai ha^{-1}) did not reduce initial annual ryegrass density (137 plants m^{-2}) 3 wk after interseeding compared to the NTC (196 plants m^{-2}); however, the herbicide rate applied was slightly lower compared to the rates applied in our current study (saflufenacil [75 g ai ha^{-1}] + dimethenamid-P [655 g ai ha^{-1}]; Table 3).

For the overseeding scenario (field soil samples collected at 70 DAT; ~V10 to VT corn growth stages; Table 4), the most injurious PRE herbicides on annual ryegrass 28 DAS were *S*-metolachlor, *S*-metolachlor + bicyclopyrone + mesotrione, atrazine + *S*-metolachlor, and atrazine + *S*-metolachlor + bicyclopyrone + mesotrione (0.057 to 0.096 g pot^{-1}) compared to the NTC (0.472 g pot^{-1}). Saflufenacil + dimethenamid-P, atrazine + acetochlor, acetochlor, clopyralid + acetochlor + mesotrione, and flumetsulam + clopyralid + acetochlor caused intermediate impacts on annual ryegrass biomass (0.259 to 0.365 g pot^{-1}). Annual rye was not injured by flumetsulam + clopyralid, simazine, atrazine, or mesotrione (0.397 to 0.424 g pot^{-1}) compared to the NTC (Table 5). Validating these results, the AUBS analysis showed that *S*-metolachlor (2.07), atrazine + *S*-metolachlor (2.22), *S*-metolachlor + bicyclopyrone + mesotrione (3.23), and atrazine + *S*-metolachlor + bicyclopyrone + mesotrione (4.83) provided the lowest AUBS values, which means that these herbicides caused the highest injury to annual ryegrass throughout the soil sampling period. The highest AUBS values were observed for atrazine (32.48), flumetsulam + clopyralid (40.99), and mesotrione (42.08), soil residual herbicides applied PRE that did not injure annual ryegrass compared to the NTC (33.43).

Interseeding and Overseeding Cereal Rye Scenario

High levels of tolerance to soil residual herbicides applied PRE were observed 28 DAS for cereal rye in the interseeding scenario (field soil samples collected at 30 DAT; ~V3 to V5 corn growth stages; Tables 4 and 6). Cereal rye biomass was not reduced by clopyralid + acetochlor + mesotrione, saflufenacil + dimethenamid-P, atrazine, acetochlor + mesotrione, mesotrione, and flumetsulam + clopyralid + acetochlor (0.652–0.857 g pot^{-1}) compared to the NTC (0.769 g pot^{-1}). Simazine, acetochlor, *S*-metolachlor + bicyclopyrone + mesotrione, and atrazine + *S*-metolachlor + bicyclopyrone + mesotrione resulted in intermediate injurious (0.559–0.595 g pot^{-1}) compared to the NTC. *S*-metolachlor (0.358 g pot^{-1}), atrazine + *S*-metolachlor (0.401 g pot^{-1}), and atrazine + acetochlor (0.476 g pot^{-1}) were the most injurious PRE herbicides (Table 6).

For the overseeding scenario (field samples collected at 70 DAT; ~V10 to VT corn growth stages; Table 4), none of the PRE herbicides tested herein negatively impacted cereal rye biomass (0.495 to 0.666 g pot^{-1}) compared to the NTC (0.530 g pot^{-1}; Table 6). The AUBS findings support the high cereal rye tolerance observed for biomass values (Table 6). Atrazine (51.85), mesotrione (61.71), acetochlor + mesotrione (54.56), saflufenacil + dimethenamid-P (56.62), flumetsulam + clopyralid (63.11), flumetsulam + clopyralid + acetochlor (61.13), and clopyralid + acetochlor + mesotrione (54.75) did not negatively impact cereal rye compared to the NTC (55.38). A previous study also reported that saflufenacil + dimethenamid-P did not injure cereal rye interseeded at 30 DAT compared to the NTC (Smith 2015). Palhano et al. (2018), in field research, described 11% of fall-seeded cereal rye emergence reduction following POST application of mesotrione (Group 27).

Interseeding and Overseeding Radish Scenario

Radish biomass 28 DAS in the interseeding scenario (field soil samples collected at 30 DAT; ~V3 to V5 corn growth stages; Table 4) was negatively impacted by flumetsulam + clopyralid + acetochlor, flumetsulam + clopyralid, atrazine + *S*-metolachlor + bicyclopyrone + mesotrione, clopyralid + acetochlor + mesotrione, *S*-metolachlor + bicyclopyrone + mesotrione, acetochlor + mesotrione, mesotrione, and saflufenacil + dimethenamid-P (0.460 to 1.096 g pot^{-1}) compared to the NTC (1.744 g pot^{-1}; Table 7). The soil residual herbicides applied PRE that contained only Groups 5 and 15 (atrazine, simazine, acetochlor, *S*-metolachlor, atrazine + *S*-metolachlor, and atrazine + acetochlor) did not injure radish (1.528 to 1.939 g pot^{-1}) compared to the NTC.

For the overseeding scenario (field samples collected at 70 DAT; ~V10 to VT corn growth stages; Table 4), radish biomass was not reduced by atrazine + *S*-metolachlor, atrazine + acetochlor, *S*-metolachlor, simazine, acetochlor, atrazine, or saflufenacil + dimethenamid-P (1.225 to 1.621 g pot^{-1}) compared to the NTC (1.344 g pot^{-1}; Table 7). The remaining treatments reduced radish biomass (0.704 to 1.119 g pot^{-1}) compared to the NTC. For the AUBS, only *S*-metolachlor (155.66), acetochlor (148.29), and atrazine + *S*-metolachlor (135.95) were not different from the NTC (139.02). Atrazine + acetochlor, atrazine, and saflufenacil + dimethenamid-P presented intermediate AUBS values (88.90 to 127.33), whereas the remaining treatments had the lowest AUBS values (45.00 to 73.80).

On the basis of these results, applications of residual herbicides containing Groups 2, 4, and 27 evaluated in this study are likely to injure radish interseeded into corn at 30 DAT (V3 or V5

Table 6. Effect of PRE herbicides on cereal rye biomass production at each sampling time and area under biomass stairs estimated for cereal rye biomass production by PRE herbicide over time in greenhouse bioassays using field-treated soil from Janesville, WI, and Lancaster, WI, in 2021 and 2022.[a,b]

	DAT in the field								
Treatment (herbicide rate)	0	10	20	30	40	50	60	70	AUBS
g ai or ae ha^{-1}	g pot^{-1} [c]								
Nontreated check	0.659 cd	0.692 bc	0.695 a–c	0.769 a–c	0.772 bc	0.687 b–d	0.623 b	0.530 cd	55.38 bc
ATZ (1,120)	0.510 e	0.492 ef	0.716 a–c	0.669 b–d	0.768 bc	0.640 cd	0.698 ab	0.531 cd	51.85 c–e
SMZ (2,240)	0.352 f	0.385 gh	0.529 e	0.559 de	0.618 e	0.730 a–c	0.504 d	0.530 cd	43.91 f
ACET (1,960)	0.299 f	0.369 h	0.567 de	0.570 de	0.659 c–e	0.655 cd	0.619 b	0.495 d	43.93 f
S-MET (1,791)	0.306 f	0.329 hi	0.364 f	0.358 g	0.505 f	0.440 f	0.518 cd	0.493 d	34.45 h
MES (175)	0.829 ab	0.889 a	0.767 ab	0.791 ab	0.752 b–d	0.689 b–d	0.748 a	0.557 b–d	61.71 a
ACET (1,971) + MES (185)	0.570 de	0.606 c–e	0.651 b–d	0.722 bc	0.787 b	0.813 a	0.609 bc	0.549 b–d	54.56 b–d
ATZ (1,310) + S-MET (1,634)	0.307 f	0.223 j	0.385 f	0.401 fg	0.512 f	0.514 ef	0.520 cd	0.560 b–d	35.55 h
ATZ (952) + ACET (2,408)	0.187 g	0.278 ij	0.512 e	0.476 ef	0.637 e	0.588 de	0.607 bc	0.515 d	34.44 g
SAFL (75) + DIM-P (655)	0.730 bc	0.602 c–f	0.712 a–c	0.653 cd	0.771 bc	0.781 ab	0.661 ab	0.608 a–c	56.62 b
FLUM (52) + CLOP (168)	0.926 a	0.816 ab	0.801 a	0.740 a–c	0.869 ab	0.837 a	0.628 b	0.512 d	63.11 a
S-MET (1,602) + BIP (45) + MES (179)	0.492 e	0.487 fg	0.655 b–d	0.595 d	0.645 de	0.745 a–c	0.634 b	0.537 cd	50.08 e
ATZ (700) + S-MET (1,498) + BIP (42) + MES (168)	0.541 de	0.570 c–f	0.616 c–e	0.568 de	0.761 bc	0.599 de	0.694 ab	0.613 a–c	51.11 de
FLUM (42) + CLOP (133) + ACET (1,315)	0.552 de	0.553 d–f	0.759 ab	0.857 a	0.945 a	0.827 a	0.764 a	0.666 a	61.13 a
CLOP (133) + ACET (1,960) + MES (210)	0.483 e	0.631 cd	0.695 a–c	0.652 cd	0.854 ab	0.726 a–c	0.631 b	0.627 ab	54.75 bc
LSD (0.05)	0.094	0.086	0.088	0.079	0.077	0.079	0.070	0.065	0.340
P-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.01	<0.001

[a]Abbreviations: ACET, acetochlor; ATZ, atrazine; AUBS, area under biomass stairs; BIP, bicyclopyrone; CLOP, clopyralid; DAT, days after treatment; DIM-P, dimethenamid-P; FLUM, flumetsulam; IFT, isoxaflutole; LSD, least significant difference; MES, mesotrione; SAFL, saflufenacil; S-MET, *S*-metolachlor; SMZ, simazine.
[b]Means within a column with the same letter are not significantly different according to Fisher's LSD test at P ≤ 0.05.
[c]Aboveground biomass 28 d after sowing the greenhouse bioassay.

Table 7. Effect of PRE herbicides on radish biomass production at each sampling time and area under biomass stairs estimated for radish biomass production by PRE herbicide over time in greenhouse bioassays using field-treated soil from Janesville, WI, and Lancaster, WI, in 2021 and 2022.[a,b]

	DAT in the field								
Treatment (herbicide rate)	0	10	20	30	40	50	60	70	AUBS
g ai or ae ha^{-1}	g pot^{-1} [c]								
Nontreated check	1.791 a	1.735 a	1.984 a	1.744 ab	1.800 cd	1.649 c	1.668 b	1.344 bc	139.02 bc
ATZ (1,120)	0.259 d–f	0.701 c	1.473 c	1.705 ab	1.871 b–d	1.675 bc	1.938 a	1.227 cd	114.06 e
SMZ (2,240)	0.071 g	0.131 f	0.335 h	1.528 b	1.638 de	1.756 bc	1.382 cd	1.468 ab	88.90 f
ACET (1,960)	1.934 a	2.088 a	1.965 a	1.914 a	1.985 a–c	1.885 bc	1.530 b–d	1.293 bc	148.29 ab
S-MET (1,791)	2.041 a	2.034 a	2.032 a	1.939 a	2.137 ab	1.960 ab	1.721 ab	1.469 ab	155.66 a
MES (175)	0.128 fg	0.294 de	0.510 fg	0.918 cd	1.073 gh	1.163 d–f	1.358 de	1.119 de	69.69 g
ACET (1,971) + MES (185)	0.228 d–f	0.356 d	0.809 d	0.853 de	1.215 fg	1.269 d	1.190 ef	1.014 e	73.80 g
ATZ (1,310) + S-MET (1,634)	0.518 bc	1.011 b	1.866 a	1.766 ab	2.275 a	2.197 a	1.664 b	1.621 a	135.95 cd
ATZ (952) + ACET (2,408)	0.691 b	0.932 bc	1.817 ab	1.827 ab	1.976 a–c	1.919 a–c	1.634 b	1.554 a	127.33 d
SAFL (75) + DIM-P (655)	0.374 cd	0.303 de	1.494 bc	1.096 c	1.429 ef	1.205 de	1.575 bc	1.225 cd	93.58 f
FLUM (52) + CLOP (168)	0.411 cd	0.409 d	0.795 de	0.531 fg	0.868 h	0.973 f	0.720 g	0.704 f	55.70 i
S-MET (1,602) + BIP (45) + MES (179)	0.081 g	0.110 f	0.713 d–f	0.648 ef	1.285 fg	1.223 de	1.103 f	0.986 e	66.47 gh
ATZ (700) + S-MET (1,498) + BIP (42) + MES (168)	0.084 g	0.157 ef	0.379 gh	0.531 fg	1.063 gh	1.012 ef	1.114 f	0.982 e	60.43 hi
FLUM (42) + CLOP (133) + ACET (1,315)	0.305 de	0.416 d	0.524 fg	0.460 g	0.632 i	0.486 g	0.758 g	0.785 f	45.00 j
CLOP (133) + ACET (1,960) + MES (210)	0.172 e–g	0.191 ef	0.579 ef	0.643 ef	1.285 fg	1.131 d–f	1.158 f	1.007 e	66.32 gh
LSD (0.05)	0.171	0.163	0.148	0.133	0.117	0.115	0.092	0.087	0.631
P-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

[a]Abbreviations: ACET, acetochlor; ATZ, atrazine; AUBS, area under biomass stairs; BIP, bicyclopyrone; CLOP, clopyralid; DAT, days after treatment; DIM-P, dimethenamid-P; FLUM, flumetsulam; IFT, isoxaflutole; LSD, least significant difference; MES, mesotrione; SAFL, saflufenacil; S-MET, *S*-metolachlor; SMZ, simazine.
[b]Means within a column with the same letter are not significantly different according to Fisher's LSD test at P ≤ 0.05.
[c]Aboveground biomass 28 d after sowing the greenhouse bioassay.

growth stage) and 70 DAT (V10 to VT growth stages) because of the short interval between herbicide application and cover crop interseeding or overseeding. Mesotrione (Group 27) and flumetsulam + clopyralid (Groups 2 and 4) herbicide labels list a 26- and 10-mo rotational restriction for canola (*Brassica napus* L.; Anonymous 2022a, 2022b), respectively, which belongs to the same family as radish and may have similar sensitivity. Brooker et al. (2020b), in a field experiment, also

https://doi.org/10.1017/wet.2023.72 Published online by Cambridge University Press

Table 8. Effect of PRE herbicides on red clover biomass production at each sampling time and area under biomass stairs estimated for red clover biomass production by PRE herbicide over time in greenhouse bioassays using field-treated soil from Janesville, WI, and Lancaster, WI, in 2021 and 2022.[a,b]

	DAT in the field								
Treatment (herbicide rate)	0	10	20	30	40	50	60	70	AUBS
g ai or ae ha^{-1}	g pot^{-1} [c]								
Nontreated check	0.223 a	0.176 a	0.212 a	0.253 a	0.203 a	0.208 ab	0.229 ab	0.296 a–c	18.94 a
ATZ (1,120)	0.019 de	0.048 c	0.111 b	0.203 a–c	0.158 ab	0.229 a	0.218 ab	0.290 bc	13.98 cd
SMZ (2,240)	0.007 e	0.008 e	0.027 e	0.168 c	0.186 ab	0.167 b–d	0.239 a	0.288 bc	11.97 e
ACET (1,960)	0.106 c	0.110 b	0.109 bc	0.239 ab	0.194 ab	0.188 a–c	0.259 a	0.309 ab	15.89 bc
S-MET (1,791)	0.125 bc	0.102 b	0.127 b	0.243 ab	0.169 ab	0.206 ab	0.231 ab	0.354 a	16.63 b
MES (175)	0.000 f	0.000 f	0.000 g	0.000 f	0.001 d	0.002 f	0.000 e	0.009 f	0.16 ij
ACET (1,971) + MES (185)	0.000 f	0.000 f	0.000 g	0.000 f	0.000 d	0.000 f	0.001 e	0.001 g	0.07 ij
ATZ (1,310) + S-MET (1,634)	0.037 d	0.029 d	0.075 cd	0.199 bc	0.177 ab	0.177 bc	0.253 a	0.254 b–d	13.04 de
ATZ (952) + ACET (2,408)	0.001 f	0.012 e	0.062 d	0.122 d	0.150 b	0.135 d	0.211 ab	0.230 d	10.02 f
SAFL (75) + DIM-P (655)	0.155 b	0.162 a	0.194 a	0.211 a–c	0.151 b	0.156 d	0.208 ab	0.251 ab	15.88 bc
FLUM (52) + CLOP (168)	0.000 f	0.000 f	0.007 f	0.027 e	0.057 c	0.131 d	0.183 b	0.231 d	7.07 g
S-MET (1,602) + BIP (45) + MES (179)	0.000 f	0.000 f	0.000 g	0.000 f	0.000 d	0.000 f	0.000 e	0.000 g	0.03 j
ATZ (700) + S-MET (1,498) + BIP (42) + MES (168)	0.000 f	0.000 f	0.000 g	0.000 f	0.002 d	0.001 f	0.009 d	0.007 f	0.25 i
FLUM (42) + CLOP (133) + ACET (1,315)	0.001 f	0.000 f	0.020 ef	0.019 e	0.048 c	0.041 e	0.095 c	0.153 e	4.51 h
CLOP (133) + ACET (1,960) + MES (210)	0.000 f	0.000 f	0.000 g	0.000 f	0.000 d	0.000 f	0.003 de	0.003 fg	0.07 ij
LSD (0.05)	0.056	0.051	0.060	0.060	0.059	0.059	0.062	0.067	0.350
P-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

[a]Abbreviations: ACET, acetochlor; ATZ, atrazine; AUBS, area under biomass stairs; BIP, bicyclopyrone; CLOP, clopyralid; DAT, days after treatment; DIM-P, dimethenamid-P; FLUM, flumetsulam; IFT, isoxaflutole; LSD, least significant difference; MES, mesotrione; SAFL, saflufenacil; S-MET, *S*-metolachlor; SMZ, simazine.
[b]Means within a column with the same letter are not significantly different according to Fisher's LSD test at $P \leq 0.05$.
[c]Aboveground biomass 28 d after sowing the greenhouse bioassay.

reported that Group 2 herbicides (flumetsulam [56 g ai ha^{-1}] and rimsulfuron [22 g ai ha^{-1}]) caused >70% radish stand reduction into corn at the V3 to V6 stages compared to the NTC. In a greenhouse experiment, these same authors observed that Group 27 (mesotrione [210 g ai ha^{-1}]) resulted in >50% biomass reduction at rates less than field use rates; the authors did not observe radish stand and biomass reduction by a Group 4 herbicide (clopyralid [105 g ai ha^{-1}]). According to our results, delaying radish planting until 70 DAT is likely to reduce injury and biomass reduction if saflufenacil + dimethenamid-P is applied. A previous study reported that fall-seeded radish was not negatively impacted by saflufenacil + dimethenamid-P (735 + 1,470 g ai ha^{-1}; Yu et al. 2015).

Interseeding and Overseeding Red Clover Scenario

Red clover biomass 28 DAS in the interseeding scenario (field soil samples collected at 30 DAT; ~V3 to V5 corn growth stages; Table 4) was negatively impacted by soil residual herbicides applied PRE that contained Groups 2, 4, and 27 (mesotrione, acetochlor + mesotrione, flumetsulam + clopyralid, *S*-metolachlor + bicyclopyrone + mesotrione, atrazine + *S*-metolachlor + bicyclopyrone + mesotrione, flumetsulam + clopyralid + acetochlor, and clopyralid + acetochlor + mesotrione) with a biomass production ranging from 0.000 to 0.027 g pot^{-1} compared to the NTC (0.253 g pot^{-1}; Table 8). *S*-metolachlor, acetochlor, saflufenacil + dimethenamid-P, and atrazine did not negatively impact red clover biomass (0.203 to 0.243 g pot^{-1}). The remaining treatments resulted in intermediate injury (0.122 to 0.199 g pot^{-1}).

For the overseeding scenario (field samples collected at 70 DAT; ~V10 to VT corn growth stages; Table 4), the PRE herbicides that contained Group 27 (mesotrione, acetochlor + mesotrione, *S*-metolachlor + bicyclopyrone + mesotrione, atrazine + *S*-metolachlor + bicyclopyrone + mesotrione, and clopyralid + acetochlor + mesotrione) still caused high injury to red clover (0.000 to 0.009 g pot^{-1}) compared to the NTC (0.296 g pot^{-1}; Table 8). PRE herbicides that contained Groups 5, 14, and 15 (atrazine, simazine, acetochlor, *S*-metolachlor, atrazine + *S*-metolachlor, and saflufenacil + dimethenamid-P) did not injure red clover (0.0.251 to 0.354 g pot^{-1}) compared to the NTC. The remaining PRE herbicide treatments resulted in intermediate injury (0.231 and 0.153 g pot^{-1}).

The AUBS results (Table 8) support the high red clover sensitivity to soil residual herbicides applied PRE that contain Groups 2, 4, and 27 (flumetsulam + clopyralid [7.07], flumetsulam + clopyralid + acetochlor [4.51], mesotrione [0.16], acetochlor + mesotrione [0.07], *S*-metolachlor + bicyclopyrone + mesotrione [0.03], atrazine + *S*-metolachlor + bicyclopyrone + mesotrione [0.25], and clopyralid + acetochlor + mesotrione [0.07]) compared to the NTC (18.94). Although none of the PRE herbicides reached an AUBS equal to the NTC, PRE herbicides containing Groups 5, 14, and 15 caused less injury than the PRE herbicides last mentioned. The PRE herbicides that caused less injury were atrazine (13.98), simazine (11.97), acetochlor (15.89), *S*-metolachlor (16.63), atrazine + acetochlor (13.04), and saflufenacil + dimethenamid-P (15.88).

The low red clover biomass reduction by atrazine and simazine after 30 DAT may be due to the fast degradation of these herbicides. Mueller et al. (2017) reported enhanced dissipation and a decrease in atrazine persistence in some locations in Wisconsin due to microbial degradation, limiting extended weed control. Our results demonstrate that red clover is highly sensitive to mesotrione

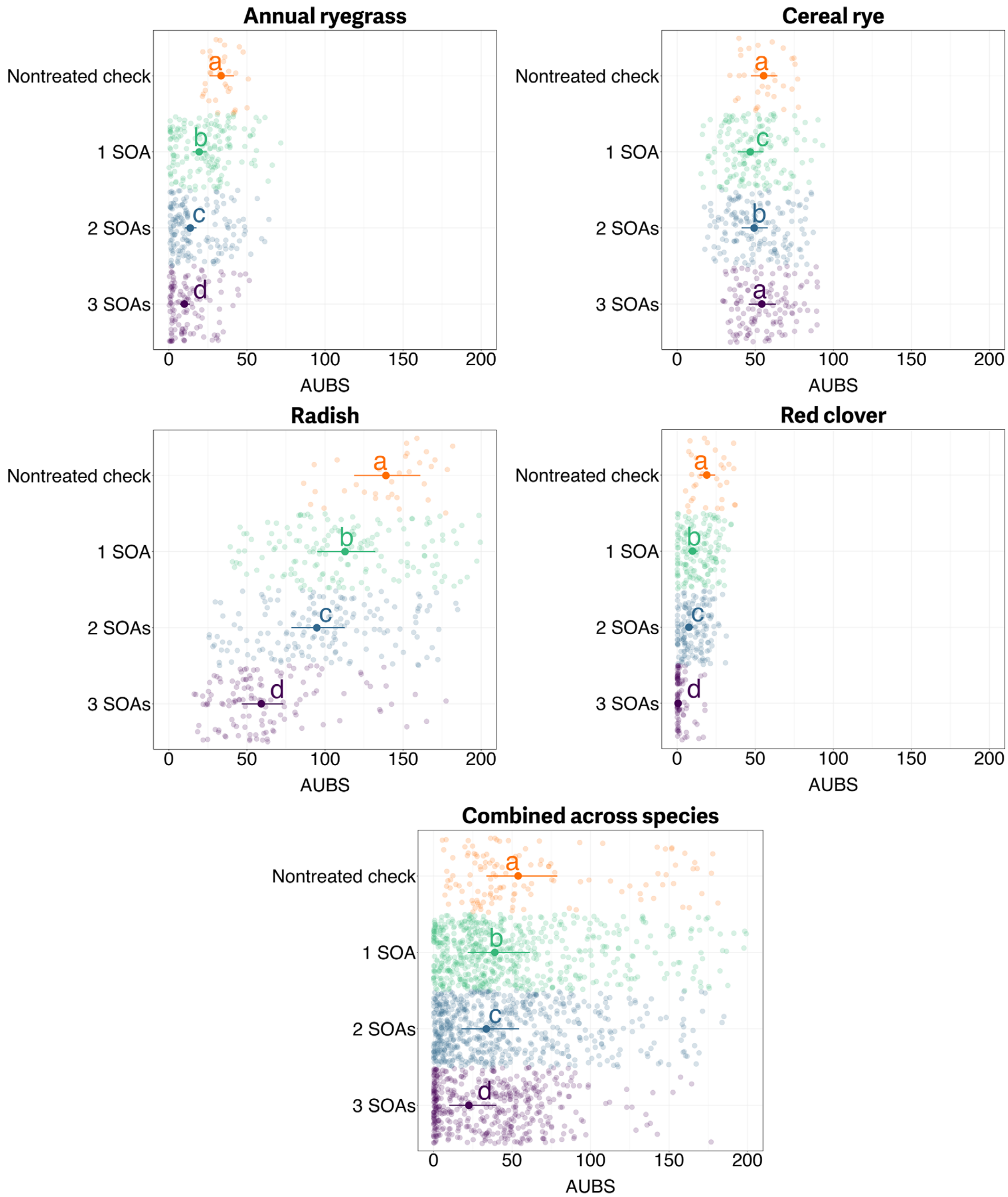

Figure 4. Area under biomass stairs (AUBS) estimated for annual ryegrass, cereal rye, radish, and red clover, and combined across species by PRE herbicide sites of action (SOAs; one, two, or three SOAs) over time in greenhouse bioassays using field-treated soil from Janesville, WI, and Lancaster, WI, in 2021 and 2022. Jittered points represent replicates, centered solid points denote the means, and error bars represent the upper and lower 95% confidence interval limits. Means were compared using Fisher's least significant difference, and herbicide treatments with the same letters are not different at $P \leq 0.05$.

(Group 27) applied solo and in the premixes even at 70 DAT. Wallace et al. (2017) reported more than 98% biomass reduction by mesotrione (188 g ai ha^{-1}) and atrazine + *S*-metolachlor + mesotrione applied PRE at a reduced rate (0.5X) compared to the NTC in silt-loam soil fields at the V3 corn growth stage. Field studies conducted in silt-loam soils have shown that the half-life of mesotrione ranges from 8 to 32 d (Dyson et al. 2002), but mesotrione may persist longer in the soil, depending on the edaphoclimatic conditions (Su et al. 2017), especially pH and organic matter (Dyson et al. 2002; Shaner et al. 2012). For example, as pH decreases, the mesotrione half-life increases (Chaabane et al. 2008; Shaner et al. 2012). Our results at 70 DAT are also supported by other studies that have demonstrated mesotrione carryover injury to rotational crops (Pintar et al. 2020) and fall-seeded cover crops (Palhano et al. 2018). Mesotrione (Group 27) also lists an 18-mo rotational restriction for red clover (Anonymous 2022a), which can explain the high sensibility of red clover up to 70 DAT in our study. These rotational herbicide label restrictions address only potential crop injury and are independent of plant-back interval (PBI) restrictions established by the Environmental Protection

https://doi.org/10.1017/wet.2023.72 Published online by Cambridge University Press

Agency (WSSA 2022). If cover crops are planted for soil health purposes, PBI restrictions do not apply. However, if cover crops are planted for livestock feeding, grazing, or human consumption, PBI restrictions must be complied with.

Cover Crops Injury by the Number of Active Ingredients

The estimated cover crop AUBS values analyzed by the number of SOAs showed that the higher the number of SOAs for the products tested is, the higher is the injury, except for cereal rye (Figure 4). For annual ryegrass, the AUBS values followed the order of NTC (33.43) followed by PRE herbicides with one SOA (19.43), two SOAs (13.68), and three SOAs (9.92). The cereal rye AUBS for PRE herbicides with three SOAs (54.2) was not different from the NTC (55.4), whereas PRE herbicides with one SOA (46.7) and two SOAs (49.3) slightly reduced growth compared to the NTC. All SOA numbers negatively impacted radish AUBS (112.8, 94.8, and 59.2 for one, two, and three SOAs, respectively) compared to the NTC (139.0). The same was observed for red clover AUBS values (9.82, 7.5, and 0.59 for one, two, and three SOAs, respectively) compared to the NTC (18.94). The AUBS combined across species followed the same trend, where PRE herbicides with a single SOA (39.1), two SOAs (33.6), and three SOAs (22.5) negatively impacted the cover crops compared to the NTC (53.9).

Although the cover crops tended to be more sensitive to the premixes with multiple SOAs than with a single SOA (Figure 4), premixes with at least two SOAs are necessary to improve weed control success (Silva et al. 2023). In this case, the selection of cover crop species can be more restricted. But acceptable levels of weed control are needed to achieve production goals and enhance the chances of successful establishment of interseeded cover crops (Wallace et al. 2017). Therefore premixes with at least two SOAs should be tested in the field on different weeds and cover crop species to carefully select a herbicide program that can provide effective weed control and successful cover crop establishment.

Practical Implications

All herbicides tested, except atrazine and simazine, resulted in biomass reduction of at least one cover crop 28 DAS in the interseeding scenario (field soil samples collected at 30 DAT; ~V3 to V5 corn growth stage) and the overseeding scenario (field samples collected at 70 DAT; ~V10 to VT corn growth stage). Consequently, species selection might be a challenge in the case of using grass–legume cover crop mixtures. Conversely, for each cover crop studied, there were soil residual herbicides applied PRE that did not negatively impact biomass. Cereal rye was the most tolerant cover crop species, followed by radish, red clover, and annual ryegrass. Cereal rye was affected by only 6 out of the 14 total PRE herbicides at 30 DAT and by none of the PRE herbicides at 70 DAT. The chances of injury were higher for annual ryegrass, radish, and red clover when the number of SOAs in a premix was higher. This trend was not observed for cereal rye. These results suggest that certain soil residual herbicides applied PRE are likely to reduce biomass of interseeded (~V3 corn growth stage) and overseeded (~VT corn growth stage) cover crops; therefore cover crop species should be carefully selected depending on the residual PRE herbicide applied. This new system can be challenging, but this study shows some potential cover crop options for farmers using the soil residual herbicides applied PRE investigated herein. Moreover, additional field studies are needed to validate these results in different environments and to support recommendations to growers interested in this system.

Acknowledgments. We thank the University of Wisconsin–Madison Lancaster Agricultural Research Station and the Rock County Farm staff and the University of Wisconsin–Madison Cropping Systems Weed Science members for their technical assistance in conducting this research. TSS's graduate research assistantship was partially sponsored by CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior–Brasil; Finance Code no. 001). The authors acknowledge Ryan DeWerff and Dan Smith (University of Wisconsin–Madison) for their assistance with protocol development and field study establishment and Luis Antonio de Avila (Mississippi State University), Alexandre Tonon Rosa (Bayer Crop Science), John M. Wallace (Pennsylvania State University), Ahmadreza Mobli (University of Wisconsin–Madison), and Christopher A. Proctor (University of Nebraska–Lincoln) for their contributions to the article. This research received no specific grant from any funding agency or the commercial or not-for-profit sectors. The authors declare no conflicts of interest.

References

Adler RL, Nelson KA (2020) Overseeding cover crops on corn and soybean response in upstate Missouri. Crop Forage Turfgrass Manage 6:e20037

Anonymous (2022a) Callisto® herbicide label. Greensboro, NC: Sygenta. 40 p

Anonymous (2022b) Hornet® WDG herbicide label. Zionsville, IN: Dow AgroSciences. 13 p

Bates D, Mächler M, Bolker B, Walker S (2015) Fitting linear mixed-effects models using lme4. J Stat Softw 67:1–48

Broeske M, Lauer J (2020) University of Wisconsin visual guide to corn development. https://ipcm.wisc.edu/wp-content/uploads/sites/54/2022/11/UW_CornDevGuide.pdf. Accessed: May 2, 2021

Brooker AP, Renner KA, Sprague CL (2020a) Interseeding cover crops in corn. Agron J 112:139–147

Brooker AP, Sprague CL, Renner KA (2020b) Interseeded annual ryegrass, oilseed radish, and crimson clover tolerance to residual herbicides commonly used in corn. Weed Technol 34:35–41

Caswell K, Wallace JM, Curran WS, Mirsky SB, Ryan MR (2019) Cover crop species and cultivars for drill-interseeding in Mid-Atlantic corn and soybean. Agron J 111:1060–1067

Chaabane H, Vulliet E, Calvayrac C, Coste CM, Cooper JF (2008) Behaviour of sulcotrione and mesotrione in two soils. Pest Manage Sci 64:86–93

Cornelius CD, Bradley KW (2017) Carryover of common corn and soybean herbicides to various cover crop species. Weed Technol 31:21–31

Curran WS, Hoover RJ, Mirsky SB, Roth GW, Ryan MR, Ackroyd VJ, Wallace JM, Dempsey MA, Pelzer CJ (2018) Evaluation of cover crops drill interseeded into corn across the Mid-Atlantic region. Agron J 110:435–443

Dyson JS, Beulke S, Brown CD, Lane MC (2002) Adsorption and degradation of the weak acid mesotrione in soil and environmental fate implications. J Environ Qual 31:613–618

Fox J, Weisberg S (2019) An R Companion to Applied Regression. 3rd ed. Thousand Oaks, CA: SAGE. 608 p

Freckleton RP, Watkinson AR (2000) Designs for greenhouse studies of interactions between plants: an analytical perspective. J Ecol 88:386–391

Galon L, Santin CO, Andres A, Basso FJ, Nonemacher F, Agazzi LR, Silva AF, Holz CM, Fernandes FF (2018) Competitive interaction between sweet sorghum with weeds. Planta Daninha 36:e018173689

Grint KR, Arneson NJ, Oliveira MC, Smith DH, Werle R (2022) Cereal rye cover crop terminated at crop planting reduces early-season weed density and biomass in Wisconsin corn–soybean production. Agrosys Geosci Environ 5: e20245

Jursík M, Kočárek M, Kolářová M, Tichý L (2020) Effect of different soil and weather conditions on efficacy, selectivity and dissipation of herbicides in sunflower. Plant Soil Environ 66:468–476

Kassambara A (2022) Package ggpubr. R package version 0.5.0. https://cran.r-project.org/web/packages/ggpubr/ggpubr.pdf. Accessed: December 17, 2022

Kladivko EJ, Kaspar TC, Jaynes DB, Malone RW, Singer J, Morin XK, Searchinger T (2014) Cover crops in the upper midwestern United States:

potential adoption and reduction of nitrate leaching in the Mississippi River Basin. J Soil Water Conserv 69:279–291
Lenth R (2022) emmeans: estimated marginal means, aka least-squares means. R package version 1.8.3. https://cran.r-project.org/web/packages/emmeans/index.html. Accessed: December 28, 2022
Mendiburu F (2022) Package agricolae. R package version 1.3.5. https://cran.r-project.org/web/packages/agricolae/agricolae.pdf. Accessed: December 28, 2022
Mueller TC, Parker ET, Steckel L, Clay SA, Owen MD, Curran WS, Currie R, Scott R, Sprague C, Stephenson DO, Miller DK, Prostko EP, James Grichar W, Martin J, Cruz LJ, Bradley K, Bernards ML, Dotray P, Knezevic S, Davis V, Klein R (2017) Enhanced atrazine degradation is widespread across the United States. Pest Manage Sci 7:1953–1961
Noland RL, Wells MS, Sheaffer CC, Baker JM, Martinson KL, Coulter JA (2018) Establishment and function of cover crops interseeded into corn. Crop Sci 58:863–887
Palhano MG, Norsworthy JK, Barber T (2018) Sensitivity and likelihood of residual herbicide carryover to cover crops. Weed Technol 32:236–243
Peterson CM, Mirsky SB, VanGessel MJ, Davis BW, Ackroyd VJ, Tully KL (2021) Evaluation of interseeding cover crop mixtures in Mid-Atlantic double-crop soybean. Agron J 113:3935–3951
Pintar A, Stipičević S, Lakić J, Barić K (2020) Phytotoxicity of mesotrione residues on sugar beet (*Beta vulgaris* L.) in agricultural soils differing in adsorption affinity. Sugar Technol 22:137–142
R Development Core Team (2022) R: A Language and Environment for Statistical Computing. Vienna, Austria: R Foundation for Statistical Computing. 16 p
Ribeiro VH, 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
Rusch HL, Coulter JA, Grossman JM, Johnson GA, Porter PM, Garcia y Garcia A (2020) Towards sustainable maize production in the US upper Midwest with interseeded cover crops. PLoS ONE 15:e0231032
Schipanski ME, Barbercheck M, Douglas MR, Finney DM, Haider K, Kaye JP, Kemanian AR, Mortensen DA, Ryan MR, Tooker J, White C (2014) A framework for evaluating ecosystem services provided by cover crops in agroecosystems. Agric Syst 125:12–22
Schmitt MB, Berti M, Samarappuli D, Ransom JK (2021) Factors affecting the establishment and growth of cover crops intersown into maize (*Zea mays* L.). Agronomy 11:712
Silva TS, Arneson NJ, DeWerff RP, Smith DH, Silva DV, Werle R (2023) Preemergence herbicide premixes reduce the risk of soil residual weed control failure in corn. Weed Technol. DOI:10.1017/wet.2023.45
Simko I, Piepho HP (2012) The area under the disease progress stairs: calculation, advantage, and application. Phytopathology 102:381–389
Smith DH (2015) Cover crops and herbicide interactions. MS thesis, University of Wisconsin–Madison. 91 p
Smith D, Ruark M (2022) Interseeding cover crops. https://ipcm.wisc.edu/blog/2022/06/interseeding-cover-crops/. Accessed: January 18, 2023
Smith HD, Moore MV, Ruark M, Silva E (2019) Interseeding cover crops in row-cultivated corn. https://learningstore.extension.wisc.edu/collections/farming/products/interseeding-cover-crops-in-row-cultivated-corn. Accessed: January 22, 2023
Stanton VL, Haramoto ER (2019) Environmental factors may influence interseeded annual ryegrass and red clover establishment and growth more than soil residual herbicide applications. Weed Technol 33:296–302
Striegel S, Oliveira MC, Arneson N, Conley SP, Stoltenberg DE, Werle R (2021) Spray solution pH and soybean injury as influenced by synthetic auxin formulation and spray additives. Weed Technol 35:113–127
Shaner DL (2014) Herbicide Handbook. 10th ed. Lawrence, KS: Weed Science Society of America. 513 p
Shaner D, Brunk G, Nissen S, Westra P, Chen W (2012) Role of soil sorption and microbial degradation on dissipation of mesotrione in plant-available soil water. J Environ Qual 41:170–178
Singer JW (2008) Corn belt assessment of cover crop management and preferences. Agron J 100:1670–1672
Su W, Hao H, Wu R, Xu H, Xue F, Lu C (2017) Degradation of mesotrione affected by environmental conditions. Bull Environ Contam Toxicol 98: 212–217
[USDA-NASS] U.S. Department of Agriculture National Agricultural Statistics Service (2019) 2017 Census of Agriculture United States summary and state data. Vol. 1. Geographic Area Series Part 51. https://www.nass.usda.gov/Publications/AgCensus/2017/Full_Report/Volume_1,_Chapter_1_US/usv1.pdf. Accessed: January 22, 2023
[USDA-SARE] U.S. Department of Agriculture Sustainable Agriculture Research and Education (2020) Annual report 2019–2020. National Cover Crop Survey Reports. https://www.sare.org/publications/cover-crops/national-cover-crop-surveys/. Accessed: January 22, 2023
Wallace JM, Curran WS, Mirsky SB, Ryan MR (2017) Tolerance of interseeded annual ryegrass and red clover cover crops to residual herbicides in Mid-Atlantic corn cropping systems. Weed Technol 31:641–650
Wallander S, Smith D, Bowman M, Claassen R (2021) Cover crop trends, programs, and practices in the United States. Economic Information Bulletin 222. https://www.ers.usda.gov/webdocs/publications/100551/eib-222.pdf. Accessed: January 22, 2023
[WSSA] Weed Science Society of America (2022) New WSSA backgrounder clarifies purpose and use of plant-back intervals. https://ucanr.edu/blogs/blogcore/postdetail.cfm?postnum=53528. Accessed: April 25, 2023
Youngerman CZ, DiTommaso A, Curran WS, Mirsky SB, Ryan MR (2018) Corn density effect on interseeded cover crops, weeds, and grain yield. Agron J 110:2478–2487
Yu L, Van Eerd LL, O'Halloran I, Sikkema PH, Robinson DE (2015) Response of four fall-seeded cover crops to residues of selected herbicides. Crop Protect 75:11–17