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TATIANE SEVERO SILVA

IMPACT OF SOIL RESIDUAL HERBICIDES ON ESTABLISHMENT OF INTERSEEDED/OVERSEEDED COVER CROPS IN CORN AND WEED CONTROL EFFICACY

MOSSORÓ
2023

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Tese apresentada ao Programa de Pós-Graduação em Fitotecnia da Universidade Federal Rural do Semi-Árido como requisito para obtenção do título de Doutora em Fitotecnia.

Linha de Pesquisa: Manejo de Plantas Daninhas

Orientador: Prof. DSc. Daniel Valadão Silva

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Dedico

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ABSTRACT

SILVA, Tatiane Severo. **Impact of soil residual herbicides on establishment of interseeded/overseeded cover crops in corn and weed control efficacy**. 2023. 89 f. Dissertation (Doctorate in Plant Science) - Universidade Federal Rural do Semi-Árido (UFERSA), Mossoró-RN, 2023.

Preemergence (PRE) herbicides with soil residual activity resurge as a foundation for early-season weed control in corn; however, there is a potential injury from soil residual PRE herbicides to interseeded/overseeded cover crops, a cultural practice of interest to corn growers. Field experiments were conducted at Janesville and Lancaster, WI in 2021 and 2022 (4 site-years) to evaluate the weed control efficacy of solo (single site of action [SOA]) and premix (two or more SOAs) PRE herbicides in conventional tillage corn systems. Greenhouse bioassays were conducted in 2021 and 2022 to assess the impact of these PRE herbicides on the establishment of interseeded cover crops. Treatments consisted of 18 PRE herbicides plus a nontreated check. Annual rye (*Lolium multiflorum* L.), cereal rye (*Secale cereale* L.), radish (*Raphanus sativus* L.), and red clover (*Trifolium pratense* L.) were used as bioindicators. PRE herbicides with two (78%) and three (81%) SOAs provided higher weed control than PRE herbicides with a single SOA (68%), indicating at least two SOAs are needed in a premix to enhance weed control. Cereal rye was the least sensitive species to PRE herbicides. Annual rye, radish, and red clover were more sensitive to PRE herbicides containing two and three SOAs than herbicides with a single SOA. PRE herbicide efficacy varied according to the weed species, but the premixes appeared as a more reliable option to improve early-season weed control in conventional tillage corn systems. However, cover crop species should be carefully selected depending on the residual PRE herbicide when interseeded or overseeded into corn. Additional field studies are needed to validate these results in different environments and support recommendations to growers interested in this system.

Keywords: bioassay, carryover, cover crop interseeding, preemergence herbicides, *Zea mays* L.

RESUMO

SILVA, Tatiane Severo. **Impacto de herbicidas residuais do solo no estabelecimento de culturas de cobertura intercaladas em milho e eficácia no controle de plantas daninhas**. 2023. 89 f. Tese (Doutorado em Fitotecnia) - Universidade Federal Rural do Semi-Árido (UFERSA), Mossoró-RN, 2023.

Herbicidas pré-emergentes (PRE) com atividade residual no solo ressurgem como base para o controle de ervas daninhas no início da estação do milho; no entanto, existe um dano potencial de herbicidas PRE residuais no solo para culturas de cobertura intercaladas, uma prática cultural de interesse para os produtores de milho. Experimentos de campo foram conduzidos em Janesville e Lancaster, WI em 2021 e 2022 (4 locais-anos) para avaliar a eficácia do controle de plantas daninhas por herbicidas PRE com um único sítio de ação (SOA) e mistura (dois ou mais SOAs) em lavoura convencional de milho. Bioensaios em casa de vegetação foram conduzidos em 2021 e 2022 para avaliar o impacto desses herbicidas PRE no estabelecimento de culturas de cobertura intercaladas. Os tratamentos consistiram em 18 herbicidas PRE mais uma testemunha não tratada. Azevém (*Lolium multiflorum* L.), centeio (*Secale cereale* L.), rabanete (*Raphanus sativus* L.) e trevo-comum (*Trifolium pratense* L.) foram utilizados como bioindicadores. Os herbicidas PRE com dois (78%) e três (81%) SOAs forneceram maior controle de plantas daninhas do que os herbicidas PRE com um único SOA (68%), indicando que pelo menos dois SOAs são necessários em uma pré-mistura para melhorar o controle de plantas daninhas. O centeio foi a espécie menos sensível aos herbicidas PRE. Azevém, rabanete e trevo-comum foram mais sensíveis a herbicidas PRE contendo dois e três SOAs do que herbicidas com um único SOA. A eficácia do herbicida PRÉ variou de acordo com as espécies de plantas daninhas, mas as pré-misturas apareceram como uma opção mais confiável para melhorar o controle de plantas daninhas no início da estação em sistemas de cultivo convencional de milho. No entanto, as espécies de plantas de cobertura devem ser cuidadosamente selecionadas, dependendo do herbicida PRE residual, quando intercaladas ou semeadas no milho. Estudos de campo adicionais são necessários para validar esses resultados em diferentes ambientes e apoiar recomendações aos produtores interessados neste sistema.

Palavras-chave: bioensaio, “carryover”, cultura de cobertura intercalada, herbicidas pré-emergentes, *Zea mays* L.

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LIST OF ABBREVIATIONS AND ACRONYMS

ACET	acetochlor
ANOVA	Analysis of variance
ATZ	atrazine
AUBS	Area under biomass stairs
AUDPS	Area under the disease progress stairs
BIP	bicyclopyrone
CLOP	clopyralid
DAP	Days after planting
DAT	Days after treatment
DICAM	dicamba
DIM-P	dimethenamid-P
FLUM	flumetsulam
IFT	isoxaflutole
LSD	Fisher's least significant difference
MES	mesotrione
NTC	Nontreated check
PYRO	pyroxasulfone
OM	Organic matter
pH	Potential Hydrogen
PRE	Preemergence
SAFL	saflufenacil
SMZ	simazine
S-MET	S-metolachlor
SOA	Site of action
TCM	thiencarbazone-methyl
WAT	Weeks after treatment
WI	Wisconsin

LIST OF SIMBOLS

C	Degree Celsius
cm	Centimeter
g	Gram
g ai ha^{-1}	Grams active ingredient per hectare
g ha^{-1}	Grams per hectare
g pot^{-1}	Grams per pot
L ha^{-1}	Litres per hectare
ppm	Parts per million
R^2	Coefficient of determination
®, TM	Registered trademark
%	Percentage

SUMMARY

GENERAL INTRODUCTION

Corn (*Zea mays*) is the primary cultivated crop in the United States, with an area of 32 million hectares harvested for grain in 2022 (USDA, 2023). The midwestern is the top producing region, representing over 85% of the harvested area in 2022 (USDA, 2023). Herbicides are the most extensively used pesticide in corn, applied to >95% of planted corn hectares in the US in 2021 (USDA, 2022) because weed management is a major challenge in corn production systems. United States corn growers rely primarily on herbicides and tillage for weed management (Grint et al., 2022a), which has led to a widespread occurrence of herbicide resistance mainly to postemergence (POST) herbicides (HEAP, 2022). An approach to minimize the overreliance on POST herbicide applications is the use of soil residual preemergence (PRE) herbicides for early-season weed control (KNEZEVIC et al., 2019). The adoption of herbicides with effective soil residual activity applied PRE provides extended period of weed control early-season, protecting crop yields during their most susceptible developmental stages to weed interference (GRINT et al. 2022b). PRE herbicides can reduce the weed density and postpone the time to POST applications, lowering the selection pressure for more resistance to POST herbicides (FALECO et al. 2022).

Effective early-season weed control with PRE herbicides can be achieved depending on physicochemical properties of the herbicide (i.e., water solubility, vapor pressure, octanol-water coefficient, acid ionization constant), physicochemical properties of the soil (i.e., pH, organic matter, soil texture), environmental conditions (i.e., pattern and amount of rainfall, temperature), and soil seedbank weed species composition and density (VARANASI et al. 2016; ZHAO et al. 2017). When some of these conditions are not favorable, the efficacy of PRE herbicides is reduced (URACH et al. 2020). Seeking to low the risk of early-season weed control failure and herbicide resistance, the use of PRE herbicide premixes containing multiple SOAs is being adopted (STRIEGEL et al. 2021a). PRE herbicides with multiple SOAs (premixes) can expand the spectrum of weed control compared to a single SOA herbicide and simultaneously target the same weed spectrum for maximum benefit (NORSWORTHY et al. 2012). Premixes efficacy is also enhanced when the active ingredients have similar soil residual activity (PALMA-BAUTISTA et al. 2021).

PRE herbicide premixes tend to have a better performance than the same active ingredients applied solo when weather conditions are not favorable (JANAK, GRICHAR, 2016). As the weather becomes more variable across the United States (LANDAU et al. 2021), PRE herbicide premixes with multiple SOAs may improve early-season weed control. In this context, PRE herbicide premixes that contain multiple SOAs can potentially become a more reliable practice for chemical weed management programs due to the widespread occurrence of herbicide resistance across the United States coupled with the more variable and extreme weather conditions. On the flip side, residual herbicides, specially premixes might have potential to injure interseeded or overseeded cover crops into corn.

Growers have adopted cover crops to improve water infiltration, reduce soil erosion, enhance nutrient cycling, and weed and insect pest suppression (WALLANDER et al. 2021). One of the main challenges for successful cover crop establishment in corn cropping systems in the Upper Midwest of the United States is the short growing season to plant and establish cover crop following corn harvest (SMITH et al. 2019). Interseeding or overseeding cover crops, while the primary crop is still in the field, extend growing time and cover crop biomass production compared to cover crop planted after harvest, enhancing the ecosystem benefits of cover cropping in corn grain rotation (CASWELL et al. 2019). Interseeding is a system where a cover crop is planted early in the growing season when corn is between the V4-V8 growth stage. In contrast, cover crop overseeding is typically aerially seeded just before or at crop maturity (KLADIVKO et al. 2014). These systems allow using legume cover crop species (e.g., crimson clover [*Trifolium incarnatum* L., Peterson et al. 2021; Youngerman et al. 2018], radish (*Raphanus sativus* L., Brooker et al. 2020b), red clover (*Trifolium pratense* L., Wallace et al. 2017) since they require early planting dates for optimal establishment before winter (Youngerman et al. 2018).

A main concern with interseeding or overseeding is whether residual PRE herbicides will injure the cover crops. Researchers have investigated the impact of residual herbicides on interseeded cover crops and reported high injuries depending on the PRE herbicide selection and cover crop species adopted (BROOKER et al. 2020b; WALLACE et al. 2017). Cover crops species selection and herbicide labels should be carefully considered in interseeding or overseeding corn systems. Additionally, studies should be done in different types of soil and regions since weather and soil conditions can vary and influence herbicide residual activity in the soil (CORNELIUS et al. 2017; JURSÍK et al. 2020).

Due to the early-season weed control challenge and the potential residual herbicides injury to cover crops interseeded and overseeded into corn; this study evaluated the weed control and the tolerance of four commonly adopted cover crop species (annual rye, cereal rye, radish, and red clover) to a comprehensive list of labeled corn residual PRE herbicides. The weed control was evaluated in the field and the cover crops tolerance was assessed via greenhouse bioassay simulating an interseeding (~V3-V5 corn growth stage) and overseeding

(~V10-VT growth stage) scenario. Results can support corn growers with more effective herbicide selection considering the key weed species present in their field and cover crops selection according to the residual weed control program and cover crop establishment goals.

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CHAPTER I - Preemergence Herbicide Premixes Reduce the Risk of Soil Residual Weed Control Failure in Corn

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Abstract

Widespread occurrence of herbicide-resistant weeds and more variable weather conditions across the United States are challenging weed control in corn. Preemergence (PRE) herbicides with soil residual activity have resurged as foundation for early-season weed control in corn. Field experiments were conducted at Janesville and Lancaster, WI in 2021 and 2022 (4 site-years) to evaluate the weed control efficacy of solo (single site of action [SOA]) and premix (two or more SOAs) PRE herbicides in conventional tillage corn systems. Treatments consisted of 18 PRE herbicides plus a non-treated check. At Janesville-2021, S-metolachlor + bicyclopyrone + mesotrione, atrazine + S-metolachlor + bicyclopyrone + mesotrione, and clopyralid + acetochlor + mesotrione provided >72% giant ragweed control. At Janesville-2022, none of the PRE herbicides evaluated provided >70% giant ragweed control due to the heavy giant ragweed density and the lack of timely rainfall for PRE herbicide activation in the soil. At Lancaster-2021, atrazine, dicamba, and flumetsulam + clopyralid provided <45%

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waterhemp control, but the remaining treatments provided >90% control. At Lancaster-2022, the efficacy of some PRE herbicides was reduced due to the high waterhemp pressure, yet most herbicides provided >75% control. At Lancaster-2021 and 2022, only dicamba and S-metolachlor did not provide >75% common lambsquarters control. PRE herbicides containing SOA group 15 provided >75% control of giant foxtail. Across weed species, PRE herbicides with two (78%) and three (81%) SOAs provided higher weed control than PRE herbicides with a single SOA (68%), indicating that at least two SOAs PRE result in better early-season weed control. The efficacy of the PRE herbicide treatments evaluated herein varied according to the soil seedbank weed community composition and environmental conditions (i.e., rainfall following application), but the premixes appeared as a more reliable option to improve early-season weed control in conventional tillage corn systems.

Nomenclature: common lambsquarters, *Chenopodium album* L.; corn, *Zea mays* L.; giant foxtail, *Setaria faberi* Herrm.; giant ragweed, *Ambrosia trifida* L.; waterhemp, *Amaranthus tuberculatus* [Moq.] J.D. Sauer

Key Words: herbicide efficacy; herbicide mixture; residual herbicide; weed management

Introduction

Corn is the most cultivated crop in the United States, with an area of 32 million hectares harvested for grain in 2022 (USDA 2023). The Midwest is the top producing region, representing over 85% of the harvested area and over 88% of the corn produced in 2022 in the United States (USDA 2023). Weed management is a major challenge in corn production systems. United States corn growers rely primarily on herbicides and tillage for weed management (Dong et al. 2017; Grint et al. 2022). As a result, herbicides are the most extensively used pesticide in corn, applied to >95% of planted corn hectares in the US in 2021 (USDA 2022). The dependence on chemical weed control has led to a widespread occurrence of herbicide resistance mainly to postemergence (POST) herbicides (Heap 2022, Jha et al. 2017). An effective chemical approach to minimize the overreliance on POST herbicide applications is to adopt soil residual preemergence (PRE) herbicides for early-season weed control (Knezevic et al. 2019). The use of herbicides with effective soil residual activity applied PRE provides extended period of weed control early-season, protecting crop yields during their most vulnerable developmental stages to weed interference (Grint et al. 2022b; Oliveira et al. 2017a). PRE herbicides can reduce the weed density and delay the time to POST applications, lowering the selection pressure for further resistance to POST herbicides (Faleco et al. 2022a; Oliveira et al. 2017b). Adopting PRE herbicides as part of an integrated weed management program brings more diversity regarding effective sites-of-action (SOA) and opportunities for broad-spectrum chemical weed control (Norsworthy et al. 2012; Somerville et al. 2017).

The residual weed control efficacy of a PRE herbicide depends on several variables, including environmental conditions (i.e., pattern and amount of rainfall following application, temperature), physicochemical properties of the herbicide (i.e., water solubility, vapor pressure, octanol-water coefficient, acid ionization constant), physicochemical properties of the soil (i.e., pH, organic matter, texture), and soil seedbank weed community composition (Varanasi et al.

2016; Zhao et al. 2017). Effective early-season weed control with PRE herbicides can be achieved when these variables are favorable to the properly selected chemical program. However, when some of these conditions are not favorable, failure in early-season weed control may occur (Hay et al. 2018; Urach Ferreira et al. 2020). For example, adequate rainfall following application increases the probability of effective waterhemp and common lambsquarters control with PRE herbicides (Landau et al. 2021a) which are two of the most troublesome weeds in Wisconsin corn cropping systems (Werle and Oliveira 2018). Low residual weed control has been commonly reported under dry weather conditions due to the lack of residual herbicide activation and availability in soil solution (Bell et al. 2015; Jursik et al. 2015; Priess et al. 2020).

A practice recommended to lower the risk of early-season weed control failure and herbicide resistance is the use of PRE herbicide premixes containing multiple effective SOAs (Striegel et al. 2021a). PRE herbicides with multiple SOAs can expand the spectrum of weed control compared to a single SOA herbicide (Carneiro et al. 2020). Besides providing broad-spectrum control, herbicides with multiple SOAs that simultaneously target the same weed spectrum can maximize weed control benefits (Norsworthy et al. 2012). Their effectiveness is also improved when the active ingredients have similar soil residual activity (Beckie and Harker 2017; Palma-Bautista et al. 2021). Jha et al. (2015) reported high control (≥72%) of kochia (*Kochia scoparia* L.), common lambsquarters, and wild buckwheat (*Polygonum convolvulus* L.) with saflufenacil + dimethenamid-P and acetochlor + pendimethalin at 63 days after treatment (DAT) compared to these herbicides applied alone (≤47%). Other studies also demonstrated high efficacy of herbicide premixes (>90%) in controlling weeds in corn-soybean cropping systems (Oliveira et al. 2017b; Sarangi and Jhala 2018; Striegel et al. 2021a).

PRE herbicide mixes tend to be more effective than the same active ingredients applied solo when weather conditions are not favorable, but the extension of this effect may vary according to water solubility and soil sorption of each herbicide in the premix (Janak and Grichar 2016; Landau et al. 2021a; Stewart et al. 2010). It is well known that each herbicide has a particular behavior in the soil depending on edaphoclimatic conditions. For instance, clopyralid and dicamba present faster dissipation in moist soils with warm temperatures whereas, under dry soils and cold temperatures, their residual activity can persist longer (Cahoon et al. 2015; Pik et al. 1977; Seefeldt et al. 2014). As the weather becomes more variable across the United States corn producing regions (Landau et al. 2021b), PRE herbicide premixes with multiple SOAs may play a significant role to provide adequate early season weed control, mainly for troublesome weeds with an extended emergence window such as giant ragweed and waterhemp (Striegel et al. 2021b). In this context, PRE herbicide premixes that contain multiple SOAs can potentially become a more reliable practice for chemical weed control programs due to the widespread occurrence of herbicide resistance across the United States coupled with the more variable and extreme weather conditions. In this study, we evaluated a comprehensive list of labeled corn residual PRE herbicides (18 products containing one or multiple SOAs) including commonly used PRE herbicides in Wisconsin corn production, a novel premix herbicide (clopyralid + pyroxasulfone + mesotrione), and a not as commonly used herbicide premix in corn (saflufenacil + dimethenamid-P). Results can support corn growers and their decision influencers with more effective PRE herbicide selection based on key weed species present in their fields.

Materials and Methods

Field Experiments

Field experiments were conducted in 2021 and 2022 at the Rock County Farm, in Janesville, WI (42.43°N, 89.01°W) and at the University of Wisconsin-Madison Lancaster Agricultural Research Station, near Lancaster, WI (42.83°N, 90.76°W) to evaluate the residual weed control efficacy of solo (single SOA) and premix (commercial products with two or more SOAs) herbicides applied PRE in conventional tillage corn. PRE herbicide rates used herein are commonly recommended by the industry and adopted by growers in WI (DeWerff et al. 2022; Table 1). The rates of the single active ingredient herbicide treatments did not necessarily match their rates when used in the premix treatments (Table 1).

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. Soil at Janesville was a Plano silt loam and at Lancaster a Fayette silt loam. Soil properties, corn hybrid, seeding rate, and planting and herbicide application dates for each site-year are described in Table 2.

The experiment was conducted as a randomized complete block design with four replications. The treatments consisted of 18 PRE herbicides plus a nontreated control (NTC; Table 1). The experimental units were 3 m wide (4 corn rows) x 9 m long. Herbicides were applied within a day after corn planting (Table 2) using a CO_2 pressurized backpack sprayer equipped with six Teejet TTI110015 flat-fan (Teejet, Springfield, IL) nozzles 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}.

Data Collection

Daily mean air temperature and total cumulative precipitation at each site-year were obtained from onsite weather stations (WatchDog 2700, Spectrum Technologies®, Aurora, IL) (Figure 1). The density of the predominant weed species at each site-year was recorded from the NTC experimental units at 6 weeks after treatment (WAT) at Janesville-2021, Lancaster-2021, and Lancaster-2022, and at 4 WAT at Janesville-2022. Weed control and weed aboveground biomass at Janesville-2021, Lancaster-2021, and Lancaster-2022 were assessed at 6 (WAT). At Janesville-2022, visual weed control and aboveground weed biomass were assessed at 4 WAT because of the high giant ragweed (*Ambrosia trifida* L.) pressure and their rapid growth. All response variables were assessed between the two center corn rows of each experimental unit. Weed control in each experimental unit was estimated using a visual scale (0 = no control, 100% = complete control). Weed aboveground biomass was collected using 2 quadrats (0.25 m^{-2}) randomly placed between the center two rows of each experimental unit. Weeds were enumerated and harvested by species. Weed biomass for each species from both quadrats within an experimental unit was combined into a single paper bag. Weed biomass was dried at 60 C until constant dry weight and then weighed. Weed biomass data were reported as percentage biomass reduction compared to the NTC:

$$\% \text{ Biomass reduction} = [NTC - T)/NTC] * 100$$

where *NTC* is the mean weed biomass (g) of the NTC across replications within a specific site-year, and *T* is the weed biomass (g) of the experimental unit of interest.

Data Analyses

All response variables (visual weed control [%] and biomass reduction [%]) were analyzed using R statistical software version 4.2.1 (R Core Team, 2022). A generalized linear mixed model (GLMM) with a beta distribution and logit family (glmmTMB package; Brooks et al. 2017) was used to analyze both response variables. PRE herbicide efficacy is known to vary by year and location because of weather and soil conditions (Gaspie et al. 2021, Landau et al. 2021a) and soil seedbank weed community composition (Striegel et al. 2021a). Therefore, data were analyzed separately by weed species and site-year. Herbicide treatments were considered as fixed effects, while replications nested within site-year were treated as a random effect. ANOVA was performed for giant ragweed control and biomass reduction in Janesville-2021 and Janesville-2022. For Lancaster-2021, ANOVA was performed for waterhemp, and common lambsquarters, whereas giant foxtail visual control and biomass reduction were also analyzed in Lancaster-2022 besides waterhemp and common lambsquarters. Evaluation of homogeneity of residual variance was carried out using Levene's test ('car' package; Fox and Weisberg 2019). When ANOVA ('glmmTMB' package) indicated a significant PRE herbicide treatment effect, means were compared using Fisher's Least Significant Difference ($p \leq 0.05$) (emmeans package; Lenth 2022).

Pearson's correlation was performed (*cor.test* function) to estimate the linear correlation between visual weed control and weed biomass reduction. A linear mixed model was also performed to analyze visual weed control (%) according to the number of herbicide SOA for each weed species and for weed species combined ("overall", site-years pooled together). The number of herbicide SOA groups in each treatment (1, 2, and 3 SOAs) were considered as fixed effects, and replications nested within site-years were included as random effect. If ANOVA indicated a significant effect of number of PRE herbicide SOA groups ($p \leq 0.05$), means were compared using Fisher's protected LSD test.

Results and Discussion

Environmental Conditions

Daily precipitation varied across site-years (Figure 1). At Janesville-2021, the first rainfall event occurred 6 DAT (30 mm) whereas 40 mm of rainfall accumulated within 15 DAT. At Janesville-2022, the first rainfall occurred 7 DAT (only 9 mm), accumulating 21 mm of rain within 15 DAT. The average air temperature in the first week after treatment was lower in 2021 (15 C) compared to 2022 (22 C). At Lancaster-2021, the first rainfall event occurred 1 DAT (2 mm), whereas 32 mm accumulated within 7 days and 35 mm within 15 DAT. At Lancaster-2022, the first rainfall occurred within 1 day (3 mm), accumulating 6 mm of rain within 7 days and 42 mm of rain within 15 DAT. The average air temperature in the first week after treatment was 15 C in 2021 and 18 C in 2022.

Weed Species composition at Each Site-year

Giant ragweed was the predominant weed species observed at Janesville in both years (24 ± 2 plants m^{-2}, average ± standard error from NTC, in 2021 [6 WAT] and 104 ± 4 plants m^{-2} in 2022 [4 WAT]). At Lancaster, common lambsquarters (109 ± 24 plants m^{-2}) and waterhemp (41 ± 13 plants m^{-2}) were the predominant weed species in 2021, and waterhemp (100 ± 18 plants m^{-2}), common lambsquarters (37 ± 12 plants m^{-2}), and giant foxtail (27 ± 9 plants m^{-2}) in 2022. The weed species present at these site-years comprise some of the most common weeds in Wisconsin corn production systems (Werle and Oliveira, 2018).

Giant Ragweed Control

At Janesville-2021, the PRE herbicide treatment effect was significant for control ($p < 0.01$) and biomass reduction ($p < 0.01$) and efficacy across treatments was low at 6 WAT (<75% of control; Figure 2). Giant ragweed control was higher with certain herbicide premixes containing two or more SOAs compared with the herbicide treatments with a single SOA (Figures 2 and 3). For instance, premixes containing mesotrione (S-metolachlor + bicyclopyrone + mesotrione, atrazine + S-metolachlor + bicyclopyrone + mesotrione, and clopyralid + acetochlor + mesotrione) provided ≥72% control and ≥60% biomass reduction of giant ragweed (Figure 2). These premixes improved giant ragweed control and biomass reduction compared with the single active ingredient mesotrione (60% and 48%), acetochlor (58% and 27%), or S-metolachlor (8% and 3%) (Figure 2). Acetochlor or S-metolachlor in premixes with atrazine (atrazine + acetochlor and atrazine + S-metolachlor) improved giant ragweed control when compared to each active ingredient sprayed separately, but the control was still poor (≤50%). The thiencarbazone-methyl + isoxaflutole premix also increased giant ragweed control (40%) compared to isoxaflutole alone (26%).

At Janesville-2022, the PRE herbicide treatment effect was significant for control ($p < 0.01$) and biomass reduction ($p < 0.01$), but none of the treatments provided ≥70% giant ragweed control and biomass reduction 4 WAT. Nevertheless, similar to Janesville-2021 results, the herbicide premixes increased giant ragweed control and biomass reduction compared to the herbicides with a single SOA (Figures 2 and 3), except for flumetsulam + clopyralid + acetochlor and acetochlor + mesotrione, where the addition of the active ingredients flumetsulam and mesotrione did not improve giant ragweed control compared to acetochlor alone. Surprisingly, dicamba alone provided the greatest level of giant ragweed control (≥60%; Figure 2).

The relatively effective level of giant ragweed (≥72%) observed in 2021 for the PRE herbicide premixes with two or more SOA containing mesotrione may be associated with the ability of mesotrione to control a wide spectrum of broadleaf species (Carles et al. 2017; Sarangi and Jhala 2018). Striegel et al. (2021a) reported high giant ragweed control at this experimental location (95%) using herbicide premixes containing mesotrione (clopyralid + acetochlor + mesotrione and S-metolachlor + bicyclopyrone + mesotrione). In our study, the mixtures containing mesotrione resulted higher average control of giant ragweed compared to mesotrione applied alone. Moreover, different SOAs in the mixture can complement each other under a range of environmental conditions providing more consistent weed control thus lowering the risk of additional weed resistance (Barbieri et al. 2022; Bollman et al. 2006; Norsworthy et al. 2012). For instance, Janak and Grichar (2016) observed that Palmer amaranth control was high with saflufenacil + dimethenamid-P (95%) compared with saflufenacil (≤72%) and dimethenamid-P applied alone (≤53%) in a field condition with no rainfall by 14 DAT; in the field that received 35 mm by 14 DAT, the efficacy of saflufenacil + dimethenamid-P was 100% and saflufenacil and dimethenamid-P applied alone was 99% and 98%, respectively.

The heavy giant ragweed pressure and the lack of timely rainfall for herbicide activation in the soil might be one of the factors leading to poor giant ragweed control (≤70%) at Janesville in 2022. The lower amount of accumulated rainfall during the first (9 mm) and second weeks (21 mm) after PRE herbicide application was probably not adequate for herbicide incorporation and proper herbicide activation thus reducing weed control (Figure 1). In 2022, the amount of rainfall between application and 15 DAT was only half the amount compared to 2021 (21 versus 40 mm; Figure 1). According to Landau et al. (2021a), 50-100 mm total rainfall in the first 15 days, depending on the herbicide and weed species, is typically required to prevent losses in control efficacy due to poor incorporation and activation of PRE herbicides.

The greater control of giant ragweed in 2022 by dicamba confirms the extended residual activity of this herbicide under limited rainfall conditions (21 mm within 15 DAT; Figure 1). This would be associated with a reduction in microbial degradation under dry conditions and reduced leaching thus more dicamba available to control germinating sensitive broadleaf weeds such as giant ragweed (Cahoon et al. 2015). Dicamba has high solubility in water, 4500 mg L^{-1} at 25 C, thus lower rainfall in 2022 can explain the greater dicamba residual activity 4 WAT (Shaner 2014). Although dicamba PRE activity did not result in giant ragweed control >70% in this study, the residual activity of dicamba appears to improve early season giant ragweed control in dry springs. Mundt et al. (2022) also observed that the residual weed control is extended if the rainfall accumulation is not enough to leach dicamba molecules through the crop residue and soil profile.

Another reason for reduced giant ragweed control in 2022 compared to 2021 was the high soil seedbank pressure (24 ± 2 plants m^{-2} in 2021 [6 WAT] compared to 104 ± 4 plants m^{-2} in 2022). A previous study also reported low giant ragweed control by PRE herbicides due to the high giant ragweed soil seedbank infestation at Janesville site in 2018 (Striegel et al. 2021a). No cases of giant ragweed resistance have been documented in Wisconsin for the PRE herbicides tested in this study (Heap 2022).

Waterhemp Control

At Lancaster-2021, the PRE herbicide treatment effect was significant for control ($p < 0.01$) and biomass reduction ($p < 0.01$) and most PRE herbicides provided ≥90% waterhemp control and biomass reduction 6 WAT, other than atrazine, dicamba, and flumetsulam + clopyralid (<45%; Figure 4). Thus, all the herbicide premixes provided effective control of waterhemp, except the premix flumetsulam + clopyralid. At Lancaster-2022, the PRE herbicide treatment effect was significant ($p < 0.01$) and the herbicides isoxaflutole, dicamba, atrazine, and simazine (single SOA) and the premix flumetsulam + clopyralid (2 SOAs) were ineffective in controlling waterhemp (≤70% control and biomass reduction; Figure 4). Atrazine + acetochlor, atrazine + S-metolachlor, and atrazine + S-metolachlor + bicyclopyrone + mesotrione premixes increased waterhemp control (98%, 92%, and 80%, respectively) and biomass reduction (96% and 84%, and 87%, respectively) compared to atrazine alone (66% of control and 56% of biomass reduction) in 2022 (Figure 4). Herbicide premixes with more than one SOA provided better waterhemp control than herbicides with a single SOA (Figure 3).

The high waterhemp control efficacy with most PRE herbicides in 2021 can be attributed to the lower waterhemp pressure at the time of data collection as the corn was planted in late-April and significant waterhemp emergence was still observed in the NTC experimental units after data collection 6 WAT (Silva, personal observation). In 2022, the corn was planted in early-May, which allowed more time for waterhemp emergence before data collection in late-June. In Wisconsin, waterhemp starts to emerge in mid to late-May, reaching >75% cumulative emergence by late-June (Striegel et al. 2021b), which explains the high waterhemp infestation during the field study evaluation in 2022. Considering that less than 20% of the Wisconsin corn crop was planted by May 8 (2017-2022; USDA 2022), the 2022 waterhemp control results might be more valuable and realistic for most Wisconsin corn growers.

The low waterhemp control by the PRE herbicide premix flumetsulam + clopyralid and atrazine may be related to waterhemp resistance to acetolactate synthase (ALS)-inhibiting herbicide (Faleco et al. 2022b) and photosystem II [PSII]-inhibiting herbicide, respectively (Faleco et al. 2022a, 2022b). ALS- and PSII-resistant waterhemp have been widely reported across the US Midwest (Evans et al. 2019; Heap 2022; Vennapusa et al. 2018). The ineffective waterhemp control by atrazine can also be a result of reduced residual activity caused by repeated use of atrazine over the years. According to previous studies, atrazine microbial degradation is enhanced in soils with a history of atrazine use compared with soils not treated with the herbicide (Mueller et al 2017; Shaner and Henry 2007).

Reduced residual waterhemp control by simazine in 2022 may be due to the rapid dissipation of this herbicide; although simazine is considered moderately persistent in soil with an average half-life of 60 days (Shaner 2014), persistence is affected by edaphoclimatic conditions and history of use with a wide range of half-life (16 to 186 d). Abit et al. (2012) observed a range of simazine half-life of 21-158 days in California vineyards and the residual weed control was reduced in the site-years where simazine was dissipated more quickly due to rapid microbial degradation.

The effective control of waterhemp in the premixes containing atrazine may be attributed to the shared active ingredients mesotrione (group 27), acetochlor (group 15), and S-metolachlor (group 15), which are herbicides recommended for small-seeded broadleaf control (DeWerf et al. 2023). PRE herbicides are typically more effective in controlling small-seeded broadleaf weeds than large-seeded broadleaf weeds (Arneson et al. 2022) in parts due to the higher seed surface area of small-seeded species exposing them to higher herbicide concentrations in the soil and smaller seedling size requiring lower herbicide amount for control (Gelviz-Gelvez et al. 2020; Schutte et al. 2012). For example, S-metolachlor and acetochlor provide high efficacy against many small-seeded weeds but have limited control of large-seeded broadleaf weeds (Keeling et al. 2013, Striegel et al. 2021b) such as giant ragweed. As a result, in our study S-metolachlor and acetochlor provided effective waterhemp control but poor giant ragweed control. Therefore, despite considered the most troublesome weed in Wisconsin cropping systems and across US corn production (Van Wychen 2020; Werle and Oliveira 2018), there are multiple effective PRE herbicides options for waterhemp management.

Common Lambsquarters Control

At Lancaster-2021, the PRE herbicide treatment effect was significant for control ($p < 0.01$) and biomass reduction ($p < 0.01$) and most PRE herbicides provided ≥90% control of common lambsquarters at 6 WAT. Dicamba resulted in the lowest control of common lambsquarters (≤64%). Acetochlor, dicamba, and saflufenacil + dimethenamid-P provided low biomass reduction (≤66%), and the remaining treatments resulted in ≥87% biomass reduction (Figure 5). In 2022, the PRE herbicide treatment effect was significant for control and biomass ($p < 0.01$) reduction ($p < 0.01$); acetochlor, saflufenacil + dimethenamid-P, isoxaflutole, and S-metolachlor resulted in ≤77% of common lambsquarters control and the remaining treatments provided effective control (≥90%). Isoxaflutole, dicamba, acetochlor, S-metolachlor, and saflufenacil + dimethenamid-P resulted in the lowest common lambsquarters biomass reduction (≤68%; Figure 5). The premixes atrazine + acetochlor, atrazine + S-metolachlor, atrazine + S-metolachlor + bicyclopyrone + mesotrione, clopyralid + acetochlor + mesotrione, S-metolachlor + bicyclopyrone + mesotrione, and flumetsulam + clopyralid + acetochlor enhanced common lambsquarters control (≥93%) compared when acetochlor (≤77%) or S-metolachlor were applied alone (≤87%) in both years (Figure 5). Similar trend was reported by

Jha et al. (2015) where the addition of pendimethalin to acetochlor improved residual control of common lambsquarters (acetochlor plus pendimethalin; 91% and 81%) compared to acetochlor applied alone at 21 and 35 DAT (51% and 45%, respectively).

The herbicide premixes resulted in effective control and biomass reduction of common lambsquarters (≥90%; Figures 3 and 5), except saflufenacil + dimethenamid-P with ≤77% control and biomass reduction. A similar result was observed by Underwood et al. (2017), where the premix saflufenacil + dimethenamid-P provided low control of common lambsquarters (65%). Although ranked in the top five most problematic weeds in US corn production (Van Wychen 2020), results of this study demonstrate that several PRE herbicides can be effective for common lambsquarters control.

Giant Foxtail Control

Giant foxtail control data were only collected at Lancaster-2022 (this species was not present in Lancaster-2021 field study location). The PRE herbicide treatment effect was significant for control ($p < 0.01$) and biomass reduction ($p < 0.01$) and only atrazine + acetochlor resulted in more than 90% control of giant foxtail 6 WAT (Figure 6). The premixes performed better than the herbicides with a single SOA (Figure 3 and Figure 6), except for acetochlor (≥87%), which provided a high level of giant foxtail control, and the premix flumetsulam + clopyralid that resulted in a low level of giant foxtail control (≤68%; Figure 6). The biomass reduction followed a similar trend, where the PRE herbicides with a single active ingredient resulted in low levels of giant foxtail biomass reduction (≤48%), except S-metolachlor and acetochlor (≥75%) with relatively effective levels of biomass reduction (Figure 6).

The premix flumetsulam + clopyralid and atrazine + S-metolachlor + bicyclopyrone + mesotrione premixes provided low giant foxtail biomass reduction (≤42%). The low biomass reduction by atrazine + S-metolachlor + bicyclopyrone + mesotrione suggests that not all herbicide premixes with multiple SOAs may provide effective weed control. The lower rate of S-metolachlor applied in this premix (1,498 g ai ha^{-1}) compared to S-metolachlor alone (1,791 g ai ha^{-1}) may have contributed to the lower giant foxtail biomass reduction in the premix treatment. Thus, it is important to consider the application rate of each active ingredient in a premix and how that compares to that same herbicide applied alone. Besides containing multiple SOAs at appropriate rates, premixes or herbicide mixtures should contain active ingredients that have similar efficacy and persistence in soil to act simultaneously on the same spectrum of weeds (Norsworthy et al. 2012).

Corroborating the visual control results, acetochlor and atrazine + acetochlor provided the highest giant foxtail biomass reduction (≥90%; Figure 6). The high giant foxtail control with acetochlor, atrazine + acetochlor (≥87%), and the relatively effective levels (≥70%) of control with the other acetochlor premixes, S-metolachlor alone, and S-metolachlor premixes might be due to the action of very long chain fatty acid (VLCFA)-inhibiting herbicides (acetochlor or S-metolachlor) since the premixes were not different from acetochlor or S-metolachlor applied alone. VLCFA-inhibitors limit the biosynthesis of VLCFAs, leading to a lack of lipids, proteins and lignin (Lamberth and Dinges 2016), causing the inhibition of shoot elongation in grasses. The VLCFA-inhibiting herbicides are effective in controlling emerging small-seeded annual grasses and small-seeded broadleaf weeds (Heap 2019; Ribeiro et al. 2022; Striegel et al. 2021a).

Pearson's Correlation

A strong positive correlation was detected between overall visual weed control and biomass reduction (R = 0.88; p <0.001; Figure 7). Despite the potential subjectivity of visual weed control ratings, the strong correlation detected herein indicates that such assessments can be a reliable measurement in chemical weed control research. Visual weed control and weed biomass reduction are important measurements in determining PRE herbicide efficacy but often times researchers will only collect visual control. According to our results, high quality visual weed control data can be used as indicators of PRE herbicides efficacy when biomass data are not available. In general, less intensive work is required to collect visual weed control data allowing for a rapid quantitative evaluation of herbicide efficacy. Despite that, biomass data are commonly required in the weed science literature to support weed control results and can be used to estimate weed seed production if such correlations (biomass and seed production) are available in the literature (Chauhan and Johnson 2010; Schwartz et al. 2016; Wilson et al. 1995).

Weed Control by the Number of Active Ingredients

The PRE herbicide comparison by the number of SOAs showed that PRE herbicide premixes (two and three SOAs) tended to result in higher control of giant ragweed, waterhemp, common lambsquarters, and giant foxtail than herbicides with a single SOA (Figure 3). The overall weed control across site-years followed the same trend, where PRE herbicides with two (78%) and three (81%) SOAs provided higher weed control than PRE herbicides with a single SOA (68%) (Figure 3).

Supporting our weed control and biomass reduction findings, these results indicate that at least two SOAs are needed in a premix to achieve higher weed control with PRE herbicides. But at the same time, more SOAs may not further improve the weed control as in this study was not observed difference between the premixes with 2 and 3 SOAs (Figure 3). Nevertheless, the strategic selection of premixes with at least 2 SOAs considering the weed seedbank community composition and the predicted environmental conditions following application can improve the diversity of the weed management program and may delay the evolution of resistance because of reduced selection pressure on single PREs and POST herbicides (Norsworthy et al. 2012).

Even when limited rainfall conditions occurred at Janesville 2022, the PRE herbicide premixes still performed better than most PRE herbicides with a single SOA (Figure 1 and 2).

Considering that more variable weather conditions and future weed resistance problems are likely to occur across the US Midwest (Landau et al. 2021a; Westwood et al. 2018), strategically selected herbicide premixes may become a standard management practice for more effective early-season weed control in corn. The premixes bring the diversity of SOAs combination to develop a more sustainable and effective corn PRE herbicide program offering a broader spectrum weed control and reducing the reliance on single PRE and POST herbicides. Our results demonstrate that the likelihood of weed control success increases when premixes with multiple SOAs are used due to the extended spectrum of activity supporting effective weed management.

Practical Implications

In summary, the results of this study provide insight into preemergence herbicide options to improve early-season weed control in conventional corn tillage systems. PRE herbicide premixes containing at least 2 SOAs appear as a reliable option for PRE herbicide programs to improve weed control compared to herbicides with a single site of action, but dominant weed species and rainfall amount and pattern are still essential factors to be considered when selecting a PRE herbicide premix. These results can support PRE herbicides selection and recommendations for weed control in Wisconsin corn production systems and beyond according to the soil seedbank weed community composition and anticipated environmental conditions.

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Conflicts of Interest

No conflicts of interest have been declared. This research received no specific grant from any funding agency, commercial, or not-for-profit sectors.

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Table 1. PRE herbicide treatments, site of action group (SOA), trade names, manufacturers, chemical families, half-lives, and rates evaluated in the corn field experiments.

Herbicide (SOA)	Trade name®	Manufacturer	Chemical Family	Half-life[a] days	Rate g ai or ae ha^{-1}
dicamba (4)	Diflexx™	Bayer CropScience[b]	Benzoates	14	560
atrazine (5)	AAtrex®	Syngenta Crop Protection[c]	Triazines	60	1120
simazine (5)	Princep® 4L	Syngenta Crop Protection[c]	Triazines	60	2240
acetochlor (15)	Harness®	Bayer CropScience[b]	α-Chloroacetamides	12	1960
s-metolachlor (15)	Dual II Magnum®	Syngenta Crop Protection[c]	α-Chloroacetamides	112-124	1791
isoxaflutole (27)	Balance® Flexx	Bayer CropScience[b]	Isoxazoles	0.5-2.4	79
mesotrione (27)	Callisto®	Syngenta Crop Protection[c]	Triketones	5-15	175
acetochlor (15) + mesotrione (27)	Harness® Max	Bayer CropScience[b]	α-Chloroacetamides + Triketones	-	1971 + 185
thiencarbazone-methyl (2) + isoxaflutole (27)	Corvus®	Bayer CropScience[b]	Triazolinones + Isoxazoles	-	34 + 85
atrazine (5) + S-metolachlor (15)	Bicep Lite II Magnum®	Syngenta Crop Protection[c]	Triazines + α-Chloroacetamides	-	1310 + 1634
atrazine (5) + acetochlor (15)	Harness® Xtra	Bayer CropScience[b]	Triazines + α-Chloroacetamides	-	952 + 2408
saflufenacil (14) + dimethenamid-P (15)	Verdict®	BASF Corporation[d]	N-Phenyl-imides + α-Chloroacetamides	-	75 + 655
flumetsulam (2) + clopyralid (4)	Hornet® WDG	Corteva Agriscience[e]	Triazolopyrimidine + Pyridine-carboxylates	-	52 + 168

S-metolachlor (15) + bicyclopyrone (27) + mesotrione (27)	Acuron® Flexi	Syngenta Crop Protection[c]	α-Chloroacetamides + Triketone + Triketone	-	1602 + 45 + 179
atrazine (5) + S-metolachlor (15) + bicyclopyrone (27) + mesotrione (27)	Acuron®	Syngenta Crop Protection[c]	Triazines + α-Chloroacetamides + Triketones + Triketones	-	700 + 1498 + 42 + 168
flumetsulam (2) + clopyralid (4) + acetochlor (15)	Surestart® II	Corteva Agriscience[e]	Triazolopyrimidine + Pyridine-carboxylates + α-Chloroacetamides	-	42 + 133 + 1315
clopyralid (4) + acetochlor (15) + mesotrione (27)	Resicore®	Corteva Agriscience[e]	Pyridine-carboxylates + α-Chloroacetamides + Triketones	-	133 + 1960 + 210
clopyralid (4) + pyroxasulfone (15) + mesotrione (27)	Maverick™	Valent[f]	Pyridine-carboxylates + Isoxazolines + Triketones	-	194 + 194 + 233

[a] Average field half-life of the herbicides, obtained from the WSSA Herbicide Handbook (10th ed.; Shaner 2014) and Pesticide Properties DataBase (PPDB 2022). Manufacturer location - [b]St. Louis, MO, [c]Greensboro, NC, [d]Durham, NC, [e]Indianapolis, IN, [f]Walnut Creek, CA.

Table 2. Soil properties, corn hybrids, seeding rates, and planting and herbicide application dates for corn field experiments.

Site-year	pH	OM [a]	Sand	Silt	Clay	Corn hybrid	Seeding rate	Planting date	Herbicide application date
		---------------%---------------					Seeds ha^{-1}		
Janesville 2021	5.4	4.1	8	68	24	NK 9653-5222EZ [b]	87600	April 26	April 28
Janesville 2022	5.9	2.6	26	63	12	NK 9653-5222EZ [b]	87600	May 10	May 11
Lancaster 2021	6.6	2.5	10	76	14	B97T04SXE [c]	80200	April 28	April 29
Lancaster 2022	5.3	4.1	18	65	18	P9998Q-N802 [d]	80200	May 11	May 13

[a] OM: organic matter. [b] Brevant®, Indianapolis, IN 46268. [c] Syngenta®, Greensboro, NC 27419. [d] Pioneer®, Johnston, IA 50131. 2021-Janesville experimental field was fertilized with 200 kg ha^{-1} of nitrogen (46-0-0); Lancaster-2021: 128 kg ha^{-1} of nitrogen (46-0-0); 2022-Janesvile: 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); 2022-Lancaster: 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)

Figure Legends

Figure 1. Mean daily air temperature and total cumulative precipitation at Janesville (left) and Lancaster (right), WI, in 2021 (top) and 2022 (bottom) during the corn field experiment.

Figure 2. Giant ragweed control (% of nontreated control; left) and biomass reduction (% of nontreated control; right) in Janesville, WI, 2021 at 6 weeks after treatment and 2022 at 4 weeks after treatment. 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 LSD, and herbicide treatments with the same letters are not different at $\alpha = 0.05$. Numbers in parentheses in the y-axis represent the site of action of each herbicide treatment. Abbreviations: DICAM = dicamba, ATZ = atrazine, SMZ = simazine, ACET = acetochlor, S-MET = S-metolachlor, IFT = isoxaflutole, MES = mesotrione, TCM = thiencarbazone-methyl, SAFL = saflufenacil, DIM-P = dimethenamid-P, FLUM = flumetsulam, CLOP = clopyralid, BIP = bicyclopyrone, PYRO = pyroxasulfone.

Figure 3. Control (% of nontreated control) of giant ragweed at Janesville (6 weeks after treatment [WAT] in 2021 and 4 WAT 2022), waterhemp (2021 and 2022), common lambsquarters (2021 and 2022), giant foxtail (2022) at Lancaster, WI (6 WAT), and all data combined across species based on herbicide treatments with a single, 2, and 3 sites of action applied PRE in corn.

Figure 4. Waterhemp control (% of nontreated control; left) and biomass reduction (% of nontreated control; right) in Lancaster, WI, 2021 and 2022 at 6 weeks after treatment. 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 LSD, and herbicide treatments with the same letters are not different at $\alpha = 0.05$. Numbers in parentheses in the y-axis represent the site of action of each herbicide. Abbreviations: DICAM = dicamba, ATZ = atrazine, SMZ = simazine, ACET = acetochlor, S-MET = S-metolachlor, IFT = isoxaflutole, MES = mesotrione, TCM = thiencarbazone- methyl, SAFL = saflufenacil,

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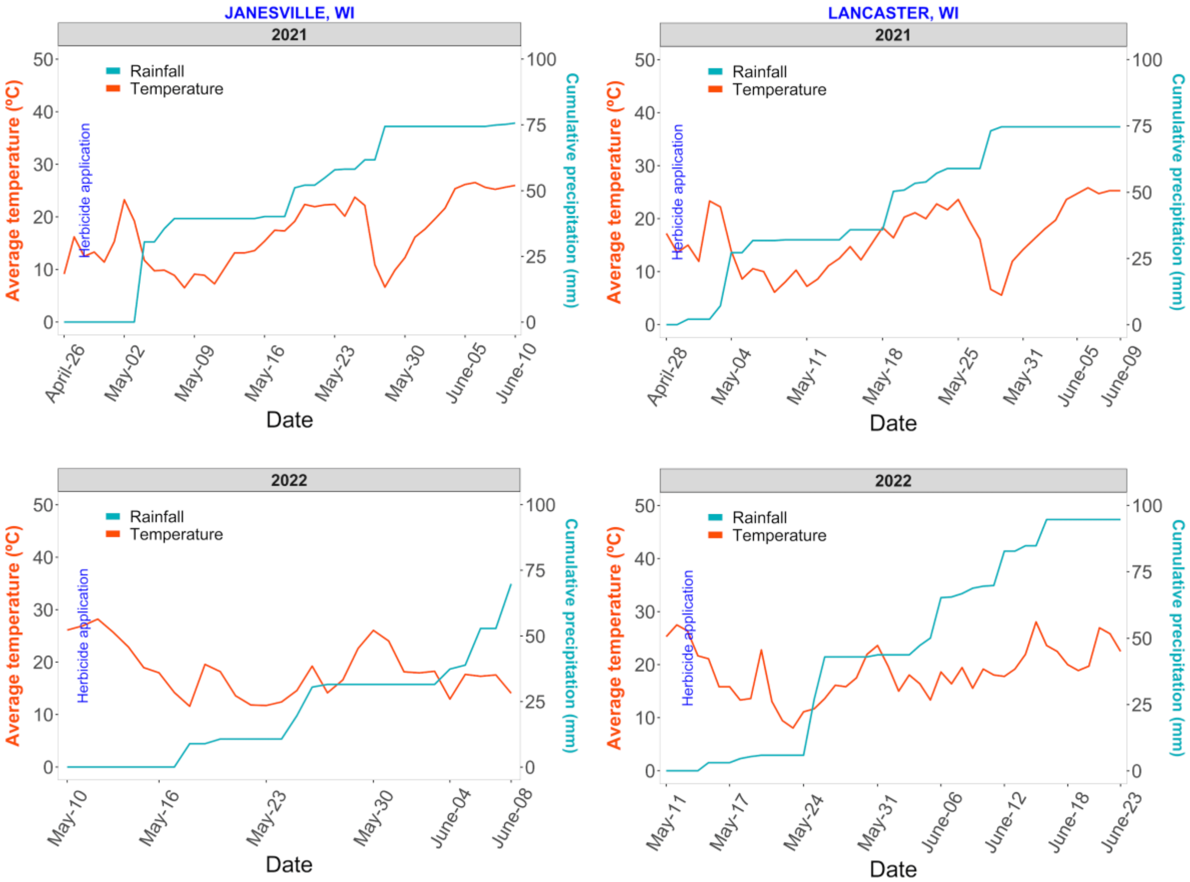
Figure 5. Common lambsquarters control (% of nontreated control; left) and biomass reduction (% of nontreated control; right) in Lancaster, WI, 2021 and 2022 at 6 weeks after treatment. Jittered points represent replicates, centered solid points denote the means, and error bars represent the upper and lower 95% confidence interval limits. Means were compared using Fisher's LSD, and herbicide treatments with the same letters are not different at α = 0.05. Numbers in parentheses in the y-axis represent the site of action of each herbicide. Abbreviations: DICAM = dicamba, ATZ = atrazine, SMZ = simazine, ACET = acetochlor, S-MET = S-metolachlor, IFT = isoxaflutole, MES = mesotrione, TCM = thiencarbazone-methyl, SAFL = saflufenacil, DIM-P = dimethenamid-P, FLUM = flumetsulam, CLOP = clopyralid, BIP = bicyclopyrone, PYRO = pyroxasulfone.

Figure 6. Giant foxtail control (% of nontreated control; left) and biomass reduction (% of nontreated control; right) in Lancaster, WI, 2022 at 6 weeks after treatment. 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 LSD, and herbicide treatments with the same letters are not different at α = 0.05. Numbers in parentheses in the y-axis represent the site of action of each herbicide. Abbreviations: DICAM = dicamba, ATZ = atrazine, SMZ = simazine, ACET = acetochlor, S-MET = S-metolachlor, IFT = isoxaflutole, MES = mesotrione, TCM = thiencarbazone-methyl, SAFL = saflufenacil, DIM-P = dimethenamid-P, FLUM = flumetsulam, CLOP = clopyralid, BIP = bicyclopyrone, PYRO = pyroxasulfone.

Figure 7. Pearson's linear correlation between weed control (% of nontreated control) and weed biomass reduction (% of nontreated control) for giant ragweed, waterhemp, common lambsquarters, and giant foxtail at Janesville and Lancaster in 2021 and 2022 combined. The

correlation (R) is 0.88 (lower confidence interval [CI] 0.86–upper CI 0.89) with *p*-value < 0.001. The blue line represents the linear trend and the shaded area the 95% CI.



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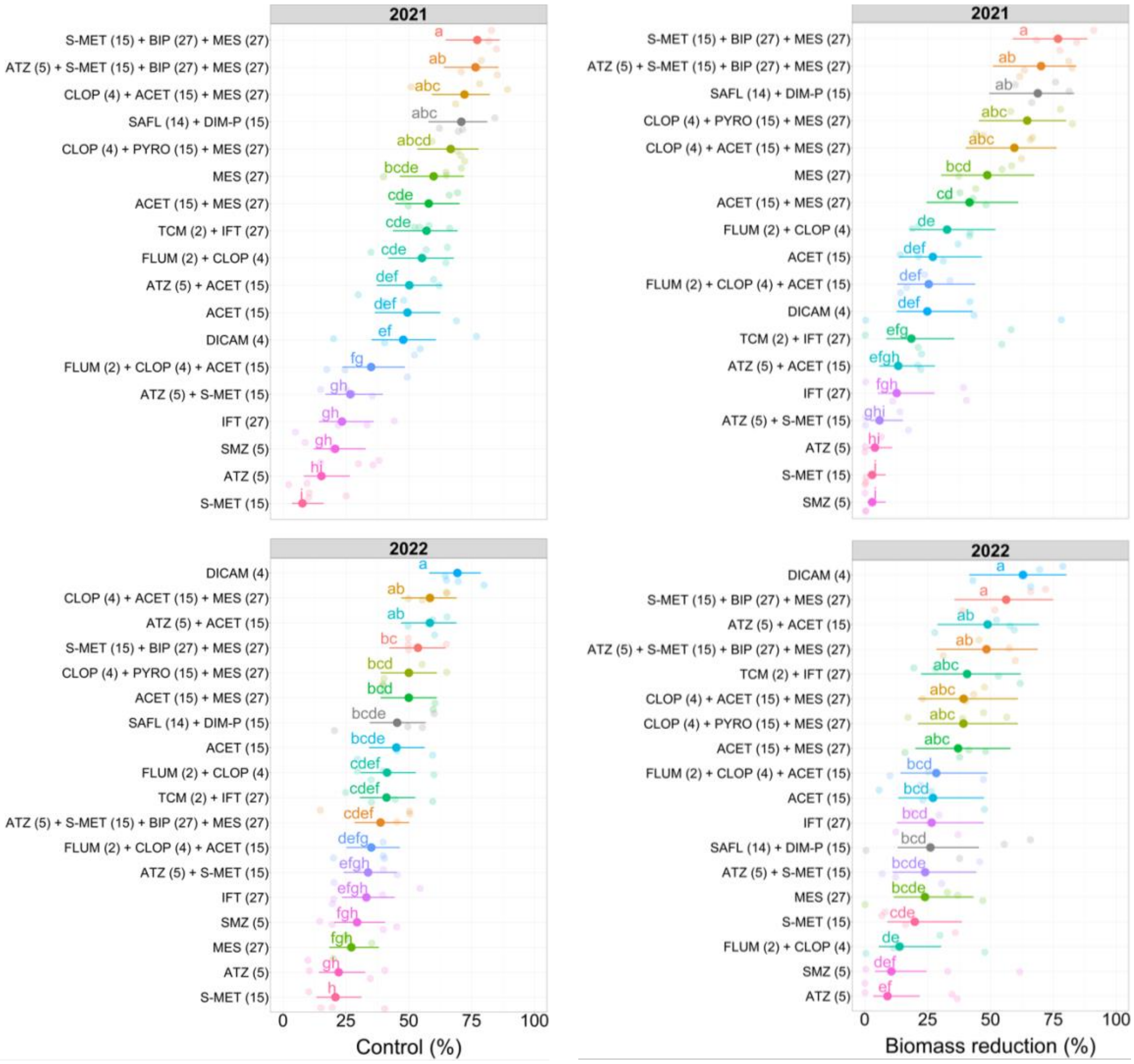

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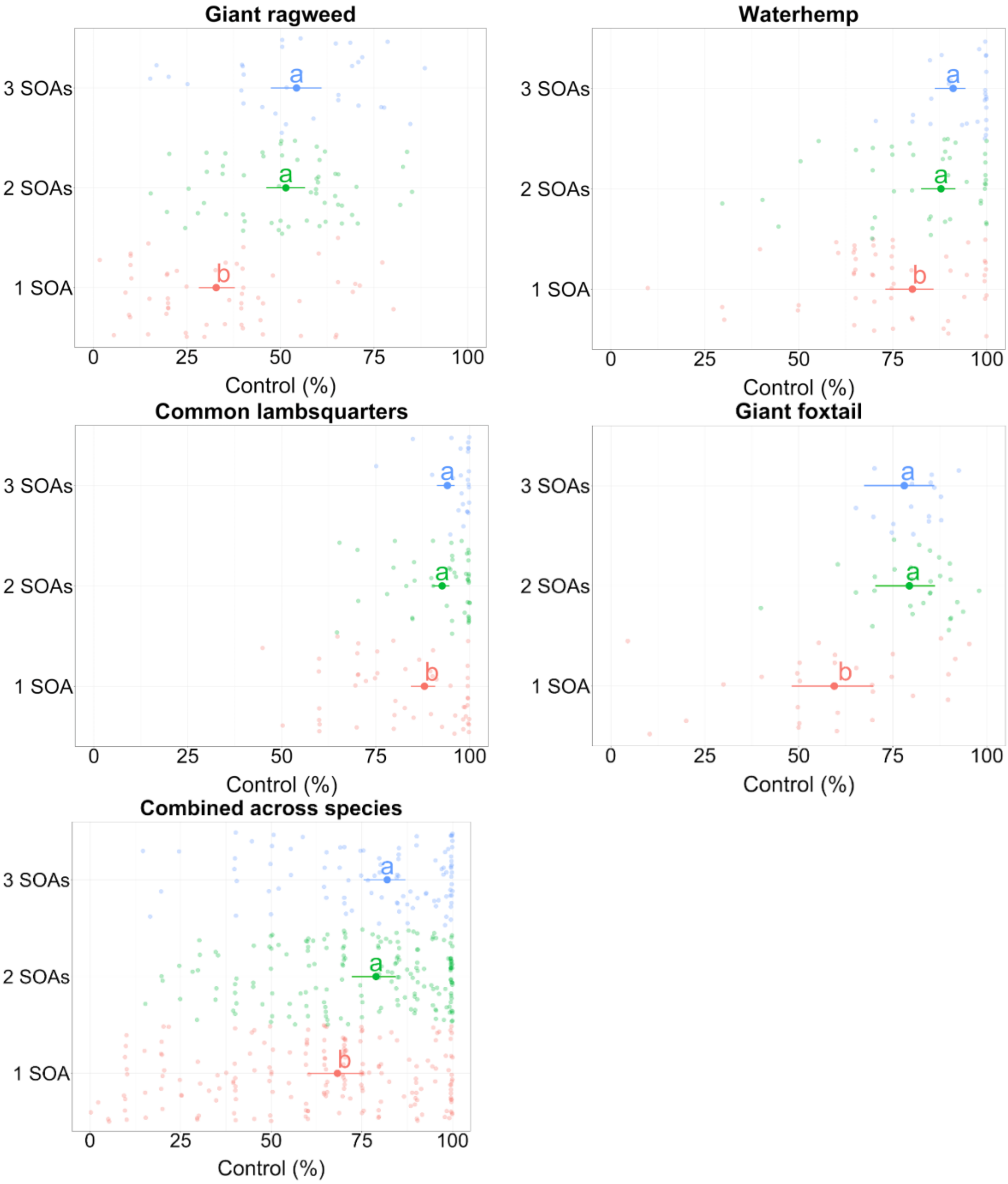

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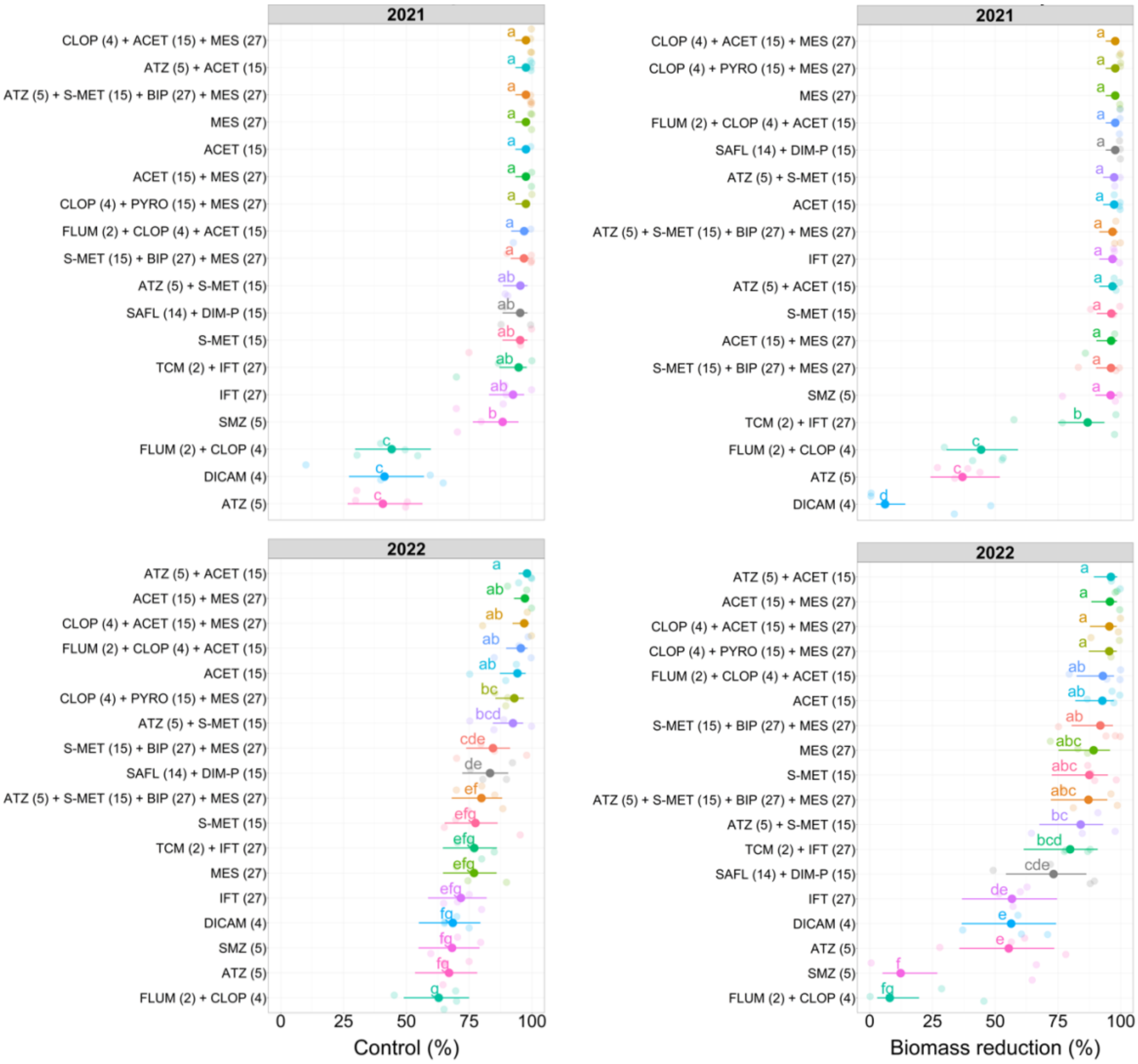

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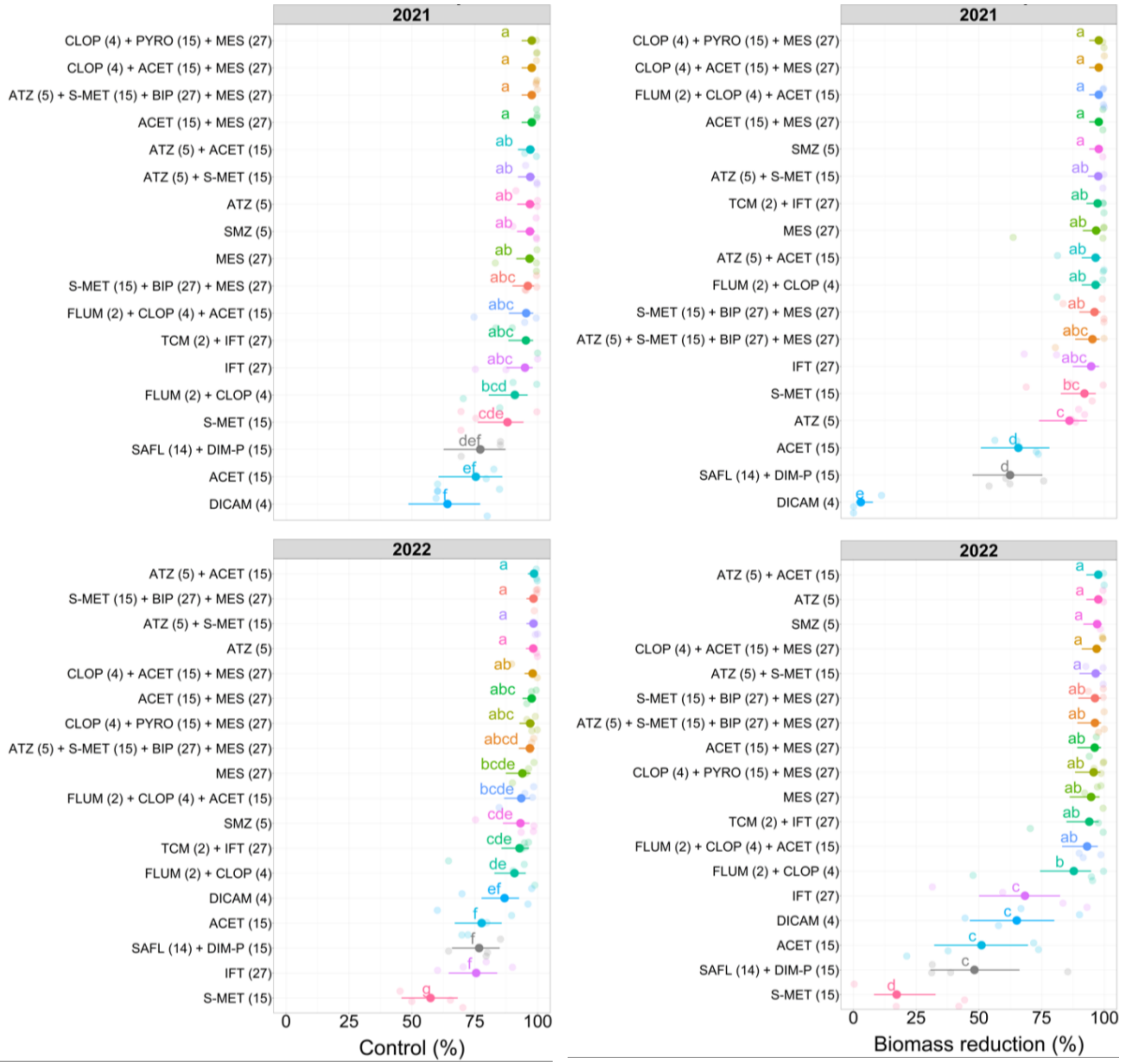

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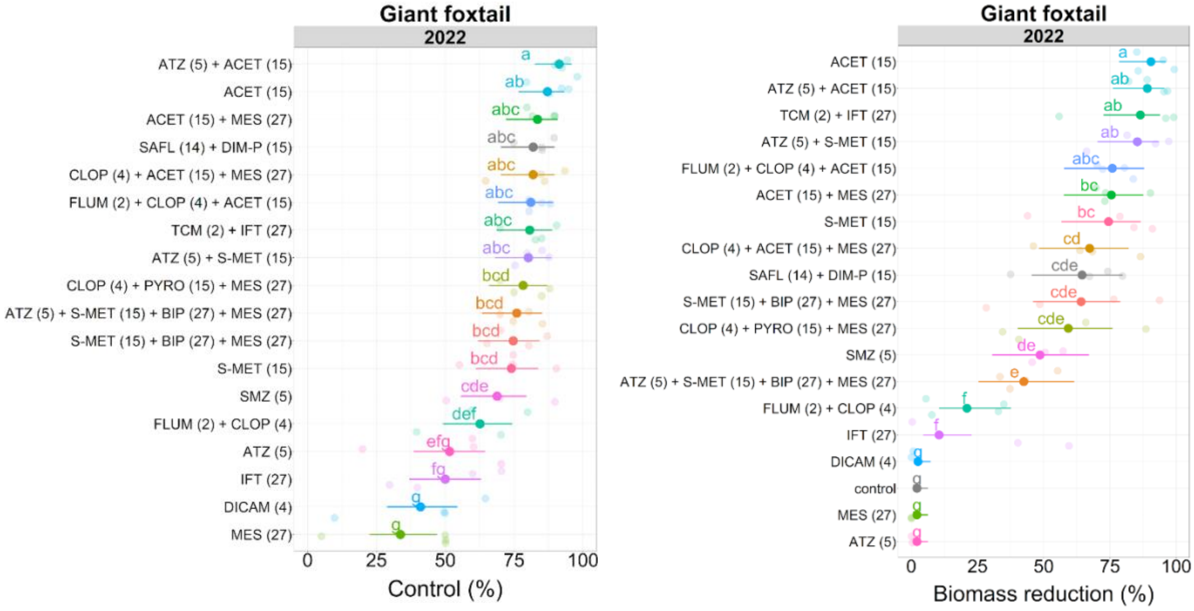

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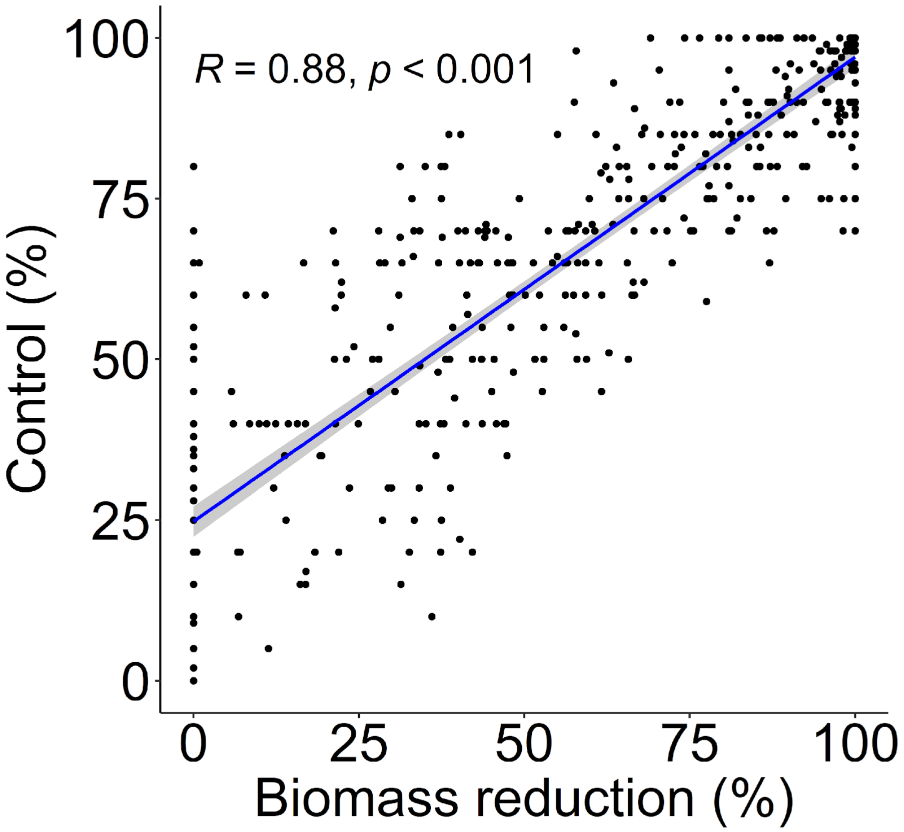

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CHAPTER II - Evaluating Cover Crop Tolerance to Corn Residual Herbicides Using Field Treated Soil in Greenhouse Bioassay

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Abstract

More growers across the US Midwest are considering interseeding and 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 and Lancaster, WI to investigate the impact of PRE residual herbicides on establishment of interseeded and overseeded cover crops via greenhouse bioassay. Soil samples (0-5 cm depth) were collected from field experiments at 0, 10, 20, 30, 40 and 50, 60, and 70 days after treatment (DAT). Treatments consisted of 14 single and multiple sites of action PRE herbicides plus a nontreated control (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 days after bioassay seeding. Cover crop species responded differently across herbicide treatments. Annual ryegrass and cereal rye were sensitive to treatments containing herbicide group 15 but not as impacted by herbicide groups 2, 4, 5, 14, and 27 when field soil was collected at 30 DAT (interseeding scenario) and 70 DAT (overseeding scenario) compared to

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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 sites of action than herbicides with a single site of action. Based on 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.

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

Key Words: biomass production; carryover; cover crop interseeding; cover crop overseeding; herbicide injury; preemergence herbicides

Introduction

The adoption of cover crops increases the crop diversity in continuous corn (*Zea mays* L.) and corn-soybean (*Glycine max* L. Merr.) rotations across the Midwest United States (Brooker et al. 2020a). Cover crops can provide a variety of 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 hectares 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 establish cover crops following corn grain harvest (Kladivko et al. 2014; Smith et al. 2019).

In a continuous corn and corn-soybean rotation, the selection of cover crop species is typically limited to winter cereals, such as cereal rye, because 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.), are sensitive or 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-V8 vegetative growth stage (Smith et al. 2019; Youngerman et al. 2018). In contrast, cover crop overseeding is typically done by aerially 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), 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 common 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-V6 corn growth stage and reported high injuries depending on the herbicide active ingredient and cover crop species. In one interseeding study established at the V5 corn growth stage 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 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 et al. 2017; Jursík et al. 2020).

Few studies have reported the potential herbicide residual injury to cover crops interseeded at V3-V5 corn growth stage and overseeded at V10-VT corn growth stage. More research is needed to support herbicide selection that provides effective weed control yet allow establishment of cover crops to 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 PRE herbicides was evaluated. The main purpose of this study was to investigate potential soil residual herbicide and cover crop combination options for interseeding (~V3-V5 corn growth stage) and overseeding (~V10-VT growth stage) scenario via greenhouse bioassay.

Materials and Methods

Field-treated soil samples were collected from a field experiment to evaluate via greenhouse bioassay how soil residual herbicides applied PRE impact cover crop establishment (simulating a scenario where cover crops are planted at different times during the corn growing season).

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 please see Severo Silva et al. (*In Review*). Briefly, soil properties, corn hybrid, and seeding rates for each location are summarized in Table 1. 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}]) was applied PRE in the previous season for the Janesville-2022 field. Sequence® (glyphosate [800 g ai ha^{-1}] + S-metolachlor [1100 g ai ha^{-1}]) 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) and are summarized in Table 2.

The field experiment was conducted in a randomized complete block design with four replications. The field experimental units were 3 m wide (4 corn rows) x 9 m long. The treatments consisted of 18 soil residual herbicides applied PRE plus a nontreated control (NTC). 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 (Teejet, Springfield, IL) nozzles 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 (0-5 cm depth) were collected from 14 residual PRE herbicide treatments (including herbicides with single and multiple sites of action - SOA) plus the nontreated control (NTC; Table 3) from field experiment conducted at the four site-years. Soil samples were collected at 0, 10, 20, 30, 40 and 50, 60, and 70 days after treatment (DAT) to evaluate cover crops response to herbicide residual over time. A handheld 6-cm-diameter soil sampler (Fiskars®, Middleton, WI) was used to collect the soil samples. At each sampling time, six soil cores were collected adjacent to the two central corn rows from each plot, combined, and placed in a plastic bag (~1000 g). Soil samples were stored in a freezer (-20 C) until the onset of the greenhouse bioassay experiment (approximately four months 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 additional 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 field-treated soil samples were used to perform the bioassay experiment (e.g., in the fall of 2021, greenhouse bioassays were conducted with the soil samples collected from Janesville-2021 and Lancaster-2021 field experiments; in the fall of 2022, greenhouse bioassays were conducted with the soil samples collected from Janesville-2022 and Lancaster-2022 field experiments). The bioassay experiment was conducted in the Walnut Street Greenhouse at the University of Wisconsin-Madison, Madison, WI. Each bioassay experimental unit consisted of a 210 cm^3 seed tray cell (6 cm length x 6 cm width x 5.9 cm depth; 804 Series T.O Plastics Inc., Clearwater, MN, USA). The four field soil samples from each treatment within a site-year and sampling time were thawed, combined across replications (creating a composite sample), and mixed to obtain eight uniform replicates (treated as 4 replications and 2 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. Seeds were sown in pots filled with soil (four replicates of 25 seeds each) and at 10 days after sowing 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. 2017) 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 per tray cell, which corresponded to 17, 34, 67, 101, 134, 168, 201, 235, 268, 302, 335, 369 plants m^{-2}. At 28 days after sowing (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 per cell for cereal rye and radish (134 plants m^{-2}), 10 plants for annual ryegrass (168 plants m^{-2}), and 18 plants per cell 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 separate experimental units (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 difference in day/night temperature and relative humidity in the greenhouses between the two experimental years was 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 16/8-h day/night photoperiod, using high-pressure sodium light bulbs (400-W) to supplement the natural light. The greenhouse environmental conditions were monitored throughout the experiment using a WatchDog® A150 logger (Spectrum Technologies, Aurora, IL, USA). 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, respectively, and 150 ppm of phosphorus. At 28 days after planting, 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, force-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 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 time.

After carefully exploring the bioassay data and observing different response trends across treatment combinations, 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 bioassays 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 treatments 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 “agricolae” package (Mendiburu 2022). The AUBS referred 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. 2020) and herbicide impact on biomass bioindicator species (Ribeiro et al. 2021). The AUDPS (herein 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 the last DAT. For instance, each biomass value was multiplied by 10 (interval between soil sampling) and the first and the 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 obtained, the lower the PRE herbicide injury on cover crops (Figure 2).

AUBS estimated values for each cover crop species by PRE herbicide treatments 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 (single, 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 fit 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 ($P \leq 0.05$).

Results and Discussion

Cover Crops Response

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 that there were negative correlations between visual injury rating values and biomass production for annual ryegrass, cereal rye, radish, and red clover at 28 DAS (Figure 3A). 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 3A). These results indicate that visual injury rating is associated with cover crop biomass production and suggests that higher visual injury occurred when lower biomass was produced; therefore, only the biomass data was 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 herbicides treatments and the nontreated control at all sampling times (Figure 3B). 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 discussions on the results from the field soil samples collected 30 and 70 DAT but the complete results are also available in Tables 5-8. Using the cover crop greenhouse bioassay data (28 DAS), we assumed a situation of interseeding at 30 DAT (~V3-V5 corn growth stage) and overseeding at 70 DAT (~V10-VT growth stage; Table 4). This decision was taken considering that cover crop interseeding (planted during the vegetative corn growth stage) in Wisconsin can be successful between V3-V7 corn growth stage (Smith and

Ruark 2022), and the overseeding is adopted just before or at corn maturity (Kladivko et al. 2014; Adler and Nelson 2020). 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 soi samples collected at 30 DAT; ~V3-V5 corn growth stage; Table 4) was reduced by most soil residual herbicides applied PRE compared to the NTC (Table 5). Atrazine + S-metolachlor (biomass = 0.005 g pot^{-1}), S-metolachlor (biomass = 0.013 g pot^{-1}), S-metolachlor + bicyclopyrone + mesotrione (biomass = 0.019 g pot^{-1}), atrazine + S-metolachlor + bicyclopyrone + mesotrione (biomass = 0.023 g pot^{-1}), atrazine + acetochlor (biomass = 0.061 g pot^{-1}), acetochlor (0.092 g pot^{-1}), and saflufenacil + dimethenamid-P (biomass = 0.097 g pot^{-1}) had the most detrimental impact on annual ryegrass when compared to the NTC (biomass = 0.419 g pot^{-1}) (Table 5). Clopyralid + acetochlor + mesotrione (biomass = 0.172 g pot^{-1}), acetochlor + mesotrione (biomass = 0.195 g pot^{-1}), and flumetsulam + clopyralid + acetochlor (biomass = 0.342 g pot^{-1}) resulted an intermediate negative impact on annual ryegrass (Table 5). Only mesotrione (biomass = 0.525 g pot^{-1}), flumetsulam + clopyralid (biomass = 0.547 g pot^{-1}), atrazine (biomass = 0.369 g pot^{-1}), and simazine (biomass = 0.369 g pot^{-1}) did not impact annual ryegrass biomass 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-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-V6 corn growth stage 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 (1790 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 (1960 g ai ha^{-1}) applied PRE in standard label rate resulted in less than 20% of annual ryegrass biomass reduction at the V5 stage, which was suggested to be acceptable levels 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 weeks after interseeding compared to the nontreated check (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-VT; Table 4), the most injurious PRE herbicides on annual ryegrass 28 DAS were S-metolachlor (biomass = 0.057 g pot^{-1}), S-metolachlor + bicyclopyrone + mesotrione (biomass = 0.057 g pot^{-1}), atrazine + S-metolachlor (biomass = 0.068 g pot^{-1}), and atrazine + S-metolachlor + bicyclopyrone + mesotrione (biomass = 0.096 g pot^{-1}) compared to the NTC (biomass = 0.472 g pot^{-1}). Saflufenacil + dimethenamid-P, atrazine + acetochlor, acetochlor, clopyralid + acetochlor + mesotrione, and flumetsulam + clopyralid + acetochlor caused intermediate impact on annual ryegrass biomass (0.259-0.365 g pot^{-1}). Annual rye was not injured by flumetsulam + clopyralid, simazine, atrazine, and mesotrione (biomass = 0.397-0.424 g pot^{-1}) compared to the NTC (Table 5). Validating these results, the AUBS analysis showed that S-metolachlor (AUBS = 2.07), atrazine + S-metolachlor (AUBS = 2.22), S-metolachlor + bicyclopyrone +

mesotrione (AUBS = 3.23), and atrazine + S-metolachlor + bicyclopyrone + mesotrione (AUBS = 4.83) provided the lowest area under biomass stairs values, which means these herbicides caused the highest injury to annual ryegrass throughout the soil sampling period. The highest AUBS values were observed for atrazine (AUBS = 32.48), flumetsulam + clopyralid (AUBS = 40.99), and mesotrione (AUBS = 42.08), soil residual herbicides applied PRE that did not injure annual ryegrass compared to the NTC (AUBS = 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-V5 corn growth stage; Tables 4 and 6). Cereal rye biomass was not reduced by clopyralid + acetochlor + mesotrione (biomass = 0.52 g pot^{-1}), saflufenacil + dimethenamid-P (biomass = 0.653 g pot^{-1}), atrazine (biomass = 0.669 g pot^{-1}), acetochlor + mesotrione (biomass = 0.722 g pot^{-1}), mesotrione (biomass = 0.791 g pot^{-1}), and flumetsulam + clopyralid + acetochlor ((biomass = 0.857 g pot^{-1}) compared to the NTC (biomass = 0.769 g pot^{-1}). Simazine (biomass = 0.559 g pot^{-1}), acetochlor (biomass = 0.570 g pot^{-1}), S-metolachlor + bicyclopyrone + mesotrione (biomass = 0.595 g pot^{-1}), atrazine + S-metolachlor + bicyclopyrone + mesotrione (biomass = 0.568 g pot^{-1}) resulted in intermediate injurious compared to the NTC. S-metolachlor (biomass = 0.358 g pot^{-1}), atrazine + S-metolachlor (biomass = 0.401 g pot^{-1}), and atrazine + acetochlor (biomass = 0.476 g pot^{-1}) were the most injurious PRE herbicides (Table 6).

For the overseeding scenario (field samples collected at 70 DAT; ~V10-VT; Table 4), none of the PRE herbicides tested herein negatively impact cereal rye biomass (0.495-0.666 g pot^{-1}) compared to the NTC (biomass = 0.530 g pot^{-1}; Table 6). The AUBS findings support the high cereal rye tolerance observed for biomass values (Table 6). Atrazine (AUBS = 51.85), mesotrione (AUBS = 61.71), acetochlor + mesotrione (AUBS = 54.56), saflufenacil +

dimethenamid-P (AUBS = 56.62), flumetsulam + clopyralid (AUBS = 63.11), flumetsulam + clopyralid + acetochlor (AUBS = 61.13), and clopyralid + acetochlor + mesotrione (AUBS = 54.75) did not negatively impact cereal rye compared to the NTC (AUBS = 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-V5 corn growth stage; Table 4) was negatively impacted by flumetsulam + clopyralid + acetochlor (biomass = 0.460 g pot^{-1}), flumetsulam + clopyralid (biomass = 0.531 g pot^{-1}), atrazine + S-metolachlor + bicyclopyrone + mesotrione (biomass = 0.531 g pot^{-1}), clopyralid + acetochlor + mesotrione (biomass = 0.643 g pot^{-1}), S-metolachlor + bicyclopyrone + mesotrione (biomass = 0.648 g pot^{-1}), acetochlor + mesotrione (biomass = 0.853 g pot^{-1}), mesotrione (biomass = 0.918 g pot^{-1}) saflufenacil + dimethenamid-P (biomass = 1.096 g pot^{-1}) compared to the NTC (biomass = 1.744 g pot^{-1}; Table 7). The soil residual herbicides applied PRE that contain only groups 5 and 15 (atrazine, simazine, acetochlor, S-metolachlor, atrazine + S-metolachlor, and atrazine + acetochlor) did not injure radish (biomass = 1.528-1.939 g pot^{-1}) compared to the NTC.

For the overseeding scenario (field samples collected at 70 DAT; ~V10-VT; Table 4), radish biomass was not reduced by atrazine + S-metolachlor (biomass = 1.621 g pot^{-1}), atrazine + acetochlor (biomass = 1.554 g pot^{-1}), S-metolachlor (biomass = 1.469 g pot^{-1}), simazine (biomass = 1.468 g pot^{-1}), acetochlor (biomass = 1.293 g pot^{-1}), atrazine (biomass = 1.227 g pot^{-1}), and saflufenacil + dimethenamid-P (biomass = 1.225 g pot^{-1}) compared to the NTC (biomass = 1.344 g pot^{-1}; Table 7). The remaining treatments reduced radish biomass (0.704-1.119 g pot^{-1}) compared to the NTC. For the AUBS, only S-metolachlor (AUBS = 155.66), acetochlor (AUBS = 148.29), and atrazine + S-metolachlor (AUBS = 135.95) were not different from the NTC (AUBS = 139.02). Atrazine + acetochlor, atrazine, and saflufenacil + dimethenamid-P, presented an intermediate AUBS (88.90-127.33), whereas the remaining treatments had the lowest AUBS (45.00-73.80).

Based on these results, applications of residual herbicides containing group 2, 4, and 27 evaluated in this study are likely to injure radish interseeded into corn at 30 DAT (V3 or V5 growth stages) and 70 DAT (V10-VT growth stages) due to the short interval between herbicide application and cover crop interseeding or overseeding. Mesotrione (group 27) and flumetsulam + clopyralid (group 2 + 4) herbicide labels list a 26- and 10-months rotational restriction for canola (*Brassica napus* L.; Anonymous 2022a and b), which belongs to the same family as radish and may have similar sensitivity. Brooker et al. (2020b) in a field experiment also 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-V6 stage compared to the NTC. In a greenhouse experiment, these same authors observed that group 27 (mesotrione [210 g ai ha^{-1}]) resulted >50% biomass reduction at rates less than field use rates; the authors did not observe radish stand and biomass reduction by 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. Previous study reported that fall-seeded radish was not negatively impacted by saflufenacil + dimethenamid-P (735 + 1470 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-V5 corn growth stage; Table 4) was negatively impacted by soil residual herbicides applied PRE that contain 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 (biomass = 0.253 g pot^{-1}; Table 8). S-metolachlor (biomass = 0.243 g pot^{-1}), acetochlor (biomass = 0.239 g pot^{-1}), saflufenacil + dimethenamid-P (biomass = 0.211 g pot^{-1}), and atrazine (biomass = 0.203 g pot^{-1}) did not negatively impact red clover biomass. The remaining treatments resulted in intermediate injury (biomass = 0.122-0.199 g pot^{-1}).

For the overseeding scenario (field samples collected at 70 DAT; ~V10-VT; Table 4), the PRE herbicides that contain 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 (biomass = 0.000-0.009 g pot^{-1}) compared to the NTC (biomass = 0.296 g pot^{-1}; Table 8). PRE herbicides that contain groups 5, 14, and 15 (atrazine, simazine, acetochlor, S-metolachlor, atrazine + S-metolachlor, and saflufenacil + dimethenamid-P) did not injure red clover (biomass = 0.0.251-0.354 g pot^{-1}) compared to the NTC. The remaining PRE herbicide treatments resulted in intermediate injury (biomass = 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 [AUBS = 7.07], flumetsulam + clopyralid + acetochlor [AUBS = 4.51], mesotrione [AUBS = 0.16], acetochlor + mesotrione [AUBS = 0.07], S-metolachlor + bicyclopyrone + mesotrione [AUBS

= 0.03], atrazine + S-metolachlor + bicyclopyrone + mesotrione [AUBS = 0.25], and clopyralid + acetochlor + mesotrione [AUBS = 0.07) compared to the NTC (AUBS = 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 (AUBS = 13.98), simazine (AUBS = 11.97), acetochlor (AUBS = 15.89), S-metolachlor (AUBS = 16.63), atrazine + acetochlor (AUBS = 13.04), and saflufenacil + dimethenamid-P (AUBS = 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 (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 plus S-metolachlor plus mesotrione applied PRE at a reduced rate (0.5×) compared to the nontreated check in silt-loam soil fields at corn V3 stage. Field studies conducted in silt-loam soils have shown that the half-life of mesotrione ranged 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 (Cornelius et al. 2018). Mesotrione (group 27) also lists 18 months rotational restriction for red clover (Anonymous 2022a), which can explain the high sensibility of red clover up to 70 DAT in our study. This rotational herbicide label restrictions only address potential crop injury and are independent of plant-back interval (PBI) restrictions established by the Environmental

Protection Agency (EPA) (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 crops AUBS analyzed by the number of SOAs showed that the higher the number of SOAs, the higher the injury, except for cereal rye (Figure 4). For annual ryegrass, the AUBS values followed the order of NTC (AUBS = 33.43), PRE herbicides with a single SOA (AUBS = 19.43), two SOAs (AUBS = 13.68), and three SOAs (AUBS = 9.92). Cereal rye AUBS for PRE herbicides with 3 SOAs (AUBS = 54.2) was not different from the NTC (AUBS = 55.4), whereas PRE herbicides with one SOA (AUBS = 46.7) and two SOAs (AUBS = 49.3) caused high injury to cereal rye compared to the NTC. All SOA numbers negatively impacted radish AUBS (AUBS = 112.8, 94.8, and 59.2 for a single, two, and three SOAs, respectively) compared to the NTC (AUBS = 139.0). The same was observed for red clover AUBS (AUBS = 9.82, 7.5, and 0.59 for a single, two, and three SOAs, respectively) compared to the NTC (AUBS = 18.94). The AUBS combined across species followed the same trend, where PRE herbicides with a single SOA (AUBS = 39.1), two (AUBS = 33.6), and three (AUBS = 22.5) negatively impacted the cover crops compared to the NTC (AUBS = 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 the chances of weed control success (Severo Silva et al. 2022, in review). 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

In summary, 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-V5 corn growth stage) and the overseeding scenario (field samples collected at 70 DAT; ~V10-VT). 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 only affected by 6 out of the 14 total PRE herbicides at 30 DAT and by none of the PRE herbicides at 70 DAT. The higher the number of SOAs in a premix, the higher the chances of injury for annual ryegrass, cereal rye, and red clover, except 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 support recommendations to growers interested in this system.

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Conflicts of Interest

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Table 1. Soil properties, corn hybrid, seeding rates for corn field experiments at Janesville and Lancaster, 2021 and 2022.

Site year	pH	OM [a]	Sand	Silt	Clay	Soil type	Corn hybrid	Seeding rate
		----------------%---------------						Seeds ha^{-1}
Janesville 2021	5.4	4.1	8	68	24	Plano silt loam	NK 9653-5222EZ [b]	87600
Janesville 2022	5.9	2.6	26	63	12	Plano silt loam	NK 9653-5222EZ [b]	87600
Lancaster 2021	6.6	2.5	10	76	14	Fayette silt loam	B97T04SXE [c]	80200
Lancaster 2022	5.3	4.1	18	65	18	Fayette silt loam	P9998Q-N802 [d]	80200

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. [a] OM: organic matter. [b] Brevant®, Indianapolis, IN 46268. [c] Syngenta®, Greensboro, NC 27419. [d] Pioneer®, Johnston, IA 50131. 2021-Janesville experimental field was fertilized with 200 kg ha^{-1} of nitrogen (46-0-0); Lancaster-2021: 128 kg ha^{-1} of nitrogen (46-0-0); 2022-Janesvile: 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); 2022-Lancaster: 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[a], and during the past 30 years[b].

	Janesville, WI			Lancaster, WI		
	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] 2021 and 2022 weather data were obtained from onsite weather stations.

[b] The 30-yr avg monthly (30 years monthly average) was obtained from the Wisconsin State Climatology Office (https://www.aos.wisc.edu/~sco/clim-history/acis_stn_meta_wi_index.htm

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

Herbicide (SOA)	Trade name	Manufacturer	Chemical Family	Half-life[a]	Rate
				days	g ai or ae ha^{-1}
atrazine (5)	AAtrex®	Syngenta Crop Protection[b]	Triazines	60	1120
simazine (5)	Princep® 4L	Syngenta Crop Protection[b]	Triazines	60	2240
acetochlor (15)	Harness®	Bayer CropScience[c]	α-Chloroacetamides	12	1960
s-metolachlor (15)	Dual II Magnum®	Syngenta Crop Protection[b]	α-Chloroacetamides	112-124	1791
mesotrione (27)	Callisto®	Syngenta Crop Protection[b]	Triketones	5-15	175
acetochlor (15) + mesotrione (27)	Harness® Max	Bayer CropScience[c]	α-Chloroacetamides +Triketones	-	1971 + 185
atrazine (5) + S-metolachlor (15)	Bicep Lite II Magnum®	Syngenta Crop Protection[b]	Triazines + α-Chloroacetamides	-	1310 + 1634
atrazine (5) + acetochlor (15)	Harness® Xtra	Bayer CropScience[c]	Triazines + α-Chloroacetamides	-	952 + 2408
saflufenacil (14) + dimethenamid-P (15)	Verdict®	BASF Corporation[d]	N-Phenyl-imides + α-Chloroacetamides	-	75 + 655
flumetsulam (2) + clopyralid (4)	Hornet® WDG	Corteva Agriscience[e]	Triazolopyrimidine + Pyridine-carboxylates	-	52 + 168
S-metolachlor (15) + bicyclopyrone (27) + mesotrione (27)	Acuron® Flexi	Syngenta Crop Protection[b]	α-Chloroacetamides + Triketone + Triketone	-	1602 + 45 + 179
atrazine (5) + S-metolachlor (15) + bicyclopyrone (27) + mesotrione (27)	Acuron®	Syngenta Crop Protection[b]	Triazines + α-Chloroacetamides + Triketones + Triketones	-	700 + 1498 + 42 + 168
flumetsulam (2) + clopyralid (4) + acetochlor (15)	Surestart® II	Corteva Agriscience[e]	Triazolopyrimidine + Pyridine-carboxylates + α-Chloroacetamides	-	42 + 133 + 1315

clopyralid (4) + acetochlor (15) + mesotrione (27)	Resicore®	Corteva Agriscience	Pyridine-carboxylates + α-Chloroacetamides + Triketones	-	133 + 1960 + 210

[a] Average field half-life of the herbicides, obtained from the WSSA Herbicide Handbook (10th ed.; Shaner 2014) and Pesticide Properties DataBase (PPDB 2022); [b] Greensboro, NC; [c] St. Louis, MO; [d] Durham, NC; [e] Indianapolis, IN.

Table 4. Corn planting and herbicide application dates for each site year and corn growth stage for each collection date in days after treatment.

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

DAT = days after treatment

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

	Annual ryegrass								
	Days after treatment in the field								
Treatment herbicide	0	10	20	30	40	50	60	70	AUBS
	Aboveground biomass 28 days after sowing the greenhouse bioassay (g pot^{-1})[a]								
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 (5)	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 (5)	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 (15)	0.020 gh	0.009 hj	0.060 fg	0.092 ef	0.156 def	0.160 e	0.317 cd	0.322 cd	12.19 e
S-MET (15)	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 (27)	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 (15) + MES (27)	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 (5) + S-MET (15)	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 (5) + ACET (15)	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 (14) + DIM-P (15)	0.019 gh	0.017 gh	0.074 efg	0.097 e	0.113 f	0.174 e	0.188 e	0.259 d	10.40 e
FLUM (2) + CLOP (4)	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 (15) + BIP (27) + MES (27)	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 (5) + S-MET (15) + BIP (27) + MES (27)	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 (2) + CLOP (4) + ACET (15)	0.106 de	0.093 e	0.162 cd	0.342 c	0.334 c	0.298 bcd	0.368 bc	0.365 bc	21.62 c
CLOP (4) + ACET (15) + MES (27)	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	0.38
P-value	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$

Abbreviations: AUBS = Area Under Biomass Stairs, ATZ = atrazine, SMZ = simazine, ACET = acetochlor, S-MET = S-metolachlor, IFT = isoxaflutole, MES = mesotrione, SAFL = saflufenacil, DIM-P = dimethenamid-P, FLUM = flumetsulam, CLOP = clopyralid, BIP = bicyclopyrone.

[a] Means within a column with the same letter are not significantly different according to Fisher's LSD test at $P \leq 0.05$.

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

	Cereal rye								
	Days after treatment in the field								
Treatment herbicide	0	10	20	30	40	50	60	70	AUBS
	Aboveground biomass 28 days after sowing the greenhouse bioassay (g pot^{-1})[a]								
Nontreated check	0.659 cd	0.692 bc	0.695 abc	0.769 abc	0.772 bc	0.687 bcd	0.623 b	0.530 cd	55.38 bc
ATZ (5)	0.510 e	0.492 ef	0.716 abc	0.669 bcd	0.768 bc	0.640 cd	0.698 ab	0.531 cd	51.85 cde
SMZ (5)	0.352 f	0.385 gh	0.529 e	0.559 de	0.618 e	0.730 abc	0.504 d	0.530 cd	43.91 f
ACET (15)	0.299 f	0.369 h	0.567 de	0.570 de	0.659 cde	0.655 cd	0.619 b	0.495 d	43.93 f
S-MET (15)	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 (27)	0.829 ab	0.889 a	0.767 ab	0.791 ab	0.752 bcd	0.689 bcd	0.748 a	0.557 bcd	61.71 a
ACET (15) + MES (27)	0.570 de	0.606 cde	0.651 bcd	0.722 bc	0.787 b	0.813 a	0.609 bc	0.549 bcd	54.56 bcd
ATZ (5) + S-MET (15)	0.307 f	0.223 j	0.385 f	0.401 fg	0.512 f	0.514 ef	0.520 cd	0.560 bcd	35.55 h
ATZ (5) + ACET (15)	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 (14) + DIM-P (15)	0.730 bc	0.602 cdef	0.712 abc	0.653 cd	0.771 bc	0.781 ab	0.661 ab	0.608 abc	56.62 b
FLUM (2) + CLOP (4)	0.926 a	0.816 ab	0.801 a	0.740 abc	0.869 ab	0.837 a	0.628 b	0.512 d	63.11 a
S-MET (15) + BIP (27) + MES (27)	0.492 e	0.487 fg	0.655 bcd	0.595 d	0.645 de	0.745 abc	0.634 b	0.537 cd	50.08 e
ATZ (5) + S-MET (15) + BIP (27) + MES (27)	0.541 de	0.570 cdef	0.616 cde	0.568 de	0.761 bc	0.599 de	0.694 ab	0.613 abc	51.11 de
FLUM (2) + CLOP (4) + ACET (15)	0.552 de	0.553 def	0.759 ab	0.857 a	0.945 a	0.827 a	0.764 a	0.666 a	61.13 a
CLOP (4) + ACET (15) + MES (27)	0.483 e	0.631 cd	0.695 abc	0.652 cd	0.854 ab	0.726 abc	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	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.01$	$P < 0.001$

Abbreviations: AUBS = Area Under Biomass Stairs, ATZ = atrazine, SMZ = simazine, ACET = acetochlor, S-MET = S-metolachlor, IFT = isoxaflutole, MES = mesotrione, SAFL = saflufenacil, DIM-P = dimethenamid-P, FLUM = flumetsulam, CLOP = clopyralid, BIP = bicyclopyrone.

[a] Means within a column with the same letter are not significantly different according to Fisher's LSD test at $P \leq 0.05$.

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

Radish									
	Days after treatment in the field								
Treatment herbicide	0	10	20	30	40	50	60	70	AUBS
	Aboveground biomass 28 days after sowing the greenhouse bioassay (g pot^{-1})[a]								
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 (5)	0.259 def	0.701 c	1.473 c	1.705 ab	1.871 bcd	1.675 bc	1.938 a	1.227 cd	114.06 e
SMZ (5)	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 (15)	1.934 a	2.088 a	1.965 a	1.914 a	1.985 abc	1.885 bc	1.530 bcd	1.293 bc	148.29 ab
S-MET (15)	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 (27)	0.128 fg	0.294 de	0.510 fg	0.918 cd	1.073 gh	1.163 def	1.358 de	1.119 de	69.69 g
ACET (15) + MES (27)	0.228 def	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 (5) + S-MET (15)	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 (5) + ACET (15)	0.691 b	0.932 bc	1.817 ab	1.827 ab	1.976 abc	1.919 abc	1.634 b	1.554 a	127.33 d
SAFL (14) + DIM-P (15)	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 (2) + CLOP (4)	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 (15) + BIP (27) + MES (27)	0.081 g	0.110 f	0.713 def	0.648 ef	1.285 fg	1.223 de	1.103 f	0.986 e	66.47 gh
ATZ (5) + S-MET (15) + BIP (27) + MES (27)	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 (2) + CLOP (4) + ACET (15)	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 (4) + ACET (15) + MES (27)	0.172 efg	0.191 ef	0.579 ef	0.643 ef	1.285 fg	1.131 def	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	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$

Abbreviations: AUBS = Area Under Biomass Stairs, ATZ = atrazine, SMZ = simazine, ACET = acetochlor, S-MET = S-metolachlor, IFT = isoxaflutole, MES = mesotrione, SAFL = saflufenacil, DIM-P = dimethenamid-P, FLUM = flumetsulam, CLOP = clopyralid, BIP = bicyclopyrone.

[a] Means within a column with the same letter are not significantly different according to Fisher's LSD test at $P \leq 0.05$.

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

	Red clover								
	Days after treatment in the field								
Treatment herbicide	0	10	20	30	40	50	60	70	AUBS
	Aboveground biomass 28 days after sowing the greenhouse bioassay (g pot^{-1})[a]								
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 abc	18.94 a
ATZ (5)	0.019 de	0.048 c	0.111 b	0.203 abc	0.158 ab	0.229 a	0.218 ab	0.290 bc	13.98 cd
SMZ (5)	0.007 e	0.008 e	0.027 e	0.168 c	0.186 ab	0.167 bcd	0.239 a	0.288 bc	11.97 e
ACET (15)	0.106 c	0.110 b	0.109 bc	0.239 ab	0.194 ab	0.188 abc	0.259 a	0.309 ab	15.89 bc
S-MET (15)	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 (27)	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 (15) + MES (27)	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 (5) + S-MET (15)	0.037 d	0.029 d	0.075 cd	0.199 bc	0.177 ab	0.177 bc	0.253 a	0.254 bcd	13.04 de
ATZ (5) + ACET (15)	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 (14) + DIM-P (15)	0.155 b	0.162 a	0.194 a	0.211 abc	0.151 b	0.156 d	0.208 ab	0.251 cd	15.88 bc
FLUM (2) + CLOP (4)	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 (15) + BIP (27) + MES (27)	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 (5) + S-MET (15) + BIP (27) + MES (27)	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 (2) + CLOP (4) + ACET (15)	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 (4) + ACET (15) + MES (27)	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	0.35
P-value	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$

Abbreviations: AUBS = Area Under Biomass Stairs, ATZ = atrazine, SMZ = simazine, ACET = acetochlor, S-MET = S-metolachlor, IFT = isoxaflutole, MES = mesotrione, SAFL = saflufenacil, DIM-P = dimethenamid-P, FLUM = flumetsulam, CLOP = clopyralid, BIP = bicyclopyrone.

[a] Means within a column with the same letter are not significantly different according to Fisher's LSD test at $P \leq 0.05$.

Figure Legends

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

Figure 2. Graphical example of clopyralid + acetochlor + mesotrione herbicide effect on annual ryegrass aboveground biomass production (28 days 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. 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.

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

Figure 4. Area under biomass stairs estimated for annual ryegrass, cereal rye, radish, and red clover, and combined across species by PRE herbicide sites of action (single, two, or three SOAs) over time in greenhouse bioassays using field-treated soil from Janesville 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 LSD, and herbicide treatments with the same letters are not different at $P \leq 0.05$.

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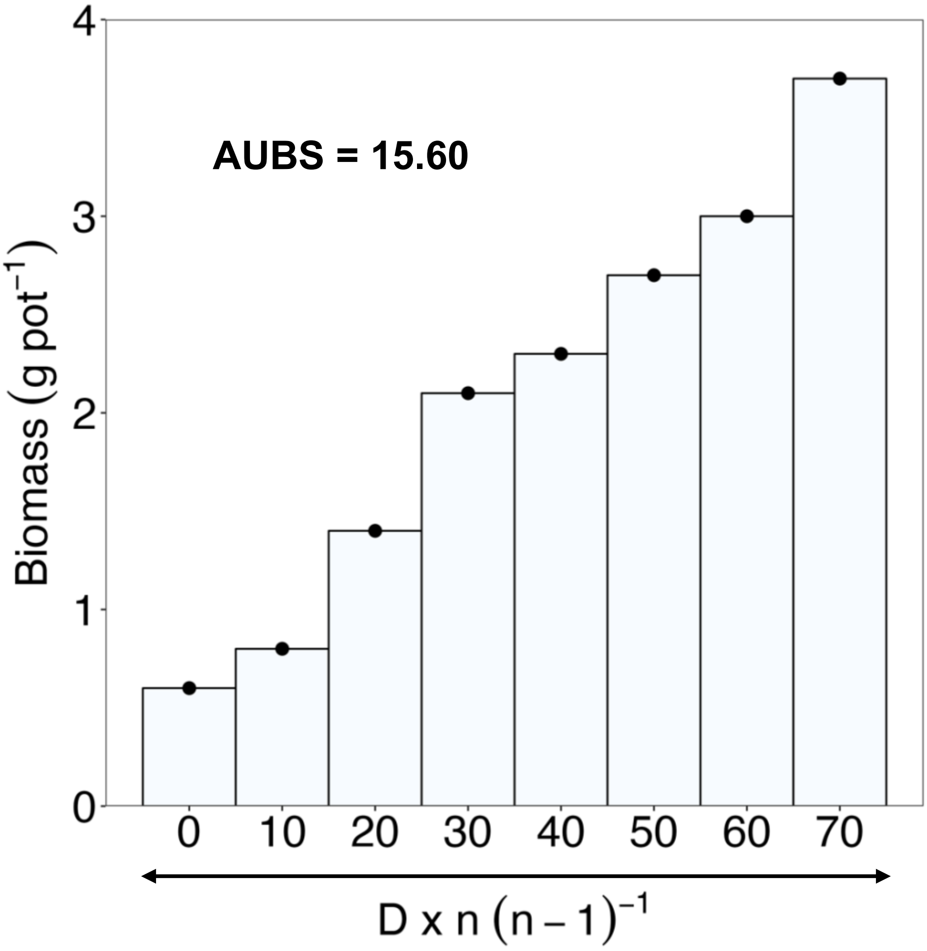

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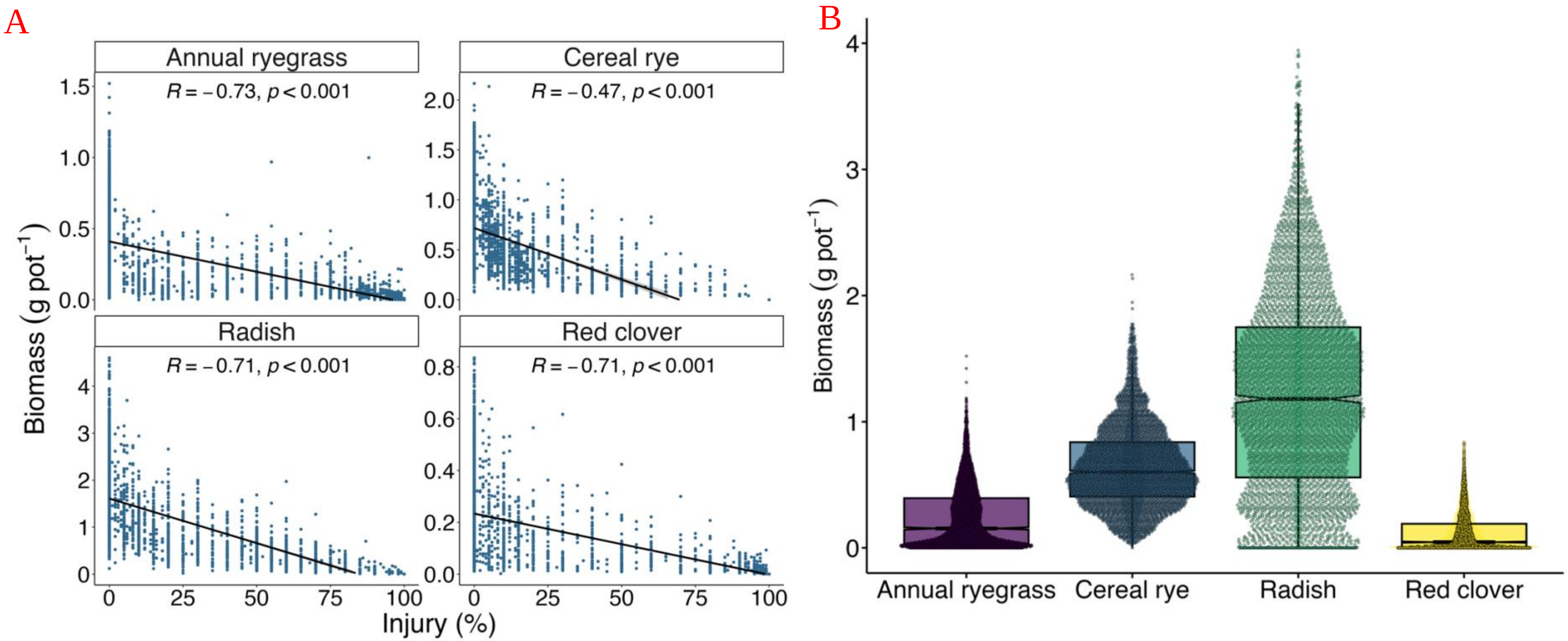

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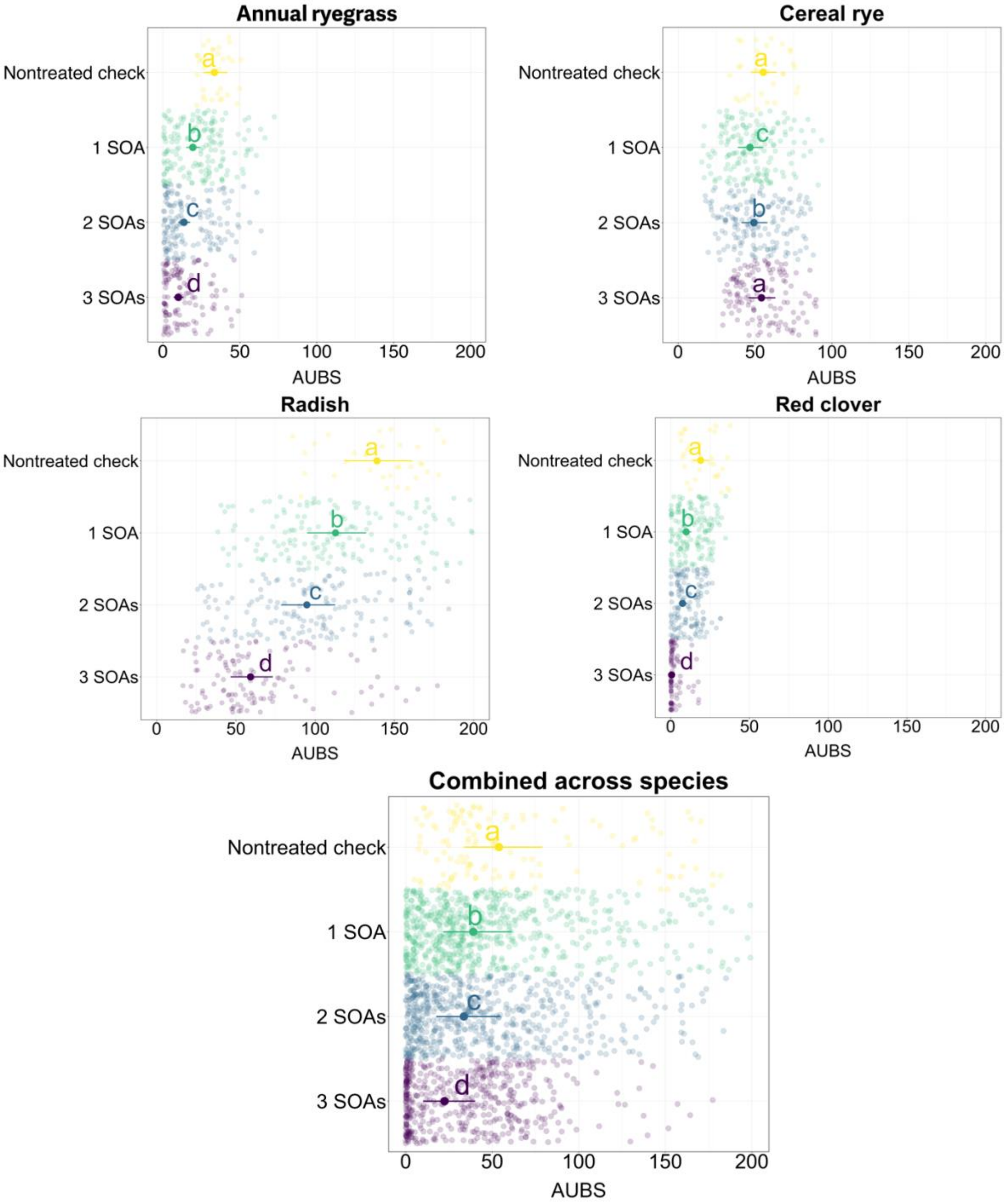

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limits. Means were compared using Fisher’s LSD, and herbicide treatments with the same letters are not different at $P \leq 0.05$.