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COVER CROP MANAGEMENT EFFECTS ON THE FATE OF SOIL-RESIDUAL HERBICIDES IN NO-TILL SYSTEMS

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ABSTRACT

Herbicide-resistant weed species in no-till crop production systems require innovative approaches for control. Integrated weed management that includes use of fall-sown cover crops has emerged as a valuable approach, where cover crops compete with weeds for essential resources during growth and surface mulch suppresses weeds after termination. The adoption of cover crops also has numerous soil health and conservation agriculture benefits. However, cover crops offer incomplete weed control that is not season long, and therefore requires integration with other herbicide-based tactics, such as use of soil-residual herbicide for more comprehensive weed control. Soil-residual herbicides applied preemergence extend the weed-free period, improve crop competitiveness, and slow resistance evolution to postemergence herbicides. Yet, integrating cover crops and herbicides pose several management challenges, including reduced soil bioavailability of residual herbicides to cover crop interference of spray deposition. Interference is likely amplified by the practice of planting green, which has gained popularity, due to greater cover crop biomass accumulation prior to herbicide application.

My thesis research aims to assess herbicide interception rates in various high-residue cover crop management scenarios to develop best management practices for integrating soil-residual herbicides and cover crops. My research also aims to more fully understand herbicide washoff patterns that result from interactions between cover crop biochemical properties and herbicide chemical properties. This research strives to improve the best management practices of residual herbicide programs integrated with high cover crop biomass, contributing to sustainable weed management practices in no-till cropping systems.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

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PROLOGUE

The overreliance on herbicide-based weed control has led to herbicide-resistant weed species in no-till crop production systems (Perotti et al., 2020). The use of gene stacking in transgenic crops is currently being used to allow for new use patterns of postemergence herbicides to manage herbicide resistant weeds. However, new stacked gene varieties provide only a short-term fix for delaying the evolution of weeds (Gressel et al., 2017; Mortensen et al. 2024).

Herbicide-resistant weeds have renewed interest in implementing integrated weed management (IWM) tactics, including physical, biological, and cultural control methods (Norsworthy et al. 2012b; Harker and O'Donovan 2013; Mortensen et al. 2024). Fall-sown cover crops are an increasingly valuable cultural IWM tactic (Teasdale and Daughtry 1993; Teasdale 1996; Teasdale et al. 2005b). Cover crops compete with weed populations for sunlight, space, soil moisture and nutrients during the period of active growth (Smith et al., 2015). After termination, cover crop biomass left on the surface in no-till cropping systems suppresses weed recruitment and growth through direct and indirect mechanisms (Teasdale et al. 2005b).

Adoption of cover cropping is increasing due to numerous soil conservation benefits, such as increased water infiltration, nutrient retention, and erosion control (CTIC, 2023). However, the use of cover crops as a cultural weed management tactic offers incomplete weed control and requires integration with preemergence or postemergence herbicide programs (Teasdale 1996; Norsworthy et al. 2012b). In a no-till cropping system, herbicide-based termination of cover crops, applied pre-plant or shortly after planting, often include residual herbicides in the tank mixture.

Residual herbicides applied at planting allow for a prolonged weed-free window, giving crops a competitive advantage over early emerging summer annual weed species (Nunes et al., 2018; Teasdale et al. 2005; Nunes et al. 2023). This makes it possible for crops to reach a size advantage and shade out weeds from getting established. By applying an effective residual herbicide program, producers have greater flexibility in the use or timing of postemergence applications. Residual herbicides also allow for diversification of herbicide sites-of-action, which allows for control of weed genotypes that are resistant to postemergence sites-of-action (Busi et al., 2020). Consequently, use of residual herbicides is an important tactic for delaying the evolution of resistance to a particular herbicide site-of-action.

One problem that has arisen with integration of cover cropping and herbicide tactics is the possible decrease in efficacy of the soil-activated residual herbicides due to cover crop interference (Khalil et al., 2018; Whalen et al. 2020; Nunes et al. 2023). Cover crops can create a physical barrier that prevents soil-activated preemergence herbicides from reaching and becoming activated in the upper soil profile where weeds germinate in no-till systems.

Planting green has become a more common practice within the Mid-Atlantic region in recent years as it aids in soil moisture and slug management (Reed et al., 2019). Planting green results in higher amounts of cover crop biomass compared to standard 14 to 21 d pre-plant burndown tactics. Greater cover crop biomass designed to create a more weed suppressive mulch in no-till will likely have significant impacts on herbicide deposition and is likely to increase when delaying cover crop termination to at or after planting. An incorporating rainfall event needs to occur in a timely manner following an herbicide application to activate herbicides (Khalil et al., 2018) but the interaction between cover crop biochemical properties and herbicide properties can result in different absorption rates when applied to living cover crops, or

adsorption and desorption rates when applied to surface mulch, which impacts soil bioavailability of herbicides (Grover & Cessna, 1990; Teasdale et al., 2003).

The objective of this thesis research is to: (1) quantify herbicide interception rates in high residue cover crop management scenarios to develop best management practices for integrating residual herbicides and cover crops; and (2) understand herbicide washoff patterns from cover crop residues after a precipitation event across different cover crop biochemical properties and herbicide active ingredients. This research will aid development of best management practices to increase the presence of a residual herbicide in the soil with high biomass accumulating cover crops.

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Chapter 1

Effects of Cover Crop Management and Herbicide Application Timing on Deposition and Washoff of a Soil-Residual Herbicide

INTRODUCTION

Advancements in herbicide-tolerant seed trait technology and the simplification of herbicide programs has resulted in many weed populations that have evolved resistance to multiple herbicide sites-of-action (Powles 2008). Integrated weed management (IWM) strategies that use non-chemical control methods to reduce herbicide inputs are necessary to slow resistance evolution and reduce environmental costs associated with increased herbicide use in the past decade (Norsworthy et al. 2012; Harker and O'Donovan 2013; Mortensen et al. 2024).

In no-till systems, cover crop adoption is increasing (Zhou et al. 2022) due to potential soil conservation benefits, which result from increased water infiltration, nutrient retention, and erosion control (Locke et al., 2015; Schipanski et al. 2014). Cover crops also offer a cultural means of weed suppression, given their ability to directly compete for resources with weeds that have an overlapping life cycle and to suppress weeds through several mechanisms when left on the surface as a mulch (Teasdale and Daughtry 1993; Teasdale et al. 2003; Teasdale et al. 2005). Interest has grown in increasing weed suppression potential from cover crops by delaying termination until after or at cash crop planting, often referred to as 'planting green', which can allow for greater biomass accumulation compared to standard management recommendations to terminate a cover crop 14-21 prior to planting (Reed et al. 2019; Mohler and Teasdale 1993; Finney et al. 2016). Planting green can also improve planting conditions in wet springs due to increased transpiration loss from growing cover crops (Reed et al. 2019). However, delaying

cover crop termination can remove early season moisture from field and impact crop establishment when planters are not equipped with adequate residue management tools.

Herbicide-based weed control in no-till production systems commonly employs a two-pass herbicide program that includes foliar preplant nonselective herbicide products and preemergent (PRE) soil-residual herbicides near planting, followed by a second herbicide application at early crop growth stages (POST) to control weed escapes that emerge after residual herbicides have dissipated below phytotoxic levels. Use of PRE soil-residual herbicides is considered a best management practice (BMP) in conservation agriculture (Norsworthy et al. 2012). By applying an effective residual herbicide program, producers reduce weed crop competition during the critical period of weed control and typically have greater flexibility in the use or timing of POST applications. Residual herbicides also allow for diversification of herbicide sites-of-action, which allows for control of weed genotypes that are resistant to POST herbicide sites-of-action (Busi et al., 2020). Consequently, use of residual herbicides is an important tactic for delaying the evolution of resistance to a particular herbicide site-of-action.

Integrating the use of planting green practices and soil-residual herbicides has the potential to further diversify weed management, which reduces the risk of weed control failures (Liebman and Gallandt 2017) and likely slows evolution of resistant biotypes. However, there is currently limited knowledge of how cover crop management practices impact the bioavailability of soil-residual herbicides in planting green systems due to high cover crop biomass accumulation. Several cover crop management factors influence deposition and washoff processes that ultimately influence soil bioavailability of residual herbicides, including (1) total surface mass and leaf area index among cover crop species (Teasdale and Mohler 2000), (2) use of residue management tools (i.e., roll-crimper) that create a surface mulch (Mirsky et al. 2013); and (3) biochemical properties of decomposing cover crop residues at the time of herbicide application.

Herbicide deposition and washoff processes. Cover crops create a physical barrier that prevents soil-residual herbicides from reaching the upper soil profile where weeds germinate in no-till cropping systems (Khalil et al. 2019; Sperry et al. 2022). Precipitation is needed to washoff intercepted herbicides from cover crop residues and activate residual herbicides in the soil (Khalil et al. 2018). Studies suggest that at least 1.25 cm of rainfall is needed to maximize herbicide washoff from the cover crop residue (Khalil et al. 2018; Khalil et al. 2019). In a planting green scenario, herbicide absorption into plant membranes of a living cover crop may reduce washoff potential (Grover & Cessna, 1990; Teasdale et al., 2003). Absorption of herbicides into living plant cells can result in herbicide translocation and metabolism processes, and thus a loss of weed control potential (Shimabukuro 1985).

If soil-residual herbicides are applied after a cover crop has been terminated, sorption of residual herbicides to cover crop residue is dependent on the cover crop decomposition stage (Whalen et al. 2020). The easily decomposable non-structural carbohydrates and hemi-cellulose of cover crops decompose quickly while the lignin and cellulose portions of the cover crop are slower to decompose(Thapa et al. 2022). This explains the mass reduction of the cover crop surface mulch. Higher relative humidity, temperatures, and frequency and amount of precipitation effect the decomposition rate of cover crop surface mulch (Thapa et al. 2022). Studies suggest that as cover crop biomass decompose, the sorption rate of herbicides increases as there are more free carbons in the cover crop mulch (Reddy et al. 1995).

Cover crop properties. Higher biomass accumulation associated with planting green can increase weed suppression potential (Ficks et al. 2022; Nunes et al. 2023), but also increases herbicide interception (Teasdale et al. 2005). Cover crops terminated at earlier growth stages are correlated with lower quantities of biomass but also represent different ratios of leaf to stem than later growth stages (Mirsky et al. 2009).

Cover crop species also differ in ecosystem service provisioning (Starovoytov et al. 2010; Schipanski et al. 2014). Some producers plant grass-legume cover crop mixtures to balance nitrogen scavenging and fixation potential prior to corn (Finney et al. 2016). Grass and legume cover crop species vary in biomass potential but also leaf area index, which effects the interception of soil activated herbicides onto the soil surface. Teasdale and Mohler (2000) quantified the relationship between these physical properties (i.e., Mass Area Index; MAI) and light penetration to the soil surface. They observed that legume cover crops had less light penetration through the cover crop canopy to the soil surface compared to cereal rye. Light penetration through surface mulch is likely positively correlated with herbicide deposition.

Residue management effects. In conventional no-till production systems, broad spectrum foliar herbicides are the primary means for cover crop termination. Roll-crimping has increased as a residue management tool when higher-biomass levels are achieved. Roll-crimping also allows for lower boom height at application timing, which has been correlated with better herbicide deposition (Simão et al. 2020). Roll-crimped surface residues may alter herbicide interception by the cover crop relative to allowing the cover crop to remain standing after termination. Little is known about soil-residual herbicide interactions with roll-crimped residues, as most studies have focused on soil-residual herbicide interactions with dead cover- or cash-crop residues under varying decomposition stages.

In this study, we quantify soil-residual herbicide deposition across varying cover crop, residue management, and herbicide application timing scenarios to develop best management practices for integrating soil-residual herbicides and cover crops. A second objective was to understand herbicide washoff patterns from cover crop residues among these scenarios. This research will aid development of management tactics to increase the efficacy of a soil-residual herbicides when integrated with cover crop surface mulch in no-till systems.

MATERIALS AND METHODS

Experimental locations. Field experiments were conducted at the Pennsylvania State University Russell E. Larson Agricultural Research Center (RELARC) near Rock Springs, PA, the University of Delaware's Research and Education Center (UDREC) near Georgetown, DE, and the Virginia Agricultural Experiment Station (VAES) Kentland Farm near Blacksburg, VA in the 2022 and 2023 growing season for a total of six site-years. Experimental sites differed in soil texture, including Hagerstown silt loam at RELARC, Hammonton loamy sand soil at UDREC, and Ross loam at VAES.

Experimental design. Experiments were designed as a two-factor complete block and arranged in a split-plot treatment structure with four replicates. Treatments were randomized at the main plot and split plot level. Main plot treatments were cover crop management tactic (CC) with six treatment levels and the split-plot treatment was herbicide application timing (HAT) with two treatment levels. Main plot size was 24 by 3 m and split plots were 12 by 3 m. Main plot treatments included cereal rye (*Secale cereale* L.) terminated and left standing at the (1) flag leaf stage (Zadoks 37-39); (2) heading stage (Zadoks 51-59); and (3) and anthesis stage (Zadoks 65-69); and cover crops terminated after roll-crimping at the anthesis stage (Zadoks 65-69), including a (4) cereal rye monoculture; (5) cereal rye and crimson clover (*Trifolium incarnatum*) mixture; and a (6) cereal rye and hairy vetch (*Vicia villosa*) mixture. Cereal rye was seeded at 67 kg ha^{-1} in monoculture treatments and at 33 kg ha^{-1} in legume mixtures. Hairy vetch and crimson clover were seeded at 33 kg ha^{-1}.

Table 1-1. Main and split plot treatments (n=12). Main plot treatments consist of cover crop species, growth stage of cereal cover crop was terminated at, and orientation of cover crop. Split plot treatments consist of pyroxasulfone application timing relative to cover crop termination.

Main Plot Treatment	Split Plot Treatment	
	0 DAT	EPOST
Cereal rye, flag leaf, standing	X	X
Cereal rye, heading, standing	X	X
Cereal rye, anthesis, standing	X	X
Hairy vetch/cereal rye, anthesis, roll-crimped	X	X
Crimson clover/cereal rye, anthesis, roll-crimped	X	X
Cereal rye, anthesis, roll-crimped	X	X

Split plot treatments included the test residual herbicide, pyroxasulfone, applied (1) at cover crop termination (0 days after termination; 0 DAT) or (2) 21 days after cereal rye anthesis, simulating an early post emergence application (EPOST). Pyroxasulfone (0.15 kg ai ha^{-1}) was used as the test residual herbicide treatment because it can be applied PRE and EPOST in both corn and soybean production. Pyroxasulfone was tank-mixed with glyphosate (1.27 kg ai ha^{-1}) and AMS (2.5% v/v) in 0 DAT treatments. For EPOST split-plot treatments, glyphosate and AMS were applied without pyroxasulfone at the appropriate cereal rye termination stage (boot, heading, anthesis) in monoculture treatments and 2,4-D LVE (0.56 kg ae ha^{-1}) was included in the tank-mix for legume mixtures.

Cover crops were established in the fall preceding the growing season using a 3.3 m no-till grain drill with 19 cm row spacing. No-till practices were used in the previous soybean crop. Roll-crimping was performed using a 3-m unit with chevron pattern. A single pass in the hydraulic float position was used for treatments requiring roll-crimping.

Petri dish assay. A bulk collection of soil cores was collected to a depth of 7.6 cm at each location from the experimental field in late spring each year. Soil cores were air dried and passed through a 2 mm sieve. Dried, sieved soil was then weighed in 50 g increments and placed in 9 cm diameter Petri dishes.

At each herbicide application timing, two Petri dishes were placed 0.75-m from the middle of the plot within two adjacent cover crop inter-rows. Location was selected based on uniformity and species composition of the stand. For early growth stages, a flag was used to denote Petri dish placement to prevent foot traffic influencing assays at the time of herbicide application. Petri dishes were placed directly on the soil surface to mimic field surface conditions. Within approximately 1 h of Petri dish placement, the residual herbicide treatment was applied using a CO_2 pressurized backpack sprayer and 3 m boom designed with 51 cm nozzle spacing, equipped with 110015 AIXR (TeeJet) nozzles, and a carrier rate of 140 L ha^{-1}. Weather data was collected at each residual herbicide application timing, including the three cereal rye cover crop growth stages (flag leaf, heading, anthesis) application timings and the EPOST timing (21 days after anthesis).

Cover crop residue was allowed to dry for two hours after the application. Each pair of Petri dishes was then collected and replaced with a new Petri dish. The second pair of Petri dishes were collected after the cumulation of 12.5 mm precipitation, which is considered an adequate incorporating rainfall for weed control. Cover crop biomass was then collected in the area of Petri dish assays using a 65 by 38 cm quadrat, which encompassed three seeded rows and the two inter-row spaces where Petri dishes were placed. Cover crop biomass was oven dried at 65 C for 5-7 days and weighed. Following their collection, Petri dishes were paired and placed in freezer bags stored in a -7 C freezer to prevent microbial degradation.

Following the completion of field assays, samples were removed from the freezer and allowed to thaw for 12 hr. Paired samples (50 g) were emptied from the Petri dishes and homogenized (100 g). A 15 g sub-sample was placed in a weighed 50 mL falcon tube and the wet soil weight was recorded. Acetone (40 mL) was added to each falcon tube as a solvent to remove herbicide from the soil sample. The falcon tubes were capped tightly and placed on a shaker table overnight (14 hr). A 3 mL syringe and 0.45 µm filter was then used to extract 1 mL of solution,

which was placed in a 1.5 mL glass vial, capped, and stored in the -7°C freezer. Falcon tubes containing the remaining solvent and soil were re-capped and stored until completion of the filtering, at which time they were uncapped and placed in a fume hood to allow the solvent to evaporate. Dried soil was weighed and used to correct the sample dry weight in herbicide concentrate calculation.

Preliminary tests were conducted with known concentrations of differing herbicide active ingredients mixed with soil using different solvents (acetone, methanol) to observe the differences in percentage recovery. In the solvents tested, acetone (Fisher Scientific, Waltham, MA, USA) optimized herbicide recovery from the soil particles. Deposition, washoff, and no cover crop control standards were analyzed as data. We analyzed pyroxasulfone concentrations using a Dionex™ ICS-6000^{+} HPIC™ System (ThermoFisher, Sunnyvale, CA, USA) coupled with a Q Exactive Oribtrap™ mass spectrometer (ThermoFisher, Bremen, Germany) through heated electrospray injection (HESI) in positive ion mode (see *Supplemental Information*).

Statistical Analysis. Data was subset and analyzed in two ways to address hypotheses. First, data were subset by treatments that included cereal rye monocultures that were left standing (n = 3) to isolate on cereal rye biomass and phenology stage effects on (1) residual herbicide deposition expressed as a proportion of the control (no cover crop); (2) residual herbicide total recovery [deposition + washoff] expressed as a proportion of the control, and the (3) washoff proportion of total recovery [washoff / total recovery]. Data were then subset by treatments that included cover crops terminated at the cereal rye anthesis stage (n = 4) to contrast residue management and cover crops species effects on residual herbicide deposition, total recovery and washoff proportion under higher biomass levels.

Total recovery and washoff proportion of total response variables were analyzed using generalized linear mixed-effects models (GLMMs) with a beta distribution (glmmTMB package; Brooks et al. 2017) in R (R Core Team, 2022). Use of a beta distribution within GLMMs allow

for analysis of continuous proportion data using the original scale, thereby producing less biased estimates relative to transformation-based statistical approaches (Douma and Weedon, 2019).

Total recovery and washoff proportion of total response variables were first modeled as categorical data, with cover crop treatment (n = 3; CC), herbicide application timing (n = 2; HAT), site-year (n = 5) and their two- and three-way interactions as fixed effects. Block and main plots nested within block were fit as random effects. Due to an incomplete dataset, the Virginia 2022 site-year was removed from models analyzing cereal rye phenological effects on deposition and total recovery.

Next, data were subset into standing cereal rye treatments (n = 3) and roll-crimped treatments (n = 3). Deposition and total recovery response variables were then modeled using beta-regression, where main effects included cover crop biomass (BIO) as a continuous variable, herbicide application timing (HAT), and their interaction (BIO x HAT) were fit as a fixed factors and site-year was fit as a random factor.

The significance of fixed effects in all models were evaluated using log-likelihood ratio tests (Wald χ^2) to compare full versus reduced models using the *anova* function. Post hoc comparisons at either the main effect level or two- and three-way interactions for ANOVA models were conducted using Tukey's contrasts within the 'emmeans' function (Lenth 2024). Model fit of beta-regression models were assessed using the marginal (R^2_m) and conditional (R^2_c) coefficient of determination (Nakagawa and Schielzeth 2012).

RESULTS

Site-year variation in incorporating rainfall events. The total amount of precipitation (mm) and duration (d) to collection of Petri-dishes varied widely among site-years (**Table 1-2**), including precipitation events that exceeded the 12.5 mm precipitation target and site-years where drought conditions either lengthened time to collection or prevented the precipitation threshold target from being reached. It is likely that total precipitation and duration to collection influenced variation in the washoff potential among sites and between years within sites but could not be controlled using our field-based assay approach.

Studies suggest that longer time lapse between herbicide application and precipitation exposes the herbicide to photodegradation, volatilization, and microbial activity (Khalil et al. 2019). In 2022, precipitation (>12.5 mm) occurred between 1 to 6 days after herbicide application among locations (**Table 1-2**). In comparison, necessary precipitation occurred between 1 to 25 days and varied considerably in amount within the 2023 growing season due to dryer spring conditions (**Table 1-2**).

Table 1-2. Total cumulative precipitation (mm) and days (d) between placement and collection of washoff Petri-dish assays by site-year and residual herbicide application timing (flag leaf, heading, anthesis, EPOST).

	Herbicide application timing							
	SECCE-flag leaf		SECCE-heading		SECCE-anthesis		EPOST	
site-year	mm	d	mm	d	mm	d	mm	d
DE22	12.7	0	17.8	0	20.3	0	12.7	1
DE23	10.7	1	12.7	1	12.7	1	12.7	1
PA22	16.5	1	10.2	3	12.7	1	11.4	6
PA23	25.4	25	25.4	14	25.4	9	19.1	6
VA22	2.5	10	2.5	1	3.8	5	13.0	14
VA23	44.5	2	17.8	4	70.4	12	16.3	10

Abbreviations: DE, Delaware; PA, Pennsylvania; VA, Virginia; SECCE, *Secale cereale* (cereal rye); EPOST, early postemergence application

Site-year variation in cover crop biomass. In analysis of cover crop biomass within cereal rye monocultures left standing, significant site-year interactions with cover crop treatment ($\chi^2 = 38$; $p < 0.001$) and residual herbicide application timing ($\chi^2 = 14.0$; $p = 0.007$) were observed. From the flag leaf to anthesis stage, cereal rye biomass increased from 1.3 to 2.5 and 2.0 to 3.6 Mg ha^{-1} in Delaware in 2022 and 2023, respectively, 3.2 to 6.0 and 3.1 to 6.2 Mg ha^{-1} in Pennsylvania, and 3.1 to 6.1 Mg ha^{-1} in Virginia (**Figure 1-1**). Mass loss between the planting green and EPOST application timing was observed among all locations, but significant differences were only detected in Pennsylvania in 2022 (17%) and Delaware in 2023 (25%).

Total cover crop biomass (Mg ha^{-1}) within treatments terminated at the cereal rye anthesis stage varied by residual herbicide application timing ($\chi^2 = 13.6$; $p < 0.001$) and site-year ($\chi^2 = 201$; $p < 0.001$; **Figure 1-2**). No significant difference in amount of biomass accumulated at anthesis timing among standing or roll-crimped cereal rye monocultures and roll-crimped legume-grass mixtures was observed ($\chi^2 = 6.5$; $p = 0.09$). Mean biomass was 5.0 and 4.1 Mg ha^{-1} within the planting green and EPOST treatments, respectively. Consequently, the mean reduction in total biomass (i.e., mass loss) between planting green and EPOST was 19%. The difference in mean cover crop biomass among the six site-years (DE, 1.9 and 3.3 Mg ha^{-1}; PA, 4.9 and 5.0 Mg ha^{-1}; VA, 5.5 and 6.1 Mg ha^{-1}) allowed for the development of regression models to correlate herbicide recovery with total cover crop biomass accumulation.

The proportion of total biomass attributed to legume species with mixtures varied considerably among locations and across years within locations. In Pennsylvania, crimson clover was <1% of mixture in 2022, but 87% in 2023 due to poor cereal rye establishment. Crimson clover in Delaware represented 76% and 29% of the mixture in 2022 and 2023, respectively, and 11% and 3% of the mixture in Virginia in 2022 and 2023, respectively. Hairy vetch represented 3 to 16% in Pennsylvania, 68 to 28% in Delaware, and 12 to 8% in Virginia in the 2022 and 2023 growing seasons, respectively.

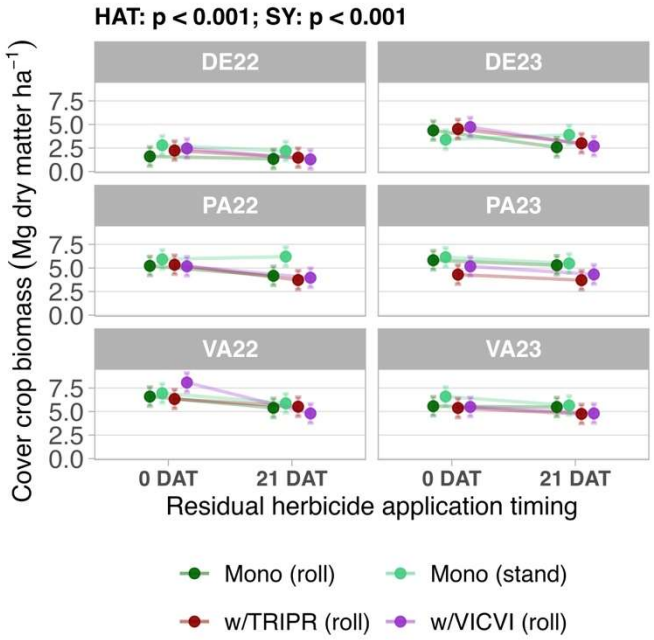

Figure 1-1. *Mean (95% CI) cover crop biomass (Mg ha^{-1}) by treatment, residual herbicide application timing, and site-year, which differed by herbicide application timing (HAT) and site-year (SY). Herbicide application timings include 0 and 21 days after cover crop termination (DAT). Cover crop treatments include cereal rye monocultures (Mono) left standing or roll-crimped (roll) and roll-crimped cereal rye mixtures with crimson clover (TRIPR) or hairy vetch (VICVI).*

Standing cereal rye management scenario. A three-way interaction between cover crop treatment, herbicide application timing and site-year ($p = 0.002$) was observed in analysis of total residual herbicide recovery within standing cereal rye. Total herbicide recovery averaged across

cereal rye growth stages ranged from 45 to 75% among locations within the 0 DAT treatment and 32 to 82% within the 21 DAT. In four of five site-years, total recovery did not differ among cover crop treatments within the 21 DAT treatment level but did differ within the 0 DAT treatment level (**Supplemental Table 1-2**). Within the flag leaf treatment, total recovery did not differ between herbicide application timings at each site-year, whereas herbicide application timing effects within both heading and anthesis treatments differed depending on site-year.

A two-way interaction between herbicide application timing and site-year ($\chi^2 = 11.0$; $p = 0.03$) was observed in analysis of the proportion of total residual herbicide recovery that was attributed to the washoff process, which ranged from 15 to 33% among site-years (**Supplemental Table 1-2**). In four of five site years, herbicide application timing did not affect the washoff proportion of total recovery but was significantly greater in the 21 DAT treatment (41%) compared to 0 DAT (16%) in Virginia (2023).

Increasing biomass levels across cereal rye growth stages resulted in a significant decrease in deposition and total recovery of residual herbicides (**Table 1-3**). Fitting this relationship by herbicide application timing, allowing for varying intercepts and slopes, did not improve models of herbicide deposition (**Table 1-3**; **Figure 1-2a**) but a significant interaction between cover crop biomass and herbicide application timing was observed in models of total herbicide recovery (**Table 1-3; Figure 1-2c**).

Table 1-3. *Results of four fitted beta-regression mixed models, including fixed effects of cover crop biomass (BIO), herbicide application timing (HAT) and their interaction on deposition (%) and total recovery (%) within treatments imposed in cereal rye monocultures left standing (n = 3) and treatments terminated at the anthesis stage (n = 4).*

	Beta regression fixed effects			
Response variables[1]	BIO	HAT	BIO*HAT	R^2_m
	---------- Wald χ^2 (*p*-value) ---------			
Deposition / standing treatments	5.8*	0.1	0.2	0.44
Total recovery / standing treatments	3.2*	0.9	3.2*	0.41
Deposition / roll-crimped treatments	13.9***	0.5	2.5	0.87
Total recovery / roll-crimped treatments	8.7**	0.1	4.3*	0.64

[1] Mixed model beta regression use site-year (n = 5) as a random intercept term; site-years differ by models of standing treatments (n = 5) and anthesis treatments (n = 6);[2] R^2_m, marginal coefficient of determination; *, p < 0.10; **, p < 0.01, ***, p < 0.001

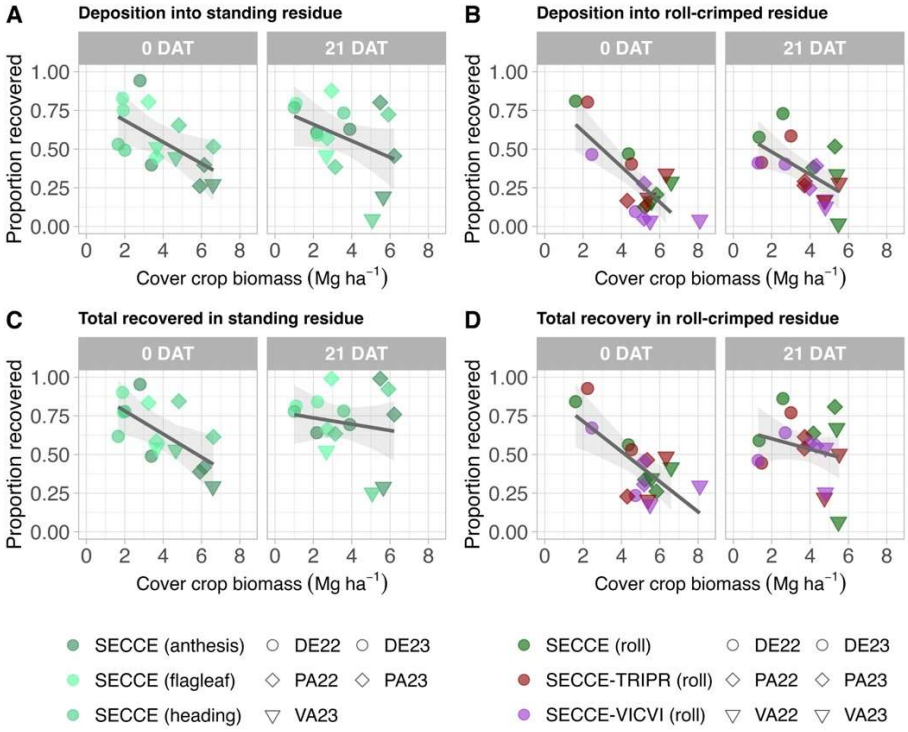

Figure 1-2. *Effect of cover crop biomass by herbicide application timing on deposition of residual herbicides, expressed as a proportion of the control, into (**A**) cereal rye left standing at three growth stages (flag leaf, heading, anthesis) and (**B**) roll-crimped cover crops terminated at the anthesis stage (cereal rye, cereal rye/ crimson clover mixture, cereal rye/ hairy vetch mixture); and total herbicide recovery, expressed as a proportion of the control, into (**C**) cereal rye left standing at three growth stages (**D**) roll-crimped cover crops terminated at the anthesis stage. Points denote mean estimates (n = 4) by cover crop treatment (color) and site-year (shape).*

Roll-crimping management scenario. In analysis of treatments terminated at the cereal rye anthesis stage (n = 4), a significant main effect of cover crop treatment ($\chi^2 = 9.8$; $p = 0.02$) was observed, where roll-crimping a cereal rye– hairy vetch mixture resulted in lower pyroxasulfone recovery compared to both cereal rye monoculture treatments (**Figure 1-3**). Roll-crimping did not significantly decrease pyroxasulfone recovery compared to cereal rye monocultures left standing. Total pyroxasulfone recovery averaged across location and herbicide application timing ranged from 42 to 61%.

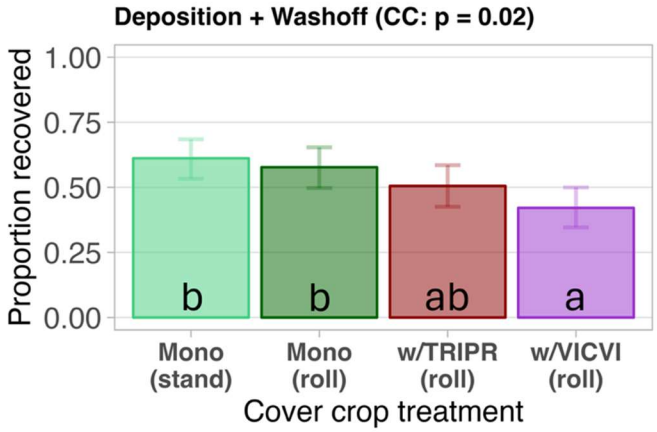

Figure 1-3. *Mean (95% CI) total herbicide recovery, expressed as the proportion recovered compared to the no cover crop control, among cover crop treatments terminated at the cereal rye anthesis stage, including cereal rye monocultures (mono) left standing (stand) or roll-crimped (roll) and roll-crimped cereal rye-legume mixtures, including with crimson clover (TRIPR) or hairy vetch (VICVI). Means are averaged across site year (n = 6) and herbicide application timing (n = 2).*

The effect of herbicide application timing on total pyroxasulfone recovery varied by site-year ($\chi^2 = 56.4$; $p < 0.001$; **Figure 1-4**). Higher total pyroxasulfone recovery was observed at the EPOST timing compared to planting green (0 DAT) in Pennsylvania in 2022 and 2023 and Delaware 2023, whereas no application timing effects were observed in Virginia 2022 and 2023

site-years. In a single site-year (Delaware 2022), there was a decrease in total recovery for the EPOST timing compared to planting green.

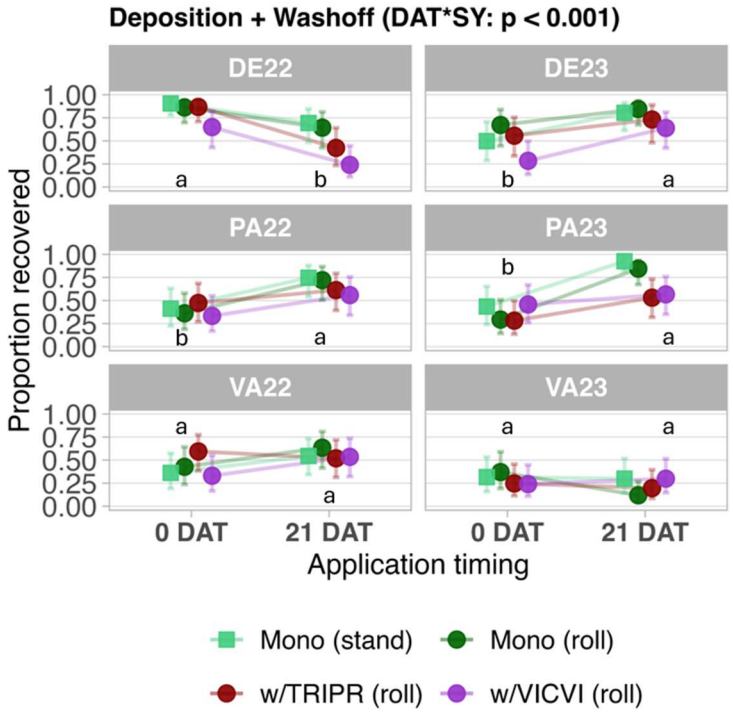

Figure 1-4. *Mean (95% CI) total herbicide recovery, expressed as the proportion recovered compared to the no cover crop control, by cover crop treatment, herbicide application timing and site-year(SY). Cover crop treatments include cereal rye monocultures (mono) left standing (stand) or roll-crimped (roll) and roll-crimped cereal rye-legume mixtures, including with crimson clover (TRIPR) or hairy vetch (VICVI). Residual herbicide application timing treatments including 0 and 21 days after cover crop termination (DAT), which occurred at the cereal rye anthesis stage. Means are averaged across replicates (n = 4).*

A two-way interaction between herbicide application timing and cover crop treatment (χ^2 = 8.2; p = 0.04) was observed in analysis of the proportion of total residual herbicide recovery that was attributed to the washoff process, which ranged from 25 to 65% among site-years (**Figure 1-5**). Pyroxasulfone application at 0 DAT within roll-crimped cereal rye– hairy vetch mixtures resulted in higher a higher proportion of total recovery coming from the washoff process compared to other treatments, but no differences among cover crop treatments were observed at the 21 DAT treatment.

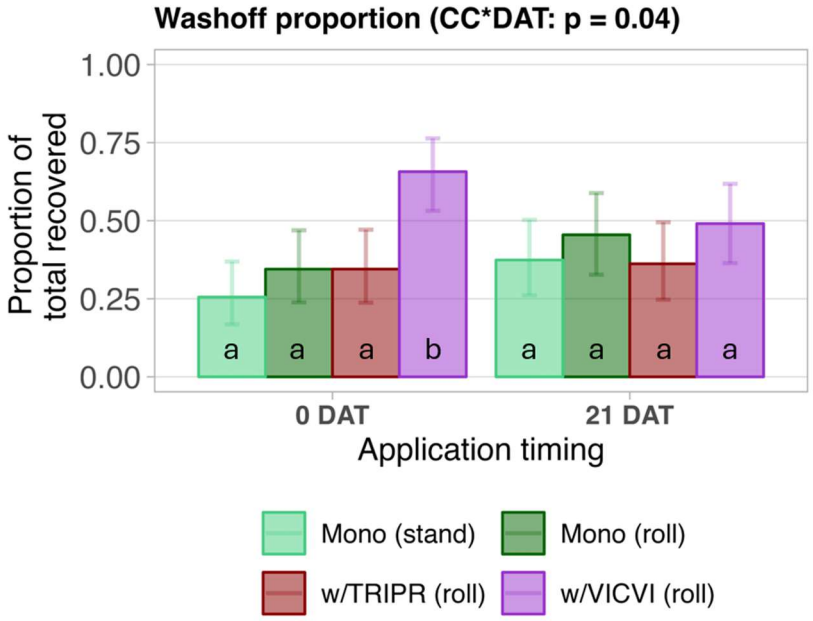

Figure 1-5. *Mean (95% CI) proportion of total herbicide recovery attributed to washoff process by cover crop treatment and herbicide application timing. Cover crop treatments include cereal rye monocultures (mono) left standing (stand) or roll-crimped (roll) and roll-crimped cereal rye-legume mixtures, including with crimson clover (TRIPR) or hairy vetch (VICVI). Residual herbicide application timing treatments including 0 and 21 days after cover crop termination (DAT), which occurred at the cereal rye anthesis stage. Means are averaged across site-years (n = 6) and replicates within site year (n = 4).*

Increasing biomass levels across roll-crimped cover crop treatments resulted in a significant decrease in deposition and total recovery of residual herbicides (**Table 1-2**). Fitting this relationship by herbicide application timing, allowing for varying intercepts and slopes, did not improve models of herbicide deposition (**Table 1-2**; **Figure 1-2b**), but a significant interaction between cover crop biomass and herbicide application timing was observed in models of total herbicide recovery (**Table 1-2; Figure 1-2d**).

DISCUSSION

In our experiment, pyroxasulfone deposition and total recovery was reduced as cover crop biomass increased. When controlling for biomass level, we found that pyroxasulfone

deposition and total recovery was lower when applied at termination (0 DAT), within a planting green scenario, compared to EPOST timing (21 DAT). After controlling for biomass level, we also found that deposition and total recovery was lower when pyroxasulfone was applied into roll-crimped cereal rye compared to standing cereal rye. These results suggest that herbicide uptake in the 0 DAT exposes pyroxasulfone to cellular processes, translocation, and metabolism, which reduced total recovery potential during the washoff process. Pyroxasulfone is weakly adsorbed to soil and can leach into ground water or runoff and contaminate surface water, though the EPA concludes levels are below levels of concern (Yamaji et al. 2016). Increased total herbicide recovery observed in EPOST application timings may have resulted from a decrease in cover crop dry matter due to decomposition compared to 0 DAT. Our findings are similar to pyroxasulfone washoff levels from plant residue observed by Kailah et al. (2018).

This study contributes to growing understanding of cover crop impacts on the fate and efficacy of PRE herbicides. Teasdale et al. (2003) observed interception by hairy vetch cover crop surface residues, which reduced soil concentration of atrazine and s-metolachlor. In our experiment, two grass legume mixtures terminated at cereal rye anthesis were compared to a cereal rye monoculture. Significantly less total recovery of pyroxasulfone was observed in the cereal rye – hairy vetch mixture than the cereal rye monoculture and a similar trend was observed for the crimson clover – cereal rye mixture compared to cereal rye monoculture. This result could be, in part, due to the growth habit and plant architecture of hairy vetch, which has a greater mass area index (MAI; Teasdale and Mohler, 1993) compared to cereal rye, and therefore, greater surface area for herbicide interception to take place.

Whalen et. al (2020) explored the termination of cover crops 7 to 21 days before planting on the fate of soil-residual herbicides applied at planting. The authors reported an inverse relationship between sulfentrazone soil concentrations and cover crop biomass accumulation

observed across the range of pre-plant termination timings. The authors also reported better weed control when the PRE was applied 21 days after cover crop termination or when the EPOST was applied 14 days after cover crop termination. We observed higher total recovery of pyroxasulfone in the EPOST application 21 days after cover crop termination compared to the application of pyroxasulfone at 0 DAT cover crop termination in half of the site-years. Differences in total recovery of pyroxasulfone among site-years could be due to differing rainfall amounts and time to rainfall events. Thus, the washoff process was not uniform among site-years, and the remainder of pyroxasulfone on the cover crop could have been exposed to photodegradation, cellular uptake or metabolism, or microbial degradation, all of which could have an impact on total recovery of the herbicide.

Nunes et al (2023) assessed the application of PREs at the cereal rye anthesis growth stage and found little difference between standing and roll-crimped residues in the spray droplet density and coverage. Our experiment found similar results applied at anthesis in standing and roll-crimped CC, with no significant difference in total recovery for both the 0 and 21 DAT. The authors also reported soil concentrations of sulfentrazone and S-metolachlor that were negatively correlated to cereal rye biomass at application and similar to our spray deposition results (Nunes et al. 2023b).

MANAGEMENT IMPLICATIONS

The results of this study demonstrate that deposition and total recovery of a soil-residual herbicide can differ based on several cover crop management tactics and herbicide application timing. The delay in cover crop termination allows for higher biomass accumulation, which is correlated with better weed suppression (Teasdale 1996). Nichols et al. (2020) suggested that a threshold of 5 Mg ha^{-1} that was needed to provide 75% weed suppression. With the use of planting green, this threshold is possible within Mid-Atlantic production region. However,

applying soil-residual PREs at termination when employing planting green tactics may result in significant reductions in the bioavailability of the herbicide in the soil. For example, at a targeted cover crop biomass threshold of 5 Mg ha^{-1}, our results indicate that bioavailability of pyroxasulfone will be reduced by 40% when applied at cereal rye termination in a planting green scenario where residue is left standing, and by 22% when pyroxasulfone is applied at an EPOST application. When roll-crimping is used as a residue management tool, total herbicide recovery is reduced by more than 55% at either application timing.

When using grass-legume mixtures, total herbicide recovery was less than 50% of our applied rate at a targeted 5 Mg ha^{-1} biomass threshold. In this scenario, producers may consider the management tradeoff between PRE efficacy and nitrogen fixation potential of legume-grass mixtures. Producers may also choose to roll-crimp as a residue management tactic despite decreases in bioavailability of soil-residual herbicides, as it provides better conditions for crop establishment (Wallace et al. 2023).

Further research needs to be conducted to evaluate the fate and efficacy of reduced concentrations of soil-residual herbicides observed in this study in the presence of a cover crop surface mulch. Studies suggest that reduced rates of residual herbicides can contribute to the evolution of herbicide resistant weed populations when reduced rates are sub-lethal (Norsworthy et al. 2012). Identifying a cover crop biomass threshold for which application of a PRE is no-longer an effective weed control tool would improve management recommendations for integrating these tactics. This study improves understanding of PRE herbicide and cover cropping tradeoffs in the context of biomass thresholds, residue management tactics, cover crop species selection, and residual herbicide application timing, which will contribute to development of best management practices.

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Chapter 2

Effects of herbicide and cereal rye residue properties on herbicide washoff potential among alternative application timings

INTRODUCTION

Integration of soil-residual herbicides applied preemergence (PREs) is increasingly needed to manage herbicide resistant weed populations and is considered a best management practice in conservation tillage systems (Norsworthy et al. 2012). Integration of soil-residual herbicides increases the number of sites of action employed in herbicide-based weed control programs, which can slow evolution of resistance to foliar-active herbicides commonly used in POST applications (Norsworthy et al. 2012). Application of soil-residual herbicides at planting can provide weed control for several weeks, allowing emerging cash crops to gain a competitive advantage against weeds.

Many herbicides with soil-residual activity have a long application window relative to cash crop growth stages, which allows for their use in PRE and postemergence (POST) herbicide programs. Using soil-residual herbicides at both application timings results in overlapping soil residual activity, which is increasingly employed to achieve season-long control of herbicide resistant weed species that have prolonged emergence windows (Chahal et al. 2018; Sarangi and Jhala 2019).

Optimizing the application timing of soil-residual herbicides in no-till crop production systems that integrate cover crops is challenging due to herbicide interception by cover crop residues, which can reduce weed control potential via reduced soil bioavailability (Teasdale et al. 2005; Khalil et al. 2019). Planting green, which is the practice of delaying cover crop termination until after planting of the cash crop, can result in greater weed suppression potential due to an increase in cover crop biomass potential compared to conventional cover crop termination before planting with less biomass (Reed et al. 2019; Ficks et al. 2022). However, this integrated weed

management (IWM) tactic is likely to further increase interception of soil residual herbicides. Previous studies have demonstrated that a proportion of herbicide intercepted on cover crop residues may become soil-activated after an incorporating rainfall event (Khalil et al. 2018). The proportion of intercepted herbicide that becomes available from this washoff process is likely to be a function of biochemical properties of the cover crop residue or chemical properties of the herbicide active ingredient.

Herbicide washoff potential will vary depending on whether it is applied to a living cover crop or a previously terminated cover crop. Application of soil-residual herbicides to living cover crops can expose these herbicide to cellular uptake, translocation, and metabolism (Shimabukuro 1985) within cover crops, which reduces the total amount of herbicide reaching the soil surface (Khalil et al. 2018). After a cover crop is terminated, decomposition results in both mass loss and changes in biochemical properties of surface residues. Studies suggest that cover crop decomposition results in an increase in lignin percentage because the hemicellulose portion of the cover crop biomass are more easily decomposed, resulting in mass loss within a cash crop growing season (Khalil et al. 2019).

The polarity and ionic (charge) properties of soil-residual herbicides influence herbicide water solubility, and thus are likely to influence herbicide washoff potential from cover crop residues (McBride 1994). The octanol-water partition coefficient (K_{ow}) is a primary measure of herbicide polarity that can range from a log K_{ow} of -3 to 6. Herbicides with a high log K_{ow} (3 to 6) have a greater affinity for lipids and can become trapped in the phospholipid-bilayer of cellular membranes. Herbicides with lower log K_{ow} (-3 to < 0) have comparatively greater affinity for water and have less potential to pass through cellular membranes (Khalil et al. 2019). Previous research suggests that herbicides with intermediate log K_{ow} values (> 0 to 3) permeate through membranes at a greater rate than herbicides with low or high polarity (Bromilow et al. 1990). Permeation rates through plasma membranes described as a function of herbicide polarity results

in a bell-shaped pattern, with peak permeation rates observed at intermediate log K_{ow} values and lower rates observed for more hydrophilic or lipophilic herbicides.

Herbicide polarity also depends on the presence or absence of an electron charge on the herbicide molecule. Ionic herbicides contain regions within the molecule that have differing charges, which creates greater potential to bond with other molecules (Wilms et al. 2020). Thus, ionic herbicides are more likely to form bonds with cover crop residues as they decompose because of the increase in free carbons that are produced (Wilms et al. 2020). In comparison, non-ionic compounds do not have regions that differ in charge, so they are relatively neutral and less likely to interact and form bonds with the cover crop surface residues.

Previous research into residual herbicide washoff from plant residues has primarily focused on cash- or cover- crop residues in advanced stages of decomposition. For example, washoff potential from wheat straw has been described for a range of herbicides such as metribuzin (Banks and Robinson 1982), oryzalin (Banks and Robinson 1984), and several non-ionic, very long-chain fatty acid inhibitors, including acetochlor, alachlor, and *S*-metolachlor (Banks and Robinson 1986), and pyroxasulfone (Khalil et al. 2020). These studies indicate that soil-residual herbicide concentrations in the soil can be reduced by 14 to 62% at wheat straw surface residue levels of 2250 to 9000 kg ha^{-1}, respectively (Banks and Robinson 1984).

The role of cumulative precipitation in maximizing herbicide washoff differs among herbicides (Khalil et al. 2019), but generally the first 5 mm of rainfall starts to wash herbicide from plant residue. Rainfall intensity has little to no effect on herbicide washoff potential from plant residues (Khalil et al. 2019).

Understanding how residual herbicide properties influence washoff potential in cover crop management scenarios that include applications into living cover crops (i.e., planting green) or cover residues that are at early decomposition stages remains a knowledge gap. In this study, we evaluate the washoff potential of soil residual herbicides that range in polarity when applied in

a planting green scenario or as POST application across varying levels of cover crop decomposition using a combination of field and greenhouse assays. The goal of this research is to improve herbicide selection and application timing to optimize weed control efficacy of residual herbicides within no-till systems that employ cover cropping and planting green management practices.

MATERIALS AND METHODS

Planting green assays. A field experiment was conducted at the Pennsylvania State University Russell E. Larson Agricultural Research Center (RELARC) near Rock Springs, PA in the 2022 and was replicated in the 2023 growing season. The soil texture consisted of Hagerstown silt loam. Cereal rye (*Secale cereale* L.) was established in the fall preceding each experimental growing season using a 10 ft no-till grain drill (Great Plains, Salina KS) with a seeding rate of 67 kg ha^{-1}.

The experimental design consisted of a two-factor complete block arranged in a split-plot treatment structure with four replicates. Main plot treatments were randomized with cover crop management strategy with two treatment levels and the split-plot treatment was the residual herbicide active ingredient with six treatment levels. Main plot treatments included cereal rye (1) left standing or (2) roll-crimped just prior to termination at the anthesis stage (Zadoks 69). Roll-crimping treatments were imposed with a front-mounted 3-m unit (I&J Manufacturing) four hours prior to herbicide treatments.

Split plot treatments included residual herbicides that differed in polarity and ionic charge (**Table 2-1**) and were applied at standard labeled rates for medium-texture soils: (1) mesotrione, 0.11 kg ai ha^{-1}, (2) pyroxasulfone, 0.15 kg ai ha^{-1}, (3) atrazine, 1.12 kg ai ha^{-1}, (4) *S*-metolachlor, 1.79 kg ai ha^{-1}, (5) pendimethalin, 1.07 kg ai ha^{-1}, and an (6) nontreated control. Each residual herbicide application was applied with Roundup PowerMAX (glyphosate) at 1.27 kg ae ha^{-1} and a

non-ionic surfactant (NIS 0.25% v/v) to terminate the cereal rye, thereby simulating a planting green scenario in which PRE herbicides are tank-mixed with foliar products necessary to terminate cereal rye. Split-plot size was 3 by 6 m in 2022 and 3 by 9 m in 2023. The residual herbicide treatment was applied using a CO_2 pressurized backpack sprayer and 3-m boom designed with 51 cm spacing, equipped with 110015 AIXR (TeeJet) nozzles, and a carrier rate of 140 L ha^{-1} to simulate common application parameters used in burndown scenarios.

Table 2-1. *Experimental herbicide treatments, application rate, and properties that influence washoff potential, including ionic charge and octanol-water partition coefficient (log K_{ow})*

Herbicide	Product	Rate (kg ai ha^{-1})	Ionic charge	log K_{ow}
mesotrione	Callisto 4SC	0.11	weak acid	0.50
pyroxasulfone	Zidua 4.17 SC	0.15	non-ionic	2.39
atrazine	Aatrex 4L	1.12	weak base	2.68
S-metolachor	Dual II Magnum	1.79	non-ionic	2.90
pendimethalin	Prowl H20	1.07	non-ionic	5.18

Cover crop biomass was sampled four hours after herbicide application by harvesting a 45 cm length of row in two adjacent cereal rye rows, resulting in a sample area of 0.17 m^{-2} per plot. The fresh sample was weighed and divided by 14 to reach equivalent biomass per area for Buchner funnels (0.012 m^2) that were employed for subsequent washoff assays. To reach the target sample weight, cereal rye samples were cut into 7.6 cm length pieces and assembled with equal representation of leaf, stem, and flower. Samples were stored in the cooler (4 C) and under dark conditions for 20 hrs and washoff assays were initiated 24 hours after application by placing samples in Buchner funnels with a 500 mL bottle placed below each funnel to collect simulated rainfall washoff. The rainfall simulator applied 190 mm of simulated precipitation over the course of 25 minutes using 15 gallon per minute cone shaped irrigation nozzles. Each 500 mL bottle was collected when a 233 mL (190 mm by volume) sample had been reached, transferred from 500 mL to 250 mL bottles to reduce headspace, and then placed in -7° C freezer to prevent herbicide

degradation. A 3 mL syringe and 0.45 um filter was used to extract 1 mL of solution from each water sample, and was then expelled in a 1.5 mL glass vial.

Surface residue decomposition assays. Additional washoff assays were conducted with use of the roll-crimping and no-residual control treatment within the planting green washoff assay experiment. This second experiment was designed as a two-factor complete block imposed using a split-plot treatment structure with four replicates. The main plot factor was cereal rye decomposition stage with four treatment levels, including (1) 0 days after termination; DAT, (2) 14 DAT, (3) 42 DAT, and (4) 84 DAT. The split plot factor was residual herbicide treatment with five treatment levels, including (1) mesotrione 0.11 kg ai ha^{-1}, (2) pyroxasulfone 0.15 kg ai ha^{-1}, (3) atrazine 1.12 kg ai ha^{-1}, (4) *S*-metolachlor 1.79 kg ai ha^{-1}, and (5) pendimethalin 1.07 kg ai ha^{-1}.

Cereal rye was harvested from roll-crimped plots receiving no soil activated residual herbicide treatment in the planting green assay (described above) approximately four hours after roll crimping by harvesting a 45 cm length of row from two rows. Samples were then placed into a 50 by 25 cm decomposition bag. Eight decomposition bags were prepared for each treatment replicate, and secured to the soil surface using landscape staples in the same area the biomass sample was taken from. Two bags were collected at each decomposition stage (0, 14, 42, and 84 DAT) and replicate block, placed in drier for 3 days at 65° C, and then stored at room temperature.

After the completion of the decomposition assay, paired samples were then homogenized. Biomass was weighed and divided into 14 sub-samples consisting of equal portions of leaf, stem, and flower at 7.6 cm length. To adjust for mass loss of the biomass from decomposition during the growing season, biomass samples were adjusted to the amount present at the lowest amount (84 DAT). Standardizing biomass levels across decomposition stages isolates treatments effects on interactions between herbicide properties and biochemical properties of the cereal rye residue.

Following preparation of experimental units, cereal rye samples were fully submerged in deionized water for 24 hours for the rehydration of the biomass. The samples were then emptied into trays composed of hardware cloth that were equal to the area of the Buchner funnel. Biomass samples were allowed to drip dry for the 30 min before the application of herbicide treatments.

Herbicide treatments were applied using a laboratory-based pneumatic track sprayer with a carrier rate of 140 L ha^{-1} equipped with Tee Jet 110015 AIXR nozzles. Sprayed trays were then placed in a dark growth chamber at 5°C and a relative humidity of 85% to reduce moisture loss. Biomass samples were removed from the trays and placed in Buchner funnels 24 hours after the herbicide treatment, with 500 mL bottles located beneath each funnel, and arranged on greenhouse benches by randomizing treatments within each experimental block. Simulated rainfall was applied at 250 mm (335 mL volume) using overhead irrigation with JetRain nozzles, which was achieved over a 35-minute period. A 1 mL of subsample was extracted using 0.45 um filters from each water sample and expelled into a 1.5 mL glass vial.

Cereal rye biochemical properties. An oven dried 10 gram subsample of equal part leaf, head, and stem was taken from each block, decomposition level, and year was ground and sent to Cumberland Valley Analytical Services (CVAS). Near-infrared (NIR) wet chemistry was used to analyze crude protein, acid detergent fiber (ADF), neutral detergent fiber (aNDF), and lignin.

Herbicide detection in water. Preliminary experiments showed that washoff water samples contained high enough concentrations to be detected using high performance liquid chromatography (HPLC). A 3 mL syringe and 0.45 um filter was used to extract 1 mL of solution, which was placed in a 1.5 mL glass vial, capped, and stored in the -7°C freezer. Preliminary tests were conducted with known concentrations of differing herbicide active ingredients mixed using different solvents (acetone, methanol) to observe the differences in

percentage recovery. In the solvents tested, acetone (Fisher Scientific, Waltham, MA, USA) optimized herbicide recovery. Acetone was used to dissolve active ingredient standards to test HPLC recovery rates (**Supplemental Table 1-1**).

Statistical analysis. Washoff recovery data were expressed as a proportion of total recovery potential based on herbicide standards in water. Washoff recovery was analyzed using generalized linear mixed-effects models (GLMMs) with a beta distribution (glmmTMB package; Brooks et al. 2017) in R (R Core Team, 2022). Use of a beta distribution within GLMMs allow for analysis of continuous proportion data using the original scale, thereby producing less biased estimates relative to transformation-based statistical approaches (Douma and Weedon, 2019). Washoff recovery data from planting green assays included cover crop treatment (n = 2; CC), herbicide (n = 5; H), and year (n = 2) and their two- and three-way interactions as fixed effects. Block and main plots nested within block were fit as random effects. Washoff recovery and lignin proportion data from decomposition assays included decomposition stage (n = 4; DAT), herbicide (n = 5; H), and year (n = 2) and their two- and three-way interactions as fixed effects. Block and main plots nested within block were fit as random effects. The significance of fixed effects in all models were evaluated using log-likelihood ratio tests (Wald χ^2) to compare full versus reduced models using the *anova* function. Post hoc comparisons at either the main effect level or two- and three-way interactions for ANOVA models were conducted using Tukey's contrasts within the 'emmeans' function (Lenth 2024).

RESULTS

Cover crop performance. Within each experimental year, cereal rye biomass and growth stage at the time of termination was the same for the planting green and decomposition assay, as each assay was initiated from the same experimental plots. Cereal rye was terminated at

anthesis (Zadoks 65-69), in both 2022 and 2023, which resulted in a mean total biomass of 5.1 ± 2.1 Mg ha^{-1} in 2022 and 4.9 ± 2.6 Mg ha^{-1} in 2023.

Herbicide washoff in planting green management scenario. A two-way interaction between herbicide treatment and year ($\chi^2 = 33.6$; $p < 0.001$) was observed in analysis of washoff recovery, expressed as a proportion of total applied (**Table 2-2**). In 2022, the highest recovery rates were observed in residual herbicides with more intermediate log K_{ow} values (pyroxasulfone, atrazine) compared to residual herbicides having low (mesotrione) or high (S-metolachlor, pendimethalin) log K_{ow} values (**Figure 2-1**). In 2023, higher recovery rates were observed in residual herbicides with low (mesotrione) to intermediate log K_{ow} (pyroxasulfone, atrazine) properties compared to S-metolachlor and pendimethalin.

A main effect of cover crop treatment was also observed ($\chi^2 = 5.3$; $p = 0.02$), where residual herbicide applied into roll-crimped cereal rye residue resulted in greater washoff recovery compared to standing cereal rye (**Table 2-2**). Though not measured in this study, it possible that roll-crimped cover crops resulted in higher herbicide interception rates in comparison to standing cover crops, and therefore have greater washoff potential.

These results suggest that the relationship between log K_{ow} herbicide properties and their permeation rate through plasma membranes may be a useful indicator of washoff potential in planting green scenarios (Bromilow 1984), where herbicides with intermediate log K_{ow} values have greater potential to be recovered from cover crop residues via the washoff process than more hydrophilic or lipophilic herbicides. Higher permeation rates through membranes allows the herbicide to both enter and leave a cell, increasing herbicide washoff potential.

Table 2-2. *Main effects and interactions of herbicide (H) and cover crop (CC) treatments and experimental year (Y) on herbicide washoff recovery following applications made to living cereal rye.*

	Herbicide washoff recovery	
ANOVA[1]	Wald χ^2	P-value
Herbicide (H)	70.86	**<0.001**
Cover crop (CC)	5.33	**0.02**
Year (Y)	8.46	**<0.01**
H x CC	3.64	0.45
H x Y	33.67	**< 0.001**
CC x Y	0.01	0.94
H x CC x Y	2.31	0.67

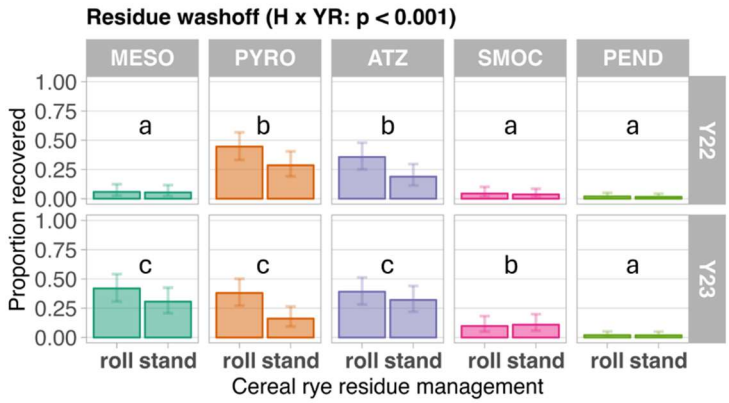

Figure 2-1. *Mean (95% CI) herbicide washoff recovery from living cover crop residue, expressed as the proportion recovered compared to a no cover crop control, among site years (2022 or 2023) and cover crop treatments terminated at the cereal rye anthesis stage, including cereal rye monocultures left standing (stand) or roll-crimped (roll). Residual herbicide treatments include mesotrione (MESO), pyroxasulfone (PYRO), atrazine (ATZ), s-metolachlor (SMOC) and pendimethalin (PEND).*

Herbicide washoff recovery from decomposing surface residues. A significant effect of decomposition stage (DAT) on washoff recovery was observed ($\chi^2 = 9.2$; $p < 0.001$, p=0.03; **Table 2-3)**. Averaged across herbicide treatments and years, no differences in washoff recovery were observed from 0 to 42 DAT, but recovery was lower 84 DAT compared 0 and 42 DAT (**Figure 2-2**).

Table 2-3 Effects of herbicide (H), days after termination (DAT), and experimental year (Y) on herbicide washoff recovery, expressed as a proportion of the no cover crop control.

	Herbicide washoff recovery	
ANOVA[1]	Wald χ^2	P-value
Herbicide (H)	41.5	< 0.001
Days after termination (DAT)	9.2	0.03
Year (Y)	7.5	< 0.01
H x DAT	11.8	0.22
H x Y	7.6	0.05
DAT x Y	4.0	0.25
H x DAT x Y	16.9	0.05

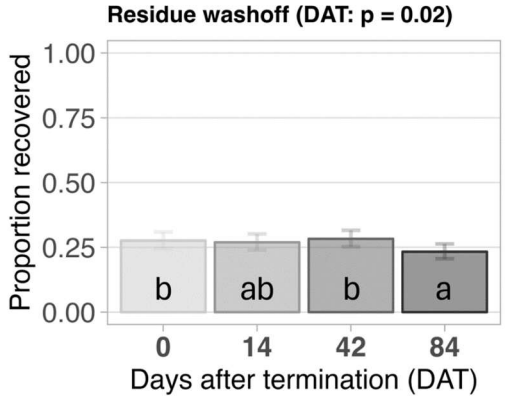

Figure 2-2. *Mean (95% CI) residual herbicide washoff recovery, expressed as the proportion recovered compared to the no cover crop control, among decomposition levels including 0, 14, 42, or 84 days after cover crop termination (DAT). Means are averaged across residual herbicides (n = 4) and site year (n = 2).*

Lower recovery at 84 DAT may be partially explained by changes in the biochemical properties of cereal rye residue at the four different levels of decomposition (0, 14, 42, and 84 DAT). The lignin percentage of cereal rye residue differed by decomposition stage ($\chi^2 = 63.3$; $p < 0.001$, $p < 0.001$) but the rank order of treatments differed by year ($\chi^2 = 8.3$; $p < 0.001$, $p=0.03$). In both years, lignin percentage was greater 84 DAT compared to earlier decomposition stages

(**Figure 2-3**). In 2023, lignin percentage was greater 42 DAT compared to earlier decomposition stages.

Higher lignin ratios within plants result in a smaller proportion of hemicellulose and is more likely to form bonds with sorbed herbicides (Dao 1991). Reduced recovery and higher lignin ratios at the 84 DAT treatment suggest that lignin content may serve as an indicator of herbicide washoff patterns in cereal rye residues. However, 84 DAT is outside of a soil-residual herbicide application window because soil-residual herbicides are typically not labeled for use that long after planting for crop safety and herbicide residue reasons (**Figure 2-2)**. However, termination of cereal rye at earlier phenological stages or incorporation of legumes in cover crop mixtures may result in higher lignin content of surface residues within postemergence application windows.

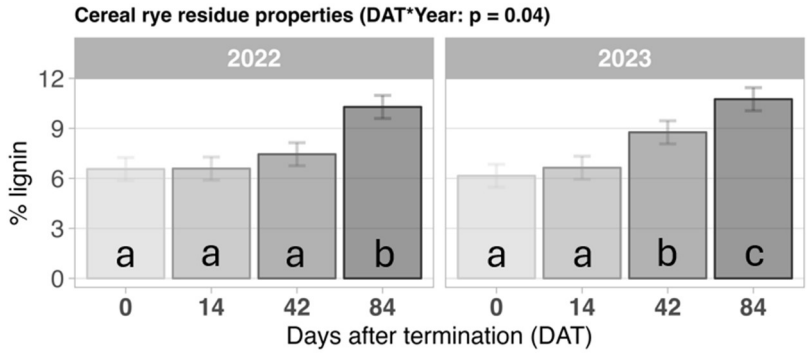

Figure 2-3. *Mean (95% CI) lignin percentage at each decomposition level (0, 14, 42, or 84 days after cover crop termination (DAT)).*

In both experimental years, recovery of pendimethalin in washoff assays was below the detection threshold of 0.02 mg L^{-1}, and thus, excluded from model fitting. Herbicide treatment had a significant main effect ($\chi^2 = 41.5$; $p < 0.001$) on washoff recovery (**Figure 2-4**). Averaged across years and decomposition stage (DAT), pyroxasulfone resulted in greater washoff recovery than other herbicides and atrazine resulted in greater washoff recovery compared to mesotrione

and S-metolachlor. Herbicide washoff recovery results had a bell curve distribution pattern, similar to washoff patterns observed in planting green assays (**Figure 2-1**).

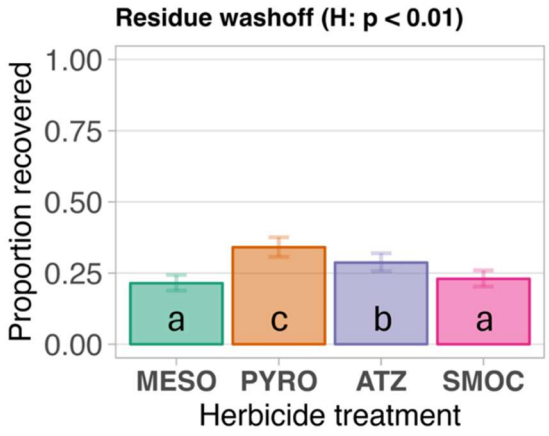

Figure 2-4. *Mean (95% CI) herbicide washoff recovery, expressed as the proportion recovered compared to the no cover crop control, among decomposition levels including 0, 14, 42, and 84 days after cover crop termination (DAT). Residual herbicide treatments include mesotrione (MESO), pyroxasulfone (PYRO), atrazine (ATZ), and s-metolachlor (SMOC). Pendimethalin reported below detection threshold. Means are averaged across site year (n = 2) and decomposition level (n = 4).*

A three-way interaction between herbicide, decomposition stage (DAT), and experimental year was also observed ($\chi^2 = 41.5$; $p < 0.001$, p=0.05; **Table 2-3**) in analysis of herbicide washoff recovery (**Figure 2-5**). In 2022, no herbicide treatment differences were observed at 14 and 84 DAT, but greater pyroxasulfone recovery was observed compared to mesotrione 0 and 42 DAT and to S-metolachlor 0 DAT. In 2023, pyroxasulfone and atrazine recovery was greater than mesotrione and S-metolachlor 0 DAT. Pyroxasulfone recovery was greater than mesotrione, but other herbicide treatment comparisons did not differ at 14, 42 and 56 DAT.

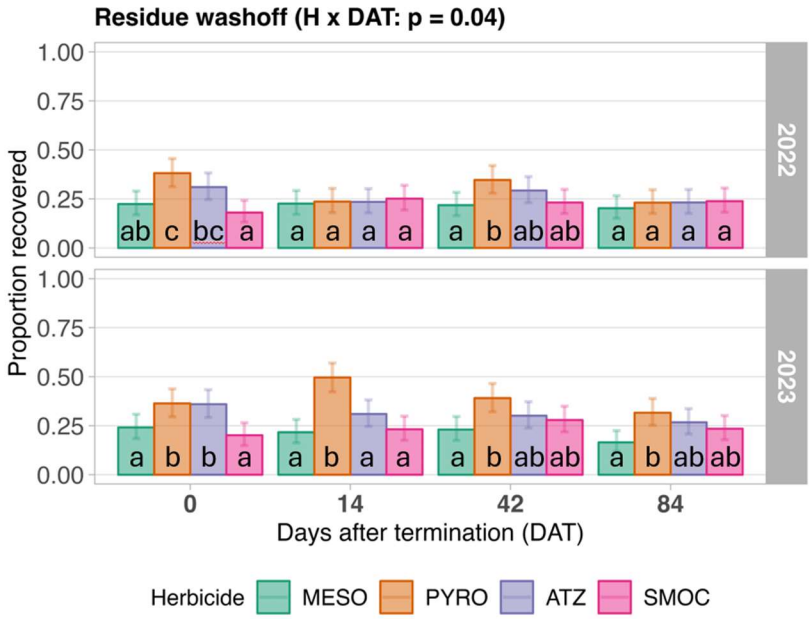

Figure 2-5. *Mean (95% CI) residual herbicide washoff recovery from residue with 0, 14, 42, or 84 days of decomposition after termination across site years (2022 or 2023). Residual herbicide treatments include mesotrione (MESO), pyroxasulfone (PYRO), atrazine (ATZ), and s-metolachlor (SMOC). Pendimethalin reported below detection threshold.*

DISCUSSION

This research contributes to growing understanding of residual herbicide fate and efficacy in systems that integrate cover crops as an integrated weed management tool. Our study provides partial support for using herbicide polarity, as measured by low K_{ow}, as indicator of herbicide washoff potential across cover cropping scenarios. Our results suggest that herbicide washoff potential is greatest in herbicides with intermediate K_{ow} properties, meaning they are neither lipophilic nor hydrophilic. Intermediate herbicide polarity results in increased permeation rates through cellular membranes, both into and out of cells, which is important in a planting green scenario where the cover crops are still living, and active transport is taking place between cells.

Few studies have investigated differences in fate among herbicides in a planting green scenario. Nunes et al. (2023) evaluated flumioxazin and pyroxasulfone dissipation in soil after

applications within planting green scenarios and reported that flumioxazin was more dependent on a timely incorporating rainfall event to be washed from the biomass than pyroxasulfone. The authors attribute this finding to flumioxazin being less water soluble than pyroxasulfone. Kahlil et al. (2018) found that decomposition of crop residues for over a year had less residual herbicide washoff potential than less decomposed residues of chickpea, canola, lupin, wheat, and barley. The authors noted that the biochemical properties of cereal crops change slowly over the course of decomposition. In our study, no significant difference in total washoff was observed among decomposition that occurred 0 to 42 DAT, but a marginal reduction in washoff potential was observed at 84 DAT (**Figure 2-2**). This result suggests that decomposition of cereal rye terminated near the anthesis stage is too slow to meaningfully impact residual herbicide washoff patterns within residual herbicide application windows.

MANAGEMENT IMPLICATIONS

Our observations suggest that herbicide polarity, measured via log K_{ow} values, can aid in development of relative rankings of washoff potential among residual herbicides used in corn and soybean systems. Intermediate log K_{ow} values result in greater washoff potential from a cereal rye cover crop regardless of management scenarios ranging from applications into living cereal rye when planting green or postemergence application into surface residues. While research needs to be done to see if these reduced rates of residual herbicides are effective at controlling weed populations, producers may consider if certain active ingredient(s) have properties that increase washoff potential when multiple residual herbicide options are available within a given weed control spectrum or use pattern.

Though lignin percentage of cereal rye residues increased across the growing season, differences within the residual herbicide application window (0 to 42 d) were likely not significant enough to influence herbicide washoff processes. However, this observation may be

limited to cereal rye monoculture terminated at anthesis. Biochemical properties of different cover crop species or grass-legume mixtures may result in different biochemical properties that have a greater impact on washoff processes of residual herbicides. Further research should be conducted to explore differences among cover crop species.

It is important to note that results of washoff assays should not be used to quantify total soil bioavailability of residual herbicides, but rather show that herbicide washoff processes differ among herbicide properties and cover crop biochemical properties in a predictable way, which can be used in combination with further understanding of cover crop management impacts on herbicide deposition processes to refine management. In conclusion, this research suggests that herbicide polarity, measured using log K_{ow} values, may be a useful indicator of herbicide washoff potential in cereal rye cover crop systems.

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EPILOGUE

The results of this research will help growers improve integrated weed management (IWM) systems that incorporate both soil-activated residual herbicides and cover crop surface mulch in no-till systems. In Chapter I, we identified the impact of (1) cereal rye biomass; (2) cereal rye – legume mixtures, (3) roll-crimping; and (4) herbicide application timing on the soil bioavailability of pyroxasulfone. Soil bioavailability was quantified by measuring both deposition through the cover crop profile and washoff of pyroxasulfone from cover crop residues after 12.5 mm of rainfall. In Chapter II, we identified the impact of (1) cover crop and (2) herbicide properties on the herbicide washoff process from cover crop residues. Cover crop properties included (1) living vs. dead residue and (2) decomposition stage. Herbicide properties included active ingredients that differed in polarity (i.e., hydrophilic vs. lipophilic).

Understanding the combined effects of cover crops and reduced levels of a residual herbicide on weed control was beyond the scope of my thesis. My results can be used to identify which management scenarios result in greater than a 50% reduction in soil bioavailability of pyroxasulfone after 12.5 mm of rainfall. This threshold is important both from an economic and herbicide resistance management perspective, given concern over reduced rates of soil-applied herbicides contributing to resistance evolution (Norsworthy et al. 2012).

The role of cover crop biomass. When pyroxasulfone was applied at cereal rye termination in a planting green scenario, soil bioavailability decreased from 86% to 33% as biomass increased from 1 to 8 Mg ha^{-1}, respectively, when cereal rye was left standing. A 50% reduction in soil bioavailability occurred between 5 and 6 Mg ha^{-1} of biomass. Across this biomass range, approximately 10% of the herbicide that is present in the soil came from the washoff process. Greater biomass production is associated with improved weed suppression

potential, soil moisture conservation, and improved soil health. These benefits should be weighed against reductions in herbicide soil bioavailability.

Effect of roll-crimping. In comparison to standing cover crops, soil bioavailability decreased from 81% to 13% as biomass increased from 1 to 8 Mg ha^{-1}, respectively, in a planting green scenario when either cereal rye or cereal rye – legume mixtures were roll-crimped prior to application of residual herbicides. A 50% reduction in soil bioavailability occurred at 4 Mg ha^{-1}. At this biomass level, 25% of herbicide present in the soil came from the washoff process. At 6 and 8 Mg ha^{-1} of roll-crimped residue, the washoff process accounted for 50% and 100%, respectively, of the herbicide present in soil. Roll-crimping can improve early season conditions for cash crop growth and these benefits should be weighed against reductions in herbicide soil bioavailability.

Effect of grass-legume mixtures. Grass-legume cover crop mixtures reduced residual herbicide concentration in the soil compared to a grass monoculture. Averaged across biomass levels, pyroxasulfone applied into roll-crimped cereal rye – hairy vetch mixtures in a planting green scenario reduced herbicide soil bioavailability by 25% compared to a cereal rye monoculture. Cereal rye – crimson clover mixtures reduced bioavailability by 12% compared to a cereal rye monoculture. Some growers may select legumes for nitrogen fixation purposes and these benefits should be weighed against reductions in herbicide soil bioavailability.

Effects of delayed applications (EPOST). Pyroxasulfone concentration in the soil was generally higher when applied in a two-pass herbicide application consisting of a burndown followed by application of the residual herbicide at an EPOST timing, after termination of the cover crop. Compared to planting green into standing cereal rye, delaying application of pyroxasulfone to EPOST increased its concentration by 10% at 4 Mg ha^{-1} cereal rye residue to

100% at 8 Mg ha^{-1}. Compared to planting green into roll-crimped cereal rye or cereal rye – legume mixtures, delaying pyroxasulfone to EPOST resulted in a marginal increase (< 3%) in concentration at 4 Mg ha^{-1} to a 300% increase at 8 Mg ha^{-1}.

Effects of herbicide properties. Our studies that compared washoff potential of alternative soil-residual herbicides with differing properties showed partial support that log K_{ow}, a measure of lipophilicity, can be used to predict washoff potential when applied into a living cover crop in a planting green scenario or to decomposing residues. In general, greater washoff potential was observed in herbicides with intermediate K_{ow} values (2 to 3).

Effects of cereal rye decomposition stage. The decomposition stage of a cereal rye cover crop that is terminated at anthesis did not affect the amount of herbicide washoff up to 42 days after termination, which is within the residual herbicide application window in corn and soybean management scenarios. Results suggest that cereal rye lignin content (%) did not change enough to affect herbicide washoff until 84 days after termination, when residual herbicides are no longer applied to crops as they are not labeled and may cause crop injury. However, herbicide washoff potential may be different for cereal rye cover crops terminated at earlier growth stages and grass-legume cover crops due to lower C:N ratios resulting in increased decomposition rates during the early crop growing season.

These findings address the knowledge gap between cover crop management and use of a preemergent residual herbicide to control weeds in no-till systems.

APPENDIX A: SUPPLEMENTAL INFORMATION

Reagents and Solutions. A herbicide standard (pyroxasulfone; West Chester, PA, USA; purity ≥ 98%) was used to test recovery rate from untreated soil from each site year. ASTM-1 quality water used in analyses was ultra-purified using a GenPure Pro UV-TOC/UF system (Waltham, MA, USA). Stock and standard solutions made by dissolving 5 mg of each standard into 50 mL 50/50 Methanol/Acetone, then further diluting to 2.5 ng/mL. All stocks and solutions were stored in the dark at 4 ˚C, and used to test accuracy of lab equipment.

Herbicide Extractions. Herbicides were extracted from soil samples using a solvent extraction method (Mueller and Senseman 2015). For each soil sample (15±5 g, corrected to 15 g based on air-dried samples), we added 40 mL of acetone. Samples were placed on a reciprocal shaker table overnight (14 hours) and 3 mL syringes and 0.45 um filters were used to extract 1 mL of solution, which was transferred to 2 mL chromatography vials, which we stored at 4˚ C until HPLC-Orbitrap analysis. In addition to unknown samples, solvent blanks, matrix blanks, and matrix spikes were used as QCs and included within each sample run.

Herbicide Detection and Quantification. We analyzed pyroxasulfone concentrations using a Dionex™ ICS-6000^{+} HPIC™ System (ThermoFisher, Sunnyvale, CA, USA) coupled with a Q Exactive Oribtrap™ mass spectrometer (ThermoFisher, Bremen, Germany) through heated electrospray injection (HESI) in positive ion mode. An aliquot of each sample (2 µL) was injected onto an Acclaim RSLC PA2 Polar Advantage II (150 × 2.1 mm 2.2-µm, ThermoFisher, Sunnyvale, CA, USA) column. Chromatographic separation was achieved using a two-phase linear gradient (22 min, 0.2

mL/min flow rate) of water (+ 0.1% formic acid and 0.025% Ammonium Formate) and methanol (+ 0.1% formic acid and 0.025% Ammonium Formate) at a column temperature of 37˚ C (additional conditions detailed in **Supplemental Table S1**). The mass spectrometer was operated in Targeted-SIM for the 5-parent ion m/z with an inclusion tolerance of ± 5ppm.

For each herbicide, the limit of detection (LOD) was 1 µg L^{-1}. The limit of quantification (LOQ) was 2 µg L^{-1}. MS counts were converted to concentration values using calibration curves (2.5 µg L^{-1} – 1000 µg L^{-1}). Any values below the calibration range were set to zero, and any value above the calibration range was excluded from statistical analyses. Reported values were adjusted based on blank and matrix values.

Supplemental Table S1. HPLC Gradient Profile and mass spectrometer settings.

Time (Min)	% Water (+buffer)	% Methanol (+buffer)
0	75	25
1.5	75	25
12	10	90
19	10	90
19	75	25

Mass spectrometer parameters	Setting
Method Duration	22 min
Runtime (start-stop)	1 – 21.5 min
Injection (start-stop)	1 – 20 min
t-SIM Range	± 2 m/z (inclusion)
Spray Voltage	3500 V
Max Spray Current	1.5 µA
Capillary Temp	280 °C
Probe Heater Temp	200 °C
S-Lens RF level	50 %
Sheath Gas pressure	32 a.u.
Auxiliary Gas pressure	7. a.u.
Sweep gas pressure	0 a.u.
MS resolution	70000
Automatic Gain Control (AGC) Target	5.0×10^6
Maximum Intensity Threshold (IT)	247 ms

Component (t-SIM mass)	Quantitation m/z	Confirmation m/z
Atrazine	216.10110	174.05410
Mesotrione	340.04860	227.99611
Metolachlor	284.14210	252.11500
Pendimethalin	282.14480	212.06858
Pyroxasulfone	392.06979	409.09698

Supplemental Table S1.2 Regression model results for standing and roll crimped cereal rye cover crop residue means for recovery of deposition and total recovery (deposition and washoff) across biomass accumulation gradient. Planting green timing (0 DAT) and EPOST (21 DAT).

Herbicide assay	Biomass Mg/ha^{-1}	Standing		Roll Crimped	
		0 DAT	21 DAT	0 DAT	21 DAT
Deposition	1	0.85	0.71	0.72	0.55
	2	0.78	0.66	0.61	0.48
	3	0.70	0.60	0.50	0.40
	4	0.63	0.55	0.38	0.33
	5	0.55	0.50	0.27	0.26
	6	0.48	0.44	0.15	0.18
	7	0.40	0.39	0.04	0.11
	8	0.33	0.33	-0.07	0.04
Total Recovery	1	0.85	0.75	0.81	0.63
	2	0.78	0.73	0.71	0.60
	3	0.70	0.71	0.61	0.56
	4	0.63	0.69	0.51	0.53
	5	0.55	0.67	0.42	0.50
	6	0.48	0.65	0.32	0.46
	7	0.40	0.63	0.22	0.43
	8	0.33	0.61	0.13	0.39

Supplemental Table S1.3 Complete forage analysis conducted across decomposition gradient (days after termination ; DAT) of cereal rye cover crop.

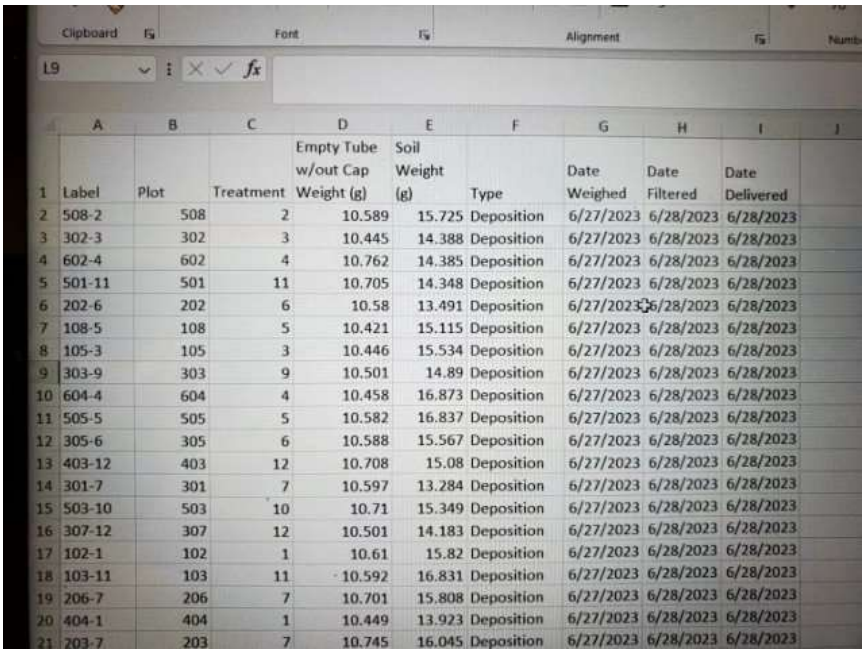
			Based on Dry Matter Content		
Year	DAT	Lignin %	ADF %	aNDF %	ADF:aNDF
2022	0	6.56	46.8	69.6	0.67
	14	6.59	50.9	75.2	0.67
	42	7.45	53.6	76.4	0.70
	84	10.29	58.5	80.0	0.73
2023	0	6.15	44.1	67.2	0.65
	14	6.64	44.1	68.1	0.64
	42	8.76	50.1	62.7	0.79
	84	10.75	54.6	76.8	0.71

APPENDIX B: SOIL EXTRACTION PROTOCOL

Day 1: Petri dishes (decomposition or washoff) were collected from the field, place in a Ziploc bag, and stored in a -7°C freezer until extractions were performed.

To begin extractions, the Petri dishes of soil were thawed overnight.

Day 2: Set up a balance to weigh the tubes and soil. Ensure that the balance is accurately calibrated and level. Create an excel sheet with columns including: Sample ID, date weighed, date filtered, empty tube weight, wet weight, and any additional notes or columns needed.



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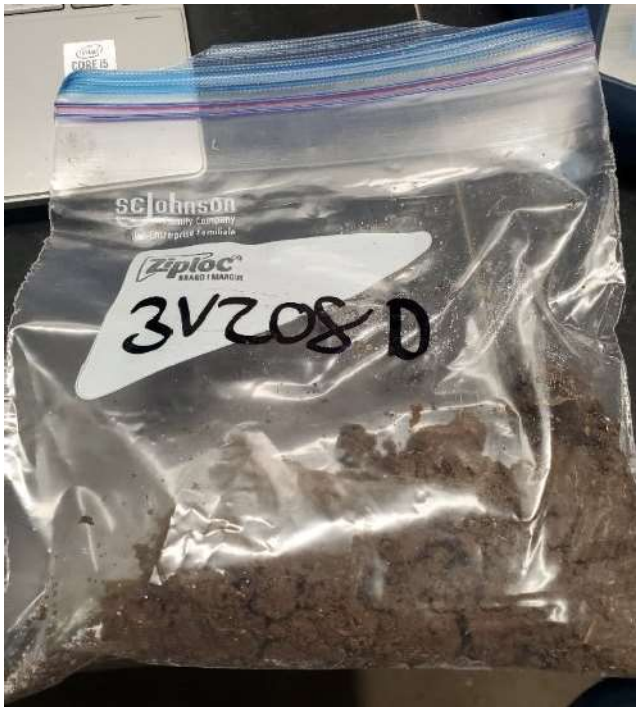
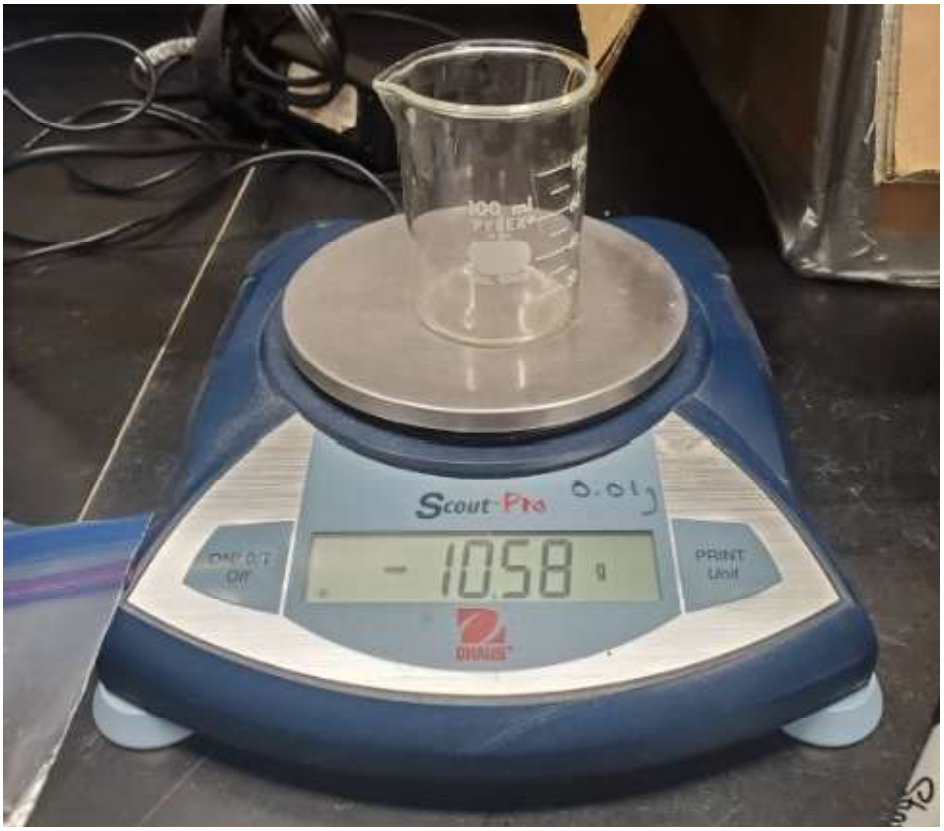
1	Label	Plot	Treatment	Empty Tube w/out Cap Weight (g)	Soil Weight (g)	Type	Date Weighed	Date Filtered	Date Delivered	
2	508-2	508	2	10.589	15.725	Deposition	6/27/2023	6/28/2023	6/28/2023	
3	302-3	302	3	10.445	14.388	Deposition	6/27/2023	6/28/2023	6/28/2023	
4	602-4	602	4	10.762	14.385	Deposition	6/27/2023	6/28/2023	6/28/2023	
5	501-11	501	11	10.705	14.348	Deposition	6/27/2023	6/28/2023	6/28/2023	
6	202-6	202	6	10.58	13.491	Deposition	6/27/2023	6/28/2023	6/28/2023	
7	108-5	108	5	10.421	15.115	Deposition	6/27/2023	6/28/2023	6/28/2023	
8	105-3	105	3	10.446	15.534	Deposition	6/27/2023	6/28/2023	6/28/2023	
9	303-9	303	9	10.501	14.89	Deposition	6/27/2023	6/28/2023	6/28/2023	
10	604-4	604	4	10.458	16.873	Deposition	6/27/2023	6/28/2023	6/28/2023	
11	505-5	505	5	10.582	16.837	Deposition	6/27/2023	6/28/2023	6/28/2023	
12	305-6	305	6	10.588	15.567	Deposition	6/27/2023	6/28/2023	6/28/2023	
13	403-12	403	12	10.708	15.08	Deposition	6/27/2023	6/28/2023	6/28/2023	
14	301-7	301	7	10.597	13.284	Deposition	6/27/2023	6/28/2023	6/28/2023	
15	503-10	503	10	10.71	15.349	Deposition	6/27/2023	6/28/2023	6/28/2023	
16	307-12	307	12	10.501	14.183	Deposition	6/27/2023	6/28/2023	6/28/2023	
17	102-1	102	1	10.61	15.82	Deposition	6/27/2023	6/28/2023	6/28/2023	
18	103-11	103	11	10.592	16.831	Deposition	6/27/2023	6/28/2023	6/28/2023	
19	206-7	206	7	10.701	15.808	Deposition	6/27/2023	6/28/2023	6/28/2023	
20	404-1	404	1	10.449	13.923	Deposition	6/27/2023	6/28/2023	6/28/2023	
21	203-7	203	7	10.745	16.045	Deposition	6/27/2023	6/28/2023	6/28/2023	

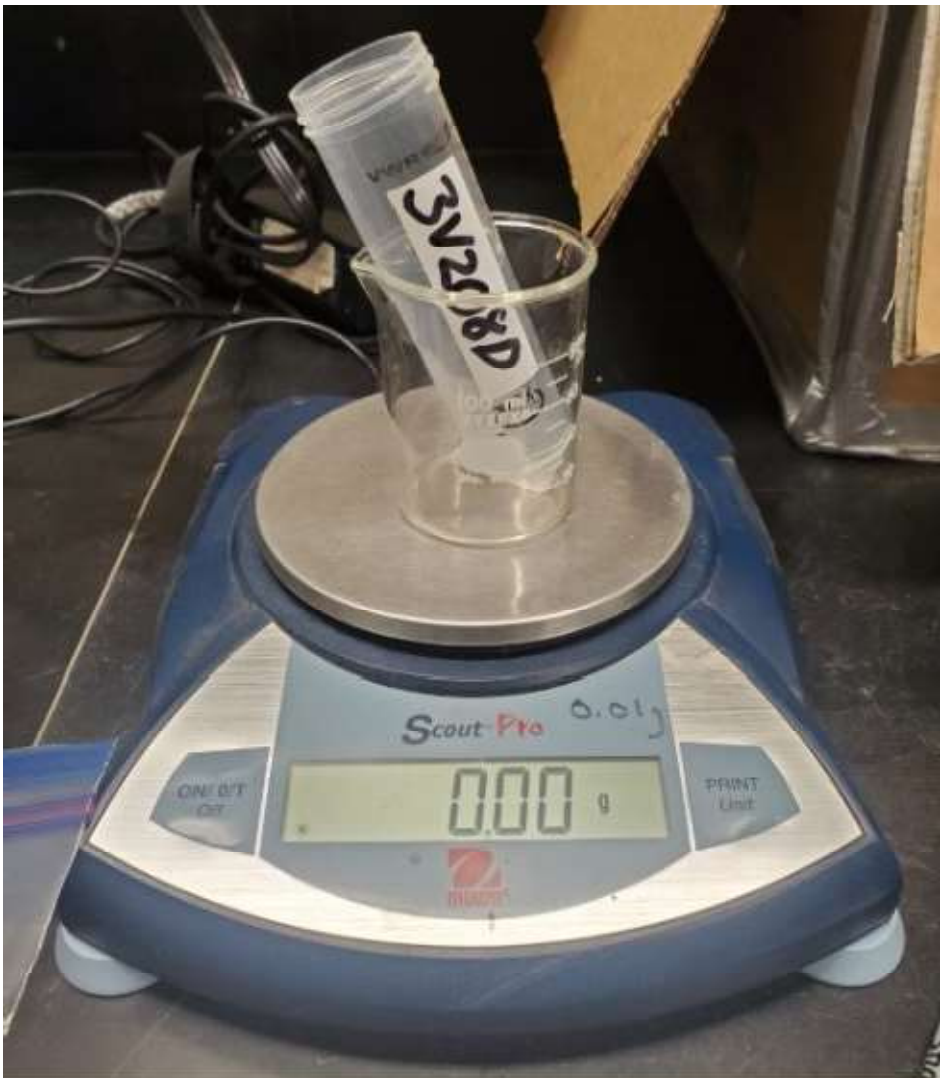
Like-samples were combined and homogenized into a labeled Ziploc bag.



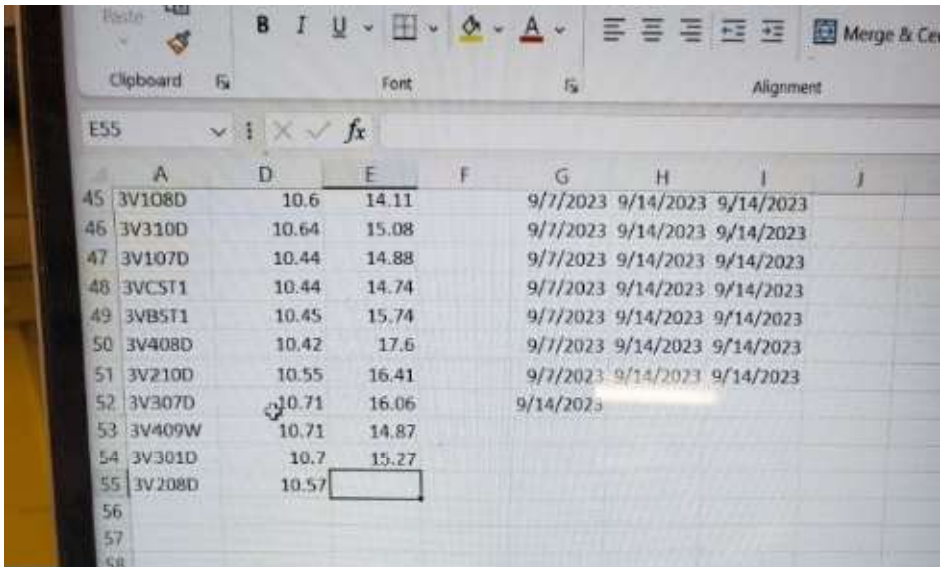
Tare the balance. Remove the cap from a 50-mL falcon tube, weigh the empty tube and document the weight of the tube. Label the empty falcon tube with one corresponding soil sample.

Place a small beaker to hold the falcon tube and tare the scale.





Add ~15 grams of soil to the falcon tube and document the final “wet” weight.



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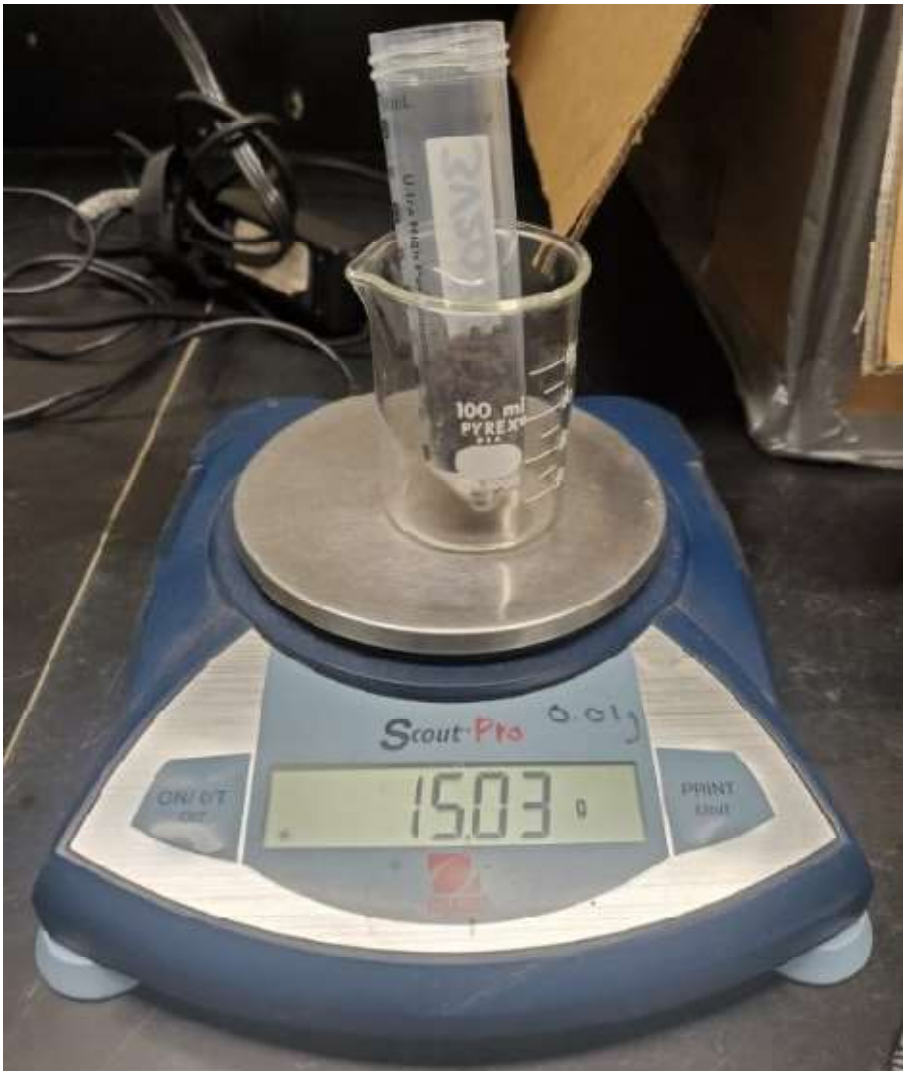
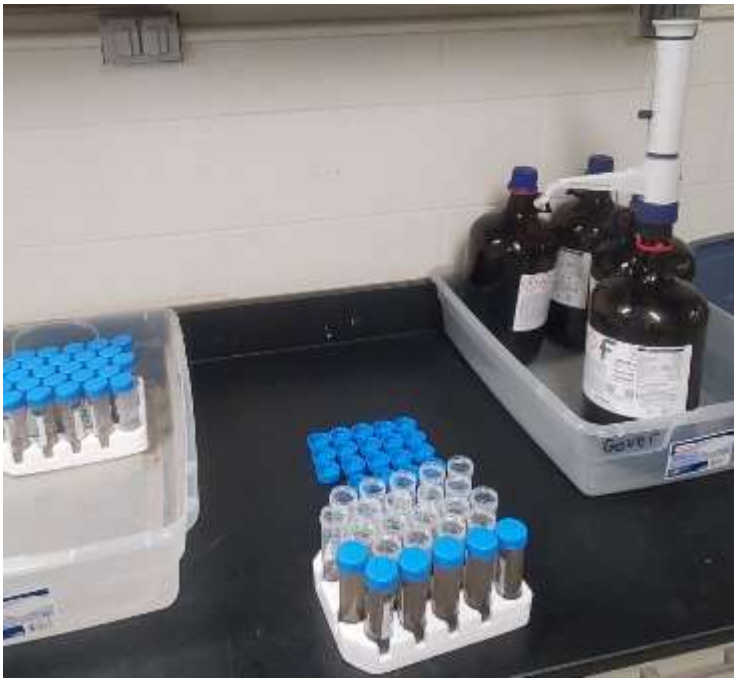
	A	D	E	F	G	H	I	J
45	3V108D	10.6	14.11		9/7/2023	9/14/2023	9/14/2023	
46	3V310D	10.64	15.08		9/7/2023	9/14/2023	9/14/2023	
47	3V107D	10.44	14.88		9/7/2023	9/14/2023	9/14/2023	
48	3VCST1	10.44	14.74		9/7/2023	9/14/2023	9/14/2023	
49	3VB5T1	10.45	15.74		9/7/2023	9/14/2023	9/14/2023	
50	3V408D	10.42	17.6		9/7/2023	9/14/2023	9/14/2023	
51	3V210D	10.55	16.41		9/7/2023	9/14/2023	9/14/2023	
52	3V307D	10.71	16.06		9/14/2023			
53	3V409W	10.71	14.87					
54	3V301D	10.7	15.27					
55	3V208D	10.57						
56								
57								



Sub-sample weight and volume may vary due to water content in the sample. ±5 grams of soil is allowable, however, it is recommended that the samples stay within 15 ± 2 grams . Cap the tube. Continue this step until all samples are in 50-mL tubes. If at any point you need to stop, store all the tubes and unused soil samples in the -7°C freezer until you are ready for the next step.

In the afternoon or evening, add 40 mL of acetone to each falcon tube with soil and replace the caps.

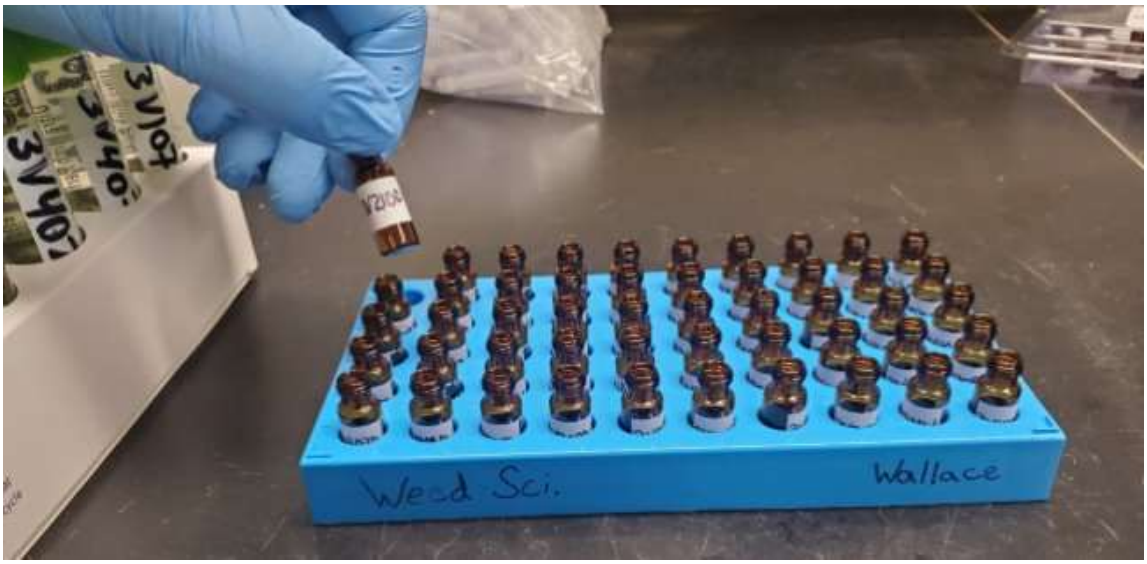


Ensure that the cap of the falcon tube is tight.

Load securely onto the shaker table, plug the shaker table in, and set it to low. Let the shaker table and soil samples run 12-14 hours, recommended overnight.

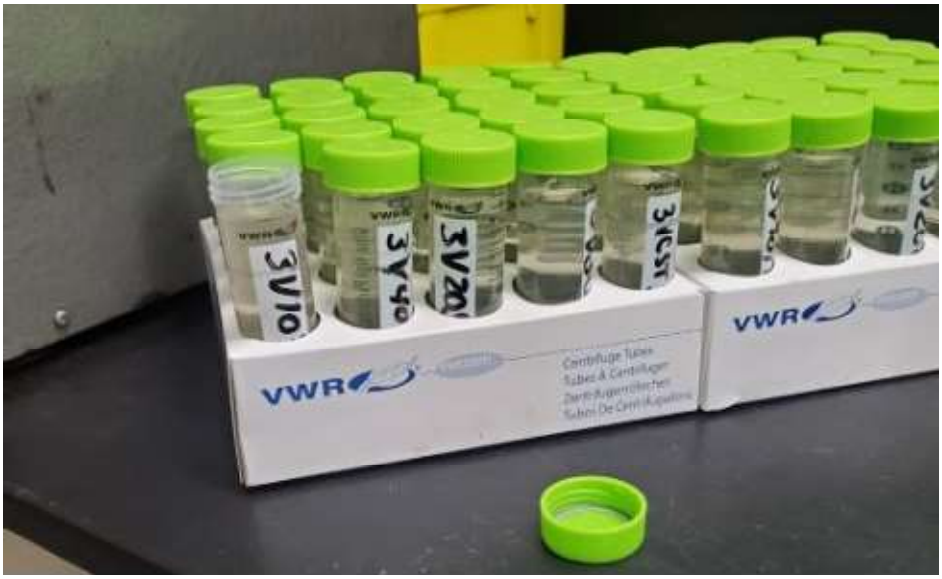
Turn off the shaker table and unplug. Remove the falcon tubes from the shaker table and place in a tube rack.

Allow the samples to sit undisturbed for 30 minutes to allow the particles to settle out of the suspension. In the mean time, label 1.5 mL vials with each individual sample ID.

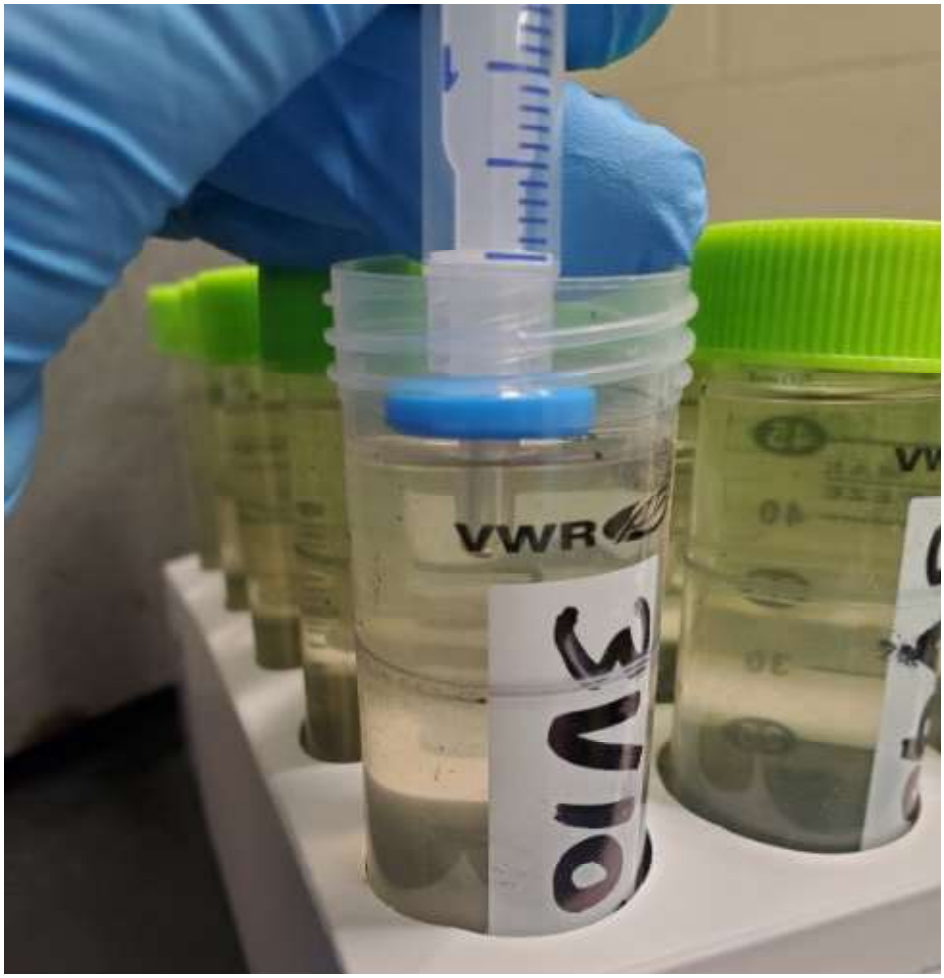


Screw 0.45 µM filters onto 3 mL syringes. Utilize a different syringe for each sample.

One at a time, remove cap from a falcon tube and carefully low the syringe into the solution.

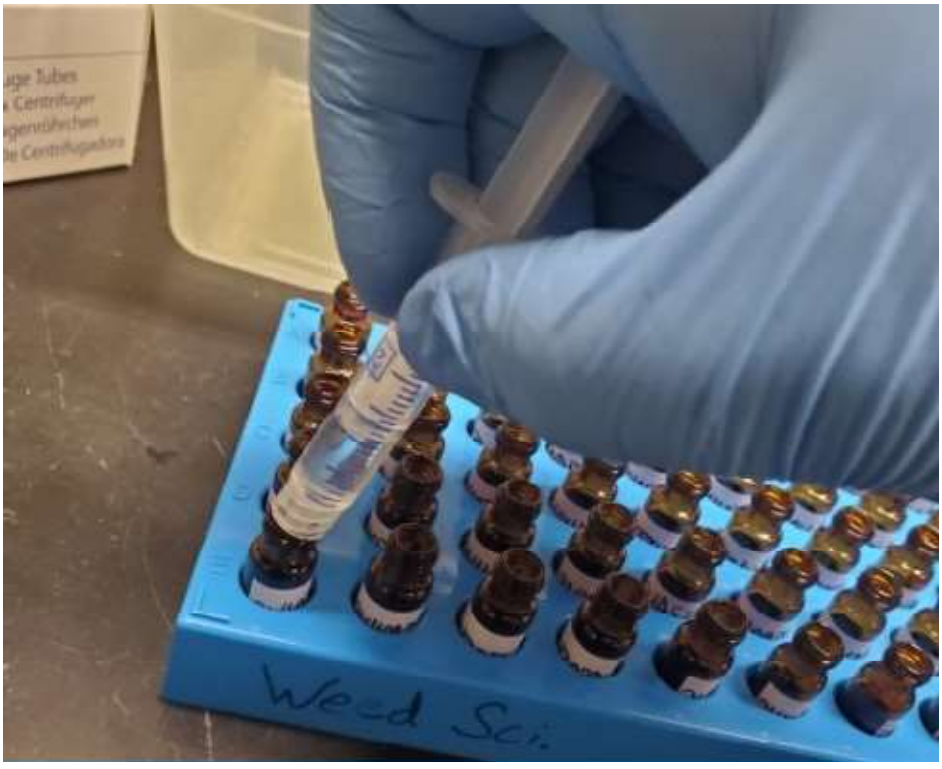


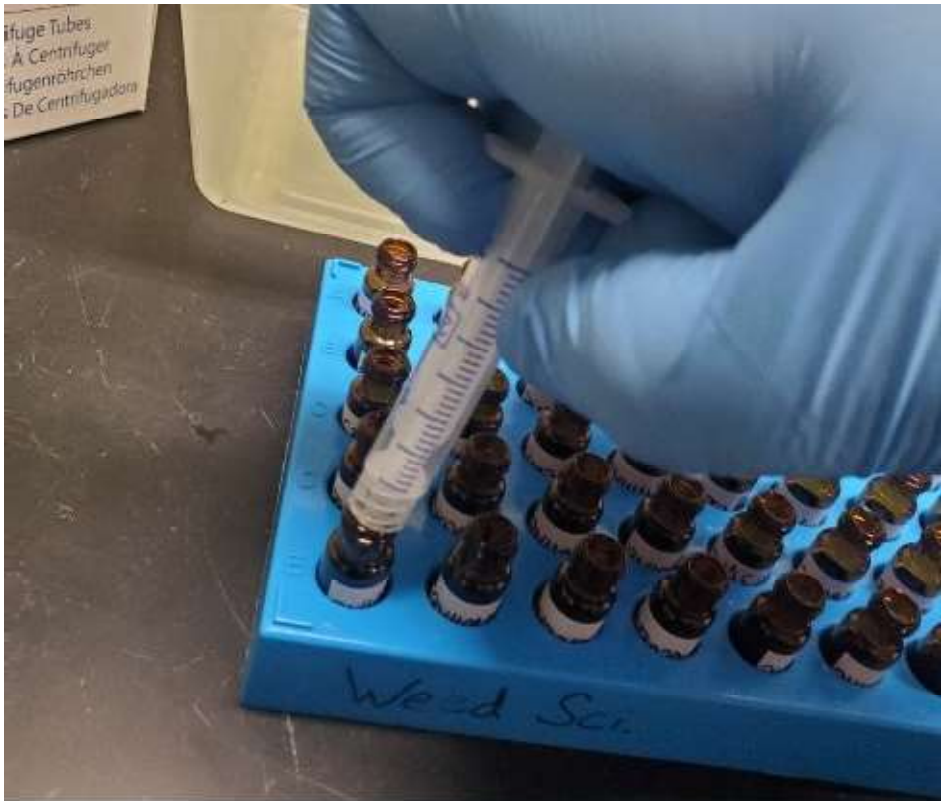
Do not allow the filter to be submerged.



Draw 1 mL of solution into the syringe and remove and discard the filter.

Expel the solution into the matching labeled vial and add a cap.

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Recap the falcon tube and proceed to the remaining samples. Return any unused sample to the freezer.

Samples are ready to be analyzed within 24-48 hours. Store in refrigerator until analysis but keep in mind that acetone will evaporate. Following analysis, evaporate the acetone out of the falcon tubes in a fume hood. Weigh the remaining soil in each falcon tube and document the weight in the spreadsheet.