Optimizing soil- and foliar-applied herbicides for weed management

by

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Dedication

I would like to dedicate my work to my parents (Joao Alberto and Rosana) and my sister (Julia).

Chapter 1 - Literature review

Integrated Approaches to Weed Management

Weeds are among the most significant biological constraints in agricultural production systems. They negatively affect crop growth and yield by competing with crops for essential resources such as nutrients, sunlight, space, and water (Chauhan and Gill, 2014). Weeds are exceptionally well-adapted to control practices due to their high phenotypic plasticity and capacity for evolutionary change (Baker, 1974; Buhler et al., 2000). Historically, weed control relied heavily on labor-intensive methods until the introduction of synthetic herbicides in the late 1940s (Oerke, 2006).

Herbicides have proven to be highly effective weed control tools, achieving 90 to >99% control of targeted weeds (Foster et al., 1993). However, the repeated use of herbicides has led to the evolution of resistance, agricultural ecosystems often create strong selection pressures that can lead to rapid evolutionary changes in weed populations (Neve et al., 2009). The first recorded case of herbicide resistance occurred in 1957 with wild carrot (*Daucus carota* L.) resistant to 2,4-D (Switzer, 1957; Whitehead and Switzer, 1963). Currently, there are 533 unique cases of herbicide-resistant weeds globally, involving 273 species (156 dicots and 117 monocots) (Heap, 2025). Weeds have developed resistance to 21 of the 31 known herbicide sites of action and to 168 different herbicides.

Integrated weed management (IWM) is an approach in which farm managers use multiple practices and tools in a combined effort to maximize weed control efficacy and reduce the selection pressure for resistance to any individual tactic used alone (Norsworthy et al., 2012). Thus, IWM helps maximize the longevity of preferred control methods, such as herbicides (Grint et al., 2022). Integrated weed management programs may incorporate preventative methods such as minimizing weed seed introductions (Scavo and Mauromicale, 2020), cultural methods such as crop rotation, mechanical methods such as cultivation, and even biological methods such as conserving the habitat of beneficial insects (Liebman and Gallandt, 1997).

In most agricultural systems, IWM also involves diversification of herbicide programs and tactics to enhance herbicide efficacy (Norsworthy et al., 2012). When multiple herbicide sites of action (SOAs) are applied sequentially or combined in premixes and tank mixes, it is more difficult for weeds resistant to a particular site of action to escape (Evans et al., 2016). Weed escapes can also be minimized by optimizing application methods, although the applicator must be careful to avoid causing crop injury (Norsworthy et al., 2012). Applied together, these strategies can help reduce or eliminate the contribution of weed seeds, especially herbicide-resistant seeds, to the soil seedbank.

This thesis consists of three studies focused on screening for herbicide resistance and characterizing glufosinate resistance in waterhemp (*Amaranthus tuberculatus*) populations, understanding the influence of soil factors on preemergence herbicide injury in soybean, and evaluating adjuvants for optimizing weed control. The following sections provide background related to these three areas.

Herbicide resistance in waterhemp (*Amaranthus tuberculatus*)

Waterhemp is a pigweed species native to North America. It is a weed of crop fields, especially soybean and corn in the Midwest (Mangold, 2020). Waterhemp has reduced corn yields from 13 to 74%, soybean seed yields by as much as 56% when it emerged with the crop, and 10% when it emerged as late as the V4 (Steckel et al., 2007). Factors contributing to its weediness include a dioecious reproductive system, prolific seed production, and evolved herbicide resistance (Mangold, 2020; Nordby et al., 2007).

Waterhemp is dioecious, meaning that its male and female flowers are on separate plants. For this reason, two plants always mix genes when the plant reproduces, increasing genetic diversity. This increase in diversity increases the potential for spreading herbicide resistance and other traits that favor survival in agronomic systems (Nordby et al., 2007). Waterhemp also has an extended emergence period and prolific seed production, which allows for rapid soil seedbank replenishment if even a few plants escape control to set seed (Hartzler et al., 2009). The plant rapidly invades disturbed areas because of this copious seed production and the formation of persistent seed banks (Costea et al., 2005).

The widespread development of resistance by waterhemp to herbicides with varying modes of action complicates weed control and increases weed control costs in many areas (Shoup et al., 2003). In the United States, waterhemp has evolved resistance to seven herbicide SOAs: acetolactate synthase (ALS), auxin mimics, photosynthesis at photosystem II – serine 264 binders (PS II), enolpyruvyl shikimate phosphate synthase (EPSPS), protoporphyrinogen oxidase (PPO), very long-chain fatty acid synthesis (VLCFA), and 4-Hydroxyphenylpyruvate dioxygenase (HPPD), through both target-site and non-target-site mechanisms (Faleco et al., 2022).

Mechanisms of herbicide resistance are divided into target-site and non-target-site mechanisms. Target-site resistance mechanisms occur when direct alterations at the herbicide's specific SOA prevent the herbicide from binding effectively, leading to resistance. These mechanisms include point mutations that change amino acids in the target protein, gene amplification that increases the quantity of the target enzyme, and overexpression of the target protein, all of which reduce herbicide efficacy and often confer resistance to herbicides with the same mode of action (Carvalho-Moore et al., 2022; Ghanizadeh and Harrington, 2017). Plants that exhibit resistance to more than one herbicide with the same mode of action are considered cross-resistant, and multiple resistance occurs when a population is resistant to two or more herbicides acting at different sites (Rubin, 1997).

Non-target site resistance mechanisms do not directly alter the target site but reduce the amount of active herbicide reaching the target site through reduced absorption, reduced translocation, or increased metabolism of the herbicide. In the case of increased metabolism, the active herbicide is broken down into non-toxic forms before it reaches the target site, thus reducing its efficacy (Shyam et al., 2021). Detoxifying enzymes, including cytochrome P450 monooxygenases (P450s), glutathione-S-transferases (GSTs), and glycosyltransferases (GTs) metabolize herbicide molecules through the processes of oxidation and conjugation with glutathione or sugars (Ghanizadeh and Harrington, 2017).

Reintroduction of pre-emergent herbicides in soybeans

Historically, soybean weed control programs included pre-emergence (soil residual) herbicides. The introduction of soybean varieties tolerant to post-emergence herbicides such as glyphosate led to the widespread abandonment of pre-emergence herbicides (Carpenter and Gianessi, 1999). However, increasing rates of resistance to commonly used post-emergence herbicides are driving increased adoption or re-adoption of pre-emergence options (Young, 2006). Particularly in fields where herbicide-resistant weeds are present or suspected, an effective pre-emergence application can go a long way toward controlling a problem weed population (Jhala, 2017a).

Pre-emergence herbicide products available for soybean can prevent weed emergence or kill weeds at the early stages of growth, setting the stage for successful season-long weed control (Salomão et al., 2021). This application timing helps protect soybean plants from competition in the initial stages of development (Santin et al., 2019). To avoid yield loss, weeds must be controlled during a portion of the growing season known as the critical period of weed control (CPWC). The CPWC represents the time interval between two separately measured crop–weed competition components: the maximum amount of time that the crop can tolerate early-season weed competition before suffering irrevocable yield reduction (a critical time for weed removal) and the minimum amount of time that the crop must remain weed-free to avoid yield loss (critical weed-free period) (Knezevic et al., 2019). Combining pre-emergence with post-emergence weed control can successfully protect crops for the entire CPWC (Knezevic et al., 2003).

One important consideration associated with pre-emergent herbicides in soybean is the possibility of crop injury. Most pre-emergence products used in soybean are either contact or systemic herbicides. For this reason, little herbicide reaches the growing point in most situations, helping the crop quickly recover from the damage and minimizing the risk of yield loss (Hartzler, 2017). In a greenhouse study using a silt loam soil, the labeled rate of 11 pre-emergence herbicides did not impact soybean development, root nodulation, and N fixation, other than reduced canopy early in the season (VC growth stage) with sulfentrazone (Ribeiro et al., 2021). PPO-inhibiting herbicides do not cause injury yearly, but they do carry some risk, as do all herbicides, depending on weather (Jhala, 2017b).

Environmental factors play a significant role in modifying the risk of herbicide injury. Cool, wet soil conditions can slow down herbicide metabolism in soybean, increasing the likelihood of injury (Hager, 2014). Soil texture and chemistry also influence herbicide adsorption, leaching, and uptake. Each herbicide has a unique set of chemical characteristics that influence its behavior under different soil conditions. Herbicide adsorption varies with soil organic matter and other soil characteristics, including clay content, mineral oxides, soil pH, and soil water content (Nordmeyer, 2015). Depending on field history, it is possible to have significant within-field variability in organic matter content, texture, and pH. Many labels for soil-applied herbicides provide different rates depending on soil texture class and soil organic matter content, making it challenging to determine what to apply in fields with great spatial variability (Gundy and Dille, 2021).

The use of adjuvants to increase herbicide performance

Post-emergence herbicide applications sometimes provide inadequate levels of efficacy in certain weeds (Brankov et al., 2023b). Improper herbicide selection or treatment errors can result in suboptimal weed control, enabling the emergence of secondary weeds later in the growing season and thereby reducing crop yield and quality (Hartzler, 2006). Furthermore, ineffective herbicide applications can accelerate the evolution of herbicide-resistant weed populations, complicating future management strategies (Norsworthy et al., 2012). Effective herbicide performance depends on overcoming a variety of barriers—morphological, biological, and environmental—to facilitate the active ingredient's entry into the plant (Pacanoski, 2010). For effective foliar application of post-emergence products, spray droplets need to reach the leaf surface and adhere to it, rather than forming beads and rolling off (Xu et al., 2010). Prolonged droplet retention on the leaf enhances the absorption and uptake of active ingredients in systemic pesticides (Xu et al., 2010).

Adjuvants are specialized products designed to enhance herbicide effectiveness by acting as activators or stabilizers that modify the physical properties of spray solutions (Bell et al., 2019). These agents play a crucial role in minimizing spray drift and improving precision of herbicide application (Brankov et al., 2023a). Proper selection and application of adjuvants represent a critical strategy to optimize herbicide efficacy, particularly under challenging environmental conditions, and reduce the required input of active ingredients (Zhang et al., 2022).

Adjuvants can be broadly classified into two categories: activator and utility modifiers (Pacanoski, 2010). Each type serves a distinct purpose within herbicide applications. Activator adjuvants enhance herbicide activity by increasing absorption, promoting spread, providing rainfastness, and reducing photodegradation of the herbicide. Common activators include surfactants, which improve coverage by reducing surface tension between spray droplets and leaf surfaces; crop oil concentrates (COCs), which offer surfactant benefits while enhancing herbicide retention and penetration through waxy cuticles; and methylated seed oils (MSOs), which dissolve the leaf cuticle to increase and accelerate herbicide uptake. High surfactant oil concentrates (HSOCs) combine the benefits of surfactants, MSOs, and COCs, making them ideal for use with herbicides like glyphosate that may be antagonized by traditional oil concentrates (Legleiter et al., 2024). Utility modifier adjuvants broaden the operational conditions under which herbicides are effective. They include emulsifiers and dispersants (ensure uniform mixing of herbicides in the spray solution), stabilizing and coupling agents (maintain formulation stability), compatibility agents and cosolvents (prevent incompatibility issues when tank-mixing multiple products), and antifoam agents and ammonium fertilizers (enhance spray solution performance) (Pacanoski, 2015).

The inclusion of adjuvants with foliar-applied herbicides is not merely a recommendation but a vital component of effective herbicide use as a component of IWM. Incorporating adjuvants into herbicide management strategies can enhance immediate weed control and extend the longevity of herbicide efficacy by improving herbicide absorption, reducing environmental losses, and optimizing spray deposition. Combining herbicides with adjuvants can significantly improve the efficacy of a diversity of weed species and enhance herbicide performance under adverse conditions, such as drought or extreme temperatures (Ramsdale and Messersmith, 2001). As agricultural practices advance toward precision and sustainability, adjuvants serve as practical tools to maximize the return on herbicide investments while safeguarding crop yield and quality (Brankov et al., 2025).

Knowledge gaps

Although the importance of tracking the spread of herbicide resistance in waterhemp, reducing the risk of soybean injury by pre-emergence herbicide applications, and identifying appropriate adjuvants for soybean herbicide applications is already well-established, many questions remain. The current extent of herbicide resistance in waterhemp is not understood in the northeast Kansas cropping system. Producers need local, specific knowledge about these trends to protect their crop yields and develop strategies for long-term resistance management. The impacts of soil texture, soil pH, and herbicide (sulfentrazone or chlorimuron-ethyl) dose on soybean injury are also not well understood. The best combinations of herbicides and adjuvants to optimize weed control still need some clarification. This thesis seeks to provide practical information about these questions to Great Plains farmers, supporting economically and environmentally sustainable programs for integrated weed management.

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Chapter 2 - Confirmation of Multiple Herbicide Resistance in Waterhemp (*Amaranthus tuberculatus*) Populations from Northeast Kansas

Abstract

Waterhemp in the United States has evolved resistance to seven different herbicide sites of action and is suspected to be resistant to glutamine synthetase (GS) inhibitors. This study aimed to investigate waterhemp resistance to multiple herbicides and evaluate potential resistance to glufosinate-ammonium. Greenhouse experiments were conducted during the summer of 2024, with six waterhemp populations from northeast Kansas fields (labeled K1 to K6) compared to one known susceptible population (S) that was screened with recommended field doses of glufosinate-ammonium, glyphosate, 2,4-D (choline salt), fomesafen, mesotrione, and lactofen. Subsequently, a dose-response study with glufosinate-ammonium was conducted on four populations (K2 to K5) and a known susceptible population (S), with doses of 0 (NT, non-treated), 147.5, 295, 590 (recommended dose 1X), and 1180 g ha^{-1}, including AMS at 1% v/v. Three weeks after treatment, the plants were visually assessed for injury. Populations K3 and K6 were cross-resistant to PPO inhibitors (lactofen and fomesafen). Populations K2, K4, and K6 were resistant to fomesafen and glyphosate. The dose-response study confirmed that population K3 was less sensitive to glufosinate-ammonium. Based on our findings, waterhemp from fields in northeast Kansas demonstrated a complex resistance profile, with varying levels of susceptibility and resistance across different herbicides. Given that glufosinate resistance in waterhemp is not confirmed yet, targeted management practices, potentially involving rotation or a mixture of herbicides with other sites of action and integrated weed management practices, are recommended.

Keywords: PPO-inhibitors, glufosinate-ammonium, multiple-resistance, cross-resistance, sensitivity

Introduction

The intensification of farming since the 1940s and changing cultivation practices, caused rapid shifts in weed communities found in crop fields (Waselkov et al., 2020). These shifts were largely influenced by the widespread adoption of monoculture systems and the heavy reliance on herbicides, which placed intense selective pressure on weed populations, favoring the evolution of herbicide-resistant species and biotypes. Additionally, changes in tillage practices, particularly the transition from conventional to conservation tillage, have further altered the weed flora composition in agricultural systems (MacLaren et al., 2021).

Waterhemp (*Amaranthus tuberculatus*) emergence is greater in no-till systems compared to those that involve tillage, as the vertical distribution and the longevity of weed seeds are significantly affected by tillage (Refsell and Hartzler, 2009). Waterhemp plants can produce as many as 1 million seeds, but 35,000 to 200,000 seeds per plant are more typical (Mohler et al., 2021). It is a very successful weed, it can grow 2 to 3 m tall and extend seed germination and seedling emergence late into the row-crop growing season. Additionally, it can survive and thrive in low-light environments (Steckel, 2007). It is a dioecious plant, that is, male and female flowers are on separate plants, and two plants always mix genes when reproduction occurs. This increases genetic diversity and increases the potential for the evolution of herbicide resistance and the spread of the traits through pollen and seed (Nordby et al., 2007).

Waterhemp has reduced corn yields from 13 to 74%, soybean yields by as much as 56% when it emerged with the crop, and 10% when it emerged as late as the V4 growth stage in soybean (Steckel, 2007). In row-crop production systems, herbicides are often the best option for controlling weeds due to their relatively low cost and ease of implementation. However, the extensive use of herbicides has resulted in the evolution of herbicide-resistant weed populations (Priess et al., 2022; Singh et al., 2023).

Waterhemp has evolved resistance to seven different herbicide sites of action (SOAs) in the United States: acetolactate synthase (ALS) inhibitors, synthetic auxins, photosynthesis at photosystem II – serine 264 binders (PS II) inhibitors, enol pyruvyl shikimate phosphate synthase (EPSPS) inhibitors, protoporphyrinogen oxidase inhibitors (PPO), very long-chain fatty acid synthesis (VLCFA) inhibitors, and 4-hydroxyphenylpyruvate dioxygenase (HPPD) inhibitors (Symington et al., 2024; Heap 2025). In Kansas, waterhemp has evolved resistance to four different SOAs: ALS inhibitors (Horak and Peterson, 1995), PS II inhibitors (Peterson, 1999), EPSPS inhibitors (Thinglum et al., 2011), and PPO inhibitors (Li et al., 2004).

Due to the increasing occurrence of new resistance cases and the confirmation of multiple herbicide resistance in waterhemp, new alternatives have been explored. One such approach is the development of two-pass weed control programs, with an effective soil-applied residual herbicide in the first pass (Symington et al., 2024). Also, the introduction of herbicide-tolerant crop technologies providing tolerance to herbicides such as glyphosate, glufosinate, dicamba, and 2,4-D has enabled in-crop use of these herbicides. These technologies offer more options for growers to diversify their weed control programs and manage herbicide resistance effectively (Singh et al., 2023).

Glufosinate-ammonium is one of the key herbicides used to manage glyphosate-resistant weeds due to its nonselective, broad-spectrum weed control (Singh et al., 2023). It is a foliar-applied, contact, herbicide that can be applied in a single application or sequentially. Glufosinate inhibits glutamine synthetase, a crucial enzyme for nitrogen metabolism in plants; this enzyme is responsible for ammonium assimilation, and its inhibition leads to ammonium accumulation in plant tissues and, as a result, photorespiration and photosynthesis are indirectly disrupted, ultimately causing plant death (Jhala et al., 2017).

Currently, there are no confirmed reports of waterhemp resistance to glufosinate in the United States. However, a recent study evaluated the sensitivity of waterhemp and Palmer amaranth (*Amaranthus palmeri*) populations to glufosinate, dicamba, and 2,4-D across multiple states in the U.S. (Singh et al., 2023). The results indicated that, although the overall efficacy of glufosinate exceeded 90%, the level of susceptibility varied among populations, with survival frequencies ranging from 0% to 32%, 2,4-D showed an overall survival of 17% across all populations, but survival frequency for some population was up to 73% (Singh et al., 2023).

In Kansas, farmers have reported difficulties in controlling certain waterhemp populations in the northeast part of the state. The objective of this study was to screen waterhemp populations from Northeast Kansas to confirm multiple herbicide resistance and investigate potential reduction in glufosinate sensitivity.

Materials and Methods

Plant materials and growing conditions

Waterhemp populations were present at the end of the 2022 growing season in corn and soybean fields in northeast KS (Pottawatomie County). From each of the six sites, approximately 30 waterhemp plants were clipped and bagged, and stored in a greenhouse over winter until threshed. Seeds were stored at room temperature until used. Six waterhemp populations were screened (K1, K2, K3, K4, K5, K6) and a known susceptible population (S) to 2,4-D from Nebraska (Bernards et al., 2012) was used in this study. Experiments were conducted during the summers of 2023 and 2024, in the Department of Agronomy, Kansas State University greenhouse. The seeds from the respective waterhemp population were broadcast into a greenhouse tray (21 by 6 by 4 cm) containing a commercial potting mix (Berger BM 6 all-purpose mix, Saint-Modeste, QC, Canada) and were placed in the greenhouse maintained at a temperature of 32/23 °C day/night (d/n) temperatures, 16 hr photoperiod with 60±10% relative humidity and 600 µmol m^{-2} s^{-1} light intensity at canopy level. Two weeks after emergence, waterhemp seedlings were individually transplanted into plastic pots (6 by 6 by 6.5 cm), maintained in the same greenhouse, and watered as needed until they reached a height of 10 cm.

Waterhemp resistance to multiple herbicides

Plants for this experiment were initiated in August 2023, and the second run was initiated in October 2023. The experiments were organized in a randomized complete block design with twelve replications per treatment and repeated over time (two experimental runs). A total of seven treatments were arranged as P × H factorial with P representing the number of populations and H the number of herbicides. When the waterhemp plants reached 10 cm tall, the herbicide application was done using a bench-type sprayer (Research Track Sprayer, De Vries Manufacturing, 86956 State Highway 251, Hollandale, MN, US) fitted with a single flat-fan nozzle (8002 Teejet tip, Spraying System Co., 100 W. Lake Drive, Glendale Heights, Wheaton, IL, US) which delivered a volume of 187 L ha^{-1} at a speed of 4.85 km h^{-1} at 207 kPa pressure. Twelve plants from each population were treated separately with different herbicides at recommended field doses and adjuvants (Table 2.1). After treatment, all the plants were placed back in the same greenhouse. At seven, 14, and 21 days after application (DAT), the plants were assessed for visual injury on a scale of 0 to 100%, where 0% means no injury and 100% means plant death, compared to their respective non-treated checks (NTC). The above-ground biomass was harvested 21 DAT and dried in an oven maintained at 60 °C for 72 hours, and dry weight was measured. The biomass data were converted into percent biomass reduction compared to the NTC using Equation 2.1 (adapted from Wortman 2014).

$$\text{Biomass Reduction (\%)} = (1 - \text{BEU/BNTC}) \times 100 \qquad \text{[Eq. 2.1]}$$

where BEU represents the dried biomass of the experimental unit (g per plant) and BNTC represents the mean of NTC biomass (g) for the respective population.

Whole-plant dose-response experiment

Plants for this experiment were initiated in April 2024 and a second run was initiated in May 2024. The experiments were organized in a randomized complete block design (in trays) with ten replications per treatment and repeated over time (two experimental runs). A total of six treatments were arranged as P X R factorial, with P representing the four populations and a known susceptible population (S) to 2,4-D from Nebraska, and R representing the five herbicide rates. When waterhemp was about 10 cm tall, plants were treated with glufosinate-ammonium (Liberty® 280 SL), including 0 (non-treated check, NTC), 147.5, 295, 590, and 1180 g ha^{-1} (where 590 g ha^{-1} is the 1X recommended field dose) along with AMS at 1% v/v. The same susceptible population S from Nebraska was used and compared to five of the KS populations: K2, K3, K4, K5, and K6. Herbicide treatments were applied using the bench-type sprayer as described before. After the herbicide application, all the plants were placed back into the greenhouse and watered as needed. Plants were assessed for visual injury 7, 14, and 21 DAT.

Statistical analysis

For waterhemp resistance to multiple herbicides, a linear model (lm) with a transformation of the response variable was used to evaluate the effects of population and herbicide on biomass reduction. Analysis of Variance (ANOVA) was performed to assess the significance of the main effects (population and herbicide) as well as their interaction on biomass reduction. Significant effects ($p < 0.05$) were followed by post-hoc pairwise comparisons to determine which specific population differed within each herbicide treatment.

For the whole-plant dose-response experiment, the injury data were subjected to a non-linear regression analysis using the drc package in R. A four-parameter log-logistic model was selected through the lack of fit test ($p>0.05$), where the relationship between the herbicide dose and injury was described in Equation 2.2:

$$Y = c + (d-c)/(1 + \exp(b \, . \, (\log(X) - \log(e))) \qquad [\text{Eq. } 2.2]$$

where Y is the injury expressed as a percentage of the NTC, X is the dose, log (x) represents the natural logarithm of x, parameter c represents the lower asymptote of the curve, the minimum value that y can take when x approaches zero, parameter d defines the upper asymptote of the curve, the maximum value that y can take when x is very large, parameter e represents the dose at which 50% of the response is observed (I50, effective dose), and parameter b is the slope for the curve. The Resistance Index (RI) is the ratio of I50 values between each tested population vs the susceptible population to give the level of resistance for each K population. The greater the index, the greater the difference in the dose required to control the potentially resistant population compared to the susceptible one.

Results and Discussion

Waterhemp resistance to multiple herbicides

None of the herbicides resulted in 100% biomass reduction because it was a post-emergence application, and the biomass present at the time of application was not measured. Populations were screened for each herbicide at the recommended field dose (Table 2.1), and the reduction in biomass (%) relative to NTC was presented in Figure 2.1. For 2,4-D, there was no statistical difference among the populations, considering 2,4-D was effective in control. For fomesafen, populations K1, K2, K3, K4, and K6 were different from S, showing them to be less sensitive to the herbicide. For glufosinate, only the K3 population was different from S, presenting less than a 50% reduction in biomass relative to NTC. For glyphosate, populations K2, K4, and K6 were different from S, and for lactofen, populations K3, K5, and K6 were less sensitive to the herbicides compared with S, and for mesotrione, no differences were found.

Populations K3 and K6 showed cross-resistance to both PPO inhibitors (lactofen and fomesafen). In the areas where seeds were collected, the use of PPO-inhibiting herbicides has been limited because of suspected resistance. These results are consistent with Shoup et al. (2003), who found a waterhemp biotype in northeast Kansas cross-resistant to all PPO-inhibiting herbicides they tested (acifluorfen, lactofen, fomesafen, and sulfentrazone).

Multiple resistance was found for populations K2, K3, K4, and K6. Population K2 and K4 showed multiple resistance to fomesafen and glyphosate, while population K3 demonstrated multiple herbicide resistance to fomesafen, lactofen, and glufosinate, and population K6 was resistant to fomesafen, glufosinate, and lactofen. This is a concerning finding that underscores the growing challenges in weed management. This suggests that these populations, besides presenting cross resistance, may be accumulating multiple resistance mechanisms, whether through continuous selection, gene flow, or the introduction of contaminated seeds (Gaines et al., 2021). This highlights the importance of IWM practices that minimize reliance on herbicides alone.

The effectiveness of post-emergence herbicides can be impacted by environmental factors occurring before, during, or after application. Factors such as relative humidity and temperature have been shown to affect the absorption and movement of herbicides applied to foliage. For example, for glufosinate, Coetzer et al (2001) noted that it was necessary to increase the herbicide dose in conditions of low humidity (35%) to achieve good control of the waterhemp compared to high humidity (90%).

Whole-plant dose-response experiment

Due to the results observed in the screening study, a dose-response experiment was conducted with glufosinate. The RI calculated on the percentage of injury at 21 DAT, populations K2 and K3 presented 2.2 and 2.3 fold resistance, respectively, relative to the susceptible population to glufosinate-ammonium. About 262 to 276 g ha^{-1} of glufosinate-ammonium was needed to achieve 50% injury. Populations K5 and K4 presented 1.4 and 1.6 fold resistance to glufosinate, respectively, needing 174 to 195 g ha^{-1} to achieve 50% injury (Table 2.2). A resistance index greater than 1 implies that the resistant population requires a higher herbicide dose for effective control, indicating some degree of resistance; a value between 1 and 2 for the RI may indicate early-stage resistance or natural variations within populations (EHRAC, 2017). According to Beckie et al. (2013) such low-level resistance could reflect minor shifts in sensitivity, potentially due to natural genetic variability or early-stage selection pressure. These subtle shifts may not immediately impact field efficacy but could signal a trend towards resistance if the same selection pressure continues (Preston and Powles, 2002).

During this study, distinct morphological differences among the populations were observed. When the plants reached the ideal height of 10 cm for herbicide application, they were already starting to develop branches. At 7 DAT, the plants appeared dead, but by 21 DAT, regrowth was evident, specifically in the areas where branching had been initiated. Glufosinate is a contact herbicide with limited translocation in plants, and the amount of translocation is affected by temperature and humidity. Also, glufosinate tends to accumulate in old leaves instead of young leaves and the apical meristem (Takano et al., 2020). Due to application issues, such as insufficient coverage or inconsistent herbicide contact on branched areas, this may have contributed to the observed regrowth; temperature and humidity were not adjusted.

Also, the rapid necrosis process of contact herbicides can cause a reduction in herbicide absorption and translocation (Meyer and Norsworthy, 2020), and it could be one of the reasons why some populations in this study were not controlled by glufosinate (Figure 2.3). But currently, there are no documented cases in the literature of plants developing resistance to glufosinate through a rapid necrosis mechanism. This resistance mechanism appears to be occur with two different herbicides, glyphosate and 2,4-D, in two distinct species: giant ragweed (*Ambrosia trifida*) and fleabane (*Conyza sumatrensis*) (Queiroz et al., 2019).

This study confirmed the presence of cross- and multiple-herbicide resistance in waterhemp populations from Northeast Kansas, with significant resistance observed to fomesafen, lactofen, and glyphosate. Some populations also displayed reduced sensitivity to glufosinate, raising questions about whether this indicates early resistance development or if environmental factors are impacting herbicide efficacy. On a positive note, herbicides such as 2,4-D and mesotrione remained highly effective against these populations. While effective chemical control options are still available, these findings highlight the critical challenge posed by herbicide-resistant waterhemp in modern agriculture. They emphasize the need for IWM practices, as reliance on chemical control alone is becoming increasingly unsustainable.

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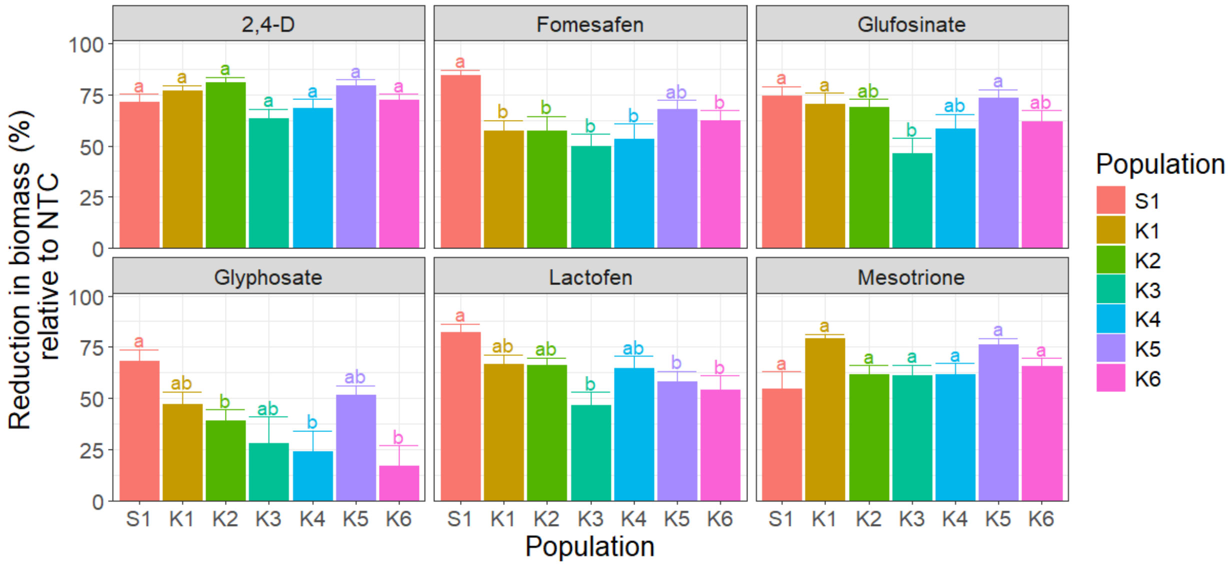

Figure 2.1. Percent of biomass reduction 21 days after treatment with different herbicides for each population from Northeast Kansas (K1, K2, K3, K4, K5, K6) and known susceptible (S1)

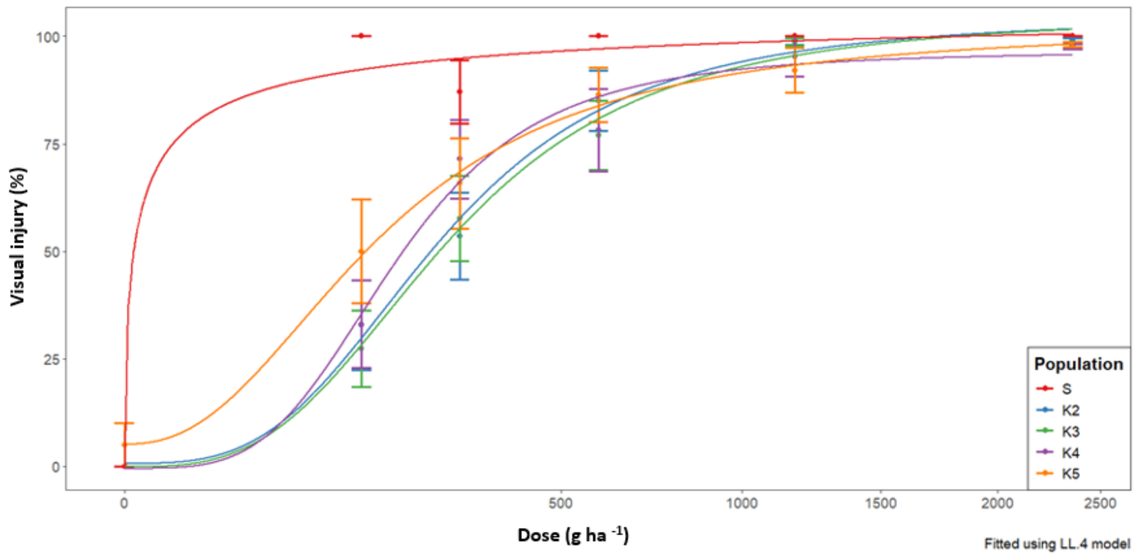

Figure 2.2. Dose-response curve comparing the percentage of injury 21 days after the treatment of the suspected glufosinate-resistant K2, K3, K4, and K5 populations and the susceptible (S)

Figure 2.3. Symptomology observed in population K4, 14 days after glufosinate application.

Table 2.1. Post-emergent herbicide treatments used to evaluate the response of waterhemp populations

Herbicide	**Trade name**	**Mechanism of action**	**Manufacturer**	**Field dose ($g\ ha^{-1}$)**
Glufosinate[a]	Liberty 280 SL	Glutamine synthetase inhibitor	BASF Corporation, 26 Davis Drive, Research Triangle Park, NC	590
Glyphosate[a]	Roundup PowerMax	EPSP synthase inhibitor	Bayer Cropscience, 800 N, Lindbergh Blvd. St. Louis, Missouri	1060
2,4-D choline	Enlist One	Synthetic auxins	Corteva Agriscience LLC, 9330 Zionsville Road, Indianapolis, IN	733
Fomesafen[ac]	Reflex	PPO Inhibitor	Syngenta Crop Protection, LLC P.O. Box 18300 Greensboro, North Carolina	281
Mesotrione[b]	Callisto	HPPD inhibitor	Syngenta Crop Protection, LLC P. O. Box 18300 Greensboro, North Carolina	105
Lactofen[b]	Cobra	PPO Inhibitor	Valent U.S.A. Corporation P.O. Box 8025 Walnut Creek CA	140

[a] AMS (1% v/v) adjuvant included
[b] COC (1% v/v) adjuvant included
[c] AMS (4% v/v) adjuvant included

Table 2.2. Regression parameter estimates (Equation 2.2) and Resistance Index (RI) for visual injury at 21 days after treatment of glufosinate to waterhemp populations from Northeast Kansas compared to a known susceptible (S)

Population	Parameter estimates		
	b	I50 (g a.i.ha^{-1})	R.I
K2	-1.6 (0.4)	262.0 (40.4)	2.2
K3	-1.6 (0.4)	275.7 (46.1)	2.3
K4	-1.9 (0.6)	195.3 (27.4)	1.6
K5	-1.1 (0.4)	174.4 (42.3)	1.4
S	-8.4 (45.0)	120.4 (155.5)	

Chapter 3 - Effects of Soil Texture and pH on Soybean Emergence and Injury to Sulfentrazone and Chlorimuron-ethyl Herbicides

Abstract

Soil-applied herbicides commonly used in soybeans, like sulfentrazone and chlorimuron-ethyl, can cause phytotoxic crop injury under certain soil conditions. This greenhouse study investigated the influence of soil texture, pH, and herbicide rate on soybean emergence and injury. The study was initiated with soil having a pH of 6 and a sand content of 16%. After four months of hydrated lime addition, the pH stabilized at the desired levels of 6.0, 6.7, and 7.0. These soils were mixed with 0, 20, 40, or 60% sand to achieve four texture levels. The experimental design was completely randomized and had a factorial arrangement of 3 pH levels, 4 textures, two herbicides, and four herbicide rates, with five replications and two experimental runs. Four soybean seeds were planted per pot, which were treated with sulfentrazone (1X = 280 g ha^{-1}) or chlorimuron-ethyl (1X = 53 g ha^{-1}) at rates of 0, 0.5, 1 (field rate), and 2X. Soybean seedling emergence and injury were assessed 21 days after treatment. The visual injury was evaluated, where 0% indicated no injury and 100% indicated plant death. For sulfentrazone, emergence was influenced by the interaction between soil texture, pH, and herbicide rate. Higher sand content (40% and 60%) negative impacted seedling emergence, particularly at higher herbicide rates (1 and 2X). Soybean injury was influenced by texture and pH; sandier soils (60%) in pH 6 and 7 presented the highest injuries. For chlorimuron-ethyl, soybean emergence was influenced by the interactions between pH and texture, indicating that the effect of pH on soybean seedling emergence depends on soil texture, the injury was influenced by the interaction between texture and rate, even at lower doses (0.5X), it was possible to observe that injury was very high for soil texture 60%. These findings emphasize the importance of soil variability in influencing the effectiveness and safety of soil-applied residual herbicides. Understanding soil-herbicide-crop interactions is critical for optimizing weed management strategies.

Keywords: soil-applied herbicides, pKa, sand, interaction

Introduction

After 1996, with the advent of glyphosate-resistant crops, the use of soil-applied residual herbicides and cultivation dramatically declined (Norsworthy et al., 2012). Due to overreliance on glyphosate and the subsequent widespread evolution of glyphosate-resistant weeds, soybean producers were once again using soil-applied herbicides as part of their weed control programs (Arsenijevic et al., 2020). Applying PRE herbicides with multiple effective sites of action (SOA) was particularly important in fields where herbicide-resistant weeds were present or suspected. Effective PRE applications can go a long way toward controlling a problem weed population, especially when post-emergence herbicide options become limited or less effective in soybean (Jhala, 2018).

Soil-applied herbicides, also referred to as "residual herbicides", provide extended control of germinating or newly emerging weeds (Jhala, 2015). Weeds that germinate in the first 3 to 4 weeks after crop planting are usually controlled. It kills the weed seedling, typically before it emerges from the soil (Nolte et al., 2011). The concentration and persistence of soil-applied herbicides depend on the chemical properties of the herbicide, weather conditions, and soil factors including moisture, pH, texture, organic matter (SOM), and cation exchange capacity (CEC) (Menalled and Dyer, 2004). Soil-applied herbicide products available for soybeans consist of either single or multiple active ingredients from multiple SOAs (Arneson et al., 2020). In addition, the use of these herbicides will provide multiple or alternative SOAs needed to manage glyphosate-resistant weeds (Knezevic et al., 2019). Control with PRE herbicides protects against crop yield loss in the early season when the crop is most vulnerable to weed competition (Arsenijevic et al., 2020).

Even though for some soil-applied herbicides the recommended application rate takes into account soil characteristics, they are normally sprayed uniformly across the entire field, based on efficacy and dose justification trials. These recommendations are based on worst-case situations and do not account for specific field situations like spatial variability of soil properties (e.g., varying soil organic matter within fields) and weed population (Nordmeyer, 2015). When PREs are applied to the soil with ample moisture but rainfall does not occur to activate the herbicide, poor weed control may result. This is because sufficient soil moisture conditions exist for weed seed germination before the herbicide is activated (Moomaw et al., 1992). For effective weed control, adequate soil moisture is necessary to make the herbicide available in soil solution, but when soil conditions are cool and wet for extended periods during crop emergence, the ability of soybeans to metabolize the soil-applied herbicides is reduced, resulting in more injury (Arsenijevic et al., 2020).

Herbicide adsorption to soil can be described using soil/solution coefficients (Kd) and adjusted for soil Kd/organic carbon (Koc). The Koc and Kd values are defined as the ratio between the amount of herbicide adsorbed and the amount in soil solution (Weber et al., 2000). Depending on field history and structure, fields vary in organic matter content, soil texture, and weed occurrence, this means that a uniform application across an entire field may not be an appropriate application of herbicides, according to small-scale soil variability, differences in herbicide adsorption and the availability of herbicides to plants can be expected (Nordmeyer, 2015). Many labels for soil-applied herbicides account for this variability by providing a specific rate based on general texture class and SOM content, making it a challenge to determine what to apply in fields with great spatial variability (Gundy and Dille, 2021).

Two common soil-applied herbicides used in soybean production include sulfentrazone, a herbicide from the triazinone chemical class, that is a protoporphyrinogen oxidase (PPO) inhibitor (Walsh et al, 2015) and chlorimuron-ethyl, from the sulfonylurea family, and is a herbicide that inhibits acetolactate synthase (ALS) (Brown and Neighbors, 1987). Sulfentrazone is a weak acid (pKa 6.56), at pH levels above the pKa, most sulfentrazone molecules will exist in the anionic form (negatively charged). This occurs because, under more alkaline conditions, there are fewer H^+ ions available to protonate the molecule, leaving it in its dissociated form. The anionic form tends to have a lower affinity for soil particles, especially in soils that also have a dominant negative charge, such as soils rich in clay or organic matter (Brandon et al., 2010), and sulfentrazone will be more soluble in water. The herbicide has high persistence, with a half-life was 146.5 days, being significantly affected by temperature; soil water contents have no significant effect (Martinez et al., 2008).

Chlorimuron-ethyl is also a weak acid (pKa 4.2), and in this study, all the treatments had a soil pH above the pKa, being prevalent in its ionized form in the soil, negatively charged, and more water-soluble. The half-life in soil is usually 7-70 days, but it depends on the soil characteristics; the persistence of chlorimuron-ethyl increases with increasing pH (Wang et al., 2018). Chlorimuron degradation is faster with higher temperatures, and slower in dry soils due to the decrease in microbial breakdown (Fuesler and Hanafey, 1990). Environmental conditions such as cool and wet soil (Arsenijevic et al., 2020), and cool and dry soil (Taylor-Lovell et al., 2001) can both increase soybean injury caused by these herbicides because of the influence in herbicide degradation and soybean metabolism and also soil pH and texture plays a role in the extent of crop damage. To better understand soybean tolerance to soil-applied herbicides, especially in the presence of variability in soil characteristics, the objective was to investigate the influence of soil texture, pH, and herbicide rate on soybean emergence and injury in response to sulfentrazone and chlorimuron-ethyl in the greenhouse.

Materials and Methods

Soil conditions

The soil used for this study was collected from the Kansas State University Department of Agronomy Research Farm located in Manhattan (North Farm) and transferred to the greenhouse. The natural soil pH was 6, and it was measured using the methodology defined by Peters et al, 2012. For texture, the soil presented a percentage of sand was 16%, silt of 50%, and clay of 34%; these parameters were calculated based on the methodology of Bouyoucos (1962). Pilot studies were conducted to determine the required amount of hydrated lime (Bonide – Hydrated Lime) and the duration of incubation needed to achieve the desired pH levels.

To raise the soil pH from 6 to 6.7, 14 g of lime per kg of soil was applied. For an increase from pH 6 to 7, 30 g of lime per kg of soil was used. For each target pH level, four plastic boxes were prepared, each containing 40 kg of soil. The soil was mixed with the appropriate amount of lime using a cement mixer for five minutes and then watered. Once the soil dried, it was mixed every 5 days by hand and watered, with this process repeated over four months. Soil pH was measured every two weeks using the 1:1 soil-to-solution method, following standard procedures. When consistent values were observed across three consecutive measurements, the pH was deemed stable. Soils with 6.0, 6.7, and 7.3 pH levels were mixed with 0, 20, 40, and 60% sand (Tech Mix – Pool Filter Sand) by weight in a cement mixer to achieve four texture levels.

Plant materials and herbicide application

Greenhouse experiments were conducted during the summer of 2024 with a temperature of 32/23 °C day/night and a 16/8 hr (d/n) photoperiod with 60±10% relative humidity, and 600 mol m^{-2} s^{-1} light intensity at canopy level. The experimental design was completely randomized and had a factorial arrangement of 3 pH levels, 4 texture levels, 2 herbicides, and 4 rates, and pots were seeded with one soybean variety (Pioneer P37A18E) for a total of five replications and two experimental runs.

Four soybean seeds were planted per square pot (6 x 6 x 6.5 cm), and herbicides were applied immediately after planting. Sulfentrazone (1X = 280 g ha^{-1}) or chlorimuron-ethyl (1X = 53 g ha^{-1}) were applied at rates of 0, 0.5, 1 (field rate), and 2X. Herbicide treatments were applied using a bench-type sprayer (Research Track Sprayer, De Vries Manufacturing, 86956 State Highway 251, Hollandale, MN, US) fitted with a single flat-fan nozzle (8002 Teejet tip, Spraying System Co., 100 W. Lake Drive, Glendale Heights, Wheaton, IL, US), which delivered a volume of 187 L ha^{-1} at a speed of 4.85 km h^{-1} at 207 kPa pressure. Soybean was assessed for the number of emerged plants and visual injury on a scale of 0 to 100%, where 0% means no injury and 100% means plant death compared to their respective non-treated checks at 7, 14, and 21 days after treatment (DAT).

Statistical analysis

The main effects of soil pH, soil texture, and herbicide rate, as well as their interactions, on soybean seedling emergence and plant injury, were analyzed separately for each herbicide using generalized linear models (GLMs) in RStudio. Data from both experimental runs were pooled for statistical analysis, as preliminary analysis indicated no significant differences between runs ($p > 0.05$). A Poisson family distribution was chosen for these models to account for the count-based nature of the emergence data and percentage of injury. NTC were excluded from the injury analysis to ensure that model assumptions, including the fit of the Poisson distribution and homogeneity of variance. Assumptions of the GLMs were thoroughly assessed using simulated residuals from the DHARMa package in R. The significance of fixed effects (soil pH, soil texture, herbicide rate, and their interactions) was evaluated using analysis of variance (ANOVA) applied to the fitted GLMs. For significant interaction effects identified in the ANOVA, post-hoc pairwise comparisons were performed to explore differences among specific levels of the factors (e.g., pH levels, soil textures, or herbicide rates). Tukey's Honest Significant Difference (HSD) adjustment was applied to account for multiple tests.

Results and Discussion

Soil types

Twelve types of soils were created by the end of this process (Table 3.1). For each one of these soils, both herbicides were tested at different rates, and the results for each one are described below.

Sulfentrazone

Soybean emergence was influenced by the interaction between texture (% of sand), pH, and sufentrazone rate (p=0.0016). For 0X seedling emergence was generally consistent with the average of 3 plants per pot across the different pH levels and textures, with no differences among the treatments, suggesting that, without the herbicide presence, pH and texture had minimal impact on seedling emergence. Soybean injury was influenced by the interaction between texture and pH (p=0.0002).

Higher sand content (40% and 60%) negative impacted seedling emergence, particularly at higher herbicide rates (1 and 2X), having 2 or fewer plants emerging per pot. During the emergence process, the herbicide probably still has a greater concentration close to the emergence zone, over time when we observe the percentage of injury it is clear to see that in the sandier soils (60%) there was more injury that can be related with the herbicide availability in a solution that was pronounced by time and texture. Lower sand content (20%) was more favorable for seedling emergence, with higher levels even at high herbicide rates (Figure 3.1).

The movement of herbicides in the soil is not solely dependent on their chemical interactions with soil particles but also on the physical characteristics of the soil, such as porosity and aggregation, these factors can slow down the movement of herbicides, keeping them in the root zone longer and potentially affecting plant emergence (Paszko et al., 2016). For pH 7 and 0% sand, it is possible to observe a small number of seedlings emerging. This can be explained by the higher herbicide availability in the soil due to the anionic form, which prevents it from binding strongly to clay particles and organic matter. Additionally, the high clay content reduces water infiltration and percolation, keeping the herbicide close to the emergence zone. This is confirmed when the injury was evaluated after 21 DAT (Figure 3.2), the herbicide could be moved along the soil, becoming less available to cause plant symptoms.

Kerr et al (2004) observed similar results that the change in pH had little effect on phytotoxicity; however, texture reduction from 23.3 cmol kg^{-1} to 8.2 cmol kg^{-1} increased the phytotoxicity by 34%. Also, we believe that a higher amount of sand increases the flow of percolating water. Ohmes and Mueller l (2007) found that soils with distinct textures presented differences in bulk densities, resulting in different pore volumes for each soil; one pore volume of additional water added to a saturated soil represented a high mobility scenario. In this study, the amount of water was not measured, which may also have been a factor influencing the results. Dalazen et al (2020) observed that under lower water availability, percolation is less pronounced, leading to a higher concentration of herbicides in the soil surface where the initial development of the plants occurs, resulting in greater plant injury.

Other researchers observed that soybean seedling length and seedling weight were reduced when sulfentrazone was applied before soybean emergence (Gazziero et al., 2005), but even when reducing the number of emerged plants, there was no significant reduction in soybean yield (Arsenijevic et al., 2020). In this study, soybean development was monitored up to 21 days after treatment, making it hard to determine whether the soybean recovered from the herbicide injury and if it kept yield or not. It is known that soybeans can compensate for lower densities through increased pre-plant productivity, especially in higher-yielding environments (Carciochi et al., 2019).

Chlorimuron-ethyl

For soybean seedling emergence with chlorimuron-ethyl, pH: texture (p<0.001) and were statistically significant. This indicates that the effect of pH on soybean seedling emergence depends on soil texture and that the impact of herbicide rate varies according to the soil texture (Figure 3.3).

The injury was influenced by the interaction between texture and rate (p=0.0019). Even at lower doses (0.5X), it was possible to observe that injury was very high for soil texture 60% (Figure 3.4), as mentioned before, soils with higher sand content have high water mobility, making the herbicide available as the solution to be absorbed by the plants. In contrast, lower rates and soils with less sand significantly reduce injury, regardless of pH.

Anderson and Barrett (1985) found that chlorsulfuron soil texture may influence persistence, and degradation would occur more rapidly in the sand loam. In this study, we observed more soybean injury in soils with higher sand content, but this can be related to the short period (21 DAT) in which we observed the soybeans and herbicide availability in soil solution. The soybean injury in the sand soils would probably decrease over time, and injury in the clay soils would increase due to the speed of the herbicide degradation in each type of soil. Santin et al. (2019) found that for different pre-emergence herbicides, soybean injury was observed until 21 DAT; after that, the culture recovered the symptoms of damage.

This study highlights the significant influence of soil properties, such as pH and texture, and their interactions with soil-applied herbicides like sulfentrazone and chlorimuron-ethyl, on soybean seedling emergence and plant injury. These findings emphasize the importance of soil variability in influencing the effectiveness and safety of pre-emergent herbicides. Understanding soil-herbicide interactions and evaluating soybean performance throughout the growth cycle is critical for optimizing weed management strategies. Future research should focus on investigating the long-term persistence of herbicides in various soil types and the impact of water availability on herbicide behavior and dynamics. Incorporating site-specific management practices that account for soil variability into precision agriculture technologies has the potential to enhance herbicide application efficiency, improve weed control, and reduce the risk of crop injury, thereby contributing to more sustainable agricultural practices.

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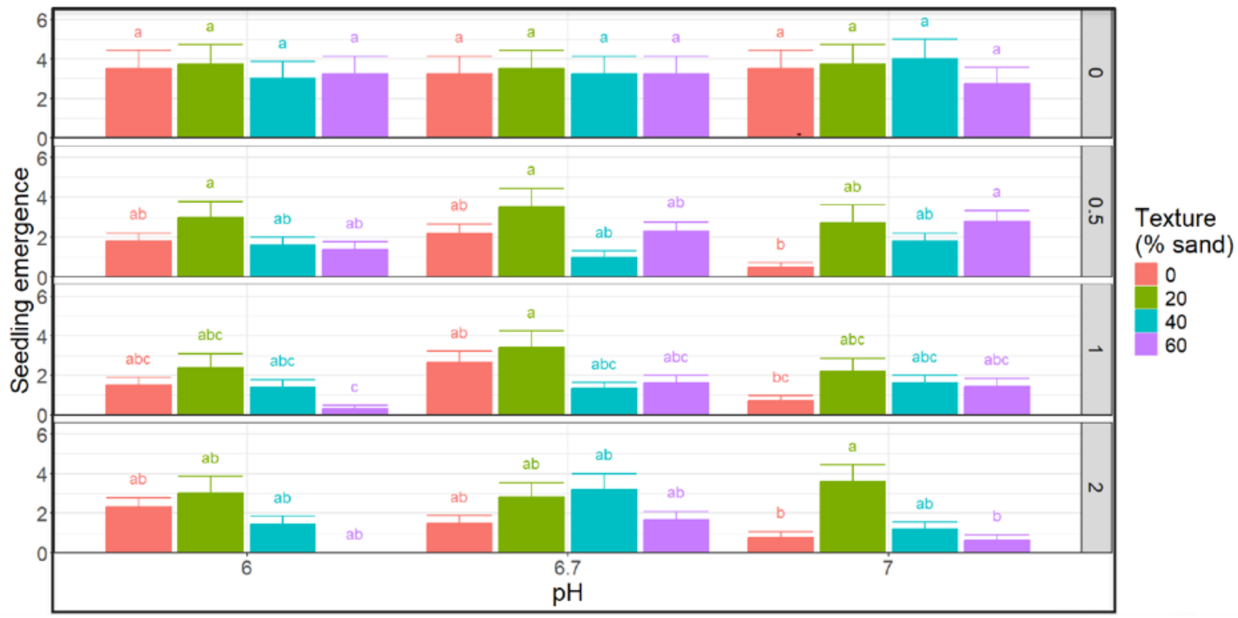

Figure 3.1. Soybean seedling emergence (number per pot) at 21 DAA in response to 0, 0.5, 1, and 2X of sulfentrazone (1X = 280 g ha^{-1}) to soils with different pH and texture treatments. Different letters for seedling emergence across pH, texture, and rate levels indicate significance at a=0.05.

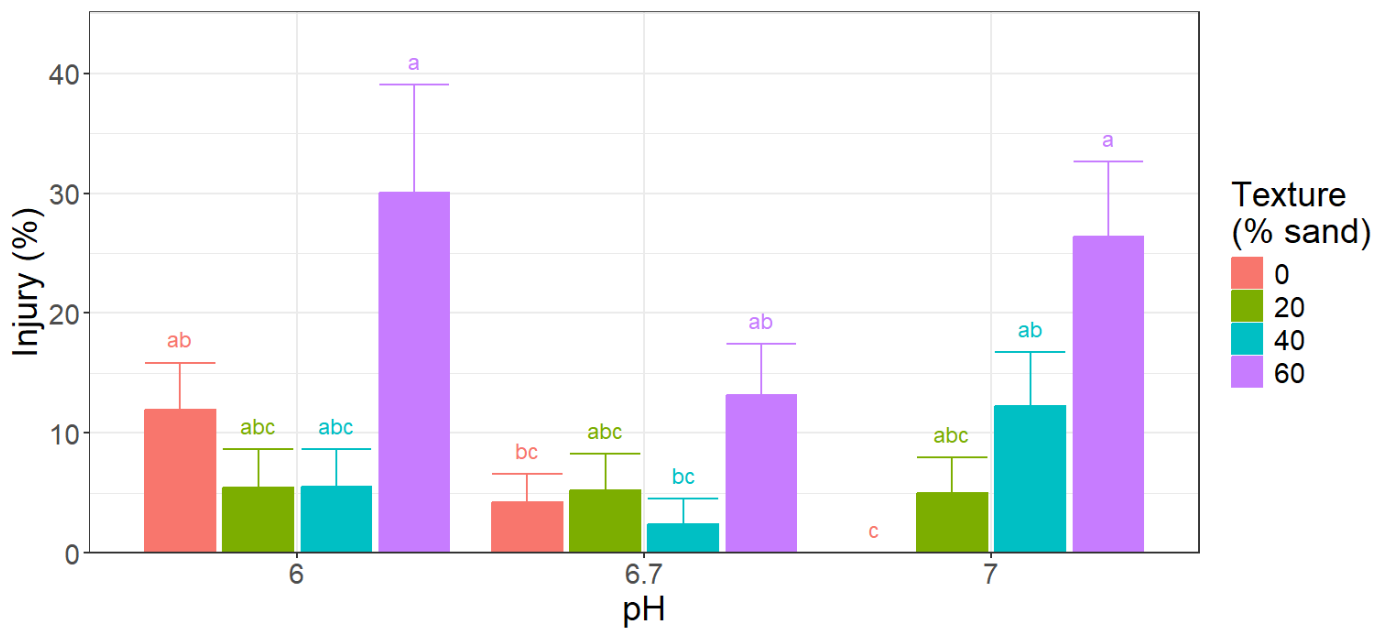

Figure 3.2. Visual percent soybean injury in response to sulfentrazone at 21 days after application to soils with different pH and soil texture treatments. Different letters for seedling emergence across pH, texture, and rate levels indicate significance at a=0.05.

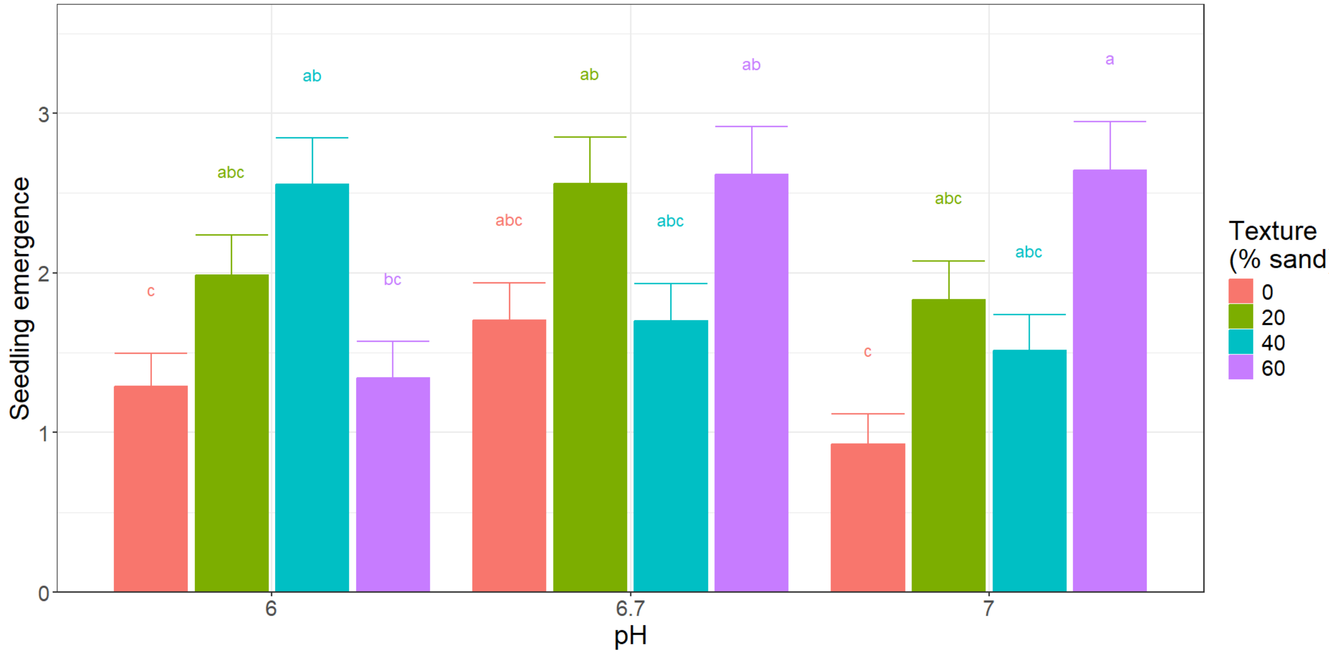

Figure 3.3. Soybean seedling emergence (number per pot) to soils with different pH and texture treatments. Different letters for seedling emergence across pH, texture, and rate levels indicate significance at a=0.05.

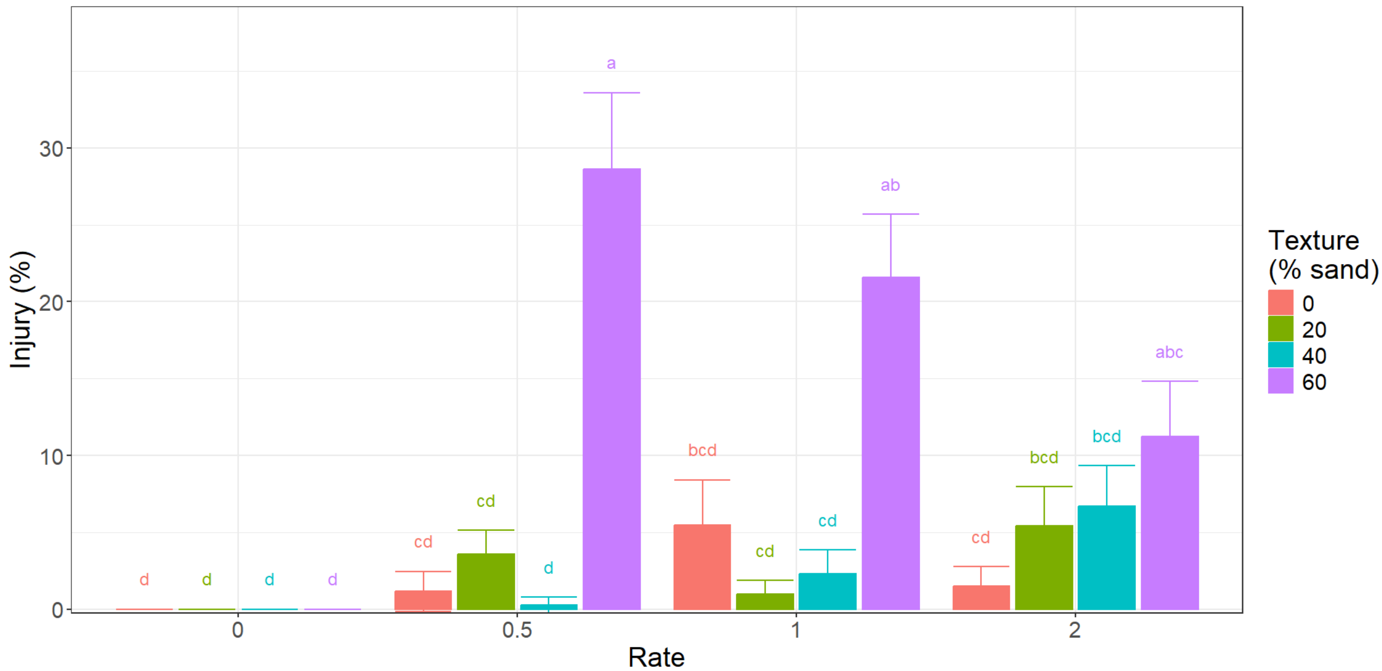

Figure 3.4. Visual percent soybean injury in response to 0.5, 1, or 2X chlorimuron-ethyl (53 g ha^{-1}) at 21 days after application to soils with different soil texture treatments. Different letters for seedling emergence across pH, texture, and rate levels indicate significance at a=0.05.

Table 3.1.Final soil characteristics of pH, CEC, sand, silt, and clay percentage after lime and sand amendments that were used to test each herbicide treatment.

Treatment	pH	CEC	Sand (%)	Silt (%)	Clay (%)
0% sand	6	17.7	17	49	34
	6.7	20.3	16	51	33
	7	20.5	16	50	34
20% sand	6	15	34	40	26
	6.7	18.1	35	37	28
	7	18	31	41	28
40% sand	6	10.9	52	28	20
	6.7	14.1	52	26	22
	7	15.0	56	24	20
60% sand	6	8.3	62	22	16
	6.7	9.2	74	14	12
	7	9.4	73	13	14

Chapter 4 - Changes in droplet characteristics and weed control when adding different adjuvants with herbicides

Abstract

Herbicide efficacy can be significantly influenced by the addition of adjuvants, which improve droplet deposition and minimize the negative effects of adverse environmental conditions on herbicide performance, enhancing weed control. This study evaluated the impact of different adjuvant combinations on spray coverage and weed control in burndown applications. A field experiment was conducted using a randomized complete block design with 12 herbicide and adjuvant combinations, along with a non-treated control. Weed control effectiveness was quantified by the percentage of weed-free area and droplet count was analyzed using spray cards and ImageJ software. The results indicated that certain adjuvant combinations improved weed control compared to herbicides applied alone. For glyphosate-based treatments, the combination with Amsol™ + Destiny® HC, Amsol + Noble®, Class ACT® NG® + Destiny® HC, and Class ACT® NG® + Strikelock demonstrated the best percentage in weed control. For saflufenacil, no adjuvant combinations resulted in weed control differences compared to the herbicide applied alone. For glyphosate, the only adjuvant combination that increased the number of droplets was Amsol™ + Destiny® HC. The other combinations had no differences compared to the herbicide applied alone. For sulfentrazone, more droplets was achieved with the combination of Amsol™ + Destiny® HC, as well as with the combinations of Class ACT® NG® + Destiny® HC and Class ACT® NG® + Destiny® HC + Interlock. It was observed in this study that when the droplet count increased for glyphosate with Amsol™ + Destiny® HC, the percentage of weed-free area also increased. For some treatments, even when the droplet count was increased, there were no differences in the percentage of weed-free area.

This study demonstrated that certain simple combinations of adjuvants can enhance the weed control effectiveness of these herbicides.

Keywords: glyphosate, saflufenacil, efficacy, droplet number.

Introduction

Achieving optimal herbicide efficacy in weed control depends on uniform droplet deposition across the leaf surface of the target weed. Adjuvants are commonly used to enhance application efficiency by reducing the liquid's surface tension, which increases the spreading area and decreases the contact angle of the droplets (Oliveira et al., 2025). In addition to improving droplet deposition, adjuvants also play a crucial role in minimizing the negative effects of adverse environmental conditions on herbicide performance, such as high temperatures, low humidity, and wind (Green and Foy, 2004). Ineffective herbicide applications can accelerate the development of herbicide-resistant weed populations, complicating future management strategies (Norsworthy et al., 2012).

Adjuvants can be classified into two groups: activator adjuvants that enhance the efficacy of an herbicide once it has been deposited on the target surface, and utility adjuvants that work on the properties of the spray solution (McMullan, 2000). An adjuvant may increase weed control with one herbicide but not with another (Zollinger, 2012); before using any adjuvant, it is necessary to understand how it may benefit the spray solution or improve control efficacy (Ferreira et al., 2020).

The interactions among herbicide-adjuvant-plant-environment results in a complex system (Paconoski, 2015). Selecting the appropriate adjuvant is crucial for enhancing herbicide efficacy, as it requires a comprehensive understanding of the specific conditions and desired improvements. Factors such as environmental conditions, weed species, and tank-mix partners play significant roles in determining the necessity and type of adjuvant to be used (Hartzler, 2017). While adjuvants can enhance herbicide efficacy, their inappropriate use may not provide significant benefits and can sometimes decrease the herbicide's effectiveness or cause harm to non-target plants (Tu et al., 2001).

Despite the widespread evolution of glyphosate-resistant weed populations, glyphosate remains an effective herbicide for the control of perennial weeds, many grass species, and in no-till farming systems (Duke and Powles, 2008). Formulated products and tank mixtures containing adjuvants can enhance the effectiveness of glyphosate, improving herbicide uptake by acting on the leaf surface, on the cuticle, and within the internal tissues (Travlos et al., 2017). The adjuvant alone cannot overcome existing herbicide resistance mechanisms, but enhancing the herbicide efficacy can contribute to more effective weed control and potentially slow the development of resistance in the future (Hartzler, 2015). It was noted that the synergistic effects of glyphosate and adjuvants were strongly influenced and depended on several factors, including the nature of the leaf surface, the relative humidity, and the physicochemical properties of the formulated product and adjuvants (Leaper and Holloway, 2000).

Saflufenacil is a PPO-inhibiting herbicide that controls broadleaf weeds that are resistant to glyphosate, used in preplant burndown applications, and has both contact and residual activity (Knezevic et al., 2009). Saflufenacil efficacy was enhanced by the addition of adjuvants (Eubank et al., 2013) and According to Knezevic et al., (2009), the burndown activity of saflufenacil was the greatest with methylated seed oil (MSO) or crop oil concentrate (COC). Given the potential to enhance weed control using adjuvants, the objective of this study was to evaluate the weed control efficacy and droplet characteristics when adding different adjuvant combinations to herbicide products for burndown applications.

Materials and methods

A field study was conducted at the Kansas State University Department of Agronomy, Ashland Bottoms Research Farm near Manhattan, KS, in 2023. The study was organized in a randomized complete block design with four replications, sprayed on May 18, 2023. The treatments consisted of two herbicides (glyphosate and saflufenacil) alone or each in combination with five adjuvants as well as a nontreated control treatment (Table 4.1). Source of products (Table 4.2) and adjuvants (Table 4.3) are provided. Each treatment had four replications. The application volume was 189 L $ha^{-1,}$ using eight banding nozzles type 40-02E set on 38.1 cm spacings, for a total of 3 m wide. Before the application process, weeds were documented by species and number in each plot, using four randomized measurements in 0.25 m^2 quadrats, and images were captured using a GoPro camera before and 7 and 14 days after treatment (DAT) to document weed control. Images were input into the Canopeo app (https://canopeoapp.com/) developed by Oklahoma State University, to compare the % reduction in greenness (Figure 4.3). At the time of application, three spray cards (SpotOn, 51 x 76 mm) were placed in each plot, and they were collected and placed in a plastic bag after the application. For each card, the droplet characteristics among treatments was analyzed using ImageJ to compare if there were differences in droplet number. For all the treatments, dicamba + diflufenzopyr was added considering the high infestation of pigweeds in the area.

Herbicides were analyzed separately, to evaluate the effects of treatments, generalized linear mixed models (GLMMs) were fitted using the glmmTMB package in R. Model fit and residual diagnostics were assessed using the DHARMa package. Post hoc comparisons of treatment means were conducted using estimated marginal means (emmeans package), followed by Tukey’s honestly significant difference (HSD) test for multiple comparisons.

Results and Discussion

Percentage of weed-free area

The presence of pigweeds and some grasses like yellow foxtail (*Setaria pumila*) and large crabgrass (*Digitaria sanguinalis*) were predominant in the area. No differences were observed for the image data describing the percentage weed-free area as calculated by the Canopeo app, before and after the application. In general, the weed-free area was more than 50%. All treatments demonstrated a significant improvement in weed control compared to the nontreated control, with varying levels of efficacy among the different adjuvant combinations (Figures 4.1 and 4.2). For glyphosate-based treatments, the combinations of Amsol™ + Destiny® HC, Amsol + Noble®, Class ACT® NG® + Destiny® HC, and Class ACT® NG® + Strikelock showed the best percentage of weed-free area (Figure 4.1). While no differences in weed-free area were observed for the combination of glyphosate with Class ACT® NG® + Destiny® HC + Interlock, the addition of adjuvants in general consistently resulted in a higher percentage of weed-free areas compared to the herbicide alone, highlighting the potential benefits of adjuvant inclusion in enhancing herbicide performance.

When glyphosate is mixed with hard water (higher concentration of dissolved minerals, notably calcium, magnesium, and iron), these positively charged cations can bind to the negatively charged glyphosate molecules, and this interaction forms complexes that are less readily absorbed by plants, thereby diminishing the herbicide's efficacy (Soltani et al., 2011). Adding ammonium sulfate (AMS) to the spray tank water before mixing in glyphosate helps condition the water and enhances weed control, even if a surfactant isn't required. The sulfate ion (SO_4^{2-}) in AMS carries a negative charge and attaches to positively charged minerals commonly found in hard water, like calcium (Ca^{2+}), magnesium (Mg^{2+}), and iron (Fe^{2+}). By binding these ions first, AMS prevents them from interacting with glyphosate, which would otherwise reduce its effectiveness in the plant (Manuchehri and Lancaster, 2017). The inclusion of surfactant makes droplet coverage on leaves better (Li et al, 2019); besides that, it increases evaporation time, allowing the glyphosate absorption to continue even after the droplets' evaporation (Travlos et al, 2017). When applied alone, even in different salt forms, it did not penetrate specific structural elements of plant surfaces (Castro et al, 2016). Various types and amounts of adjuvant additives, either included in the formulated products or added to the tank mixture, have been found to improve glyphosate performance in different ways (Travlos et al, 2017). For saflufenacil, no adjuvant combinations demonstrated differences compared to the herbicide applied alone. Our results go in the opposite direction from those found by Knezevic et al (2009), who observed that the best control for weeds was achieved with the inclusion of MSO (Destiny® HC and Noble®) with saflufenacil.

Droplet count

For glyphosate, the only adjuvant combinations that increased the number of droplets were Amsol™ + Destiny® HC. The other combinations showed no differences compared to the herbicide applied alone. For saflufenacil, the same result was achieved with the combination of Amsol™ + Destiny® HC, and also with the combinations of Class ACT® NG® + Destiny® HC and Class ACT® NG® + Destiny® HC + Interlock.

When the droplet count increased for glyphosate in combination with Amsol™ + Destiny® HC, the percentage of weed-free area also increased. For some treatments, even when the droplet count was increased, there were no differences in the percentage of weed-free areas. Contrary to the results of this study, Bouse et al (1990) found that different droplet spectra had no influence on systemic herbicides, but highly influenced contact herbicides.

Droplet size was not directly measured in this study. However, since the total application volume remained constant while the number of droplets increased, it can be mathematically determined that the droplet size was reduced. Milanowski et al (2022) found that some adjuvants added in tank mixes reduced droplet volume median diameter (VMD), leading to a higher proportion of fine droplets susceptible to drift.

This study demonstrated that certain simple combinations of adjuvants can enhance the weed control effectiveness of these herbicides. A more detailed study conducted in a controlled environment could help clarify key aspects, such as the changes in droplet size induced by some of these adjuvants, improvements in plant coverage, and their potential impact on drift.

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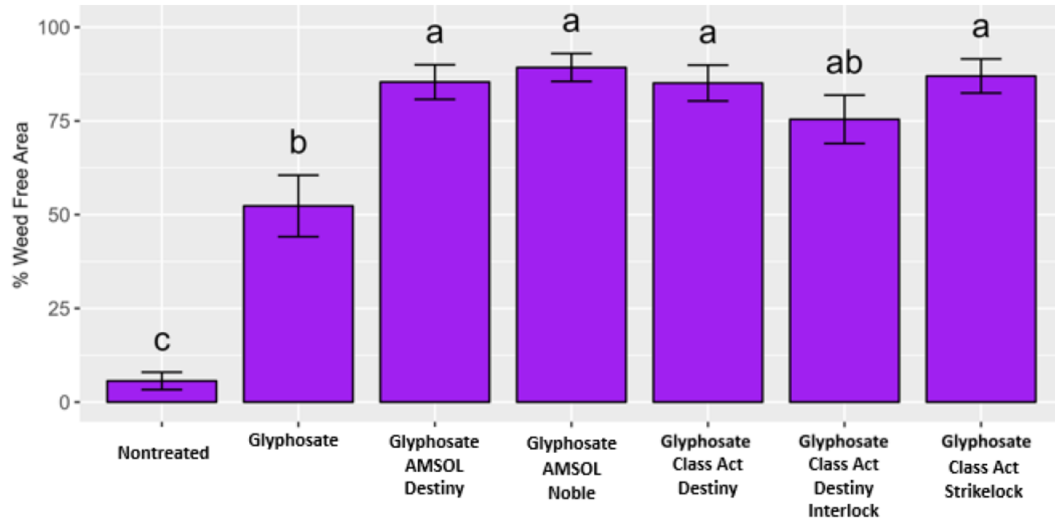

Figure 4.1. Percentage of weed-free area for glyphosate in combination with different adjuvants

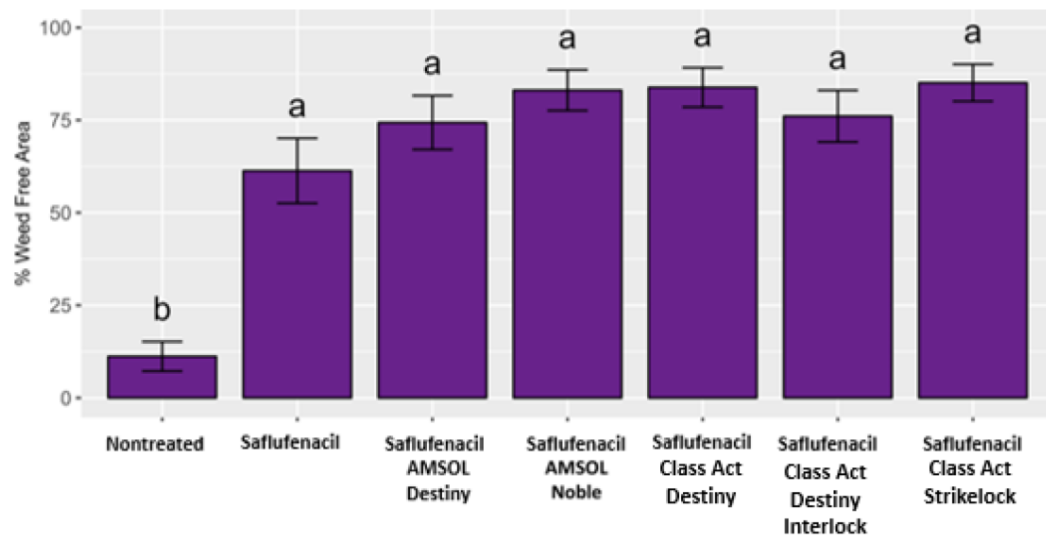

Figure 4.2.Percentage of weed-free area for saflufenacil in combinations with different adjuvants

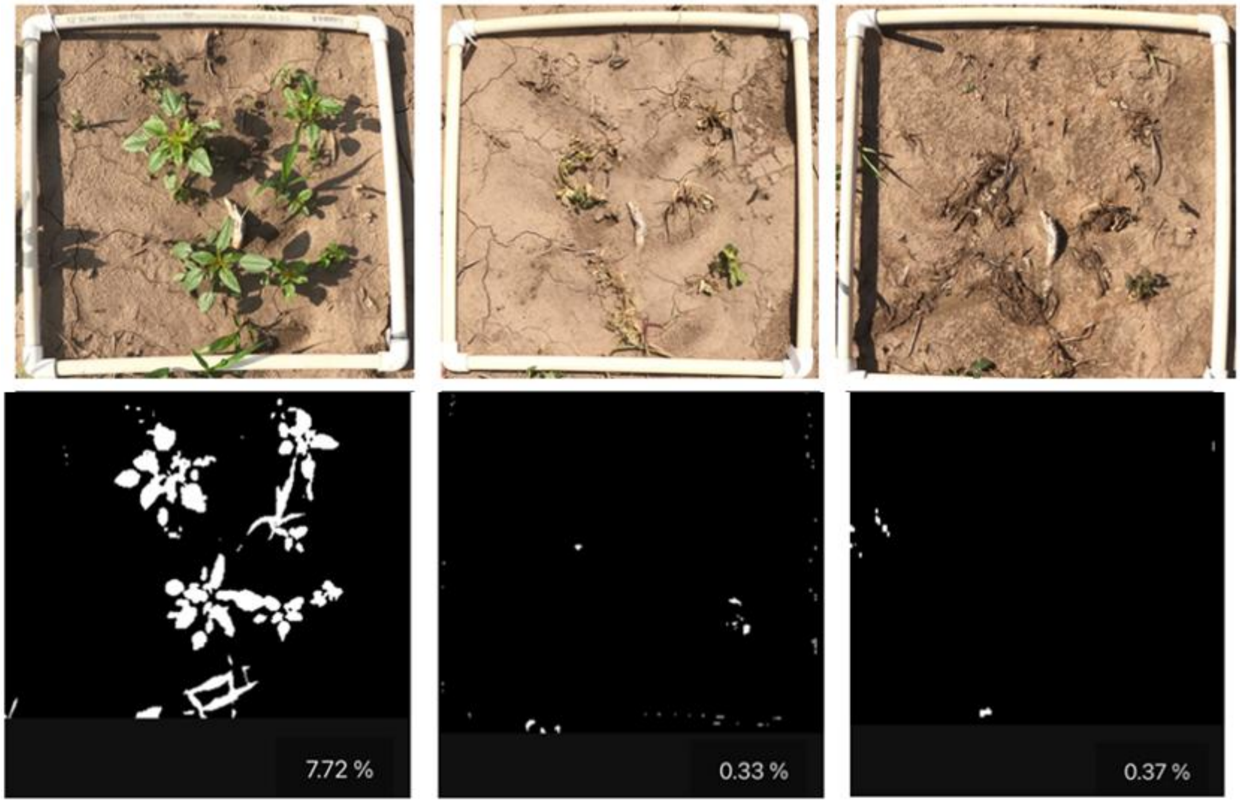

Figure 4.3 Comparison of images using the Canopeo app before the application and at 7 and 14 days after treatment application to evaluate percentage reduction in greenness. The example shown is for the treatment Powermax + Status + Amsol + Noble MSO**.**

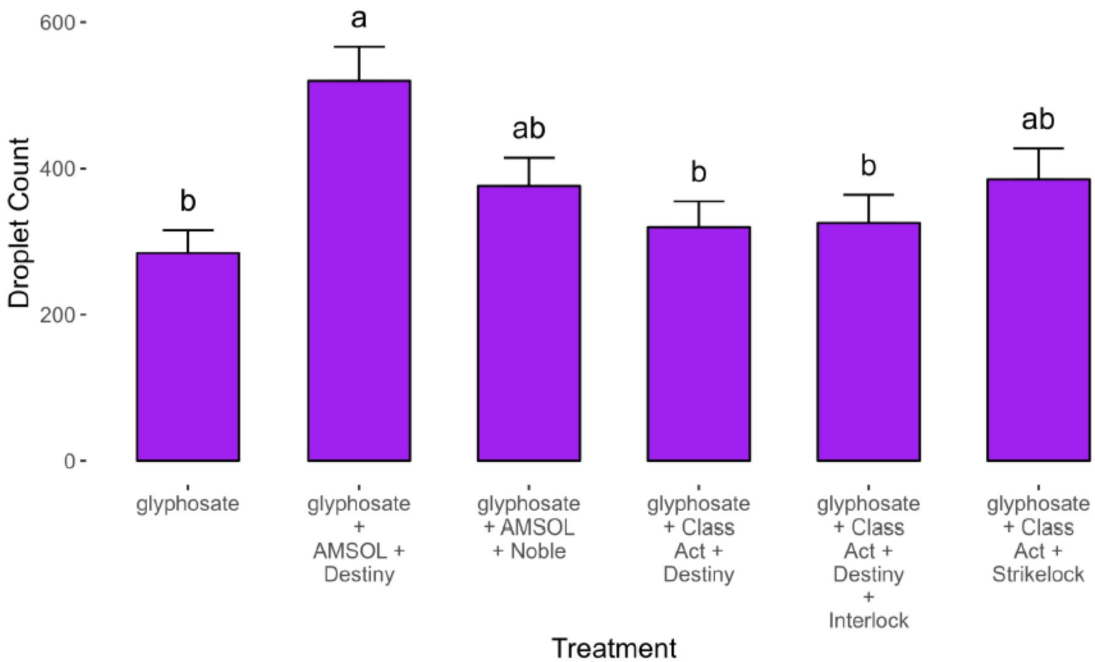

Figure 4.4 Droplet count for glyphosate in combination with different adjuvants

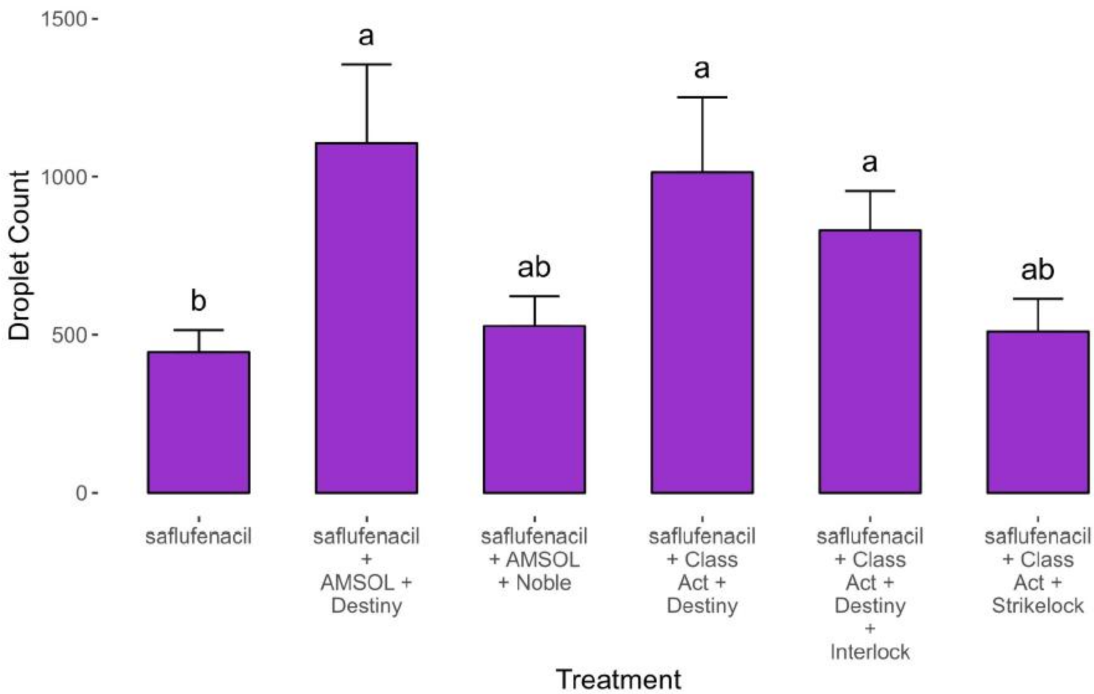

Figure 4.5 Droplet count for saflufenacil in combination with different adjuvants

Table 4.1. Treatments description

Treatments
Sharpen + Status
Sharpen + Status + Amsol + Noble MSO
Sharpen + Status + Amsol + Destiny HC
Sharpen + Status + Class Act NG + Destiny HC
Sharpen + Status + Class Act NG + Destiny HC + Interlock
Sharpen + Status + Class Act NG + StrikeLock
Powermax + Status
Powermax + Status + Amsol + Noble MSO
Powermax + Status + Amsol + Destiny HC
Powermax + Status + Class Act NG + Destiny HC
Powermax + Status + Class Act NG + Destiny HC + Interlock
Powermax + Status + Class Act NG + StrikeLock
Nontreated

Table 4.2. Herbicides used for the study

Herbicide	Trade name	Mechanism of action	Manufacturer	Field doses (g ha^{-1})
Glyphosate	Roundup Powermax	EPSP synthase inhibitor	Bayer Cropscience, 800 N, Lindbergh Blvd. St. Louis, Missouri	1261
Dicamba + Diflufenzopyr	Status 56 WDG	Growth regulator inhibitors	BASF Corporation, 26 Davis Drive Research Triangle Park, NC 27709	224 + 90
Saflufenacil	Sharpen	PPO Inhibitor	BASF Corporation, 26 Davis Drive Research Triangle Park, NC 27709	75

Table 4.3. Adjuvants used for the study were from Winfield Solutions, LLC.

Adjuvant	Principal Function Agent	Use Rate
Class ACT® NG®	NIS + water conditioner + anti-foam	2.5 % v/v
Amsol™	Water conditioner + drift reduction	2.5 % v/v
InterLock®	Drift reduction + coverage enhancer	1% v/v
Noble®	(MSO) Methylated seed oil	1% v/v
Destiny® HC	COC / MSO	0.5 % v/v
StrikeLock®	Drift reduction	0.5 % v/v

Conclusions and Future Directions

Optimizing weed control is becoming increasingly important due to the growing challenges resulting from the lack of efficient and viable management. Discovering strategies to make current chemical management practices more efficient and sustainable from a weed resistance perspective is essential. This study focused on three major improvements that can be done to address the problem.

Results from the chapter to identify the presence of cross- and multiple-herbicide resistance in waterhemp populations from Northeast Kansas, were crucial to helping farmers make informed decisions about their chemical programs. While some populations exhibited reduced sensitivity to glufosinate, they remained susceptible to 2,4-D. Future studies are needed to determine whether this reduced susceptibility signals early resistance development or if environmental factors are affecting herbicide efficacy.

Understanding the influence of soil properties, such as pH and texture, and their interactions with soil-applied herbicides on soybean emergence and injury is crucial for selecting the most appropriate herbicide. Sulfentrazone and chlorimuron-ethyl tend to have a negative impact on soybeans emergence and cause injury in sandy soils, particularly up to 21 days after planting and herbicide application. Further studies are needed to assess the extent of this negative effect later in the season, to determine whether soybeans can recover and maintain yield. Also, it would be important to evaluate if the herbicide's effectiveness for weed control changes in soils with different characteristics.

Results demonstrated that some adjuvants in combination with glyphosate and saflufenacil can enhance weed control. For glyphosate, the treatment that showed the best weed control also showed the highest droplet count. Future studies are necessary to supplement information like weather conditions.

This thesis integrates three complementary aspects of weed management, demonstrating that small adjustments can maintain or even enhance weed control over time. Further research will be essential to address unresolved questions and assess these strategies on a field scale, particularly the use of soil-applied herbicides in variable soil conditions. It will help develop practical management recommendations to support farmers in making informed decisions.