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Challenges and Strategies in Weed and Herbicide Management for Industrial Hemp

Milos Zaric
University of Nebraska-Lincoln, milos.zaric@huskers.unl.edu

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CHALLENGES AND STRATEGIES IN WEED AND HERBICIDE MANAGEMENT FOR INDUSTRIAL HEMP

by

Milos Zaric

A DISSERTATION

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The Graduate College at the University of Nebraska
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For the Degree of Doctor of Philosophy

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(Weed Science)

Under the Supervision of Professor Samuel Wortman

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CHALLENGES AND STRATEGIES IN WEED AND HERBICIDE MANAGEMENT FOR INDUSTRIAL HEMP

Milos Zaric, Ph.D.

University of Nebraska, 2023

Advisor: Samuel Wortman

Renewed interest in industrial hemp (Rosales, Cannabinaceae, *Cannabis sativa* L.) production in the United States, driven by recent legislative changes including the Agriculture Act of 2014 and Agriculture Improvement Act of 2018, has sparked opportunities across various industries, from textiles to pharmaceuticals. With a specific focus on the High Plains climate, this research aims to inform growers about the feasibility of integrating industrial hemp into or nearby existing corn and soybean fields, crucial for those considering starting or expanding their production.

The research is structured into three primary studies:

1. **Herbicide Drift Sensitivity (Chapter 2)**: Assessing the risk of physical herbicide drift (through physical movement of droplets) from corn and soybean fields adjacent to hemp. Results underscore the high-sensitivity because of hemp exposure to herbicides commonly used in these crops, indicating a high risk of biomass reduction and economic losses.
2. **Hemp Tolerance to ACCase Inhibitors (Chapter 3)**: Evaluating the response of different hemp cultivars to Group 1 (ACCase inhibitor) herbicides. The

study reveals varying levels of cultivar sensitivity, with hereby assessed cultivars displaying minor effects even when exposed to herbicide doses exceeding typical usage. These insights are crucial for developing tailored management strategies and understanding the interaction between ACCase inhibitors and hemp.

3. **Volunteer Hemp Management in Soybean (Chapter 4) and Corn (Chapter 5) Cultivation**: Investigating the response of early-stage volunteer hemp to weed control methods widely used in Nebraska in these crops. This study contributes to the strategic development of herbicide programs, enhancing the effectiveness of volunteer hemp control within standard soybean and corn rotations.

Together, these studies provide essential insights for growers, outlining the opportunities and challenges of incorporating hemp into existing crop rotation systems. The results assist in informed decision-making choices regarding the use of herbicides in adjacent crops, managing risks with anticipated herbicide application (limited to ACCase inhibitors), and decision-making for comprehensive volunteer hemp control strategies in subsequent crop rotations.

Dedication

To my family, whose faith in me has been both my anchor and inspiration. This achievement is as much yours as it is mine.

In memory of Srđan Ćirović (Срђан Ћировић), your memory lives on,
a silent but powerful inspiration.

Acknowledgments

Embarking on this PhD journey in August 2020, I first extend my deepest gratitude to Dr. Greg Kruger. Dr. Kruger's initial guidance through my master’s program at Pesticide Application Technology laid the foundation for my research and academic growth. I indeed appreciate trust in my potential and the academic freedom he provided which allowed me to explore and delve deeply into different research topics. His consistent support, resources, and mentorship have been instrumental in shaping my development as a scientist. However, his transition from academia to industry was a pivotal moment in my journey, necessitating a change in supervision. This change brought me under the mentorship of Dr. Sam Wortman, to whom I owe immense thanks for taking me over.

Dr. Wortman’s advice and the directions he offered have influenced my thinking and approach to various scientific tasks. Over the two years he played a crucial role in sharpening my ability to broaden my perspective to consider the greater context of my research. His expertise not only guided me through the technical aspects but also encouraged a deeper appreciation for finding coherence and significance within complicated datasets. This skill has been invaluable in framing my research and in refining my capabilities as a critical thinker and a pragmatic researcher. I also wish to express my sincere appreciation to Drs. Chris Proctor and Julie Peterson for their invaluable time, dedication, and contributions as members of my committee. Their commitment and involvement significantly enriched my experience and learning as a PhD student. Their collective wisdom, guidance, and support have been crucial in helping me navigate the challenges and triumphs of this academic expedition. I am eternally grateful for their mentorship and the pivotal roles they have played in my development and success.

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I extend special thanks to Kelly Bruns, Director of the West Central Research, Extension, and Education Center in North Platte, NE, Bob Skates, Facilities Manager, and to the team at the Greater NE Business Center. Special mention goes to Stephanie Cole, Angie Mohr, Amanda Wuehler, Susan O'Keefe, and Alecia Hothan from the IANR Finance & Personnel Office. Their unwavering support and guidance have been instrumental in my continuous learning about the University of Nebraska-Lincoln system. I am appreciative of their detailed attention in reviewing my research proposals. Their keen eyes ensured that no oversight went unnoticed, ultimately keeping me on the right track. Furthermore, I must express my appreciation for Cathy Fox, whose expertise in swiftly resolving technological issues was invaluable, especially during a critical moment when my laptop stopped working. Cathy, you may not always realize the enormous value of your contributions, but they have significantly impacted on me and my work. I am also deeply grateful to Turner Door and his family (Bridget, Boone, Silas, Briar, and River) for their presence and the enriching discussions we have shared during breaks and major holidays. Their friendship and perceptions have added greatly to my experience here in North Platte, Nebraska.

Last, but certainly not least, I need to thank my family and sisters Marija, Milica, Stanika, and Danijela—and my nieces (Vanja, Andjelija, and Nikolina (who will bless our world in late December or early January)) and nephews (Dusan, Marko, Stefan, Nemanja, Darko, and Vladimir) for all their love and support since I started my journey. Special thanks must be directed to my dad and mom, Sreten and Ljubica Zaric. Without their sacrifices and efforts to help me find the right path, teach me respect, show me how to be hard-working, and be persistent in achieving my goals, I would not be where I am now. Sometimes, I may have missed a few of our 'noon' calls, but those were always followed by a message, just so I knew that both of you were thinking of me. Finally, I would like to thank Andrea; your love and support, especially on the difficult days, meant so much to me!

THANK YOU ALL FOR BEING A PART OF THIS JOURNEY!!!

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CHAPTER 1

Overview and Purpose of Research Work

The expansion of industrial hemp (*Cannabis sativa* L.) cultivation and its numerous applications caused a substantial shift in agricultural and industrial practices globally as well as in the United States (US). Since the transformative Agriculture Act of 2014 and the Agricultural Improvement Act of 2018, hemp has emerged not only as a versatile crop in the agricultural sector but also as an important section in diverse industries such as textiles, food, construction, and pharmaceuticals. Renewed interest is evident from the substantial legal cultivation across 48 continental US states and in over 30 nations worldwide, signifying a remarkable turnaround in policy and public perception globally (Adesina et al., 2020; Amaducci et al., 2015; Mark et al., 2020). In the US, states like Colorado and Montana lead in production, and the market value of hemp products increased from $700 million in 2018 to $821 million in 2021, underlining the crop economic potential (Johnson, 2019; USDA NASS, 2022). However, currently the US still relies significantly on imports of raw materials, especially for hemp grain where imports between 2015 to 2020, went from $51.2 to $79.9 million, with Canada being a major supplier (UNCTAD, 2022).

Growth in requirements for hemp seeds in the US, combined with supporting regulations, suggests an encouraging future for hemp grain cultivation on a global scale, particularly within the continental US. However, the integration of hemp into established crop rotations, particularly with corn (*Zea mays* L.) and soybean (*Glycine max* (L.) Merr.), poses several unknowns. Understanding the potential pros and cons of industrial hemp production is crucial for local growers interested in starting or expanding their production efforts. This dissertation will provide research findings addressing the following: (1) Herbicide spray drift effects on industrial hemp in a low-speed wind tunnel, (2) Comparison of Acetyl CoA Carboxylase inhibiting herbicides for use in industrial hemp, and (3) Volunteer hemp tolerance to early-season herbicides in soybean and corn.

Sensitivity to the physical drift of herbicides registered for use in corn and/or soybean

The prevalent use of herbicide-tolerant crops in the US has led to a dependency on herbicides for post-emergence (POST) weed control, strengthening concerns about herbicide drift, defined as the movement of herbicides away from intended target, primarily through wind-driven droplets (Al-Khatib and Peterson, 1999; Alves et al., 2020; Dodson, 2020). In general, about 70% of sprayed herbicides reach their target, the rest being susceptible to drift or runoff, highlighting the need for improvements in pesticide delivery methods (Pivato et al., 2015; Van der Werf, 1996). Numerous factors, including environmental conditions, sprayer configuration, and droplet size influenced by nozzle type, play a fundamental role in determining drift potential (Hewitt, 2000; Nuyttens et al., 2007; Vieira et al., 2020).

In the context of industrial hemp, a crop without any currently approved POST herbicides in the US, advanced understanding of the implications of herbicide drift are critical. Past research has focused on the impact of direct herbicide application at sub-lethal doses, revealing substantial visual injury as well as high potential for biomass reduction in hemp (Flessner et al., 2020; Ortmeier-Clarke et al., 2022). Therefore, our study aimed to investigate the effects of herbicide drift on industrial hemp in a controlled, low-speed wind tunnel environment, examining how different nozzle types and herbicides affect droplet formation, spray deposition, and ultimately, hemp biomass accumulation.

Evaluation of hemp tolerance and the possibilities for diversification of Group 1 (ACCase inhibitors) herbicides

The limited number of POST herbicides approved for hemp in the US, highlights the need for expanding chemical weed management options in hemp (Amaducci et al., 2015). Figure 1.1 illustrates a sample of hemp grain extracted from a grain bin in Nebraska, displaying hemp seeds mixed with a diversity of weed seeds, thereby highlighting the necessity of developing efficient herbicide treatments for use in industrial hemp production. Initial screenings of pre-emergence and POST herbicides revealed that many, while reasonably effective herbicides in crops like corn and soybean, caused significant injury to hemp (Flessner et al. 2020; Ortmeier-Clarke et al. 2022). An exception within these screenings was the Group 1 herbicides, ACCase inhibitors, which hemp can tolerate with minimal impact on biomass (Flessner et al., 2020; Ortmeier-Clarke et al., 2022). ACCase inhibitors, comprised of aryloxyphenoxypropionates (FOPs), cyclohexanediones (DIMs), and phenylpyrazoline (DENs), target an enzyme crucial in lipid biosynthesis, primarily affecting monocot weeds while typically sparing dicotyledonous crops like hemp (Bough and Dayan, 2022; Kaundun et al., 2012; Takano et al., 2020).

Despite the allowance of specific ACCase herbicides like quizalofop in Canada and Australia for hemp cultivation, the lack of comprehensive data on the impact of various ACCase inhibitors across different hemp cultivars and environmental conditions is evident (AGVET 2022; Santo 2022; Van Wychen 2022). Current studies, including Flessner et al. (2020), Ortmeier-Clarke et al. (2022), and Lingenfelter (2018 and 2019), showed variations in hemp tolerance to different ACCase herbicides. In addition, the work by Savic et al. (2020) particularly draws attention to the contrasted responses of hemp to ACCase herbicides, emphasizing the need for broader, more comprehensive evaluations across cultivars.

This study, therefore, aims to address this critical research gap by assessing the tolerance of specific hemp cultivars to seven ACCase inhibitors from different chemical families (FOPs, DIMs, and DENs) under controlled conditions. This effort might enhance understanding of hemp response to these herbicides but as well as also potentially lay the groundwork for broader, more effective ACCase herbicide programs in hemp cultivation, with a focus on sustainable, safe, and effective weed management.

Volunteer hemp responses to early-stage herbicides used in corn and soybean cultivation

One of the main challenges with currently available hemp grain cultivars includes the indeterminate flowering trait that as a result has varying seed maturation within a seed head. The occurrence of this trait might result in significant numbers of volunteer hemp seeds left in following crop rotations. Initial studies, confined to just two sites and ten samples per site collected during 2022, indicate the possibility of achieving 5,000 to

10,000 plants per square meter as shown in Figure 1.2. Alongside the limited information on effective herbicide treatments for volunteer hemp, particularly in soybean and corn cultivation, implies a critical knowledge gap. Despite the widespread adoption of herbicide-tolerant crop varieties and the primary reliance on herbicides in these systems, the effectiveness of commonly used herbicides against volunteer hemp remains largely unexplored (Chahal and Johnson, 2012; USDA ERS, 2022).

Past research indicates intensified sensitivity of hemp to various active ingredients, yet such studies primarily focused on direct application to individual plants, not considering the dynamics of dense, field-grown volunteer hemp populations. Initial findings suggest that certain herbicides, such as clethodim and clopyralid (Ortmeier-Clarke et al., 2022), may be less detrimental to hemp biomass, presenting a potentially less concerning management issue in soybean and corn rotations. However, high sensitivity to glyphosate (90% or greater biomass reduction compared to non-treated) was previously observed for volunteer hemp control (Horowitz, 1977; Mettler, 2021; Ortmeier-Clarke et al., 2022). Additional challenges associated with a constant over-reliance on a single mode of action herbicides for weed control historically have led to the evolution of herbicide-resistant biotypes (Heap, 2014). Moreover, supporting diverse chemical control options is critical and requires further evaluation to avoid the development of volunteer hemp-resistant populations.

Given the substantial potential for hemp seed loss and the consequential emergence of volunteer hemp, coupled with the limited current understanding of herbicide efficacy under field conditions. This study aimed to bridge the critical knowledge gap by evaluating the effectiveness of various early-season herbicides used in soybean and corn cultivation against high-density volunteer hemp. This research not only contributes significantly to sustainable agricultural practices but also aids in formulating well-coordinated control programs for managing volunteer hemp in subsequent soybean and corn rotations.

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Figure 1.1. Post-harvest industrial hemp grain from grain bin displaying weed seed contamination.

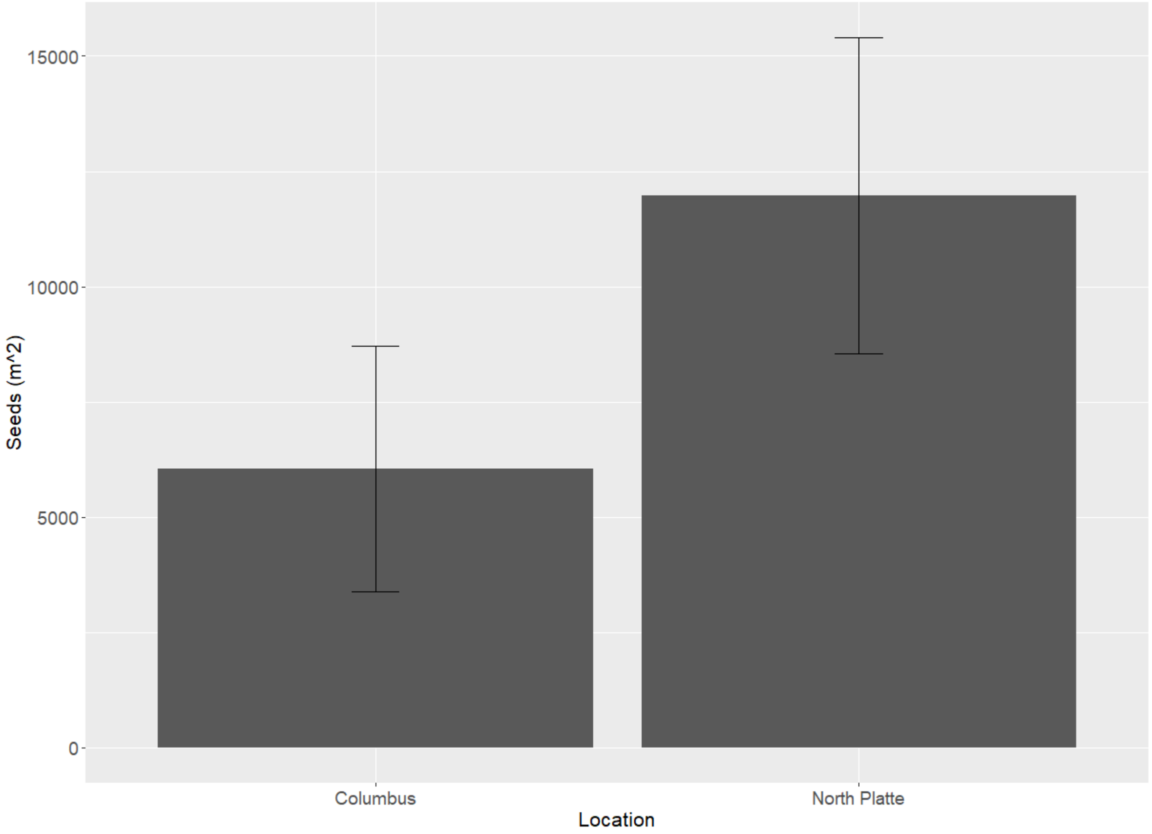

Figure 1.2. Preliminary estimations of volunteer hemp in seeds per meter squared (with standard deviation) in Columbus and North Platte, Nebraska. Ten samples per location were gathered post-hemp harvest in October 2022.

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Chapter 2

Herbicide Spray Drift Effects on Industrial Hemp in a Low-speed Wind Tunnel

Abstract

The establishment of industrial hemp (*Cannabis sativa* L.) fields in areas adjacent to row crops has raised questions about the possible adverse effects of herbicide drift on hemp biomass production. This study aimed to examine industrial hemp susceptibility to the physical drift of herbicides registered for use in corn and/or soybean. Herbicide solutions (2,4-D, dicamba, glufosinate, glyphosate, imazethapyr, lactofen, and mesotrione) were applied separately in the wind tunnel (3.6 m s^{-1} airspeed) with conventional TP95015EVS (TP) and air inclusion AI95015EVS (AI) flat fan nozzles calibrated to deliver 140 L ha^{-1} carrier volume at 230 kPa. At application time, 20-25 cm tall industrial hemp plants (two-three pairs of true leaves stage) were positioned at downwind distances up to 12 m from the nozzle alongside mylar cards. Results indicated that nozzle design influenced herbicide deposition; 5% of spray deposits from TP nozzles reached 5.9 m downwind compared to 2.0 m for AI nozzles. The greatest susceptibility was observed for glyphosate, glufosinate, and mesotrione herbicide drift. Estimations for 50% hemp biomass reduction for TP were at 19.3, 8.7, and 9.3 m, while for AI, at 4.1, 4.0, and 2.9 m downwind, for the three herbicides. Herbicide application from adjacent fields at 3.6 m s^{-1} or greater wind speed must be considered a high-risk situation when industrial hemp is nearby.

2.1. Introduction

Opportunity for growth and utilization in various industries has led to the adoption of industrial hemp (*Cannabis sativa* L.) in over 30 nations worldwide (Adesina et al., 2020). Since 2018, industrial hemp can be legally grown for grain, fiber, and pharmaceuticals (i.e., cannabinoids) (Amaducci et al., 2015; Mark et al., 2020) in at least 48 continental United States (US) (Karus and Vogt, 2004; Olson et al., 2020). Annual hemp product sales for 2018 and 2021 are estimated at nearly $700 and $821 million in the US, respectively (Johnson, 2018; USDA NASS, 2022). As of 2022, Colorado (4087 ha) and Montana (3197 ha) are the two leading states in industrial hemp production, with a significant increase in planted areas since 2018 (USDA NASS, 2022). Specifically, in the North Central growing region of the US, it is expected that there will be an increase in industrial hemp grown over the next five years and that industrial hemp will be grown in fields adjacent to herbicide-tolerant row crops.

The wide adoption of herbicide-tolerant crops in the US has resulted in an increased overreliance on herbicides for post-emergence (POST) weed management (Dodson, 2020; Harker and O’Donovan, 2013; Kniss, 2018; Reddy, 2001). The abundance of POST and broad-spectrum herbicide applications, including glyphosate, glufosinate, dicamba, and 2,4-D, has raised concerns regarding off-target movement (Al-Khatib and Peterson, 1999; Alves et al., 2020; Derksen, 1989; Jones et al., 2019; Roider et al., 2007). Common sources of off-target movement from row crops such as corn and soybean are through herbicide physical or vapor drift that occurs at or after application (Alves et al., 2020; Soltani et al., 2020; Werle et al., 2018). This research predominantly focuses on providing insights into industrial hemp susceptibility to herbicide particle drift (hereafter referred to as herbicide drift), which is commonly defined as a part of the herbicide application that moves away from the target area by wind-carried droplets (Briand et al., 2002; Matthews et al., 2014). Previous studies suggest that about 70% of the sprayed herbicide solution reaches the final target, while the remaining 30% may be lost due to off-target movement and/or runoff (Pivato et al., 2015; Van der Werf, 1996). Therefore, proper selection of application technology parameters must be considered to improve application efficiency while reducing herbicide drift potential (Matthews et al., 2014).

Herbicide application is a complex process influenced by many environmental conditions, including wind velocity, temperature, and relative humidity (Alves et al., 2017; Hewitt, 2000). In addition, sprayer setup, product formulation, tank-mix additives, nozzle type, operational pressure, and boom height can influence herbicide drift potential (Creech et al., 2015; Dorr et al., 2013; Havens et al., 2018; Nordby and Skuterud, 1974; Rodrigues et al., 2016; Vieira et al., 2020). From all variables, it has been considered that spray droplet size directly affected by nozzle selection represents the most critical variable associated with herbicide drift reduction and subsequent consequences (Nuyttens et al., 2007).

Some earlier-developed (standard) flat fan nozzles are characterized by a higher proportion of finer droplets compared to new drift-reducing nozzles. Although standard and drift-reducing nozzles can have similar performance in terms of effectiveness depending on the product used (Nuyttens et al., 2009; Ramsdale and Messersmith, 2001; Souza and Moretti, 2020) finer droplets (less than 200 µm) are typically more prone to drift (Yates et al., 1985). Therefore, to minimize herbicide drift, nozzle manufacturers developed drift-reducing nozzle types that generate lower percentages of driftable fines with coarser droplets (Alves et al., 2017; Dorr et al., 2013; Nuyttens et al., 2007; Perine et al., 2021). One of the differences in drift potential among nozzle types is due to pre-orifice and/or the venturi air-inclusion ports featured in some nozzle designs. In both cases, the aim is to increase droplet size, which will reduce drift potential (Derksen et al., 1999; Dorr et al., 2013; Guler et al., 2007) and downwind spray deposition (Alves et al., 2017; Creech et al., 2015; Vieira et al., 2019) as well as consequences to non-target crops.

As an emerging crop, there are no POST herbicides currently registered for use in industrial hemp in the US (US EPA, 2022). Previous research by Tsaliki et al. (2021) reported a strong positive correlation between total biomass and both hemp fiber (R^2 = 0.86) and stem biomass yield (R^2 = 0.97). Hemp plants exposed to herbicide drift may experience reduced growth and yield, which could lead to decreased total biomass, fiber yield, and stem biomass yield. The strong positive correlation between total biomass and these yield parameters suggests that herbicide drift could have a negative impact on hemp crops and ultimately reduce their economic value. Currently, there is limited information on how much industrial hemp biomass production and final yield could be impacted by herbicide drift from adjacent crops (Ortmeier-Clarke et al., 2022). Moreover, if herbicide residues are detected in plant parts used for direct consumption, it could result in severe economic loss or even crop destruction (Michlig et al., 2021; Seltenrich, 2019). Adverse economic outcomes from off-target pesticide movement have already been reported when high-value, specialty, and food-grade crops are planted near row crops (Bales and Sprague, 2020; Buol et al., 2019; Calzolari et al., 2017; Dintelmann et al., 2019; Johnson et al., 2012). Similar outcomes could be expected for industrial hemp, where consistency in the quantity and quality of harvested materials are critical for either end-industrial use or further processing (Amaducci et al., 2015; Andre et al., 2016; Michlig et al., 2021).

Previous research on the evaluation of industrial hemp tolerance to herbicides has been conducted with direct applications over the top of plants using just a single (Flessner et al., 2020) or series of herbicide doses (Ortmeier-Clarke et al., 2022) under diverse herbicide application settings. As a result, it was determined that most of the evaluated POST herbicide programs, when applied at recommended rates, resulted in detrimental industrial hemp injury and biomass reduction. Ortmeier-Clarke et al. (2022) reported an estimate of the effective dose for 10% industrial hemp biomass reduction for mesotrione (0.005 g ai ha^{-1}), imazethapyr (1.5 g ai ha^{-1}), lactofen (0.3 to 4.8 g ai ha^{-1}), and glufosinate (21 g ai ha^{-1}). All earlier studies have evaluated industrial hemp herbicide susceptibility by simulating drift with sub-labeled doses of herbicides applied directly over the top of the plants. However, it is essential to understand the potential impact of herbicide drift applied at recommended labeled rates and drift toward industrial hemp at various distances from the nozzle (i.e., actual simulation of herbicide drift instead of simulated through sub-labeled doses) under high and low herbicide drift potential.

This research aimed to perform droplet size analysis and quantify the impact of herbicide drift on spray deposition and industrial hemp biomass reduction from commonly used herbicides in corn and/or soybean in a low-speed wind tunnel. The hypotheses were that the nozzle type (with distinct drift potentials) and herbicide used would influence droplet formation, spray deposition, and industrial hemp biomass reduction.

2.2. Materials and Methods

Study site and plant material

All studies were conducted during the summer and fall of 2020 at the Pesticide Application Technology Laboratory (University of Nebraska-Lincoln, West Central Research, Extension and Education Center in North Platte, NE, USA). A multi-purpose (high-cannabidiol, fiber, and grain) variety of industrial hemp (NWG2730, New West Genetics Inc., Fort Collins, CO, USA) used in this study was obtained as a part of the material transfer agreement (*2020-0369A*) between both institutions. In addition, special permission to grow industrial hemp for research purposes for the 2020 growing season was obtained through the Nebraska Department of Agriculture, granted under the *Industrial Hemp Pilot Research Project* (no special license required).

Growing conditions

Industrial hemp seeds were planted in 1 L plastic pots containing commercial potting mix (Pro-Mix BX5, Premier Tech Horticulture Ltd, Rivière-du-Loup, Canada). Pots were maintained under greenhouse conditions (30° C during the day and 20° C during the night) and irrigated daily with tap water. Plants were fertilized using fertilizer blended with water at 0.2% v v^{-1} (UNL 5-1-4, Wilbur-Ellis Agribusiness, Aurora, CO, USA) as needed. Supplemental lighting was provided using LED lights (520 µmol s^{-1}, Philips

Lighting, Somerset, NJ, USA) to ensure a 16-h photoperiod and keep plants in a vegetative stage.

Droplet size measurements

Spray droplet size was quantified in a low-speed wind tunnel with all herbicide solutions prepared as described in Table 2.1. Herbicide solutions (2,4-D, dicamba, glufosinate, glyphosate, imazethapyr, lactofen, and mesotrione) were separately sprayed using highest recommended field labeled rates at 140 L ha^{-1} and two nozzle types including TP95015EVS and AI95015EVS (TeeJet Technologies Spraying Systems Co., Glendale Heights, IL, USA) at 230 kPa. The droplet size analysis for each treatment combination evaluated for this study was measured three times using a laser diffraction instrument in a wind tunnel with a constant airspeed of 6.7 m s^{-1}. Each replication consisted of a complete spray plume passing through the measurement area. More information about procedures and wind tunnel setup, operation, study methods, and concept clarifications are described by Creech et al. (2016) and Vieira et al. (2018). Recorded values included $D_{V0.1}$, $D_{V0.5}$, and $D_{V0.9}$ (droplet diameters such that 10, 50, and 90% of the total spray volume is in droplets of lesser diameter, respectively). The percentage of driftable fines, defined as droplet diameters 200 µm or less, was reported as a proportion of the total spray volume. The spray classifications were based on curves from reference nozzles spraying water alone per ASABE S572.3 standard (ASABE, 2020).

Wind tunnel herbicide drift study

Simulated herbicide drift under controlled conditions was conducted to understand the impact of nozzle selection and herbicide solutions on spray deposition and industrial hemp biomass reduction. Integration of simulated drift in a low-speed wind tunnel as a study method have been determined to nearly-generates data observed under the field conditions when similar nozzles were used (Vieira et al., 2018). Spray deposition and industrial hemp herbicide drift studies were conducted in a complete randomized design with four replications. The study was conducted twice (summer and fall), resulting in two experimental runs. Industrial hemp was used as a bioindicator plant to assess herbicide drift implications on biomass reduction in a wind tunnel following similar approach reported in previous studies (Brankov et al., 2023; Vieira et al., 2019)

The seven herbicide solutions (2,4-D, dicamba, glufosinate, glyphosate, imazethapyr, lactofen, and mesotrione) were prepared as previously described in Table 2.1. with the addition of 1,3,6,8-pyrene tetra sulfonic acid tetrasodium salt (PTSA) as a fluorescent tracer (Spectra Colors Corporation, Kearny, NJ, USA) at a concentration of 3 g L^{-1}. Herbicide solutions were sprayed at 140 L ha^{-1} and 230 kPa using TP95015EVS (TP) and AI95015EVS (AI) nozzle types under a 3.6 m s^{-1} airspeed in a wind tunnel. The selection of TP and AI nozzle types allowed the evaluation of industrial hemp response to two distinct herbicide drift scenarios with high and relatively low-risk potential, respectively. Spray deposition collectors and hemp plants were placed at downwind distances of 1, 2, 3, 6, 9, and 12 m from the spray nozzle (Figure 2.1.). Mylar cards (10 cm by 10 cm) (Grafix Plastics, Cleveland, OH, USA) were used as spray deposition collectors. Industrial hemp plants 20-25 cm tall (two or three pairs of true leaves) were used as bioindicator plants and 51 cm underneath the nozzle. Each replication encompassed a uniform two-second duration of continuous application, regulated by a digital auto shut-off timer switch (Intermatic Inc., EI 400C, Spring Grove, IL, USA). Mylar cards and plants were removed from the wind tunnel two minutes after herbicide application. The average air temperature and relative humidity during this study were 22-25° C and 45-50%, respectively.

Mylar card processing and plant maintenance

To avoid photodegradation of PTSA after application, mylar cards were kept separate in individual pre-labeled bags under dark conditions. Spray deposition for each herbicide solution was quantified by fluorometric analysis. Mylar cards were washed in 40 mL of 10% alcohol solution (91% isopropyl alcohol, PL Developments, Clinton, SC, USA) prepared with distilled water. After the cards were washed, a 1.5 mL aliquot was removed from each sample bag to fill a glass cuvette. The cuvette was placed inside a fluorimeter (Turner Designs, Trilogy, Sunnyvale, CA, USA) equipped with a PTSA module that uses ultraviolet light to obtain relative fluorescence data (RFU). RFU was converted into ɳg cm^{-2} to obtain spray deposition percentage and compared to the theoretical application rate of 140 L ha^{-1}. Additional information about conversion procedures is described by Alves et al. (2017) and Vieira et al. (2019).

After application, hemp plants were maintained in the greenhouse under growing conditions described previously. Plant aboveground biomass was harvested 21 days after application. Biomass was dried in an air-forced dryer at 65 ˚C to reach a constant weight. Dry biomass weights were recorded and converted into percentage of biomass reduction using equation 1:

$$br = [(nt-t)/nt] * 100 \quad [1]$$

where *br* represents biomass reduction (%), *nt* is dry biomass (g) of non-treated plants, and *t* is dry biomass (g) of plants exposed to herbicide drift.

Statistical analysis

The droplet size dataset was subjected to analysis of variance using a generalized linear mixed model (PROC GLIMMIX) in SAS (Statistical Analysis Software, v9.4, Cary, NC, USA). All comparisons were performed within and across nozzle types at $\alpha = 0.05$ significance using a Tukey's Least Significant Difference test.

For herbicide spray deposition and industrial hemp biomass reduction, the model selection function *mselect* tool in R software (R Foundation for Statistical Computing, Vienna, Austria) was used to compare several non-linear model candidates to fit the data (Oliveira et al., 2018; Ortmeier-Clarke et al., 2022). The four-parameter log-logistic function was selected as the best-fit model based on Akaike's information criterion (data not shown), which were analyzed using the *drc* package in R software following equation 2:

$$y = c + \{d - c/1 + \exp [\ b\ (\log x - \log e)]\} \quad [2]$$

where y represents spray deposition or biomass reduction (%), b is the slope at the inflection point, c is the lower limit of the model (fixed to 0%), d is the upper limit (fixed to 100%), and e is the inflection point (distance to 50% spray deposition (m) or biomass reduction (%)) (Knezevic et al., 2007; Ritz et al., 2015). Data from the two experimental runs were combined.

2.3. Results & Discussion

Droplet size distribution

Nozzle design and herbicide solution influenced $D_{V0.1}$, $D_{V0.5}$, $D_{V0.9}$, and the percent of driftable fines values ($P < 0.0001$). The TP nozzle had lower droplet size (Table 2.2.) and greater proportion of driftable fines than the AI nozzle across herbicides (Figure 2.2.). In general, the TP nozzle had a “fine” spray classification with the lowest $D_{V0.5}$ values observed for glufosinate (182 µm) and glyphosate (201 µm) (Table 2.2.). For TP nozzle, the lowest drift potential was observed for 2,4-D and lactofen across herbicides. Application of herbicides with AI nozzle resulted in the formation of larger droplets (“extremely coarse” and “ultra coarse” spray classifications), which, as a result, had an impact on a substantial decrease in drift potential. Glufosinate had the highest potential for drift across all herbicide solutions tested for the TP nozzle (56.6%) and the AI nozzle (2.6%) (Figure 2.2.). Similarly, Creech et al. (2015) reported a decrease in $Dv_{0.5}$ of about 18% for glufosinate compared to water-alone treatment pooled across nozzles. Nozzle design and herbicide solution significantly impact droplet size distribution and driftable fines values, with the TP nozzle producing finer droplets and higher drift potential than the AI nozzle. The differences in drift potential and droplet size categories demonstrate the influence of both nozzle type and the specific herbicide used, which may result in variations in spray deposition. As previously reported the nozzle design emerged as the most critical determinant, in contrast to the composition of the herbicide solution which exhibited a comparatively minimal effect, corroborating findings from previous research (Creech et al., 2015; Door et al., 2013; Vieira et al., 2018; Vieira et al., 2019)

Spray deposition is dependent on nozzle type and herbicide

Pooling herbicides across nozzles indicates that TP and AI nozzles had 5% of the spray deposits reaching 5.9 and 2.0 m downwind, respectively. Similar results have been reported in the literature, where employment of nozzles with an air-inclusion port decreased drift potential (Alves et al., 2017; Creech et al., 2016; Vieira et al., 2019). Adding a pre-orifice and air-inclusion port allows pressure to drop within a nozzle and the introduction of air into the herbicide solution, directly increasing droplet size and decreasing drift potential (Derksen et al., 1999; Dorr et al., 2013).

Tracer spray deposition results for comparison made across nozzles shows higher drift profile for the TP nozzle (Figure 2.3. A) compared to the Ai nozzle (Figure 2.3. B). To determine the inflection point, slope (*b*), and distance to 50% application spray deposition (*e*), log-logistic model was used with parameters presented in Table 2.3. A larger *e* value indicates that greater distances were required to observe 50% spray deposition, therefore indicating more spray drift potential. Results indicate that distance to 50% application spray deposition (parameter *e*) decreased at least 25% for most of the evaluated herbicides by changing the nozzle from TP to AI. No difference was found between the distances where 50% spray deposition was estimated for glufosinate herbicide, even when the nozzle type was changed. Similar trend was observed in droplet size distribution study with the lowest $D_{V0.5}$ values (Table 2.2.) followed by the greatest proportion of percent of fines <200 µm (Figure 2.2.) for glufosinate. Those findings may be partially attributed to the interaction of nozzle type with herbicide (product) formulation (Vieira et al., 2022; Creech et al., 2015; Meyer et al., 2016;

Mueller and Womac, 1997). As previously reported, product formulation types could affect droplet size and drift reduction by changes in physical properties (including surface tension and viscosity) that directly influence atomization through specific nozzle types (Hilz and Vermeer, 2013; Mueller and Womac, 1997). Therefore, identifying products is especially important when reproducing or extending previous research findings, as relying solely on active ingredients may not provide enough detail to replicate experimental conditions accurately.

Herbicide drift affected industrial hemp biomass

The susceptibility of industrial hemp biomass reduction greatly depended on nozzle type and herbicide solution. For TP (Figure 2.4. A) and AI (Figure 2.4. B), the nozzles used for herbicide drift simulation results indicate that as downwind distance increased, there was less impact on overall industrial hemp biomass as observed for spray deposition (Figure 2.5. and Figure 2.6.).

Log-logistic model parameter estimates indicate the greatest susceptibility of industrial hemp to glyphosate, mesotrione, and glufosinate (Table 4). Changing the nozzle from TP to AI reduced the distance at which 50% biomass reduction occurred from 19.31 (±4.32) to 4.14 (±0.21) for glyphosate. Because the furthest distance evaluated in the study was 12 m due to limitations of wind tunnel length, the distance for 50% biomass reduction for glyphosate was extrapolated based on the data points collected. Sensitivity to glyphosate is due to interference with the shikimic acid production pathway by inhibiting 5-enolpyruvylshikimate-3-phosphate synthase (Ghosh et al., 2012) necessary for the normal development of plants and synthesis of aromatic substances like lignin (Gandolfi et al., 2013; Liu et al., 2018; Stevulova et al., 2014). Industrial hemp susceptibility to glyphosate has been previously documented, even when applied at sub-labeled rates (Horowitz, 1977; Sosnoskie and Maloney, 2021; Ortmeier-Clarke et al., 2022). Ortmeier-Clarke et al. (2022) were unable to fit a dose-response model for industrial hemp due to high susceptibility associated with POST application of glyphosate across a range of doses (157.5 to 1260 g ai ha^{-1}). The susceptibility to glyphosate has been documented in other commodity crops, including wheat (Roider et al., 2007), corn (non-glyphosate-tolerant) (Barnes et al., 2020; Reddy et al., 2010), and rice (Koger et al., 2005). In these crops, final biomass reduction depended on the hybrid or cultivar selected and the growth stage at the time of exposure. Given the limited information available on hemp in the literature, the impact of glyphosate on different hemp cultivars and growth stages remains uncertain. Therefore, the results of this study should be interpreted with caution and may vary depending on the hemp cultivars and growth stages used. Reddy et al. (2010) findings associated with field drift from an aerial application of glyphosate on non-glyphosate-tolerant corn show that at wind speed of 3.11 m s^{-1}, the distance for 50% of shoot dry weight reduction was estimated to be at 19.42 m (±0.46) from the sprayed area three weeks after application. Following similar susceptibility observed with other commodities, hemp deserves special care in the predominant corn and soybean cropping systems where glyphosate applications are still dominant (Fernandez-Cornejo, 2015).

Switching from TP to AI nozzles significantly reduced the distance at which 50% biomass reduction occurred for mesotrione and glufosinate, underscoring the importance of nozzle selection in herbicide application. For mesotrione and glufosinate, changing the nozzle from TP to AI reduced the distance at which 50% biomass reduction occurred by about 3.3- and 2.1-fold, respectively. Despite the significant decrease in estimated 50% biomass reduction distance using the AI nozzle, industrial hemp exhibited higher sensitivity levels compared to other tested herbicides, particularly to mesotrione and glufosinate. Ortmeier-Clarke et al. (2022) found that high sensitivity to mesotrione and glufosinate resulted in a 10% biomass reduction at effective doses of 0.005 (±0.03) and 21.0 (±10.1) g ai ha^{-1}, respectively, when applied POST. This indicates that hemp is highly susceptible to mesotrione and glufosinate, as low doses can significantly reduce biomass accumulation. Consequently, the selection of appropriate application parameters is crucial to prevent negative impact on hemp growth and development. It is also important to highlight the anticipated introduction of the next generation of genetically modified next generation of soybean traits, currently known as the HT5 trait. This trait encompasses tolerance to a range of herbicides including glyphosate, glufosinate, dicamba, 2,4-D, glufosinate, 4-Hydroxyphenylpyruvate dioxygenase (HPPD) and Protoporphyrinogen Oxidase Inhibitors (PPO) inhibitors (Reither, 2021). The emergence of these traits will likely lead to an intensified use of listed herbicide-traits, necessary to consider due to the observed sensitivity of hemp. Such an increase poses a significant risk of herbicide drift, potentially affecting susceptible crops including hemp.

Results indicate that industrial hemp was more susceptible to dicamba compared to 2,4-D. According to the inflection point-slope (parameter *b*), for both nozzles tested, there is a faster curve decay for 2,4-D than for dicamba (Table 2.4.). Even though a multi-purpose variety of hemp was used (CRS-1 for food, fiber, and CBD; Hemp Genetics International, Saskatoon, SK, Canada), the findings from this study do not corroborate with Ortmeier-Clarke et al. (2022), who found the effective dose for 50% biomass reduction to be at 79.7 (±6.8) and 122.6 (±11.0) g ae ha^{-1} for 2,4-D and dicamba, respectively. The discrepancy in the results may be genetic variability due to selective or artificial breeding selection methods to meet specific environmental needs. In addition, most previous studies assessed industrial hemp susceptibility to direct herbicide application over the top of the plants instead of indirect exposure to herbicide drift (Flessner et al., 2020; Ortmeier-Clarke et al., 2022).

Regardless of the nozzle used, imazethapyr and lactofen resulted in lower hemp biomass reduction levels when compared to the other herbicides tested. Even under high drift scenarios with the TP nozzle, the estimated distances for 50% biomass reduction for imazethapyr and lactofen were no greater than 2.43 to 2.91 m downwind, respectively. The observed findings for these two active ingredients do not consistently align with published literature. For example, Flessner et al. (2020) reported no impact on height when imazethapyr (200 g ai ha^{-1}) was applied POST to 25-30 cm tall industrial hemp plants, but there was a 51% biomass reduction relative to the non-treated control. The differing application rates between Flessner et al. (2020) and the current study (200 vs. 70 g ai ha^{-1}) might explain the varied responses. Similarly, hemp tolerance varied for other (PPO Inhibitors; WSSA Group 14). Ortmeier-Clarke et al. (2022) observed biomass reduction above 75% and 35-65% for two hemp cultivars when lactofen was applied POST at 220 and 27.5 g ai ha^{-1}, respectively. High sensitivity (approximately 70% biomass reduction) to direct application of fomesafen and acifluorfen has been reported in the literature (Flessner et al., 2020; Ortmeier-Clarke et al., 2022). These findings underscore the complexity and variability in hemp response to various PPO inhibiting herbicides, emphasizing the necessity for ongoing research. As agricultural practices and herbicide technologies progress, including the development of the HT5 soybean trait, there is an increasing need to adapt and respond to these changes in a well-informed manner (Reither, 2021).

Previous studies conducted on herbicide efficacy have shown that responses are influenced by carrier volume (Butts et al., 2018; Creech et al., 2015a). Nonetheless, research on industrial hemp susceptibility to herbicides, including the studies by Flessner et al. (2020) and Ortmeier-Clarke et al. (2022), has primarily focused on sub-labeled doses applied directly over the plants. However, the impact of actual herbicide drift, even at similar dose ranges, might differ significantly from these controlled applications. Wang and Liu (2007) emphasized that the concentration of active ingredients within droplets might influence the diffusion process during foliar uptake.

The present study examines the effects of systemic and contact herbicides on hemp. As previously reported systemic herbicides including glyphosate, mesotrione, 2,4-D, and dicamba, which are effective at lower carrier volumes and coverage (Butts et al., 2018; Vranjes et al., 2019). However, their systemic nature presents a distinct risk in drift scenarios, as even low doses can be absorbed and translocated within plants, potentially causing a greater impact on non-target species like hemp. Increased activity on cotton with low doses of 2,4-D and dicamba was observed when used in lower carrier volumes with more concentrated droplets (Smith et al., 2017). Oppositely, for contact herbicides examined glufosinate, lactofen, and imazethapyr typically require higher carrier volumes with greater coverage being crucial for effectiveness. The drift implications for these herbicides differ; reduced coverage may reduce their impact (Butts et al., 2018; Creech et al., 2015). As a result of non-systemic activity indicates they are less likely to significantly impact non-target plants. The findings underscore the importance of considering both herbicide activity and application method, particularly in the context of potential drift, to minimize unintended environmental consequences.

Besides employing additional drift-mitigation techniques (i.e., nozzle, adjuvants, or others), current and future commercial and private pesticide applicators should follow the labeled application recommendations and buffer zone requirements of 73.2 m and 9.2 m for dicamba (Anonymous, 2020) and 2,4-D (Anonymous, 2022), respectively. For herbicides lacking specified buffer zones, determining appropriate buffer widths depends on risk assessment and drift potential related to the chosen pesticide application parameters. Unless otherwise specified on product label it is recommended that industrial hemp fields maintain no-spray buffer zones of at least 15 m (i.e., the standard buffer width for non-organic fields, potentially representing a worst-case scenario) to protect surrounding vegetation when employing herbicides without designated buffer zones (USDA AMS, 2022). However, no-spray buffer zones may increase based on risk assessment after site inspection to avoid unintended area exposure to prohibited active ingredients even when drift-mitigating nozzles are used (USDA AMS, 2022). Some of the additional tactics that can help with the buffer zone width changes may include additional no-spray barriers consisting of row crops such as corn (Vieira et al., 2018), hedgerows (Lazzaro et al., 2008), and the employment of hooded sprayers to mitigate drift potential (Foster et al., 2018; Vieira et al., 2021). As a result, all the previously mentioned practices would aim to avoid unintended off-target movement and adverse effects on industrial hemp growth and development.

2.4. Conclusions

In conclusion, this study highlights the critical need for herbicide drift mitigation techniques in industrial hemp production to prevent adverse effects on biomass production and ensure high-quality plant materials for processing. This research provides valuable insights into the impact of nozzle design and herbicide solution on droplet size distribution, driftable fines, spray deposition, and industrial hemp biomass accumulation. Notably, nozzle type and herbicide solution significantly influence droplet size distribution, drift potential, and herbicide damage to industrial hemp. The substitution of TP nozzles with AI nozzles can reduce spray drift and negative impacts on industrial hemp biomass reduction, particularly when exposed to glyphosate, mesotrione, and glufosinate drift. Imazethapyr and lactofen spray drift resulted in lower hemp biomass reduction levels when compared to the other herbicides tested in this study. These findings have practical implications for pesticide applicators in agriculture to make informed decisions when selecting nozzle types and herbicide solutions to minimize off-target movement and avoid potential crop damage. If additional drift-reduction techniques (drift-reducing adjuvants or hooded sprayers) or buffer zones are not employed, overall plant biomass (presumably yield of flowers, fiber, and grain) might be reduced. Ongoing research is necessary to develop effective practices for agricultural pest management, including the implementation of drift-reduction techniques and no-spray buffer zones when hemp is planted in adjacent fields. Additionally, future studies should examine the impact of herbicide drift on industrial hemp during the reproductive stage and potential changes in tetrahydrocannabinol, other cannabinoids, and herbicide residues accumulation in plants. Overall, these findings can help promote sustainable agricultural practices that balance effective pest management with environmental stewardship and economic viability.

Acknowledgments

We thank Wendy Mosher (President and Chief Executive Officer) and Dr. Rich Flecher (Chief Technology Officer) with New West Genetics, Inc. (Fort Collins, CO, USA) for providing seed supplies (NWG2730) for this experiment. This research received no specific grant from public, commercial, or not-for-profit funding agencies. The Pesticide Application Technology Laboratory (PAT Lab), University of Nebraska-Lincoln, WCREEC, North Platte, Nebraska Research and Extension program provided funding and resources from internal PAT Lab service centers. We thank chemical suppliers from BASF Corporation, Bayer CropScience, Corteva Agriscience, Syngenta Crop Protection, and Valent for providing herbicides used in this study. Representatives from companies were not involved in the study design, collection, data analysis, and interpretation of the data. Finally, we thank all the undergraduate and graduate research assistants and research support staff who assisted with this study.

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Figure 2.1. Interior of low-speed wind tunnel with spray deposition collectors and industrial hemp plants positioned at downwind distances from the nozzle.

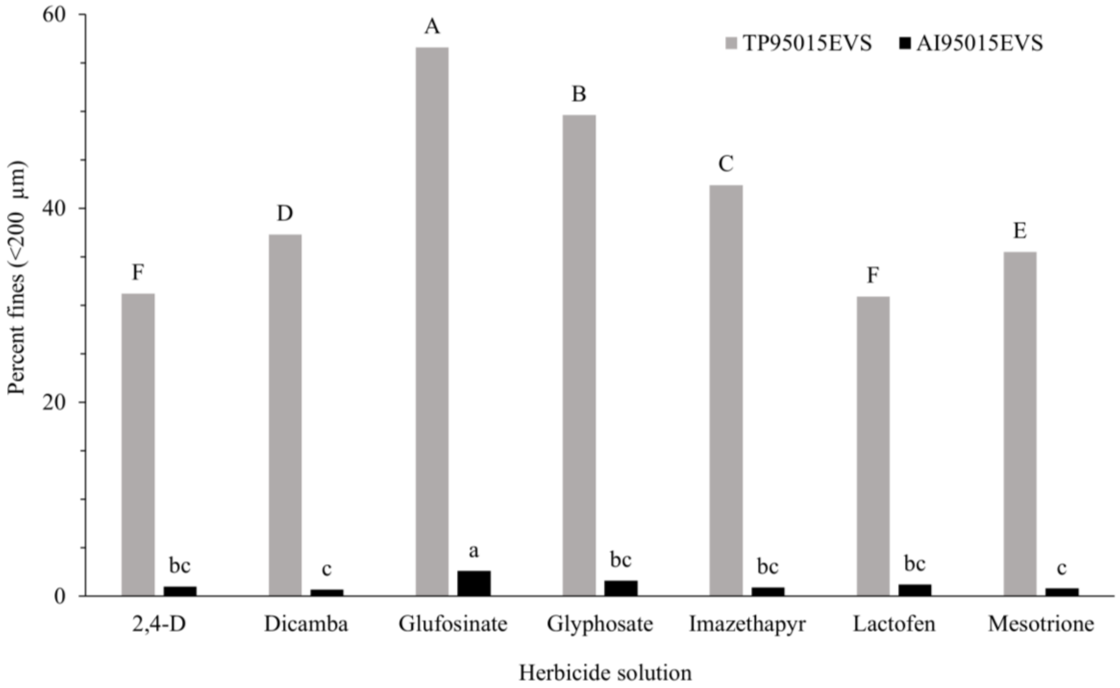

Figure 2.2. Percentage of droplets smaller than 200 µm produced by seven herbicide solutions sprayed with two different nozzle types. TP95015EVS and AI95015EVS nozzles (TeeJet Technologies Spraying Systems Co., Glendale Heights, IL, USA) at 230 kPa. There was a significant impact on percent of fines (<200 µm) ($P < 0.0001$) between two nozzle types tested for all hereby assessed herbicide solutions. Differences between herbicide solutions within TP95015EVS nozzle are indicated by different uppercase letters, whereas differences between AI95015EVS are indicated by lowercase letters. Means by the same letter within a column are not different ($P \geq 0.05$).

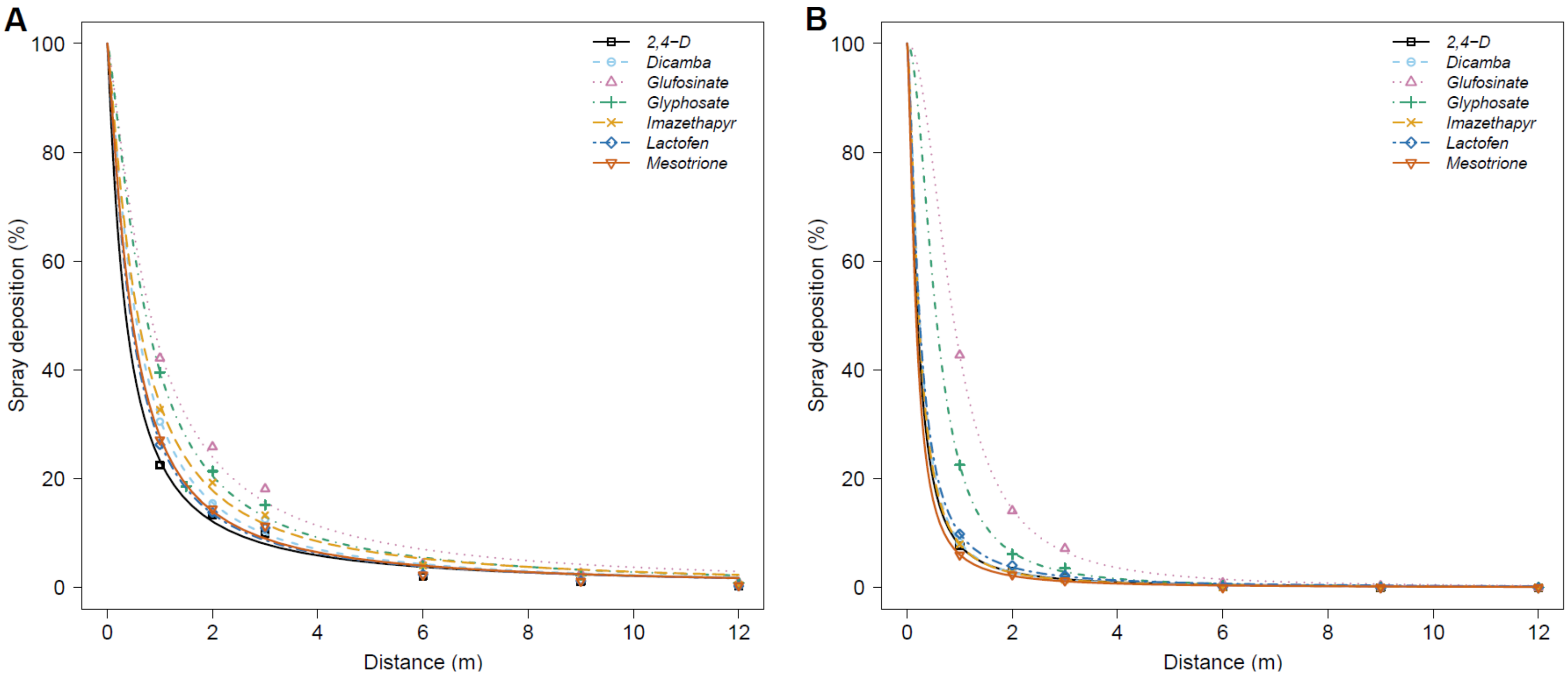

Figure 2.3. Spray deposition on mylar cards from herbicide drift in a low-speed wind tunnel using (A) TP95015EVS and (B) AI95015EVS nozzles (TeeJet Technologies Spraying Systems Co., Glendale Heights, IL, USA) at 230 kPa. Mean estimates were determined from eight replications across two experimental runs. Parameter estimates for the models are in Table 2.3.

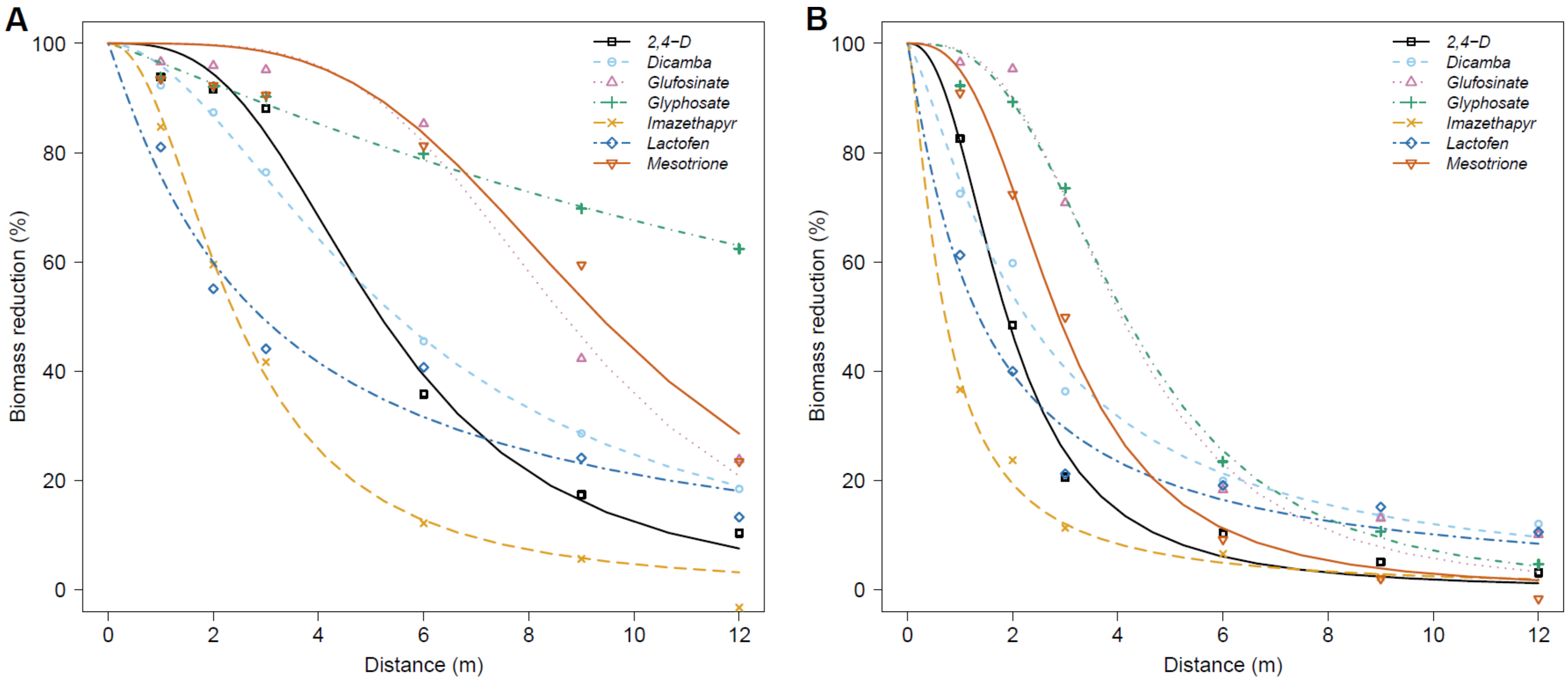

Figure 2.4. Industrial hemp biomass reduction caused by herbicide drift in a low-speed wind tunnel from applications with (A) TP95015EVS and (B) AI95015EVS nozzles (TeeJet Technologies Spraying Systems Co., Glendale Heights, IL, USA) at 230 kPa. Mean estimates were determined from eight replications across two experimental runs. Parameter estimates for the models are in Table 2.4.

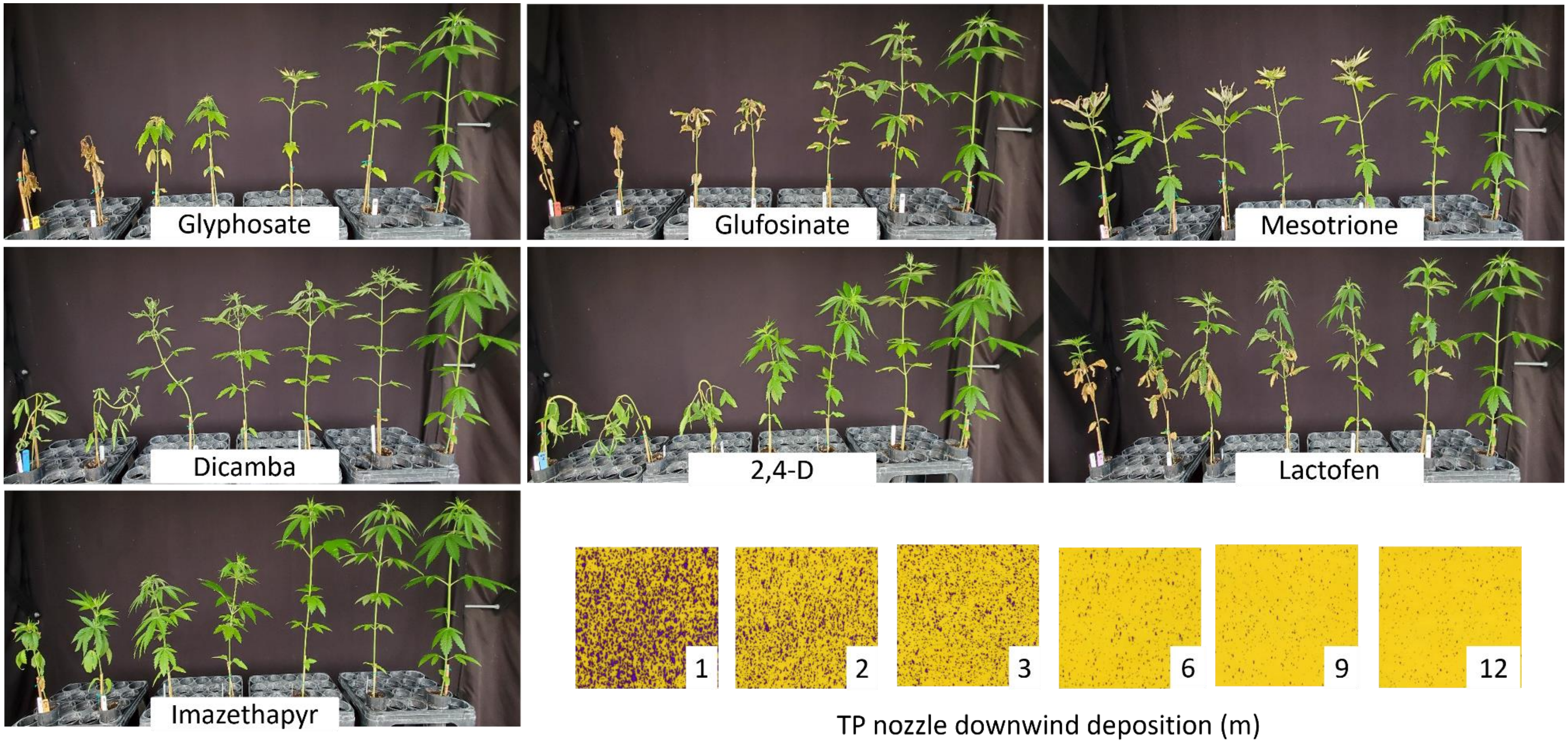

Figure 2.5. Industrial hemp response to herbicide drift for listed herbicides from application with TP95015EVS (TeeJet Technologies Spraying Systems Co., Glendale Heights, IL, USA) nozzle at 230 kPa. Plants order in meters 1, 2, 3, 6, 9, 12, and non-treated control 21 days after exposure, from left to right, respectively. Parameter estimates for the models are in Tables 2.3. and 2.4. Note: water sensitive cards are used only for visual reference without any data collection and analysis.

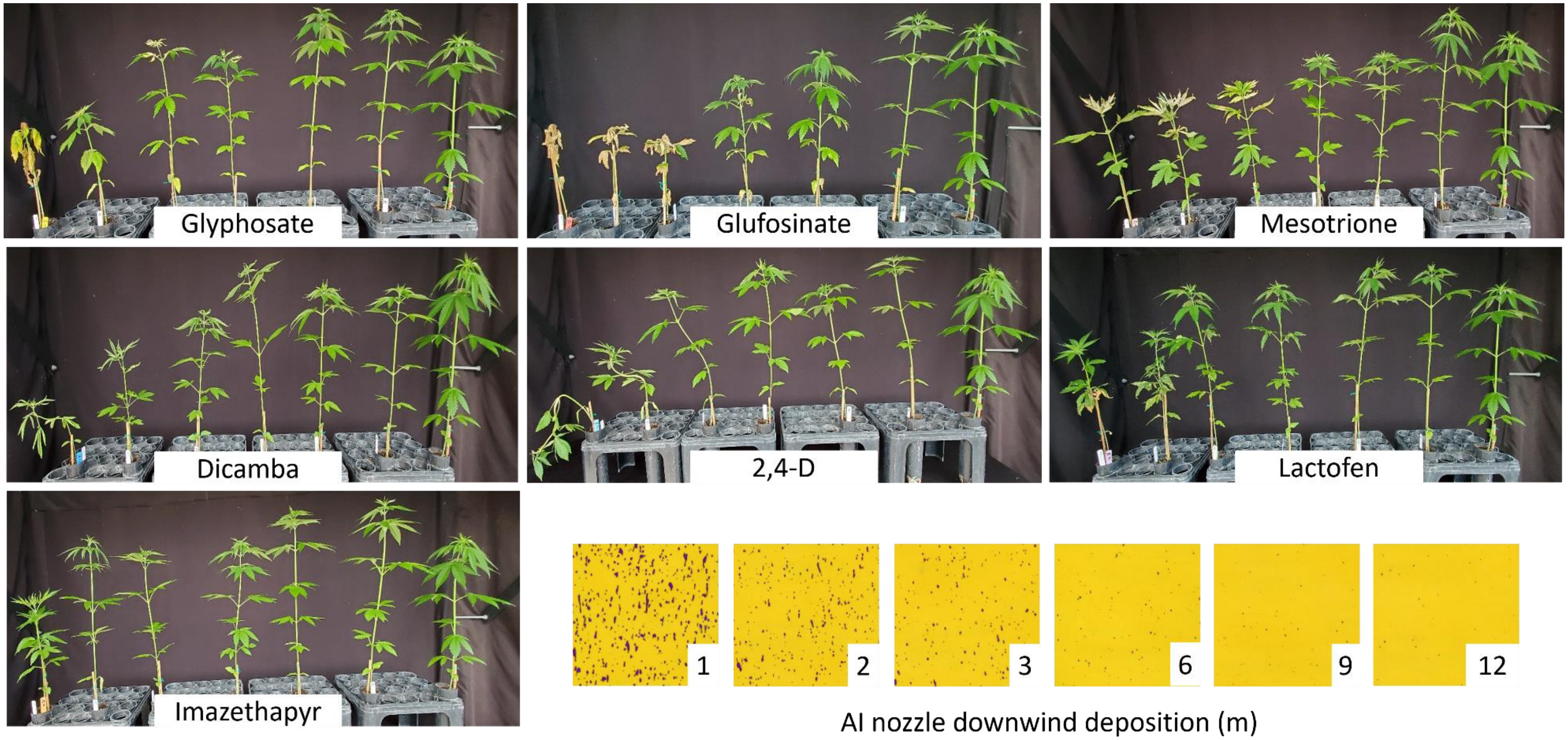

Figure 2.6. Industrial hemp response to herbicide drift for listed herbicides from application with AI95015EVS (TeeJet Technologies Spraying Systems Co., Glendale Heights, IL, USA) nozzle at 230 kPa. Plants order in meters 1, 2, 3, 6, 9, 12, and non-treated control 21 days after exposure, from left to right, respectively. Parameter estimates for the models are in Tables 2.3. and 2.4. Note: water sensitive cards are used only for visual reference without any data collection and analysis.

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Table 2.1. Herbicide solution, trade name, and application rate for solutions evaluated in the spray drift study[a].

Herbicide solution	Trade name	WSSA Group[b]	Application Rate[c,d] g ai/ae ha^{-1}	Manufacturer
2,4-D	Enlist One®	4	1065	Corteva AgriSciences
Dicamba	Xtendimax®	4	560	Bayer CropScience
Glufosinate	Liberty® 280 SL	12	645	BASF Corporation
Glyphosate	Roundup WeatherMAX®	9	1260	Bayer CropScience
Imazethapyr	Pursuit®	2	70	BASF Corporation
Lactofen	Cobra®	14	220	Valent U.S.A. Corporation
Mesotrione	Callisto®	27	105	Syngenta Crop Protection

[a] A fluorescent tracer 1,3,6,8-pyrene tetra sulfonic acid tetra sodium salt (PTSA) was added at 3 g L^{-1}.

[b] Herbicide mode of action as listed by Weed Science Society of America (WSSA).

[c] Application rate shown as grams of active ingredient (ai) or acid equivalent (ae) per hectare.

[d] Selected dose as the highest labeled field use rate for labeled crops.

Table 2.2. Droplet size distribution for TP95015EVS and AI95015EVS nozzles and herbicide solutions evaluated in the spray drift study.

Herbicide solution	TP95015EVS[a]				AI95015EVS[a]			
	$D_{V0.1}$	$D_{V0.5}$	$D_{V0.9}$	SC[b]	$D_{V0.1}$	$D_{V0.5}$	$D_{V0.9}$	SC[b]
	µm				µm			
2,4-D	129 A	251 A	394 A	F	391 C	740 D	1089 E	EC
Dicamba	114 C	236 C	396 A	F	433 B	836 A	1262 A	UC
Glufosinate	79 F	182 F	342 C	F	335 F	720 E	1118 D	EC
Glyphosate	88 E	201 E	354 BC	F	385 D	796 C	1169 C	EC
Imazethapyr	103 D	220 D	370 AB	F	441 A	845 A	1254 A	UC
Lactofen	132 A	250 AB	385 A	F	375 E	708 F	1048 F	EC
Mesotrione	120 B	240 BC	392 A	F	437 A	820 B	1201 B	UC

[a] $D_{V0.1}$, $D_{V0.5}$, and $D_{V0.9}$ represent the droplet size such that 10, 50, and 90% of the spray volume is contained in droplets equal or lesser diameters, respectively. There was a significant difference of nozzle design on $D_{V0.1}$, $D_{V0.5}$, and $D_{V0.9}$ ($P < 0.0001$) across all herbicide solutions tested. Means by the same letter within a column are not different ($P \geq 0.05$).

[b] The spray classifications (SC) for this study were based on reference curves created from reference nozzle data at the Pesticide Application Technology Laboratory as described by ASABE S572.3 where F = Fine, EC = Extremely Coarse, and UC = Ultra Coarse.

Table 2.3. Log-logistic model parameters and standard errors for TP95015EVS (A) and AI95015EVS (B) nozzles and herbicide solutions evaluated in the spray deposition study.

Herbicide solution	Log-logistic model parameters[a]			
	TP95015EVS		AI95015EVS	
	b	e	B	E
		m		M
2,4-D	1.14 ± 0.08	0.35 ± 0.04	1.53 ± 0.19	0.20 ± 0.04
Dicamba	1.28 ± 0.07	0.54 ± 0.03	1.66 ± 0.20	0.24 ± 0.04
Glufosinate	1.30 ± 0.05	0.82 ± 0.03	2.16 ± 0.05	0.87 ± 0.01
Glyphosate	1.35 ± 0.06	0.73 ± 0.03	2.07 ± 0.10	0.55 ± 0.02
Imazethapyr	1.23 ± 0.06	0.57 ± 0.03	1.63 ± 0.20	0.22 ± 0.04
Lactofen	1.22 ± 0.08	0.44 ± 0.04	1.51 ± 0.15	0.23 ± 0.04
Mesotrione	1.23 ± 0.07	0.46 ± 0.04	1.55 ± 0.25	0.17 ± 0.05

[a] b parameter corresponds to the slope at the inflection point; e parameter corresponds to the distance estimated for 50% of spray deposition.

Table 2.4. Log-logistic model parameters and standard errors for TP95015EVS and AI95015EVS nozzles and herbicide solutions evaluated in the biomass reduction study.

Herbicide solution	Log-logistic model parameters[a]			
	TP95015EVS		AI95015EVS	
	b	e	B	E
		m		M
2,4-D	2.97 ± 0.36	5.18 ± 0.25	2.37 ± 0.34	1.89 ± 0.11
Dicamba	1.86 ± 0.20	5.49 ± 0.32	1.33 ± 0.15	2.25 ± 0.18
Glufosinate	4.10 ± 0.60	8.67 ± 0.28	3.11 ± 0.39	4.06 ± 0.21
Glyphosate	1.12 ± 0.25	19.31 ± 4.31	2.91 ± 0.34	4.14 ± 0.21
Imazethapyr	2.13 ± 0.24	2.43 ± 0.14	1.39 ± 0.25	0.72 ± 0.12
Lactofen	1.06 ± 0.11	2.91 ± 0.25	1.09 ± 0.16	1.35 ± 0.16
Mesotrione	3.66 ± 0.68	9.34 ± 0.33	2.81 ± 0.36	2.87 ± 0.14

[a] b parameter corresponds to the slope at the inflection point; e parameter corresponds to the distance estimated for 50% industrial hemp biomass reduction.

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Chapter 3

Comparison of Acetyl CoA Carboxylase Inhibiting Herbicides for use in Industrial Hemp

Abstract

Currently there are limited herbicides registered for post-emergence use in industrial hemp in the United States, which complicates weed management. The potential of Acetyl CoA Carboxylase inhibiting (ACCase) herbicides to control monocot weeds in hemp is promising, but there is limited knowledge about hemp tolerance. To date, only quizalofop residue trials are underway. If approved for hemp grown for fiber and seed, an increase in the growing area might lead to an overreliance on quizalofop, raising concerns about developing resistant grass-weed biotypes. This study aimed to evaluate crop tolerance and explore diversification possibilities of ACCase herbicides for industrial hemp use. Herbicides were applied at varying doses with active ingredients, including clethodim, pinoxaden, sethoxydim, fluazifop, fenoxaprop, fluazifop+fenoxaprop, and quizalofop on two hemp cultivars, including a high-cannabidiol, fiber, and grain cultivar (cultivar A) and high-grain and fiber cultivar (cultivar B). Herbicides chosen for this study were applied at doses of 0.5, 1, 2, and 4X from the maximum label rate in labeled crops. Applications occurred when plants were 20-25 cm tall (with two or three pairs of true leaves) using a single DG9502EVS nozzle. The spray chamber was calibrated to deliver 140 L ha^{-1}. The research in 2022 and 2023 was conducted in a randomized complete block design involving seven replications per experimental run. Visual symptoms and biomass accumulation were measured for two hemp cultivars under greenhouse conditions up to 21 days after application. After drying, the reduction in biomass was recorded. The most significant visual symptoms and biomass reduction appeared in treatments with clethodim and pinoxaden. Estimates for just 5% hemp biomass reduction with clethodim were at ~131 and 192 g ai ha^{-1}, while pinoxaden had 60 and 39 g ai ha^{-1} for cultivars A and B, respectively. Therefore, biomass reduction implications varied by cultivar. Oppositely, other herbicides like sethoxydim, fluazifop, and quizalofop showed minimal adverse effects, indicating they might be potential ACCase active ingredients for hemp. Embracing a diversified approach in herbicide use in hemp might decrease the threat of weed resistance, thereby ensuring sustainable and effective weed management in the rapidly expanding hemp cultivation sector.

3.1. Introduction

Industrial hemp (*Cannabis sativa* L.; Rosales; Rosidae; Cannabaceae) cultivation has gained substantial global momentum. Multifaceted hemp uses and expansion in area grown has been observed in over 30 countries worldwide (Adesina et al., 2020). Notably, the United States (US) historic decision came with the introduction of the 2018 Farm Bill that recognized hemp as a versatile crop legal to grow for grain, fiber, and pharmaceutical derivatives (Amaducci et al., 2015; Mark et al., 2020). At present, Colorado (4100 ha), and Montana (3200 ha) comprise the largest growing hemp growing areas in the US (USDA NASS, 2022). Adopting diversified uses has led to a growth in value from $700 million in 2018 to $821 million in 2021 (Johnson, 2019; USDA NASS, 2022). Focusing on hemp grain, reports indicate a significant rise in US imports between 2015 to 2020, from $51.2 to $79.9 million, according to UNCTAD (2022). As noted by the USDA ERS (2020), Canada has established itself as the predominant supplier of this commodity to the US. Growth in demand for hemp seeds, combined with supporting regulations, suggests an encouraging future for hemp grain cultivation globally, particularly within the US.

Hemp holds significant potential as a grain crop in the US, with yields ranging from 0.98 to 2.00 tons ha^{-1} (Conley et al., 2018; USDA ERS, n.d.). While hemp cultivation for fiber is widely recognized for rapid growth and ability to outcompete weeds, mainly when sown at high densities of about 2.5 million plants per hectare in narrow rows (≤ 20 cm), the dynamics change when hemp is cultivated for grain. Specifically, grain cultivars require lower planting densities, from 100 to 150k plants per hectare, and wider row spacing. Such a configuration provides weeds more opportunity for establishment, leading to intensified competition. This direct contest for resources like light, water, and nutrients might severely inhibit hemp yields, an occurrence observed across various crops (Kaur et al., 2018). Corroborating this, studies have shown that without weed management, hemp yields are 25% lower compared to weed-free maintained plots (Mettler, 2021). Despite challenges facing the US hemp industry, there is a gap in knowledge about the quality standards for grain and fiber hemp.

Conversely, Canada has set rigorous standards, demanding a 99.9% purity level to prevent contamination from other crops or foreign impurities (AGRIC, 2014). To ensure a clean harvest, the application of ethafluralin, classified under the Herbicide Resistance Action Committee (HRAC, 2022) or the Weed Science Society of America (WSSA, 2021) as Site of Action (SOA) Group 3, is permitted in Canada. Mirroring this strategy, the US, rejuvenated by its interest in hemp farming, has approved ethafluralin beginning April 2023 as the exclusive herbicide for pre-planting hemp weed control (EPA, 2023; US EPA, 2023a). Such limitations on available early season herbicide use leaves weed management in hemp cultivation particularly demanding and results in producers for the reminder of the growing season largely reliant on mechanical and cultural strategies. The expansion of hemp cultivation areas, combined with the absence of information on herbicide safety for this crop, emphasizes the pressing need to diversify chemical weed management strategies.

Recent initiatives aimed to diversify herbicide programs through both laboratory and field screening to a range of pre-emergence and post-emergence (POST) herbicides indicate a lack of effective solutions for weed management in hemp cultivation (Flessner et al., 2020; Ortmeier-Clarke et al., 2022). It was found that many herbicide programs designated for effective broadleaf weed control in crops like corn and soybean, when applied at their currently recommended labeled rates, led to significant hemp injury and biomass reduction. Among the different herbicides, the acetyl-CoA carboxylase (ACCase) inhibitors (SOA 1) emerged as a notable exception. These compounds showcased relative tolerance by hemp plants and minimal detrimental effects on their biomass.

The ACCase group consists of three chemically distinct chemical families, including the aryloxyphenoxypropionates (FOPs), cyclohexanediones (DIMs), and phenylpyrazoline (DENs). Regardless of the chemical family, ACCase-inhibiting herbicides hinder the growth and development of monocot (grass) weeds by inhibition of an essential enzyme in lipid biosynthesis, while, in most cases, leaving dicotyledonous (broadleaf) plants (including weeds and crops) unaffected (Bough and Dayan, 2022; Kaundun et al., 2012; Takano et al., 2020). Therefore, changes in fatty acid composition ultimately leads to the death of the plant by preventing the production of the necessary lipids required for normal growth and development. Hemp being a broadleaf crop, it is expected to exhibit target-site insensitivity to these herbicides (Kukorelli et al., 2013). Currently, from ACCase herbicides, agricultural use of quizalofop is allowed in Canada (including hemp grown for seed, fiber, and oil production) with a use rate of 36 to 72 grams active ingredient per hectare (g ai ha^{-1}) (Anonymous, 2023). Furthermore, in Australia, use of clethodim 120 g ai ha^{-1}, fluazifop 24 g ai ha^{-1}, haloxyfop 52 g ai ha^{-1}, and quizalofop 100 g ai ha^{-1} was granted for control of grass weeds in hemp at the two- to eight-true leaf stage until 2024 (AGVET, 2022; Santo, 2022). However, even with granted permits and possibilities for applying those herbicides in hemp, there is no information on how hemp growth and development, including various cultivars across environments, may be impacted. To date, in the US only quizalofop residue trials are currently underway within Interregional Research Project #4 (IR-4, North Carolina State University, Raleigh, NC). Therefore, if approved for use after 2024 in hemp (Van Wychen, 2022) cultivated for fiber, seed, and oil, an increase in the hemp growing area is anticipated to contribute to the continuous overreliance on quizalofop.

In existing cropping systems, the agricultural use of quizalofop throughout the US is associated with corn (Enlist™), soybean, rice (Provisia™), grain sorghum (FirstAct™), and wheat (CoAXium®) (Anonymous, 2021; Anonymous, 2021a; Anonymous, 2022; Anonymous, 2023a; Anonymous, 2023b). The forthcoming US approval of quizalofop, already widely used in crops, with estimates prior to the release of some quizalofop-tolerant traits about ~68k kg ai year^{-1}, raises weed resistance risks (USGS, 2018). Historically, over-reliance on a single herbicide or herbicide(s) with the same site of action has led to shifts in weed species composition and the evolution of herbicide-resistant weed biotypes (Heap, 2014). There are seven reports in the US (a total of 40 worldwide) of grass weeds resistant to quizalofop (Heap, 2023). Considering the lack of studies investigating hemp response to alternative ACCase herbicides in the United States and the ongoing quizalofop residue trials, it becomes increasingly critical to implement diversified ACCase herbicide strategies. This proactive approach is essential to prevent the overreliance on quizalofop, a trend that has historically resulted in the development of weed resistance problems across different agricultural systems.

Hemp sensitivity to alternative ACCase herbicides in the US is understudied and there is limited knowledge of hemp cultivar tolerance. While ACCase inhibitors from the WSSA group 1 are generally considered safe for broadleaf crops, initial studies indicate potential concerns with specific active ingredients. In research conducted by Flessner et al. (2020), POST herbicides sethoxydim (300 g ai ha^{-1}) and quizalofop (77 g ai ha^{-1}) showed less than 20% injury with grain yields no different from non-treated controls under field conditions. Similarly, Ortmeier-Clarke et al. (2022) indicated that, even with biomass reduction estimates of 5 to 25% associated with 9.55 to 76.4 g ai ha^{-1} from greenhouse tests conducted on hemp plants at 5 to 10 cm in height, clethodim was suggested as a possible POST herbicide for further field evaluations. Notably, these tests revealed no significant differences in herbicide response among the tested hemp cultivars, underscoring the potential of clethodim for broader application. Lingenfelter (2018 and 2019) and Pearce and Carter (2018 and 2019) regulated studies found that both quizalofop (up to 77 g ai ha^{-1}) and clethodim (up to 272 g ai ha^{-1}) exhibited minimal to no injury. Our preliminary research indicated that clethodim dosages ranging from 136 to 1088 g ai ha^{-1} could cause visual injuries between 17 to 46%, with estimations for a 20% reduction in biomass accumulation at 166 g ai ha^{-1} for high-CBD, grain, and fiber industrial hemp cultivar thereby tested under controlled environment, respectively (Savic et al., 2020). Even though available literature on hemp tolerance to ACCase herbicides has resulted in mixed findings, there is a clear need to evaluate further its response across different ACCase chemical families and integration of more cultivars. While the suggested studies offer a preliminary understanding of hemp differential sensitivity to various ACCase herbicides, prior experiments were confined to one or two distinct active ingredients at varying concentrations. At present, there is limited evidence detailing the reactions of hemp cultivars, intended for diverse applications, to the spectrum of ACCase herbicides typically employed in other crops.

This study aimed to assess the safety of seven ACCase inhibitors across three distinct chemical families: FOPs, DIMs, and DENs. The evaluation was conducted on two specific hemp cultivars under controlled conditions. Our primary objective was to investigate the hemp response to these ACCase inhibitors, to support subsequent research on residue testing, and to contribute to the availability of a broader spectrum of ACCase herbicide programs tailored for use in hemp cultivation.

3.2. Materials and methods

Study site and plant material

The study was conducted at the Pesticide Application Technology Laboratory (University of Nebraska-Lincoln, West Central Research, Extension and Education Center in North Platte, NE, USA). Two cultivars of industrial hemp, including NWG2730 (high-cannabidiol, fiber, and grain; hereafter referred as cultivar A) and NWG452 (high-grain and fiber; hereafter referred as cultivar B) (New West Genetics Inc., Fort Collins, CO, USA) were used in this study. Industrial hemp varieties were obtained as a part of the material transfer agreement (*MTA 2020-0369A Amendment 02&03*). In addition, special permission to grow industrial hemp for research purposes for the 2022 and 2023 growing seasons was obtained through the Nebraska Department of Agriculture - Hemp Program, granted under the *Industrial Hemp Research Project* (Cultivator License #31_0105).

Greenhouse growing conditions

Industrial hemp seeds were planted in 1 L plastic pots containing commercial potting mix (Pro-Mix BX5, Premier Tech Horticulture Ltd, Rivière-du-Loup, Canada). Pots were maintained under greenhouse conditions (set at 30° C during the day and 20° C at night) and irrigated daily using water blended with fertilizer at 0.2% v v^{-1} (UNL 5-1-4, Wilbur-Ellis Agribusiness, Aurora, CO, USA). Supplemental lighting was provided using LED lights (520 µmol s^{-1}, Philips Lighting, Somerset, NJ, USA) to ensure a 16-h photoperiod and keep plants in a vegetative stage.

ACCase inhibitors screening

Studies under controlled conditions were conducted during 2022 and 2023 (April through June) in a randomized complete block design with a factorial arrangement (including active ingredient, application rate, and cultivar) of treatments. Treatment factors included active ingredients: clethodim, pinoxaden, sethoxydim, fluazifop, fenoxaprop, and fluazifop+fenoxaprop, and quizalofop associated application at four rates (0.5-, 1-, 2-, and 4-X) relative to the fractions of the highest labeled rate (Table 3.1.) to investigate tolerance of two industrial hemp varieties NWG2730 and NWG452 with seven replications and two experimental runs. The dose representing a 1-X rate was selected as the maximum labeled rate by the product label for approved use crops.

Herbicide application (Figure 3.1.) was performed when plants were 20-25 cm in height (two to three pairs of true leaves) using a single DG9502EVS (TeeJet® Technologies, Wheaton, IL, USA) nozzle in a spray chamber (DeVries Manufacturing Inc., Hollandale, MN, USA). Industrial hemp plants were positioned 30.5 cm below the nozzle to deliver 140 L ha^{-1} at 1.34 m s^{-1} sprayer traveling velocity. ACCase applications made sequentially over the top of the plants did not contain any spray adjuvant except ones that product manufacturers used for product formulation. In addition, it is important to note, that the type and quantity of spray additive included in the product formulation was not disclosed by product manufacturers.

The visual evaluation of symptoms was conducted 21 days after application (DAA) and is detailed in Table 3.2. This evaluation used a scale ranging from 0 to 100%, where 0% indicated no visible symptoms and 100% represented complete plant death. In addition, at 21 DAA, aboveground biomass accumulation data were collected. After harvesting aboveground biomass, plants were dried in an air-forced dryer at 65° C until they reached a constant weight. Dry biomass weights were recorded and converted into a percentage of biomass reduction using equation 1:

$$br = [(nt-t)/nt] * 100 \quad [1]$$

where *br* represents industrial hemp biomass reduction (%) compared to non-treated control, *nt* is dry biomass (g) of non-treated plants, and *t* is dry biomass (g) of plants exposed to the herbicide.

Data analysis

Dose-response parameters were generated in R software (R Foundation for Statistical Computing, Vienna, Austria) for the percent of observed symptomology at 21 DAA and biomass reduction using the *drc* package. The model selection function in R software *mselect* was used to compare models, and the three-parameter log-logistic function was selected as the best-fit model based on Akaike's information criterion (data not shown) for herbicide spray deposition and industrial hemp biomass reduction, which were analyzed in R software following equation 2:

$$y = c + \{d - c/1 + \exp [\, b (\log x - \log e)]\} \quad [2]$$

where y represents either symptomology (%) at 21 DAA or biomass reduction (%), b is the slope at the inflection point, c is the lower limit of the model (by default 0%), d is the upper limit, and e is the inflection point (dose to 5, 10, and 25% visual symptomology 21 DAA or biomass reduction) (Knezevic et al., 2007; Ritz et al., 2015). Two hierarchical models were established for every active ingredient examined: one detailed model that accounted for each individual hemp cultivar and a simpler model that combined the data for two hemp varieties. The ANOVA function from the *drc* package was utilized to conduct the F-test. If the resulting P-value from the F-test exceeded 0.05, the null hypothesis was upheld, suggesting that the simpler model is more appropriate (i.e., a single model is suitable for the two varieties). Conversely, if it is less, it implies varying responses from the hemp varieties under a specific herbicide treatment (Oliveira et al. 2018). Data from the two experimental runs were combined with replications and experimental runs considered as random effects.

3.3. Results and Discussion

Dose-response studies indicated the varied reactions of hemp to ACCase herbicides, including pinoxaden, clethodim, and quizalofop. In addition, findings indicate that different hemp cultivars possess distinct sensitivities to these herbicides, with significant variations in visual symptoms and biomass reduction upon exposure. Notably, some of the tested active ingredients, even at doses exceeding standard recommendations, failed to result in observable effects in hemp. These findings might be paramount in refining and understanding of ACCase impacts on hemp and tailoring diversified management strategies accordingly.

Visual symptoms 21 days after herbicide application indicates different hemp cultivars possess distinct sensitivities to ACCase herbicides

The susceptibility of industrial hemp visual symptoms 21 days after application (DAA) was greatly influenced by pinoxaden dose, with response being different between hemp cultivars (P-value = 0.008) (Figure 3.2.). As the dose of pinoxaden increased, a shift in visual symptoms was evident and can be characterized by changes in growth compared to plants at the 0 g ai ha^{-1} dose non-treated control (Figure 3.3.). Pinoxaden is a commonly used herbicide for POST control of grass weeds, especially in crops such as wheat and barley (Anonymous, 2022a). Our study evaluated its potential impact on two hemp varieties, providing additional insights into the selectivity profile and potential risks for non-target crops. The effective doses, estimated using the log-logistic model parameter (*e*), highlighted significant differences between the two hemp cultivars (Table 3.3.). The effective dose inducing 5 to 25% visual symptoms for hemp cultivar A was estimated to be 86 to 130 g ai ha^{-1} . This suggests a greater tolerance of cultivar A to pinoxaden when compared to cultivar B, which exhibited visual symptoms at effective doses of 5% and 25%, ranging from 35 to 79 g ai ha^{-1}, respectively. Such differential response emphasizes the need for caution when considering the application of pinoxaden in fields adjacent to hemp cultivation areas. Despite its widespread use in controlling grass weeds, pinoxaden safety under field conditions, particularly for hemp, remains uncertain. Our findings are consistent with the concerns raised by the US Environmental Protection Agency (EPA, 2005) in their Pesticide Fact Sheet, where the EPA highlights potential risks to broadleaved species upon exposure to pinoxaden, either through runoff or drift during aerial or ground-based applications (Anonymous, 2022a). This risk, coupled with the absence of corroborating research on the safety of pinoxaden in greenhouse conditions, underscores the importance of its careful application.

Investigations into the sensitivity of industrial hemp POST clethodim and quizalofop applications have provided valuable insights (Figure 3.4). For both clethodim and quizalofop, no significant difference was observed between the sensitivities of the two tested hemp varieties, with P-values of 0.92 and 0.91, respectively. The visual response of industrial hemp varied with increasing clethodim doses, ranging from 0 to 1087 g ai ha^{-1}. Interestingly, data for clethodim at lower doses indicated possibilities of causing the noticeable visual response. Research on clethodim effect on hemp growth and development is limited despite the observed variability in visual responses to its increasing doses (Savic et al. 2020). This absence of information underscores the need for further studies (including greenhouse and field) to understand its potential implications if used in hemp.

Quizalofop doses ranging from 0 to 375 g ai ha^{-1} suggest consistent visual symptoms across the dose spectrum in hemp (Figure 3.5.). Findings indicate possibilities for elevated visual symptoms up to 15% 21 DAA (i.e., bleaching) associated with dose 375 g ai ha^{-1}. Even though quizalofop is systemic herbicide symptoms were localized and limited only to leaves present at exposure time, while new growth 21 DAA was not affected. These findings are in partial agreement with existing literature. Lingenfelter (2018) reported no injury to industrial hemp upon the application of quizalofop (77 g ai ha^{-1}) tank-mixed with crop oil concentrate (COC) at 1% v v^{-1}, 21 days after application. Flessner (2017 and 2018) found that a similar dosage of quizalofop, combined with a non-ionic surfactant (NIS) at 0.25% v v-1, resulted in only a 5% visual symptom, and this observation remained consistent over the two years. Furthermore, when extending the observation beyond hemp to other broadleaf crops, previous literature reports have highlighted the temporary nature of quizalofop in terms of visual symptoms observed on tested plants. Soltani et al. (2006) illustrated this in adzuki beans, where quizalofop doses of 72 g ai ha^{-1} and 144 g ai ha^{-1} caused less than 1.5% and 2.5% visual injury, respectively, up to 28 days post-application. Despite a greater injury at higher doses, the effects diminished over time (Soltani et al., 2006).

POST application effects of clethodim and quizalofop on hemp, effective doses were determined for 5%, 10%, and 25% visual symptoms 21 days after application (Table 3.4.). Results, pooled across two hemp cultivars using a log-logistic model, for clethodim, the doses required were 80 g ai ha^{-1} to 187 g ai ha^{-1} to produce 5% to 25% visual symptoms, respectively. Conversely, for quizalofop, the hemp plants demonstrated remarkable tolerance. For quizalofop, a dose exceeding 370 g ai ha^{-1} was necessary to induce 5% or greater visual symptoms according to datapoints collected. Given the lack of prior research under greenhouse conditions, our findings provide a foundation for future studies. However, it is essential to note that while our results provide valuable insights into hemp response to these active ingredients, the specific outcomes might differ under field conditions. Environmental factors such as light intensity, temperature, and relative humidity that are controlled in a greenhouse setting might interact with the active ingredients differently in open fields (Matzrafi et al., 2016).

The POST application of sethoxydim, fluazifop, fenoxaprop, and the pre-mix of fluazifop+fenoxaprop on industrial hemp indicated no or minimal visual symptoms 21 DAA even at doses up to four times the standard label recommendations (Appendix Figure 3.1.). This observation suggests an innate tolerance of hemp to these ACCase herbicides, which are designed to target and control annual and perennial grass weeds in crops such as hemp. This tolerance is especially noteworthy when considering the specificity and mode of action of ACCase herbicides. A corroborating study by Flessner (2018) also found that the application of sethoxydim at 315 g ai ha^{-1}, with the addition of COC at 1%, resulted in no detectable visual changes to the treated hemp plants. This lack of visual response was consistent with the present findings, strengthening the certainty of hemp tolerance to certain active ingredients. Moreover, exploring the implications of sethoxydim application on another broadleaf crop, adzuki bean, further emphasizes this trend of tolerance. Soltani et al. (2006) observed that even at doses of 500 and 1000 g ai ha-1, sethoxydim resulted in minimal visual symptoms, not greater than 5%, over a span of 28 days (Soltani et al., 2006).

ACCase herbicide selection affected biomass accumulation

Industrial hemp biomass was notably affected by the doses of clethodim and pinoxaden, with significant differences (P-value = 0.0001) observed among the hemp cultivars tested (Figure 3.6.). As the dose increased, a more prominent differential response was observed between the cultivars. Our recent findings have indicated that the application of clethodim may have implications beyond the previously mentioned visual symptoms (Figure 3.7). Specifically, the anomalous growth patterns observed at dosages of 544 and 1088 g ai ha^{-1} suggest a potential disruption in hormonal balances initiated by the herbicide itself. This aligns with prior research that included corn, where clethodim had demonstrated noteworthy impacts. Radwan and Soltan (2012) found that an adverse impact on the leaves was observed when clethodim was applied at concentrations ranging from 50-1000 ppm. These impacts manifested as changes in physiological, biochemical, and metabolic activities, which subsequently led to noticeable morphological (including pigment changes and necrosis) alterations. Such parallel observations across different studies emphasize the importance of understanding the broader implications of clethodim usage, especially concerning potential disruptions in plant hormonal balances and growth patterns (Radwan and Soltan, 2012).

Similarly, when considering pinoxaden, both hemp varieties showed similar responses at up to half the recommended labeled rate. Beyond this point, a marked difference in sensitivity between the two cultivars was evident. Notably, while both cultivars A and B were affected by increasing dosages, differential responses between the cultivars were observed for visual symptoms. The distinct responses to clethodim and pinoxaden across the hemp cultivars underscore the necessity for dose recommendations in hemp.

The implications of POST application on hemp, particularly in the context of clethodim and pinoxaden effects on biomass accumulation, provide essential perspectives on varietal sensitivity. Employing the log-logistic model parameters to determine effective doses on two hemp varieties, A and B, revealed differences in their sensitivity (Table 3.5.). For clethodim, the effective doses exhibited notable variation. In cultivar A, the effective doses ranged from 131 g ai ha^{-1} to 398 g ai ha^{-1}. For cultivar B, the range was narrower, spanning 192 g ai ha^{-1} to 346 g ai ha^{-1}. A critical observation is the 131 g ai ha^{-1} dose for clethodim in cultivar A, derived from a three-parameter log-logistic model. It is important to note that this value was extrapolated, as the lowest applied dose was 136 g ai ha^{-1}. This extrapolation necessitates caution in interpreting the results. The extrapolation approach could potentially introduce flaws, especially in lower dose ranges. It raises questions about the reliability of these extrapolated values and suggests a need for further validation, which might be addressed possibly through additional experiments focusing on lower dose ranges. These results contrast the recent findings of Ortmeier-Clarke et al. (2022). Their study on clethodim indicated that a dose of 76.4 g ai ha-1, regardless of the hemp cultivar, led to a 10% biomass reduction, equating to 7.6 g ai ha-1. They further found that doses exceeding 306 g ai ha^{-1} were necessary to achieve 50% and 90% biomass reductions. This discrepancy suggests that our results might be specific to the conditions and cultivars we tested, underscoring the variability in response to clethodim across different hemp cultivars. This variability highlights the importance of considering cultivar-specific responses in herbicide efficacy studies. Pinoxaden showcased a different dose-response, with cultivar A having effective doses between 60 g ai ha^{-1} and 116 g ai ha^{-1}, and cultivar B exhibiting a range from 39 g ai ha^{-1} to 59 g ai ha^{-1}.

Active ingredients, including sethoxydim, fluazifop, fenoxaprop, fluazifop+fenoxaprop, and quizalofop exhibited minimal effects on biomass accumulation under greenhouse conditions. Moreover, subsequent F-test revealed no significant interaction (P-value > 0.05) between the cultivars regarding the response to the herbicides, justifying the pooling of data (Figure 3.8.). Employing a three-parameter log-logistic model led to insightful findings (Table 3.6.) highlighting the biomass reduction percentages in industrial hemp upon exposure to various doses of the acetyl-CoA carboxylase inhibitors. However, no corroborating research is available for most of the examined active ingredients except quizalofop. For fluazifop, as one of the tested ACCase inhibitors, the estimated effective doses were 86, 98, and 117 g ai ha^{-1} for 5%, 10%, and 25% biomass reduction, respectively. Oppositely, the combination of fluazifop+fenoxaprop required a higher dose of 648 g ai ha^{-1} to achieve just a 5% biomass reduction, with estimated doses exceeding 841 g ai ha^{-1} necessary for any biomass reduction above 10%. Even though quizalofop is the most evaluated product in the US due to registration in Canada, most evaluation is only limited to assessment through visual symptoms (R. Batts, personal communication, October 18, 2023). These studies predominantly confirm findings of minimal to no visual response. One corroborating study, including other broadleaf crops, indicated that even though there was an initial crop injury with quizalofop application up to 144 g ai ha^{-1}, there were no adverse effects on plant height, above ground dry weight, or yield of adzuki bean (Soltani et al., 2006).

3.4. Conclusions

This study provided valuable insights into the response of different hemp cultivars to ACCase herbicides, explicitly for active ingredients including pinoxaden, clethodim, and quizalofop. The observed differences in hemp sensitivities to these active ingredients emphasize the complexity of various factors, including selected dose, active ingredient, and cultivar (genetics). Notably, some tested active ingredients, even at doses considerably higher than current maximum label rates for other crops, exhibited minimal to no effect on hemp, suggesting a tolerance.

Our results support the idea that hemp cultivars distinctly differ in their sensitivities to active ingredients, particularly pinoxaden. The differential susceptibility to pinoxaden among the studied cultivars aligns with concerns expressed by agencies such as the US Environmental Protection Agency about potential risks to broadleaf species (EPA, 2005). Moreover, it is apparent that even active ingredients like clethodim and quizalofop, might lead to variable biomass accumulation impacts based on the specific cultivar. Such findings emphasize the necessity of more targeted, cultivar-specific management as part of the decision-making considered for weed control in hemp cultivation.

Interestingly, the POST application of sethoxydim, fluazifop, fenoxaprop, and fluazifop+fenoxaprop on hemp presented a marginal visual response, even with an application at higher than recommended doses in labeled crops. This could suggest a possible innate tolerance of hemp to these active ingredients, a feature that warrants further exploration, especially considering the specificity and mode of action of ACCase herbicides. The discrepancies observed in our study compared to findings from previous research, such as Ortmeier-Clarke et al. (2022), highlight the complexities of herbicide responses. Factors including environmental conditions, genetic variations, and experimental methodologies might play a pivotal role in influencing the observed results.

In conclusion, while our findings enrich the understanding of hemp response to ACCase herbicides under controlled greenhouse conditions, extrapolating these outcomes to open-field scenarios necessitates caution. The potential interactions of environmental factors like light intensity, temperature, and humidity with the active ingredients might yield different outcomes under field conditions. As the global hemp industry continues expanding, our research underscores the importance of informed decision-making and a diversified approach to herbicide management in hemp cultivation. Future research endeavors could further explore the intricate relationships between herbicide types, doses, and hemp cultivar sensitivities, carried out under controlled and field conditions.

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LIST OF FIGURES

Figure 3.1. Interior of a single-nozzle spray chamber with industrial hemp plants prior to exposure to one of the acetyl-CoA carboxylase inhibitor herbicides tested in the dose response experiment.

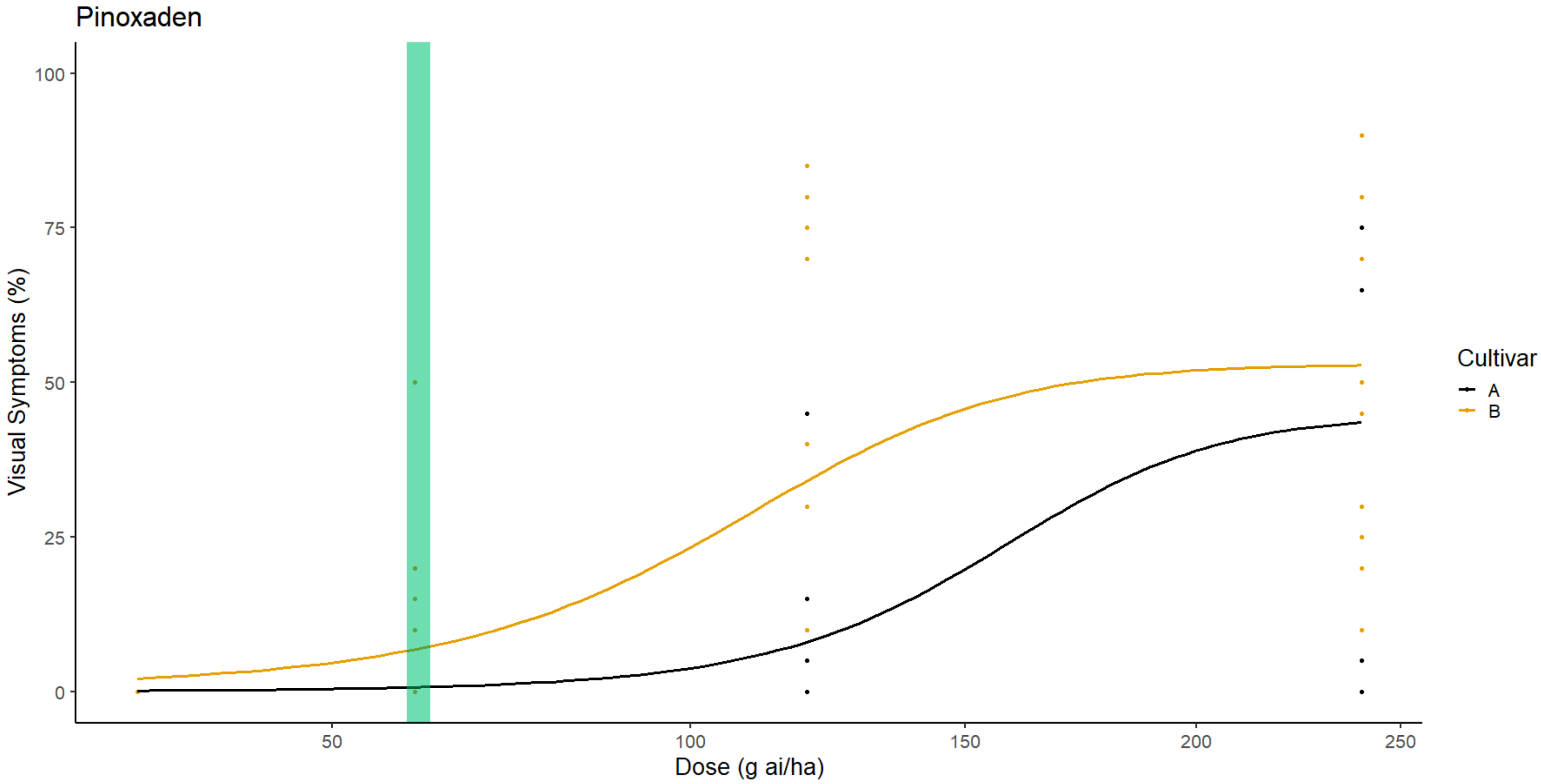


Figure 3.2. Industrial hemp visual symptoms (%) 21 days after application as influenced by pinoxaden dose for hemp cultivars A (high-CBD, grain, and fiber) and B (high-grain and fiber) using a three-parameter log-logistic model. The green shaded region represents the range of recommended labeled doses. Trade name, manufacturer, and application doses are presented in Table 3.1. Visual symptoms assessment scale for industrial hemp at 21 days after application are presented in Table 3.2. Model parameter estimates are presented in Table 3.3. Abbreviations: g, gram; ai, active ingredient; ha, hectare.

Figure 3.3. Visual symptoms 21 days after application of pinoxaden for cultivar A (left) & cultivar B (right). High-CBD, grain, and fiber (cultivar A) and high-grain and fiber (cultivar B). Industrial hemp plants were exposed to incremental doses of pinoxaden, ranging from 0 to 240 g ai ha^{-1} (within a cultivar from left to right). Trade name, manufacturer, and application rate are presented in Table 3.1. Visual symptoms assessment scale for industrial hemp at 21 days after application are presented in Table 3.2.

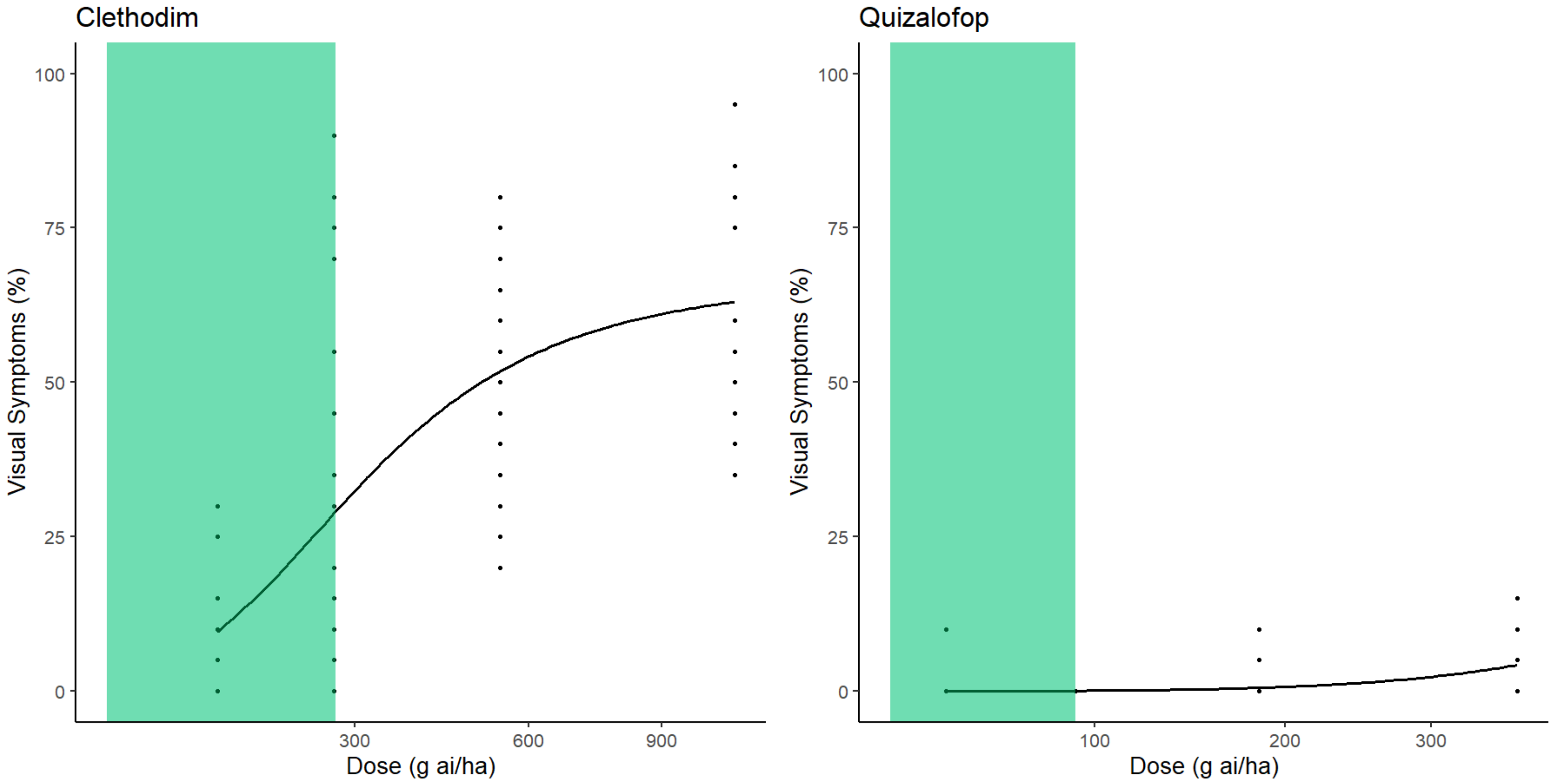


Figure 3.4. Industrial hemp visual symptoms (%) at 21 days after application as influenced by clethodim (left) and quizalofop (right) dose with response pooled across hemp varieties using a three-parameter log-logistic model. The green shaded region of the graph represents the range of recommended labeled doses. Trade name, manufacturer, and application doses are presented in Table 3.1. Visual symptoms assessment scale for industrial hemp at 21 days after application are presented in Table 3.2. Model parameter estimates are presented in Table 3.4. Abbreviations: g, gram; ai, active ingredient; ha, hectare.

Figure 3.5. Industrial hemp visual symptoms at 7 (left) and 21 (right) days after application as influenced by quizalofop dose of 370 g ai ha^{-1}. Trade name, manufacturer, and application doses are presented in Table 3.1. Visual symptoms assessment scale for industrial hemp at 21 days after application are presented in Table 3.2.

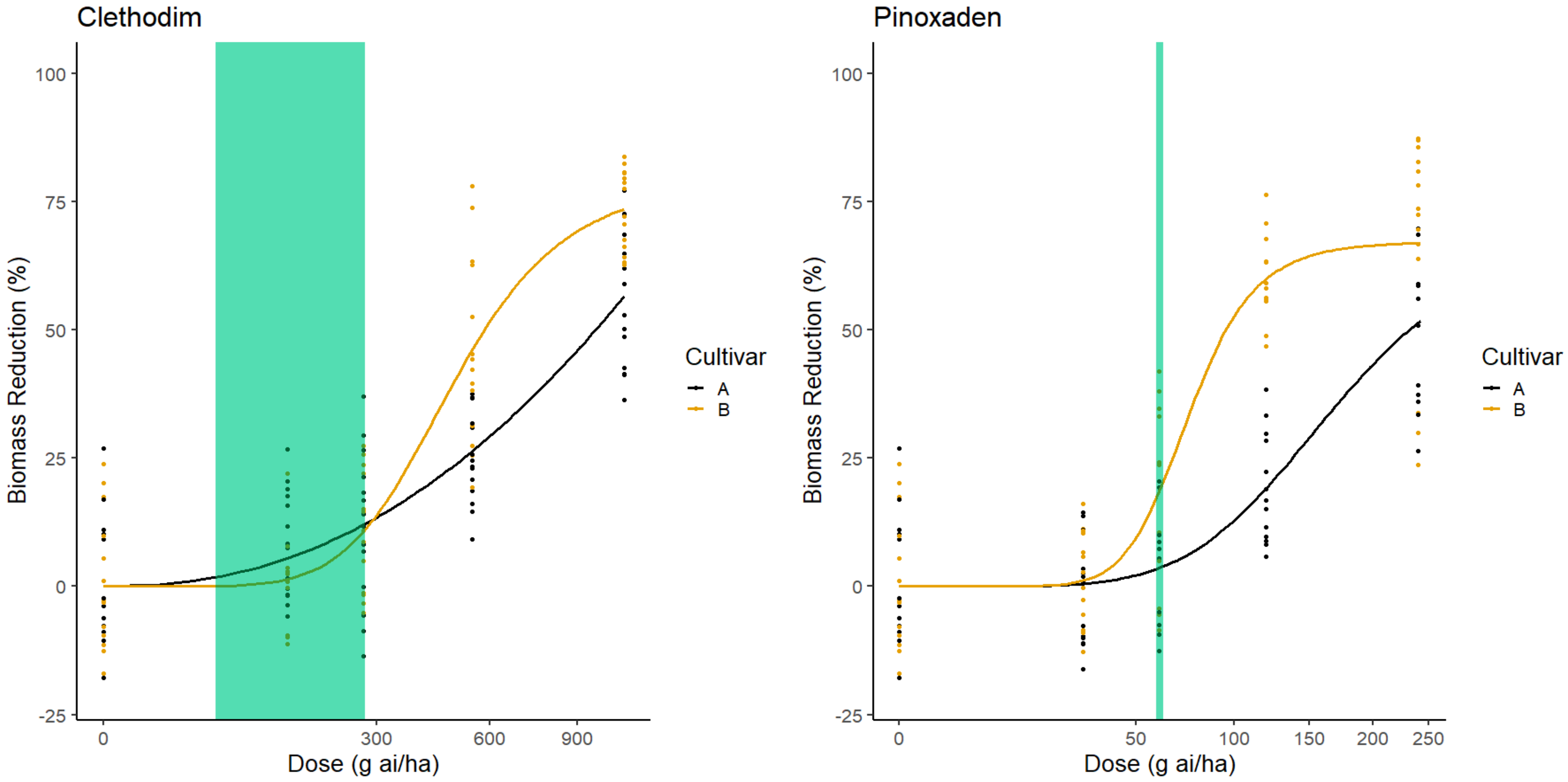


Figure 3.6. Industrial hemp biomass reduction (%) as influenced by clethodim (left) and pinoxaden (right) dose for hemp cultivar A (high-CBD, grain, and fiber) and B (high-grain and fiber) using a three-parameter log-logistic model. The green shaded region of the graph represents the range of recommended labeled doses. Trade name, manufacturer, and application doses are presented in Table 3.1. Model parameter estimates are presented in Table 3.5. Abbreviations: g, gram; ai, active ingredient; ha, hectare.

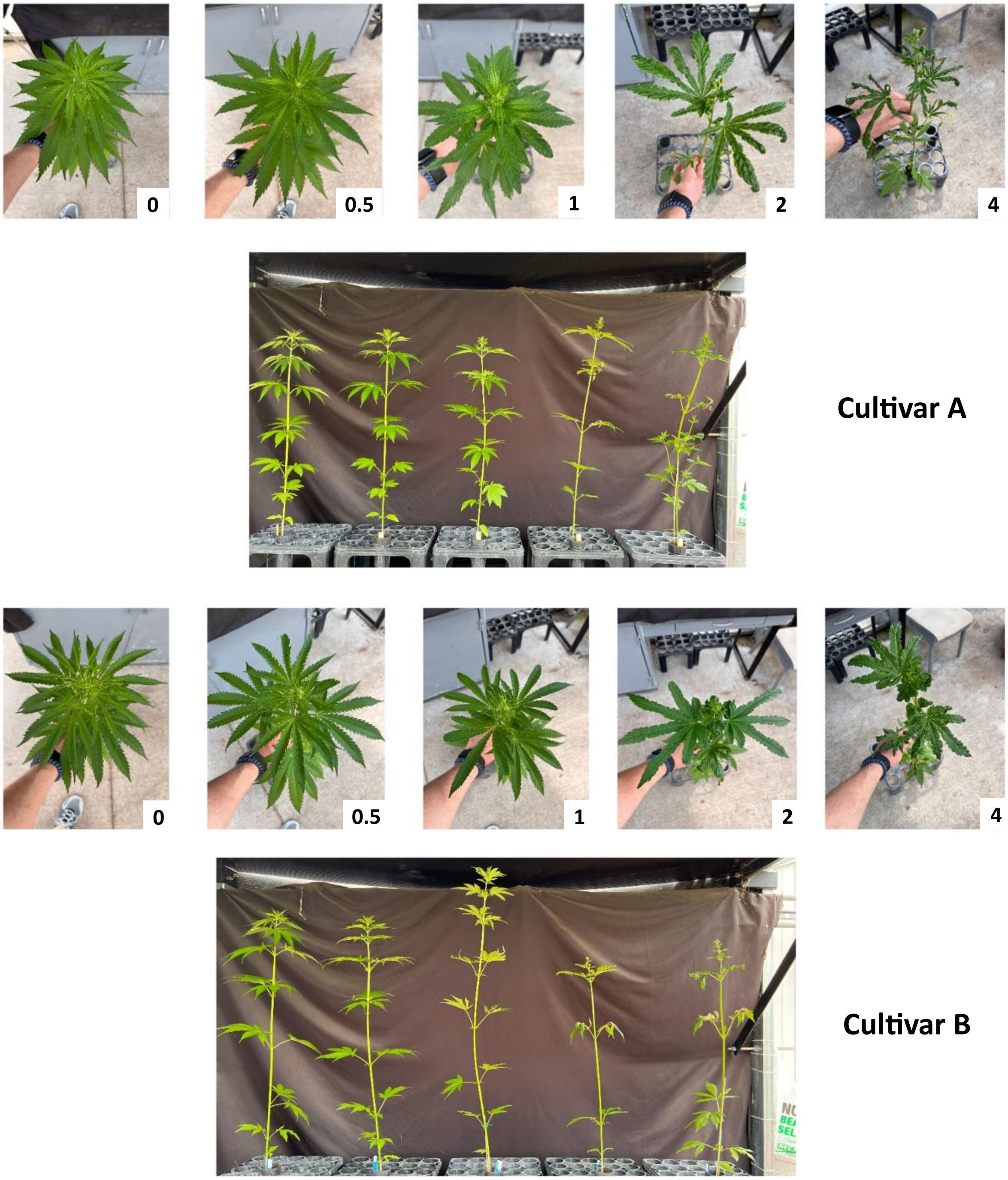


Figure 3.7. Clethodim impact on high-CBD, grain, and fiber (cultivar A - TOP) and high-grain and fiber (cultivar B – BOTTOM) directly above and horizontal view of industrial hemp plants. Clethodim dose from left to right 0, 136, 272, 544, 1088 g ai ha^{-1} or as fraction of applied dose 0-4x, respectively. Trade name, manufacturer, and application rate (1x) are presented in Table 3.1. Visual symptoms assessment scale for industrial hemp at 21 days after application are presented in Table 3.2.

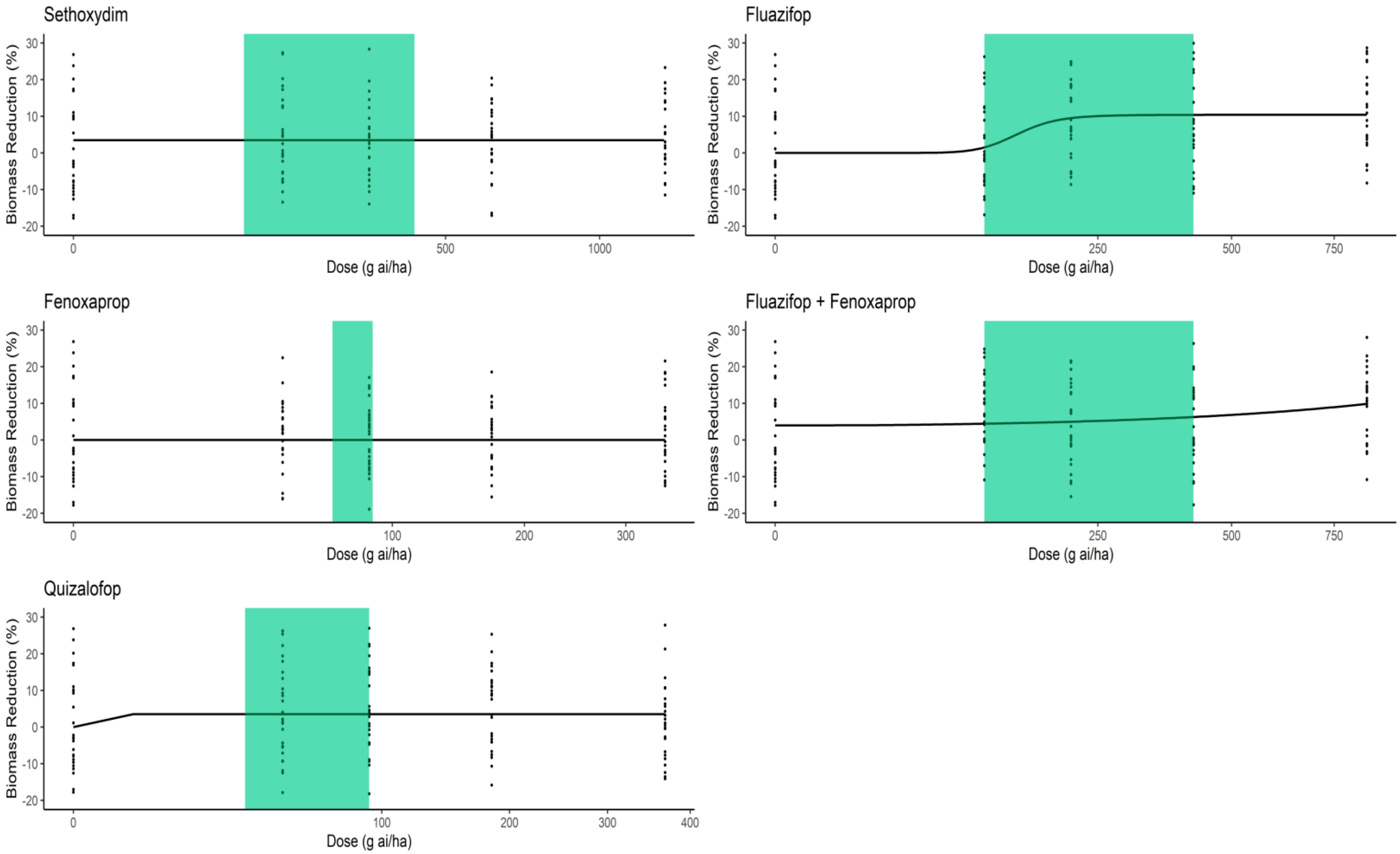
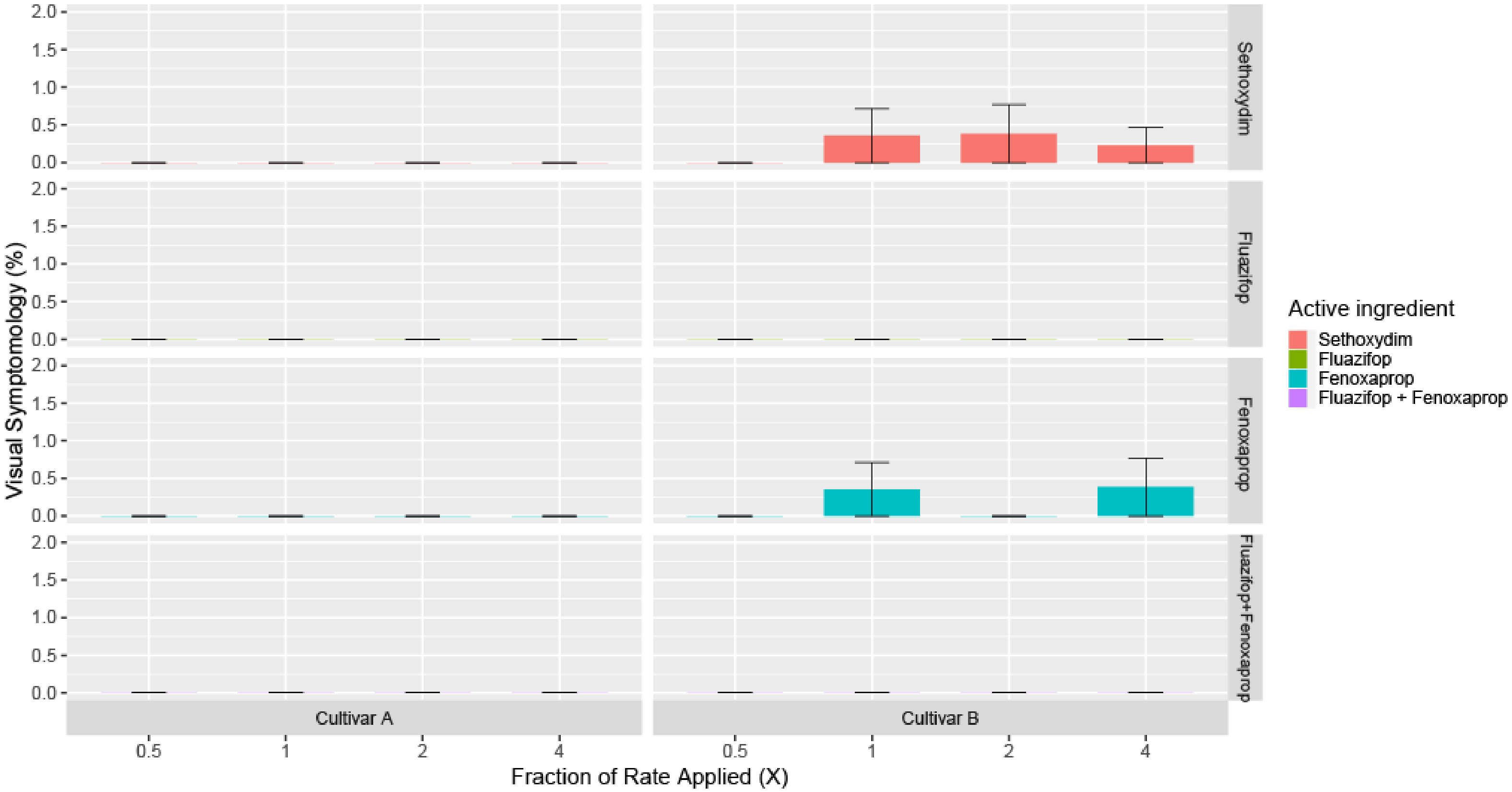


Figure 3.8. Industrial hemp biomass reduction (%) as influenced by tested acetyl-CoA carboxylase inhibitors dose with response pooled across hemp varieties using a three-parameter log-logistic model. The green shaded region of the graph represents the range of recommended labeled doses. Trade name, manufacturer, and application doses are presented in Table 3.1. Model parameter estimates are presented in Table 3.6. Abbreviations: g, gram; ai, active ingredient; ha, hectare.

LIST OF APPENDIX FIGURES



Appendix Figure 3.1. Visual symptoms 21 days after application (with standard deviations) for sethoxydim, fluazifop, fenoxaprop, and fluazifop+fenoxaprop for cultivar A (left) and cultivar B (right). High-CBD, grain, and fiber (cultivar A) and high-grain and fiber (cultivar B). Industrial hemp plants were exposed to incremental doses of aforementioned active ingredients, ranging from 0.5 to 4x (within a cultivar from left to right) fraction doses equivalent of selected label 1x dose. Trade name, manufacturer, and application rate are presented in Table 3.1. Visual symptoms assessment scale for industrial hemp at 21 days after application are presented in Table 3.2.

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Table 3.1. Active ingredients, trade names, manufacturers, and application rates of acetyl-CoA carboxylase inhibitor herbicides tested in the dose response experiment.

Active ingredient[a]	Trade Name	Manufacturer	Dose[b,c]	
			g ai ha^{-1}	fl oz ac^{-1}
Clethodim	Select Max®	Valent U.S.A. LLC	272	32
Pinoxaden	Axial XL®	Syngenta Crop Protection, LLC	60	16.4
Sethoxydim	Poast Plus®	BASF Corporation	315	36
Fluazifop	Fusilade® DX	Syngenta Crop Protection, LLC	210	12
Fenoxaprop	Ricestar® HT	Bayer CropScience LP	86	17
Fluazifop+Fenoxaprop	Fusion®	Syngenta Crop Protection, LLC	210 + 59	12
Quizalofop	Assure® II	AMVAC Chemical Corporation	92	12

[a] All-active ingredients belong to Site of Action Group 1 herbicide.

[b] Treatments applied using an application volume of 140 L ha^{-1} or 15 gal ac^{-1} using a single-nozzle spray chamber equipped with DG9502EVS (TeeJet® Technologies, Wheaton, IL, USA) nozzle at 276 kPa.

[c] Selected labeled (1x) dose as the highest labeled field use dose for labeled crops. Industrial hemp screening test completed at fraction doses equivalent to 0.5, 1, 2, and 4x of selected label 1x dose.

Abbreviations: g, gram; ai, active ingredient; ha, hectare; fl oz, fluid ounce; ac, acre.

Table 3.2. Visual symptoms assessment scale for industrial hemp at 21 days after application of acetyl-CoA carboxylase inhibitor herbicides tested in the dose response experiment. [a]

Visual Symptoms Level	Description of Symptoms
0[a] %	No visible symptoms. Plant appears healthy with vibrant green leaves, no discoloration, curling, or texture changes.
5 %	Very slight textural changes in leaves. No discoloration or leaf deformation is visible. Plant overall appears healthy.
10 %	Minor curling or wrinkling of leaf edges. Leaves may be less glossy. No significant color change is evident.
20 %	Moderate leaf curling, especially at the tips and margins. Slight yellowing (chlorosis) may begin to show on some leaves.
40 %	Pronounced leaf curling and twisting. Yellowing and possibly browning (necrosis) at leaf tips and blades. Some leaves may exhibit stunted growth or deformation and slight axillary shoot growth observed.
60 %	Severe leaf curling and twisting affecting most leaves. Discoloration is more widespread with clear signs of chlorosis and necrosis. No expansion of terminal leaf, second leaf size one-half or less that of control, moderate axillary shoot growth observed.
80 %	Extreme leaf deformation with significant necrosis. Plants show signs of stunted growth and wilting. Many leaves are yellow or brown. Terminal bud dead, strongly malformed, and extreme axillary shoot growth.
100 %	Complete plant death.

[a] Trade name, manufacturer, and application rate are presented in Table 3.1.

[b] Baseline established through observation of a non-treated control on the evaluation date.

Table 3.3. Effective dose to cause 5, 10, and 25% visual symptoms 21 days after application on two hemp cultivars for post-emergence application of pinoxaden.

Cultivar	Log-logistic model parameter (e)[a] by hemp cultivar[b]		
	5 (±SE)	10 (±SE)	25 (±SE)
	——— g ai ha^{-1} ———		
Cultivar A	86 (±63)	104 (±38)	130 (±46)
Cultivar B	35 (±27)	53 (±21)	79 (±14)

[a] Log-logistic model parameter (e) estimates dose to 5, 10, and 25% visual symptoms 21 days after application with standard errors (SE) as influenced by pinoxaden.

[b] High-CBD, grain, and fiber (cultivar A) and high-grain and fiber (cultivar B).

Trade name, manufacturer, and application rate are presented in Table 3.1.

Abbreviations: g, gram; ai, active ingredient; ha, hectare.

Table 3.4. Effective dose to cause 5, 10, and 25% visual symptoms 21 days after application for post-emergence application of clethodim and quizalofop with response pooled across hemp varieties.

Active ingredient	Log-logistic model parameter (e)[a, b]		
	5 (±SE)	10 (±SE)	25 (±SE)
		g ai ha^{-1}	
Clethodim	80 (±22)	112 (±21)	187 (±22)
Quizalofop		> 370	

[a] Log-logistic model parameter (e) estimates dose to 5, 10, and 25% visual symptoms 21 days after application with standard errors (SE) as influenced by active ingredient.
[b] The use of > indicates that the estimated dose was above the highest dose tested.
Trade name, manufacturer, and application rate are presented in Table 3.1.
Abbreviations: g, gram; ai, active ingredient; ha, hectare.

Table 3.5. Effective dose to cause 5, 10, and 25% biomass reduction on two hemp cultivars for post-emergence application of clethodim and pinoxaden.

Active ingredient	Log-logistic model parameter (e)[a] by hemp cultivar[b]					
	Cultivar A			Cultivar B		
	5 (±SE)	10 (±SE)	25 (±SE)	5 (±SE)	10 (±SE)	25 (±SE)
	g ai ha^{-1}					
Clethodim	-[c]	131 (±63)	398 (±68)	192 (±32)	244 (±30)	346 (±26)
Pinoxaden	60 (±12)	79 (±13)	116 (±39)	39 (±6)	46 (±5)	59 (±4)

[a] Log-logistic model parameter (e) estimates dose to 5, 10, and 25% biomass reduction with standard errors (SE) as influenced by active ingredient.

[b] High-CBD, grain, and fiber (Cultivar A) and high-grain and fiber (Cultivar B).

[c] No estimation available.

Trade name, manufacturer, and application rate are presented in Table 3.1.

Abbreviations: g, gram; ai, active ingredient; ha, hectare.

Table 3.6. Effective dose to cause 5, 10, and 25% biomass reduction for post-emergence application of various acetyl-CoA carboxylase inhibitors with response pooled across hemp varieties.

Active ingredient	Log-logistic model parameter (e)[a, b]		
	5 (±SE)	10 (±SE)	25 (±SE)
	g ai ha^{-1}		
Sethoxydim	> 1261		
Fluazifop	86 (±37)	98 (±33)	117 (±32)
Fenoxaprop	> 345		
Fluazifop+Fenoxaprop	648 (±135)	> 841	
Quizalofop	> 370		

[a] Log-logistic model parameter (e) estimates dose to 5, 10, and 25% biomass reduction with standard errors (SE) as influenced by active ingredient.

[b] The use of > indicates that the estimated dose was above the highest dose tested. Trade name, manufacturer, and application rate are presented in Table 3.1. Abbreviations: g, gram; ai, active ingredient; ha, hectare.

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Chapters 4 and 5

Volunteer Hemp Tolerance to Early-Season Herbicides in Soybean and Corn

Abstract

The inclusion of industrial hemp grown for grain in diverse crop rotations has prompted concerns regarding the appearance of volunteer hemp because of indeterminate inflorescence. Current regulations for volunteer hemp control in the US have not yet been published, and knowledge of available volunteer hemp herbicide control options in diverse cropping systems is limited. The objective of this study was to evaluate volunteer hemp tolerance to commonly used herbicides for spring burndown in soybean (2,4-D-tolerant) and corn. Field trials were conducted in 2021 and 2022 in a randomized complete block design with four replications, including 21 soybean and 40 corn spring burndown treatments. All 61 treatments were applied at 140 L ha^{-1} using an AIXR11002 nozzle at 221 kPa. At application time, volunteer hemp was 10-20 cm in height with a volunteer hemp density of 1003 (± 129) plants per m^2. Visual evaluations were collected up to 28 to 35 days after application (DAA) for soybean and corn, respectively. At 35 DAA, biomass was harvested from an area of 0.093 m^2 and oven-dried at 65° C until constant weight was reached. The dry weights were recorded and used for further analysis. Data were analyzed in SAS, with all comparisons made using a Tukey-Kramer's test at significance level $\alpha = 0.05$. Among treatments examined for soybean, volunteer hemp exhibited the greatest sensitivity to glyphosate, with about 90% biomass reduction when applied alone or in a tank-mix compared to non-treated. In general, 2,4-D treatments were more effective when sprayed in a tank-mix with other herbicides. The pyroxasulfone alone resulted in about 10 hemp biomass reduction, while a tank-mix with saflufenacil and imazethapyr resulted in a 25% biomass reduction. Similar sensitivity of volunteer hemp to glyphosate was also observed for assessed treatment in corn. In addition, treatments containing mesotrione resulted in biomass reduction greater than 90%. Atrazine alone resulted in about 70% biomass reduction. In addition, tank-mixing acetochlor or isoxaflutole with atrazine increased biomass reduction to 95%. Fluthiacet was the only treatment that did not cause more than 4% biomass reduction, being the least harmful active ingredient on volunteer hemp across all examined treatments. Results indicate that various control options could be utilized for volunteer hemp control in subsequent soybean or corn crop rotations. However, none of the treatments resulted in 100% volunteer hemp control, suggesting that the application of only spring burndown herbicides may not be sufficient. Although findings indicate increased hemp tolerance to several active ingredients, further assessment is required to understand the consequences when used in-crop situations.

4.1 & 5.1 Introduction

Renewed interest in the production of industrial hemp (Cannabinaceae, *Cannabis sativa* L.) in the United States (US) arose with (1) the implementation of the Agriculture Act of 2014 and Agriculture Improvement Act of 2018 and (2) the opportunities to incorporate industrial hemp (hereafter referred to as hemp) across different industries including textile, food, construction, and pharmaceuticals (Agricultural Act of 2014; Agricultural Improvement Act of 2018; Amaducci et al., 2015; Johnson, 2014; Karus and Vogt, 2004). Changes in the federal regulations in the US led to an increase in hemp cultivated areas, reaching 16.3 thousand hectares (ha^{-1}) in 2021 (USDA NASS, 2022). Compared to previous years, in addition to a hemp product sale increase to almost $821 million, it was noted more diversity in production with partition 3.3 + 1.4 (grain + seed), 5.1 (fiber), and 6.5 (floral) thousand ha^{-1} (USDA NASS, 2022). Despite the significant increase in cultivation area to meet national demands, the US currently imports hemp grain from various countries worldwide (USDA ERS, 2020).

Interest in hemp grain comes along with possibilities to be used as food, livestock feed, or grain-derived oil that can be incorporated within the framework of a variety of industry products or as biodiesel (Amaducci et al., 2015; Crini et al., 2020; Das et al., 2020; Salentijn et al., 2015; Vonapartis et al., 2015). From 2015 to 2020, US hemp grain imports increased substantially from $51.2 to $79.9 million (UNCTAD, 2022). Canada is currently the largest supplier of hemp grain to the US market, with other major suppliers including China and several European countries (USDA ERS, 2020). The increasing demand and supportive regulations are likely to promote further growth of hemp grain cultivation globally, including in the US.

A major challenge associated with commercial hemp grain varieties globally is the indeterminate flowering trait, which leads to varying grain maturity within the same inflorescence (Amaducci et al., 2015; Elias et al., 2020; Salentijn et al., 2019). The occurrence of this trait increases the potential for the appearance of volunteer hemp plants in sequential cropping rotations due to grain shed from the hemp plants before or at harvest. Up-to-date recommendations to avoid grain loss include adjusting harvest windows when 70 - 80% maturity is reached (OMAFRA, 2020; CHTA, 2019; Kaiser et al., 2015). According to the USDA NASS (2022), hemp grain yield potential has been estimated at 594 kg ha^{-1}. Contemplating a possibility for seed loss of only 15% either because seed shattering or reduced harvest efficiency, this may contribute to hemp grain loss in the 89 kg ha^{-1}. Using an approach similar to the Alms et al. (2016) who assessed corn grain losses, under the assumption 17 to 36 g per 1000 hemp seeds^{-1} with 95% germination (and 30% mortality rates), there is a potential for at least 497 to 235 volunteer hemp seeds m^{-2} in the following cropping sequence, respectively (Bócsa and Karus, 1998; OMAFRA, 2020; PCDF, 2012). Therefore, the possible losses of this magnitude are of concern for production sustainability as well as implications associated with volunteer hemp appearance in subsequent crop rotations.

Similar implications had been previously reported with canola (Brassicaceae, *Brassica napus* L.) because of prolonged pod maturation. For a small-seed crop like canola, even small yield losses (less than 3%) can result in the presence of a large population of volunteer canola plants in the following cropping sequence (Gulden et al., 2003). According to Gulden et al. (2003), from field surveys, seed loss after harvest operations is determined to range from 1680 to 5000 canola seeds m^{-2}, representing up to 56-fold seeding rates of canola, respectively. In addition, Simard et al. (2002) and Beckie and Warwick's (2010) results suggest that the volunteer canola may persist for 5-7 years, making management options difficult. Research on yield losses in wheat has been reported previously, in which a volunteer canola density of 261 plants m^{-2} resulted in 68% wheat (Poaceae, *Triticum aestivum* L.) yield reduction (Krato and Petersen, 2012). Irrespective of the volunteer canola trait (non-herbicide-tolerant or herbicide-tolerant, including single or multiple traits), successful herbicide programs were identified in subsequent crop rotations for cereals and broadleaf crops (Beckie et al., 2004). Conversely, for volunteer hemp, there is no information concerning potential hemp grain loss and there is limited knowledge about effective herbicide programs.

In general, the impact of volunteer hemp management challenges in the subsequent crop rotation with soybean (Fabaceae, *Glycine max* (L.) Merr.) and corn (Poaceae, *Zea mays* L.) is not well understood. In Nebraska, about 2.3 and 3.6 million ha^{-1} are annually planted under soybean and corn with reasonable expectation for hemp inclusion as part of the crop rotation, respectively (USDA ESMIS, 2022). In addition, most of the land is maintained under no- or reduced-till with approximately 95% soybean and 90% corn area comprised of herbicide-tolerance cultivars or hybrids (USDA ERS, 2022). In cropping systems like those, herbicides are considered a primary option for weed control (Chahal and Johnson, 2012; Harker and O'Donovan, 2013). Considering that no herbicide-tolerant hemp varieties are commercially available the prevalence of various herbicide-tolerant traits adopted in soybean and corn permits the inclusion of various herbicides as part of management in the later crop rotations. However, there is limited information concerning the effectiveness of commonly used early-season (including pre-emergence and post-emergence (POST)) soybean and corn herbicides for volunteer hemp control. Different from Canada where volunteer hemp plants must be under control by cutting, pulling, cultivation, and/or herbicides in the fields following hemp production (OMAFRA, 2020), in the US (except Montana) current regulations for volunteer hemp control have not yet been published (ARM, 2021; USDA FAS, 2019). However, even with the lengthy history (since 1998) of hemp production in Canada the effectiveness of various herbicide treatments has not yet been evaluated for volunteer hemp control under field conditions nor herbicides are available that specify volunteer hemp on their label (USDA FAS, 2019). Several studies are available to address limited herbicide options available in hemp as a crop as part of the greenhouse or field screening to application of pre-emergence or POST thus allowing general insights into hemp tolerance to diverse active ingredients.

Previous research conducted as part of herbicide screening on hemp indicates elevated hemp sensitivity to a wide range of soybean and corn active ingredients commonly used in the US and Canada (Flessner et al., 2020; Howatt and Mettler, 2018; Mettler, 2021; Ortmeier-Clarke et al., 2022). Ortmeier-Clarke et al. (2022) reported from all tested pre-emergence and POST herbicide programs, only clethodim (site of action [SOA] Group 1) and clopyralid (SOA 4) caused less than 75% biomass reduction, suggesting that management of volunteer hemp in soybean and corn is not expected to be of concern. In addition, including hemp in crop rotation with either glyphosate- or glufosinate-tolerance crops has typically been considered as a common management strategy in Canada and North Dakota for volunteer hemp control (CHTA, 2019; Howatt and Mettler, 2018). High sensitivity to glyphosate and glufosinate (90% or greater biomass reduction compared to non-treated) was previously observed for volunteer hemp control (Horowitz, 1977; Mettler, 2021; Sosnoskie and Maloney, 2021; Ortmeier-Clarke et al., 2022). However, findings associated with glufosinate efficacy may be limited only to early stages (less than 10 cm) because of increased tolerance at later stages (CHTA, 2019). Furthermore, challenges associated with a constant over-reliance on a single mode of action herbicides for weed control historically have led to the evolution of herbicide-resistant biotypes (Heap, 2014). Moreover, supporting diverse chemical control options is critical and requires further evaluation to avoid the development of volunteer hemp-resistant populations. Even though earlier research provides general insights into hemp sensitivity, most experiments were completed performing herbicide application simultaneously over individual plants. Currently, no research is available on the efficacy of active ingredients alone or in various combinations commonly used in soybean and corn to control volunteer hemp plants present at high density under the field conditions. Therefore, this study aimed to determine the effectiveness of various early-season herbicide programs currently used in soybean and corn to help with future management decisions and development well-coordinated volunteer hemp control programs in succeeding crops.

Chapter 4

Volunteer Hemp Tolerance to Early-Season Herbicides in Soybean

4.2. Materials and Methods for Volunteer Hemp Control in Soybean

Study site description. Field research was conducted during the 2021 and 2022 growing seasons at the West Central Research and Extension Center in North Platte, Nebraska (41°05’17.2” N - 100°46’40.7” W). The soil type was Sandy Loam, with a pH of 7.5 and 2.1% organic matter. Monthly data about environmental conditions (mean air temperature and rainfall) for 2021 and 2022 growing seasons for the duration of the studies can be found in Table 4.1.

A volunteer hemp control study in soybean was initiated in crop rotation after hemp. Both hemp and soybeans were grown in a no-till system without additional maintenance. For 2021 and 2022, observed volunteer hemp density (n= 5) was 1163 (± 138) and 842 (± 121) plants per m^2, respectively. A relatively high density of volunteer hemp plants in subsequent crop rotation at the study location can be attributed to the harvest of only 20% area per plot in 2020 and 2021, leaving a significant amount of hemp seed in the field. The underlying reasons for this were (1) the inability to market hemp grain per the hemp research license agreement and (2) to ensure the establishment of research plots on volunteer hemp control in soybean following the growing season. The 2,4-D-tolerant soybean (P26T23E, Pioneer, Corteva Agroscience, Indianapolis, IN, USA) cultivar was planted at 346.000 seeds per hectare in rows spaced 0.76 m apart on May 21, 2021, and May 26, 2022. No specific maintenance practices were applied before the soybean establishment, so the performance of applied herbicide treatments can be evaluated.

Experimental and treatment design. The experiments were arranged in a randomized complete block design with four replications (following historically known pH gradients in the field from east to west). Experimental units (plots) were 3 m wide and 3 m long, consisting of four soybean rows. Evaluated treatments included 20 herbicides and a non-treated control (Table 4.2.). Herbicide treatments including glyphosate, 2,4-D, sulfentrazone, cloransulam, metribuzin, chlorimuron, carfentrazone, flumioxazin, pyroxasulfone, saflufenacil, and imazethapyr were applied alone or as part of a tank-mix to evaluate volunteer hemp response. As a result of different recommendations for adjuvant types and associated rates, no adjuvants were used in the tank-mix with the herbicide treatments.

Herbicide application. Herbicide treatments were applied in the assigned field when volunteer hemp plants were 10-20 cm in height (developed two to four true leaves). The application was performed on May 14 for both years using a CO_2 backpack sprayer equipped with a four-nozzle (51 cm apart) boom equipped with Air Induction Extended Range (AIXR, TeeJet Spraying Systems Co., Wheaton, IL, USA) 11002 nozzles calibrated to deliver 140 L ha^{-1} of a solution at 221 kPa. More details about environmental conditions throughout May for the 2021 and 2022 growing seasons can be found in Figure 4.1.

Data collection. Visual estimations of volunteer hemp control were collected 7, 14, and 28 days after application (DAA). Visual control was estimated based on the entire plot response using a scale of 0 – 100%, where 0% represented no visual response and 100% was complete plant death. At 28 DAA, biomass was harvested from an area of 0.093 m^2 and oven-dried at 65° C until a constant weight was reached. Dry biomass weights were recorded and converted into percentage of biomass reduction compared to non-treated control using equation 1 (in which NT represents the mean biomass of non-treated plants and T represents the biomass of the treated plants):

$$\%\ Biomass\ reduction = \left[\frac{(NT - T)}{NT}\right] * 100 \tag{1}$$

Data Analysis. The dataset (visual estimations of volunteer hemp control over time and biomass reduction) was subjected to analysis of variance (ANOVA) using a generalized linear mixed model (PROC GLIMMIX) in SAS (Statistical Analysis Software, version 9.4, Cary, NC, USA). Studentized residual plots generated in PROC GLIMMIX and the Shapiro-Wilk statistic normality test generated in PROC UNIVARIATE were used to check for normality and deviations from the assumption of variance homogeneity. Visual estimations of volunteer hemp control up to 28 DAA met the assumptions for ANOVA analysis when arcsine-square root transformed, while biomass reduction data did not require transformation. For evaluated response variables, herbicide treatment was considered fixed effects, while blocks (replications) were nested within an experimental run (year) and considered a random effect. In addition, for visual estimations of volunteer hemp control, treatment by time interactions were included as part of the statistical analysis. Assessment of this interaction allowed for identifying individual treatment differences at specific evaluation times and assessing changes in volunteer hemp tolerance to applied herbicides over time using the "*SLICE*" option in SAS (Winer 1971). Where applicable data means were back transformed to the data scale of actual values for comparison. Means were separated at the α= 0.05 level of significance using a Tukey-Kramer's *test*.

4.3. Results and Discussion for Volunteer Hemp Control in Soybeans

Visual control. Volunteer hemp control was influenced by herbicide treatment*time (P=0.0028) interaction (Appendix Table 4.1). Visual estimations of volunteer hemp control (hereafter referred to as control) of applied treatments were evaluated 7, 14, and 28 days after application (DAA), with findings presented in Table 4.3.

The experiment results demonstrate that, irrespective of whether glyphosate was applied independently or in combination with other herbicides, it facilitated more rapid and greater weed control compared to 2,4-D application. In most cases, glyphosate-containing treatments exhibited a difference between 7 and 14 days after application (DAA) evaluation periods, while no difference was detected afterward indicating low tolerance (or rapid high injury) of volunteer hemp. Conversely, treatments containing 2,4-D typically displayed a notable difference between 7 and 28 DAA, with the 28 DAA evaluation producing greater volunteer hemp control. Among the treatments with minimal changes throughout the evaluation over the time include glyphosate + 2,4-D (94% control), flumioxazin + glyphosate (93%), sulfentrazone + metribuzin + 2,4-D (90%), carfentrazone (54%), and pyroxasulfone (11%). Consequently, critical trends regarding the performance of individual treatments are examined, focusing on variations associated with specific evaluation time or the effectiveness of these herbicides when incorporated into tank-mixtures (Appendix Figure 4.1).

The application of glyphosate alone at 7 DAA resulted in 90% control, which increased to 98% for 14 DAA and remained unchanged for 28 DAA. These findings are consistent with previous studies where high sensitivity of hemp was observed following POST application of glyphosate at 800 g ae ha^{-1}, resulting in 80 to 98% injury on hemp at 7 to 19 DAA, respectively (Mettler, 2021). Similar outcomes were observed across various tank-mixtures containing glyphosate, with the overall finding at 28 DAA being that volunteer hemp control exceeded 96%. Consequently, for the treatment combinations evaluated in this study, there were no changes in glyphosate efficacy when present in diverse tank-mixtures.

The application of herbicides, such as sulfentrazone + cloransulam, sulfentrazone + chlorimuron, and sulfentrazone + chlorimuron + carfentrazone, caused similar weed control (approximately 53%) at 7 days after application (DAA). Although incorporating 2,4-D into the tank-mixture increased control to approximately 72%, no significant difference was observed in comparison to the application of 2,4-D alone. Past research has shown similar sensitivity for POST application of 2,4-D (amine), resulting in nearly 64% hemp visual injury at 7 DAA (Mettler, 2021). The application of sulfentrazone + metribuzin (72%) and sulfentrazone + metribuzin + 2,4-D (86%) demonstrated a difference only at 7 DAA evaluation timing, while no difference was observed later (up to 92%) at 28 DAA. Previous studies indicate that using sulfentrazone as preemergence herbicide at 158 g ai ha^{-1} resulted in 50% visual injury at 15 DAA (Mettler, 2021). Moreover, metribuzin POST application at 210 g ai ha^{-1} has been shown to cause up to 62% hemp injury.

In contrast to previous observations, the 2,4-D tank-mix with various herbicide programs enhanced weed control after 14 DAA. For instance, a tank-mix of flumioxazin + chlorimuron resulted in 88% control, while the addition of 2,4-D increased control to 94%. Cuvaka et al. (2020) and Flessner et al. (2020) found that the application of flumioxazin at 280 g ai ha^{-1} was relatively safe across multiple hemp varieties and environments. Meanwhile, Mettler (2021) observed up to 18% visual injury with a rate of 277 g ai ha^{-1}, suggesting varying hemp responses across different studies. Similarly, the herbicide treatment comprising sulfentrazone + chlorimuron + carfentrazone benefited from the addition of 2,4-D in the tank-mix. When applied alone, volunteer hemp control was 86%, while the inclusion of 2,4-D increased control to 96% at 28 DAA. Interestingly, carfentrazone contribution to volunteer hemp control appears to be minimal (55%). The addition of carfentrazone in a tank-mix with sulfentrazone + chlorimuron did not enhance control. Howatt and Mettler (2018) previously reported relatively low levels of carfentrazone injury of 15% at 25 DAA with an application rate of 12 g ai ha^{-1}, indicating hemp's relative tolerance. The application of sulfentrazone + cloransulam (220 + 30 g ai ha^{-1}) resulted in 65-75% control, while incorporating 2,4-D in the tank-mix increased control to 85-92% for evaluations at 14-28 DAA. This is consistent with previous studies that reported cloransulam application at 18 or 36 g ai ha^{-1} leading to up to 84% visual injury 21 DAA under field conditions (Mettler, 2021).

Overall, only two evaluated herbicide programs containing pyroxasulfone and saflufenacil + imazethapyr + pyroxasulfone did not result in satisfactory control levels (below 33% at 28 DAA). In comparison to previous reports that utilized single active ingredients reduced effectiveness can be associated with several reasons including possibility for antagonistic interactions between the active ingredients in the herbicide mixtures or differences in application rates, timing, or environmental conditions. The application of saflufenacil as a POST herbicide program for hemp has not been assessed yet. Mettler (2021) found that imazethapyr application at 35 g ai ha^{-1} led to the 38% visual injury at 19 DAA. Furthermore, higher application rates of 200 g ai ha^{-1} resulted in 49 to 53% visual injury for 14 and 28 DAA, respectively (Byrd, 2019; Flessner et al., 2020). The application of pyroxasulfone alone at 150 g ai ha^{-1} in our study caused only 11% control over time, suggesting a minimal impact on volunteer hemp for application after plant emergence. Consequently, the application of pyroxasulfone and saflufenacil + imazethapyr + pyroxasulfone should be avoided for volunteer hemp control unless tank-mixed with glyphosate, where control exceeded 94%.

Biomass reduction. The herbicide treatment greatly influenced volunteer hemp biomass reduction (P<0.0001) as indicated in Appendix Table 4.1. Biomass reduction of volunteer hemp compared to non-treated control findings are presented in Figure 4.2. Overall findings indicate that most of the evaluated herbicide treatments, 11 out of 20 resulted in greater than 82% volunteer hemp biomass reduction.

The application of glyphosate alone led to an 89% biomass reduction. Furthermore, most treatment combinations benefited the addition of glyphosate. No difference was observed among tank-mixtures that contained glyphosate including 2,4-D, flumioxazin, flumioxazin + chlorimuron, pyroxasulfone, and saflufenacil + imazethapyr + pyroxasulfone. For example, the application of flumioxazin + chlorimuron alone (80 + 30 g ai ha^{-1}) resulted in 58% biomass reduction while the addition of glyphosate enhanced biomass reduction to 85%. A similar impact on volunteer hemp biomass reduction was observed with the addition of 2,4-D in tank-mix with flumioxazin + chlorimuron. Previous literature on the effects of flumioxazin and chlorimuron on hemp is limited to preemergence application. Flumioxazin was found to cause no biomass reduction when applied at 89 g ai ha^{-1} (Mettler, 2021) and up to 49% with 100 g ai ha^{-1} (Byrd, 2019; Flessner et al., 2020). Additionally, Ortmeier-Clarke et al. (2022) estimated dose for 50% biomass reduction associated w with the preemergence application of flumioxazin was found to be cultivar-dependent, with estimates up to 61.4 g ai ha^{-1}.

The application of 2,4-D alone resulted in 68% volunteer hemp biomass reduction associated with application at 1060 g ae ha^{-1}. According to Ortmeier-Clarke et al. (2022), application of 2,4-D (choline at 800 g ae ha^{-1}) under greenhouse conditions caused less than 75% biomass reduction associated with the POST application. However, another resource indicates possibilities for formulation differences where the application of 2,4-D (amine at 280 g ae ha^{-1}) resulted in 67% biomass reduction (Mettler, 2021). The addition of glyphosate with 2,4-D application increased biomass reduction to 82%. Similar trends regarding the performance increase were noted for 2,4-D as well for most of the herbicide treatments examined hereby. Treatment combinations that benefited from addition of 2,4-D in the tank-mix include sulfentrazone + chlorimuron +

carfentrazone where biomass reduction increased from 65 to 80% without or with 2,4-D, respectively. Instances where the addition of 2,4-D did not increase biomass reduction were observed in sulfentrazone + cloransulam. Sulfentrazone + cloransulam performance alone or with 2,4-D was no different from the application of 2,4-D alone. However, the treatment containing sulfentrazone + metribuzin outperformed the application of 2,4-D, suggesting that the performance difference may arise from the integration of metribuzin in the tank-mix. Our results indicate that sulfentrazone + metribuzin (180 + 260 g ai ha^{-1}) caused an 84% biomass reduction when applied after volunteer hemp emergence. Mettler (2021) previously reported that application of metribuzin as part of preemergence (277 g ai ha^{-1}) or POST (210 g ai ha^{-1}) programs resulted in 83 or 68% biomass reduction, respectively. Similarly, Byrd (2019) and Flessner et al. (2020) observed that the preemergence application of metribuzin at 600 g ai ha^{-1} resulted in 84% biomass reduction as well as 47% plant height reduction, at eight weeks after herbicide application. Therefore, the use of 2,4-D alone for early-season volunteer hemp control should not be practiced unless tank-mixed with glyphosate or other evaluated herbicide treatments that enhanced herbicide performance.

Treatments including carfentrazone, saflufenacil + imazethapyr + pyroxasulfone, and pyroxasulfone alone resulted in biomass reduction less than 46% when compared across the evaluated treatments in this study. Carfentrazone at 20 g ha^{-1} resulted in a 46% biomass reduction; however, these findings do not corroborate with previous studies. According to Mettler (2021), the application of 9 g ai ha^{-1} resulted in a 67% biomass reduction when ammonium sulfate was added at a concentration of 10.2 g L^{-1}.

Therefore, increased effectiveness at lower used rates may be explained by the inclusion of adjuvants like ammonium sulfate as part of the tank-mixture. Previous studies have reported that the inclusion of adjuvants in tank-mixes can improve herbicide efficacy (Brankov et al., 2023; Moraes et al., 2019; Polli et al., 2022).

Our findings suggest that the application of saflufenacil (21 g ai ha^{-1}) + imazethapyr (58 g ai ha^{-1}) + pyroxasulfone (100 g ai ha^{-1}) resulted in a 25% reduction in volunteer hemp biomass. Limited research conducted on volunteer hemp response to these herbicides, particularly in combination, makes it difficult to draw definitive conclusions. The results indicate variable sensitivity of hemp to the preemergence application of saflufenacil, as demonstrated by the following studies. Ortmeier-Clarke et al. (2022) found that applying saflufenacil at 25 g ha^{-1} resulted in >50% biomass reduction. Mettler (2021) reported no difference in biomass accumulation between treated (38 g ai ha^{-1}) and non-treated controls. Knezevic et al. (2020) observed temporary plant injury (up to 25%) and similar performance across hemp varieties at 25 g ai ha^{-1}. Furthermore, the literature reveals high variability in hemp response to pyroxasulfone applications across different environments. Under greenhouse conditions Maxwell (2016), Ortmeier-Clarke et al. (2022) and Flessner et al. (2020) reported 75-80% biomass reduction with pyroxasulfone application rates of 119 and 900 g ai ha^{-1}. Oppositely under field conditions Mettler (2021) found no significant difference in biomass accumulation between treated (109 and 218 g ai ha^{-1}) and non-treated plots. All studies, including pyroxasulfone, were tested as part of the preemergence application.

However, our findings indicate a minimal impact (up to 10% visual control and 4% biomass reduction) on volunteer hemp associated with early POST application.

As a result, it is recommended to avoid the application of saflufenacil, imazethapyr, pyroxasulfone herbicides alone for volunteer hemp control during early POST. To improve control, it might be beneficial to consider tank-mixing pyroxasulfone or the saflufenacil + imazethapyr + pyroxasulfone combination with glyphosate. Glyphosate could potentially enhance the overall performance of these herbicide programs when applied in combination, ensuring more effective control of volunteer hemp. The effectiveness of herbicide combinations involving saflufenacil, imazethapyr, and pyroxasulfone for controlling volunteer hemp biomass appears to be highly variable and dependent on factors such as application rate, environment, and hemp variety.

Chapter 5

Volunteer Hemp Tolerance to Early-Season Herbicides in Corn

5.2. Materials and Methods for Volunteer Hemp Control in Corn

Study site description. Two field studies were conducted during the 2022 growing season at the West Central Research and Extension Center in North Platte, NE (Lincoln County) and Columbus, NE (Platte County) locations. The soil type at the North Platte site was Sandy-Loam with a pH of 7.5 and 2.1% organic matter, while at the Columbus site Silty Clay-Loam with a pH of 5.3 and organic matter content of 4.1%. In addition, a volunteer hemp control study in corn for both locations was initiated after hemp. Monthly data about environmental conditions (mean air temperature and rainfall) for the 2022 growing season for the duration of the studies at North Platte and Columbus can be found in Table 5.1.

North Platte. Hemp and corn were grown in a no-till system without additional maintenance ahead of the growing season. Volunteer hemp density (n= 5) was 816 (± 163) plants per m^2 with plant height ranging from 8-12 cm. Corn hybrid (P0662Q, Pioneer, Johnston, IA, USA) was planted at 79.080 seeds per hectare in rows spaced 0.76 m apart on May 10, 2022.

Columbus. Hemp and corn were grown in a reduced-till system with additional plot maintenance prior to corn establishment. In the spring of 2022, ahead of corn planting hemp residues and emerged volunteer hemp plants were incorporated using shallow disking. Volunteer hemp density (n= 5) was 510 (± 131) plants per m^2 with plant height ranging from 12-16 cm. Corn hybrid (P1548AM, Pioneer, Johnston, IA, USA) was planted at 69.200 seeds per hectare in rows spaced 0.91 m apart on May 14, 2022.

Experimental and treatment design. The experiments were arranged in a randomized complete block design with four replications. Experimental units (plots) were 3 m wide and 3 m long that consisted of three (Columbus) and four (North Platte) corn rows. Evaluated 40 herbicide treatments included either as premix or tank-mix (based upon availability) to evaluate volunteer hemp response (Table 5.2.). Even though, with pronounced differences among soil properties, for the sake of the experiment repeatability, product rates for both locations were kept the same. No adjuvants were used in the tank-mix with the herbicide treatments.

Herbicide application. The application was performed on May 10 (North Platte, NE) and May 13 (Columbus, NE) 2022 using a CO_2 backpack sprayer equipped with a four-nozzle (51 cm apart) boom equipped with Air Induction Extended Range (AIXR, TeeJet Spraying Systems Co., Wheaton, IL, USA) 110015 nozzles calibrated to deliver 140 L ha^{-1} of a solution at 276 kPa. More details about environmental conditions for May 2022 at study locations can be found in Figure 5.1.

Data collection. Visual control evaluations were collected 7, 21, and 35 days after application (DAA). Visual control was estimated based on the entire plot response using a scale of 0 – 100%, where 0% represented no visual response and 100% was complete plant death. At 35 DAA, biomass was harvested from an area of 0.093 m^2 and oven-dried at 65° C until a constant weight was reached. Dry biomass weights were recorded and converted into a percentage of biomass reduction as previously described for the soybean study (Equation 1).

Data analysis. The dataset was subjected to ANOVA using PROC GLIMMIX in SAS. Visual estimations of hemp control (7, 21, and 35 DAA) and biomass reduction required arcsine square root transformation to meet the assumptions of the variance analysis. Visual estimations of volunteer hemp control, herbicide treatment, timing, and location were analyzed as fixed effects, while replication (block) was considered random. The three-way herbicide treatment by timing by location (P<0.0001) interaction was significant for visual estimations of volunteer hemp control. Data were analyzed and presented separately for each location; therefore, allowing the possibility to evaluate herbicide treatment by timing interaction allows assessment of changes in volunteer hemp tolerance to herbicides at 7, 21, and 35 DAA as well overall performance over time as previously described using "*SLICE*" option (Winer, 1971). For the biomass reduction dataset, herbicide treatment and location were analyzed as fixed effects, while replication (block) was considered a random effect. The two-way herbicide treatment by location (P<0.0001) interaction was significant for biomass reduction of volunteer hemp.

5.3. Results and Discussion for Volunteer Hemp Control in Corn

Visual control at North Platte location. Volunteer hemp control was influenced by herbicide treatment*time (P<0.0001) interaction (Appendix Table 5.1.). As a result of the complexity associated with herbicide treatment performance over time only important trends are discussed (Table 5.3.).

The application of S-metolachlor alone at 1620 g ai ha^{-1} resulted in 20% control and remained unchanged over time. Similarly, findings associated with the preemergence application of S-metolachlor under the field conditions caused less than 15% injury when applied at 1600 g ai ha^{-1} (Byrd, 2019; Flessner et al., 2020). Inclusion of diverse active ingredients with S-metolachlor improved treatment performance. For example, the addition of atrazine in tank-mix with S-metolachlor resulted in control increase to 65% at 7 DAA, which increased to 99% at 35DAA. Corroborating results suggests with POST atrazine application at 280-560 g ai ha^{-1} caused 34-60% hemp visual injury across diverse environments 19 DAA, respectively. Even with initial differences observed at 7 DAA (61-88%) treatments consisting of S-metolachlor + atrazine + mesotrione or S-metolachlor + atrazine + mesotrione + bicyclopyrone resulted in no to minor difference in volunteer hemp control 35 DAA (98-100%). The only exception involves treatment consisting of S-metolachlor + atrazine + glyphosate which resulted in 65% control with no changes over time. The observed reduction of effectiveness may be associated with possible antagonistic interaction among glyphosate and atrazine. Previous studies suggest tank-mix of glyphosate and atrazine can reduce activity because of physical binding of glyphosate to inert components of atrazine formulation (Appleby and Somabhi, 1978). However, this was not reported for application of S-metolachlor and glyphosate. Clewis et al. (2006) findings indicate for comparison made across two glyphosate formulations (isopropylamine- or trimethylsulfonium-salt) increased control with the addition of S-metolachlor across various troublesome weed species including common lambsquarters (Chenopodiaceae, *Chenopodium album* L.), common ragweed

(Asteraceae, *Ambrosia artemisiifolia* L.), Palmer amaranth (Amaranthaceae, *Amaranthus palmeri* S. Watson), and velvetleaf (Malvaceae, *Abutilon theophrasti* Medik.). Incidence of possible antagonistic interaction are supported as well by our findings where application of glyphosate, atrazine, or S-metolachlor + atrazine alone provided about 98% volunteer hemp control at 35 DAA.

Application of acetochlor was formulation dependent where acetochlor (1) resulted in 84% control, while acetochlor (2) in 32% for early evaluation timing. Interestingly, volunteer hemp control increased over time to 96% for acetochlor (2), while for acetochlor (1) remained unchanged. Differences in control may be attributed to used rates 2450 and 4260 g ai ha^{-1} as well as formulation differences having acetochlor (1) and (2) being non-encapsulated versus encapsulated, respectively. In addition, the observed differences in control levels between the two formulations could be due to various factors such as release rate of active ingredient or the presence of additional additives in the formulations, which might influence overall herbicide performance. Regardless of formulation and used rates herbicide treatment consisting of acetochlor + atrazine (1-3) no difference was observed for comparison made within and across evaluation timings with steady control increase from 7 to 28 DAA; with 28 DAA resulting in more volunteer hemp control. This could indicate that the combination of acetochlor and atrazine has a slow, but consistent effect on controlling volunteer hemp growth. The steady increase in control over time might be attributed to the herbicides mode of action by environment interaction, which require time for their full effect to be observed.

Application of mesotrione, isoxaflutole, and tembotrione provided 97, 98, and 45% control at 35 DAA, indicating differences with commonly used corn application rates of 190, 90, and 80 g ai ha^{-1}, respectively. In addition, it is important to note that final performance was based on tank-mixture. For example, isoxaflutole alone at 7 DAA resulted in 46%, while the addition of atrazine, thiencarbazone-methyl, or atrazine + thiencarbazone-methyl increased control up to 63%. At 21 DAA indicates performance of isoxaflutole alone (88%) was not different from treatments containing either atrazine, thiocarbazone-methyl, and atrazine + thiocarbazone-methyl. Findings associated with tembotrione application suggest decreased levels (47%) of volunteer hemp control without major changes for included evaluation timings. Therefore, application in tank-mix with dicamba should be recommended for volunteer hemp control increase. Differential hemp sensitivity associated with application of mesotrione, isoxaflutole, and tembotrione has been previously reported in literature for hemp (Ortmeier-Clarke et al., 2022). As part of the preemergence application of mesotrione (105 g ai ha^{-1}) or isoxaflutole (53 g ai ha^{-1}) there was 91 and 51% visual injury observed 25 DAA, directly indicating separation in hemp sensitivity to different active ingredients within the same herbicide site of action even when different hemp varieties were evaluated (Maxwell, 2016; Mettler, 2021; Ortmeier-Clarke et al., 2022).

Treatments that had the least impact on volunteer hemp control except S-metolachlor include dimethenamid-P, clopyralid, pyroxasulfone, and fluthiacet at 7 DAA. The additional evaluations at 21 and 35 DAA indicate control increase for clopyralid, pyroxasulfone, and fluthiacet. Therefore, at 35 DAA less than 30% volunteer hemp control was only observed with S-metolachlor (17% applied at 1620 g ai ha^{-1}) and dimethenamid-P (27% applied at 1100 g ai ha^{-1}). Findings for dimethenamid-P do not corroborate with literature. Previous research associated with preemergence herbicide application of dimethenamid-P under the greenhouse conditions resulted in 39-49% visual injury with application of 700-840 g ai ha^{-1} at evaluation timing 28DAA (Mettler, 2021; Flessner et al., 2020). Furthermore, our results suggest the addition of saflufenacil with dimethenamid-P increased control to 93%. Similar was observed for clopyralid (43%) where the addition of flumetsulam increased control to 90% with no performance difference from flumetsulam alone. Clopyralid findings corroborate with previous reports indicating relative hemp tolerance (Byrd, 2019; Mettler, 2021; Flessner et al., 2020). However, this was not observed for flumetsulam findings. According to Knezevic et al. (2020) and Cuvaka et al. (2020) only temporary injury was observed (up to 25%) at 21 DAA associated with application of 26 g ai ha^{-1} applied as part of preemergence program. Oppositely, our findings indicate up to 90% injury at 35 DAA with application of 20 g ai ha^{-1} associated with POST application. Therefore, inconsistency in volunteer hemp control may be associated with utilized herbicide rates as well application timing (POST opposed to pre-emergence).

Biomass reduction at North Platte location. The herbicide treatment influenced volunteer hemp biomass reduction ($P<0.0001$) as indicated in Appendix Table 5.1. Findings associated with volunteer hemp biomass reduction for North Platte location are presented in Figure 5.2. From evaluated herbicide treatments 24 out of 39 resulted in volunteer hemp biomass reduction greater than 87% indicating high sensitivity of volunteer hemp to evaluated herbicides commonly used in corn (Appendix Figure 5.1.).

The application of S-metolachlor alone at 1620 g ai ha^{-1} resulted in a 48% biomass reduction. According to Ortmeier-Clarke et al. (2022) effective dose to cause 50 to 90% biomass reduction for preemergence application ranged from 327 to 1737 g ai ha^{-1}, respectively. Therefore, findings like this suggest that volunteer hemp tolerance may increase as volunteer plants establish in the field. The addition of atrazine (1820 g ai ha^{-1}) in the tank-mix with S-metolachlor increased biomass reduction to 92%. Previous findings indicate application of atrazine as part of the preemergence program would require 288 g ai ha^{-1} for 90% biomass reduction (Ortmeier-Clarke et al., 2022). In addition, according to Mettler (2021) application of atrazine either 280 or 560 g ai ha^{-1} even with an increase in visual injury from 34 – 60% there was no impact on plant height. Therefore, S-metolachlor was most effective when used in combination with other herbicides. However, this was not observed for all tank-mixtures. Observations made for volunteer hemp visual control suggests for treatment that included S-metolachlor + atrazine + glyphosate (77%) in tank-mix resulted in a comparably lower biomass reduction than stand-alone application of atrazine (94%), glyphosate (88%), and a combination of S-metolachlor + atrazine (92%). As previously mentioned this finding can be supported with antagonistic interaction of atrazine and glyphosate (Appleby and Somabhi 1978); while not with S-metolachlor (Clewis et al., 2006).

The application of mesotrione alone or S-metolachlor + mesotrione resulted in similar biomass reduction (>90%) even when used with more diversified herbicide treatments consisting of S-metolachlor + atrazine + mesotrione and S-metolachlor + atrazine + mesotrione + bicyclopyrone. Similar change has been observed in acetochlor, where volunteer hemp exhibited high sensitivity regardless of application alone (89%) or in combination with other actives, including atrazine, mesotrione, and clopyralid resulting in 91, 84, and 89% biomass reduction, respectively. Fairly high levels of sensitivity have been previously reported with mesotrione where application of 105 g ai ha^{-1} preemergence or POST resulted in equal or greater than 80% biomass reduction (Mettler, 2021; Ortmeier-Clarke et al., 2022). Therefore, mesotrione and acetochlor, either alone or in combination with other herbicides, are effective in controlling volunteer hemp. The high level of biomass reduction provided by these herbicides, even in more complex mixtures, supports their potential use in integrated weed management strategies.

Application of isoxaflutole alone or in a tank-mix with atrazine, thiocarbazone-methyl, and atrazine + thiocarbazone-methyl resulted in 90-93% biomass reduction. However, an instance where the difference was observed included the assessment of the application of rimsulfuron alone or in combination with isoxaflutole. Addition of isoxaflutole with the application of rimsulfuron benefited treatment performance with increased biomass reduction from 81 to 91%. Previous findings associated with isoxaflutole have been reported to cause severe damage in preemergence and POST applications with response as variety dependent. According to Ortmeier-Clarke et al. (2022) estimated isoxaflutole dose for 90% biomass reduction for preemergence application was 15.1 g ai ha^{-1} while for POST was up to 38.3 g ai ha^{-1} suggesting that hemp tolerance over time may increase. Conversely, findings associated with tembotrione application suggest decreased levels of volunteer hemp biomass reduction; therefore, application along with dicamba for enhancement of volunteer hemp biomass reduction is recommended.

No difference among S-metolachlor, pyroxasulfone, pyroxasulfone + carfentrazone-ethyl, and dimethenamid-P providing less than 57% biomass reduction. As previously mentioned, S-metolachlor performance may be improved by incorporation of various active ingredients in tank-mix. Even with moderate impact of dimethenamid-P on volunteer hemp biomass accumulation the addition of saflufenacil improved biomass reduction from 56 to 94%. At North Platte location application of saflufenacil alone resulted in 89% biomass reduction with no difference when found in tank-mix with glyphosate. From all evaluated herbicide treatments fluthiacet was the only treatment that resulted in only 8% biomass reduction. Therefore, application of fluthiacet alone should not be recommended for early-season POST volunteer hemp control.

Visual control at Columbus location. Volunteer hemp control was influenced by herbicide treatment*time (P<0.0001) interaction (Appendix Table 5.1.). As a result of the complexity associated with herbicide treatment performance over time important trends are discussed (Table 5.4.).

Application of S-metolachlor alone provided 24% control at 7 DAA; however, by 35 DAA it decreased to 7%, suggesting volunteer hemp recovery. Similar findings regarding the low impact of hemp have been previously reported at the North Platte location up to 17% at 35 DAA. Differences among locations can be attributed to the presence taller plants at the Columbus location up 16 cm at application timing. Furthermore, S-metolachlor is labeled for use in corn as part of the pre-plant, at-planting, preemergence, and/or POST program in which greatest phytotoxicity is expected only prior to weeds emergence (Anonymous, 2020). Therefore, limited impact was observed with volunteer hemp associated with POST application. The addition of atrazine in tank-mix with S-metolachlor improved control to 90% resulting in being different from the application of atrazine alone (71% at 35 DAA). This finding suggests that the combination of S-metolachlor and atrazine can provide more effective control of volunteer hemp than atrazine used independently. As noted for the North Platte location, a tank-mix of S-metolachlor + atrazine + glyphosate resulted in significantly lower control with more differences observed for later evaluation timing (<20%). This finding indicates that the addition of atrazine to the tank-mix might have an antagonistic effect on the performance of glyphosate in controlling volunteer hemp. Therefore, using this treatment combination for volunteer hemp control may not be suitable.

The inclusion of more diversified herbicide treatments consisting of S-metolachlor + atrazine + mesotrione or S-metolachlor + atrazine + mesotrione + bicyclopyrone resulted in volunteer hemp control increase for 21 and 35 DAA (97 - 100%). Similarly, high levels of sensitivity were observed for glyphosate and saflufenacil application alone or in a tank-mix with a control of 91-100%. Application of saflufenacil at 25 g ai ha^{-1} previously was shown to be safe if used as part of the preemergence program (Cuvaka et al., 2020). However, our results indicate application after hemp emergence at 70 g ai ha^{-1} may cause greater than 90% control. These results indicate that the rate of saflufenacil application is an important factor influencing its efficacy in controlling volunteer hemp. When applied at a higher rate during the POST stage, saflufenacil appears to be more effective in controlling volunteer hemp indicating that saflufenacil could be a valuable herbicide for spring-burndown.

The performance of treatments containing acetochlor was highly influenced by both formulation and the selection of tank-mix partners. Comparing the application of acetochlor (1) and acetochlor (2) resulted in 37% and 62% control at 35 DAA, respectively. The increased control for acetochlor (2) could be attributed to the higher application rate of 4260 g ai ha^{-1} compared to 2450 g ai ha^{-1} for acetochlor (1). This observation is consistent with previous studies where an increase in acetochlor rate from 1050 to 2100 g ai ha^{-1} led to an increase in injury from 16% to 31% (Mettler, 2021). The addition of atrazine, mesotrione, and mesotrione + clopyralid in tank-mix with acetochlor for most evaluated treatments resulted in control increase to greater than 98%. The only exception of acetochlor + atrazine (1) that had reduced control to 83% control at 35 DAA. Control decrease can be associated with use of encapsulated formulation of acetochlor which limited volunteer hemp control at Columbus location. However, available literature findings on encapsulated acetochlor vary across studies. Byrd (2019) and Flessner et al. (2020) found that encapsulated acetochlor applied at 3400 g ai ha^{-1} resulted in 28% injury 28DAA without any height reduction. However, Ortmeier-Clarke et al. (2022) findings suggest application of 87 g ai ha^{-1} caused considerable injury followed with greater than 75% biomass reduction for studies conducted in the greenhouse. These contrasting results may be attributed to various factors, such as differences in environmental conditions, experimental setup, or plant sensitivity (associated with different hemp varieties) between field and greenhouse studies. Furthermore, greenhouse studies can sometimes produce different outcomes compared to field studies, as conditions tend to be more controlled and may not fully represent the complex interactions that occur in the field environment.

Application of mesotrione, isoxaflutole, and tembotrione resulted in 93, 69, and 32% volunteer hemp control at 35 DAA, indicating differences among active ingredients tested. In general, as observed at North Platte location, volunteer hemp exhibited the greatest sensitivity to mesotrione. Regardless of application mesotrione alone or in tank-mix with other active ingredients performance at 35 DAA was greater than 93%. Application of isoxaflutole alone at 35 DAA resulted in 69%, while with the application of atrazine or thiencarbazone-methyl in tank-mix increased control to 100%. Conversely, there was no enhancement in volunteer hemp control with application of isoxaflutole + thiencarbazone-methyl + atrazine resulting in only 25% control at 35 DAA at Columbus location. Those findings indicate that application of product at later stage may alter hemp tolerance 12-16 cm, as opposed to 8-12 for Columbus to North Platte location, respectively. Similarly, to observations made at North Platte location findings associated with tembotrione application suggests low levels (32%) of volunteer hemp control. It has been noted that the addition of dicamba with application can improve efficacy to 82%, with no performance difference observed from stand-alone application of dicamba.

Across all treatments, including S-metolachlor, dimethenamid-P, clopyralid, and fluthiacet, there was generally low volunteer hemp control (less than 16%) observed at

35 days after application. Even when the level of volunteer hemp control was increased to 21%, applying pyroxasulfone alone was no more effective than applying a combination of pyroxasulfone and fluthiacet (22%) or fluthiacet alone (13%). Based on these results, it is not recommended to use these products alone for early-season volunteer hemp control. Instead, employing them together as a tank-mix partner with other more effective herbicides to achieve superior control is recommended. Tank-mixing different herbicides can offer several advantages, such as improved weed control, reduced chances of developing herbicide resistance, and a broader spectrum of control for different weed species.

Biomass reduction at Columbus location. The herbicide treatment influenced volunteer hemp biomass reduction ($P<0.0001$) as indicated in Appendix Table 5.1. The results on volunteer hemp biomass reductions for Columbus locations are presented in Figure 5.3. From all evaluated herbicide treatments, 15 out of 39 tested treatments resulted in greater than 89% of volunteer hemp biomass reduction.

The application of glyphosate alone or in combination with saflufenacil resulted in 100% volunteer hemp biomass reduction. The application of S-metolachlor alone resulted in only 13% biomass reduction. S-metolachlor has been used in China as viable option as preemergence weed control program in hemp fields at suggested rates of 3 L ha^{-1} (using 65% emulsion) (Amaducci et al., 2015). Therefore, increased hemp tolerance may be an underlying reason for the limited effect on volunteer hemp. As observed at North Platte location lower biomass reduction was observed among treatment combination that included S-metolachlor + atrazine + glyphosate (28%) being no different from application of S-metolachlor. General findings associated with the application of acetochlor on volunteer hemp biomass reduction were formulation dependent. Acetochlor (1) resulted in a 19% biomass reduction, while acetochlor (2) led to a more substantial 42% reduction. The addition of other active ingredients, such as atrazine, mesotrione, or a combination of mesotrione and clopyralid, to the tank-mix significantly improved volunteer hemp control, with reductions ranging from 70% to 100%. Formulation-dependent findings in acetochlor application highlight the importance of selecting the appropriate herbicide combinations for effective volunteer hemp control. Furthermore, these results suggest that the use of appropriate acetochlor formulations in combination with other herbicides can lead to more effective volunteer hemp control.

Comparison among the same site of action (SOA 27) highlights the difference among active ingredients tested for volunteer hemp control. Across active ingredients, volunteer hemp biomass reduction from herbicides (from highest to lowest): mesotrione>isoxaflutole>tembotrione. Those findings corroborate with previous studies (Ortmeier-Clarke et al., 2022). In general, application of mesotrione alone resulted in biomass reduction of 93%. Furthermore, the addition of either S-metolachlor and atrazine + mesotrione or atrazine + mesotrione + bicyclopyrone resulted in biomass reduction increase to 96 – 100%. More notable changes in performance were observed with isoxaflutole. The application of isoxaflutole alone resulted in 52% biomass reduction while the addition of either atrazine or thiocarbazone-methyl increased efficacy to about 98%. However, this was not determined in case with three-way tank-mix that included isoxaflutole + thiencarbazone-methyl + atrazine or tank-mixture with rimsulfuron. This unexpected decrease in control when using all three active ingredients together could be due to a few factors, such as antagonistic interactions among the herbicides, application rates and timing, or site-specific environmental conditions. Moreover, earlier research has indicated that the combination of an herbicide HPPD inhibitor and an ALS inhibitor can have antagonistic effects, potentially due to a decrease in the absorption or movement of the herbicide (Jhala et al., 2022; Kaastra et al., 2008; Schuster er al., 2007). Lastly, tembotrione was only tested with dicamba mixture. General findings suggest that there was no difference in treatment performance among mixtures that included dicamba application alone or dicamba + tembotrione; however, those tank-mixtures outperformed tembotrione application resulting in more than 20% biomass reduction increase.

S-metolachlor, pyroxasulfone, fluthiacet, and pyroxasulfone + fluthiacet resulted in the least volunteer hemp biomass reduction less than 13%. Therefore, the application of those active ingredients should not be recommended for volunteer hemp control. Like observations for the North Platte location, S-metolachlor performance can be improved by incorporating various active ingredients. In addition, even with observed 20 - 30% of volunteer hemp biomass reduction with clopyralid and dimethenamid-P the addition of either flumetsulam or saflufenacil may help with volunteer hemp control.

4.4. & 5.4. Conclusions

Overall findings contribute valuable information regarding the effectiveness of various herbicide programs currently available in soybean and corn that can help with future management decisions and development of well-coordinated volunteer hemp control programs. The results demonstrate the relatively high effectiveness of various herbicide programs currently available in soybean (2,4-D-tolerant) and corn under field conditions. Upon assessment of the herbicide treatments in soybeans, it was found that 55% of treatments led to a biomass reduction exceeding 82%. Regarding volunteer hemp control, the findings demonstrated that glyphosate application, whether used alone or in combination with other herbicides, was more effective and faster than 2,4-D. Moreover, adding glyphosate combined with diverse herbicide programs can improve their overall performance, resulting in the more effective control of volunteer hemp. An approach that includes multiple active ingredients in a tank-mix can be used effectively for volunteer hemp control in soybean without compromising efficacy. Conversely, the response of volunteer hemp to the herbicide treatments evaluated in corn was contingent upon the location. In North Platte, 62% of the treatments resulted in a biomass reduction surpassing 87%, while in Columbus, only 38% of evaluated treatments exhibited a similar effect. In summary, for both North Platte and Columbus locations, observations made regarding herbicide efficacy in volunteer hemp control include that glyphosate was highly effective in North Platte, providing 100% biomass reduction when used alone or combined with saflufenacil. Oppositely, findings for S-metolachlor indicate low effectiveness when used alone (13% reduction in North Platte and 48% reduction in Columbus), but its efficacy improved significantly when combined with other herbicides like atrazine. Furthermore, acetochlor efficacy was formulation-dependent, with improved volunteer hemp control when combined with other herbicides, such as atrazine, mesotrione, or mesotrione and clopyralid, resulting in 70-100% biomass reduction in North Platte. Mesotrione, isoxaflutole, and tembotrione showed varying levels of efficacy in reducing volunteer hemp biomass. Among tested herbicides from SOA 27 mesotrione was the most effective active ingredient, both when used alone or in combinations across both locations. Conversely, in Columbus, the use of mesotrione and acetochlor, either alone or in combination with other herbicides, effectively controlled volunteer hemp, with biomass reductions over 80%, supporting their potential use in integrated weed management strategies. Integration of various active ingredients is critical because it lowers the chance of developing resistance and expands the range of weeds (including volunteer hemp) that can be controlled at different stages. Although all herbicide treatments were applied after the emergence of volunteer hemp, the differences in control levels between locations may be attributed to variations in soil type (clay and organic matter content), as well as local environmental conditions, such as temperature, rainfall, soil moisture levels at the time of application, and other factors that can affect herbicide performance. It is important to note that the selection of herbicide formulation, the amount of product applied, and the combination of tank-mix can significantly impact the effectiveness of herbicide options for controlling volunteer hemp. Additionally, factors such as state, region, and site-specific conditions may also limit the performance of herbicides. Therefore, careful consideration and proper selection are important for successful volunteer hemp control. S-metolachlor, fluthiacet, and pyroxasulfone were treatments that were the least harmful active ingredients on volunteer hemp across all examined treatments. However, the final effects on volunteer hemp control and biomass reduction were environment dependent. Even though findings indicated increased volunteer hemp tolerance to those active ingredients, to be considered as potential candidates for use in hemp, further assessment is required to generate data points regarding the residue and crop safety data when used in-crop situations across diverse hemp cultivars. Further research regarding the volunteer hemp control should focus on optimizing application rates, timings, and environmental conditions for these herbicides, as well as assessing the potential for developing herbicide resistance in volunteer hemp populations. Additionally, exploring effectiveness of alternative weed management approaches, such as mechanical (e.g., roller crimpers, flame weeding, etc.) control methods, can help ensure long-term control success.

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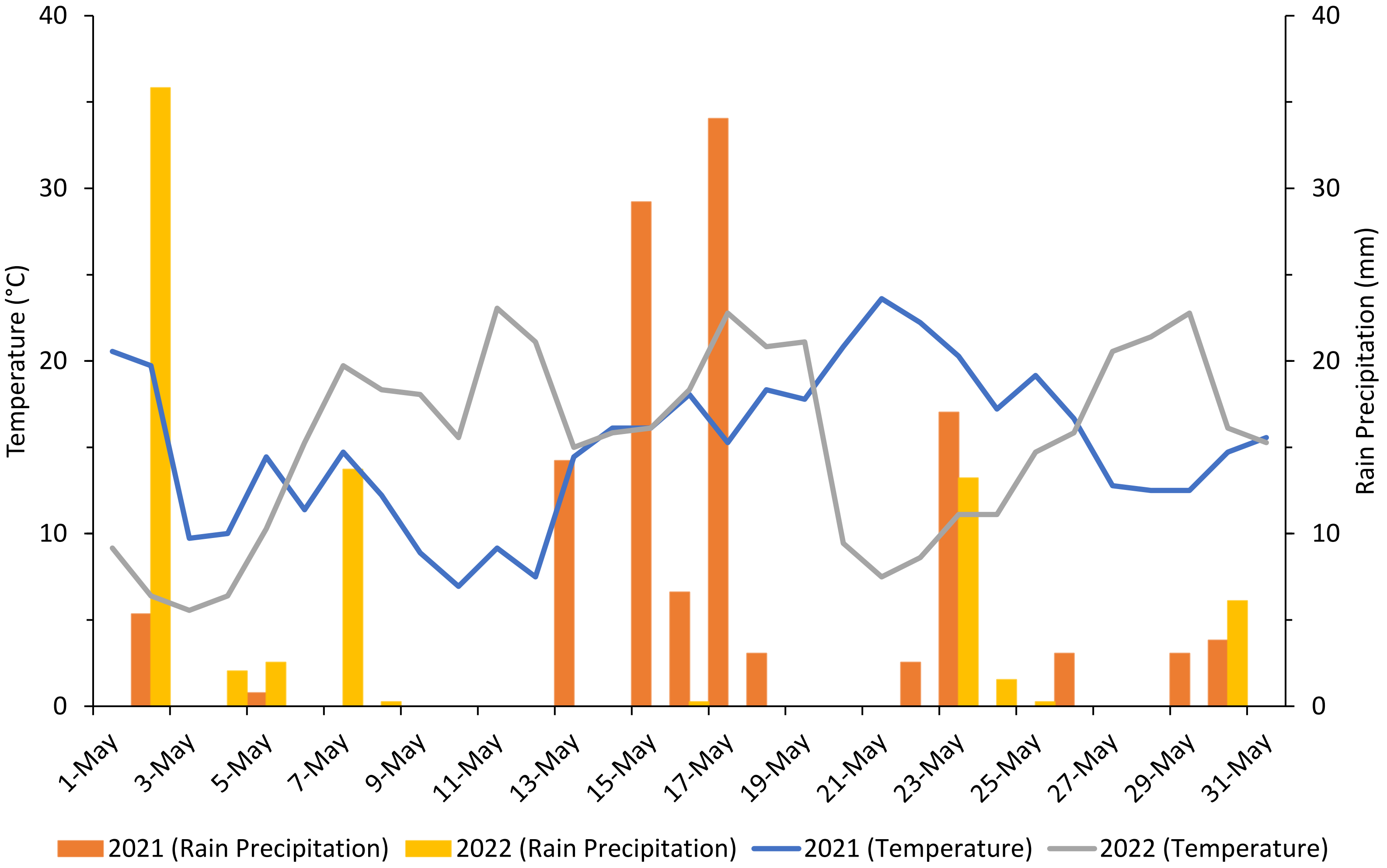

Figure 4.1. Temperature and rain precipitation for May North Platte, NE, in 2021 and 2022. Data obtained from National Weather Service (https://www.weather.gov/).

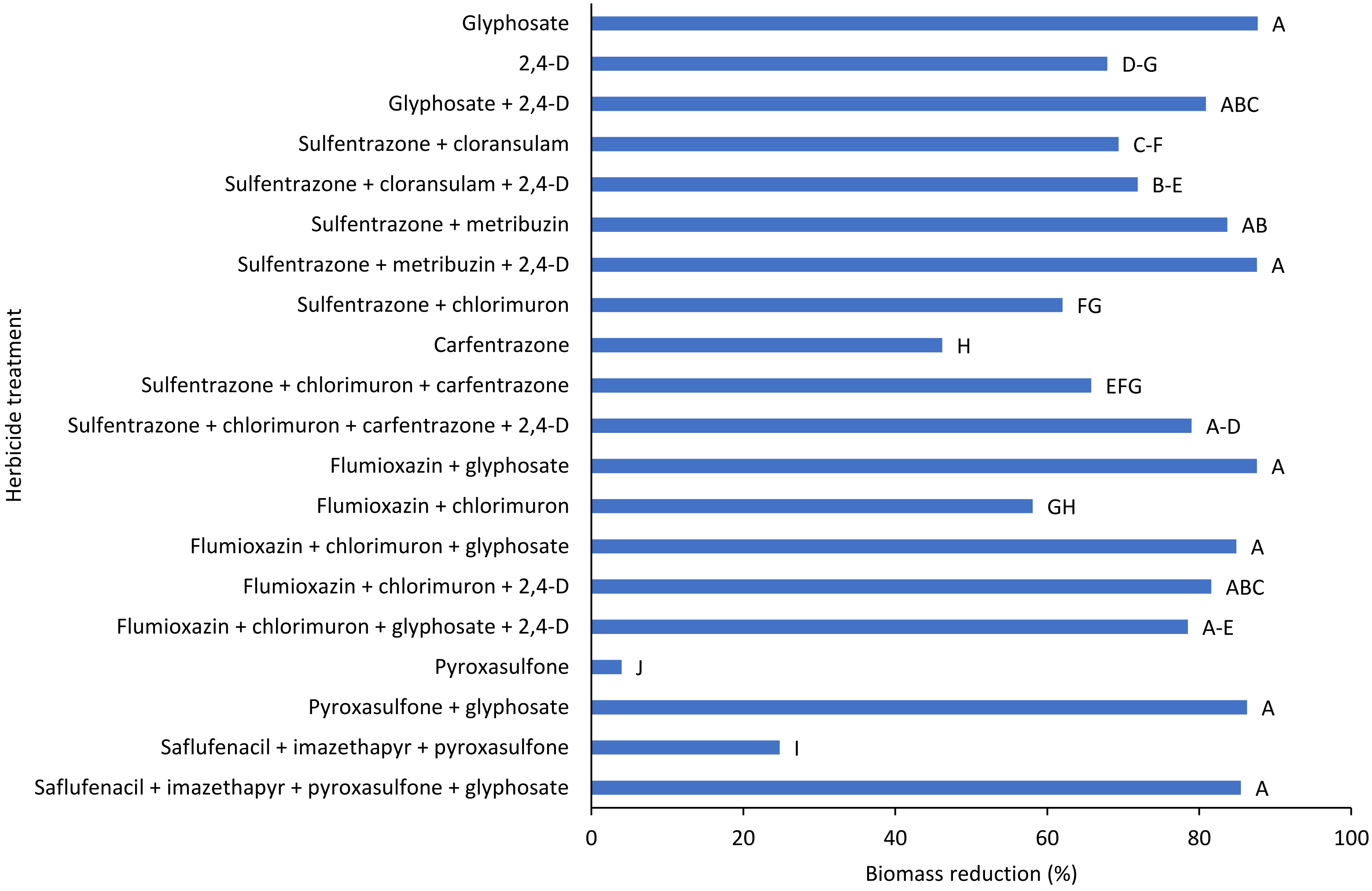

Figure 4.2. Effects of herbicide treatments on volunteer hemp biomass reduction in soybean field experiment conducted in North Platte, Nebraska in 2021 and 2022. Herbicide treatments with the same letter(s) do not differ from each other using Tukey-Kramer's test. Trade name and active ingredient rate of all herbicide treatments are displayed in Table 4.2.

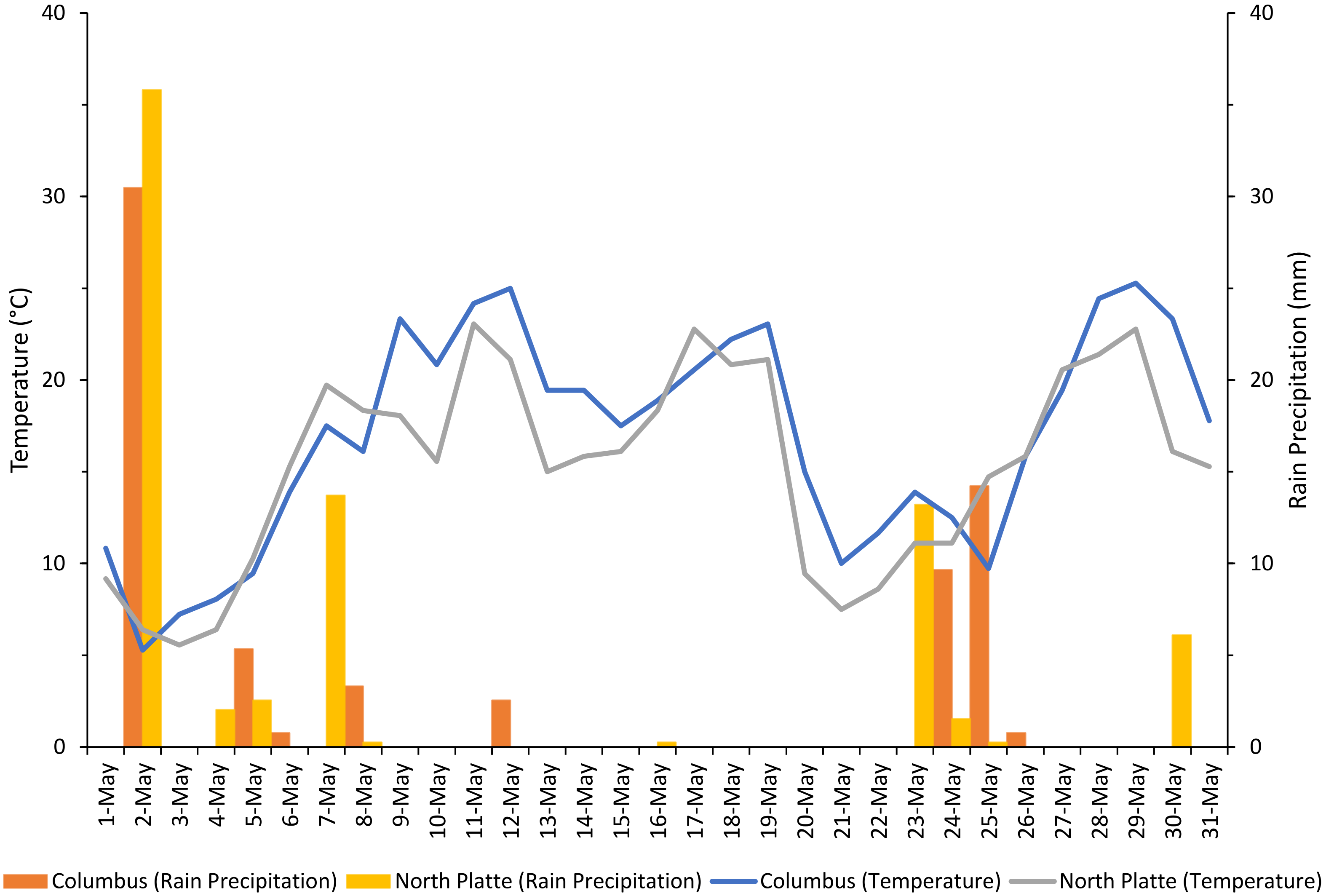

Figure 5.1. Temperature and rain precipitation for May 2022 for North Platte and Columbus, Nebraska. Data obtained from National Weather Service (https://www.weather.gov/).

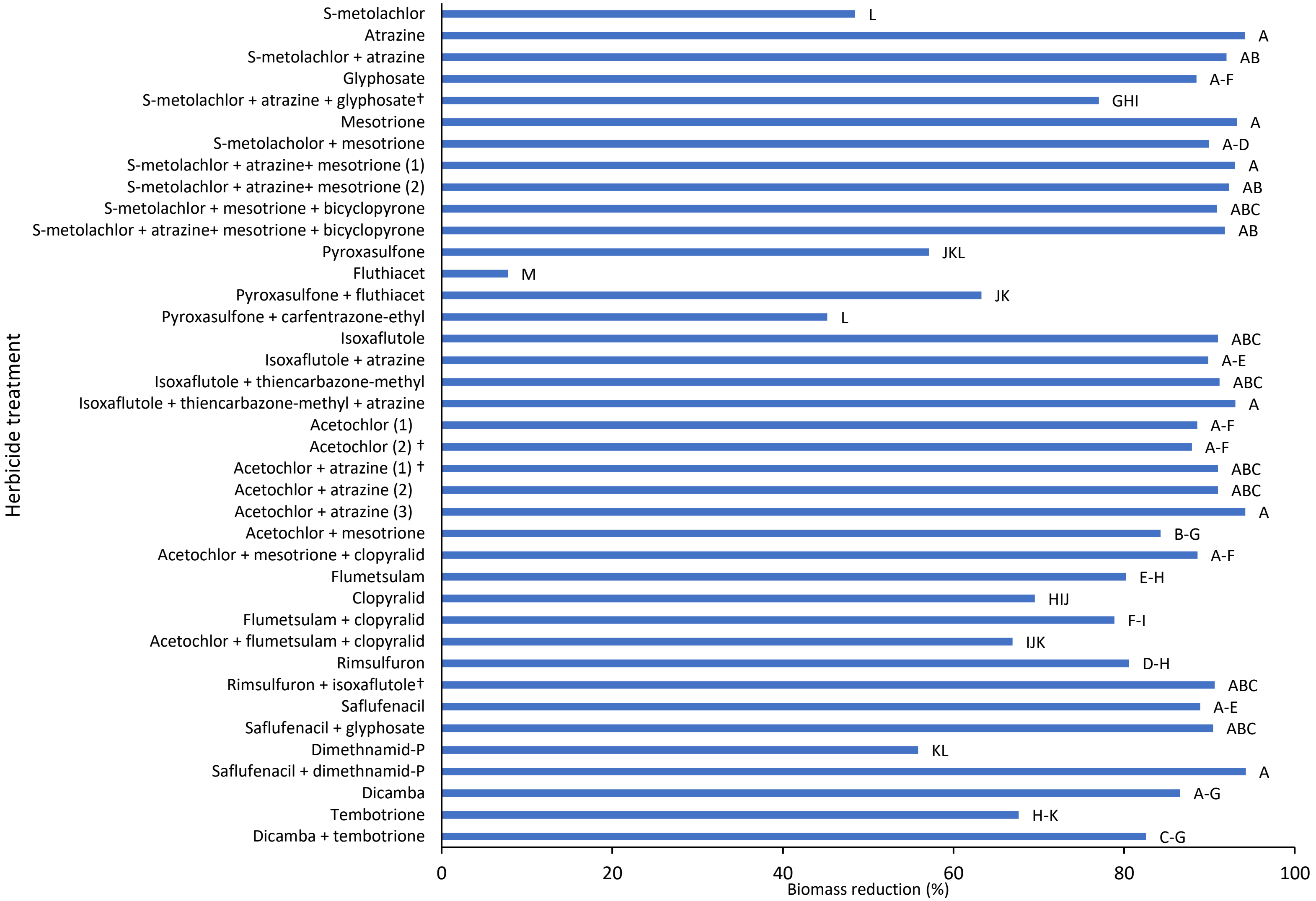

Figure 5.2. Effects of herbicide treatments on volunteer hemp biomass reduction in corn field experiment conducted in North Platte, Nebraska. Herbicide treatments with the same letter(s) do not differ from each other using Tukey-Kramer's test. Trade name and active ingredient rate of all herbicide treatments are displayed in Table 5.2. († Herbicide tank-mix otherwise premixes; Number in parentheses indicates formulation difference).

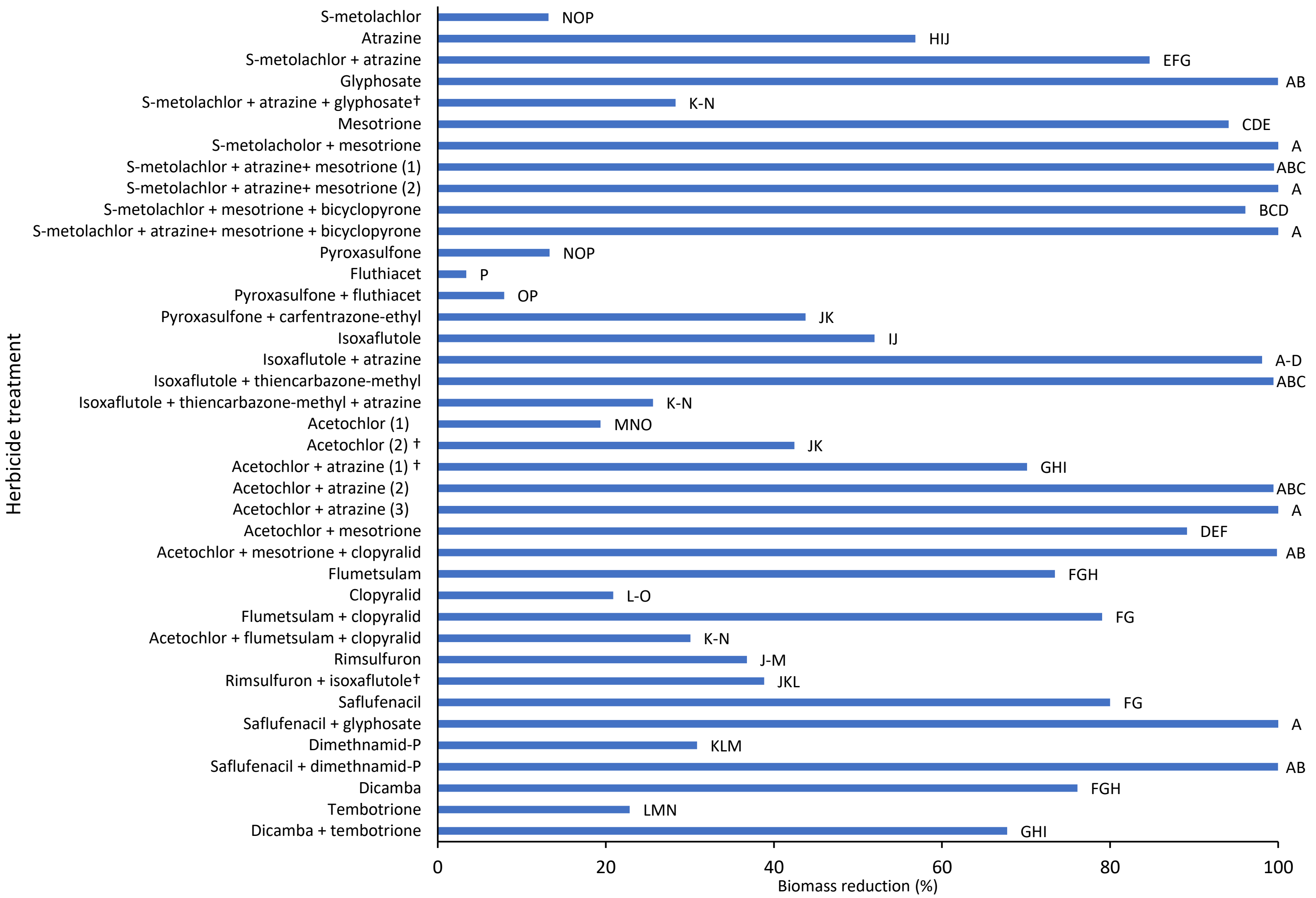

Figure 5.3. Effects of herbicide treatments on volunteer hemp biomass reduction in corn field experiment conducted in Columbus, Nebraska. Herbicide treatments with the same letter(s) do not differ from each other using Tukey-Kramer's test. Trade name and active ingredient rate of all herbicide treatments are displayed in Table 5.2. († Herbicide tank-mix otherwise premixes; Number in parentheses indicates formulation difference).

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May 2021 June 2021

Appendix Figure 4.1. Volunteer hemp tolerance to selected early-season soybean herbicides in North Platte, Nebraska, 2021. Caption: Left: Field layout before herbicide application. Right: Aerial view 32 days post-application. The red rectangle highlights the initial range of applied herbicide treatments.

May 2022

June 2022

Appendix Figure 5.1. Volunteer hemp tolerance to selected early-season corn herbicides, North Platte, Nebraska, 2022. Caption: Left: Field layout soon after application herbicide application. Right: Aerial view 35 days post-application. The red rectangle highlights the initial range of applied herbicide treatments.

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Table 4.1. Monthly rain precipitation and temperature at North Platte, NE, in 2021 and 2022[a].

Month	Rainfall		Temperature	
	2021	2022	2021	2022
	mm		°C	
April	38.6	36.3	8.3	9.6
May[b]	122.7	75.7	15.2	15.3
June	40.1	10.9	23.0	23.3
July	66.0	102.4	24.7	26.1
Total[c]	267.5	225.3	71.1	74.2

[a] Data obtained from National Weather Service (https://www.weather.gov/).

[b] Herbicide Application.

[c] Totals for the duration of study.

Table 4.2. Trade names, herbicide treatments, site of action group, and rate of products used in volunteer hemp control study in soybean field experiment conducted in North Platte, Nebraska in 2021 and 2022.

Trade Name[#]	Herbicide treatment	Site of Action[§]	Rate
		#	kg* or g ai ha^{-1}
None	Non-treated control	n/a	n/a
Roundup PowerMAX[a] (RPM)	Glyphosate	9	1.54*
Enlist One[b] (EO)	2,4-D	4	1.06*
RPM[a] + EO[b]	Glyphosate + 2,4-D	9 + 4	1.54* + 1.06*
Authority First[c]	Sulfentrazone + cloransulam	14 + 2	220 + 30
Authority First[c] + EO[b]	Sulfentrazone + cloransulam + 2,4-D	14 + 2 + 4	220 + 30 + 1.06*
Authority MTZ[c]	Sulfentrazone + metribuzin	14 + 5	180 + 260
Authority MTZ[c] + EO[b]	Sulfentrazone + metribuzin + 2,4-D	14 + 5 + 4	180 + 260 + 1.06*
Authority Maxx[c]	Sulfentrazone + chlorimuron	14 + 2	177 + 10
Aim[c]	Carfentrazone	14	20
Authority Maxx[c] + Aim[c]	Sulfentrazone + chlorimuron + carfentrazone	14 + 2 + 14	170 + 10 + 20
Authority Maxx[c] + Aim[c] + EO[b]	Sulfentrazone + chlorimuron + carfentrazone + 2,4-D	14 + 2 + 14 + 4	170 + 10 + 20 + 1.06*
Valor SX[d] + RPM[a]	Flumioxazin + glyphosate	14 + 9	90 + 1.54*
Valor XLT[d]	Flumioxazin + chlorimuron	14 + 14	80 + 30
Valor XLT[d] + RPM[a]	Flumioxazin + chlorimuron + glyphosate	14 + 2 + 9	80 + 30 + 1.54*
Valor XLT[d] + EO[b]	Flumioxazin + chlorimuron + 2,4-D	14 + 2 + 4	80 + 30 + 1.06*
Valor XLT[d] + RPM[a] + EO[b]	Flumioxazin + chlorimuron + glyphosate + 2,4-D	14 + 2 + 9 + 4	80 + 30 + 1.54* + 1.06*
Zidua SC[e]	Pyroxasulfone	15	150
Zidua SC[e] + RPM[a]	Pyroxasulfone + glyphosate	15 + 9	150 + 1.54*
Zidua PRO[e]	Saflufenacil + imazethapyr + pyroxasulfone	14 + 2 + 15	21 + 58 + 100
Zidua PRO[e] + RPM[a]	Saflufenacil + imazethapyr + pyroxasulfone + glyphosate	14 + 2 + 15 + 9	21 + 58 + 100 + 1.54*

[#] Product manufacturer information denoted with different letters. [a] Bayer CropScience LP, St. Louis, MO; [b] Corteva AgroSciences, Indianapolis, IN; [c] FMC Corporation, Philadelphia, PA; [d] Valent U.S.A. Corporation, Walnut Creek, CA; [e] BASF Corporation, Research Triangle Park, NC.

[§] SOA, herbicide site of action group according to Weed Science Society of America.

Table 4.3. Effects of herbicide treatments on volunteer hemp control in soybean field experiment conducted in North Platte, Nebraska in 2021 and 2022.

Herbicide treatment	Visual Control[a]								
	7 DAA			14 DAA			28 DAA		
				%					
Non-treated control[b]	0			0			0		
Glyphosate	90	b	AB	98	a	AB	98	a	ABC
2,4-D	73	b	DE	79	ab	G	86	a	GHI
Glyphosate + 2,4-D	92	a	AB	94	a	A-D	96	a	B-F
Sulfentrazone + cloransulam	47	b	F	65	a	GH	75	a	I
Sulfentrazone + cloransulam + 2,4-D	72	b	DE	85	a	EF	92	a	D-G
Sulfentrazone + metribuzin	72	b	DE	84	ab	EF	91	a	EFG
Sulfentrazone + metribuzin + 2,4-D	86	a	ABC	91	a	B-E	92	a	EFG
Sulfentrazone + chlorimuron	54	b	F	65	b	GH	79	a	HI
Carfentrazone	52	a	F	56	a	H	55	a	J
Sulfentrazone + chlorimuron + carfentrazone	59	b	EF	77	a	FG	86	a	GH
Sulfentrazone + chlorimuron + carfentrazone + 2,4-D	82	b	BCD	90	ab	CDE	96	a	B-E
Flumioxazin + glyphosate	89	a	AB	94	a	A-D	96	a	B-F
Flumioxazin + chlorimuron	45	b	F	57	b	H	88	a	FGH
Flumioxazin + chlorimuron + glyphosate	91	b	AB	98	a	A	100	a	A
Flumioxazin + chlorimuron + 2,4-D	76	b	CD	87	ab	DEF	94	a	C-G
Flumioxazin + chlorimuron + glyphosate + 2,4-D	93	b	A	96	b	ABC	100	a	A
Pyroxasulfone	7	a	G	10	a	I	15	a	L
Pyroxasulfone + glyphosate	88	b	AB	96	a	ABC	99	a	AB
Saflufenacil + imazethapyr + pyroxasulfone	51	a	F	52	a	H	33	b	K
Saflufenacil + imazethapyr + pyroxasulfone + glyphosate	90	b	AB	94	ab	A-D	98	a	A-D

[a] Visual control in days after application (DAA). Means within columns (uppercase) and within rows (lowercase) with no common letter(s) are significantly different according to Tukey-Kramer's test at $P < 0.05$.

[b] The percent control (0%) data of non-treated control were not included in analysis.

Trade name and active ingredient rate of all herbicide treatments are displayed in Table 4.2.

Table 5.1. Monthly rain precipitation and temperature for North Platte and Columbus, NE, in 2022[a].

Month	Rainfall		Temperature	
	North Platte	Columbus	North Platte	Columbus
	mm		°C	
April	36.3	72.6	9.6	9.4
May[b]	75.7	67.1	15.3	16.8
June	10.9	38.6	23.3	23.8
July	102.4	62.0	26.1	25.1
Total[c]	225.3	240.3	74.2	75.1

[a] Data obtained from National Weather Service (https://www.weather.gov/).

[b] Herbicide Application.

[c] Totals for the duration of study.

Table 5.2. Trade names, treatments, site of action group, and rate of products used in volunteer hemp control study in corn field experiment conducted in North Platte and Columbus, Nebraska in 2022.

Trade name[#]	Herbicide treatment[†, ‡]	Site of Action[§]	Rate kg* or g ai ha^{-1}
Dual II Magnum[a] (DM)	S-metolachlor	15	1.62*
Aatrex 4L[a] (A)	Atrazine	5	1.20*
Bicep II Magnum FC[a]	S-metolachlor + atrazine	15 + 5	1.41* + 1.82*
Buccaner Plus[b] (BP)	Glyphosate	9	840
DM + A + BP[s1]	S-metolachlor + atrazine + glyphosate[†]	15 + 5 + 9	1.41* + 1.82* + 840
Callisto[a]	Mesotrione	27	190
Zemax[a]	S-metolacholor + mesotrione	27 + 15	190 + 1.87*
Lexar EZ[a]	S-metolachlor + atrazine+ mesotrione (1)	14 + 15 + 27	1.46* + 1.46* + 190
Lumax EZ[a]	S-metolachlor + atrazine+ mesotrione (2)	14 + 15 + 27	1.88* + 710 + 190
Acuron Flexi[a]	S-metolachlor + mesotrione + bicyclopyrone	15 + 27 + 27	2.00* + 220 + 60
Accuron[a]	S-metolachlor + atrazine+ mesotrione + bicyclopyrone	15 + 5 + 27 + 27	1.50* + 700 + 170 + 40
Zidua WG[c]	Pyroxasulfone	15	180
Cadet[d]	Fluthiacet	14	10
Anthem Maxx[d]	Pyroxasulfone + fluthiacet	15 + 14	180 + 10
Anthem Flex[d]	Pyroxasulfone + carfentrazone-ethyl	15 + 14	160 + 10
Balance Flex[e] (BF)	Isoxaflutole	27	90
Balance Flex[e] + Aatrex 4L[a]	Isoxaflutole + atrazine	27 + 5	90 + 1.31*
Corvus[e]	Isoxaflutole + thiencarbazone-methyl	27 + 2	90 + 40
Corvus[e] + Aatrex 4L[a]	Isoxaflutole + thiencarbazone-methyl + atrazine	27 + 2 + 5	90 + 40 + 1.12*
Surpass NXT[f]	Acetochlor (1)	15	2.45*
Warrant[e, s2, #]	Acetochlor (2) [†, #]	15	4.26*
Warrant[e] + Aatrex 4L[a, s3, #]	Acetochlor + atrazine (1) [†, #]	15 + 5	2.65* + 1.31*
Harness Xtra[e]	Acetochlor + atrazine (2)	15 + 5	2.89* + 1.14*
Keystone NXT[f]	Acetochlor + atrazine (3)	15 + 5	2.43* + 1.96*
Harness Max[e]	Acetochlor + mesotrione	15 + 27	2.47* + 230
Resicore[f]	Acetochlor + mesotrione + clopyralid	15 + 27 + 4	1.96 + 210 + 130
Python WDG[f]	Flumetsulam	2	60
Stinger[f]	Clopyralid	4	20
Hornet WDG[f]	Flumetsulam + clopyralid	2 + 4	60 + 20
SureStart II[f]	Acetochlor + flumetsulam + clopyralid	15 + 2 + 4	920 + 30 + 90
Resolve SG [g] (R)	Rimsulfuron	2	3
R + BF [s4]	Rimsulfuron + isoxaflutole[†]	2 + 27	3 + 4
Sharpen[c]	Saflufenacil	14	70
Sharpen[c] + BP	Saflufenacil + glyphosate	14 + 9	70 + 840
Outlook[c]	Dimethnamid-P	15	1.10*
Verdict[c]	Saflufenacil + dimethnamid-P	14 + 15	70 + 660
DiFlexx[e]	Dicamba	4	520
Laudis[e]	Tembotrione	27	80
DiFlexx DUO[e]	Dicamba + tembotrione	4 + 27	520 + 80

Product manufacturer information denoted with different letters. [a] Syngenta Crop Protection LLC, Greensboro, NC; [b] Tenkoz Inc., Alpharetta, GA; [c] BASF Corporation, Research Triangle Park, NC; [d] FMC Corporation, Philadelphia, PA; [e] Bayer CropScience LP, St. Louis, MO; [f] Corteva AgroSciences, Indianapolis, IN; [g] DuPont, Wilmington, DE.

† Herbicide tank-mix otherwise premixes; ‡ Number in parentheses indicates formulation difference; # Encapsulated formulation.

S [1-4] Substitutes due to lack of formulated products; [1] Expert[a] (S-metolachlor + atrazine + glyphosate, 1.41* + 1.82* + 840), [2] Degree[e] (Acetochlor, 4.26*), [3] DegreeXtra[e] (Acetochlor + atrazine, 2.65* + 1.31*), and [4] Prequel[g] (Rimsulfuron + isoxaflutole, 3 + 4). All rates in kg* or g ai ha^{-1} adjusted accordingly to recommended rated of formulated products.

§ SOA, herbicide site of action group according to Weed Science Society of America.

Table 5.3. Effects of herbicide treatments on volunteer hemp control in corn field experiment conducted in North Platte, Nebraska.

Herbicide treatment[†, ‡]	Visual Control[a]								
	7 DAA			21 DAA			35 DAA		
	%								
None[b]	0			0			0		
S-metolachlor	20	a	O	17	a	M	17	a	R
Atrazine	58	c	EFG	89	b	D-F	98	a	B-E
S-metolachlor + atrazine	65	c	CDE	93	b	A-F	99	a	A-D
Glyphosate	85	b	A	91	b	B-F	97	a	D-H
S-metolachlor + atrazine + glyphosate[†]	68	a	CDE	65	a	J	64	a	N
Mesotrione	65	c	CDE	92	b	B-F	97	a	D-G
S-metolachlor + mesotrione	88	c	A	98	b	A	100	a	A
S-metolachlor + atrazine+ mesotrione (1)	75	c	BC	96	b	ABC	100	a	ABC
S-metolachlor + atrazine+ mesotrione (2)	70	c	CD	95	b	A-D	98	a	C-F
S-metolachlor + mesotrione + bicyclopyrone	61	c	DEF	92	b	B-F	99	a	A-E
S-metolachlor + atrazine+ mesotrione + bicyclopyrone	70	c	CD	97	b	AB	100	a	ABC
Pyroxasulfone	28	b	L-O	43	a	K	35	ab	OPQ
Fluthiacet	18	b	O	21	ab	M	32	a	OPQ
Pyroxasulfone + fluthiacet	24	b	MNO	48	a	K	26	b	QR
Pyroxasulfone + carfentrazone-ethyl	42	a	H-K	40	a	KL	31	a	PQ
Isoxaflutole	46	c	G-J	88	b	EFG	98	a	B-E
Isoxaflutole + atrazine	66	c	CDE	94	b	A-E	100	a	AB
Isoxaflutole + thiencarbazone-methyl	60	c	DEF	86	b	FGH	100	a	A
Isoxaflutole + thiencarbazone-methyl + atrazine	63	c	DEF	90	b	C-F	100	a	A
Acetochlor (1)	84	a	AB	80	a	GHI	75	a	MN
Acetochlor (2) [†, #]	32	c	K-N	75	b	IJ	96	a	D-H
Acetochlor + atrazine (1) [†, #]	61	c	DEF	90	b	C-F	100	a	ABC
Acetochlor + atrazine (2)	65	c	CDE	96	b	ABC	100	a	A
Acetochlor + atrazine (3)	70	c	CD	95	b	A-E	100	a	A
Acetochlor + mesotrione	62	c	DEF	75	b	IJ	92	a	G-L
Acetochlor + mesotrione + clopyralid	59	b	DEF	86	a	FGH	89	a	KL
Flumetsulam	34	c	K-N	68	b	J	90	a	JKL
Clopyralid	23	b	NO	40	a	KL	43	a	OP
Flumetsulam + clopyralid	35	c	J-M	70	b	IJ	90	a	I-L
Acetochlor + flumetsulam + clopyralid	38	a	JKL	47	a	K	38	a	OPQ
Rimsulfuron	40	c	I-L	65	b	J	87	a	KL
Rimsulfuron + isoxaflutole[†]	32	c	K-N	68	b	J	85	a	LM
Saflufenacil	35	c	J-M	68	b	J	90	a	I-L
Saflufenacil + glyphosate	91	a	A	96	a	ABC	96	a	D-H
Dimethnamid-P	19	a	O	28	a	LM	27	a	R
Saflufenacil + dimethnamid-P	51	b	F-I	90	a	B-F	93	a	F-K
Dicamba	53	c	FGH	74	b	IJ	92	a	H-L
Tembotrione	41	a	H-K	47	a	K	45	a	O
Dicamba + tembotrione	53	c	FGH	79	b	HI	90	a	I-L

[†] Herbicide tank-mix otherwise premixes; [‡] Number in parentheses indicates formulation difference; [#] Encapsulated formulation.

[a] Visual control in days after application (DAA). Means within columns (uppercase) and within rows (lowercase) with no common letter(s) are significantly different according to Tukey-Kramer's test at $P < 0.05$.

[b] The percent control (0%) data of nontreated control were not included in analysis.

Trade name and active ingredient rate of all herbicide treatments are displayed in Table 5.2.

Table 5.4. Effects of herbicide treatments on volunteer hemp control in corn field experiment conducted in Columbus, Nebraska.

Herbicide treatment[†, ‡]	Visual Control[a]								
	7 DAA			21 DAA			35 DAA		
	%								
None[b]	0			0			0		
S-metolachlor	24	a	NOP	9	b	QR	7	b	OP
Atrazine	65	ab	HI	50	b	JKL	71	a	EFG
S-metolachlor + atrazine	83	a	A-D	81	a	FGH	90	a	CD
Glyphosate	93	b	A	96	b	BCD	100	a	A
S-metolachlor + atrazine + glyphosate[†]	69	a	E-I	20	b	NO	16	b	MNO
Mesotrione	59	b	IJ	92	a	DEF	93	a	BC
S-metolachlor + mesotrione	89	b	ABC	100	a	AB	100	a	A
S-metolachlor + atrazine+ mesotrione (1)	86	b	A-D	100	a	AB	100	a	A
S-metolachlor + atrazine+ mesotrione (2)	85	b	A-D	100	a	A	100	a	A
S-metolachlor + mesotrione + bicyclopyrone	67	b	GHI	97	a	BCD	98	a	AB
S-metolachlor + atrazine+ mesotrione + bicyclopyrone	80	b	C-G	100	a	A	100	a	A
Pyroxasulfone	31	a	L-O	17	b	OP	21	ab	K-N
Fluthiacet	13	a	P	9	b	R	13	a	NOP
Pyroxasulfone + fluthiacet	21	a	NOP	14	a	OP	22	a	J-N
Pyroxasulfone + carfentrazone-ethyl	56	a	IJK	55	a	IJK	35	b	IJK
Isoxaflutole	44	b	JKL	55	ab	JK	69	a	FG
Isoxaflutole + atrazine	81	c	CDE	94	b	CDE	100	a	A
Isoxaflutole + thiencarbazone-methyl	41	c	KLM	94	b	CDE	100	a	A
Isoxaflutole + thiencarbazone-methyl + atrazine	65	a	HI	39	b	KLM	25	b	J-M
Acetochlor (1)	81	a	C-F	36	b	LMN	37	b	IJ
Acetochlor (2) [†, #]	37	b	LMN	32	b	MN	62	a	GH
Acetochlor + atrazine (1) [†, #]	67	b	F-I	71	ab	HI	83	B	C-F
Acetochlor + atrazine (2)	83	b	B-D	100	a	A	100	A	A
Acetochlor + atrazine (3)	88	b	ABC	99	a	ABC	100	a	A
Acetochlor + mesotrione	61	c	HI	88	b	EFG	98	a	AB
Acetochlor + mesotrione + clopyralid	62	b	HI	96	a	B-E	98	a	AB
Flumetsulam	42	b	KLM	80	a	GH	89	a	CD
Clopyralid	22	a	NOP	9	b	OPQ	16	ab	L-O
Flumetsulam + clopyralid	30	b	L-O	77	a	GH	84	a	CDE
Acetochlor + flumetsulam + clopyralid	34	b	LMN	35	b	MN	56	a	GH
Rimsulfuron	41	a	KLM	39	a	KLM	47	a	HI
Rimsulfuron + isoxaflutole[†]	19	b	OP	32	ab	MN	50	a	HI
Saflufenacil	54	b	IJK	82	a	FGH	91	a	BCD
Saflufenacil + glyphosate	92	b	AB	95	b	CDE	100	a	A
Dimethnamid-P	26	a	M-P	8	b	PQR	5	b	P
Saflufenacil + dimethnamid-P	69	b	E-I	97	a	A-D	100	a	A
Dicamba	68	b	E-I	61	b	IJ	85	a	CDE
Tembotrione	58	a	IJK	38	b	KLM	32	b	I-L
Dicamba + tembotrione	75	a	D-H	82	a	FGH	82	a	DEF

[†] Herbicide tank-mix otherwise premixes; [‡] Number in parentheses indicates formulation difference; [#] Encapsulated formulation.

[a] Visual control in days after application (DAA). Means within columns (uppercase) and within rows (lowercase) with no common letter(s) are significantly different according to Tukey-Kramer's test at $P < 0.05$.

[b] The percent control (0%) data of nontreated control were not included in analysis.

Trade name and active ingredient rate of all herbicide treatments are displayed in Table 5.2.

LIST OF APPENDIX TABLES

Appendix table 4.1. Analysis of variance for evaluated parameters herbicide treatments on volunteer hemp visual control and biomass reduction in soybean field experiment.

Factor	Visual Control		
	$F_{(Num\ DF,\ Den\ DF)}$[a]	Fc[b]	ρ-value[c]
Treatment	$F_{(19,411)}$	76.72	<.0001
Time	$F_{(2,411)}$	68.99	<.0001
Treatment*Time	$F_{(38,411)}$	1.82	0.0028
	Biomass Reduction		
Treatment	$F_{(19,133)}$	17.58	<.0001

[a] Num DF – The number of degrees of freedom in the model; Den DF – The number of degrees of freedom associated with the model errors.

[b] Fc: Calculated F-value.

[c] At the α= 0.05 level of significance using a Tukey-Kramer's *test*

Appendix table 5.1. Analysis of variance for evaluated parameters herbicide treatments on volunteer hemp visual control and biomass reduction in corn field experiment conducted for North Platte and Columbus locations.

Factor	North Platte			Columbus		
	Visual Control					
	$F_{(\text{Num DF, Den DF})}$[a]	Fc[b]	ρ-value	$F_{(\text{Num DF, Den DF})}$[a]	Fc[b]	ρ-value
Treatment	$F_{(38,368)}$	109.90	<0.0001	$F_{(38,354)}$	109.56	<0.0001
Time	$F_{(2,368)}$	82.43	<0.0001	$F_{(2,354)}$	96.10	<0.0001
Treatment*Time	$F_{(76,368)}$	10.30	<0.0001	$F_{(76,354)}$	10.19	<0.0001
	Biomass Reduction					
Treatment	$F_{(38,96)}$	21.08	<0.0001	$F_{(38,103)}$	19.53	<0.0001

[a] Num DF – The number of degrees of freedom in the model; Den DF – The number of degrees of freedom associated with the model errors.

[b] Fc: Calculated F-value.

[c] At the α= 0.05 level of significance using a Tukey-Kramer's *test*.

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CHAPTER 6

Summary of Findings and Suggested Future Work

Alteration in public perception and policy alongside the versatility of hemp as crop has led to significant shifts across diverse industries, from textiles to pharmaceuticals, while its economic impact is underscored by increase in market value. Since its resurgence in the United States in 2019, industrial hemp has significantly transformed the agricultural and industrial sectors, influencing everything from the production of raw materials to their incorporation into various products. The increasing trends in hemp cultivation, along with its expanding markets, present significant economic opportunities for growers, investors, and businesses within the agricultural and industrial sectors. However, these come with agricultural challenges, particularly regarding the integration of hemp into traditional crop rotations, that opened opportunities for diversified research in the United States (US). This dissertation examined the pros and cons of the integration of hemp into US agricultural systems, focusing primarily on interactions with conventional crops like corn and soybean through three targeted studies.

Firstly, an assessment of hemp sensitivity to herbicide drift from adjacent corn and soybean fields has revealed its susceptibility to damage, which could lead to significant economic losses. These findings underscore the necessity for implementing herbicide drift mitigation techniques in the cultivation of industrial hemp to preserve biomass yield. Furthermore, consistent with previous knowledge, current research indicates that the design of the nozzle and the choice of herbicide play a crucial role in controlling drift, thereby minimizing the potential damage to nearby plants in critical. Additionally, the study highlights that different herbicides can cause varying levels of biomass reduction, with imazethapyr and lactofen having a relatively lower impact. These insights are crucial for pesticide applicators as they provide crucial guidance on how to minimize off-target damage, particularly from herbicides like glyphosate, mesotrione, glufosinate, 2,4-D, and dicamba. However, it should be noted that these simulations were conducted in a controlled environment. Consequently, more field studies are necessary to fully understand the long-term effects of herbicide drift on hemp, especially during its reproductive stages. Future research following this study should also include an evaluation of a range of drift reduction technologies under various environmental conditions to identify the most effective methods for reducing herbicide drift in scenarios where hemp is grown next to herbicide-tolerant crops.

The second study provides an evaluation of the tolerance of two hemp cultivars to ACCase-inhibiting herbicides. Overall findings suggest a spectrum of responses that are critical for developing effective weed management strategies in the growing hemp industry. The findings indicate that the sensitivity of different hemp cultivars to specific ACCase herbicides, including pinoxaden, clethodim, and quizalofop, differs significantly. This variance in responses suggests that the effectiveness of these herbicides may be influenced by factors such as dose applied, the type of herbicide (including product formulation), and the genetic profile of the cultivar. Some herbicides, like sethoxydim, appear to have a minimal impact on hemp, suggesting an inherent tolerance. However, the study underscores the necessity for caution when extrapolating controlled environment results to field conditions. Environmental factors can significantly alter the outcome, highlighting the importance of conducting field-level research.

Considering the findings from greenhouse settings, it becomes clear that while the basic understanding of hemp response to ACCase herbicides has advanced, predicting field application outcomes remains complex due to environmental influences. The research thus advocates for refined, knowledge-based use of herbicides in hemp farming, tailored to specific cultivars and more realistic environmental conditions. Moreover, it remains imperative to investigate how the application of ACCase inhibitors might impact the herbicide residues within harvested hemp material. Understanding the subsequent effects on the fatty acid profile, phytochemical composition, including the levels of tetrahydrocannabinol (aka THC) and other cannabinoids, is essential for ensuring the safety and quality of hemp products. This future research might be vital not only for agricultural best practices but also for consumer safety and regulatory compliance.

Investigating the control of volunteer hemp in soybean and corn rotations is essential for developing effective herbicide programs. This research has contributed valuable insights, particularly in the context of soybean fields, including soybean cultivars tolerant to 2,4-D. The research revealed that more than half of the early-season solutions tested significantly reduced the biomass of volunteer hemp, indicating that current herbicide strategies in soybeans are successful. Glyphosate use alone or in combination with other herbicides was notably effective, underlining its crucial role in controlling volunteer hemp. The incorporation of glyphosate into herbicide programs could therefore enhance their overall efficacy.

In contrast, the effectiveness of herbicide treatments in corn varied and appeared to be highly location-dependent, suggesting that environmental conditions and soil types might influence herbicide activity. For example, in North Platte, most treatments were highly-effective, with some combinations, such as glyphosate and saflufenacil, achieving complete biomass reduction. This highlights the potential benefits of customizing herbicide combinations to specific field conditions to optimize control. However, some herbicides, such as S-metolachlor, fluthiacet, and pyroxasulfone, demonstrated limited effectiveness on volunteer hemp when used alone.

The success of herbicide treatments is contingent upon several factors, including application timing, environmental conditions, and soil properties. Combining multiple herbicides might be a more effective approach for managing volunteer hemp, particularly in soybean fields, and could help in preventing herbicide resistance. This approach promotes the sustainability of long-term weed control. The study underscores the need for additional research to refine our understanding of herbicide responses under various environmental conditions. Future research should focus on optimizing application rates and timings for those herbicides with limited efficacy and exploring their roles within broader herbicide programs. The scope of our study focused solely on the effectiveness of applied herbicide programs for controlling volunteer hemp due to limited research space, consequently not capturing yield data for soybean and corn, which grants opportunities for future studies to explore this aspect. Moreover, the integration of alternative weed management methods, such as mechanical (roller crimping and/or propane flaming) tools, remains an important facet of integrated weed management that would require further assessment. These additional management strategies can complement herbicide programs, ensuring sustained success in controlling volunteer hemp.

The comprehensive examination of hemp integration into US agricultural systems through three targeted studies offers vital insights for the hemp industry evolution. The findings underscore the importance of mitigating herbicide drift to preserve hemp quality and yield while highlighting the role of nozzle design and herbicide selection in drift control. Additionally, the study emphasizes the need for caution when extrapolating controlled environment results to field conditions, necessitating further research in realistic settings. The evaluation of hemp cultivar tolerance to herbicides emphasizes the complexity of predicting field outcomes, contributing to refined, cultivar-specific herbicide uses under real-world environmental conditions. Furthermore, the control of volunteer hemp in soybean and corn rotations suggests successful herbicide strategies, with customization to specific conditions and consideration of alternative weed management methods.

Collectively, these studies provide crucial guidance for the agricultural industry as it adapts to the inclusion of hemp in crop rotations, ensuring both the hemp viability and the continuation of established farming practices.