HERBICIDE SCREENING IN INDUSTRIAL HEMP (*CANNABIS SATIVA* L.) PRODUCTION

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The Supervisory Committee certifies that this ***disquisition*** complies with North Dakota State University's regulations and meets the accepted standards for the degree of

MASTER OF SCIENCE

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ABSTRACT

Lack of labeled herbicides for industrial hemp (*Cannabis sativa* L.) production in the U.S. accentuates urgency to identify herbicides for weed control. Weed competition reduced seed yield by 25 to 32%. Pre- and post-emergence herbicide experiments were conducted to evaluate hemp tolerance to herbicides and support efforts for herbicide registrations. For post-emergence herbicides, hemp response to clopyralid (105 g ae ha^{-1}) and bromoxynil (280 g ae ha^{-1}) exhibited the lowest phytotoxicity, up to 38%, with no reduction of yield compared with the hand-weeded check. Pre-emergence herbicides were more viable options because pendimethalin (1120 g ai ha^{-1}), trifluralin (840 g ai ha^{-1}), saflufenacil (38 g ai ha^{-1}), and pyroxasulfone (109 g ai ha^{-1}) resulted in less than 17% visible hemp injury and increased seed yield by 18 to 33% compared with the non-treated, weedy check.

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INTRODUCTION

Industrial hemp (*Cannabis sativa* L.) is a recently rejuvenated agricultural crop that is grown for its fiber, oil, and seed (McPartland 2018; Teh and Birch 2013). Under the Agricultural Act of 2014, the U.S. differentiated industrial hemp and marijuana, which allowed pilot programs and hemp research to be conducted by state departments of agriculture and universities (Williams and Mundell 2018). Federal legalization to cultivate hemp was achieved through the passage of the Agriculture Improvement Act of 2018 (U.S. Government 2018). This Act no longer classified hemp as a Schedule 1 controlled substance as in the Controlled Substance Act and allowed agricultural production of hemp. Hemp production has increased since 2018, with 183,000 hectares of hemp in production in July 2020 (Farm Service Agency 2020).

As production increased, the industry became more aware of the problems and challenges of growing hemp. One of the challenges growers face is crop competition for resources against weeds (Cole and Zurbo 2008; Baxter and Scheifele 2000). Competition between weeds and hemp seedlings has been demonstrated to affect crop performance and result in severe yield loss (Hall et al. 2014; Jankauskiene et al. 2014). Published literature describing or specifically examining the direct relationship, without confounding factors, of weed densities and their influence on yield are not well understood (Sandler and Gibson 2019). More research is needed to better understand this relationship between weed competition and yield.

Hemp has been observed to emerge and establish quickly and develop dense canopies that have the ability to suppress weeds (Amaducci et al 2015; Fortenbery and Bennett 2004). When hemp is grown for seed (50 to 150 plants m^{-2}), the leaf canopy does not become as dense due to fewer established plants than for fiber hemp (200 to 250 plants m^{-2}), and weed control may become necessary (Hall et al. 2014; Fike 2016; Baxter and Scheifele 2000). Increased light penetration through a less dense hemp canopy allows more weeds to germinate and compete with hemp for soil nutrients, water, and space. Research has suggested that weeds are adequately suppressed when hemp density reaches 200 to 250 plants m^{-2} in fiber hemp production. Increasing the seeding rate of oilseed hemp from 20 to 60 or 80 kg ha^{-1} reduced weed density and size by 33% and 34%, respectively, and increased hemp biomass and seed yield by 23% and 34%, respectively (Vera et al. 2006). Planting hemp for seed at 22 to 33 kg ha^{-1} or achieving a final plant density of 110 to 130 plants m^{-2} is common practice in the U.S. and Canada (HGI 2014; Legacy Hemp 2020). Hemp seed for planting costs approximately $9 per kilogram. Increasing the seeding rate of hemp three- or four-fold to reduce weed competition and increase seed yields is a large investment comparatively to more cost-effective herbicide options. Effective weed control practices are needed at the current recommended seeding rate in order to maintain hemp yield and make growing a hemp crop economically feasible.

Chemical weed control methods are a viable option that could be used to suppress and control weeds. The herbicides ethalfluralin (85 to 140 g ai ha^{-1}), quizalofop-p-ethyl (93 g ai ha^{-1}), and paraquat (545 to 1135 g ai ha^{-1}) are already labelled for use in Canada (AMVAC 2020a; Gowan 2018; Syngenta 2020). Hemp production in Europe utilizes minimal amounts of herbicides; however, the literature has shown that encapsulated forms of s-metolachlor (65% at 3 L ha^{-1}) and pendimethalin (30% at 3 L ha^{-1}) in addition to acetochlor (65% at 0.75 L ha^{-1}) have been used pre-emergence in China (Amaducci 2005; Amaducci et al. 2015). Linuron (37.6% w/w) has been used in the Czech Republic after planting to control broadleaf weeds within research plots (Tang et al. 2016). Research conducted in Moldova suggested that hemp was relatively tolerant to acetochlor (2200 g ai ha^{-1}) and metolachlor (1500 g ai ha^{-1}) when applied pre-emergence and flusiphor-p-butyl (1000 g ai ha^{-1}) when applied post-emergence (Chiriţă N

2008). There are no herbicides with Section 3 registration for use in the industrial hemp crop in the U.S. (Flessner et al. 2020; Gray et al. 2017; Anderson 2018).

During the developmental stage of our research and at the onset of its implementation, there were very few published research papers that studied herbicide use in hemp. There was some research conducted which led to herbicide registrations in other countries, as mentioned previously, but very limited peer-reviewed papers available that evaluated hemp response to herbicides within the U.S.

Prior to the onset of this experiment, a single greenhouse and a few field experiments evaluated pre-emergence herbicide use in hemp. The greenhouse experiment was an unpublished Master's thesis from Virginia Tech that included evaluations of herbicides in site of action Groups 2, 3, 5, 7, 12, 13, 14, and 15 (Byrd 2019). The experiment was conducted using silt loam soil. Chlorimuron-ethyl (40 g ai ha^{-1}), a Group 2 acetolactate synthase (ALS) inhibitor herbicide, and pendimethalin (1600 g ai ha^{1}), a Group 3 seedling root inhibitor herbicide, both resulted in 17 to 23% visible hemp injury (Byrd 2019). Diuron (2300 g ai ha^{-1}), metribuzin (600 g ai ha^{-1}), and linuron (1400 g ai ha^{-1}), Group 5 photosynthesis inhibitor herbicides, resulted in 15 to 20%, 34 to 45%, and 14 to 24% injury, respectively. Norflurazon (2800 g ai ha^{-1}), Group 12, and clomazone (1400 g ai ha^{-1}), Group 13, proved to be quite injurious with nearly 80% injury and negatively affected emergence. Hemp injury from Group 14 herbicides that inhibit protoporphyrinogen oxidase (PPO) and Group 15 herbicides that inhibit seedling shoot growth ranged from 23 to 50% injury in the greenhouse. Field experiments included similar herbicides along with additional modes of action.

Two seedling root growth inhibitors have been evaluated in the field for use in hemp: ethalfluralin and pendimethalin. Granular ethalfuralin applied at 850 to 3400 g ha^{-1} resulted in less than 26% injury across multiple experiments with an average injury below 10% (Byeongseok and Jean-François 2016; Byeongseok and Ulrich 2014a, 2016a, 2016b). Pendimethalin at 1065 to 1600 g ai ha^{-1} resulted in a wide range of injury response (0 to 70%) across experiments. In some experiments, pendimethalin resulted in lower hemp density and biomass compared with the non-treated check, but in others the result was 15 to 20% injury to hemp with no negative effects on plant height or seed yield (Anderson 2018; Maxwell 2016; Pearce and Carter 2018; Woosley et al. 2015). In another unpublished Master's thesis from Western Kentucky University, pendimethalin (1120 g ha^{-1}) resulted in a 21% reduction in hemp establishment compared to the non-treated (Anderson 2018). In the same experiment, plant height of established plants was reduced early in the season, but did not differ from control plants 34 days after planting. There was also a 24% increase in seed yield over the non-treated hemp. Results from experiments evaluating hemp response to trifluralin were not available at the onset of this experiment; therefore, trifluralin, being in the same mode of action, was viewed as a viable herbicide to explore in our research.

At the beginning of our research, herbicides in Groups 5, 13, and 14 had received little attention in the U.S. Metribuzin (278 g ha^{-1}), Group 5, applied pre-emergence on a silt loam in Moldova resulted in approximately 50% injury across all evaluation timings (Chiriţă N 2008). Clomazone (114 to 225 g ai ha^{-1}), a Group 13 carotenoid biosynthesis inhibitor, was evaluated by the University of Kentucky where less than 12% visible necrosis to hemp was observed (Pearce and Carter 2018). Hemp injury with fomesafen-sodium (350 to 400 g ai ha^{-1}), a Group 14 PPO inhibitor, ranged between 11 and 86% (Maxwell 2016; Woosley et al. 2015). The range of hemp injury with fomesafen was partly attributed to the difference between cultivars under similar soil conditions. A single experiment evaluated hemp tolerance to saflufenacil (25 g ai ha^{-1}), Group

14, where pre-emergence use resulted in 0 to 2% hemp injury (Pearce and Carter 2018). Sulfentrazone (158 to 313 g ai ha^{-1}), another Group 14 herbicide, resulted in 0 to 25% hemp injury (Willenborg and Johnson 2018).

Group 15 herbicides that have been evaluated in the field prior to this research included s-metolachlor and pyroxasulfone, both resulting in an average hemp injury of approximately 25% (Chiriţă 2008; Maxwell 2016). Mesotrione, a Group 27 herbicide, resulted in greater than 80% injury at various rates and should not be considered for use as a pre-emergence herbicide in hemp (Maxwell 2016; Woosley et al. 2015).

The majority of pre-emergence herbicide experiments with hemp have been conducted on silt loam soils. Hemp response to pre-emergence herbicides can differ due to soil type and environmental conditions. The North Dakota soil, edaphic factors, and environment are very different from many locations where hemp response to herbicides has been conducted. As such, additional work is warranted to evaluate possible herbicide options for this region. There is also a need for replication and verification of hemp response to herbicides that have only been evaluated in a singular experiment, in addition to evaluating hemp response to herbicides that have not been screened yet. The most viable and least injurious pre-emergence herbicides to hemp should be evaluated in the field environment to accurately represent the production environment. Herbicides applied post-emergence will also be evaluated for hemp safety. Some research was already conducted on various post-emergence herbicides prior to this experiment.

Prior to conducting this research, no greenhouse experiments could be found that evaluated hemp response to post-emergence herbicides. Various herbicide site of action groups were evaluated in the field as post-emergence applications. Group 1, acetyl-CoA carboxylase inhibitors, primarily used to control monocot weeds, were relatively safe to use in hemp and often resulted in less than 10% injury (Lingenfelter 2018; Pearce and Carter 2018). Herbicides evaluated included clethodim (136 to 272 g ai ha^{-1}) and quizalofop (185 g ha^{-1}).

Hemp injury response was highly variable (11 to 90%) within Group 2 herbicides (Maxwell 2016; Woosley et al 2015). Group 2 herbicides previously evaluated included bispyribac-sodium (20 g ai ha^{-1}), flazasulfuron (110 g ai ha^{-1}), rimsulfuron (70 g ai ha^{-1}), and trifloxysulfuron (7 g ai ha^{-1}), leaving many more to be evaluated. At the onset of this research, research conducted in the U.S. was not available that evaluated Group 4 herbicides.

Bromoxynil (140 to 560 g ae ha^{-1}), a Group 6 herbicide, has been one of the most extensively studied broadleaf herbicides used post-emergence in hemp and has typically resulted in less than 15% hemp injury (Byeongseok and Ulrich 2014b; Howatt and Mettler 2018; Lingenfelter 2018; Maxwell 2016; Pearce and Carter 2018; Willenborg and Johnson 2018; Woosley et al 2015). Bromoxynil was included in our research efforts.

Research was not found that included post-emergence herbicides from Groups 9, 10, 14, and 15. This is likely due to the fact that glyphosate (Group 9) and glufosinate-ammonium (Group 10) are non-selective herbicides. Non-selective herbicides were included in the preliminary greenhouse experiment to provide a baseline of data and prove their efficacy for control of hemp. Herbicides from Group 14 and 15 were also included in the initial greenhouse screening, due to the lack of research.

Monosodium methanearsonate (2,270 to 4,540 g ai ha^{-1}) was the only group 17 herbicide previously evaluated and resulted in less than 10% injury (Maxwell 2016; Woosley et al. 2019). This herbicide was not included in the greenhouse experiment because it is not commonly used in North Dakota and has a limited weed control spectrum, although it is an approved organic weed control product.

In Group 27, mesotrione caused significant injury (70 to 80%) when applied at 105 g ai ha^{-1}. However, topramezone (18.4 g ai $ha^{-}1$) has been reported to cause less than 5% visible injury to hemp (Howatt and Mettler 2018; Lingenfelter 2018). Past research shows variability in hemp response to Group 27 herbicides.

Hemp growth stage at the time of post-emergence herbicide application likely influences hemp response to the herbicide. Larger plants might be more resilient to the effects of herbicide and metabolize the herbicide more rapidly, or could be exposed to a larger dose of the herbicide because of more leaf area exposed. The specific response could also be influenced by the herbicide site of action in question. Experiments have described the hemp stage at the time of application in terms of hemp height, number of leaves, and leaf stage. Timing of post-emergence herbicide applications have ranged from 5 to 30 cm tall hemp, and two- to ten-leaf stage. The environment, especially light intensity and quality, can greatly influence the height of hemp plants at any specific leaf stage. Also, there appeared to be discrepancy, or at least uncertainty, that leaf staging was done accurately and correctly. There appears to be little consistency in application timing and hemp stage description. Evaluation of herbicide timings and consistent descriptions of hemp stage would be beneficial in moving forward with herbicide registration. Comparison of hemp response to herbicides when applied at a consistent growth stage is important for valid comparison of relative risks. There is also a need for replication and verification of hemp response to herbicides that have only been evaluated in a singular experiment, in addition to evaluating hemp response to herbicides that have not been screened yet. The most viable and least injurious post-emergence herbicides to hemp need to be evaluated in the field environment for valid production evaluation.

The objective of this research was to evaluate hemp for tolerance to various pre- and post-emergence herbicides under consistent conditions. Field experiments were conducted to test the null hypothesis that hemp would not exhibit plant injury or reduced emergence, height, light interception, and/or yield as a response to various herbicide treatments relative to the weed-free and non-treated check. Determining the relative safety of herbicides on hemp is important to aid and focus future research. Results from this research will also help identify and support herbicide registration and future management practices of the hemp industry in the U.S.

Objectives

- Determine pre- and post-emergence herbicide safety on hemp by evaluating herbicide influence on plant density, visible injury, height, canopy light interception, and yield.
- Identify pre- and post-emergence herbicides to be further evaluated for registration and labeled use in industrial hemp.

Hypothesis

- H_0: Both pre- and post-emergence herbicide applications will have no effect on: plant density, visible injury, height, canopy light interception, and yield.

CHAPTER 1. REVIEW OF LITERATURE

1.1. Characterization of Industrial Hemp

Industrial hemp (*Cannabis sativa* L.) is a recently rejuvenated agricultural crop that is primarily grown for its fiber and oil. Hemp is a member of the *Cannabaceae* botanical family with two primary subspecies, *Cannabis sativa* subsp. *sativa* and *Cannabis sativa* subsp. *indica* (McPartland 2018). *Cannabis indica* L. is more commonly referred to as marijuana and is known for its medicinal properties. *Cannabis sativa* is similar morphologically to marijuana, but differs significantly in its makeup of chemical compounds, specifically in the content of the psychoactive ingredient delta-9-tetrahydrocannabinol (THC) (Fortenbery and Bennett 2004). *Cannabis sativa* tends to have greater amounts of cannabinol (CBD) and lesser amounts of THC compared with marijuana, which typically contains 3 to 15% THC on a dry-weight basis. *Cannabis sativa,* herein referred to simply as hemp, tends to have a THC content of less than 1%. In order for a hemp cultivar to be grown and produced legally in the European Union, Canada, or the U.S., the THC level of any part of the hemp plant must be below 0.3% (Johnson 2014).

Hemp is primarily an annual, indeterminate, dioecious plant. However, monoecious cultivars have been developed and are utilized in hemp seed production (AAF 2015). Plant height varies from 150 to 250 cm tall, depending on the cultivar grown and whether it is being grown for seed or fiber. Fiber types are typically taller. The stem diameter of the plant ranges from 4 to 11 mm in diameter and consists of an inner hurd and outer bark (Anderson 2018). According to Fike (2016), hemp can grow up to 6 m tall. Plant morphology varies with plant density and develops more lateral branches at lower densities. Branching is also dependent upon cultivar, sex, and availability of nutrients and water (Bócsa and Karus 1998). Male plants typically produce fewer branches and are 10 to 15% taller than female plants.

The arrangement of the palmately compound leaves is opposite toward the base of the stem, during the vegetative phase, and become more alternate toward the top of the plant when it switches to reproductive growth (Small and Cronquist 1976). The number of leaflets per leaf can vary (Bócsa and Karus 1998). The first true leaves are unifoliate and only have one leaflet. The second pair of leaves tends to have three leaflets while the third pair of leaves typically has five leaflets. The older the hemp, the more pairs of leaves and the more leaflets there are per leaf. Fully developed leaves can have 5 to 13 leaflets. The leaflets of the cannabis plant are dark green with a lighter green underside, lanceolate, and serrated with a point at the tip (Anderson 2018). Leaflets are 5 to 15 cm long and 1 to 2 cm wide. Leaves are supported by 3- to 15-cm long petioles.

A standardized decimal scale has been developed for recording the growth stages of hemp (Mediavilla et al. 1998). Figure 1.1 below depicts the decimal code system for hemp as presented in the Journal of the International Hemp Association. The first digit of the code represents the general categories of germination and emergence (0), vegetative stage (1), flowering and seed formation (2), and senescence (3). Vegetative growth stages are described in terms of the number of leaf pairs. When pairs of leaves have leaflets at least 1 cm long, the hemp stage advances to the next leaf pair. The flowering and seed formation stages are slightly different for dioecious and monoecious hemp. Unified use of this decimal scale would benefit the hemp industry and prove helpful for agronomists and researchers.

Table 1.1. Definitions and codes of growth stages of hemp plants.

Code[a]	Definition	Remarks
Germination and emergence		
0	Dry seed	
1	Radicle apparent	
2	Emergence of hypocotyl	
3	Cotyledons unfolded	
Vegetative stage refers to main stem. Leaves are considered as unfolded when leaflets are at least one cm long.		
1002	1st leaf pair	1 leaflet
1004	2nd leaf pair	3 leaflets
1006	3rd leaf pair	5 leaflets
1008	4th leaf pair	7 leaflets
1010	5th leaf pair	
	:	:
10XX	11th leaf pair	XX = 2(nth leaf pair)
Flowering and seed formation refers to the main stem including branches		
2000	GV point	Change of phyllotaxis on the main stem from opposite to alternate. Distance between petioles of alternate leaves at least 0.5 cm
2001	Flower primordia	Sex nearly indistinguishable
	Dioecious Plant	
	male	
2100	Flower formation	First closed staminate flowers
2101	Beginning of flowering	First opened staminate flowers
2102	Flowering	50% open staminate flowers
2103	End of flowering	95% of staminate flowers open or withered
	female	
2200	Flower formation	First pistillate flowers Bract with no styles
2201	Beginning of flowering	Styles on first female flowers
2202	Flowering	50% of bracts formed
2203	Beginning od seed maturity	First seeds hard
2204	Seed maturity	50% of seeds hard
2205	End of seed maturity	95% of seeds hard or shattered
	Monoecious Plant	
2300	Female flower formation	First pistillate flowers Perigonal bracts with no styles
2301	Beginning of female flowering	First styles visible
2302	Female flowering	50% of bracts formed
2303	Male flower formation	First closed staminate flowers
2304	Male flowering	Most staminate flowers open
2305	Beginning of seed maturity	First seeds hard
2306	Seed maturity	50% of seed hard
2307	End of seed maturity	95% of seeds hard or shattered
Senescence		
3001	Leaf desiccation	Leaves dry
3002	Stem desiccation	Leaves dropped
3003	Stem decomposition	Bast fibers free

[a] Decimal code system for hemp as presented in the Journal of the International Hemp Association.

The architecture of the hemp plant also depends on day length. Hemp is a "short-day cycle" plant, where the plant will not enter the reproductive phase unless the daily exposure to sunlight is shorter than the critical day length period to induce reproductive growth (Bócsa and Karus 1998). As a result, cultivars can vary in growth, dependent on the geographical latitude in which they are grown. Most hemp cultivars are photoperiod sensitive and begin flowering in Canada in late June or early July when a maximum day length of approximately 14 h is reached (Prade et al. 2011). Male plants are taller, thinner, and die shortly after shedding pollen (Fike 2016). Hemp plants are prolific pollen producers and produce the largest amount of pollen of all cultivated plants (Bócsa and Karus 1998). Hemp fields can produce dense clouds of pollen that can travel via wind up to 12 km. Thirty to forty grams of pollen can be produced from a single robust male plant. Female plants are even more robust and produce more leaves and terminal inflorescences (Fike 2016). Seeds are surrounded by bracts covered in hairs and have glands that produce resinous compounds (Clarke and Merlin 2016). Delta-9-tetrahydrocannabinol content of the plant is most concentrated in the inflorescence or buds prior to fertilization. Seeds develop and mature in an asynchronous matter on the inflorescence.

The variable rate of maturity between male and female plants along with the variable levels of seed maturity within a single inflorescence can create harvest challenges and yield loss due to seed shatter. Hemp seed, as they are commonly called, are nuts that are an achene that contains a single seed (Bócsa and Karus 1998). The thousand seed weight ranges from 2 to 70 g and varies by subspecies and cultivar. Seed size and the presence of mottled or marbled seed was thought to be a key feature in identifying subspecies and varieties of *Cannabis sativa* L. (Small and Cronquist 1976). Subspecies *sativa var*. *sativa* and *var*. *spontanea*, have relatively small seed (<3.8 mm long), weighing 15 to 20 g per thousand, and were thought to be characteristically mottled. *Cannabis sativa* L. *var. indica* have larger seeds (>3.8 mm long) that were said to be absent of mottling. However, the marbling later proved to not be a genetic characteristic and is a result of a colored imprint of the surrounding bracts that can be rubbed off (Bócsa and Karus 1998). Hemp seed has an oil content of 30 to 32%. Hemp seed germination is around 95% the year of harvest, but decreases to 80% the following year, and the year after is rendered unsuitable for commercial sowing unless stored in a freezer to extend its viability.

Hemp plants have a large, primary taproot which can reach to a depth of 2 to 2.5 m in well-drained soils (Anderson 2018; Bócsa and Karus 1998). The numerous branched, secondary roots can extend out 60 to 80 cm. According to Fortenbery and Bennett (2004), hemp grows best on loamy, well-drained, high organic matter soils. Optimum mean daily temperatures for hemp production are around 13 to 22°C. At 16°C plants can enter a rapid growth phase, where plants can grow 4 to 6 cm each day. Early-maturing cultivars require 1,600 to 1,700 growing degree days (Bócsa and Karus 1998). Hemp plants are rather hardy and can handle colder and warmer climates. Young hemp plants up to the fifth leaf pair can withstand light frosts down to -6°C (Bócsa and Karus 1998; Burczyk 2008). Hemp requires significant moisture, approximately 25- to 36-cm of rainfall during the first six weeks of growth and a total around 71 cm for the season (Fortenbery and Bennett 2004). However, once the root system is well established, hemp can tolerate periods of water stress and may only need 20- to 30-cm of rainfall throughout the entire growing season (Burczyk 2008). It is an adaptable plant that can be grown in a variety of climates.

1.2. Origin and History of Hemp

Cannabis sativa L. likely originated from southern Europe, India, and Central Asia (Gilmore et al. 2007). Cannabis higher in THC such as *Cannabis sativa* subsp. *indica* and subsp.

afghanica likely originated from India, Afghanistan, and China. Subspecies grown for fiber such as *Cannabis sativa* subsp. s*ativa* and subsp. *chinensis* can be traced more closely to European or East Asian origin. Evolutionary history between *Cannabis* and human interactions have allowed for the development of many cultivars, each selected for various traits such as fiber, food, or psychological compounds (Clarke and Merlin 2016). *Cannabis* plants underwent phenotypic changes during the selection and domestication process. Hemp plants grown and selected for fiber tend to have longer spaces between internodes and fewer branches. The opposite being true for plants grown for seed, which resulted in plants relatively short in stature and more prone to branching. Over time, cultivated *Cannabis* has been selected for larger seeds, quick and even germination, earlier maturity, greater resin production, low and high THC content, and reduced seed shattering.

Hemp was first cultivated as a fiber crop in Central Asia, then developed in Europe as an important cash crop by the 1600s and is believed to have first arrived in North America in 1606 where it was grown for a few hundred years as a source of fiber (Cherney and Small 2016). United States presidents George Washington and Thomas Jefferson even encouraged hemp cultivation (Small and Marcus 2002). By the mid-1800s, states such as Kentucky, Missouri, and Illinois were in the middle of a thriving hemp industry, producing fiber for cordage and sailcloth for the U.S. Navy (Fortenbery and Bennett 2004). At this time, Kentucky had over 160 factories manufacturing hemp bagging, bale, and rope. However, the hemp industry did not thrive for long with the invention of the cotton (*Gossypium hirsutum* L.) gin, carbon-fuel powered ships, and the ability to import cheaper sources of fiber. After the completion of the Civil War and the abolishment of slavery, there was a reduced work force for the labor-intensive hemp crop which limited its practicality (Cherney and Small 2016).

During the mid-1880s, hemp production had expanded west to states such as Missouri and Illinois (Fortenberry and Bennett 2001). Hemp production declined, but again increased during World War I, waned, and once again increased during World War II. Just prior to World War II, the Marijuana Tax Act of 1937 required special permission from the U.S. Drug Enforcement Administration to cultivate hemp, essentially making cannabis production unprofitable (Cherney and Small 2016). Canada passed similar legislation in 1938 making the cultivation of all cannabis illegal. In 1970, all cannabis became illegal to grow and possess in the U.S. with the passage of the Controlled Substance Act (Williams and Mundell 2018). This act declared all cannabis (hemp and marijuana) as a Schedule 1 controlled substance alongside heroin and cocaine. The U.S. interest in legalizing hemp returned in the mid-1990s once European countries and Canada began to allow hemp production (Fike 2016).

Canada led the way in North America by issuing licenses to allow research to be conducted on hemp beginning in 1994 (Cherney and Small 2016). By 1998, Canada started issuing licenses for the commercial cultivation of hemp. From 2010 to 2015, cultivated hemp acreage in Canada grew 25% annually, with 43,706 ha grown in 2014 (AAF 2015). Under the Agricultural Act of 2014, the U.S. followed suit and legally differentiated industrial hemp and marijuana. As a result of this Act, the U.S. allowed pilot programs and hemp research to be conducted as long as the research was conducted by state departments of agriculture or universities (Williams and Mundell 2018). After 1970, but prior to 2014, hemp production in the U.S. did exist, but under highly regulated circumstances with no processing facilities available due to regulation (Malone and Gomez 2019).

Public curiosity and interest in hemp and hemp products grew, resulting in imported hemp product sales to reach about $500 million in 2015 and $600 million in 2016 and was projected to reach $1.5 billion by 2020 (Hemp Biz Journal 2016, Das et al. 2017). In order for the hemp industry to move forward and achieve its potential, there was a need for processing facilities with localized access. More agricultural knowledge regarding cultural and management practices was also necessary. The number of registered pilot program hectares in the U.S. increased from 2,700 to 10,400 ha from 2015 to 2017, respectively (Malone and Gomez 2019). States such as Colorado, Kentucky, and Oregon had the largest amount of land area in production during that time. North Dakota also saw a significant increase in hemp acres, jumping from 28 ha in 2016 to over 1,200 ha in 2017. By 2019, North Dakota had 64 registered hemp growers on 1,594 ha (Smith 2021). Total hemp hectares in 2020 decreased slightly to 1,372 ha for 81 hemp growers. As more information on hemp agricultural practices became available through these pilot programs, the U.S. government was also able to loosen restrictions and allow for the commercial production of industrial hemp.

Federal legalization was achieved through the passage of the Agriculture Improvement Act of 2018 (U.S. Government 2018). This act specifically defined hemp, regardless of the species and subspecies of *cannabis*, as “the *cannabis* plant, or any part thereof, including extracts and cannabinoids, having a THC concentration of not more than 0.3% on a dry weight basis”. This Act no longer classified hemp as a Schedule 1 controlled substance as in the Controlled Substance Act and allowed the agricultural production of hemp. Hemp producers are still required to be licensed and meet specific requirements to identify the crop location, test for THC content, and dispose of any non-compliant plants (Mead 2019). Such regulations are state specific. The Act did not prohibit or restrict interstate commerce of hemp and has allowed states the authority to legislate more restrictive production parameters.

1.3. Industrial Hemp Uses and By-products

The products derived from hemp are dependent on the cultivar, which can be grown for fiber, biomass, or seed production (Clarke and Merlin 2016). Agricultural practices also influence what parts of the hemp plant will be harvested. Basically, every part of the hemp plant above ground can be used to make a commercial product, whether it is derived from the stems, leaves, inflorescence, or seeds.

Hemp was historically harvested for the fiber that comes from the stems of the plant and was often used to produce cordage, building materials, paper, and other various products (Ruspasinghe et al. 2020). Hemp produces hurd and bast fibers (Cherney and Small 2016). Xylem fibers, known as hurd fibers, constitute the inner core of the stem and are the short, stiff, supportive fibers of the plant. Hurd fibers are of poorer quality and are often used for animal bedding, hemp-lime construction, and fiberboard. Bast fibers are the long, outer phloem fibers and are much more valuable than the hurd fibers. Bast fibers are 5 to 40 mm long and have many desirable characteristics such as tear resistance and wet strength capacity, which is important for specialty products such as paper currency, tea bags, filters, and hygiene products (Fike 2016). Bast fibers are also used in plastic-molded products such as biodegradable landscape matting, and fine textiles (Small and Marcus 2002). In order to transform hemp fibers in these end-products, hemp must go through a process of separating the hurd from the bast fibers called retting (Cherney and Small 2016).

The world hemp fiber market is predominantly in Europe with little infrastructure readily available in the U.S. Cotton is the dominant crop in the natural fiber textile market, accounting for about 85% of the world market (Cherney and Small 2016). Hemp fiber accounts for less than 0.5%. Hemp maintains a greater share (15%) of automobile bio-composites in the European

Union. Cherney and Small (2016) note that heavily subsidized European hemp fiber production has not greatly expanded and that U.S. hemp fiber imports have been relatively stagnant compared with hemp seed imports. Yet, optimism surrounds the hemp industry. Once hemp fiber production establishes in the U.S., many markets and opportunities could develop.

Hemp has great yield potential adding to its economic feasibility when compared with other crops (Fike 2016). Hemp yield is higher compared to other crops grown for fiber. Hemp can yield up to 250% more fiber than cotton and greater than 600% more fiber than flax (*Linum usitatissimum* L.) per unit area (Ruspasinghe et al. 2020). Hemp production and processing methods currently make hemp less practical and competitive than wood or cotton (Fike 2016). Approximately 10 to 15 metric tons of hemp biomass can be produced per hectare and used as a source of fiber and animal bedding or converted into bioenergy (Burczyk 2008).

Production for bioenergy can use the entire plant or a fraction that is not used by another industry. Hemp can be burned as is or processed to make charcoal, methanol, methane, gasoline, or cellulosic-based ethanol (Cherney and Small 2016). The main competitors for the development of hemp as a biogas crop are wood, sugar-beet (*Beta vulgaris* L.), and corn (*Zea mays* L.). Hemp is an above-average energy crop with large potential. Hemp biogas and biomass energy yield is similar to maize, sugar-beet, switchgrass (*Panicum virgatum* L.), and sorghum (*Sorghum bicolor* L.) (Prade et al. 2011; Das et al. 2017). Usable harvested raw material from hemp for bioenergy purposes is approximately 10.5 tons ha^{-1} (Kraszkiewicz et al. 2019). Hemp biomass yield is highly variable and is dependent on the cultivar grown. Some research indicated that hemp biomass yield is closer to 6 tons ha^{-1}, similar to switchgrass and sorghum (Das et al. 2017).

Hemp raw biomass or shredded plant material can be processed into hemp bricks and even pellets to be sold by energy companies. Physical and chemical properties are very comparable with some of the best plants currently used for energy. Hemp has a favorable ratio of energy produced compared with the energy used. In part, due to relatively low production inputs compared with other energy crops. Hemp has a relatively high heat of combustion near 18 to 19 MJ kg^{-1}, which is comparable to oak (Quercus spp.) wood and slightly higher than sorghum and switchgrass (Das et al. 2017; Kraszkiewicz et al. 2019; OMAFRA 2020). Hemp also has a relatively low ash content of 2.5% and high lignin content (21.9%) compared with kenaf (*Hibiscus cannabinus* L.) (20.3%), switchgrass (20.1%) and sorghum (18.3%), which suggests improved profit potential (Das et al. 2017). Ash and carbon content are qualitative characteristics for biomass crops. The lower the ash content, the less by-product to dispose of or repurpose. According to research conducted by Sasserde and Adamovics (2012), ash content for hemp ranges from 2.5 to 3.8% while carbon content varies from around 40 to 45%. In a comparative study, ash levels for energy crops such as flax, linseed (*Linum usitatissimum*), hemp, sunflower (*Helianthus annuus* L.), and reed canarygrass (*Phalaris arundinaceae* L.) were 2.7, 3.5, 4.3, 6.7, and 11.9%, respectively (Komlajeva et al. 2012). Hemp can yield 310 L of ethanol per ton of hemp stems (Das et al. 2017). Cost analysis indicated that industrial hemp could generate higher gross profit per hectare than most other biomass crops if grown for seed in conjunction with biomass (Prade et al. 2011; Das et al. 2017). However, it was observed through an assortment of emissions collected from various combustion tests that hemp combustion, especially with the use of grate-type heating devices, resulted in emissions that had an unacceptable load of CO_2, NO, and SO_2 per environmental standards (Kraszkiewicz et al. 2019). This can also be the case with other bioenergy crops as well and is dependent on the quality of the biomass. Not all hemp materials, whether it be shredded raw material, hemp bricks, or pellets, have the same combustion properties. With increased efforts in breeding to select for biomass yield characteristics, hemp could be competitive in this market.

Cultivars grown for seed production can be utilized as human food, animal feed, and in some cases, pharmaceuticals (Clarke and Merlin 2016). Harvested hemp seed containing about 35% oil can be pressed and the oil harvested and prepared for fuel and human consumption (Liang et al. 2015). Comparatively, flax and canola (*Brassica napus* L.) seeds contain approximately 40 and 42% oil, respectively (Teh and Birch 2013). According to Izzo et al. (2020), hemp seed contains 25 to 35% lipid, 20 to 25% protein and 30% carbohydrates. Hemp seed oil has approximately 80% polyunsaturated (healthy) fatty acids and 20% saturated fatty acids. Hemp seed is also characterized by having the ideal 1:3 ratio of omega-3 to omega-6 essential fatty acids for optimal human health. Other oil seed crops such as flax, canola, and soybean [*Glycine max* (L.) Merr.] have ratios of 1:4, 1:2, and 1:7, respectively (Teh and Birch 2013).

Hemp oil is also made up of many other ingredients including polyphenols, carotenoids, tocopherols, phytosterols peptides, and cannabidiol (CBD) (Teh and Birch 2013). When comparing hemp, flax, and canola oils, hemp seed oil had the highest concentrations of γ-tocopherol (antioxidative), chlorophyll pigments, linoleic acid, total phenolic, and flavonoid compounds. Overall, hemp, flax, and canola oil qualities were considered desirable. Cannabidiol, as a component of hemp oil, can be separated from other oil components to create a CBD-based oil derivative. Cannabidiol has proven to have antibacterial activity (Khan et al. 2014). Several experiments with various extracts of different hemp plant parts have resulted in some degree of growth reduction, and in some cases growth inhibition of bacteria such as *Staphylococcus* *aureus*, *P. aeruginosa*, *V. cholera*, *B. subtilis,* and *E. coli*. Hemp grown in northern latitudes has been reported to have produced higher CBD content and resulted in greater antimicrobial activity. The CBD oil can be used in biomedical applications to create antimicrobial polymer composites, cosmetics, and antimicrobial food packaging products. The application possibilities in the medical industry are endless. There are an estimated 25,000 different uses for hemp and by-products from hemp (INRED 1998). One way the hemp industry can become more competitive and economical is to maximize yields. This is done by providing the optimal amount of resources for the hemp plants to grow, which means minimizing disease and competition from weeds.

1.4. Hemp Pests

Hemp has been thought of as a crop with limited pest problems, resulting in reduced pesticide inputs. However, it has been noted in European literature that there is an assortment of insects and diseases that can cause significant damage to hemp. Problematic insect pests include hemp flea beetle (*Psylliodes attenuate*), hemp borer (*Grapholita delineana*), European corn borer (*Ostrinia nubilalis*), hemp greenflies (*Phorodon cannabis*), and the northern root-knot nematode (*Meloidogyne hapla*) (Bócsa and Karus 1998). Under extreme defoliation (>50%), or damage to the hemp crop, a 50% solution of methyl parathion at a rate of 0.5 to 1.6 L ha^{-1} has been applied along field borders in attempts to control the pests. Up to 80% leaf defoliation has been observed on hemp due to grasshoppers (*Caelifera* spp.) (J Mettler, personal observation). Although hemp is consumed by many insects, few are considered pests to the point of economic loss (Fike 2016). Studies have shown that a small amount of leaf defoliation or boring action in the stem can actually be beneficial, stimulating more hemp tillers and increased branching, which might result in increased seed yield.

Over 90 species of fungi attack *Cannabis*, with the most severe being grey mold (*Botrytis cinerea*), hemp canker (*Sclerotinia sclerotiorum*), and *Pythium* species (McPartland 1996). Like most fungal diseases, grey mold thrives in environments with cool to moderate temperature with high levels of humidity. Gray mold can completely destroy a hemp crop within a week, depending on the growth stage of the plant. Hemp canker occurs in wet soils and affects the base of the plant and the stems. (Bócsa and Karus 1998). Up to 40% field loss has been reported due to hemp canker. Hemp has been known to have uneven emergence as farmers have noticed symptoms of plants damping off shortly after emergence. This is likely a result of hemp susceptibility to *Pythium* species, that under wet soil conditions cause a soft rot leading to death of young, newly emerged hemp plants. Avoiding wet environments and planting hemp in well drained soils would significantly reduce the risk of disease. Hemp can also be affected by leaf spot diseases, rusts, and mildews (McPartland 1996). Fungal diseases are not uncommon in hemp and rarely (unless given ideal growing conditions) result in devastating crop loss.

The threat or severity of such pests is being discovered in the U.S. as many producers have been introduced to hemp as a crop with few pests and little need for pesticide use. However, as hemp production increases, conversations within the industry have revolved around increased pest issues. Several biopesticides have been registered for use in hemp in the U.S. as insecticides, fungicides, and miticides (EPA 2021). These products have active ingredients that include various salts, acids, oils, and strains of bacteria that are not as effective as synthetic chemical products at controlling insects, fungi, and mites. The hemp food industry is hesitant to use more effective chemicals to control pests due to the unknown residue amounts on plant material that may transfer to consumable products.

The food-grade standards for hemp seed are very high and, with few options to combat field-borne pathogens, most producers have been struggling to meet such quality specs. (Bryan Parr, INDHemp, personal communication, 2020). There has been increased demand and interest from producers to develop tools to combat weeds and fungal diseases with herbicides and fungicides.

1.5. Weed Control in Hemp

Current weed control practices in the U.S. include a variety of cultural and mechanical control methods such as crop rotation, planting date, plant density, pre-plant herbicide burndown, pre-plant tillage, and inter-row cultivation (Maxwell 2016). Having monocot crops such as wheat (*Triticum aestivum* L.) and corn in a rotation with hemp will allow for better control of broadleaf weeds that may be problematic when growing hemp the following year. Volunteer cash crops such as corn, sunflower, canola, pea (*Pisum sativum* L.), and wheat can also act as weeds and are necessary to control as they compete with hemp plants for resources (NDDA 2019).

Competition between weeds and hemp seedlings have been demonstrated to affect crop performance and resulted in severe yield losses (Hall et al. 2014; Jankauskiene et al. 2014). However, published literature describing or specifically examining the direct relationship, without confounding factors, of weed densities and their influence on yield are not well understood (Sandler and Gibson 2019). More research is needed to better understand this relationship between weed competition and seed yield. Problematic weed species in hemp have been identified and include wild buckwheat (*Fallopia convolvulus* L.), bindweed (*Convolvulus*), wild oat (*Avena fatua* L.), rapeseed (*Brassica napus* L.), common lambsquarters (*chenopodium album* L.), morning glory (*Ipomoea* spp. L.), hemp nettle (*Galeopsis tetrahit* L), Canada thistle

(*Cirsium arvense* (L.) *Scop*.), and pigweed (*Amaranthus* spp.) (AAF 2015; Fike 2016; Jankauskiéné et al. 2014).

In North Dakota and Minnesota, hemp is typically planted between mid-May and early June, once the soil has reached 10°C (NDDA 2019). Hemp seed can begin to germinate at 1 to 2°C, but it is best to wait for the ground temperature to reach 8 to 10°C (Bócsa and Karus 1998). Planting in temperatures much colder than that can result in poor and uneven hemp emergence. However, volunteer hemp has been observed to be among the first weeds to emerge in the spring and could be a problem for subsequent crops (J Mettler, personal observation).

Hemp has been observed to emerge and establish quickly and develop dense canopies that have the ability to suppress weeds (Amaducci et al 2015; Fortenbery and Bennett 2004). When hemp is grown for seed (50 to 150 plants m^{-2}), the leaf canopy does not become as dense due to fewer established plants than for fiber hemp (200 to 250 plants m^{-2}) and weed control may become necessary (Hall et al. 2014; Fike 2016; Baxter and Scheifele 2000). Increased light penetration through a less dense hemp canopy allows more weeds to germinate and compete for soil nutrients, water, and space. Research has suggested that weeds are adequately suppressed when hemp density reaches 200 to 250 plants m^{-2} in fiber hemp production. Increasing the seeding rate of oilseed hemp from 20 to 60 or 80 kg ha^{-1} reduced weed density and size by 33% and 34%, respectively, and increased hemp biomass and seed yield by 23% and 34%, respectively (Vera et al. 2006). Planting hemp for seed at 22 to 33 kg ha^{-1} or achieving a final plant density of 110 to 130 plants m^{-2} is common practice in the U.S. and Canada (HGI 2014; Legacy Hemp 2020). Unfortunately, effective weed control practices are likely needed to maintain hemp seed yield at that seeding rate.

Inter-row cultivation would reduce weed pressure and allow time for hemp to shade out competing weeds with rapid growth beginning from the appearance of the sixth leaf pair (Jankauskiene et al. 2014). Mechanical methods of controlling weeds after hemp emergence are difficult without causing injury to the hemp plants (Bócsa and Karus 1998). Cultivating just prior to planting would help reduce the number of competing weeds without causing injury. Hemp's ability to outcompete weeds is dependent on controllable management strategies and unknown climatic conditions (Fike 2016). In general, there could be fewer herbicides used in hemp cropping systems compared with other crops.

A few herbicides are labeled for use in hemp in Canada. Ethalfluralin (85 to 140 g ai ha^{-1}), a seedling inhibitor herbicide, is a granular pre-plant incorporated product called Edge™ that is manufactured by Gowan Agro Canada (Gowan 2018). Quizalofop-p-ethyl (93 g ai ha^{-1}), an acetyl-CoA carboxylase (ACCase) inhibitor herbicide, is labeled as a post-emergence product called Assure II, manufactured by AMVAC (formally DuPont Canada) for control of grasses and volunteer cereal plants in hemp (AMVAC 2020a). In Canada and the U.S., paraquat (545 to 1135 g ai ha^{-1}), is allowed pre-plant to kill emerged weeds (Syngenta 2020). Glyphosate-potassium (840 to 2095 g ae ha^{-1}), is also allowed to be sprayed to kill existing vegetation but must be applied 30 days prior to planting (Monsanto 2018). Spraying a foliar herbicide with no residual control in the soil 30 days prior to planting is not manageable from a weed control standpoint.

Hemp is grown in Europe with minimal herbicide use (Amaducci et al. 2015). The hemp production guide for Italy suggests no herbicide, pesticide, or fertilizer use in hemp (Amaducci 2005). Linuron, a photosystem II inhibitor, has been used in the Czech Republic after planting to control broadleaf weeds within research plots (Tang et al. 2016). China recommends s-metolachlor, acetochlor, and pendimethalin, two seedling shoot inhibitor herbicides and a seedling root inhibitor herbicide, respectively, to be used pre-emergence (Amaducci et al. 2015).

There are no herbicides with Section 3 registration for use in the industrial hemp crop in the U.S. (Flessner et al. 2020; Gray et al. 2017; Anderson 2018). In 2020, states have been able to petition for Section 24(c) registration, a special local need, of quizalofop as Assure II (AMVAC 2020b). The state of Montana will have the ability to control grassy weeds with Assure II in hemp grown for fiber only in 2021. Until recent years, there has been very little research conducted on the effects of herbicides on industrial hemp. This led to a flush of greenhouse herbicide screening experiments on a plethora of herbicides to get an idea of what herbicide sites of action may be feasible to use in hemp.

Greenhouse experiments have included evaluations of pre-emergence herbicides in site of action Groups 2, 3, 4, 5, 7, 12, 13, 14, 15, and 27. The majority of these experiments were conducted using silt loam soil. Chlorimuron-ethyl (40 g ai ha^{-1}), flumetsulam (26 g ai ha^{-1}), and halosulfuron-methyl (35 g ai ha^{-1}), Group 2 acetolactate synthase (ALS) inhibitor herbicides, resulted in 4 to 25% visible hemp injury (Cuvaca et al. 2020; Flessner et al. 2020). Halosulfuron was the least injurious to hemp and resulted in 4% visible injury 21 days after treatment (DAT). Pendimethalin (1600 g ai ha^{1}) was the only Group 3 seedling root inhibitor herbicide studied in the greenhouse and resulted in 0 to 23% injury at various evaluation timings (Byrd 2019; Cuvaca et al. 2020; Flessner et al. 2020). Diuron (2300 g ai ha^{-1}), metribuzin (600 g ai ha^{-1}), and linuron (1400 g ai ha^{-1}), Group 5 photosynthesis inhibitor herbicides, resulted in 15 to 20%, 20 to 100%, and 15 to 24% injury, respectively. Norflurazon (2800 g ai ha^{-1}), Group 12, and clomazone (1400 g ai ha^{-1}), Group 13, proved to be quite injurious with nearly 80% injury and negatively affected emergence (Flessner et al. 2020). Group 14 herbicides that inhibit protoporphyrinogen oxidase

(PPO) resulted in 3 to 50% injury, while Group 15 herbicides that inhibit seedling shoot growth ranged from 17 to 50% injury (Byrd 2019; Cuvaca et al. 2020; Flessner et al. 2020). Isoxaflutole (105 g ai ha^{-1}), a Group 27 pigment inhibitor herbicide, resulted in plant death by 21 DAT.

Greenhouse experiments evaluating hemp response to post-emergence herbicides have been conducted on various site of action groups. Visible percent hemp injury, plant height, and biomass were typically collected 21 DAT, but up to 56 DAT. Group 1 herbicides that inhibit ACCase typically resulted in no plant height or biomass difference compared with the non-treated check (Byrd 2019; Savic et al. 2020). Sethoxydim (300 to 630 g ai ha^{-1}) and quizalofop (92 to 1000 g ha^{-1}), herbicides specific to controlling monocots, have resulted in 40 to 60% visible hemp injury in the greenhouse, yet deemed suitable for use in hemp due to minimal injury observed in the field. All Group 2, ALS, herbicides evaluated resulted in 40 to 70% injury (Byrd 2019; Flessner et al. 2020). Clopyralid (100 to 160 g ae ha^{-1}) and quinclorac (75 to 200 g ai ha^{-1}), Group 4 synthetic auxin herbicides, resulted in 30 to 60% and 5 to 30%, visible hemp injury, respectively (Byrd 2019; Flessner et al. 2020; Ortmeier-Clark et al. 2020; Willenborg and Johnson 2018). Bromoxynil (250 to 300 g ae ha^{-1}) and bentazon-sodium (560 g ai ha^{-1}), Group 6 Photosystem II (PSII) inhibitors, resulted in 30 to 65% visible hemp injury (Byrd 2019; Flessner et al. 2020; Willenborg and Johnson 2018). Linuron (1400 g ai ha^{-1}), was slightly more injurious than the other photosynthesis inhibitor herbicides. Acifluorfen-sodium (2200 g ai ha^{-1}), a PPO inhibitor, the only Group 14 herbicide evaluated, resulted in 68 to 73% visible injury (Flessner et al. 2020). Overall, post-emergence herbicides used on hemp in the greenhouse resulted in significant injury. However, hemp plants were able to recover from initial injury (<35%) caused by the herbicides.

Pre-emergence and pre-plant incorporated experiments have been conducted in the field primarily on silt loam or loam soils on individual active ingredients from several sites of action. A few experiments have examined tank mixes or combinations of active ingredients (Knezevic et al. 2020; Willenborg and Johnson 2018). Acetolactate synthase inhibitors, resulted in greater than 25% injury on a least one evaluation timing, but flumetsulam (26 g ha^{-1}) and halosulfuron (35 g ha $^{-1}$) both resulted negligible injury by 28 DAT (Knezevic et al. 2020; Scott et al. 2020).

Three seedling root growth inhibitors have been evaluated in the field for use in hemp: ethalfluralin, pendimethalin, and trifluralin. Granular ethalfuralin (850 to 3400 g ha^{-1}) resulted in less than 26% visible hemp injury across multiple experiments with an average injury below 10% (Byeongseok and Jean-François 2016; Byeongseok and Ulrich 2014a, 2016a, 2016b). Visible hemp injury from pendimethalin (1065 to 1600 g ha^{-1}) ranged from 0 to 70% across experiments. In some experiments, pendimethalin resulted in lower hemp density and biomass compared with the non-treated check (Anderson 2018; Flessner et al. 2020), but in others, it resulted in 15 to 20% injury to hemp with no negative effects on plant height or seed yield (Lingenfelter 2018, Maxwell 2016; Pearce and Carter 2018; Scott et al. 2020; Woosley et al. 2015). Trifluralin applied at 450, 840, and 1680 g ha^{-1} resulted in 6 to 17%, 8%, and 25% hemp injury, respectively (Pearce and Carter 2018; Howatt and Mettler 2020).

Experiments have also evaluated hemp tolerance to other herbicides applied pre-emergence. Clopyralid (30 g ha^{-1}), a synthetic auxin that is typically applied post-emergence, did not result in hemp injury when applied pre-emergence (Knezevic et al. 2020). Group 5, PSII inhibitor herbicides, resulted in a wide range of response. Metribuzin (278 g ha^{-1}) applied to 'CFX-2' hemp on a silt loam soil resulted in greater than 95% injury across all evaluation timings (Lingenfelter 2018). Metribuzin applied to 'X-59' on a silty clay loam 15 DAT resulted in 25% hemp injury, but by 49 DAT injury was near 0% (Scott et al. 2020). Linuron evaluated in a 2-year field study in loam soil resulted in 60 to 80% injury in the first year and 10 to 15% injury in the second year (Flessner et al 2020). Linuron was applied at 1400 g ha^{-1} in both years, but authors concluded that environments played a large role in hemp tolerance.

Clomazone, a Group 13 carotenoid biosynthesis inhibitor herbicide, was evaluated across four different experiments. When clomazone was applied at 570 g ha^{-1} in sandy or silt loam soils, visible necrosis and injury to hemp was 12% or less (Pearce and Carter 2018, 2019; Scott et al. 2020). When clomazone was applied at 840 g ha^{-1} in a silty clay soil, visible hemp injury was greater than 75% (Scott et al. 2020).

Hemp injury with Group 14, PPO inhibitor, herbicides ranged between 5 and 95%. Flumioxazin applied between 86 and 170 g ai ha^{-1} on silt or sandy loam soils resulted in greater than 95% visible injury to hemp (Lingenfelter 2018; Waters and Burke 2019). Another experiment with flumioxazin at 210 and 430 g ha^{-1} on a silt loam soil resulted in 0 to 10% hemp injury (Pearce and Carter 2019). Differences in hemp tolerance depended on the soil texture, herbicide rate applied, and the hemp cultivar. Hemp injury with fomesafen-sodium applied at 350 or 400 g ai ha^{-1} on silt loam soils resulted in 8 to 96% visible injury (Byrd 2019; Lingenfelter 2018; Maxwell 2016; Woosley et al. 2015). The range of hemp injury with fomesafen was partly attributed to the difference between cultivars under similar soil conditions (Byrd 2019; Maxwell 2016). Two experiments (soil types unknown) that evaluated hemp tolerance to saflufenacil (70 g ai ha^{-1}) resulted in 0 to 2% hemp injury (Knezevic et al. 2020; Pearce and Carter 2018). Sulfentrazone (158 to 313 g ai ha^{-1}) resulted in 0 to 25% injury, unless applied in sandy loam soil where emergence was reduced by 50% (Pearce and Carter 2019; Scott et al. 2020; Waters and Burke 2019; Willenborg and Johnson 2018).

The primary Group 15 herbicides that have been evaluated in the field included acetochlor, s-metolachlor, and pyroxasulfone with average visible hemp injury of 13, 17, and 30%, respectively (Chiriţă 2008; Flessner et al. 2020; Knezevic et al. 2020; Lingenfelter 2018; Maxwell 2016; Pearce and Carter 2019; Waters and Burke 2019). Group 16 and 27 herbicides resulted in greater than 80% injury at various rates and should not be considered for use pre-emergence in hemp (Lingenfelter 2018; Maxwell 2016; Scott et al. 2020; Woosley et al. 2015).

Hemp response to pre-emergence herbicides depended on many factors including soil type, environment, and hemp cultivar (Flessner et al. 2020). The majority of pre-emergence herbicide experiments with hemp have been conducted on loam (Byeongseok A and Ulrich D 2014a; Flessner et al. 2020) and silt loam soils (Anderson 2018; Byrd 2019; Byeongseok and Ulrich D 2016a; Lingenfelter 2018; Maxwell 2016; Pearce and Carter 2019; Woosley et al. 2015), with one experiment conducted on a sandy loam (Waters and Burke 2019) and one on a silty clay loam soil (Scott et al. 2020). Hemp response or tolerance to pre-emergence herbicides can differ due to soil type, environment, climate, and cultivar.

Various herbicide sites of action were also evaluated in the field as post-emergence applications. Group 1, ACCase inhibitors primarily used to control monocot weeds, were relatively safe to use in hemp and often resulted in less than 10% injury (Byrd 2019; Flessner et al. 2020; Lingenfelter 2018; Lingenfelter and Wallace 2019; Pearce and Carter 2018, 2019; Post 2020; Waters and Burke 2019). Injury response was highly variable (10 to 85%) within Group 2 herbicides, but typically resulted in 30 to 60% visible hemp injury (Byrd 2019; Flessner et al. 2020; Howatt and Mettler 2018; Lingenfelter 2018; Lingenfelter and Wallace 2019; Maxwell 2016; Ostlie 2019; Scott et al 2020; Woosley et al 2015). Hemp response to Group 4 herbicides was also widely variable, 0 to 95% injury (Flessner et al. 2020; Howatt and Mettler 2018; Pearce and Carter 2018; Scott et al 2020; Willenborg and Johnson 2018).

Hemp was most tolerant to clopyralid (70 to 300 g ha^{-1}), 2,4-DB (250 g ai ha^{-1}), and quinclorac (840 g ha^{-1}), with 0 to 35% injury, while fluroxypyr-meptyl (40 to 160 g ai ha^{-1}), fomesafen (85 g ha^{-1}), and metribuzin (85 g ha^{-1}) resulted in greater than 60% injury (Howatt and Mettler 2018; Scott et al. 2020). Bromoxynil (210 to 560 g ha^{-1}) has been one of the most extensively studied broadleaf herbicides used post-emergence and has typically resulted in less than 15% hemp injury (Byeongseok and Ulrich 2014b; Flessner et al. 2020; Howatt and Mettler 2018; Lingenfelter 2018; Lingenfelter and Wallace 2019; Maxwell 2016; Pearce and Carter 2018, 2019; Post 2020; Waters and Burke 2019; Willenborg and Johnson 2018; Woosley et al. 2015).

Pyroxasulfone, one of the Group 15 herbicides evaluated, resulted in less than 5% injury when applied at a rate of 90 g ai ha^{-1}, but up to 20% injury when applied at 180 g ai ha^{-1} (Pearce and Carter 2019; Post 2020; Waters and Burke 2019). Pyroxasulfone is applied more predominantly as a pre-emergence herbicide and does not control emerged weeds. Monosodium acid methanearsonate (MSMA) (2270 to 4540 g ai ha^{-1}), a Group 17 herbicide, resulted in less than 10% injury (Maxwell 2016; Woosley et al. 2019).

In Group 27, mesotrione applied at 105 g ai ha^{-1} resulted in 70 to 80% visible hemp injury, while topramezone (18.4 g ha^{-}1) has been reported to cause less than 5% visible injury to hemp (Howatt and Mettler 2018; Lingenfelter 2018). This range of observed hemp response to topramezone observed could be attributed to the variable tolerance among cultivars (Flessner et al. 2020; Maxwell 2016). Cultivar choice appears to be an important factor in the agronomic success of hemp (Amaducci et al. 2015; Tang et al. 2016).

In many of these experiments, the authors noted that hemp had the ability to overcome moderate hemp injury due to herbicides (Maxwell 2016; Byrd 2019; Flessner et al 2020). The Canadian Hemp Trade Alliance (2019) confirmed the ability of hemp to overcome the effect of contact herbicides if applied when hemp was greater than 5-cm tall. Hemp has the ability to recover from herbicide leaf necrosis and will continue to develop at any uninjured nodes. Environments, cultivars, and application timings have varied among experiments that have evaluated herbicide use in hemp and resulted in varied response information. Evaluation of herbicide timings and consistent descriptions of hemp stage would be beneficial in moving forward with any herbicide registration.

Control of broadleaf weeds without the use of such herbicides could be difficult. In addition to controlling weeds within a hemp production system, control of volunteer hemp in a successive crop is equally important. Hemp is a very competitive plant. According to Reisinger et al. (2005), wild hemp (*Cannabis sativa* ssp. *spontanea*) is a problematic weed which can germinate in early spring, become a nuisance in wheat fields, and require control with a post-emergence herbicide if the population becomes too large. Volunteer hemp is a concern with the increasing amount of hemp acres in production. Hemp is susceptible to many soil-applied herbicides used to control glyphosate-tolerant volunteer crops (CHTA 2019). Glyphosate or glufosinate-ammonium applied as a pre-plant burndown herbicide is also an effective method to control volunteer hemp. Rotating hemp in succession with soybean or corn that have glyphosate- or glufosinate-resistance traits could be a viable option to control volunteer hemp from the previous year (CHTA 2019; Howatt and Mettler 2018).

CHAPTER 2. PRE-EMERGENCE HERBICIDES

2.1. Materials and Methods

2.1.1. Preliminary Greenhouse Experiment

A preliminary greenhouse experiment to identify the least injurious pre-emergence herbicides to hemp was conducted in the winter of 2019 in Fargo, ND. The purpose of this greenhouse experiment was to identify herbicides which could be studied further in the field for use in hemp. Treatments included herbicides with various sites of action (Table 2.1). The selection of herbicides and the rates of each were determined by the commonly used rates in wheat, corn, and soybean cropping systems. Chemical companies and their contact information is provided in Table 2.2.

Table 2.1. Pre-emergence herbicide treatments to determine hemp tolerance in the greenhouse.

Active ingredient	Rate		Product	Source[a]	Site of action
	g ae or ai ha^{-1}				Group
Imazethapyr-ammonium	44	ae	Pursuit	BASF	2
Pendimethalin	1120	ai	Prowl H_2O	BASF	3
Trifluralin	560	ai	Treflan HFP	Gowan	3
Quinclorac-methyl	289	ae	Facet L	BASF	4
Atrazine	426	ai	AAtrex 4L	Syngenta	5
Metribuzin	277	ai	TriCor 75 DF	UPL	5
Flumioxazin	89	ai	Valor EZ	Valent	14
Saflufenacil	38	ai	Sharpen 4F	BASF	14
Sulfentrazone	158	ai	Spartan	FMC	14
Acetochlor	1053	ai	Harness	Bayer	15
Dimethenamid-P	840	ai	Outlook	BASF	15
Ethofumesate	3361	ai	Ethofumesate 4SC	Willowood	15
S-Metolachlor	1597	ai	Dual Magnum	Syngenta	15
Pyroxasulfone	109	ai	Zidua SC	BASF	15
Isoxaflutole	53	ai	Balance Flexx	Bayer	27
Mesotrione	105	ai	Callisto	Syngenta	27

[a] Source or company information was provided in Table 2.2.

Table 2.2. Chemical company contact information.

Company	Address	City	State	Zip code	Phone number
ALBAUGH, LLC	1525 NE 36th Street	Ankeny	IA	50021	1-816-238-3377
AMVAC Chemical Corporation	4695 MacArthur Court, Suite 1200	Newport Beach	CA	92660	1-888-462-6822
BASF Corporation	26 Davis Drive	Research Triangle Park	NC	27709	1-800-832-4357
Bayer CropScience	800 N. Lindberg Blvd.	St. Louis	MO	63167	1-866-992-2937
Corteva agriscience[a]	P.O. Box 80735	Wilmington	DE	19805	1-833-367-8382
FMC Corporation	2929 Walnut Street	Philadelphia	PA	19104	1-215-299-6000
Gowan Company	P.O. Box 5569	Yuma	AZ	85366	1-800-883-1844
Nufarm, Inc.	11901 S. Austin Avenue	Alsip	IL	60803	1-708-377-1330
Syngenta Crop Protection, LLC	P.O. Box 18300	Greensboro	NC	27419	1-800-334-9481
United Phosphorous, Inc (UPL)	630 Freedom Business Center, Suite 402	King of Prussia	PA	19406	1-866-761-9397
Valent U.S.A. LLC	P.O. Box 5075	San Ramon	CA	94583	1-800-682-5368
Willowood, LLC[b]	385 Interlocken Crescent, Suite #240	Broomfield	CO	80021	1-866-396-0465
Winfield Solutions, LLC	P.O. Box 64589	St. Paul	MN	55164	1-936-870-5516

[a] Corevta agriscience formally Dow AgroSciences, DuPont and Pioneer.
[b] Willowood, LLC assets were acquired by Generic Crop Science in 2019.

Hemp ‘CFX-2’ (*Cannabis sativa* L. Hemp Genetics International) was selected to be grown in these experiments. CFX-2 is a short, dioecious, high-yielding, moderately large-seeded cultivar with a relatively short growing season of about 103 days (HGI 2014).

Hemp seed were planted into 10.5-cm by 10.5-cm square plastic containers (SKU: 691405, Beldon Plastics, St. Paul, MN.), hereafter called pots, that were 8.9 cm deep with a holding capacity of 750 mL. Each pot was an experimental unit. A multi-fold brown paper towel was folded in thirds and placed on the bottom of the pot to prevent the loss of soil through the holes on the bottom of the pot and to help retain soil moisture during the experiment. A volume of 300 mL of dry potting mix (Pro-mix® Bx, Premier Tech Horticulture, Quakertown, PA) was placed in each pot on top of the paper towel and then lightly packed. Approximately 150 mL of dry non-treated mineral soil was poured on top of the potting mix to achieve a mineral soil depth of 1.9 cm. The mineral soil texture was classified as a sandy loam with a pH of 7.4 and 39 g kg^{-1} organic matter (S&S Landscaping Co., Inc. Fargo, ND). The mineral soil was autoclaved (PSS5-K-MSSD, Primus Sterilizer Company, Omaha, NE) at 122.8°C for 120 minutes prior to being used in order to prevent foreign weeds and pathogens from confounding the experimental process and results.

In preparation for applying the soil treatments, 1000 mL of non-treated soil was placed in 25.7-cm by 25.7-cm square standard plastic inserts (ST1-201, TO Plastics, Clearwater, MN.) with roughly the same surface area of five replicates of a single treatment. Each tray of soil was sprayed with a single treatment. Treatments were applied using a chamber sprayer (DeVries Manufacturing. Hollendale, MN. Serial number SB8-131) equipped with Turbo TeeJet 11001 nozzle tip and traveling 2.82 km h^{-1} to deliver 159 L ha^{-1} at 276 kPa (Spraying Systems Company 2014). A 11001 nozzle was used to create a fine droplet size to help evenly disperse the herbicide across the soil surface. Immediately after the herbicide treatment was applied, the soil in an insert was transferred into a 7.6 L plastic bag and gently rolled end over end for 1 minute. The herbicide was thus incorporated and dispersed throughout the soil to mimic field incorporation through cultivation or rain event. Since trifluralin required incorporation, as per the label, all herbicides were incorporated to maintain consistency across treatments.

In order to achieve a planting depth of 2 cm, a glass beaker was used to measure a volume of 200 mL of treated soil that was gently poured over 10 evenly spaced hemp seeds. A different beaker was used for each treatment to avoid contamination. Germination of the CFX-2 seed lot used was approximately 85%, so it was anticipated that eight to nine plants would establish if the herbicide had no negative impact on emergence. All water applications were completed using reverse osmosis water and each pot or experimental unit received the same amount during each watering event. Due to the hydrophobic nature of this soil created by the autoclave, the first water application of 60 mL was a 5% solution of non-ionic surfactant in water to aid in water infiltration. The first watering event was conducted the same day as herbicide applications and seeding. Hemp plants were watered as needed to maintain a moist soil profile.

The number of plants emerged were recorded 5 and 25 days after emergence (DAE). Days after emergence was considered the number of days after emergence of non-treated hemp. The first evaluation timing included all plants that emerged from the soil, including plants that were necrotic and dead, to assess percent emergence. At the last evaluation, the number of plants more than 3 cm tall were counted to determine the percent of plants that successfully established for calculation of the effective percent plant loss (Equation 2.1) due to the herbicide application. Hemp plants less than 3 cm were not included in the final plant counts as such plants were significantly delayed physiologically to the point where the plants would likely not contribute to fiber or seed yield in a competitive field setting.

$$Percent\ plant\ loss = 1 - \left(\frac{\#\ of\ plants\ at\ 25\ DAE}{\#\ of\ plants\ at\ 5\ DAE}\right) * 100 \qquad (2.1)$$

Hemp plants were evaluated for percent injury at 5, 15, and 25 DAE. Injury included visible herbicide symptoms including, but not limited to, stunting, chlorosis, leaf cupping, necrotic spots, and plant death. Specific injury varied depending on site of action of herbicide applied. Hemp injury evaluations were on a scale of 0 to 100% with 0% representing no visible injury and 100% representing complete plant death. Plant death in this experiment was defined as the lack of visible, above ground, photosynthetically active (green) plant tissue. Root damage due to herbicide application was not quantified in this experiment.

After all visible injury ratings were completed, plants were harvested. All plants in each pot were cut at the soil surface, placed into a paper bag (S&G Brown #8, Dacotah Paper Co., Fargo, ND) and then weighed using an electronic scale tared for the bag weight (A&D Company, Limited, San Jose, CA. GX-200, 50004). The samples were then placed into a drier at an average temperature of 38°C until weight stabilized. Once removed from the drier, dry weights were recorded and samples discarded.

Automated greenhouse controls (Argus Control Systems Ltd. Surrey, British Columbia, Canada) were utilized to control the lighting, cooling, and heating systems of the greenhouse. High pressure sodium light fixtures (P.L. Light Systems, Inc., Beamsville, Ontario, Canada) delivering 400 µmol m^{-2} s^{-1} were utilized to supplement natural light and set to a 16-h photoperiod to prevent the photoperiod-sensitive hemp from flowering (Anderson 2018). The greenhouse room was set to 21°C. Hemp was fertilized with Peters General Purpose 20 to 20 to 20 fertilizer (JR Peters, Inc., Allentown, PA). Nitrogen was applied at concentrations of 200 mg L^{-1} when the hemp was less than 5 inches tall, increasing to 600 mg L^{-1} as the plants increased in size. Each pot received 80 ml of fertilizer solution weekly. Pots were rearranged biweekly to minimize the effect of microenvironment and uneven lightening in the greenhouse.

Greenhouse experiments were conducted in a randomized complete block design (RCBD) with five replicates of each treatment. The experiment was conducted twice, months apart, establishing two environments. All data from each environment was combined for analysis of variance in SAS 9.4 (Statistical Analysis Software 2003, version 9.4, SAS Institute, Inc., Cary, NC 27513). Environment and replicates were considered random effects and herbicide treatment as a fixed effect. Environments were combined for analysis. Means were separated by Fisher's protected LSD with α=0.05. Injury and plant count data from each evaluation timing were analyzed separately. Fresh and dry weights were adjusted in SAS with plant number as a covariate since not all pots had the same number of plants. Treatments that resulted in less than

40% visible hemp injury were considered for further investigation in a variable rate field experiment.

2.1.2. Field Experiment

The primary goal of the experiment was to identify pre-emergence herbicides to be further evaluated and developed for registration and use in industrial hemp. Evaluation of hemp tolerance to pre-emergence herbicides in the field was conducted in 2019 and 2020 near Fargo, Prosper, Casselton, and Hillsboro, North Dakota. Soil fertility and mechanical analysis for the four locations are provided in Table 2.3 and 2.4, respectively. Nitrogen was applied via urea fertilizer to provide 112 kg ha^{-1} nitrogen at the start of each growing season. The Hillsboro 2019 site was the only site that did not receive the nitrogen fertilizer.

Table 2.3. Fertility analysis for 2019 and 2020 experiment locations.

Location	Year	Depth	NO_3-N[a]	P	K	S	pH	EC	CEC	OM
		- cm -	kg ha^{-1}	ppm	ppm	kg ha^{-1}		mmhos cm^{-1}	Meq $100g^{-1}$	g kg^{-1}
Casselton	2019	0 - 30	47	15	671	n/a[b]	7.4	n/a	n/a	45
Fargo	2019	0 - 30	66	14	445	18	7.6	0.56	35.4	63
Hillsboro	2019	0 - 30	26	22	420	17	7.1	0.25	18.4	38
Prosper	2019	0 - 15	80	60	358	n/a	8.2	n/a	22.5	40
Fargo	2020	0 - 30	50	23	935	8	7.5	0.60	46.9	48
Hillsboro	2020	0 - 30	27	23	306	10	6.6	0.20	18.0	26
Prosper	2020	0 - 30	62	50	643	17	6.7	0.46	24.7	38

[a] Soil analysis type: pH in water; NO^3-N extracted with water; Organic Matter (OM) percent by ignition; Phosphorus (P) by Olson procedure; Potassium (K) by 1N ammonium acetate; SO_4-S extracted with 500 ppm P as monobasic calcium phosphate soluble salts (EC) in 1:1 soil:water; Cation Exchange Capacity (CEC).
[b] Particular soil analysis data was not available (n/a) for every location.

Table 2.4. Soil mechanical analysis by location.

Location	Sand	Silt	Clay	Texture[a]
	--------------------	%	-----------------	
Casselton	6.2	54.4	39.4	Silty Clay Loam
Fargo	2.8	48.1	49.1	Silty Clay
Hillsboro	59.9	28.7	11.4	Sandy Loam
Prosper	23.0	52.5	24.5	Silt Loam

[a] Mechanical analysis by hydrometer method.

Herbicides selected for this field experiment (Table 2.5.) were a collection of the safest herbicides applied in the preliminary greenhouse study previously described in Chapter 2. In addition to the safest herbicides, a few other herbicides were included to represent additional herbicide sites of action. Two herbicide rates were applied in the field experiment. The low rate of each herbicide was determined by the commonly used rate in wheat, corn, or soybean cropping systems. In the treatment list and for identification hereafter, the number 1 following an active ingredient represents the low rate while the number 2 represents the high rate. The high rate was double the amount of active ingredient in the low rate. The high rate was included in the experiment to represent areas of herbicide overlap that could be present in the field, and also to demonstrate hemp safety beyond the standard use rates. Each experiment had a non-treated check plot, as well as a hand-weeded check in each replicate.

Table 2.5. Pre-emergence herbicide treatments and associated site of action group for evaluation of hemp tolerance.

Treatment	Rate		Product	Source[a]	Site of action
	g ae or ai ha^{-1}				Group
Non-treated	n/a	n/a	n/a	n/a	n/a
Hand-weeded	n/a	n/a	n/a	n/a	n/a
Imazethapyr-ammonium-1[b]	44	ae	Pursuit	BASF	2
Imazethapyr- ammonium-2	88	ae	Pursuit	BASF	2
Pendimethalin-1	1120	ai	Prowl H_2O	BASF	3
Pendimethalin-2	2240	ai	Prowl H_2O	BASF	3
Trifluralin-1	840	ai	Treflan HFP	Gowan	3
Trifluralin-2	1680	ai	Treflan HFP	Gowan	3
Quinclorac-methyl-1	290	ai	Facet L	BASF	4
Quinclorac-methyl-2	580	ai	Facet L	BASF	4
Saflufenacil-1	38	ai	Sharpen	BASF	14
Saflufenacil-2	76	ai	Sharpen	BASF	14
Acetochlor-1	1050	ai	Harness	Bayer	15
Acetochlor-2	2100	ai	Harness	Bayer	15
Pyroxasulfone-1	109	ai	Zidua SC	BASF	15
Pyroxasulfone-2	218	ai	Zidua SC	BASF	15

[a] Source or company information was provided in Table 2.2.

[b] Herbicide active ingredient followed by a numerical digit represent a low (1X) and high (2X) rate of that active ingredient based on use patterns in other crops.

Hemp 'X-59' was selected for use in this experiment (*Cannabis sativa* L., Legacy Hemp LLC.). This cultivar is a dioecious seed/fiber cultivar that grows approximately 2 m tall with a growing season of about 110 days. X-59 originated from Russian lines 'in 50' and 'in 29'and was further developed in Canada for early maturity, early flowering males, shorter height, preferred fatty acid profile, and high yield (CFIA 2021). X-59 is also promoted for its large seed size, superb shatter resistance, and sweet nutty flavor (Legacy Hemp 2020). The expected yield for this cultivar ranges from 890 to 1680 kg ha^{-1}, but can reach up to 2240 kg ha^{-1} in productive soils.

Experimental units (plots) were established on recently tilled ground with a dimension of 3.7 m wide by 9.1 m long. Plots consisted of eight hemp rows spaced 38 cm apart and were seeded using a Great Plains 3P600 drill to achieve 206 seeds per m^2, a desired plant population of 46 plants per m of row. Seeding rate was determined by calibrating the seed output of the planter and the germination percentage of seed stock and then adjusted for an expected 25% plant mortality rate. A standard row spacing for hemp being grown for seed is 18 cm (Darby et al. 2017). However, a wider spacing was used in this study in the anticipation of 38 cm becoming the standard spacing to increase air movement within the hemp rows in order to reduce disease incidence.

Herbicide treatments were applied using a CO_2-pressurized backpack sprayer and hand-boom. The hand-boom was 2.53 m wide with five nozzles spaced 50.8 cm apart, effectively spraying 3 m wide and leaving 0.67 m on the edge of each plot or one row of hemp as non-treated material for comparison in addition to the non-treated check plot for treatment analysis. Herbicide applications were made with Tee Jet 11002 nozzle tips at 276 kPa to deliver 159 L ha^{-1} at a walking speed of 5.63 km h^{-1} (Spraying Systems Company 2014). The hand-boom was held approximately 50 cm above the soil surface during the application. No adjuvants were used in conjunction with the pre-emergence herbicide treatments.

Trifluralin required mechanical incorporation. Trifluralin was applied to the designated plots, and then the entire study area was tilled using a John Deere 670 roto-tiller to thoroughly incorporate trifluralin while maintaining a consistent seedbed throughout the entire experiment. Once the cultivation was complete, hemp was seeded and then the remaining herbicides were applied. Experiments were established just prior to a rain event when possible to aid in rapid plant establishment and herbicide availability. No post-emergence herbicides were applied to these experiments. The hand-weeded check plots were weeded as often as necessary to maintain a weed-free plot throughout the growing season, resulting in minimal weed competition.

Data collected from each of the plots included visible hemp injury, density, height, photosynthetically active radiation (PAR), and seed yield. Due to the labor and time necessary to hand-harvest plots, seed yield was only obtained from the plots that received the low rate of herbicides. Hemp plants were evaluated for percent visible crop injury compared to the controls at 7, 14, and 28 DAE on a scale of 0 to 100%, with 100% representing complete plant death and 0% representing no visible herbicide injury. Herbicide symptoms included stunting, necrotic spots, yellowing, epinasty, or some combination of symptoms. Specific injury expected varied depending on the site of action of the herbicide applied.

In order to facilitate repeat measurements of hemp density and plant height, two 1-m segments of row within hemp rows three and six were flagged and designated for data collection. One segment was located in the front half of the plot and the other in the back half of the plot. Hemp density was determined by counting the number of plants in each meter segment in the two inner rows. Density measurements took place 21 and 49 DAE. Plant height measurements were obtained from each plot 21, 49 (pollen shed), and 70 (post male senescence) DAE. Hemp height measurements were collected on three tagged plants from each meter segment with a plot, totaling six height measurements from each plot. Heights were measured to the nearest centimeter from the soil surface to the top of the terminal growth.

Photosynthetically active radiation interception, herein referred to as light interception (LI), was measured 14, 28, 49, and 84 DAE. The 14 DAE measurement was a baseline measurement prior to canopy closure between the rows. The last measurement was taken just days before harvesting the plots. Light interception was measured using a ceptometer (AccuPAR model LP-80 PAR/LAI, METER Group, Inc., Pullman, Washington, USA) and collected within 2 hrs of either side of solar noon. The ceptometer measures PAR both above and below the canopy to determine canopy growth and light interception (METER Group, Inc. 2020). The below canopy bar (Figure 2.1) consisted of 80 independent sensors spaced 1 cm apart to measure the PAR in the 400 to 700 nm wave band.

Figure 2.1. Ceptometer placement between hemp rows.

The light collection bar was placed diagonally between hemp rows at the height of the weed canopy during the moments of data collection, measuring the LI of the effective weed control canopy (Figure 2.1). For example, when measuring LI for the hand-weeded check plots, the probe was at ground level because there were no weeds present. When measuring LI on the non-treated check the measurements were often taken within a foot from the top of the hemp canopy because that was the height of the weed canopy. If the measurement was obtained consistently at ground level, below the weed canopy, the weed canopy would have confounded the reading. Weeds commonly growing within the study areas included redroot pigweed (*Amaranthus retroflexus L.*), waterhemp (*Amarathus tuberculatus),* common lambsquarters (*Chenopodium album* L.), common ragweed (*Ambrosia artimisiifolia* L*.),* common purslane (*Portulaca oleracea* L.), common cocklebur (*Xanthium strumarium* L.), common sunflower (*Helianthus annus* L.), and Venice mallow (*Hibiscus trionum* L.). Quizalofop, labelled for use in Canada, was applied at 93 g ha^{-1} post-emergence at locations where monocot weeds were problematic, as this study focused on herbicides that control broadleaf weeds (AMVAC 2020a).

Equation 2.2 below was used to determine the LI of hemp canopy after obtaining the PAR values. Light interception was expressed as a percent, ranging from 0 to 100%. Results of 0% indicated that there was no hemp canopy as a result of herbicide injury or weeds were taller than the hemp, leaving no effective weed control canopy (Purcell 2000). An LI value of 100% indicated that all light within the 400 to 700 nm wave length was absorbed by a dense and lush hemp canopy to the point where no light was available to the weeds, in theory, effectively controlling the weeds.

$$LI = [1 - (PAR\ beneath\ canopy)\ X\ (PAR\ above\ canopy)^{-1}]\ X\ 100 \qquad (2.2)$$

Approximately 102 days after sowing or 85 to 95 DAE, when the seed was 18 to 25% moisture, plots with low rates of each herbicide along with the check plots were harvested for seed by hand. All hemp seed heads within rows three and six were harvested, put in a cloth bag, and placed in a drier. The hemp seed heads were dried to approximately 8% moisture at 20 to 25°C. The seed heads were then put through a stationary plot thresher (Low Profile LPR-G, Almaco, Nevada, IA 50201) to separate the hemp seeds from vegetation. In 2019, percent moisture (Equation 2.3) was determined for the seed harvested from each plot, which was adjusted to an 8% storage moisture to determine yield. In 2020, a GAC 2100 moisture tester (Dickey-John Corp., Minneapolis, MN) was calibrated to determine percent moisture of the seed.

$$Grain\ Moisture = [(Fresh\ Wt - Dry\ Wt)\ X\ Dry\ Wt^{-1}]\ X\ 100 \qquad (2.3)$$

All data was combined for analysis of variance in SAS 9.4 (Statistical Analysis Software 2003, version 9.4, SAS Institute, Inc., Cary, NC 27513) using Proc Glimmix with normal distribution. Experiments were conducted in a RCBD with four replicates. Experimental environments, and replicates were considered random effects and herbicide treatment as a fixed effect. Year and location were combined as a random “environment” effect. Combined analysis occurred across environments (year and location) when the mean square error values were within a factor of ten and when variables passed the Bartlett’s Chi-square test for homogeneity. Means were separated by Fisher’s protected LSD with α=0.05. For the purpose of this experiment, treatment by time interactions were not considered in the statistical analysis, as individual treatment differences were sufficient to make conclusions regarding hemp tolerance to herbicides.

2.2. Results and Discussion

2.2.1. Preliminary Greenhouse Experiment

The pre-emergence herbicide screening in the greenhouse was conducted as a precursor to field evaluations and, therefore, data analysis did not evaluate time and time by treatment interactions for any data type. Pre-emergence herbicide effect on percent emergence and percent plant loss was evaluated at 5 and 25 days after DAE, respectively (Table 2.6). If herbicides inhibited hemp emergence by more than five days, or resulted in seed mortality, that would be identified in the 5 DAE evaluation timing.

Table 2.6. Visible injury, emergence, and plant loss data of industrial hemp as a result of pre-emergence herbicide treatments in the greenhouse.

	Visible injury[a]			Emergence[b]	Plant loss[c]
Treatment	5 DAE[d]	15 DAE	25 DAE	5 DAE	25 DAE
	---------------------- % ----------------------			--------------- % --------------	
Non-treated	0 a[e]	0 a	0 a	84 a	2 ab
Imazethapyr-ammonium	9 ab	14 ab	15 b	77 abc	3 ab
Pendimethalin	2 a	6 ab	12 ab	80 a	3 ab
Trifluralin	4 a	13 ab	14 ab	76 abc	1 a
Quinclorac-methyl	1 a	8 ab	16 b	78 ab	0 a
Atrazine	5 a	51 c	50 def	82 a	43 f
Metribuzin	25 de	98 g	99 g	85 a	100 h
Ethofumesate	96 i	96 g	96 g	46 d	99 h
Flumioxazin	18 bcd	18 b	24 bc	77 abc	8 abc
Saflufenacil	65 h	58 cd	38 cd	72 abc	43 f
Sulfentrazone	27 def	50 c	37 cd	73 abc	19 cde
Acetochlor	32 ef	69 de	64 f	65 bc	17 b-e
Dimethenamid-P	21 cd	62 cde	49 de	77 abc	9 abc
S-Metolachlor	25 de	57 cd	42 d	75 abc	12 a-d
Pyroxasulfone	37 f	77 ef	89 g	64 c	26 de
Isoxaflutole	10 abc	59 cd	51 ef	77 abc	32 ef
Mesotrione	49 g	89 fg	91 g	79 a	80 g

[a] Evaluation on a scale of 0% (no injury) to 100% (plant death). Visual evaluation of herbicide symptoms.

[b] Percent emergence determined on number of hemp plants out of the 10 planted per pot.

[c] Percent plant loss determined by the difference between hemp count at 25 DAE and 5 DAE, percentage plants that died after emergence.

[d] Days after emergence (DAE) of the non-treated hemp.

[e] Means within column followed by the same letter are similar according to F-protected LSD at $\alpha=0.05$ level of significance

The non-treated check exhibited 84% germination and emergence (Table 2.6). Three pre-emergence herbicides resulted in less emergence than the non-treated check. Group 15, long chain fatty acid inhibiting herbicides, acetochlor, pyroxasulfone, and ethofumesate resulted in 65, 64, and 46% emergence, respectively, compared to the non-treated hemp. All three of the herbicides that resulted in reduced emergence inhibit very-long-chain fatty acid (VLCFA) biosynthesis. While not influencing germination per se, VLCFA-inhibiting herbicides can inhibit emergence from the soil or severely stunt emerged plants (Cobb and Reade 2010). It is interesting to note that all other herbicides, including other Group 15 herbicides such as s-metolachlor and dimethenamid-p, resulted in similar emergence to the non-treated. Non-encapsulated formulations of Group 15 herbicides were used to ensure maximum interaction of hemp with herbicide. Therefore, differences in the hemp response to Group 15 herbicides were likely due to the ability of hemp to metabolize the particular active ingredient or influence of the difference in sorption coefficients (K_d) of the herbicides.

In order for pre-emergence herbicides to cause plant injury, the herbicide must be available to the plant for uptake, typically in the water fraction of soil. Herbicide properties such as water solubility, half-life, mobility, and K_d influence the herbicidal efficacy because of the way they interact with soil properties to affect availability to plants. With the Group 15 herbicides evaluated, the $K_{d,}$ at a similar level of organic matter in the greenhouse soil, was 8.24, 4.60, and 3.26 L kg^{-1} of s-metolachlor, dimethenamid-p, and pyroxasulfone, respectively (Westra 2012). The higher the K_d the more tightly the herbicide is bound to the soil, making it less available to the hemp plant. This could partially explain why hemp emergence was less impacted by s-metolachlor and dimethenamid-p as compared to pyroxasulfone. Incorporating all pre-emergence herbicides in the greenhouse experiment could have also influenced the herbicide efficacy as well. Incorporation moves the herbicide closer to the germinating seed and seedling structures for more access than if left on the soil surface. If this experiment was to be conducted again, only those herbicides that require incorporation should be incorporated, to better simulate what would happen in the field.

Plant loss per pot was evaluated to determine delayed hemp death and capture any natural damping off of hemp plants. Percent plant loss was determined by the difference between hemp number at 25 DAE and 5 DAE. About 2.2% of initial established hemp in the non-treated soil died before 25 DAE. Imazethapyr, pendimethalin, trifluralin, quinclorac, flumioxazin, acetochlor, dimethenamid-p, and s-metolachlor resulted in similar plant loss to the non-treated. Applications of isoxaflutole, pyroxasulfone, and sulfentrazone resulted in 17 to 32% plant loss. Saflufenacil and atrazine resulted in a 43% reduction. Hemp plants experienced severe plant losses of 80% or greater with metribuzin (Group 5), ethofumesate (Group 15), and mesotrione (Group 27).

Group 27, carotenoid biosynthesis inhibitors, inhibit plastoquinone biosynthesis and result in a reduction in photosynthetic electron flow leading to bleached leaves (Figure 2.2) (Cobb and Reade 2010). Isoxaflutole was not as injurious as mesotrione (Table 2.6). Isoxaflutole is a pro-herbicide that must be metabolized or chemically converted to form the hydroxyl phenylpyruvate dioxygenase (HPPD) inhibiting compound. The difference in our observations might have been influenced by this intermediary step in phytotoxic expression.

Figure 2.2. Bleaching symptoms on hemp with mesotrione 5 days after emergence.

The Group 5 photosystem II inhibitors atrazine and metribuzin resulted in 43 and 100% plant loss, respectively. Photosystem II inhibitors disrupt the photosynthetic electron flow, producing toxic triplet chlorophyll and singlet reactive oxygen species that result in lipid peroxidation (Cobb and Reade 2010). This lipid peroxidation results in damaged cellular structure once photosynthesis takes place, which explains why no reduction in emergence was overserved with Group 5 herbicides. Symptoms included chlorosis and necrosis at the leaf margins.

Visible hemp injury was evaluated at 5, 15, and 25 DAE (Table 2.6). Injury symptoms observed included necrosis, chlorosis, epinasty, and stunting as compared to the non-treated. Specific injury varied depending on site of action of herbicide applied. The progression of the level of injury can be seen by comparing the percent injury of the three evaluation timings. Figures 2.3 and 2.4 depict a visual scale of the percent visible injury ranging from 0% for the non-treated (unlabeled) to 99% injury at 25 DAE. As a preliminary screening trial to determine overall herbicide safety, 25 DAE is a practical evaluation time to discuss as hemp plants were already recovering from initial herbicide symptoms.

Figure 2.3. Percent visible hemp injury (0 to 50%) from pre-emergence herbicides 25 days after emergence.

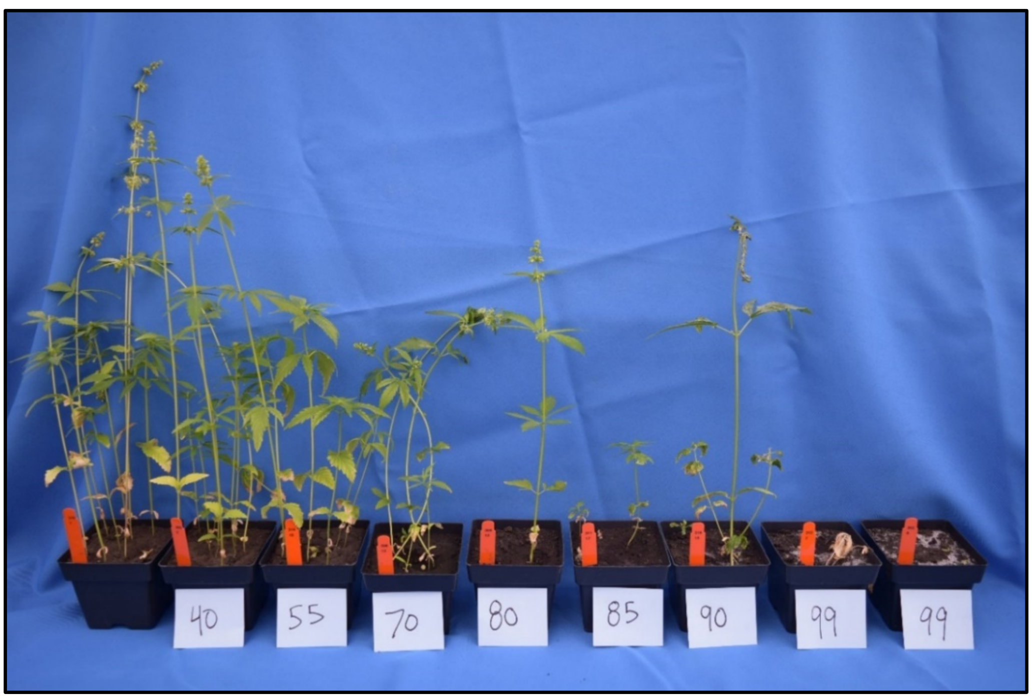

Figure 2.4. Percent visible hemp injury (40 to 99%) from pre-emergence herbicides 25 days after emergence.

Pendimethalin and trifluralin effects on hemp were similar to the non-treated at 25 DAE, although they resulted in 12 and 14% injury, respectively (Table 2.6). Hemp response to imazethapyr and quinclorac were similar at injuries of 15 and 16%, respectively. Sulfentrazone, saflufenacil, s-metolachlor, dimethenamid-p, atrazine, and isoxaflutole, in ascending order, resulted in 37 to 51% injury to hemp. Saflufenacil, acetochlor, and pyroxasulfone in similar greenhouse experiments have resulted in 0 to 5%, 4 to 17%, and 46 to 50% visible injury, respectively (Cuvaca et al. 2020). Hemp response to acetochlor in this experiment resulted in 64% injury. Comparatively, greater hemp injury was observed in this experiment than similarly conducted greenhouse experiments. Metribuzin, ethofumesate, pyroxasulfone, and mesotrione resulted in severely injured hemp, greater than 80%. Differences in hemp response can vary between greenhouse environments and the type of soil used, and the scale of visible injury can also vary between observers.

Fresh biomass was collected per pot at 25 DAE and dried to determine dry weight biomass (Table 2.7). In terms of fresh weights, all herbicides apart from atrazine, metribuzin, ethofumesate, pyroxasulfone, isoxaflutole, and mesotrione were similar to the non-treated. The non-treated resulted in 8.9 grams of fresh weight. Protoporphyrinogen oxidase (PPO) and VLCFA inhibitors typically resulted in fresh weights similar to the non-treated, but dry weights that were less than the non-treated. Hyroxyphenyl pyruvate dioxygenase (HPPD) herbicides such as isoxaflutole and mesotrione resulted in lower fresh and dry weights compared to the non-treated. The non-treated resulted in 2.3 grams of dry plant material per pot. Pots with hemp plants that resulted in greater than 1.9 grams of dry biomass were considered similar to the non-treated. Those herbicides included imazethapyr, pendimethalin, trifluralin, quinclorac, and saflufenacil. S-metolachlor, dimethenamid-p, acetochlor, and sulfentrazone resulted in hemp weights of 1.2 to 1.6 grams per pot. In addition to the HPPD herbicides, pyroxasulfone, ethofumesate, metribuzin, and atrazine all resulted in less than 1.0 gram of dry biomass, half of the weight of the non-treated hemp.

Table 2.7. Fresh and dry biomass of surviving plants per pot for pre-emergence greenhouse experiment.

Treatment	Biomass[a]	
	Fresh	Dry
	---------- g pot^{-1} ----------	
Non-treated	8.9 a[b]	2.3 a
Imazethapyr-ammonium	8.8 a	2.2 ab
Pendimethalin	9.0 a	2.3 a
Trifluralin	8.2 a	2.1 a-d
Quinclorac-methyl	9.0 a	2.3 a
Atrazine	3.9 cd	0.9 fgh
Metribuzin	1.4 d	0.4 h
Ethofumesate	2.2 d	0.6 gh
Flumioxazin	8.6 a	2.2 abc
Saflufenacil	7.9 ab	1.9 a-d
Sulfentrazone	6.6 ab	1.6 b-e
Acetochlor	4.6 abc	1.2 efg
Dimethenamid-P	6.3 abc	1.5 c-f
S-metolachlor	5.9 abc	1.5 def
Pyroxasulfone	3.4 cd	0.9 fgh
Isoxaflutole	4.3 cd	0.8 fgh
Mesotrione	1.8 d	0.5 h

[a] Fresh above ground biomass was recorded at 25 DAE and dried to determine dry biomass weight.

[b] Means within column followed by the same letter are similar according to F-protected LSD at α=0.05 level of significance.

Pendimethalin and trifluralin were the safest pre-emergence herbicides across all variables and were similar to the non-treated. Similar greenhouse experiments have observed that pendimethalin was one of the safest options for use as a pre-emergence herbicide in hemp (Byrd 2019; Flessner et al. 2020). The same experiment indicated that hemp was tolerant to the same rate of s-metolachlor (1,600 g ai ha^{-1}) which was contrary to the results of this experiment. Imazethapyr, quinclorac, and saflufenacil also resulted in similar biomass, percent emergence, and plant loss compared to the non-treated; however, these herbicides were slightly more injurious to hemp. As much as 25% visible injury on hemp did not result in less biomass compared to the non-treated, as observed with flumioxazin. Percent emergence and plant loss as a result of an application of s-metolachlor, dimethenamid-p, and flumioxazin were also similar to the non-treated, although these herbicides resulted in up to 50% visible injury (Table 2.6).

Generalizations based off of percent visible injury by herbicide site of action groups can be made to help focus future research. Herbicides from site of action Groups 5, 15, and 27 were injurious to hemp, while Groups 2, 3, 4, and 14 were relatively safe and should be investigated further (Table 2.8).

Table 2.8. Pre-emergence herbicide visible injury summary based on herbicide site of action in greenhouse.

		5 DAE[a]		25 DAE	
Site of action	Site of action	Range	Average	Range	Average
	Group	--------- % --------		---------- % ---------	
ALS Inhibitors	2	n/a[b]	9	n/a	15
Mitotic Inhibitors	3	2-4	3	12-15	14
Synthetic Auxins	4	n/a	1	n/a	16
Photosystem II Inhibitors	5	5-25	15	50-99	75
PPO Inhibitors	14	18-65	37	24-38	33
Long-Chain Fatty Acid Inhibitors	15	21-96	42	42-96	68
HPPD Inhibitors	27	10-49	30	51-91	71

[a] Days after emergence (DAE) of the non-treated hemp.
[b] Only one herbicide was evaluated for ALS Inhibitors and Synthetic Auxins, so range not applicable (n/a).

Little research has been conducted with the use of trifluralin, imazethapyr, and quinclorac as a pre-emergence herbicide in hemp. The safest herbicides from each of the site of action groups were represented in a subsequent field experiment. Multiple sites of action were represented due to the desire to have a diverse list of possible herbicide options for use in hemp. Other contributing factors of herbicide selection for the field included commercial use and weed control efficacy of herbicides within the Upper Midwest. For example, acetochlor was selected rather than s-metolachlor because of comparable popularity in the region, even though acetochlor proved to be more injurious in the greenhouse. The following herbicides were included in the field experiment: imazethapyr, pendimethalin, trifluralin, quinclorac, saflufenacil, acetochlor, and pyroxasulfone.

2.2.2. Field Experiment

Statistical analysis was conducted through SAS, combining seven distinct environments. Year by treatment and location by treatment interactions were not observed. The decision to analyze data by environments came down to the idea that if a product or herbicide becomes registered for hemp it would likely be labeled by state or region, consisting of many different locations with various soil types and field characteristics. It was anticipated that a registrant would like to know the broader picture of hemp response to a herbicide rather than location or climate specific responses, as that is something even the farmers often do not have much control over. Results from this research were intended to be extrapolated or generalized across any year and random locations, ultimately any environmental growing conditions, in order to support herbicide registration. Treatment by time interactions were present for nearly every variable measured (Table 2.9 and 2.10), but treatment only comparisons were often more practical for discussion towards the end goal of this research.

In the analysis of variance (ANOVA) tables there was almost always a treatment and environment effect. There are some large differences in the environments between years' worth noting. Germination and establishment of hemp in 2019 was excellent, achieving our desired plant density. Hemp density was nearly half of the desired population in 2020 due to soil crusting and poor establishment. This impacted plant density, light interception and likely yield data, which contributed to the significant differences in environments observed when environment was analyzed as a fixed effect (Table 2.9 and 2.10). Environmental effects in height, light interception, and yield can be attributed to various levels of moisture and soil fertility at the different locations. Visible injury differences between environments were likely impacted by the soil type for the pre-emergence field experiment. These differences between years and locations aided in the decision to combine and create an environmental factor, but also to analyze environment as a random effect and focus on the treatment response.

Table 2.9. ANOVA table depicting degrees of freedom (df), mean square (MS), and *p*-value (P) for plant density, visible injury, and yield at evaluation timings.

	Timing 1			Timing 2			Timing 3		
SOV	df	MS	P	df	MS	P	df	MS	P
					Density[a]				
Env	5	47784	*	2	4217	*	-[b]	-	-
Rep(Env)	18	317	*	9	110	NS	-	-	-
Trt	15	560	*	15	463	*	-	-	-
Trt x Env	75	206	NS	30	158	*	-	-	-
Error	268	358	-	135	173	-	-	-	-
Total	381	-	-	191	-	-	-	-	-
					Visible injury[c]				
Env	6	0.528	*	6	0.298	*	5	0.171	*
Rep(Env)	21	0.017	*	21	0.174	*	18	0.009	*
Trt	15	0.218	*	15	0.251	*	15	0.164	*
Trt x Env	90	0.012	*	90	0.028	*	75	0.031	*
Error	313	0.002	-	313	0.004	-	268	0.005	-
Total	445	-	-	445	-	-	381	-	-
					Yield[d]				
Env	3	8291733	*	-	-	-	-	-	-
Rep(Env)	12	210110	NS	-	-	-	-	-	-
Trt	8	663514	*	-	-	-	-	-	-
Trt x Env	24	150212	NS	-	-	-	-	-	-
Error	94	171628	-	-	-	-	-	-	-
Total	141	-	-	-	-	-	-	-	-

* Data is significantly different according to f-protected LSD at α=0.05 level of significance using PROC GLM.
[a] Density was evaluated at 21 and 49 days after emergence (DAE).
[b] Places in the table with a dishes (-) indicate that data was not available.
[c] Visible hemp injury was evaluated 7, 14, and 28 DAE.
[d] Yield was obtained at 102 DAE.

Table 2.10. ANOVA table depicting degrees of freedom, mean square, and *p*-value for height and light interception at evaluation timings.

	Timing 1			Timing 2			Timing 3			Timing 4		
SOV	df	MS	P	df	MS	P	df	MS	P	df	MS	P
							Height[a]					
Env	5	3694	*	4	43393	*	3	15268	*	-[b]	-	-
Rep(Env)	18	101	*	15	410	*	12	483	NS	-	-	-
Trt	15	398	*	15	317	NS	15	894	NS	-	-	-
Trt x Env	75	62	*	60	320	*	45	491	NS	-	-	-
Error	268	131	-	225	504	-	171	347	-	-	-	-
Total	381	-	-	319	-	-	246	-	-	-	-	-
							Light interception[c]					
Env	6	1.671	*	4	1.433	*	4	2.828	*	3	1.763	*
Rep(Env)	21	0.009	*	15	0.039	*	15	0.048	*	12	0.030	NS
Trt	15	0.021	*	15	0.080	*	15	0.105	*	15	0.148	*
Trt x Env	90	0.005	*	60	0.032	*	60	0.033	*	45	0.038	*
Error	313	0.004	-	225	0.012	-	225	0.068	-	180	0.018	-
Total	445	-	-	319	-	-	319	-	-	255	-	-

* Data is significantly different according to f-protected LSD at α=0.05 level of significance using PROC GLM.
[a] Height measurements were collected at 21, 49, and 72 days after emergence (DAE).
[b] Places in the table with a dishes (-) indicate that data was not available.
[c] Light interception data was collected at 14, 28, 49, and 84 DAE.

Monthly and season long average temperatures and total precipitation were obtained (Table 2.11) for each environment for the time in which the experiments were conducted to ensure random factors of year and location were within normal ranges (NDAWN 2020). In general, monthly average temperature in 2020 was slightly warmer than the 30-yr average. Both years, 2019 in particular, experienced greater than average precipitation during the months of July and August. The amount of precipitation and warmer temperatures compared to the 30-yr average generally favored hemp growth and yield. However, the heavier soil types at the Fargo and Casselton locations did not allow for proper drainage in the lower areas of the field where experiments were conducted to meet hemp moisture preference. Therefore, evaluations and data collection were limited at Prosper (P-19) and Fargo (F-19) in 2019 due to standing water and the negative hemp response to saturated soils.

Table 2.11. Monthly and seasonal average temperature and total precipitation by environment.

	May		June		July		August		May – Aug.
Environment[a]	Temp	Precip.	Temp	Precip.	Temp	Precip.	Temp	Precip.	Precip.
Loc[b]-yr	°C	cm	°C	cm	°C	cm	°C	cm	cm
C-19	10.1	8.4	19.3	8.6	21.7	13.1	18.6	7.4	37.5
F-19	11.7	7.0	19.4	8.3	22.2	12.1	20.0	9.0	36.3
P-19	10.6	6.0	18.9	12.2	21.7	15.6	18.3	10.2	44.0
H-19	11.1	4.5	18.9	6.6	21.1	11.3	18.3	10.7	33.0
F-20	12.8	3.8	21.6	6.7	23.3	13.3	21.1	12.2	36.0
P-20	12.2	4.1	21.1	7.9	22.2	12.3	20.5	11.6	35.8
H-20	11.6	5.4	21.1	9.3	22.2	12.8	20.0	16.7	44.2
30-yr Avg[c]	13.3	7.7	18.9	10.0	21.1	8.8	20.6	6.6	33.1

[a] Environment was a function of location and year. Casselton 2019 (C-19), Fargo 2019 (F-19), Prosper 2019 (P-19), Hillsboro 2019 (H-19), Fargo 2020 (F-20), Prosper 2020 (P-20), and Hillsboro 2020 (H-20).

[b] Weather stations were located 1.5, 5.0, 0.5, and 12.5 km from Casselton, Fargo, Prosper, and Hillsboro experiment sites.

[c] The 30-yr average is represented by the Prosper location as it is centrally located among the study locations.

In both years, timely rain events during the month of June (Figure 2.5) allowed for an incorporating rainfall of 1.5 cm or greater within 11 days of herbicide application. Most pre-emergence herbicides need to either be mechanically incorporated or receive adequate rainfall in order to position the herbicide for effective weed control. Having this relative consistency of herbicide incorporation across all environments provided additional confidence in the statistical analysis to combine year and location to form the random environmental factor.

Excess precipitation could have contributed to hemp's ability to overcome initial herbicide injury at some environments, potentially flushing the herbicide past the hemp germination zone. Hemp injury could have also been amplified if the herbicide concentration increased at the depth the hemp seed was planted. Neither is probable, as weed control and hemp injury response did not vary significantly within the same treatment across environments.

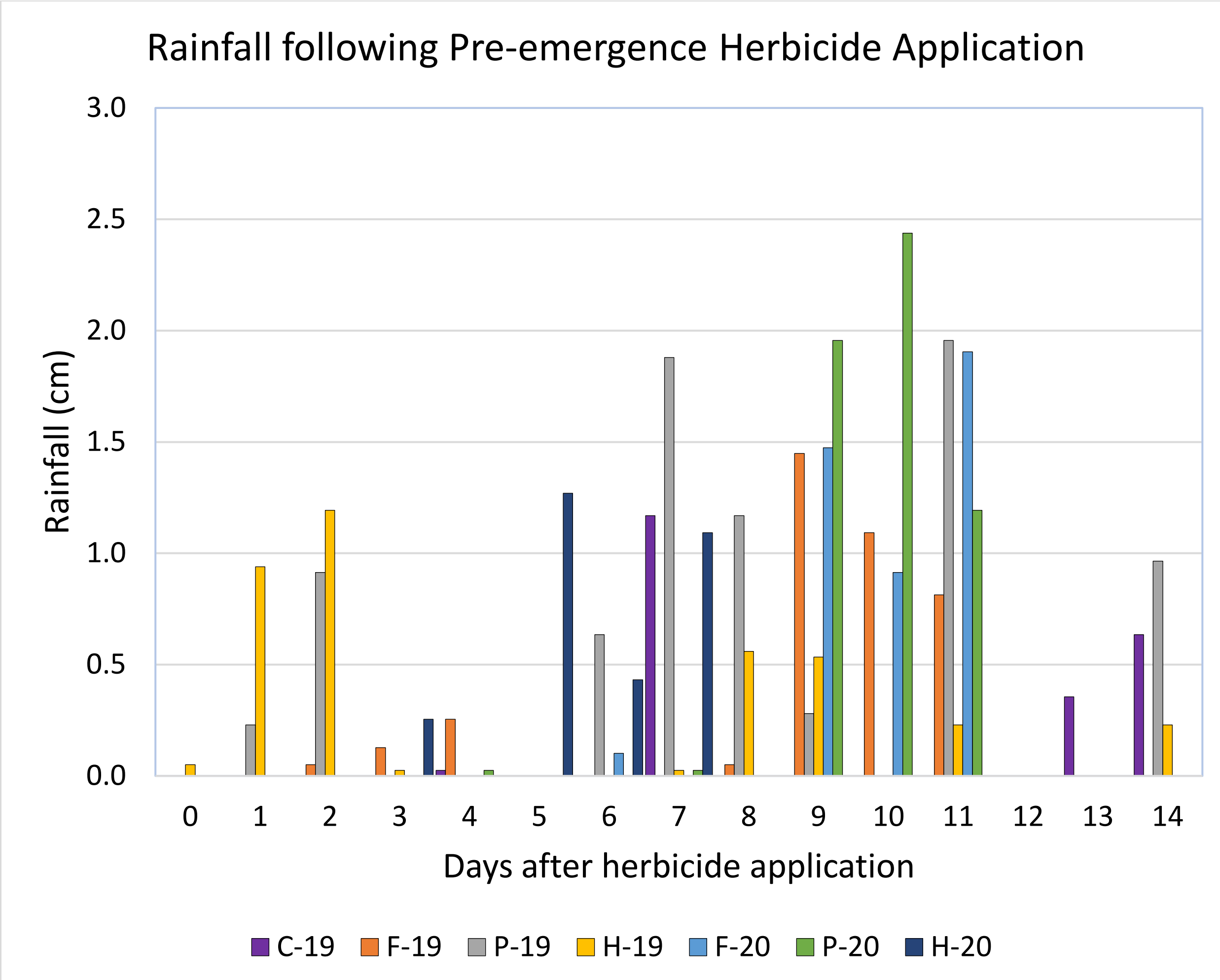

Figure 2.5. Rainfall following pre-emergence herbicide application.

In 2019, density measurements were recorded only at 21 DAE. In 2020, an additional density measurement took place at 49 DAE. In general, 2020 hemp density was roughly half of the density observed in 2019 (data not shown). Lower plant density in 2020 was attributed to planting into moist soil and near immediate rainfall and then not receiving additional precipitation for about a week. At locations with heavier soil types the soil surface dried and sealed during that germination period. Hemp seedlings were unable to break through the soil resulting in reduced emergence.

By 21 DAE, any reduction in hemp emergence would have occurred. Hemp that was not treated with a herbicide resulted in 70 plants per 2 m of row (Table 2.12). Hemp that did not show a reduced emergence with herbicide application were those that were treated with imazethapyr-1, imazethapyr-2, pendimethalin-1, quinclorac-1, and pyroxasulfone-2.

Table 2.12. Hemp density response to pre-emergence herbicides averaged across environments 21 and 49 days after emergence (DAE).

	Density	
Treatment	21 DAE	49 DAE[a]
	--- plants in 2 m of row ---	
Non-treated	70 a[b]	45 ab
Hand-weeded	70 a	44 ab
Imazethapyr-ammonium-1[c]	68 ab	51 a
Imazethapyr-ammonium-2	64 abc	41 ab
Pendimethalin-1	62 a-d	41 ab
Pendimethalin-2	60 b-e	37 bc
Trifluralin-1	62 b-e	37 bc
Trifluralin-2	58 cde	42 ab
Quinclorac-methyl-1	67 ab	43 ab
Quinclorac-methyl-2	61 b-e	37 bc
Saflufenacil-1	60 b-e	37 bc
Saflufenacil-2	58 cde	36 bc
Acetochlor-1	55 de	27 cd
Acetochlor-2	54 e	26 d
Pyroxasulfone-1	61 b-e	38 b
Pyroxasulfone-2	63 a-d	39 b

[a] 49 DAE data only collected in 2020, therefore comparisons between timings is limited.
[b] Means within column followed by the same letter are similar according to F-protected LSD at α=0.05 level of significance.
[c] Herbicide active ingredient followed by a numerical digit represent a low (1X) and high (2X) rate of that active ingredient based on use patterns in other crops.

Both rates of trifluralin, saflufenacil, and acetochlor reduced hemp emergence by 11 to 23% at 21 DAE compared to the non-treated hemp (Table 2.12). The low rates of the same three herbicides also resulted in 10 to 23% reduction in hemp emergence in the greenhouse experiment (Table 2.6). No other research has evaluated the effects of trifluralin on hemp emergence. Pendimethalin, another Group 3 herbicide similar to trifluralin, has been studied more extensively, likely because certain formulations do not require mechanical incorporation and is therefore more practical for growers. Pendimethalin applied at 1120 and 1600 g ai ha^{-1} has been observed to decrease hemp emergence by 21 and 70%, respectively (Anderson 2018; Flessner et al. 2020). Pendimethalin in this experiment was safer to hemp. Hemp population was similar to the non-treated at all rates and timings, except for the high rate of 2240 g ai ha^{-1} at 21 DAE, where a 9% reduction in emergence was observed (Table 2.12). As the season progressed, natural thinning within the non-treated hemp brought the population of hemp in the non-treated and pendimethalin at high rate to similar value. The inconsistency of hemp response to pendimethalin in various research could partially be due to the planting depth of the hemp. In this experiment, hemp was seeded at a depth of 2 cm, while in the other experiments mentioned above the seeding depth was only 1 cm. The hemp industry recommends planting hemp at a depth of 1.3 to 2.5 cm (HGI 2021). When the hemp seed is planted deeper into the soil, it can be placed outside of the effective herbicide zone, resulting in less herbicide injury. At a seeding depth of only 1 cm, it is likely that the hemp seed was exposed to greater concentrations of herbicide than if it was planted deeper.

By 49 DAE, any delayed plant death as a result of a pre-emergence herbicide would have occurred. Pre-emergence herbicides that resulted in similar hemp densities compared to the non-treated check at 49 DAE included both rates of imazethapyr, pendimethalin, trifluralin, quinclorac, saflufenacil, and pyroxasulfone (Table 2.12). The only herbicide that resulted in lower hemp density at 49 DAE was acetochlor at both the low and high rate. The lack of differences between hemp that was treated with herbicides and hemp that was not could be partly attributed to the self-thinning nature of hemp (Fike 2016; Struik et al. 2000). Hemp naturally self-thins in dense populations and environments with high soil nitrogen. Even if the herbicides initially reduced emergence, the non-treated and hand-weeded hemp could have self-thinned to the densities similar to the hemp that received a herbicide application. Therefore, hemp density response to pre-emergence herbicides at the current recommended seeding rates may not be a good indicator of herbicide tolerance.

Hemp plants were evaluated for percent visible crop injury compared to the checks at 7, 14, and 28 DAE. Visible injury was expressed with various symptoms and ranged from 0 to 40% injury across the three evaluations (Table 2.13). An ideal evaluation timing to compare herbicide tolerance was at 14 DAE when maximum hemp injury had occurred.

Hemp response at 14 DAE was similar to the checks when pendimethalin-1, trifluralin-1, quinclorac-1, quinclorac-2, and pyroxasulfone-1 were applied (Table 2.13). These herbicides resulted in 6 to 11% injury. This level of hemp injury would be considered acceptable by the hemp industry, registrants, and growers, if there was a positive return on investment. Low rates of imazethapyr, saflufenacil, and acetochlor were more injurious compared to the checks at 14 DAE, resulting in 15 to 18% injury. Visible injury as a result of the high rates imazethapyr, acetochlor, and saflufenacil decrease the likelihood and practicality of those herbicides getting registered for use in hemp. It was observed, visually, that hemp was able to overcome most herbicide injury by the end of the growing season.

Table 2.13. Visible hemp injury response averaged across environments to pre-emergence herbicides 7, 14, and 28 days after emergence (DAE).

	Visible injury[a]		
Treatment	7 DAE	14 DAE	28 DAE
	------------------ % ------------------		
Untreated	2 a[b]	2 a	0 a
Hand-weeded	2 ab	2 a	1 a
Imazethapyr-ammonium-1[c]	8 bc	17 cde	15 bc
Imazethapyr-ammonium-2	9 c	23 ef	23 cde
Pendimethalin-1	7 abc	9 abc	8 ab
Pendimethalin-2	8 bc	14 bcd	12 b
Trifluralin-1	8 bc	10 a-d	7 ab
Trifluralin-2	8 bc	11 bcd	11 b
Quinclorac-methyl-1	6 abc	6 ab	8 ab
Quinclorac-methyl-2	9 c	10 a-d	11 b
Saflufenacil-1	17 d	15 b-e	10 ab
Saflufenacil-2	28 e	31 fg	26 de
Acetochlor-1	20 d	18 de	16 bcd
Acetochlor-2	34 e	37 g	31 e
Pyroxasulfone-1	11 c	10 a-d	7 ab
Pyroxasulfone-2	11 c	13 bcd	14 bc

[a] Visual evaluation of herbicide symptoms on a scale of 0% (no injury) to 100% (plant death).

[b] Means within column followed by the same letter are similar according to F-protected LSD at α=0.05 level of significance.

[c] Herbicide active ingredient followed by a numerical digit represent a low (1X) and high (2X) rate of that active ingredient based on use patterns in other crops.

No previous research was found on the use of imazethapyr for pre-emergence use in hemp. At 7 and 14 DAE, imazethapyr-1 resulted in 9 and 23% injury, respectively, and remained at 23% injury at 28 DAE (Table 2.13). Injury symptoms included general plant chlorosis, especially on the inner whorl and new growth of hemp from the higher rate (88 g ae ha^{-1}) (Figure 2.6). By 49 DAE, hemp from the lower rate of imazethapyr (44 g ha^{-1}) appeared relatively unaffected by the herbicide compared to the check plots. Excellent weed control was achieved (Figure 2.7.) (data not shown). Imazethapyr is a Group 2, ALS inhibitor, that inhibits the ALS enzyme, reducing the biosynthesis of key amino acids (Cobb and Reade 2010). Metabolism-based tolerance to imazethapyr occurs in legumes including soybean, and perhaps could occur in hemp at selective rates.

Figure 2.6. Hemp chlorosis at meristem regions with imazethapyr, 21 days after emergence.

Figure 2.7. Understory of hemp and weed control with imazethapyr applied pre-emergence, 49 days after emergence.

Research also was not found on the use of quinclorac pre-emergence in hemp. Quinclorac resulted in 6 to 11% injury at the different evaluation timings (Table 2.13). This consistent response is likely a result of quinclorac being a Group 4 synthetic auxin herbicide that takes time to translocate and display symptoms (WSSA 2020). It was observed even late into the season that quinclorac continually expressed injury in the newest growth, even noticeable just prior to harvest. Initial symptoms included some necrosis of the older leaves, but later developed into noticeable distorted leaves. Quinclorac consistently had 5 to 10% injury during the course of the season (data not shown). In addition to sustained visible hemp injury, weed control in plots treated with quinclorac was observed to be rather poor (Figure 2.8) (data not shown).

Figure 2.8. Limited weed control 28 days after emergence with quinclorac.

Two Group 3 mitosis inhibitors were included in this field experiment. Pendimethalin has garnished attention from several researchers since the onset of this experiment, and trifluralin has been studied very little. Both herbicides resulted in 8 to 15% hemp injury in this experiment (Table 2.13). Pendimethalin resulted in 0 to 84% visible hemp injury across various experiments, averaging around 30% injury (Flessner et al. 2020; Lingenfelter 2018; Maxwell 2016; Pearce and Carter 2018; Scott et al 2020; Woosley et al 2015). Pre-emergence mitosis inhibitors interfere with the structure and functions of microtubules in the roots; therefore, seed placement as previously discussed is rather important and likely played a role in the inconsistencies across experiments with the use of pendimethalin (Cobb and Reade 2010). Some researchers view pendimethalin as a viable option that showed no differences in biomass or seed yield when compared to the non-treated (Anderson 2018; Maxwell 2016). S-metolachlor, a herbicide not evaluated in this experiment was also deemed as a viable pre-emergence herbicide (Flessner et al. 2020).

Trifluralin resulted in 1 to 25% injury across two other experiments, with an average of 9% visible hemp injury (Howat and Mettler 2020; Pearce and Carter 2018). Experiments using trifluralin appear to be more consistent in terms of hemp response, perhaps due to mechanically incorporating and evenly distributing the herbicide within the root zone prior to planting. Overall symptoms, in varying degrees, for these two mitosis inhibiting herbicides included slight chlorosis, some stunting, and leaf twisting compared to the non-treated hemp (Figures 2.9 and 2.10). New growth was generally less injured from trifluralin than pendimethalin. Reasonable weed control was achieved with both herbicides (data not shown), but the plots would have benefited from a post-emergence application to control weeds prior to hemp canopy closure.

Figure 2.9. Hemp response to pre-emergence pendimethalin, 14 days after emergence.

Figure 2.10. Hemp response to pre-emergence trifluralin, 28 days after emergence.

Saflufenacil, the only PPO inhibitor (Group 14) in this experiment, resulted in 17 to 28% visible hemp injury 7 DAE, and 10 to 26% injury 28 DAE (Table 2.13). Two other experiments evaluated hemp response to saflufenacil as 0 to 2%, both at a rate of 25 g ai ha^{-1} (Knezevic et al. 2020; Pearce and Carter 2018). This experiment evaluated saflufenacil at 38 and 76 g ai ha^{-1}, slightly higher rates to provide some residual weed control benefit. These higher rates might explain why hemp injury with saflufenacil in this research reached nearly 30%. Hemp injury symptoms included severe necrosis on the older plant tissue with normal new growth. Initial hemp necrosis was greater with the high rate of saflufenacil (Figure 2.11). In addition, the higher rate resulted in stunting, chlorosis, and stand loss. Saflufenacil resulted in excellent weed control (data not shown), unless grassy weeds were present (Figure 2.12). Saflufenacil is labelled for selective control of annual broadleaf weeds, so control of grass weeds was not expected (BASF 2019b). Saflufenacil inhibits the PPO enzyme leading to lipid peroxidation, loss of chlorophyll, and leaky membranes causing the observed necrosis (WSSA 2020).

Figure 2.11. Necrosis caused by saflufenacil is focused to more photosynthetically active leaves, 14 days after emergence.

Figure 2.12. Weed control in hemp with saflufenacil, one week prior to harvest.

Acetochlor and pyroxasulfone are Group 15 herbicides that were included in this experiment. Acetochlor at 1050 and 2100 g ai ha^{-1} resulted in 16 to 37% injury, sustaining near 20% injury at the low rate at all evaluation timings (Table 2.13). Acetochlor has only been studied in two other experiments. When applied at 811 g ha^{-1}, acetochlor resulted in 4 to 17% hemp injury, while 21 to 34% hemp injury was observed when acetochlor was applied at 3400 g ha^{-1} (Chirita 2008; Knezevic et al. 2020). This experiment aligns with other experiments in terms of the amount of hemp injury observed and the rate of herbicide applied. Hemp injury symptoms included major stunting, minor leaf twisting, and yellowing of the whorl (Figure 2.13). Acetochlor resulted in excellent weed control (Figure 2.14) (data not shown).

Figure 2.13. Plot of hemp 28 days after emergence with acetochlor applied pre-emergence.

Figure 2.14. Weed control in hemp with pre-emergence acetochlor, one week prior to harvest.

Pyroxasulfone when applied at 109 or 218 g ai ha^{-1} was more safe to hemp than acetochlor, resulting in 7 to 14% visible hemp injury (Table 2.13). Pyroxasulfone was also the more studied herbicide of the two. Hemp has responded in a wide range of injury (0 to 90%) to pyroxasulfone (Lingenfelter 2018; Maxwell 2016; Pearce 2019; Waters and Burke 2019). All experiments applied 109 g ha^{-1} pyroxasulfone or less. Experiments have shown that pyroxasulfone resulted in an average hemp injury of 36% when applied to silt loam soils and an average of 72% when applied to sandy loam soils. Seed yield was similar to the non-treated check when hemp injury was 45% or less with pyroxasulfone (Maxwell 2106). Injury symptoms were necrotic spots on the leaves, yellow whorls, and stunting, in addition to reduction in plant density. Pyroxasulfone at the higher rate resulted in fewer hemp plants, but more robust plants (Table 2.12). Weed control with pyroxasulfone appeared not as good as with acetochlor (data not shown).

Soil texture and cation exchange capacity (CEC) have been well documented to influence the effectiveness and relative availability of pre-emergence herbicides for plant uptake. It was noted that the herbicide imazamox (similar to imazethapyr used in this experiment) increased in availability in more course, sandy soils and decreased in availability in soils with more clay and a higher CEC (Pannacci et al. 2005). The same pattern was observed in this experiment as the greatest average hemp injury from imazethapyr (data not shown) across all evaluations occurred at the Hillsboro location under sandy loam soil and the least injury at the Fargo location under silty clay soil. Another experiment demonstrated increased sunflower injury to pre-emergence herbicides, including s-metolachlor and pendimethalin, with reduced soil CEC often a characteristic of sandier soils (Jursík et al. 2020). Sunflower injury was least in the soils that had higher CEC. In this research, the soil CEC (Table 2.3) of the research locations seemed to not have a direct relationship on the plant uptake of acetochlor or pendimethalin as the average visible hemp injury response (data not shown) was rather consistent across locations.

Other research had observed the importance of soil texture on herbicide dissipation, indicating that herbicides tend to dissipate more slowly in soils with more clay content (Carretta et al. 2018). This coincides with the concept that herbicides are generally more available in sandier soils comparatively to soils with a greater clay content or CEC. Carretta et al. (2018) did observe that soil texture did not impact mesotrione dissipation and that every herbicide is unique in its chemical and physical properties. Different herbicides react differently to changes in soil texture. Similar trends were observed in our research.

Although location differences were not specifically analyzed with the creation of the environment factor, soil texture (Table 2.4) and CEC (Table 2.3) did vary among locations and hemp response to herbicides responded accordingly. The Fargo location had the finest soil texture (silty clay loam) and the highest CEC (35 to 46 Meq 100 g^{-1}) followed by the Casselton, Prosper, and Hillsboro locations, in descending order. Herbicide injury was expected to be greater in the more course soil locations, such as Hillsboro. The Hillsboro location had a sandy loam texture with a CEC of 18 Meq 100 g^{-1}.

Many different environmental factors influence the overall visible hemp injury observed at a given environment, but some general trends can be made in regards to soil texture. It is important to note that the average level of injury observed in P-20 was elevated due to an early season wind and rain event that tattered the plants and resulted in hemp injury that was difficult to distinguish from some of the pre-emergence herbicide injury (Table 2.13). Therefore, environment P-19 would be a better environment to represent soil texture response for a silt loam. Hemp grown in the F-19 environment was also under additional stress due to saturated soil moisture conditions, which slightly accentuated hemp response to herbicides. Overall, at most of the evaluation timings, environments with more fine textured soils tended to result in overall less hemp injury (Table 2.14). Apart from the P-20 environment, both environments at the Hillsboro location with the sandy loam soil resulted in the most hemp injury, specifically at 14 and 28 DAE. Environment in the primary analysis of the data, however, were viewed as a random effect to reflect the end goals of our research.

Table 2.14. Average visible hemp injury response by environment to pre-emergence herbicides 7, 14, and 28 days after emergence (DAE).

		Visible injury[a]		
Environment[b]	Soil texture	7 DAE	14 DAE	28 DAE
Loc-yr		--------------------	%	------------------
H-20[c]	sandy loam	11.0 b[d]	13.5 c	10.8 b
H-19	sandy loam	7.8 c	16.9 b	19.0 a
P-19	silt loam	7.3 c	16.9 b	n/a[e]
P-20	silt loam	32.0 a	27.3 a	18.6 a
C-19	silty clay loam	7.6 c	7.1 d	7.9 cd
F-19	silty clay	10.5 b	6.9 d	7.2 d
F-20	silty clay	6.2 c	14.0 c	10.5 bc

[a] Visual evaluation of herbicide symptoms on a scale of 0% (no injury) to 100% (plant death).

[b] Environment was a function of location and year. Casselton 2019 (C-19), Fargo 2019 (F-19), Prosper 2019 (P-19), Hillsboro 2019 (H-19), Fargo 2020 (F-20), Prosper 2020 (P-20), and Hillsboro 2020 (H-20).

[c] Environments are listed from most course soil texture to least course in descending order.

[d] Means within column followed by the same letter are similar according to F-protected LSD at α=0.05 level of significance.

[e] Data not available (n/a).

Upon reviewing the data with environment (loc + year) as a fixed effect rather than a random effect, differences in overall visible hemp injury (combined across all treatments) among the environments was observed (Table 2.14). At 7 DAE, the injury response was variable and did not follow the expected trend where the more course the soil, the greater the herbicide injury. This could in part be explained by the speed at which various herbicide sites of action express symptoms. For example, symptoms from a PPO inhibiting herbicide, such as saflufenacil, that requires photosynthetic plant material for herbicidal activity would be slower to develop than a seedling root inhibiting herbicide, such as trifluralin, that targets developing roots before shoots emerge from the soil. By 14 DAE, loam soils had greater average hemp injury compared to soils with a greater CEC and clay content. All symptoms of herbicide injury had fully developed by14 DAE, and by 28 DAE, hemp plants already began to overcome and outgrow herbicide symptoms.

Soil texture could also play a role in the ability and rate of a hemp plant to recover from herbicide injury. Greater herbicide injury occurs in more course textured and low CEC soils with less soil surface area and binding sites, resulting in more herbicide being available for plant uptake (Jursík et al. 2020). The degree of hemp injury response to most herbicides was similar among similar soil textures (data not shown), despite the different environments. The greatest range of injury within similar soil textures occurred with imazethapyr and acetochlor. Visible hemp injury from imazethapyr in silt loam and sandy loam soils ranged from 5 to 23% and 8 to 33%, respectively; however, silty clay soils consistently resulted in 5 to 15% visible injury at any environment. Visible hemp injury response to acetochlor-2 in silt loam soil ranged from 14 to 70% and 14 to 30% in other soil textures. It is inconclusive as to why these particular herbicides resulted in a more variable injury response compared to the other pre-emergence herbicides in this experiment.

One of the limitations or short falls of the visible hemp injury evaluations in this experiment was that height reduction, since it was a visual effect of the herbicide, was also included in the percent visible injury values. A reduction in hemp height is not necessarily a bad thing when producing hemp for grain, as long as seed yield is not reduced. Shorter hemp plants result in less hemp stalk volume and length to wrap around combine machinery during harvest and less post-harvest crop residue to manage.

Plant height measurements were obtained from each plot 21, 49 (pollen initiation), and 70 (post male senescence) DAE. Differences in plant height were observed at 21 DAE, but by 49 DAE there were no differences in plant height among the treatments compared to the non-treated and hand-weeded hemp (Table 2.15). A reduction in height was observed at 21 DAE with herbicide applications of imazethapyr-2, quinclorac-2, saflufenacil-2, and both rates of acetochlor. Another experiment had similar results where pre-emergence herbicides reduced hemp height by 21% 34 days after planting, but did not differ in height at later evaluation dates (Anderson 2018). Hemp plants were able to recover from initial growth inhibition caused by herbicides, even when up to 25% visible injury or a 15% density reduction occurred (Table 2.12 and 2.13).

Table 2.15. Hemp height response to pre-emergence herbicides averaged across environments 21, 49, and 72 days after emergence (DAE).

Treatment	Height[a]		
	21 DAE	49 DAE	72 DAE
	------------------ cm ------------------		
Non-treated	34 a[b]	100	100
Hand-weeded	34 ab	104	113
Imazethapyr-ammonium-1[c]	27 cd	103	113
Imazethapyr-ammonium-2	24 de	100	111
Pendimethalin-1	32 abc	107	117
Pendimethalin-2	31 abc	105	121
Trifluralin-1	31 abc	107	128
Trifluralin-2	30 bc	104	109
Quinclorac-methyl-1	31 abc	98	107
Quinclorac-methyl-2	30 bc	92	98
Saflufenacil-1	29 c	103	119
Saflufenacil-2	24 de	100	119
Acetochlor-1	24 de	99	117
Acetochlor-2	20 e	100	107
Pyroxasulfone-1	31 abc	104	119
Pyroxasulfone-2	29 c	106	117

[a] Total plant height was measured to the nearest cm from the base of the plant to the end of terminal growth.

[b] Means within column followed by the same letter are similar according to F-protected LSD at α=0.05 level of significance.

[c] Herbicide active ingredient followed by a numerical digit represent a low (1X) and high (2X) rate of that active ingredient based on use patterns in other crops.

Light Interception (LI) was measured 14, 28, 49, 70, and 84 DAE. Light interception values were maximized (maximum effective weed control) at 49 DAE, as a result of the hemp canopy (Table 2.16). When determining the overall hemp response or tolerance to pre-emergence herbicides, 49 DAE is therefore an ideal timing to draw conclusions and point out differences.

Table 2.16. Light interception response averaged across environments to pre-emergence herbicides 14, 28, 49, and 84 days after emergence (DAE).

	Light interception[a]			
Treatment	14 DAE	28 DAE	49 DAE	84 DAE
	----------------------------	%	----------------------------	
Untreated	23 a[b]	48 abc	54 e	45 h
Hand-weeded	21 abc	55 a	78 a	72 a-c
Imazethapyr-ammonium-1[c]	16 de	50 abc	76 a	76 a
Imazethapyr-ammonium-2	15 e	40 cde	74 ab	79 a
Pendimethalin-1	20 a-d	52 a	66 a-d	58 c-h
Pendimethalin-2	19 bcd	48 abc	69 a-c	68 a-e
Trifluralin-1	21 abc	51 abc	72 a-c	70 a-d
Trifluralin-2	17 de	46 a-d	70 a-c	73 ab
Quinclorac-methyl-1	21 ab	47 a-d	64 b-e	53 fgh
Quinclorac-methyl-2	22 ab	43 bcd	57 de	55 e-h
Saflufenacil-1	21 abc	51 abc	73 abc	67 a-f
Saflufenacil-2	17 cde	35 de	69 abc	67 a-f
Acetochlor-1	17 cde	43 b-e	62 cde	59 b-g
Acetochlor-2	15 e	31 e	56 de	51 gh
Pyroxasulfone-1	23 ab	51 a-c	67 a-d	58 d-h
Pyroxasulfone-2	19 a-d	44 a-d	74 abc	65 a-f

[a] Evaluation on a scale of 0% to 100% (complete light interception via hemp canopy). Data collected with ceptometer.

[b] Means with variables sharing the same letter are similar according to F-protected LSD at p=0.05 level of significance.

[c] Herbicide active ingredient followed by a numerical digit represent a low (1X) and high (2X) rate of that active ingredient based on use patterns in other crops.

The way herbicides have influenced LI can be broken down into two categories: those that were similar to the hand-weeded hemp, and those that were similar to the non-treated, weedy check (Table 2.16). Low and high rates of imazethapyr, pendimethalin, trifluralin, saflufenacil, and pyroxasulfone resulted in hemp that had similar LI to the hand-weeded check 49 DAE. Both rates of quinclorac and acetochlor reduced LI of the hemp to levels similar to the non-treated. There were no height differences in the hemp at 49 DAE (Table 2.15), but when a reduction in plant density as observed as from acetochlor (Table 2.12), the overall LI was reduced. When LI was reduced on a whole plot basis, individual plants could have a greater LI and photosynthetic capacity. Reduced hemp density, as a result of herbicide, promoted greater amounts of branching on hemp plants. Hemp that had greater LI compared to the hand-weeded at 84 DAE, likely experienced delayed plant development or delayed male senescence due to the herbicide.

Approximately 102 days after planting, hemp treated with the low rate of each herbicide and the checks were harvested. The hand-weeded check resulted in a seed yield of 2110 kg ha^{-1} (Table 2.17). The non-treated hemp resulted in an average yield of 1565 kg ha^{-1}, 545 kg less than the hand-weeded. The presence of weeds within the non-treated reduced yield by 25% compared to the hand-weeded.

The use of pre-emergence herbicides imazethapyr, pendimethalin, trifluralin, saflufenacil, and pyroxasulfone preserved yield similar to the hand-weeded hemp (Table 2.17). This experiment demonstrated that seed yield comparable to weed-free hemp can be achieved and maintained with the use of a pre-emergence herbicide. Quinclorac and acetochlor resulted in yield similar to the non-treated hemp. Even though quinclorac resulted in 15% injury or less to hemp and minimal differences in density and height, hemp yield was reduced (Table 2.13-2.17). Limited weed control and sustained visible herbicide injury throughout the season from quinclorac could have attributed to the reduced hemp seed yield. Hemp grown with a pre-emergence application of imazethapyr, trifluralin, or saflufenacil resulted in yield greater than 2000 kg ha^{-1}.

The overall hemp response to quinclorac and pyroxasulfone limit the possibility for their registration. Imazethapyr and acetochlor are not practical candidates for pre-emergence use in hemp given the amount of visible injury observed (Table 2.13), even though they demonstrated desirable yield response (Table 2.17). The majority of experiments evaluating hemp tolerance to herbicides did not collect yield data. More data is needed to substantiate results of the research presented here and confirm seed yield response to herbicide use in industrial hemp. According to this experiment, the best pre-emergence herbicide options moving forward for registration are pendimethalin, trifluralin, saflufenacil and, perhaps, pyroxasulfone.

Table 2.17. Yield response averaged across environments to standard (1X) field rates of pre-emergence herbicides.

Treatment	Yield
	--- kg ha^{-1} ---
Non-treated	1565 d[a]
Hand-weeded	2110 ab
Imazethapyr-ammonium	2118 a
Pendimethalin	1845 bcd
Trifluralin	2029 abc
Quinclorac-methyl	1670 d
Saflufenacil	2088 abc
Acetochlor	1802 cd
Pyroxasulfone	1863 a-d

[a] Means within column followed by the same letter are similar according to F-protected LSD at α=0.05 level of significance.

A simple correlation analysis was conducted by SAS for combinations among hemp injury, LI, and yield. If the correlation was strong enough between visible hemp injury and LI, one could consider measuring LI as a proxy for the more subjective visible evaluation. Light interception and hemp injury were both collected at 14 and 28 DAE and resulted in R-values of -0.25 and -0.39, respectively (data not shown). Hemp injury was negatively correlated with LI. The greater the hemp injury from the herbicide, the less light was intercepted by hemp within the plot as a whole. This reflects loss in hemp density and height as a result of herbicide injury or the lack of weed control within the plot. It was concluded that the correlation of visible hemp injury to LI was not strong enough to justify further research into the concept. Hemp injury was also negatively correlated to seed yield with R-values of -0.1 and -0.27 at 14 and 28 DAE, respectively (data not shown). The greater the hemp injury, the greater the yield loss. It was surprising that the correlation between hemp injury and seed yield was not a stronger relationship. The weak correlation provides additional confidence in claiming the resilient nature of hemp to overcome herbicide injury.

One of the complications when collecting LI in this experiment was the confounding effect of the lack of weed control and hemp injury. Both influenced the amount of light intercepted by the hemp plants. Excellent weed control from the herbicide often resulted in greater amounts of hemp injury which reduced LI. Likewise, when weeds were not controlled very well, LI decreased as the ceptometer placement was adjusted in order to measure the hemp interception above the weed canopy. It was observed by mid-way through the season that hemp naturally lost its lower leaves allowing more light to be available to the remaining weeds below the hemp canopy. When weeds were not adequately controlled, it was possible that weed and hemp light interception could both simultaneously be measured, unless we collected the data in the fashion described in the materials and methods section.

If LI was strongly correlated to seed yield, it could be used as a tool to predict yield or aid in-season management practices. The more light intercepted by hemp plants within a plot, the greater the yield. Light interception had a positive correlation to yield at 28 and 49 DAE, along with just prior to harvest with moderate R-values of 0.43 and 0.56 at 28 and 49 DAE, respectively. The strongest correlation or R-value of 0.77 occurred just prior to harvest. Based on the results of this experiment, the relationship between LI and yield could warrant further study.

The main decision factors in progressing in herbicide registration is visible injury and yield. If the hemp industry can tolerate 10 to 15% visible hemp injury, weed control and higher seed yield can be achieved. Successful registration and adaptation of pre-emergence herbicide use in hemp seed production can result in an economic return of \$460 ha^{-1}. When trifluralin was applied pre-plant incorporated (PPI), average seed yield was 2029 kg ha^{-1}, a 420 kg ha^{-1} increase from the weedy check, but 185 kg ha^{-1} short of the absolute value measured for weed-free yield potential. The going rate for non-organic hemp is approximately \$1.10 kg^{-1} (Roger Gussiaas, Healthy Oilseeds, Personal Communication). A weed control program that includes trifluralin PPI increases production costs approximately \$25 per hectare. The investment of including trifluralin PPI in hemp production has the potential to result in a profit of \$435 per hectare.

Future research should focus on the narrow list of pre-emergence herbicide possibilities, and investigate hemp tolerance to different cultivars under different soil types and environments, while obtaining herbicide residue data. Hemp is a competitive crop, but not as competitive when planted in 38-cm row spacing and at densities grown for seed production compared to fiber. Research looking at more narrow rows in combination with the safer pre-emergence herbicides would also be valuable research.

CHAPTER 3. POST-EMERGENCE HERBICIDES

3.1. Materials and Methods

3.1.1. Preliminary Greenhouse Experiment

A preliminary greenhouse experiment to identify the least and most injurious post-emergence herbicides to industrial hemp was conducted in the winter of 2019 in Fargo, ND. The purpose of this greenhouse research was to identify herbicides which could be studied further in the field for use in hemp, but also to identify herbicide options for volunteer hemp control in succeeding crops. Treatments included herbicides with various sites of action. Dicamba, a synthetic auxin (Group 4); glyphosate, an enolpyruvyl shikimate-3-phosphate (EPSP) synthase inhibitor (Group 9); glufosinate, a glutamine synthetase inhibitor (Group 10); and paraquat, a cell membrane disruptor (Group 22), were included in the herbicide treatment list to be confirmed as a method to control volunteer hemp before seeding another crop or use in dicamba-, glyphosate-, or glufosinate-resistant crops. The selection of herbicides and the rates of each were determined by the commonly used rates in wheat, corn, and soybean cropping systems. Adjuvants and rates abided by product labels. Similiar to the pre-emergence greenhouse experiment, ‘CFX-2’ hemp seeds were used.

Hemp seeds were planted into 10.5-cm by 10.5-cm square plastic containers (SKU: 691525, Beldon Plastics, St. Paul, MN.), herein referred to as pots, that were 12.7 cm deep with a total holding capacity of 1040 mL. Each pot was an experimental unit. Deeper pots were used in the post-emergence experiment compared to the pre-emergence experiment due to greater anticipated root volume and overall size of the hemp plants because the experiment duration would be longer due to post-emergence herbicide application. A volume of 900 mL of dry potting mix (Pro-mix® Bx, Premier Tech Horticulture, Quakertown, PA) was placed in each pot and lightly packed to create a firm seed bed. Two hemp seeds were placed diagonally towards the center of the pot, approximately 2.5 cm from each corner totaling eight seeds per pot (Figure 3.1). Once the hemp seeds were placed on top of the soil inside the pot, a volume of 200 mL of dry potting mix was added to the pot and slightly packed creating a seed depth of 2 cm. A few days after the hemp plants emerged, each pot was thinned so that four, evenly-spaced hemp plants remained in each pot.

Figure 3.1. Hemp seed placement in greenhouse pots.

Once hemp had three leaf pairs greater than 1 cm in length herbicide treatments were applied. At that time hemp plants were approximately 8 to 12 cm tall. Treatments (Table 3.1) were applied using a chamber sprayer (DeVries Manufacturing. Hollandale, MN. Serial number SB8-131) equipped with a Turbo TeeJet 11001 nozzle tip and traveled 5.63 km h^{-1} to deliver 80 L ha^{-1} at 276 kPa (Spraying Systems Company 2014). Chemical and adjuvant companies and their contact information is provided in Table 2.2 and Table 3.2, respectively. Adjuvants were included in herbicide treatments as per product label recommendations.

Table 3.1. Herbicides applied to hemp post-emergence in the greenhouse.

Active ingredient[a]	Rate		Product	Source[b]	Site of action
	g ae or ai ha^{-1}				Group
Cloransulam-methyl	17.5	ai	FirstRate	Corteva	2
Flucarbazone-sodium	15.7	ai	Everest 3.0	UPL	2
Halosulfuron-methyl	35.2	ai	Permit	Gowan	2
Imazamox-ammonium	35.0	ae	Raptor	BASF	2
Imazethapyr-ammonium	35.0	ae	Pursuit	BASF	2
Nicosulfuron	25.6	ai	Accent Q	Corteva	2
Pyroxsulam	15.1	ai	TeamMate	Corteva	2
Tribenuron-methyl	8.8	ai	Express SG	FMC	2
Triflusulfuron-methyl	31.5	ai	Upbeet	FMC	2
2, 4-D Amine 4	280.0	ae	Shredder	Winfield	4
Clopyralid	105	ae	Stinger	Corteva	4
Dicamba	70	ae	Xtendimax	Bayer	4
Fluroxypyr-methyl	140	ae	Starene Ultra	Corteva	4
Halauxifen-methyl	5.3	ai	Elevore	Corteva	4
Atrazine	280.0	ai	AAtrex 4L	Syngenta	5
Metribuzin	210.0	ai	Tricor 75 DF	UPL	5
Bentazon-sodium	280.0	ai	Basagran 5L	BASF	6
Bromoxynil	280.0	ai	Brox 2EC	Albaugh	6
Glyphosate-potassium	840.0	ae	Roundup PowerMax	Bayer	9
Glufosinate-ammonium	655.0	ai	Liberty 280 SL	BASF	10
Carfentrazone-ethyl	9.0	ai	Aim EC	FMC	14
Fomesafen-sodium	198.0	ai	Flexstar	Syngenta	14
Oxyfluorfen	1120.4	ai	GoalTender	Nufarm	14
Paraquat	280.0	ai	Gramoxone SL 2.0	Syngenta	22
Tembotrione	92.0	ai	Laudis	Bayer	27
Topramezone	12.3	ae	Impact	AMVAC	27

[a] All post-emergence treatments included adjuvants and/or ammonium sulfate as per product label recommendations. Cloransulm, flucarbazone, halosulfuron, imazamox, imazethapyr, nicosulfuron, pyroxsulam, tribenuron, triflusulfuron, metribuzin, bentazon, glyphosate, carfentrazone, fomesafen, and paraquat included nonionic surfactant (Prefer 90) at 0.25% (v/v). Halauxifen, tembotrione, and topramezone included methylated seed oil (Super Spread) at 1.0% (v/v). Atrazine included petroleum oil (Prime Oil) at 1.0% (v/v). Cloransulm, flucarbazone, imazamox, imazethapyr, metribuzin, bentazon, glyphosate, glufosinate, carfentrazone, fomesafen, oxyfluorfen, tembotrione, and topramezone included ammonium sulfate (AMS) at 1.02 kg 100 L^{-1}. Halosulfuron, nicosulfuron, pyroxsulam, tribenuron, and glufosinate included AMS at 3.36 kg ha^{-1}. 2,4-D Amine, clopyralid, dicamba, fluroxypyr, and bromoxynil did not include an adjuvant.

[b] Source or company information was provided in Table 2.2 and Table 3.2.

Table 3.2. Adjuvant company contact information.

Company	Product	Address	City	State	Zip code	Phone number
West Central Ag Services	Prefer 90	2700 Trott Ave. SW	Willmar	MN	56201	1-800-594-8560
Winfield Solutions, LLC	Prime Oil	P.O. Box 64589	St. Paul	MN	55164	1-715-294-2789
Wilbur-Ellis	Super Spread MSO	P.O. Box 16458	Fresno	CA	93755	1-559-442-1220
CHS, Inc.	Ammonium sulfate	P.O. Box 310	Post Falls	ID	83877	1-208-773-4522

Plants were evaluated for percent visible crop injury at 7 and 19 days after treatment (DAT). Days after treatment is a reference to the number of days after the herbicide application was made. Percent hemp injury evaluations along with fresh and dry weighs of hemp plants at 19 DAT were conducted similar to the pre-emergence greenhouse experiment (Chapter 2.1.1).

Hemp plants and environmental conditions were maintained in the same fashion as in the pre-emergence greenhouse experiment (Chapter 2.1.1). Experimental design and statistical analysis also mirrored the pre-emergence experiment, except that plant number was not a covariate in the fresh and dry weight analysis, as each treatment had the same number of plants per pot. Treatments that resulted in less than 40% hemp injury were considered for further investigation in a variable rate field experiment. Treatments that resulted in 85% control or greater of hemp were included in field experiments to control volunteer hemp. Volunteer hemp control experiments were conducted independently of this work.

3.1.2. Field Experiment

A study to evaluate hemp tolerance to post-emergence herbicides was conducted in 2019 and 2020 near Fargo, Prosper, Casselton, and Hillsboro, North Dakota. Soil fertility and mechanical analysis for the four locations were provided in Tables 2.3 and 2.4, respectively. Nitrogen was applied via urea fertilizer to provide 112 kg ha^{-1} nitrogen at the start of each growing season. The Hillsboro 2019 site was the only site that did not receive the nitrogen application due to a communication error.

The primary goal of the study was to identify post-emergence herbicides to be further evaluated and developed for registration and use in industrial hemp. Hemp cultivar, herbicides, and herbicide rates were determined in a similar fashion to the pre-emergence field experiment (Chapter 2.1.2). Various sites of action were represented in the field experiment. Herbicides from Groups 9, 10, and 27 were removed from the treatment list as the herbicides were far too injurious in the greenhouse to warrant including them in this field experiment. Unlike the pre-emergence experiment, adjuvants such as a non-ionic surfactant (NIS), ammonium sulfate (AMS), or methylated seed oil (MSO) were included in treatments per product label recommendations. Chemical and adjuvant companies and their contact information is provided in Table 2.2 and Table 3.2, respectively. Experimental units were the same size and established similarly to the pre-emergence experiment (Chapter 2.1.2).

Herbicide treatments (Table 3.3) were applied using a CO_2-pressurized backpack sprayer and hand-boom. The hand-boom was 2.53 m wide with five nozzles spaced evenly 50.8 cm apart, effectively spraying 3 m wide and leaving 0.67 m on the edge of each plot, or one row of hemp, as a non-treated running check for comparison in addition to the full non-treated plot for data collection. Herbicide applications were made with Tee Jet 11001 nozzle tips at 276 kPa to deliver 79.5 L ha^{-1} at a walking speed of 5.63 km h^{-1} (Spraying Systems Company 2014). Hemp at the time of application measured 8 to 12 cm tall with 3 to 4 leaf pairs. The hand-boom was held 50 cm above the hemp foliage during the application. Pre-emergence herbicides were not applied to these experiments. The hand-weeded check plots were weeded as often as necessary to maintain a weed-free plot throughout the growing season, resulting in minimal weed competition.

Table 3.3. Post-emergence herbicide treatments and associated site of action group for evaluation of hemp tolerance in the field.

Treatment[a]	Rate	Product	Source[b]	Site of action
	-- g ae or ai ha^{-1} --			Group
Non-treated	n/a[c]	n/a	n/a	n/a
Hand-weeded	n/a	n/a	n/a	n/a
Cloransulam-methyl-1[d]	18 ai	FirstRate	Corteva	2
Cloransulam-methyl-2	36 ai	FirstRate	Corteva	2
Imazamox-ammonium-1	35 ae	Raptor	BASF	2
Imazamox-ammonium-2	70 ae	Raptor	BASF	2
Clopyralid-1	105 ae	Stinger	Corteva	4
Clopyralid-2	210 ae	Stinger	Corteva	4
Quinclorac-1	290 ai	Facet L	BASF	4
Quinclorac-2	580 ai	Facet L	BASF	4
Bromoxynil-1	280 ae	Brox 2EC	Albaugh	6
Bromoxynil-2	560 ae	Brox 2EC	Albaugh	6
Atrazine-1	280 ai	AAtrex 4L	Syngenta	5
Atrazine-2	560 ai	AAtrex 4L	Syngenta	5
Oxyfluorfen-1	1120 ai	GoalTender	Nufarm	14
Oxyfluorfen-2	2240 ai	GoalTender	Nufarm	14

[a] All herbicide treatments included an adjuvant as per product label recommendations. Cloransulam and imazamox included nonionic surfactant Prefer 90 at 0.25% (v/v) and ammonium sulfate (AMS) at 1.02 kg 100 L-1. Quinclorac included methylated seed oil Super Spread at 2.2% (v/v). Atrazine included Prime Oil at 1% (v/v). Oxyflurofen included AMS at 3.36 kg ha-1. Clopyralid and bromoxynil did not include a surfactant.

[b] Source or company information was provided in Table 2.2.

[c] Abbreviation for not applicable (n/a).

[d] Herbicide active ingredient followed by a numerical digit represent a low (1X) and high (2X) rate of that active ingredient based on use patterns in other crops.

The methodology in which hemp injury, hemp density, plant height, light interception and seed yield were obtained or measured were the same as in the pre-emergence experiment (Chapter 2.1.2). The only difference was the timing of the evaluations and data collection in terms of days after treatment rather than days after emergence. Hemp plants were evaluated for percent visible crop injury compared to the control at 7 and 21 DAT. Hemp density was determined 7 and 35 DAT. The 7 DAT plant density was taken as a baseline prior to significant herbicide injury. Plant density recorded at 35 DAT was representative of reduction due to plant mortality as a result of the herbicide. Plant height measurements were obtained from each plot 7, 35 (pollen shed), and 65 (post male senescence, approximately 2 weeks before harvest) DAT. Photosynthetically active radiation (PAR) interception or light interception (LI) was measured 7, 21, 35, and 72 DAT. Approximately 102 days after sowing or 79 to 85 DAT, plots with low rates of each herbicide along with the check plots were harvested for seed.

Similar to the pre-emergence experiment all data was combined for analysis of variance in SAS 9.4 (Statistical Analysis Software 2003, version 9.4, SAS Institute, Inc., Cary, NC 27513) using Proc Glimmix with normal distribution with the same random and fixed effects (Chapter 2.2.2).

3.2. Results and Discussion

3.2.1. Preliminary Greenhouse Experiment

The post-emergence herbicide screening in the greenhouse was conducted as a precursor to field evaluations and, therefore, data analysis did not evaluate time and time by treatment interactions for any data type. This preliminary experiment focused on treatment effect to aid in the selection of herbicides to be applied in the field experiment.

Post-emergence herbicide effect to hemp as visible injury was evaluated at 7 and 19 DAT, respectively. Injury symptoms observed included necrosis, chlorosis, epinasty, and stunting as compared to the non-treated. Specific injury varied depending on the site of action of the herbicide applied (Table 3.4). The progression of the level of injury can be seen by comparing the percent injury of the two evaluation timings. Figures 3.2, 3.3, and 3.4 depict a visual scale of the percent visible injury ranging from 0% for the non-treated (unlabeled) to 99%

injury at 19 DAT. Injury at 19 DAT and the dry weight provided good characterization of overall hemp response to post-emergence herbicides (Table 3.4).

Table 3.4. Visible injury and biomass data for industrial hemp response to post-emergence herbicide treatments in the greenhouse.

	Visible Injury[a]		Biomass[b]	
Treatment[c]	7 DAT	19 DAT	Fresh	Dry
	------------- % -------------		----------- grams ------------	
Non-treated	0 a[d]	0 a	25.6 abc	6.6 ab
Cloransulam-methyl	4 c	37 cde	23.1 a-d	5.3 bcd
Flucarbazone-sodium	68 hi	77 jk	8.2 h-k	1.7 g-j
Halosulfuron-methyl	56 de	47 efg	20.8 cde	4.2 de
Imazamox-ammonium	66 fgh	50 fgh	18.9 def	3.3 ef
Imazethapyr-ammonium	58 d-g	38 cde	21.8 cde	3.9 e
Nicosulfuron	57 def	55 ghi	21.9 bcd	4.1 de
Pyroxsulam	60 d-h	53 ghi	19.2 def	3.9 e
Tribenuron-methyl	58 def	61 i	16.1 efg	3.3 ef
Triflusulfuron-methyl	41 c	28 c	25.7 abc	5.8 bc
2, 4-D Amine 4	64 e-h	62 i	13.5 fgh	2.2 fg
Clopyralid	3 a	5 ab	28.3 a	7.5 a
Dicamba	36 c	43 ef	26.2 abc	6.3 ab
Fluroxypyr-methyl	82 jk	84 jkl	10.4 g-j	1.4 g-j
Halauxifen-methyl	83 jkl	84 jkl	8.0 h-k	1.0 g-j
Atrazine	53 d	31 cd	19.3 de	4.3 de
Metribuzin	82 jk	62 i	10.4 g-j	2.1 f-i
Bentazon-sodium	62 d-h	40 def	18.3 def	4.3 de
Bromoxynil	19 b	14 b	27.2 ab	7.3 a
Glyphosate-potassium	80 jk	98 n	1.8 l	0.6 j
Glufosinate-ammonium	95 m	96 mn	2.5 kl	0.5 j
Carfentrazone-ethyl	89 klm	60 hi	11.4 g-i	2.2 fgh
Fomesafen-sodium	93 m	75 j	6.4 h-l	1.2 g-j
Oxyfluorfen	67 gh	44 efg	19.8 de	4.5 cde
Paraquat	92 m	90 lmn	3.0 kl	0.8 ij
Tembotrione	81 jk	86 klm	5.7 i-l	1.1 g-j
Topramezone	77 ij	88 lmn	5.3 j-l	0.9 hij

[a] Evaluation on a scale of 0% (no injury) to 100% (plant death). Visual evaluation of herbicide symptoms.

[b] Fresh above ground biomass was collected at 19 DAT and dried to determine dry biomass by weight.

[c] Treatments included adjuvants and/or ammonium sulfate as per product label recommendations. Cloransulm, flucarbazone, halosulfuron, imazamox, imazethapyr, nicosulfuron, pyroxsulam, tribenuron, triflusulfuron, metribuzin, bentazon, glyphosate, carfentrazone, fomesafen, and paraquat included nonionic surfactant (Prefer 90) at 0.25% (v/v). Halauxifen, tembotrione, and topramezone included methylated seed oil (Super Spread) at 1.0% (v/v). Atrazine included petroleum oil (Prime Oil) at 1.0% (v/v). Cloransulm, flucarbazone, imazamox, imazethapyr, metribuzin, bentazon, glyphosate, glufosinate, carfentrazone, fomesafen, oxyfluorfen, tembotrione, and topramezone included ammonium sulfate (AMS) at 1.02 kg 100 L^{-1}. Halosulfuron, nicosulfuron, pyroxsulam, tribenuron, and glufosinate included AMS at 3.36 kg ha^{-1}. 2,4-D Amine, clopyralid, dicamba, fluroxypyr, and bromoxynil did not include an adjuvant.

[d] Means within column followed by the same letter are similar according to F-protected LSD at α=0.05 level of significance.

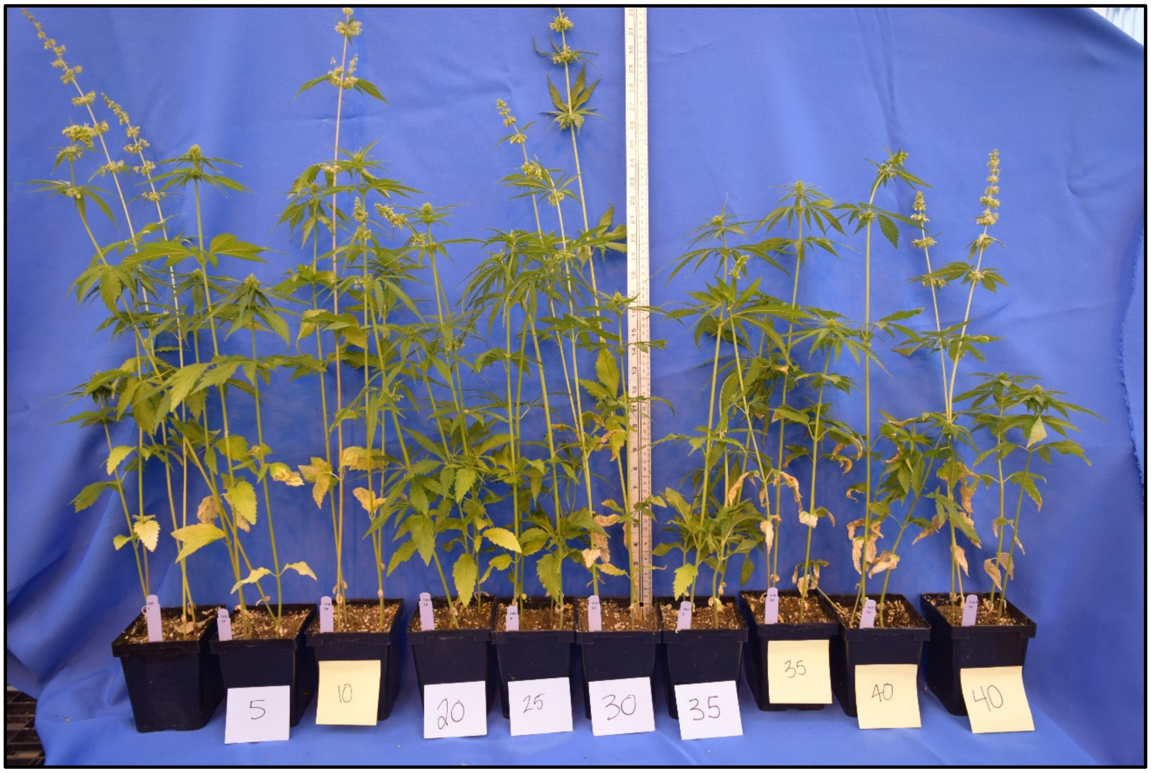

Figure 3.2. Percent visible hemp injury (5 to 40%) with post-emergence herbicides 19 days after treatment.

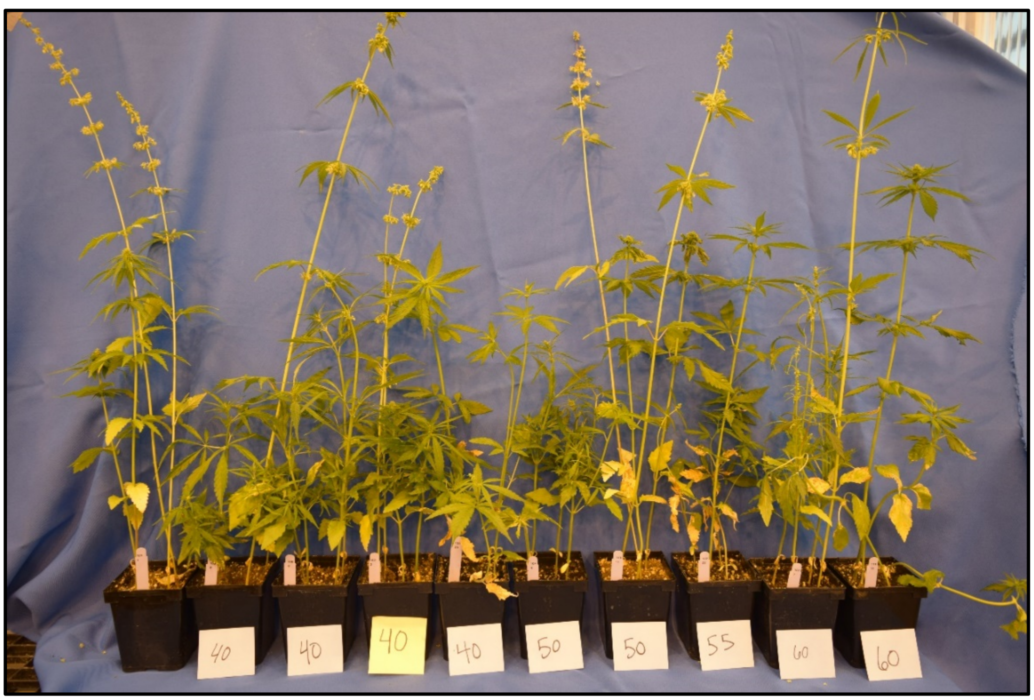

Figure 3.3. Percent visible hemp injury (40 to 60%) with post-emergence herbicides 19 days after treatment.

Figure 3.4. Percent visible hemp injury (80 to 99%) with post-emergence herbicides 19 days after treatment.

Hemp injury caused by clopyralid (Group 4) was similar to that of the non-treated (Table 3.4). All other herbicides resulted in greater injury. Other Group 4 synthetic auxins, such as 2, 4-D amine, halauxifen-methyl, and fluroxypyr-methyl resulted in hemp epinasty and chlorosis (Figure 3.5). The epinasty caused by synthetic auxin herbicides is a result of increased cell division and abnormal apical growth due to too much auxin-like compound and ethylene production in the plants (Cobb and Reade 2010). The photosynthetic rate of plants hours following an application of synthetic auxin actually increases to provide energy for the rapid, irregular growth.

Figure 3.5. Non-treated hemp (left) compared to hemp treated with 2,4-D amine (right) resulted in epinasty 7 days after treatment.

Bromoxynil, a Group 6 photosystem (PS) II inhibitor, resulted in 14% hemp injury, while triflusulfuron-methyl, a Group 2 sulfonylurea herbicide, resulted in 28% injury (Table 3.4). Triflusulfuron resulted primarily in shortened hemp plants with chlorotic new growth (Figure 3.6). Atrazine, cloransulam-methyl, imazethapyr-ammonium, bentazon-sodium, dicamba, and halosulfuron-methyl (in ascending order) resulted in 28 to 47% injury (Table 3.4). Group 5 herbicides atrazine and metribuzin resulted in necrotic or burnt leaf tips and yellowing around the leaf margins (Figure 3.7). New growth appeared to be less affected by the herbicide application. All remaining herbicides resulted in greater than 50% injury (Table 3.4). Hyroxyphenyl pyruvate dioxygenase (HPPD) herbicides tembotrione and topramezone resulted in severe visible injury (86 to 88%) 19 DAT. Non-selective herbicides such as paraquat, glufosinate-ammonium, and glyphosate-potassium resulted in greater than 90% injury.

Figure 3.6. Non-treated hemp (left) compared to hemp treated with triflusulfuron (right) that resulted in stunting and chlorotic new growth 7 days after treatment.

Figure 3.7. Non-treated hemp (left) compared to hemp treated with atrazine (right) that resulted in stunting, necrotic leaf tips, and chlorotic leaf margins 7 days after treatment.

Fresh biomass was collected at 19 DAT and dried to determine dry weight biomass. Herbicides that resulted in greater hemp injury typically resulted in less fresh and dry biomass weights compared to other herbicides (Table 3.4). The non-treated check resulted in 25.6 g of fresh weight and 6.6 g of dry weight. Herbicides that resulted in fresh biomass of greater than 20 g included halosulfuron, imazethapyr, nicosulfuron, cloransulam, triflusulfuron, dicamba, bromoxynil, and clopyralid. The later five having similar fresh biomass to the non-treated. When clopyralid, bromoxynil, dicamba, and cloransulam were applied to hemp, dry hemp biomass was similar to the non-treated. Clopyralid and bromoxynil actually had numerically greater dry biomass than the non-treated, 7.5 and 7.3 g, respectively. Hemp treated with Group 2 acetolactate synthase (ALS) inhibitors resulted in dry biomass that ranged from 1.7 to 5.8 g and averaged 4.2 g. Only Group 6 herbicides resulted in greater dry biomass than the Group 2 herbicide, 5.8 g. The remaining groups resulted in less than 4 g of dry biomass.

Generalizations based off of percent visible injury by herbicide site of action groups can be made to help focus future research (Table 3.5). Some synthetic auxin herbicides, such as clopyralid, appear to be safe for use in hemp, while others seem to cause significant injury. Hemp showed a wide range of injury response to group 4 herbicides, similar to other greenhouse research where hemp injury ranged from 10 to 75% (Byrd 2019; Flessner et al. 2020; Willenborg and Johnson 2018). Similar greenhouse research available at the conclusion of this research suggests that clopyralid is likely more injurious than what this screening portrays. Clopyralid applied at 100 g ae ha^{-1} resulted in 44% visible injury 14 DAT (Flessner et al. 2020). Another greenhouse experiment reported low levels of hemp injury from clopyralid with anticipated hemp recovery from a biomass reduction of 16 to 22% (Ortmeier-Clarke et al. 2020).

Photosystem II inhibitors had the lowest average injury across both evaluation timings. Protoporphyrinogen oxidase (PPO) and HPPD inhibitor herbicides, along with non-selective herbicides, resulted in consistent, substantial visible injury of greater than 65%. Other research was not found that evaluated hemp response to either Group 14 or 27 herbicides in the greenhouse, so corroboration will require additional work. Later research did confirm high levels (~70%) of visible hemp injury from acifluorfen when applied at 2.2 g ai ha^{-1}, another PS II herbivide. Hemp biomass response to herbicides within the same site of action group were highly variable.

Table 3.5. Post-emergence visible herbicide injury summary based on herbicide site of action in greenhouse.

		7 DAT[a]		19 DAT	
Site of action	Site of action	Range	Average	Range	Average
	Group	----------- % -----------		----------- % ----------	
ALS Inhibitors	2	4-68	52	28-77	49
Synthetic Auxins	4	3-83	54	5-84	56
Photosystem II Inhibitors	5	53-82	68	31-62	47
Photosystem II Inhibitors	6	19-62	41	14-40	27
EPSP Synthase Inhibitor	9	n/a[b]	80	n/a	98
Glutamine Synthesis Inhibitor	10	n/a	95	n/a	96
PPO Inhibitors	14	67-93	85	44-90	67
HPPD Inhibitors	27	77-81	76	86-88	87

[a] Days after treatment (DAT).

[b] Only one herbicide was evaluated for EPSP Synthase and Glutamine Synthesis inhibitors, so range not applicable (n/a).

Very few post-emergence herbicides were safe enough to be considered for field evaluation. Clopyralid was the safest herbicide as hemp injury and biomass were similar to the non-treated check (Table 3.4). Hemp was fairly resilient to moderate herbicide injury, up to 35%, in the greenhouse without loss of biomass production. The safest herbicides from each of the sites of action groups were represented in subsequent field trials.

Contributing factors of herbicide selection for the field included the general commercial use and weed control efficacy of herbicides within the Upper Midwest. For example, imazamox was selected rather than imazethapyr because it is more commonly used and has a better weed control spectrum than imazethapyr for this geography. Cloransulam and oxyfluorfen were selected to help represent a diverse set of sites of action. Quinclorac was relatively safe to hemp when applied pre-emergence, and due to the limited options for post-emergence it was added to the field experiment. The following herbicides were included in field experiments: cloransulam, imazamox, clopyralid, quinclorac, bromoxynil, atrazine, and oxyfluorfen.

3.2.2. Field Experiment

Mean square error values for all variables were within a factor of 10 and passed the Bartlett's Chi-square test for homogeneity; therefore, data were combined and analyzed through SAS accordingly. Since year and location were considered random effects and combined to form seven environments for analysis, year by treatment and location by treatment interactions were not evaluated. Similar to the pre-emergence field experiment (Chapter 2.2.2), the analysis of variance (ANOVA) tables almost always indicated a treatment by environment effect in the post-emergence experiment (Tables 3.6 and 3.7). These effects can, in part, be explained by the environmental conditions of each year. Comments in regards to weather conditions can be found in the discussion of field results with pre-emergence herbicides (Chapter 2.2.2). Results from this research were intended to be extrapolated or generalized across any year and random locations, ultimately any environmental growing conditions, in order to support a Section 3 herbicide registration. Treatment by time interactions were observed for nearly every variable measured (Table 3.6 and 3.7), but treatment only comparisons were often more practical for discussion towards the end goal of this research.

In general, 2020 hemp density was roughly half of the density observed in 2019 (data not shown). Lower plant density in 2020 was attributed to planting into moist soil and then not receiving precipitation for about a week. Hemp typically emerges within 5 days and at locations with heavier soil types the soil surface dried and sealed during that germination period. Hemp seedlings were unable to break through the sealed soil resulting in reduced emergence.

Table 3.6. ANOVA table depicting degrees of freedom, mean square, and *p-value* for plant density, visible injury, and yield at evaluation timings.

	Timing 1			Timing 2		
SOV	df	MS	P	df	MS	P
			Density[a]			
Env	6	105	*	5	18214	*
Rep(Env)	21	280	*	18	294	NS
Trt	15	222	NS	15	3246	*
Trt x Env	90	170	NS	75	361	*
Error	313	146	-[b]	270	361	-
Total	445	-	-	383	-	-
			Visible injury[c]			
Env	6	0.213	*	5	0.129	*
Rep(Env)	21	0.019	NS	18	0.014	NS
Trt	15	2.672	*	15	2.188	*
Trt x Env	90	0.028	*	75	0.033	*
Error	314	0.034	-	267	0.033	-
Total	446	-	-	380	-	-
			Yield[d]			
Env	4	4962647	*	-	-	-
Rep(Env)	15	405768	*	-	-	-
Trt	8	4382459	*	-	-	-
Trt x Env	32	748768	*	-	-	-
Error	117	116222	-	-	-	-
Total	176	-	-	-	-	-

* Data is significantly different according to f-protected LSD at α=0.05 level of significance using PROC GLM.

[a] Density was evaluated at 7 and 35 days after treatment (DAT).

[b] Places in the table with a dishes (-) indicate that data was not available.

[c] Visible hemp injury was evaluated 7, and 21 DAT.

[d] Yield was obtained at 100 DAT.

Table 3.7. ANOVA table depicting degrees of freedom (df), mean square (MS), and *p-value* (P) for height and light interception at evaluation timings.

	Timing 1			Timing 2			Timing 3			Timing 4		
SOV	df	M	P	df	MS	P	df	MS	P	df	MS	P
						Height[a]						
Env	6	1038	*	5	16383	*	4	18617	*	-[b]	-	-
Rep(Env)	21	72	*	18	326	NS	15	445	NS	-	-	-
Trt	15	1693	*	15	11378	*	15	6310	*	-	-	-
Trt x Env	90	52	*	75	841	*	59	730	*	-	-	-
Error	299	51	-	253	233	-	198	393	-	-	-	-
Total	431	-	-	366	-	-	291	-	-	-	-	-
						Light interception[c]						
Env	6	0.176	*	6	1.258	*	5	0.719	*	4	1.577	*
Rep(Env)	21	0.019	*	21	0.023	NS	18	0.027	*	15	0.070	*
Trt	15	0.238	*	15	0.758	*	15	1.146	*	15	0.625	*
Trt x Env	90	0.019	*	90	0.075	*	75	0.091	*	60	0.089	*
Error	300	0.005	-	311	0.017	-	265	0.102	-	221	0.018	-
Total	432	-	-	443	-	-	378	-	-	315	-	-

* Data is significantly different according to f-protected LSD at α=0.05 level of significance using PROC GLM.

[a] Height measurements were collected at 7, 35, and 72 days after treatment (DAT).

[b] Places in the table with a dishes (-) indicate that data was not available.

[c] Light interception data was collected at 7, 21, 35, and 72 DAT.

Hemp density at 7 DAT was similar across all plots and ranged from 56 to 66 plants per 2 m of row (Table 3.8). Similar density at 7 DAT was expected, and indicated hemp establishment across the experiment was consistent. At 7 DAT, all plants, even those severely injured by herbicides, were counted to establish a baseline prior to the 35 DAT evaluation. At 35 DAT, the non-treated and hand-weeded had densities of 52 and 55 plants per 2 m of row, respectively. Hemp naturally self-thins in dense plant communities and environments with high soil nitrogen (Fike 2016; Struik et al. 2000). Atrazine-1 along with both rates of cloransulam and oxyfluorfen reduced hemp densities at 35 DAT. Atrazine-2, Cloransulam-1, and oxyfluorfen-1 reduced hemp density by 15, 34, and 37%, respectively, compared to the non-treated hemp.

Table 3.8. Hemp density response averaged across environments to post-emergence herbicides 7 and 35 days after treatment (DAT).

Treatment	Density 7 DAT[a]	35 DAT[b]
	-- Plants in 2 m of row --	
Non-treated	66	52 abc[c]
Hand-weeded	64	55 ab
Cloransulam-methyl-1[d]	61	34 def
Cloransulam-methyl-2	62	31 f
Imazamox-ammonium-1	64	56 ab
Imazamox-ammonium-2	66	59 a
Clopyralid-1	65	51 abc
Clopyralid-2	64	53 abc
Quinclorac-methyl-1	66	51 abc
Quinclorac-methyl-2	60	52 abc
Bromoxynil-1	61	51 abc
Bromoxynil-2	58	45 bcd
Atrazine-1	56	44 cde
Atrazine-2	60	34 ef
Oxyfluorfen-1	61	29 fg
Oxyfluorfen-2	59	19 g

[a] 7 DAT counted all plants that would have been living plants prior to the herbicide application, as a baseline hemp density.

[b] 35 DAT represents a reduced hemp density as a result of the herbicide application.

[c] Means within column followed by the same letter are similar according to F-protected LSD at α=0.05 level of significance.

[d] Herbicide active ingredient followed by a numerical digit represent a low (1X) and high (2X) rate of that active ingredient based on use patterns in other crops.

Hemp plants were evaluated for percent visible injury compared to the checks at 7 and 21 DAT. Hemp responded quickly to post-emergence herbicides, with all herbicides resulting in hemp injury at 7 DAT (Table 3.9). Atrazine and oxyfluorfen resulted in greater than 50 and 90% injury, respectively, primarily expressed as necrosis on the leaves that were present during the time of herbicide application. However, hemp was already recovering from atrazine damage at 7 DAT through production of healthy new leaf tissue. Cloransulam resulted in greater than 60% injury, mostly due to chlorosis at 7 DAT. Injury symptoms to quinclorac were primarily distorted leaves, with some chlorosis.

Table 3.9. Visible hemp injury response averaged across environments to post-emergence herbicides 7 and 21 days after treatment (DAT).

	Visible injury[a]	
Treatment	7 DAT	21 DAT
	--------------- % -------------	
Non-treated	2 a[b]	1 a
Hand-weeded	3 a	1 a
Cloransulam-methyl-1[c]	74 e	76 h
Cloransulam-methyl-2	76 e	84 hi
Imazamox-ammonium-1	62 d	46 ef
Imazamox-ammonium-2	62 d	50 fg
Clopyralid-1	19 b	12 b
Clopyralid-2	17 b	10 ab
Quinclorac-methyl-1	24 b	33 cd
Quinclorac-methyl-2	22 b	41 def
Bromoxynil-1	38 c	28 c
Bromoxynil-2	55 d	38 cde
Atrazine-1	56 d	34 cd
Atrazine-2	78 e	60 g
Oxyfluorfen-1	93 f	89 ij
Oxyfluorfen-2	96 f	95 j

[a] Visual evaluation of herbicide symptoms on a scale of 0% (no injury) to 100% (plant death).

[b] Means within column followed by the same letter are similar according to F-protected LSD at α=0.05 level of significance.

[c] Herbicide active ingredient followed by a numerical digit represent a low (1X) and high (2X) rate of that active ingredient based on use patterns in other crops.

An ideal evaluation timing to compare herbicide tolerance in this experiment was at 21 DAT, after hemp had the opportunity to recover from initial damage. The statistical analysis was not conducted in a fashion in order to draw conclusions based of the changes in visible hemp response between evaluation timings; however, the level of hemp injury at 21 DAT when compared to the hemp injury at 7 DAT can be a good indicator of hemp's ability to overcome particular herbicide injury.

At 21 DAT, non-treated hemp plants ranged from 75 to 140 cm tall (data not shown) with 7 to 11 leaf pairs, depending on the location (Figure 3.8). Visible injury to hemp at 21 DAT ranged from 0 to 95% (Table 3.9). Acetolactate synthase inhibitors cloransulam and imazamox resulted in 46 to 84% injury, respectively. Injury symptoms included severe stunting and chlorosis accompanied by death of the terminal growing point and sometimes entire plants. Death of terminal growing point led to a visible increase in the amount of lateral branching compared to the non-treated hemp. Acetolactate synthase inhibitors lead to growth inhibition by reducing the biosynthesis of amino acids leucine, isoleucine, and valine (Cobb and Reade 2010). It was noted during evaluations, that hemp treated with either cloransulam or imazamox only grew 15 to 60 cm after herbicide application, primarily from axillary buds (Figure 3.9). New growth appeared relatively normal with slight chlorosis at 21 DAT.

Figure 3.8. Non-treated hemp 21 days after treatment.

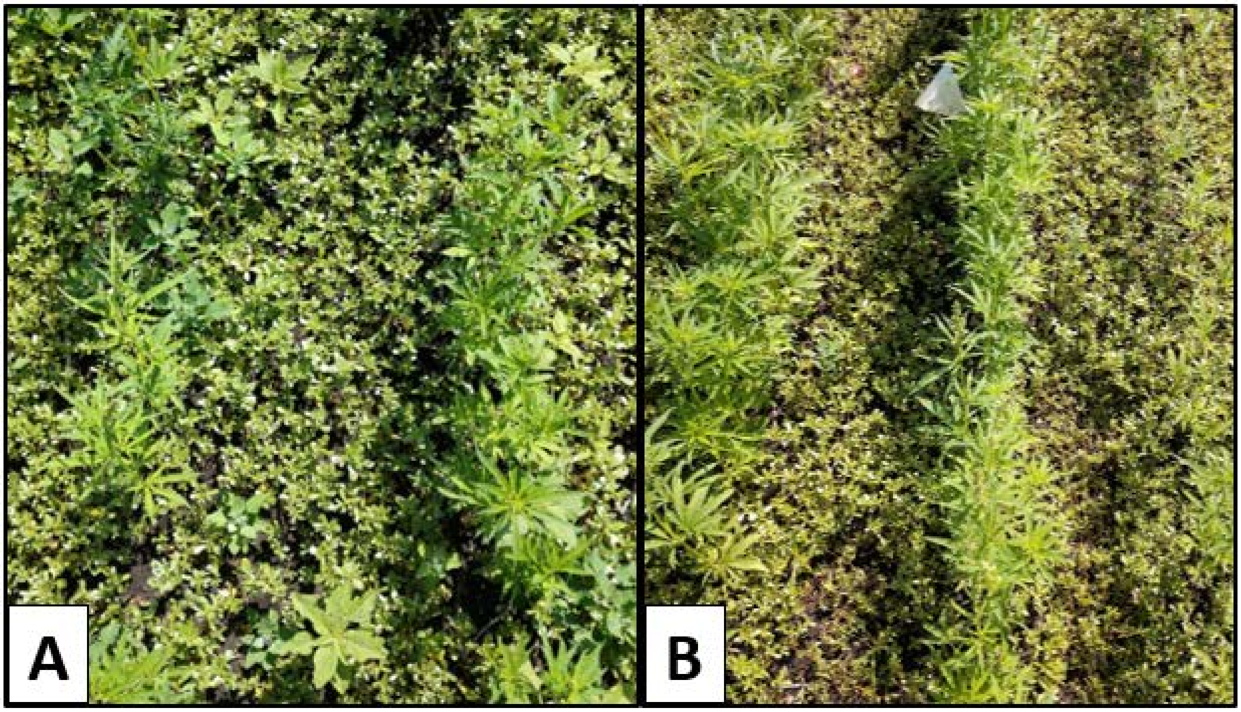

Figure 3.9. Hemp stunting and chlorosis from cloransulam (A) and imazamox (B) 21 days after treatment.

Very little research was previously conducted to evaluate hemp response to cloransulam or imazamox in the field. No research was available at the onset of this experiment. Cloransulam at 19.6 g ai ha^{-1} resulted in 60 to 87% hemp injury in an exploratory experiment conducted at the University of Nebraska Lincoln (Scott et al. 2020). Less visible hemp injury was observed in response to imazamox (14.2 g ae ha^{-1}) (Howatt and Mettler 2018). This research also showed cloransulam as the more injurious ALS inhibitor (Table 3.9). Other ALS inhibitors that have been studied as a post-emergent in recent years include chlorimuron-ethyl (5.8 g ai ha^{-1}), flazaulfuron (110 g ai ha^{-1}), halosulfuron (35 to 50 g ai ha^{-1}), nicosulfuron (34.4 g ai ha^{-1}), rimsulfuron (70 g ai ha^{-1}), thifensulfuron-methyl (3.5 to 6 g ai ha^{-1}), trifloxysulfuron (7 g ai ha^{-1}), and triflusulfuron-methyl (14.2 g ha^{-1}) (Flessner et al. 2020; Howatt and Mettler 2018; Lingenfelter 2018; Lingenfelter and Wallace 2019; Maxwell 2016; Scott et al. 2020; Woosley et al 2015). Visible hemp injury response ranged from 0 to 86%, with early evaluations such as 7

DAT typically being less injurious than later evaluations. Average hemp injury response from ALS inhibitors was approximately 58%, with triflusulfuron generally resulting the least amount of injury (Lingenfelter 2018; Lingenfelter and Wallace 2019).

Visible hemp injury 21 DAT from the low rates of synthetic auxins (Group 4) clopyralid-1 and quinclorac-1 resulted in 12 and 33% injury, respectively (Table 3.9). It was observed during the injury evaluation that some degree of stunting with minimal yellowing of new growth along with light leaf crinkling occurred due to clopyralid (Figure 3.10). Hemp metabolism allowed for minimal symptomology by harvest. Visible hemp injury from quinclorac expressed itself as deformed leaves and buds along with slight stunting and a bluish tint to the leaves. In more severe cases quinclorac resulted in yellowing of the mid-rib and alligator-backing on the outer third individual leaflets (Figure 3.11). Hemp plants never truly recovered from the injury caused by the herbicide application, which persisted to some degree all season long.

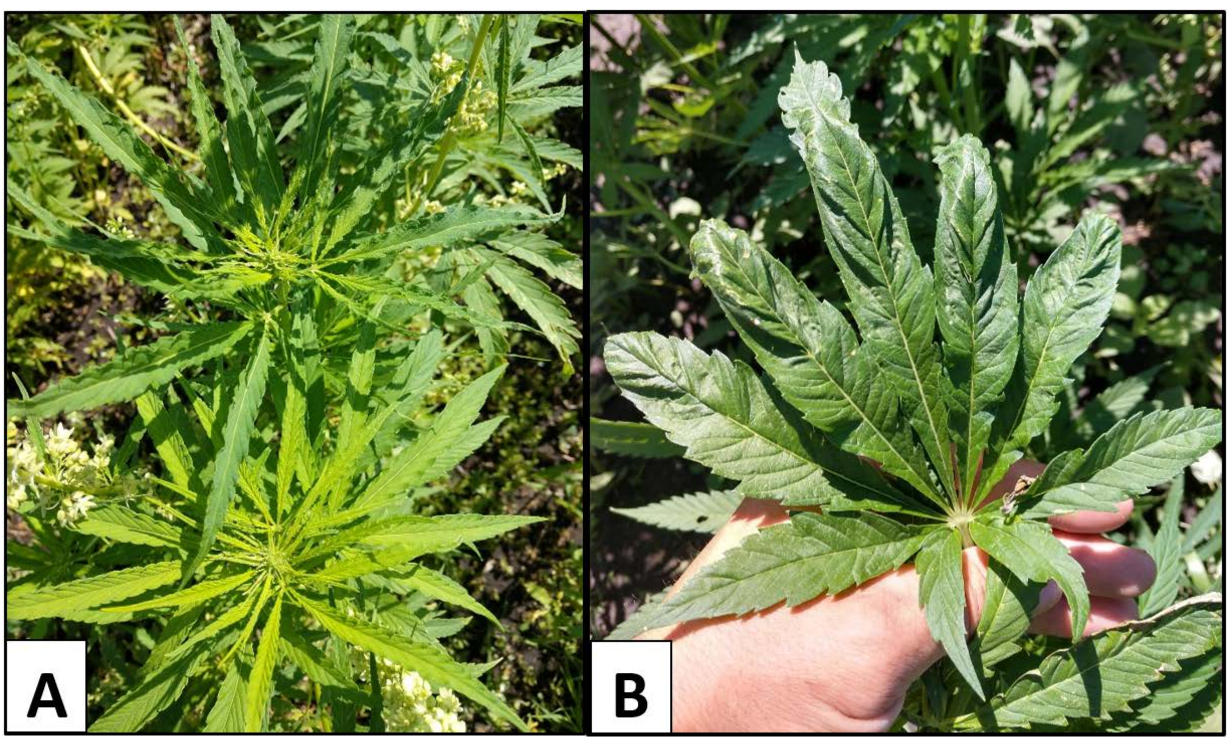

Figure 3.10. Hemp injury response to clopyralid at 105 (A) and 210 g ae ha^{-1} (B) 21 days after treatment.

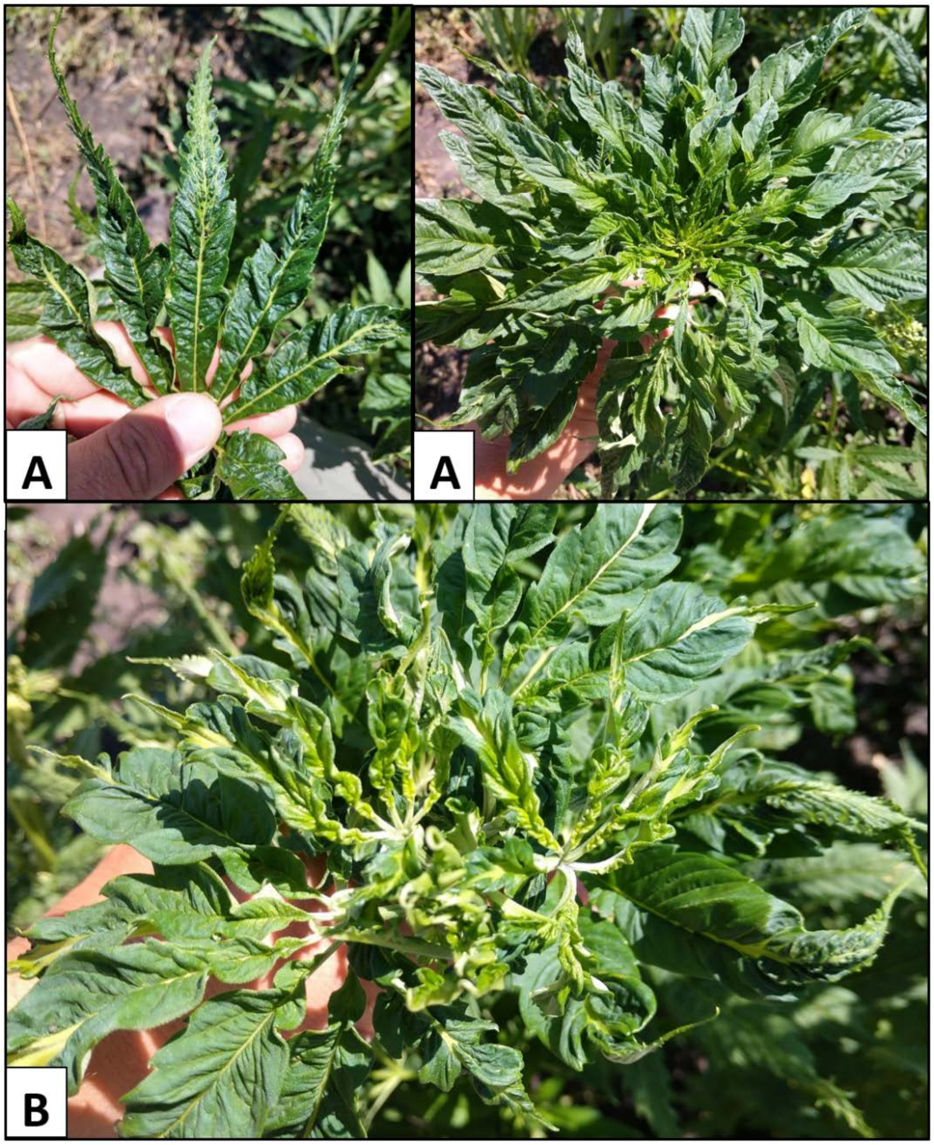

Figure 3.11. Hemp injury response to quinclorac at 290 (A) and 580 g ai ha^{-1} (B) 21 days after treatment.

Synthetic auxin herbicides affect cell division, differentiation, and elongation which can result in uncontrolled growth, leaf cupping, and epinasty in young plant tissue (Cobb and Reade 2010). Visible hemp injury in this experiment from synthetic auxins reflected this typical injury response. Research conducted at the University of California evaluated herbicide symptomology on hemp using 25% of normal herbicide rates and also observed abnormal growth in the form of leaf cupping from 2,4-D and stem epinasty from triclopyr (Light and Hanson 2021).

One other experiment evaluated quinclorac as a post-emergence weed control option in hemp. Visible hemp injury from quinclorac at 842 g ha^{-1} with 3.3% (v/v) MSO ranged from 7 to 20% injury (Scott et al. 2020). In our experiment quinclorac was applied at 290 and 580 g ha^{-1} with 2.2% (v/v) MSO and resulted in approximately 20% more visible injury, ranging from 24 to 41% (Table 3.9). Typically, the addition or higher rates of MSO would result in greater injury. The opposite was true when comparing hemp response to quinclorac between these two experiments. Other possible factors such as environmental conditions during the time of herbicide application could have also influenced the efficacy of quinclorac.

The variability in hemp response to quinclorac within and between experiments could be explained by looking further into the mechanism of herbicide action in conjunction with the weather conditions following the herbicide application. In order for a plant to support the irregular and rapid growth caused by a synthetic auxin herbicide the plant needs to rapidly increase its photosynthetic rate (Cobb and Reade 2010). The stimulation of photosynthesis can occur within hours of the herbicide application. If weather conditions are cloudy during and following herbicide application, the photosynthetic response and rapid growth symptomology as a result of the herbicide would be slower than if the herbicide application was made during conditions more conducive to photosynthesis. This delayed or prolonged herbicide response could allow for the plant to counter act or compensate physiological changes caused by the herbicide, effectively reducing the overall amount of visual injury via metabolism. In this research, there was approximately 20% cloud cover on average during the time of application across all environments. Therefore, the environmental conditions, particularly cloud cover, at the time of herbicide application was a minimal factor in accounting for the variable hemp injury response to quinclorac within this experiment.

In North Dakota, clopyralid is typically applied without the use of an adjuvant as was done in this experiment. Other research has shown that clopyralid applied with an adjuvant resulted in significant hemp injury and some authors anticipated difficulty in its registration for use in hemp (Willenborg and Johnson 2018). Based on the research available, when clopyralid was applied to hemp at less than 100 g ae ha^{-1}, 0 to 10% visible hemp injury occurs (Byrd 2019; Flessner et al. 2020; Willenborg and Johnson 2018). Visible hemp injury increased to 3 to 35% with 150 g ha^{-1} and 10 to 35% injury with 300 g ha^{-1} clopyralid (Flessner et al. 2020; Scott et al. 2020; Willenborg and Johnson 2018). The amount of hemp injury due to clopyralid (105 and 210 g ha^{-1}) from our research coincides with other research findings (Table 3.9). Hemp treated with MSO (10% v/v), without a herbicide, resulted in yellow and necrotic burns on the leaves similar to what is observed from contact herbicides which could explain why some research resulted in greater amounts of injury when clopyralid was applied with MSO (Light and Hanson 2021). Other studies have suggested that registration of clopyralid could be pursued because hemp height, density, and yield did not differ from non-treated and hand-weeded plots (Howatt and Mettler 2018; Bryd 2019).

Other Group 4 herbicides that have been evaluated in the field at standard use rates for use in hemp include fluroxypyr-methyl (43 to 157 g ae ha^{-1}), fomesafen (86 g ai ha^{-1}), and halauxifen-methyl (2.1 g ai ha^{-1}) which resulted in 72 to 92%, 85%, and 35% hemp injury respectively (Howatt and Mettler 2018; Scott et al. 2020). Among synthetic auxin herbicides, clopyralid appears to be the least injurious to hemp.

In this research, bromoxynil resulted in 28 to 55% hemp injury across evaluation timings (Table 3.9). At 7 DAT, bromoxynil-1 (280 g ha^{-1}) resulted in 38% injury (Figure 3.12) and 28% injury at 21 DAT (Table 3.9). Bromoxynil is a Group 6, PS II inhibitor, that displaces plastoquinone, an electron transporter in the PS II pathway, to prevent electron (energy) flow and results in photosynthetic inhibition (Cobb and Reade 2010). This disruption of electrons leads to excess energy and, ultimately, active oxygen species that can damage cellular membranes leading to the characteristic necrotic spots and lesions on leaves that have been in contact with a Group 6 herbicide. At 21 DAT, hemp that was treated with bromoxynil was slightly stunted and had some necrotic spots midway up the plants compared to the non-treated hemp. A greater degree of height reduction and leaf deformation became apparent with the higher (560 g ha^{-1}) bromoxynil rate. The extent of bromoxynil-1 injury to hemp occurred early on and by 28 DAT symptomology was minimal given the natural leaf senescence of the lower leaves where the majority of the visible injury had occurred.

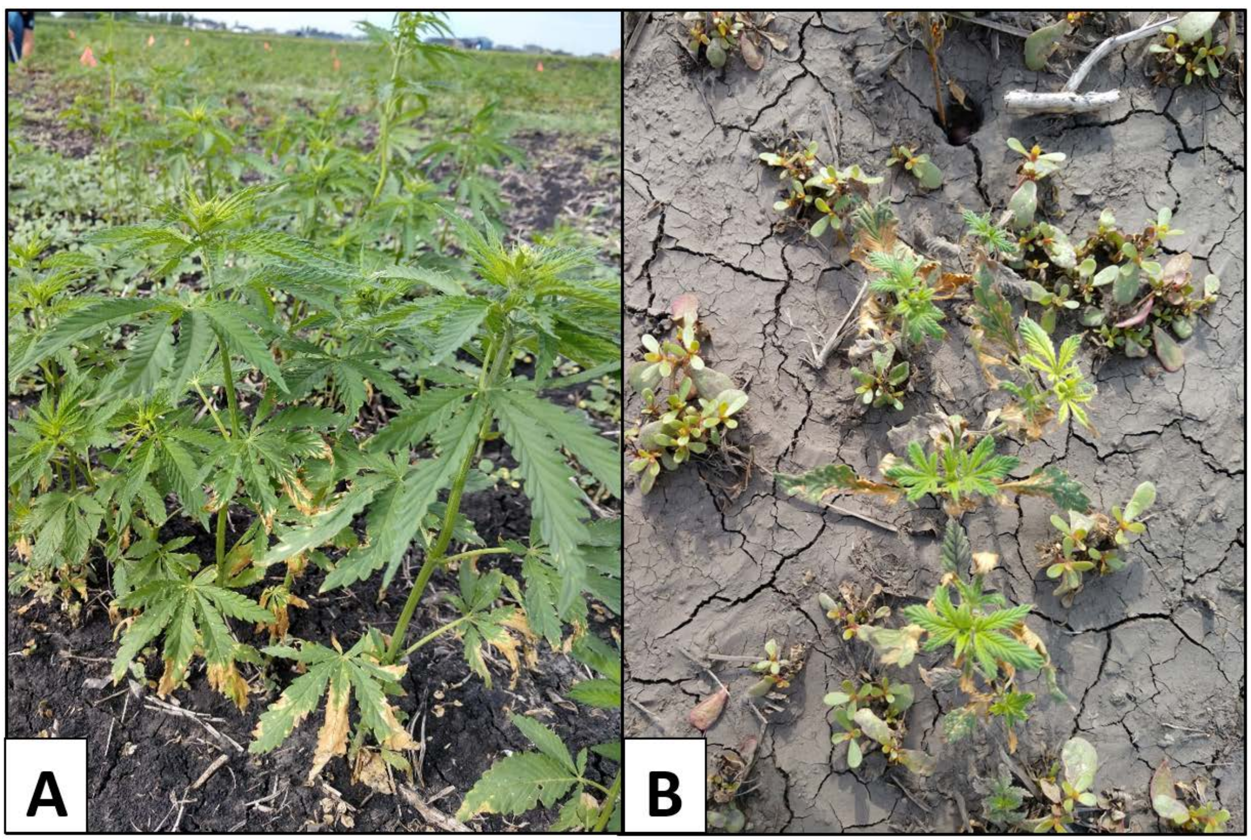

Figure 3.12. Hemp injury response to bromoxynil at 280 (A) and 560 g ae ha^{-1} (B) 7 days after treatment.

Bromoxynil has been one of the more extensively studied herbicide options for use in industrial hemp. When bromoxynil was applied at a rate of 100 to 150 g ha^{-1}, hemp injury ranged from 0 to 10% (Howatt and Mettler 2018; Byeongseok and Ulrich 2014b). When bromoxynil rates increased to near 300 g ha^{-1}, up to 20% visible hemp injury was observed (Flessner et al. 2020; Lingenfelter 2018; Lingenfelter and Wallace 2019; Pearce and Carter 2019; Willenborg and Johnson 2018). At a rate of 560 g ha^{-1}, hemp injury ranged from 5 to 20%, with certain hemp cultivars reaching up to 40% injury (Pearce and Carter 2019; Post 2020; Willenborg and Johnson 2018). Bromoxynil has been evaluated at a maximum rate of 1120 g ha^{-1}, basically four times the typical commercial use rate, which resulted in 18 to 28% injury (Post 2020). At the onset of this experiment, it was reported that bromoxynil typically resulted in less than 15% visible injury to hemp; however, with more recent research, that value is probably closer to 25% injury or less and is dependent on the hemp cultivar.

Similar conclusions could be drawn for bromoxynil and clopyralid use in hemp, which could become a viable weed control option if registrants and producers accepted initial injury and focus on the end results. Industry professionals have discussed that ‘X-59’, the cultivar used in these experiments, is more susceptible to herbicides than other cultivars (personal communication). This could explain the greater degree of hemp injury observed with bromoxynil, and even quinclorac, in this research compared to other publications. Research has not been conducted to specifically quantify the herbicide tolerance differences among cultivars. Research conducted in Canada did show that ‘Canda’, ‘CFX-2’, ‘CFX-1’, ‘CRS-1’, ‘Delores’, ‘Grande’, ‘Joey’, ‘Katani’, and ‘Piccolo’ exhibited acceptable tolerance to bromoxynil; however, higher injury ratings were observed in X-59 (Willenborg and Johnson 2018). Additional screening of various cultivars with clopyralid and bromoxynil, along with herbicide application timing experiments, are needed to fully develop these herbicide use patterns in hemp.

Atrazine-1 resulted in 34% hemp injury at 21 DAT with nearly twice as much injury present with the high rate (Table 3.9). The primary injury response was necrosis. It was visually easy to observe the reduction in hemp height and density as a result of the herbicide application. It is important to note that, by the end of the growing season, plots that experienced a reduction in hemp density had more branched and robust plants. Relatively good weed control was achieved with atrazine-1 and atrazine-2 across all weed species and locations in 2020 at 21 DAT, up to 75% control (data not shown). Atrazine, similar to bromoxynil, is a PS II inhibitor but is more persistent in the soil, which extends the length of adequate weed control (Cobb and Reade 2010). To date, no other studies examined atrazine use in hemp. Metribuzin, another Group 5 herbicide, resulted in 82% and 63% injury at 14 and 28 DAT, respectively (Howatt and Mettler 2018).

Hemp was severely injured, greater than 85%, with applications of the Group 14 herbicide, oxyfluorfen (Table 3.9). Hemp treated with oxyfluorfen expressed large amounts of leaf chlorosis and necrosis, which led to severe terminal growth injury, but development of more lateral branches compared to less injured hemp (Figure 3.13). However, oxyfluorfen did provide greater than 96% control of all weed species present within the plots in 2020 (data not shown). Due to the degree of hemp injury, oxyfluorfen should not be considered further for use as a weed control option in industrial hemp. Apart from this study, oxyfluorfen has not been evaluated as a herbicide option in hemp. Carfentrazone-ethyl, another Group 14 herbicide, resulted in 15 to 23% and 73 to 82% injury at rates of 3.6 and 5.3 g ai ha^{-1}, respectively, in other research (Howatt and Mettler 2018; Lingenfelter 2019).

Figure 3.13. Hemp injury from oxyfluorfen 21 days after treatment.

Post-emergence herbicides had an immediate impact on height, as expected since the herbicides were applied just prior to the rapid growth and stem cell elongation of hemp. Differences in height were observed at each evaluation time (Table 3.10). Applications of clopyralid and quinclorac had a relatively minimal effect on hemp growth and height, with hemp size similar to the non-treated and hand-weeded checks. At 7 DAT, clopyralid and quinclorac were the only herbicides that resulted in hemp height similar to the hand-weeded. In general, by 35 DAT, hemp recovered from the initial stunting that resulted from bromoxynil and atrazine. By 72 DAT, all hemp heights were similar to the non-treated and hand-weeded apart from hemp treated with cloransulam or oxyfluorfen. The hemp that was similar ranged in height from approximately 90 to 110 cm. Hemp plants were able to recover from initial height reductions caused by herbicides, unless height was initially reduced by greater than 60%.

Additional data was collected during height measurements to determine the difference in height between male and female hemp plants and also the occurrence of males within the population. The male plants within the sampling population were measured 7 and 35 DAT. By

72 DAT all male plants had naturally senesced and only female heights were recorded. Male plants at 35 DAT (maximum male height) were on average 21% or 18.5 cm taller than female plants (data not shown). Male plants consisted of 35% of the hemp population in 2019 and 45% of the population in 2020 (data not shown). The occurrence of male to female hemp plants within this dioecious crop is close to a 1:1 ratio, but female plants do tend to predominate (Bócsa and Karus 1998). It is unknown as to the cause of the different levels of male incidence between years within this study. Perhaps the slightly higher temperatures and lower amount of precipitation in 2020 (Table 2.11) resulted in more stressed hemp plants leading to the development of fewer female plants. The proportion of male to female plants can vary as a result of geographical races, the variety, and environmental conditions (Bócsa and Karus 1998).

Table 3.10. Hemp height response to post-emergence herbicides averaged across environments at 7, 35, and 72 days after treatment (DAT).

	Height[a]		
Treatment	7 DAT	35 DAT	72 DAT
	------------------- cm -----------------------		
Untreated	31 a[b]	102 ab	102 ab
Hand-weeded	30 ab	103 a	113 a
Cloransulam-methyl-1[c]	10 f	54 d	69 c
Cloransulam-methyl-2	12 ef	40 de	52 cd
Imazamox-ammonium-1	12 def	85 bc	98 ab
Imazamox-ammonium-2	11 f	84 c	92 b
Clopyralid-1	27 ab	94 abc	101 ab
Clopyralid-2	27 ab	100 abc	104 ab
Quinclorac-methyl-1	27 b	94 abc	94 b
Quinclorac-methyl-2	27 b	92 abc	99 ab
Bromoxynil-1	20 c	89 abc	101 ab
Bromoxynil-2	15 de	91 abc	99 ab
Atrazine-1	16 d	86 abc	100 ab
Atrazine-2	12 def	86 abc	107 ab
Oxyfluorfen-1	9.3 f	40 de	59 cd
Oxyfluorfen-2	11 f	25 e	45 d

[a] Total plant height was measured to the nearest cm from the base of the plant to the end of terminal growth.

[b] Means within column followed by the same letter are similar according to F-protected LSD at α=0.05 level of significance.

[c] Herbicide active ingredient followed by a numerical digit represent a low (1X) and high (2X) rate of that active ingredient based on use patterns in other crops.

The sampling size of six hemp plants per plot for determining hemp height had its challenges and resulted in greater levels of variability or poorer plot representation than anticipated. At 7 DAT, individual plant sex was unidentifiable so all plants accounted for the height observation and plants selected for continual observation were selected at random. For example, some plots had five male plants and one female plant selected to represent the plot, which was discovered at the 35 DAT measurement. That left only one female plant remaining at 72 DAT to determine the average height for that plot. Since male plants were also taller on average, that particular plot could have a slightly taller average height compared to the other replicates of that treatment.

Degradable tags were stringed around the base of hemp stems to identify the same hemp plants for height measurements at each evaluation timing. It worked well to measure the same six plants to develop a true growth pattern and allow for comparisons between treatments even though the statistical analysis was not conducted in a way that allowed for comparisons through evaluation timings. However, sometimes the strings tightened essentially girdling the plant, resulting in plant death and therefore reducing the number of measurable hemp plants within a plot. Combining the statistical analysis across all environments reduced the impact such plots had on the overall outcome of this research.

Similar to hemp height, light interception (LI) differences were observed at each evaluation timing. Hemp canopy LI was similar to the checks at 7 DAT when either rates of clopyralid and quinclorac were applied (Table 3.11). All other herbicides initially reduced LI by at least 35%. At 7 DAT, oxyfluorfen, resulted in as much as, 74% less LI by hemp compared to the non-treated. By 21 DAT, hemp treated with several herbicides had similar LI compared with the non-treated and hand-weeded checks.

Light interception was maximized 35 DAT and is an ideal timing to draw conclusions. Light interception between the non-treated and the hand-weeded began to differ at 21 DAT and were no longer considered similar at 35 DAT (Table 3.11). At 35 DAT, the LI for non-treated and hand-weeded plots were 51 and 78%, respectively. The hand-weeded plots were essentially weed-free, while the non-treated plots often had weeds half as tall at the hemp at 35 DAT, reducing the effective weed control canopy and hemp canopy LI. The non-treated hemp allowed 34% more light to the weeds below the hemp canopy than the hand-weeded. Hemp that received the low rate of bromoxynil intercepted light at a similar level to the hand-weeded, and 32% more than the non-treated check at 35 DAT. All other herbicides, apart from cloransulam and oxyfluorfen, resulted in similar LI (60 to 66%) to both the non-treated and hand-weeded. Cloransulam and oxyfluorfen resulted in 27% LI or less 35 DAT. Low LI from oxyfluorfen was due to hemp injury and not weed control, which was 99% in 2019 (99% data not shown). Low hemp LI with cloransulam was due to a combination of hemp injury and poor weed control of less than 40% (data not shown).

Hemp LI that was similar to the hand-weeded, yet different than the non-treated at 72 DAT, was hemp treated with imazamox-2 and both rates of bromoxynil (Table 3.11). Imazamox-1 and both rates of clopyralid, quinclorac, and atrazine were similar to the hand-weeded and the non-treated checks at 72 DAT. Many herbicides that were different from the checks at 21 DAT ended up being similar to the checks at 72 DAT, attributing to the ability of hemp to recover from herbicide injury.

Table 3.11. Light interception response averaged across environments to post-emergence herbicides 7, 21, 35, and 72 days after treatment (DAT).

	Light interception[a]			
Treatment	7 DAT	21 DAT	35 DAT	72 DAT
	---------------------------------- % --------------------------------			
Non-treated	36 a[b]	47 abc	51 b	44 bc
Hand-weeded	34 a	57 a	78 a	68 a
Cloransulam-methyl-1[c]	13 de	17 e	20 c	19 d
Cloransulam-methyl-2	12 de	9 f	13 c	16 d
Imazamox-ammonium-1	17 cde	41 bcd	61 ab	55 ab
Imazamox-ammonium-2	14 de	37 cd	66 ab	66 a
Clopyralid-1	32 a	53 ab	66 ab	50 abc
Clopyralid-2	31 a	53 ab	63 ab	53 ab
Quinclorac-1	30 ab	48 abc	66 ab	55 ab
Quinclorac-2	32 a	46 abc	66 ab	60 ab
Bromoxynil-1	22 bc	46 abc	75 a	65 a
Bromoxynil-2	16 cde	40 bcd	66 ab	64 a
Atrazine-1	18 cd	40 bcd	66 ab	57 ab
Atrazine-2	12 de	29 de	64 ab	61 ab
Oxyfluorfen-1	10 e	9 f	27 c	32 cd
Oxyfluorfen-2	9 e	9 f	14 c	19 d

[a] Evaluation on a scale of 0% to 100% (complete light interception via hemp canopy). Data collected with ceptometer.

[b] Means within column followed by the same letter are similar according to F-protected LSD at α=0.05 level of significance.

[c] Herbicide active ingredient followed by a numerical digit represent a low (1X) and high (2X) rate of that active ingredient based on use patterns in other crops.

Approximately 100 days after planting, hemp treated with the low rates of each herbicide and the checks were harvested. The hand-weeded check resulted in a seed yield of 2037 kg ha^{-1} (Table 3.12). The non-treated resulted in a yield of 1385 kg ha^{-1}, 32% less than the hand-weeded. Imazamox, clopyralid, quinclorac, bromoxynil, and atrazine resulted in hemp seed yield similar to the hand-weeded hemp. Oxyfluorfen resulted in yield similar to the non-treated hemp. Oxyfluorfen controlled the weeds present, but at the expense of herbicide damage to hemp. In the field, cloransulam and oxyfluorfen caused substantial hemp injury and reduced plant densities and yields, and, therefore, should not be further considered for use in industrial hemp. Hemp treated with cloransulam produced 73% less seed yield then hand-weeded hemp. Hemp with a post-emergence application of bromoxynil resulted in the largest numerical yield of 1940 kg ha^{-1} among treatments with herbicide.

Table 3.12. Yield response averaged across environments to standard (1X) field rates to post-emergence herbicides.

Treatment	Yield
	kg ha^{-1}
Non-treated	1385 bc[a]
Hand-weeded	2037 a
Cloransulam-methyl	557 d
Imazamox-ammonium	1540 ab
Clopyralid	1632 ab
Quinclorac-methyl	1498 ab
Bromoxynil	1941 ab
Atrazine	1712 ab
Oxyfluorfen	919 bc

[a] Means within column followed by the same letter are similar according to F-protected LSD at α=0.05 level of significance.

Average seed yield for all post-emergence treatments in 2019 and 2020 was 1295 kg ha^{-1} and 1588 kg ha^{-1}, respectively (data not shown). Interestingly, higher average seed yield was obtained in 2020 with more male plants and fewer total hemp plants per plot. Male plants consisted of 35% of the hemp population in 2019 and 45% of the population in 2020, yet seed production was greater in 2020 (data not shown). There were on average only 19 female plants per 2 m row in 2020 comparatively to 36 female plants per 2 m row in 2019 (data not shown). Other research had concluded the opposite, where increased seeding rates of oilseed hemp led to increased plant density that resulted in 23% and 34% increased seed yield (Vera et al. 2006). Mean temperature in 2020 was generally 1 to 2°C warmer than in 2019; however, precipitation was similar among environments (Table 2.11). With fewer plants per plot in 2020, more nutrients were available to each plant in 2020 compared to 2019. Perhaps this allowed for the development of more robust seed heads; more seeds per head and greater weight per seed.

Similar to the pre-emergence herbicide experiment, a correlation analysis was conducted by SAS among hemp injury, LI, and yield. If the correlation was strong enough between visible hemp injury and LI one could consider measuring LI as a proxy for the more subjective visible evaluation. Light interception and hemp injury were both collected at 7 and 21 DAT and resulted in R-values near -0.70 at both evaluation timings (data not shown). Hemp injury was negatively correlated to light interception. This negative correlation was stronger in the post-emergence experiments compared to the pre-emergence experiments, which had a correlation of -0.25 and -0.39 at 14 and 28 days after emergence, respectively (data not shown). This could be attributed to the greater range and extent of hemp injury observed with post-emergence herbicides. Further research into this concept could be justified for post-emergence herbicide applications in hemp.

Hemp injury was also negatively correlated to seed yield with R-values of -0.07 and -0.16 at 7 and 21 DAT, respectively (data not shown). The correlation between hemp injury and seed yield was similar in the pre-emergence experiments. It was expected that greater amounts of hemp injury would result in greater seed yield loss, but that was not the case in this research.

Light interception had a positive correlation to yield at all evaluation timings. However not as strong of a correlation as the pre-emergence data. If light interception was strongly correlated to seed yield, it could be used as a tool to predict yields or aid in-season management practices. In the post-emergence experiments, the confounding factors determining LI, such as weed control and hemp injury, had a greater impact on the data collected. In general, hemp density as a result of the post-emergence herbicide was lower compared to the pre-emergence experiment, which allowed for more light availability for the weeds below the hemp canopy. Light interception was positively correlated to yield with R-values of 0.12, 0.29, 0.28, 0.43 at 7, 21, 35 DAT, and just prior to harvest, respectively (data not shown). The R-value increased during the course of the season, as the hemp canopy became more dense, absorbing more light in the 400 to 700 nm wave length. Due to the weak relationship or correlation between LI and yield, further study of this relationship does not seem warranted relative to post-emergence herbicides.

Hemp has been very resilient and can make up for early season phytotoxicity (Anderson 2018; Bryd 2019). Hemp seed production for X-59 has ranged from 900 to 1680 kg ha^{-1} in North Dakota (Legacy Hemp 2020), which was achieved or exceeded by numerous treatments in this work. Post-emergence application of bromoxynil or atrazine exceeded that yield, and hand-weeded check plots yielded greater than 2,000 kg ha^{-1}. That is quite exceptional yield and profit potential if hemp can be grown weed free with assistance of herbicides. Hemp expressed the most tolerance to clopyralid and bromoxynil.

Future research and opportunity for post-emergence herbicide use in industrial hemp is limited given the extent and variability of hemp injury that has been observed thus far. There are economic benefits for the growers, but limited interest from herbicide registrants to support a label. For example, a successful registration and adaptation of bromoxynil as a post-emergence herbicide in hemp seed production could result in an economic return of $585 per hectare, if a producer and registrant could accept up to 40% visible hemp injury. There are also political implications because of the diverse end-products derived from hemp. This research focused on hemp seed and fiber, but hemp can also be grown for consumable CBD products and pharmaceuticals, which likely have more strict regulations (Clarke and Merlin 2016). Future research should focus on conducting residue studies to determine if any of the herbicides that resulted in the least amount of hemp injury manifests in the seeds of hemp, jeopardizing the use of herbicides in hemp. This would help in the decision of whether or not registrants would consider moving forward with the expensive and rigorous testing needed to support a herbicide registration in hemp.

CHAPTER 4. CONCLUSION

4.1. Conclusion

Visible injury and yield are the primary and preliminary factors registrants consider when evaluating herbicides for registration for use in industrial hemp. All other data collected in these experiments were designed to support those two factors. If too much visible injury occurs due to a herbicide, hemp producers fear yield losses, while registrants fear producer complaints and lawsuits.

The suggested injury threshold or level of injury tolerance for hemp, based on this research and others, could be approximately 25%. Less than 25% visible injury from pre- or post-emergence herbicides resulted in similar seed yields compared to the non-treated (Flessner et al. 2020). Another experiment showed that bromoxynil and Monosodium methanearsonate applied post-emergence along with pendimethalin and pyroxasulfone applied pre-emergence resulted in less than 13% visible injury and similar yield to non-treated hemp (Maxwell 2016).

In this experiment, the majority of post-emergence herbicides resulted in greater than 35% visible injury to hemp at 21 days after treatment (DAT) (Table 3.9). Clopyralid-1 resulted in the least amount of injury (10 to 12%) to hemp followed by quinclorac-1 (24 to 33%), and bromoxynil-1 (28 to 38%). Willenborg and Johnson (2018) evaluated the use of clopyralid (75 to 300 g ae ha^{-1}), quinclorac-methyl (100 to 200 g ai ha^{-1}), and bromoxynil (280 to 560 g ae ha^{-1}) in ten hemp cultivars across multiple years. When clopyralid was applied at 75 to 150 g ha^{-1}, hemp injury was less than 10%. The standard rate of clopyralid is 105 g ha^{-1}. When the rate was increased to 300 g ha^{-1} hemp injury increased to 25%. Seed yield was similar to the non-treated across all rates of clopyralid, yet hemp was concluded to have insufficient tolerance to clopyralid in their research.

Quinclorac applied at 100 to 200 g ai ha^{-1} elicited sustained injury throughout the season (20 to 40%), similar to our experiment (290 to 580 g ha^{-1}), yet resulted in similar yield to the non-treated and hand-weeded (Willenborg and Johnson 2018). Hemp tolerance to quinclorac was insufficient by industry standards.

Bromoxynil applied at the same rates as in this experiment typically resulted in 15 to 30% visible injury initially, but fully recovered by 19 DAT and with similar yield to the non-treated check (Willenborg and Johnson 2018). In their study, out of the 10 cultivars evaluated, 'X-59' expressed the least amount of tolerance to herbicides. The hemp injury response in our experiment, based on X-59 could be slightly elevated in comparison to a similar study done using other hemp cultivars that possess more tolerance, in general, to herbicides.

The safest post-emergence herbicide options in this study and others for use in hemp still exhibited a range of hemp injury that is dependent on the hemp cultivar and the environment. There is likely too much variation in injury response for registrants to willingly register a post-emergence herbicide that can control broadleaf weeds without some other entity assuming the risk and financial liability through an indemnified label. Therefore, a less injurious pre-emergence herbicide is a more viable option moving forward.

According to this experiment, the best pre-emergence herbicide options for registration were pendimethalin, trifluralin, and pyroxasulfone, which all expressed less than 15% visible injury at either the low or high rate when averaged across environments (Table 2.13). Saflufenacil resulted in less than 17% visible injury at the low rate, but expressed near 30% injury at the high rate through 28 DAT. In other experiments, pendimethalin elicited an average response of 30% injury and reduced emergence, but seed yield was similar to the non-treated and hand-weeded checks (Flessner et al. 2020; Howatt and Mettler 2020; Maxwell 2016; Pearce and

Carter 2018; Scott et al. 2020; Woosley et al. 2015). Trifluralin has only been evaluated a handful of times and has resulted in less than 25% injury with yield similar to the non-treated check (Howatt and Mettler 2020; Pearce and Carter 2018). Hemp response to pyroxasulfone has been variable across experiments, likely limiting chances of registration (Lingenfelter 2018; Maxwell 2016, Pearce and Carter 2019; Waters and Burke 2019). Saflufenacil has resulted in low hemp injury (0-2%) in other experiments, with limited complementary yield data (Knezevic et al. 2019; Pearce and Carter 2018). In our experiment, saflufenacil resulted in 10 to 30% hemp injury (Table 2.13).

The hand-weeded check in the pre-emergence field experiment resulted in a yield of 2110 kg ha^{-1}, 35% greater than the non-treated, weedy check (Table 2.17) Hemp planted for seed at established plant densities of 50 to 150 plants m^{-2} cannot suppress weeds to the same degree as hemp planted for fiber (200 to 250 plants m^{-}2) (Hall et al. 2014; Fike 2016; Baxter and Scheifele 2000). This experiment confirms the need to have herbicides available to adequately control weeds and increase yield.

Pendimethalin, trifluralin, pyroxasulfone, and saflufenacil resulted in hemp seed yield similar to the hand-weeded check. Pendimethalin and pyroxasulfone were also similar to the non-treated check, but improved yield numerically by 18 and 19%, respectively (Table 2.17). Trifluralin (2029 kg ai ha^{-1}) and saflufenacil (2088 kg ai ha^{-1}) increased seed yield by 30 and 33% compared to the non-treated. The pre-emergence herbicides mentioned above increased yield by 25% on average compared to the non-treated. Successful registration and adoption of pre-emergence herbicide use in hemp seed production could result in an economic return of $435 ha^{-1} above the cost of herbicide and application.

A single pre-emergence herbicide application is often not adequate for season long weed control. In 2019 pendimethalin, trifluralin, pyroxasulfone, and saflufenacil resulted in an average weed control rating of 24, 43, 48 and 72%, respectively 21 days after emergence (data not shown). The species of weeds included redroot pigweed (*Amaranthus retroflexus L.*), common lambsquarters (*Chenopodium album* L.), common purslane (*Portulaca oleracea* L.), and Venice mallow (*Hibiscus trionum* L.). Given the relative safety of pre-emergence herbicides, such as pendimethalin, trifluralin, and pyroxasulfone, the hemp industry would benefit from an investigation of pre-emergence herbicides applied after hemp emergence to extend the length of weed control following a traditional pre-emergence herbicide application.

A program that includes trifluralin PPI or similar pre-emergence herbicide with a sequential pre-emergence action herbicide applied early post-emergence to the crop but before subsequent weeds emerge, increases production costs approximately $50 per hectare. The cost to apply trifluralin PPI can result in a $435 per hectare profit. An additional profit of $200 per hectare could be achieved with the sequential pre-emergence herbicide that would cost approximately $25 per hectare, totaling more than $635 per hectare increase in net profits as a result of the herbicide program that controls all weeds. There are pre-emergence herbicides that are currently labeled for early season post-emergence application in other crops, pyroxasulfone as Zidua SC for example (BASF 2019a). In addition to the safest herbicides from this research, acetochlor would also be a viable option to evaluate as a subsequent pre-emergence application. An encapsulated version of acetochlor should limit hemp injury when applied post-emergence.

The proposed research in addition to herbicide residue studies would be beneficial to the hemp industry and registration efforts. These would demonstrate a program approach to effectively control weeds within industrial hemp.

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