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Effects of Herbicides on Industrial Hemp (Cannabis Sativa) Phytotoxicity, Biomass, and Seed Yield

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EFFECTS OF HERBICIDES ON INDUSTRIAL HEMP (*Cannabis sativa*)
PHYTOTOXICITY, BIOMASS, AND SEED YIELD

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EFFECTS OF HERBICIDES ON INDUSTRIAL HEMP (*Cannabis sativa*)
PHYTOTOXICITY, BIOMASS, AND SEED YIELD

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I dedicate this thesis to my parents, Scott and Wendy Maxwell. I would not be where or who I am today without your constant love and support.

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PREFACE

In 2014, Western Kentucky University first participated in the Hemp Pilot Program which was a particularly dry year. Due to the lack of moisture the hemp crop poorly established. This led to bare patches of soil resulting in heavy weed pressure. Perhaps, if the hemp had a better chance to establish and create a good canopy then, the weed pressure would not have been as great. It was also determined that if industrial hemp were to be legalized to grow as a commercial crop, producers would want a solution to weed issues in the crop, especially in seed or cannabidiol production where row spacing is wider and plant populations are lower. For these reasons, this research seemed necessary. The goal of this study was to test different herbicides in industrial hemp in order to identify what herbicides might be used in the future if the crop is legalized in the United States.

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Field studies were established in 2015 at Bowling Green and Lexington, KY to evaluate industrial hemp (*Cannabis sativa*) tolerance to various herbicides. Hemp was planted into conventionally tilled soils in mid to late June at a seeding rate of 39 kg/ha in Bowling Green and 22 kg/ha in Lexington. Five herbicide active ingredients were applied preemergence (PRE) the day of planting and six postemergence (POST) treatments were applied to 30 cm hemp with a CO_2-backpack sprayer delivering 140 L/ha. Plots were 3.1 m wide by 6.1 m long and were sprayed with a 2.1 m boom sprayer leaving a 0.46 m visual check on either side of the sprayed area. A weed free check and a non-treated control were included and all treatments were replicated four times in a randomized complete block design.

Hemp phytotoxicity was evaluated at 14 days after treatment for both PREs and POSTs. Hemp above-ground biomass, weed above-ground biomass, and seed yield were also evaluated. PRE herbicides did not injure hemp as much as POST herbicides, especially at the Bowling Green location. Mesotrione was the most injurious PRE evaluated (> 90%) while bromoxynil and MSMA applications resulted in low phytotoxicity (< 15%). Above-ground biomass was higher in the PRE treated plots, with the exceptions of bromoxynil and MSMA. Weed above-ground biomass was higher in the POST treated plots with the exception of mesotrione. At Bowling Green, PRE

herbicides resulted in comparable yields to the weed-free check, except mesotrione. Metolachlor increased seed yield compared to the weed-free check and MSMA and bromoxynil had comparable yields to the weed-free check at both locations. Results identified possible herbicides to include in a future integrated pest management weed control program for industrial hemp.

LITERATURE REVIEW

A. Characteristics of Industrial Hemp

History of Industrial Hemp

Cannabis sativa L. originated in central Asia and has been utilized for multiple products from the time it was domesticated. *Cannabis sativa* L. is divided into two subspecies, hemp and marijuana. They are similar in appearance, however the ratios of cannabidiol (CBD) and tetrahydrocannabinol (THC) are different. Hemp has more CBD than THC while marijuana has more THC than CBD. Legally, in order for a cultivar to be designated as hemp, it must have a THC content < 0.3 %. The use of hemp for fiber began around 2800 BCE by the Japanese. In the 7^{th} century, the Japanese began to make paper from hemp (NRHA, 2014). It spread rapidly west to Europe where it was grown across the northern latitudes for its narcotic value. Hemp became an important crop in many societies, from a food ingredient to a rope source. Hemp made its way to North America around 1606 and was grown in Kentucky as early as 1775 (Kentucky Department of Agriculture, 2016). Between 1840 and 1860, hemp flourished in Kentucky, Missouri, and Illinois due to its use in sailcloth and cordage. After the Civil War, the production of industrial hemp declined in the United States. In Post-Civil War U.S.A., most of the hemp was grown in Kentucky. In 1915, 8,400 acres of hemp was grown in the U.S., of that amount Kentucky was responsible for 6,500 acres. In 1937, the Marihuana Tax Act caused hemp production to almost cease completely until the Second World War which saw a brief resurgence in hemp production (Marcus and Small, 2014). During World War II, the "Hemp For Victory" campaign (1942-1945) encouraged farmers to grow hemp to aid in the war effort. By 1943 acreage of hemp increased to

146,200 acres. The increased production was short lived and after the war only Wisconsin continued to grow hemp until 1958. In 1970 the Controlled Substance Act classified *Cannabis sativa* as an illegal Schedule I drug. At this point it was illegal to grow hemp in the United States without a DEA permit because the act did not differentiate between hemp and marihuana. The restrictions to grow hemp were so strenuous that only one grower was able to register with the DEA (Shweitzer, 2014). Canada made hemp illegal to grow under the Opium and Narcotics Act in 1938 while Europe and Asia continued to grow and export hemp. It was not until 1998 that Canada passed new legislation under the Controlled Drugs and Substances Act that allowed for commercial development of a hemp industry (Marcus and Small, 2014). Currently there are about 30 countries in Europe, Asia, and North and South America that produce hemp. In 2011, 200,000 acres were grown globally and pounds of hemp produced has increased since 1999. China is one of the largest producers followed by an active market in Europe where production is centered in France and Great Britain. In 2010, the EU was reported to produce 26,000 acres of hemp (Johnson, 2015).

Industrial Hemp Taxonomy and Morphology

Industrial hemp (*Cannabis sativa L.*) belongs to the family Cannabaceae and is an annual wind pollinated broadleaf (ITIS, 2014). Plants in the Cannabaceae family contain molecules called cannabinoids. Two of the main cannabinoids are CBD and THC. CBD is usually found in higher concentrations in hemp than in marijuana and is used in pharmaceuticals. THC levels are significantly lower in hemp than in marijuana. THC is used for its narcotic effects. Hemp is any cultivar of *Cannabis sativa* that has a THC level $< 0.3\%$ THC by weight. Most marijuana cultivars have approximately 1.0% or more

THC and are used for both medicinal and recreational purposes (NAIHC, 2014). Most hemp cultivars are dioecious aside from a few monoecious fiber cultivars that originate from Europe. The plant has a laterally branched taproot that penetrates 30-60 cm into the soil but may reach 2.5 m in loose soil. Plant heights vary among cultivars, ranging from 1-5 m (Hemp Oil Canada, 2014). The stem is erect with a woody interior which possess secondary fibers called hurds. The primary fibers or bast fibers make up the exterior of the stalk.

Agronomy

Industrial hemp thrives in soils that are favorable to corn production. Industrial hemp prefers light to medium textured soil with a pH of 6.0 to 7.5. Hemp should not be rotated into a field that has recently been used to grow corn, oilseeds, wheat, or rye due to the fact that these crops are known vectors for disease. Common diseases include: Downy mildew, powdery mildew, gray mold, *Fusarium* canker, *Fusarium* wilt, *Anthracnose*, and *Fusarium* root rot. Hemp best follows alfalfa incorporated as a green manure or summer fallows. Planting is recommended when soil temperature is 7.7-10 C and germination is expected in about 4 to7 days (Hemp Oil Canada, 2014). Target seeding rates are 20-40 kg/ha, approximately 1.3 to 2.5 million seed/ha. Seeds are planted at a 1-2 cm depth (Baxter, 2013). Recent fertility studies in Canada have found that hemp benefited from applications up to 200 kg of nitrogen/ha where neither a maximum nor a plateau were found. The authors concluded that more nitrogen may be needed for hemp crops than previously thought and more research is still required on this topic. This study also found that applications of phosphorus and potassium had little to no effect on the hemp when applied to soils with high initial soil fertility (Aubin, et al., 2015)

B. Uses of Industrial Hemp

Fiber Production

Hemp cultivars utilized for fiber are generally taller with less branching than those used for seed. Fiber cultivars are planted at higher seed rates (35-40 kg/ha) than cultivars used for seed. The fiber is classified into two parts, primary fiber and core fiber. Primary fibers are separated by a process known as retting, whereby humidity and bacteria are used to break down the fiber-bonding pectins. Retting can also be accomplished through chemical means. Fibers can be used for a myriad of products including automotive parts, paper and floor coverings (Alberta Agriculture and Foresty, 2015). Fibers work well for these products because of their antimildew and antimicrobial properties. Hurds are utilized by animal owners as a form of ultra-absorbent bedding. These fibers are also utilized in many other products such as cements and plastics (NAIHC, 2014).

Seed/Oil Production

When Industrial hemp is marketed for its seeds and oil, it is planted more sparsely (20 kg/ha) than cultivars used for fiber. Cultivars exist that are utilized for both seed and fiber, these are planted at populations similar to those intended for seed/oil production. Although cultivars for both seed and fiber will supply two marketable products instead of one, they do not maximize either fiber or seed production. Hemp seed is used for a multitude of products including human food and animal feeds. Seed oil can also be used for animal and human consumption as well as body creams and oils, plastics, and paints.

Hemp oil is low in fat meaning it is heart healthy resulting in increasing popularity as a healthy alternative to other oils. A 30 g serving of hemp seed has only 14 g of total fat and contains 11 g of protein (Global Hemp, 2005). Hemp seeds contain all the amino acids and fatty acids that humans require to maintain a healthy life. They are high in protein which is made up of 65% globulin edistin and is easily processed by the human body (Osburn, 1992).

Cannabidiol Production

Cannabidiol (CBD) is a substance that is found naturally in industrial hemp and is categorized as a cannabinoid; THC falls into this category as well. CBD has a 3,4-trans ring junction with a double bond at the Δ^1 position (Razdan, 2007). CBD as well as other cannabinoids have been found to have medicinal uses. CBD has been found to aid children with epilepsy and other illnesses. Cannabinoids are found in sessile- and capitate-stalked secretory glands that are located throughout the plant, the highest concentrations are found in the female inflorescence (Mahlberg, 2004). Cannabinoids are believed to be synthesized in the plant by two pathways, the deoxyxylulose phosphate pathway and the mevalonate pathway (Fellermeier, et al., 2001). Much is still unknown about cannabinoids and their production. However, some studies have found that the environment can play a role in the production of different cannabinoids in the plant namely THC and CBD. Soil fertility can affect the production of cannabinoids. The amount of nitrogen taken up by the plant had a positive correlation to THC and CBD concentrations (Coffman and Gentner, 1975). Light also influences cannabinoid production; plants that were exposed to high concentrations of UV-B light also produced more THC, while plants that were exposed to lower concentrations of UV-B light tended to produce more CBD (Lydon, et al., 1987). Industrial hemp grown for CBD production is planted differently than hemp for fiber production. Instead of being grown in denser populations and tight row spacing like fiber cultivars, hemp grown for CBD is grown similarly to grain cultivars in wide rows and much lower plant populations. Hemp grown for CBD or grain can have row spacing as wide as 80 cm and a plant population of 60 – 80 plants/m^2 (EIO, 2016)

C. Weed Control in Industrial Hemp

Cultural Control

Cultural control is defined as the changing of a cropping system to reduce pest populations or to avoid pest injury to crops. It is popular belief that cultural controls are sufficient for the production of industrial hemp. Crop rotation is one way to control different kinds of weeds. Since industrial hemp is a dicot plant, producers will have more issues with dicot weeds due to the fact that herbicides used to control dicot weeds will most likely injure the hemp. If a grass crop precedes hemp dicot weeds can be more easily controlled and will thus be less of an issue in the succeeding hemp crop. Altering planting date is another cultural control. Hemp planted at the appropriate time will be more competitive with weed species. Hemp grows quickly and rapidly forms a canopy which shades emerged weeds and prevents germination of some weed species. This practice can be seen utilized in other crops. One study showed that when turmeric was planted later (May and June) that it was more competitive with weed species due to the fact that the soil temperature was higher and the turmeric was able to grow longer shoots faster. Using this practice does reduce yield due to the fact that there is a shorter growing period but this can be an option used by organic farmers (Hossain, 2005). Plant population can also be used as an effective way to combat weed pressure. When plants are seeded at dense populations, the resulting crop canopy will suppress weeds more effectively than at wider plant spacing. Populations for fiber cultivars should be around 40-50 kg/ha in order to compete with weed species. Vera, et al. (2002) recommends hemp grown organically to have an even higher seeding population of 60-80 kg/ha. Another cultural control is mulching. There are different types of mulches that can be used that are known to suppress weeds. Woodchips were used in lentils as an effective weed suppressant (Wang, et al, 2012). Living mulches can also be utilized to compete with weeds. A study in corn showed that when hairy vetch was interseeded, weed competition was reduced and crop yield was not decreased (Mohammadi, 2010). Another type of mulch is polyethylene which is effective at suppressing weeds by providing a smothering effect (Subrahmaniyan, et al., 2002).

Mechanical Control

Mechanical controls in industrial hemp for fiber are unlikely to be used due to the fact that plant populations are so high and that fiber is produced on a larger scale than hemp used for seed or CBD. In smaller scale operations such as greenhouses, manual hoeing or hand pulling may be viable options. However, in larger scale operations, in-row cultivation and pre-plant tillage can be used to reduce weed competition. In some cases pre-emerge tilling practices may result in lower weed populations (Johnson and Holm, 2009).

Chemical Control

Chemical control of weeds is the most popular and most practical method of weed control in most traditional crops. However, there are no herbicides in the USA registered for hemp. This means that any herbicides that could be potentially be used are not legal to apply to industrial hemp. The only chemical controls that extension agencies in Canada recommend are non-selective products, such as paraquat (0.55-1.1 L/ha) or glyphosate (0.75-4.68 L/ha), and even in this case they are only recommended as a pre-plant herbicide for site treatment (Guide to Weed Control, 2014). The only active ingredient that is recommended by Canadian extension agencies to be applied post-emergence (POST) is quizalofop-p-ethyl (0.036-0.07 kg/ha), used to control grass weeds in hemp grown for fiber (Guide to Weed Control, 2014).

D. Current Legislation and Future of Industrial Hemp

Current Legislation

Two pieces of legislation have recently been passed that allow Industrial hemp production in Kentucky and other parts of the United States for certain individuals. In 2013 the Kentucky Senate passed Senate Bill 50 which exempted industrial hemp from the state Controlled Substances Act (Kentucky Department of Agriculture, 2016). Although this legislation stated that industrial hemp was not a controlled substance the bill also stated that Kentucky must follow all federal rules and regulations regarding industrial hemp. On February 7, 2014 the Federal Farm Bill was signed into law. The Federal Farm Bill allowed state departments of agriculture in states where industrial hemp is legal to start pilot programs for research and development purposes. To date, 24 states are approved to grow industrial hemp (NAIHC, 2014).

Future of Industrial Hemp

The future of industrial hemp in the United States will depend on a rapidly growing market and the installation of processing plants and other infrastructure. Processing of hemp has been a concern among those wanting to invest in industrial hemp. Hemp fiber is difficult to transport and process, and because of the lack of production in the last few decades, resources needed to start a hemp market are not available. Hemp processing has been improved by the work of Adrian Clarke. Clarke has developed a mobile hemp "decoricator." This machine is able to process the hemp in the field eliminating the need to transport hemp long distances to processing plants. He has developed technologies that separate the fibers from the hemp without the use of the "retting" process. This process can produce fiber that can be spun by cotton systems, making the hemp fiber cost similar to cotton production (Bryant, 2014). If more technology, innovations, and research are able to be developed, the future of hemp will become much more clear.

MATERIALS AND METHODS

Field Experiments were conducted in two locations, Bowling Green, and Lexington, Kentucky. In Bowling Green, a Crider silt loam soil was roto-tilled and cultipacked, it was further compacted to provide a firm seedbed and after planting a drag chain was used to cover the seed. In Lexington, a Maury silt loam was roto-tilled and cultipacked and was seeded with a research grain drill. In Bowling Green, seed depth was 1.3 cm with row spacing 7.6 cm while planting rate was 39 kg/ha, which is approximately 2,335,144 seed/ha. In Lexington, planting depth was 0.6 cm, row spacing was 40.6 cm, and seeding rate was 22 kg/ha, which is approximately 1,334,367 seed/ha. In Bowling Green a mixture of Italian cultivars was used while in Lexington cv. Finola was used. The trial was seeded on 18 June 2015 in Bowling Green and 24 June 2015 in Lexington. Herbicide treatments consisted of five pre-emergent (PRE) and six post-emergent (POST) treatments; a weed-free check and an untreated check (Table 1). Plots were 3 m by 6 m and treatments were replicated four times in a randomized complete block design. A carbon dioxide backpack sprayer was used to apply herbicides at 140 L/ha. PRE herbicides were applied on the day of planting while POST herbicides were applied 10 July in Bowling Green, 22 days after seeding (DAS) and 16 July in Lexington (24 DAS). At both locations POST herbicides were applied when plants were approximately 30.5 cm and at the 8-10 leaf stage. Hemp phytotoxicity was evaluated visually on a scale of 0 to 100, where 100 was complete control of the hemp and 0 representing no crop injury. Phytotoxicity evaluations for PREs were taken 4 weeks after treatment (WAT) while evaluations for POSTs were taken 2 WAT. Hemp biomass, weed biomass, and hemp seed yields were evaluated by taking samples from a 1.5 m^2 area in the center of each plot. Biomass and seed yield data were collected 90 DAS. Seed yield was evaluated by harvesting hemp with pruning shears, buds containing seed were stripped from the stalk by hand, to a constant weight, and dried at 32℃. Seeds were then cleaned by passing through a screen and re-dried before weighing. Data was analyzed with SAS PROC GLM and means were separated using Duncan's Multiple range test at $\alpha = 0.05$.

Table 1. Preemergence and Postemergence Herbicides Applied in Bowling Green and Lexington

Treatment #	Active Ingredient	Application Timing	Rate of Fomulated Product/ha
1	Pendimethalin	PRE	2.80 L/ha
2	Pyroxasulfone	PRE	70 g/ha
3	Metolachlor	PRE	1.95 L/ha
4	Fomesafen	PRE	1.52 L/ha
5	Mesotrione	PRE	0.39 L/ha
6	Bromoxynil	POST	0.58 L/ha
7	Flazasulfuron	POST	110 g/ha
7	NIS	POST	0.25% v/v
8	Trifloxysulfuron	POST	7 g/ha
8	NIS	POST	0.25% v/v
9	Rimsulfuron	POST	70 g/ha
9	NIS	POST	0.25% v/v
10	Bispyribac-Na	POST	20 g/ha
11	MSMA	POST	3.16 L/ha
12	Weed-free	----	----
13	Untreated	----	----

RESULTS AND DISCUSSION

Phyotoxicity

At the Bowling Green location, POST herbicides were more injurious than the PRE herbicides with the exception of mesotrione (Table 3). Trifloxysulfuron caused considerable injury (90%). Mesotrione, flazsulfuron, rimsulfuron and bispyribac-Na all resulted in equivalent injury. Metolachlor, fomesafen, and pyroxasulfone were not as injurious as the previously listed treatments but displayed significantly more crop injury than MSMA, bromoxynil, pendimethalin, and the untreated plots. MSMA, bromoxynil, and pendimethalin did not significantly injure hemp. All PRE herbicides except for mesotrione resulted in minimal crop injury (< 13%).

Table 2. Analysis of Variance Statistics for Hemp Phytotoxicity Data.

	Bowling Green		Lexington	
	Pre-emergent	Post-emergent	Pre-emergent	Post-emergent
ANOVA Statistic				
P-Value Model	<0.0001	<0.0001	<0.0001	<0.0001
P-Value Replication	0.3387	0.0776	0.0622	0.3074
P-Value Herbicide	<0.0001	<0.0001	<0.0001	<0.0001
CV	42.62	41.13	40.49	42.82

Table 3. Hemp Phytotoxicity at Bowling Green as Influenced by Herbicide Treatment

Treatment	Phytotoxicity (%) 28 DAT*	PRE/POST
Trifloxysulfuron + NIS	90.00a	POST
Mesotrione	88.75a	PRE
Flazasulfuron + NIS	86.25a	POST
Rimsulfuron + NIS	85.00a	POST
Bispyribac-Na	85.00a	POST
Metolachlor	12.5b	PRE
Fomesafen	11.25bc	PRE
Pyroxasulfone	7.50bc	PRE
MSMA	6.25bcd	POST
Bromoxynil	6.25bcd	POST
Pendimethalin	5.00cd	PRE
Weed-free	0d	NA
Untreated	0d	NA

** means sharing the same letter are not different ($p < 0.05$)*

At the Lexington location PRE herbicides were in most cases more injurious than POST herbicides (Table 4). Mesotrione, fomesafen and metolachlor all resulted in the highest crop injuries in Lexington (>78%). Trifloxysulfuron, pyroxasulfone, pendimethalin, flazasulfuron, and rimsulfuron all showed an unacceptable amount of injury (52.5-28.75%) being significantly more injurious than the untreated checks, however they were not as injurious as the previously stated treatments. Treatments that displayed an acceptable amount of crop injury included bispyribac-Na, bromoxynil, and MSMA. Crop injury in this grouping was < 12%.

Table 4. Hemp Phytotoxicity at Lexington as Influenced by Herbicide Treatment

Treatment	Phytotoxicity (%) 28 DAT*	PRE/POST
Mesotrione	95.00a	PRE
Fomesafen	86.25a	PRE
Metolachlor	78.75a	PRE
Trifloxysulfuron + NIS	52.50b	POST
Pyroxasulfone	45.25b	PRE
Pendimethalin	45.00b	PRE
Flazasulfuron + NIS	28.75bc	POST
Rimsulfuron + NIS	28.75bc	POST
Bispyribac-Na	11.25cd	POST
Bromoxynil	5.00cd	POST
MSMA	5.00cd	POST
Weed-free	0d	NA
Untreated	0d	NA

** means sharing the same letter are not different ($p < 0.05$)*

Hemp Biomass

Hemp biomass at Bowling Green tended to be inversely related to phytotoxicity whereby treatments that were most injurious to the hemp tended to have the lowest hemp biomass (Table 6). Mesotrione, trifloxysulfuron, bispyribac-Na, and rimsulfuron reduced biomass more than other treatments; they also showed significant injury when compared to the other treatments (Table 3). Fomesafen and flazasulfuron resulted in significantly less hemp biomass than the weed-free check however they had significantly more hemp biomass than mesotrione and trifloxysulfuron. MSMA, pendimethalin, bromoxynil, metolachlor, and pyroxasulfone did not reduce biomass. Minimal phytotoxicity likely resulted in higher hemp biomass (Tables 2,4).

Table 5. Analysis of Variance of Statistics for Hemp Biomass Data.

	Bowling Green	Lexington
ANOVA Statistic		
P-Value Model	<0.0001	<0.0001
P-Value Replication	0.0565	0.0879
P-Value Herbicide	<0.0001	<0.0001
CV	33.25	38.44

Table 6. Hemp Biomass in Bowling Green as Influenced by Herbicide Treatment

Treatment	Biomass (kg/1.5 m^2)*	PRE/POST
Weed-free	2.73a	NA
MSMA	2.67a	POST
Pendimethalin	2.49ab	PRE
Untreated	2.37ab	NA
Bromoxynil	2.33ab	POST
Metolachlor	2.29ab	PRE
Pyroxasulfone	2.26ab	PRE
Fomesafen	1.87bc	PRE
Flazasulfuron + NIS	1.37cd	POST
Rimsulfuron + NIS	1.10de	POST
Bispyribac-Na	0.73de	POST
Trifloxysulfuron + NIS	0.51e	POST
Mesotrione	0.50e	PRE

** means sharing the same letter are not different ($p < 0.05$)*

Hemp in Lexington also tended to display an inverse relationship between phytotoxicity and biomass. POST herbicides tended reduce to hemp biomass less than PRE herbicides (Table 7). Mesotrione and trifloxysulfuron, fomesafen, pendimethalin, and metolachlor resulted in the lowest hemp biomass ($\leq$ 0.55 kg/1.5 m^2). Treatments resulting in biomass significantly lower than the weed-free check and higher than mesotrione were pyroxasulfone, rimsulfuron, and flazasulfuron. MSMA, bromoxynil, and bispyribac did not reduce hemp biomass in comparison to the weed-free check. MSMA and bromoxynil did not reduce hemp biomass at either location.

Table 7. Hemp Biomass in Lexington as Influenced by Herbicide Treatment

Treatment	Biomass (kg/1.5 m^2)*	PRE/POST
Weed-free	1.40a	NA
MSMA	1.32ab	POST
Bromoxynil	1.12abc	POST
Untreated	0.98abcd	NA
Bispyribac-Na	0.95abcd	POST
Pyroxasulfone	0.88bcde	PRE
Rimsulfuron + NIS	0.82cde	POST
Flazasulfuron + NIS	0.76cdef	POST
Metolachlor	0.55defg	PRE
Pendimethalin	0.41efg	PRE
Trifloxysulfuron + NIS	0.30fg	POST
Fomesafen	0.25g	PRE
Mesotrione	0.08g	PRE

** means sharing the same letter are not different ($p < 0.05$)*

Weed Biomass

In Bowling Green, bispyribac-Na treated plots had the highest weed biomass. (Table 9) All other treatments resulted in lower hemp biomass. This may be because bispyribac-Na was not effective at controlling weeds and injured the crop (Table 3). A reduction in crop stand combined with the lack of control by bispyribac-Na allowed weeds to grow unhindered.

Table 8. Analysis of Variance Statistics for Weed Biomass Data.

	Bowling Green	Lexington
ANOVA Statistic		
P-Value Model	<0.0017	<0.0033
P-Value Replication	0.6945	0.0529
P-Value Herbicide	<0.0017	<0.0033
CV	131.02	96.14

Table 9. Weed Biomass in Bowling Green as Influenced by Herbicide Treatment

Treatment	Biomass (kg/1.5 m^2)	PRE/POST
Bispyribac-Na	1.03a	POST
Rimsulfuron + NIS	0.51b	POST
Trifloxysulfuron + NIS	0.46b	POST
Mesotrione	0.41b	PRE
Flazasulfuron + NIS	0.21b	POST
Untreated	0.17b	NA
Bromoxynil	0.09b	POST
Weed-free	0.07b	NA
Fomesafen	0.02b	PRE
Pendimethalin	0.02b	PRE
MSMA	0.02b	POST
Pyroxasulfone	0.01b	PRE
Metolachlor	0.00b	PRE

** means sharing the same letter are not different ($p < 0.05$)*

Bispyribac-Na, bromoxynil, MSMA, and pendimethalin treated plots resulted in significantly more weed biomass than plots treated with other herbicides (Table 10). This difference between the results shown in Bowling Green versus Lexington may be explained by the difference in row spacing and seeding density. In Bowling Green, a higher seed population and narrower row spacing provided hemp an advantage against weeds by allowing the crop to create a quicker canopy and shade out competing weeds. In Lexington, row spacing was more than 5 times wider, and the amount of seed used in Lexington was ~50% that of the seed used in Bowling Green. This row spacing and seed population resulted in barer ground which gave weeds more competitive advantage.

Table 10. Weed Biomass in Lexington as Influenced by Herbicide Treatment

Treatment	Biomass (kg/1.5 m^2)*	PRE/POST
Untreated	1.97a	NA
Bispyribac-Na	1.64ab	POST
Bromoxynil	1.11abc	POST
MSMA	0.91abc	POST
Pendimethalin	0.90abc	PRE
Mesotrione	0.86bc	PRE
Trifloxysulfuron + NIS	0.79bc	POST
Rimsulfuron + NIS	0.45c	POST
Flazasulfuron + NIS	0.33c	POST
Fomesafen	0.24c	PRE
Metolachlor	0.11c	PRE
Pyroxasulfone	0.08c	PRE
Weed-free	0.02c	NA

** means sharing the same letter are not different ($p < 0.05$)*

Hemp Seed Yields

In Bowling Green, all pre-emergent herbicides resulted in comparable yields to the weed-free check except mesotrione (Table 12). Similar results were observed in Lexington, where both mesotrione and fomesafen reduced seed yield (Table 13). MSMA and bromoxynil had comparable seed yields to the weed-free check at both locations. Flazasulfuron, mesotrione, bispyribac-Na, and trifloxysulfuron reduced yield in Bowling Green. In Lexington, flazasulfuron, rimsulfuron, fomesafen, trifoxysulfuron, and mesotrione reduced yield. In Bowling Green, metolachlor resulted in increased seed yield compared to the weed-free check and in Lexington, MSMA resulted in increased seed yield compared to the weed-free check. This may be explained by the fact that although neither of these two treatments were overly injurious there was some damage to the hemp. This led to more space between plants in the row than in the weed-free plot. The extra space may have allowed for more light penetration and thus more lateral growth to occur. The difference in fomesafen reducing yield in Lexington but not in Bowling Green may be attributed to the fact that fomesafen was more injurious in Lexington than it was in Bowling Green (Tables 3, 4).

Table 11. Analysis of Variance Statistics for Hemp Phytotoxicity Data.

	Bowling Green	Lexington
ANOVA Statistic		
P-Value Model	<0.0001	<0.0001
P-Value Replication	0.0016	0.0152
P-Value Herbicide	<0.0001	<0.0001
CV	23.36	30.59

Table 12. Hemp Seed Yields in Bowling Green as Influenced by Herbicide Treatment

Treatment	Yield (kg/ha)*	PRE/POST
Metolachlor	996.85a	PRE
Pendimethalin	770.8ab	PRE
Fomesafen	744.25ab	PRE
Weed-free	703.47bc	NA
Untreated	699.25bc	NA
MSMA	617.77bcd	POST
Pyroxasulfone	543.07bcde	PRE
Bromoxynil	512.75bcde	POST
Rimsulfuron + NIS	460.08cde	POST
Flazasulfuron + NIS	407.08def	POST
Mesotrione	390.63def	PRE
Bispyribac-Na	295.6ef	POST
Trifloyxsulfuron + NIS	167.7f	POST

** means sharing the same letter are not different ($p < 0.05$)*

Table 13. Hemp Seed Yields in Lexington as Influenced by Herbicide Treatment

Treatment	Yield (kg/ha)*	PRE/POST
MSMA	1136.6a	POST
Bromoxynil	1034.25ab	POST
Bispyribac-Na	934.18abc	POST
Weed-free	888.6bcd	NA
Untreated	717.23cdef	NA
Pendimethalin	639.65cde	PRE
Metolachlor	686.0def	PRE
Pyroxasulfone	654.83def	PRE
Flazasulfuron + NIS	623.03ef	POST
Rimsulfuron + NIS	534.55f	POST
Fomesafen	284.97g	PRE
Trifloxysulfuron + NIS	123.22gh	POST
Mesotrione	51.93h	PRE

** means sharing the same letter are not different ($p < 0.05$)*

Conclusion

This experiment identified some herbicides that worked well with hemp and some herbicides that should simply not be used in industrial hemp. Mesotrione and trifloxysulfuron are two herbicides that should not be used in industrial hemp due to the considerable injury they caused in both locations. However, MSMA, bromoxynil, and pendimethalin showed promise in this experiment and should be considered for further investigation. There were many differences in results between the two locations. The PREs tended to be more injurious in Lexington than in Bowling Green and the opposite was true for the POSTs. The differences in row spacing, seeding population and cultivar could account for the differences between the two locations. In the future, experiments should be conducted where seeding rate, row spacing, and cultivar are constant in order to acquire data that can be effectively used to determine what herbicides are going to be acceptable for industrial hemp.

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