II. Research Proposal

The regulations require the completion of a research proposal before your research license application can be considered. If you completed a Pre-Application and Pre-Application Research Proposal and want to certify your Pre-Application Research Proposal for use as your research proposal on this research license application, you must complete all provisions of the research license application and submit a copy of your Pre-Application Research Proposal. As the Primary Applicant, you will be given the opportunity to certify your request in the Acknowledgment Section below. **To be able to certify your Pre-Application Research Proposal, the Primary Applicant must be the same individual that completed the Pre-Application and Pre-Application Research Proposal.**

Your research proposal must explain the research you plan to conduct on behalf of the Kansas Department of Agriculture. Your research proposal will play an important role in the evaluation of your research license application and approval to participate in the Program.

Below are possible research topics that may be useful areas of study. Once you have determined the area of your research, explain in detail, on the following pages, the specific subject your research will cover.

- Agronomic research and analysis of soils, growing conditions or water usage required to successfully grow industrial hemp.
- Research on types of industrial hemp seed that are best suited to be grown in Kansas, including seed availability, creation of hybrid seed types, in-the-ground variety trials and seed production.
- Agronomic research and analysis of the most efficient types of equipment and techniques for seeding, tillage or harvest.
- Agronomic research and analysis of the most effective, economical and environmentally beneficial pest control or fertilization products or methods.
- Analysis of the management techniques and/or environmental factors that impact the delta-9 tetrahydrocannabinol (THC) concentration in industrial hemp.
- A study on the feasibility of attracting federal and private funding for industrial hemp research.
- Analysis of the economic feasibility of developing markets for the various types of industrial hemp that can be grown in Kansas.
- Research and analysis of the most efficient types of equipment and techniques for transporting industrial hemp plants, plant parts, grain or seeds.
- Analysis of the estimated value-added benefits, including environmental benefits, that Kansas businesses would reap by having an industrial hemp market of Kansas-grown industrial hemp varieties.
- Research and analysis of the most efficient types of equipment and techniques for processing industrial hemp plants, plant parts or grain.
- Analysis of the economic feasibility of developing markets for varieties of industrial hemp seed in and beyond Kansas.
- Research into the development of national and international markets for Kansas-grown industrial hemp and industrial hemp products.
- Analysis of the most efficient and economical methods for distributing and transporting Kansas-grown industrial hemp and industrial hemp products.
- Other types of research into the economic development, cultivation, market analysis, manufacturing, distribution and transportation of industrial hemp and industrial hemp products.

Research Proposal Instructions:

- **Complete the following sections**, either by utilizing the fillable form or by providing your answers on a separate page(s). *Handwritten submissions are strongly discouraged.*
- **If answering on a separate document**, please label each section (A-F).
- **Be concise**: Your research proposal should be as concise as possible; **not to exceed 2 pages total.**
- **For more information on how to write a research proposal**, please see the Technical Bulletin on How to Conduct Research on Your Farm or Ranch by visiting https://www.sare.org/Learning-Center/Bulletins/How-to-Conduct-Research-on-Your-Farm-or-Ranch.

SECTION A: Identify your research question and objective, including a statement of the type of research to be conducted and its purpose.

Q1. Which of the approved varieties are best suited for fiber, grain, or CBD production in KS?
Q2. Will different plasticulture systems grow high quality hemp?
Q3. Does fertilizer form and rate influence hemp production?
Q4. Is irrigation necessary to grow a successful hemp crop in south-central KS?
The objective of the research is to answer the above questions in field growing conditions in south-central Kansas. The purpose is to generate baseline data evaluating some basic production questions to be able to make some recommendations to new hemp growers.

SECTION B: Identify your experimental design.

All experiments will be conducted in a Randomized Complete Block Design with a minimum of four replications per treatment. Variety trials will be planted in 8ft x 20ft plots using a grain drill. Plots will be monitored for growth, weed presence, and insect presence. Plasticulture systems will use drip irrigation and different color plastic and paper to exclude weeds and moderate soil temperature. This is a more intensively managed system for higher value plants. To investigate fertilizer requirement, different rates of conventional and organic fertilizers will be applied to the soil and incorporated prior to planting. We will monitor growth and plant health throughout the growing season. To investigate irrigation, a larger field will be segregated into different irrigation regimes. We will monitor soil moisture and measure plant growth throughout the season. We will also be able to measure plant stress.

SECTION C: Explain what will be measured and what data will be collected.

We plan to measure establishment (plants/m^2), final plant height (m), biomass production (ton/acre), and grain yield (lb/acre) of the fiber and grain varieties. In the plasticulture system, we will measure final plant biomass (lbs/plant dry wt.), flower yield (inflorescense/plant), and resin content (CBD and THC content). In the fertilizer study we will measure final plant biomass, flower yield, and resin content. We will also look at chlorophyll content using a chlorophyll meter and leaf size (cm^2/leaf). In the irrigation project will will collect similar data as the fiber and grain study. Additionally, we will install soil moisture probes to monitor soil water status.

SECTION D: Explain how the project will be implemented, including location and size of your anticipated research areas (in acres or square feet), duration of your research operations and variety of industrial hemp that will be used in your research.
The fiber and grain variety trial will be conducted in field IH1. Varieties for CBD will be tested in IH2. We hope to plant in early May and harvest at the onset of pollen dispersal (fiber) or when 70% of grain has matured (grain). We don't know exactly when that will be in KS yet. The plasticulture experiment will be conducted in fields IH3 and IH4. Planting will occur in mid-May and harvest will occur when trichomes have turned tan/amber in color. We don't yet know when this will happen in KS. The fertilizer experiment will be run in fields IH3 and IH4. Planting will occur in mid-May and the study will be terminated with the trichomes on female plants have turned tan/amber in color. We don't know when that will happen. The irrigation study will be conducted in field IH6. It will be planted in early May. Harvest will occur at the onset of pollen dispersal.
SECTION E: Explain how research data will be collected, recorded and analyzed.
Data will be collected by employees of the John C. Pair Horticultural Center consistent with our procedures for collecting data from other projects. Data will be written on paper with pencil then transcribed to Excel spreadsheets. Handwritten data will then be filed and saved in case of computer failure. Data will be analyzed with a common statistical analysis software such as SAS. Data will be subjected to analysis of variance and means will be subjected to a common means separation tool when appropriate.
SECTION F: Explain how the data will be interpreted and how conclusions will be drawn, including anticipated results.
Data will be interpreted after all statistical analyses are completed. Conclusions will be based on that statistical analysis in a manner consistent with academic research ethics standards. We anticipate finding large varietal differences among our research plots. It is expected that some of the approved varieties may not be well adapted to Kansas and result in near crop failure of the variety. We anticipate large, highly productive plants, with numerous inflorescence in the plasticulture system. Based on conversations with colleagues at other institutions, we anticipate plants fertilized with organic fertilizer to outperform conventional fertilizers. They report improved plant growth in organic systems. We anticipate improved productivity in irrigated plots. However, we will wait to see if the increased productivity justifies the increased monetary and environmental expense of irrigating.

2019 K-State Industrial Hemp CBD Variety Trial

John C. Pair Horticultural Center[1]

Jason Griffin, Michael Shelton, Clint Wilson, Tami Myers

K·STATE
Research and Extension
John C. Pair Horticultural Center

[1]College of Agriculture, Department of Horticulture & Natural Resources
1901 E. 95th Street South, Haysville, KS
Corresponding author: jgriffin@ksu.edu
With support from K-State Research & Extension and the USDA National Institute of Food and Agriculture, Hatch-Multistate project 1019339: Industrial Hemp Production, Processing and Marketing in the U.S.
The authors wish to thank Colorado Hemp Genetics and Craiger Enterprises for generous donation of germplasm, and the Kansas Department of Agriculture for collaborative support throughout the project.

Introduction:

Hemp is a broad term used to describe the many varieties of *Cannabis sativa* L. that produce less than 0.3% tetrahydrocannabinol (THC). The crop is globally significant, but only recently was allowed to be grown once again in the United States. Varieties have been selected and are currently grown with a wide cannabinoid profile. Cannabinoids are of high interest for their putative medical and therapeutic role in humans and companion pets. Cannabidiol (CBD) and THC are the two cannabinoids of primary interest. THC is of interest because it determines whether the final product is considered hemp (<0.3 THC) or marijuana (>0.3% THC). CBD is of interest because of its potential therapeutic properties and its legal status across many states. Currently, there is no information available regarding adaptability or cannabinoid production of these varieties in Kansas.

In 2019 Kansans were allowed to apply for research licenses to grow industrial hemp. It was assumed the crop would grow well throughout KS since there are wild remnant populations of *C. sativa* flourishing at numerous locations across the state. However, controlled variety trials are necessary to determine which varieties are best adapted to Kansas. Currently, growers must rely on information generated from other states with vastly different growing conditions than KS. Variety selection is vital in CBD hemp production considering environmental conditions strongly influence cannabinoid ratios and ultimately, total cannabinoid content.

The objective of this study was to evaluate commercially available varieties of CBD hemp in south-central KS grown in containers outdoors or inside a high tunnel. Outdoor hemp production is of interest because of reduced infrastructure cost. However, pollination is a concern with outdoor hemp. Unpollinated female flowers contain the highest concentration of cannabinoids. When flowers are pollinated and seed are produced, the total concentration of all cannabinoids is greatly diminished. In Kansas naturalized populations of *C. sativa* can be found throughout the state. With pollen easily travelling as far as 3 miles there is concern regarding the viability of outdoor CBD hemp potential. We wanted to test whether a covered high tunnel could effectively reduce pollination of the plants within.

Methods:

On 17 June 2019 clones (from rooted cuttings) of each variety (Table 1) were potted from 1 gal plastic pots into 7 gal plastic pots or 7 gal fabric bags and filled with a soilless potting substrate composed of composted pine bark (HappiGro) and ½ in screened pinebark nuggets (1:1 by vol) ammended with 2 lbs/yd^3 dolomitic lime. Plants received one of two fertilizer treatments that were incorporated into the potting substrate. The first was a conventional controlled release fertilizer at 14.5 lbs/yd^3 (Osmocote plus 15-9-12) that included micronutrients. The second was an organic chicken manure based fertilzer (Turf-Mate 4-2-3) incorporated at 65 lbs/yd^3. A micronutrient fertilizer (Micromax) was added to the substrate at 1.5 lbs/yd^3 to compensate for the lack of micronutrients in the organic

Figure 1. Clones growing in containers or bags in a high tunnel.

fertilizer. Fertilizer rates were determined to ensure an equal amount of nitrogen in both treatments. Plants were then placed outdoors on a container production pad or in an adjacent high tunnel. Outdoor plants were placed on a black fabric ground cloth and fastened to a trellis wire to prevent blowover. Plants were in rows 6 ft apart with 4 ft spacing in the row. The high tunnel was 20 ft x 99 ft with the same black fabric ground cloth on the soil surface (Fig 1). Clear polyethelyene covered the top of the high tunnel with 30% white shade cloth over that. The sidewalls and end walls were covered with insect exclusion screen to prevent insect pollinators from easily entering the high tunnel. Plants were spaced on a 4 ft x 4 ft grid pattern. On 21 June, each terminal growing point was removed (pinched) to encourage lateral branching. The plants had been pinched twice previously while in the 1 gal plastic pots. Throughout July plants were hand watered every other day. For the remainder of the season plants were watered daily via a micro-irrigation spray stake in each container.

By late July, outdoor plants fertilized with the organic fertilizer were smaller and displayed symptoms of nitrogen deficiency (chlorosis) (Fig 2) compared to plants with conventional fertilizer. To prevent crop loss, outdoor and high tunnel plants with organic fertilizer were topdressed with a conventional controlled release fertilizer (Harrell's 17-6-12 Plus) at 120 grams per container on 29 July. Prior to fertilizer application, chlorophyll readings were taken on all plants with a hand-held chlorophyll meter (SPAD meter). One measurement was taken on each of three individual fully mature leaves per plant and averaged to obtain one chlorophyll reading per plant.

On 7 October plant height was measured from the container substrate surface to the terminal growing shoot. Additionally, the number of primary colas (central flower cluster on main stems) per plant were counted and recorded. Five colas per variety were harvested randomly from the outdoor plants and the high tunnel plants for THC and CBD analysis. Finally, total plant fresh weight was obtained by cutting each plant at the base and weighing.

The experimental design was a randomized complete block design with a split plot arrangement of treatments. Whole plot was high tunnel or outdoors while subplots were container type and fertilizer form. Data were analyzed using ANOVA and means separated with Fisher's Protected LSD.

Results & Discussion:

Figure 2. Plants with organic fertilizer (right) showed chlorotic symptoms and were generally smaller than plants with conventional fertilizer (left).

There was no influence of container type on any of the growth data collected (data not shown) so data presented are averaged over container type (Fig 3). Chlorophyll content (SPAD) of all varieties were influenced by fertilizer form (organic or conventional) (Table 2). Plants growing in the organic fertilizer had lower chlorophyll concentrations than plants fertilized with the conventional fertilizer. An interaction between the fertilizer form and exposure (high tunnel or outdoors) was observed in varieties ACDC, Cherry, and The Wife. In these three varieties there was a decrease of chlorophyll concentration between conventional and organic fertilizer, however, the difference was magnified outdoors compared to high tunnel plants. In fact, the chlorophyll concentration of plants in the high tunnel compared to outdoor plants fertilized with conventional fertilizer were nearly identical.

Only ACDC and Cherry height was affected by fertilizer form (Table 3). However, their response was opposite. Plants of ACDC were taller with organic fertilizer whereas, Cherry plants were taller when fertilized with conventional fertilizer. Plant height of all varieties was highly influenced by exposure. High tunnel plants were much taller than outdoor plants. This result was not surprising given the relatively protected environment of the high tunnel. Plants in the high tunnel likely experienced much less wind and reduced photoinhibition compared to outdoor plants. However, for CBD hemp, plant height is less important than the amount of floral material. The number of primary colas per plant was strongly influenced by the high tunnel environment in all varieties (Table 4). Both Cherry and Super CBD were only influenced by the high tunnel, whereas, the other varieties were also influenced by the fertilizer form or an interaction was observed. All Cherry plants in the outdoor plot had completely senesced by experiment termination while the high tunnel plants were alive and appeared as expected at harvest time. Conventional fertilizer increased cola number in ACDC, Otto II Stout, and The Wife. There was over a 100% increase in cola number in high tunnel The Wife plants with conventional compared to organic fertilizer (68.6 compared to 32.6, respectively).

Considering the difference in plant height and number of primary colas, it is not surprising that plants in the high tunnel had greater fresh weight at harvest time (Table 5). In many instances fresh weight was doubled in the high tunnel compared to outdoor plants. Additionally, the conventional fertilizer also positively influenced plant fresh weight. The combination of the protected environment of the high tunnel that produced taller plants and the improved nutrient source that created more highly branched plants (evidenced by increased number of colas) resulted in overall increased plant biomass at harvest.

Figure 3. Plants fertilized with organic fertilizer (left) were generally smaller and more chlorotic than plants fertilized with conventional fertilizer (right), regardless of container type.

Cannabinoid production in outdoor plants was low overall (Table 6). This was not unexpected. There was a fiber and dual-purpose variety trial plot approximately 400 ft north of the high tunnel and outdoor CBD variety plot. High rates of pollination were anticipated. The highest outdoor CBD content (4.6%) was detected in The Wife. This variety also appeared to have the most well-developed female flower buds and lowest quantity of seed. THC concentration was well below the 0.3% threshold for industrial hemp in all varieties. One goal of the project was to test the efficacy of the insect screening to exclude wind borne pollen from the high tunnel and therefore improve cannabinoid production. While some seed development was observed in the high tunnel, there was much less pollination compared to plants in the outdoor plot. Cannabinoid concentration also confirms a lower rate of pollination inside the high tunnel. CBD concentration reached as high as 9.9% and 10.5% in The Wife and Cherry, respectively. THC was detected in most of the samples and was below or at 0.3% for industrial hemp. However, THC in ACDC was reported at 0.4%.

Although data was not collected, significant pest were noted throughout the growing season. In the high tunnel army worms were a nuisance and were primarily controlled by scouting and hand removal. Their presence did not appear to significantly impact the plants. There was also an outbreak of russet mites that were extensive on individual plants (Fig 4). Heavily infested plants were removed and replaced and lightly infested plants were pruned to remove infested branches. Protective sprays (2x weekly for two weeks) of a pyrethrin (Pyganic) and azadirachtin (Azera) tank mix seemed to control the pest for the remainder of the growing season. Outdoor plots were affected by spotted cucumber beetle early in the season. Damage was mostly cosmetic and restricted primarily to foliar feeding. Eurasian hemp borer was noticed on several outdoor plants causing damage to the flower buds. No control efforts were attempted for this pest

Figure 4. Visual symptoms of russet mite (left) include up curling leaf margin, stunted growth, and overall 'tan' color to the bud. Under a dissecting microscope (right), mites can be seen covering the infested bud.

This was the first year of industrial hemp research in Kansas and there is a great need for further variety and production based research. Improving yield per plant, managing pests and weeds, and identifying production systems to decrease labor and increase productivity will be important for industrial hemp in the future.

Table 1. Clonal varieties and source of industrial hemp (*Cannabis sativa*) for CBD variety trial at the K-State John C. Pair Horticultural Center in 2019.

Variety Name	Source
ACDC	Craiger Enterprises
Cherry	Craiger Enterprises
Otto II Stout	Colorado Hemp Genetics
Super CBD	Craiger Enterprises
The Wife	Craiger Enterprises

Table 2. Chlorophyll reading (SPAD values) of *Cannabis sativa* varieties fertilized with conventional (Osmocote 15-9-12) or organic (Turf-Mate 4-2-3) fertilizer and grown in a high tunnel (HT) or outdoors (Out).

	Out		HT		Significance		
Variety	Org	Conv	Org	Conv	Exp	Fert	Inter
ACDC	37.0	57.9	44.1	56.9	**	**	**
Cherry	45.6	62.4	56.0	62.1	**	**	**
Otto II Stout	33.5	49.8	39.5	55.1	**	**	NS
Super CBD	39.4	55.6	45.0	57.6	**	**	NS
The Wife	41.0	54.9	47.6	55.8	**	**	**

Values are a mean of 8 plants.
Exp=Exposure (HT or Out) significant at $P<0.01$ (**).
Fert=Fertilizer (Org or Conv) significant at $P<0.01$ (**).
Inter=Interaction of Ext*Fert significant at $P<0.01$ (**) or not significant (NS).

Table 3. Plant height (cm) at harvest of *Cannabis sativa* varieties fertilized with conventional (Osmocote 15-9-12) or organic (Turf-Mate 4-2-3) fertilizer and grown in a high tunnel (HT) or outdoors (Out).

	Out		HT		Significance		
Variety	Org	Conv	Org	Conv	Exp	Fert	Inter
ACDC	140	123	191	184	**	*	NS
Cherry	76	84	109	124	**	**	NS
Otto II Stout	96	99	148	143	**	NS	NS
Super CBD	115	133	213	197	**	NS	NS
The Wife	88	94	131	144	**	NS	NS

Values are a mean of 8 plants.
Exp=Exposure (HT or Out) significant at $P<0.01$ (**).
Fert=Fertilizer (Org or Conv) significant at $P<0.05$ (*), $P<0.01$ (**), or not significant (NS).
Inter=Interaction of Ext*Fert was not significant (NS).

Table 4. Number of primary colas on *Cannabis sativa* varieties fertilized with conventional (Osmocote 15-9-12) or organic (Turf-Mate 4-2-3) fertilizer and grown in a high tunnel (HT) or outdoors (Out).

	Out		HT		Significance		
Variety	Org	Conv	Org	Conv	Exp	Fert	Inter
ACDC	6.4	12.3	35.4	44	**	*	NS
Cherry	0.0	0.0	19.4	23.1	**	NS	NS
Otto II Stout	8.9	16.0	30.0	51.1	**	**	**
Super CBD	3.9	17.5	47.9	52.9	**	NS	NS
The Wife	8.5	11.3	32.6	68.6	**	**	*

Values are a mean of 8 plants.
Exp=Exposure (HT or Out) significant at $P<0.01$ (**).
Fert=Fertilizer (Org or Conv) significant at $P<0.05$ (*), $P<0.01$ (**), or not significant (NS).
Inter=Interaction of Ext*Fert was significant at $P<0.05$ (*), $P<0.01$ (**) or not significant (NS).

Table 5. Fresh weight (kg) at harvest of *Cannabis sativa* varieties fertilized with conventional (Osmocote 15-9-12) or organic (Turf-Mate 4-2-3) fertilizer and grown in a high tunnel (HT) or outdoors (Out).

	Out		HT		Significance		
Variety	Org	Conv	Org	Conv	Exp	Fert	Inter
ACDC	1.0	1.3	1.6	2.3	*	NS	NS
Cherry	0.2	0.3	0.9	1.3	**	**	NS
Otto II Stout	0.8	1.3	1.7	2.9	**	**	**
Super CBD	0.7	1.6	2.1	2.7	**	**	NS
The Wife	0.7	1.2	1.5	2.5	**	**	NS

Values are a mean of 8 plants.
Exp=Exposure (HT or Out) significant at $P<0.05$ (*) or $P<0.01$ (**).
Fert=Fertilizer (Org or Conv) significant at $P<0.01$ (**) or not significant (NS).
Inter=Interaction of Ext*Fert was significant at $P<0.01$ (**) or not significant (NS).

Table 6. Concentration (% dry weight) cannabidiol (CBD) and tetrahydrocannabinol (THC) of *Cannabis sativa* varieties fertilized with conventional (Osmocote 15-9-12) fertilizer and grown in a high tunnel (HT) or outdoors (Out).

	Out		HT	
Variety	CBD (%)	THC (%)	CBD (%)	THC (%)
ACDC	2.2	0.1	6.1	0.4
Cherry	3.5	0.0	10.5	0.1
Otto II Stout	1.9	0.0	4.0	0.3
Super CBD	2.5	0.0	5.5	0.0
The Wife	4.6	0.0	9.9	0.1