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Jason Griffin

Kansas State University, jgriffin@ksu.edu

Michael J. Shelton

Kansas State University, mshelton@ksu.edu

Clint Wilson

Kansas State University, cdwilson@ksu.edu

See next page for additional authors

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2019 Kansas State University Industrial Hemp CBD Variety Trial

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Authors

Jason Griffin, Michael J. Shelton, Clint Wilson, and Tami Myers

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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 allowed to be grown again in the United States. Varieties have been selected and are currently grown with a wide range of cannabinoid profiles. Cannabinoids are of interest for their putative medical and therapeutic role in humans and 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 Kansas 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 Kansas. Variety selection is vital in CBD hemp production considering that 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 Kansas grown in containers outdoors or inside of 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 seeds 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 traveling 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.

¹ John C. Pair Horticultural Center, Kansas State University College of Agriculture, Department of Horticulture and Natural Resources, Haysville, KS.

Methods

On June 17, 2019, clones (from rooted cuttings) of each variety (Table 1) were potted from 1 gal plastic pots into 7 gal plastic pots or into 7 gal fabric bags. Pots and bags were filled with a soilless potting substrate composed of composted pine bark (HappiGro) and half inch screened pinebark nuggets (1:1 by vol) amended with 2 lbs/yd³ 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/yd³ (Osmocote plus 15-9-12) that included micronutrients. The second was an organic chicken manure-based fertilizer (Turf-Mate 4-2-3) incorporated at 65 lbs/yd³. A micronutrient fertilizer (Micromax) was added to the substrate at 1.5 lbs/yd³ to compensate for the lack of micronutrients in the organic 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 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 × 99 ft with the same black fabric ground cloth on the soil surface (Fig 1). Clear polyethylene 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 × 4 ft grid pattern. On June 21, 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 July 29. 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 October 7 plant height was measured from the container substrate surface to the terminal growing shoot. Additionally, the numbers 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 and Discussion

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 was influenced by fertilizer form (organic or conventional; Table 2). Plants growing with 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 fertilized with conventional fertilizer in the high tunnel compared to outdoor plants fertilized with conventional fertilizer were nearly identical.

Only ACDC and Cherry height were 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 greater than 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.

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. The 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 were not collected, significant pests were noted throughout the growing season. In the high tunnel, army worms were a nuisance and were primarily controlled by scouting and removal by hand. Their presence did not appear to significantly impact the plants. Russet mites were extensive on individual plants during an outbreak (Fig 4). Heavily infested plants were removed and replaced, and lightly infested plants were pruned to remove infested branches. Protective sprays (2 times 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.

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 are important for industrial hemp.

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Table 1. Clonal varieties and source of industrial hemp (*Cannabis sativa*) for CBD variety trial at the Kansas State University 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)

Variety	Outdoors		High tunnel		Significance		
	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 Exp \times 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)

Variety	Outdoors		High tunnel		Significance		
	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 Exp \times 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)

Variety	Outdoors		High tunnel		Significance		
	Org	Conv	Org	Conv	Exp	Fert	Inter
ACDC	6.4	12.3	35.4	44.0	**	*	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 Exp \times 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)

Variety	Outdoors		High tunnel		Significance		
	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 Exp \times Fert was significant at $P < 0.01$ (**) or not significant (NS).

Table 6. Concentration (% dry weight) of 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)

Variety	Outdoors		High tunnel	
	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



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

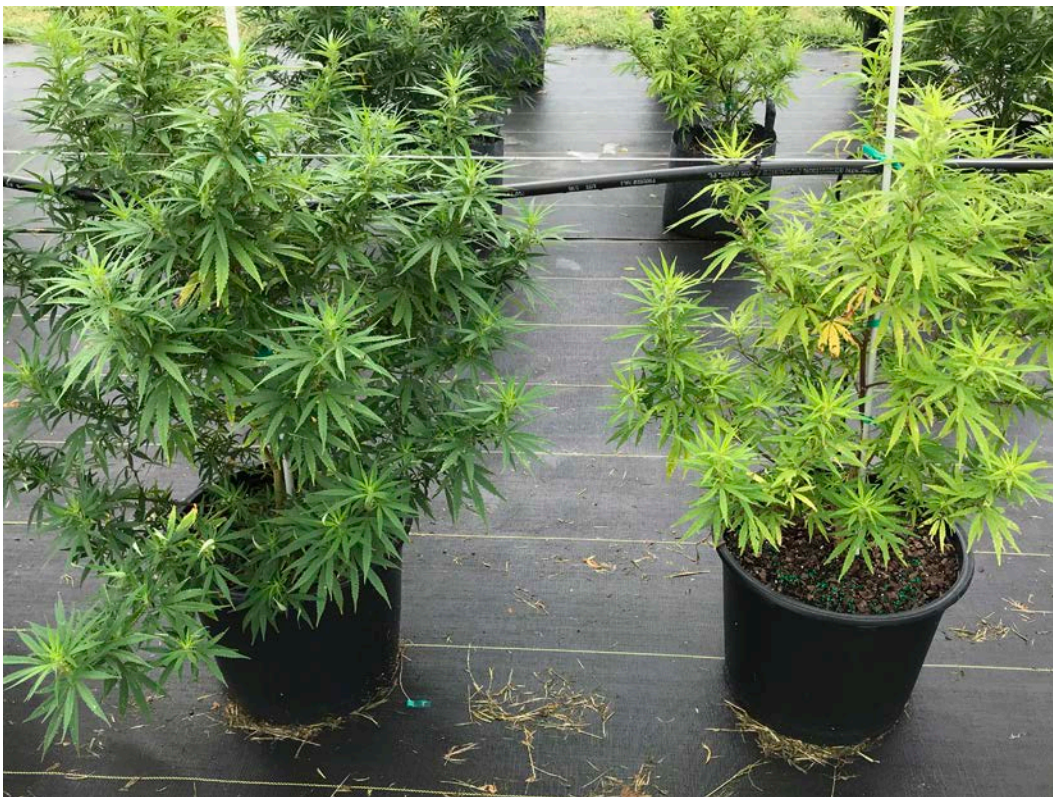


Figure 2. Plants with organic fertilizer (right) showed chlorotic symptoms and were generally smaller than plants with conventional fertilizer (left).



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.



Figure 4. Visual symptoms of russet mite (left) include upward 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.