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Screening Commercial Hemp Cultivars for Cannabinoid Analysis

Abstract

Twenty-eight essential oil-type cultivars of industrial hemp (*Cannabis sativa L.*) were grown to maturity in a controlled environment. Data were collected on various plant growth parameters to assess cultivar characteristics with the goal of identifying suitable cultivars for commercial hemp producers. Cannabinoid analysis identified concentrations throughout different plant strata to further validate the regulatory sampling protocol mandated by the USDA. Results of this study indicate plant strata do not affect cannabinoid concentrations in mature hemp plants. Commercial hemp producers should choose cultivars with desirable traits specific to their operation.

Keywords: *Cannabis sativa*, auto flower, photoperiod, greenhouse, strain, medicinal plants

Introduction

Industrial hemp (*Cannabis sativa* L.) plants are regulated by the USDA under the Agricultural Improvement Act of 2018 and must fall below a threshold of 0.3% total tetrahydrocannabinol (THC) concentration on a dry weight basis. Any samples found to exceed 0.3% THC are classified as marijuana and subject to destruction of the crop.

The USDA enforces standard and performance-based sampling guidelines for the measurement of total THC content, which determine whether the specimens are hemp or marijuana (USDA, 2021). The samples are intended to be representative of the total THC content in a “lot” of hemp crop as specified by the producer. The THC content of hemp generally peaks as the plant developmentally matures, therefore the timing of sampling the target plant is important to accurately measure total THC concentration and ensure compliance with the USDA hemp production program. The program mandates all samples “must be collected from the flowering tops of the plant by cutting the top five to eight inches from the ‘main stem’ (which includes the leaves and flowers), ‘terminal bud’ (located at the end of a stem) or ‘central cola’ (cut stem that can develop into a bud) of the flowering top of the plant” (USDA, 2021).

The objective of this study was to assess the growth and characteristics of 28 cultivars of essential oil-type hemp plants grown to full maturity. Floral bud samples were collected on a weekly basis to quantify the seasonal development of the major cannabinoids and the acid precursors CBDa, CBD, THCa, THCV, and Δ 9-THC. Cannabigerol (CBG) was quantified only in cultivars containing detectable concentrations of this cannabinoid. The goal of this sampling procedure was to identify an optimum harvest window for THC compliancy when producing commercial hemp. Additional samples were collected to assess the spatial distribution of cannabinoids within the plant at the time of bud maturity. Defining potential differences or similarities in cannabinoid concentrations between different plant strata on a singular plant will help solidify current sampling techniques or potentially enhance regulatory sampling protocols for THC compliancy.

Materials and Methods

Location and plant material

This project is a subset of a multistate trial lead by Kentucky State University (USDA #S1084 to B. Jampala et al.). Twenty-eight cultivars of essential oil-type hemp (*C.*

sativa) were grown to maturity in a greenhouse located at the Plant Materials Center on the Central Research Station in Baton Rouge, Louisiana (30.3606107, -91.1771971).

Daylength Sensitive		Day Neutral
Berry Blossom	Sour Lifter	Auto CBG
Bubbatonic	Special Sauce	Dr. Chunk
Forbidden Five	Stem Cell CBG	Maverick
Fruity Petals	Super Cinco	Pipeline
Hawaiian Haze	Super Sour Space Candy	Sour Citron
Lady Lee	Truckoo	Sour RNA Seedless
Lifter Seedless	Umpqua	
Rincon	Valerie 29	
Rogue	White CBG	
Santiam	White CBG Seedless	
Sour Kush		

Experimental design

Hemp plants were seeded into four-inch cow pots[1] filled with FoxFarm Ocean Forest Potting Soil[2] at a rate of one seed per pot. Each cultivar had a total of five plants, or replicates. The micro drip irrigation system was set to water for 30 second intervals every hour from 06:00 to 18:00. Once the plants grew their first true leaves the transplants were fertilized every other day with two fluid ounces of solution made with Miracle Gro Water-Soluble All-Purpose Plant Food[3] following the recommended application rate of one tablespoon per gallon of water. Twenty-eight days after seeding, plants were transplanted into five-gallon plastic nursery containers[4] filled with Miracle-Gro Potting Mix[5] and fitted with spaghetti tubing emitters for irrigation. Irrigation was set to six times a day at three-minute intervals, with one hour and 30 minutes in between each watering. After two weeks, irrigation was increased to four-minute intervals to accommodate for increased plant biomass. Plants were given structural support *via* 48-inch plastic garden stakes[6]. Each plant received five ounces of fertilizer solution each

[1] CowPots, LLC, East Canaan, Connecticut
[2] FoxFarm Soil and Fertilizer Co., Arcada, California
[3] Scotts Miracle Gro Company, Marysville, Ohio
[4] Pro Cal Innovations LLC, Alpena, Michigan
[5] Scotts Company, LLC, Marysville, Ohio
[6] Sheldon Manufacturing, INC, Cornelius, Oregon

week using Jack's Nutrients fertilizer (15-0-0)[7] at a rate of one tablespoon per gallon of water. Plants were not pinched or pruned throughout the duration of this trial. Plants were grown in ambient lighting plus supplemental lighting provided by 1000W high-pressure sodium lamps[8] that were placed eight feet above the benchtop. The twelve 1000-watt bulbs were on for 18 hours and off for six hours, creating an 18:6 photoperiod. Supplemental lights were turned off 60 days after sowing seed to induce flower initiation. The greenhouse was automatically programmed to heat at 21°C and cool at 28°C both day and night year-round.

Data collection

Cannabinoid concentrations were analyzed three to four weeks post-anthesis, depending on the visible signs of floral maturity, all the way to plant senescence. Flower samples were collected weekly from 14 cultivars: Berry Blossom, Bubbatonic, Forbidden Five, Fruity Petals, Hawaiian Haze, Lady Lee, Lifter Seedless, Rincon, Rogue, Santiam, Sour Kush, Sour Lifter, Special Sauce, and Truckoo. Samples were taken by randomly selecting various locations of floral tissue from each of the five replicates to construct one representative sample per cultivar per week. To screen for phenotypic variability among cultivars, individual samples were collected from each replicate of four randomly chosen cultivars: Berry Blossom, Fruity Petals, Lifter Seedless, and Maverick. Each replicate was sampled separately, for a total of five samples per cultivar per week (n=5). Weekly sampling of these individual plants resulted in most bud tissue being removed throughout the duration of this sampling phase. Subsequently, the cultivar replicates that were sampled individually resulted in insufficient floral tissue for all essential laboratory processes in year one of the study. In year two of this study, only composite samples were taken from all cultivars to ensure there was enough plant tissue biomass for laboratory processes. Five to seven grams of floral tissue (fresh weight) were collected in each sample in year two of the study to ensure enough plant tissue for further laboratory analysis. Stratified sampling took place

[7] Jack's Nutrients, Allentown, Pennsylvania
[8] P.L. Light Systems NXT2; Ontario, Canada

to analyze the distribution of cannabinoids at plant maturity. These samples were collected from the upper third, middle third, and lower third of the plant. Individual plants of the following cultivars were sampled for laboratory analysis: Berry Blossom, Fruity Petals, Lifter Seedless and Santiam. All remaining flowers were harvested, and the dry weight was recorded as the total yield. Once dried to a moisture content below 12% as per 2018 USDA Farm Bill protocol, the flowers were hand-trimmed.

Drying hemp material

In year one of the greenhouse trial, flowers were dried on wire racks in ambient temperatures, between 21°C and 28°C, for three days. In year two, the plant material was dried in a Shel Lab forced air oven 7 at 55°C until plants reached a moisture content of 12% or less. The drying protocol was modified between years to ensure all samples were dried uniformly and to provide a replicable protocol for future studies. Percent moisture content of each sample was quantified using the Mettler Toledo HC103 moisture analyzer[9] using the parameters listed in Table 1. Samples were ground, homogenized (size and material) and distributed evenly on the sample pan prior to moisture analysis.

Table 1. Mettler Toledo HC103 moisture analyzer parameters used during the quantification of percent moisture content of dried hemp bud grown in south Louisiana.

Parameter	Specification
Drying Program	Standard
Drying Program	60°C
Switch Off Criteria	5 (1mg/140s)
Switch Off Criteria	% MC
Start Weight	0.500 g
Start Weight Tolerance	10%

[9] Mettler Toledo, LLC, Columbus, Ohio

Cannabinoid extraction

Dried plant tissue was ground with a Magic Bullet blender appliance[10] and extracted according to the Louisiana Department of Agriculture and Forestry (LDAF) hemp extraction protocol. This protocol consisted of adding 200 mg ±0.5 mg of freeze-dried tissue to a 50 mL plastic centrifuge tube (Corning 430828) followed by adding 25 mL of HPLC grade methanol. The mixture was vortexed for one minute and sonicated for 15 minutes, with a one-minute pause in vortexing for every five minutes of sonication. The mixture was then centrifuged for five minutes at 1230 G. 0.2 µm of the supernatant was filtered into a 15 mL centrifuge tube (Corning 430790) using a 0.2 µm polyvinylidene difluoride (PVDF) syringe filter (Whatman 6873-2502). The filtered supernatant was diluted at a ratio of 1:10 with one-part HPLC grade water and three-parts 0.1% formic acid and placed into autosampler vials.

Cannabinoid analysis

Samples were prepared by mixing 100 µl of the sample extract solution with 600 µl of the mobile phase (58% acetonitrile in water containing 0.1% formic acid). The standard stock solution contained CBDa at the concentration of 200 µg/mL and THCV, CBG, 9-THC, 8-THC and 9-THCa at the concentration of 20 µg/mL. The calibration curve was created by a series of different concentrations made from 200/20 µg/mL to 100/10, 50/5.0, 25/2.5, 12.5/1.25 and 6.25/0.625 µg/mL.

Analysis of the cannabinoids CBD, CBDV, CBDa, CBG, Δ 9-THC, THVa and THCV was performed with a Dionex ICS-3000 system, which includes Dionex Ultimate 3000 pump, Ultimate 3000 Autosampler, Ultimate 3000 column compartment and Ultimate 3000 Photodiode Array Detector which were controlled by Chromeleon 6.8 software. The samples were separated on a Waters Cortecs T3 column with the mobile phase of 58% acetonitrile containing 0.1% formic acid (B) in 0.1% formic acid in water (A) at 38 °C. The mobile phase gradient used during the separation of the solutes is shown in Table

[10] Homeland Housewares, LLC, Los Angeles, CA

2. The flow rate was 1.0 ml/min with a detection wavelength at 228 nm. Injection volume was 20 µl.

Table 2. High-Performance Liquid Chromatography mobile phase gradient during the separation of hemp solutes for the quantification of cannabinoids.

Time (min.)	Mobile Phase A (0.1% formic acid in water)	Mobile Phase B (0.1% formic acid)
0.0	42	58
15.0	42	58
16.0	5	95
19.0	5	95
19.2	42	58
23.0	42	58

Statistical analysis

Data analysis was completed using R (R Core Team, 2017) and RStudio (RStudio Team, 2020) as well as the following packages: Lme4 (Bates *et al*., 2015), Agricolae (de Mendiburu, 2021), lmerTest (Kuznetsova, 2017), Emmeans (Lenth, 2020) and tidyverse (Wickham *et al*., 2019).

Results

Limitations of the study

In year one of the greenhouse trial there was variable water pressure amongst the irrigation fittings, despite the irrigation being uniformly automated. As a result, several replicates did not receive adequate water and yields were significantly reduced. These plants were excluded from the analysis as outliers. During year two of this trial, an irrigation leak resulted in insufficient water for several transplants that did not recover

beyond their permanent wilting point. Thus, the trial was conducted with less than five replicates (reps) for the following cultivars: Forbidden Five (three reps), Metolius (three reps), Rogue (four reps), and Truckoo (four reps). The remaining cultivars had the intended number of replications (n=5).

Leaf chlorophyll content

Average leaf chlorophyll contents were compared using the post-Analysis of Variance (ANOVA) Tukey test with a threshold p-value of 0.05. The averages did not differ significantly between most cultivars (Table 3). The cultivars with the highest chlorophyll content, or SPAD readings, were Bubbatonic (117.37), Hawaiian Haze (111.64) and Special Sauce (125.65) and were statistically significantly different than the rest.

Final plant height

A General Linear Model with Mixed Effects (GLMMX) was implemented in R (using RStudio and the package lme) to compare the mean plant height for the various Cultivars (fixed effect), with the variable “Year” being considered as a random effect. This was followed by a Tukey post-hoc analysis at $|p|<0.05$ (n=5). The final heights of the plants were significantly different among hemp cultivars grown in greenhouse conditions. Table 4 displays these differences and clearly shows that cultivar type affects the final height of hemp plants ($p=2.2e-16$). The daylength-neutral cultivars (Auto CBG, Dr. Chunk, Maverick, Pipeline and Sour RNA Seedless) were among the shortest in height. The two shortest cultivars in the study were Auto CBG and Sour Citron.

Table 3. Average leaf chlorophyll content (SPAD value) of greenhouse-grown essential oil-type hemp plants by cultivar, combined years (2020-2021), including Tukey groups.

Cultivar	Chlorophyll Content (SPAD value)	Significance
Auto CBG	42.31	A
Berry Blossom	52.12	A
Bubbatonic	117.37	B
Dr. Chunk	41.57	A
Forbidden Five	46.12	A
Fruity Petals	62.15	A
Hawaiian Haze	111.64	B
Lady Lee	49.64	A
Lifter Seedless	67.31	A
Maverick	46.94	A
Metolius	53.74	A
Pipeline	48.94	A
Rincon	33.95	A
Rogue	57.18	A
Santiam	66.99	A
Sour Citron	56.21	A
Sour Kush	56.03	A
Sour Lifter	66.71	A
Sour RNA Seedless	82.42	A
Special Sauce	125.65	B
Stem Cell CBG	55.65	A
Super Cinco	55.23	A
Super Sour Space Candy	55.22	A
Truckoo	63.31	A
Umpqua	67.91	A
Valerie Twenty-Nine	48.85	A
White CBG	54.58	A
White CBG Seedless	66.84	A

Table 4. Average final height of greenhouse-grown essential oil-type hemp by cultivar, combined years (2020-2021), including Tukey groups.

Cultivar	Final height (in)	Significance
Auto CBG	13.93	A
Sour Citron	17.57	A
Maverick	23.0	AB
Pipeline	21.31	AB
Sour RNA Seedless	22.4	AB
Dr. Chunk	29.94	BC
Valerie Twenty-Nine	34.7	CD
Rincon	36.33	CDE
Berry Blossom	37.58	CDEF
Fruity Petals	37.0	CDEF
Lady Lee	39.08	CDEF
Rogue	38.78	CDEF
Special Sauce	38.55	CDEF
Super Cinco	37.5	CDEF
Stem Cell CBG	39.85	DEFG
Umpqua	40.23	DEFG
Bubbatonic	42.7	DEFGH
Forbidden Five	40.47	DEFGH
Hawaiian Haze	43.55	DEFGH
Metolius	40.56	DEFGH
Santiam	43.15	DEFGH
Sour Kush	42.25	DEFGH
Sour Lifter	43.25	DEFGH
Super Sour Space Candy	42.33	DEFGH
White CBG Seedless	45.33	EFGH
White CBG	45.81	FGH
Lifter Seedless	48.53	GH
Truckoo	50.17	H

Maverick, Pipeline and Sour RNA Seedless were also shorter than the remaining cultivars grown in this greenhouse study. The tallest hemp plants were White CBG, Lifter Seedless and Truckoo. The remainder cultivars did not differ in average height of plants grown in the greenhouse.

Cultivar yield

The cultivar type influences the final bud weight (p= 1.112e-11) (Table 5). GLMMX was implemented in R (using RStudio and the package lme) to compare the mean bud weight for the various cultivars (fixed effect), with the variable "Year" being considered as a random effect. This was followed by a Tukey post-hoc analysis at $|p|<0.05$ (n=5). Umpqua was the highest yielding amongst all 28 cultivars. The total yields of Sour Lifter, Lifter Seedless and White CBG Seedless cultivars ranked second, third and fourth, respectively. The remaining cultivars produced comparable yields.

Final height and yield correlation

A linear regression was used to determine the correlation between the final height and yield among all cultivars combined (Figure 1). The correlation coefficient between them was 0.5126, and the corresponding linear regression equation was: $\mathrm{Weight} = \mathrm{Final\ Height} * 0.54399 + 1.38445$. The corresponding analysis of variance (ANOVA) table for the regression yielded F (1, 145) = 51.712, p = 3.156e-11. The corresponding R squared value was 0.2628 and the root mean square error was 9.980089.

The coefficient of the final height implies that the linear regression might be a good model for the interaction between final height and bud weight of hemp plants, but more replications are needed as well as separate models for each cultivar. The three tallest cultivars were Lifter Seedless, White CBG and Truckoo, and the cultivars with the three highest yields were Lifter Seedless, Sour Lifter and Umpqua. White CBG and Truckoo were among the higher-yielding cultivars. The three shortest cultivars were Auto CBG, Sour Citron and Maverick, while the three cultivars with the lowest yields were Auto CBG, Berry Blossom and Sour RNA Seedless.

Table 5. Average bud weight of greenhouse-grown essential oil-type hemp by cultivar, combined years (2020-2021), including Tukey groups.

Cultivar	Bud Weight (g)	Significance
Auto CBG	8.53 A	A
Berry Blossom	7.76 A	A
Sour RNA Seedless	9.56	A
Fruity Petals	11.98	AB
Lady Lee	13.56	ABC
Maverick	13.63	ABC
Rincon	12.92	ABC
Bubbatonic	18.6	ABCD
Metolius	18.86	ABCD
Pipeline	16.7	ABCD
Santiam	19.74	ABCD
Sour Citron	10.09	ABCD
Sour Kush	18.62	ABCD
Stem Cell CBG	20.18	ABCD
Super Sour Space Candy	17.6	ABCD
Forbidden Five	22.82	ABCDE
Hawaiian Haze	22.73	ABCDE
Rogue	25.22	ABCDE
Special Sauce	25.22	ABCDE
Super Cinco	24.38	ABCDE
Truckoo	25.7	ABCDE
Valerie Twenty-Nine	25.58	ABCDE
White CBG	28.7	ABCDE
White CBG Seedless	32.26	BCDE
Lifter Seedless	32.76	CDE
Sour Lifter	35.94	DE
Umpqua	42.88	E

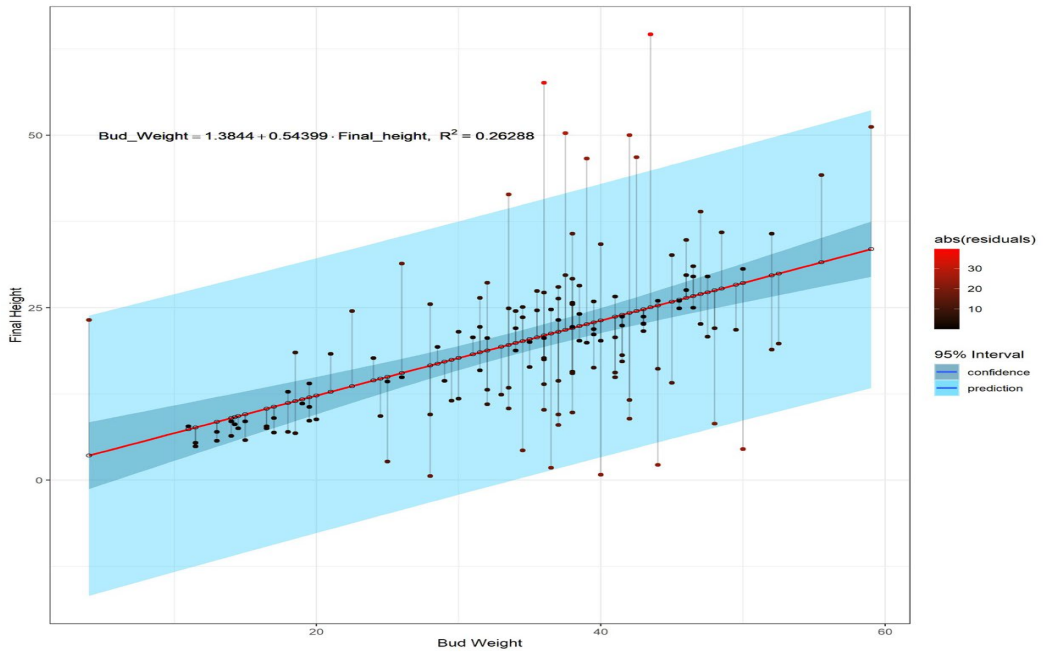

Figure 1. Linear regression analysis between the final height (inches) and bud weight (grams) of greenhouse grown hemp plants in south Louisiana with combined years (2020 and 2021).

Final cannabinoid concentration

Due to the high number of repetitions that are required for the type of statistical analysis this project requires, significant differences were unable to be generated for the final cannabinoid concentrations at plant maturity. Insufficient repetitions (n≤5) in this study resulted from the large number of cultivars (n=16) evaluated and spatial limitations in the greenhouse. Despite lack of statistical differences in cannabinoid concentrations, producers and industry personnel may gather useful observations from this information. It is important to note that any cannabinoid concentration level of ‘0 ’does not mean that the cannabinoid is not present in the plant; it simply signifies that the concentration was below detectable concentrations. Table 6 displays the final cannabinoid concentrations in individual cultivars tested in this project.

Table 6. Average final concentration of CBDa, CBD, THCa, Δ 9-THC, THCV, and CBG in mature essential oil-type hemp cultivars grown in the greenhouse across two consecutive years (2021-2022).

Cannabinoid Concentrations (% dry weight)												
Cultivar Year	CBDa Y1	CBDa Y2	CBD Y1	CBD Y2	THCa Y1	THCa Y2	D9-THC Y1	D9-THC Y2	THCV Y1	THCV Y2	CBG Y1	CBG Y2
Auto CBG	2.2	0	.02	0	.07	0	0	0	.34	0	0	0
Berry Blossom	13.9	4.7	0.4	5.9	0.3	0.2	0	0.5	2.1	1.3	0	0.4
Bubbatonic	6.6	4.3	0.4	5.4	0.2	0.3	0	0.4	1.6	0.7	0	0
Forbidden Five	2.9	2.3	0.1	2.0	0.08	0.1	0	0.2	1.0	0.7	0	0
Fruity Petals	12.3	5.4	6.0	9.3	0.2	0.3	0.5	0.8	4.1	0.4	0.9	0
Hawaiian Haze	10.3	6.6	0.4	8.8	0.3	0.3	.03	0.8	2.5	1.5	0	0
Lady Lee	7.1	4.6	0.4	8.1	0.2	0.4	.03	0.6	1.4	0.6	0	0
Lifter Seedless	5.2	3.7	6.3	6.0	0.2	0.2	0.5	0.5	0.6	0.7	0	0
Maverick	2.4	5.2	0.2	0.4	.07	0.2	0	.04	0.3	0.9	0	0
Rincon	3.4	4.6	4.1	0.3	0.2	0.1	0.3	.05	0.3	0.3	0	0
Rogue	5.9	3.6	0.3	6.0	0.2	0.2	0	0.5	2.6	0.4	0	0
Santiam	2.1	3.3	0.1	4.6	0.1	0.1	0	0.4	3.4	0.3	0	0
Sour Kush	.01	7.7	6.0	0.3	0.2	0.2	0.5	0	4.1	1.3	.90	0
Sour Lifter	6.3	3.7	0.3	6.0	0.2	0.2	.02	0.5	1.8	0.7	0	0
Special Sauce	8.8	1.7	0.5	2.6	0.2	0.2	0.0	0.3	1.6	15.1	0	1.7
Truckoo	7.3	3.4	0.3	4.7	0.2	0.1	.03	0.4	1.3	0.8	0	0

Values of cannabinoids are compared within columns, not between rows.

Although not significantly, Berry Blossom cultivar had the highest final concentration of CBDa (13.900%) in year one of the study, followed by Fruity Petals (12.332%),

Hawaiian Haze (10.290%) and Special Sauce (8.829%) (Table 6). In year two cultivars Sour Kush (7.710%), Hawaiian Haze (6.629%), Fruity Petals (5.358%) and Maverick (5.196%) had the highest concentrations of CBDa. The average concentration of CBDa in year one was 39% higher than the average concentration in year two. Although not significant, the cultivar Lifter Seedless had the highest final concentration of CBD (6.327%) in year one of the study, followed by Sour Kush (5.952%) and Fruity Petals (5.952%) (Table 3.8). In year two cultivars Fruity Petals (5.952%), Hawaiian Haze (8.764%) and Lady Lee (8.129%) had the highest concentration of this cannabinoid. The average concentration of CBD in year one was 93% higher than the average concentration in year two. The higher concentrations in cannabinoids may be the result of inconsistent sampling periods in years one and two of this study. Future studies will use a quantifiable indicator to ensure accurate and replicable sampling periods.

Compliancy is determined by combining the concentration of THCa, and Δ 9-THC and cannot be solely identified by THCa or Δ 9-THC alone. The cultivars with the least concentration of THCa in both years, although not significant, were Auto CBG, Forbidden Five, Maverick and Santiam (Table 6). Auto CBG was the cultivar with the lowest concentration of Δ 9-THC in both years of this study (Table 6). These cultivars should be evaluated further to see if different environmental conditions affect the concentration of this cannabinoid in the plant.

The cultivar Special Sauce had the highest final concentration of THCV in year one of the study (15.103%) followed by Fruity Petals (4.128%) and Sour Kush (4.128%). The cultivars with the lowest concentration of THCV were Rincon, Maverick, and Rogue.

Cannabinoid concentration by plant strata

Figure 2 displays the final concentrations of CBD, CBDa, CBDV, Δ9-THC and THCV between plant locations (top, middle and bottom strata of the entire plant) of ‘Berry Blossom’ hemp grown in the greenhouse. A General Linear Model with mixed effects was used by R and RStudio to compare the means among various locations using Year as a random effect, with a Tukey post-hoc analysis at $|p|<0.05$. Mean comparisons across plant locations through a one-way ANOVA with Tukey post-hoc analysis at $P \leq$

0.05 were conducted for the cannabinoids not present in both years (n=5). In both years, the concentrations of all cannabinoids did not differ among the top, middle and bottom portions of the plant.

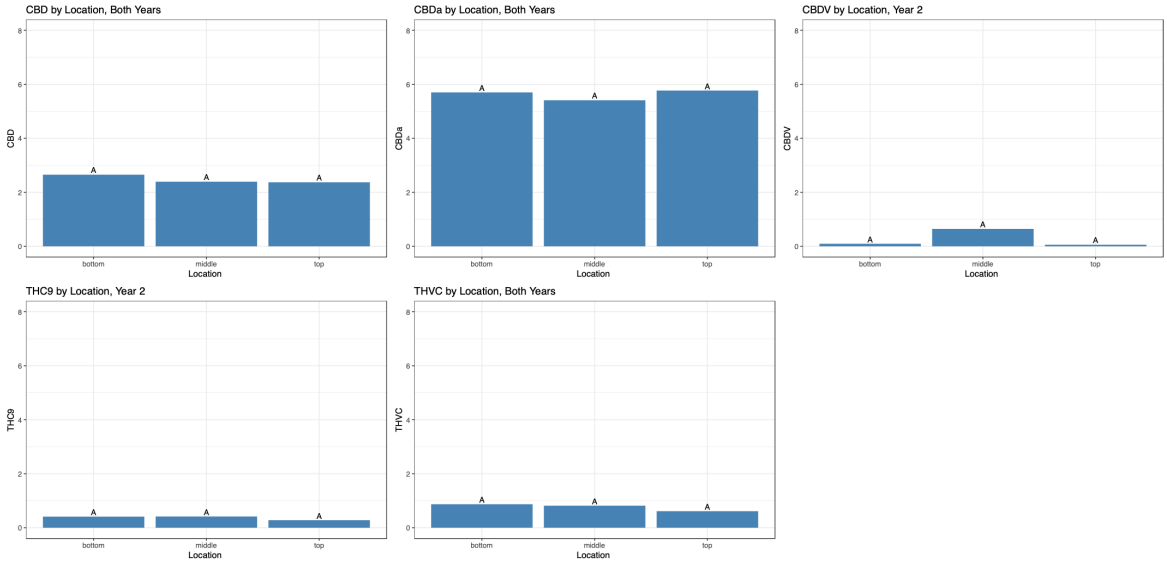

Figure 2. Final cannabinoid concentrations (CBD, CBDa, CBDV, Δ9-THC and THCV) between plant locations (top, middle and bottom strata of the entire plant) of 'Berry Blossom' essential oil-type hemp grown in the greenhouse.

Figure 3 displays the final concentrations of CBD, CBDa, CBDV, Δ9-THC and THCV between plant locations (top, middle and bottom strata of the entire plant) of the Fruity Petals cultivar in both years one and two of this study. A General Linear Model with mixed effects was used by R and RStudio to compare the means among various locations using Year as a random effect, with a Tukey post-hoc analysis at $|p|<0.05$. (n=5) for all figures. CBDa in the top portion of the Fruity Petals cultivar was higher than the bottom portion of the plant. The concentrations of CBD, CBDV, Δ9-THC and THCV did not differ in the top, middle and bottom portions of the plant. Although CBDa was higher in the top portion of the plant, regulatory sampling is only concerned with THC concentrations, specifically, Δ9-THC and THCa.

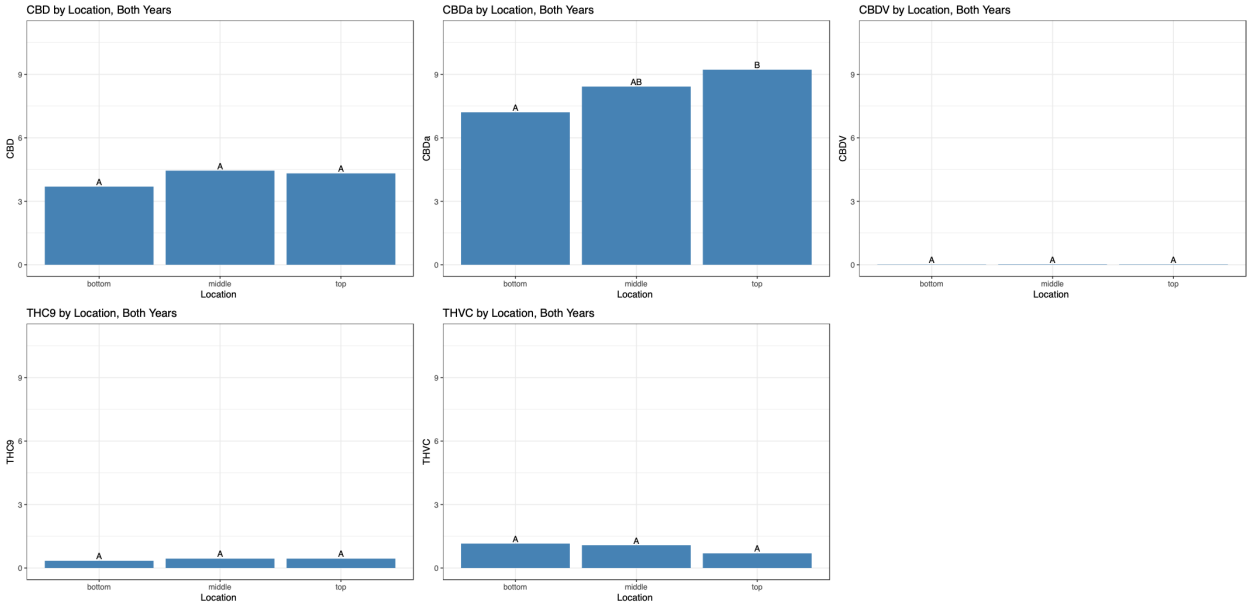

Figure 3. Final cannabinoid concentrations (CBD, CBDa, CBDV, Δ9-THC and THCV) between plant locations (top, middle and bottom strata of the entire plant) of 'Fruity Petals' essential oil-type hemp grown in the greenhouse.

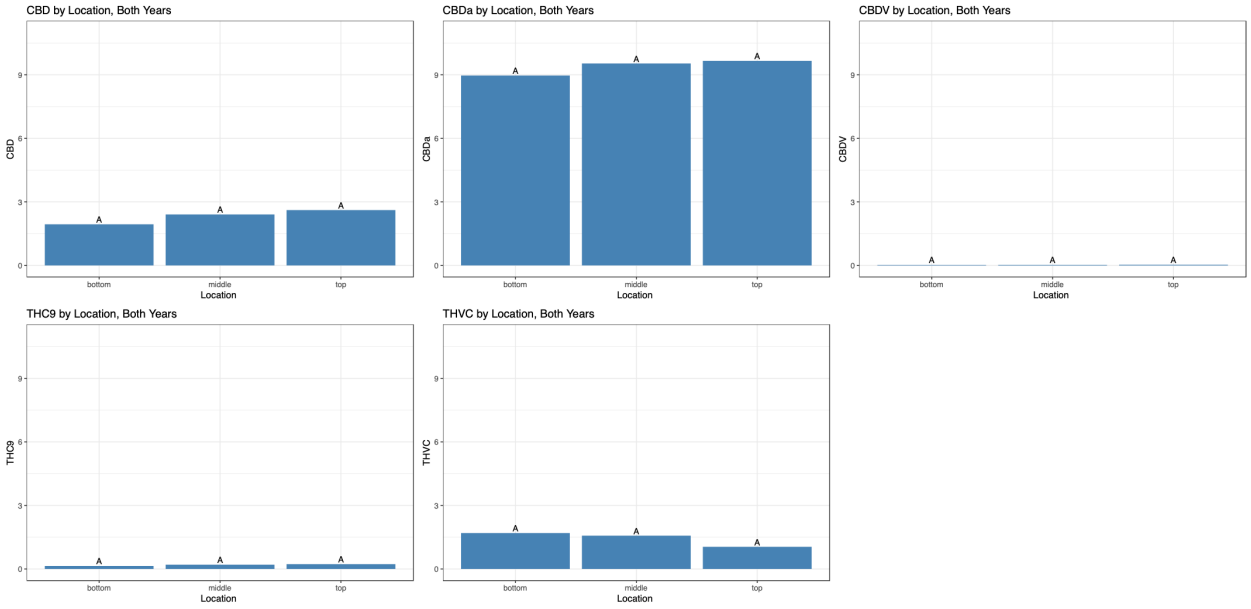

Figure 4. Final cannabinoid concentrations (CBD, CBDa, CBDV, Δ9-THC and THCV) between plant locations (top, middle and bottom strata of the entire plant) of 'Lifter Seedless' essential oil-type hemp grown in the greenhouse.

Figure 4 displays the final concentrations of CBD, CBDa, CBDV, Δ9-THC and THCV between plant locations (top, middle, and bottom strata of the entire plant) of the cultivar Lifter Seedless hemp in both years one and two of this study. The concentrations of all cannabinoids did not differ from the top, middle and bottom portions of Lifter Seedless.

Figure 5 displays the final concentrations of CBD, CBDa, CBDV, Δ9-THC and THCV between plant locations (top, middle and bottom strata of the entire plant) of ‘Santiam’ hemp in both years one and two of this study. Concentrations of all cannabinoids did not differ in the top, middle and bottom portions of the plant. Results conclude that stratified sampling might not be a necessary component of regulatory sampling protocols for THC compliancy in the cultivars Berry Blossom, Fruity Petals, Lifter Seedless and Santiam.

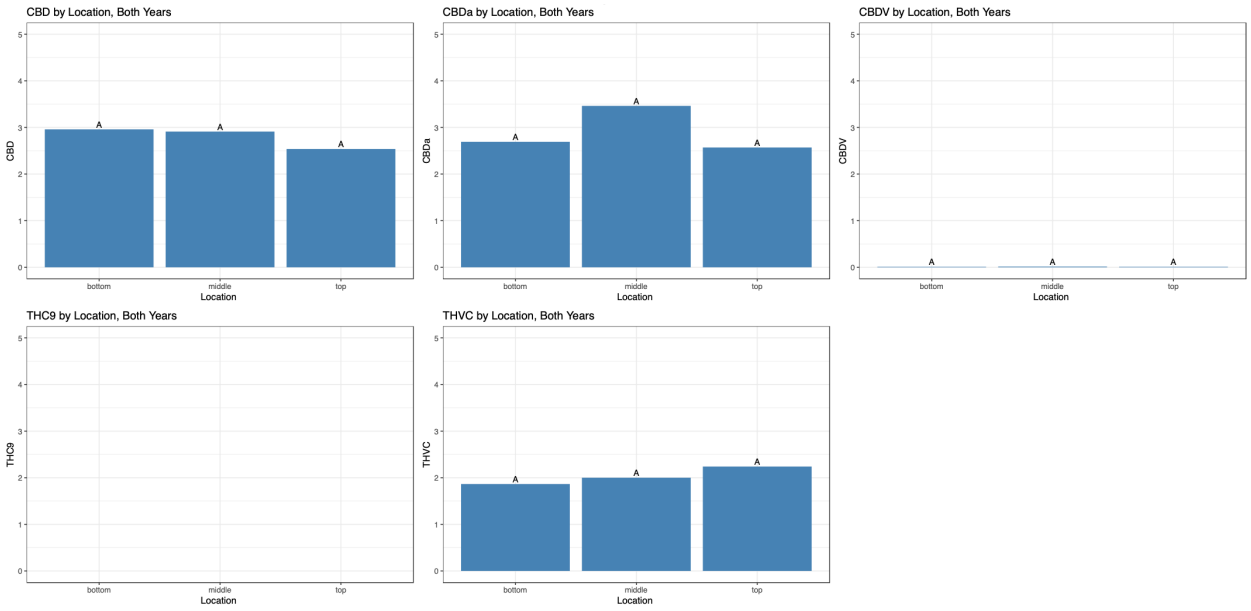

Figure 5. Final cannabinoid concentrations (CBD, CBDa, CBDV, Δ9-THC and THCV) between plant locations (top, middle and bottom strata of the entire plant) of ‘Santiam’ essential oil-type hemp grown in the greenhouse.

Discussion

The lack of differences in chlorophyll content of most cultivars were likely because all readings were collected during the same time period. SPAD differences are generally noticed when taken within the same plant but at different stages of growth, such as the

vegetative and reproductive phases of growth. Notably, SPAD readings in corn tend to correlate with total grain yield (Kandel, 2020). In this study, we see differences in yield but few differences in SPAD readings (Table 3). Lack of differences may be explained by the fact that all hemp plants in this study were grown in a highly controlled environment. The greenhouse was fitted with automated irrigation, maintained an average temperature range between 21°C to 28°C, and fertilization was consistent among all plants. This differs from most field trials where changes in soil structure, soil fertility, drainage, and environmental factors such as amount of rain, wind and relative humidity take place. Despite SPAD readings being similar in most cultivars, the final heights of the plants were significantly different among hemp cultivars grown in greenhouse conditions.

The differences in plant height among cultivars may be explained by the shorter period of vegetative growth daylength-neutral cultivars naturally receive compared to the remaining daylength-sensitive cultivars. Additionally, there are several considerations to note with respect to the low-yielding cultivars. Daylength-neutral cultivars (Auto CBG, Maverick, Sour Citron, and Sour RNA Seedless) began flowering four weeks (plus or minus three days) before daylength-sensitive cultivars. Reducing vegetative growth by one month can subsequently reduce yields, however these cultivars required less production time which may be beneficial for producers looking for a crop with a shorter production period. Pipeline was the highest-yielding daylength-neutral cultivar in this study and may be optimal for producers.

It is important to note that the cultivars selected for stratified sampling (Berry Blossom, Fruity Petals, Lifter Seedless and Santiam) had greater amounts of floral tissue removed throughout the duration of this study. As a result, these cultivars would have higher yields than accounted for in the data (approximately 21–28g) had the stratified sampling not taken place. Despite collecting stratified sampling throughout the growing season in year one, Lifter Seedless was the third highest-yielding cultivar. The cultivar Maverick had many replications infected with fusarium head blight (*Fusarium oxysporum*) in both years of this trial, which likely affected plant growth and subsequent yields. Despite Berry Blossom's optimal growth, this cultivar was not compliant and

exceeded 0.3% THC. Producers wanting to grow ‘Berry Blossom ’should carefully monitor this cultivar and consider harvesting it early to ensure it remains compliant.

Based on the results of this greenhouse study, stratified sampling might not be a necessary component of regulatory sampling protocols for THC compliancy and the maximizing of desirable cannabinoids. With the exception of CBDa, in the top portion of Fruity Petals, all cannabinoid concentrations in Berry Blossom, Fruity Petals, Lifter Seedless and Santiam cultivars were the same in each stratum throughout both years of this greenhouse trial (Figures 2–5). These results support the current regulatory sampling guidelines as per the 2018 Farm Bill by demonstrating that the top stratum of the plant is representative of the middle and bottom strata of the plant. Future studies should sample throughout the flowering period to see if flower maturity affects the concentration of cannabinoids within the different plant strata.

Conclusions

The concentrations of cannabinoids were similar in the top, middle and bottom portions of the plant, with the exception of CBDa, which was higher in the top portion of the cultivar Fruity Petals. Based on these results, stratified sampling might not be a relevant component of regulatory sampling protocols for THC compliancy. Regardless of the sampling location on the plant, the concentration of cannabinoids will be representative of the entire plant based on the analyses in this study. White CBG and Truckoo were among the higher-yielding cultivars and indicate that the final height and yield of cultivars are positively correlated. Berry Blossom, Bubbatonic, Forbidden Five, Hawaiian Haze, Lifter Seedless, Metolius, Santiam, Sour Kush, Sour Lifter, Super Sour Space Candy, Truckoo, White CBG and White CBG Seedless were the highest yielding cultivars and may be optimal cultivars for Louisiana hemp production.

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