Development of Cannabinoids in Flowers of Industrial Hemp
(*Cannabis sativa* L.): A Pilot Study

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**ABSTRACT:** A field study was performed to investigate the development of cannabinoids in flowers of industrial hemp using three day-length-sensitive and two day-length-neutral varieties. Flower samples were analyzed for cannabinoids on a weekly basis from 2 to 4 weeks postanthesis to plant senescence. Results indicate that total THC, CBD, and CBG significantly increased as flowers matured, reaching the greatest concentration during 6 to 7 weeks postanthesis. After a plateau stage of varied length for different varieties, the peak concentrations declined as plants senesced. Total THC was above the 0.3% threshold from 4 weeks postanthesis to the end of the growing season for day-length-sensitive varieties, but this only occurred during 6 to 7 weeks postanthesis for day-length-neutral varieties. The CBD/THC ratio in flowers dynamically changed during the entire reproductive stage for all of the evaluated varieties. The current study provides vital information for successful cultivation of industrial hemp.

**KEYWORDS:** cannabis sativa, THC, CBD, CBG, CBD/THC ratio

**INTRODUCTION**

Cannabis (*Cannabis Sativa* L.) has been domesticated and cultivated by human beings for over 4000 years as a source of food, fiber, and medicine.

Cannabis is characterized by a distinctive class of terpeno phenolic compounds named cannabinoids. To date, more than 100 cannabinoids have been reported, including tetrahydrocannabinol (THC), cannabidiol (CBD), and cannabigerol (CBG).

Although taxonomically and morphologically similar, cannabis can be distinguished into two unique groups, industrial hemp and marijuana, based on phytochemical profiles. Cannabis plants that contain a total THC concentration of ≤0.3% on a dry weight basis are defined as "industrial hemp" by law in the United States. Total THC is defined by the following formula:

\[
\text{concentration}_{\Delta-9-\text{THC}} + (\text{concentration}_{\Delta-9-\text{THCA}} \\
\times 0.877)
\]

\(\Delta-9\)-Tetrahydrocannabinolic acid (THCA) is the molecular precursor to \(\Delta-9\)-THC and is often more abundant in raw plant material. When the plant material is exposed to heat, light, or alkaline conditions, THCA will convert to \(\Delta-9\)-THC through decarboxylation. The decarboxylated forms are biologically active for medicinal or recreational use, while the acidic precursors do not share the same activity. Determining total THC content allows for the quantification of all potential \(\Delta-9\)-THC. The same rule also applies to other cannabinoids, such as CBD and CBG.

Industrial hemp usually contains nonpsychoactive cannabinoids, such as CBD and CBG as major constituents. Of the few cannabinoids that have been extensively studied, CBD is primarily used for pharmaceutical and medicinal purposes. As a vigorous antioxidative and anti-inflammatory agent, CBD may provide neuroprotection in acute and chronic cases of neurodegeneration.

It is also reported as a promising antiepileptic agent for treatment of intractable pediatric epilepsy, with U.S. Food and Drug Administration (FDA) approval of the first drug comprised of CBD for this indication, Epidiolex, in 2018. Although industrial hemp is traditionally cultivated in Eurasia as a source of fiber and grain, some strains have been selected and bred for high CBD content.

The interest in obtaining CBD from industrial hemp surged since the 2018 Farm Bill in the United States removed industrial hemp from the Controlled Substances Act (CSA) and regulated it as a "normal" crop. For this market, the value of the crop is determined by the cannabinoid content in the flowers. To maximize profit, it would be critical to harvest flowers when these compounds are at or near their maximum concentrations. Another critical factor to consider is the THC concentration. Currently, the interim final rule for industrial hemp cultivation proposed by U.S. Department of Agriculture (USDA) requires floral materials to be tested within 15 days prior to the anticipated harvest date. All plants that exceed 0.3% total THC must be disposed of in accordance with the CSA. Information regarding development of cannabinoids in flowers of industrial hemp will be helpful for determining the date of THC regulatory testing, as well as the date of harvest.
for maximum profit. Unfortunately, few modern, replicated, refereed studies have explored this topic. A few investigators have tracked the evolution of cannabinoids in flowers of marijuana or in the leaves of cannabis plants cultivated in vitro and in greenhouse settings, but none of the cannabis accessions evaluated in these studies were high-CBD industrial hemp varieties that are currently cultivated in the United States. The objective of this study was to investigate the development of cannabinoids in the flowers of industrial hemp using high-CBD varieties under open field conditions.

**MATERIALS AND METHODS**

**General Experimental Procedure.** A field study was performed at University of Florida’s North Florida Research and Education Center at Quincy, FL (30.54°N, 84.60°W) in 2019. The experimental design was a randomized complete block design with four replications. Three day-length-sensitive (DLS) varieties, including Cherry Blossom (CBL), CherryXT1 (CT1), and Cherry Wine (CW) obtained from Green Point Research (Fort Lauderdale, FL), and two day-length-neutral (DLN) varieties, including Pipeline (P) and Maverick (M) obtained from Kayagene, LLC (Salinas, CA), were evaluated. Feminized seeds were sown in the greenhouse into 128-cell seedling trays filled with PRO-MIX HP growth medium (Premier Horticulture Inc, Quakertown, PA) on June 14, 2019. Seedlings were grown under high pressure sodium light (~50,000 lm) at 16-h light and 8-h dark to maintain vegetative growth of the DLS varieties. Irrigation was supplied as needed using overhead irrigation. Uniform seedlings of each variety were transplanted to the field on July 3, 2019. The field setup was plasticulture production with 76 cm-wide and 20 cm-high raised beds. The spacing between rows and between plants within a row was 1.8 and 1.5 m, respectively; therefore, the plant density was ~3600 plants per hectare, which is typical for current outdoor industrial hemp production in the United States. Anthesis, which was determined when 50% plants within a plot showed the first distinguishable pistillate flowers, occurred immediately after transplanting for the DLN varieties (July 3, 2019) and on August 7, 2019 for the DLS varieties when day length was ~13.5 h. Flower samples were taken on a weekly basis from 2 to 4 weeks postanthesis until the plants fully senesced. Flower samples (50–60 g on a fresh weight basis) were taken from the top one-third of 5 uniform plants within a plot, dried in an oven at 55 °C for 72 h, trimmed to remove stems and leaves, and ground into fine powder using a mortar and a pestle for cannabinoid analysis. In addition, 4 uniform plants within each plot were harvested for yield determination when flowers reached full maturity as indicated by the orange/brown color of pistils (August 22 and September 26, 2019) for the first distinguishable pistillate flowers were then ground into fine powder using a small coffee grinder for cannabinoid analysis.

Soils in the test site were a mixture of Tifton loamy fine sand (fine-loamy, kaolinitic, thermic Plinthic Kandiudults) and Norfolk loamy fine sand (fine-loamy, kaolinitic, thermic Typic Kandiudults) and were both well-drained. Maximum and minimum daily air temperature as well as daily rainfall was obtained from Florida Automated Weather Network (FAWN) weather station located within 2 km of the test site and illustrated in Figure 1. Total rainfall from transplanting (July 3, 2019) to harvest of the DLS varieties (September 26, 2019) was 524.5 mm. Irrigation was supplied using drip tapes under the plastic mulch based on a generalized irrigation (September 26, 2019) was 524.5 mm. Irrigation was supplied using a small co

![Figure 1. Maximum (max.) and minimum (min.) daily air temperature and rainfall at North Florida Research and Education Center in Quincy, FL, in 2019.](https://dx.doi.org/10.1021/acs.jafc.0c01211)

with irrigation as needed throughout the season based on an accumulated rate of 56 kg N ha⁻¹. Southern blight and corn earworm were observed in the test site, but neither was prevalent to cause severe damage.

**UPLC-MS/MS Analysis of Samples.** Extraction and analysis of cannabinoid was performed in a laboratory permitted under the University of Florida Institute of Food and Agricultural Sciences (UF/IFAS) Industrial Hemp Pilot Project’s U.S. Drug Enforcement Administration (DEA) registration to conduct chemical analysis of industrial hemp; therefore, there was not unexpected, new, and/or significant hazards or risks associated with the reported work. In brief, ground plant materials were spun with extraction solvent of methanol and water (95/5, v/v) acidified with 0.005% formic acid (plant material/solvent ratio = 1/100, w/v) using a vortex mixer for 5 min, sonicated for 5 min, and centrifuged at 3220 g for 10 min at 4 °C. The supernatant was further diluted by 500-times using the same extraction solvent. Three analytical replicates were used for each sample. Commercially available calibration standards for CBD, THC, CBG, cannabidiolic acid (CBDA), THCA, and cannabinolic acid (CBGA) as well as deuterated internal standards including delta-9-tetrahydrocannabinol-D3 (Δ-9-THC-D3) and 11-nor-9-carboxy-Δ-9-THC-D9 (11-nor-9-COOH-Δ-9-THC-D9) were obtained from Cerilliant (Round Rock, TX) and prepared using the same procedure. Samples were then analyzed using a Waters I-Class Acquity ultraperformance liquid chromatograph equipped with a Waters Xevo TQS Micro triple-quadrupole mass spectrometer (UPLC-MS/MS, Waters Corp, Milford, MA). Positive electrospray ionization (ESI⁺) was used for neutral cannabinoids (e.g., CBD, CBG, and THC) while negative electrospray ionization (ESI⁻) was used for acidic forms (e.g., CBDA, THCA, and CBGA). The analytes were separated on an Acquity BEH C18 column (2.1 × 100 mm, 1.7 μm, Waters Corp, Milford, MA) at a flow rate of 0.35 mL min⁻¹ using a gradient elution for 6 min. The mobile phase included water containing 0.1% formic acid (A) and methanol (B). Initially, 11% A and 89% B was held for 0.5 min, and then solvent B was linearly increased to 100% until 5.5 min followed by a sharp decrease back to the initial conditions for another 0.5 min to re-equilibrate the column. MassLynx 4.2 software (Waters Corp, Milford, MA) was used to quantify each cannabinoid using a 1/x² weighing method with coefficient of determination (r²) > 0.99 for all cannabinoids. This method has been validated in accordance with International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH) guidelines for analytical procedure validation. The recovery percentage was 98.0–114.1% for different cannabinoids at different concentrations, which was considered satisfactory. Total cannabinoid was calculated as the sum of its neutral form plus its acidic form × 0.877 and reported on a dry weight basis.

**Statistical Analysis.** Data were analyzed using mixed model methodology. Variety was treated as a fixed effect, while sampling date was treated as a repeated measure. Block and block × variety
interaction were random effects. To test for differences among varieties, sampling dates, and their interactions, Tukey’s honestly significant difference (HSD) test was performed using SAS 9.4 software at $\alpha = 0.05$ level (SAS Institute Inc., Cary, NC). Figures were composed using SigmaPlot 14.0 software (Systat Software, San Jose, CA).

**RESULTS AND DISCUSSION**

Significant variety × sampling date interaction was observed for all the evaluated cannabinoids ($P < 0.001$). Therefore, development of cannabinoids was plotted separately for each variety.

**CBD.** For all evaluated varieties, total CBD increased as flowers matured and reached the greatest concentration at 6 weeks postanthesis, but the trends differed afterward for different varieties (Figure 2A). For CW and the two DLN varieties, total CBD significantly dropped by 26.5, 25.1, and 17.8%, respectively, 2 weeks following the peak (8 weeks postanthesis). In contrast, a plateau of 4 and 6 weeks existed for CBL and CT1 before total CBD began to decline (Figure 2A), allowing a longer harvest window without loss of profit. Similar patterns also applied to CBDA (Figure 2C). However, the development of CBD in the DLS varieties was different from total CBD and CBDA. As shown in Figure 2B, neutral CBD initially decreased and remained relatively consistent for 5−6 weeks before it began to increase at 10 weeks postanthesis. Since the average concentration of CBDA across the whole growing season was 3- and 1.7-times greater than neutral CBD for the DLS and DLN varieties, it may be assumed that the development pattern of total CBD is primarily determined by CBDA.

The development of neutral CBD and neutral CBD/total CBD ratio synchronized (Figure 2B,D). At 6 weeks postanthesis, when total CBD peaked, neutral CBD accounted for ~17 and 40% of total CBD for the DLS and DLN varieties (Figure 2D).

**THC.** The development of total THC approximated total CBD. Using marijuana cultivated under greenhouse settings, De Backer et al. also found that total THC content increased strongly with plant age and reached the highest level during 5−6 weeks postanthesis. Total THC went above the 0.3% threshold at 4 weeks postanthesis and stayed above the threshold for the rest of the season for all DLS varieties (Figure 3A). Though concentration of $\Delta^9$-THC gradually increased as flowers matured (Figure 3B), its contribution to total THC sharply dropped from >90 to <40% (Figure 3D). The development of THCA was similar to total THC except for a more evident peak at 10 weeks postanthesis (Figure 3C). Both total THC and $\Delta^9$-THC in the DLS varieties was above the threshold at 6−7 weeks postanthesis (Figure 3A,B). Unlike total THC and $\Delta^9$-THC, which immediately dropped following the peak, THCA in the two DLN varieties remained relatively steady until senescence (Figure 3C). In the DLN varieties, $\Delta^9$-THC accounted for ~80% of the total THC at 6 weeks postanthesis, compared to ~31% in the DLS varieties (Figure 3D).

To maximize profit by harvesting plants when total CBD peaked at 6 weeks postanthesis, plants should be sampled and tested at 4 weeks postanthesis according to the interim final rule for industrial hemp cultivation proposed by the USDA. At that time point, however, total THC was 0.339, 0.450, and 0.402% for CBL, CT1, and CW, respectively (Figure 3A). In such a situation, all plants may need to be disposed of and...
growers could lose most of the economic value of their investment. To minimize the possibility of a “false positive” test result, the USDA allows a measurement of uncertainty (i.e., “analytical error”). As long as the 0.3% threshold falls within the range of the measurement of uncertainty, the samples will be considered “having acceptable hemp THC level”. However, each state may have a different interpretation of this rule. Furthermore, sampling at 15-days prior to anticipated harvest does not guarantee a federally compliant crop at harvest as total THC concentration continued to increase from 4 to 6 weeks postanthesis (Figure 3A).

**CBG.** Development of total CBG was slightly different from total CBD and THC with more fluctuations (Figure 4A–C). This could be due to competition for CBGA among cannabinoids. CBGA is the precursor for both THCA and CBDA, as well as cannabichromenic acid (CBCA), so a significant increase in THCA and CBDA during 5–6 weeks postanthesis may have resulted in the decrease in CBGA and thus neutral CBG and total CBG at 5 weeks postanthesis (Figure 4A–C). After reaching the peak at the seventh week postanthesis, total CBG significantly decreased by 43.5, 37.9, and 65.3% within 2 weeks for CBL, CT1, and CW, respectively (Figure 4A). As shown in Figure 4B,C, the development of CBGA resembled total CBG, but neutral CBG remained relatively consistent from maturity to senescence (8–13 weeks postanthesis), indicating that the CBGA synthase may be saturated. Future research on kinetics of key enzymes involved in biosynthesis of cannabinoids is necessary to better understand this topic. The evolution of neutral CBG/total CBG ratio (Figure 4D) was similar to neutral CBD/total CBD ratio (Figure 2D).

**CBD/THC Ratio.** For the DLS varieties, the CBD/THC ratio gradually decreased throughout the entire reproductive growing stage and followed a two-stage linear regression using combined data (Figure 4). The first stage occurred during 2–6 weeks postanthesis and the CBD/THC ratio significantly dropped from 27.7 to 18.0 (\( y = -2.48x + 32.09, r^2 = 0.93 \)). During 7–13 weeks postanthesis, this ratio further declined from 19.4 to 14.9 with a less steep slope (\( y = -0.77x + 24.31, r^2 = 0.63 \)). In contrast, an approximately quadratic pattern was observed for the DLN varieties. The CBD/THC ratio initially decreased by \( \sim 15\% \) as flowers matured, remained relatively steady for 3 weeks (5–7 weeks postanthesis), and then significantly increased to >20 as flowers senesced (Figure 5). At 6 weeks postanthesis when both the CBD and THC peaked, the CBD/THC ratio was \( \sim 18 \) and 14 for the DLS and DLN varieties.

Previous studies reported that the CBD/THC ratio was fairly constant throughout the plant’s entire life cycle, which is inconsistent with results from the current study, indicating development of cannabinoids may follow different patterns for different chemotypes of cannabis and/or different hemp varieties. Cannabis can be assigned to different chemotypes based on \( \log_{10}(\text{CBD}/\text{THC}) \), with values <0.0 being Type I (“drug type”) and >0.0 being Type II/III (“intermediate/fiber type”). It is clear from our data that, based on this criterion, all of the varieties evaluated in the current study were Type III plants during the entire reproductive stage, indicating chemotype of the cannabis plants is fairly stable despite of the ever-changing CBD/THC values during the reproductive growth stage.

**Yield.** CBL tended to produce greater shoot biomass and flower yield than CW and CT1, but the harvest index was...
significantly lower (Table 1). Flower yield of 0.45 kg plant\(^{-1}\) (i.e., 1 lb plant\(^{-1}\)) is generally considered optimal for DLS varieties. Flower yield for the DLS varieties in the present study was >0.7 kg plant\(^{-1}\) despite of the late planting date compared to what is typically used in other states in the southeastern United States (e.g., late May to early June).\(^{12}\) Total THC, CBD, and CBG tended to be greater in CT1 relative to CBL and CW (Table 1). The two DLN varieties did not compete with the DLS varieties for flower yield or CBD content, but they showed greater harvester index than the DLS varieties. Maverick had greater yield than Pipeline, but total cannabinoid content was not significantly different (Table 1).

Total THC content in all evaluated varieties except for Pipeline were above the 0.3% threshold at harvest, which was expected as they were all harvested at 7 weeks postanthesis when both total CBD and THC were at or near their maximum concentrations based on their development curves (Figure 2 and 3). Similar results were observed during the 2019 season in North Carolina, where total THC content in flowers of CBL and CW at full maturity averaged 0.52 and 0.54%.\(^{12}\) However, the same varieties have tested below the THC threshold at harvest in South Carolina (Gilbert Miller, personal communication). The varieties evaluated in the current study have the required certificate of analysis (CoA) for a THC level of \(\leq\)0.3% for parent material, but all went above threshold during reproductive growth. Without uniform testing standards, which have now been proposed by the USDA,\(^2\) it is unclear when and how industrial hemp varieties were sampled and tested to obtain the CoA. There are clearly periods early in the reproductive phase of industrial hemp varieties when THC is below the critical threshold.

It is not uncommon that industrial hemp strains have tested above the THC threshold under different environmental conditions. Of the 227 high-CBD industrial hemp varieties tested in Kentucky, 141 varieties (62%), including CBL and CW are defined as “Prohibited Variety” or “Variety of Concern”, which means that they had at least one THC test result of >0.3%.\(^{13}\) About 61% of the high-CBD varieties that were tested by Cornell University had total THC concentration >0.3%.\(^{14}\) Although it has been reported that biosynthesis of cannabinoids is primarily under genetic control,\(^{15,16}\)

Figure 4. Development of total CBG (A), CBG (B), CBGA (C), and CBG/total CBG ratio (D) in flowers of Cherry Blossom (CBL), Cherry Wine (CBL), Cherry×T1 (CT1), Pipeline (P), and Maverick (M). Data represents means ± SE (\(n = 4\)). Means subscribed with different lowercase letters among sampling dates within each variety indicate significant differences at \(\alpha = 0.05\).

Figure 5. Development of total CBD/total THC ratio in flowers of Cherry Blossom (CBL), Cherry Wine (CBL), Cherry×T1 (CT1), Pipeline (P), and Maverick (M). Data represents means ± SE (\(n = 4\)). Means subscribed with different lowercase letters among sampling dates within each variety indicate significant differences at \(\alpha = 0.05\).
the present study as well as results recently reported by other researchers indicates that industrial hemp accessions cultivated under different environmental conditions are expected to have different cannabinoid content. Impact of the genetic (G), environment (E), and genotype-by-environment (GxE) interaction on fiber quality of industrial hemp has been reported, but few studies have addressed this issue regarding cannabinoid content. Campbell et al. reported that only 1.7 and 6% of the variation in THC and CBD were explained by environment; however, irrigation was the only environmental factors evaluated in this study and fiber/grain varieties instead of high-CBD varieties were used. Future endeavors will be essential to better understand the stability of cannabinoid content in high-CBD varieties across different environments and thus improve the success of breeding programs.

In conclusion, cannabis production under open field conditions solely for the harvest of cannabinoids is a completely new agricultural endeavor in the United States; therefore, both growers and policy makers should be aware that environmental factors may play a role in biosynthesis of cannabinoids. Since development of cannabinoids in different hemp varieties may not follow exactly the same pattern, growers should carefully monitor content of cannabinoids postanthesis to maximize the profit and minimize the risk of above-threshold THC content. The current study indicates that the CBD/THC ratio in flowers dynamically changes during the whole reproductive growth stage. Future studies are necessary to verify these results using a larger population of hemp varieties under different environmental conditions.

### ACKNOWLEDGMENTS

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### REFERENCES

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### Table 1. Shoot Biomass, Flower Yield, and Total Cannabinoid Content in Flowers of Cherry Blossom (CBL), Cherry Wine (CBL), CherryXT1 (CT1), Pipeline (P), and Maverick (M) at Full Maturity

<table>
<thead>
<tr>
<th>variety</th>
<th>shoot biomass (kg plant$^{-1}$)</th>
<th>flower yield (kg plant$^{-1}$)</th>
<th>harvest index</th>
<th>THC (%)</th>
<th>CBD (%)</th>
<th>CBG (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CBL</td>
<td>1.13a</td>
<td>0.86a</td>
<td>0.65c</td>
<td>0.521ab</td>
<td>9.619a</td>
<td>0.197ab</td>
</tr>
<tr>
<td>CW</td>
<td>1.03b</td>
<td>0.74b</td>
<td>0.72b</td>
<td>0.474b</td>
<td>8.927a</td>
<td>0.189b</td>
</tr>
<tr>
<td>CT1</td>
<td>1.10ab</td>
<td>0.76ab</td>
<td>0.71b</td>
<td>0.582a</td>
<td>10.254a</td>
<td>0.260a</td>
</tr>
<tr>
<td>P</td>
<td>0.06d</td>
<td>0.02d</td>
<td>0.85a</td>
<td>0.286c</td>
<td>4.543c</td>
<td>0.200b</td>
</tr>
<tr>
<td>M</td>
<td>0.12c</td>
<td>0.05c</td>
<td>0.83a</td>
<td>0.314c</td>
<td>5.562c</td>
<td>0.219b</td>
</tr>
</tbody>
</table>

Means ($n = 16$) followed with different lowercase letters within each column indicate significant differences among varieties at $\alpha = 0.05$. Day-length-neutral varieties and day-length-sensitive were harvested on August 22 and September 26 in 2019. *Harvest index is calculated as the ratio of flower yield to shoot biomass.