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Cannabidiol industrial hemp growth, biomass, and temporal cannabinoids accumulation under different planting dates in southern Florida

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Abstract

Due to limited information, identifying suitable cannabidiol (CBD) dominant industrial hemp (*Cannabis sativa* L.) cultivars and optimal planting date are critical for hemp commercialization for CBD production in Florida. Two field trials were conducted with seven cultivars planted on three different dates from late April to late June. The cultivars were received from two different suppliers (Kentucky and Colorado) representing their adaptation. Plant growth parameters such as plant height and green canopy cover were recorded at 2–3 weeks intervals during crop growth. Temporal CBD and Δ -9-tetrahydrocannabinol (THC) concentrations in developing flowers were analyzed weekly beginning two weeks after flowering until maturity. The final floral biomass yield was measured at harvest. CBD and THC concentration curve over crop growing season varied among the tested cultivars. High positive correlations between CBD and THC concentrations ($R^2 = 0.88$ – 0.98) were observed in all cultivars. This resulted in similar CBD/THC ratios across all planting conditions. Earlier planting resulted in higher CBD, THC, and floral biomass yield in a few cultivars, with no significant effect in most cultivars. Cultivars adapted in Kentucky performed better compared to Colorado in floral biomass and CBD yield. However, THC concentrations in the cultivars adapted in Kentucky mostly exceeded the acceptable legal threshold (0.3% THC) at harvest. Results indicate cultivar performance was affected more by genetics compared to planting date. Screening more cultivars adapted to regions of similar latitudes and environmental conditions is necessary to identify suitable hemp cultivars for Florida or similar climatic conditions.

Abbreviations: CBD, cannabidiol; CBDA, cannabidiolic acid; THC, Δ -9-tetrahydrocannabinol; THCA, tetrahydrocannabinolic acid; USSC, United States Sugar Corporation.

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1 | INTRODUCTION

Industrial hemp (*Cannabis sativa* L.) is rapidly emerging as an alternative crop because of its diverse use for food, biofuel (from biomass), and industrial and medicinal uses (Adesina et al., 2020; Rehman et al., 2021). Cannabis plants are categorized as industrial hemp or marijuana based on Δ -9-tetrahydrocannabinol (THC) concentration, which is the main psychoactive cannabinoid present in the plant. In the United States, cannabis plants with total THC concentration below 0.3% are defined as industrial hemp, and plants with total THC concentration above 0.3% are defined as marijuana (USDA, 2019). Among more than 100 identified cannabinoids in cannabis, THC and cannabidiol (CBD) are extensively studied because of their psychoactive and medicinal properties, respectively. CBD is widely used for pharmaceutical and medicinal purposes because of its antioxidative, anti-inflammatory, and neuroprotective properties (Devinsky et al., 2014; Hampson et al., 1998). Hence, there has been significant interest in cultivating CBD-dominant hemp cultivars for their medicinal uses. Many studies are being conducted to identify suitable cultivars and cultural practices for CBD-dominant industrial hemp cultivation in different agroclimatic regions including Florida (Anderson et al., 2021; Yang et al., 2020; Zhang et al., 2021). However, in several areas such as Florida, high CBD yielding cultivars are also accompanied with high THC concentrations (Yang et al., 2020). As a result, THC concentration often exceeds the legal limit, which remains a bottleneck for hemp cultivation in Florida. Studies have demonstrated that CBDA synthase (CBDAS) enzyme also produces small quantities of THC as a side product during CBD synthesis (~26 CBD:1 THC ratio) (Linder et al., 2022a; Zirpel et al., 2018), which could explain more THC concentration in high CBD yielding cultivars. Furthermore, most hemp cultivars are photoperiod sensitive short-day plants, and flowering begins when day length (photoperiod) decreases to a certain threshold. Summer day length is relatively shorter in the tropical regions such as Florida as compared to northern latitudes. As a result, hemp cultivars adapted to areas with longer photoperiod tend to flower very early in the season in Florida without accumulating much biomass (Zhang et al., 2021). Because of the early flowering, less biomass accumulation is another major challenge for hemp commercialization in Florida (Zhang et al., 2021). Therefore, it is important to identify suitable CBD-dominant hemp cultivars for the different soil types, climatic, and photoperiod conditions in Florida.

Cannabinoids are produced from the glandular trichomes and accumulated as a resin mostly in the floral parts of the plants (Tanney et al., 2021). It is believed to be produced as a defensive response to biotic and abiotic environmental stresses, suggesting environmental factors could play an important role in cannabinoids production (Pate, 1994). Few

Core Ideas

- Identifying suitable cultivars and optimal planting date is critical for hemp commercialization.
- Cultivar performance was largely affected by genetics compared to planting date.
- Cultivars adapted in Kentucky performed better compared with those adapted in Colorado in southern Florida.
- Suitable cultivars with THC level below the limit remain the major challenge in southern Florida.
- CBD and THC were positively correlated in all the studied cultivars.

studies have previously evaluated the effect of environmental factors on CBD, THC, and other cannabinoids accumulation patterns in the hemp cultivars primarily grown for CBD production. Recently, Toth et al. (2021) studied cannabinoid profiles in CBD-dominant hemp under different biotic and abiotic stresses (flooding, ethephon, powdery mildew, herbicide, and physical wounding). They reported less CBD and THC under herbicide treatments with other stresses having nonsignificant effect on cannabinoid profiles. However, several studies in marijuana, grain, fiber, and dual-purpose industrial hemp cultivars suggested environmental conditions could significantly influence cannabinoids production. Studies have reported higher CBD and THC production when plants were under some environmental stress. Caplan et al. (2019) reported drought stress increased CBD and THC concentrations and yield. Sikora et al. (2011), using fiber and marijuana cultivars, also found that precipitation had a negative impact on CBD and THC accumulation. However, Campbell et al. (2019), using both fiber and dual-purpose (grain and fiber) industrial hemp cultivars, reported less CBD and THC concentration under water-limiting environments. Sikora et al. (2011) reported soil temperature had a positive effect on CBD, but no effect on THC, while air humidity had a positive effect on THC, but no effect on CBD accumulation. Hence, there is ample evidence from previous studies on marijuana, grain, and fiber hemp cultivars to suggest that environmental conditions could significantly influence CBD and THC accumulation patterns. However, similar information is not available in CBD-dominant hemp cultivars.

Identifying environmental conditions that can induce favorable CBD and THC ratios would be helpful for hemp commercialization in many areas including southern Florida. Planting date is an important crop management practice that could largely determine crop growing period, resource availability, and the environmental conditions during crop growth. Earlier planting in Florida could extend the vegetative grow-

TABLE 1 Cannabinoids cultivars and their source, planting, and flowering dates at two locations in southern Florida, 2020.

Cultivar	Source	Planting date ^a			Date of 50% flowering ^b		
		Planting I	Planting II	Planting III	Planting I	Planting II	Planting III
BaOX	Kentucky	5/12	5/29	6/25	6/10	6/15	7/21
Wife	Kentucky	5/12	5/29	6/25	7/20	7/27	8/11
T1	Kentucky	5/12	5/29	6/25	6/8	6/10	7/21
Stout	Kentucky	5/12	5/29	6/25	6/10	6/15	7/28
Abacus	Colorado	4/29	5/29	6/25	5/11	6/15	7/6
Cherry Abacus	Colorado	4/29	5/29	6/25	5/18	6/15	7/10
Early Bird	Colorado	4/29	5/29	6/25	5/8	6/15	6/29

^aAbacus, Cherry Abacus, and Early Bird were not used in first planting at Lykes location.

^bFlowering dates were averaged across both the locations as there were no significant differences in flowering times between two locations.

ing period and consequently result in higher biomass accumulation. Planting date effects on crop growth, physiology, yield, biomass, and seed composition have been documented in several crops (Chiluwal et al., 2018; Mourtzinis et al., 2017; Tsimba et al., 2013). A recent study in industrial hemp also evaluated the planting date effect on its floral biomass (Linder et al., 2022b). However, the effect of planting date on CBD and THC accumulation pattern over crop growing season in CBD-dominant hemp cultivars is still unknown.

This field study was conducted at two locations in Florida using seven CBD-dominant hemp cultivars adapted in Kentucky (BaOX, Wife, T1, and Stout) and Colorado (Abacus, Cherry Abacus, and Early Bird), USA (Table 1) to (1) evaluate the effects of three different planting dates on crop growth, biomass yield, and temporal CBD and THC accumulation pattern, and (2) identify suitable industrial hemp cultivars for CBD production in sandy soil in southern Florida.

We hypothesized that plants at different planting dates will be exposed to different environmental conditions during CBD and THC accumulation. This could potentially alter their concentration and consequently their relative proportion and biomass yield. We also hypothesized that cultivars from Kentucky will adapt and perform better in Florida's environment due to comparatively less variation in their latitude (Florida ~27°N; Kentucky ~38°N; Colorado ~40°N) and climatic conditions (temperature) than Colorado.

2 | MATERIALS AND METHODS

2.1 | Experimental sites

Field trials were conducted in 2020 at two locations in southern Florida: Lykes Bros. farm in Glades County near Basinger (27°12'44.6" N, 81°05'08.9" W), and the United States Sugar Corporation (USSC) farm in Hendry County near Clewiston (26°44'25.3" N, 80°58'56.3" W). The soil in both experimental fields was classified as Immokalee sand (Sandy, siliceous,

hyperthermic Arenic Alaquods) with >97% sand and 0%–2% slopes (USDA-NRCS, 2021). Both experimental sites have a humid subtropical climate with hot and wet conditions during the summer (growing season of hemp). The weather data at both locations including precipitation and minimum and maximum air temperatures are shown in Figure S1.

2.2 | Crop establishment and management

Raised beds approximately 76 cm in width and 20 cm in height were prepared in the field. Two drip tapes were laid out on each bed for irrigation and then the beds were tightly covered with white plastic. Bed-to-bed distance (center to center) was 1.8 m (6 ft). Half of the beds were fumigated with chloropicrin (Tripic 100 at 134 kg per treated ha) to control soil-borne insects and pests, a common practice in high value crop production. Hemp cultivars were planted in greenhouse on April 08, 2020 (cultivars from Colorado) and April 21, 2022 (cultivars from Kentucky) for the first planting; May 08, 2020 for the second planting; and June 04, 2020 for the third planting. Seedling was raised until 3 weeks after emergence. Uniform seedlings (20 per plot) of each cultivar were then transplanted manually into the beds at 1.2 m (4 ft) plant spacing. Nitrogen and K fertilizers were injected at weekly interval through the drip at 0.42 kg N and 0.42 K ha⁻¹ day⁻¹ until 1 month after transplanting. The rates were increased to 1.4 kg N ha⁻¹ day⁻¹ and 1.96 kg K ha⁻¹ day⁻¹ afterward. The total fertilizer amount varied depending on the crop growing period under different planting dates. Urea, magnesium nitrate, and potassium nitrate were source of N and K. The phosphorus level was sufficient in the soil, hence supplemental P was not provided.

2.3 | Experimental design and treatments

The experimental design was a split-split plot randomized complete block design with three replications. Planting date

was the main plot, fumigation as sub-plot, and cultivars as sub-sub plot. There were three planting dates in the study (Table 1). The first planting was done during the last week of April (cultivars from Colorado) or the second week of May (cultivars from Kentucky). Similarly, the second and third planting was done during the last week of May and June, respectively. Four cultivars from Kentucky (BaOX, Wife, T1, and Stout) and three cultivars from Colorado (Abacus, Cherry Abacus, and Early Bird) were planted at both locations (Table 1).

2.4 | Data collection

Plant height and green canopy cover were measured every 2–3 weeks during crop growth from three randomly selected plants in each plot. Plant height was measured from the base of the plant to the tip of the main stem. The percentage of green canopy cover was obtained using the mobile phone application Canopeo, which was developed using Matlab (Mathworks, Inc.), that differentiates pixel in the images based on red-to-green (R/G) and blue-to-green (B/G) color ratios and an excess green index ($2G-R-B$) (Patrignani & Ochsner, 2015). The app was installed on an iPhone 8 Plus (Apple) and pictures of each plant were taken from about 1.5 m vertical distance from the ground right above the canopy. Dates of flowering were recorded when flower initiation occurred in $\geq 50\%$ plants (10 plants) in the plot. Flower initiation was considered when a pair of white stigmas started to come out from the green calyx. Beginning 2 weeks after flowering, destructive sampling of flowers was conducted at weekly intervals to measure temporal CBD and THC concentrations. The top 25-cm portion of two randomly selected plants per plot was clipped and dried at 50°C for 1 day. The dried samples were analyzed using ultra high-performance liquid chromatography following the method developed by ACS Laboratory, Sun City Center, FL, which is a US Drug Enforcement Administration licensed laboratory designated by the Florida Department of Agriculture for cannabinoids analysis in hemp. The samples were analyzed for CBD, cannabidiolic acid (CBDA), THC, tetrahydrocannabinolic acid (THCA). Total CBD and total THC were calculated following the Equations (1) and (2), respectively (Yang et al., 2020).

$$\text{Total CBD} = \text{CBD} + (0.877 \times \text{CBDA}) \quad (1)$$

$$\text{Total THC} = \text{THC} + (0.877 \times \text{THCA}) \quad (2)$$

The plants used for sampling were tagged and excluded from future data collection and sampling. The number of samples and in-season data were different across the treatments due to variation in plant growth and development rate as plants

took less days to mature in later plantings and resulted in less data/sample collection points. Three plants were sampled on each sampling date. At maturity, three plants were harvested from each replication and dried at 50°C for 3 days to estimate the dry floral biomass yield of whole plants. Total CBD and total THC from the top 25-cm portion of the plants at the time of harvest were also analyzed following the same procedure as above.

2.5 | Statistical analysis

Data were analyzed with an ANOVA using Proc Glimmix in SAS version 9.4 (SAS Institute, 2013). The effect of fumigation was nonsignificant in both locations and all three planting dates, indicating no significant soil-borne disease and pest infestation in the experimental fields. Hence, data across both fumigation treatments were combined, analyzed, and presented in the results. Location, planting date, cultivar, and their interactions were considered as fixed factors. Block or replication and its interaction with other fixed factors were considered as random factors. Means were separated by Tukey's honestly significant difference test when treatments and interactions were significant at $p \leq 0.05$.

3 | RESULTS

3.1 | CBD and THC accumulation curve

The cannabinoids accumulation patterns or curve over the crop growing season varied among the tested cultivars (Figure 1). In BaOX (Figure 1a) and T1 (Figure 1c), CBD and THC accumulation increased with crop growth but slightly decreased at the end of the growing season. However, CBD and THC accumulation in Wife increased until the end of crop growth with an exception in the second planting at USSC (Figure 1b). A similar trend was seen in Abacus except in the third planting at USSC (Figure 1e). The opposite trend was observed in Early Bird, in which both CBD and THC concentrations were higher at the early stages then decreased at later stages (Figure 1g). In the Cherry Abacus, large fluctuations in CBD and THC concentrations with crop growth were observed in the first planting, while little to no variation in CBD and THC concentrations was observed in later plantings (Figure 1f). Except for Early Bird and Cherry Abacus (second and third plantings), all of the tested cultivars exceeded the maximum allowable total THC concentration (0.3%) at some stage of their growth period (Figure 1). The THC concentration in Abacus was only slightly higher than the limit and never exceeded 0.4% in any crop growth stages (Figure 1e).

The CBD and THC accumulation curve was similar between the two locations in all cultivars except Stout

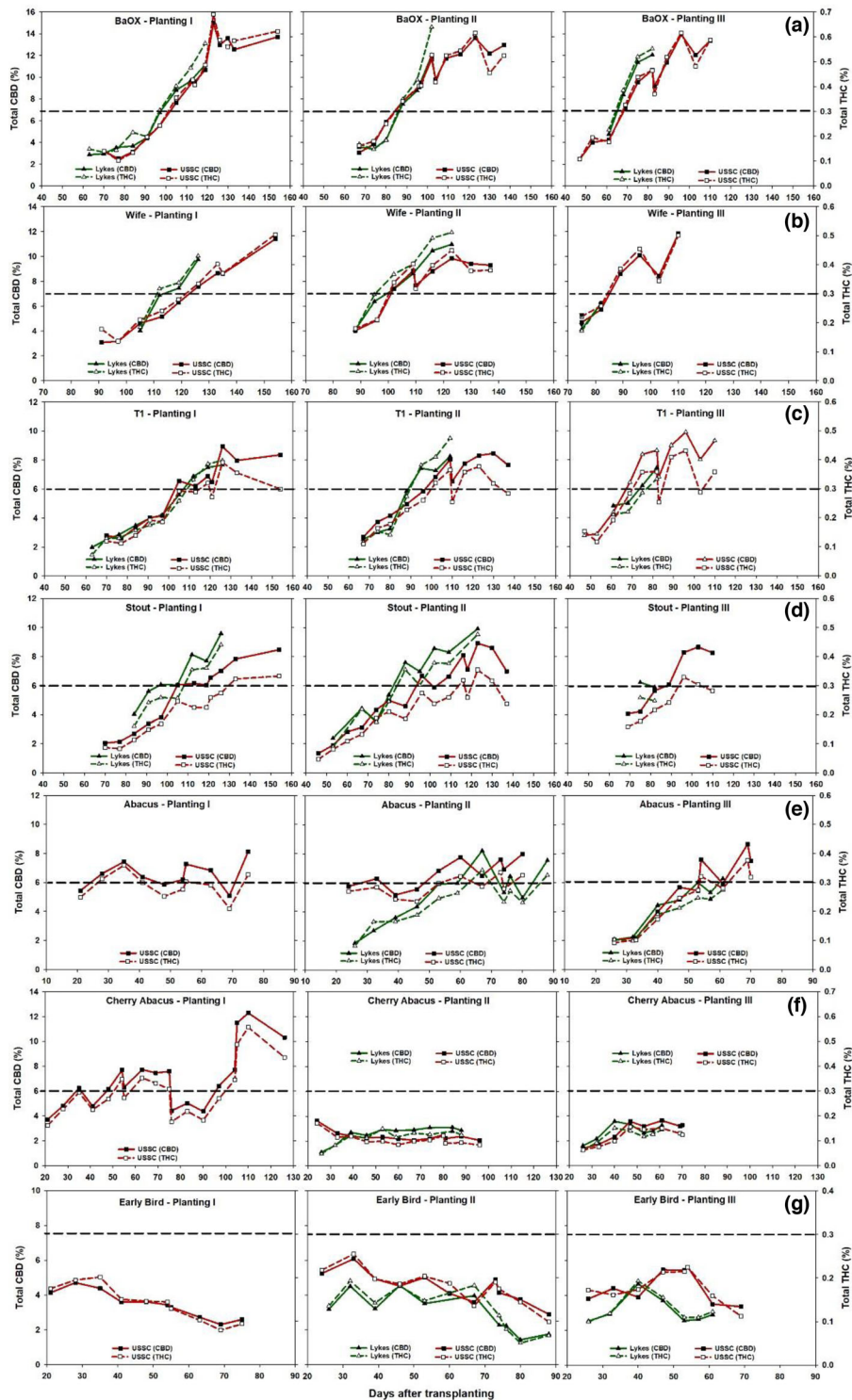


FIGURE 1 Cannabidiol (CBD) and Δ -9-tetrahydrocannabinol (THC) concentration curve of seven industrial hemp cultivars in each planting date and location in southern Florida, 2020. Dashed line is drawn at 0.3% THC, the maximum threshold THC for industrial hemp in United States. Solid line is drawn at 10% CBD as a reference to facilitate visual comparison among the cultivars.

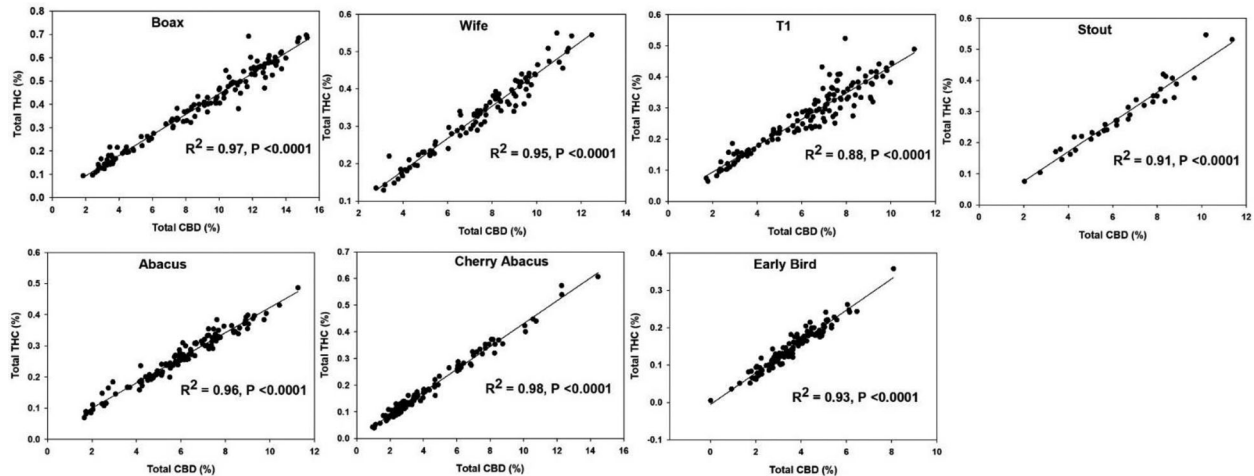


FIGURE 2 Relationship between cannabidiol (CBD) and Δ -9-tetrahydrocannabinol (THC) in seven industrial hemp cultivars across two locations in southern Florida, 2020.

TABLE 2 Analysis of variance for cannabidiol (CBD), Δ -9-tetrahydrocannabinol (THC), CBD/THC, and biomass yield at harvest of seven industrial hemp cultivars at two locations in southern Florida, 2020.

Source	Total CBD	Total THC	CBD/THC	Floral biomass yield
Location	NS	NS	***	—
Planting date	***	***	NS	***
Cultivar	***	***	***	***
Location \times planting date	***	***	NS	—
Location \times cultivar	*	***	***	—
Planting date \times cultivar	***	***	NS	***
Location \times planting date \times cultivar	**	**	NS	—

Abbreviations: NS, not significant.

*, **, and *** represent significance at the 0.05, 0.01, and 0.001 probability level, respectively.

(Figure 1). The CBD and THC concentrations increased until the end of the growing season at the Lykes farm and slightly plateaued or decreased at the USSC farm (Figure 1d). Similarly, CBD and THC accumulation curve was similar among the planting dates in all cultivars except Cherry Abacus, where CBD and THC concentrations were higher in the early than in the later plantings (Figure 1f). The CBD and THC accumulation follows similar patterns in all the cultivars (Figure 1), resulting in strong correlation between total CBD and THC concentrations ($R^2 = 0.88$ – 0.98) in all cultivars (Figure 2).

3.2 | CBD and THC concentrations and their ratio at harvest

The total CBD and THC concentrations at harvest were significantly affected by planting date, cultivar, the planting date by cultivar interaction, and their two-way and three-way inter-

actions with location (Table 2). The main effect of location on total CBD and THC concentrations was nonsignificant (Table 2).

Similarly, no significant differences in CBD and THC concentrations between the two locations were observed in most cultivars and planting date combinations (Figure 3). In the combination, where the location effect was significant, CBD concentration was higher at USSC (Figure 3a–d), while the THC concentration was higher at Lykes (Figure 3h–k) in most cases (Figure 3).

There was a significant planting date main effect on total CBD and THC concentrations (Table 2). However, no significant differences in CBD and THC concentrations among the planting dates were observed in most of the cultivars in both locations (Figure 4). Significant differences in CBD concentrations across the planting dates were observed only in Stout at Lykes (Figure 4d), Cherry Abacus at USSC (Figure 4f), and Wife at Lykes (Figure 4b). Similarly, THC concentration significantly varied across the planting dates in Stout at Lykes

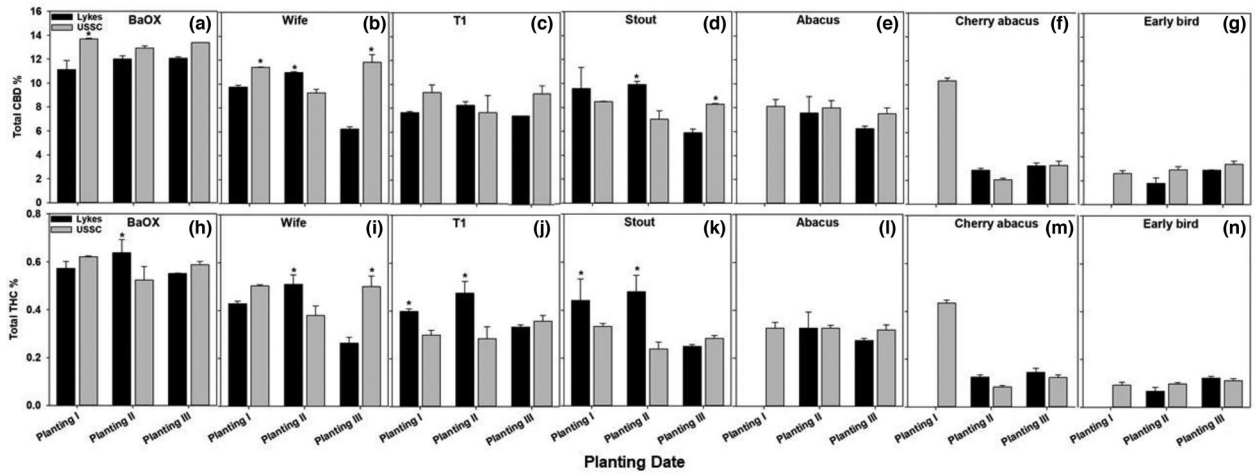


FIGURE 3 Effect of location on total cannabidiol (CBD) and Δ -9-tetrahydrocannabinol (THC) concentration at harvest in seven industrial hemp cultivar within a planting date in southern Florida, 2020. *Significantly higher CBD or THC concentration in the location compared to the other location at 0.05 probability level. Error bars represent standard error of the mean.

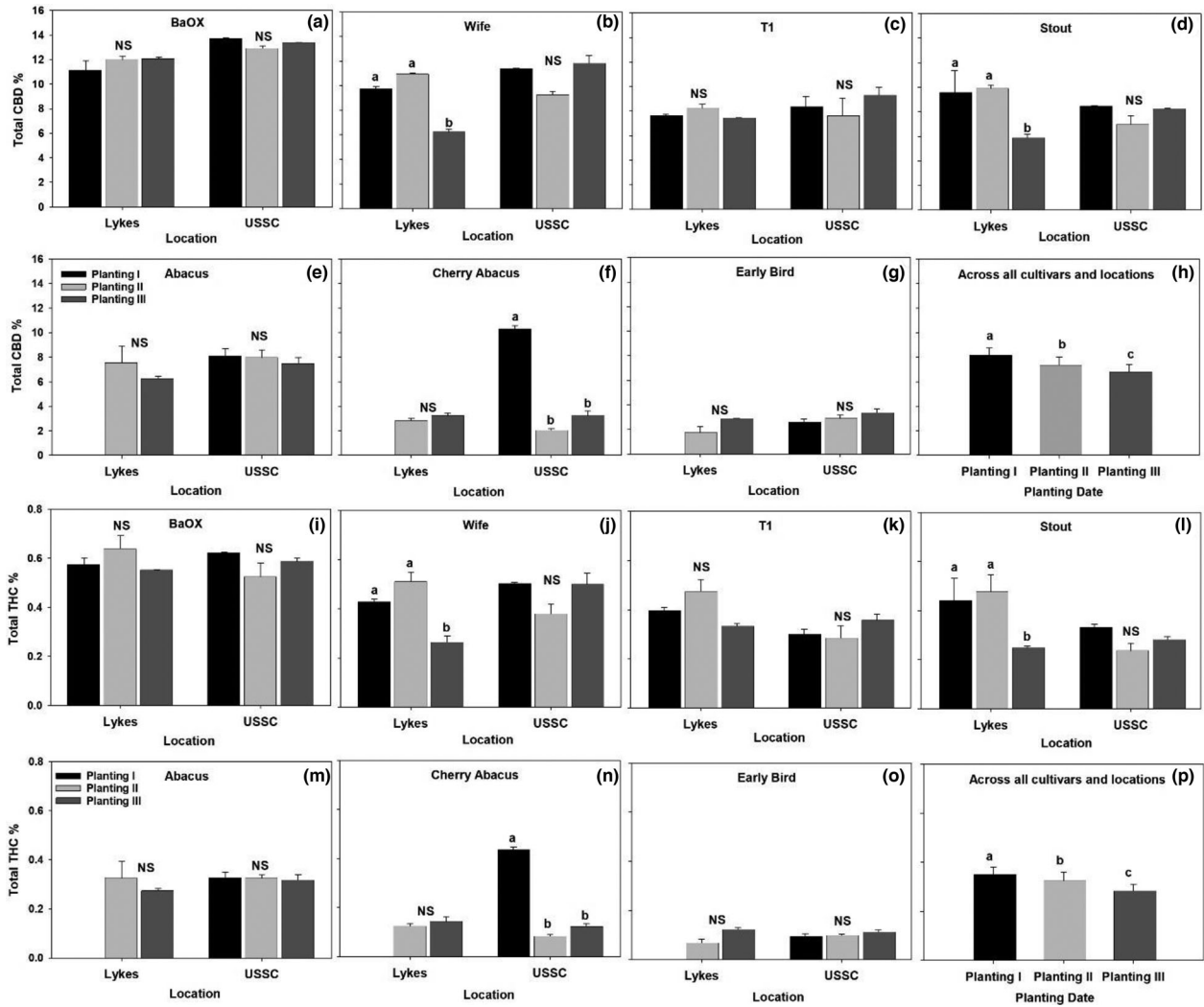


FIGURE 4 Effect of planting dates on total cannabidiol (CBD) and Δ -9-tetrahydrocannabinol (THC) concentrations at harvest in seven industrial hemp cultivars within a location in southern Florida, 2020. NS = not significant, and bars with different letters within a group indicate a significant difference at 0.05 probability level. Error bars represent standard error of the mean.

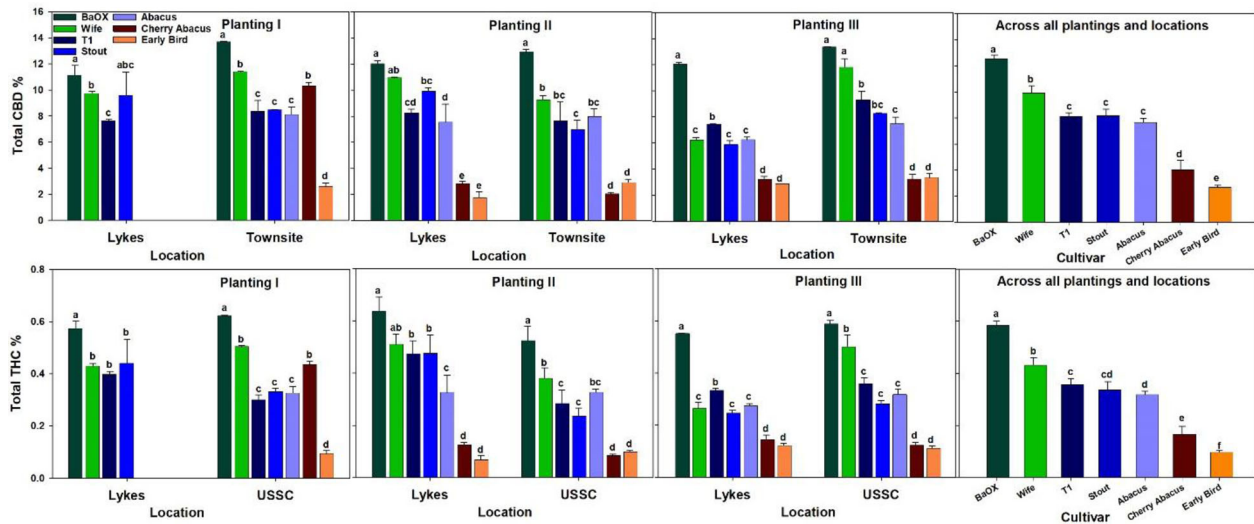


FIGURE 5 Cultivar differences in total cannabidiol (CBD) and Δ -9-tetrahydrocannabinol (THC) concentrations in seven industrial hemp cultivars at harvest in each location in southern Florida, 2020. Bars with different letters within a group indicate significant difference at 0.05 probability level. Error bars represent standard error of the mean.

(Figure 4i), Cherry Abacus at USSC (Figure 4n), and Wife at Lykes (Figure 4j). In the cultivars where the effect was significant, earlier plantings recorded higher CBD and THC concentrations than later plantings (Figure 4). Both CBD and THC concentrations in the first planting were higher than in other plantings in Cherry Abacus and were higher in the first and second plantings than the third planting in Wife and Stout (Figure 4).

Large variations in CBD and THC concentrations were observed among the cultivars within each planting date and location. CBD and THC concentrations in BaOX were higher than other cultivars in the first and second plantings at USSC, and in the third plantings at Lykes (Figure 5). The CBD and THC concentrations in Early Bird and Cherry Abacus were lower than other cultivars within each planting date and location with an exception in the first planting at USSC (Figure 5). Overall, CBD and THC concentrations level was high in BaOX and Wife, intermediate in T1, Stout and Abacus, and low in Cherry Abacus and Early Bird (Figure 5d,h).

The CBD/THC ratio was affected by location, cultivar, and their interaction (Table 2). However, there was no significant effect of planting date on the ratio (Table 2). Each cultivar produced higher CBD/THC ratio at USSC compared to Lykes (Figure 6b). On average, CBD/THC ratios were 21.9 in Lykes and 22.7 in USSC (Figure 6a). In each location (Figure 6b) and average across both locations (Figure 6c), cultivars with higher CBD and THC concentrations had lower ratios while cultivars with lower CBD and THC concentrations had higher ratios.

3.3 | Final floral biomass yield and crop growth parameters at USSC

The hemp floral biomass yield was affected by planting dates, cultivar, and their interaction (Table 2). Planting date had a significant effect in three (BaOX, Stout, and Cherry Abacus) of seven cultivars (Figure 7b), where biomass yield was higher in the first planting compared to the other two. Significant cultivar differences in biomass yield were observed in all three planting dates (Figure 7a). BaOX, Wife, and Stout produced higher biomass than other cultivars in all plantings (Figure 7a,b). On average across all planting, BaOX, Wife, and Stout produced 1388, 1170, and 924 kg ha⁻¹ final dried floral biomass yield, respectively. Those cultivars also recorded higher plant height (Figure 8a–c) and green canopy cover (Figure 8a–c) compared to others.

4 | DISCUSSION

In our study, we examined the effects of planting dates and locations on CBD and THC accumulation curves and growth and floral biomass yield of seven CBD-dominant hemp cultivars obtained from two different sources. We found no significant effect of planting dates on final cannabinoid concentrations and floral biomass yield in majority of the cultivars (four out of seven). Previous studies have reported that environmental factors, especially temperature and moisture stress increased (Caplan et al., 2019; Sikora et al., 2011)

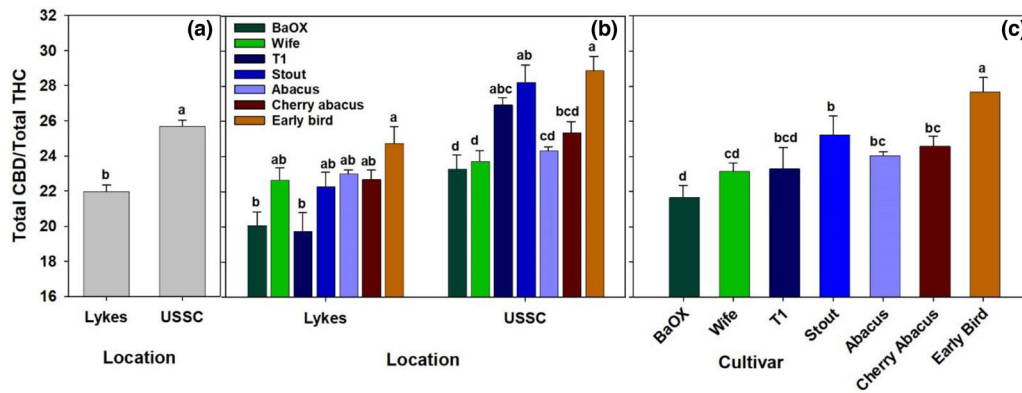


FIGURE 6 Cannabidiol (CBD)/ Δ -9-tetrahydrocannabinol (THC) ratios in seven industrial hemp cultivars at harvest in each southern Florida location across all varieties (a), in each cultivar within a location (b), and in each cultivar across both locations (c), 2020. Bars with different letters within a group indicate significant difference at 0.05 probability level. Error bars represent standard error of the mean.

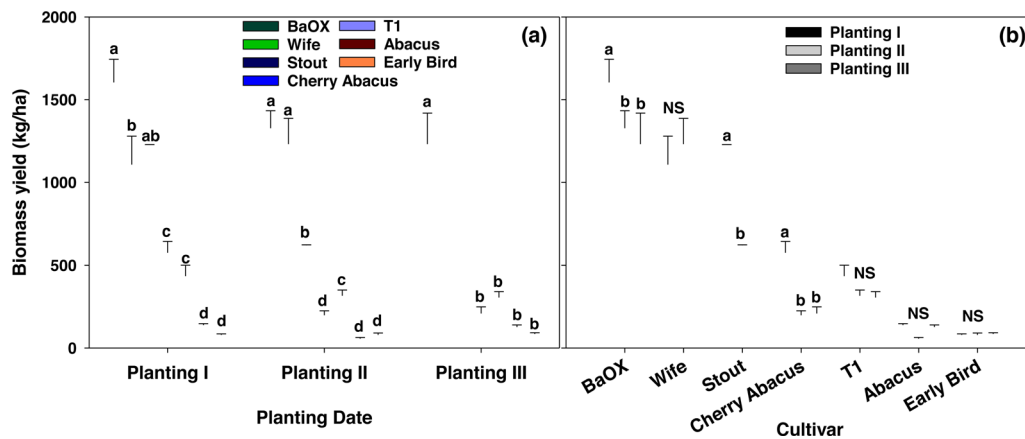


FIGURE 7 Cultivar differences in floral biomass yield in each planting date (a), and effect of planting date on biomass yield in each seven industrial hemp cultivar (b) at the USSC location in southern Florida, 2020. NS indicates not significant, and bars with different letters within a group indicate a significant difference at 0.05 probability level. Error bars represent standard error of the mean.

or decreased (Campbell et al., 2019) CBD and THC concentrations. As expected, there was some difference in the time of flowering among the planting dates for all cultivars (Table 1). As a result, cannabinoid accumulation occurred during different time periods of the growing season for the three different planting dates. However, there was ample precipitation throughout the crop-growing season, hence little supplemental irrigation was required during the entire crop-growing period (Figure S1c). So, plants may not have experienced any water limitations throughout their growth under any planting conditions. Furthermore, there was little variation in temperature among months, especially from June to September (Figure S1b) when most of the CBD and THC accumulation occurred in the cultivars across all planting dates. Hence, lack of drought stress and little variation in temperature during cannabinoid accumulation might have resulted in similar CBD and THC concentrations and ratios among the planting dates in majority of the cultivars.

In a few cultivars, where the planting date effect was significant, earlier planting resulted in higher total CBD and THC concentrations. This suggests earlier planting may offer some advantages compared to later planting dates in those cultivars. However, both CBD and THC concentrations varied similarly in response to planting dates. As a result, the CBD/THC ratio was not affected by planting dates in any of the cultivars (Table 2). There was a high and significant positive correlation between CBD and THC in all cultivars (Figure 2), consistent with the findings from Campbell et al. (2019). The results suggest that manipulating planting dates in hemp may not be useful to alter the relative proportion of CBD and THC concentrations in southern Florida and other locations with similar weather conditions under nonstressed conditions. On the other hand, higher floral biomass under the first planting compared to later plantings in some cultivars suggests earlier planting could be a useful practice to improve floral biomass yield, which is a major challenge in Florida’s environments.

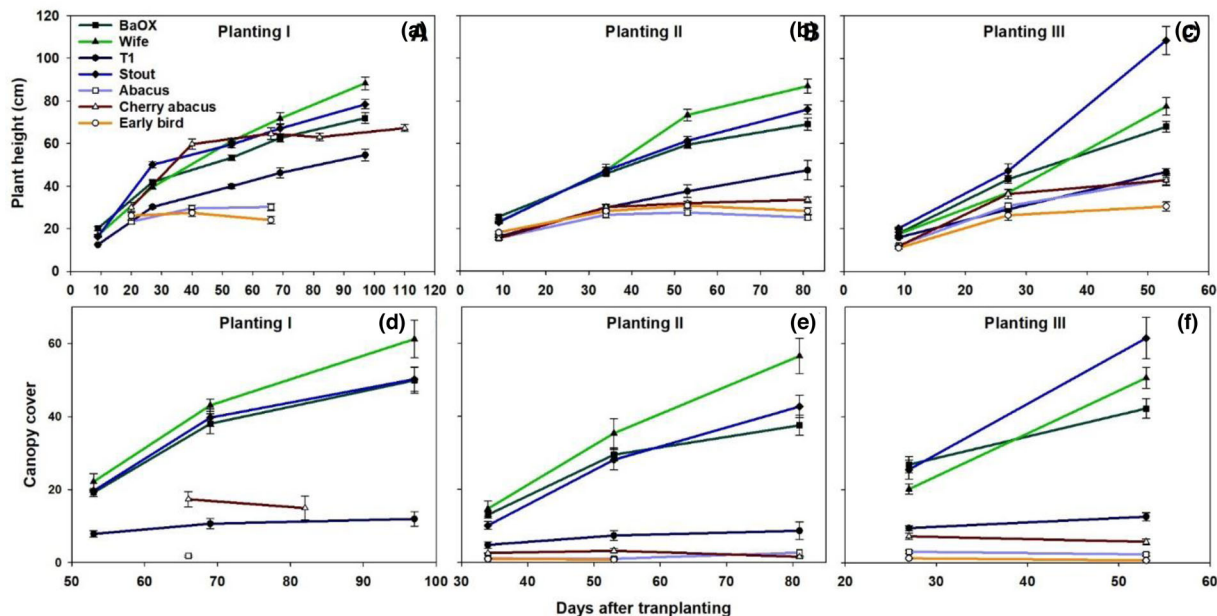


FIGURE 8 Plant height (a–c) and canopy cover (d–f) of seven industrial hemp cultivars within a planting date at USSC location in southern Florida, 2020. Error bars represent standard error of the mean.

Testing cultivars under a wider range of planting dates such as early April (1 month before our first planting date) would be interesting research undertaking in the future.

The variation in CBD and THC accumulation curves among the tested cultivars was consistent with previous studies. Yang et al. (2020) using both day length sensitive and neutral cultivars also reported different CBD and THC accumulation patterns among the cultivars. They found both CBD and THC concentrations increased rapidly until 6 weeks after anthesis. After that, its concentration remained either similar, higher, or lower depending on cultivars. Calzolari et al. (2017) also reported both CBD and THC concentrations increased gradually until maturity in some locations while slightly decreased after reaching their peak in others. De Backer et al. (2012) also found similar results, where THC concentrations increased rapidly after flowering and then either plateaued or slightly dropped at maturity. Similar results have also been documented in other studies where both CBD and THC concentrations increased gradually and then started declining at the end of the growing season (Latta & Eaton, 1975; Pacifico et al., 2008). Linder et al. (2022a) also reported THC concentration increased gradually and then slightly decreased when the plant approached maturity. However, no such decrease in CBD concentration was observed in their study. In this study, CBD and THC accumulation pattern in Early Bird was different from other cultivars and reports from most of the previous studies as their concentration decreased as plant growth progressed (Figure 1). Early Bird flowered earliest (Table 1) and produced lowest cannabinoids (Figure 5) and floral biomass (Figure 7). The results indicate that among the tested culti-

vars, Early Bird is the least suited in Florida's locations, which could be one of the reasons for its unusual CBD and THC concentration curve.

Identifying suitable CBD-dominant cultivars with THC concentration below the 0.3% threshold limit and sufficient floral biomass is the major challenge for hemp commercialization in Florida and elsewhere. A recent study in Quincy, Florida, using both day-length sensitive and neutral cultivars found THC concentrations exceeded the legal limit at 4 weeks and 6–7 weeks after anthesis in day length sensitive and neutral cultivars, respectively (Yang et al., 2020). In this study, only Early Bird and Cherry Abacus (in the second and third planting) cultivars had THC concentrations below the limit at maturity. However, they contained very low CBD concentration and also produced less biomass. Hence, these cultivars will not likely be economically profitable to grow for CBD production in southern Florida. On the other hand, T1, Stout, and Abacus had higher CBD concentrations, but biomass yield was still comparatively low in those cultivars. Furthermore, THC concentration also exceeded its limit during some stages of crop growth making them also not ideal cultivars for cultivation.

In BaOX and Wife, both CBD concentration and biomass yield were highest among the tested cultivars. However, they also exceeded legal THC concentration. Harvesting these cultivars before THC reaches its legal limit could be an alternative. However, floral biomass yield during that time is an important factor that would ultimately determine whether it is economically profitable practice or not. Future studies with temporal CBD and THC concentrations along with floral

biomass accumulation patterns over the crop growing season could provide more insight. Overall, supporting our hypothesis, we found cultivars obtained from Kentucky (BaOX, Wife, T1, and Stout) performed much better in Florida's environments compared to the cultivars obtained from Colorado (Abacus, Cherry Abacus, and Early Bird) in terms of CBD concentration and biomass yield. Hence, screening more cultivars adapted to a similar latitude and environmental conditions as Florida is key to identify and develop suitable CBD-dominant hemp cultivars in Florida's environments.

To our knowledge, this is the first study which attempted to evaluate the effect of different planting dates on temporal cannabinoids accumulation in CBD-dominant industrial hemp. One of the goals of planting date treatments was to provide different environmental conditions, especially temperature and moisture during cannabinoid accumulation. However, abundant rainfall throughout the growing season and little change in temperature resulted in similar growing conditions at different planting dates. We acknowledge that lack of replication over time is the limitation of this research. But further research with methods replicated here in different years and distant locations will better understand the effect of planting date and consequently different environmental conditions on CBD-dominant industrial hemp growth and cannabinoid accumulation. We believe the results of this study serve as a model for other researchers to evaluate similar questions in different agroclimatic regions.

5 | CONCLUSIONS

We found no significant effect of planting date on relative proportion of CBD and THC in any of the tested cultivars. Earlier planting offered a little or no advantage on CBD concentration and floral biomass yield depending on the cultivars. Cultivar performance was largely affected by genetics rather than planting date. Cultivars adapted in Kentucky produced more biomass and cannabinoid yield compared to cultivars adapted in Colorado in Florida's environment. However, THC concentrations in those cultivars also exceeded the legal threshold limit. As a result, identifying suitable CBD-dominant cultivars with THC concentration below the legal threshold remains a major challenge for industrial hemp cultivation in southern Florida. Screening more cultivars from similar climatic conditions would be helpful to identify suitable cultivars for Florida's environments.

AUTHOR CONTRIBUTIONS

Anuj Chiluwal: Data curation; Formal analysis; Investigation; Writing – original draft; Writing – review & editing. **Saroop Sandhu:** Investigation; Project administration; Writing – review & editing. **Hardev Sandhu:** Conceptualization; Funding acquisition; Project administration; Supervision;

Validation; Writing – review & editing. **Mike Irely:** Conceptualization; Funding acquisition; Project administration; Supervision; Validation; Writing – review & editing. **Flint Johns:** Investigation; Project administration; Writing – review & editing. **Richard Sanchez:** Investigation; Project administration; Writing – review & editing.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflict of interest.

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REFERENCES

- Adesina, I., Bhowmik, A., Sharma, H., & Shahbazi, A. (2020). A review on the current state of knowledge of growing conditions, agronomic soil health practices and utilities of hemp in the United States. *Agriculture*, *10*, 129. <https://doi.org/10.3390/agriculture10040129>
- Anderson, S. L., Pearson, B., Kjelgren, R., & Brym, Z. (2021). Response of essential oil hemp (*Cannabis sativa* L.) growth, biomass, and cannabinoid profiles to varying fertigation rates. *PLoS ONE*, *16*, 0252985. <https://doi.org/10.1371/journal.pone.0252985>
- Calzolari, D., Magagnini, G., Lucini, L., Grassi, G., Appendino, G. B., & Amaducci, S. (2017). High added-value compounds from cannabis threshing residues. *Industrial Crops and Products*, *108*, 558–563. <https://doi.org/10.1016/j.indcrop.2017.06.063>
- Campbell, B. J., Berrada, A. F., Hudalla, C., Amaducci, S., & McKay, J. K. (2019). Genotype × environment interactions of industrial hemp cultivars highlight diverse responses to environmental factors. *AgroSystems, Geosciences & Environment*, *2*, 1–11. <https://doi.org/10.2134/age2018.11.0057>
- Caplan, D., Dixon, M., & Zheng, Y. (2019). Increasing inflorescence dry weight and cannabinoid content in medical cannabis using controlled drought stress. *Hortscience*, *54*, 964–969. <https://doi.org/10.21273/HORTSCI13510-18>
- Chiluwal, A., Bheemanahalli, R., Perumal, R., Asebedo, A. R., Bashir, E., Lamsal, A., Sebela, D., Shetty, N. J., & Jagadish, S. V. K. (2018). Integrated aerial and destructive phenotyping differentiates chilling stress tolerance during early seedling growth in sorghum. *Field Crops Research*, *227*, 1–10. <https://doi.org/10.1016/j.fcr.2018.07.011>
- De Backer, B., Maebe, K., Verstraete, A. G., & Charlier, C. (2012). Evolution of the content of THC and other major cannabinoids in drug-type cannabis cuttings and seedlings during growth of plants. *Journal of Forensic Science*, *57*, 918–922. <https://doi.org/10.1111/j.1556-4029.2012.02068.x>

- Devinsky, O., Cilio, M. R., Cross, H., Fernandez-Ruiz, J., French, J., Hill, C., Katz, R., Di Marzo, V., Jutras-Aswad, D., Notcutt, W. G., Martinez-Orgado, J., Robson, P. J., Rohrback, B. G., Thiele, E., Whalley, B., & Friedman, D. (2014). Cannabidiol: Pharmacology and potential therapeutic role in epilepsy and other neuropsychiatric disorders. *Epilepsia*, *55*, 791–802. <https://doi.org/10.1111/epi.12631>
- Hampson, A. J., Grimaldi, M., Axelrod, J., & Wink, D. (1998). Cannabidiol and (-) delta9-tetrahydrocannabinol are neuroprotective antioxidants. *PNAS*, *95*, 8268–8273. <https://doi.org/10.1073/pnas.95.14.8268>
- Latta, R. P., & Eaton, B. J. (1975). Seasonal fluctuations in cannabinoid content of Kansas Marijuana. *Economic Botany*, *29*, 153–163. <https://doi.org/10.1007/BF02863315>
- Linder, E. R., Young, S., Li, X. U., Henriquez Inoa, S., & Suchoff, D. H. (2022a). The effect of harvest date on temporal cannabinoid and biomass production in the floral hemp (*Cannabis sativa* L.) cultivars BaOx and cherry wine. *Horticulturae*, *8*, 959. <https://doi.org/10.3390/horticulturae8100959>
- Linder, E. R., Young, S., Li, X. U., Henriquez Inoa, S., & Suchoff, D. H. (2022b). The effect of transplant date and plant spacing on biomass production for floral hemp (*Cannabis sativa* L.). *Agronomy*, *12*, 1856. <https://doi.org/10.3390/agronomy12081856>
- Mourtzinis, S., Gaspar, A. P., Naeve, S. L., & Conley, S. P. (2017). Planting date, maturity, and temperature effects on soybean seed yield and composition. *Agronomy Journal*, *109*, 2040–2049. <https://doi.org/10.2134/agronj2017.05.0247>
- Pacifico, D., Miselli, F., Carboni, A., Moschella, A., & Mandolino, G. (2008). Time course of cannabinoid accumulation and chemotype development during the growth of *Cannabis sativa* L. *Euphytica*, *160*, 231–240. <https://doi.org/10.1007/s10681-007-9543-y>
- Pate, D. W. (1994). Chemical ecology of Cannabis. *Journal of International Hemp Association*, *1*, 29–37.
- Patrignani, A., & Ochsner, T. E. (2015). Canopeo: A powerful new tool for measuring fractional green canopy cover. *Agronomy Journal*, *107*, 2312–2320. <https://doi.org/10.2134/agronj15.0150>
- Rehman, M., Fahad, S., Du, G., Cheng, X., Yang, Y., Tang, K., Liu, L., Liu, F. -H. U., & Deng, G. (2021). Evaluation of hemp (*Cannabis sativa* L.) as an industrial crop: A review. *Environmental Science and Pollution Research*, *28*, 52832–52843. <https://doi.org/10.1007/s11356-021-16264-5>
- SAS Institute. (2013). *The SAS system for windows*. Release 9.4. SAS Institute, Cary, NC.
- Sikora, V., Berenji, J., & Latković, D. (2011). Influence of agroclimatic conditions on content of main cannabinoids in industrial hemp (*Cannabis sativa* L.). *Genetika-Belgrade*, *43*, 449–456. <https://doi.org/10.2298/GENSR1103449S>
- Tanney, C. A. S., Backer, R., Geitmann, A., & Smith, D. L. (2021). Cannabis glandular trichomes: A cellular metabolite factory. *Frontiers in Plant Science*, *12*, 721986. <https://doi.org/10.3389/fpls.2021.721986>
- Toth, J. A., Smart, L. B., Smart, C. D., Stack, G. M., Carlson, C. H., Philippe, G., & Rose, J. K. C. (2021). Limited effect of environmental stress on cannabinoid profiles in high-cannabidiol hemp (*Cannabis sativa* L.). *GCB Bioenergy*, *13*, 1666–1674. <https://doi.org/10.1111/gcbb.12880>
- Tsimba, R., Edmeades, G. O., Millner, J. P., & Kemp, P. D. (2013). The effect of planting date on maize grain yields and yield components. *Field Crops Research*, *150*, 135–144. <https://doi.org/10.1016/j.fcr.2013.05.028>
- USDA-Agricultural Marketing Service. (2019). Establishment of a domestic hemp production program. *Federal Register*, *84*, 58522–58564.
- USDA-NRCS. (2021). *Custom soil resource report*. USDA, Natural Resources Conservation Service. <https://www.nrcs.usda.gov/wps/portal/nrcs/main/soils/survey/>
- Yang, R., Berthold, E. C., Mccurdy, C. R., Da Silva Benevenuto, S., Brym, Z. T., & Freeman, J. H. (2020). Development of cannabinoids in flowers of industrial hemp (*Cannabis sativa* L.): A pilot study. *Journal of Agricultural and Food Chemistry*, *68*, 6058–6064. <https://doi.org/10.1021/acs.jafc.0c01211>
- Zhang, M., Anderson, S. L., Brym, Z., & Pearson, B. J. (2021). Photoperiodic flowering response of essential oil, grain, and fiber hemp (*Cannabis sativa* L.) cultivars. *BioRxiv*. <https://doi.org/10.1101/2021.05.13.444025>
- Zirpel, B., Kayser, O., & Stehle, F. (2018). Elucidation of structure-function relationship of THCA and CBDA synthase from *cannabis sativa* L. *Journal of Biotechnology*, *284*, 17–26. <https://doi.org/10.1016/j.jbiotec.2018.07.031>

SUPPORTING INFORMATION

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