FLORIDA INTERNATIONAL UNIVERSITY

Miami, Florida

EVALUATION OF HARVESTING TIME FOR INDUSTRIAL HEMP (*CANNABIS SATIVA L.*) PILOT PROJECT VARIETIES GROWN IN SOUTH FLORIDA

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To: Dean Michael R. Heithaus
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This thesis, written by Jordan William Prats, and entitled Evaluation of Harvesting Time for Industrial Hemp (*Cannabis sativa L*,) Pilot Project Varieties Grown in South Florida, having been approved in respect to style and intellectual content, is referred to you for judgment.

We have read this thesis and recommend that it be approved.

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ABSTRACT OF THE THESIS

EVALUATION OF HARVESTING TIME FOR INDUSTRIAL HEMP (*CANNABIS SATIVA L.*) PILOT PROJECT VARIETIES GROWN IN SOUTH FLORIDA

by

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Florida International University, 2020

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A field study was conducted to observe the natural development of cannabinoids in three day-length sensitive industrial hemp varieties Bubba Kush (BK), Emerald Flower (EF), and Golden Sunset (GS). Plants were configured in a randomized block design with 3 replications. Once 50% of the plants within a variety reached reproductive growth, plants were sampled weekly until senescence and analyzed through a HPLC-UV/DAD. The results from the study indicate that all three varieties of industrial hemp tested reached reproductive growth within the first week of transplanting. The transition into reproductive growth occurred early due to a 12-hour day-length at the time and the varieties being daylight sensitive. Data suggests that total CBD and THC reached their peak concentration at 5-7 weeks after anthesis. After seven weeks, the degradation and transformation of secondary metabolites occurred, causing a decrease in cannabinoid concentration. The federal limit of total THC was reached in the BK variety three weeks post-anthesis, while EF and GS reached their limit at 5-7 weeks. Although the fluctuation of cannabinoids was dynamic within each variety, the study provides information and insights on the proper management and cultivation of industrial hemp in South Florida.

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1. INTRODUCTION

Cannabis (*Cannabis sativa L.*) is a plant genus belonging to the Cannabaceae family. Throughout history, this crop has been cultivated to produce food, fiber, building materials, and medicinal products (Small, 2015). Cannabis is characterized by the presence of terpenophenolic compounds known as cannabinoids, which gives the plant its distinct phytochemical characteristics. Although scientists have identified more than a hundred different cannabinoids, *C. sativa* has been selectively bred to primarily produce cannabidiol (CBD), cannabigerol (CBG) and tetrahydrocannabinol (THC) (Andre, Hausman & Guerriero, 2016). While cannabis is predominantly known for its psychoactive compound THC, there are other non-psychoactive cannabinoids such as CBD and CBG that accumulate in the plant. These cannabinoids have generated interest in the commercial industry, leading to further research of cannabis for its secondary metabolite production. Cannabidiol (CBD) is the most studied cannabinoid because of its application in pharmaceutical and medical industries (Jones et al., 2011). The production of CBD dominant industrial hemp grew after the 2018 Farm Bill that legally defined and differentiated industrial hemp from marijuana, excluding it from the Controlled Substance Act (USDA, 2018). Despite being the same species of plant, industrial hemp and marijuana are only differentiated by the concentration of Δ-9-tetrahydrocannabinol (Δ-9-THC) within the plant. Crops containing a total THC concentration ≤0.3% Δ-9-THC were considered "industrial hemp," while plants that exceeded the limit were deemed "marijuana" and identified as federally illegal to grow and cultivate (USDA, 2018).

The observation and regulation of Total THC is imperative for the proper production of industrial hemp. Total THC is calculated by implementing the following formula: ConcentrationΔ-9-THC + (ConcentrationΔ-9-THCA × 0.877) (USDA, 2018). Δ-9-THC is not abundant in plant varieties recommended for industrial hemp production, but its precursor molecule, tetrahydrocannabinolic acid (THCA), is often found in raw plant material. Most cannabinoids accumulate in their acidic form until decarboxylated. The decarboxylation process removes the carboxylic acid from the compound converting THCA into Δ-9-THC (Wang et al., 2016). Determining total THC allows for the accurate quantification of potential psychoactive compounds accumulated in the plant. The same formula for total THC can be utilized to calculate total CBD. Total CBD is calculated by replacing the THC compounds with CBD compounds within the equation: Concentration CBD + (Concentration CBDA × 0.877) (USDA, 2018).

The value of a CBD crop is determined by the total concentration of cannabidiol (CBD) within the plant material. Cannabidiol (CBD) and tetrahydrocannabinol (THC) reach peak concentrations in the plant within the same time frame (between 5-7 weeks after transplanting) (Stack et al., 2021). Observing cannabinoid levels throughout the growth cycle is crucial to maximize cannabinoid harvest while remaining federally compliant. Data collected from plants post-anthesis can help determine a harvesting period for cannabinoid production within farming operations suitable to south Florida climatic conditions.

As a result of industrial hemp's novelty in the U. S market, research on *C.sativa* is limited. The objective of the present research was to observe the development and accumulation of cannabinoids within CBD industrial hemp varieties grown in South

Florida through HPLC analysis. The present study evaluates the change of total CBD and total THC post-anthesis in three different industrial hemp varieties. The varieties tested were selected for their CBD production, and grown in an open field to represent growing conditions farmers would encounter in South Florida.

2. OBJECTIVES

Due to the federally imposed total THC limit and fluctuations in cannabinoid accumulation, a study was conducted to observe the change of total CBD and total THC post-anthesis in three different industrial hemp varieties. The main goal of this research was to monitor the development and accumulation of cannabinoids within CBD industrial hemp varieties grown in South Florida through HPLC analysis. The objectives of this research were:

1. To identify harvesting periods for industrial hemp varieties grown under field conditions.
2. To assess how varying harvesting intervals can be correlated to oil/cannabinoid concentration.
3. To monitor the growth and development of industrial hemp cultivars in South Florida to find suitable varieties adapted to subtropical conditions.

3. LITERATURE REVIEW

3.1 Industrial Hemp Pilot Program

In 2014, the United States government signed an amendment to the Farm Bill, which defined industrial hemp as the *Cannabis sativa L.* with a Δ-9-tetrahydrocannabinol (Δ-9-THC) concentration of 0.3% or lower on a dry weight basis (Lucas, 2014). The

amendment also created the Industrial Hemp Pilot Program throughout the United States, granting universities and other institutions an opportunity to conduct research on cannabis. As a result of prior regulations, there is limited research on the cultivation and production of *C. sativa L* within the U.S. The goal of the Industrial Hemp Pilot Program was to fill this gap by tasking universities to study a) the crop's agro-economic potential, b) management and cultivation practices, and c) identify varieties suitable for legal commercialization. The amendment was enacted to examine an alternative crop for farmers to cultivate, intending to help alleviate loss from other failing crop markets.

In 2018, the Farm Bill, declassified C. *sativa L.* (industrial hemp) from a schedule 1 substance, thus federally legalizing the commercialization of this new agricultural commodity (Conaway, 2018). The bill provided a regulatory framework for how the government would manage compliance in the commercialization process. With the approval from the USDA, state governments were granted the ability to maintain and regulate their local hemp industries.

Florida was granted USDA approval for its industrial hemp production program on April 16th, 2020. The state of Florida is ranked 2nd in national agricultural production, and it is projected to become a leading producer in the industrial hemp market (BDS Analytical, 2019). Additionally, the state's subtropical climate and photoperiod length, allows the potential for 3 to 4 outdoor harvests annually, as opposed to once or twice in other states (Moher, Jones, & Zheng, 2020). Favorable growing conditions and multiple harvesting periods make industrial hemp an appealing and promising crop for cultivation in South Florida. For this reason, the Florida Department of Agriculture and Consumer Services (FDACS) has implemented the University Pilot Program to aid in the regulation

process for cultivation, harvesting, testing, and marketing. Extensive testing on different varieties of *C. sativa L.* is needed to determine the proper cultivation and management practices appropriate for economic and legal viability. By law, only approved varieties from the federal or state government may be used for cultivation and production in the United States. Data generated from pilot program research helps the government in the selection of varieties apt for legal cultivation.

To assist FDACS goals, FIU's Agroecology program created the "Florida International University Industrial Hemp Pilot Project." The primary goal of the pilot project is to research and develop cultivation and management practices for industrial hemp cultivation in Florida. The program also monitors the phytochemical production of industrial hemp to collect data on legal and economic viability of the varieties tested. Lastly, the program aims to identify industrial hemp varieties that are fit for cultivation under South Florida's unique environmental conditions. The data collected from this pilot project will be provided to the federal and state government, to inform about the agronomic properties of industrial hemp and suitable varieties for legal cultivation.

3.2 *Cannabis sativa L.*

Cannabis is described as an herbaceous crop with 3 to 13 palmately compound serrated leaflets, surrounding a flexible main stem composed of branches and nodes (Chandra, Lata, & ElSohly, 2018). As an annual crop, cannabis is primarily dependent on photoperiod to transition through its life cycle (Moher, Jones, & Zheng, 2020). A minimum of 14 hours of light is needed to maintain vegetative growth in most cannabis cultivars (Moher, Jones, & Zheng, 2020). Once the day length shortens, *C. sativa L.*

enters reproductive growth. There are some unique varieties that are not dependent on day length called auto-flowering. The auto-flowering varieties reach reproductive growth at a certain number of days after germination regardless of the photoperiod (Small, 2017).

Cannabis is also a dioecious crop which differentiates between male and female plants at the reproductive stage (Small, 2017). Female plants form pistillate flowers arranged in uniform clusters of bracts covered in glandular trichomes (Dayanandan & Kaufman, 1976). The chemical expression of cannabis mainly occurs within glandular trichomes. These small bulbous resin glands are found primarily in the flowering sections of the crop (Dayanandan & Kaufman, 1976). They act as a phytochemical power plant producing cannabinoids, terpenes, and flavonoids (Small, 2014; Dayanandan & Kaufman, 1976). These secondary metabolites are accumulated in the gland head of the glandular trichome, deterring pests and predators from consuming or damaging the crop (Stack et al., 2021). Male cannabis plants form staminate flowers composed of five petals concealing pollen sacs (Raman et al., 2017). As the pollen is dispersed by wind, it is deposited on the pistils of the female flower, leading to seed production (Raman et al., 2017). Given the lack of trichome abundance, male cannabis does not have the same rate of secondary metabolite production as female plants (Small, 2014). On occasion, cannabis has also been observed as monoecious, having both female and male sex organs within the same plant. Monoecious plants have the ability to self-pollinate and reproduce (Moliterni et al., 2004). In this type, 99% of seeds would result as female plants because of the absence of a Y chromosome (Moliterni et al., 2004).

Over a time, cannabis split into three species, *Cannabis indica*, *Cannabis sativa* and the uncommon *Cannabis ruderalis* (Chandra, Lata, & ElSohly, 2018). Each has their

own distinct chemotypic and phenotypic expressions that differentiate them from one another (Aizpurua-Olaizola et al., 2016; Small, 2017). For example, *Cannabis indica* is described as a short and bushy plant with broad leaves. It is adapted to a temperate climate because of its ability to survive in cold short seasons (Chandra, Lata, & ElSohly, 2018). *Cannabis sativa L.* is characterized as a tall and slender plant with narrow leaves (Chandra, Lata, & ElSohly, 2018). It has been traced to areas with a warmer climate and associated with longer seasons (Chandra, Lata, & ElSohly, 2018). As cannabis was dispersed through different climatic zones, the crop developed adaptations to help alleviate stressors in the environment (Russo, 2007). Adaptations differentiated cannabis into varietals, which are subsections of a given species characterized by a common set of physical or chemical traits expressed (Mechoulam & Hanuš, 2000). These varieties are utilized in modern day hemp production for their numerous commercial applications.

3.3 Cannabis Cultivation

Varieties are grouped within three main facets of production: fiber, grain or oil (phytochemicals). These categories represent the raw material that can be collected from a cannabis crop. The selection of a cannabis variety for cultivation is determined by the intended market a producer intends to fulfill.

The fiber industry utilizes cannabis varieties that grow a large and narrow stem. These plants range anywhere from 7 to 18 feet in height. Producers exploit the bast and hurd fibers from the inner and outer portions of the stem, to be utilized for construction, textile, biofuels, food, etc. (Taura et al, 1996; Shahzad, 2011). Fiber varieties are cultivated at a high plant density, growing from 25 lbs to 40 lbs of seeds per acre, similar

to corn production. Plant spacing between rows is typically 0.3 meters (Conley et al., 2018). The utilization of high-density planting promotes elongation of the stem, producing longer fibers (Conley et al., 2018). Fiber varieties are primarily grown outdoors only under vegetative growth and are harvested before flowering. On average cannabis fiber producers receive approximately $300 per ton of fiber (USDA, 2018).

Cannabis grain varieties are only cultivated for their seeds. Fatty acids, proteins and vitamins make up the interior of these seeds and are utilized in biofuel, cosmetics, and food production (Horner et al., 2019). Grain varieties can grow from 1.2 m to 2.1 m in height and are selected based on branching structure and seed yields (Horner et al., 2019). Internodal branching throughout the plant provides space for reproductive growth (Wortmann & Dweikat, 2020). Ample spacing in-between plants is important. For this reason, cannabis cultivated for grain is planted at a rate of 20 to 30lbs seeding rate per acre (Wortmann & Dweikat, 2020). The seeding rate provides adequate spacing between plants to mitigate shading competition. Grain varieties are grown until 70 to 80% of seeds are mature before harvest. Subsequently, crops are harvested, dried, and separated to be utilized in commercial industries (Horner et al., 2019; Wortmann & Dweikat, 2020). On average cannabis grain farmers grow 1000 lbs of grain per acre, receiving $0.60 to $0.65 per pound of grain.

Varieties cultivated for oil (phytochemicals) are the most prominent and sought after in the cannabis industry. Compounds such as cannabinoids, terpenes, and flavonoids are of economic interest to pharmaceutical, self-care, recreational and manufacturing industries (Cherney & Small, 2016; Andre, Hausman, & Guerriero, 2016). Cannabigerol (CBG), cannabidiol (CBD) and tetrahydrocannabinol (THC) are of special interest

because of their abundance in cannabis plants and high market value (ElSohly & Slade, 2005). Cannabis varieties dedicated to oil production are small to medium size plants (4 to 7ft) that have networks of branches to maximize flowering yield (Harper, et al. 2018). Most of the secondary metabolite accumulation occurs within the flowering sections of the plant (Dayanandan & Kaufman, 1976). Flowers are harvested to extract cannabinoids from their glandular trichomes. To increase maximum yield, crops are exclusively selected as female and never get pollinated. By eliminating seed production, producers can extend the accumulation time of phytochemicals (Small, 2017; Harper et al., 2018). Plant density and population is significantly lower compared to fiber and grain farms when cultivating cannabis for oil. This provides the farmers enough space to pay close attention to flower development and oil/trichome production. Cannabis is harvested 5 to 8 weeks post anthesis to provide proper accumulation time of secondary metabolites (Harper et al., 2018). Once harvested, cannabis is dried and extracted for its essential oils. On an average, a producer of CBD oil crop receives approximately $4.00 per % of total CBD per pound of biomass, and $1,500 per kilo of crude oil (USDA, 2018).

3.4 Cannabis Chemotypes

Cannabis varieties can be further categorized for their intended market by their chemotype. The classification is defined by the synthesis of specific cannabinoids represented as Chemotype I, Chemotype II, Chemotype III, and Chemotype IV. The classification was first referenced in Fetterman et al. (1971) study and modified by Small & Beckstead (1973) to provide an accurate evaluation of the chemical distribution of cannabis.

Chemotype I is known as the “drug” or THC dominant type. Cannabis plants identified as chemotype I have a total THC concentration greater than 0.3% (Fetterman et al, 1971). As a consequence of the compound’s psychoactive properties, THC is the most known cannabinoid within *C. sativa*. In chemotype I plants, CBGA (Cannabigerolic acid) is converted to THCA (tetrahydrocannabinolic-acid) through the tetrahydrocannabinolic-acid synthase enzyme (Fetterman et al, 1971; Shoyama et al., 2012). Since THCA is a precursor for Δ-9-THC, a federally controlled substance, there is limited studies focusing on THC compounds and their isomers. While several claims have been made in support of THC’s medicinal properties, more research is needed to confirm the compound’s viability as a pharmaceutical product. Some states have legalized the recreational use of marijuana, leading to anecdotal evidence on chemotype I plants cultivation practices.

Chemotype II is considered an intermediate between chemotype I and chemotype III plants. In chemotype II plants, THCA and CBDA concentrations are both synthesized through their respective synthase enzymes from CBGA (Small & Beckstead, 1973; Taura, Morimoto, & Shoyama, 1996; Shoyama et al., 2012). Chemotype II reflects *C. sativa’s* original chemical make-up before selective breeding enhanced the production of specific compounds. Chemotype II contains a wide array of cannabinoids that accumulate throughout the plant parts at varying concentrations (Small & Beckstead, 1973; Taura, Morimoto, & Shoyama, 1996; Shoyama et al., 2012;). Although chemotype II is not as commonly cultivated as the other hemp types, they are important for building stable genetics in breeding and cultivation.

Chemotype III is known as the “medicinal” or CBD dominant type, and it is utilized in various pharmaceutical and cosmetic industries (Stott & Guy, 2004).

Cannabidiol (CBD) is the most abundant compound found in industrial hemp (*Cannabis. sativa L.*). It is a non-psychoactive compound that acts as an anti-inflammatory and neuroprotective agent for epilepsy (Jones et al., 2011). According to the World Health Organization (WHO), CBD is nonaddictive as a medication as opposed to THC. In chemotype III, CBGA (Cannabigerolic acid) is converted to CBDA (Cannabidiolic acid) through the Cannabidiolic acid synthase enzyme (Taura, Morimoto, & Shoyama, 1996). Although the usage of CBD in the commercial markets is new, it has already been projected to become a profitable industry within the next five years (USDA, 2018).

Finally, Chemotype IV is known as the CBGA type. CBGA is the non-psychoactive precursor molecule to all phytocannabinoids, including THCA and CBDA (Small & Beckstead, 1973; Morimoto et al., 1998). Cannabigerolic acid is normally found in low concentrations in young plants and Chemotype III varieties (Morimoto et al., 1998). Due to economic interest, selective breeding has produced varieties with high concentrations of CBGA. In these varieties, CBGA is never converted to THCA or CBDA because of a lack of synthase enzymes needed to make the compounds (Morimoto et al., 1998). Research on CBGA is lacking, but preliminary studies have shown that it contains medicinal and therapeutic properties (Citti et al., 2018). As industrial hemp cultivation continues to expand, new cannabinoids and chemotypes could create economic interest.

3.5 Harvesting Period for Industrial Hemp

Harvesting time of a crop is one of the most important aspects of agricultural production. Determining the correct time to harvest industrial hemp is imperative for

compliance of federally mandated total THC limits (Conaway, 2018). Monitoring phytocannabinoid accumulation during industrial hemp cultivation allows farmers to maximize yields before surpassing the total THC limits (Andre, Hausman, & Guerriero, 2016; Conaway, 2018). The value of a CBD crop is primarily determined by the total cannabidiol (CBD) concentration within the plant material (Mark et al., 2020). Additionally, industrial hemp produces other compounds of economic value such as flavonoids, phenolics, and terpenes that are utilized in other commercial industries (Mark et al., 2020). As mentioned before, phytochemical accumulation reaches peak concentration in between 5 to 8 weeks post-anthesis (Pacifico et al., 2007). As a result, total THC has the potential to surpass the legal limit of 0.3% before CBD can reach a profitable range (Arnall, Bushong, & Lofton, 2019). Moreover, the concentration of cannabinoids differs because of growth conditions and genetic differences among varieties (Mechoulam & Hanuš, 2000; Moher, Jones, & Zheng, 2020). Thus, making the cultivation and management of industrial hemp's agro-economic viability difficult to balance. Varieties utilized for industrial hemp oil production must be bred for uniformity and stability, with low levels of total THC throughout the growth period to provide the crop sufficient time to reach a profitable CBD concentration (Andre, Hausman, & Guerriero, 2016).

In a study conducted by the Swiss Federal Research Station in 1998, they observed factors that influenced the yield and quality of hemp (*Cannabis sativa L.)* essential oil. They tested four different cannabis varieties/strains from reproductive growth to senescence in a greenhouse and outdoor setting and monitored their oil concentration (Meier & Mediavilla, 1998). Although Meier and Mediavilla's study did

not not calculate cannabinoid concentrations of the varieties tested, it displays the quantity of terpenoids throughout the accumulation cycle. The study demonstrated that the production of secondary metabolites within cannabis have a rising accumulation rate for the first 5 to 8 weeks post-anthesis (Meier & Mediavilla, 1998). After that period, concentration of secondary metabolites begins to decrease as a result of the transformation and loss of compounds cannabinoids, terpenes, and flavonoids (Meier & Mediavilla, 1998).

Similar results were observed in an experiment conducted by the University of Florida. Yang et al. (2020) monitored the development of cannabinoids in industrial hemp flowers. Yang et al. (2020) conducted a field study at their North Florida Research and Education Center in Quincy, FL, monitoring five varieties for their cannabinoid production. Varieties tested were germinated and grown briefly in a greenhouse. Once rooted, plants were transplanted to an outdoor raised bed system (Yang et al., 2020). Flower samples were taken on a weekly basis from reproductive growth to senescence (Yang et al., 2020). The data showed that the varieties tested reached a peak concentration of cannabinoids 5 to 6 weeks post-anthesis (Yang et al., 2020). Despite differences within environmental conditions, genetics and management, several studies concur that cannabis follows the same pattern of secondary metabolite accumulation (Yang et al., 2020; Stack et al., 2021). Even though some varieties had a total THC concentration above the legal limit four weeks post-anthesis, other varieties were able to reach peak CBD concentration before surpassing the legal limit (Yang et al., 2020).

Lastly, fluctuation of cannabinoid levels as a function of plant variety can be observed in the study conducted by Stack et.al (2021). Stack et al. (2021) monitored

season-long characterization of high cannabinoid hemp, revealing variation in cannabinoid accumulation, flowering time, and disease resistance. Thirty different varieties were grown and harvested at varying intervals to determine the change of cannabinoids over time (Stack et al., 2021). Varieties were grouped into chemotypes categorized by cannabinoids accumulation to correlate change between each type. Chemotypes I and II exceeded the THC threshold before CBD could reach its peak concentration at 6-8 weeks (Stack et al., 2021). Chemotype III and IV had CBD:THC ratios from 20:1 - 30:1 depending on the cultivar (Stack et al., 2021). Most plants from chemotype III and IV did not surpass the legal limit, making them the most viable for commercial cultivation (Stack et al., 2021). Despite the data showing a positive result, fluctuation is present within the varieties (Stack et al., 2021). Meaning more breeding practices are necessary to build stable genetic varieties.

4. METHODOLOGY

GS3	GS3	GS3	GS3	GS3	GS3
EF3	EF3	EF3	EF3	EF3	EF3
BK3	BK3	BK3	BK3	BK3	BK3
GS2	GS2	GS2	GS2	GS2	GS2
EF2	EF2	EF2	EF2	EF2	EF2
BK2	BK2	BK2	BK2	BK2	BK2
GS1	GS1	GS1	GS1	GS1	GS1
EF1	EF1	EF1	EF1	EF1	EF1
BK1	BK1	BK1	BK1	BK1	BK1

B. Random Block Design Arrangement

Figure 1 *Arrangement of Experimental plots at FIU Industrial Hemp Pilot Project.* a) Image of experiment. experimental plots located in Homestead, FL. b) Random block design arrangement of variety in field.

4.1 Site Description

The study was conducted at the Green Point Research Project Partner Farm (Fig 1), located in 22400 SW 266th St., Homestead, FL 33031; Latitude: 25.5180260 Longitude: -80.5567780. This 8,100 sq meter farm is home to FIU Industrial Hemp Pilot Project.

4.2 Experimental Design

A field study was conducted on three varieties of industrial hemp (540 plants), configured in a randomized block design with three replications. Three day-length-sensitive varieties were chosen. Seedlings of Bubba Kush (BK), Emerald Flower (EF) and Golden Sunset (GS) varieties obtained from Green Point Research and evaluated. Varieties were seeded in 72-cell liners containing potting mix for germination. Seedlings

were grown under high pressure sodium halide bulbs for a minimum of 14 hours daily to maintain vegetative growth. Overhead irrigation was applied to seedlings to provide sufficient moisture to growing media. Once seedlings were established, liners were removed from the greenhouse to allow gradual light penetration from 35% to 55%. This aided in the hardening process for future outdoor transition. Three weeks after germination, seedlings were transplanted to the field site on September 11th, 2020.

The field site consisted of a plasticulture raised beds system 30 inches wide and 8 inches high. Spacing between plants was 1 ft, with a row spacing of 40 inches wide. Irrigation in the field was done by a piping system within the raised beds. Soil at the field site was made up of a sandy loam, which is representative of farming operations in South Florida. Prior to planting, the Helena Professional Slow-release fertilizer NPK (6-12-12) was applied at a rate of 800 pounds per acre to the field.

4.3 Field Sampling and Data Collection

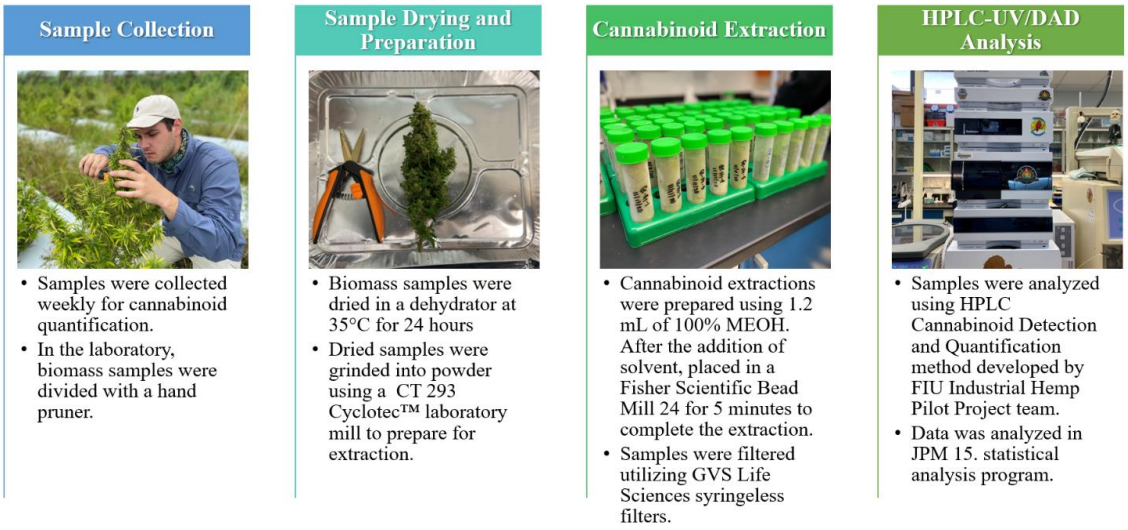
4.3.1 Leaf Chlorophyll Concentration, Plant Height and Dry Biomass Weight

To monitor the growth and development of the varieties cultivated, leaf chlorophyll concentrations were measured bi-weekly utilizing a Soil-Plant Analyses Development (SPAD) 502 Plus Chlorophyll Meter. Thirty-six plants were randomly sampled per variety per week utilizing Freidenreich et al. (2019) SPAD sampling method. The “SPAD meter” was utilized to determine chlorophyll concentrations and monitor the health of the plant. Height was also measured weekly from transplants across all varieties. During the last week of the experiment, biomass samples were collected to record biomass weight. Stems and leaves were separated and then dried in a Thermo

Scientific Precision Economy Oven at 65ºC for 72 hours. Subsequently, dry samples were weighed and recorded the weights.

4.3.2 Flower Collection and Sample Preparation for HPLC analysis

Figure 2 *Sample Collection and Preparation methods for HPLC-UV/DAD analysis.*



Once 50% of plants within a variety displayed their first pistillate flowers (post-anthesis), samples of the variety were collected weekly. All three varieties of industrial hemp tested in the field study reached reproductive growth within the first week of transplanting. This is the result of a 12-hour day-length and the varieties being daylight sensitive. Samples were collected utilizing a modified version of FDACS sampling protocols (FDACS, 2019). In short, ten random samples were collected from each variety every week. Samples were taken from the primary stem, measuring 8 to 10 inches from the tip of the stem. These samples included all flowers, leaves, stalks, and stems present in this section of the stem. Size and weight of the sample varied depending on the variety and harvest date, but this expected under current FDACS guidelines.

The biomass was dried in a forced air dehydrator at 35°C for 24 hours to avoid decarboxylation of cannabinoids from heat. Before drying, samples were divided and placed in aluminum trays to accelerate the drying process. After samples were fully dried, they were transferred to a CT 293 Cyclotec™ laboratory mill. Samples were randomly paired together to create 5 composite samples per variety. Samples were ground to a fine powder and passed through a 1.0 mm sieve, to produce a uniform particle size. Sieve size was selected to avoid resin accumulation within the mill. After composite plant samples were ground, they were transferred to 20 mL tubes for storage until extraction.

4.4 HPLC-UV/DAD Analysis

4.4.1 Cannabinoid Extraction

A standard protocol (Standard Operating Procedure) for cannabinoid extraction was developed by the Florida International University Industrial Hemp Pilot Project research team. During the extraction process, 30mg of each dried plant material was separated and placed into a 2ml screw-cap tube. Four ceramic beads were added to each tube, along with 1.2ml of 100% methanol (MEOH). Subsequently, tubes were transferred and placed in a Fisher Scientific Bead Mill 24 for five minutes to complete the extraction. After the extraction is finished, the vials are placed in a Thermo Scientific Sorvall Legend Micro 21 Centrifuge for one minute to separate cell debris from supernatant. Finally, supernatant is transferred to 0.2-micron PTFE GVS Life Sciences syringeless filters to prepare for HPLC analysis.

4.4.2 HPLC Analysis

Calibration standards for cannabidiolic acid (CBDA), cannabidiol (CBD), tetrahydrocannabinolic acid (THCA), Delta-9-tetrahydrocannabinol (Δ-9-THC) were obtained from Cayman Chemical and Restek. HPLC-UV/DAD analyses were carried out with an Agilent Technologies (Waldbronn, Germany) modular model 1100 system, with a vacuum degasser, quaternary pump, a temperature controlled auto-sampler, and a diode array detector (UV/DAD). The chromatographs were observed and recorded through the Agilent Open Lab - ChemStation LC and LC-MS systems software. The mobile system consisted of (B) H2O/ Acetonitrile 5%/ Formic Acid/ Ammonium Formate, (C) Acetonitrile/ Formic Acid, and (D) Acetonitrile. Solvent (D) (100% ACN) was used to flush and stabilize the system between samples. The final gradient elution was: 0.00- 6.00 min 25% B and 75% C, 6.00- 8.00 min 33% B and 67% C; and 8.00- 13.00 min 100% D; utilizing a Restek Raptor C18 Column 2.7µl (1.50 x 4.6mm) (Belforte, PA). The flowrate was a constant 1.7mL/min with an injection volume of 5 µl. Post-run equilibration time was 7 min in-between samples. The chromatograms were acquired at 200 nm to 240 nm. Concentrations of all cannabinoids evaluated were calculated utilizing integrated peak area in combination with standard calibration curves. Finally, total THC and total CBD were calculated using the equations below.

$$\text{Total THC concentration} = (\text{THCA}*0.877) + \Delta\text{-9-THC}$$

Equation 1 *Total THC Equation for accumulation.*

$$\text{Total CBD concentration} = (\text{CBDA}*0.877) + \text{CBD}$$

Equation 2 Total CBD Equation for accumulation.

4.3 Statistical Analysis

Data analysis was performed in JPM 15. One-way and two-way ANOVAs were done to detect any differences or changes in total THC and total CBD accumulation over time and by variety. Additionally, a multiple linear regression analysis was conducted to determine the effects of time and variety interactions had on total THC and total CBD concentrations. Finally, p-value was considered statistically significant at 0.05.

5. RESULTS

5.1 Physical Parameters

5.1.1 Leaf Chlorophyll Concentrations

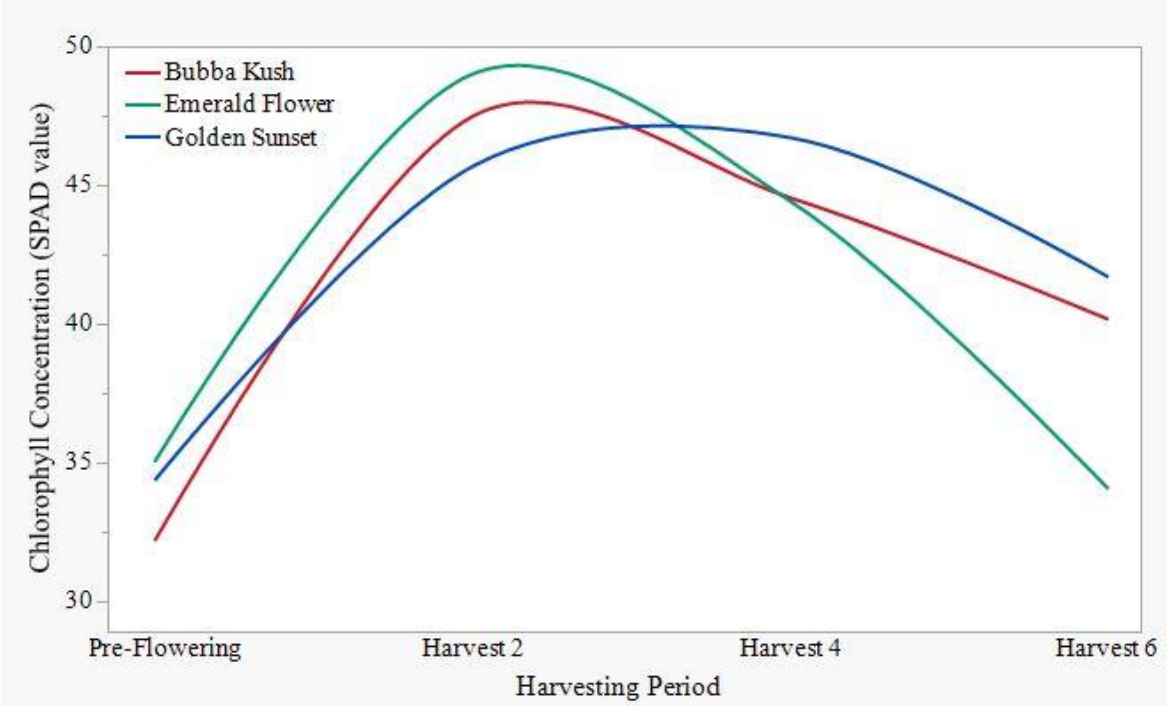


Figure 3 *Mean Leaf Chlorophyll concentrations across time and variety*. Lines represent mean leaf chlorophyll concentrations expressed as SPAD values. Varieties are distinguished by colors a) Bubba Kush= Red, b) Emerald Flower= Green and c) Golden Sunset= Blue. For this graph harvesting periods represent a two-week time interval. One-way ANOVAs were conducted to determine the effects of time and variety on chlorophyll concentrations. P-value < 0.05 was considered significant.

Changes in chlorophyll concentrations were monitored through a Soil-Plant Analyses Development (SPAD) 502 Plus Chlorophyll Meter every two weeks. Chlorophyll concentrations varied significantly ($p < 0.05$) over time for all varieties (Fig 3). Generally, all varieties experienced the highest chlorophyll levels during the month of October in Harvest 2 (47.39 ± 7.8 SPAD) and Harvest 4 (45.23 ± 4.8 SPAD) (Fig 3). The lowest chlorophyll concentrations were recorded during Pre-flowering (33.86 ± 16.2 SPAD) and Harvest 6 (38.65 ± 9.6 SPAD) which correspond with transplanting and the start of senescing, respectively (Fig 3). Moreover, total chlorophyll concentrations were not significantly ($p > 0.05$) different across varieties evaluated.

5.1.2 Plant Height and Biomass

Plant height was monitored weekly. The greatest stem heights recorded were observed in the Golden Sunset variety (55 ± 12.1 cm), followed by the Bubba Kush variety (53.12 ± 11.2 cm). The Emerald Flower variety had significantly lower ($p < 0.05$) stem heights (43.17 ± 8.4 cm), compared to Bubba Kush and Golden Sunset. Differences in overall biomass production was also recorded for each variety. High variation in total dry weight was observed throughout the sampled population. The Golden Sunset variety had significantly heavier ($p < 0.05$) plants (30.2 ± 12.1 g) than Bubba Kush (10 ± 8 g) and Emerald Flower (8 ± 6.8 g) varieties.

5.1.3 Mortality

Mortality rates for all varieties were recorded. Plants with >65% dried leaves and flowers were considered dead and not sampled for chemical analysis. Throughout the experiment, 40.4% of all cannabis crops planted perished. The highest mortality rate was

observed in Emerald Flower populations (51.7%), followed by Bubba Kush (45.6%). The Golden Sunset variety had the lowest mortality rate, losing only 29.4% of individuals planted. Mortality rates may have been influenced by, weather, temperature, pests, or disease.

5.2 HPLC-UV/DAD Analysis for Cannabinoid Quantification in Industrial Hemp Varieties

5.2.1 Total THC % and Total CBD % Accumulation in Varieties tested

Cannabinoid accumulation was monitored for all varieties across harvesting periods through HPLC-UV/DAD. Total THC% and Total CBD % values are expressed as "milligrams per gram of dried plant material". Cannabinoid concentrations fluctuate across time and variety. In general, varieties experienced their highest total THC accumulation in between Harvest 5 and Harvest 7 surpassing the current 0.3% THC federal limit (Fig 4). Total THC levels started to decrease towards Harvest 8 (0.334 ± 0.33 % total THC) (Fig 4). Similarly, total CBD levels for each variety reach their peak during Harvest 6 and Harvest 7. Highest concentration of CBD detected was during Harvest 7 at 3.957 ± .71 % total CBD (Fig 5). Total CBD began to decrease in Harvest 8 (3.484 ± 0.77 % total CBD), which corresponded with a decrease in total THC across all the varieties.

The varieties evaluated had different mean total THC and total CBD levels (Fig 6, Fig 7). Emerald Flower (0.1979 ± 0.12 % total THC) and Golden Sunset (0.190 ± 0.13 % total THC) varieties had comparable levels of total THC within the sampling population (Fig 6). Bubba Kush plants had significantly higher concentration of total THC at 0.411 ±

.50 %, which was twice the amount detected in Emerald Flower and Golden Sunset varieties (Fig 6).

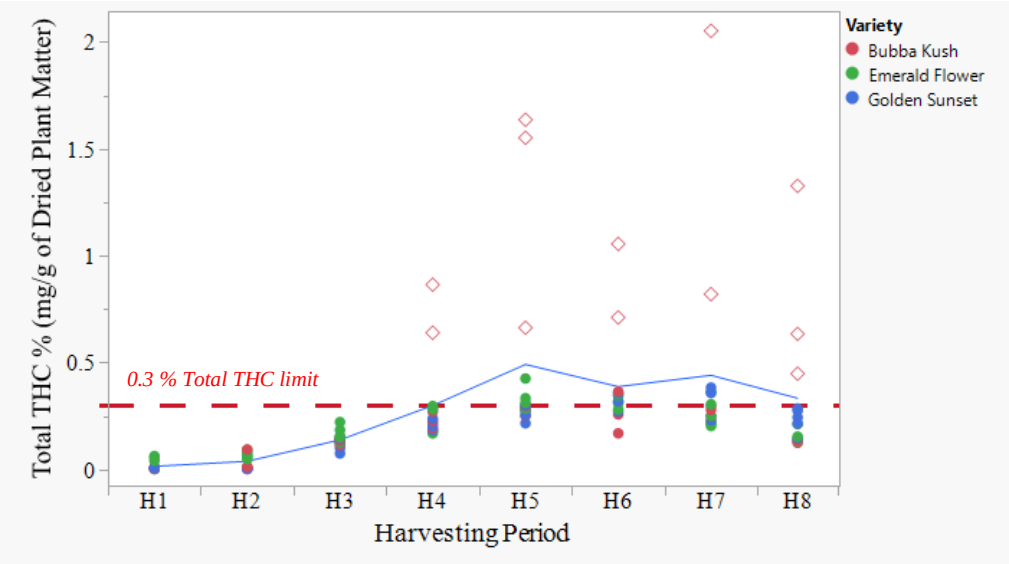


Figure 4. *Mean Total THC concentrations across all harvest periods*. Points in the graph represent the total THC concentration for individual samples. Varieties are distinguished by colors a) Bubba Kush= Red, b) Emerald Flower= Green and c) Golden Sunset= Blue. Each harvesting period represents one week transpired. Red- dotted line denotes 0.3% total THC federal limit. Due to the high variation in total THC concentrations within the Bubba Kush population, outliers were identified with a red diamond. One-way ANOVAs were conducted to determine the effects of time in total THC concentration. P-value < 0.05 was considered significant.

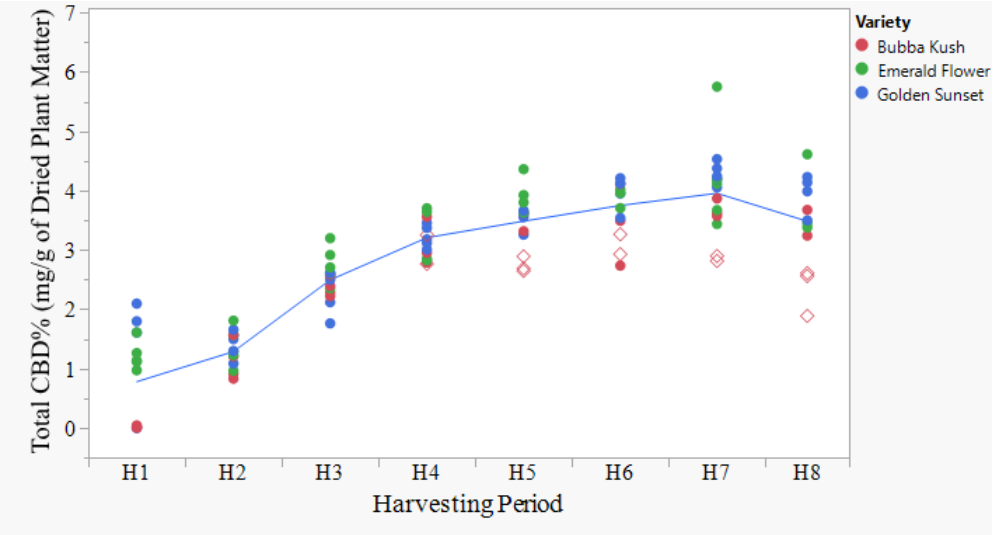


Figure 5 *Figure Mean Total CBD concentrations across all harvest periods. Points* in the graph represents the total CBD concentration for individual samples. Varieties are distinguished by colors a) Bubba Kush= Red, b) Emerald Flower= Green and c) Golden Sunset= Blue. Each harvesting period represents one week transpired. Individuals with high concentration of total THC in Bubba Kush varieties were identified with a red diamond to showcase relationship in cannabinoid production One-way ANOVAs were conducted to determine the effects of time in total THC concentration. P-value < 0.05 was considered significant.

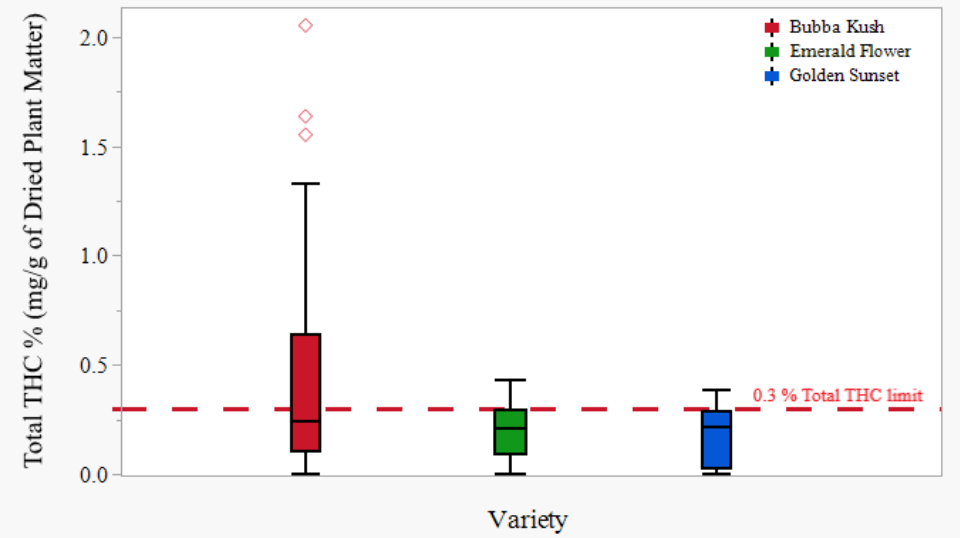


Figure 6 *Mean Total THC concentrations across all variety types*. Boxplots demonstrate mean total THC % concentration. Varieties are distinguished by colors a) Bubba Kush= Red, b) Emerald Flower= Green and c) Golden Sunset= Blue. Error bars one standard deviation from the mean. Red- dotted line denotes 0.3% total THC federal limit. Outliers in Bubba Kush variety were identified with a red diamond. One-way ANOVAs were conducted to determine the effects of variety in total THC concentration. P-value < 0.05 was considered significant.

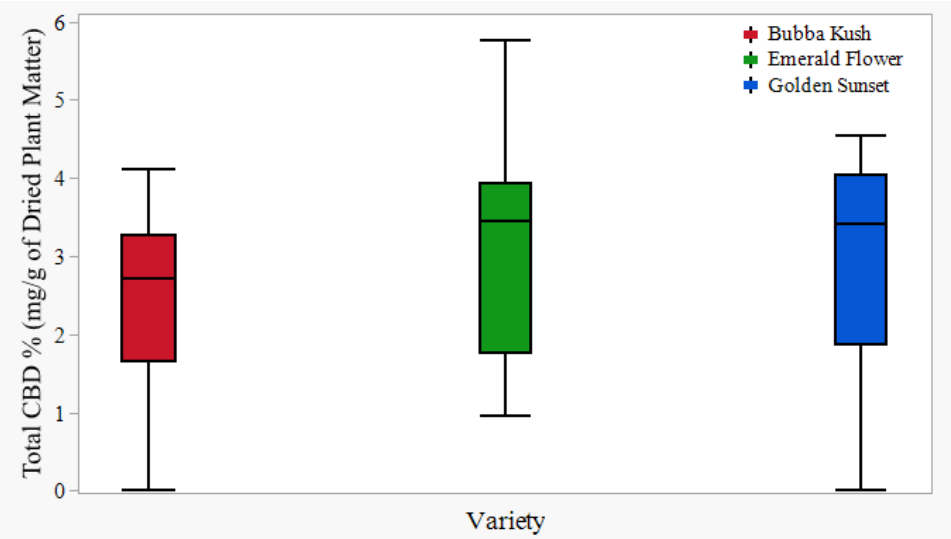


Figure 7 Mean Total CBD concentrations across all variety types. Boxplots demonstrate mean total THC % concentration. Varieties are distinguished by colors a) Bubba Kush= Red, b) Emerald Flower= Green and c) Golden Sunset= Blue. Error bars are one standard deviation from the mean. One-way ANOVAs were conducted to determine the effects of variety in total CBD concentration. P-value < 0.05 was considered significant.

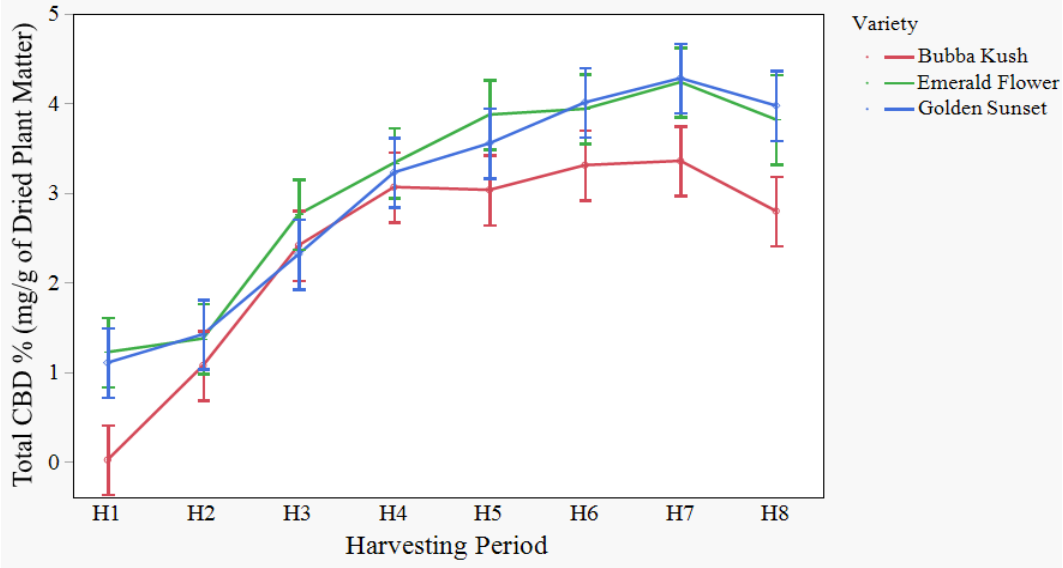


Figure 8 *Least Squares Means Plot demonstrating the effect of the interactions between harvest period and variety in Total THC concentrations with the factors transposed.* Line represents mean total THC concentration. Varieties are distinguished by colors a) Bubba Kush= Red, b) Emerald Flower= Green and c) Golden Sunset= Blue. Error bars represent confident limits of our regression model. P-value < 0.05 was considered significant. A regression analysis was conducted to determine the effects of time and variety interactions had on total THC concentrations. P-value < 0.05 was considered significant.

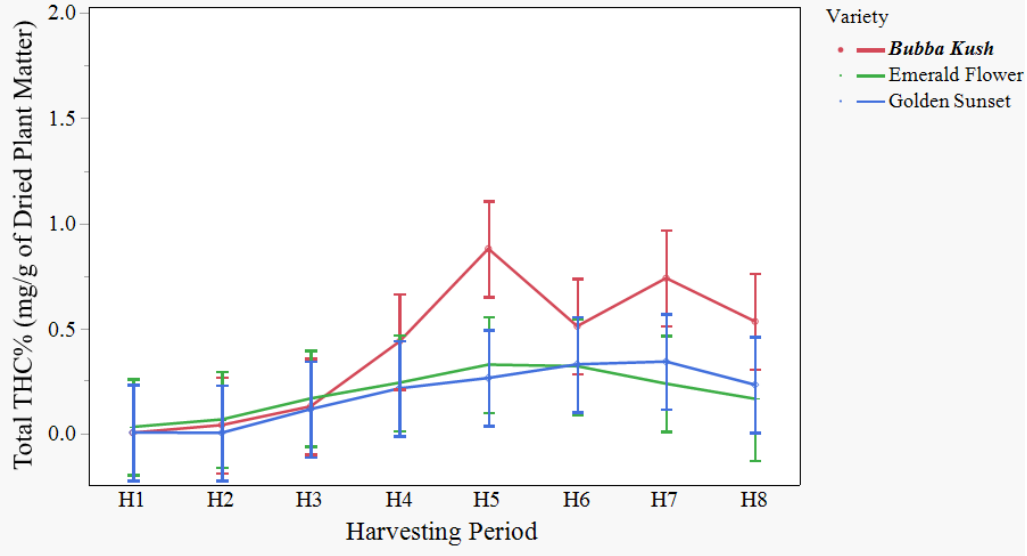


Figure 9 *Least Squares Means Plot demonstrating the effect of the interactions between harvest period and variety in Total CBD concentrations with the factors transposed.* Line represents mean total CBD concentration. Varieties are distinguished by colors a) Bubba Kush= Red, b) Emerald Flower= Green and c) Golden Sunset= Blue. Error bars represent confident limits of our regression model. P-value < 0.05 was considered significant. A regression analysis was conducted to determine the effects of time and variety interactions had on total CBD concentrations. P-value < 0.05 was considered significant.

A multiple linear regression analysis was preformed utilizing a fit model to test the effects of variety and harvesting period on total THC and total CBD concentrations. The regression analysis demonstrated that 38% of the variation of THC% across the experimental samples was explained by the combined effect of variety and harvesting period ($R^2=0.39$, $F=7.64$, $P>0.0001$). In total CBD, 89% of the variation in total CBD% was observed ($R^2=0.89$, $F=84$, $P<0.0001$*). Meaning that variety and harvesting period were significant predictors of total THC and total CBD in an industrial hemp crop. However, interactions between variety and harvesting time were not significant. The interaction between variety and harvesting period was not significant ($p > 0.05$) because of the similar pattern of accumulation of total THC and total CBD all varieties experienced (Fig 8, Fig 9). For this reason the model with the variety-harvesting period interaction was excluded of the analysis. In the following sections, total THC and total CBD are discussed by individual variety.

5.2.2 Bubba Kush

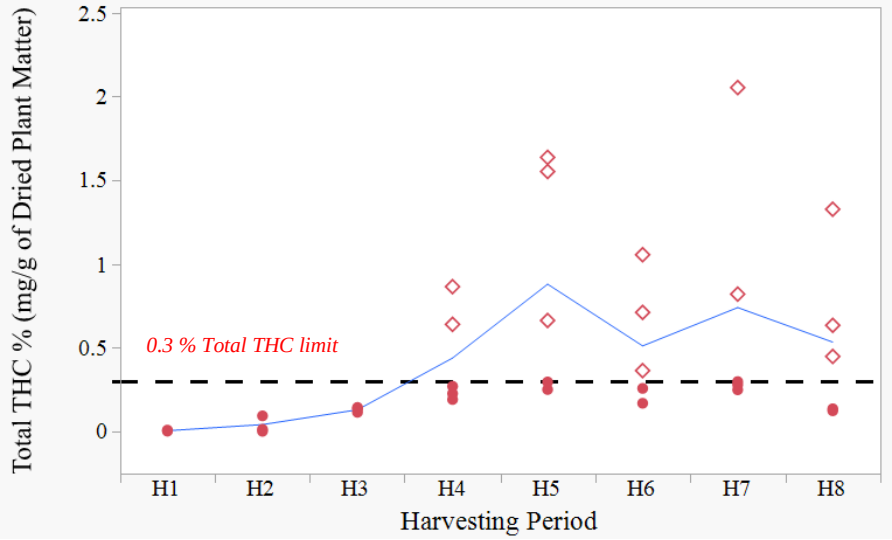


Figure 10 *Bubba Kush total THC concentration change over time.* Points in the graph represents total THC concentration for an individual sample. This was done to demonstrate the relationship between high total THC and low total CBD accumulation. A one-way ANOVAs were conducted to determine the effects of time in total THC concentration. P-value < 0.05 was considered significant.

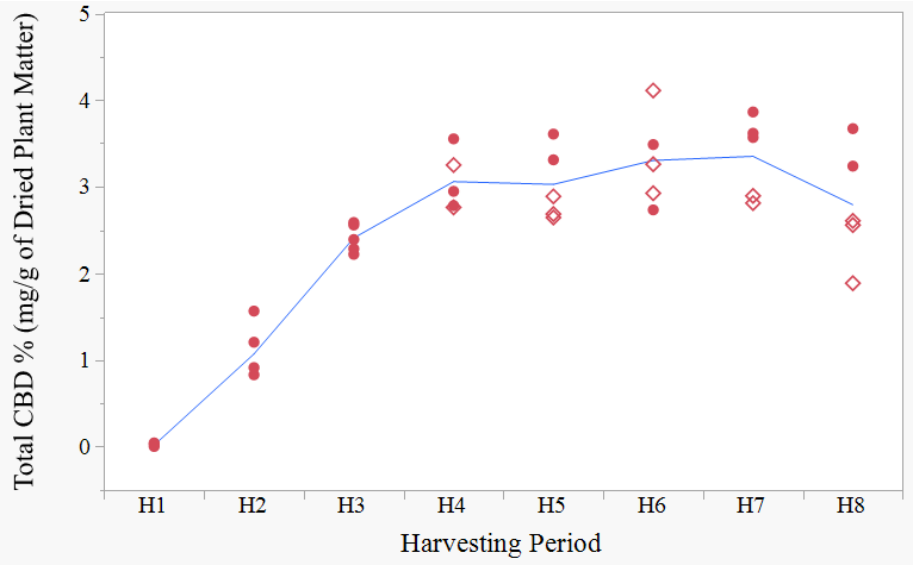


Figure 11 *Bubba Kush total CBD concentration change over time.* Points in the graph represents total THC concentration for an individual sample. Samples over the 0.3% total THC federal limit are identified with a red diamond. This was done to demonstrate the relationship between high total THC and low total CBD accumulation. A one-way ANOVAs were conducted to determine the effects of time in total CBD concentration. P-value < 0.05 was considered significant.

Individuals from the Bubba Kush population had a high variability in total THC concentration. THC federal threshold was surpassed at Harvest 4 (0.439 ± 0.30 % total THC) (Fig 10). Peak accumulation of total THC occurred during Harvest 5 (0.880 ± .67 % total THC). Although total THC concentrations began decreasing by Harvest 6 (0.512 ± 0.37 % total THC), a sharp increase was detected in Harvest 7 (0.745 ± 0.77 % total THC) (Fig 10). The increase in mean total THC concentration in Harvest 7 was influenced by a single outlier with a concentration of 2.05% total THC. Finally, total THC levels decreased in Harvest 8 (0.535 ± 0.49 total THC) (Fig 10). Total CBD accumulation was at its highest in Harvest 7 (3.355 ± 0.47 % total CBD) (Fig 11). The highest concentrations of total CBD in Bubba Kush occurred 3 weeks after the total THC federal limit was exceeded. To reach compliance, Bubba Kush must be harvested during Harvest 3 (2.410 ± 0.16 total CBD), which had significantly lower ($p < 0.0001$) total CBD concentrations in comparison to Harvest 7 (Fig 10, Fig 11). Furthermore, individuals with high total THC concentrations also experienced decreased production of total CBD. This relationship can be observed by comparing the diamond-shaped data points in the total THC % and total CBD % Bubba Kush graphs (Fig 10, Fig 11).

5.2.3 Emerald Flower

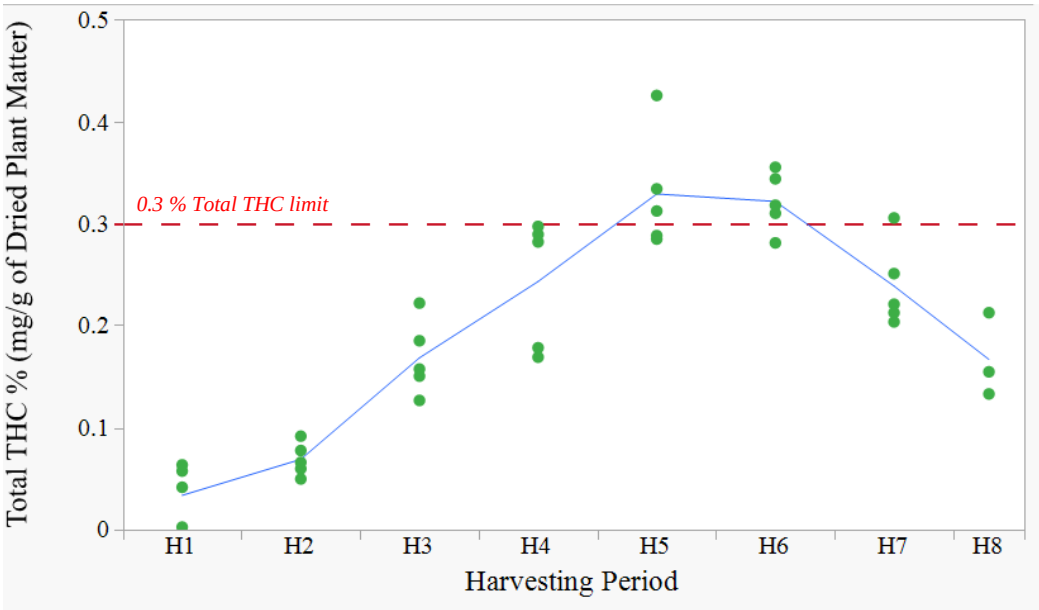


Figure 12 *Figure Emerald Flower total THC concentration change over time.* Points in the graph represents total THC concentration for an individual sample. Red- dotted line denotes 0.3% total THC federal limit. A one-way ANOVAs were conducted to determine the effects of time in total THC concentration. P-value < 0.05 was considered significant.

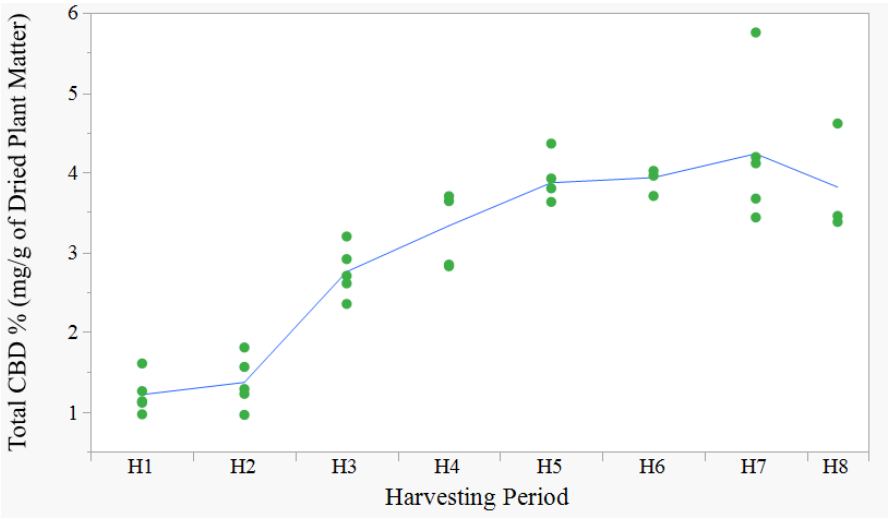


Figure 13 *Emerald Flower total CBD concentration change over time.* Points in the graph represents total THC concentration for an individual sample. A one-way ANOVAs were conducted to determine the effects of time in total CBD concentration. P-value < 0.05 was considered significant.

Emerald Flower plants experienced maximum concentration of total THC in Harvest 5, surpassing the total THC federal limit at 0.393 ± 0.06 % total THC. Two weeks after (Harvest 7), total THC levels decreased below the federal limit to 0.239 ± 0.041% total THC (Fig 12). Total CBD concentrations for the Emerald Flower crop reached peak concentration at Harvest 7 (4.235 ± 0.91 % total CBD) (Fig 13). Inadvertently, maximum concentrations of total CBD occurred as the total THC levels decreased below the federal limit once again. Therefore, Emerald Flower plants can be harvested during maximum CBD accumulation.

5.2.4 Golden Sunset

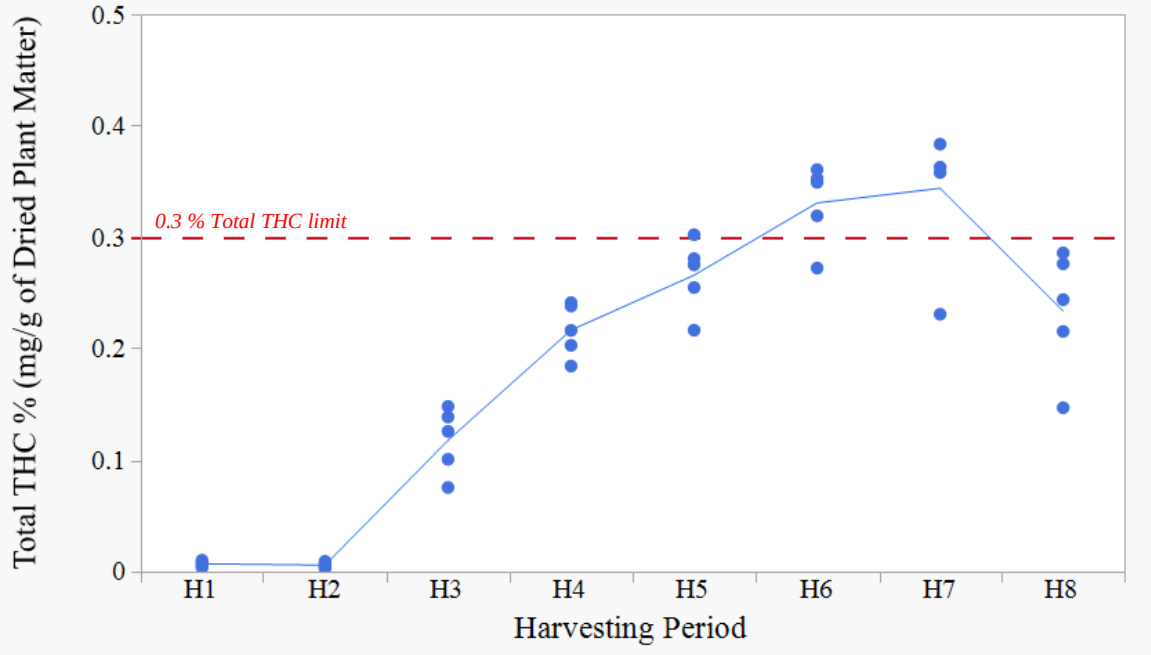


Figure 14 *Golden Sunset total THC concentration change over time*. Points in the graph represents total THC concentration for an individual sample. Red- dotted line denotes 0.3% total THC federal limit. A one-way ANOVAs were conducted to determine the effects of time in total THC concentration. P-value < 0.05 was considered significant.

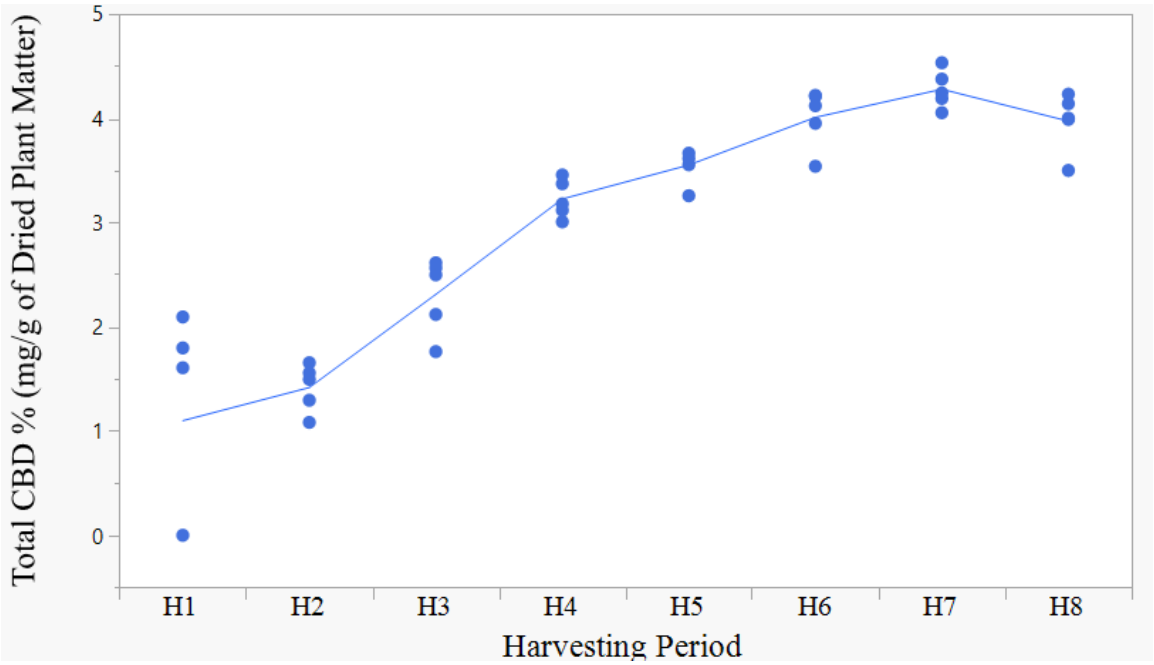


Figure 15 *Golden Sunset total CBD concentration change over time*. Points in the graph represents total THC concentration for an individual sample. A one-way ANOVAs were conducted to determine the effects of time in total CBD concentration. P-value < 0.05 was considered significant.

Golden Sunset plants surpassed the federal THC limit in Harvest 6 (0.330 ± 0.04 % total THC) (Fig 14). The sampled population experienced maximum concentration of total THC at Harvest 7 (0.344 ± 0.06 % total THC). In the following harvest period (Harvest 8), total THC concentration decreased below the federal level (0.234 ± 0.06 % total THC) (Fig 14). Total CBD concentrations peak at Harvest 7 (4.279 ± 0.18 % total CBD) (Fig 15). Maximum total CBD concentrations coincided with peak total THC concentrations in the Golden Sunset variety (Fig 14, Fig 15). The following week (Harvest 8), total THC concentrations were below the federal limit, allowing for the potential legal harvest of the crop. During Harvest 8, total CBD levels were at 3.973 ± 0.28 %, which was not significantly different ($p > 0.05$) than the peak concentration observed (Fig 15).

6. DISCUSSION

6.1 Physical Parameters of *C. sativa* Varieties Tested.

Overall, Golden Sunset cannabis plants had greater chlorophyll levels, taller stems, and heavier biomass. Physical parameters measured indicated that Golden Sunset can be used for agricultural production in Southern Florida during the fall season. Additionally, Golden Sunset also had the lowest mortality rate compared to Bubba Kush and Emerald Flower varieties.

Chlorophyll levels were measured with a "SPAD meter". This chlorophyll meter provides "SPAD values" as a measure for chlorophyll concentrations in the plant. Chlorophyll levels are a useful indicator for plant health (Ling et al, 2011; Percival et al., 2008). Stressors such as nutrient deficiencies, dehydration and diseases all produce changes in chlorophyll concentrations and appearance of the leaf (Percival et al., 2008). Biweekly chlorophyll measures did not significantly differ ($p > 0.05$) among varieties tested. However, chlorophyll concentrations significantly changed ($p < 0.05$) over time. Peak concentration for chlorophyll production occurred during October, in-between Harvest 2 and Harvest 4. The lowest SPAD values were generally observed during "Pre-flowering" and Harvest 6. Pre-Flowering data collection occurred during September, three weeks after transplanting. The average temperature in Homestead, Fl in September range from 72° F/ 22.2°C to 89° F/31.7°C. Stressors such as high temperatures and transplanting of the crop might have influenced chlorophyll production during "pre-flowering". Additionally, SPAD values for Harvest 6 were taken 2 weeks before the experiment ended. At this time, several plants from the Emerald Flower and Bubba Kush varieties had started to senesce. During senescence, plants experience programed cell

death which allows them to break-down compounds like chlorophyll and sugars and relocate nutrients to other organs (Mayta et al., 2019; Thomas, 2012).

Bubba Kush Emerald Flower Golden Sunset

Figure 16 *Branching Structure and Biomass Production Differences among Varieties Tested.*

Plant height and dry biomass weight was recorded to compare growth and development of the varieties tested. Golden Sunset plants had the largest stems and heaviest biomass recorded. Differences among dry biomass weight might be attributed to Golden Sunset's height, fan leaf production, internodal branching and abundance of flowers (Fig 16). Generally, Golden Sunset plants had lateral branching throughout the main stem, which provided better coverage, structure, and space for flower development (Bozzolo & Siemens, 2021; Kocjan Ačko, Flajšman & Trdan, 2019). In comparison, Bubba Kush and Emerald Flower did not performed as well in the field trial. Due to variable genetics, the Bubba Kush and Emerald Flower varieties displayed two distinct morphological structures (type A and type B) within their populations (Fig 16, Fig 17). The majority of Bubba Kush and Emerald Flower plants were identified as "Type A".

Type A plants grew a main stem with no lateral branching and various fan leaves. As a result, Type A plants produced a large singular cluster of flowers on top of the main stem (Fig 16, Fig 17). “Type B” individuals had a similar morphological structure to Golden Sunset plants (Fig 16, Fig 17). Alike Golden Sunset, Type B plants had lateral branching with the presence of multiple flower clusters. This physical structure was present in low percentages of the Bubba Kush and Emerald Flower populations.

Varieties tested lost more than a quarter of their population during the field study. Substantial loss of individuals in Bubba Kush and Emerald Flower varieties may be attributed to plant their structure, inclement weather conditions and weekly biomass sampling. Golden Sunset had the lowest mortality rate among the varieties tested.

Bubba Kush **“Type A”** Plant Bubba Kush **“Type B”** Plant

Figure 17 *Images demonstrating differences between “Type A” and “Type B” branching structures.*

Golden Sunset’s branching structure and abundant fan leaves may have protected the crop from environmental stressors (Bernstein, Gorelick, & Koch, 2019). The structure

of Bubba Kush and Emerald Flower varieties could have left them more susceptible to abiotic stressors and disease-causing pathogens. Heavy rainfall and high temperatures throughout the experiment caused several plants to perish. Furthermore, excess moisture led to the development of bud rot (*Botrytis cinerea*), which caused the decay of many cannabis flowers. Mortality rates might have also been influenced by the sampling protocols employed during weekly harvest. Following FDACS sampling protocol, the first 8 to 10 inches from the top portion of the main stem was harvested for cannabinoid testing. Sampling such a substantial portion of the main stem might have caused death or led pathogen infection. Lastly, hurricane Etna produced strong winds and torrential downpours in Homestead, Fl on November 8th, 2020. The inclement weather toppled and stripped several plants, increasing the total mortality rate. As a result, the experiment was concluded at Harvest 8 to avoid any confounding variables within our data.

6.2 Total THC and Total CBD Concentrations Across Time and Variety

Among the varieties tested, total THC and total CBD fluctuated across time and variety. Data demonstrated that the pattern of accumulation for total THC and total CBD was similar throughout time. Both compounds experienced their maximum accumulation rates 5 to 7 weeks post-anthesis. Emerald Flower and Golden Sunset varieties were at peak total CBD accumulation without surpassing the total THC limit. This would allow for the legal harvest of both crops during maximum CBD concentration, increasing overall profitability of the crop. Industrial hemp's ability to be legally cultivated is dependent on the total THC concentration within the plant. Furthermore, total CBD concentrations directly influences profitability of the crop. Bubba Kush plants had

variable concentrations of total THC within the same harvesting periods. However, total CBD concentrations in Bubba Kush plants followed the same pattern of accumulation as the other varieties. Several similar studies including Pacifico et al. (2007), Ascrizzi et al. (2019), and Baldini et al. (2018), evaluated the accumulation of cannabinoids over time. Despite varying environmental conditions and genetic differences, the aforementioned studies concur that maximum cannabinoid accumulation occurs 5 to 7 weeks post-reproductive growth. These results are consistent with the FIU Industrial Hemp Pilot Project cannabinoid data.

Several factors can influence the total accumulation of THC and CBD in industrial hemp plants. Factors such as environmental conditions, genetic traits, stress, and time can alter cannabinoid accumulation rates (Vanhove et al., 2011). As a result, legal cultivation of industrial hemp is difficult to achieve without the acquisition of proper genetic varieties for agricultural production (Sikora et al., 2011). The regression analysis demonstrated that variety and harvesting interval were significant predictors of total CBD and total THC in the crops tested. Some varieties may have limitations on total THC and total CBD production. Petit et al. (2020) noted that limited production of THC and CBD may be attributed to genetics of a particular variety. Cannabinoid accumulation is also influenced by harvesting period. Total THC and total CBD rapidly accumulate post-anthesis (Small, 2018). Ingallina et al., (2020) observed the relationship between harvesting period and CBD /THC concentration in monecious varieties in Italy. Results suggest that continuous monitoring of cannabinoid productions could maximize total CBD yields while avoiding the total THC federal limit.

6.3 Assessment of Bubba Kush, Emerald Flower and Golden Sunset Varieties

The Bubba Kush variety had a high variability in the production of total THC. Bubba Kush would be considered a chemotype II variety because of the high accumulation of CBD and THC throughout reproductive growth. In a study conducted by Small and Beckstead (1973), they observed that chemotype II varieties produced total CBD and total THC in quantities over 0.5% mg/g of dried plant matter. Maximum accumulation of CBD occurs after total THC has surpassed the federal limit (Harvest 4). For this reason, Bubba Kush plants should be harvested at 3 weeks post-reproductive growth (Harvest 3) for federal compliance. Harvesting before maximum total CBD accumulation would represent a loss in cannabinoid yield. Additionally, flowers may not be mature or large enough to support sufficient trichome development for agricultural production.

The Emerald Flower variety displayed chemical characteristics of chemotype III cannabis plants. Chemotype III plants have a total CBD accumulation greater than 0.5 % and a total THC accumulation less than 0.3% (Welling et al., 2016). Overall, the Emerald Flower plants had the highest concentration of total CBD throughout the experiment. However, physical parameters confirmed that Emerald Flower plants produced the lowest amount of biomass. Furthermore, Emerald Flower populations experienced a higher mortality rate compared to Bubba Kush and Golden Sunset varieties.

Finally, the Golden Sunset variety was also considered a chemotype II crop. Maximum accumulation of total CBD occurred at the same time as total THC concentrations surpassed the federal limit (Harvest 7). The following week (Harvest 8), total THC concentrations decrease below the federal limit, allowing the legal harvest of

the crop. Additionally, Golden Sunset plants produced the greatest amount of biomass. Turner et al. (1980) observed that plants with higher bract and leaf area throughout the plant produced a higher concentration of cannabinoids. This is attributed to a larger surface area for trichomes development.

7. CONCLUSION

Total THC and total CBD production were significantly affected by harvesting time and variety. Generally, all varieties followed similar patterns of accumulation for total CBD. Maximum accumulation for total CBD occurred around 5 to 7 weeks post-anthesis. Similarly, total THC experienced maximum accumulation between Harvest 5 to Harvest 7, surpassing the 0.3% federal limit in all varieties.

The Bubba Kush variety experienced highly varied total THC concentrations within their population. To avoid surpassing the total THC federal limit, Bubba Kush flowers must be harvested 3 weeks after post-anthesis. At this time, flowers might be underdeveloped, decreasing the amount of trichome abundance. Based on this field trial, the combination of early harvesting time and high THC production might render this variety unfit for outdoor cultivation in South Florida. More studies need to be conducted on this crop to confirm its viability.

Emerald Flower produced the highest total CBD across all varieties tested. Despite high CBD concentrations, physical parameters demonstrated decreased biomass development. Moreover, Emerald Flower experienced the highest mortality rate out of all varieties tested. Results of this field trial indicate that Emerald Flower might not be fit for

outdoor field production in South Florida. Additional studies are needed to confirm the viability of this crop.

Finally, the Golden Sunset variety had the most favorable chemical and physical traits for South Florida outdoor field cultivation. Golden Sunset plants had ample lateral branching, providing space for increased flower development. Additionally, the Golden Sunset crop can be legally harvested during peak levels of CBD accumulation. The results from this study indicate that Golden Sunset may be utilized for outdoor industrial hemp production in South Florida. Although, more studies are needed to confirm its viability throughout the year.

Disclaimer:

All industrial hemp crops planted in this experimental field study were left the FDACS approved on-site to senesce. Additionally, no plants from the study were harvested. Plant samples were solely collected for experimental purposes such as biomass weight or cannabinoid quantification. Lastly, industrial hemp samples were stored in approved FDACS-FIU facilities. All samples that exceeded the 0.3% total THC were disposed of by Florida International University Environmental Health and Safety at the end of analysis.

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