ABSTRACT

LINDER, ERIC REINHOLD. Establishing Transplant Date, Plant Spacing and Harvest Date Recommendations for Floral Hemp Production. (Under the direction of Dr. David Suchoff).

The cultivation of industrial hemp (*Cannabis sativa* L) has increased in recent years due to the passing of The American Agricultural Improvement Act of 2018 (2018 Farm Bill). Floral hemp is mainly cultivated for the extraction non-psychoactive secondary metabolites known as cannabinoids. Data-backed information regarding best agronomic practices for the cultivation of floral hemp is scarce. Farmers growing floral hemp for extraction purposes are compensated based on total biomass and cannabidiol (CBD) concentrations. To maximize profit farmers can either increase the amount of biomass produced on a per hectare basis and/or produce the highest CBD concentration floral material. The first objective of this work was to establish optimal transplant dates and plant spacing recommendations to maximize biomass production of floral hemp. The second object of this work was to model the temporal accumulation of CBD and THC in field grown floral hemp in North Carolina to establish harvest timing recommendations and minimize non-compliant crop production. To address these objectives two separate field trials were conducted in 2020 and 2021.

The first trial evaluated the effect of five transplant dates and four plant spacing treatments. This trial included day-length sensitive floral hemp cultivar ‘BaOx’ and was conducted over three sight years. Trials utilized a split-plot randomized complete block design with transplant date as the main-plot and plant spacing as the sub-plot. Transplant date treatments occurred on May 11, May 25, June 8, June 22, and July 7 (+-1 d). Plant spacing treatments were 0.91, 1.21, 1.52, and 1.83 m between plants. Weekly height and width data were collected throughout the vegetative growing period. Leaf and floral dry biomass was recorded following harvest. The main effect of planting date was significant on plant height and plant

width. Additionally, the main effect of plant spacing was significant only on plant width. Earlier planting date treatments resulted in taller and wider plants while larger plant spacing treatments resulted in wider plants. Biomass production on per-plant basis increased with earlier transplant dates and larger plant spacing. However, the reverse trend occurred for biomass production on a per-hectare basis where earlier transplant dates and smaller plant spacing resulted in the greatest biomass production. Our results indicate that an early transplant date and small plant spacing are the driving factors for maximizing biomass on a per-hectare basis.

The second trial investigated the effect of harvest date on temporal biomass, CBD and tetrahydrocannabinol (THC) accumulation for day-length sensitive floral hemp cultivars ‘BaOx’ and ‘Cherry Wine’. The two main cannabinoids of economic and legal concern are CBD and THC. Floral hemp is primarily cultivated for the non-psychoactive cannabinoid CBD however, farmers also be aware of temporal THC accumulation due to the USDA compliance threshold of 0.3% total THC by dry weight. Trials utilized a split-plot randomized complete block design with cultivar as the main-plot and harvest date as the split-plot. Harvest events started 2 weeks after floral initiation and occurred every two weeks for 12 weeks. Per-plant threshed biomass accumulation exhibited a linear plateau trend. The best fit model for temporal accumulation of total THC was a beta growth curve. As harvest date was delayed total THC concentrations increased until concentrations reached their maximum, concentrations then decreased as plants approached senescence. Logistic regression was the best fit model for temporal accumulation of total CBD. Total CBD concentrations increased with later harvest dates. Unlike THC concentrations, there was no decline in total CBD concentrations. To minimize risk, growers should test their crop as early as possible within the USDA’s 30-day compliance window. We observed ‘BaOx’ and ‘Cherry Wine’ exceeding the compliance threshold at 50 and 41 DAFI,

respectively. Therefore, to have a crop test below the compliance threshold and remain there at the time of harvest, farmers growing these cultivars should have samples collected no later than 20 DAFI.

Establishing Transplant Date, Plant Spacing and Harvest Date Recommendations for Floral Hemp Production.

by
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DEDICATION

To my Partner in life, Cassondra Newman. Thank you for always being there for me, supporting my dreams, and inspiring me to do better. You make life an adventure, I am so grateful for you.

BIOGRAPHY

Eric R. Linder was born in South Field, Michigan on September 24, 1989, to Natalie Whitcomb and Gerhard Linder. Growing up in Metro Detroit, Eric spent most of his weekends in rural northern Michigan. Over the course of several years, Eric his father and grandfather worked together building a house in northern Michigan where his father now lives. Throughout this period, Eric spent a considerable amount of time exploring the woods and farmland of the surrounding area. It was during these years where Eric developed a passion for nature, plants, and agriculture.

In 2014 Eric started his Associates Degree and became a research technician at the University of Florida Citrus Research and Education Center where he participated in projects focused on managing and slowing the spread of citrus greening disease. Interacting with local growers and observing the impact research had on the citrus industry brought Eric great joy. After finishing his Associates Degree Eric knew he wanted to have a career in agriculture. He then transferred to the University of Florida (UF). During his time at UF, Eric became a lab technician at UF's Forest Pathology Lab and the Sweet Corn Genetics and Genomics Lab. He graduated cum laude with a bachelor's degree in Agricultural Operations and Management. Following graduation, Eric spent a year as a field manager for BASF's exploratory soybean breading program. He then took the opportunity to start a master's degree under Dr. David Suchoff at North Carolina State University in 2020.

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CHAPTER 1

The Effect of Transplant Date and Plant Spacing on Biomass Production for Floral Hemp (*Cannabis sativa L.*)

Abstract

Floral hemp (*Cannabis sativa L.*) cultivated for the extraction of cannabinoids is a new crop in the United States. Information regarding agronomic practices for field-grown floral hemp is scarce. The aim of this study to develop agronomic recommendations for plant spacing and transplant timing to maximize field-grown floral hemp biomass production in North Carolina. Field trials were conducted in 2020 and 2021 with the day-length sensitive floral hemp cultivar BaOx. Trials utilized a split-plot randomized complete block design with transplant date as the main-plot and plant spacing as the sub-plot. Transplant date treatments occurred on May 11, May 25, June 8, June 22, and July 7 (+-1 d). Plant spacing treatments were 0.91, 1.21, 1.52, and 1.83 m between plants. Weekly height and width data were collected throughout the vegetative growing period. Leaf and floral dry biomass was recorded following harvest. The main effect of planting date was significant on plant height and plant width. Additionally, the main effect of plant spacing was significant only on plant width. Earlier planting date treatments resulted in taller and wider plants while larger plant spacing treatments resulted in wider plants. Biomass production on per-plant basis increased with earlier transplant dates and larger plant spacing. However, the reverse trend occurred for biomass production on a per-hectare basis where earlier transplant dates and smaller plant spacing resulted in the greatest biomass production. Our results indicate that an early transplant date and small plant spacing are the driving factors for maximizing biomass on a per-hectare basis.

Introduction

Cannabis sativa L. is a short day, predominantly dioecious, annual crop that initiates reproductive growth when day length becomes shorter than the critical photo period (12-16 h depending on cultivar; Hall et al., 2012). The American Agricultural Improvement Act of 2018 (2018 Farm Bill) categorized Cannabis into two legally distinguishable categories depending on the plant's concentration of total tetrahydrocannabinol (THC). Total THC is calculated as Δ^9-THC + 0.877 × tetrahydrocannabinolic acid (THCA) and is reported on a dry weight basis. Cannabis is classified as marijuana (federally illegal) if it produces more than 0.3% total THC on a dry weight basis, and industrial hemp (IH) if it produces less than 0.3% total THC on a dry weight basis (federally legal). Industrial hemp may be cultivated for either seed, fiber, or floral material for the secondary metabolites called cannabinoids. Traditionally, IH has been cultivated for seed and fiber in Canada, parts of Europe, and Asian countries. In recent years, interest in the potential therapeutic and medical properties of non-psychoactive cannabinoids such as cannabidiol (CBD), cannabichromene (CBC), and cannabigerol (CBG) has led to an increase in domestic IH production for the plants floral and leaf material which contain these compounds (Abrams, 2018; Levinsohn & Hill, 2020).

Farmers growing floral hemp for extraction purposes are compensated based on total biomass and CBD concentrations. Thus, to maximize profitability farmers can either produce the highest CBD concentration floral material and/or increase the amount of biomass produced on a per hectare basis. Cannabinoid concentrations are predominantly determined and limited by genetics and the time of harvest (Campbell et al., 2019; Burgel et al., 2020; Toth et al., 2021). Additionally, post-anthesis total THC concentrations in floral hemp exceed the 0.3% threshold before peak CBD concentration is attained (Yang et al., 2020). Furthermore, Zirpel et al. (2018)

demonstrated that CBDA synthase is a promiscuous enzyme and produces approximately a 20:1 CBD:THC ratio. Taken together, farmers cannot increase profits based purely on maximum CBD production as it comes with a non-compliant concentration of THC. Therefore, farmers should seek to implement cultural practices that maximizes biomass production to increase profitability.

As a day length sensitive crop, earlier transplant dates may result in a prolonged vegetative growing period, allowing for increased biomass production. Research has shown that earlier planting dates resulted in taller plants for IH cultivated for fiber production (Sengloung et al., 2009). There is a scarcity of research for establishing the effect of planting date on floral hemp; however, the effect of planting for other photoperiod sensitive short-day crops such as rice (*Oryza sativa* L.) has been extensively studied. A study examining the effect of planting date on agro-morphological traits of rice by Satapathy et al. (2021) identified a decrease in grain yield, plant height, tiller production, and dry matter production (biomass) as planting date was delayed.

Planting density may also improve total biomass production on a per hectare basis. Studies on IH cultivated for fiber have demonstrated how variations in planting density influence fiber hemps morphology, growth rate, and stem yield. Higher planting densities with fiber hemp resulted in faster initial growth rates and increased stem yield (Amaducci et al., 2002; Tang et al., 2017). However, production systems for floral and fiber hemp are vastly different. Floral hemp is usually grown similar to high-value vegetable crops at much lower densities (3,589 - 7,173 plant/ha) than fiber hemp (~2,000,000 seed/ha). Few scientific studies assessing the effect of planting density on floral hemp biomass production have been conducted. Benevenute et al. (2021) observed flower yield decreasing on a per plant basis with increasing planting density but yield on a per-hectare base gradually increased. However, in this study plants were transplanted relatively late in the

growing season and only flower yield was quantified and not total marketable biomass (leaves and floral material).

There is a current knowledge gap for growers regarding ideal agronomic production practices for floral hemp cultivated for cannabinoids. The objectives of this study were to develop farmer recommendations for plant spacing and transplant timing to maximize field-grown floral hemp biomass production in North Carolina.

Materials and Methods

Experimental Design

Field experiments were conducted during the 2020 and 2021 growing season. Trial locations in 2020 were in Kinston at the Cunningham Research Station on a Norfolk loamy sand (fine-loam, kaolinitic, thermic Type Kandiudults), and in Salisbury, NC at the Piedmont Research Station on a Clay Loam (Fine, kaolinitic, thermic Type Rhodic Kanhapludults). A third location was added in 2021 at the Sandhills Research Station on a Loamy Sand (Loamy, kaolinitic, thermic Type Arenic Kanhapludults) resulting in five unique year × location environments. Asexually propagated clones of the CBD hemp cultivar BaOx (Triangle Hemp, Durham, NC) were used at all field locations for both growing seasons.

Fields were prepared with pre-bedding primary and secondary tillage according to research stations practices. Before bedding, and depending on soil test reports, all fields received a broadcast application of urea, diammonium phosphate and potash (Weaver 17-17-17; Winston Weaver Co, Winston-Salem, NC) (Utrasol MOP 0-0-60; Sociedad Quimica y Minera, Santiago, Chile) at a rate of up to 67 Kg N ha^{-1}, 67 Kg P ha^{-1}, and 134.5 Kg K ha^{-1}. Simple on/off drip tape connector valves were placed at each connection point between the layflat and each section of drip tape so plots could be independently irrigated or fertigated.

The Kinston and Salisbury locations utilized white 1.25 mm polyethylene plastic mulch on raised beds with a 1.5 m center to center row spacing with drip tape (Netafim Streamline 10 mil with 30.48 cm emitter spacing .908 LPH) laid underneath. Fertigation events supplying nitrogen and boron occurred every two weeks with calcium nitrate (Yaraliva Calcinit 15.5-0-0; Yara International, Oslo, Norway) and disodium boron (Borate 21%B; Borates Plus Inc., Apopka, FL) until floral initiation. Season total fertilizer application was 134.5 Kg N ha^{-1}, 67 Kg P ha^{-1},134.5 Kg K ha^{-1} and 1.12 Kg B ha^{-1}.

The Jackson Springs location utilized raised beds on bare ground with a row spacing of 1.2 m and was irrigated by an overhead linear track irrigation system at the discretion of the research stations superintendent. Granular fertilizer was applied with three applications, a pre-plant application then at 14 days after transplanting (DAT), and at 28 DAT by a side dressing application. The fertilizer was a custom blended containing urea, ammonium sulfate, mono-ammonium phosphate, muriate of potash, and calcium carbonate (17-17-17; FCI Fertilizer Division, Parkton, NC). Season total fertilizer application was 133.8 Kg N ha^{-1}.

After bedding and seven days before transplanting an herbicide application of Paraquat (Gramoxone SL 3.0; Syngenta, Basel, Switzerland) at 1.55 kg active ingredient (ai) ha^{-1}, Napropamide (Devrinol 2 XT; United Phosphorus, Inc., King of Prussia, PA) at 1.68 kg ai ha^{-1}, and Pendimethalin (Prowl H2O; BASF, Ludwigshafen, Germany) at 0.53 kg ai/ha^{-1} was applied to the bare ground. Transplants were hand planted at the appropriate spacing for all locations.

Experimental Design

Trials were arranged in a split-plot randomized complete block design with transplant date as the main-plot and plant spacing as the sub-plot. Each location contained four blocks. The study evaluated five transplant date and four plant spacing treatments. Transplant dates were the

same for 2020 and 2021, *i.e.*, May 11, May 25, June 8, June 22, and July 7 (±1 d). The four plant spacing treatments were 0.91, 1.21, 1.52, and 1.83 m between plants. The transplant date treatments were randomized to each block and spacing treatments randomized to plots within the unique block × transplant date main-plot. Spacing treatment plots contained ten plants with a buffer space of 1.52 m between plots.

Data and Sample Collection

Height and width data were collected weekly starting two weeks after transplant on five representative plants. To avoid any potential border effect, measurements were taken from plants within the plot, starting from the third plant in. Plant height was measured from the base of the plant to the tip of the apical meristem. Width was measured from the widest part of the plant, followed by a second width measurement perpendicular to the first. These two were averaged to give an average plant width. Height and width data collection ceased once vegetative growth stopped and plants shifted to the reproductive phase, which occurred the third week in August. Floral initiation was determined based on the onset of pre-terminal and terminal pistil development and internode shortening as described by Carlson et al. (2021). A final set of height and width data were collected on two representative plants per plot 6 weeks after floral initiation, which was the standard harvest timing used by hemp farmers in the region.

At harvest plants were cut at the base of the stalk approximately 5 cm above the soil line. Plants were placed in forced air tobacco curing barns at 48.8 °C for 5 days. Once dry, the floral and leaf material was stripped from the stalk by hand and the resulting biomass was recorded. These biomass data were used to calculate individual plant biomass production and biomass production on a per hectare basis.

Statistical Analysis

Data were plotted and inspected for any outliers and to determine treatment response trends. The nlme and nlraa packages in R were utilized for all analyses (Miguez, 2021; Pinheiro et al., 2021; R Core Team, 2018). Plant height and width data showed a distinct sigmoid trend, common for plant growth data (Arcontoulis & Miguez, 2014). Multiple sigmoid functions were fit to the data and compared using their respective corrected Akaike Information Criteria (AICc) and Bayesian Information Criteria (BIC). The model with the lowest AICc and BIC was selected. The best fitting model for height and width data was the four-parameter logistic model (Table 1.1), expressed as:

$$Y = A + \frac{B-A}{1+\exp\left(\frac{xmid-x}{scale}\right)}$$

Where *A* is the lower asymptote, *B* is the upper asymptote, *xmid* is the value of *x* at the inflection point, and *scale* is a numeric scale parameter which describes the overall width of the fit curve (Pinheiro & Bates, 2000).

For modeling plant height and width data, transplant date, plant spacing, and their interaction were treated as discrete fixed effects for all four model parameters. Year, location within year, block, and block × transplant date were treated as random effects. To account for the non-independent nature of plant height data, the within-subject variance was modeled using a diagonal positive-definite variance-covariance matrix (Pinheiro & Bates, 2000). Tukey's honestly significant difference test was employed for all fixed effects found to be significant ($P < 0.05$) using the emmeans package (Lenth et al., 2022).

Plotted biomass data showed a linear response to transplant date. Similar to the plant height and width data, transplant date, plant spacing, and their interaction were treated as fixed effects. However, we treated transplant date as a continuous variable by transforming each date

to its respective Julian date. Higher order polynomial regression model was fit to the data. The final model selection was based on the significant highest degree term as well as a Lack-of-fit (LOF) test, resulting in the most parsimonious model fit for the data.

Results and Discussion

Transplant Date Effecting Plant Height

We did not observe a significant interaction between transplant date and plant spacing nor main effect of plant spacing on plant height ($p > 0.05$). However, all four parameters of the four-parameter logistic model (FPLM) describing plant height over time were affected by transplant date ($p < 0.0001$; Fig. 1.1, Table 1.2). The parameter estimate *B*, describing the upper asymptote or maximum height achieved during the growing season, was highest in the 11 May transplant date. Final plant height decreased as transplant date was delayed. The final height of plants transplanted on 25 May was similar to 8 June transplant date. Similarly, the 8 June final plant height was not different from the 22 June transplant date, with the July 6 transplant date resulting in the shortest final plant height (Fig. 1.1, Table 1.2).

The effect of transplant date on *xmid* followed a similar trend to *B* (Table 1.2). Values were highest in the early transplant dates and decreased as transplant date was delayed. The observed trend indicates that earlier transplant dates require more time to attain their maximum growth rate (*scale*) in comparison to later transplant dates. The 11 May transplant date required the longest amount of time (38.23 d) to reach its max growth rate while the latest transplant date (6 July) needed the least amount of time (28.95 d). The 11 May and 25 May transplant dates were statistically similar, while the 8 June transplant date required significantly less time. The 22 June and 6 July were statistically comparable but required significantly less time than the 8 June, May 25, and May 11 transplant dates to reach their maximum growth rate.

The *scale* parameter followed a similar trend observed in the effect of transplant date on parameter *B* and *xmid*. The 11 May transplant date had the largest growth rate while the 6 July transplant date had the smallest growth rate. The 25 May, 8 June, and 22 June transplant date were statistically equivalent.

Unlike the prior three parameter estimates, estimates for parameter *A* tended to increase with later transplant dates (Table 1.2). The 11 May transplant date had the lowest value (-1.87) and the 6 July transplant date had the highest value (11.48). The middle three transplant dates were all statistically similar. Height measurements began two week after transplanting. Since the lower asymptote occurs before the two-week mark the model must extrapolate beyond the collected data to determine the height. We did not measure transplant height upon receiving the clones and thus cannot determine whether the differences in the estimates of *A* are due to differences from clone producer or the effects of transplant date.

Industrial hemp is a short-day crop, when day length shortens past a certain threshold (12-16 h depending on cultivar) the plant transitions from vegetative to reproductive growth (Hall et al., 2012). Later transplant date treatments exhibited a decrease in final plant height (Fig 1.1, Table 1.2), which is most likely due to these treatments receiving a truncated vegetative growth period before day lengths reached the reproductive threshold. The shortened growing period that later transplant date treatments experienced impacted the length of the vegetative growing phase and the plants maximum growth rate. The earlier transplant dates had a longer growing season where they were exposed to a greater number of long days, which resulted in more vegetative growth and taller plants (parameter *B*; Table 1.2). Taller plants may exhibit in an increased number of internodes and leaves which could increase floral biomass production. Our observed response to transplant date agrees with Darby et al. (2018) who conducted a one-year

planting date and planting density trial in Vermont where they observed taller plants with earlier planting dates.

Earlier transplant dates required more time (*xmid*) to reach maximum growth rate (*scale*; Table 1.1). This may be due to a combination of two factors. First, the relatively short days experienced by the 11 May and 25 May transplant dates early in the season could have resulted in slower initial growth rates as compared to those experienced by later transplant dates. Second, the limited number of long days experienced by the later transplant date treatments resulted in truncated vegetative growth, thus reducing the time necessary to reach rate of growth.

Previous studies examining the effect of planting date on growth and development of fiber hemp found similar trends with the growth parameters *B*, *xmid*, and *scale*. Sengloung et al. (2008) described the effect of reduced maximum stem height, shorter time until maximum growth rate (inflection point) and reduced maximum growth rate (slope at inflection point) for later sowing dates.

Transplant Date Affecting Plant Width

No significant interaction was observed between transplant date and plant spacing on plant width. However, both main effects had a significant effect on plant width.

All four parameters of the FPLM describing plant width over time were affected by transplant date (Fig 1.2, Table 1.3). Estimates for parameter *B*, representing the maximum width attained during the growing season was greatest for the 11 May transplant date (148.35 cm). Average plant width decreased with subsequent transplant dates. The final width of clones transplanted on 25 May was less than the 11 May transplant date. There was no significant difference in final plant width between the 8 June and 22 June transplant dates. The least wide plants were found in the last transplant date July 6 (99.09 cm; Table 1.3).

Similar to parameter *B*, *xmid* exhibited a decreasing trend with later transplant dates (Table 1.3). Similar to transplant date affecting plant height, the results suggest that earlier transplant dates require more time to reach their maximum growth rate in contrast to later transplant dates. The 11 May and 26 May transplant dates were statistically similar requiring the most amount of time to attain their maximum growth rate. The 8 June, June 22, and 6 July transplant dates were all similar requiring the least amount of time to reach their maximum growth rates.

Estimates for the *scale* parameter exhibited similar trends as *B* and *xmid* estimates. The maximum growth rate was greatest for earlier transplant date and decreased with subsequent transplant dates. The 11 May transplant date had the greatest maximum growth rate. The 25 May, 8 June, and 22 June transplant dates were comparable. The final 6 July transplant date had the lowest growth rate. Parameter *A*, the height of the lower asymptote exhibited an opposite trend to the three prior parameters. Values tended to increase with later transplant dates (Table 1.3). The initial 11 May transplant has the lowest value (-25.59) and the final 6 July transplant date had the highest value (-0.10). The May 11, May 25, and June 8 transplant dates were statistically equal. The May 25, June 8, and June 22 produced plants that were similar. For parameter *A* the model is attempting to calculate the height of the lower asymptote before the two-week mark.

The effect of transplant date on plant width are similar to the effect of transplant date on plant height. The trends and statistical differences found among treatments for the effect of transplant date on plant height and width are nearly the same for all four parameters of the FPLM (Table 1.1, 1.2). These relationships indicate that the factors regulating growth potential for plant height and width with respect to transplant date are closely related.

Similar to the effect of transplant date on plant height, earlier transplant date treatments displayed the widest final plant width (Parameter *B*; Table 1.2, Fig. 1.3). This effect is most likely the due to the treatments receiving a longer vegetative growing period. Earlier transplant dates required more time (*xmid*) to reach maximum growth rate (*scale*) (Table 1.2, Figure 1.3). Temperature may be another factor affecting these parameters. Hemp's rate of photosynthesis and cellular respiration can be significantly affected by day and night temperature. A study in a controlled environment tested four fiber hemp cultivars and three THC producing cultivars to determine ideal temperatures for optimal photosynthetic rates. Optimal photosynthetic rates for six of the cultivars were at temperatures between 25-30 °C, while one of the cultivars had an optimal photosynthetic rate between 30-35 °C. All the cultivars showed a twofold increase rate in cellular respiration with an increase in temperature from 20-40 °C (Chandra et al., 2011). The relatively cooler temperatures that earlier transplant dates experienced early in the growing season (Figure 1.4) may have delayed early season development resulting in more time to reach maximum growth rate

Plant Spacing Affecting Plant Width

The parameters *B*, *xmid* and *scale* of the FPLM describing plant width over time were affected by plant spacing (Fig 1.3, Table 1.4). Parameter *A* was not affected by plant spacing ($p > 0.05$). The widest plants (parameter *B*) were observed in the 1.83 m plant spacing treatment (142.38 cm; Table 1.3). Plant width decreased significantly as plant spacing was reduced. The 1.52 m spacing was comparable to the 1.83 m. The 1.22 m spacing was similar to the 1.52 m spacing. The least wide plants were observed in the smallest plant spacing treatment of 0.91 m (118.94 cm; Table 1.4).

The parameter *xmid* estimates displayed a similar trend to parameter *B*. Wider plant spacing treatments exhibited higher values which decreased with smaller plant spacing treatments. This trend suggests that plants with wider spacing treatments require additional time to obtain their maximum growth rate (*scale*) in comparison to plants with narrower spacing treatments. The 1.83 m spacing required the most amount of time to attain maximum growth (33.38 d) (Table 1.4). The 1.83 m, 1.52 m, and 1.22 m spacing treatments were statistically comparable, these treatments required the longest time to obtain maximum growth rate. The 1.22 m and 0.91 m treatments were statistically similar. The 0.91m treatment required the least amount of time to reach maximum growth rate (29.22 d) (Table 1.4).

The estimates for parameter *scale* exhibited a similar trend as the previous two parameters. As plant spacing decreased so did the maximum growth rate. The 1.83 m spacing had the largest growth rate which was statistically comparable to the 1.52 m and 1.22 m treatments. The 1.52 m, 1.22 m, and 0.91 m plant spacing's had comparable growth rates. The 0.91 m treatment had the lowest maximum growth rate.

Larger plant spacing treatments resulted in wider plants, while smaller plant spacing resulted in narrower plants (Figure 1.3, Table 1.4). This trend is likely an effect from inter-plant competition for light, water, and nutrients. During the growing season nutrient and water availability were not directly measured; however, due to the fertilization rate and regular irrigation interplant competition can mostly be attributed to light interception. Furthermore, we did not observe any nutrient deficiencies in the field nor were the plants water stressed.

Inter-plant competition resulting from increasing planting density can influence plant morphology and growth rates. When fiber hemp is cultivated at higher planting densities canopy closure occurs faster than plantings at lower planting densities (Tang et al., 2017). Amaducci et

al. (2002) fitted early season light interception data from multiple fiber hemp planting densities to a logistic model and reported canopy closure was reached in less time with higher planting densities and higher planting densities required significantly less time to reach the inflection point (maximum growth rate) than lower panting densities.

Narrower plant spacing treatments required less time (*xmid*) to reach their maximum growth rate (*scale*; Figure 1.3, Table 1.4). Consequently, we observed smaller plant spacing treatments achieving canopy closure earlier than larger spacing treatments. Canopy closure for the 11 May 0.91 m spacing was achieved by June 28 at the Jackson spring's location, July 7 at the Kinston location and August 2 at the Salisbury location. This effect may have potentially been induced by increased light competition experienced by smaller plant spacing treatments. For bare ground systems, the reduced time needed for canopy closure may be advantageous for increasing the crop's competitive ability with weed competition. Similar results have been found in fiber hemp planting density studies (van der Werf et al., 1995). However, in a system utilizing plastic mulch the weed-competitive benefits of crop canopy closure are not as relevant.

Biomass Affected by Transplant Date and Plant Spacing

There was no significant interaction between transplant date and plant spacing on per-plant biomass production. However, both main effects affected biomass production and following a linear regression ($p < 0.0001$, LOF $p = 0.6832$; Fig. 1.5).

The common slope of the regression represents the main effect of transplant date, which was -6.38 g $plant^{-1}$ d^{-1} (Table 1.5). The slope implies that for every day planting is delayed after the initial May 11 transplant date an average of 6.38 g of potential biomass is lost.

The different y intercepts represent the main effect of plant spacing. On a per plant basis, there is a decreasing trend with decreasing plant spacing (Fig. 1.5, Table 1.5). Biomass yields

were highest in the 1.83 m and 1.52 m treatments, which were not different from one another (Table 1.4). The 1.83 m spacing treatment yielded significantly more than the 1.22 m and 0.91 m treatments. By doubling the space from 0.91 m to 1.83 m, we saw a 14.2% increase in biomass yield.

Yield results on a per hectare basis showed the same linear trend with significant main effects ($p < 0.0001$, LOF $p = 0.6751$; Fig. 1.6, Table 1.6). For all the planting date treatments and regardless of plant spacing we observed a slope of -31.98 kg ha^{-1} d^{-1}. The slope indicates that, regardless of plant spacing, we lost on average 31.98 kg of biomass for every day we delayed transplanting after 11 May.

We observed a reverse trend in the plant spacing treatment effect on per hectare yield compared to per plant yield; decreasing plant spacing resulted in significantly higher yield per hectare (Fig 1.6, Table 1.6). The 1.83 m spacing had the smallest y intercept (7,049.04 kg ha^{-1}), the 1.52 m spacing was slightly larger but statistically similar (7,218.70 kg ha^{-1}). The 0.91 m spacing treatment yielded the highest amount of biomass on a per hectare bases compared to all other treatments. Here, doubling the space between plants from 0.91 m to 1.83 m, resulted in an 8.8% decrease in yield on a per hectare basis (Table 1.6). These results indicate that, while increased space between plants increases the amount of biomass produced on a per plant basis (Table 1.5), the drivers for maximum yield on a per-hectare basis are increasing plant density and early planting (Table 1.6).

Our planting spacing results concur with Benevenute et al. (2021) who tested different planting density (3,000, 4,000, 6,000, 12,000 plants ha^{-1}) with the day length sensitive cultivar Cherry Wine. We observed similar trends regarding biomass production where lower planting densities resulted in more biomass on a per plant basis while higher planting densities resulted in

more biomass produced on a per-hectare basis. Additionally, a study conducted by Anderson et al. (2021) identified a strong correlation between increased growth rates and taller final plant height ($r = 0.97$) likewise, taller plants at flower initiation were correlated to an increase in final biomass. In our study, we observed the greatest growth rate for plant height and width with our earliest 11 May transplant date (Table 1.2, 1.3). When compared to all other treatments, our 0.91 m plant spacing, and 11 May transplant date treatments yielded the greatest biomass on a per-hectare basis. Our results along with Benevenute et al. (2021) and Anderson et al. (2021) reinforces the hypothesis that small plant spacing, and early transplant date are both key driving factors for maximizing biomass on a per-hectare basis.

Research regarding the effect of transplant date on floral hemp is limited. However, in other day-length sensitive short day crops such as rice similar results are found. Satapathy et al. (2021) conducted a trial examining the effect of planting date on agro-morphological traits of rice. The trial utilized three planting dates over a 14 d period. Satapathy et al. (2021) identified that earlier planting dates resulted in more grain yield, higher panicle weight, increased plant height, and increased biomass production. Bashir et al. (2010) found similar results with a planting date study on rice. Bashir et al. (2010) observed a decrease in final plant height and above ground biomass as planting date was delayed. Concluding that earlier planting dates produced taller plants as a result of experiencing a longer growing season due to the photoperiod response.

We did not quantify cannabinoid in our trial; however, Benevenute et al. (2021) did not find any evidence of planting density influencing cannabinoid concentration. Cannabinoid synthesis of THC and CBD is primarily regulated by genotype. Campbell et al. (2019) investigated the effect of genotype by environment for 13 industrial hemp cultivars and found

that 80% and 83% of variance for THC and CBD could be explained by genotype while 1.7% and 6% of variance could be explained by environment. Additionally, field trials have shown that cannabinoid content and the CBD to THC ratio in high CBD hemp cultivars are regulated by genetics and are not influenced by environmental stress (Toth et al., 2021). Therefore, maximum potential cannabinoid concentration will always be limited by plant genetics. Burgel et al. (2020) demonstrated that cannabinoids are highly dependent on genotype and growth stage. Yang et al. (2020) tracked the development of cannabinoids in inflorescent of IH in a pilot study and observed a general increase in THC, CBD, and CBG as flowers matured. Day length sensitive cultivars THC exceeded the legal 0.3 percent threshold at about 4-weeks post anthesis. Total CBD continued to increase reaching its greatest concentration at 6-week post anthesis. Since THC concentrations exceed 0.3% before maximum CBD production the maximum CBD concentration growers could attain will always be limited by the legal threshold.

Plant nutrition can influence morphology and growth however, it has a limited influence on cannabinoid production. In a study conducted in a controlled environment with five CBD hemp cultivars, Anderson et al. (2021) observed optimal growth and biomass production at 50 ppm nitrogen, increasing fertilizer rates resulted in reduced growth and cannabinoid content. The observed reduction in cannabinoids was a response to salinity stress associated with overall plant decline. Caplan et al. (2017) observed maximum plant growth and biomass production on a drug type cultivar with a fertilizer rate of 389 mg N/L however there was no effect of fertilizer rate on cannabinoid concentrations. Field studies in North Carolina have demonstrated that nitrogen fertility levels influence plant growth and biomass production but does not affect cannabinoid concentrations. Additionally, Potassium levels differently impacted plant growth and cannabinoid concentrations depending on the environment and soil texture. In clay environments

a rate of 110 kg K_2O ha^{-1} maximized biomass yield but did not affect cannabinoids. In sand environments potassium rates did not affect biomass however, a THC and CBD concentrations showed a linear decrease of less than one percent as K_2O rates increased from 0 to 224 kg K_2O ha^{-1} (Short, 2021). Considering the results from these authors there is a consensus that CBD production potential is largely regulated by genetics and harvest timing. Since compensation is based on a percent CBD pound basis a grower's main mode of increasing profitability would be to maximize marketable biomass production through cultural practices.

The floral hemp industry in the United States is still relatively young. There are many uncertainties and challenges facing the industry. Little information is available regarding the demand and price for inputs, hemp biomass, and hemp derived products. The market data that is available is provided by private sources such as PanXchange, Hemp Benchmarks, and The Jacobsen; however, none of these sources are publicly available. As with most new alternative crop commodity markets as the United States hemp market continues to develop and expand it is expected that the market will also become increasingly volatile (Mark et al., 2020). Hemp biomass prices have dropped from a high of greater than $4 per percentage CBD pound to below $1 (Skorbiansky et al., 2021). Additionally, the price of inputs such as seed, and clones is variable. Economics is an important factor when considering planting densities. However, due to the lack of reliable market data, instability of the hemp market, and fluctuation for cost of inputs drafting a dependable production budget is not feasible.

When planting at higher densities depending on the location and climate of the field location there may be possibility for an increased risk for foliar plant pathogens to develop. In a survey of plant pathogen affecting hemp in North Carolina Thiessen et al. (2020) identified common pathogens by surveying plant sample submissions from the North Carolina Department

of Agriculture and Consumer and the North Carolina Plant Disease and Insect Clinic. The paper identified pathogens which are commonly experienced by growers in field and controlled environments. Common pathogens identified in field environments include Fusarium foliar and flower blights (Fusarium graminearum), Fusarium wilt (Fusarium oxysporum), and Helminthosporium leaf spot (Exserohilum rostratum). We speculate that high planting densities may influence disease susceptibility by creating a micro-climate where reduced airflow allows for water and humid conditions to persist. Since hemp is a new crop to the United States agricultural landscape there is a need for more research so we can better understand factors influencing disease susceptibility.

Conclusions

The aim of this study was to identify cultural practices that maximize floral hemp biomass production. Earlier transplant dates resulted in prolonged vegetative growth, producing taller and wider plants. Furthermore, increased plant spacing resulted in wider plants. These physiological result

On a per plant basis, we observed the most marketable biomass produced with earlier transplant dates and larger plant spacing treatments. However, on a per-hectare basis the most marketable biomass was produced with earlier planting dates and smaller plant spacing treatments.

Farmers growing floral hemp for extraction purposes are compensated based on total biomass and CBD concentrations. Since cannabinoid concentrations are largely determined and limited by genetics and the time of harvest (Campbell et al., 2019; Burgel et al., 2020; Toth et al., 2021). According to the results from this trial the key cultural practices for maximizing marketable biomass production are planting as early as possible while avoiding the potential for frost damage and utilizing small plant spacing to maximize planting density.

'BaOx' was the only cultivar examined in this study. It should be noted that the growth and structure of different hemp cultivars may vary, therefore, the effect of transplant date and planting density may differ. Furthermore, these studies were conducted in the Southeast at a latitude of approximately 35.7° N. Farmers in more southerly or northerly latitudes with differing summer day lengths may require early or later transplanting, respectively, based on their unique weather and day length conditions.

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Tables

Table 1.1. Goodness-of-Fit criteria for mixed nonlinear sigmoid models describing the relationship between floral hemp plant height and width over time.

Response	**Model**	**Goodness-of-Fit Criteria[z]**	
		AICc	BIC
Plant height	Logistic	23,776.61	23,820.53
	Four-Parameter Logistic	23,761.87	23,818.33
	Gompertz	24,498.32	24,542.24
	Beta growth function	25,340.37	25,384.29
	Four-parameter Beta growth function	25,756.81	25,813.27
Plant width	Logistic	26,629.02	26,672.68
	Four-Parameter Logistic	26,298.21	26,341.87
	Gompertz	26,611.26	26,667.38
	Beta growth function	NC[y]	NC
	Four-parameter Beta growth function	27,376.02	27,419.68

[z]AICc = Corrected Akaike's Information Criterion; BIC = Bayesian Information Criterion.

[y]NC = No convergence.

Table 1.2. Parameters of the four-parameter logistic model for temporal floral hemp plant height as affected by transplant date.

	Parameter			
Transplant date	***A***	***B***	***xmid***	***scale***
	cm	cm	days	cm day^{-1}
May 11	-1.87 c[z]	105.54 a	38.23 a	15.05 a
May 25	3.02 b	100.63 b	37.42 a	11.20 b
June 8	2.36 b	98.72 bc	33.87 b	10.88 b
June 22	3.25 b	95.64 c	30.59 c	10.78 b
July 6	11.48 a	80.98 d	28.95 c	9.15 c
P-value	<0.0001	<0.0001	<0.0001	<0.0001

[z]Letters indicate significant differences within a parameter estimate using Turkey's HSD (α = 0.05).

Table 1.3. Parameters of the four-parameter logistic model for temporal floral hemp plant width as affected by transplant date.

	Parameter			
Transplant date	***A***	***B***	***xmid***	***scale***
	cm	cm	days	cm day^{-1}
May 11	-25.59 c[z]	148.38 a	36.20 a	20.57 a
May 25	-15.72 bc	136.39 b	35.59 a	15.94 b
June 8	-18.49 bc	127.78 c	28.76 b	14.90 b
June 22	-12.58 b	124.99 c	27.25 b	14.07 b
July 6	-0.10 a	99.09 d	26.84 b	11.36 c
P-value	<0.0001	<0.0001	<0.0001	<0.0001

[z]Letters indicate significant differences within a parameter estimate using Turkey's HSD (α = 0.05).

Table 1.4. Parameters of the four-parameter logistic model for temporal floral hemp plant width as affected by transplant plant spacing.

	Parameter			
Spacing	***A***	***B***	***xmid***	***scale***
(m)	cm	cm	days	cm day^{-1}
0.91	-14.83	118.94 c[z]	29.22 b	15.33 b
1.22	-17.68	133.09 b	31.45 ab	16.46 ab
1.52	-15.94	136.32 ab	33.06 a	16.65 ab
1.83	-18.17	142.38 a	33.38 a	17.49 a
P-value	NS	<0.0001	<0.0001	0.0158

[z]Letters indicate significant differences within a parameter estimate using Turkey's HSD (α = 0.05).

Table 1.5. Linear regression for individual plant marketable biomass affected by transplant date and spacing.

Spacing	**Intercept**	**Slope**
(m)	g plant^{-1}	g plant^{-1} day^{-1}
0.91	1389.00 c[z]	-6.38
1.22	1473.00 b	-6.38
1.52	1530.99 ab	-6.38
1.83	1585.71 a	-6.38

[z]Letters indicate significant differences using Turkey's HSD ($\alpha = 0.05$)

Table 1.6. Linear regression for biomass yield per hectare affected by transplant date and spacing.

Spacing	**Intercept**	**Slope**
(m)	kg ha^{-1}	kg ha^{-1} day^{-1}
0.91	7,727.8 a[z]	-31.98
1.22	7,453.7 b	-31.98
1.52	7,218.7 bc	-31.98
1.83	7,049.04 c	-31.98

[z]Letters indicate significant differences using Turkey's HSD ($\alpha = 0.05$)

Figures

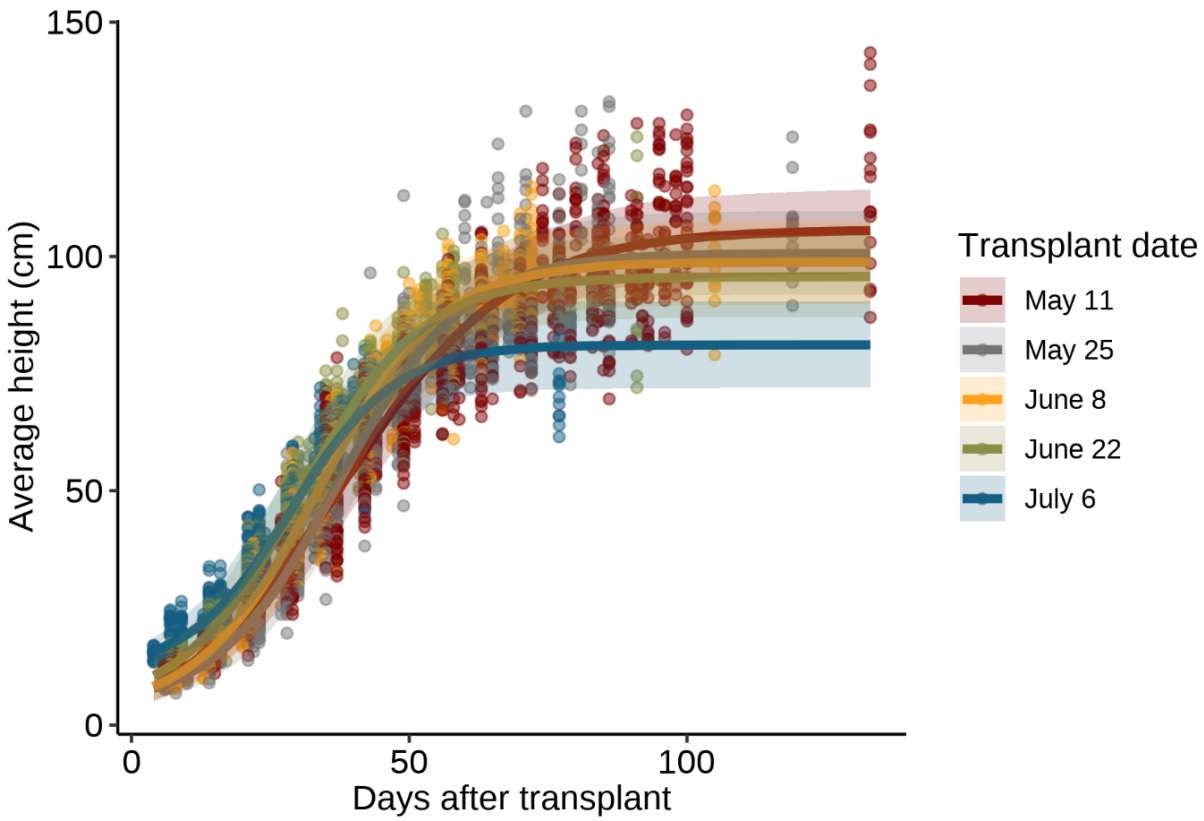

Figure 1.1. Four-parameter logistic regression of temporal plant height in floral hemp as affected by transplant date throughout the vegetative growing period. Shaded area represents 95% confidence interval. Day 0 represents initial day of transplant. Circles represent plot-level data points.

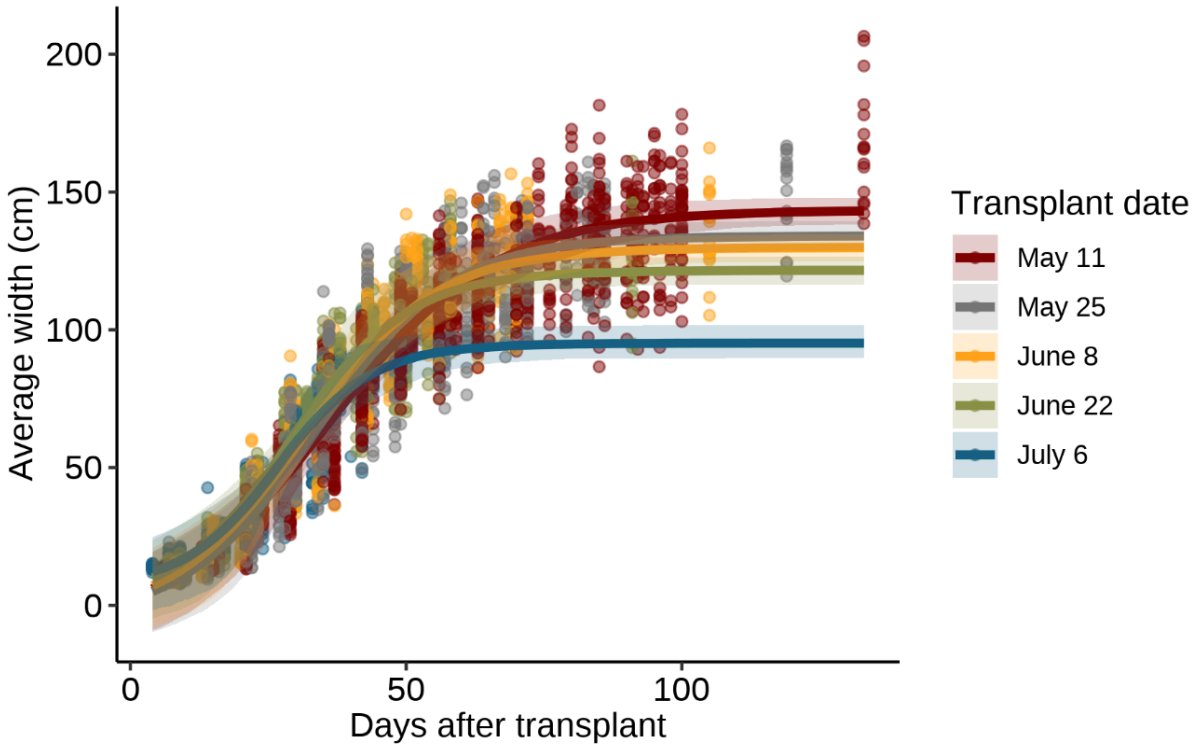

Figure 1.2. Four-parameter logistic regression of temporal plant width in floral hemp as affected by transplant date throughout the vegetative growing period. Shaded area represents 95% confidence interval. Day 0 represents initial day of transplant. Circles represent plot-level data points.

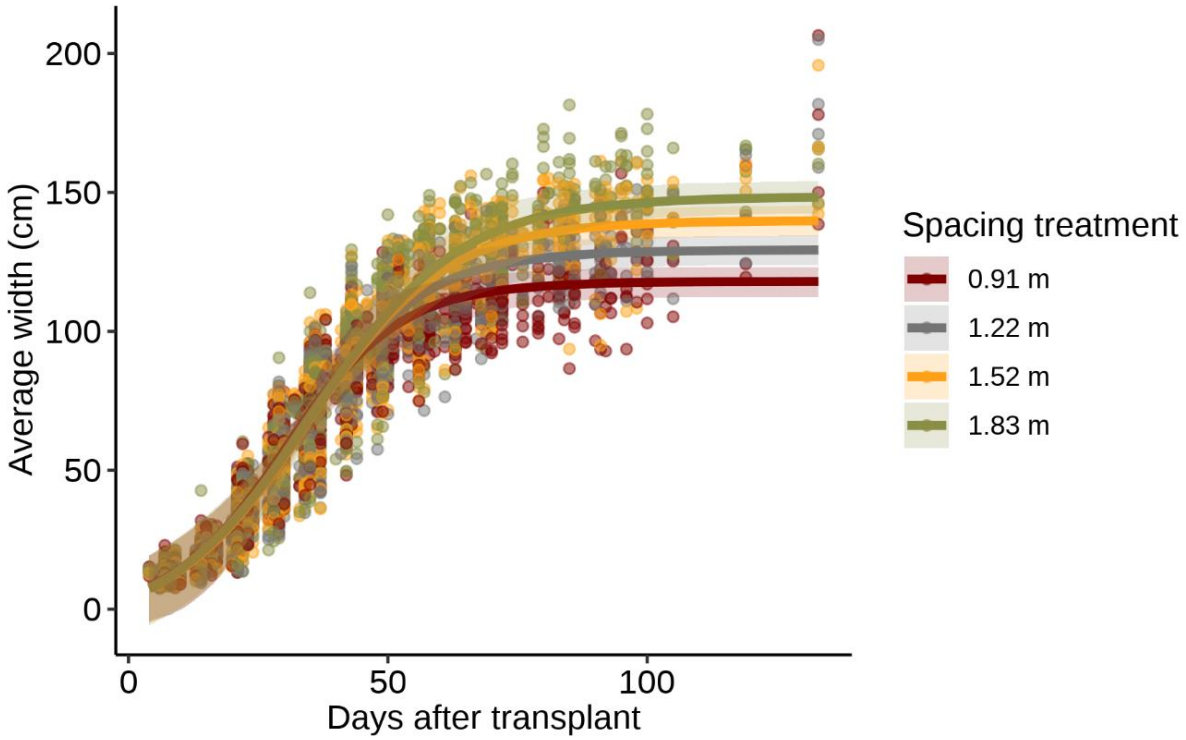

Figure 1.3. Four-parameter logistic regression of temporal plant width in floral hemp as affected by plant spacing throughout he vegetative growing period. Shaded area represents 95% confidence interval. Day 0 represents initial day of transplant. Circles represent plot-level data points.

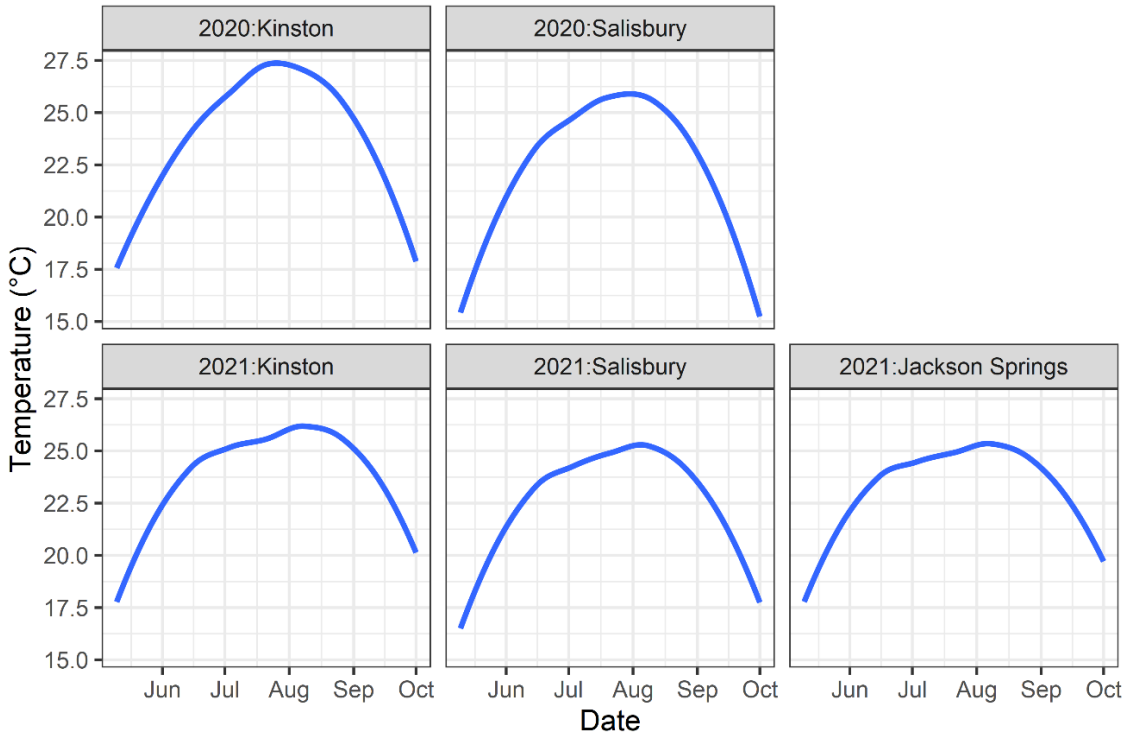

Figure 1.4. Average daily air temperature for the 2020 and 2021 growing seasons (planting through harvest) at field trial locations in Kinston, Salisbury, and Jackson springs, NC.

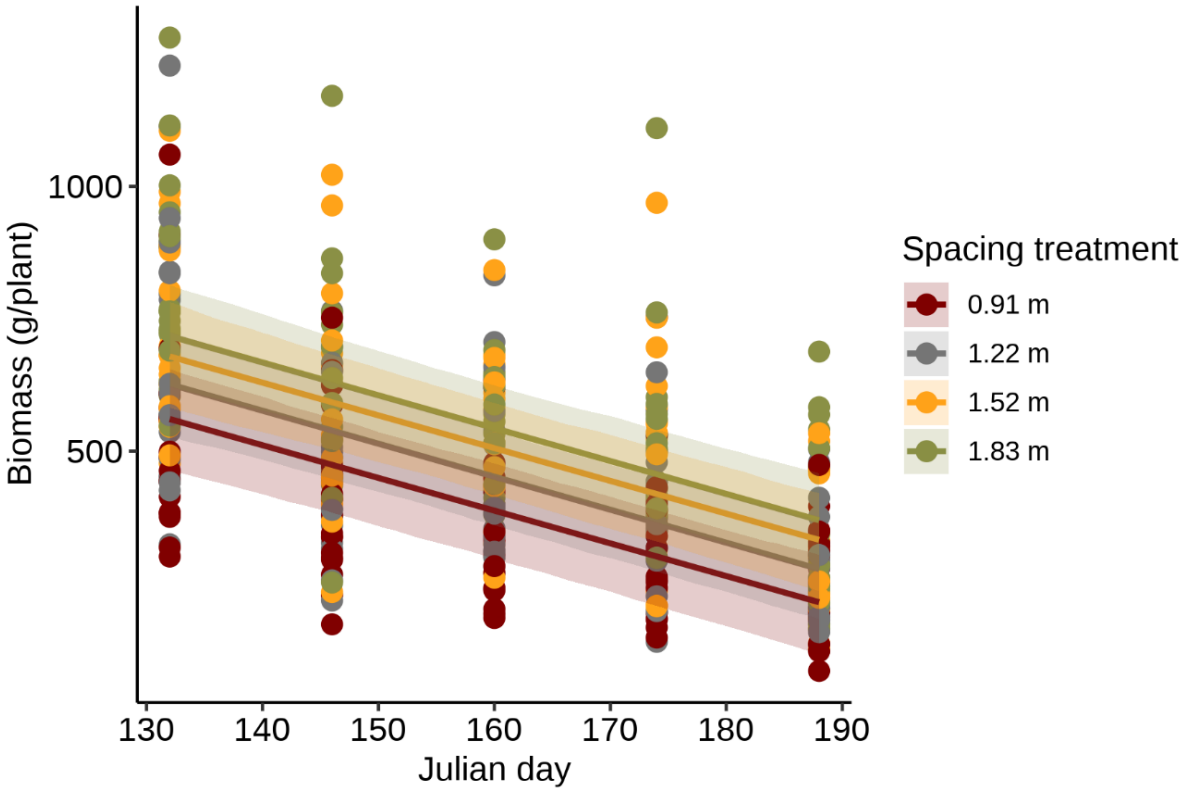

Figure 1.5. Linear response to individual floral hemp plant biomass affected by transplant date and spacing. Dates reported in Julian calendar days. 11 May transplant date = 132 Julian day, 25 May transplant date = 146 Julian date, 8 June transplant date = 160 Julian date, 22 June = 174 Julian date, and 6 July transplant date = 166 Julian date. Shaded area represents 95% confidence interval. Circles represent plot-level data points.

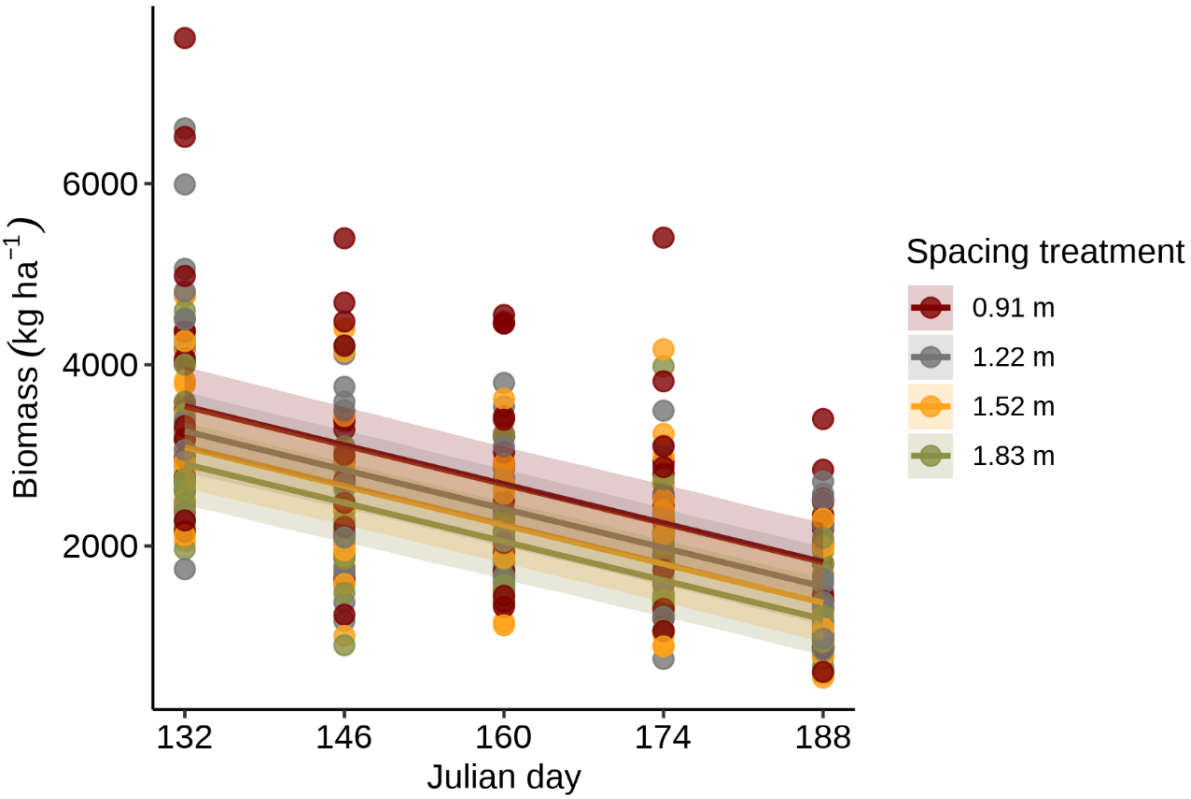

Figure 1.6. Liner response to biomass yield per hectare in floral hemp affected by transplant date and spacing. Dates reported in Julian calendar days. 11 May transplant date = 132 Julian day, 25 May transplant date = 146 Julian date, 8 June transplant date = 160 Julian date, 22 June = 174 Julian date, and 6 July transplant date = 166 Julian date. Shaded area represents 95% confidence interval. Circles represent plot-level data points.

Chapter 2:

The Effect of Harvest Date on Temporal Cannabinoid and Biomass Production in Floral Hemp (*Cannabis sativa* L.)

Abstract

During North Carolina's Hemp Pilot Program, approximately 10% of all tested fields were non-compliant due to farmers basing harvest timing on trichrome coloration. The objectives of this study were to model the temporal accumulation of CBD and THC in field-grown floral hemp in North Carolina and establish harvest timing recommendations to minimize non-compliant crop production. Field trials were conducted in 2020 and 2021 with the cultivar's 'BaOx' and 'Cherry Wine'. Trials utilized a split-plot randomized complete block design with cultivar as the main-plot and harvest date as the split-plot. Harvest events started 2 weeks after floral initiation and occurred every two weeks for 12 weeks. Per-plant threshed biomass accumulation exhibited a linear plateau trend. The best fit model for temporal accumulation of total THC was a beta growth curve. As harvest date was delayed total THC concentrations increased until concentrations reached their maximum, concentrations then decreased as plants approached senescence. Logistic regression was the best fit model for temporal accumulation of total CBD. Total CBD concentrations increased with later harvest dates. Unlike THC concentrations, there was no decline in total CBD concentrations. To minimize risk, growers should test their crop as early as possible within the USDA's 30 day compliance window. We observed 'BaOx' and 'Cherry Wine' exceeding the compliance threshold at 50 and 41days after flower initiation (DAFI), respectively. Therefore, to have a crop test below the compliance threshold, farmers growing these cultivars should have samples collected no later than 20 DAFI.

Introduction

The cultivation of *Cannabis sativa* L. for non-psychoactive cannabinoids has increased in recent years due to changes in legislation. With the passing of The American Agricultural Improvement Act of 2018 (2018 Farm Bill), *Cannabis sativa* L. plants containing less than 0.3% total tetrahydrocannabinol (THC) is classified as hemp and is federally legal to cultivate. Total THC is calculated as Δ^9-THC + 0.877 × tetrahydrocannabinolic acid (THCA) and is reported on a dry weight basis. Of the approximately 125 documented cannabinoids (Radwan et al., 2021), industry focus has been primarily on cannabidiol (CBD).

Cannabis is primarily a dioecious plant and when grown for cannabinoid extraction only female plants are cultivated due to the high concentration of glandular trichomes found on the female flowers. These glandular trichomes are the site of cannabinoid biosynthesis and storage (Happyana et al., 2013). Cannabidiolic acid (CBDA), Tetrahydrocannabinolic acid (THCA), and Cannabichromeneic acid (CBCA) are the three most abundant cannabinoids found in Cannabis. These acidic cannabinoids are synthesized from Cannabigerolic acid (CBGA) by their respective enzymes CBDA, THCA, and CBCA synthase (Perrotin-Brunel et al., 2011). Acidic cannabinoids may be decarboxylated in the presence of heat or light to their respective neutral counterpart's CBD, Δ^9-THC, and cannabichromene (CBC) (Dussy et al., 2005; Pellati et al., 2018; Perrotin-Brunel et al., 2011).

Depending on cannabinoid content, Cannabis can be classified into three major chemical types ("chemotypes"). Inheritance of chemotype can be modeled as a monogenic trait at one locus (*B* locus) with two codominant alleles (B_T & B_D). Chemotype I plants mainly produce THC and are classified as marijuana; these plants are homozygous with the B_T alleles. Chemotype II

plants produce roughly equal amounts of THC and CBD and are heterozygous with B_T /B_D alleles. Chemotype III plants are typically classified as hemp and produce mostly CBD with very low amounts of THC; individuals of this chemotype homozygous with B_D alleles (de Meijer et al., 2003).

High CBD:THC ratios are indicative of chemotype III Cannabis. Stack et al. (2020) conducted a study characterizing high cannabinoid hemp, by screening 30 cultivars with the majority being chemotype III Cannabis. Out of the purely chemotype III cultivars tested, CBD:THC ratios ranged from 22.09:1 to 27.45:1 (Stack et al., 2020). Chemotype III cultivars produce a small amount of THC which could potentially be problematic for growers since the legal THC compliant threshold is extremely low at 0.3% total THC on a dry weight basis. Zirpel et al. (2018) demonstrated that CBDA synthase (CBDAS) and THCA synthase (THCAS) are indiscriminate and produce multiple cannabinoids as a side product during the synthesis of their target product. Specifically, they demonstrated that CBDAS produces CBDA and THCA molecules at a ratio of approximately 20:1. This enzymatic promiscuity poses a significant challenge to floral hemp producers seeking to maximize profits via high CBD production while still maintaining a compliant crop (<0.3% total THC).

There have been relatively few modern replicated studies exploring temporal cannabinoid accumulation for high CBD yielding chemotype III cultivars. In general, the concentration of CBD and THC increase within the floral material after reproductive growth initiates; however, temporal accumulation trends may differ by cultivar (Aizpurua-Olaizola et al., 2016; De Baker et al., 2012; Massuela et al., 2016; Stack et al., 2020). At the onset of the legal hemp production, lacking any evidence based recommendations, farmers looked towards the marijuana industry for harvest timing recommendations. Marijuana growers historically base harvest timing on

trichome coloration (Rosenthal, 2017). As the plant matures, trichrome coloration transitions from clear to opaque and then amber in color with harvest occurring between the opaque and amber color transition. However, this method is highly subjective and would often result in a non-compliant crop; during the North Carolina Hemp Pilot Program, approximately 10% of all tested fields were non-compliant due to farmers utilizing this harvest timing method (Paul Adams, NCDA, personal communication).

The market value of floral hemp is determined on a percent CBD by weight basis. For growers to maximize profit and reduce risk it is crucial to have a harvest date that maximizes CBD concentration while ensuring the crop remains compliant. There is a current knowledge gap for growers regarding ideal harvest timing for floral hemp cultivated for CBD. The objective of this study were to: 1) model the temporal accumulation of CBD and THC in field-grown floral hemp in North Carolina and 2) establish harvest timing recommendations to minimize non-compliant crop production.

Materials and Methods

Experimental Design

Field trials were conducted during the 2020 and 2021 growing seasons at the Cunningham Research Station in Kinston, NC (CRS) on a Norfolk loamy sand (fine-loam, kaolinitic, thermic Type Kandiudults), and at the North Carolina Department of Agriculture & Consumer Services (NCDA&CS) Piedmont Research Station (PRS) in Salisbury, NC on a Clay Loam (Fine, kaolinitic, thermic Type Rhodic Kanhapludults).

Asexually propagated clones of the CBD hemp cultivars BaOx and Cherry Wine (Ryes Greenhouses; Broadway, NC) were used in both locations and growing seasons. Field trials were

arranged in a split-plot randomized complete block design with cultivar as the main-plot and harvest date being the split-plot. Each location contained four blocks. Transplanting occurred on 1 and 2 June of each year in Salisbury and Kinston, respectively. Clones were transplanted into raised beds covered in white 1.25 mm polyethylene plastic mulch with tape (Netafim Streamline 10 mil with 30.48 cm emitter spacing .908 LPH) laid under the plastic. Each plot contained 20 plants with a 1.5 m in-row and between row spacing.

Field Management

Seven days before transplanting herbicide was applied to the bare ground between rows. Herbicide applications utilized Paraquat (1.55 kg ai ha^{-}1, Gramoxone SL 3.0; Syngenta Basel, Switzerland), Napropamide (1.68 kg ai ha^{-}1, Devrinol 2 XT; United Phosphorus, Inc., King of Prussia, PA), and Pendimethalin (0.53 kg ai/ha^{-1}, Prowl H2O; BASF Ludwigshafen, Germany).

Season total fertilizer application was 134.5 Kg N ha^{-1}, 67 Kg P ha^{-1},134.5 Kg K ha^{-1} and 1.12 Kg B ha^{-1}. Half of the nitrogen, phosphorus and all the potassium was applied and incorporated as a pre-planting application. Calcium nitrate (Yaraliva Calcinit 15.5-0-0; Yara International, Oslo, Norway) and boron (Borate 21%B; Borates Plus Inc., Apopka, FL) was injected biweekly through fertigation to meet the remaining nitrogen, phosphorous and boron requirements.

Data Collection

Harvesting and data collection began when plants transitioned from vegetative to reproductive growth. We determined flower initiation when at least 50% of the plants showed visible pistillate inflorescence at the apical meristem as well as the lateral shoots. Three plants were randomly harvested within a plot starting 2 weeks after floral initiation and every two weeks after until 12 weeks after floral initiation (n = 6 harvest times). Plants were cut at the base

of the stalk approximately 5 cm form the soil line. Plants were then transported to a tobacco barn where they were dried under forced air at temperatures less than 48.8 °C for five days. Once dry, the floral and leaf material was stripped from the stalk by hand and the stalk was discarded. The weight of the floral and leaf material was recorded and a representative sample was submitted for cannabinoid analysis. Samples were analyzed for total CBD (CBD + 0.877×CBDA) and total THC (Δ^9 THC + 0.877×THCA) by Delta 9 Analytical (Raleigh, NC) and North Carolina State University Environmental and Agricultural Testing Services (EATS) Laboratory in 2020 and at the EATS Laboratory in 2021 by using UHPLC/MS/MS analysis. In short, the protocol for the analysis went as follows: 0.1 grams of dried ground plant material was added to a 15 mL centrifuge tube with 5mL of extraction solution consisting of 80% HPLC grade methanol and 20% HPLC grade water and was agitated for about 30 seconds with a vortex mixer. Then 1mL of sample extract was filtered through 0.2 μM syringe filter and loaded into an auto sampler vial. Two auto sampler vials were then prepared for the extract of each hemp sample, one at a dilution factor of 1:5 (df5) and the other at a dilution factor of 1:100 (df100). For the df5, 200 μL of sample extract and 800 μL of sample dilutant solution was added. For the df100, 10 μL of sample extract and 990 μL of sample dilutant solution was added. Sample dilutant solution consisted of 29% of HPLC water with LC/MS grade Formic Acid, and 71% HPLC grade Acetonitrile. Before the instrument analysis, a new calibration curve was made. Calibration curves were made using commercially available calibration standards containing 100 μg ml^{-1} of CBD, CBDA, CBN, CBG, THC, & THCA in methanol. After the calibration curve was made a quality control check sample was completed to verify recoveries were 100% ± 15% for each cannabinoid using commercial standards. Samples were analyzed in batches of 15 samples at their respective dilution factors, the column was washed, and a quality control verification was

completed between each batch of samples. Analysis was completed with a liquid chromatography equipped with photodiode array detector, the instrument parameters were as follows; Run time: 8.0 minutes, Mobile Phase: 29% Solvent 1 (HPLC Water): 71% Solvent 2 (MeCN), Injection volume: 10 µL, Flow rate: 1.0 mL/min, Tray Temperature 18 °C ± 2.0 °C, Column oven: 40 °C ± 2.0 °C, Column parameters: PerkinElmer Quasar SPP C18 column (150mm x 3.0mm, particle size 2.6 µm), UV/VIS wavelength: 228nm.

Statistical Analysis

Data were plotted and inspected for any outliers and to determine treatment response trends. The nlme and nlraa packages in R were utilized for all analyses (Miguez, 2021; Pinheiro et al., 2021; R Core Team, 2018). In all instances, cultivar and days after flower initiation (DAFI) were treated as fixed effects whereas environment (unique year × location combination), block nested in environment, and block × cultivar treated as random effects.

Biomass, total THC, and total CBD results showed strong nonlinear trends. Biomass data showed an asymptotic/plateau trend whereas total THC and CBD showed a distinct sigmoid trend. In all cases, multiple models were fit to these data (Tables 2.2 and 2.3) and resultant corrected Akaike Information Critera (AICc) and Bayesian Information Criteria (BIC) compared to select the best fitting model. Residual diagnostic plots were investigated, and heteroscedasticity was observed for the biomass data. The power variance structure ("varPower") ameliorated this heterogeneity and resulted in a better fit model (lower AICc and BIC compared to original model).

A linear mixed model was employed to investigate the total THC and total CBD relationship between cultivars. A similar approach was taken to compare total potential THC (total THC + CBN) and total CBD. The inclusion of CBN when calculating total potential THC

provides a more appropriate estimation of total enzymatic production of THC via CBDA synthase since CBN is the degradative product resulting from THC oxidation over time (Stack et al., 2021). Heteroskedasticity was observed in the residual diagnostic plots for both analyses and ameliorated by employing an exponential variance function structure ("varExp").

Results and Discussion

Biomass Accumulation

Logistic, linear-plateau, quadratic-plateau, and asymptotic functions were fit to the data and compared using their individual corrected Akaike Information Criteria (AICc) and Bayesian Information Criteria (BIC). The linear-plateau model had the lowest AICc and BIC (Table 1.1) and was selected as the best fit model for threshed biomass accumulation on a per-plant basis. The linear-plateau model is expressed as

$$Y = \begin{cases} A + B\text{x if } x < XS \\ Y_m \text{ if } x \geq XS \end{cases}$$

Where *Y* represents the response variable, *A* is the intercept, *B* is the slope, XS is the threshold level of *x* where the model plateaus (Y_m).

The effect of cultivar was not significant for the parameters estimates of *A* and *XS,* however, it was significant for the estimate of *B* (Table 2.3, Fig. 2.1). The estimate of parameter *B* or slope of the regression for the cultivar BaOx was 7.4 g day^{-1} 4.1 g day^{-1} for Cherry Wine. The effect of cultivar was not significant on the parameter *XS* and both reached maximum biomass at approximately 74 DAFI (Fig. 2.1).

There is a scarcity of information in the literature regarding post-anthesis temporal accumulation of biomass for floral hemp. Massuela et al. (2022) conducted a study in a controlled environment with chemotype III plants and found a significant affected of harvest time on inflorescence production. The general trend was an increase in inflorescences yield from 5 weeks post-anthesis when harvest events started to 11-week post-anthesis when the final harvest concluded. Interestingly, Massuela et al. (2022) did not observe a yield plateau in this study; however, the cultural practices applied in this study most likely influenced yield outcomes. Plants received a truncated growing period were vegetatively growth lasted for 28 days, which resulted in relatively small plants. Additionally, 95% of natural light was blocked with a shade cloth and only artificial light was used to induce flowering and throughout the remainder of the experiment. These cultural practices most likely limited yield potential and temporal biomass accumulation for the plants used in this study. Additional studies are needed to better characterize post-anthesis biomass accumulation of floral hemp.

Temporal Accumulation of Cannabinoids

Multiple sigmoid functions were fit to the temporal cannabinoid data and were compared using their respective corrected Akaike Information Criteria (AICc) and Bayesian Information Criteria (BIC). The best fit model for these data was a beta growth function (Table 2.2) which is expressed as:

$$w = W.max\left(1 + \frac{Te-x}{Te-Tm}\right)\left(\frac{x}{Te}\right)^{\frac{Te}{Te-Tm}} \text{ with } 0 \leq Tm < Te$$

Where *W.max* is the maximum observed THC concentration, *Te* is the time point at which *W.max* occurs, *Tm* is the time point where the maximum THC accumulation rate is obtained, and *x* is DAFI.

The effect of cultivar was significant on the parameters *W.max*, *Te*, and *Tm* (Table 2.4). Maximum total THC (*W.max*) was highest in 'BaOx' (0.454%) compared to 'Cherry Wine' (0.367%; Table 2.4, Figure 2.2). Time required to reach *W.max* (*Te*) for 'Cherry Wine' and 'BaOx' was 63.2 and 75.2 DAFI, respectively. A comparable trend was observed for the effect of cultivar on the estimate of the parameter *Tm* or the time at which the maximum THC accumulation rate is reached: 'Cherry Wine' reached its maximum accumulation rate before 'BaOx' at 16.5 and 44.7 DAFI, respectively. Additionally, 'Cherry Wine' reached the USDA THC compliance threshold at an earlier date at approximately 41 DAFI whereas 'BaOx' reached this threshold at 50 DAFI (Figure 2.2).

We observed a decline in total THC concentration with later harvest dates (Figure 2.2). THC is susceptible to non-enzymatic oxidation by exposure to oxygen, heat, and light. The products of the oxidative degradation of THCA and Δ^9 THC are Cannabinol (CBN) and to a lesser extent the isomer Delta-8 THC (Dussy et al., 2005; Moreno-Sanz 2016; Pellati et al., 2018). The decline in THC concentration associated with later harvest times is likely the result of the oxidization of THC.

Postanthesis temporal total CBD accumulation trends are comparable to total THC: a strong sigmoid relationship between DAFI and total CBD was observed (Fig. 2.3). Unlike total THC, we did not observe a decrease in total CBD concentration at the end of the trial. The best fit model for describing the relationship between DAFI and total CBD concentration was a logistic regression (Table 1.2), which is expressed as:

$$Y = \frac{W.max}{1 + e^{-scale(x - Xmid)}}$$

Where *Y* is total CBD concentration, *W.max* denotes the maximum CBD concentration, *scale* is the maximum CBD accumulation rate, *Xmid* the time point at the maximum accumulation rate, and *x* is DAFI.

The effect of cultivar was not significant on the parameters *W.max* or *scale* (Table 2.5), which were 13.54% and 12.4% $DAFI^{-1}$, respectively (Fig. 2.3). However, the effect of cultivar was significant for the estimate of *Xmid*; 'BaOx' reached the maximum CBD accumulation rate at 45.5 DAFI compared to 26.9 DAFI in 'Cherry Wine' (Table 2.5). We observed a CBD concentration plateau for the cultivar 'Cherry Wine'; however, we did not observe a plateau with 'BaOx' (Fig. 2.3).

Total CBD accumulation trends during reproductive growth were cultivar dependent (Fig. 2.3, Table 2.5). Neither of the trends presented a post plateau decline in total CBD concentration as observed in total THC (Fig. 2.2). Temporal accumulation of total CBD and total THC for day length sensitive chemotype III cultivars has been sparsely documented. Stack et al. (2021), Aizpurua-Olaizola et al. (2016), and Yang et al. (2020) reported temporal accumulation of THC that generally fits a beta growth curve model. Specifically, these authors showed a strong sigmoid accumulation pattern in total THC followed by a decline towards the end of their studies. Additionally, temporal accumulation of THC for day length sensitive chemotype I cultivars exhibit a similar growth curve (De Baker et al., 2012). However, in the literature there is considerable variation in trends for the temporal accumulation of total CBD for day length sensitive chemotype III cultivars. Yang et al. (2020) who conducted trials in Florida observed a decrease in total CBD concentrations after concentrations plateaued. Aizpurua-Olaizole et al. (2016) did not observe a post-plateau decline in total CBD concentrations, but rather a continued increase in concentrations until plants were harvested. Stack et al. (2021) tested 30 hemp

cultivars and observed three separate trends in total CBD accumulation where concentrations either plateaued, continued to increase until harvest, or decreased after plateauing. Our results, in combination with prior published work, indicate that total CBD accumulation rates differ by genotype.

Yang et al. (2020) reported a post plateau decline in CBD concentrations for the cultivar 'Cherry Wine' starting at approximately 6 weeks postanthesis. In our study we did not observe the same trend. Instead, we observed CBD concentrations leveling off after they had reached a plateau. Studies have shown that cannabinoid concentrations are primarily controlled by genotype (Campbell et al., 2019) and are generally not influenced by environmental stress (Toth et al., 2021). It is likely that the variation found between studies for the cultivar Cherry Wine may have been due to inconsistent genetics. Unfortunately, there are many 'Cherry Wine' floral hemp cultivars sold and not all of them come from the same stock. This complicates research and, more importantly, can have negative implications for farmers expecting one cultivar that is not true to type.

In our study, the absence of post plateau decline with CBD may be related to the stability of the compound. While there is a lack of literature regarding the stability of CBD within the plant, there have been several studies that investigate the stability of cannabis oil and cannabinoids outside the plant. Yangsud et al. (2021) isolated and purified Δ^9 THC, CBD, and CBN from seized drug type *Cannabis sativa* L. and investigated each compound's stability against multiple modes of degradation. When compared with THC, CBD is slightly more stable against oxidation and thermal degradation. However, CBD was considerably less stable than THC when exposed to acid and alkaline degradation. Additionally, CBD was slightly less stable than THC when exposed to photo-degradation. Trofin et al. (2012) observed the decay of CBD

and Δ^9 THC in seized cannabis oil over a period of four years in darkness at 4°C and exposed to laboratory light at 22°C. Over the four-year period the decay of THC amounted to an 83.75% loss at 4°C in darkness, and 89.58% loss at 22°C with light exposure. The decay of CBD amounted to a 40.81% loss at 4°C in darkness, and 44.85% loss at 22°C with light exposure. The results from both studies indicate that CBD is less susceptible than THC to degradation under normal field conditions. This reduced affinity for degradation may explain why we did not observe a post plateau decline in total CBD concentration as the plants began to senesce.

Cannabinoid Ratios

As discussed, Δ^9 THC and THCA can be converted to CBN and Δ^8 THC by non-enzymatic oxidative degradation. Therefore, we determined the CBD:THC ratio by two different methods. First, by including total potential THC (Δ^9 THC + 0.877×THCA+CBN) and second, by only including total THC (Δ^9 THC + 0.877×THCA). No Δ^8 THC was found in any of the samples, thus not included when calculating total potential THC. Multiple linear regression was used to model the relationships between cultivar, total CBD, total THC, and total potential THC. The linear regression describing the CBD:THCP relationship indicated a significant interaction between THCP and cultivar (Table 2.6; Fig. 2.4A). This interaction indicates that the relationship between total potential THC and CBD is cultivar dependent. The slope of the regression indicates a CBD:THCP ratio of 16.2:1 for the cultivar BaOx and 18.2:1 for Cherry wine (Table 2.6).

There was no significant interaction between total THC and cultivar on CBD for the linear regression explaining the CBD:total THC relationship (Table 2.6). However, both main effects of total THC and cultivar significantly affected total CBD. Both cultivars had a shared

slope of 31.0 (Table 2.6, Fig. 2.4B). The mutual slopes for both cultivars indicate that 'BaOx' and 'Cherry Wine' share a CBD:total THC ratio of 31:1. The two cultivars had a slightly different y-intercepts: 'BaOx' had a y-intercept of -0.253% while 'Cherry Wine' had a y-intercept of -0.728% (Table 2.6). The slightly higher y-intercept observed with 'BaOx' may indicate that the cultivar is predisposed to produce slightly more CBD.

Both CBDA and THCA are produced from the substrate cannabigerolic acid (CBGA) by CBDA synthase (CBDAS) and THCA synthase (THCAS), respectively (Sirikantaramas et al., 2004; Taura et al., 2007). Cannabinoid content and chemotype is largely determined by the expression of synthase enzymes CBDAS and THCAS. Until recently, chemotype was largely modeled as a monogenic trait through Mendelian genetics. de Meijer et al. (2003) observed a 1:2:1 proportion when a crossing a type I chemotype (high THC) with type III chemotype (high CBD) and proposed a model involving one locus with two codominant alleles. Recently, the genetic structure of these cannabinoid biosynthesis genes was mapped (Grassa et al., 2018; Laverty et al., 2019). The biosynthesis genes responsible for THCAS and CBDAS are non-allelic, generally un-linked and are not at equivalent loci. Furthermore, these genes are concentrated near the ends of chromosomes and are contained in regions where chromosomal scaffolds are physically linked and very non-homologous which leads to low recombination rates (Laverty et al., 2019).

Zirpel et al. (2018) demonstrated that CBDAS produces CBCA and THCA as side products during the synthesis of CBD, each of these side products are produced at about 5% of the CBDA amount. At optimal pH the *in vitro* expression of wild-type CBDAS resulted in the production of a CBD:THC ratio very close to 20:1 (Zirpel et al., 2018). The high CBD producing cultivars used in this study are often referred to as chemotype III cultivars. In chemotype III

plants, THCA is produced predominantly as a side product through the action of CBDAS (Toth et al., 2020). Therefore, the CBD:THC ratio can be interpreted as a metric depicting the efficiency of the CBDAS enzyme for the given chemotype III cultivar. Depending on the harvest date and cultivar, excluding CBN from the THC fraction of the CBD:THC ratio could result in an exaggerated ratio. Without including CBN, our CBD:THC ratio was 31:1 which is significantly higher than the average ratio reported in Zirpel et al. (2018). This inflated ratio is likely the result of harvest events occurring after THC concentrations plateaued and began to decline (Fig. 2.2). After including CBN our CBD:THCP ratio was closer to 20:1 with ratios at 16.2:1, and 18.2:1 for 'BaOx' and 'Cherry Wine', respectively. Excluding CBN form the THC fraction of the CBD:THC ratio resulted in an inflated ratio which masked the interaction between the main effects found in the regression associated with the CBD:THCP (Table 2.6). An accurate CBD:THC ratio is an essential tool for hemp breeders and growers as it indicates the efficiency of CBDAS.

Originally, the American Agricultural Improvement Act of 2018 (2018 Farm Bill) outlined the regulatory framework where growers had a 15-day window between compliance testing and time of harvest. Recently, the USDA published a final rule which became effective on March 22, 2021. This final rule provides revised regulations for the domestic production of hemp. As part of this final rule the window between compliance testing and harvest was extended from 15 to 30 days. To minimize risk, growers should have their crop tested as early as possible within this 30 day window. We observed 'BaOx' and 'Cherry Wine' exceeding the compliance threshold at 50 and 41 DAFI, respectively (Figure 2.2). Therefore, to have a crop test below the compliance threshold and remain there at the time of harvest, farmers growing 'BaOx' and 'Cherry Wine' should have samples collected no later than 20 DAFI

Conclusions

The purpose of this study was to model the temporal accumulation of CBD and THC in floral hemp so we could establish harvest timing recommendations to minimize non-compliant crop production. Per-plant threshed biomass accumulation exhibited a linear plateau trend. As harvest date was delayed the cultivar 'BaOx' accumulated biomass at a rate 7.4 g day^{-1} and 'Cherry Wine' accumulated 4.1 g day^{-1}. The threshed biomass accumulation rate plateaued for both cultivars at approximately 74 DAFI (Fig. 2.1).

The best fit model for postanthesis temporal accumulation of total THC was a beta growth curve. As Harvest date was delayed total THC concentrations increased until concentrations reached their maximum. After concentrations peaked, we observed a decrease in THC concentration as plants approached senescence. This decline in THC concentrations can be attributed to non-enzymatic oxidative degradation of the THC molecule (Dussy et al., 2005; Moreno-Sanz 2016; Pellati et al., 2018). The cultivar 'BaOx' achieved a slightly higher maximum THC concentration than the cultivar 'Cherry Wine' at 0.454% and 0.367% (Table 2.4, Figure 2.2) respectively. 'Cherry Wine' reached its maximum accumulation rate before 'BaOx' at 16.5 and 44.7 DAFI, respectively. Furthermore, 'Cherry Wine' reached the USDA THC compliance threshold at an earlier date than 'BaOx' at 41 DAFI and 50 DAFI (Figure 2.2) respectively.

The best fit model for postanthesis temporal accumulation of total CBD was a logistic regression. Total CBD concentrations increased as harvest date was delayed. Unlike THC concentrations, we did not observe a decline in total CBD concentrations with later harvest dates. Both 'BaOx' and 'Cherry Wine' had similar maximum total CBD concentrations at 13.54% and 12.4% $DAFI^{-1}$, respectively (Fig. 2.3). However, temporal accumulation trends differed by

cultivar. ‘Cherry Wine’ reached its maximum CBD accumulation rate much earlier than ‘BaOx” at 26.9 DAFI compared to 45.5 DAFI (Table 2.5). Additionally, ‘Cherry Wine’ reached a maximum CBD concentration plateau before senescence however, we did not observe a maximum CBD concentration plateau for ‘BaOx’. In the literature there is considerable variation in trends for the temporal accumulation of total CBD for day length sensitive chemotype III cultivars (Aizpurua-Olaizole et al., 2016; Stack et al., 2021; Yang et al., 2020). Ultimately, more research is warranted to better characterize temporal CBD accumulation trends as they relate to cultivar.

THC degrades through non enzymatic degradation oxidation to produce CBN. The CBD:THCP ratio includes CBN in the THC fraction of the ratio. The relationship between total potential THC and CBD is cultivar dependent. We observed a CBD:THCP ratio of 16.2:1 for the cultivar BaOx and 18.2:1 for Cherry wine (Table 2.6). Excluding CBN from the THC fraction of the CBD:THC ratio resulted in an inflated ratio of 31.0:1 for both cultivars. By including CBN in the THC fraction of the CBD:THCP ratio we observed a ratio much closer to 20:1 ratio achieved by the in vitro expression of wild-type CBDAS at optimal pH (Zirpel et al., 2018). Having an accurate CBD:THC ratio is an essential tool for breeders and growers as it indicates the efficiency of CBDAS.

The USDA’s final rule extended the time frame between compliance testing and harvest from 15 days to 30 days. To minimize risk, growers should have their crop tested as early as possible within this 30 day window. Our results showed that ‘BaOx’ and ‘Cherry Wine’ exceeding the compliance threshold at 50 and 41 DAFI, respectively (Figure 2.2). Therefore, to have a crop test below the compliance threshold and remain there at the time of harvest, farmers growing ‘BaOx’ and ‘Cherry Wine’ should have samples collected no later than 20 DAFI. It

should be noted that these results may not be applicable to other hemp cultivars. Furthermore, these trials were conducted in the Southeast at a latitude of approximately 35.7° N. producers in more southerly or northerly latitudes with differing summer day lengths may require early or later harvest timing, respectively, based on their unique weather and day length conditions.

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Tables

Table 2.1. Goodness-of-Fit criteria for mixed nonlinear asymptotic models describing temporal production of floral hemp threshed biomass.

Model	**Goodness-of-Fit Criteria[z]**	
	AICc	BIC
Logistic	6,885.408	6,919.173
Linear-plateau	6,877.525	6,911.289
Quadratic-plateau	6,880.481	6,922.607
Asymptotic	6,917.306	6,946.878

[z]AICc = Corrected Akaike's Information Criterion; BIC = Bayesian Infromation Criterion.

Table 2.2. Goodness-of-Fit criteria for mixed nonlinear sigmoid models describing the relationship between floral hemp total THC and CBD concentrations over time.

Response[z]	**Model**	**Goodness-of-Fit Criteria**[y]	
		AICc	BIC
Total THC %	Logistic	-1,403.124	-1,365.121
	Four-Parameter Logistic	-1,381.136	-1,351.522
	Gompertz	-1,372.822	-1,343.208
	Beta growth function	-1,432.406	-1,394.403
	Four-parameter Beta growth function	-1,422.664	-1,393.051
Total CBD %	Logistic	2,057.301	2,095.305
	Four-Parameter Logistic	2,136.931	2,166.54
	Gompertz	NC[x]	NC
	Beta growth function	2,161.043	2,199.047
	Four-parameter Beta growth function	2,186.523	2,216.137

[z]Total CBD % calculated as %CBD + 0.877 × CBDA; Total THC % calculated as Δ^9-THC + 0.877 × THCA.
[y]AICc = Corrected Akaike's Information Criterion; BIC = Bayesian Infromation Criterion.
[x]NC = No convergence.

Table 2.3. Parameter estimates of the linear plateau nonlinear regression for individual plant biomass production over time for the floral hemp cultivars BaOx and Cherry Wine.

	Parameter		
Cultivar	***A*** (g plant^{-1})	***B*** (g day^{-1})	***XS*** (DAFI)
BaOx	271.2	7.4	77.3
Cherry Wine	410.5	4.1	64.1
P-value	0.5923	<0.0001	0.1510

[a]Days after floral initiation.

Table 2.4. Parameters estimates of the beta growth curve for floral hemp total THC concentration over time for the floral hemp cultivars BaOx and Cherry Wine.

	Parameter		
Cultivar	***W.max*** (%)	***TE*** (DAFI)	***TM*** (DAFI)
BaOx	0.45	75.20	44.67
Cherry Wine	0.36	63.19	16.54
P-value	0.0187	<0.0001	<0.0001

[a]Days after floral initiation.

Table 2.5. Parameter estimates of the logistic model for floral hemp total CBD concentration over time for the floral hemp cultivars BaOx and Cherry Wine.

	Parameter		
Cultivar	***W.max*** (%)	***Xmid*** (DAFI[a])	***Scale*** (% DAFI^{-1})
BaOx	15.20	45.5	13.0
Cherry Wine	11.88	26.9	11.8
P-value	0.9958	<0.0001	0.3925

[a]Days after floral initiation.

Table 2.6. Linear regression coefficient estimates for models describing the linear relationship between floral hemp cultivars, total THC concentration, total potential THC concentration, and total CBD concentrations.

Cultivar	**Intercept**	**Slope**	**Intercept**	**Slope**
	----Total Potential THC[a]----		------Total THC[b]-----	
	%CBD	%CBD %THCP^{-1}	%CBD	%CBD %THC^{-1}
BaOx	-0.196	16.2	-0.253	30.6
Cherry Wine	-0.987	18.2	-0.728	31.5
P-value	0.0669	<0.0001	0.0340	0.0975

[a]Total potential THC calculated as Δ^9 THC + 0.877×THCA+CBN.

[b]Total THC calculated as Δ^9 THC + 0.877×THCA.

Figures

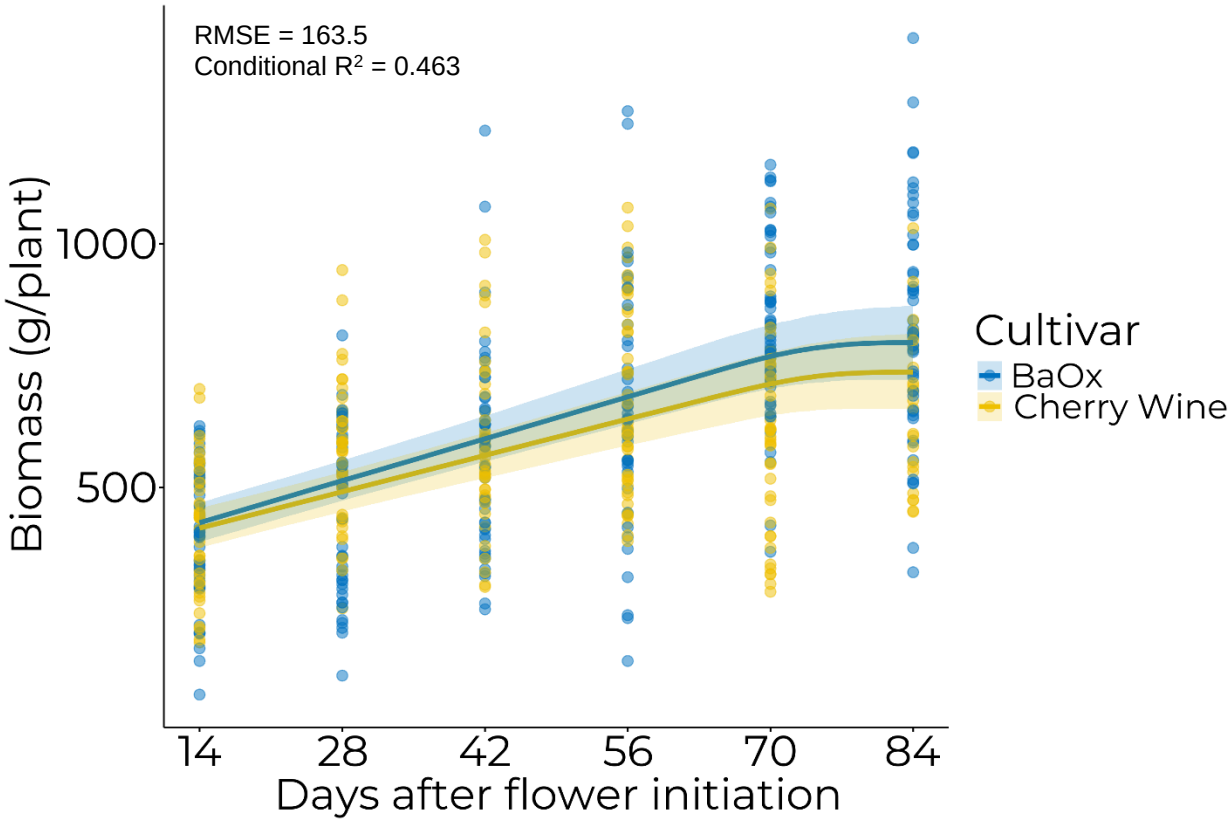

Figure 2.1. Linear-plateau regression for per-plant biomass over time as affected by days after flower initiation. Shaded area represents 95% confidence interval for the predicted model. Circles represent plot-level data points. RMSE = Root Mean Square Error

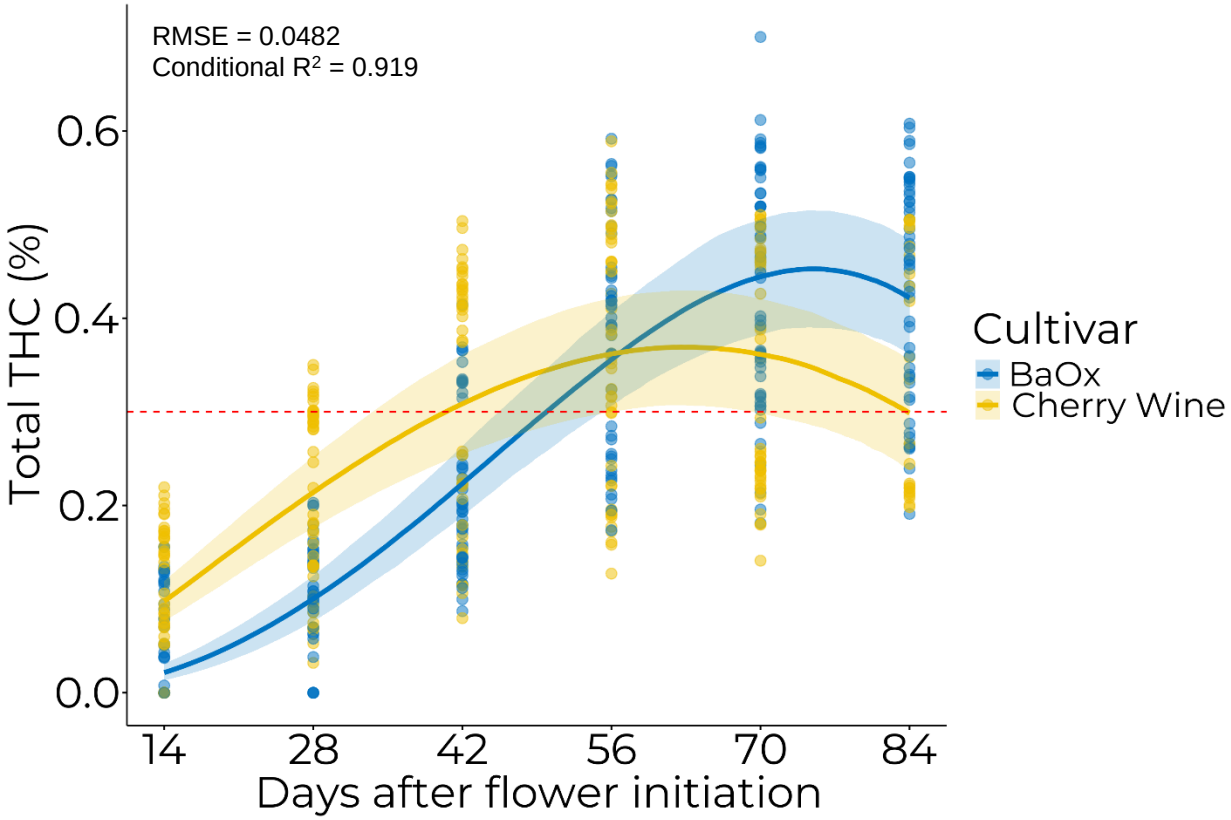

Figure 2.2. Beta growth curve nonlinear function for floral hemp total THC concentration over time as affected by cultivar. Red dashed line at 0.3% total THC represents the legal threshold. Shaded area represents 95% confidence interval for the predicted model. Circles represent plot-level data points. RMSE = Root Mean Square Error.

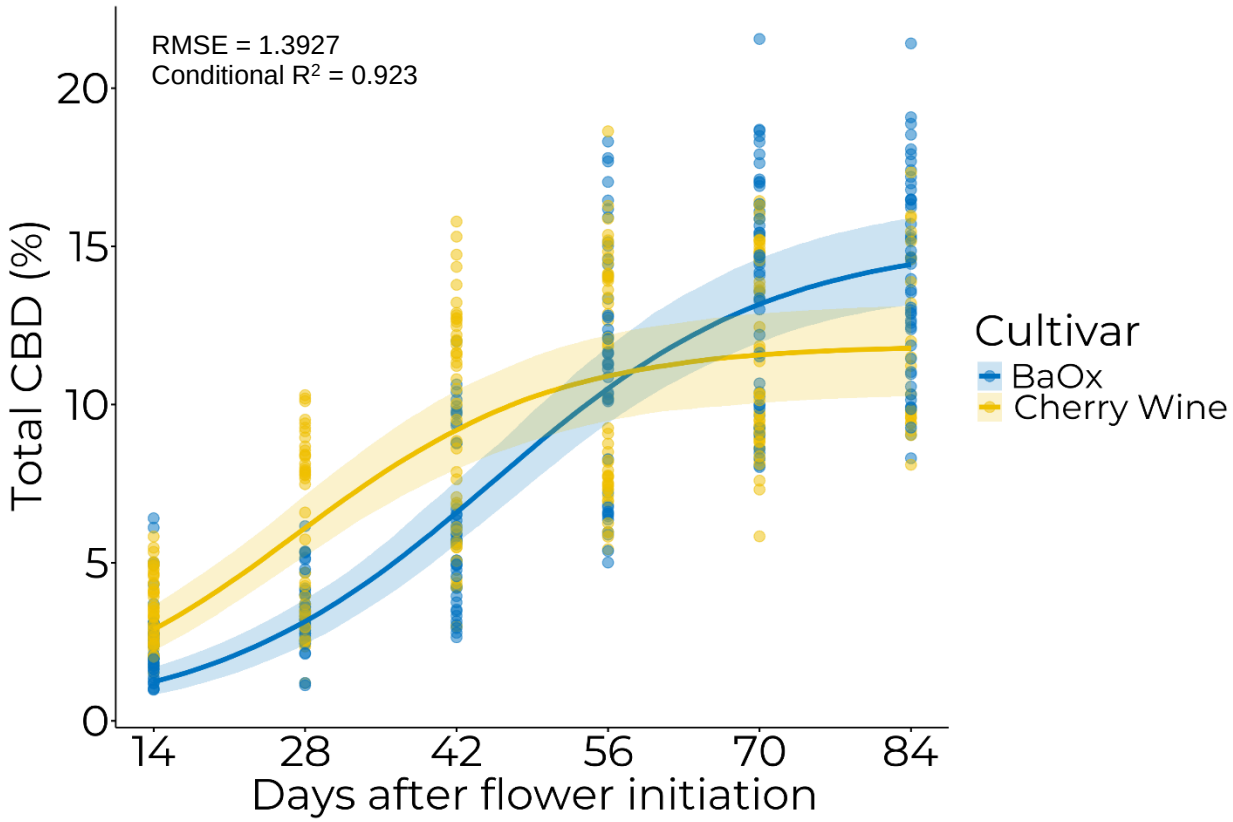

Figure 2.3. Nonlinear logistic regression for floral hemp total CBD concentration over time as affected by cultivar. Shaded area represents 95% confidence interval for the predicted model. Circles represent plot-level data points. RMSE = Root Mean Square Error.

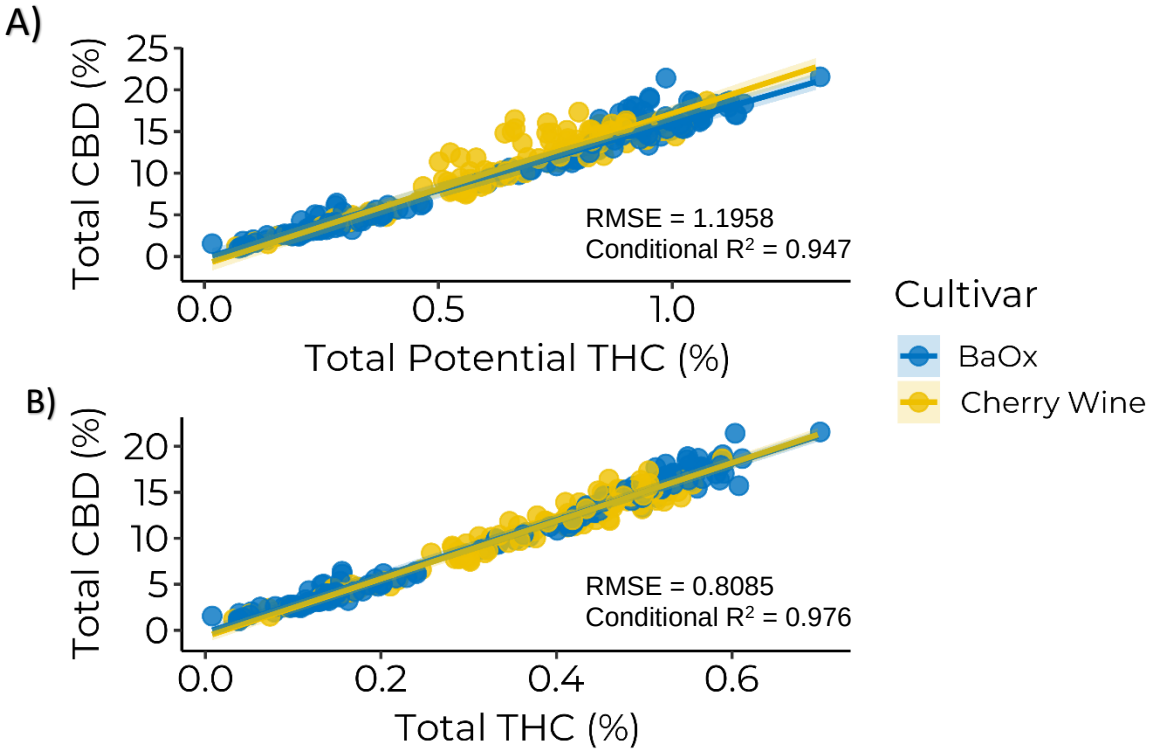

Figure 2.4. Linear regression for the effect of cultivar and total potential THC (A) and total THC (B) on total CBD. Total potential THC concentration calculated as Δ^9THC + 0.877×THCA + CBN, total THC concentration calculated as Δ^9THC + 0.877×THCA. Shaded area represents 95% confidence interval for the predicted model. Circles represent plot-level data points. RMSE = Root Mean Square Error.

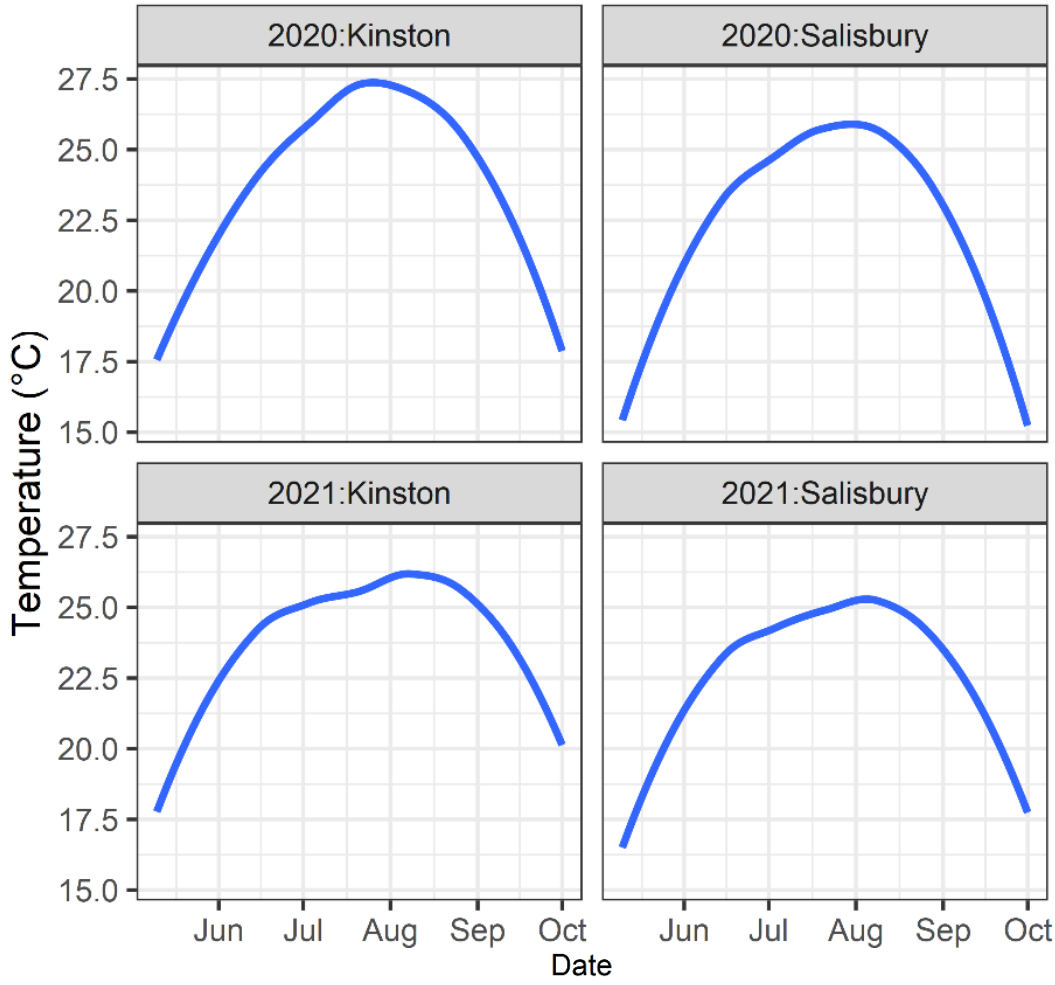

Figure 2.5. Average daily air temperature for the 2020 and 2021 growing seasons (planting through harvest) at field trial locations in Kinston, and Salisbury, NC.