ABSTRACT

HALKER, CHINA ALLISSA PAIGE. Development of Sustainable Fertility Options in Floral Hemp and Investigation of New Fiber Hemp Genetics for North Carolina. (Under the direction of Dr. David Suchoff).

Preliminary floral hemp (*Cannabis sativa* L. <0.30% total tetrahydrocannabinol) studies establishing conventional fertilizer rates, as well as adequate planting and harvesting times for Southeastern environments have been explored. In addition to the growing body of knowledge from past literature, we sought to explore ways to meet the everchanging environmental stressors of modern-day agriculture. The first objective of this study was to investigate fall planted leguminous cover crops as a full or partial organic nitrogen replacement for floral hemp production systems. The second objective was to compare bare ground and plasticulture bedding with and without the use of leguminous cover crops. The final objective was to develop grower recommendations that allow for maximum yield, quality, and reduce overall grower inputs. In general, the combination of leguminous cover crops with plasticulture bedding helped maintain plant available nitrogen throughout the season. Particularly, hairy vetch as a leguminous cover crop combined with plastic beds; together achieved 79% of our highest organic nitrogen control biomass yields. Our results documented the effects of organic nitrogen and bare ground versus plasticulture bedding systems on floral hemp, and should be utilized for future grower recommendations.

Interest in fiber hemp continues to rise across the U.S; however, lack of regionally-appropriate genetics for the Southeast has been a limiting factor. The first objective of this study was to investigate a broad panel of Chinese fiber hemp genetics intended for textile-grade fiber production. The second objective was to compare planting dates (mid-March, mid-April, and mid-May) for all genetics investigated. The final objective was to develop grower recommendations that allow for maximum crop yield and quality. In general, we achieved higher yield biomass and thicker stem width compared to previously-reported European genetics. Obtaining thinner stems may be possible with earlier harvest dates, although this point requires further evaluation. Planting date differences were minimal, and freezing temperatures experienced in our early planting date treatment showed no negative effect on crop growth. Taken together, fiber hemp has a wide planting window in North Carolina, affording growers flexibility when deciding when to plant. We saw minimal differences among the Chinese fiber hemp genetics investigated. Much of the differences appear to be due to seed quality and stand establishment than inherent genetic differences.

Development of Sustainable Fertility Options in Floral Hemp and Investigation of New Fiber Hemp Genetics for North Carolina

by
China Allissa Paige Halker

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DEDICATION

This is dedicated to our fallen brothers and sisters from NCSU. Your presence, and impact on the community will never be forgotten. To all the LGBTQIA+ identifying peoples, women in STEM, and underserved populations in our education systems, your persistence in these spaces fuels the change needed in this world. To my wonderfully loving, supportive, friends and family; without the acknowledgment and care you've all offered me over the years, I would not be where I am today. Thank you, from the bottom of my heart, this is for you all.

BIOGRAPHY

China Allissa Paige Halker was born on July 14, 1998 to Tonya Baker and Zachary Halker in Thomasville, North Carolina. Allissa graduated from First Flight High School in 2016, where she transitioned to college at UNC Asheville for one year and was enrolled in environmental exploratory studies. The combination of growing up on the Outer Banks and the year spent in Asheville fueled her passion for sustainability and the environment; where she would make the transition to North Carolina State University and major in Horticulture for her Undergraduate, being awarded her degree in 2020. While working on her undergraduate career, Allissa held numerous internships and made the dean's list her final four semesters. Throughout her internship opportunities she worked in many greenhouse environments with crops such as; clary sage, floral hemp, tobacco, and turf grass. Which shortly thereafter, led her to begin a Master of Science in Crop and Soil Science working with the Alternative Crops Lab at NCSU. Upon completion of this degree, Allissa hopes to continue her efforts implementing textile grade fiber hemp production as an alternative crop in North Carolina.

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TABLE OF CONTENTS

Chapter 1: Evaluation of Leguminous Cover Crops as a Nitrogen Source for Floral Hemp (*Cannabis sativa* L.) in Bare-Ground and Plasticulture Systems

Chapter 2: Establishing Planting Dates and Variety Recommendations for Fiber hemp (Cannabis sativa L.) Grown in North Carolina

LIST OF TABLES

Chapter 1: Evaluation of Leguminous Cover Crops as a Nitrogen Source for Floral Hemp (Cannabis sativa L.) in Bare-Ground and Plasticulture Systems

Chapter 2: Establishing Planting Dates and Variety Recommendations for Fiber hemp (Cannabis sativa L.) Grown in North Carolina

LIST OF FIGURES

Chapter 1: Evaluation of Leguminous Cover Crops as a Nitrogen Source for Floral Hemp (Cannabis sativa L.) in Bare-Ground and Plasticulture Systems

Chapter 2: Establishing Planting Dates and Variety Recommendations for Fiber hemp (Cannabis sativa L.) Grown in North Carolina

CHAPTER 1

Evaluation of Leguminous Cover Crops as a Nitrogen Source for Floral Hemp (*Cannabis sativa* L.) in Bare-Ground and Plasticulture Systems

Abstract

Field trials were conducted in 2021 and 2022 to evaluate three fall-planted leguminous cover crops (Austrian Winter Pea [*Pisum sativum* spp. *sativum*], Crimson Clover [*Trifolium incarnatum*], and Hairy Vetch [*Vicia villosa*]) as a full or partial replacement for external nitrogen fertilizer. Two fertilizer treatments (0 kg N ha^{-1} and 168 kg N ha^{-1}) were also included. Trials were conducted in Clinton, Kinston, and Salisbury, North Carolina. Cover crop treatments were planted in early November, 2020, and mid-October, 2021, and terminated two weeks prior to bedding and transplanting (early May, 2021, and mid-May, 2022). Each location was arranged as a strip-plot randomized complete block design with four blocks where the cover crop was the main plot and the bedding system (bare ground with irrigation or plastic mulch with irrigation) the subplot. Asexually propagated transplants of the CBD floral hemp cultivar BaOx were used at all locations. Data collection included cover crop biomass and nitrogen concentration at termination, plant available nitrogen and tissue nitrogen content throughout the season, plant height and width at harvest, bucked biomass and cannabinoid profiles. In general, we saw the use of plastic mulch combined with leguminous cover crops as a nitrogen source can help maintain plant available nitrogen throughout the season. In particular, we found that using hairy vetch in combination with plastic mulch can result in 79% of the biomass yields achieved with 168 kg N ha^{-1} control. These results indicate that external nitrogen inputs can be significantly reduced for floral hemp production when proper cover crop and mulch systems are utilized.

Introduction

Floral hemp ([FH]; *Cannabis sativa* L. <0.30% total tetrahydrocannabinol) is a newly legal crop produced for the high concentrations of cannabinoids in the female flowers. Initial research efforts have focused on developing sound agronomic practices that maximize floral hemp yield and quality. Ideal transplant dates and harvest timing for Southeastern production have been established (Linder et al. 2022a, 2022b) using bedded polyethylene mulch and drip systems (“plasticulture”). Conventional fertilizer rates for floral hemp have been established, mimicking flue-cured tobacco (*Nicotiana tabacum* L.) production systems (James et al. 2023).

Controlled environment investigations with floral hemp have facilitated a baseline for hemp nutrient requirements. Greenhouse studies characterizing nutrient disorders created a model for growers, helping them develop a sense of understanding, regarding nutrient deficiency and toxicity symptoms, optimum synthetic fertilizer requirements in greenhouse production, and varying synthetic nitrogen fertigation effects on cannabinoid profiles (Anderson et al., 2021; Cockson et al., 2019a; Landis et al., 2019b). Field trials in the Southeast are limited, though these studies set the foundation for larger field operations. Synthetic nitrogen recommendations for floral hemp biomass production in NC has been reported to plateau at a rate 86.8 kg ha^{-1} (James et al. 2021). This finding allows producers to fine tune input requirements, while still maximizing biomass production. Next steps for hemp production require finding sustainable solutions to meet the nutrient demand to lessen producer inputs and integrate more climate-smart practices.

The utilization of leguminous cover crops in the Southeast has been explored as an organic nitrogen source for subsequent cash crops (Parr et al., 2011; Poffenbarger et al., 2015; Vann et al., 2019). Parr et al. (2011) studied the biological nitrogen fixation potential and total nitrogen accumulation among sixteen winter annual cover crops prior to no-till organic corn (*Zea mays* L.) production systems in North Carolina. The authors observed maximum corn yield obtained from a hairy vetch ([HV];*Vicia villosa* ‘Roth’) monoculture or combined in cereal rye ([CR];*Secale cereale* L.) biculture. Poffenbarger et al. (2015) explored biomass production and nitrogen content of HV and CR in Maryland. The authors found the combination of no more than 50% HV in a CR:HV biculture increased nitrogen content and decreased C:N ratio compared to CR alone. Vann et al. (2019) conducted a study investigating five winter pea (*Pisum sativum* L.) genotypes, one crimson clover (*Trifolium incarnatum*) cultivar, one HV cultivar, in combination with barley (*Hordeum vulgare*), oats (*Avena sativa*), and wheat (*Triticum aestivum*) across environments in Maryland and North Carolina. The authors reported HV having the highest percent cover (visual estimation) leguminous cover crop, regardless of environment; in some cases, out competing small-grain production. The authors found variability in total biomass among cover crops and location, suggesting the need for tailored species selection with growing environment. Although leguminous cover crops have been well-studied in the Southeast, research into the integration of this climate-smart practice in floral hemp systems does not exist.

Plasticulture is used in agriculture for a multitude of reasons including minimizing in-row weed emergence, regulating soil temperatures, and to improve soil moisture retention (Mormile et al., 2017). Plasticulture research is extensive in many horticultural crops, including but not limited to; sweet corn (*Zea mays* L. var. *saccharate),* strawberries (*Fragaria* x *ananassa* L.), tomatoes (*Solanum lycopersicum* L.), peppers (*Capsicum annuum* L.), radish (*Raphanus sativus* L.), and watermelon ([*Citrullus lanatus* var. *lanatus*]; Díaz-Pérez et al., 2022; Gazula et al., 2009; Nnamdi et al., 2023; Poling et al., 2005; Zhao et al., 2020). Although grower-use and research involving plasticulture in row crops is limited, interest exists. Machanoff et al. (2022)

investigated four colors of polyethylene mulch with flue-cured tobacco and determined that weed emergence was significantly reduced with the use of opaque mulches. The authors also found differences between their mulched tobacco yields compared to their bare-ground plots, where mulch yields increased by 290% during one year of their study. Dong et al. (2009) conducted a polyethylene mulch study with cotton (*Gossypium hirsutum* L.) in China and found that mulch helped with plant establishment and growth throughout the season compared to their bare ground control. Wang et al. (2021) examined soil temperature, root structure, yield, and quality of maize production in combination with biodegradable and polyethylene mulch. Their study concluded that, regardless of mulch type, no differences in yield were observed, suggesting that not all agriculture commodities are guaranteed to benefit from the use of plasticulture.

Currently, no research exists in floral hemp production comparing the effects of yield and quality from organic fertilizer sources with both bare-ground and plasticulture systems. Furthermore, a knowledge gap exists regarding the use of leguminous cover crops as a full- or partial nitrogen replacement for floral hemp. As such, the objectives of this study were to: 1) investigate three fall-planted leguminous cover crops as a full- or partial nitrogen replacement for floral hemp; 2) compare plasticulture and bare ground systems with- and without leguminous cover crops and; 3) develop farmer recommendations that allow for maximum yield, quality, and reduce overall farmer inputs.

Materials and Methods

Field Layout

Field experiments were conducted at the Cunningham Research Station (CRS) in Kinston (35°17'54.0"N 77°34'21.2"W) on Norfolk loamy sand (fine-loamy, siliceous, semiactive, acid, Type Fluvaquentic Endoaquepts), and the Horticultural Crops Research Station (HCRS) in Clinton (35°01'21.2"N 78°16'54.3"W) on Orangeburg loamy sand (fine-loamy, kaolinitic Type Kandiudults) in 2021 and 2022 and at the Piedmont Research Station (PRS) in Salisbury (35°41'47.8"N 80°37'23.2"W), North Carolina on Lloyd clay loam (fine, kaolinitic, Type Rhodic Kanhapludults) in 2022 (n=5 site years).

Five fertility treatments and two plasticulture treatments were evaluated. The fertility treatments included three fall-planted leguminous cover crop species (Austrian winter pea [Pisum sativum spp. arvense], crimson clover [Trifolium incarnatum], and hairy vetch [Vicia villosa]), and two bare-ground controls. Each of the five fertility treatments were randomly assigned to the block and planted in 15.2 × 6 m plots. Austrian winter pea (seeding rate 15 kg ha^{-1}), crimson cover (seeding rate 9 kg ha^{-1}), and hairy vetch (seeding rate 9 kg ha^{-1}) were planted using a grain drill with 19.05 cm spacing the first week of November 2020 and the second week of October 2021. The two controls were; bare ground (winter fallow) receiving 0 kg N ha^{-1} and bare ground receiving 168 kg N ha^{-1} (15-0-2, micro pelletized Allganic Nitrogen Plus, SQM Specialty Plant Nutrition, Atlanta, GA). All cover crops were terminated the first week of May 2021 and the second week of May 2022 using a rotary tiller, left to partially decompose for approximately two weeks before bedding and transplanting. All treatments received 100 kg K ha^{-1} (0-0-52, Allganic Soluble Potassium Sulfate, SQM Specialty Plant Nutrition, Atlanta, GA) and 1 kg B ha^{-1} (Borate 21%B, Borates Plus Inc., Apopka, FL), which were applied before bedding. The 168 kg N ha^{-1} bare ground control received 79 kg K ha^{-1} to account for the potassium added through the fertilizer source.

Two bedding treatments were also investigated: bare ground or plasticulture. Both bedding treatments contained drip irrigation lines (Streamline Plus 8 MIL Drip Tape with Flap, .20 GPH 30.48 cm Spacing, Berry Hill Irrigation, Buffalo Jct., VA). The plasticulture treatment contained both drip irrigation and plastic mulch (white/black, 152.4 cm x 1219.2 m, High Strength Embossed Polyethylene Film, Berry Hill Irrigation, Buffalo Jct., VA). Each bedding treatment was randomized within the block and was applied in two rows.

Experimental Design

Experimental design followed a strip-plot randomized complete block design with four blocks in both years at CRS and HCRS and three blocks in 2022 at PRS. Plots consisted of two rows each 15.2 long and 1.5 wide with a 3 m alley included in the final field layout to distinguish between fall-planted fertility treatments. Asexually propagated clones of the high CBD cultivar BaOx were used in all locations and provided by Triangle Hemp LLC (Durham, NC) in 2021 and Harrell Hemp Services (Hobgood, NC) in 2022. We hand transplanted ten clones per treatment row with 1.5 m between plants two weeks after termination of the fall planted cover crop. Plants in the 2022 season 168 kg ha^{-1} plasticulture treatments were replaced. Damage observed among all locations; 100% mortality experienced in Kinston, 70% in Clinton, and 50% in Salisbury, respectively, not in all plots.

Data Collection

Before the cover crop termination, biomass from each fertility treatment was collected. Crimson clover was at 90% floral senescence in 2021 and 2022 at the time of harvest, Austrian winter pea and hairy vetch were both at 50% floral senescence at the time of harvest in 2021 and

2022. We collected 1 m^2 of cover crop biomass before termination. This material was dried for 72 h at 60 °C, ground, and submitted to the North Carolina Department of Agriculture and Consumer Services (NCDA&CS) Agronomic Division for quantification of carbon and nitrogen. Soil temperature data were collected using Bluetooth-enabled data loggers (HOBO Tidbit MX Temperature 400', ONSET Brands, Bourne, MA). Data loggers were programmed to take soil temperature every hour and placed 25 mm into the ground at the beginning of each plot on the day of transplanting at HCRS and CRS in 2021 and 2022, and PRS in 2022. Data loggers in plasticulture rows were placed into the ground and then covered back up with plastic mulch to ensure temperature data accuracy. We collected the data loggers following the final harvest.

Soil samples were collected to quantify soil nitrogen status throughout the season. Four 20 cm soil cores were collected from the center of each plot starting at transplant and every other week until termination for both years at all locations. Soils were stored in a freezer (0 °C) until processes. Samples were extracted with 10 M KCl solution and submitted to EATS for nitrate nitrogen (NO3-N), nitrite nitrogen (NO2-N), and ammonium nitrogen (NH4-N) analysis using Lachat Quikchem Flow Injection.

Three weeks after transplant and every other week until termination, fifteen mature leaves (petiole included) per plot were removed from the axillary branch to quantify foliar tissue nitrogen. All leaves were removed from plants located in data rows (the inner row of a plot) to ensure data accuracy. Leaves were dried for 24 h at 60 °C, ground, and submitted to the NC State Environmental and Agricultural Testing Services (EATS) for quantification of leaf nitrate nitrogen (NO3-N) and nitrite nitrogen (NO2-N) nitrogen.

Leaf chlorophyll content was quantified in 2022 using a chlorophyll meter (Apogee Instruments MC-100, Apogee Instruments, INC., Logan, UT). Ten leaves (excluding the first and last plants from either row of the bedding treatment) were measured to give an average chlorophyll reading on a per-plot basis. Measurements were taken from the most recent fully expanded leaf at all locations starting roughly three weeks after transplant and every other week until harvest to coincide with foliar tissue nitrogen quantification.

Plants were harvested approximately 5 weeks after floral initiation (September 15, 2021 at CRS, September 20, 2021 at HCRS, August 29, 2022 at PRS, August 31, 2022 at CRS, and September 2, 2022 at HCRS). Three plants from the data row were randomly selected, plant height (measured from base to apical meristem), two widths (first measured from the widest apices of the plant, second measurement perpendicular to the first), cut at the base and dried in modified tobacco driers at 45 °C for approximately 24 h. Once dried, all leaf and floral material was stripped from stems by hand and the resultant biomass was weighed. A subsample of the threshed material was submitted for cannabinoid analysis at Delta 9 Analytical LLC (Raleigh, NC).

Statistical Analysis

All data were analyzed in SAS v. 9.4 (SAS Institute, Cary, NC) with the GLIMMIX procedure. Fertility and bedding treatments were treated as fixed effects. Yield, cannabinoid, plant height and width data were originally analyzed with environment (the unique year × location combination) treated as a fixed effect. No significant treatment × environment interaction was found, thus we decided to treat environment as random to increase our inference space. Block nested within environment, block × fertility treatment, and block × bedding treatment were treated as random effects. The latter two random effects were included in the model as error terms for the strip-plot design. Model residuals were investigated to ensure that the assumptions of ANOVA were met. Residuals for soils data in 2022 showed strong heteroscedasticity. We employed a log transformation of these data, which ameliorated the issue. Soil and plant tissue nitrogen results were analyzed by environment since sampling dates differed between years and among locations. These data were analyzed as repeated measures with a first-order autoregressive (AR(1)) variance-covariance matrix structure. This variance-covariance matrix structure was chosen as it resulted in the lowest corrected Akaike Information Criterion (AICc) when compared to other structures. When appropriate, least squares mean estimates were compared using Tukey's Honestly Significant Difference *post-hoc* means separation test.

Results and Discussion

Field Conditions

Weather patterns including total rainfall (cm) and average monthly temperature (°C) for both growing seasons were retrieved from the nearest Econet stations using North Carolina State Climate Office (NCSCO; Table 1.1). CRS and HCRS seasonal temperatures in 2021 were similar. Total rainfall from both locations in 2021 differed by 1.5 cm, with the latter of the two having the most abundant amount (75.8 cm) among all locations and years. CRS and HCRS temperatures in 2022 were comparable to each other. PRS temperatures were consistently lower across the growing season. Rainfall data results in 2022 were similar during the first month of the study, with the latter two locations being comparable and the former location being lower. HCRS had the highest total rainfall (44.9), followed by PRS (40.3), and CRS (39.7 cm).

Leguminous Cover Crop Biomass

Differences in biomass were not observed among leguminous cover crop treatments ($p = 0.5201$). Nitrogen content (%) among leguminous cover crop treatments were different ($p < 0.0001$; Table 1.2). Austrian winter pea (2.7% N) and hairy vetch (2.9% N) were not different from one another, but both were higher than crimson clover (1.7% N). Though differences in nitrogen content were observed, this did not result in different total nitrogen (kg N ha^{-1}) among the leguminous cover crop treatments ($p = 0.49$). Similarly, carbon content among leguminous cover crop treatments did not differ ($p = 0.07$). Finally, C:N ratio differed among the leguminous cover crop treatments ($p < 0.0001$); crimson clover had the highest ratio (32.9:1), followed by Austrian winter pea (17.9:1), and hairy vetch (17.7:1). No differences were observed between the latter two treatments.

Our leguminous cover crop biomass yields were lower than reported for the area. Parr et al. (2011) investigated biomass production, ideal termination date, and nitrogen accumulation from sixteen winter annual cover crop cultivars in organic corn production systems. The authors reported differences in average biomass among the cover crops investigated; hairy vetch and crimson clover produced 5.7 Mg ha^{-1} and 5.4 Mg ha^{-1}, respectively. To achieve maximum total N from early-maturing legume varieties (e.g., crimson clover) required termination from mid-to late April. Subsequently, the authors observed a precipitous decline of total N production at the onset of seed production and floral senescence. On the contrary, the authors observed late-maturing legume varieties, (e.g., Austrian winter pea and hairy vetch), achieve total N content early- to late-May. Reberg-Horton et al. (2012) reported corresponding ideal termination time-lines. Vann et al. (2020) reported earlier floral initiation in a study done with leguminous cover crops in both NC and MD. These trends in peak total N production are crucial in determining which leguminous cover crop species are ideal to grow in the Southeast, from a production standpoint, and give insight on ideal termination times for succeeding cash crops. Parr et al. (2011) hypothesized differences in leguminous cover crop biomass yield to be species-dependent on the mineralization rates of each cover crop. Specifically, nitrogen mineralization rates could be dependent on the quality (total N, C:N ratio, hemicellulose, lignin, etc.) within individual residue biomass. Incorporating crop residue places biomass closer to the general vicinity of soil microbes that help decompose plant material. In turn, plant available nitrogen may be released faster, Gaskell et al. (2007). Supplemental nitrogen or a combination of grass-legume bicultures might be necessary to coordinate improved nitrogen synchronicity (Ranells et al. 1997).

In a study investigating leguminous cover crops in a flue-cured tobacco system, Hahn et al. (2021) reported comparable, yet inconsistent total nitrogen results. For example, one year their studies concluded no differentiation among the total nitrogen rates, and the next year their studies concluded differences between Austrian winter pea, hairy vetch, and crimson clover. The former two were not different from one another and produced more total nitrogen than crimson clover. The differences observed in their studies were due to the age and maturity of the crimson clover plant tissue at harvest. In a 1992 study conducted by Ranells and Wagner, crimson clover growth stages were reported to determine nitrogen released over the season. Their investigations concluded that the longer crimson clover is left in the field, the more likely an increase in C:N, cellulose, hemicellulose, and lignin content. Lignin is known to be resistant to microorganism decomposition (Szegi et al. 1988). Carbon : nitrogen ratios over 30:1 will require biological production of CO_2 to lower C:N to 20:1 or less for the N to be considered plant-available (Allison, 1966). Studies over the past decade have shown crimson clover C:N values to be significantly higher than Austrian winter pea and hairy vetch, our results are analogous (Hahn et al., 2021; Parr et al., 2011).

Notably, over the duration of our study, we were able to achieve 79% of our 168 kg N ha^{-1} control yields using HV alone. Previous research has investigated the utilization of leguminous cover crops as an organic source of nitrogen for subsequent cash crops (Parr et al., 2011; Poffenbarger et al., 2015; Vann et al., 2019). The authors of these studies found that the combination of either monoculture or biculture HV with other winter annual species was advantageous - improving maize yields (Parr et al., 2011) and outcompeting weed biomass (Vann et al. 2019). Further research is required to determine how HV in a small-grain biculture mix compares with monoculture HV for use in floral hemp systems. The increased C:N ratio of the biculture (Parr et al., 2011) may retard the biomass decomposition, improving the crop demand and nitrogen mineralization synchrony.

Soil Total Available Nitrogen Over Time

Soil total available nitrogen (TAN) in 2021 at both locations were affected by the interaction of time × bedding × fertility treatments ($p < 0.0001$; Figure 1.1). Interaction was sliced by bedding × time to determine fertility treatment differences within a given time and bedding treatment. Early season (late May, 2021) results in Clinton (Fig. 1.1A) and Kinston (Fig. 1.1C) bare-ground treatments showed differences between the 168 kg N ha^{-1} treatment and all other fertility treatments. Similar trends were observed in plasticulture (Fig. 1.1B, 1.1D), with 168 kg N ha^{-1} treatment providing more TAN than all other fertility treatments. Mid-season (July 2021) trends at both locations showed no differences among fertility treatments. End-of-season (August 2021) trends at both locations showed similar results, although TAN had a slight increase in Clinton that were not observed in Kinston. The plasticulture treatment in both locations appeared to maintain TAN in the 168 kg N ha^{-1} for longer than the bare ground treatments.

Soil TAN in Clinton 2022 was affected by the interaction of time × bedding treatment ($p < 0.0001$; Figure 1.2A) and fertility treatment × bedding treatment ($p = 0.0034$; Figure 1.2B). Differences in TAN were not consistent throughout the season between bedding treatments (Fig. 1.2A). The bare ground treatment resulted in higher TAN on 9 June (31.6) and 5 July (61.3 mg

L^{-1}); however, later in the season, the plastic treatments provided higher TAN than bare ground. Differences in TAN were only observed in the 168 kg N ha^{-1} and hairy vetch treatments (Fig. 1.2B). TAN from the 168 kg N ha^{-1} treatment was highest in the bare ground (73.8) treatment compared to plasticulture (54.5 mg L^{-1}). The opposite was observed in the hairy vetch treatment; TAN was higher in the plasticulture (38.4) treatment than in bare ground (31.2 mg L^{-1}). Overall, TAN was much higher in the 168 kg N ha^{-1} than in all other fertility treatments.

Soil TAN in Kinston 2022 was affected by the interaction of time × bedding treatment ($p = 0.0009$; Figure 1.3A) and the main effect of fertility treatments ($p < 0.0001$; Figure 1.3B). Differences were only observed on 20 July and 2 August, in which the bare ground treatment resulted in higher TAN than plasticulture (Fig. 1.3A). The 168 kg N ha^{-1} (199.7 mg L^{-1}) provided more TAN than all other fertility treatments (Fig. 1.3B). Austrian winter pea (29.4), hairy vetch (26.2), crimson clover (21.4), and 0 kg N ha^{-1} (18.4 mg L^{-1}) showed no differences.

Salisbury 2022 TAN was affected by the interaction of time × bedding ($p < 0.0001$; Figure 1.4A) and the main effect of fertility treatment ($p < 0.0001$; Figure 1.4B). Differences between bedding treatments were observed on 18 July, 1 August, and 17 August; TAN from the bare ground treatment was higher than the plasticulture treatment on all three dates (Fig. 1.4A). Similar to Kinston 2022, 168 kg N ha^{-1} (268.4 mg L^{-1}) resulted in more TAN than all other fertility treatments (Fig. 1.4B).

Our 2021 soil TAN data follow trends reported in other literature. Machanoff et al. 2022 reported on the use of different colored polyethylene mulches to bare ground treatments in organic flue-cured tobacco production. The fertilizer source in their study was an organic poultry litter product similar to that used in our 168 kg N ha^{-1} control. The authors found that soil TAN dropped precipitously in the bare ground treatments, which they attributed to heavy precipitation and course soil texture that is prone to leaching. Precipitation in the 2021 field season was higher than 2022 (Table 1.1), which may explain why soil TAN from the bare ground 168 kg ha^{-1} treatment (Fig. 1.1A, 1.1C) dropped at a faster rate than the same treatment under plasticulture (Fig. 1.1B, 1.1D). Similar results were reported by Filipović et al. (2016) in a vegetable plasticulture study. The authors investigated four commonly used colors of plastic mulch and one control (bare-ground). The authors reported lower plant available nitrogen in bare-ground treatments compared to the plastic mulch, which they also attributed to leaching.

Soil TAN results were variable across years and locations. Early season sampling in 2022 at both Kinston and Salisbury showed plastic mulch TAN higher than bare-ground (Fig. 1.3A, 1.4B), though they were not different from one another. End of season sampling showed greater differences between bedding treatments, none consistent enough to draw conclusions. These specific differences experienced between years and locations could have been due to changes in weather, as previously mentioned, or experimental procedure. For example, 168 kg N ha^{-1} plots in 2021 received nitrogen approximately one week prior to planting at each location. In 2022 they received nitrogen the same day as planting. This resulted in nitrogen burn on the transplants and, in some locations, we replaced as many as 100% of transplants due to the nitrogen toxicity. Little research exists on nutrient toxicity in hemp. In a floral hemp variety study focused on fertigation rates in greenhouses, Anderson et al. (2021) reported nitrogen toxicity at rates between 450-600 mg L^{-1}; however, this was variety-dependent.

Cover crop biomass yield results for combined years were low overall but followed trends reported for southeastern production (Hahn et al., 2021). Nitrogen mineralization from cover crops is known to be directly correlated to soil temperature, moisture, and chemical properties (Miller & Geisseler, 2018). Each year we experienced different amounts of rainfall

(Table 1), which could explain some of the soil TAN differences between years. If cover crop termination and cash crop planting are not synchronized to optimize nitrogen release and crop use, then plant available nitrogen will be lost (Balkcom et al. 2016). Machanoff et al. (2020) reported a steep decline in total soil nitrogen from organic fertilizer applied to bare-ground tobacco compared to plasticulture systems. Though we did not have consistent trends between years with regards to bedding, one trend was prominent: the 168 kg N ha^{-1} control provided more TAN than leguminous cover crops (Fig. 1.1A, 1.1B,1.2B,1.3B,1.4B).

Foliar Nutrient Concentration Over Time

Foliar nitrogen in Clinton 2021 was affected by the interaction of time × fertility × bedding treatment (p = 0.0095 ; Figure 1.5). We sliced this interaction by time × bedding to compare fertility treatments. Early season results showed foliar nitrogen in bare ground treatments differentiated between the 168 kg N ha^{-1} (4.0) control and Austrian winter pea (2.8%; Fig. 1.5A). Fertility treatments in plastic showed mid-season differences among 168 kg N ha^{-1} (4.8), hairy vetch (3.4), 0 kg N ha^{-1} (3.3), and Austrian winter pea (2.8%; Fig. 1.5B). The latter three treatments were not different from one another. End-of-season fertility treatments in plastic showed differences between 168 kg N ha^{-1} (3.1) and hairy vetch (2.2%). The only fertility source in the bare ground treatment resulting in foliar tissue nitrogen within the NCDA sufficiency range was the 168 kg N ha^{-1} control on July 7, 2021. After July 7, 2021, all treatments fell below the sufficiency range. Fertility treatments in the plasticulture treatment maintained foliar leaf nitrogen content within the NCDA sufficiency range during the first two sampling dates (7 July and 20 July), after which all foliar tissue nitrogen levels fell below the lower end of the sufficiency range.

Kinston 2021 foliar tissue results were affected by the main effects of time and fertility treatment ($p < 0.0001$; Figure 1,6A, $p = 0.00289$; Figure 1.6B). Foliar nitrogen declined over time, with each sequential sampling date being lower than the previous date (Fig. 1.6A). Foliar nitrogen was highest in the 168 kg N ha^{-1} (3.8), significantly higher than 0 kg N ha^{-1} control (3.3%; Fig. 1.6B), but not different from the three cover crop treatments. All treatments except the 0 kg N ha^{-1} control resulted in foliar nitrogen content within the NCDA sufficiency range.

Foliar nitrogen in Clinton 2022 were affected by the interaction of time × fertility treatments ($p < 0.0001$; Figure 1.7A), and time × bedding treatments ($p < 0.0001$; Figure 1.7B). Hairy vetch (4.1) and 168 kg N ha^{-1} (4.1) differed from 0 kg N ha^{-1} (3.0%) on 22 July. The former two were not different. The 0 kg N ha^{-1}, 168 kg N ha^{-1}, and hairy vetch treatments were within the NCDA sufficiency range on 22 July. The 168 kg N ha^{-1} (3.9) and hairy vetch (3.6) differed from crimson clover (3.2), winter pea (3.2), and 0 kg N ha^{-1} (2.9%) on 3 August. The former two and latter three did not differ from each other. The 168 kg N ha^{-1} (4.6%) differed from all other fertility treatments on 16 August. Hairy vetch (4.2) and Austrian winter pea (4.0) differed from 0 kg N ha^{-1} (3.6%). The former two did not differ. Crimson clover (3.9%; Fig. 1.7A), Austrian winter pea, and 0 kg N ha^{-1} did not differ. The hairy vetch, 168 kg N ha^{-1}, crimson clover, and Austrian winter pea treatments fell within the NCDA sufficiency range for the entirety of the study.

Bare (4.3 – 3.6) and plastic (3.8 - 3.1%) bedding treatments differed on 22 July, 3 August, and 16 August. Bedding treatments fell within the NCDA sufficiency range on 22 July and 15 August (Fig. 1.7B).

Foliar nitrogen results in Kinston 2022 were affected by the interaction of time × fertility ($p = 0.0010$; Figure 1.8A), time × bedding ($p < 0.0001$; Figure 1.8B), and bedding × fertility (p =

0.0001; Figure 1.8C). We sliced the time × fertility interaction by time to compare fertility treatments on a given date. All fertility and bedding treatments except the 15 August sampling date stayed within the NCDA sufficiency range. Hairy vetch (5.1) and 168 kg N ha^{-1} (4.4%) differed on the 7 July sampling date. The 168 kg N ha^{-1} (4.5 – 4.2), 0 kg N ha^{-1} (4.1 – 3.8), and hairy vetch (3.8%) differed 20 July and 2 August. Finally, the 168 kg N ha^{-1} (3.9%) was higher than all other fertility treatments on 15 August (Fig. 1.8A).

We sliced the time × bedding by time to compare bedding treatments at a given date. Bare and plastic treatments differed on sampling dates 7 July and 2 August (Fig. 1.8B).

Finally, we sliced the bedding × fertility interaction by fertility treatment to compare bedding treatments within a given fertility treatment. Plastic 168 kg N ha^{-1} (4.6) differed from plastic hairy vetch (3.8), while the opposite occurred in bare ground treatments. Bare hairy vetch (4.2) differed from bare 168 kg N ha^{-1} (3.9%). Plastic 168 kg N ha^{-1} and bare hairy vetch, as well as, bare 168 kg N ha^{-1} and plastic hairy vetch did not differ (Fig 1.8C).

Foliar nitrogen in Salisbury 2022 were only affected by the interaction of time × bedding treatments ($p = 0.0023$; Figure 1.9). Plastic (5.0) and bare (4.7%) treatments differed on the first sampling date. After 6 July each sequential sampling date were lower than the previous. Both bedding treatments fell within the NCDA sufficiency range over the season.

Short et al. (2021) reported similar foliar nitrogen results from a study with floral hemp where the effect of five different nitrogen and potassium fertilizer rates were examined. The authors reported differences between sand and clay environments where 168 kg N ha^{-1} consistently resulted in the highest foliar nitrogen; we saw comparable results across environments. The authors reported one instance where they saw a decline in vegetative growth (defined as the average of both height and width measurements) which they attributed to finite nutrient supply throughout the season, and potential reallocation of nutrients due to floral production. The authors also split-applied their fertilizer, similar to a tobacco-production system. Our fertilization schedule consisted of one initial fertilizer application and no layby applications, which may explain why we saw a steady decline in foliar nitrogen. Split application of fertilizer throughout the season may be a necessary step to maintain nitrogen availability for the crop through the length of the season by limiting losses related to leaching. Furthermore, applying all nitrogen up front can potentially cause nitrogen toxicity as experienced in 2022.

Landis et al. (2019) reported foliar nutrient differences among five CBD hemp cultivars. The authors observed mean differences in certain varieties lower than the average greenhouse range reported by Bryson and Mills (2014), comparatively, our mean nitrogen value across years x locations x sampling dates was 3.75%. This value falls within the floral hemp foliar nitrogen sufficiency range reported by the North Carolina Department of Agriculture and Consumer Services.' Cockson et al. (2019) conducted a six-week greenhouse study examining deficiency symptoms in floral hemp cultivar T1 and reported 1.62% N in their foliar deficient plants. According to findings reported by authors previously mentioned, our data show results that conclude we had nitrogen deficiency in Clinton, on July 20, 2021 (FIG #).

Foliar nitrogen concentrations resembled those reported by Atoloye et al. (2021) who studied the effects of nitrogen fertilization rate (0, 56, 112, 224 kg N ha^{-1}) on two CBD hemp cultivars (Spectrum and Therapy). They reported that the interaction of growth (mid [4 weeks after transplant] and late [8 weeks after transplant] vegetative stage) × fertilizer × year did not impact the foliar nitrogen concentrations, though differences between varieties were observed. Our mean foliar nitrogen was slightly lower (3.75%) than previously reported variety Therapy (3.86).

Foliar Chlorophyll Content Over Time

Foliar chlorophyll content (FCC) in Clinton were affected by the interaction of time × fertility treatments ($p < 0.0001$; Figure 1.10A), and time × bedding treatments ($p < 0.0001$; Figure 1.10B). July 22, 2022 chlorophyll measurements (CM) showed differences between hairy vetch (273.0) and 0 kg N ha^{-1} (223.3). August 3, 2022, showed differences among 168 kg N ha^{-1} (185.4), crimson clover (126.1), and 0 kg N ha^{-1} (117.1 µmol m^{-2}; Fig. 1.10A). The latter two treatments did not differ. Differences between CM in bedding treatments appeared on 22 July and 3 August. With the bare treatment showing higher FCC on both dates (Fig. 1.10B).

Foliar chlorophyll content in Kinston were affected by the interaction of time × fertility treatments ($p = 0.0015$; Figure 1.11A), and bedding × fertility treatments ($p = 0.0109$; Figure 1.11B). August 2, 2022, is the only sampling date to show differences in CM among fertility treatments. Control, 168 kg N ha^{-1} (302.4 µmol m^{-2}; Fig. 1.11A) differs from all other fertility treatments on this date. Bareground FCC showed no differences among fertility treatments (Fig. 1.11B). Plastic FCC showed differences between 168 kg N ha^{-1} (276.4) and 0 kg N ha^{-1} (237.5 µmol m^{-2}, Fig. 1.11B).

Foliar chlorophyll content in Salisbury were only affected by the main interaction of time ($p < 0.0001$; Figure 1.12). August 1, 2022 (260.7) CM differed from 6 July (193.1) and 18 July (195.8 µmol m^{-2}). The latter two treatments showed no differences.

Healthy foliar chlorophyll content has not yet to be defined for floral hemp, making production standards for farmers difficult. Maļceva et al. (2011) reported nitrogen fertilizer dose effects on the phenotypic habits of hemp grown for both seed and fiber. The authors concluded from a sub-sample of greenhouse genetics that leaves with higher foliar nitrogen content frequently had equivalent leaf greenness (as defined by SPAD units), these data were not influenced by additional nitrogen compared to their no nitrogen control. Our chlorophyll data are not consistent with Maļceva et al. (2011). We saw differences among nitrogen sources: higher chlorophyll content in 168 kg N ha^{-1} treatment compared to the other fertility treatments (Fig. 1.10A, 1.11B). These data aligned with our soil TAN results, suggesting chlorophyll readings may be a quicker, cheaper alternative to submission of leaf tissue for nitrogen analysis. It's important to note that chlorophyll measurements are from only one year. Further research is required to validate these results.

Floral Hemp Height and Width

End-of-season plant height ($p < 0.0001$) and width ($p= 0.0001$) were affected by the interaction of fertility and bedding treatments (Table 1.3). Height of plants in the plastic treatments were not. Average height in bare ground treatments were different between 0 kg N ha^{-1} (86.7) and 168 kg N ha^{-1} (76.7 cm). The largest differences in height occurred between plastic hairy vetch (97.8) and bare 168 kg N ha^{-1} (76.7 cm). More variability was observed in the plant width results. Average width in plastic treatments differed between 168 kg N ha^{-1} (136.2) and 0 kg N ha^{-1} (108 cm). Average width in bare-ground treatments did not differ from one another. The largest differences in width occurred between plastic 168 kg N ha^{-1} (136.2) and bare 0 kg N ha^{-1} (92.5 cm).

Insufficient research has been reported on cultural practices impacting floral hemp height and width. Linder et al. (2022) reported on spacing and transplant date impacts on 'BaOx' grown in North Carolina. The authors reported larger variability within transplant date and spacing on plant width compared to height. Although no clear trends were observed in our bedding results, the plasticulture treatment tended to result in wider plants than the bare ground treatments (Table 1.1). Within our plasticulture treatment the 168 kg N ha^{-1} resulted in the widest plant (136.2 cm).

Our plant average heights and width fell short of those reported by Linder et al. (2022). However, the authors supplied fertilizer via drip irrigation throughout the duration of the field season, which could be the cause of variability reported. Short et al. (2021) reported the impact of nitrogen and potassium rates on floral hemp growth index (Height × [{Width1 × Width2}/2]). The authors reported the highest growth index from nitrogen rates between 168 and 224 kg N ha^{-1}.

Bedding and Fertility Source Effect on Yield

End-of-season floral hemp biomass was affected by the interaction of fertility source and bedding treatment (p= 0.0016; Figure 1.13). The plastic 168 kg N ha^{-1} control produced the highest yields (464.2), which was significantly higher than all other treatments except for the hairy vetch plastic treatment (367.5 g). No differences were observed among the fertility sources within the bare-ground treatments and the lowest yields were observed in bare-ground crimson clover (183.7) and bare-ground 0 kg N ha^{-1} (184.4 g). We found no significant interaction nor main effects of bedding or fertility treatments on CBD and THC concentrations. Across all site years the total CBD content was 5.23 and total THC content was 0.23%.

Our floral hemp biomass data follow trends that have been reported in other literature. Bruce et al. (2022) reported on the impact of four organic fertility sources on floral hemp biomass and cannabinoid concentrations over time. The authors reported their lowest per plant biomass to be from their control plots that were not fertilized we saw comparable results with our 0 kg N ha^{-1} control. The authors also found that regardless of increasing the fertility treatment, which increased biomass, they saw no effect on the ratio of CBD:THC over time. James et al. (2023) reported on different nitrogen and potassium fertilizer rates for maximizing floral hemp biomass and CBD production, where they found a yield plateau was reached at 86.8 and 84.2 kg

N ha^{-1}. The authors reported a slight bell-shaped curve response in CBD to increasing N application. Whether these results are of true biologic relevance, or simply a statistical phenomenon, require further investigation as they go against a growing body of evidence that cannabinoid concentrations are not impacted by management or environmental conditions. Toth et al. (2021) investigated the impact of herbicide damage, flooding, wounding, ethephon, and Powdery Mildew on cannabinoid production. The authors found minimal impact of these stresses on cannabinoids except for a lower total THC content in the herbicide treated plants, which they attributed to overall poor growth. Regardless, none of these treatments affected the CBD:THC ratio. Similarly, Bruce et al. (2022) found that organic fertility source did not impact CBD:THC ratios. Our results are in agreement with the aforementioned studies and strengthen the growing body of knowledge that cannabinoid concentrations are not impacted by cultural practices and environmental conditions.

Our CBD:THC ratio across locations and years (20.5:1) are in line with recent reports. Stack et al. (2021) described cannabinoid accumulation, disease resistance and phenotypic performance on 30 commonly used CBD floral cultivars. The authors stated that of the five common chemotypes reported for cannabis, their chemotype III cultivars (responsible for CBD(A) production) had ratios between 20:1 and 30:1, respectively. The variability observed was due to differences in cultivar and sampling time. Chiluwal et al. (2023) conducted field trials in Florida where they investigated three planting dates and seven different cultivars. The authors reported an average CBD:THC ratio between 21.9:1 and 22.7:1. The enzyme responsible for CBD synthesis (CBDA synthase) has been shown to be a promiscuous enzyme, *in vitro* (Zirpel et al. 2018). The enzyme produces a ratio of approximately 26:1 CBD:THC. As such, our results are in line with those published for both *in vitro* (Zirpel et al. 2018) and *in planta* (Chiluwal et al., 2023; Stack et al., 2021) for CBD:THC ratios. Furthermore, the lack of significant bedding of fertility treatment on total CBD and total THC concentrations as well as CBD:THC ratios adds to the growing body of research demonstrating that cannabinoid synthesis is strongly linked to genetics and phenology, not environmental or cultural effects (Toth et al. 2021).

Conclusion

The use of leguminous cover crops as a nitrogen source can be variable. In general, we saw the use of leguminous cover crops in combination with plasticulture can help maintain nitrogen within beds, allowing for nitrogen availability throughout the season.

We saw no impact from the main effects of fertility or bedding treatments on our cannabinoid concentrations or CBD:THC ratios. These results are in line with previously reported data stating that environmental conditions and cultural practices do not influence cannabinoid concentrations.

Hairy vetch appears to be a better leguminous cover crop for use in floral hemp in the Southeast, our results show that by incorporating hairy vetch alone into floral hemp production systems with plasticulture, we were able to obtain 79% of our organic 168 kg N ha $^{-1}$ control. Further research is required to determine the necessity of incorporating grass-legume bicultures to prolong nitrogen availability in the Southeast. Similarly, more work should be conducted investigating fertigation as a supplement to incorporated leguminous cover crops to extend crop-available nutrients further into the season.

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Table 1.1. Average Monthly Temperature and Total Precipitation in 2021 and 2022.

Environment [z]	**May**		**June**		**July**		**August**		**September**		**Season Total**
	Total Rainfall (cm)	Average Monthly Temperature (°C)	Total Rainfall (cm)	Average Monthly Temperature (°C)	Total Rainfall (cm)	Average Monthly Temperature (°C)	Total Rainfall (cm)	Average Monthly Temperature (°C)	Total Rainfall (cm)	Average Monthly Temperature (°C)	Total Rainfall (cm)
CRS 2021	4.5	20.1	25.3	24.3	21.3	25.7	21.2	26.3	3.6	22.4	75.8
HCRS 2021	5.4	20.4	14.5	24.6	33.4	25.7	15.5	26.2	5.5	22.2	74.3
CRS 2022	10.0	22.1	4.5	25.0	14.1	26.9	11.2	25.2	- [y]	-	39.7
HCRS 2022	10.5	22.1	7.7	25.0	11.6	26.0	8.0	25.2	7.2	22.2	44.9
PRS 2022	9.5	20.5	0.8	24.4	20.7	25.7	9.4	24.1	-	-	40.3

[z] Cunningham research station (CRS), Horticultural crops research station (HCRS), Piedmont research station (PRS) average monthly temperature and total precipitation. Data were retrieved from the nearest Econet stations using North Carolina State Climate Office (NCSCO) weather resources.

[y] Dashes represent data points that were not retrieved. CRS (August 29, 2022) and PRS (August 31, 2022) were harvested prior to HCRS (September 2, 2022). The former two locations were not pertinent data points to include.

Table 1.2. Leguminous Cover Crop Biomass and Nutrient Content.

Cover Crop	Biomass [x]	Nitrogen Content	Total N	Carbon Content	C:N Ratio
	kg ha^{-1}	(%)	kg ha^{-1}	(%)	
Crimson Clover	3314.4	1.7 b [w]	56.0	42.1	32.9 a
Hairy Vetch	2770.4	2.9 a	69.9	43.2	17.7 b
Austrian Winter Pea	2830.4	2.7 a	66.0	42.1	17.9 b
p-value	0.5201	<0.0001	0.4910	0.0756	<0.0001

[x] Prior to cover crop termination biomass from each fertility treatment was taken. Crimson clover was taken at 90% floral senescence, hairy vetch, and Austrian winter pea were taken at 50% floral senescence in 2021 and 2022.

[w] Means followed by the same letter within a column are not significantly different ($p > 0.05$) and represent one sample x four replicants x five environments (n = 20 data points per mean)

Table 1.3. Interaction Effect of Bedding and Fertility Source Treatments on Floral Hemp Height and Width at Harvest.

Bedding Treatment	**Fertility Treatments**	**Response** [z]	
		Height (cm)	Width (cm)
Bare ground	0 kg N ha $^{-1}$	86.7 ab[y]	92.5 e
	168 kg N ha^{-1}	76.7 c	92.9 de
	Crimson Clover	84.5 bc	93.3 cde
	Hairy Vetch	91.5 ab	108.2 bcde
	Austrian Winter Pea	85.3 bc	94.7 cde
Plastic	0 kg N ha $^{-1}$	90.0 ab	108.0 bcde
	168 kg N ha^{-1}	95.5 ab	136.2 a
	Crimson Clover	90.2 ab	115.0 bcd
	Hairy Vetch	97.8 a	124.8 ab
	Austrian Winter Pea	93.5 ab	115.0 bc
p-value		<0.0001	0.0001

[z] Height and width were measured on three random plant subsamples per plot approximately 5 weeks after floral initiation. Height was measured from the base of the plant to the apical meristem. Two widths were taken; the first was measured from the widest apices of the plant and the second measurement was perpendicular to the first. These widths were averaged together to give the numbers seen in the table above.

[y] Means followed by the same letter within a column are not significantly different ($p > 0.05$) and represent three subsamples x four replicates x five environments ($n = 60$ data points per mean).

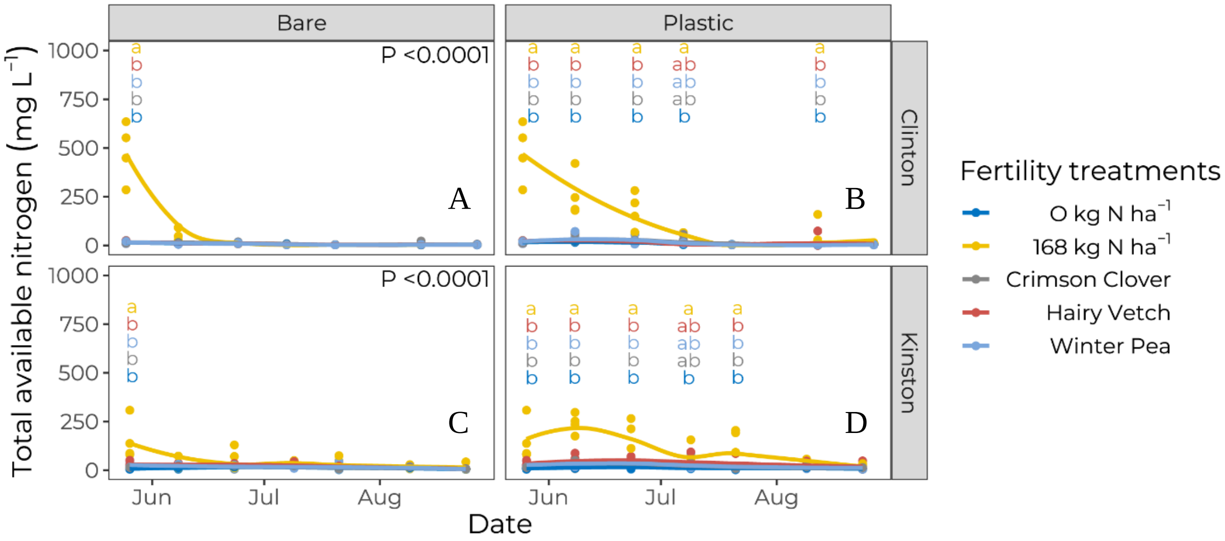

Figure 1.1. The influence of bedding × fertility treatments × time interaction on soil total available nitrogen at Clinton ($p < 0.0001$; A, B) and Kinston ($p < 0.0001$; C, D), 2021. Total available nitrogen was calculated as the sum of soil nitrate and ammonium nitrogen. Means followed by the same letter are not significantly different ($p > 0.05$).

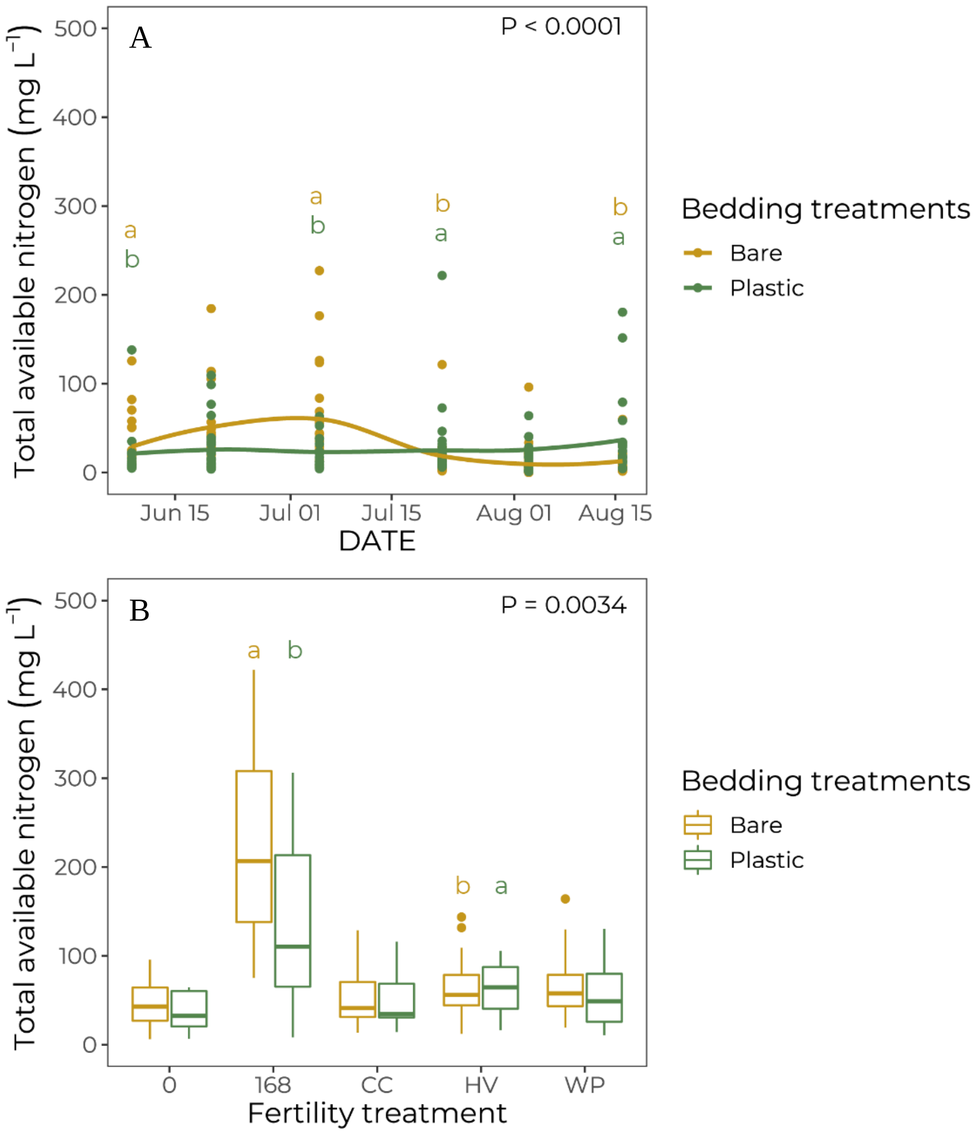

Figure 1.2. The influence of time × bedding (p = 0.0001; A) and fertility × bedding treatment (p < 0.0034; B) in soil total available nitrogen, Clinton, 2022. Total available nitrogen was calculated as the sum of soil nitrate and ammonium nitrogen. Mean separation letters are color coded to bedding treatments and represent differences in either; a specific date, or fertility treatment. Absence of mean separation letters indicates no significant differences (p > 0.05).

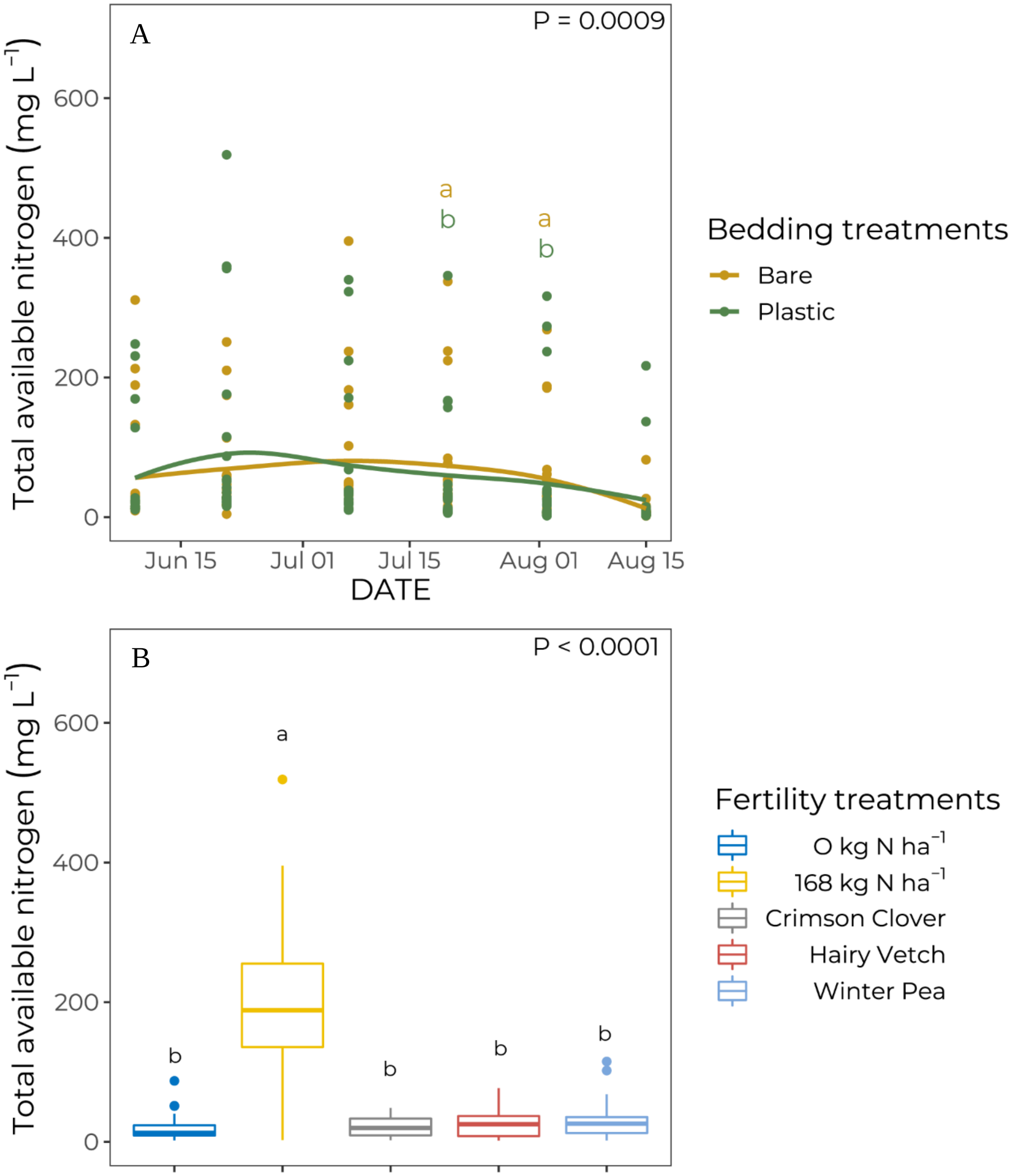

Figure 1.3. The influence of time × bedding ($p = 0.0009$; A) and differences among fertility treatments ($p < 0.0001$; B) in soil total available nitrogen, Kinston, 2022. Total available nitrogen was calculated as the sum of soil nitrate and ammonium nitrogen. Absence of mean separation letters indicates no significant differences ($p > 0.05$).

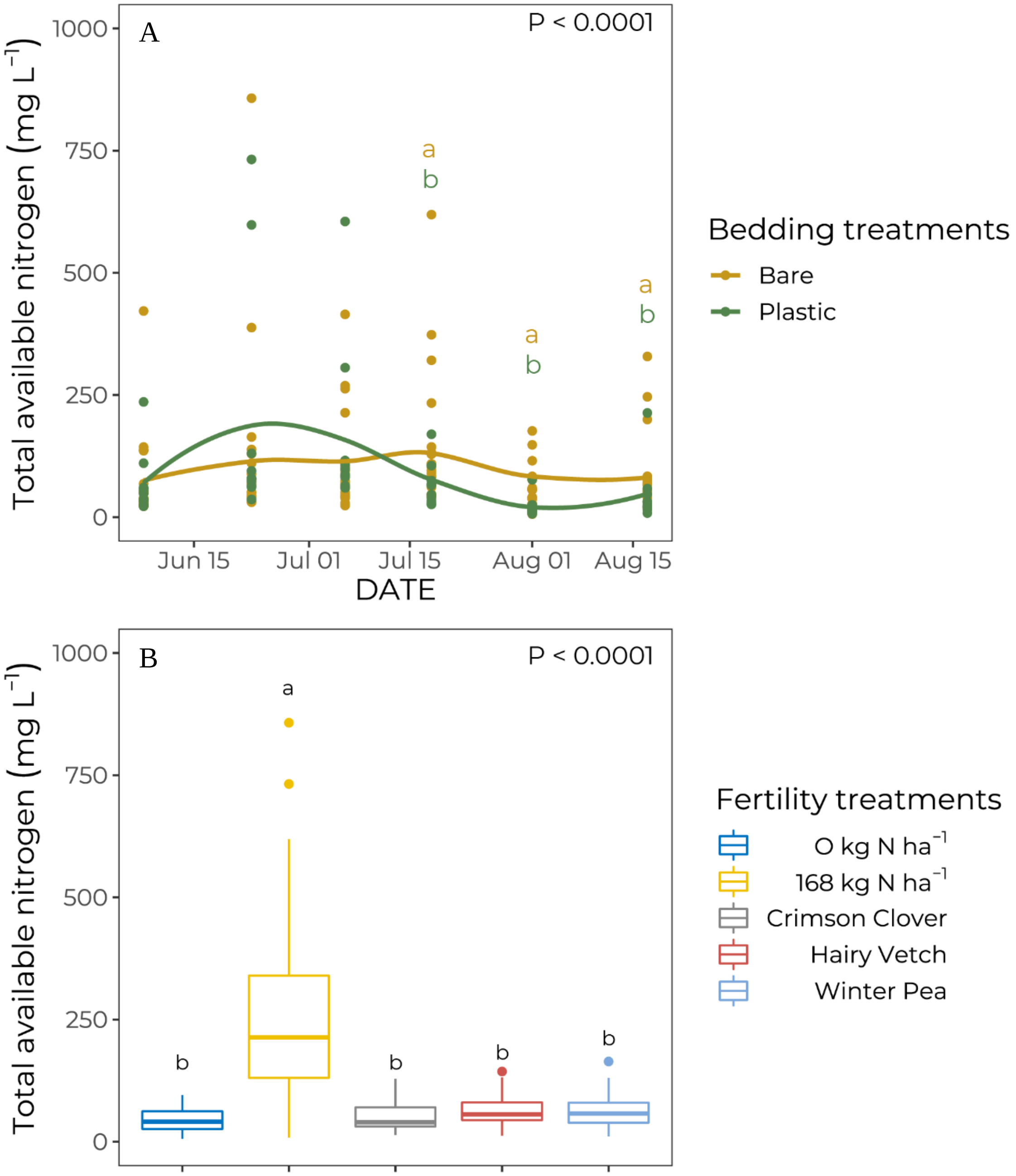

Figure 1.4. The influence of time × bedding ($p < 0.0001$; A) and differences among fertility treatments ($p < 0.0001$; B) in soil total available nitrogen, Salisbury, 2022. Total available nitrogen was calculated as the sum of soil nitrate and ammonium nitrogen. Absence of mean separation letters indicates no significant differences ($p > 0.05$).

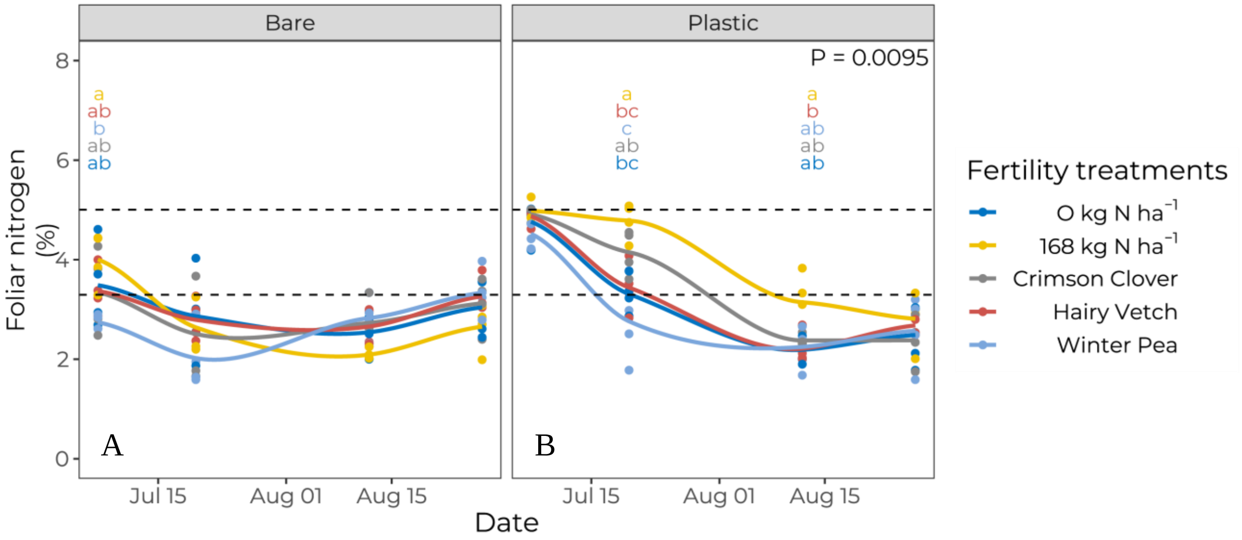

Figure 1.5. The influence of bedding × fertility × time on foliar tissue nitrogen values in Clinton, 2021 ($p = 0.0095$). Dashed lines represent the upper and lower bounds of the North Carolina Department of Agriculture and Consumer Services' floral hemp foliar nitrogen sufficiency range. Mean separation letters are color coded to fertility treatment and represent the differences within a location at that specific date, separated between bedding treatments (Bare; Fig. 1.6A, Plastic; Fig. 1.6B). Absence of mean separation letters indicated no difference among treatments ($p > 0.05$).

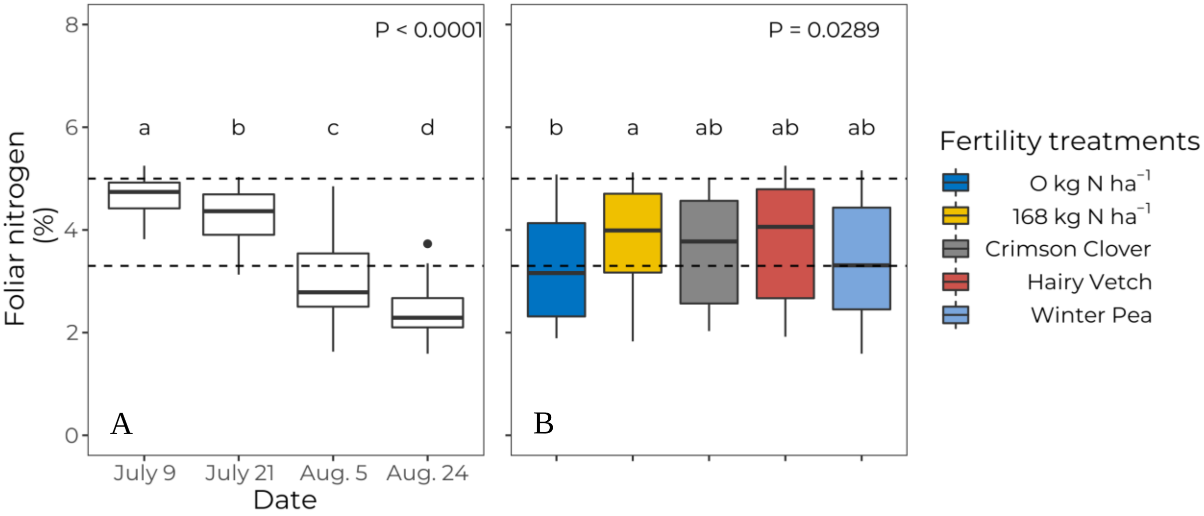

Figure 1.6. The influence of fertility ($p = 0.0289$; A) and time ($p < 0.0001$; B) on foliar tissue nitrogen in Kinston, 2021. Dashed lines represent the upper and lower bounds of the North Carolina Department of Agriculture and Consumer Services' floral hemp foliar nitrogen sufficiency range. Means that share a letter within the response are not different ($p > 0.05$).

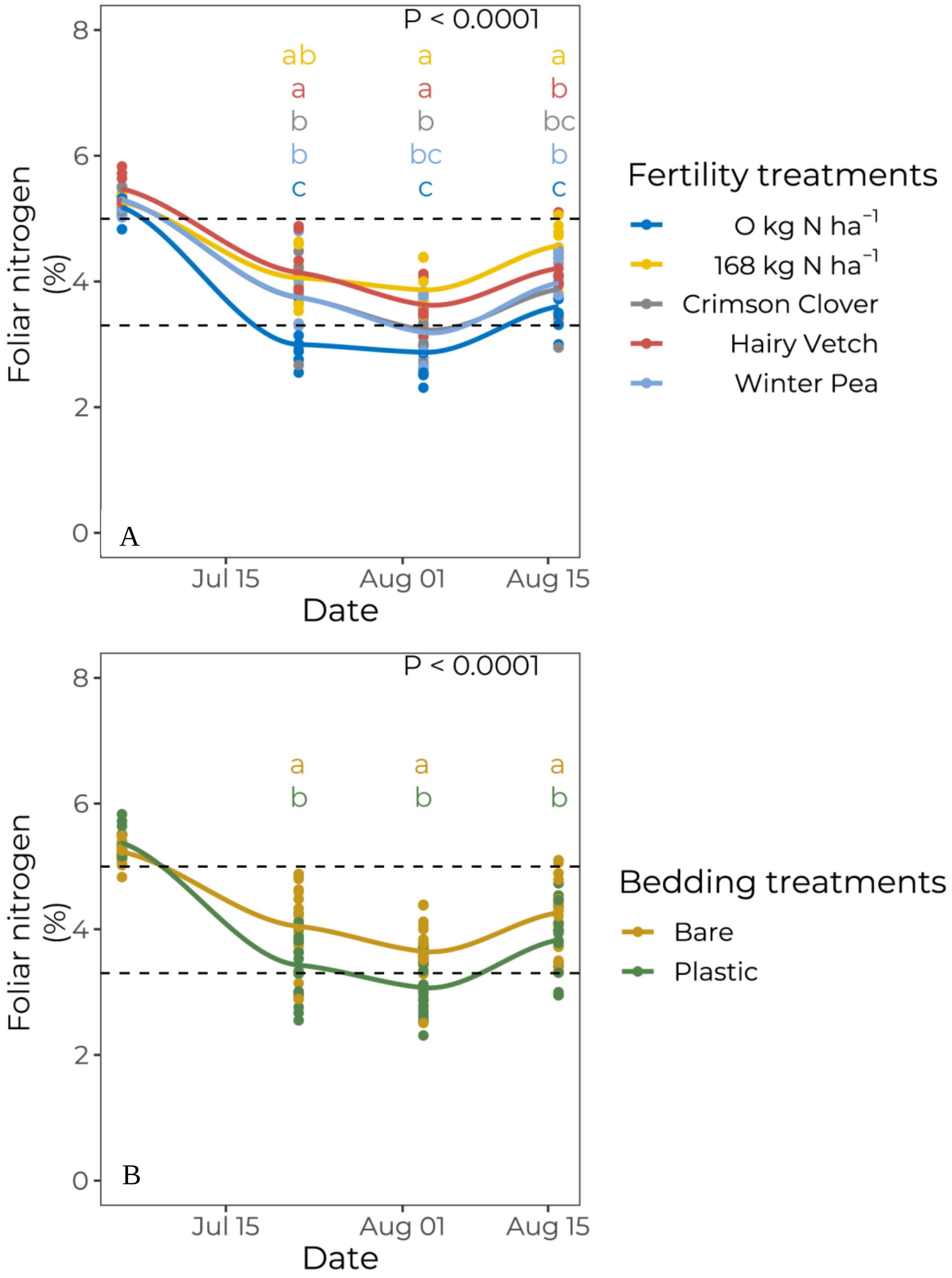

Figure 1.7. The influence of time × fertility treatment ($p < 0.0001$; A) and time × bedding treatment ($p < 0.0001$; B) on foliar tissue nitrogen values in Clinton, 2022. Dashed lines represent the upper and lower bounds of the North Carolina Department of Agriculture and Consumer Services' floral hemp foliar nitrogen sufficiency range. Absence of mean separation letters indicates no significant differences among treatments ($p > 0.05$).

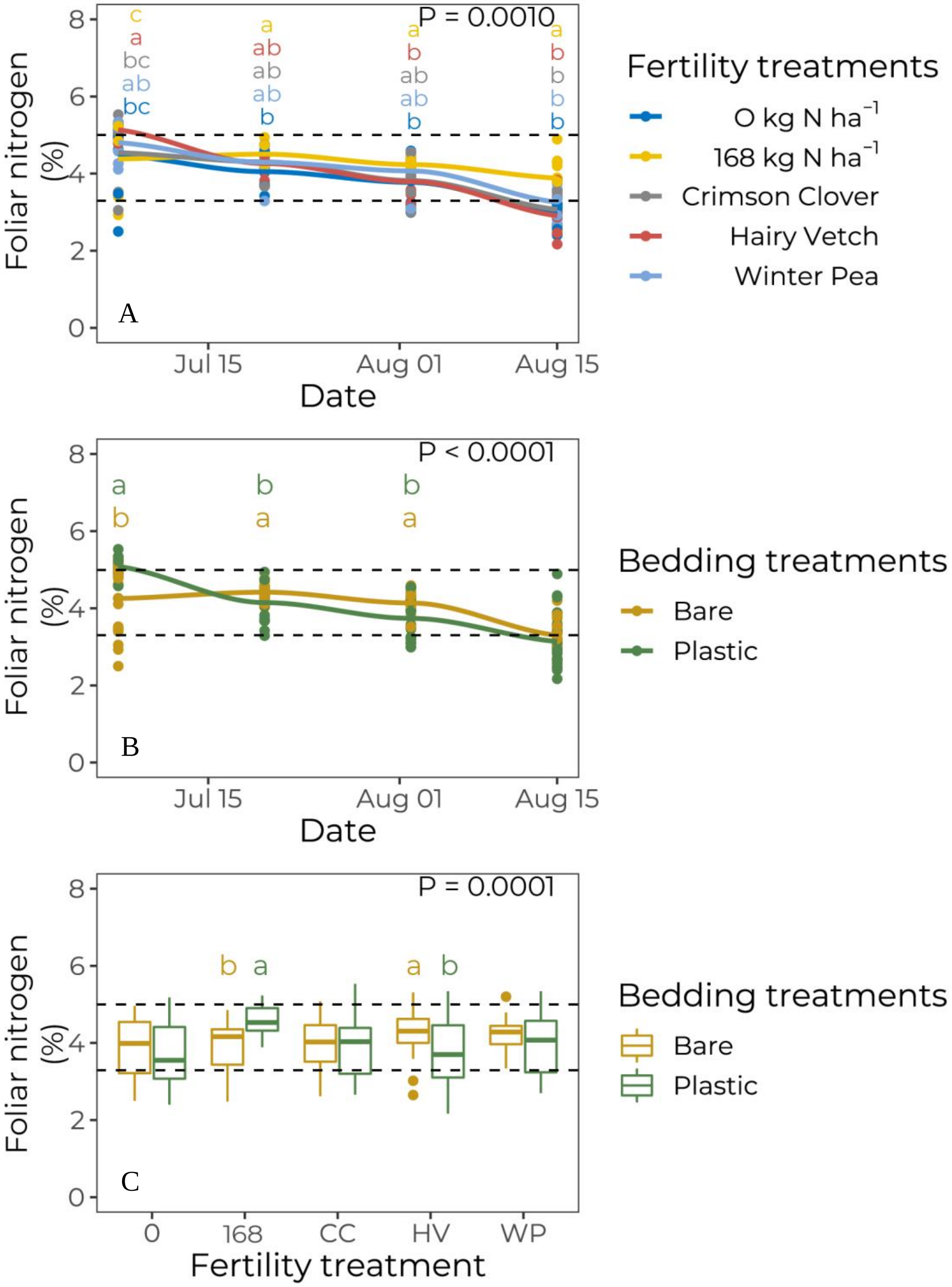

Figure 1.8. The influence of time × fertility treatments ($p = 0.0010$; A), time × bedding treatments ($p < 0.0001$; B), and bedding × fertility treatments ($p = 0.0001$; C) on foliar tissue nitrogen values in Kinston, 2022. Dashed lines represent the upper and lower bounds of the North Carolina Department of Agriculture and Consumer Services' floral hemp foliar nitrogen sufficiency range. Absence of mean separation letters indicates no significant differences among treatments ($p > 0.05$).

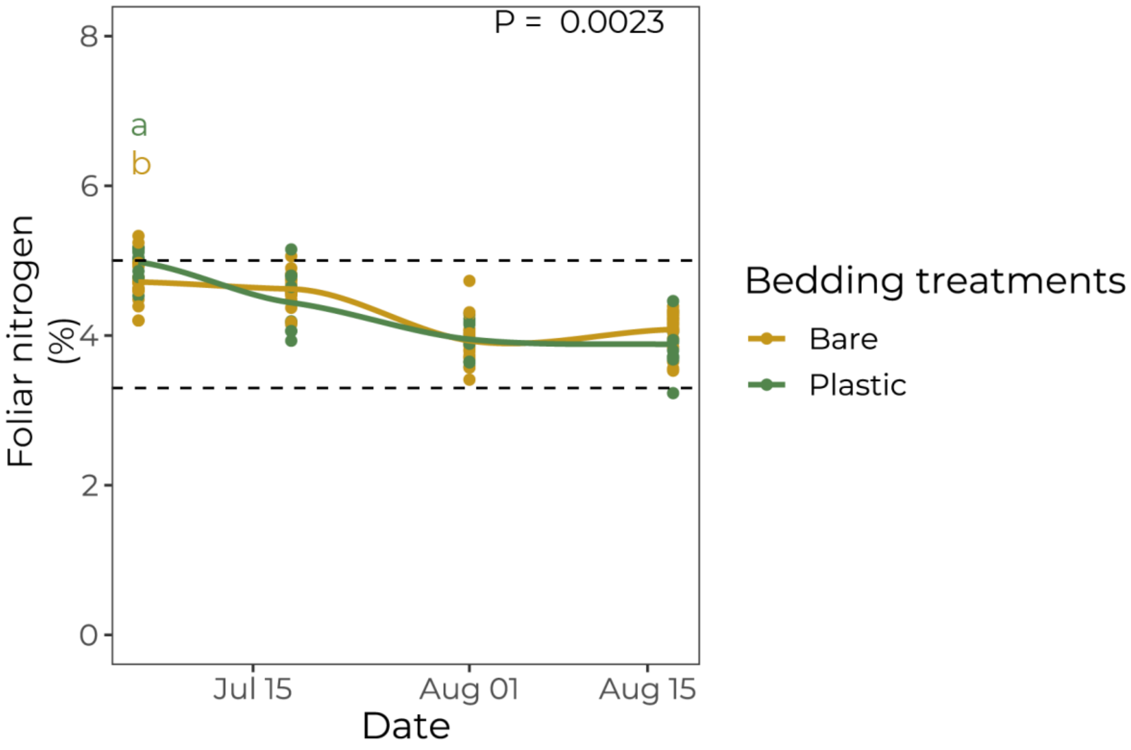

Figure 1.9. The influence of time × bedding treatment (p = 0.0023) on foliar tissue nitrogen values in Salisbury, 2022. Dashed lines represent the upper and lower bounds of the North Carolina Department of Agriculture and Consumer Services' floral hemp foliar nitrogen sufficiency range. Mean separation letters are color-coded to represent differences within a location and bedding treatment at that specific date. Absence of mean separation letters indicates no significant differences among treatments (p > 0.05).

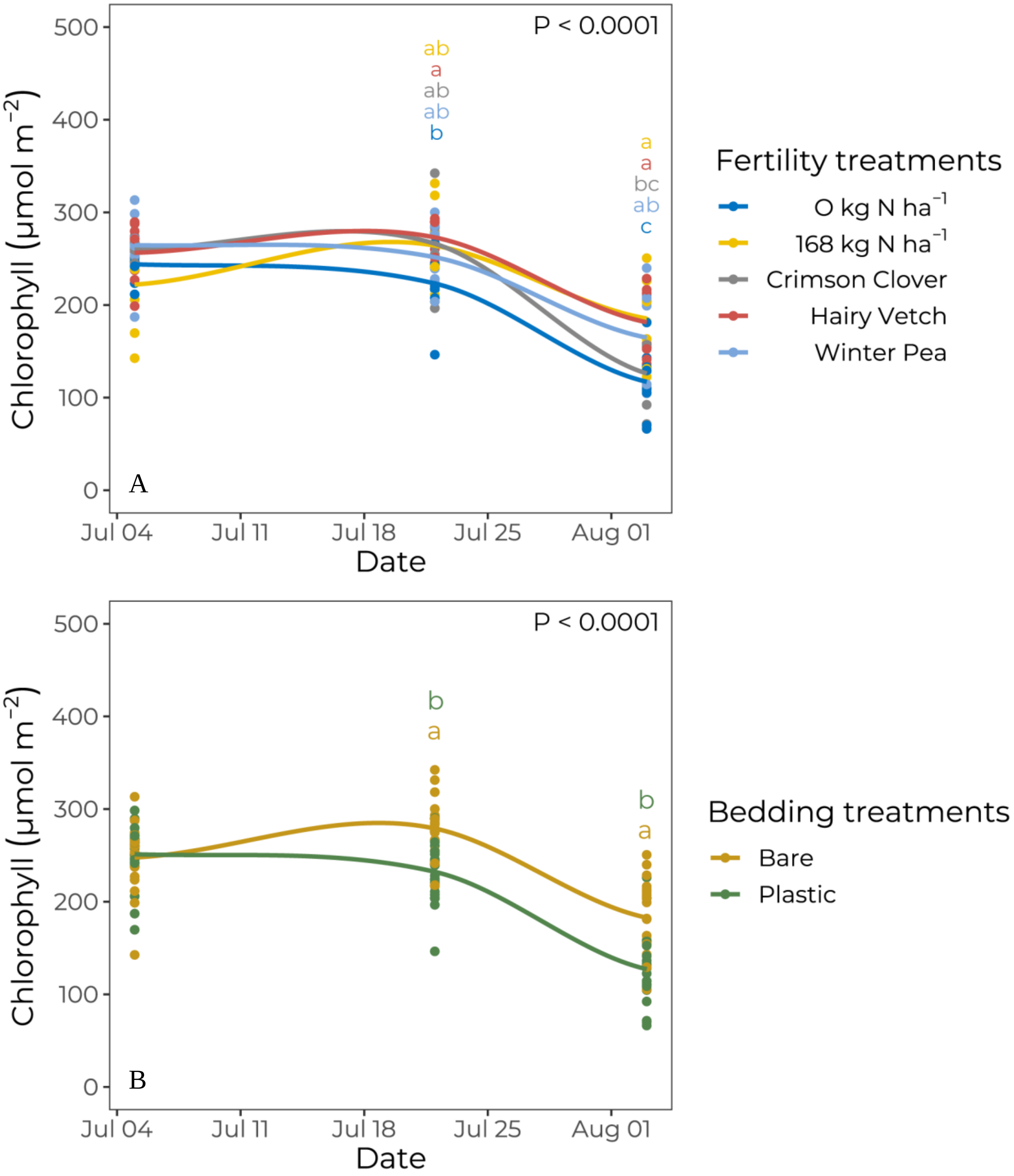

Figure 1.10. The influence of the interaction between time × fertility treatments ($p < 0.0001$; A) and time × bedding treatments ($p < 0.0001$; B) on chlorophyll concentrations in Clinton, 2022. Absence of mean separation letters indicates no significant differences among treatments ($p > 0.05$).

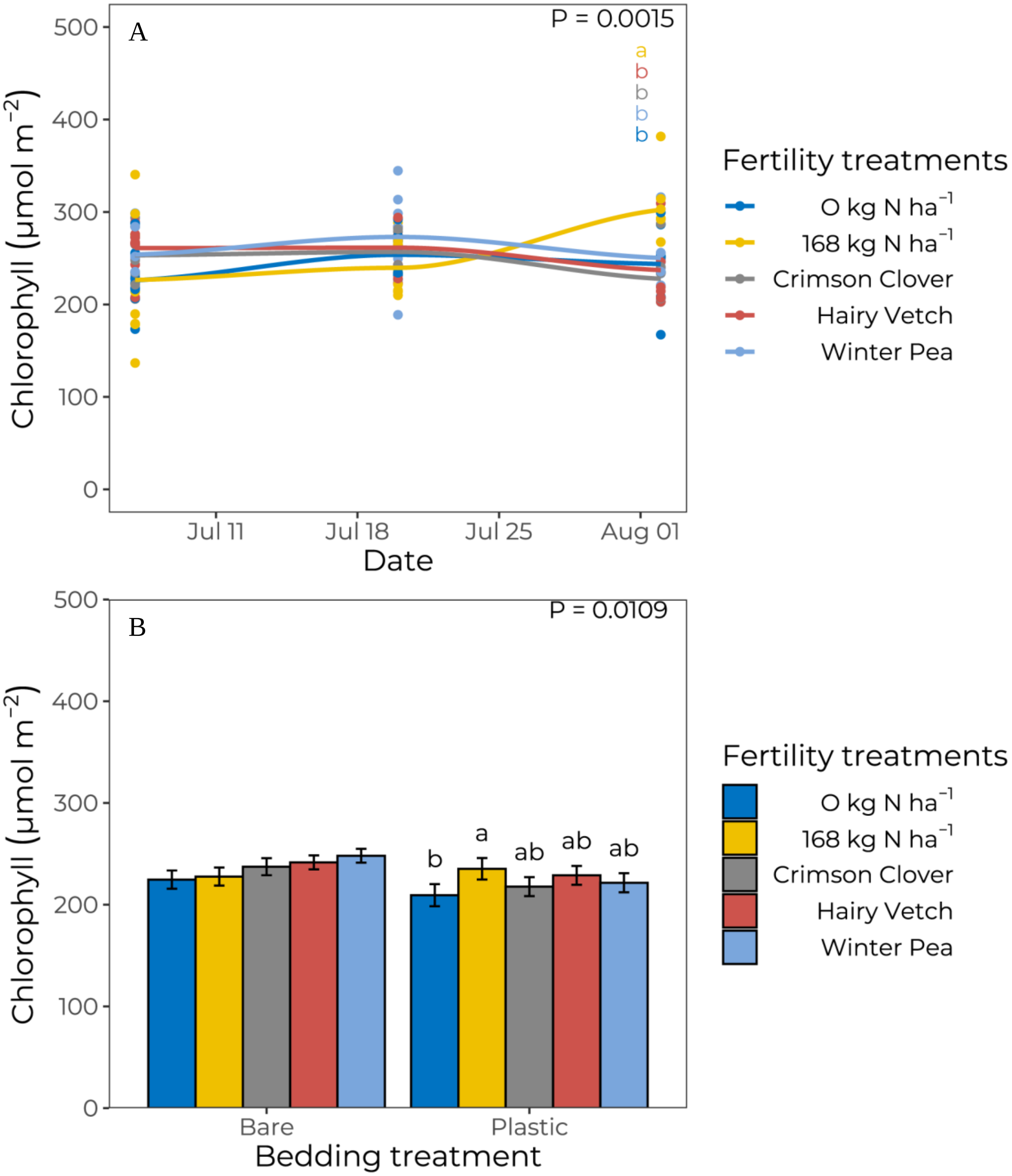

Figure 1.11. The influence of the interaction between time × fertility treatments (p = 0.0015; A) and bedding × fertility treatments (p < 0.0109; B) on chlorophyll concentrations in Kinston, 2022. Absence of mean separation letters indicates no significant differences among treatments (p > 0.05).

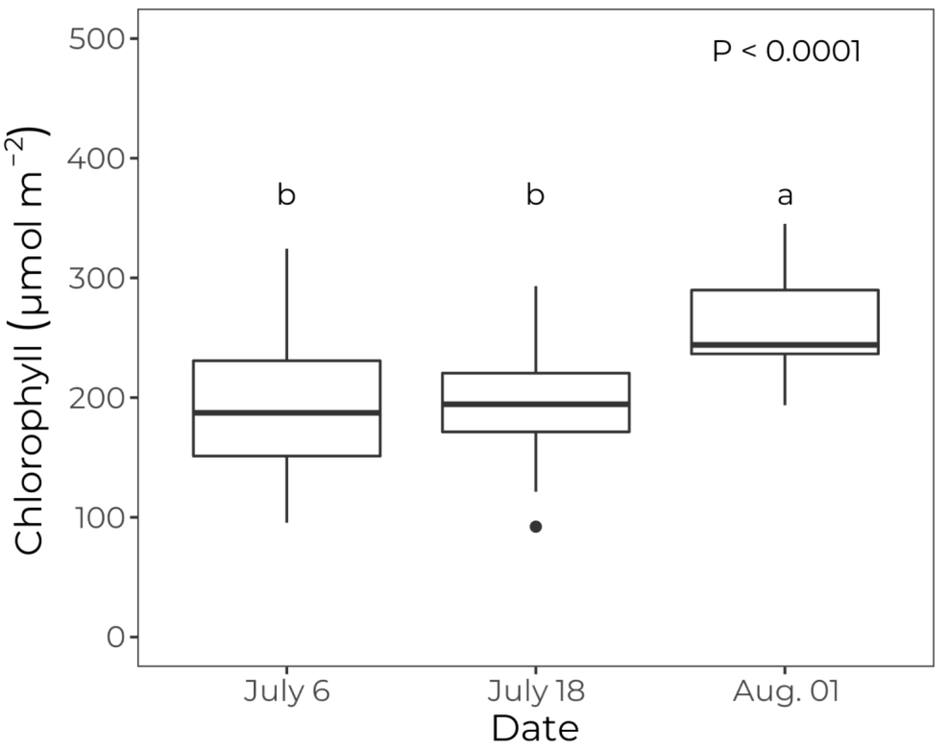

Figure 1.12. The effect of time ($p < 0.0001$) on chlorophyll concentrations in Salisbury, 2022.

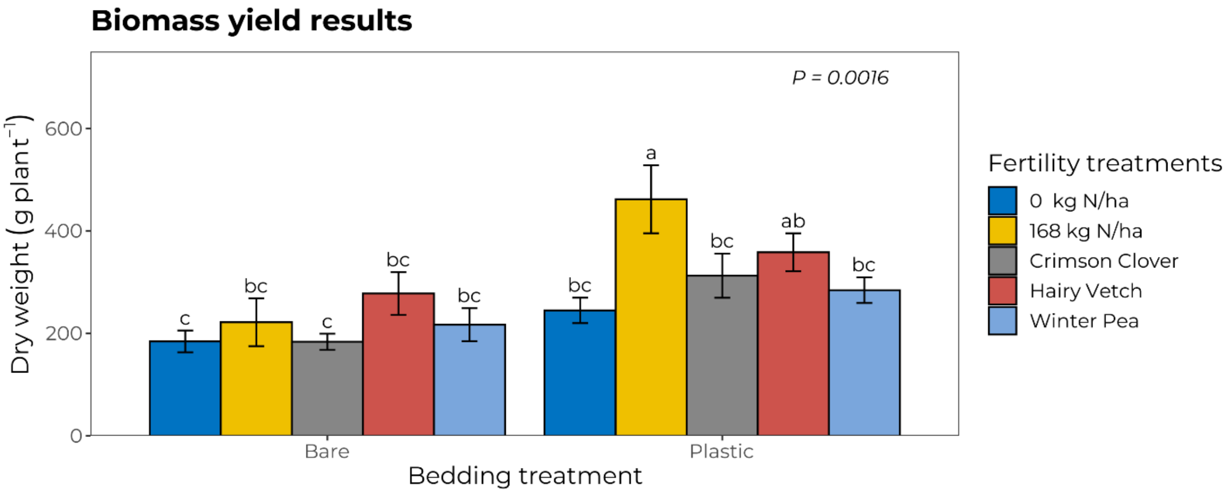

Figure 1.13. The influence of bedding and fertility treatments on the dry biomass yield of floral hemp. Dry biomass was calculated by averaging end-of-season threshed samples. Results include combined data; years x location. Means followed by the same letter are not significantly different ($p > 0.05$) and represent the average of three subsamples x four replicants x five environments (n =60).

CHAPTER 2

Establishing Planting Dates and Variety Recommendations for Fiber hemp (*Cannabis sativa* L.) Grown in North Carolina

ABSTRACT

Field trials were conducted in 2021 and 2022 to evaluate the effects of planting date (Mid-March, Mid-April, and Mid-May) on eleven fiber hemp (*Cannabis sativa* L. <0.3% total THC) varieties. Trials were conducted in Goldsboro, Kinston, and Salisbury, North Carolina. Each location followed a split-plot randomized complete block design with at least three blocks where planting date was the main-plot and variety the sub-plot. Varieties investigated originated in China and Australia (2021 only). Data collection included flowering time, end of season stand counts, stem height, diameter, and final retted dry straw yield. We found differences among the varieties investigated in both years, however no distinct trend was observed across years. All varieties investigated flowered at the end of August and beginning of September, allowing for a long growing season and ability to produce abundant biomass. In general, the Chinese genetics yielded higher stem biomass compared to previously-reported European genetics. Stem thickness was greater than 7.5 mm, which is generally considered the maximum width for textile-grade fiber production. To achieve thinner stems from the varieties investigated, harvesting prior to male-flower initiation may be required. The crop experienced temperatures below freezing in both years with no signs of damage. Differences among planting dates were minimal regarding final crop yield and stem measurements. Taken together, farmers seeking to plant fiber hemp in North Carolina have a wide planting window from mid-March to mid-May using new genotypes from similar latitudes to our growing region.

Introduction

Growing interest exists in North Carolina towards the introduction of fiber hemp (*Cannabis sativa* L. <0.3% total tetrahydrocannabinol [THC]) as an alternative crop. Dingha et al. (2019) conducted a study among certified organic farmers in NC, and found that 93% of respondents indicated that if they had assurance towards market profitability, they would grow hemp for certified fiber and seed. The majority of hemp grown in NC was high-cannabinoid floral hemp for extraction of smoking purposes. However, since the 2018 Farm bill was passed there has been a decrease in the acreage of land licensed floral hemp in NC (Quaicoe et al., 2023). Though interest in fiber hemp has grown in NC, appropriate genetics for the region has been a limiting factor. Preliminary fiber hemp field trials in NC utilized genetics sourced from more northerly latitude regions of Europe. Overbaugh et al. (2019) investigated different planting densities using early (‘CFX2’) and late (‘Felina32’; ’Carmagnola’) flowering cultivars from Canada and Northern Europe. The varieties investigated in these trials flowered mid- to late-July, resulting in suboptimal biomass production. Fiber hemp is harvested at the onset of flowering, consequently varieties with a longer vegetative cycle (shorter critical photoperiod) are required for profitable biomass production in the Southeast.

Region specific genetic research is imperative to the success of fiber hemp as an agriculture commodity in the US. Genetics have been known to play a role in the outcome of plant growth factors such as stem diameter (Campiglia et al., 2017; Darby et al., 2018), flowering time (Overbaugh et al. 2019), height (Herppich et al., 2020; Papastylianou et al., 2018; Westerhuis et al., 2019), and stands (Darby et al., 2018; Overbaugh et al., 2019; Wilsie et al., 1944). Eight CBD, fiber, and dual cultivars from China (‘Bama’, ’Puma-3’, ’Yuma-2’), Europe (’Carmagnola Selezionata’, ’Eletta Campana’, ’Tygra’), and the US (‘Cherry Blossom x T1’, ‘Cherry Blossom’) were examined under controlled environments with supplemental lighting in Florida (Desaeger et al., 2022). Coolong et al. (2021) determined the use of supplemental lighting prolonged the vegetative state in all their varieties, and stated their Chinese cultivars never went to flower. Chinese (‘Jin Ma’) and Polish (‘Bialobrezeskie’) varieties were compared in Georgia, where the authors found ‘Jin Ma’ stayed in a vegetative state longer than ‘Bialobrezeskie’ . The authors reported ‘Bialobrezekie’ was harvested at the mid-August, while ‘Jin Ma’ was harvested mid-September. Dry retted straw yields were evaluated among 28 fiber and dual cultivars over the course of four years in New York (Smart et al., 2022). The authors found differences in yield among cultivars across location and year, strengthening the importance of matching genetics with environment.

Fiber hemp is a short-day, photoperiod-sensitive plant. A large body of research exists examining hemp’s photoperiodic responses across Europe, Australia, and parts of the US (Lisson et al., 2000; Mediavilla et al., 2001; van der Werf et al., 1994; Zhang et al., 2021). Two cultivars (Kompolti and Futura 77) were examined in growth chambers, with photoperiods ranging from 8 to 16 h in Australia (Lisson et al., 2000). The authors reported different responses to flowering time when the daylength was shortened; rapid flower development with daylength shorter than 14 h in both genotypes. Fiber morphology (female v. male flowers) and growth yield formation parameters were investigated on ‘Kompolti’ in a field trial located in Switzerland (Mediavilla et al. 2001). The authors found strong correlations between the amount of fiber yield with stem and bark development throughout the season. The authors also suggested harvesting at the time of ‘technical maturity’ which was defined as the end of male flowering, or beginning of female flowering, to achieve maximum fiber yields. Technical maturity was reported between 25 August and 2 September in the aforementioned study. Later floral development has been achieved when investigating 24 h daylength in the Netherlands (van der Werf et al. 1994). The authors of this study examined two cultivars (‘Fédrina 74’; ‘Kompolti Hybrid TC’), where their field conditions consisted of 24 h daylength over the course of two field seasons. The authors stated that higher stem yields are achievable with late flowering varieties in the Netherlands, although higher stem yields have the potential to diminish stem quality. Similar to Lisson et al. (2000), Zhang et al. (2021) determined critical photoperiod for fiber and grain cultivars grown under controlled lighting. They found that those genetics originating from European countries in more northerly latitudes flowered once days shortened below 15 h. The Chinese genetics investigated (‘HAN-FN-H’, ‘HAN-NE’, and ‘HAN-NW’) flowered once days shortened below 14h, and ‘PUMA-3’ and ‘PUMA-4’ flowered once days shortened below 12 h. These findings suggest that Chinese fiber hemp genetics sourced from more Southerly latitudes may be a better fit for southeast US hemp production.

Currently, little to no research exists investigating proper genetics for fiber hemp in the Southeastern region. With grower interest and market value on the rise, there is a novel curiosity for investigation on this crop as a means to break pest and disease cycles and add to the rising diversity of NC agriculture. As such, the objectives of this study were to: 1) investigate a broad panel of Chinese fiber hemp genetics for textile-grade fiber production; 2) compare planting dates and density across the state and; 3) develop grower recommendations that allow for maximum yield and crop quality.

Materials and Methods

Field Layout

Field experiments were conducted at the Caswell Research Farm (KIN), in Kinston, NC (35°17'54.0"N 77°34'21.2"W) on Norfolk loamy sand (fine-loamy, siliceous, semiactive, acid, Type Fluvaquentic Endoaquepts), and the Piedmont Research Station (SAL) in Salisbury, NC (35°41'08.7"N 80°36'07.3"W) on Mecklenburg clay loam (fine, mixed, active, Type Ultic Hapludalfs) in 2021. In 2022, the trials were conducted in SAL and at the Cherry Research Farm (GBS) in Goldsboro (35°22'59.7"N 78°02'04.0"W), NC on Dragston loamy sand (coarse-loamy, mixed, semiactive, Type Aeric Endoaquults) (n = 4 site years).

Eleven fiber hemp varieties were evaluated in 2021 and seven varieties in 2022. The eleven varieties assessed in 2021 comprised Chinese genotypes; seven of the highest yielding varieties were evaluated in 2022 (Table 2.1.). The Chinese genetics were sourced from Zhangpu Zhonglong Kenaf Seeds Co in Fujian Province, China. We also investigated three planting dates: mid-March, mid-April, and mid-May. The mid-March planting date was included to evaluate fiber hemp's tolerance to colder temperatures. In 2021 we were unable to plant the mid-March treatment at KIN due to prolonged rain and wet soils. In 2022, the mid-May planting date at SAL was delayed until June 22 due to extreme drought (Table 2.2.). Actual planting dates in the 2021 season were 14 April, and 18 May at KIN and 12 March, 12 April, and 17 May at SAL. Planting dates in the 2022 season were 28 March, 21 April, and 22 June at SAL, and 23 March, 27 April, and 25 May at GBS.

Fields for each planting date were prepared approximately one week prior to planting. Soils were deep tilled, a pre-emergent herbicide applied (Sonalan 0.99 L a.i. Ethalfluralin ha^{-1}, Dow Agrosciences, Indianapolis IN), and half of the fertilizer (34 kg N ha^{-1}; 15-0-2, micro pelletized Allganic Nitrogen Plus, SQM Specialty Plant Nutrition, Atlanta, GA) applied by hand. The remaining 34 kg N ha^{-1} was applied approximately four weeks after emergence.

Experimental Design

Experimental design followed a split-plot randomized complete block design with three blocks at SAL in 2021, four blocks at KIN 2021, GBS 2022, and SAL 2022. Planting date was the main-plot and variety the sub-plot. Each variety was randomly assigned to 6 m × 1.5 m plots. Seeds were direct seeded using a grain drill (Plot Motion XL, Wintersteiger, Ried im Innkreis, Austria) with 19.05 cm row spacing. Seed depth was dependent on soil type and moisture and ranged between 0.6 cm and 1.3 cm. In 2021 we planted at a rate of 1,878,000 Pure Live Seed (PLS) ha^{-1}, based on seed provider recommendations, and in 2022 we increased this rate to 2,471,053 PLS ha^{-1}. We increased the planting rate due to lower-than-expected stands in 2021.

Data Collection

Throughout the season plants were examined weekly to note time of flower development. All varieties included in the study were dioecious, so floral initiation was defined as the presence of unopened staminate flowers (growth code 2100; Mediavilla et al., 1998).

Approximately one week after floral initiation, 1 m^2 of plants per plot were harvested using a professional trimmer with a circular saw blade attachment (Figure 2.1.). All plants were cut, roughly 4 cm above the ground. Plants from each 1 m^2 were counted to calculate final stand counts. Stem height and diameter from ten representative plants were recorded. Heights were measured from the cut site to apical meristem. Stem diameter was measured 10 cm above the cut site. After these data were collected, we bundled the stems together and left to ret in the field. In 2021, bundles were left in the field for one week and then flipped and left for another week. In 2022, bundles were left in the field for two weeks before flipping and left for another week. Finally, stems were collected and dried in modified tobacco barn at 48° C for 48-72 h and final straw dry weight collected.

Statistical Analysis

All data were analyzed in SAS v. 9.4 (SAS Institute, Cary, NC) with the GLIMMIX procedure. We analyzed each environment (unique location × year combination) separately due to differences in planting dates. Variety and planting date were treated as fixed effects. Block and block × planting date were treated as random effects. When appropriate, least squares mean estimates were compared using Tukey's Honestly Significant Difference *post-hoc* means separation test. Finally, we conducted a correlation among stand count, stem diameter, stem height, and stem dry weight to understand better any potential relationship.

Results and Discussion

Field Conditions

All locations experienced temperatures below freezing in the month of March and in May for SAL 2021 (Figure 2.2.). We saw no visible negative effects from experiencing below freezing temperatures. At the time of this publication, this research is the first in the US to observe below freezing field conditions while producing fiber hemp. Mayer et al. (2015) investigated nine Canadian varieties in controlled environments to ascertain cold-stress response among four freezing temperatures (-3 °C, -5 °C, -7 °C, -9 °C). The authors observed varietal differences in the ability to acclimate to freezing temperatures; categorizing their varieties among three groups - (1) cold-acclimated, (2) acclimated in at least one freezing temperature, or (3) not-acclimated. Their research showed that certain varieties can withstand freezing conditions and still recover in controlled environments. Comparatively, our research adds to the growing body of knowledge on fiber hemp field production. Our data support Mayer et al. (2015) finding that fiber hemp is capable of withstanding freezing field temperatures, specifically at planting; with no visible negative impact on growth. Overall, average monthly temperatures were higher in

2022 at both locations, with August and September being exceptions (Table 2.2.). Kinston experienced generous amounts of rainfall in March, 2021 (37.1 cm total), which hindered our ability to plant the mid-March treatment; KIN had the highest season total rainfall (116 cm), followed by SAL 2022 (84 cm). Salisbury 2021 (70.7 cm) and GBS 2022 (71.5 cm) had comparable season total rainfall patterns. Unseasonably low precipitation occurred during the month of June, 2022 in SAL (0.8 cm; Table 2.2.).

Flowering date

All varieties and planting dates showed staminate flowers during the last week of August and first week of September in both years of the trial. The day length at this time of year in North Carolina is approximately 13 hr. These results are in agreement with Zhang et al. (2021). The authors found that ‘Puma-3’ and ‘Puma-4’ reached 100% flower initiation at a photoperiod of 12 h 30 min. Overbaugh et al. (2019) conducted initial fiber hemp variety trials in North Carolina using the varieties Felina32, Carmagnola, and CFX2. The authors reported pistillate flower maturity, defined as the time when 50% of pistillate plants had reached flowering, ranging from 14 July to 4 August. Day length at these dates ranged from 14:25 to 13:54. These differences are invariably due to the latitudinal differences of the seed sources.

Stand Counts

Plant stands were affected by the interaction of planting date × variety in KIN 2021 ($p < 0.0001$; Table 2.3.). Varieties planted on the 14 April had statistically equivalent stands. Yuma-1 (655,000 plants ha^{-1}) and Yuma-2 (452,000 plants ha^{-1}) planted on the 18 May had the highest stands at KIN in 2021.

Plant stands were affected by the main effects of planting date ($p = 0.0012$) and variety ($p < 0.0001$) in SAL 2021 (Table 2.4.). The 12 April planting date had the highest stands (298,125 plants ha^{-1}), followed by 12 March (139,777 plants ha^{-1}) and 17 May (134,088 plants ha^{-1}); the latter two dates were not different. Yuma-2 (418,817 plants ha^{-1}) and Yuma-1 (377,775 plants ha^{-1}) had the highest stands and were not statistically different from one another.

Plant stands were affected by variety in GBS 2022 (p = 0.0388; Table 2.5.). We saw greater differences among the stands at this location. Yuma-3 (740,833 plants ha^{-1}) had the highest stands, but was not statistically different from Yuma-1 (704,500 plants ha^{-1}), Yuma-0 (696,667 plants ha^{-1}), or Yuma-2 (659,167 plants ha^{-1}). Puma (570,000 plants ha^{-1}) and Yuma-4 (567,500 plants ha^{-1}), and Bama (558,333 plants ha^{-1}) had the lowest stands. Similarly, SAL 2022 plant stands were affected by variety (p = 0.0193; Table 2.5.). Yuma-1 (670,833 plants ha^{-1}) had the highest stands but was not different from Yuma-0 (551,667 plants ha^{-1}), Yuma-2 (545,833 plants ha^{-1}), or Yuma-3 (628,540 plants ha^{-1}). Bama (412,141 plants ha^{-1}), Puma (519,167 plants ha^{-1}), and Yuma-4 (474,167 plants ha^{-1}) were not different from one another. The latter three varieties had the lowest stand estimate at SAL in 2022.

Overall, stands were higher in 2022 than in 2021, likely due to the increase in seeding rate and overall better seed quality. However, stands were still not reaching the initial population of 1,878,000 plants ha^{-1} in 2021 nor 2,471,053 PLS ha^{-1} in 2022.

Limited research exists on fiber hemp in the US due to only recently being made a legal commodity. Overall plant stands throughout literature can be highly variable based on genetics; our plant stands were lower than those reported previously in literature (Darby et al., 2018; Overbaugh et al., 2019; Wilsie et al., 1944), apart from van der Werf et al. (1995). It should be noted the former three studies were conducted over one year, and appropriate genetics have yet to be established for Southeastern production. Overbaugh et al. (2019) investigated three cultivars (‘CFX2’, ’Carmagnola’, ’Felina32’) across three seeding rates (16.8, 18, and 39.2 kg ha^{-1}) in Salisbury, NC. The authors reported differences between the main effects. It should be noted that the cultivars used were not well-suited for southern latitudes. The authors reported their planting date for this study to be 16 June 2017 and each cultivar bloomed and was harvested on 17 August, 2017. Comparatively, our crop did not start to flower until the last week August and first week of September. Consequently, these Chinese genetics provide a more regionally-appropriate fiber hemp alternative for growers in the Southeast, allowing them to produce biomass yields comparable to other fiber-producing regions of the world. Wilsie et al. (1944) investigated two varieties, ‘Chilean’ and ‘Kentucky’, across three seeding rates at two locations in Wisconsin. Their seeding rates were among, and not limited to, 36 – 124 kg ha^{-1} (seeding rates were based on the weight of seed, not the exact number of seeds being planted), this study took place for a single season in 1942. The authors averaged their harvest plant stands across all three seeding rates, where they reported a mean for desirable (above 1.2 m tall) stands to be 1,208,247 plants ha^{-1} (authors reported in plants/sqft), higher stand counts than we saw. The authors saw a negative correlation between stem height and increased seeding rate at two of their locations. van der Werf et al. (1995) tested the effects of self-thinning among four planting densities and two cultivars (‘Kompolti Hybrid TC’ and ‘Kozuhara zairai’) to ascertain differences in fiber hemp biomass yield, plant mortality and morphology in the Netherlands. The authors reported that increasing the plant density at or above 90 plants m^{-2} also resulted in self-thinning through inter-row competition. van der Werf et al. (1955) reported the differences among initial plant density and cultivar on above ground dry biomass at harvest. The authors reported dry matter among harvests to range from 0.36 – 17.5 t ha^{-1}, cultivar differences ranged from 1.16 – 19.4. Our stands were higher than those reported by the previous authors, which may have been influenced due to initial PLS rates, or genetic factors. Darby et al. (2018) investigated twelve varieties of fiber hemp in Vermont to determine proper genetics for the region. The authors reported mean harvest populations of 1,628,984 plants ha^{-1}, which are higher than our reported stand values. Although, formerly mentioned values are from one year's worth of data.

Average Height

Stem heights in in KIN 2021 and GBS 2022 were not affected by the main effects or interaction of variety and planting date ($p > 0.05$). Average height in KIN 2021 was 266.94 cm, and 246.21 cm in GBS 2022.

Average height in SAL 2021 was affected by the main effect of variety ($p = 0.0257$; Table 2.6.). Yuma-4 (336.30 cm) produced the tallest plants, although they were not different from Bama (316.31 cm), MS77 (320.22 cm), Puma (326.57 cm), Yuma-0 (317.94 cm), or Yuma-3 (304.47 cm). Yuma-1 (300.38 cm) and Yuma-2 (298.71 cm) produced the shortest plants and were not different from former varieties listed.

Average height in SAL 2022 was affected by the main effect of planting date ($p = 0.0009$; Table 2.7.). The 28 March (283.27 cm) and 21 April (277.39 cm) produced the tallest plants, while 22 June (204.91 cm) produced the shortest at this location.

Very few studies exist investigating the sole effect of Chinese fiber hemp genetics and plant height. Those studies that have investigated genetic impacts on plant height tend to utilize European genetics. Our overall height results are comparable to Papastylianou et al. (2018) who studied the effects of four nitrogen fertilization rates on five different varieties of fiber hemp in Greece. In general, the authors saw a positive response between increased N and stem height, although the main effects alone were not significant. The authors reported the differences in height among their fertilization rates across each variety; heights ranged between 1.6 m to 1.7 m, respectively. Our height results are comparable, and were slightly taller than those reported by

Papastylianou et al. (2018), apart from our 22 June, 2022 planting date in SALS (Table 2.7.). Herppich et al. (2020) tested the physiological effects of extreme drought and severe temperature conditions on two high-yielding fiber hemp cultivars ‘Ivory’ and ‘Santhica-27’ in Germany. The authors reported taller plants, and more variability in height when comparing ‘Santhica-27’ to ‘Ivory’, 90 days after planting. We saw differences in height when comparing our varieties (Table 2.6.), like Herppich et al. (2020), suggesting that average height could be driven by genetic factors such as variety. Our overall height results were a bit taller compared to their tallest producing cultivar ‘Santhica-27’. Taller plants (1.5 m or taller) may not be ideal for textile-grade fiber hemp production due to the likelihood of secondary fiber production (Westerhuis et al. 2019). Tang et al. (2022) conducted a fertilization and planting density study where three fertilization rates, two NPK ratios, and two planting densities were assessed. The authors tested these effects on fiber hemp yield in China across three locations; each location tested a different cultivar. The authors reported total rainfall between field seasons and locations to range from 262 -1059 mm. The former average values being much lower than our reported total rainfall values. The authors reported average plant heights ranging from 217 to 500 cm in height, respectively; where one location in 2016, and a different location in 2017 showed significant positive correlations. Our average height data is in line with what has been reported by Tang et al. (2022), where they experienced much less rainfall at some locations, comparatively; the vast range of precipitation fiber hemp can withstand is worth noting. Livingstone et al. (2022) investigated the relationship of four planting densities across two row spacing treatments with two fiber hemp cultivars (‘Fasamo’ and ‘Ferimon-12’) in New Zealand. Similar to van der Werf et al. (1995), the authors of this study reported that high densities (above 80 plants m^{-2}) resulted in shorter plants. The authors reported plant heights to be significantly influenced by location; our average plant heights were comparable to their ‘Fasamo’ cultivar (~198.3 cm), and most were taller than their ‘Farimon-12’ cultivar (~151.1 cm).

Average Stem Width

Average stem width in KIN 2021 was affected by the main effects of planting date ($p = 0.0074$) and variety ($p < 0.0001$; Table 2.8.). We saw 14 April (23.74 mm) produce thicker stems compared to the 18 May (15.33 mm) planting date. The thinnest plants were found from Yuma-1 (12.79 mm), though they were not different from Yuma-2 (13.34 mm), Yuma-3 (19.04 mm), or Yuma-4 (19.18). The thickest stems were found from Bama (24.64 mm), MS77 (20.85 mm), Puma (24.03 mm), Puma-3 (21.14 mm), and Yuma-0 (20.78 mm).

Average stem width in SAL 2021 was affected by the interaction of planting date × variety ($p = 0.0038$; Table 2.9.). Thinnest stems were found in Yuma-1 and Yuma-2 in 12 April and 12 March, though they were not different from MS77 (20.22 mm) or Yuma-3 (20.79 mm). Puma (30.83 mm) and Yuma-4 (29.60 mm) produced the thickest stems, and were not different from Bama (23.42 mm) or Yuma-0 (25.26 mm). All varieties from our 17 May planting date were statistically the same with the thinnest stem coming from Yuma-2 (15.59 mm), and the thickest stems coming from Yuma-4 (22.95 mm). Puma planted 12 March produced the thickest stems.

The main effects of variety and planting date, as well as their interaction were not significant at either location in 2022 (tables not included). Goldsboro 2022 had an average stem width (ASW) of 10.80, and SALS 2022 had an ASW of 10.58 mm.

Campiglia et al. (2017) investigated seven fiber hemp genotypes, three planting densities, and two N fertilization rates in Central Italy. The authors reported differences among the interactions of genotype × N fertilizer, and plant density × N fertilizer; where the differences among genotypes were greater compared to density. The authors reported stem diameter in genotypes ranged from 5.0 - 6.7 mm, dependent N fertilizer rate. Stem diameter showed an inverse correlation with plant density, the authors reported widths from 4.6 mm (highest density, lowest N) – 6.9 mm (lowest density, highest N). Comparatively, we also saw differences in our stem width when comparing varieties; our average widths in 2021 (Table 2.8; Table 2.9.) were much larger than those reported by Campiglia et al. (2017). When our seed rate was increased in 2022, we observed thinner stems (data not significant), though still double those reported by the previous authors. Our stem widths were comparable to those reported by Tang et al. (2017). The authors observed stem diameters ranging from 13.9 – 16.6 mm across their locations. Darby et al. (2018) evaluated twelve varieties during one season in Vermont to determine genetic recommendations for their region. The authors reported a trial mean for stem diameter of 4.31 mm, which was much lower than any of our reported widths. The authors did see differences based on variety, which were similar to our study; although, the former values were only from one year's worth of data, and genetics were sourced from different growing regions.

In general, the stem diameter from the harvested material was thicker than what the textile-grade fiber hemp industry is seeking. A stem diameter the width of a #2 pencil (7.5 mm) is desirable and anything thicker generally results in more hurd and no gain in fiber or fiber quality (Guy Carpenter, Bear Fibers, *personal communication*). Stem diameter increases throughout the season (Westerhuis et al., 2019). Though the initiation of flowering is generally regarded as the time to harvest, the long growing season of the Chinese fiber hemp genetics investigated may warrant earlier harvest timing to meet industry specifications.

Dry Retted Straw Yield

Yields in SAL 2021 were affected by the interaction of planting date × variety ($p = 0.0017$; Table 2.10.). Our 12 March planting date showed Yuma-0 (35,124 kg ha^{-1}) produced the highest yields (ha^{-1}) from all planting dates at this location, though not statistically different from Bama (22,309 kg ha^{-1}). April 12 planting date showed Yuma-0 (21,809 kg ha^{-1}) and Yuma-3 (22,449 kg ha^{-1}) produced the highest yields, though not different from any other variety. May 17 planting date showed Yuma-0 (24,559) with the highest yield, though like our 12 April planting date, not different from any other variety. Our lowest yield came from Bama planted on 17 May (3,709.80).

Yields in SAL 2022 were affected by the main effect of planting date ($p < 0.0001$; Table 2.11.). Our 28 March (18,648) and 21 April (17,636) planting dates were not different from one another but were higher than our latest, 22 June (4,824.54) planting date.

The main effects of planting date and variety, as well as their interaction were not significant in KIN 2021, or GBS 2022 (tables not included). Dry retted straw yields (DRSY) for KIN 2021 were 18,755 kg ha^{-1}, and 11,740 kg ha^{-1} in GBS 2022.

Our stem yields compare favorably to previously published work. Mediavilla et al. (2001) investigated the effect of harvest time on fiber yield, quality, stem proportion and the percentage of female versus male flowers using 60 kg ha^{-1} ‘Kompolti’ in Switzerland. The authors reported their maximum stem yield to be 14.8 t ha^{-1}. Campiglia et al. (2017) reported differences in stem yield, ranging from 3.48 t ha^{-1} in their lowest density (‘Santhica-27’) to 7.19 t ha^{-1} in their highest density (‘Santhica-27’) at 50 kg N ha^{-1}. Comparatively, their 100 kg N ha^{-1} treatment showed higher stem yields ranging from 3.92 t ha^{-1} in their lowest density (‘Ferimon-17’) to 8.20 t ha^{-1} in their highest density (‘Santhica-27’). Our yields were higher, with the exceptions of

Yuma-3 on 12 March, and Bama, MS77, and Puma on 17 May, 2021 (Table 2.10.). As well as our 22 June, 2022 planting date (Table 2.11.). Overbaugh et al. (2019) conducted one of the first studies exploring fiber hemp production in NC. The authors investigated three varieties and three planting densities over the course of one field season in Salisbury. Where they reported biomass at maturity ranging anywhere from 1,207 – 7,884 kg ha^{-1}, differences were seen among varieties which the authors attributed to staminate flowering date. Our DRSW was much higher than those reported by Overbaugh et al. (2019), which is likely due to the longer growing season of the Chinese genetics and lower critical day length threshold.

Correlation Data

Correlation between stem yield and planting density among the four environments was only significant SAL 2022 (r = 0.332; Figure XXXX). However, when data were combined, there was an overall significant negative correlation (r = -0.225; FIGURE#). Our overall stem yield and planting density correlation results are comparable to those reported in previous literature. van der Werf et al. (1995) reported stem yield to be negatively correlated with planting density. The authors of this study reported self-thinning to be associated with densities above 90 m^{-2}.

Among the four environments there was only a significant correlation between stem diameter and stem yield in GBS (0.330) and SAL, 2022 (0.371); however, when combined there was a significant positive correlation (r = 0.395). Tsaliki et al. (2021) observed similar, albeit non-significant correlation between stem diameter and fiber yields; as stem diameter increased, fiber yields increased.

Stem density and stem height showed a significant negative, weak correlation in two of the combined environments (SAL 2021 [r = -0.272] and GBS 2022 [-0.300]). When combined, this significant, weak negative correlation remained (r = -0.377). The correlation between stem density and stem height seems to be highly variable across environments. Campiglia et al. (2017) investigated the relationship among genotype, planting density and N fertilization. The authors also saw a negative correlation with planting density and plant height, similar to our overall negative correlation. The authors reported as much as a 41% decrease in height when comparing their highest density treatment (40 plants m^{-2}) to their lowest density treatment (120 plants m^{-2}), due to early reproductive maturity associated with their higher density. Wilsie et al. (1944) reported a somewhat negative trend in relation to increased density and shorter plants at one of their locations; however, they did not observe this trend at their second location. This may indicate a complex environmental interaction in determining plant development throughout the season.

All environments showed a significant positive correlation between height and yield. Similarly, when combined this relationship was significant (r = 0.504). Amaducci et al. (2008) found that delaying harvest increased stem height and consequently biomass. However, Tsaliki et al. (2021) observed a non-significant, negative trend in stem height and bast fiber production. However, these authors did not investigate the relationship between stem height and total biomass produced.

Similar to the relationship between height and yield, a common, significant positive correlation was observed between height and stem diameter, Pearson correlation coefficients ranged from 0.503 to 0.778, and all were significant. When combined across environments, this significant positive relationship held true (r = 0.622). Campbell et al. (2019) reported that one of their grain cultivars had a strong, positive correlation between plant height and stem diameter values. The authors stated that they also noticed a strong positive correlation among plant height, stem diameter, and water uptake within the plants. Although we did not test water uptake in this study, this is certainly worth further investigation.

Finally, the relationship between plant density and stem diameter showed a significant negative correlation for all environments. When combined, this relationship showed the strongest correlation (r = -0.727) among all metrics evaluated. Our plant density and stem diameter correlation results are comparable to other literature. Campiglia et al. (2017) found a negative correlation between their plant density and stem diameter results. Darby et al. (2018) found that their thickest stem diameters came from their least dense stands. Hall et al. (2014) also reported that they saw a significant inverse relationship between plant density and stem diameter. Our results add to the growing literature that genetic and environmental factors both play important roles in the determination of plant density and stem diameter.

Cannabinoid Results

Cannabinoid results did not get replicated over locations; thus, no formal statistical analysis was possible to determine differences among varieties. Some of our varieties were approaching the negligent 1% rate, including; Yuma-2 in 2021, and Yuma-0 in 2022 (Table 2.12.). Clarke et al. (2020) saw two of their Chinese varieties (‘Bama’ [0.5060 % dry weight] and ‘Yuma’ [0.7230 % dry weight]) tested above the allowed thresholds. High total THC values is worrisome. If values are above 0.30% total THC, farmers must remediate their crop, which adds an economic burden (USDA AMS, 2021). If the values are above 1.0% the farmer receives a negligent violation. Receiving three negligent violations in a five-year span will result in a five-year suspension of licensure.

Conclusions

All Chinese varieties proved to be a good fit for North Carolina’s latitude given the lower critical day length threshold and later flowering. This is the first study of its kind to investigate proper genetics fit for fiber hemp production in the Southeastern US. We found differences among varieties, but there were not common trends throughout years and location, likely due to differences in seed quality and stand counts between years. Planting date also appeared to have minimal effects on final crop yield and stem measurements. This gives farmers a large planting window and may even allow for inclusion of fiber hemp in a double-cropping system. Though biomass yields were high, the stems produced may not have been of optimal quality for the textile industry given the thick stems. The extremely long vegetative growing season of these Chinese varieties may warrant different harvest timing to meet the specific quality needs of the textiles industry. Some of our varieties tested above the 0.3% THC threshold, proving that more work is needed to develop compliant varieties. We found strong correlations between plant density and many agronomic traits at harvest. Further work is needed to determine optimum planting density that incorporates yield, quality, and economic return.

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Table 2.1. Eleven fiber hemp varieties evaluated in 2021, as well as the seven highest yielding varieties evaluated in 2022.

Year	Variety
2021	
	Bama (B)
	Bama-4 (B4) *
	Bama-5 (B5) *
	MS77 (MS77)
	Puma (P)
	Puma-3 (P3)*
	Yuma-0 (Y0)
	Yuma-1 (Y1)
	Yuma-2 (Y2)
	Yuma-3 (Y3)
	Yuma-4 (Y4)
2022	
	Bama (B)
	Puma (P)
	Yuma-0 (Y0)
	Yuma-1 (Y1)
	Yuma-2 (Y2)
	Yuma-3 (Y3)
	Yuma-4 (Y4)

* Varieties were planted but dropped from the trial due to very poor germination and stands.

Table 2.2. Average Monthly Temperature and Total Precipitation in 2021 and 2022.

Environment[z]	March		April		May		June		July		August		September		Total
	Rainfall (cm)	Temperature (°C)	Rainfall (cm)	Temperature (°C)	Rainfall (cm)	Temperature (°C)	Rainfall (cm)	Temperature (°C)	Rainfall (cm)	Temperature (°C)	Rainfall (cm)	Temperature (°C)	Rainfall (cm)	Temperature (°C)	Rainfall (cm)
KIN 2021	37.1	10.2	2.9	16.5	4.5	20.1	25.3	24.2	19.7	25.7	22.4	26.3	4.1	22.7	116.0
SAL 2021	30.6	8.20	2.7	15.0	7.7	18.8	12.2	23.3	9.40	25.0	8.10	25.0	-[y]	-	70.7
GBS 2022	9.50	11.6	6.7	16.5	5.1	22.0	3.2	25.3	22.8	27.0	11.4	25.4	12.8	22.2	71.5
SAL 2022	20.6	9.70	7.4	15.0	9.5	20.5	0.8	24.4	20.7	25.6	9.40	24.4	15.6	20.3	84.0

[z] Caswell research farm (KIN), Cherry research farm (GBS), and Piedmont research station (SAL) average monthly temperatures and total precipitation. Data were retrieved from the nearest Econet stations using North Carolina State Climate Office (NCSCO) weather resources.

[y] Study in SAL 2021 terminated at the end of August.

Table 2.3. Analysis of plant stands from Kinston, 2021 influenced by the interaction of planting date × variety ($p<0.0001$).

Planting Date	Variety	Plant Stands (± SE) [z] (plants ha^{-1})
4/14/2021	Bama	7,658 (45,877) c
	MS77	34,362 (45,924) c
	Puma	27,041 (45,851) c
	Puma-3	47,990 (39,673) c
	Yuma-0	27,500 (39,673) c
	Yuma-1	210,000 (39,673) c
	Yuma-2	162,500 (39,673) c
	Yuma-3	21,028 (45,924) c
	Yuma-4	38,030 (56,285) c
5/18/2021		
	Bama	112,500 (39,741) c
	MS77	210,000 (39,741) c
	Puma	82,500 (39,741) c
	Puma-3	140,000 (39,741) c
	Yuma-0	117,500 (39,741) c
	Yuma-1	655,000 (39,741) a
	Yuma-2	452,500 (39,741) b
	Yuma-3	115,000 (39,741) c
	Yuma-4	117,500 (39,741) c
	p-value	<0.0001

[z] shared letter values indicate no significance (p = > 0.05)

Table 2.4. Analysis of plant stands from Salisbury, 2021 influenced by the main effects of planting date ($p = 0.0012$) and variety ($p<0.0001$).

Planting Date	Plant Stands (± SE) [z] (Plants ha^{-1})
3/12/2021	139,777 (23,028) b
4/12/2021	298,125 (19,883) a
5/17/2021	134,088 (28,272) b
p-value	0.0012
Variety	
Bama	127,508 (38,714) b
MS77	152,416 (32,603) b
Puma	95,793 (42,190) b
Yuma-0	142,036 (42,072) b
Yuma-1	377,775 (28,660) a
Yuma-2	418,817 (28,654) a
Yuma-3	93,545 (35,225) b
Yuma-4	116,618 (32,914) b
p-value	< 0.0001

[z] shared letter values indicate no significance (p = > 0.05)

Table 2.5. Analysis of plant stands from Goldsboro (p = 0.0388) and Salisbury (0.0193), 2022 influenced by the main effect of variety.

Location	Variety	Plant Stands (± SE) [z] (Plants ha^{-1})
GBS	Bama	558,333 (68,804) c
	Puma	570,000 (68,804) bc
	Yuma-0	696,667 (68,804) ab
	Yuma-1	704,500 (68,804) ab
	Yuma-2	659,167 (68,804) abc
	Yuma-3	740,833 (68,804) a
	Yuma-4	567,500 (68,804) bc
	p-value	0.0388
SAL	Bama	412,141 (57,200) c
	Puma	519,167 (51,700) bc
	Yuma-0	551,667 (51,700) abc
	Yuma-1	670,833 (51,700) a
	Yuma-2	545,833 (51,700) abc
	Yuma-3	628,540 (59,754) ab
	Yuma-4	474,167 (51,700) c
	p-value	0.0193

[z] shared letter values indicate no significance (p = > 0.05)

Table 2.6. Analysis of height from Salisbury, 2021 influenced by the main effect of variety (p = 0.0257).

Variety	Stem Height (± SE) [z] (cm)
Bama	316.31 (13.59) ab
MS77	320.22 (12.11) ab
Puma	326.57 (14.41) ab
Yuma-0	317.94 (14.05) ab
Yuma-1	300.38 (11.22) b
Yuma-2	298.71 (11.21) b
Yuma-3	304.47 (12.59) ab
Yuma-4	336.30 (12.10) a
p-value	0.0257

[z] shared letter values indicate no significance (p = > 0.05)

Table 2.7. Analysis of height from Salisbury, 2022 influenced by the main effect of planting date (p = 0.0009).

Planting Date	Stem Height (± SE) [z] (cm)
3/23/2022	283.27 (10.80) a
4/21/2022	277.39 (10.80) a
6/22/2022	204.91 (10.80) b
p-value	0.0009

Analysis of height from Salisbury, 2022 influenced by the main effect of planting date (p = 0.0009). Plant height was taken from ten representative plants / plot.

[z] shared letter values indicate no significance (p = > 0.05)

Table 2.8. Analysis of stem width from Kinston, 2021 influenced by the main effects of planting date (p = 0.074) and variety (p < 0.0001).

Planting Date	Stem width (± SE) [z] (mm)
4/14/2021	23.74 (1.25) a [y]
5/18/2021	15.33 (1.17) b
p-value	0.0074
Variety	
Bama	24.64 (1.92) a
MS77	20.85 (1.92) ab
Puma	24.03 (1.92) a
Puma-3	21.14 (1.82) a
Yuma-0	20.78 (1.79) ab
Yuma-1	12.79 (1.79) c
Yuma-2	13.34 (1.79) bc
Yuma-3	19.04 (1.92) abc
Yuma-4	19.18 (2.16) abc
p-value	<0.0001

[z] Stem diameter was collected from 10 plants per plot and measured 10 cm above the cut site at harvest.

[y] Means within a main effect followed by the same letter are not significantly different (p > 0.05).

Table 2.9. Analysis of stem width from Salisbury, 2021 influenced by the interaction of main effects planting date × variety (p = 0.0038).

Planting Date	Variety	Stem width (± SE) [z] (mm)
3/12/2021		
	Bama	23.42 (1.73) ab
	MS77	20.22 (1.92) abc
	Puma	30.83 (2.23) a
	Yuma-0	25.26 (2.23) ab
	Yuma-1	14.71 (1.73) c
	Yuma-2	14.48 (1.73) c
	Yuma-3	20.79 (2.23) abc
	Yuma-4	29.60 (1.91) a
4/12/2021		
	Bama	17.35 (1.73) bc
	MS77	15.67 (1.73) bc
	Puma	18.37 (1.73) bc
	Yuma-0	20.31 (1.17) abc
	Yuma-1	13.68 (1.73) c
	Yuma-2	13.32 (1.73) c
	Yuma-3	20.35 (1.73) abc
	Yuma-4	17.34 (1.73) bc
5/17/2021		
	Bama	19.01 (3.14) abc
	MS77	19.04 (2.30) abc
	Puma	21.64 (3.19) abc
	Yuma-0	21.21 (3.07) abc
	Yuma-1	16.68 (1.94) bc
	Yuma-2	15.59 (1.94) bc
	Yuma-3	21.14 (2.30) abc
	Yuma-4	22.95 (2.30) abc
	p-value	0.0038

[z] Stem diameter was collected from 10 plants per plot and measured 10 cm above the cut site at harvest.

[y] Means within a main effect followed by the same letter are not significantly different ($p > 0.05$).

Table 2.10. Analysis of dry retted stem yield in Salisbury, 2021 influenced by the interaction of planting date × variety (p =0.0017).

Planting Date	Variety	Weight (± SE) ^{z} (Kg ha^{-1})
Planting date × Variety		
3/12/2021	Bama	22,309 (2,543.96) ab
	MS77	17,520 (2,853.94) b
	Puma	14,945 (3,386.66) b
	Yuma-0	35,124 (3,386.39) a
	Yuma-1	16,054 (2,543.96) b
	Yuma-2	19,219 (2,543.96) b
	Yuma-3	6,117.16 (3,386.66) b
	Yuma-4	16,601 (2,853.14) b
4/12/2021		
	Bama	18,354 (2,543.96) b
	MS77	19,254 (2,543.96) b
	Puma	16,919 (2,543.96) b
	Yuma-0	21,809 (2,543.96) ab
	Yuma-1	19,929 (2,543.96) b
	Yuma-2	19,869 (2,543.96) b
	Yuma-3	22,449 (2,543.96) ab
	Yuma-4	17,639 (2,543.96) b
5/17/2021		
	Bama	3,709.80 (4,629.63) b
	MS77	7,042.00 (3,386.66) b
	Puma	8,731.00 (4.635.73) b
	Yuma-0	24,559 (4,625.92) ab
	Yuma-1	11,915 (2,852.04) b
	Yuma-2	18,973 (2,853.94) b
	Yuma-3	14,464 (3,386.39) b
	Yuma-4	13,557 (3,386.71) b
	p-value	<0.0017

^{z} shared letter values indicate no significance ($p > 0.05$)

Table 2.11. Analysis of dry retted stem yield in Salisbury, 2022 influenced by the main effect of planting date ($p < 0.0001$).

Planting Date	Weight (± SE) [z] (Kg ha^{-1})
3/23/2022	18,648 (798.49) a
4/21/2022	17,636 (798.49) a
6/22/2022	4,824.54 (798.49) b
p-value	<0.0001

[z] shared letter values indicate no significance ($p > 0.05$)

Table 2.12. Total THC % by dry weight volume across each year. These data were not replicated across locations therefore we can only report on the values alone, and not their significance.

	Bama	Puma	Yuma-0	Yuma-1	Yuma-2	Yuma-3	Yuma-4	MS77
2021	0.4067	0.6699	0.2961	0.2117	0.9276	0.2261	0.2544	0.1803
2022	0.0658	0.0850	0.9498	0.0328	0.0343	0.0228	0.0248	

Figure 2.1. Fiber hemp harvest technique. Professional trimmer with a circular saw blade attachment was used.

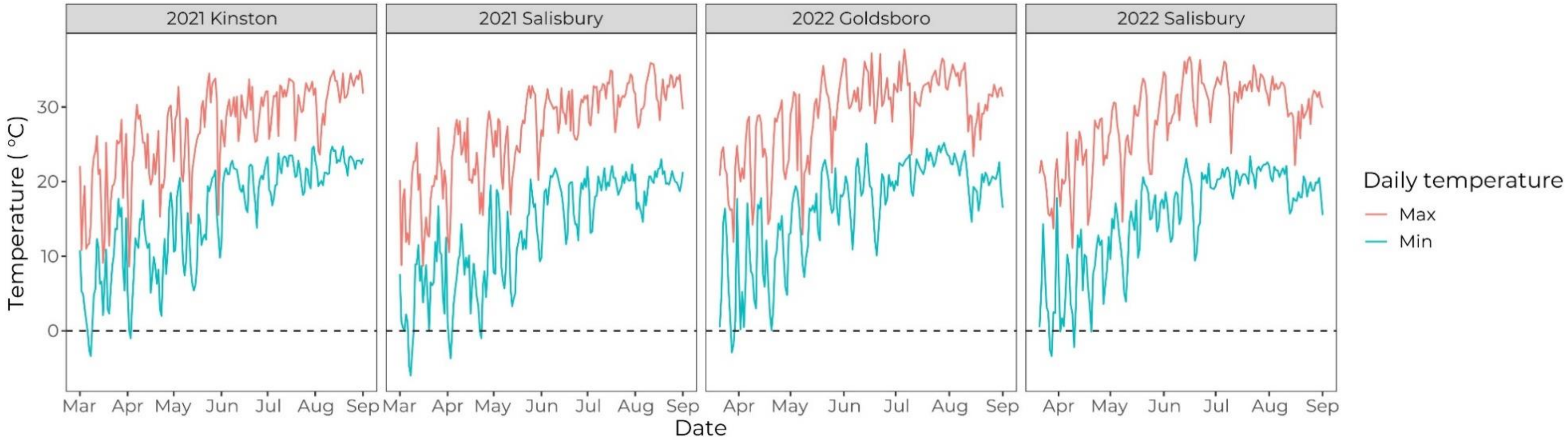

Figure 2.2. Daily minimum and maximum temperatures; 2021 Kinston (A), 2021 Salisbury (B), 2022 Goldsboro (C), 2022 Salisbury (D). Data were retrieved from the nearest Econet stations using North Carolina State Climate Office (NCSCO) weather resources.

Figure 2.3. Correlation matrix among fiber hemp growth metrics. Our strongest correlation from this data set seemed to be stem diameter (mm) and density (plants/ha; r = -0.727).