

ABSTRACT

OVERBAUGH, EZEKIAL. The Effect of Seeding Rate, Fertility and Cultivar on the Production of Industrial Hemp (*Cannabis sativa* L.) in North Carolina (Under the direction of Dr. Angela Post and Dr. Keith Edmisten).

Industrial hemp, (*Cannabis sativa* L.) is an emerging crop in North Carolina, with optimum cultural management still in development for the region. Production methods differ depending on target market, with most growers producing either seed, fiber, or cannabinoids. In 2017 and 2018, industrial hemp trials were planted in NC to evaluate yield responses to seeding rate and fertility in fiber and seed cultivars.

Seeding rate is the best management practice available to growers for managing crop density at the farm scale. In 2017 and 2018, crop density, seed yield and stem biomass of industrial hemp were tested in response to seeding rate for seed and fiber cultivars. In 2017, cultivars CFX2, Carmagnola and Felina32 were seeded at 16.8, 28.0, and 39.2 kg seed ha⁻¹. In 2018, CFX2 and Carmagnola were evaluated in separate trials. CFX2 was seeded at 100, 200, 300, and 400 pure live seed m⁻² (16.1, 32.2, 48.3, 64.5 kg seed ha⁻¹ equivalents). Carmagnola was seeded at 300, 400, 500, and 600 pure live seed m⁻² (54.8, 73.1, 91.4, 109.6 kg seed ha⁻¹ equivalents). The 2017 population trials took place in Salisbury, NC and the 2018 trials took place in Salisbury, Rocky Mount, and Oxford, NC. Results from both years and all locations indicate differences in biomass and seed yield were limited even as crop density increased significantly in response to seeding rate. Under weed free conditions, producers of seed and stem biomass can choose the lowest of the tested rates to optimize yield: 100 and 300 pure live seed m⁻² for cultivars CFX2 and Carmagnola, respectively. Higher rates may be necessary when competing against weeds. Textile hemp producers will also need to consider the impact of plant

density to fiber quality. Higher seeding rates have been reported to increase the proportion of bast fiber yield important for textiles (Meijer et al. 1995).

Fertility information is lacking for field grown hemp in North Carolina. In 2017 and 2018, fertility trials were established to test seed cultivars for responses to nitrogen (N), potassium (K), and sulfur (S) fertilization. In 2017, seed yield of cultivar CRS1 was tested in response to N rates of 0, 56, 112, 168 and 224 kg N ha⁻¹ and S rates of 0 and 39 kg S ha⁻¹. In 2018, seed yield of cultivar CFX2 was tested in response to N rates of 0, 56, 112, 168, and 224 kg N ha⁻¹, K rates of 0 and 56 kg K ha⁻¹, and S rates of 0 and 39 kg S ha⁻¹. 2017 fertility trials were conducted in Salisbury and Rocky Mount and 2018 trials were conducted in Salisbury and Waynesville, NC. The 2017 fertility trials failed to find seed yield differences in response to treatments. In 2018, seed yield of CFX2 was significantly greater in response to additions of K on a soil with relatively low extractable K content in Waynesville, NC. No significant response was found to N or S. While the lack of N response is inconsistent with previous hemp research, the K response fits with seed yield improvements reported in other crops. More research should be conducted to establish the threshold K requirements of hemp and to find the appropriate N fertilization rate in North Carolina.

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The Effect of Seeding Rate, Fertility and Cultivar on the Production of Industrial Hemp
(*Cannabis sativa* L.) in North Carolina

by
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A thesis submitted to the Graduate Faculty of
North Carolina State University
in partial fulfillment of the
requirements for the degree of
Master of Science

Crop Science

Raleigh, North Carolina
2019

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DEDICATION

In dedication to Eric and Melissa Brown, the truest farmers I know. Thank you for showing me a different way of life.

BIOGRAPHY

Ezekial Overbaugh was born November 28th, 1990 to Hettie Lynn Overbaugh and Kenneth Allen Overbaugh. He enjoyed growing up at the beach and on the river in Sneads Ferry, NC. Ezekial received his B.S. in Plant Biology and a B.A. in Anthropology from North Carolina State University in 2014. As an undergraduate, he had the opportunity to work with the NCSU Rubus Breeding Program. After college, he worked in commercial raspberry production as well as private and non-profit farm management. Ezekial began his Master's program in 2017 and helped conduct the inaugural season of industrial hemp research at NCSU.

ACKNOWLEDGMENTS

I'd like to acknowledge the support of the people who worked and drove long hours all over the state with me. Eliza, Jeremy, Ian, Andrew, Kaitlyn, Ryan, Megan, Phil, Shep, Ethan and Blake, thanks for helping figure this hemp thing out. I'm also thankful for the station staff: Brad, Hunter, Creig, Joanne, Margaret, and Robbie. I am glad other researchers have you on their side. As for Leo, I would be missing a chapter if it was not for your attention to detail and patience.

I appreciate Dr. Keith Edmisten and Dr. Angela Post for creating time and space for me as they took on the responsibility of the first Industrial Hemp Research Program at NC State. I also appreciate Dr. Amy Johnson's willingness to sit on my committee and for allowing me to assist her Ag Institute lab. I had no idea I liked teaching. Last not but least, Dr. Grady Miller went above and beyond his duties as a professor to selflessly guide me through experimental statistics and SAS.

Funding for this research was provided by the North Carolina Bioenergy Research Initiative and the North Carolina Agricultural Foundation.

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Literature Review

The focus of this work has been on hemp fiber and seed production. A review of literature as it relates to diversity, crop density and fertility in fiber and seed production will better inform primary investigations and recommendations for growers.

Cannabis Diversity:

Without a review of the diversity in *Cannabis*, it is difficult to understand the factors affecting seed and fiber production. *Cannabis* as a genus is characterized by erect, summer annuals with palmately compound, serrate leaves with elongated stipules (Reed 1914). Changes in phyllotaxy within an individual occur mainly as a factor of reproductive maturity, with opposite leaves having been reported as the more vegetative condition, at least in seed grown plants (Schaffner 1926). Stems are terete to longitudinally furrowed, with a pithy to wood-like cortex and a durable bast component (Bosca and Karus 1998a; personal observations 2017, 2018). Plants range between 1 - 5 meters in height and possess a taproot when originating from seed (Clarke 1999).

Staminate and pistillate flowers are borne on inflorescences on separate plants, unless monoecism has been bred into a generation (Bosca and Karus 1998b). A thorough hemp floral dissection was described by Reed (1914) writing from Iowa State University. The ovary position of pistillate plants was determined to be lateral, with the ovule developing in the vascularized, fertile half of two fused carpels. Pistillate flowers have green, trichome covered tepals from which emerge two elongated pistils originating from the fused carpels. Staminate flowers have five radial petals with five stamens (Reed 1914). Natural flowering is initiated by long

uninterrupted night cycles (Bosca and Karus 1998a; Clarke 1999). Inflorescences occur in clusters in leaf axils. Staminate plants senesce soon after pollen shed.

Cannabis classification at the species level has been subject to greater dispute. As a historical summary, 19th and early 20th century taxonomists including Lamarck, Vavilov and Janischesvky broadened the early Linnaean classification to include multiple species based on geographically consistent morphology (Small 1979; Bosca and Karus 1998a; Clarke 1999). The ability of the ecotypes to hybridize successfully, however, led Dewey (1914) and later Small (1979; 2015) to conclude all populations belong to one species, *Cannabis sativa* L. This perspective fits a standard set by other angiosperm phylogeny experts, and demonstrates a conservative approach prioritizing integrity of the species concept and ease of communication (Stevens 2001; Small 2015). Due to the high degree of outcrossing arising from dioecism, even cultivated populations are more appropriately considered “selections” instead of stable cultivars. Cloning, as is done with cannabinoid market types, must be employed to maintain germplasm integrity (Bosca and Karus 1998b; Clarke 1999). Even geographically and morphologically consistent chemotypes based on cannabinoid profiles are not reliable for taxonomy because of the simple inheritance pattern of tetrahydrocannabinolic acid (THCA) and cannabidiolic acid (CBDA) profiles (de Meijer et al. 2003).

Nonetheless, modern hemp which has been selected for fiber, seed, or cannabinoid markets has distinct characteristics. Fiber hemp from European and Asian populations is typically taller (3-4m) with less branching than hemp grown for seed or cannabinoid production (Bosca and Karus 1998b; de Meijer 1999). Seed hemp from North American and European populations has heavy seed set and shorter stature (<2m) (Ranalli 1999; de Meijer 1999; Vera et al. 2004). Both fiber and seed types historically have THCA: CBDA ratios less than 1 (Hillig and

Mahlberg 2004). Cannabinoid rich types, traditionally cultivated for drug use in central Asia and the Indian subcontinent, tend to have broader leaflets, more branching, and more profuse trichome production (Clarke 1999). Historically, drug types had THCA: CBDA ratios well above 1 (Hillig and Mahlberg 2004). Modern drug types subjected to crossing and selection of cannabinoid profiles can have extremes in content in either direction, with THCA or CBDA making up to 25% of the dry weight of inflorescences. A conscious effort has been made to use the label “drug type” for populations selected for cannabinoid use, as this term encompasses all pharmacological uses whether therapeutic, recreational or otherwise.

Cultural practices also influence the morphology of *Cannabis*. Seed and fiber hemp are direct seeded with grain drills on narrowly spaced rows on large acreages similar to flax (Amaducci et al. 2014). Planting from seed allows for the development of a taproot and the higher density crops exhibit reduced branching (Meijer et al. 1995; Bosca and Karus 1998c; Clarke 1999). Clones of drug types are often transplanted outdoors at rates less than 10 plants m⁻² and may never close canopy in the field (Amaducci et al. 2014). The lack of tap root makes clones more susceptible to lodging (Edmisten, personal communication, 2019).

While hemp is a short day flowering plant, wide variation exists in maturity (Vera et al. 2006; Amaducci et al. 2014; Campiglia et al. 2017). Stable regional selections exhibit strong latitude specific flowering periods (Bosca and Karus 1998b). Earlier flowering cultivars tend to be higher latitude adapted, and have been selected for seed production to prevent harvest from interfering with fall field preparations (Bosca and Karus 1998b). Bosca and Karus (1998a) reported Mediterranean fiber types flower nearly 6 weeks later than northern European seed types grown at the same locations. Later flowering allows for increases in height and accumulation of mass in the stem (Meijer et al. 1995; Campiglia et al. 2017). This makes the

later flowering, lower latitude adjusted populations more fit for fiber production (Amaducci et al. 2014).

Sex expression of the dioecious plants also plays a role in yield and harvest timing. Staminate plants senesce after flowering, resulting in populations nearly half that of the growing season. This leads fiber producers to harvest at anthesis to maximize stem yield (Bosca and Karus 1998c). Seed or dual purpose producers must necessarily wait for seed development (Bosca and Karus 1998c). Dual purpose crops are those harvested for both the seed yield and the stem. Lower yields of each component are expected in dual purpose types (Amaducci et al. 2014). Monoecious lines were developed to increase seed yields in traditional seed cultivars as well as increase harvestable stem populations for dual purpose crops (Bosca and Karus 1998c).

Hybridization has been important in hemp breeding and production, especially for seed and fiber types. USDA botanist Lyster Dewey (1914) made it clear that Chinese selections were continuously being introduced to Kentucky fiber populations by the late 19th century. A short, heavy seed producing type otherwise emerged as dominant in the Kentucky populations of European origin (Dewey 1914). Ranalli (1999) concurs that heterosis in fiber hemp is well achieved by first generation crosses of East Asian wild types and western European commercial selections. Ivan Bosca, credited with the development of several lines, has published pedigrees and detailed accounts of hybrid, monoecious, and unisexual type breeding in Eastern Europe in the 20th century (Bosca and Karus 1998b).

Specialized seed lines are a more recent development than fiber or dual purpose types (Bosca and Karus 1998b; Ranalli 1999). Modern Canadian seed producers use relatively short, early maturing cultivars with seed yields of sufficient value to justify leaving biomass as residue in the field (Vera et al. 2004; Vera et al. 2009; Amaducci et al. 2014; Canadian Hemp Trade

Alliance 2019). The shorter stature of these seed cultivars aids harvest by combine. With taller plants, combine headers must be raised high to harvest the seed heads while minimizing the intake of fibrous material which may bind equipment (Post, personal communication 2018).

Crop Density:

An optimum crop density maximizes photosynthetic and resource use efficiency and ensures sufficient partitioning of carbon products to desired yield components at the field scale (Deng et al. 2012). Broadleaf plants grown at higher density typically close canopy more quickly and have a higher leaf area to ground area ratio (Leaf Area Index, LAI) than those at low density (Meek 1984; Board et al. 1992). A higher LAI allows plants to intercept more solar radiation per ground area, potentially increasing photosynthetic efficiency and net primary production (Larcher 1975b; Monteith 1994; Gitelson and Gamon 2015). However, interception of light by upper canopy leaves reduces the quantity of photosynthetically active radiation reaching understory vegetation. The radiation that *is* reflected by the upper canopy is richer in far red light, which has further inhibitory effects on the understory (Kunzelmann and Schafer 1985).

Predictive modelling of optimum crop density based on these factors has relied on assumptions about the stability of resource availability over time, especially soil resources (Larcher 1975a, 1975b; Deng et al. 2012). These models are complicated by obvious environmental factors such as precipitation but also by feedback loops between productivity and resource use. For example, soil moisture and nutrient availability are impacted by plant density as soil solution is subject to mass flow towards individual plant roots (Matimati et al. 2014). Water absorption at the root surface is the result of a water potential created by photosynthetic demand and transpiration of the individual. The growth of new root surface area capable of

absorbing soil solution and the loss of water to transpiration and photosynthesis are in turn mediated by above ground production and the ability to compete for sunlight (Black et al. 1969; Matimati et al. 2014). Thus, overcrowding of crop plants reduces the quantity and quality of light as well as the availability of soil resources to crop neighbors. If photosynthesis of carbohydrates and oxygen is insufficient to supply maintenance respiration and growth under these competitive conditions, assimilation of carbohydrates into economically desirable components may be limited (Larcher 1975a, 1975b).

In terms of hemp, the traditional yield components affected by carbohydrate production are seeds and stems. Hemp seeds have oil contents upwards of 30% and are rich in gamma linolenic fatty acids (Vera et al. 2006). Although not used for cooking, the seed oil is used in condiments, dietary supplements or in skincare products (Pate 1999b). Hemp seed oil has also been considered by some authors for use in biodiesel production (Li et al. 2010; Gill et al. 2011). Hemp stems are composed of an outer bast fiber and a woody core or “hurd”. The bast fibers have traditionally been processed for textiles and cordage (Bosca and Karus 1998d). Crônier et al. (2005) studied the structure and chemistry of hemp bast fiber in detail. They report that hemp bast fibers are lignocellulose-rich primary and secondary cells that surround the core xylem. The primary fibers originate from the procambium and reach up to 100mm in length and 15 μ m in diameter. The secondary fibers are generated from the cambium and are generally much shorter. The fibers form tightly bound bundles that run parallel to the stem and provide support. The inner hurd is made up of xylem cells that are oriented and bonded in a way to give rigidity (Crônier et al. 2005). The hurd is nearly 70% of the stem biomass and has a cellulose content as high as 61% (Sponner et al. 2005; Barta et al. 2010).

The cellulose rich nature of hemp stems has prompted research into their conversion to biofuel. Barta et al. (2010) tested the recovery of sugars and ethanol from hemp hurds. They found that at steam pretreatments of 210°C, glucose and ethanol yields were maximized at 336 g kg⁻¹ and 141 g kg⁻¹ of dry hurd, respectively (Barta et al. 2010). Kreuger et al. (2011) tested methane yields from the anaerobic digestion of entire hemp stems. They recovered a maximum of 122 GJ of energy in the form of combustible methane per hectare of hemp harvested (Kreuger et al. 2011). While the textile fiber has historically been the more profitable yield component of the stem, Sponner et al. (2005) indicated a trend of increased industrial consumption of the hurd in the 21st century. If conversion of hemp biomass to biofuels can be achieved commercially, this trend may continue to increase. Defining optimum density in hemp depends on which of these markets producers are targeting.

Early recommendations regarding optimum crop density for hemp in the eastern United States were largely aimed at producing textile fiber. The handwritten farm notes of Thomas Jefferson dating to 1826 and published in full facsimile in 1953 state that 1 bushel of hemp seed to the acre was adequate for a textile crop (Betts 1953). At least as early as 1856, a bushel of hempseed was legally recognized as 44 lb (Legislative Council 2019). For context, hemp seed weights range from 25,000 - 30,000 seeds lb⁻¹ (55,000 – 66,000 seeds kg⁻¹) depending on cultivar (Bosca and Karus 1998a; personal observations 2017, 2018). Jefferson was making the 1 bushel ac⁻¹ recommendation for multiple farms in the Virginia piedmont, the soils of which are predominately ultisols of clay and silt loam texture (Soil Survey Staff 2017). In 1914, Dewey (1914) stated that 3 to 6 pecks (33 – 66lb), depending on soil quality, was sufficient to produce a fiber stand in Kentucky. Dewey (1914) also stated the average total yield of fresh stems was 15,000 lb acre⁻¹ or about 6,000 lb acre⁻¹ after field retting. In terms of effect on quality, Hessler

(1945) demonstrated that as plant density increased from 3 to 5 pecks (33 – 55lb), so did tensile strength of the resulting bast fiber. Hesseler (1945) concluded this response was either a result of reduced retting in the denser harvest swaths or that plant density may have affected stem growth characteristics. Decreased diameter, increased internode length, and decreases in volume to surface area ratio have since been observed at higher density hemp plantings (van der Werf et al. 1995; Meijer et al. 1995; Campiglia et al. 2017).

Modern crop density research in hemp has been conducted in different parts of Canada, China and Europe and has included effects on seed yield. The differences in soil, photoperiod and weather between the southeastern United States and these locations, however, may limit the applicability of the results. Further, some disagreement exists between the authors in this field. In the Netherlands, Meijer et al. (1995) established that increased seeding rate led to faster rates of canopy closure but at higher densities the measure of dry matter produced per unit of solar energy decreased. Total biomass was not significantly different at the end of the season between plots that had been seeded in a range from 20 to 140 kg seed ha⁻¹. Meijer et al. (1995) postulated that density induced mortality (self-thinning) throughout the season was the cause of reduced differences in harvestable biomass and reduced values of radiation use efficiency. Van der Werf et al. (1995) also concluded self-thinning limited differences in final biomass in response to seeding rate in hemp.

In Italy, Campiglia et al. (2017) corroborated the trend of earlier canopy closure at high density. However, the authors reported no density induced mortality in their trials in which plots were manually thinned to 40, 80, 120 plants m⁻² post emergence. Contradicting the Dutch trials further, Campiglia et al. (2017) measured significant differences in total biomass in response to increased population. Decreases in height, weight and diameter of individuals at higher densities

were also found, though the biomass contributed by a greater number of individuals evidently outweighed the effects of these trends (Campiglia et al. 2017). Canadian studies by Vera et al. (2006) concurred with those of Campiglia et al. (2017), and reported increased densities and biomass as a result of increased seeding rates. They tested seeding rates of 20, 60 and 80 kg seed ha⁻¹. Vera et al. (2006) also found that seeding rate corresponded to higher seed yields and seed oil content.

As far as impact to seed yield, Campiglia et al. (2017) found that cultivar played a larger role than density, whereby earlier flowering cultivars produced more seed at the same densities than did later flowering cultivars. Later flowering cultivars did, however, produce greater biomass (Campiglia et al. 2017). The trend of higher biomass production as a result of vegetative duration, a factor tied to genotype, is well documented in hemp (Bosca and Karus 1998b, Ranalli 1999, Faux et al. 2013; Amaducci et al. 2014).

In a geographically comprehensive study, Tang et al. (2017) tested plant densities of two medium to late flowering cultivars in Italy, Latvia, Czech Republic and France. Increasing planting density from 30 to 120 plants m⁻² increased biomass by 32%. Unlike the studies conducted by Vera et al. (2004), Tang et al. (2017) did not find that the seed yield was significantly affected by planting density between 30 and 240 plants m⁻². The authors concluded that for a dual-purpose crop a density of 90-150 plants m⁻² is suitable for any location. It is worth noting that the row spacing varied by location in the study conducted by Tang et al. (2017). The row spacing also varied between several of the previously discussed studies, which influences the spatial arrangement of density. If a constant number of plants is targeted within a unit of area, yet the row spacing increases, there must be a greater linear density. As has been shown previously in hemp as well as in other bast crops like kenaf, row spacing has a significant impact

on biomass and final population densities (White et al. 1970; Neill and Kurtz 1994; van der Werf et al. 1995).

Other factors contributing to the varying trends between studies of hemp density in the literature are the maturity differences of the cultivars tested, the interaction between cultivar and latitude, and differences in planting techniques or rates. The results of Vera et al. (2006) were achieved by using seeding rate as a treatment without thinning. Other authors thinned plots to exact densities (Campiglia et al. 2017; Tang et al. 2017). Both methods have their advantages. Using seeding rate as treatment directly reflects the technique employed by farmers to manage crop density at the field scale but may lead to higher variation in experimental densities. Conversely, thinning plots to desired densities is more precise, but incurs uneven compaction in plots and poorly translates to strategies for farmers. Within studies that used exact densities, unless there is overlap in the treatment range, it is difficult to compare trends. For example, Campiglia et al. (2017) and Tang et al. (2017) were able to detect increases in biomass at their overlapping rates. Yet the range at which Meijer et al. (1995) and van der Werf et al. (1995) failed to find biomass responses was much higher and achieved with different row spacing. Regarding the interaction of cultivar, maturity and location, Bosca and Karus (1998b, 1998c) reported that cultivars adapted to lower latitudes failed to reach seed maturity when grown at higher latitudes. Differences in soil physical properties such as water holding capacity and pH may also influence the effect of plant population on yields.

While yield quantity has been of primary interest in most hemp populations studies, quality of the target yield component should also be considered. The higher densities of textile production, regardless of their effect on total biomass, decrease the stem diameter of individual plants and therefore increase the surface area to volume ratio of the individual (Bosca and Karus

1998c; Campiglia et al. 2017). An increased surface area to volume ratio of the stems has reportedly improved tensile strength and fineness of the bast fiber (Hesseler 1945; Ranalli 1999). If the higher populations persist to harvest without self-thinning, the yield proportion of desirable bast fiber for textile production has then also increased per unit ground area (Bosca and Karus 1998c).

Concluding with a broader review of density trends in related crops will provide context for interpreting results from future hemp studies. Like hemp, kenaf (*Hibiscus cannabinus*) is a tall, erect broadleaf with a fibrous bast and wood-like core (Sponner et al. 2005). Originally a subtropical crop of Africa and Asia, kenaf was tested extensively as a timber pulp alternative in the central and southern United States beginning in the late 1950s. G.A. White and colleagues (1970) summarized and presented the results of these kenaf trials in a USDA production report. Several of the trials concerned plant population and row spacing. A trial in Florida showed that while total biomass in response to population varied by cultivar, there was no significant difference in biomass whether these cultivars were planted on 19in or 38in row widths, even when living populations were nearly double in the closer spacing (White et al. 1970). A trial in Georgia evaluated populations with 2 and 4 plants per row foot at three different row widths. Row width impacted yield, but there was no significant difference between the biomass yields of rows having either 2 or 4 plants per foot (White et al. 1970). In a North Carolina study from 1966 using a 7in row width, stand counts were similar by harvest, regardless of differences in initial seeding rate (White et al. 1970). A later series of kenaf trials were relaunched in the 1980s in Mississippi, again as part of the development of alternative crops. In these population trials, Neill and Kurtz (1994) reported mortality at higher densities as well as thickening of individual

stems and branching at lower densities. All the populations still produced similar biomass yields (Neill and Kurtz 1994).

As for general effects of plant density on seed production, it is helpful to look at other broadleaf, small seeded crops with an erect and mildly-branching habit. Flax and sesame are similar to hemp in this regard. From 1988 to 1990, Stevenson and Wright (1996) found that in weed free conditions flax seed yield was not affected by seeding rates from 300 to 900 seeds m^{-2} in Saskatchewan. Under weedy conditions, however, seed yield at the highest seeding rate increased by 180 kg seed ha^{-1} over the lowest rate. Stevenson and Wright (1996) conclude that higher seeding rates allow the crop to establish a weed suppressing canopy more quickly, an advantage not as clear when weeds are absent. For sesame, trials in Brazil conducted by Rodrigues et al. (2016) showed that changes in seed yield are not linear in response to changes in population. Doubling the population from 55,555 to 111,111 plants ha^{-1} increased the seed yield less than 25% (Rodrigues et al. 2016).

Soil Fertility:

Improvements to plant yield when nutrients are applied to deficient soils have been demonstrated widely, including for cereals, legumes, vegetables and other specialty crops (Zubriski et al. 1970; Rabuffetti and Kamprath 1977; Fageria et al. 2011; Simmone et al. 2017; May 2018). However, nutrients are sometimes applied in excess of what can be utilized by a crop in a given time frame or at rates whose return in yield do not justify the cost (Glass 2003; Kant et al. 2011). Optimum nutrient rates are an important tool for not only maximizing yield but also limiting nutrient costs.

Fertility research for field grown hemp has traditionally taken place in Europe and Canada, regions with existing commercial markets for the seed and fiber. For hemp studies in Italy, Campiglia et al. (2017) found an increase in stem biomass from 4.86 to 5.84 t ha⁻¹ when the nitrogen (N) rate was increased from 50 to 100 kg N ha⁻¹. As context, commercial yields are expected to reach 7 to 10 t ha⁻¹ (Bosca and Karus 1998c; Ranalli 1999). Campiglia et al. (2017) did not find a response in seed yield to N rate. This is in contrast to Canadian studies, where Vera et al. (2004, 2009) detected both biomass and seed yield increases in response to N applications, as described below.

The trials carried out by Vera et al. (2004, 2009) took place in Saskatchewan from 2000-2002 and again from 2006-2008. In the earlier trials, Vera et al. (2004) evaluated cultivar responses to N rate and phosphorus (P) rate. Height, biomass and seed yield increased in response to N rate in a quadratic fashion with the response plateauing within the N range tested (0, 40, 80 and 120 kg N ha⁻¹). The mean seed yields in response to the increasing N rates were 401, 542, 625, and 657 kg seed ha⁻¹. Seed yield response differed by cultivar, with the estimated maximum yield for Finola occurring at an N rate of 100 kg N ha⁻¹. The later flowering cultivar Fasamo did not reach an estimated maximum yield within the 120 kg N ha⁻¹ range. In response to P, a decrease in seed yield and biomass occurred in both cultivars at the tested P rates (0, 20, 40, 60, 80 kg P ha⁻¹) (Vera et al. 2004).

In the later studies, Vera et al. (2009) tested cultivar yield responses to N, P and sulfur (S) rates. In the later trials, a wider N range was tested (0, 50, 100, 150 and 200 kg N ha⁻¹). Vera et al. (2009) found that for both cultivars, height and biomass were maximized at the N application rate of 150 kg N ha⁻¹, though Finola achieved the highest seed yield at an N rate of 200 kg N ha⁻¹. This N requirement for maximum Finola seed yield is nearly double what had been found in

the previous study conducted at a different location (Vera et al. 2004; Vera et al. 2009). Average seed yield across cultivars in the later study was 570 kg seed ha⁻¹ at the control rate; average seed yields at each positive nutrient rate were not reported. There was no effect on yield at the S rates applied, even on S deficient soil. The results led Vera et al. (2009) to suggest that hemp may have low S requirements compared to other crops. Phosphorus had a positive impact to height but not seed yield or biomass at the rates tested (20, 40, 60, 80 kg P ha⁻¹) (Vera et al. 2009).

In Dutch trials, van der Werf et al. (1995) adjusted soil levels of extractable N to 80 and 200 kg N ha⁻¹. Throughout the season, they found that biomass was consistently higher at the extractable soil N of 200 kg ha⁻¹. However, at the higher soil N level, self-thinning was more severe. The authors concluded that the biomass lost to self-thinning was compensated by the increase in growth and mass accumulation achieved with more available N (van der Werf 1995).

In Irish trials, Finnan and Burke (2013) applied K at rates of 0, 50, 90, 120, and 150 kg K ha⁻¹ to multiple cultivars. Potassium uptake differed widely by cultivar, with the late maturity cultivar, Futura75, having the highest uptake. Finnan and Burke (2013) also found that up to 75% of plant tissue K was located in the stem as opposed to leaves or inflorescences. They suggested that if stems are left to rot in the fields, much of this K can be returned to the soil. The effect of K on seed yield was not measured. There was no biomass difference in response to K rate, though K tissue content was higher when hemp was grown on soils with higher K levels. Finnan and Burke (2013) concluded that hemp might respond to K applications if tested on a soil with low K content.

In Eastern Canada, Aubin et al. (2015) tested biomass and seed yield responses to N, P, and K applications. In a range of N rates from 0 to 200 kg N ha⁻¹, seed yield improved with each successive rate from 519 to 1340 kg seed ha⁻¹. Biomass also improved from 1674 to 4209 kg ha⁻¹.

¹. The cultivar responses to N were similar to those found by Vera et al. (2004, 2009) in which the later flowering type had a greater biomass increase per unit N applied (Aubin et al. 2015). Aubin et al. (2015) did not find a plateau in yield parameters in response to N rate, and the authors did not suggest an optimum N fertilization recommendation. Further, a yield response to K was not found when K was applied at rates ranging from 0 - 200 kg K ha⁻¹. The soil levels of K at the test locations, however, were relatively high, with the lowest soil K level being 195 kg ha⁻¹. It was not specified if this was total or extractable soil K. Like Vera et al. (2004, 2009), Aubin et al. (2015) reported a positive response in height to P applications, and this was achieved at the lower end of the tested rates (0, 25, 50, 75, and 100 kg P ha⁻¹).

Taken together, these studies suggest seed and fiber hemp yields respond to P, K, and S applications within a narrow range. Nitrogen applications were concluded to increase stem biomass over a wide range but at different rates depending on location and cultivar. The response of seed yield to N applications varied with location and cultivar, and none of these nutrient factors have been definitively investigated for hemp production in the southeastern United States.

Despite the nuances of specific crop sufficiency ranges, the general functions of nutrients in plants are understood. In the 2017 and 2018 hemp fertility trials in North Carolina, N, K, and S were the factors of study, and a literature review of nutrient physiology will be limited to those nutrients. For N, the primary forms absorbed by plant roots are nitrate (NO₃⁻) and ammonium (NH₄⁺) (Le Deunff 2014). Reductions to amine (NH₂⁻) within the cell allow for the formation of nucleic and amino acids, components of the genetic material that code for proteins as well as the polypeptide chains of proteins themselves (Fageria 2014). Since proteins and enzymes provide structure and carry out metabolic activity for respiration, as well as mediate transport of nutrients

across membranes, their importance for growth cannot be over stated. Additionally, nitrate concentrations are directly involved in cellular signaling, especially for modulation of hormones in the root (Fageria 2014). Nitrogen is also an essential component of chlorophyll, the primary pigment involved in photosynthesis, the process by which all carbohydrates in plants are produced by transformations of light energy (Greenwood et al. 1986). An optimum supply of N for photosynthesis facilitates the partitioning of carbohydrates into desirable yield components (Larcher 1975b; Muchow and Sinclair 1994).

Nitrogen applications generally improve the mass of vegetative portions of many crops, though the impact to reproductive structures is less direct (Fageria 2014). Carbohydrate, nutrient and protein accumulation in seeds is limited by the production and availability of these nutrients in nearby vegetative structures (Gastal and Lamare 2002; Fageria 2014). In rice, Yoshida (1972) relates how spikelet length and grains per spikelet, both important yield factors, are positively correlated with total N absorbed before initiation of flowering. It was concluded that production of carbohydrates for grain fill per unit of N supplied was greater for N applications made before flowering as opposed to after flowering (Yoshida 1972). Cassman et al. (1992) also observed positive impacts on resource partitioning to wheat grains in response to increased N rates early in the season. Late season applications of N, however, led to inefficient partitioning of protein content to grain and led to decreased responses in harvest indices in relation to total N applied. Like hemp, sesame (*Sesamum indicum* L.) is a broadleaf, oil seed crop. Couch et al. (2017) report a wide range (31-66%) for the amount of N remobilized from leaves to seeds in sesame. Their final conclusion is that the N applications made after germination and prior to flowering have the greatest impact to seed yield (Couch et al. 2017).

Potassium is absorbed by plant roots as the K^+ ion. Potassium performs functions in the plant related to its ionic nature (Leonard 1985). As a monovalent cation, K is essential in creating charge and solute gradients necessary for driving transport of numerous substances across membranes in plant cells, including osmotic control of water movement into guard cells for the opening of stomata. This serves gas and water vapor exchange and occurs in reaction to light but is also mediated by drought response signaling (Beringer and Nothdurft 1985). This has a downstream effect on carbon dioxide concentration for photosynthesis as well as water loss. Also related to its ionic nature and atomic radii, K plays an activation role in numerous enzymes in which its binding affinity to certain substrates affects necessary conformational changes (Beringer and Nothdurft 1985). As it relates to growth and respiration, K has a role in the active transport (enzymatic ion pumping) of carbohydrates and the enzymatic breakdown of sugars for respiration (Huber 1985).

Sulfur is absorbed by plants primarily from soil in the inorganic sulfate form, SO_4^{-2} (Bohn et al. 1986). Some exchange of sulfide gas can take place through stomata. In cultivated soils of the southeastern United States, SO_4^{-2} typically accumulates in subsoil because liming at the soil surface increases pH and reduces adsorption to soil particles (Kamprath and Jones 1986). While not needed in the quantities of other macronutrients (usually <1% w/w), S is an essential constituent of the amino acids cysteine and methionine. The disulfide bonds between adjacent cysteine compounds provide the secondary and tertiary structure of proteins necessary for certain functional conformations. Outside of this role, SO_4^{-2} in plant cells is necessary for reduction of nitrates for their later use (Duke and Reisenauer 1986). The rate of plant uptake of SO_4^{-2} is limited by the concentrations of SO_4^{-2} within root cells. While plants respond positively to

additions of S on low SO_4^{2-} soils, typically yield increases are not continuous over a broad application range (Kamprath and Jones 1986).

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Chapter 1

The Effect of Seeding Rate and Cultivar on Plant Density, Biomass and Seed Yield in Industrial Hemp (*Cannabis sativa* L.)

Introduction:

Industrial hemp (*Cannabis sativa* L.) is produced commercially for three major markets: seed, fiber and metabolites (Clarke 1999; Amaducci et al. 2014). Conversion of stem biomass and seed oil to various biofuels has also been demonstrated (Kreuger et al. 2011; Barta et al. 2010; Gill et al. 2011). In practice, seeding rates differ by target product. For example, bast fiber for textiles has been reported to require higher crop densities than general biomass or seed production (Meijer et al. 1995; Amaducci et al. 2014). However, some disagreement persists concerning the effect of seeding rate on crop density, biomass and seed yield (Meijer et al. 1995; van der Werf 1995; Vera et al. 2006; Amaducci et al. 2014; Campiglia et al. 2017; Tang et al. 2017). Further, industrial hemp seeding rates have not been tested in the soils and climate of North Carolina. The objective of this study was to determine the optimum seeding rate for biomass and seed yield of industrial hemp grown in North Carolina. This information will assist producers of all types, including those interested in biofuel production.

Hemp seeds have oil contents upwards of 30% and are rich in gamma linolenic fatty acids (Vera et al. 2006). Although not used for cooking, the seed oil is used in condiments, dietary supplements or in skincare products (Pate 1999b). Hemp seed oil has also been considered by some authors for use in biodiesel production (Li, et al. 2010; Gill et al. 2011). Hemp stems are composed of an outer bast fiber and a woody core or “hurd”. The bast fibers have traditionally been processed for textiles and cordage (Bosca and Karus 1998d). The hurd, however, is nearly 70% of the stem biomass and has a cellulose content as high as 61% (Crônier et al. 2005; Sponner et al. 2005; Barta et al 2010). Barta et al. (2010) tested the recovery of

sugars and ethanol from hemp hurds. They found that at steam pretreatments of 210°C, glucose and ethanol yields were maximized at 336 g kg⁻¹ and 141 g kg⁻¹ dry hurd, respectively (Barta et al. 2010). Kreuger et al. (2011) tested methane yields from the anaerobic digestion of entire hemp stems including bast fiber. They recovered a maximum of 122 GJ energy in the form of combustible methane per hectare of hemp biomass digested (Kreuger et al. 2011). While the textile fiber has historically been the more profitable yield component of the stem, Sponner et al. (2005) indicated a 21st century trend of increased industrial demand for the hurd. If conversion of hemp biomass to biofuels can be achieved commercially, this trend may continue.

Hemp cropping density research as it affects seeds and stem biomass has been conducted in different parts of Canada, China and Europe. The differences in soil, photoperiod and weather between the southeastern United States and these locations may limit the applicability of the results. There also exists some inconsistency in the literature regarding the densities at which biomass and seed yields reach a maximum. In the Netherlands, Meijer et al. (1995) found that total biomass was not significantly different at the end of the season between plots that had been thinned to an equivalent density range of 20 to 140 kg seed ha⁻¹. Higher densities led to faster rates of canopy closure but the measure of dry matter produced per unit of solar energy decreased (Meijer et al. 1995). The authors postulated that density induced mortality (self-thinning) throughout the season was the cause of reduced differences in harvestable biomass and reduced values of radiation use efficiency. Van der Werf et al. (1995) also concluded self-thinning limited differences in final biomass in response to seeding rate in hemp.

In Italy, Campiglia et al. (2017) corroborated the trend of early canopy closure at high density. However, they reported no density induced mortality in their trials in which plots were manually thinned to 40, 80, 120 plants m⁻² post emergence. Contradicting the Dutch trials

further, Campiglia et al. (2017) reported significant differences in total biomass in response to increased crop density. Decreases in height, weight and diameter of individuals at higher densities were also found, though the biomass contributed by a greater number of individuals evidently outweighed the effects of these trends (Campiglia et al. 2017).

Canadian studies by Vera et al. (2006) reported increased densities (174%) and increased biomass (23%) as a result of increased seeding rates. Vera et al. (2006) used seeding rates of 20, 60 and 80 kg seed ha⁻¹ with early flowering cultivars selected for seed production in Canada. Vera et al. (2006) also found that increased seeding rate corresponded to significantly higher seed yields (34%) and seed oil content. As far as impact to seed yield, Campiglia et al. (2017) reported that cultivar played a larger role than density, whereby earlier flowering cultivars produced more seed at the same densities than did later flowering cultivars. Later flowering cultivars did, however, produced greater biomass (Campiglia et al. 2017). The trend of higher biomass production as a result of vegetative duration, a factor tied to genotype, is well documented in hemp (Bosca and Karus 1998b; Faux et al. 2013; Amaducci et al. 2014; Campiglia et al. 2017).

In a geographically comprehensive study, Tang et al. (2017) studied plant densities of two intermediate flowering cultivars in Italy, Latvia, Czech Republic and France. Increasing planting density from 30 to 120 plants m⁻² increased biomass by 32%. Tang et al. (2017) did not find that seed yield was affected by crop density in a range from 30 to 240 plants m⁻². The authors concluded that for a dual-purpose crop a density of 90-150 plants m⁻² is suitable for any location. It is worth noting that the row spacing varied between locations evaluated by Tang et al. (2017). The row spacing also varied between several of the previously discussed studies, which influences the spatial arrangement of density. If a constant number of plants is targeted within a

unit of area, yet the row spacing increases, there must be a greater linear density. As has been shown previously in hemp as well as in other bast crops like kenaf, row spacing has a significant impact on biomass and final population densities (White et al. 1970; Neill and Kurtz 1994; van der Werf et al. 1995). Other factors contributing to the varying trends found in the literature are the maturity differences of the cultivars tested, the interaction between cultivar and latitude, and differences in planting techniques or rates.

The results of Vera et al. (2006) were achieved by using seeding rate as a treatment without thinning. Other authors thinned plots to exact densities (Campiglia et al. 2017; Tang et al. 2017). Both methods have their advantages. Seeding rate directly reflects the technique employed by farmers to manage crop density at the field scale but may lead to higher variation in experimental densities. Conversely, thinning plots to desired densities is more precise but incurs uneven compaction in plots and poorly translates to strategies for farmers. Within studies that used exact densities, it is difficult to compare trends unless there is overlap in the treatment range. For example, Campiglia et al. (2017) and Tang et al. (2017) were able to detect increases in biomass at their overlapping rates, yet the common range at which Meijer et al. (1995) and van der Werf et al. (1995) failed to find biomass responses was much higher.

Regarding the interaction of cultivar, maturity and latitude, Bosca and Karus (1998b, 1998c) reported the failure of lower latitude adapted cultivars to mature when grown at higher latitudes. And while nutrient application rates or soil nutrient content can be standardized across locations, locations with different soil physical properties such as pH or water holding capacity may have different nutrient availability. Care should be practiced when making recommendations for regions in which hemp responses to density have not been previously tested. It is the objective of this study to test crop density, biomass and seed yield in response to

seeding rate for both early flowering and late flowering cultivars grown on the highly weathered Ultisols of North Carolina.

Methods and Materials:

The 2017 density trial was planted in Salisbury, NC. Cultivars Felina32, Carmagnola and CFX2 were seeded at three rates in a randomized complete block design with four replications for a total of 36 plots. Salisbury is in USDA hardiness zone 7b. Plots were planted on June 16th, 2017 on a Lloyd clay loam (Fine, kaolinitic, thermic Rhodic Kanhapludults). Field preparation included glyphosate application to spring weeds and shallow surface tillage for preparation of the seed bed. Seeding rates were calculated equivalents to 16.8, 28.0, and 39.2 kg ha⁻¹ (15, 25 and 35 lb ac⁻¹). Plots were planted using an Almaco cone drill at 19 cm row spacing with plot dimensions of 1.52m x 8.34m. The target seeding rates were calculated using plot dimensions and average seed weight adjusted for labelled germination of each cultivar. Seed depth was approximately 1.27cm. Granular ammonium nitrate was applied by station spreader at a rate of 28 kg N ha⁻¹ on the day planting. The remaining 140 kg N ha⁻¹ was applied 4 weeks after planting (WAP). Phosphorous (P) and potassium (K) had been annually managed to meet NCDA recommendations for maize/soybean rotations. After crop emergence Charger Max by Winfield AgriSolutions (S - metolachlor) was applied at a rate of 1071 g a.i. ha⁻¹ for control of weeds. Existing weeds were manually removed 4WAP.

At 4WAP, stand counts along 1 row-meter and height for one representative plant per plot were recorded. Visual assessments of relative canopy closure were recorded at 4 and 8WAP. The validity of relative visual assessments for general crop monitoring is supported by Buchi et al. (2018). Two Julian dates were recorded per plot, one when approximately 50% of pistillate

plants were showing styles and another when approximately 50% of staminate plants were shedding pollen. Flowering status was monitored twice a week leading up to maturity. On August 17th, 2017, hemp biomass was collected by manually cutting stems approximately 2.5cm from soil surface within 1m². Biomass was dried at 50°C for 48 hours and weighed.

The 2018 trials took place in Salisbury (USDA zone 7b), Oxford (7b), and Rocky Mount (8a), North Carolina. At all locations, Carmagnola was planted in a factorial arrangement of four seeding rates by two planting dates, in a randomized complete block design. Plots were blocked by planting for ease of planting and harvest as well as blocked by replication within each planting date. Rates were 300, 400, 500 and 600 pure live seed m⁻² with four replications for a total of 32 plots. The Salisbury experiments were planted June 7th and June 24th on a Lloyd clay loam (Fine, kaolinitic, thermic Rhodic Kanhapludults). Oxford was planted June 13th and July 2nd on a Vance sandy loam (Fine, mixed, semiactive, thermic Typic Hapludults). Rocky Mount was planted June 13th and July 2nd on a Norfolk loamy sand (Fine-loamy, kaolinitic, thermic Typic Kandiudults). Also at each location, CFX2 was planted at four rates for one planting date in a randomized complete block design with four replications. Rates were 100, 200, 300, 400 pure live seed m⁻². The total number of plots in each CFX2 trial was 16 and these were planted on June 7th in Salisbury, June 13th in Oxford and on June 13th in Rocky Mount. Field preparation at all three locations included conventional tillage and surface cultivation for a smooth seedbed.

Plots were planted with a Wintersteiger XXL Plotseeder on 19cm spacing with plot dimensions of 1.52m x 8.34m. Per plot equivalents to the target seeding rate were calculated based on the plot dimensions, row spacing, and seed counts adjusted for labelled germination of each cultivar. Seed depth was approximately 1.27cm. Phosphorous and potassium were managed annually at each station according to respective NCDA soil test recommendations. 56 kg N ha⁻¹

was surface applied as ammonium nitrate at planting and the remaining 112 kg N ha⁻¹ was applied 4WAP. Charger Max by Winfield AgriSolutions (S - metolachlor) was applied at a rate of 1071 g a.i. ha⁻¹ after planting but before crop emergence in Salisbury for weed control. Oxford and Rocky Mount did not receive herbicide. Existing weeds were removed manually from all locations approximately 4WAP.

At 4 and 8 WAP, stand counts along 1 row-meter and height for one representative plant per plot were recorded. Row-meters were flagged in an attempt to recount the same line. Julian dates for staminate and pistillate flowering were recorded as in year one. Biomass was harvested within 1m² by manually cutting 2.5cm above soil surface when approximately 75% of staminate plants were shedding pollen. Biomass was weighed fresh and dry after 48 hours at 50°C. Stems from the 1m² sample were counted. For a random subsample of 10 stems, diameters were measured approximately 1 cm from the base. Seed yield was harvested with a Wintersteiger Delta plot combine when approximately 75% of the seed was in the brown stage and able to be threshed by hand but not yet shattering. Whole plot seed yield was dried for 24 hours at 50°C, cleaned with an Almaco table top seed cleaner, and allowed to adjust to ambient moisture. Seed moisture was measured using a Dickey John 2100 on canola settings. Seed weight was recorded after reaching stability (~6% moisture).

Analysis of variance was performed on the data from 2017 using the procedure for general linear models in SAS Version 9.4 (SAS, Inc. Cary, NC) ($\alpha=0.05$). Mean separation was performed with Fisher's LSD ($\alpha=0.05$). Due to incomplete data sets from stand failures or logistical limits to collection in 2018, a Type 3 test of fixed effects was performed on that year's data using the procedure for mixed effects models in SAS. Seeding rate and planting date were held as fixed effects and location and replication were held as random effects. Location was

removed from the random statement when testing those dependent variables for which there was only one location's worth of data. The SAS procedure for mixed models by default uses restricted maximum likelihood to generate probabilities for the Type 3 test of fixed effects ($\alpha = 0.05$). Mean separation was also performed using least squares means with a Tukey adjustment ($\alpha = 0.05$).

Results:

In 2017, there was no seeding rate by cultivar interaction ($\alpha = 0.05$) (Table 1-1). Increases in crop density averaged across cultivars were significant in response to seeding rate ($p < 0.0001$) (Table 1-1). Four weeks after planting, densities averaged across cultivars were approximately 392280, 684099, and 975917 plants ha^{-1} in response to the 16.8, 28.0, and 39.2 kg ha^{-1} seeding rates (Table 1-2). However, biomass at maturity was not significantly different in response to seeding rate ($\alpha = 0.05$), and the average across cultivars ranged from 4498 to 4900 kg ha^{-1} (Table 1-2). Differences in both crop density and biomass were significant in response to cultivar ($p = 0.0086$; $p < 0.0001$) (Table 1-1). The later flowering cultivars, Felina32 and Carmagnola, accumulated nearly 4 times the biomass of the early flowering cultivar CFX2 (Table 1-3). The difference in flower maturity was significant in response to cultivar ($p < 0.0001$) (Table 1-1), with 19 days between staminate plant maturity of CFX2 and the latest flowering cultivar Carmagnola (Table 1-3). Four weeks after planting, heights were significantly different in response to cultivar ($p = 0.005$) but not seeding rate (Table 1-1). Four weeks after planting, Felina32 had an average height of 50cm and was significantly taller than Carmagnola at 30 cm and CFX2 at 38cm (Table 1-3). Four weeks after planting, canopy closure was significantly different in response to seeding

rate ($p=0.017$) (Table 1-1). Canopy closure was highest for Felina32 at 61%, while Carmagnola averaged 32% and CFX2 averaged 27% (Table 1-3).

For the Carmagnola trial in 2018, there was no seeding rate by planting date interaction. Densities 4WAP, 8WAP, and at maturity were significantly different in response to seeding rate averaged across planting date ($p<0.0369$; $p=0.0149$; $p<0.0168$) (Table 1-4). At harvest, densities averaged across planting date were approximately 291652, 432486, 664569, and 657902 plants ha^{-1} in response to the 300, 400, 500, and 600 pure live seed m^{-2} seeding rates (Table 1-5). Seed yield and biomass were not significantly different in response to seeding rate ($\alpha =0.05$) (Table 1-4). Seed yields were 285, 266, 295, and 317 $kg\ ha^{-1}$ in response to the 300, 400, 500, and 600 pure live seed m^{-2} seeding rates (Table 1-5). Biomass values were 4306, 4664, 4885, and 4967 $kg\ ha^{-1}$ in response to the 300, 400, 500, and 600 pure live seed m^{-2} seeding rates (Table 1-5).

For Carmagnola in 2018, biomass was significant in response to planting date ($p=0.0052$) (Table 1-4). The biomass for plants seeded on June 13th was 5,805 $kg\ ha^{-1}$ and the biomass of plants seeded on June 24th was 3,606 $kg\ ha^{-1}$ (Table 1-6). Heights 4WAP were significantly different in response to planting date ($p=0.0125$) (Table 1-4). 4 weeks after the June 13th planting, heights were 27cm on average while 4 weeks after the June 24th planting, plants were 50cm on average (Table 1-6). Stem diameters at maturity were significantly different in response to planting date ($p=0.0365$) (Table 1-4; Table 1-6).

For the CFX2 trials in 2018, densities at harvest in Salisbury were significantly different in response to seeding rate ($p=0.008$) (Table 1-7). Densities at harvest in Salisbury were approximately 155000, 407500, 607500, and 720000 plants ha^{-1} in response to the 100, 200, 300, and 400 pure live seed m^{-2} seeding rates (Table 1-8). Differences in stem diameter at harvest in Salisbury were significantly different in response to seeding rate ($p=0.009$) (Table 1-7). Stem

diameters in Salisbury were 4.17, 3.36, 2.5, and 2.29 mm in response to the 100, 200, 300, and 400 pure live seed m⁻² seeding rates (Table 1-8). Seed yield and height in all locations, as well as biomass in Salisbury were not significantly different in response to seeding rate ($\alpha = 0.05$) (Table 1-7).

Discussion:

In 2017, only densities 4 weeks after planting (WAP) were recorded. Densities were significantly higher for all cultivars at each successive seeding rate (Table 1-3). In 2018, densities were recorded at 4WAP, 8WAP, and at maturity when approximately 75% of staminate plants were shedding pollen. Density measurements at this “technical maturity” as described by Bosca and Karus (1998c) is the most relevant timing for stem producers because it ensures the highest harvestable population before staminate plants senesce. In 2018, densities at maturity increased in response to seeding rate for both CFX2 and Carmagnola at all locations. Only two distinct groupings of densities in response to seeding rate were detectable by LSD analysis for both those cultivars in 2018, as opposed to the three distinct groups of densities achieved in 2017. Larger intervals between treatment rates in 2017 likely explain the greater distinction in the response. The increases in density in response to increased seeding rates in both 2017 and 2018 support the efficacy of seeding rate in controlling population in industrial hemp, especially for farmers (Table 1-2). These trends in density fit with existing expectations in commercial hemp production (Bosca and Karus 1998c; Canadian Hemp Trade Alliance 2019). Additional work is needed to quantify the difference between the targeted and emerged populations.

In 2017, canopy closure and height 4 weeks after planting increased significantly with increased seeding rate. Higher canopy closure rates could have led to competition for light,

resulting in taller plants such as has been described with other species (Larcher 1975; Board et al. 1992). The faster canopy closure at higher densities may allow suppression of weed competition. More work is needed to determine the optimum seeding rate when weeds are present. Under weed pressure, a higher seeding rate may prove useful.

The difference in the biomass yields between cultivars in 2017 may be attributable to staminate flowering date (Table 1-3). The staminate plants of the two later flowering cultivars, Felina32 and Carmagnola had not yet finished shedding pollen at the time of sampling, while those of CFX2 had senesced and shed all leaves, branches, inflorescences, and considerable portions of their main stalk. Only living pistillate plants were collected. The difference in biomass between the medium flowering Felina32 and the latest flowering Carmagnola may also be explained by the increased duration of the vegetative stage. The higher radiation use efficiency and net primary productivity of vegetative phases of hemp compared to reproductive phases has been described in detail by van der Werf et al. (1995, 1999). It is well accepted that later flowering hemp cultivars produce more biomass within the same time frame than earlier flowering cultivars (Bosca and Karus 1998b; van der Werf et al. 1999; Clarke 1999; Faux et al. 2013; Campiglia et al. 2017).

Interestingly, the cultivar with the most biomass, Carmagnola, did not have heights or canopy closure ratings that were significantly different from the lowest biomass producer, CFX2, when measured 4 weeks after planting (Table 1-3). It is possible that although limited in biomass production by its vegetative duration, CFX2 has rates of growth in the early season similar to late flowering cultivars. These early maturing cultivars would be best for producing seed in a timely manner if fields were intended for other small grain planting in the fall (Bosca and Karus 1998c). At the end of the season, they are also considerably shorter, which facilitates harvest by combine.

The cultivar of medium maturity, Felina32, had significantly greater heights and canopy closure 4WAP than the other cultivars. Medium maturity cultivars are likely the best choices for producing a dual crop of biomass and seed when field turnaround time is limited (Ranalli 1999).

In 2017 and 2018, and for all cultivars and locations, biomass and seed yield did not differ significantly in response to the seeding rates tested. This trend remained even as density increased, which suggests intracrop competition limited the accumulation of mass in stems and seeds in individual plants. To capture the effect of competition on individual stems in 2018, diameters were analyzed for a response to seeding rate. In CFX2, stem diameters decreased significantly in response to seeding rate (Table 1-8). There was no detectable trend in diameter for Carmagnola. It was not able to be determined if branches and leaves shed throughout the growing season would have contributed to final biomass. It has been reported that low density plants accumulate considerable mass in lateral branches compared to high density plantings (van der Werf 1995; Meijer et al. 1995). It is also possible that at the much higher rates tested for Carmagnola, a maximum density was being approached and individual stems had reached their minimum diameters. This conclusion is supported by the fact that the density averaged across locations and planting dates actually decreased numerically for the highest seeding rate of Carmagnola in 2018 (Table 1-5). Additionally, the final densities at maturity were less than those measured 8 weeks after planting. These trends may support the concept of self-thinning as described by van der Werf et al. (1995).

Within 2018, the earlier planting date had a significantly higher biomass yield and a numerically higher seed yield (Table 1-6). This too may be explained by a longer vegetative duration. The differences in heights and canopy closure 4 weeks after planting between the earlier and later planting dates may be attributable to precipitation differences within this critical

growth stage of hemp (Table 1-9). The first planting dates occurred in early to mid-June, a month when only 2.6cm of rain fell in 2018 in Salisbury. The second planting date occurred at the end of June, followed by 19.5 cm of rainfall in July. The difference in heights between the early planting and late planting were was not detected in height measurements made 8WAP, suggesting growth rates leveled out as both stands matured.

Conclusion:

For CFX2 grown in North Carolina under weed free conditions, approximately 16.8 kg ha⁻¹ seed or 100 pure live seed m⁻² is the rate that yields the most seed and biomass with the minimal seed input. Under competition from weeds, higher rates may be necessary. In Canada, seeding rates of approximately 25 kg ha⁻¹ are recommended when no herbicide is used to control weeds (Canadian Hemp Trade Alliance 2019). For Carmagnola grown in North Carolina under weed free conditions in 2018, approximately 55 kg seed ha⁻¹ or 300 pure live seed m⁻² was the seeding rate that maximized biomass and seed yield with the minimum seed input. The optimum Carmagnola rate could be lower given the results from 2017 in which 16.8 kg seed ha⁻¹ produced similar biomass and seed yields as the 39.2 kg seed ha⁻¹. A seeding rate recommendation for Carmagnola of 55 kg seed ha⁻¹ would be conservative in light of the difference in the tested rates in 2017 and 2018 and the possible confounding effect of precipitation differences between the two years. Textile hemp producers will need to consider the impact of plant density to fiber quality. Higher seeding rates have been reported to increase the proportion of bast fiber yield of the stem important for textiles (Meijer et al. 1995)

In 2018, an earlier planting date resulted in higher harvest biomass and seed yield than a planting date just two weeks later. Greater precipitation during the first four weeks of the second

planting date, however, may have led to increased height and canopy closure and improved population survival when measurements were made 4 weeks after planting (WAP). Both of the planting dates were relatively late in 2018, and were in drier months of the year. Hemp is sown with success in other countries as soon as the threat of frost has passed and well before dry summer conditions (Bosca and Karus 1998c; Canadian Hemp Trade Alliance 2019). The same should be recommended for North Carolina.

Growers specifically looking to produce hemp seed in North Carolina would benefit by using shorter stature, early flowering cultivars such as CFX2 or others derived from seed programs in Canada and northern Europe. A shorter stature will aid combine harvest and an early maturity limits interference with preparations for subsequent fall/winter rotations. For early maturing cultivars, early planting dates may be particularly important to ensure sufficient vegetative growth within a timely manner to support reproductive development. Bast fiber, biomass or dual purpose growers should consider later flowering, lower latitude adapted cultivars such as Felina32 or Carmagnola for their potential to attain greater stem height and mass.

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Table 1-1 Crop density, height, canopy closure (cc), Julian dates of flower maturity†, and biomass in response to seeding rate and cultivar. Salisbury, NC. 2017.

Cultivar	Seeding Rate	Density 4WAP	Biomass at Maturity	Height 4WAP	CC 4WAP	Pistillate Maturity	Staminate Maturity
	kg ha ⁻¹	plants ha ⁻¹	kg ha ⁻¹	cm	%	JD	JD
CFX2	16.8	272,683	2,273	33	11	195	195
	28	502,310	1,207	36	25	195	195
	39.2	975,917	1,492	46	45	195	195
Carmagnola	16.8	401,848	6,108	26	18	216	214
	28	545,365	7,884	31	35	216	212
	39.2	774,993	6,605	34	44	216	214
Felina32	16.8	502,310	5,895	49	45	204	204
	28	1,004,620	4,403	49	63	204	204
	39.2	1,176,841	6,605	53	78	206	206

ANOVA††

<i>Source of Variation</i>							
Seeding Rate		p<0.0001	ns	ns	p=0.0017	ns	ns
Cultivar		p=0.0086	p<0.0001	p=0.005	p=0.0002	p<0.0001	p<0.0001
Seeding Rate * Cultivar		ns	ns	ns	ns	ns	ns
Block		ns	p=0.0025	ns	ns	ns	ns
CV, %		38.24	39.32	27.07	45.73	1.05	1.41

† Flower maturity recorded when 50% of pistillate or staminate plants in the plot had reached flowering

†† $\alpha = 0.05$

Table 1-2 Density, biomass, and canopy closure (cc) 4 weeks after planting (WAP) in response to industrial hemp seeding rate averaged across all cultivars. Salisbury, NC. 2017

Seeding Rate	Density 4WAP	Biomass at Maturity	CC 4WAP
kg ha ⁻¹	plants ha ⁻¹	kg ha ⁻¹	%
39.2	975,917 a†	4,900 a	55 a
28	684,099 b	4,498 a	41 a
16.8	392,280 c	4,758 a	25 b

† Within columns, means followed by the same letter are not significantly different according to Fisher's LSD ($\alpha = 0.05$)

Table 1-3 Biomass, Julian date of staminate flower maturity†, canopy closure (CC) and height 4 weeks after planting (WAP) in response to cultivar averaged across all seeding rates. Salisbury, NC. 2017

Cultivar	Biomass at Maturity	Staminate Maturity	CC 4WAP	Height 4WAP
	kg ha ⁻¹	JD	%	cm
Carmagnola	6,865 a††	213 a	32 a	30 a
Felina32	5,634 a	204 b	61 b	50 b
CFX2	1,657 b	195 c	27 a	38 a

† Flower maturity recorded when 50% of pistillate or staminate plants in the plot had reached flowering

†† Within columns, means followed by the same letter are not significantly different according to LSD ($\alpha = 0.05$)

Table 1-4 Density and height four and eight weeks after planting (WAP), seed yield, biomass, and stem diameter at maturity in response to seeding rate as pure live seed (PLS m⁻²) and planting date (PD) for cultivar Carmagnola. Salisbury, Oxford and Rocky Mount, NC. 2018.

Location	PD	Seeding Rate	Density 4WAP	Height 4WAP	Density 8WAP	Height 8WAP	Seed Yield†	Biomass	Density	Stem Diameter
		PLS m ⁻²	plants ha ⁻¹	cm	plants ha ⁻¹	cm	kg ha ⁻¹	kg ha ⁻¹	plants ha ⁻¹	mm
Salisbury	June 6th	300	688,883	28	688,883	206	373	9,653	487,500	8.16
		400	660,179	30	1,062,027	203	405	9,840	680,000	6.71
		500	1,033,324	29	1,506,930	199	395	9,745	780,000	6.88
		600	1,062,027	29	1,894,427	208	385	9,850	807,500	7.42
	June 24th	300	975,917	64	774,993	221	445	6,755	532,500	6.19
		400	1,363,413	60	1,478,227	196	365	7,393	687,500	5.84
		500	1,851,372	64	1,176,841	207	499	8,015	1,060,000	5.13
		600	1,707,855	72	1,549,986	204	532	8,070	1,047,500	5.47
Oxford	June 13th	300	200,924	14	172,221	96	154	3,725	255,000	3.76
		400	301,386	20	258,331	118	154	4,200	365,000	3.49
		500	617,124	20	487,959	102	168	4,000	405,000	2.56
		600	545,365	20	588,420	101	202	3,875	470,000	3.07
	July 2nd	300	272,683	24	229,628	164	169	.	.	.
		400	243,979	25	243,979	139	142	.	.	.
		500	373,145	30	344,441	150	121	.	.	.
		600	774,993	31	545,366	129	153	.	.	.

Table 1-4 (continued).

Rocky	June 13th	300	114,814	34	100,462	166	.	4,050	137,500	6.04
Mount		400	287,034	35	200,924	115	.	3,625	215,000	5.17
		500	258,331	38	401,848	106	.	3,375	350,000	3.99
		600	215,276	34	229,628	110	.	3,725	255,000	5.43

Type 3 Test of Fixed Effects††

<i>Effect</i>									
Seeding Rate		p=0.0369	ns	p=0.0149	ns	ns	ns	p=0.0168	ns
Planting Date		ns	p=0.0125	ns	ns	ns	p=0.0052	ns	p=0.0365
Seeding Rate*Planting Date		ns	ns	ns	ns	ns	ns		ns

† Stand failure and logistical issues prevented data collection for values indicated by a "."

†† $\alpha = 0.05$

Table 1-5 Density eight weeks after planting (WAP) and at maturity, biomass at maturity and seed yield in response to seeding rate as pure live seed (PLS) of cultivar Carmagnola averaged across locations and planting dates. 2018.

Seeding Rate	Density 8WAP	Density at Maturity	Biomass at Maturity	Seed Yield
PLS m ⁻²	plants ha ⁻¹	plants ha ⁻¹	kg ha ⁻¹	kg ha ⁻¹
300	314,832 a†	291,652 a	4,306 a	285 a
400	587,515 ab	432,486 ab	4,664 a	266 a
500	683,193 ab	664,569 b	4,885 a	295 a
600	879,333 b	657,902 b	4,967 a	317 a

† Within columns, means followed by the same letter are not significantly different according to Tukey adjusted least squares means ($\alpha = 0.05$)

Table 1-6 Height four weeks after planting (WAP), density, biomass, stem diameter at maturity, and seed yield in response to planting date for Carmagnola averaged across locations and seeding rates. 2018.

Planting Date	Height 4WAP	Density at Maturity	Biomass at Maturity	Diameter at Maturity	Seed Yield
	cm	plants ha ⁻¹	kg ha ⁻¹	mm	kg ha ⁻¹
June 13th	27 a†	433,958 a	5,805 a	5.22 a	303 a
June 24th	50 b	589,347 a	3,606 b	3.65 b	278 a

† Within columns, means followed by the same letter are not significantly different according to Tukey adjusted least squares means ($\alpha = 0.05$)

Table 1-7 Density and height four and eight weeks after planting (WAP), biomass, density and stem diameter at maturity, and seed yield in response to seeding rate as pure live seed (PLS) of CFX2.Salisbury, Oxford, and Rocky Mount, NC. 2018.

Location	Seeding Rate	Density 4WAP	Height 4WAP	Density 8WAP	Height 8WAP	Seed Yield†	Biomass at Maturity	Density at Maturity	Diameter at Maturity
	PLS m ⁻²	plants ha ⁻¹	cm	plants ha ⁻¹	cm	kg ha ⁻¹	kg ha ⁻¹	plants ha ⁻¹	mm
Salisbury	100	272,683	32	157,869	71	56	3,820	155,000	4.17
	200	1,090,731	31	344,441	67	44	4,415	407,500	3.35
	300	789,345	29	617,124	58	82	4,525	607,500	2.5
	400	1,377,765	29	947,214	60	54	4,575	720,000	2.33
Oxford	100	172,221	29	129,166	34
	200	315,738	24	186,572	24
	300	330,090	21	114,814	30
	400	157,869	20	143,517	25
Rocky Mount	100	57,407	39	43,055	28
	200	86,111	39	28,704	32
	300	71,759	41	43,055	39
	400	43,055	37	57,407	32

Type 3 Test of Fixed Effects††

<i>Effect</i>									
Seeding Rate		ns	ns	ns	ns	ns	ns	p=0.008	p=0.0069

† Stand failure and logistical issues prevented data collection for values indicated by a "."

†† $\alpha=0.05$

Table 1-8 Density and stem diameter at maturity in response to seeding rate as pure live seed (PLS) of CFX2. Salisbury, NC. 2018

Seeding Rate	Density at Maturity	Diameter at Maturity	Biomass
PLS m ⁻²	plants ha ⁻¹	kg ha ⁻¹	kg ha ⁻¹
100	155,000 a†	4.17 a	3820 a
200	407,500 ab	3.36 ab	4415 a
300	607,500 b	2.50 b	4525 a
400	720,000 b	2.29 b	4575 a

† Within columns, means followed by the same letter are not significantly different according to Tukey adjusted least squares means ($\alpha = 0.05$)

Table 1-9 Soil texture and sum of monthly precipitation at each research station location in the 2017 and 2018 industrial hemp trials.

Location	Soil Type/Text.	Year	Precipitation			
			cm			
			June	July	Aug.	Sep.
Salisbury	Lloyd clay loam	2017	18.4	11.5	6.5	13
		2018	3.6	19.5	10.7	18.1
Oxford	Vance sandy loam	2017	13.9	4.1	8.9	6
		2018	14.8	12.1	15.6	21.4
Rocky Mount	Norfolk loamy sand	2017	12.2	15.1	17.6	7.6
		2018	7.3	18.1	9.4	13
Waynesville	Braddock clay loam	2017	9	3.4	10.8	11.5
		2018	6.9	4.8	12.9	11.8

Chapter 2

The Effect of Nitrogen, Potassium, and Sulfur Fertilization on Seed Yield in Industrial Hemp (*Cannabis sativa* L.)

Introduction:

Farmers in the southeastern United States do not have contemporary soil fertility guidelines for producing industrial hemp. Nutrient application rates for hemp have been studied in other countries, though recommendations differ by target market and responses differ by cultivar and environment (Ranalli 1999; Vera et al. 2004; Finnan and Burke 2013; Amaducci et al. 2014; Aubin et al. 2015). Nutrition information is valuable because improvements to yield with soil fertilization have been demonstrated widely, including for cereals, legumes, vegetables and other specialty crops (Zubriski et al. 1970; Rabuffetti and Kamprath 1977; Fageria et al. 2011; Simone et al. 2017; May 2018). However, nutrients are sometimes applied in excess of what can be utilized by a crop in a given time frame (Glass 2003; Kant et al. 2011). Appropriate nutrient applications are an important tool for not only maximizing yield but also limiting nutrient costs.

Fertility research for field grown hemp has traditionally taken place in Europe and Canada, regions with existing commercial markets for the seed and fiber. For hemp studies in Italy, Campiglia et al. (2017) found an increase in stem biomass from 4.86 to 5.84 t ha⁻¹ when the nitrogen (N) rate was increased from 50 to 100 kg N ha⁻¹. As context, commercial yields are expected to reach 7 to 10 t ha⁻¹ (Bosca and Karus 1998b; Amaducci et al. 2014). Campiglia et al. (2017) did not find a response in seed yield to N rate. This is in contrast to Canadian studies, where Vera et al. (2004, 2009) detected both biomass and seed yield increases in response to N

applications. The trials executed by Vera et al. (2004, 2009) took place in Saskatchewan from 2000-2002 and again from 2006-2008.

In the earlier studies, Vera et al. (2004) evaluated plant height, stem biomass and seed yield of different cultivars in response to N and phosphorus (P). Height, biomass and seed yield increased in response to N rate in a quadratic fashion with the response plateauing within the range of N rates evaluated (0, 40, 80 and 120 kg N ha⁻¹). The mean yields in response to the increasing N rates were 401, 542, 625, and 657 kg seed ha⁻¹. Seed yield response differed by cultivar, with the estimated maximum yield for Finola occurring in response to the N rate of 100 kg N ha⁻¹. Fasamo, the later flowering cultivar, did not reach an estimated maximum yield within the 120 kg N ha⁻¹ range. In response to P, a decrease in seed yield and biomass occurred in both cultivars at the tested P rates (0, 20, 40, 60, 80 kg P ha⁻¹) (Vera et al. 2004).

In the later studies, Vera et al. (2009) evaluated cultivar yield responses to N, P and sulfur (S) rates. In those trials, a wider N range was tested (0, 50, 100, 150 and 200 kg N ha⁻¹). Vera et al. (2009) found that across cultivars, height and biomass were maximized at the N application rate of 150 kg N ha⁻¹, though Finola achieved the highest seed yield at an N rate of 200 kg N ha⁻¹. This N requirement for maximum Finola seed yield is nearly double what had been found in the previous study conducted at a different location (Vera et al. 2004, 2009). Average seed yield across cultivar in the later study was 570 kg seed ha⁻¹ at the control rate; average seed yields at each positive nutrient rate were not reported. There was no effect on yield at the S rates applied, even on S deficient soil. The results led Vera et al. (2009) to suggest that hemp may have low S requirements compared to other crops. P had a positive impact on height but not seed yield or biomass at the rates tested (20, 40, 60, 80 kg P ha⁻¹) (Vera et al. 2009).

In Dutch trials, van der Werf et al. (1995) adjusted soil levels of extractable N to 80 and 200 kg N ha⁻¹. Throughout the season, they found biomass was consistently higher at soil N level of 200 kg N ha⁻¹. However, at the higher soil N level, self-thinning was more severe. The authors concluded that biomass lost to self-thinning was compensated for by the increase in growth and mass accumulation achieved with more available N (van der Werf et al. 1995).

In Irish trials, Finnan and Burke (2013) applied potassium (K) at rates of 0, 50, 90, 120, and 150 kg K ha⁻¹ to multiple cultivars. Potassium uptake differed widely by cultivar, with the late maturity cultivar, Futura75, having the highest uptake. Finnan and Burke (2013) also found that up to 75% of absorbed K was located in the stem tissue as opposed to leaves or inflorescences. They suggested that if stems are left to rot in the fields, much of this K can be returned to the soil. The effect of K on seed yield was not tested. There was no biomass difference in response to K rate, though K tissue content was higher when hemp was grown on soils with higher K levels. The authors concluded that hemp might respond to K applications if tested on a soil with low K content (Finnan and Burke 2013).

In Eastern Canada, Aubin et al. (2015) evaluated biomass and seed yield responses to N, P, and K applications. In response to a range of N rates from 0 to 200 kg N ha⁻¹, seed yield improved with each successive rate from 519 to 1340 kg seed ha⁻¹. Biomass also improved from 1674 to 4209 kg ha⁻¹. The cultivar responses to N were similar to those found by Vera et al. (2004, 2009), in which the later flowering type had a greater biomass increase per unit N applied. Aubin et al. (2015) did not find a plateau in yield parameters in response to N rate, and the authors did not suggest an optimum N fertilization recommendation. Further, a yield response to K was not found when K was applied at rates ranging from 0 - 200 kg K ha⁻¹. The soil levels of K at the test locations, however, were relatively high, with the lowest soil K level being 195 kg

ha⁻¹. It was not specified if this was total or extractable soil K. Like Vera et al. (2004, 2009), Aubin et al. (2015) reported a positive response in height to P applications, and this was achieved at the lower end of the tested rates (0, 25, 50, 75, and 100 kg P ha⁻¹).

Taken together, these studies suggest seed and fiber hemp yields respond to P, K, and S applications within a narrow range. Nitrogen applications were concluded to increase stem biomass over a wide range but at different rates depending on location and cultivar. The response of seed yield to N applications varied with location and cultivar, and none of these nutrient factors have been definitively investigated for hemp production in the southeastern United States.

In North Carolina, farmers have recently been licensed the opportunity to produce hemp for fiber, seed, and cannabinoids. As a summer crop, hemp could serve as a break rotation. More so than for fiber and cannabinoids, the equipment required for hemp seed production is familiar to farmers in the southeast, particularly small grain growers. Planting and processing equipment similar to that used for wheat or maize can be used for hemp (Post, personal communication 2018; Canadian Hemp Trade Alliance 2019). Considering the lack of nutrient information and the potential to transition from similar small grains to hemp seed production, the objective of this study was to test the effect of N, K, and S rate on hemp seed yield in North Carolina.

Methods and Materials:

The first year of fertility trials took place in 2017. On June 12th 2017, a trial was planted in Salisbury, NC on a Lloyd clay loam (USDA hardiness zone 7b; soil class: Fine, kaolinitic, thermic Rhodic Kanhapludults) and on June 14th, 2017 in Rocky Mount, NC on a Norfolk loamy sand (8a; Fine-loamy, kaolinitic, thermic Typic Kandiudults). Soil samples taken prior to the

experiment were analyzed by the NCDA soil testing laboratory using Mehlich III extraction. Interpretation of nutrient concentrations in the soil followed published guidelines (Hardy 2014). In Salisbury in 2017, extractable phosphorus was present at 290 kg P ha⁻¹, potassium at 258 kg K ha⁻¹, and sulfur at 58 kg S ha⁻¹. Soil pH was 5.4 with a cation exchange capacity (CEC) of 8.5. In Rocky Mount, extractable phosphorus was present at 127 kg P ha⁻¹, potassium at 187 kg K ha⁻¹ and sulfur at 42 kg S ha⁻¹. Soil pH was 5.8 with a CEC of 6.3. Soil phosphorus and potassium levels were amended by station staff according to NCDA soil test recommendations for maize in Salisbury and for cotton in Rocky Mount after soil sampling but prior to the experiment in 2017 (Hardy et al. 2014).

Field preparation included glyphosate application to spring weeds in Salisbury and disking with shallow vertical tillage at both locations. Seed cultivar, CRS1, was seeded at 39.23 kg ha⁻¹ (35 lb acre⁻¹) using a John Deere 8000 series seed drill calibrated for hemp seed. Row spacing was 19.05 cm (7.5in). Seed depth was approximately 1.2cm (0.5in). After crop emergence Charger Max by Winfield AgriSolutions (S - metolachlor) was applied at a rate of 1071 g a.i. ha⁻¹ for weed control. Emerged weeds were manually removed 4 weeks after planting (WAP).

In 2017, nitrogen (N) and sulfur (S) treatments were surface applied 4WAP. Plot dimensions were 3.05m x 8.53m (10ft x 28ft). Five rates of N (0, 56, 112, 168, 224 kg N ha⁻¹) and 2 rates of S (0, 39 kg S ha⁻¹) were applied in a factorial arrangement for a total of 10 treatments. A randomized complete block design was used with four replications. While 0 N:0 S controls did exist in each rep, there was no 0 N:+S treatment because S was applied as ammonium sulfate, bringing a credit of 35 kg N ha⁻¹. Nitrogen was applied as ammonium nitrate and was adjusted for the credit from ammonium sulfate when necessary. Fertilizers were in

granular form, premeasured by plot, and broadcast by Solo brand multipurpose chest mount spreader.

At 4WAP, stand counts along 1 row meter and height for one representative plant per plot were recorded. Visual assessments of relative canopy closure were recorded at 4 and 8WAP. The validity of relative visual assessments for general crop monitoring is supported by Buchi et al. (2018). Julian dates were recorded for flowering, one when approximately 50% of pistillate plants were showing styles and another when approximately 50% of staminate plants were shedding pollen. Plots were harvested on August 27th, 2017 in Salisbury and on August 24th, 2017 in Rocky Mount. A Wintersteiger Delta plot combine was used to cut a 5 foot wide swath through the entire length of the plot. Whole plot samples were collected and dried on racks indoors at 20°C with 50% air humidity. Moisture was monitored using a Dicky John 2100 Grain Analysis Computer set to read canola samples. Once stable moisture (~8%) was met for all samples, moisture and sample weight were recorded.

The 2018 fertility trials were planted on June 7th in Salisbury, NC on a Lloyd clay loam (USDA hardiness zone 7b; Fine, kaolinitic, thermic Rhodic Kanhapludults), on June 14th in Waynesville, NC on a Braddock clay loam (6b; Fine, mixed, semiactive, mesic Typic Hapludults), and on June 15th, 2018 in Oxford, NC on a Vance sandy loam (7b; Fine, mixed, semiactive, thermic Typic Hapludults). Soil samples prior to the experiment were analyzed by Water's Agricultural Laboratory using NCDA standards (Mehlich-III extraction) and were interpreted following published guidelines (Hardy et al. 2014). In Salisbury, extractable phosphorus was present at 180 kg P ha⁻¹, potassium at 199 kg K ha⁻¹, and sulfur at 24 kg S ha⁻¹. Soil pH was 5.8 with a CEC of 7.3 meq 100g⁻¹. In Waynesville, extractable phosphorus was present at 40 kg P ha⁻¹, potassium at 66 kg K ha⁻¹ and sulfur at 22 kg S ha⁻¹. Soil pH was 6.2 with

a CEC of 8.6 meq 100g⁻¹. In Oxford, extractable phosphorus was present at 129 kg P ha⁻¹, potassium at 82 kg K ha⁻¹ and sulfur at 26 kg S ha⁻¹. Soil pH was 5.5 with a CEC of 3.8 meq 100g⁻¹. No nutrient additions were made between soil testing and the experiment in 2018.

Field preparation included shallow vertical tillage in Salisbury and disking followed shallow vertical tillage in Waynesville and Oxford. Seeds of CFX2 were planted as in 2017. For weed control in Salisbury, Charger Max by Winfield AgriSolutions (S - metolachlor) was applied at a rate of 1071 g a.i. ha⁻¹ after planting but before crop emergence, as opposed to after emergence as was done in 2017. Oxford and Waynesville did not receive herbicide. Emerged weeds were removed manually approximately 4WAP.

In 2018, N, S and potassium (K) treatments were broadcast at planting. Plots dimension were 2.13m x 9.144m (7ft x 30ft). Five N rates (0, 56, 112, 168, 224 kg N ha⁻¹), two K rates (0, 56 kg K ha⁻¹) and two S rates (0, 39 kg S ha⁻¹) were applied in a factorial arrangement for a total of 20 treatments. A randomized complete block design was used with four replications. Nitrogen was band applied as urea ammonium nitrate with a CO₂ powered backpack sprayer and boom equipped with streamer nozzles. Potassium was applied as granular potassium chloride and S as granular elemental. Potassium and sulfur were premeasured by plot and applied by multipurpose hand spreaders available at each research station.

In 2018, stand failure in Oxford prevented data collection at that site. At Salisbury and Waynesville, stand counts along 1 row meter and height for one representative plant per plot were recorded 4 and 8 WAP. Row meters were flagged so as to recount the same line. Julian dates for flowering were recorded as in year one. Seed yield was harvested by combine as in year one. Salisbury was harvested on August 27th, 2018 and Waynesville on August 16th, 2018. Entire samples were dried for 24 hours at 50°C, cleaned with an Almaco table top seed cleaner, and

allowed to stabilize at 20°C and 50% humidity. Once stable moisture (~6%) was met for all samples, moisture and sample weight were recorded. In 2018, the most recent mature leaves (RML) were sampled randomly from 12 plants within each plot for tissue nutrient analysis by the NCDA Tissue Laboratory. In Salisbury, leaf samples were taken on July 30th when plants were in bloom and August 13th when plants were in fruit. In Waynesville, leaf samples were taken on July 18th and August 1st when plants were in bloom and August 15th when plants were in fruit.

In both years, SAS version 9.4 (SAS, Inc. Cary, NC) was used to perform analyses of variance with the procedure for general linear models. Treatment factors were listed in the class statement as well as the model statement. Each location was analyzed separately because of interactions between treatments and locations. Fisher's LSD means separation tests were performed at $\alpha=0.05$. Regression analysis was also performed with the procedure for general linear model using SAS v. 9.4. Only two level factors were kept in the class statement and a solution request was made in the model statement to generate parameter estimates. In 2018, Pearson's correlation statistics were generated using the correlation procedure.

Results:

ANOVA:

In 2017, there was no significant difference in seed yield in response to nitrogen (N) or sulfur (S) rate in Salisbury (Table 2-1). There was a significant interaction between N rate and S rate in Salisbury ($p=0.0262$) (Table 2-1). There was no significant difference in seed yield in response to N rate, S rate, or their interaction in Rocky Mount (Table 2-2).

At the Salisbury location in 2018, there were no significant differences in seed yield, density, or height in response to N rate, K rate, or S rate. Tissue N content during late bloom

(July 30th) was significantly different in response to K rate ($p=0.0028$). Tissue N content during fruit (Aug. 13th) was significantly different in response to N rate ($p=0.0186$) (Table 2-3). Tissue K content at late bloom (July 30th) was significantly different in response to K rate ($p=0.001$) and the N, K, S interaction ($p=0.0236$) (Table 2-3). At late bloom (July 30th) tissue K was higher with each successive increase in N rate, with the lowest and highest K contents of 1.33% and 1.57% being statistically distinct (Table 2-4). Tissue K at late bloom (July 30th) was also higher by 0.22% for plants that had received K (Table 2-5). Conversely, tissue N at late bloom was 0.21% lower for the plants that had received K (Table 2-5). During fruit set (Aug. 13th), tissue N was higher at each successive increase in N rate, with the lowest and highest N contents of 2.70% and 2.91% being statistically distinct (Table 2-4).

For Waynesville in 2018, seed yield was significantly higher in response to K rate ($p=0.0278$), (Table 2-6). At the 0 K rate, seed yield was 878 kg ha⁻¹ when averaged across other factors (Table 2-8). With the addition of 56 kg ha⁻¹ of K, the seed yield was 1043 kg ha⁻¹ when averaged across other factors (Table 2-8). Heights 4WAP were significantly higher in response to K rate ($p<0.0001$), and decreased in response to S rate ($p=0.0065$) and the interaction of N rate and S rate ($p=0.00156$) (Table 2-6). Height 8WAP was also significantly higher in response to K rate ($p=0.0001$) (Table 2-6). Tissue N content during late bloom (Aug. 1) was significantly different in response to the interaction of N rate, K rate, and S, rate ($p=0.0007$) (Table 2-7). 4WAP, plants in Waynesville that had received the addition of K were 11 cm on average taller than the control K rate (Table 2-8). At 8 WAP, the advantage over the control rate of K was 15cm. Heights 4WAP and 8WAP were lower by 3cm and 1 cm in response to the addition of S in Waynesville (Table 2-9).

Correlation:

Significant correlations existed between several variables in the 2018 fertility trials in Waynesville. There was a positive correlation between K rate and height 4WAP ($r=0.59$, $p<0.0001$) and a positive correlation between K rate and height 8WAP ($r = 0.60$, $p<0.0001$). There was a positive correlation between height 4WAP and 8WAP ($r = 0.73$, $p<0.0001$). There was a positive correlation between height 8WAP and seed yield ($r = 0.53$, $p<0.0001$) and a positive correlation between K rate and seed yield ($r = 0.25$, $p=0.0245$). For tissue content, there was a positive correlation between N content and S content at early bloom (July 18th) ($r = 0.40$, $p=0.0002$). In Salisbury in 2018, there was a significant positive correlation between tissue N and S content at late bloom (July 30th) ($r =0.75$, $p<0.0001$).

Regression:

For the 2017 fertility trial in Salisbury, the complete multiple regression model including N rate, S rate and the N*S interaction did not reliably estimate observed seed yield at the $\alpha=0.05$ level ($p=0.5669$, $R^2 =0.0540$). Though the slopes are not significantly different from zero, the fitted equations for the 2017 Salisbury datasets are: seed yield without S addition = $381.4757 + (0.3691)(N \text{ rate})$; seed yield with S addition = $399.2659 + (-0.1489)(N \text{ rate})$.

For the 2017 fertility trial in Rocky Mount, the complete multiple regression model including N rate, S rate and the N*S interaction did not reliably estimate observed seed yield at the $\alpha=0.05$ level ($p=0.2983$; $R^2 =0.0958$). Though the slopes are not significantly different from zero, the fitted equations for the 2017 Rocky Mount dataset are: seed yield without S addition = $42.8207 + (0.1542)(N \text{ rate})$; seed yield with S addition = $70.9141 + (-0.1373)(N \text{ rate})$.

For the 2018 fertility trial in Salisbury, the complete multiple regression model including N rate, K rate, S rate and the N*K*S interaction did not reliably estimate observed seed yield at

the $\alpha=0.05$ level ($p=0.8321$, $R^2=0.0488$). Though the slopes are not significantly different from zero, the fitted equations for the 2018 Salisbury dataset are: seed yield without K and without S = $51.2012 + (-0.05989)(N \text{ rate})$; seed yield without K and with S = $29.6817 + (0.0718)(N \text{ rate})$; seed yield with K and without S = $40.3084 + (0.0212)(N \text{ rate})$; seed yield with K and with S = $(33.8250) + (0.0212)(N \text{ rate})$.

For the 2018 fertility trial in Waynesville, the complete multiple regression model including N rate, K rate, S rate and the N*K*S interaction did not reliably estimate observed seed yield at the $\alpha=0.05$ level ($p=0.3486$, $R^2=0.1023$). Though the slopes are not significantly different than 0, the fitted equations for the 2018 Waynesville dataset are: seed yield without K and without S = $825.4601 + (0.6651)(N \text{ rate})$; seed yield without K and with S = $872 + (-0.1159)(N \text{ rate})$; seed yield with K and without S = $956.46 + (1.121)(N \text{ rate})$; seed yield with K and with S = $915.1538 + (1.0415)(N \text{ rate})$.

Discussion:

Linear regression is a technique used to model the relationship between an independent variable and the observed values of a response variable in a data set. When multiple independent variables are analyzed, the technique is considered multiple linear regression. In multiple regression analysis, when the probability of the result of an overall F test is below a predetermined probability value, one concludes that the model which includes the treatment factors more reliably estimates values of the response variable than an intercept only model (UCLA 2019; Christensen 2019). Further, if the results of the t-tests for the individual factors are of significant probability, one concludes that variation in each of those factors reliably estimated some portion of the variation in the response, and that the coefficients of variation (slope) of

those factors is significantly different than zero (UCLA 2019; SAS, Inc. 2019). The overall F test and individual t-tests of an analysis of variance (ANOVA), though calculated differently, similarly evaluate whether variation in the response values is explained by variation in the treatment factors. Discrepancies between the results of regression analysis and ANOVA arise when factors are held as categorical variables in ANOVA to answer an optimum treatment question more directly, forcing the calculation of the tests to differ between methods (Grace-Martin 2019a).

In agricultural extension work, regression models alone may not be sufficient for making recommendations to growers concerning a treatment factor. While responses to a continuous independent variable such as fertilizer rate might be better estimated by a regression model, a regression analysis does not provide statistics for a comparative interpretation of responses to treatments (Grace-Martin 2019b). It is more cautious to make recommendations based on ANOVA. ANOVA coupled with mean separation tests or contrasts allows for conclusions as to whether a particular treatment is better or worse considering circumstances relevant to farmers. Nevertheless, plots of regression models may provide insight into the relationship between continuous variables that may be useful for future agronomic research.

While none of the fertility trials had significant regression models, data from Waynesville in 2018 was plotted because it was the only location where yields met industry standards and showed responses to treatments as determined through ANOVA. Such visualizations should be interpreted with caution, as the slopes of the responses are numerical trends and are not significantly different from zero ($\alpha=0.05$). Seed yield in response to nitrogen (N) rate without the addition of sulfur (S) and grouped by potassium (K) rate in Waynesville suggests that with the addition of K, seed yield was higher at every N rate (Figure 2-1). This trend held true with the

addition of S (Figure 2-2). These trends are consistent with the ANOVA and with results in other seed crops (Mitchell et al. 1976; Bailey and Soper 1985). Seed yield in response to N rate without the addition of K and grouped by S rate for Waynesville in 2018 suggests S negatively affected seed yield response to N rate (Figure 2-3). Even with the addition of K, seed yield was lower at every rate of N when S had been applied (Figure 2-4). Although the mean seed yield was numerically lower with the S application than without it, the difference in means was not significant using ANOVA and Fisher's LSD mean separation. Further, a significant negative interaction between N rate and the addition of S would be unusual, as the precedent in other crops is that the interaction between S rate and N rate has a positive impact to seed yield (Rabuffetti and Kamprath 1977; Kamprath and Jones 1986). Additional experiments may be required to evaluate the effect of S on seed yield response to N.

Commercially, hemp seed yields range from 750 to 1000 kg seed ha⁻¹ (Bosca and Karus 1998c, Amaducci et al. 2014). The mean seed yields in 2017 were 449 kg seed ha⁻¹ in Salisbury and 64 kg seed ha⁻¹ in Rocky Mount (Table 2-10). In 2018, the mean seed yield was 962 kg seed ha⁻¹ in Waynesville and 36 kg seed ha⁻¹ in Salisbury (Table 2-10). In both years, late planting may have played a role in reduced yields. Harvesting after seed shatter in Salisbury in 2018 also reduced yields drastically, increasing the variability in seed yield and possibly preventing detection of N responses at that location. Unlike many cereals and legumes, domestication in hemp has not eliminated the shattering trait (Small 2015). Breeding efforts into controlling the abscission of the seed from the enveloping bract are ongoing (Schlutenhofer 2017). It is important to monitor the crop closely at the green seed stage. Maturity of the seed head will not be uniform. A conservative decision to harvest should be made while some seeds remain green

and a majority are brown but not yet released from the bract (Canadian Hemp Trade Alliance 2019).

Surface crusting during emergence was observed in the sandy soils of Rocky Mount and Oxford, and may have played a role in poor stand establishment and low yield at those sites in 2017 and 2018, respectively. Stand loss in Oxford in 2018 may have been a result of residual herbicide damage from clomazone used in previous tobacco cycles. Research station staff confirmed seedling bleaching that is symptomatic of clomazone carryover damage (Fred Smith, personal communication, 2018). Clomazone persistence has been determined by injury symptoms and soil testing up to 12 months after use in other crops (Ahrens and Fuerst 1990; Cumming et al. 2009).

In Canada, seed cultivars like CRS1 and CFX2 are sown into soil in late April or early May (Amaducci et al. 2014; Canadian Hemp Trade Alliance 2019). Since hemp is a short day flowering plant, the mid-June plantings in the North Carolina tests reduced the potential vegetative period by up to six weeks. In 2017, nutrient applications were made 4 weeks after planting, right as pistillate plants were initiating flower. The 2017 results can be interpreted as finding no difference in seed yield in response to N or S applications made at the initiation of flower to a CRS1 crop planted mid-June. This is not without precedent. In determinate cereals like wheat and rice, limited seed yield responses to N applications during reproduction are expected because carbohydrates are primarily remobilized from vegetative tissue mature enough to serve as a net exporter by the time reproductive source-sink dynamics began (Yoshida 1972; Cassman et al. 1992). Applications of N at flower primarily affect seed quality, as can be the case with protein content in wheat (Cassman et al. 1992).

In an attempt to make nutrients available during vegetative growth in 2018, N applications were made at planting. The lack of height, density, and seed yield response to nitrogen (N) at all locations in 2018 breaks with the precedent set by other hemp trials in which these yield responses were affected positively when N was applied at planting (Vera et al. 2004; Vera et al. 2009). However, in Italian studies, Campiglia et al. (2017) only found biomass responses to N applications, without increases in seed yield. Since biomass was not recovered for NC fertility trials, this trend cannot be corroborated.

Consideration should also be given to why populations were lower in Salisbury than in Waynesville in 2018 (Table 2-3; Table 2-6). Locations were not analyzed together in 2018 because of interactions, however, the highest population in Salisbury 8 weeks after planting (WAP) was only 200,924 plants ha⁻¹ (table 2-3) while in Waynesville it was 1,089,230 plants ha⁻¹. In both Salisbury and Waynesville, care was taken to calibrate seed drills before planting. No discrepancy between the target seeding rate of 39 kg ha⁻¹ and the amount of seed consumed by the drill during planting was observed. Both locations have similar soil types, and extractable soil nutrients were actually higher at the initiation of the experiment in Salisbury than in Waynesville. The effect of weather is difficult to establish, because while Salisbury received less precipitation than Waynesville in June, it received substantially more in July (Table 2-11). The application of S - metolachlor before crop emergence in Salisbury, and the lack of S - metolachlor use in Waynesville, could possibly explain the lower population in Salisbury. Leaf cupping on emerging plants consistent with S - metolachlor damage was observed in Salisbury in 2018. No research is published for the crop safety of metolachlor use in *Cannabis*.

While there was no observable height, density or seed yield responses to fertilizer rates in Salisbury in 2018, several of the tissue nutrient changes were significant in response to fertilizer

rates. Tissue K at late bloom (July 30th) was higher by 0.22% for plants that had received K (Table 2-5). At the same sampling time, tissue K was also higher with each successive increase in N rate, with the lowest and highest K contents of 1.33% and 1.57% being statistically distinct (Table 2-4). Conversely, tissue N at late bloom was 0.21% lower for the plants that had received K (Table 2-5). During fruit set (Aug. 13th), tissue N was higher at each successive increase in N rate, with the lowest and highest N contents of 2.70% and 2.91% being statistically distinct (Table 2-4). Considering these tissue results from Salisbury, the applications of K may have inhibited N tissue accumulation, but applications of N did not inhibit K tissue accumulation. Alternatively, applications of K may have facilitated the remobilization of N out of the most recent mature leaves at the stages of maturity at which leaves were sampled. Confidence in such minor but statistically significant changes in tissue nutrient content is bolstered by low coefficients of variation for nutrient content (~15%, Table 2-3) These narrow ranges for tissue content also correspond to values for *Cannabis* mineral content previously reported (Landi 1997).

In Waynesville, seed yield and height 4WAP and 8WAP increased significantly in response to the addition of K in 2018 (Table 2-8). The mean increase in seed yield was 165 kg seed ha⁻¹ over the zero K rate. The initial extractable soil K levels in Waynesville were low (66 kg K ha⁻¹) compared to the levels in Salisbury (199 kg K ha⁻¹). These seed results differ from those of Finnan and Burke (2013), in which there was no hemp biomass response to K rates, and those of Aubin et al. (2015), in which there was no biomass or seed yield response to K rate. However, in the trials conducted by Aubin et al. (2015) in eastern Canada, the lowest soil K content was 195 kg K ha⁻¹, nearly three times that of the initial soil K content in Waynesville in 2018. While Finnan and Burke (2013) did conduct trials on a site with only 67 kg K ha⁻¹ in

Ireland, they did not collect seed yield. These authors also suggested that because K tissue content was considerably higher at sites where K soil levels were high, hemp may be capable of luxury K uptake with low threshold requirements (Finnan and Burke 2013). Finnan and Burke (2013) concluded that hemp may show responses to K applications under different environments or when K available for plant uptake is less than 65 kg K ha^{-1} . Therefore, the seed yield response to the addition of K is reasonable in Waynesville where it is unlikely that all of the 66 kg ha^{-1} of extractable soil K was available to the plant. Positive seed yield responses to K additions on deficient soils have been shown for other determinate, non-leguminous broadleaves like sesame, flax, and sunflower (Mitchell et al. 1976; Bailey and Soper 1985). Indeterminate broadleaves such as cotton also show positive yield and quality responses at threshold K levels, especially under dry conditions (Kerby and Adams 1985).

The crucial role played by K ions in plant cells likely explains the increased heights and seed yield in response to K applications in Waynesville. K^+ ions facilitate the creation of charged solute gradients that drive water transportation into vacuoles that expand cells and allow growth (Leonard 1985; Beringer and Nothdurft 1985). This may explain the increases in height found in the 2018 trial. Caution should be expressed over the reliability of using one height measurement per plot. Ideally, multiple height measurements from a plot at a single sampling time reduces observer bias. Significant correlations between the heights taken at multiple sampling times does return confidence to the interpretation that height responds positively to the addition of K. Seed yield may have been improved by upstream effects of K ions on photosynthesis, such as increased carbon dioxide gas exchange from K^+ mediated stomatal activity, as well as improved photosynthate transport via the phloem and to reproductive structures and seeds (Mengel et al. 1981; Mengel 1985; Huber 1985).

Conclusion:

Positive stem growth and seed yield responses to a potassium (K) addition (56 kg K ha^{-1}) on a soil with a relatively low extractable K content ($66 \text{ kg ha}^{-1} \text{ K}$) in Waynesville, NC warrant consideration that hemp for seed production has at least a threshold K uptake requirement. This uptake requirement may be similar to that proposed by Finnan and Burke (2013) in Irish trials (65 kg K ha^{-1}). A 2015 report by the Economics and Competitiveness Division of the Alberta Department of Agriculture and Forestry listed a return price in Canadian dollars of $\$0.74 \text{ lb}^{-1}$ ($\$1.68 \text{ kg}^{-1}$) for hemp seed (Laate 2015). Following the Waynesville results, a seed increase of $165 \text{ kg seed ha}^{-1}$ in response to the addition of 56 kg K ha^{-1} equates to an additional gross income of $\$268.62 \text{ ha}^{-1}$ (Canadian dollars). Such increases justify further investigation into the impact of K on seed yield in hemp. Future researchers should determine K uptake levels in relation to extractable soil K, and whether the potential increase in yield warrants the cost of K fertilization. Soil type and climate will likely play a large role in yield responses to K (Finnan and Burke 2013, Aubin et al. 2015). Trials in North Carolina should be repeated with more consideration for existing soil nutrients and improved consistency in stand establishment and harvest timing. Future studies should collect both total biomass and seed yield to calculate harvest indices in hemp. If possible, sites with low soil K should be chosen to replicate the results of the 2018 trial in Waynesville. Responses to nitrogen (N) are also expected, and attempts should be made to reproduce the N response results of previous researchers. Such efforts will lend validity to new developments concerning K requirements of hemp for seed production as well as help to establish N requirements for North Carolina.

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Table 2-1 Seed yield of cultivar CRS1 in response to nitrogen (N) and sulfur (S) rates. Salisbury and Rocky Mount, NC. 2017.

S rate kg ha ⁻¹	N rate kg ha ⁻¹	Seed Yield	
		kg ha ⁻¹	
		Salisbury	Rocky Mount
0	0	434	51
	56	504	45
	112	386	78
	168	460	73
	224	559	80
39	35	447	84
	56	388	56
	112	510	74
	168	399	49
	224	403	52
ANOVA†			
<i>Source of Variation</i>			
S rate		ns	ns
N rate		ns	ns
S rate* N rate		P=0.0262	ns
CV, %		20.53	56.47

† $\alpha = 0.05$

Table 2-2 Seed yield, crop density and height four/eight weeks after planting (WAP) of cultivar CFX2 in response to nitrogen (N), potassium (K) and sulfur (S) rate. Salisbury, NC. 2018.

S rate	K rate	N rate	Seed Yield†	Density 4WAP	Density 8WAP	Height 4WAP	Height 8WAP	
kg ha ⁻¹	kg ha ⁻¹	kg ha ⁻¹	kg ha ⁻¹	plants ha ⁻¹	plants ha ⁻¹	cm	cm	
0	0	0	43	100,462	172,221	28	61	
		56	43	129,166	114,814	29	71	
		112	35	86,111	86,111	29	68	
		168	30	157,869	71,759	26	71	
		224	34	129,166	100,462	28	75	
	56	0	38	186,572	200,924	28	67	
		56	47	215,276	172,221	29	79	
		112	29	143,517	86,111	31	67	
		168	33	100,462	143,518	31	71	
		224	35	57,407	186,572	28	67	
	39	0	0	31	129,166	186,573	31	75
			56	35	229,628	157,869	29	72
			112	34	215,276	157,869	31	74
			168	38	157,869	143,518	29	70
			224	47	86,110	157,869	29	79
56		0	33	215,276	172,221	30	70	
		56	28	57,407	71,759	29	68	
		112	42	100,462	157,869	27	72	
		168	38	172,221	71,759	25	74	
		224	35	57,407	86,110	29	73	
ANOVA††								
<i>Source of Variation</i>								
N rate			ns	ns	ns	ns	ns	
K rate			ns	ns	ns	ns	ns	
S rate			ns	ns	ns	ns	ns	
N*K			ns	ns	ns	ns	ns	
N*S			ns	ns	ns	ns	ns	
K*S			ns	ns	ns	ns	ns	
N*K*S			ns	ns	ns	ns	ns	
CV, %			45.62	84.02	89.42	12.68	12.94	

†Seed yields were reduced by grain shattering in 2018

†† $\alpha = 0.05$

Table 2-3 Tissue nitrogen (N), potassium (K), and sulfur (S) content of cultivar CFX2 during the 2018 season in response to N, K and S rate. Salisbury, NC. 2018

S rate kg ha ⁻¹	K rate kg ha ⁻¹	N rate kg ha ⁻¹	Tissue N		Tissue K		Tissue S		
			July 30	Aug. 13	July 30	Aug. 13	July 30	Aug. 13	
0	0	0	3.77	2.76	1.23	1.16	0.23	0.17	
		56	3.51	2.78	1.33	1.35	0.24	0.19	
		112	3.52	2.77	1.32	1.16	0.24	0.18	
		168	3.44	2.9	1.34	1.23	0.21	0.19	
		224	3.64	3.03	1.48	1.24	0.25	0.19	
	56	0	3.23	2.88	1.39	1.21	0.21	0.18	
		56	3.21	2.51	1.53	1.42	0.23	0.17	
		112	3.57	2.75	1.6	1.2	0.23	0.17	
		168	3.36	2.92	1.83	1.22	0.23	0.17	
		224	3.41	2.92	1.58	1.38	0.23	0.18	
	39	0	0	3.21	2.6	1.11	1.16	0.22	0.18
			56	3.49	2.74	1.4	1.23	0.21	0.17
			112	3.23	2.71	1.66	1.44	0.23	0.2
			168	3.6	2.93	1.33	1.36	0.24	0.19
			224	3.68	2.79	1.29	1.15	0.23	0.19
56		0	3.27	2.6	1.61	1.28	0.21	0.19	
		56	3.08	2.78	1.47	1.4	0.2	0.19	
		112	3.17	2.75	1.26	1.35	0.22	0.18	
		168	3.05	2.75	1.56	1.32	0.23	0.19	
		224	3.6	2.92	1.87	1.26	0.23	0.17	

ANOVA†

<i>Source of Variation</i>							
N rate	ns	p=0.0186	ns	ns	ns	ns	ns
K rate	p=0.0028	ns	p=0.001	ns	ns	ns	ns
S rate	ns	ns	ns	ns	ns	ns	ns
N*K	ns	ns	ns	ns	ns	ns	ns
N*S	ns	ns	ns	ns	ns	ns	ns
K*S	ns	ns	ns	ns	ns	ns	ns
N*K*S	ns	ns	p=0.0236	ns	ns	ns	ns
CV, %	9.22	7.8	17.48	18.48	10.49	9.31	

† $\alpha=0.05$

Table 2-4 Tissue potassium (K) content during late bloom (July 30th) and tissue nitrogen (N) content during fruit (Aug. 13) of cultivar CFX2 in response to N rate. Salisbury, NC. 2018.

N rate kg ha ⁻¹	K Content July 30 (%)	N Content Aug. 13 (%)
224	1.57 a†	2.91 a
168	1.51 a	2.87 ab
112	1.46 ab	2.74 bc
56	1.40 ab	2.70c
0	1.33 b	2.70 c

†Within columns, means followed by the same letter are not significantly different according to Fisher's LSD ($\alpha = 0.05$)

Table 2-5 Tissue potassium (K) content during late bloom (July 30th) and nitrogen (N) tissue content during late bloom (July 30th) of cultivar CFX2 in response to K rate. Salisbury, NC. 2018.

K rate kg ha ⁻¹	K content July 30 (%)	N content July 30 (%)
56	1.56 a†	3.29 b
0	1.34 b	3.50 a

†Within columns, means followed by the same letter are not significantly different according to Fisher's LSD ($\alpha = 0.05$)

Table 2-6 Seed yield, density and height four and eight weeks after planting (WAP) for cultivar CFX2 in response to N, K, and S rate. Waynesville, NC. 2018

S rate	K rate	N rate	Seed Yield	Density 4WAP	Density 8WAP	Height 4WAP	Height 8WAP
kg ha ⁻¹	plants ha ⁻¹	cm	cm				
0	0	0	761	905,504	813,642	44	53
		56	942	1,207,339	944,874	46	58
		112	902	853,011	643,039	41	59
		168	912	944,874	813,642	38	53
		224	948	1,076,107	761,149	38	50
	56	0	1,078	1,089,230	971,121	49	65
		56	962	813,642	931,751	49	69
		112	1,003	892,381	761,149	47	69
		168	922	669,286	800,518	45	66
		224	1,378	1,010,490	1,089,230	52	77
39	0	0	962	826,765	853,011	36	60
		56	803	839,888	813,642	32	54
		112	760	1,141,723	787,395	36	51
		168	886	1,062,983	892,381	32	47
		224	892	721,779	879,258	31	50
	56	0	856	1,036,737	944,874	43	64
		56	947	1,023,614	1,076,107	50	65
		112	1,205	971,121	748,025	50	78
		168	997	1,010,490	1,036,737	49	70
		224	1,092	918,628	918,628	48	67

ANOVA†

<i>Source of Variation</i>						
N rate			ns	ns	ns	ns
K rate			p=0.0278	ns	ns	p<0.0001
S rate			ns	ns	ns	p=0.0065
N*K			ns	ns	ns	ns
N*S			ns	ns	ns	ns
K*S			ns	ns	ns	p=0.00156
N*K*S			ns	ns	ns	ns
CV, %			30.93	15.99	31.73	15.99

†α=0.05

Table 2-7 Tissue nitrogen (N), potassium (K), and sulfur (S) content of cultivar CFX2 during the 2018 season in response to N, K, and S rate. Waynesville, NC.

S rate kg ha ⁻¹	K rate kg ha ⁻¹	N rate kg ha ⁻¹	Tissue N			Tissue K			Tissue S			
			July 18	Aug. 1	Aug. 15	July 18	Aug. 1	Aug. 15	July 18	Aug. 1	Aug. 15	
0	0	0	5.08	3.33	2.35	1.03	0.54	0.67	0.35	0.23	0.28	
		56	5.08	3.16	2.36	0.94	0.5	0.68	0.36	0.23	0.26	
		112	4.93	3	2.36	1	0.55	0.67	0.37	0.22	0.26	
		168	4.93	3.27	2.4	1.47	0.78	0.74	0.35	0.19	0.24	
		224	5.14	3.16	2.34	1.29	0.59	0.64	0.39	0.24	0.25	
	56	0	5.22	3.09	2.2	1.13	0.64	0.74	0.36	0.21	0.29	
		56	4.89	3.27	2.44	1.16	0.66	0.75	0.36	0.24	0.26	
		112	5.45	3.34	2.58	1.16	0.56	0.61	0.36	0.22	0.23	
		168	5.05	3.35	2.34	1.32	0.68	0.72	0.35	0.21	0.24	
		224	4.75	3.25	2.3	1.22	0.64	0.72	0.35	0.21	0.27	
	39	0	0	5.22	3.14	2.51	1.18	0.66	0.73	0.36	0.19	0.29
			56	5.28	3.33	2.69	1.05	0.62	0.67	0.37	0.2	0.26
			112	5.16	3.46	2.43	1.24	0.71	0.8	0.37	0.22	0.26
			168	5.28	3.32	2.42	1.43	0.71	0.69	0.36	0.2	0.24
			224	5.06	3.24	2.7	0.98	0.54	0.64	0.36	0.2	0.24
56		0	5.04	3.4	2.52	1.09	0.62	0.75	0.37	0.22	0.27	
		56	4.86	2.89	1.97	1.41	0.85	0.88	0.34	0.21	0.28	
		112	5.1	2.98	2.41	1.16	0.67	0.81	0.36	0.19	0.27	
		168	4.88	3.39	2.29	1.26	0.69	0.72	0.36	0.22	0.26	
		224	5.29	3.53	2.46	1.31	0.57	0.72	0.37	0.25	0.25	

Table 2-7 (continued).

		ANOVA [†]							
<i>Source of Variation</i>									
N rate	ns	ns	ns	ns	ns	ns	ns	ns	ns
K rate	ns	ns	ns	ns	ns	ns	ns	ns	ns
S rate	ns	ns	ns	ns	ns	ns	ns	ns	ns
N*K	ns	ns	ns	ns	ns	ns	ns	ns	ns
N*S	ns	ns	ns	ns	ns	ns	ns	ns	ns
K*S	ns	ns	ns	ns	ns	ns	ns	ns	ns
N*K*S	ns	p=0.0007	ns	ns	ns	ns	ns	ns	ns
CV, %	7.02	7.06	12.73	30.18	33	18.81	5.47	14.82	11.94

[†] $\alpha =$
0.05

Table 2-8 Height four and eight weeks after planting (WAP) and seed yield of cultivar CFX2 in response to potassium (K) rate. Waynesville, NC. 2018

K rate kg ha ⁻¹	Height 4WAP cm	Height 8WAP cm	Seed Yield kg ha ⁻¹
0	37 a†	53 a	878 a
56	48 b	69 b	1043 b

†Within columns, means followed by the same letter are not significantly different according to Fisher's LSD ($\alpha = 0.05$)

Table 2-9 Height four and eight weeks after planting (WAP) and seed yield of cultivar CFX2 in response to sulfur rate. Waynesville, NC. 2018

S rate kg ha ⁻¹	Height 4WAP cm	Height 8WAP cm	Seed Yield kg ha ⁻¹
0	45 a†	61 a	987 a
39	41 b	60 b	939 a

†Within columns, means followed by the same letter are not significantly different according to Fisher's LSD ($\alpha = 0.05$)

Table 2-10 Soil type, initial Mehlich-III extractable soil nutrient concentration, sum of monthly precipitation and mean seed yield at each trial location in the 2017 and 2018 industrial hemp fertility trials.

Location	Soil Type/Text.	Year	Soil P [†] kg ha ⁻¹	Soil K kg ha ⁻¹	Soil S kg ha ⁻¹	Precipitation				Seed Yield kg ha ⁻¹
						cm				
						June	July	Aug.	Sep.	
Salisbury	Lloyd clay loam	2017	290	258	58	18.4	11.5	6.5	13	449
		2018	180	199	22	3.6	19.5	10.7	18.1	36
Rocky Mount	Norfolk loamy sand	2017	127	187	42	12.2	15.1	17.6	7.6	64
		2018	.	.	.	7.3	18.1	9.4	13	-
Waynesville	Braddock clay loam	2017	.	.	.	9	3.4	10.8	11.5	-
		2018	40	66	22	6.9	4.8	12.9	11.8	962

[†]Locations and years for which an experiment was not conducted were not sampled for soil analysis and do not have seed yield data and are indicated by a “.”

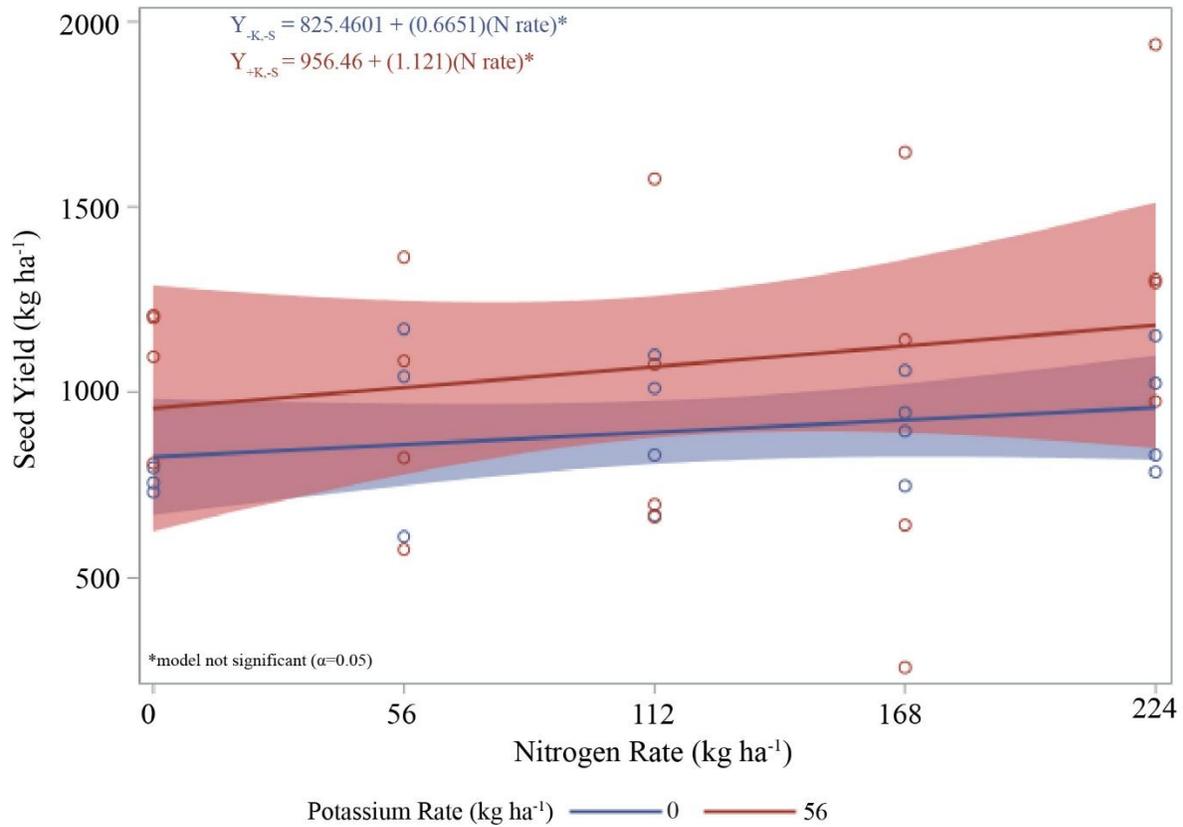


Figure 2-1 Seed yield in response to nitrogen rate (N) at the zero and 56 kg ha⁻¹ (K) rate without the addition of sulfur (S) in Waynesville, NC in 2018. Regression model not significant and slopes of lines not significantly different from zero.

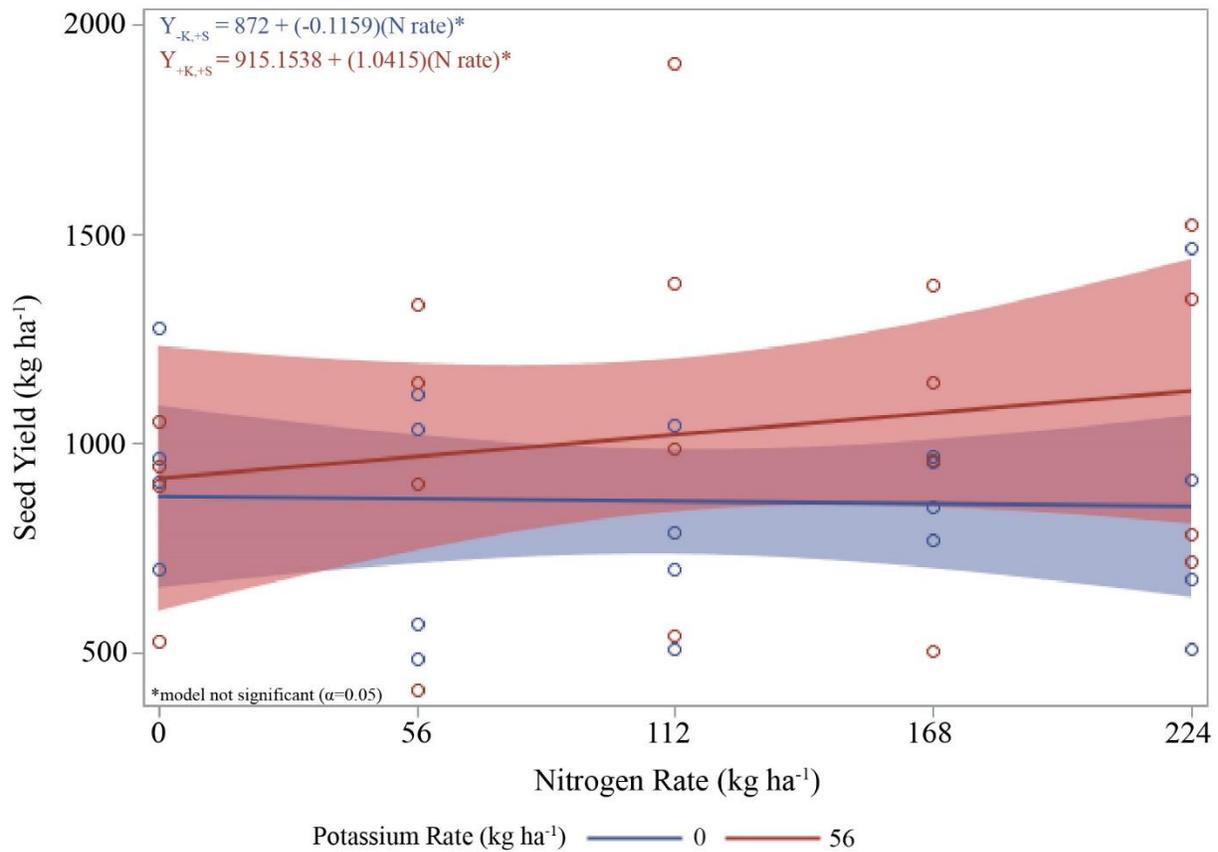


Figure 2-2 Seed yield in response to nitrogen rate (N) at the zero and 56 kg ha⁻¹ potassium (K) rate with the addition of sulfur (S) in Waynesville, NC in 2018. Regression model not significant and slopes of lines not significantly different from zero.

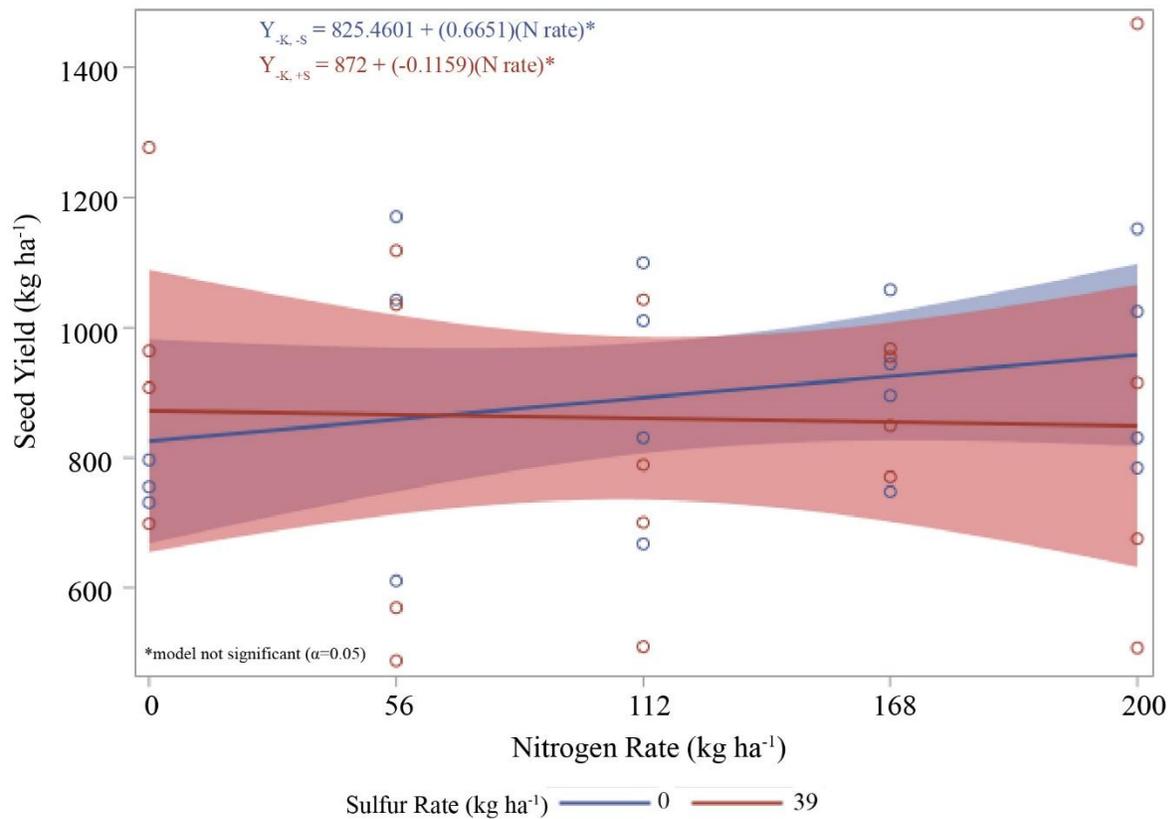


Figure 2-3 Seed yield in response to nitrogen rate (N) at the zero and 39 kg ha⁻¹ sulfur (S) rate without the addition of potassium (K) in Waynesville, NC in 2018. Regression model not significant and slopes of lines not significantly different from zero.

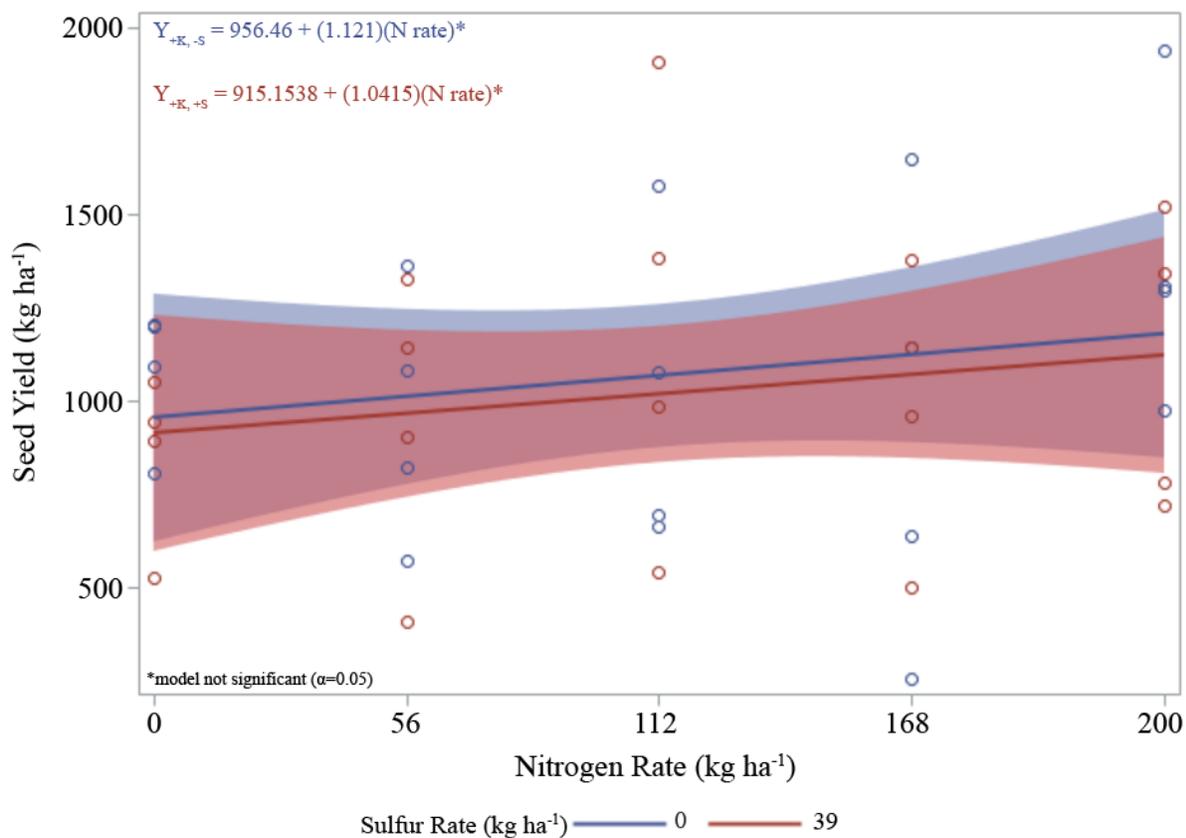


Figure 2-4 Seed yield in response to nitrogen rate (N) at the zero and 39 kg ha⁻¹ sulfur (S) rate with the addition of potassium (K) in Waynesville, NC in 2018. Regression model not significant and slopes of lines not significantly different from zero.