ABSTRACT

GRANDON, BENJAMIN SETH ROSEN. A Survey of Nutrient Levels in Floral Hemp (*Cannabis sativa* L.) Grown for Cannabinoids and the Influence of Leaf Position for Determining Nutrient Deficiencies.

Domestic production of hemp (*Cannabis sativa* L.) has increased dramatically since its legalization under the 2018 Farm Bill that allowed production of hemp varieties producing less than 0.3% delta-9-tetrahydrocannabinol (THC). This recent expansion of the crop has posed several production issues, including the lack of established leaf tissue nutrient guidelines for key growth phases, which allow producers to actively monitor the health of plants based on their ability to absorb nutrients from their environment. This study surveyed 537 plants within North Carolina and Vermont, to measure the nutrient concentrations of the most recently mature leaves from plants during their early and late vegetative growth phases. North Carolina Cooperative Extension Service Agents and an agronomist with the North Carolina Department of Agriculture and Consumer Services collected samples from healthy, floral hemp plantings being produced for their cannabinoid content, predominantly cannabidiol (CBD) and cannabigerol (CBG). Eleven percent of these samples were collected from plant material grown at the University of Vermont. This study examined differences between nutrient demands in young, actively growing plant material (early vegetative) and plants that were further into their development, but still producing vegetative growth (late vegetative). We found that leaf tissue nutrient concentrations varied throughout the plant's life cycle, but differences between early and late season vegetative growth were not large. Typical concentrations were similar to previous leaf tissue nutrient studies, with slight adjustments to be considered for the refinement of those values for the two vegetative growth phases. The most significant of these refinements was within the concentration of calcium in the plant material. These values were greatly diverse and worthy of future study. This survey continued by examining the progression of leaf tissue nutrient concentrations in the upper portion of the actively growing floral hemp plant. By analyzing the variation that exists in the most recently mature leaves, it is possible to determine if there is a risk of misanalysis if the key leaf position is not sampled. This study surveyed most recently mature leaves of the floral hemp plant, focusing on high cannabinoid varieties bred for their high cannabidiol (CBD)/low tetrahydrocannabinol (THC) ratios. Analysis was made in order to determine the relative differences for each of the key plant required nutrients between leaves positioned in the third,

fourth, and fifth nodal position away from the apical meristem of the plant. These samples were taken in the later part of the growing season, prior to transition into flowering. It was concluded that although nutrient levels did vary between leaf positions, it is unlikely that misrepresentation of the plants leaf tissue nutrient concentration would be found, as long as those leaves sampled were within three to five nodes away from the apical meristem.

A Survey of Nutrient Levels in Floral Hemp (*Cannabis sativa* L.) Grown for Cannabinoids and the Influence of Leaf Position for Determining Nutrient Deficiencies

by
Benjamin Seth Rosen Grandon

A thesis submitted to the Graduate Faculty of
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APPROVED BY:

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DEDICATION

This thesis is dedicated to my wonderful wife, Keli L. Grandon, who has supported me continuously throughout my exploration for higher learning. I am truly thankful for her love, patience, wisdom, and support which has enabled me to see the light through the darkness. Additionally, I would like to extend this dedication to my parents, Dr. Jane Rosen-Grandon and Dr. Gary Grandon, who instilled in me a true love for the educational process and a persistent character which has kept me driving forward.

BIOGRAPHY

Benjamin (Ben) Seth Rosen Grandon was born in the summer of 1982 to Jane Rosen-Grandon and Gary Grandon. Moving to Greensboro, North Carolina in 1985, Ben grew up in a small town that has since seen rapid urbanization. As a young man, Ben developed a love of the outdoors and a major appreciation for the complexities of the natural world.

After High School, Ben attended the University of Florida where he received his Bachelor of Science in Horticultural Sciences. After graduation, Ben traveled and worked in a number of locations including, but not limited to, NaanDan Irrigation in Israel, Woody Ranch Orchards in California, the Maryland Democratic Party in Maryland, and New Garden Nursery and Landscape in North Carolina.

Ben continued his education at the University of North Carolina at Greensboro, where he received his Master of Business Administration with a concentration in Finance. After which, Ben joined the North Carolina Cooperative Extension in 2013, where he has worked in Randolph County and Guilford County as an Agricultural Extension Agent.

It is Ben’s goal to remain with the North Carolina Cooperative Extension Service, where he will continue to educate and assist agricultural producers in their understanding of the natural world.

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I would like to begin by acknowledging my wife, family, and friends for their continued encouragement and support throughout my graduate experience. I would not have been able to accomplish this without them.

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Additionally, I would like to extend my gratitude to Brandon Poole with NCDA&CS and all of the participating Cooperative Extension agents who helped collect leaf samples throughout the state. Without your assistance, I would not have been able to access the quantity or quality of data needed for these robust studies. I extend this thanks to the NCDA&CS Plant Tissue Laboratory for assistance in processing samples and organizing data into a palatable form.

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Survey of Leaf Nutrient Levels in Floral Hemp (*Cannabis sativa* L.)

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Abstract:

GRANDON, BENJAMIN SETH ROSEN. Survey of Leaf Nutrients in Floral Hemp (*Cannabis sativa* L.). (Under the direction of Dr. Keith Edmisten)

Domestic production of hemp (*Cannabis sativa* L.) has increased dramatically since its legalization under the 2018 Farm Bill that allowed production of hemp varieties producing less than 0.3% delta-9-tetrahydrocannabinol (THC). This recent expansion of the crop has posed several production issues, including the lack of established leaf tissue nutrient guidelines for key growth phases, which allow producers to actively monitor the health of plants based on their ability to absorb nutrients from their environment. This study surveyed 537 plants within North Carolina and Vermont, to measure the nutrient concentrations of the most recently mature leaves from plants during their early and late vegetative growth phases. North Carolina Cooperative Extension Service Agents and an agronomist with the North Carolina Department of Agriculture and Consumer Services collected samples from healthy, floral hemp plantings being produced for their cannabinoid content, predominantly cannabidiol (CBD) and cannabigerol (CBG). Eleven percent of these samples were collected from plant material grown at the University of Vermont. This study examined differences between nutrient demands in young, actively growing plant material (early vegetative) and plants that were further into their development, but still producing vegetative growth (late vegetative). We found that leaf tissue nutrient concentrations varied throughout the plant’s life cycle, but differences between early and late season vegetative growth were not large. Typical concentrations were similar to previous leaf tissue nutrient studies, with slight adjustments to be considered for the refinement of those values for the two vegetative growth phases. The most significant of these refinements was within the concentration of calcium in the plant material. These values were greatly diverse and worthy of future study. Future studies would also be valuable in determining the sufficiency levels for leaf tissue nutrient levels, measured in coordination with yield and cannabinoid concentrations, which may reveal finer concentration requirements for optimal production. These survey ranges provide a necessary tool for the refinement of the currently established leaf tissue nutrient ranges and the determination of whether leaf nutrient concentrations vary to the extent that differentiation of early and late vegetative sample timing needs to be considered while analyzing leaf nutrient concentration values.

Introduction:

Utilizing leaf tissue nutrient analysis for fine-tuning nutrient applications is common practice in larger farming operations throughout the world. Understanding how plants are actively utilizing nutrients informs growers of those nutrients that may be deficient due to absence or unavailability. Leaf tissue nutrient analysis is based on a survey of healthy plant material to serve as a baseline to compare with samples of a similar crop with unknown health. This survey establishes a range that indicates where nutrient levels are typically found. When comparing an outside sample with this baseline, differences can be seen if there is any level that is dramatically different. Survey nutrient levels only provide information on whether a sample is different from the baseline but not how to make an accurate fertilizer application that might increase overall yield. Sufficiency values provide more information on the ability for an additional level of nutrient to positively affect the yield for the given crop. These values are more heavily focused on production and overall yield versus the baseline of the survey ranges. Creation of survey ranges is the first step to understanding the nutrient levels in leaf tissue and providing information on the plant's ability to access and absorb nutrients from its environment (Bryson and Mills, 2014).

Over recent years, several studies have analyzed leaf tissue nutrient ranges in hemp (*Cannabis sativa* L.). Hemp, as defined by the United States Department of Agriculture is *Cannabis sativa* L. with a delta-9 tetrahydrocannabinol (THC) concentration of not more than 0.3 percent on a dry weight basis (2018). The Plant Analysis Handbook IV (Bryson and Mills, 2014) is a well-known guide for leaf tissue nutrient analysis. This publication reviews both survey and sufficiency ranges for plants ranging from agronomic crops to houseplants. Bryson and Mills (2014) documented a survey range for cannabis that is reportedly from a production nursery (they did not indicate if this was a hemp or high-THC cannabis nursery). The sampling was from 25 mature leaves from new growth taken prior to the flowering cycle. While this text does establish a framework for cannabis, it is not clear how accurate this information is for new varieties of hemp that are currently being grown in North Carolina as high cannabinoid floral varieties, as opposed to grain, fiber, or high-THC varieties (Bryson and Mills, 2014).

Kalinowski et al. (2020) studied floral hemp leaf nutrient levels in plants grown under greenhouse conditions. The study focused on 13 varieties of CBD-dominant hemp plants, used for clonal propagation. Differences were found in the nutrient levels amongst varieties and the authors speculated on nutrient requirements differing based on lineage of the plant based on leaf shape. These can be used to analyze potential differences in greenhouse grown plants compared to those grown in outdoor field environments.

Suchoff et al. (2021) further defined floral hemp leaf tissue nutrient ranges using 6,119 leaf tissue samples sent to the North Carolina Department of Agriculture and Consumer Services (NCDA&CS) Agronomic Division during 2017-2020. Many of the samples were submitted by growers who were problem-solving production issues, and likely included many unhealthy samples.

Utilizing the middle two quartiles of data for the creation and analysis of leaf tissue nutrient ranges has been established in previously published research (Bryson and Mills, 2014; Suchoff et al., 2021). This process eliminates the outer 50% of data on the upper and lower ends in order to reduce the effect of outliers and increase the accuracy of the range.

Floral hemp growth stages have not been assessed in as great of depth as many other agronomic crops. Crops such as corn (*Zea mays L.*), soybeans (*Glycine max L.*), and wheat (*Triticum aestivum L.*) have been studied for decades and the sufficiency levels for nutrients at various growth stages have been determined (Lancashire et al., 1991; Large, E.C. 1954; Robins and Domingo, 1953). As research continues, floral hemp growth stages will be better defined, and recommendations will align with the stage of growth the plant is currently in. *Cannabis sativa* is a short-day crop, referring to the attribute that its initiation into flowering is triggered by a shortening of day length that is naturally observed in the late summer of the year (Chandra et al., 2017). This characteristic has been the focus of modern breeding projects, with the goal of creating lines that are day neutral, which would enter flowering based on plant age versus daylength. (Dowling et al., 2021). This change further emphasizes the need for differentiation between nutrient needs during vegetative growth and the flowering cycle. A uniform decimal code system for plant growth stages developed by Lancashire et al. (1991) was proposed as a universal measure that can be transferred between crops and is inclusive enough to include agronomic crops, flowers, and grasses. This system, which assigns a two-digit code to various growth stages based on developmental changes, may be useful for hemp. Traditional production of agronomic crops focuses on fertility regiments that include preplant fertilizer applications, with subsequent applications made through topdressing with granular or liquid nutrients. Soil testing is typically the best measure for plant available nutrients before crop establishment, while plant tissue testing allows for the analysis of nutrients throughout the growing season. To best fit the needs of the producer, the most critical timing for plant tissue testing in most crops is during active vegetative plant development, before flower initiation, so that additional nutrient applications can be made before deficiencies can negatively affect plant growth during the later stages of development (Havlin et al., 2016).

Determining the proper amount of nutrients needed to maximize production is key to increase profitability and reduce unnecessary fertilizer applications. Liebig's law of the minimum states that plant growth is not simply dictated by total resources available, but by the scarcest nutrient (Brady and Weil, 2007). This makes identifying the scarcest nutrient important for developing healthy plant growth throughout the life of the crop. Plant nutrient tissue testing allows for the continual monitoring of plants, aiding in the detection of potential deficiencies or toxicities.

Using modern fertilization technologies, such as slow-release fertilizers and fertigation, fertilization schedules are developed with the goal of maximizing yield and reducing unneeded fertilizer applications. There are many specialty fertilizers currently marketed for hemp production, but fertilization requirements needed for maximum yield have not been adequately researched. Fertilization calendars, which are typically based on weeks after transplant, focus heavily on nitrogen in the early plant development phases, followed by high levels of phosphorus and low nitrogen applications during the flowering phase. Other specialty fertilizers focus on combination fertilizers such as Calmag, which is predominantly calcium, magnesium, and iron, often chelated to make the elements readily available for plant uptake. Many specialty floral hemp fertilizers were developed for container or hydroponic systems, where micronutrients required for plant growth must be included. These products can also have high levels of silica, microbial inoculants, and sugars, potentially increasing plant growth through the feeding of the soil's microbiology.

Materials and Methods:

Leaf samples from actively growing, healthy appearing, cannabigerol (CBG) and CBD dominant, field and greenhouse grown floral hemp plants were collected to determine the most common levels of plant nutrient concentrations. Samples were collected throughout the growing seasons of 2019 and 2020. The 2019 samples were collected by university and NCDA&CS staff members from research plots on the North Carolina State University Campus in Raleigh, NC, North Carolina Agricultural and Technical State University Campus in Greensboro, NC, and from North Carolina agricultural research stations located across the state. These samples predominately came from variety trials to evaluate the suitability and production levels of floral hemp varieties in North Carolina. Samples from the 2020 growing season were collected by County Cooperative Extension Agents and NCDA&CS Regional Agronomists, both on research stations as well as on private farms participating in the NC Industrial Hemp Pilot Program (North Carolina Department of Agriculture and Consumer Services, n.d.). An additional set of 59 samples, 11% of the total samples, were received during the 2020 growing season from University of Vermont research specialists. Samplers were recruited through a request for involvement that allowed them to receive nutritional information, free of cost to the farmer, with the disclaimer that values should be used only as a base line, as opposed to a sufficiency level that would allow growers to make additional fertilizer applications based on the results. Samplers were given instructions on sampling and submitting samples to the NCDA&CS Agronomic Division plant tissue testing laboratory located in Raleigh, NC. A key instruction was that only healthy plant materials were to be sampled to help ensure that the data represented plants receiving adequate nutrition. The main information requested with these samples was the growth stage that the plants were in during sampling, location of field, and whether the plants were growing in a field or greenhouse. Many of the samples also had corresponding variety names, nitrogen rate, and cultivation method, but this was not consistent for all samples.

A total of 537 floral hemp samples were collected from actively managed field and greenhouse settings. Sampling blocks consisted of 20-30 plants with one most recently mature leaf (MRML) taken per plant. The MRML was noted as typically being found at the fifth leaf growing away from the apical meristem. Once collected, samples were packaged in paper bags and paper shipping materials and sent by mail service to the NCDA&CS Agronomic Division in Raleigh, NC for analysis. Upon receipt, the samples were promptly dried for 12-24 hours at 80 degrees Celsius. Dried samples were homogenized, utilizing a stainless-steel grinder, to the point that the material could pass through a 20-mesh screen. Once homogenized, the samples were stored at room temperature until analyzed. Standard analytical methods for nutrient measurement were implemented according to procedures commonly used by the NCDA&CS plant tissue testing laboratory (North Carolina Department of Agriculture & Consumer Services, 2015).

The goal of this study was to confirm existing leaf tissue nutrient ranges for floral hemp and explore the need for a more detailed set of ranges focused on the plant's growth phase. Cannabis growth phases have not been officially named as of yet, but in this study, they are referred to as early vegetative and late vegetative, with a major distinction being made around the time of the summer solstice, which is believed to represent a major shift in growth behavior for most photoperiod sensitive floral hemp varieties, from vegetative growth to its flowering phase (Chandra et al., 2017). Samples taken at the flowering growth phase were not included because traditional agronomic practices encourage supplemental nutrient applications to be made before the transition to flowering (Bryson and Mills, 2014). The task of differentiating between early vegetative growth and late vegetative growth was given to the sampler as opposed to setting specific dates. This was to ensure that the growth stage best fit into the actual development of the plant material as opposed to an arbitrary determination based on daylength or calendar date. Data were analyzed using Proc Means in the SAS software version 9.4 (SAS Institute, Cary, NC, USA). Similar to the method reported by Bryson and Mills (2014) and Suchoff et al. (2021), a quartiles approach was used to calculate the nutrient survey ranges for each element, which represented the middle 50% of the data collected.

Results and Discussion:

Nitrogen

Bryson and Mills (2014) noted that adequate MRML tissue tests for nitrogen in cannabis are typically between 3.3%-4.8% of dry weight. Suchoff et al. (2021) adjusted those numbers slightly higher to 3.5%-5.0% of dry weight. In this study (Table 1.1), we found that early growth samples (n=316) were 4.49%-5.28% of dry weight while the late vegetative samples (n=221) decreased to 3.77%-4.93% of dry weight. This implied that as plants got closer to vegetative maturity, nitrogen levels within the plant decreased. Even with this decrease in nitrogen levels, we found most of the samples in this study exceeded levels reported by Bryson and Mills (2014), and the lower level of adequate nutrition by Suchoff et al. (2021) of 3.3% and 3.5% of dry weight, respectively. The higher range of this study showed that nitrogen levels in healthy hemp plants exceeded the 5.0% threshold set forth by Suchoff et al. (2021) to an upper level of 5.28% for less mature plant material but would be accurate for those crops nearing vegetative maturity.

Comparing results between years, for both the early and late vegetative growth, provides information that may be helpful in future operations. Nitrogen in late season vegetative growth in the first year (3.36%-4.37%) was lower than that observed from the second year (4.33%-5.49%). This change between years for the nitrogen values was not present in the early season vegetative growth samples (Table 1.2). While both 2019 and 2020 had higher than average rainfall amount (125.3 cm), North Carolina rainfall totals were higher during the second growing season (169.0 cm in 2020) than during the first growing season (129.9 cm in 2019) (National Oceanic and Atmospheric Administration, 2023). This could have affected the plants' ability to absorb nitrogen, resulting in lower nitrogen concentrations within the leaf material.

To examine differences between cultivars of floral hemp, samples of two varieties were isolated and ranges for nutrient concentrations were established (Table 1.3). Cherrywine and Baox were the two most prevalent varieties in this nutrient survey range sampling. Consistently higher leaf tissue ranges within a variety would suggest that the variety may require more nitrogen than other varieties. There was not, however, a dramatic difference in nitrogen levels between Baox, Cherrywine, and other samples in this study in early or late vegetative tissue. Most fell within previously published ranges (Table 1.1) suggesting that producers can utilize similar nitrogen fertilization for different varieties of floral hemp. Comparisons were also made between samples collected within North Carolina and those from Vermont. These ranges were found to be similar, with no significant differences found for nitrogen or any other nutrient, between states (results not shown).

Phosphorus

Phosphorus levels previously described and refined by Bryson and Mills (2014), and Suchoff et al. (2021) were very similar to those collected for this study (Table 1.1) and ranged from 0.30%-

0.46%. of dry weight. Vegetative stage had no effect. The phosphorus levels observed were also consistent between floral hemp varieties as well as between the two years of sampling (Table 1.3).

Potassium

Potassium levels in this study were very similar to those reported in previous studies (Table 1.1). The similarities of these studies, especially on the lower end of the range, indicate the lower level of potassium required for adequate plant growth. Excessive potassium levels are not as concerning to agricultural operations, but it is important to recognize when additional applications of potassium may not be readily absorbed by the plant, negating the need for excessive potassium, and reducing the overall cost of fertilizer applications (Havlin et al., 2016). Many plants have the tendency to absorb more potassium than is typically needed for adequate growth. This luxury consumption causes issues with soil fertility and typically results in a greater need for potassium fertilization in future crops in that soil (Brady and Weil, 2007).

Calcium

In this study, the lower range of calcium found in plant samples was elevated compared to the studies performed by Bryson and Mills (2014) and Suchoff et al. (2021) (Table 1.1). This study found calcium levels in early vegetative growth were between 2.03%-5.21% of dry weight, with late season vegetative growth ranging from 2.1%-6.9% of dry weight. These numbers contrast with Bryson and Mills (2014) who documented levels for cannabis at 1.5%-4.4% of dry weight and Suchoff et al. (2021) who defined those levels as 1.5%-2.9% dry weight. Based on these results, adequate calcium is between 1.5% -2% of dry weight, while it is possible to see much higher levels without observable toxicity.

Calcium concentrations were the most variable of all the nutrients studied in this survey. Calcium is critical to soil health and is often added as lime (calcium oxides and calcium hydroxides) at high levels to adjust soil pH (Havlin et al., 2016). This recommended practice could lead to higher tissue levels that may or may not be vital to healthy growth of the cannabis plant. In many of the survey samples, calcium was found at higher concentrations in the late vegetative samples. This is consistent with the manner that calcium is absorbed and translocated throughout the plant (White and Broadly, 2003). It is unknown what effect these higher calcium levels might have on final crop yield and final product quality.

Magnesium

Magnesium survey ranges established by Bryson and Mills (2014) were set between 0.4%-0.81% of dry weight. Suchoff et al. (2021) reduced this range to 0.3%-0.65% of dry weight (Table 1.1). This study found very similar levels in early season vegetative growth samples at 0.29%-0.65% of dry weight, but our late season vegetative growth survey ranges found higher rates of magnesium at 0.45%-.90% of dry weight.

Sulfur, Iron, Manganese, Zinc, Copper, and Boron

Sulfur ranges were fairly consistent with previously published values (Tables 1.1-1.3) The micronutrients examined in this survey also did not vary greatly from the existing nutrient ranges set by Bryson and Mills (2014) or Suchoff et al. (2021) (Table 1.4-1.6). With the exception of copper, the micronutrients were found in extremely low concentrations and did not vary greatly throughout the samplings. Copper, however, was much higher in the second year than the first year (Table 1.5). Micronutrients are vital to productive plant growth and samples found outside of the determined values could be at risk of deficiency or toxicity, compromising key biomechanical actions within the plant (Havlin et al., 2016).

Conclusions:

This plant tissue nutrient survey served to increase the knowledge base of nutrient levels within healthy, actively growing floral hemp plants. Through the separation of samples into early and late vegetative growth, information is provided on how leaf tissue nutrient concentrations vary throughout the vegetative portion of the plant’s lifecycle. This study found small differences between these growth phases that could be used to better understand the true nutrient needs for the plant based on its developmental phase. Future studies will be needed to further refine these values, possibly factoring in variables such as yield response, fertilizer application schedules, fertilizer formulations, and precipitation to develop sufficiency ranges to create fertilizer recommendations.

Table 1.1. *Cannabis sativa* L. leaf macronutrient ranges. Current study (Grandon) compared to prior published research.

Reference Ranges	Nitrogen	Phosphorus	Potassium	Calcium	Magnesium	Sulfur
	(% of dry matter)					
Bryson and Mills (2014) [A]	3.3-4.8	0.24-0.49	1.8-2.4	1.5-4.4	0.4-0.81	0.17-0.26
Kalinowski et al. (2020) [B]	3.3-5.0	0.26-0.43	2.1-3.4	1.5-5.3	0.35-0.70	0.25-0.37
Suchoff et al. (2021) [C]	3.5-5.0	0.27-0.48	1.8-2.7	1.5-2.9	0.30-0.65	0.25-0.36
Grandon, Early Vegetative (n=316) [D]	4.49-5.28	0.32-0.46	1.98-2.81	2.03-5.21	0.29-0.65	0.27-0.34
Grandon, Late Vegetative (n=221) [D]	3.77-4.93	0.30-0.41	1.92-2.62	2.1-6.9	0.45-0.90	0.28-0.34

A: Bryson and Mills (2014) leaf nutrient range established from 25 unspecified *Cannabis sativa* plants in a production nursery.
B: Kalinowski et al. (2020) leaf nutrient range established from 13 cultivars of greenhouse grown floral hemp.
C: Suchoff et al. (2021) leaf nutrient range established from a survey of 6,119 *Cannabis sativa* plants submitted to the North Carolina Department of Agriculture and Consumer Services Agronomic Division between 2017-2020.
D: Data combined over years. All ranges represent the middle two quartiles of data.

Table 1.2. *Cannabis sativa* L. leaf macronutrient ranges by year and growth stage.

Year	Nitrogen	Phosphorus	Potassium	Calcium	Magnesium	Sulfur
	(% of dry matter)					
Year 1: Early Vegetative Growth Stage (n=56)	4.9-5.19	0.46-0.58	2.89-3.3	4.9-5.98	0.67-0.84	0.31-0.36
Year 2: Early Vegetative Growth Stage (n=260)	4.23-5.33	0.3-0.41	1.94-2.56	1.84-4.52	0.26-0.51	0.26-0.32
Year 1: Late Vegetative Growth Stage (n=104)	3.36-4.37	0.31-0.40	1.46-2.58	2.64-8.43	0.48-1.24	0.28-0.32
Year 2: Late Vegetative Growth Stage (n=117)	4.33-5.49	0.3-0.43	2.19-2.65	1.96-3.31	0.44-0.68	0.28-0.36

All ranges represent the middle two quartiles of data.

Table 1.3. *Cannabis sativa* L. leaf macronutrient ranges by variety.

Variety, Growth Stage	Nitrogen	Phosphorus	Potassium	Calcium	Magnesium	Sulfur
	(% of dry matter)					
Baox, Early Vegetative (n=31)	4.15-5.52	0.34-0.45	2.06-2.67	1.36-2.48	0.36-0.74	0.25-0.38
Baox, Late Vegetative (n=6)	4.19-5.45	0.31-.37	2.3-3.22	2.1-7.1	0.42-0.59	0.29-0.32
Cherrywine, Early Vegetative (n=8)	4.53-5.67	0.31-0.39	2.38-2.62	1.9-3.0	0.49-0.98	0.28-0.40
Cherrywine, Late Vegetative (n=4)	3.5-5.16	0.37-0.39	2.4-2.88	1.84-3.1	0.39-0.51	0.25-0.35
All Other Varieties, Early Vegetative (n=277)	4.51-5.22	0.31-0.35	2.0-2.83	2.1-5.33	0.28-0.61	0.27-0.33
All Other Varieties, Late Vegetative (n=211)	3.74-4.93	0.3-0.42	1.87-2.62	2.1-6.9	0.46-0.96	0.28-0.34

Data combined over years. All ranges represent the middle two quartiles of data.

Table 1.4. *Cannabis sativa* L. leaf micronutrient ranges. Current study (Grandon) compared to prior published research.

Reference Ranges	Iron	Manganese	Zinc	Copper	Boron
	(ppm)				
Bryson and Mills (2014) [A]	100-150	41-93	24-52	5.0-7.1	56-105
Kalinowski et al. (2020) [B]	84-169	67-264	26-55	1.6-7.0	26-91
Suchoff et al. (2021) [C]	70-150	40-158	33-60	5-11	30-90
Grandon, Early Vegetative (n=316) [D]	91-136	44.5-136.5	30-54	6.2-11	32-48
Grandon, Late Vegetative (n=221) [D]	101-144	91-215	36-56	3.7-10.1	36-86

A: Bryson and Mills (2014) leaf nutrient range established from 25 unspecified *Cannabis sativa* plants in a production nursery.
B: Kalinowski et al. (2020) leaf nutrient range established from 13 cultivars of greenhouse grown floral hemp.
C: Suchoff et al. (2021) leaf nutrient range established from a survey of 6,119 *Cannabis sativa* plants submitted to the North Carolina Department of Agriculture and Consumer Services Agronomic Division between 2017-2020.
D: Data combined over years. All ranges represent the middle two quartiles of data.

Table 1.5. *Cannabis sativa* L. leaf micronutrient ranges by year and growth stage.

Year	Iron	Manganese	Zinc	Copper	Boron
	(ppm)				
Year 1: Early Vegetative Growth Stage (n=56)	77-113	138-202	52-65	1.98-2.75	38-53
Year 2: Early Vegetative Growth Stage (n=260)	92-141	42-97	29-47	7.7-11.4	31-48
Year 1: Late Vegetative Growth Stage (n=104)	105-131	128-262	39-59	2.77-4.51	41-131
Year 2: Late Vegetative Growth Stage (n=117)	98-153	56-162	33-50	8.6-11.5	33-47

All ranges represent the middle two quartiles of data.

Table 1.6. *Cannabis sativa* L. leaf micronutrient ranges by variety.

Variety, Growth Stage	Iron	Manganese	Zinc	Copper	Boron
	(ppm)				
Baox, Early Vegetative (n=31)	94-132	42-128	31-63	8.4-13	30-63
Baox, Late Vegetative (n=6)	107-137	96-308	33.5-39	7.9-10.6	32-45
Cherrywine, Early Vegetative (n=8)	103-142	57-92	38-46	10-15	27-51
Cherrywine, Late Vegetative (n=4)	65-122	47-90	40-50	9-14	37-45
All Other Varieties, Early Vegetative (n=277)	89-136	45-137	30-54	6.9-10.7	32-48
All Other Varieties, Late Vegetative (n=211)	102-148	93-216	36-58	3.6-10	36-90

Data combined over years. All ranges represent the middle two quartiles of data.

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Determining Optimum Leaf Position for Nutrient Analysis in Floral Hemp (*Cannabis sativa* L.)

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Abstract:

GRANDON, BENJAMIN SETH ROSEN. Determining Optimum Leaf Position for Nutrient Analysis in Floral Hemp (*Cannabis sativa* L.). (Under the direction of Dr. Keith Edmisten)

Leaf tissue nutrient analysis is a crucial tool in understanding the ability for a plant to absorb nutrients from its environment. Since the introduction of hemp (*Cannabis sativa* L.) as a legal crop in North Carolina, basic agronomic research is needed to understand the optimal growing conditions required for maximum production. This study examined the progression of leaf tissue nutrient concentrations in the upper portion of the actively growing floral hemp plant. By analyzing the variation that exists in the most recently mature leaves, it is possible to determine if there is a risk of misanalysis if the key leaf position is not sampled. This study surveyed most recently mature leaves of the floral hemp plant, focusing on high cannabinoid varieties bred for their high cannabidiol (CBD)/low tetrahydrocannabinol (THC) ratios. Analysis was made in order to determine the relative differences for each of the key plant required nutrients between leaves positioned in the third, fourth, and fifth nodal position away from the apical meristem of the plant. These samples were taken in the later part of the growing season, prior to transition into flowering. It was concluded that although nutrient levels did vary between leaf positions, it is unlikely that misrepresentation of the plants leaf tissue nutrient concentration would be found, as long as those leaves sampled were within three to five nodes away from the apical meristem.

Introduction:

The 2018, the United States Farm Bill created a framework for the legal production of hemp (*Cannabis sativa* L.*)* and defined hemp as *Cannabis sativa* which contains less than 0.3% delta-9 tetrahydrocannabinol (delta-9 THC) (United States Dept. of Agriculture, 2018). There are three types of hemp; fiber, seed, and floral. They have different growth habits and consumer end products. Floral hemp is grown primarily as a source of various cannabinoids, which can be extracted from foliage and floral structures. With the recent reintroduction of hemp to the modern agricultural world, many agronomic related details about how this plant reacts to its environment are yet to be determined. Optimizing soil fertility and nutrient application are one of the main tools used by modern agriculture to produce a high quality and quantity of material, while maintaining profits and mitigating negative environmental effects. As with any newly introduced crop, hemp has a severe lack of scientifically produced production knowledge and farmers have been left estimating the overall fertility needs of their hemp plantings.

According to Bryson and Mills (2014), it is vital to analyze the appropriate leaf material to properly estimate the nutrients that may be in deficiency or overabundance. As young leaves expand, they accumulate nutrients taken up by the plant from the soil and redistributed from other plant portions. As leaves age, they are used as storage organs that can redistribute nutrients to the actively growing portions of the plant to reduce the sensitivity of the plant to deficiencies that may be present in the soil. This shift of nutrient concentrations can create confusion over the true levels of nutrients that are available to the plant (Taiz and Zeiger, 2003). Consistently focusing on a specific leaf position when nutrient sampling is key to receiving meaningful results that can be accurately compared to established nutrient guidelines.

The most recently matured leaf (MRML) is often used as the standard plant portion sampled for leaf nutrient testing in floral hemp crops. The practice of using the MRML for tissue testing in floral hemp was established by Bryson and Mills (2014) and continued with Suchoff et al. (2021). The use of the MRML for nutrient tissue sampling is common for many other crops including rice (*Oryza sativa* L.), soybeans (*Glycine max* L.), pinto beans (*Phaseolus vulgaris* L.), lemons (*Citrus x limon* L.), and oranges (*Citrus x sinesis* L.) (Bryson and Mills, 2014; Cerda et al., 1995; Chapman and Brown, 1950).

The objective of this survey was to determine if the MRML in leaf position four is the appropriate leaf position to be collected for plant nutrient tissue testing in floral hemp.

Methods and Materials:

For this study, MRML's were collected from named varieties of floral hemp plants, ranging in position from three, four, and five nodes away from the apical meristem. In 2019, samples were collected to survey floral hemp plants within university variety trials at the Mountain Horticultural Crops Research and Extension Center in Mills River, NC and the Piedmont Research Station in Salisbury, NC. In 2020, samples were collected at the Central Crops Research Station in Clayton, North Carolina from floral hemp plants planted for use in a post-harvest drying study. Fertilizers were applied based on soil tests and North Carolina Department of Agriculture and Consumer Services (NCDA&CS) fertility recommendations for floral hemp (McGinnis and Suchoff, 2020).

Within each of the varieties surveyed, plants were in groups of five in 2019 and groups of 15 in 2020. Samples of leaf positions three, four, and five were separately collected and stored in paper bags before transport to the NCDA&CS Plant Tissue Testing laboratory in Raleigh, NC. Samples were dried, homogenized, and ground to pass through a 20-mesh screen. Dried samples were then processed and analyzed according to NCDA&CS tissue testing protocols (NCDA&CS, 2015). Samples were collected between late July and early August of each year, placing them in the late vegetative growth stage (Grandon, 2023).

In 2019, eight varieties were surveyed at the Piedmont Research Station. These were Baox, Cherryblossom, Cherrywine, Electra, Endurance, Suver Haze, Sweeten, and T1. At the Mountain Horticultural Crops Research and Extension Center, Baox, Cherrywine, Suver Haze and Sweeten were sampled. Two samples of Cherrywine were submitted from each location due to the large amount of plant material available during the sampling events. Leaf position samples were collected from 14 groups of 5 plants. This resulted in 42 individual samples, with 14 originating from each of the sampled leaf positions. Samples from leaf positions were all taken from the same group of plants so that differences, if present, between leaf positions could be accurately analyzed. Since the required amount of leaf material was not able to be taken from a single plant, direct analysis of the nutrients within a plant was not analyzed but leaves within plant groups were compared.

In 2020, two varieties, Cherrywine and Baox, were sampled at the Central Crops Research Station located in Clayton, NC. During the second year of study, replications within varieties were also employed to ensure that the results were accurate and transferable throughout the study. Within each variety, leaf position samples were collected from four groups of 15 plants. This resulted in 24 unique samples, with 8 samples of each leaf position, across two varieties of floral hemp, utilizing approximately 120 individual plants. Leaves that were damaged by environmental or biological factors were avoided, focusing on healthy, intact samples.

Data were analyzed through the Means procedure in the SAS software version 9.4 (SAS institute, Cary, NC, USA) which allowed for comparison with the sample ranges found in previously published studies (Bryson and Mills, 2014; Suchoff et al., 2021). For these data, the two middle quartiles were used to create a range which represented 50% of the data set. Removal of the outer two quartiles allowed for the elimination of outliers that could potentially skew the resulting nutrient ranges. This practice is consistent with previous studies focused on cannabis leaf tissue nutrient concentrations and ranges (Bryson and Mills, 2014; Suchoff et al., 2021).

Results and Discussion:

The emphasis of this study focused on the ranges found within the sampling years as well as on two varieties (Baox and Cherrywine) that were sampled over the two-year period. Analyzing the ranges that were found for these data sets, allowed for the determination on whether the fourth position most accurately fit the sample as well as if the results could be skewed due to the collection of slightly older or younger leaf material.

Nitrogen and Phosphorus

The result from this survey showed that both nitrogen and phosphorus levels gradually decreased as the leaf position extended further from the apical meristem (Table 2.1). This reduction in nitrogen and phosphorus was consistent with current understanding of plant physiology and nutrient distributions found in other agronomically sampled crops (Bryson and Mills, 2014; Lancashire et al., 1991; Large et al., 1953). The nitrogen concentrations were consistently higher than the minimum thresholds established in our earlier floral hemp leaf tissue survey (Grandon, 2023), but phosphorus levels in the older leaf material were found to be slightly lower, with younger leaves still within previously published ranges (Table 2.1). The range of phosphorus levels within the sampled leaves was greatly varied, with many of the leaves in position three containing almost double the phosphorus concentration of those leaves in position five.

Nitrogen levels between the two years did differ, with the second year’s levels exceeding those levels reported in the earlier floral hemp nutrient survey (Grandon, 2023). Although there were higher levels found in the second year of testing (Table 2.1), the results between leaf positions were consistently high and their range can be seen as normal. It was also found that differences between varieties were noticeable, with Baox having consistently higher ranges for all leaf positions.

As with nitrogen, results for phosphorus in the first year of testing were distinctly lower than those samples taken in the second year of the study (Table 2.1). Phosphorus levels in the second year of testing fit well with the nutrient survey data from our earlier study (Grandon, 2023), while the first year's data were slightly below those levels observed. While the variation between years was distinct, the variation between leaves was observed to be very low, indicating an insignificant variation of nutrient levels between most recently mature leaves within positions three, four, and five.

This study also showed a strong distinction between the two varieties tested. Baox nitrogen and phosphorus concentrations were higher than those found in Cherrywine (Table 2.1), potentially signaling the greater need or ability for certain varieties to access and absorb nutrients. This is in contrast to what we found in our earlier floral hemp nutrient survey (Grandon, 2023), indicating that further study is needed.

Potassium

In year one, potassium levels decreased slightly from leaf position three to five (Table 2.1). This decrease was not as dramatic as those found with the other macronutrients, but it was still noticeable. Leaf positions three and four were very similar to each other in year one, while overall levels of potassium in position five began to reach the minimum levels found in previously published leaf tissue nutrient ranges (Bryson and Mills, 2014; Suchoff et al., 2021). Potassium levels in the second year were higher than those in year one (Table 2.1) but were consistent across the three leaf positions. Potassium leaf tissue samples also differed between the two varieties, with Baox having higher levels than Cherrywine.

Calcium and Magnesium

Calcium and magnesium levels constantly increased as leaf position extended further away from the apical meristem (Table 2.1). This increase of nutrient level was dramatic but would not likely lead to misanalysis due to the large survey range established in previously published nutrient surveys (Bryson and Mills, 2014; Grandon, 2023; Suchoff et al., 2021).

Samples taken in the first year of the study were consistently lower in calcium and magnesium than those taken in the second year. This is consistent with the other nutrients found in this study.

Differences between varieties were less noticeable with calcium and magnesium as opposed to the other nutrients discussed. Baox and Cherrywine were able to absorb comparable levels of calcium and magnesium, showing a consistent increase in leaf concentrations as the position extended further away from the apical meristem.

Sulfur, Iron, and Zinc

Although there were differences between the sampling years and the varieties sampled, the overall change in leaf tissue nutrient concentrations for sulfur, iron, and zinc were minimal across the sampled leaf positions (Table 2.1 and 2.2). These consistent nutrient levels among leaf positions shows that there is very low risk of misanalysis between leaf positions three, four and five away from the apical meristem.

As with other nutrients examined in this study, nutrient levels for sulfur, iron, and zinc were consistently higher in the Baox samples compared to the Cherrywine samples. This consistency shows further evidence that the Baox variety was able to absorb a higher level of these nutrients than those plants in the Cherrywine variety.

Copper and Boron

Copper levels decreased slightly as the leaf position increased away from the apical meristem, whereas boron levels increased (Table 2.2). The ranges found for copper were consistently higher than previously established ranges, while those for boron were found to be below those levels previously established as minimum concentrations for healthy growth. Copper and boron are both critical to the proper function of the plant but are both found in very low concentrations within the leaf material. Boron in particular, appears to be highly variable from survey to survey.

Copper and boron concentrations were consistently higher in the Baox variety as opposed to Cherrywine. This increase in nutrient concentration was consistent with other sampled nutrients.

Conclusion:

This study clearly shows that nutrient levels between commonly sampled most recently mature leaves in floral hemp are similar in their nutrient concentration between the leaf positions three, four, and five nodes away from the apical meristem. These similarities are consistent amongst individual plant required nutrients, with small variations which coincide with documented leaf nutrient allocation and nutrient movement commonly observed as leaves age. Variation between leaves would reveal that potential misanalysis could take place if the sampled leaf position were to vary greatly from those used to create the leaf tissue nutrient guidelines, primarily leaf position four. Nutrient levels remained consistent between leaf positions for most of the plant required nutrients, but phosphorus, calcium, magnesium, and manganese had larger ranges, which could lead to misanalysis. This study solidifies the theory that leaves within the positions three, four, and five can be utilized as the most recent mature leaf without the risk of misanalysis, potentially corrupting the validity of the leaf nutrient tissue test.

Differences were identified between the leaf nutrient concentrations for the two prominent varieties used in this study. Baox samples consistently had higher nutrient concentrations than those of the Cherrywine variety, indicating that Baox has higher nutrient demands than Cherrywine. This difference between varieties was not found in our previous leaf nutrient survey (Grandon, 2023) but is clear in this study. Changes in the nutrient demand could affect overall production and health of a crop, but future research is necessary to determine if this larger nutrient concentration has any effect on the overall yield of those varieties.

Variations were also found between the sampling years, which lead to the observation that annual weather conditions or other outside factors may also affect the overall ability for the floral hemp plant to properly absorb nutrients. This variation should be researched in greater detail to properly identify other factors, such as precipitation, that may affect the leaf tissue nutrient concentration.

As future research on floral hemp production continues, the creation of sufficiency ranges, which bases nutrient recommendations on potential yield gain, would greatly increase the understanding of how the floral hemp plant utilizes nutrients to increase flower production and overall yield.

Table 2.1. Essential macronutrients by leaf position in floral hemp (*Cannabis sativa* L.).

Nutrient	Leaf Position	Bryson & Mills (2014) [A]	Suchoff et al. (2021) [B]	Grandon Late Vegetative (2023) [C]	Year 2019 (14 samples per leaf position) [D]	Year 2020 (8 samples per leaf position) [E]	Baox 2019-2020 (6 samples per leaf position) [F]	Cherrywine 2019-2020 (8 samples per leaf position) [G]
		(% of dry weight)						
Nitrogen	3				4.18-4.83	5.85-6.94	5.39-7.0	4.28-5.85
	4	3.3-4.8	3.5-5.0	3.8-4.9	3.95-4.57	5.67-6.74	5.43-6.77	4.06-5.67
	5				3.83-4.46	5.47-6.46	4.94-6.53	4.14-5.47
Phosphorus	3				0.29-0.41	0.45-0.56	0.52-0.57	0.36-0.47
	4	0.24-0.49	0.27-0.48	0.30-0.41	0.23-0.33	0.37-0.44	0.44-0.44	0.30-0.41
	5				0.21-0.28	0.32-0.36	0.34-0.37	0.26-0.32
Potassium	3				1.96-2.37	2.52-2.69	2.37-2.75	1.95-2.52
	4	1.8-2.4	1.8-2.7	1.9-2.6	1.86-2.32	2.62-2.81	2.25-2.86	1.78-2.62
	5				1.77-2.27	2.51-2.90	2.36-2.99	1.73-2.62
Calcium	3				1.33-1.95	1.82-2.17	1.62-1.95	1.46-2.17
	4	1.5-4.4	1.5-2.9	2.1-6.9	1.61-2.26	2.38-2.81	2.23-2.63	1.70-2.81
	5				1.83-2.67	3.05-3.57	2.62-3.48	1.90-3.50
Magnesium	3				0.31-0.43	0.68-0.77	0.44-0.69	0.36-0.76
	4	0.4-0.81	0.30-0.65	0.45-0.90	0.34-0.49	0.85-1.01	0.53-0.88	0.40-0.98
	5				0.39-0.58	1.06-1.18	0.58-1.12	0.43-1.18
Sulfur	3				0.21-0.27	0.41-0.45	0.27-0.45	0.22-0.41
	4	0.17-0.26	0.25-0.36	0.28-0.34	0.19-0.24	0.40-0.45	0.25-0.45	0.21-0.40
	5				0.19-0.23	0.40-0.43	0.24-0.43	0.21-0.40

A: Bryson and Mills (2014) leaf nutrient range established from 25 unspecified *Cannabis sativa* plants in a production nursery.

B: Suchoff et al. (2021) leaf nutrient range established from a survey of 6,119 *Cannabis sativa* plants submitted to the North Carolina Department of Agriculture and Consumer Services Agronomic Division between 2017-2020.

C: Grandon (2023) Late Vegetative range established from 537 healthy, vegetatively growing, floral hemp samples between 2019-2020.

D: Combined means of all varieties sampled from Piedmont Research Station and Mountain Horticultural Crops Research and Extension Center; Leaf position samples were collected from 14 groups of 5 plants.

E: Combined means of all varieties sampled from Central Crops Research Station; Leaf position samples were collected from eight groups of 15 plants.

F: Combination of two samples from 2019 originating from two groups of five plants and four samples from 2020 originating from four groups of 15 plants.

G: Combination of four samples from 2019 originating from four groups of five plants and four samples from 2020 originating from four groups of 15 plants.

All ranges represent the middle two quartiles of data.

Table 2.2. Essential micronutrients by leaf position in floral hemp (*Cannabis sativa* L.).

Nutrient	Leaf Position	Bryson & Mills (2014) A	Suchoff et al. (2021) B	Grandon Late Vegetative (2023) C	Year 2019 (14 samples per leaf position) D	Year 2020 (8 samples per leaf position) E	Baox 2019-2020 (6 samples per leaf position) F	Cherrywine 2019-2020 (8 samples per leaf position) G
		(ppm of dry weight)						
Manganese	3				29.0-44.2	72.4-90.2	52.0-92.2	37.0-72.4
	4	41-93	40-158	91-215	28.0-52.9	86.9-121.7	63.6-125.8	36.8-86.9
	5				29.0-50.6	91.8-149.8	50.6-153.3	38.9-91.8
Iron	3				86.0-118.0	123.8-134.8	125.0-133.3	87.5-126.4
	4	100-150	70-150	101-143	82.3-107.0	124.0-133.8	125.1-130.1	89.5-130.2
	5				87.0-108.0	123.1-137.3	116.0-132.1	94.5-137.3
Zinc	3				31.9-37.2	53.3-81.7	41.4-83.9	35.4-53.3
	4	24-52	33-60	36-56	28.3-33.0	44.2-76.8	37.6-81.2	30.8-44.2
	5				24.8-32.1	39.9-65.7	32.1-67.8	27.5-39.9
Copper	3				12.9-17.7	15.7-18.4	17.9-19.8	14.0-16.0
	4	5.0-7.1	5-11	3.7-10.1	11.7-15.1	14.6-17.4	16.4-18.5	12.0-14.9
	5				10.2-13.9	12.8-14.1	13.4-14.8	10.6-13.1
Boron	3				19.1-28.3	26.2-31.6	31.4-31.9	19.9-27.8
	4	56-105	30-90	36-86	21.7-32.9	27.0-34.7	33.8-37.0	19.8-31.2
	5				23.7-35.4	28.4-37.8	33.3-38.8	20.5-30.3

A: Bryson and Mills (2014) leaf nutrient range established from 25 unspecified *Cannabis sativa* plants in a production nursery.

B: Suchoff et al. (2021) leaf nutrient range established from a survey of 6,119 *Cannabis sativa* plants submitted to the North Carolina Department of Agriculture and Consumer Services Agronomic Division between 2017-2020.

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