Industrial hemp agronomic management for grain, fiber, and forage

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ABSTRACT (ACADEMIC)

This research involved testing several aspects of industrial hemp (*Cannabis sativa* L.) production, including the impact of tillage on seed and fiber production, optimal harvest time for seed yield and quality, the response of seed yield to nitrogen fertility rates, and the potential of hemp as a forage crop.

A three-year study was conducted in Blacksburg and Orange of Virginia State to assess the effects of tillage management and production systems (e.g., seed, dual, and fiber) on hemp establishment and productivity. Two cultivars, Joey (a dual-purpose variety) and EcoFibre (bred specifically for fiber), were planted into seedbeds prepared with conventional tillage and no-till management. The cultivar Joey, lower plant populations under seed production systems resulted in taller plants ($P = 0.0002$) compared to the dual-purpose production systems in 2020. Greater plant heights ($P < 0.0001$) with fiber production systems in 2021 and 2022 were due to differences between cultivars and their time of flowering.

Conventional tillage resulted in greater ($P \leq 0.0161$) plant populations than no-tillage for all production systems in each year, and this response was more pronounced with fiber management in 2020 (tillage × production systems interaction; $P = 0.0007$). Greater ($P < 0.001$) yields with fiber systems observed in 2021 and 2022 were largely driven by the more productive EcoFibre cultivar. Despite treatment differences in population density, biomass and seed yields varied less by tillage management and production systems. Lower plant population density was associated with greater biomass and seed yields per plant. However, for desired fiber quality and mechanical harvest feasibility, a higher plant population density is recommended.

A second study aimed to determine the optimum harvest time for seed yield of two hemp cultivars. ‘Joey’, and ‘Grandi,’, were established in Blacksburg and Orange, Virginia in mid-May/early June of 2021 and 2022. The experiment was conducted as a randomized complete block design with a repeated measurement arrangement and four replicates. Plants were harvested four times at one-week intervals starting in mid-summer. Harvest date significantly affected seed yield, with the response differing by cultivar (cultivar × date interaction; $P = 0.001$) in 2022 at the Orange site. In Blacksburg, seed yields were similar for the two cultivars and greatest at the second harvest each season (July 22, 2021, and July 25, 2022), although they were substantially lower in 2022 due to drought (1750 vs. 480 kg ha^{-1}; $P < 0.0001$). In Orange, in 2021, as planting occurred late, harvests were also deferred until August 17, and seed yields were greatest at this first harvest (1180 kg ha^{-1}; $P<0.0001$). In 2022, yields at the Orange location were highest for Grandi at the first harvest (July 21; 1510 kg ha^{-1}) and for Joey at the second harvest (July 28; 1280 kg ha^{-1}) (Harvest Time by Cultivar interaction, $P = 0.0010$). Over the subsequent weeks of harvest, yields drastically declined (16 to 41% in 2021 and 27 to 47% in 2022 in Blacksburg; 52% to 91% in 2021 and 28% to 65% in 2022 in Orange, compared to the highest yield). Harvest timing is critical to achieving optimum seed yield, and it varies with cultivar, eco-physiographic location, and weather (e.g., rainfall). Fatty acids (FA) varied by cultivar, location, and harvest timing, but patterns of response were not consistent across FA. Gamma-linolenic ($P \leq 0.002$) and oleic acids ($P \leq 0.023$) were generally greater in Joey, with greater arachidic acid ($P \leq 0.013$) concentrations in Grandi. Stearidonic acid concentrations declined with later harvest date in Orange location ($P \leq 0.0034$).

A third study aimed to measure hemp's response to different N rates and to determine the ability to predict plant N content and seed yield based on UAV-based multispectral imagery. Two hemp cultivars, 'Joey' and 'Grandi', were planted and five N rates (0, 60, 120, 180, 240 kg N ha^{-1}) were tested in Blacksburg, Virginia in 2020, 2021, 2022. Aerial image acquisition occurred at three different growth stages in 2021 using dji M 300 drones mounted with multispectral sensors. Red/Blue index (R^2=0.89), near-infrared (NIR) band (R^2=0.84) and Enhanced vegetation index (EVI) (R^2=0.81) were better predictors of N content in leaf samples than other vegetation indices that were evaluated. Green normalized difference vegetation index (GNDVI) was the better predictor of hemp seed yield (R^2=0.58) than other evaluated vegetation indices. The seed yield of hemp was influenced ($P \leq 0.0177$) by the N input in all three experimental years. In 2020, seed yield did not increase steadily with the increase of N rate; the highest seed yield, 1640 kg ha^{-1}, was observed at 120 kg N ha^{-1}. In 2021, maximum seed yield of 2500 kg ha^{-1} occurred at the maximum N rate (240 kg N ha^{-1}). In 2022, a weak response to N rate was observed; maximum seed yield was 380 kg $ha^{-1,}$ again at 240 kg N ha^{-1}. The overall growth of the hemp plants was affected by limited rainfall and weed pressures in 2022, leading to a significant reduction in seed yield. Response to N rate will vary depending on other factors such as available soil moisture during the growing season, weed pressure, and growing period.

A fourth study examined the yield and nutritive value of three hemp cultivars, 'Grandi', 'Joey', and 'EcoFibre' as potential forage crops when harvested at weekly intervals in Blacksburg, VA. The greatest biomass and TDN yields across cultivars were 3.17 Mg ha^{-1} and 2.08 Mg ha^{-1} respectively, at two months after establishment in 2021. In the dry 2022 season, biomass and TDN yield were 1.9 Mg ha^{-1} and 1.03 Mg ha^{-1}, respectively, two months after establishment. Hemp nutritive value measures varied by cultivar and harvest time ($P < 0.05$).

Depending on the cultivar and harvest time, hemp plant biomass contained 13 to 32% CP, 22 to 45% NDF, 20 to 38% ADF, 4 to 9% lignin, and 52 to 80% TDN (cultivar × time interaction; $P < 0.05$). Hemp CP and TDN decreased gradually with maturation while ADF, NDF, and lignin increased ($P<0.0001$); however, this decline with maturity did not appear as severe as occurs with many other forages. These preliminary results suggest that hemp has the potential to be used as a forage crop. More research is needed to address hemp management and utilization, including field establishment and production, harvest timing for optimum tonnage and forage quality, and animal intake and performance studies.

These findings provide new insights into industrial hemp production in the mid-Atlantic region of the United States. Optimal tillage practices, precise harvest timing, appropriate N fertility rates, and proper management techniques all are crucial for maximizing hemp seed and fiber production and quality. Furthermore, hemp shows promise as a forage crop with its adaptability and favorable nutritional properties. Further research is warranted to refine cultivation techniques, improve crop quality, and explore the full potential of hemp in various industries.

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ABSTRACT (GENERAL AUDIENCE)

Industrial hemp (*Cannabis sativa* L.) is a versatile crop with numerous applications in various industries, but much work must be done to understand crop responses to management practices and improve its potential as a crop for greater sustainability. In this study, we explored different aspects of hemp agronomic management.

Hemp traditionally has been planted into tilled fields, which increases the chance for soil erosion. We examined whether hemp could be established without tillage and found that although tilled fields generally had great populations of taller plants; total biomass and seed yields were not as influenced by tillage. Our research suggests that with some tweaking, hemp can be successfully established without soil tillage.

Next, we investigated the optimal time to harvest hemp for maximum seed yield. Harvesting at the right moment is crucial, as seeds ripen unevenly, resulting in varying quality and yield. By carefully timing the harvest, we can maximize seed yield and ensure high-quality seeds. Our cultivars were best harvested in a late July to early August time frame. Under favorable weather conditions, we observed seed yields ranging from 1,180 to 2,510 kilograms per hectare, depending on the hemp cultivar and location.

Additionally, we studied the response of hemp seed yield to nitrogen fertilization rates. Nitrogen is an essential nutrient for plant growth, and we found that nitrogen significantly influenced seed yield, although the pattern of response varied by growing conditions. Over three

years, seed yields ranged from 380 to 2,510 kilograms per hectare. Yields generally increased with nitrogen inputs but were highly affected by available moisture. Fertility studies help farmers determine the ideal nitrogen levels for their hemp crops, promoting healthy growth, maximizing yield, and minimizing environmental contamination. Within this study, we also evaluated aerial imagery technologies to monitor plant nitrogen status and we observed that remote sensing technologies are promising for building predictive nutrient management tools.

Lastly, we explored the potential of hemp as a forage crop. Hemp plants have unique nutritional properties (e.g., protein, fatty acids) and can be used as feed for livestock. We investigated the best time to harvest hemp for maximum biomass and nutrient content, important factors for animal nutrition. Hemp plants grow rapidly and within two months after establishment they yielded up to 3.17 metric tons of biomass per hectare, with relatively high nutritional value.

Overall, these studies provide valuable insights into hemp production, including the importance of tillage practices, optimal harvest timing, and appropriate nutrient management. By applying these findings, farmers can enhance their hemp cultivation techniques, resulting in higher yields, improved crop quality, and better environmental outcomes.

DEDICATIONS

To my lovely wife, daughter, and my parents.

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CHAPTER I

INTRODUCTION

Industrial hemp (*Cannabis sativa* L.) is a versatile crop that has garnered significant attention in recent years due to its numerous applications across various industries. Hemp cultivation dates back thousands of years (Visković et al., 2023) and the plant's fibers and seeds are considered valuable resource for textiles, construction materials, food, and health products (Nath, 2022). Hemp cultivation offers several advantages, including its adaptability to different climates, fast growth rate, and potential for sustainable agricultural practices.

Legalizing hemp cultivation in many countries has led to increased interest and research in understanding the agronomic practices, crop management strategies, and potential applications of this versatile plant (Adesina et al., 2020). This thesis aims to contribute to the existing knowledge base by investigating several key aspects of industrial hemp production, including the impact of tillage practices on hemp seed and fiber production, optimal harvest timing for seed yield and quality, the response of hemp seed yield to nitrogen fertilization rates, and the potential of hemp as a forage crop.

Industrial hemp belongs to the *Cannabis sativa* species, but it is set apart from marijuana by its minimal levels (< 0.3%) of tetrahydrocannabinol (THC), the psychoactive compound responsible for the intoxicating effects commonly associated with marijuana use (Small and Marcus, 2003). Hemp varieties are specifically bred to produce low levels of THC, while focusing on other desirable traits, such as high fiber content, nutritional value, or oil production (Salentijn et al., 2015). This distinction has allowed for the legal cultivation of industrial hemp in many countries for industrial purposes.

The cultivation of industrial hemp offers numerous potential benefits (Ely et al., 2022). Hemp fibers have long been recognized for their strength and durability, making them suitable for a range of textile applications (Ranalli, 1999). The seeds of hemp are highly nutritious, containing essential fatty acids, protein, and other beneficial compounds, making them a valuable component of various food and health products (Leizer et al., 2000). Hemp oil derived from the seeds is rich in omega-3 and omega-6 fatty acids, further adding to its nutritional value (Matran et al., 2009). Additionally, hemp biomass can be utilized as a source of renewable energy and as a raw material for the production of bioplastics and construction materials (Ahmed et al., 2022).

While industrial hemp presents significant opportunities, several challenges must be addressed to promote its widespread cultivation and utilization (Ely et al., 2022). One of the key challenges is establishing optimal cultivation practices to maximize seed and fiber production. Factors such as tillage practices, cultivar selection, and production system (e.g., seed vs. dual-puprose) can influence crop performance and overall yield. Understanding the impact of these factors on hemp production is crucial for optimizing cultivation techniques and enhancing profitability.

Another challenge lies in determining the optimal timing for hemp harvest to achieve maximum seed yield and quality (Schluttenhofer and Yuan, 2017). Hemp seeds ripen at different rates, making it difficult to harvest uniformly mature seeds. Early harvesting may result in lower yields, while delayed harvesting may lead to seed shattering and decreased quality (Blandinières and Amaducci, 2022). Identifying the optimal harvest window is essential for optimizing yield and ensuring seed quality.

Furthermore, understanding the response of hemp seed yield to nitrogen fertility rates is crucial for efficient nutrient management and sustainable production (Kaur et al., 2023). Nitrogen is an essential nutrient for plant growth, and proper nitrogen fertilization can significantly impact crop yield and quality. Determining the appropriate nitrogen rates and timing of application is vital to maximize seed yield while minimizing environmental impacts (Kaur et al., 2023).

Additionally, exploring the potential of hemp as a forage crop can provide alternative opportunities for its utilization (Ely et al., 2022). Hemp has the potential to serve as a valuable feed source for livestock due to its nutritional properties and rapid summer growth (Adesina et al., 2020; Ely and Fike, 2022), but study of this use is limited. Evaluating the yield and nutritive value of hemp biomass at different growth stages can inform farmers about the feasibility of integrating hemp into their forage production systems.

This thesis aims to address these challenges and explore the potential of industrial hemp through the following objectives: assess the impact of tillage practices on hemp seed and fiber production; determine the optimal harvest timing for hemp seed yield and quality; investigate the response of hemp seed yield to nitrogen fertility rates; and evaluate the potential of hemp as a forage crop, considering its yield and nutritive value. By addressing these objectives, this research aims to provide practical guidance for hemp farmers, agronomists, and researchers to enhance cultivation practices, improve productivity, and explore the potential of industrial hemp across various industries.

In conclusion, industrial hemp presents significant opportunities for sustainable agriculture by increased carbon sequestration, economic development, and diversification of industries. Through this thesis, I aim to contribute to the existing knowledge base by investigating key aspects of hemp production, including the impact of tillage practices, optimal harvest timing, nitrogen fertility rates, and the potential of hemp as a forage crop. By enhancing our understanding of these factors, we can promote the adoption of sustainable hemp cultivation practices, maximize crop productivity, and harness the full potential of this versatile and valuable plant.

CHAPTER II

LITERATURE REVIEW

Industrial hemp, *Cannabis sativa*, is a versatile and ancient crop that has been cultivated for thousands of years (Visković et al., 2023). Its historical significance and diverse uses have shaped societies and economies across various regions of the world. Unlike its controversial cousin, marijuana, industrial hemp is cultivated for industrial and commercial purposes and contains negligible amounts of the psychoactive compound, delta-9-tetrahydrocannabinol (THC) (Block, 2023). This key distinction has prompted significant regulatory and legal considerations surrounding its cultivation and utilization (Malone and Gomez, 2019).

History

The history of industrial hemp dates back to ancient civilizations, where it was a vital agricultural crop (Lash, 2002). Archaeological evidence suggests that hemp was first cultivated in Asia around 10,000 years ago, with its use gradually spreading to Europe, the Middle East, and eventually reaching the Americas (Bilalis et al., 2019; Zohary et al., 2012). Ancient civilizations, such as the Chinese, Egyptians, and Greeks, harnessed the plant's fibrous stalks to produce ropes, textiles, and paper (Karche, 2019). In colonial America, authorities actively encouraged hemp cultivation, and it played a critical role in early agricultural practices (Howe, 1892).

Types of hemp

Cannabis sativa encompasses various subspecies and strains, leading to distinct types of hemp suited for different applications (Adesina et al., 2020). Some common types of industrial hemp include:

Fiber Hemp: Cultivated for its long, strong fibers, fiber hemp is primarily used in producing textiles, ropes, and paper. Seed Hemp: This type of hemp is grown for its nutritious and protein-rich seeds, which are utilized in foods, health products, and cosmetics. CBD Hemp: With the increasing interest in cannabidiol (CBD) products, certain hemp varieties are cultivated for their high CBD content in the flowers, which is extracted for medicinal and therapeutic purposes (Adesina et al., 2020).

Regulatory issues and legalization

Despite its industrial applications and minimal psychoactive properties, industrial hemp has faced regulatory challenges and legal restrictions due to its association with marijuana. The confusion between the two plants led to widespread prohibition and strict regulations on hemp cultivation in various countries during the 20th century (Malone and Gomez, 2019).

However, in recent years, a gradual shift in perception and understanding of hemp's potential benefits has relaxed laws in many regions (Fortenbery and Mick 2014). With the growing recognition of hemp as a valuable agricultural commodity, several countries and U.S. states have taken steps to legalize and regulate its cultivation and commercial use. Legalization often comes with specific stipulations regarding THC content limits, licensing requirements, and oversight to ensure compliance with applicable laws (Fortenbery and Mick 2014).

Importance of hemp

Industrial hemp crops are of paramount importance due to their remarkable versatility and diverse applications across numerous industries (Tilkat et al., 2023). The fibrous stalks of the hemp plant are utilized in the production of textiles, paper, and building materials (e.g., hempcrete, bio-composite, insulation, and particleboard) making it an invaluable resource for the construction, manufacturing, and fashion sectors (Nath, 2022; Ely et al., 2022). Simultaneously, industrial hemp seeds are a treasure trove of nutritious oils and protein-rich foods, offering consumers a sustainable and health-conscious option (Burton et al., 2022).

Furthermore, the recent surge in the popularity of hemp-derived CBD products has sparked a revolution in the medicinal and therapeutic industries. CBD, a non-psychoactive compound found abundantly in certain hemp varieties, is believed to provide various health benefits, ranging from pain relief and anxiety reduction to aiding in epilepsy treatment (Fiani et al., 2020).

Beyond its commercial value, industrial hemp plays a significant role in promoting sustainable agriculture (Ranalli and Venturi, 2004). Its deep root system helps prevent soil erosion and enhances soil health. Moreover, hemp efficiently absorbs atmospheric carbon dioxide during its rapid growth, thus mitigating climate change (Adesin et al., 2020).

Furthermore, the cultivation of industrial hemp can support biodiversity by providing habitat and food sources for various beneficial insects and wildlife (Phipps and Schluttenhofer, 2022). Its pollen-rich flowers attract bees and other pollinators, bolstering essential ecosystems and contributing to preserving biodiversity.

Another remarkable feature of hemp is its potential for soil remediation. Through a process called phytoremediation, certain hemp fiber varieties can help cleanse soil contaminated with heavy metals and pollutants, making it a promising tool for environmental cleanup efforts (Adesin et al., 2020). By offering sustainable alternatives to traditional non-renewable resources, such as fossil fuels and synthetic fibers, industrial hemp supports the transition towards greener and more environmentally friendly industries.

General production systems

Hemp, being an oilseed, is most suitable for inclusion in a crop rotation alongside a cereal crop or, ideally, a legume, similar to other field crops' rotational practices (Jeff and Williams, 2019). Typically, hemp is sown around the same time as corn, coinciding with soil temperatures reaching approximately 15°C (Ely et al., 2022). Earlier seeding is recommended to get higher vegetative growth. However, the soil needs to reach adequate moisture and temperature. In North America, seeding may start in late April and continue through late June.

The seeding rate varies with the production systems. For hemp fiber production, distance between rows needs to be 7 to 20 cm as higher plant density is required to produce quality fiber. For seed production, row distance is maintained at 15 to 18 cm as low plant density is required for seed production. For flower-oil production, hemp plants are transplanted following a horticulture model with a 1 m row distance as wider row spacing allow them to produce vigorously with high branching. Depending on seed size and desired plant population density, the recommended seeding rates are 45 to 70 kg ha^{-1} for fiber hemp and 10 to 45 kg ha^{-1} for seed seed hemp.

Effective weed control is crucial for successfully cultivating hemp for seed and fiber. As of now, there are no registered herbicides approved for use on hemp in the United States (Jeff and Williams, 2019). Consequently, farmers must rely on mechanical and cultural methods to manage weeds while leveraging hemp's natural ability to outperform weeds when appropriately established (Jeff and Williams, 2019).

Additionally, the limited availability of pesticides for hemp crops underscores the importance of careful field selection with well-drained soils and minimal weed competition. Adequate fertility is necessary for optimal yields, and harvesting hemp can be particularly demanding, often requiring equipment modifications and appropriate post-harvest storage facilities (Jeff and Williams, 2019).

Types of Hemp production systems

There are four different types of production systems of hemp based on harvesting practices. **CBD hemp**, also called flower hemp, is grown for the purpose of extracting essential oil from hemp flowers. Through transplanting, only the female plant population is maintained in that system, and any males are eradicated to avoid pollination. **Fiber hemp** is taller and harvested before flowering. **Grain or seed hemp** is mainly a monoecious type where male and female flowers belong in the same plant though there are some dioicous cultivars too. Seed is harvested once the seed starts shattering from intermediate inflorescences. For **dual-purpose** production, hemp cultivars are grown to harvest hemp seed and fiber. Top heads are harvested for seed, and bottom stalks are harvested for fiber (Ely et al., 2022).

Photoperiod sensitivity

As a crop sensitive to day length, hemp demonstrates consistent flowering patterns on the calendar, regardless of the seeding time. However, the precise timing of flowering can be influenced by various factors, including fertility stress, weed pressure, and moisture conditions (Jeff and Williams, 2019).

Hemp plants are photoperiodic, so they need longer days to produce higher vegetative growth, especially for fiber production. There are some photoperiod-insensitive cultivars too, e.g. Finola, which start flowering once they reach to adequate growth (Cherney and Small, 2016). Hemp plants may produce higher biomass if Southern-originated cultivars are grown in the northern region. On the other hand, the opposite may happen if Northern-originated cultivars are grown in the southern region.

Hemp crops grown for fiber are harvested soon after female flowering begins. The timing of harvest plays a crucial role in fiber quality. As hemp plants enter reproductive growth, additional lignin is produced, strengthening the stem and reducing the risk of lodging caused by the weight of the flower/seed head at the top of the stem, a common problem in seed crops, including hemp (Jeff and Williams, 2019).

Field establishment issues

Field establishment of hemp plants is challenging as emergence response varies with the various soil factors, including soil texture, soil moisture, soil crust, seeding depth, and rain events after seeding, etc. Rain events followed by seeding influence the formation of soil crust in high clay soils. Field/seedbed preparation and seeding depth are also crucial for seed emergence.

Hemp exhibits poor seedling vigor compared to other commodity crops, posing challenges in establishing a profitable stand under stressful environmental conditions (Jeff and Williams, 2019). Factors like excessive precipitation leading to soil crusting or waterlogging can further hinder seedling growth (Jeff and Williams, 2019).

Hemp plants grown at high plant density exhibited shorter plant height (approximately 41%) compared to those at low plant density (40 vs. 120 plants m^{-2}), as the higher density caused the plants to enter the reproductive stage earlier (Campiglia et al., 2017).

Establishing hemp fields poses numerous challenges due to the sensitivity of hemp seeds to various soil conditions such as soil moisture, texture, temperature, and seeding depth. The general recommendation is to opt for conventional tillage systems when planting hemp seeds (Ely et al., 2022). However, even in conventional tillage systems, there are potential issues, particularly when heavy rain occurs after planting. The soil may become compacted or develop a crust on the surface, which can hinder the emergence of hemp seeds. These seeds are particularly vulnerable to soil compaction and crust formation, making it difficult for them to emerge successfully (Roth et al., 2018).

In recent times, the adoption of no-tillage production has been on the rise due to its benefits, such as high soil carbon sequestration, lower input costs, and improved sustainability (Gozubuyuk et al., 2020; Stošić et al., 2021). Nevertheless, establishing hemp in a no-tillage system also presents challenges. The seeds may struggle to emerge from deeper soil depths, or if planted too shallow, they might not receive adequate moisture for germination (Ely et al., 2022). In essence, whether under conventional or no-tillage systems, establishing hemp fields requires careful management. Addressing the specific challenges posed by each tillage system is crucial for successful hemp cultivation.

Harvest Time

Female or hermaphrodite hemp plants have a terminal inflorescence where seeds start maturing from the bottom, with earlier-matured seeds in the lower part of plants tending to shatter earlier. However, some seeds in the top part of the plants may still remain in unfilled or dough stages as they form later. The uneven seed maturation poses challenges during harvest.

Birds, such as doves, prefer feeding on hemp seeds, which can significantly reduce seed yield. Bird predation becomes a concern for hemp cultivation as it can result in substantial portions of the seed yield being lost (Ely et al., 2022).

One notable characteristic of hemp plants is that their leaves and stems remain green even at 70 to 80% seed maturity. This persistence of greenness adds to the challenges of harvesting, as visual assessment of seed maturity becomes difficult due to the presence of green plant material (Ely et al., 2022).

Hemp is sensitive to day length, and its flowering patterns consistently follow a calendar schedule, regardless of the seeding time (Jeff and Williams, 2019). However, the precise timing of flowering can be influenced by various factors that include fertility stress, weed pressure, and moisture conditions. Environmental stress or low fertility conditions can trigger earlier flowering in hemp plants (Jeff and Williams, 2019). Hemp seed formation and ripening also follow that flowering time. This means early flowering plants will need to be harvested earlier.

Harvesting hemp seed poses unique challenges due to uneven seed maturation, bird predation, and the persistence of green leaves and stems add other challenges in using mechanical harvesting. Understanding the effects of these factors on hemp seed yield is crucial for optimizing harvest timing and implementing appropriate management strategies. Delayed harvest may lead to seed yield loss by shattering issues.

Seed quality

Hemp seeds have high food value as they contain high nutritive components and antioxidant properties (Irakli et al., 2019, Gołaszewski, 2018). Hemp seeds contain different proteins, fatty acids, dietary fiber, vitamins, and minerals. Fatty acid, carbohydrate, oil, and protein content in the hemp seed varied with the cultivars, where antioxidant and phytochemical components varied with growing condition or year (Irakli et al., 2019, Gołaszewski, 2018). Typically, hemp seed contains over 30% oil in over 80% polyunsaturated fatty acids, a rich source of two essential fatty acids- omega 3 and omega 6 (Callaway, 2004).

Harvesting time significantly impacts the quality of hempseed oil (Marzocchi and Caboni, 2020). The composition of the fatty acids in hemp seed varies during ripening, indicating changes in nutritional and functional properties (Marzocchi and Caboni, 2020). Linoleic acid is the predominant fatty acid, followed by oleic, α-linolenic, and palmitic acid. The ratio of linoleic to α-linolenic acid decreased, and the polyunsaturated to saturated fatty acid ratios increased with the ripening of the hemp seeds (Marzocchi and Caboni, 2020)

N management

Nitrogen (N) plays a vital role in hemp seed production like as other plants, supporting various essential processes such as chlorophyll synthesis, protein production, photosynthesis, amino acid formation, nutrient uptake, and energy systems (Jeff and Williams, 2019). However, the optimal N fertilization rate for hemp cultivation varies based on factors like hemp type, geographical location, and soil conditions (Adesina et al., 2020). Generally, the recommended N fertilization rate for seed or dual-purpose hemp ranges from 112 to 224 kg ha^{-1} (Jeff and Williams, 2019).

Specific recommendations for N fertilization differ depending on the intended use of hemp. Adequate seed production typically requires an application rate of 110 kg N ha^{-1}, while fiber hemp may benefit from higher rates, up to 240 kg N ha^{-1} (Papastylianou et al., 2018). However, caution must be exercised with heavy nitrogen fertilization for hemp fiber crops, as it can negatively affect growth rates and fiber quality (Jeff and Williams, 2019). Excessive nitrogen leads to rapid cell elongation, weakening bast fiber due to thinner cell walls (Jeff and Williams, 2019). Consequently, a recommended application rate of 56 kg ha^{-1} is suggested for hemp fiber crops, typically applied pre-planting (Jeff and Williams, 2019).

Research conducted in Québec, Canada, demonstrated that applying 200 kg N ha^{-1} significantly increased biomass and seed yields across different cultivars and environments. Biomass yield rose from 1674 to 4209 kg ha^{-1}, while seed yield increased from 519 to 1340 kg ha^{-1} (Aubin et al., 2015).

Previous studies conducted in Western Canada by Vera et al. (2004, 2010) found a positive relationship between N fertilization and hemp production. Increasing the N fertilization rate benefited biomass yield, plant height, and seed yield, with the recommended range falling between 100 and 150 kg N ha^{-1}.

Similarly, Papastylianou et al. (2018) observed that applying 240 kg N ha^{-1} resulted in significant improvements in biomass yield, stem dry weight, and inflorescence weight, with respective increments of 37.3%, 48.2%, and 16%.

Under Mediterranean conditions in central Italy, Campiglia et al. (2017) discovered that higher N fertilization levels (100 kg N ha^{-1}) contributed to increased stem yields (28%), while inflorescence and seed yields saw smaller improvements of 17% and 4%, respectively.

Overall, the findings from various researchers emphasize the importance of nitrogen fertilization for hemp production, with optimal rates varying based on specific factors such as cultivation purpose, location, and cultivar. Proper nutrient management, including appropriate N fertilization, can help maximize biomass and seed yields, supporting the successful cultivation of hemp.

UAV imagery-based N management and yield prediction

Remote sensing and drone imagery have emerged as valuable tools in modern agriculture, offering efficient and non-destructive methods for assessing crop health, monitoring key parameters such as nutrient status in plants, and predicting yield. By capturing high-resolution images and utilizing advanced data analysis techniques, these technologies enable accurate predictions of N content in plant tissues and yield estimation (Vergara-Díaz et al., 2016). The recent advancements and applications of remote sensing and drone imagery in this field are presented here.

In the agricultural sector, drones have become valuable tools for crop monitoring and yield prediction due to their ability to capture high-resolution imagery and provide real-time data. Different types of drones are commonly used, each offering specific capabilities and features to meet the diverse needs of agricultural applications.

Fixed-wing drones drones resemble traditional airplanes and are known for their long flight endurance and large coverage area. Fixed-wing drones are ideal for large-scale agricultural operations and can efficiently capture high-resolution aerial imagery over vast areas, allowing for comprehensive crop monitoring and analysis (Dileep et al., 2020).

Multirotor drones, such as quadcopters and hexacopters, are popular due to their agility, stability, and vertical take-off and landing capabilities. These drones can hover and maneuver in tight spaces, making them suitable for capturing detailed imagery of crops in smaller or irregularly shaped fields (Dileep et al., 2020).

Different types of sensors are commonly integrated into drones to capture specific data for crop monitoring and yield prediction. These sensors detect and measure plants' various physical and biological properties, providing valuable insights into crop health and performance.

Capturing visible light imagery akin to human vision, an RGB camera is utilized for plant health analysis, disease or stress symptom identification, and crop growth stage monitoring. High-resolution ortho-mosaic maps for accurate field mapping and vegetation analysis can also be generated by processing RGB images (Rejeb et al., 2022).

Multispectral cameras capture imagery in specific spectral bands beyond the visible range, such as near-infrared (NIR) and redEdge. These cameras allow for assessing plant vigor, chlorophyll content, and stress detection. By measuring reflectance in different bands, multispectral cameras enable the calculation of vegetation indices like the Normalized Difference Vegetation Index (NDVI), Enhanced Vegetation Index (EVI), which provides valuable information on crop health, nutrient status in plant and biomass estimation (Rejeb et al., 2022, and Pande et al., 2023).

Hyperspectral cameras capture imagery in numerous narrow and contiguous spectral bands, offering more detailed spectral information compared to multispectral cameras. Hyperspectral imaging enables precise identification and characterization of crop diseases, nutrient deficiencies, and other physiological conditions. It provides a comprehensive analysis of the biochemical and physiological properties of plants, enhancing the accuracy of crop monitoring and yield prediction models (Rejeb et al., 2022).

Unmanned Aerial Vehicle (UAV) imagery-based data analytics techniques and algorithms have revolutionized crop monitoring and N management practices, enabling accurate prediction of N status, biomass, and seed yield in crops. These advanced approaches utilize the high-resolution imagery captured by drones to extract valuable information, supporting decision-making in precision agriculture (Rejeb et al., 2022).

Machine learning algorithms, including Random Forest, Support Vector Machines, and Artificial Neural Networks, are employed for predicting N status, biomass, and seed yield in crops (Wang et al., 2022). These algorithms leverage historical data, such as spectral reflectance, weather conditions, and soil parameters, to build predictive models. Machine learning algorithms provide accurate predictions by learning the complex relationships between input features and target variables, facilitating informed decision-making in N management and yield optimization.

Crop growth models integrate UAV imagery with biophysical processes to simulate crop growth and predict yield. By incorporating factors such as plant growth stages, N uptake, and biomass accumulation, these models enable accurate predictions of crop growth, N status, and yield. Crop growth models assist in optimizing N fertilizer rates, determining optimal harvest timing, and estimating crop productivity.

Hyperspectral imaging captures the spectral signatures of crops in numerous narrow and contiguous spectral bands. Spectral unmixing techniques are then applied to decompose the hyperspectral data into specific components, such as vegetation, soil, and background. This allows for precise quantification of crop parameters, including N concentration and biomass. Hyperspectral imaging and spectral unmixing offer detailed information about crop health and N status, facilitating targeted N management strategies (Rejeb et al., 2022).

Deep learning techniques, particularly Convolutional Neural Networks (CNN), have gained popularity in analyzing UAV imagery for crop monitoring and yield prediction. CNN models automatically learn and extract features from images, enabling plant disease detection, leaf area estimation, and yield mapping tasks (Wang et al., 2022). These deep learning algorithms improve the accuracy and efficiency of crop monitoring and N management practices.

Han et al. (2022) utilized various combinations of band reflectance, vegetation indices (VIs), and phenology indicators (PIs) in random forest models to evaluate feature importance and build accurate models for estimating key wheat growth parameters. Their study demonstrated the potential of these models for effective monitoring and estimation of crop growth using UAV multispectral imagery, with R^2 values ranging from 0.60 to 0.79 and NRMSE values ranging from 10.51 to 15.83%.

Impollonia et al., 2022 conducted a study that uses UAV remote sensing and the PROSAIL model to estimate hemp cultivars' leaf area index (LAI) and leaf chlorophyll content (LCC) under varying nitrogen fertilization levels. They reported that hybrid regression methods outperformed look-up table (LUT) methods for LAI and LCC estimation, with random forest and Gaussian process regression achieving the best accuracy, respectively. High-throughput phenotyping revealed significant differences in LAI and LCC dynamics among hemp cultivars and nitrogen levels.

In another study Impollonia, 2022b investigated using UAV remote sensing for high-throughput phenotyping of hemp and miscanthus traits, estimating various characteristics like LAI, LCC, light interception, plant height, green leaf biomass, standing biomass, and moisture content. Machine learning algorithms and the PROSAIL model inversion are employed for trait estimation, while the generalized additive model (GAM) is used to analyze trait dynamics during the growing season. Combining these methods proves to be a powerful tool for effective high-throughput phenotyping.

Forage Hemp

When evaluating hemp as a forage crop, considering factors beyond yield and environmental compatibility is important, such as the nutritive value of the forage (Ball et al., 2007; Stringer, 2018). Hemp plant tissues have structural components (hemicellulose, cellulose, and lignin) and non-structural components (protein, sugar, and starch; Ball et al., 2007; Stringer, 2018). Ruminants can digest certain structural components, and laboratory testing measures digestibility through NDF, ADF, and ADL (Ball et al., 2007; Stringer, 2018).

Hemp fiber crops are primarily cultivated for their stalks, bast fibers (longer and stronger fiber), and core fibers (shorter and denser; Chabbert et al., 2013; Smalls and Marcus, 2002). Fiber crops accumulate more lignin and cellulose as they mature, reducing digestibility (Ball et al., 2007; Amaducci et al., 2008). Crude protein content declines with maturity, but frequent cuttings of hemp cultivars can help maintain protein levels (Ball et al., 2007; Stringer, 2018).

In a study by Stringer (2018), interactions between forage type, planting date, and harvest time influenced biomass yield, fiber, and lignin content. The planting and harvest dates affected NDF, ADF, digestibility, and crude protein (Stringer, 2018). The nutritive value decreased with plant maturation, and later seeding dates resulted in reduced leaf content, yield, and overall nutritive value (Stringer, 2018).

Many hemp waste products are available, especially from CBD hemp production and processing systems. Growers are interested in feeding the hemp-spent biomass (SHB) (post-extracted floral biomass) to animals. However, hemp is not currently legalized as animal feed because of concerns about possible contamination of meat and milk by cannabinoids. This indicates that research efforts are needed to focus on hemp and its by-products as a potential source of animal feed. A feeding trial conducted by Parker et al., 2022 stated that feeding lambs SHB showed no major detrimental effects on performance, meat quality, or animal health. SHB had comparable nutritive quality to alfalfa and potential as a feed ingredient, although higher inclusion levels negatively impacted feed intake and increased purge and cook loss of meat from lambs fed SHB (Parker et al., 2022). Further research is needed to determine optimal inclusion levels and long-term effects of SHB feeding (Parker et al., 2022).

CHAPTER III

CONVENTIONAL TILLAGE VS. NO-TILLAGE FOR HEMP FIBER AND SEED ESTABLISHMENT

ABSTRACT

Industrial hemp (*Cannabis sativa* L.) is a multi-purpose commercial crop that can potentially be grown for multiple uses, including food, feed, fiber, forage, and fuel. Several agronomic challenges are associated with industrial hemp cultivation, however, among which difficulty in establishing crops in the field has been a major concern. A three-year study was conducted to determine the effects of tillage management and production systems (e.g., seed, fiber, and dual) on hemp productivity within Ridge/Valley (Blacksburg) and Northern Piedmont (Orange) ecophysiographic regions of Virginia. Two cultivars, Joey (a dual-purpose variety) and EcoFibre (bred specifically for fiber), were planted into seedbeds prepared with conventional tillage or no-till management. Cultivar planting rates were based on recommendations for seed, dual-purpose and fiber production. The study was a randomized complete block design with a split plot treatment arrangement and four replicates; tillage was the main plot and production system was the subplot. In 2020, a single cultivar, Joey, was used for both production systems and fiber cultivar EcoFibre was used for fiber production systems in 2021 and 2022. Hemp was planted during mid-May using a small plot planter or no-till drill, depending on site. Plant height was not affected by tillage ($P \geq 1233$), and no interaction was observed between tillage and production systems ($P \geq 2570$). Seed production management resulted in taller plants compared to dual purpose management. Greater plant heights ($P < 0.0001$) with fiber production systems in 2021 and 2022 were a due to differences between cultivars. Conventional tillage resulted in greater ($P \leq 0.0161$) plant populations than no tillage for all production systems in each year, and the increased plant populations with fiber management were generally greater with conventional tillage management as observed in 2020 (tillage × production systems interaction; $P = 0.0007$). Greater ($P < 0.001$) yields with fiber systems observed in 2021 and 2022 were largely driven by the more productive EcoFibre cultivar. Although plant population density varied, seed yields and biomass yields varied less by tillage management and production systems. Lower plant population density was associated with greater biomass and seed yields per plant. However, for desired fiber quality and mechanical harvest feasibility, a higher plant population density is recommended.

INTRODUCTION

Industrial hemp (*Cannabis sativa* L.) is a multipurpose crop with potential commercial value for food, feed, fiber, forage, flowers (pharmaceuticals), and fuel. There is growing interest in hemp cultivation worldwide due to its multiple possible uses and benefits to humankind. However, it faces some agronomic challenges, as field establishment can be difficult. Most hemp establishment guides recommend tillage for field preparation as well as cultivating and firming soils before planting (Ely et al., 2022). The development of large-scale hemp enterprises, which would necessitate the tilling of thousands of acres for successful establishment, would go against present efforts to promote no-till farming methods (NRCS 2016). No-till farming reduces erosion and compaction (Mchunu et al., 2011; Komissarov and Klik, 2020; Carretta et al., 2021), increases soil moisture, carbon, and biological activity (Jemison et al., 2019; Somasundaram et al., 2020), and reduces labor, fuel consumption, and emissions (Gozubuyuk et al., 2020; Stošić et al., 2021). As part of a road to sustainable intensification, no-till management offers the potential for enhanced environmental and economic benefits (e.g., Nunes et al., 2018; Cusser et al., 2020). However, whether within conventional tillage or no-tillage regimes, hemp field establishment is challenging because hemps seeds are particularly sensitive to conditions at establishment. In conventionally tilled fields, seeds may fail to emerge if they are planted too deep or in wet soils with high clay content where soil compaction and crusting are common issues (Roth et al., 2018). Also, tilled soils that are not sufficiently firm at planting can dry out, leaving emerging seedlings subject to moisture stress (Mark and Will, 2019). In a no-tillage system, placing the seeds in optimal depth is challenging given more variable soil conditions; shallow-planted seeds may not get enough moisture for germination and emergence while hemp seeds may fail to emerge from deeper planting (Mark and Will, 2019).

Seeding rate recommendations for hemp vary with the end use of the given production systems. Higher plant populations generally are needed for fiber hemp production systems and seeding rate recommendations typically are in the range of 45 to 70 kg ha^{-1} depending on seed weight. The high plant population discourages branching and ensures thinner stems with greater fiber quality, and it is easier to harvest slender plants at uniform height (Roseberg et al., 2019). In contrast, hemp seeding rates for seed production system are much lower (10 to 45 kg ha^{-1}) than fiber production systems. Higher branching with moderate to low plant populations supports greater flowering and seed production. However, plant architecture (with thick, highly branched plants) may negatively affect combine harvest efficiency. The wide range of seeding rates for each hemp production system not only reflects differences in management approach, but also the substantial variation in seed weight, vigor, and emergence, and, for seed/dual-purposes crops, differences in plant sexual system (i.e., monoecious vs. dioecious varieties). Studies of the best methods of stand establishment in either tillage regime (conventional and no-tillage) for seed and fiber production systems are limited. The objectives of this study were to evaluate field establishment and productivity within three different hemp production systems (i.e., seed, dual-purpose, and fiber), in relationship to tillage managements.

MATERIALS AND METHODS

Study Sites and Experimental Design

This study was conducted at three different locations - Virginia Tech's Urban Horticulture Center in 2020 and 2021, and Kentland Farm in 2022 (both in Blacksburg, VA) and the Northern Piedmont Center (Orange, VA) in 2020, 2021, and 2022. Soils at the Urban Horticulture Center are predominantly loam of Duffield-Ernest complex (fine-loamy mixed active mesic Ultic Halpludalfs and mixed superactive mesic Auqic Fragiudults at the Urban Horticulture Center) and of Groseclose and Poplimiento series (fine mixed semiactive mesic Typic Hapludults. Soils at the Orange location are heavier and predominantly Drayke and Fauquier clay loam (fine mixed semiactive mesic Typic Rhodudults) along with Meadowville silt loam (fine-loamy mixed semi-active mesic Typic Hapludult). The experiment was conducted as a randomized complete block design with a split-plot treatment arrangement and four replications. Tillage system (conventional or no-till) was the main plot, and the production systems (seed or fiber) was considered a subplot. Plots were 6 m long with nine rows 15.2 cm spacing.

Weather Data

Daily mean ambient temperatures, maximum and minimum temperatures, and precipitation data for all locations were downloaded from Virginia Tech's WeatherSTEM Data

Mining Tool (http://vt-arec.weatherstem.com) for the entire study period for 2020, 2021, and 2022 (accessed on 13 January 2023).

Cultivars and Seeding Rate

Two hemp cultivars, ‘Joey’ (a dual-purpose variety) and ‘EcoFibre’ (a fiber variety) were used for the study. The germination percentage of these hemp cultivars was determined in the lab by ragdoll test (Chambliss, 2002) prior to the study and used as a correction factor. Seeds were planted to 1-cm depth using a no-till drill.

Table 3 - 1: Cultivars, uses, seeding rates, sites, planting and harvesting dates at two Virginia locations for tests of hemp production systems under conventional and no-till management.

Cultivars, uses, seeding rates, and intended populations					Sites, planting and harvest date			
Year	Cultivar	Use	Seeding rate (kg ha^{-1})	Population (seeds ha^{-1})	Sites	Planting date	Harvest date	
2020	Joey	Seed	28	1.5 M	Blacksburg	4-Jun	2-Sep	
	Joey	Fiber	56	3.0 M	Orange	3-Jun	8-Sep	
							Seed	Fiber
2021	Joey	Seed	28	1.5 M	Blacksburg	12-May	5-Aug	24-Sep
	EcoFibre	Fiber	78	2.7 M	Orange	8-Jun	17-Aug	7-Sep
2022	Joey	Seed	28	1.5 M	Blacksburg	17-May	2-Aug	29-Sep
	Joey	Fiber	56	3.0 M	Orange	18-May	8-Aug	15-Sep
	EcoFibre	Fiber	78	2.7 M				

Planting was conducted at two locations, Blacksburg and Orange, on different dates in each year of the study. In 2020, planting occurred on June 4th at Blacksburg and on June 3rd at Orange. The following year, planting was carried out on May 13th at Blacksburg and June 8th at Orange. In 2022, planting was done on May 18th at Orange and May 20th at Blacksburg.

Harvesting was done on September 2nd, 2020 at Blacksburg and on September 8th, 2020 at Orange. In 2021, seed harvesting was conducted on August 5th at Blacksburg and August 17th at Orange, while fiber harvesting occurred on September 24th at Blacksburg and September 7th at Orange. Finally, in 2022, seed hemp harvesting was completed on August 2nd at Blacksburg and August 8th at Orange, while fiber harvesting was done on September 29th at Blacksburg and September 15th at Orange.

Field Measurements and Harvesting

Plant heights were recorded from each plot before harvesting. Four measures were recorded randomly with measuring sticks. Seed harvests from seed/dual purpose plots were determined by when bracts at the bottom of the inflorescences began opening, releasing mature seed (i.e., initial stage of shattering). Fiber from dual purpose plots (cv Joey) was harvested in conjunction with seed harvest. Plots of dedicated fiber (cv EcoFibre) were harvested based on flowering stage. Harvesting was done by hand using a garden shear at 5 to 6 cm height above the ground. All plants from within three, 0.25-m^2 quadrates (75-m^2 total area) placed randomly within each plot were harvested and composited. After harvest, the numbers of male and female plants were counted, and the fresh weights of the composited samples were recorded. With plants under seed or dual-purpose management, inflorescences were cut at the approximate base of the inflorescence to separate from the fiber stalk.

Post-Harvest Processing

Fiber stalks were dried at 45 °C in a forced draft dryer and seed heads were air-dried in the greenhouse. Dry weights were recorded for both fiber and seed samples and was used to determine the total biomass. Seed was separated from the inflorescences by hand and a sieve was used to remove the stems, twigs, and leaves. The seed was separated from the chaff using a vacuum cleaner and a homemade seed separation chamber. Seed and chaff weights were recorded, and seed weights were used to calculate seed yield.

Statistical Analysis

Data were statistically analyzed using proc mix model ANOVA in SAS Studio (3.8, Cary, NC, USA) to determine the effects of tillage and production systems on plant heights, plant population density, biomass yield, seed yield, and biomass per plant. Data were analyzed separately by year due to the inclusion of fiber type cultivar and the difference in production systems tested. Tillage and production systems were considered fixed effects while replicate was considered a random effect. LS-means and Tukey's adjusted differences were calculated. Results were considered statistically significant at $P < 0.05$ and considered as trend at $P>0.05$ to $P<0.1$. Regression analysis was used to determine the biomass yield and seed yield. A power regression model was used where plant population density was included as an independent variable, and dependent variables were biomass yield and seed yield.

RESULTS DISCUSSION

Weather Data

Growing season averages and totals are provided in Table 3-2 with distribution patterns in Figure 3-1. In general, growing conditions were moderate, but cooler in Blacksburg than in Orange and with Blacksburg having more variable precipitation across the three growing seasons.

Table 3 - 2: Growing season averages, maximum and minimum temperature and total precipitation for the study site Blacksburg, VA, and Orange, VA, in 2020, 2021, and 2022.

Year	Site	Seasonal Temp. (°C)			Seasonal Precip. (mm)
		Mean	Max	Min	Total
2020	Blacksburg	22.8	28.5	17.1	710.0
	Orange	25.1	30.6	19.7	465.0
2021	Blacksburg	21.9	28.2	15.5	250.0
	Orange	24.6	30.5	18.7	357.6
2022	Blacksburg	22.6	29.0	16.1	160.0
	Orange	24.0	29.2	18.8	525.0

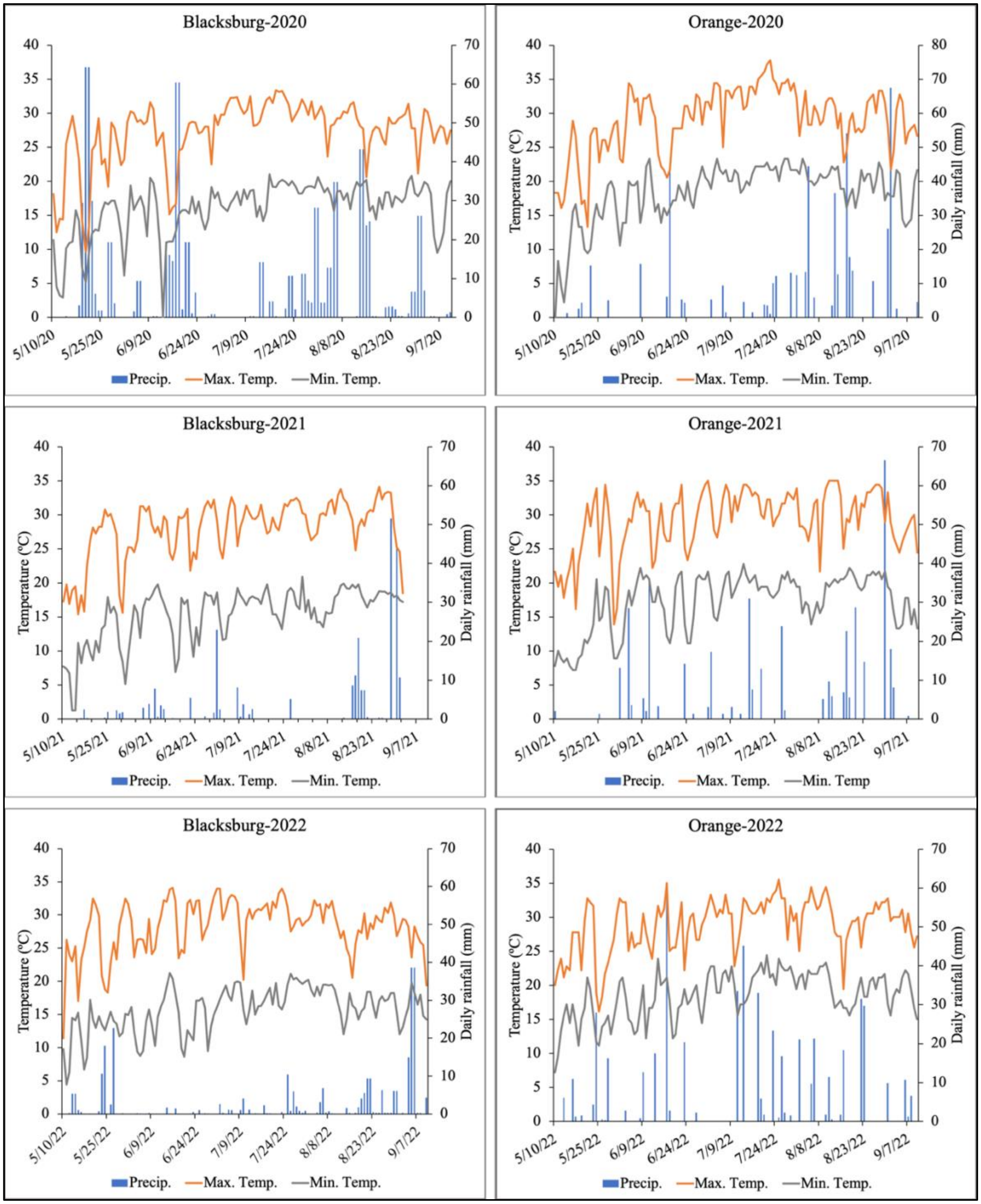


Figure 3 - 1: Daily maximum and minimum temperature and precipitation for the study site Blacksburg, VA, and Orange, VA, in 2020, 2021, and 2022.

Plants Height

Plant height was not affected ($P \geq 0.1233$) by tillage, and there was no interaction observed between tillage and production systems ($P \geq 0.2570$). However, a significant location × tillage interaction ($P = 0.0104$) was observed in 2020, with Joey planted for seed being taller with tillage at Orange and not different by tillage in Blacksburg. However, no tillage management × location interactions ($P \geq 0.3152$) were observed in 2021 or 2022. Plant height varied only by location in 2022 ($P < 0.0001$; Figure 3-2), whereas no variation was observed by location in 2020 and 2021. Plant height was shorter at Blacksburg compared to Orange ($P < 0.0001$).

Production system (seed, dual purpose, or fiber) had significant effects ($P \leq 0.0002$) on plant height throughout the study (Figure 3-2). In 2020, for seed production, the cultivar Joey produced taller plants (1.16 m) compared to plants managed for dual use production (1.00 m) ($P = 0.0002$). The shorter plant heights for Joey under fiber management suggest high seeding rates created more competition and reduced plant growth. This response in 2021 and 2022 was necessarily confounded by use of the taller fiber cultivar. EcoFibre produced taller plants (2.65 m) than Joey (1.02 m), as it continued its vegetative growth until the end of the harvest period. This cultivar originates from a southern latitude. Growing at a more northern latitude with longer day lengths supports greater height and biomass yield by delaying exposure to short nights, thus allowing the plant to continue its vegetative phase until the harvest period.

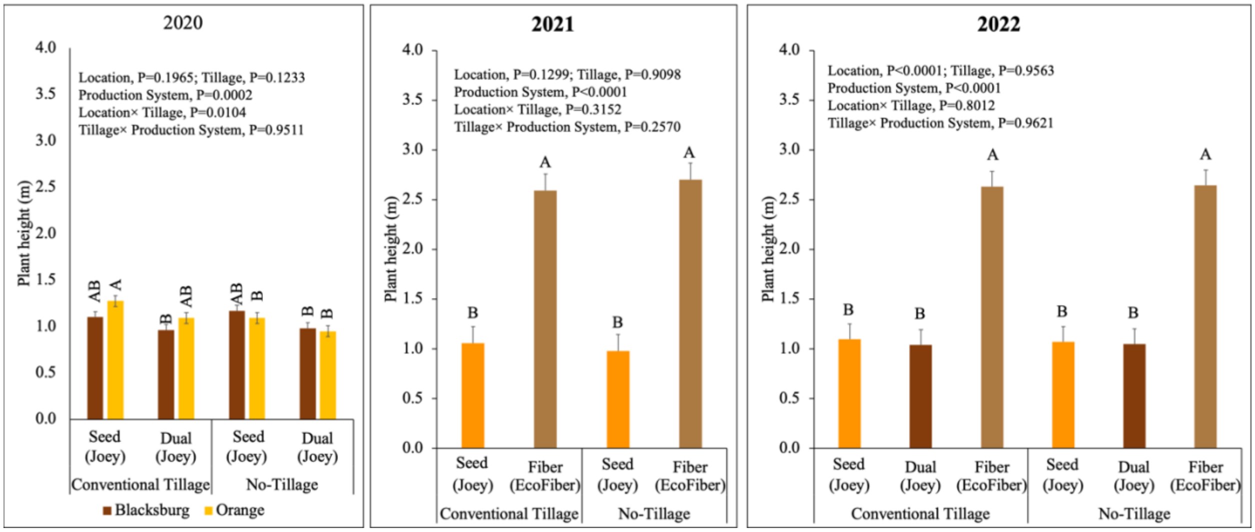


Figure 3 - 2: Mean plant height of different cultivars of Hemp under conventional tillage and no-tillage at harvest time in 2020, 2021, and 2022 in Blacksburg, and Orange, VA. Bars with different letter designations differ by production systems under both tillage ($P<0.05$).

Plant Population

Conventional tillage resulted in greater ($P \leq 0.0161$) plant population compared to no tillage for all production systems in each year (Figure 3-3). However, seeding rates did not result in planned populations (Table 3-1) in any treatment combination. In 2020, while the seeding rate for the dual purpose production system was double that of the seed production system (56 vs. 28 kg ha^{-1}), the established plant population was about five 5 times greater under fiber system (192 vs. 37 plants m^{-2}; $P < 0.0001$) with conventional seedbed establishment, and about three times greater when established using no-till (103 and 34 plants m^{-2}; tillage × production system interaction; $P = 0.0007$) (Figure 3-3).

Plant population density did not differ ($P = 0.2336$) by production system in 2021. Average plant population density across both production systems was 47 plants per square meter under conventional tillage and 29 plants per square meter under no tillage, which when calculated as a percent of planned population density ranged from 42% (in a seed production system) to 13% (fiber production). The lower plant population for EcoFibre, despite having twice the seeding rate compared to Joey grown for seed. might be attributed to the low seed vigor of EcoFibre. Preliminary work in our lab suggests that even if seed germination percent is acceptable, establishment can be reduced by low seed vigor.

In 2022, the greatest plant populations ($P < 0.0001$) were observed for conventional and no-till fiber (EcoFibre) production (123 and 88 plants m^{-2}), which were 55 and 40% of the targeted population. Populations of Joey as a dual-purpose crop were intermediate (73 and 57 plants m^{-2}), and were only 33 and 26% of desired populations. For seed production, plants per area (40 and 27 plants m^{-2}) under conventional and no tillage, respectively, were 36 and 24% of desired plant populations. Although the seeding rate was the same for fiber and dual-purpose production systems, a higher plant population density was observed for EcoFibre. This may in part be associated with using a new, higher vigor seed lot, or the difference might also be because of the larger seed size of the EcoFibre cultivar (Mi et al., 2020).

However, no hemp system × tillage management interactions ($P \geq 0.4926$) were observed for plant population density in 2021 or 2022. Additionally, there was no interaction between location and tillage ($P \geq 0.1920$) on plant population throughout the study. However, across tillage and production systems, plant populations were greater ($P \leq 0.0094$) at Blacksburg in 2020 (107 vs. 76 plants m^{-2}) and 2022 (97 vs. 39 plants m^{-2}) and plant population was greater ($P < 0.0001$) at Orange in 2021 (45 vs. 31 plants m^{-2}).

Field establishment, in terms of plant population density, is an important aspect to consider when evaluating the suitability of conventional tillage or no till systems for different production systems (seed, dual, or fiber). Results of this study suggest that conventional tillage is more favorable for achieving desired plant population density across various production systems. However, no tillage may be acceptable for dual or fiber production systems where a higher seeding rate can ensure an adequate plant population density. It is important to consider the additional cost associated with a higher seeding rate, which might be offset by cost savings from adopting no tillage practices where there is no expense for field cultivation.

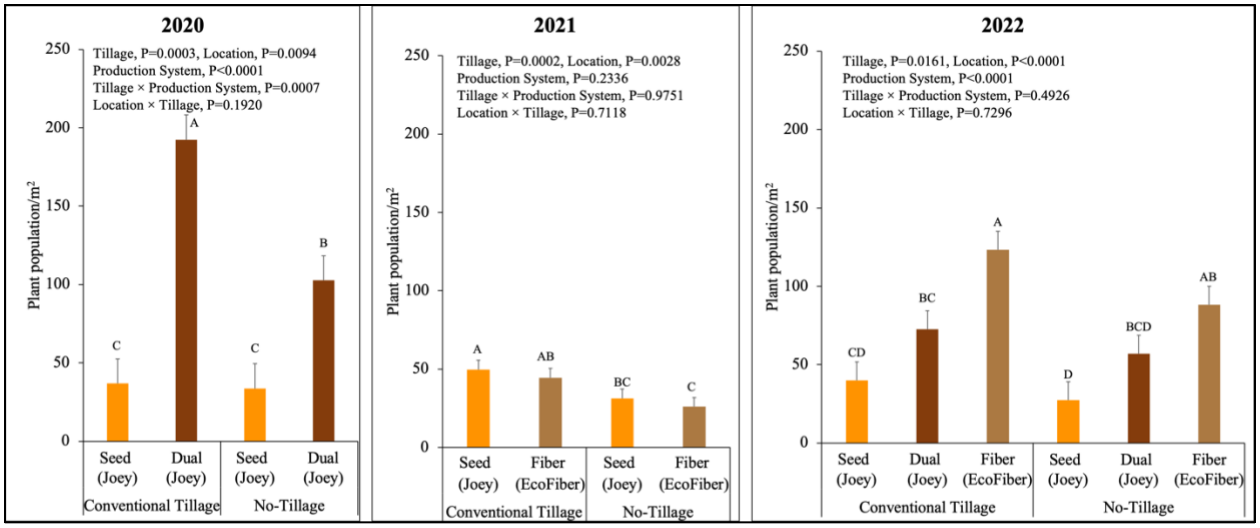


Figure 3 - 3: Mean plant population density (plants/m^2) of different cultivars of Hemp under conventional tillage and no-tillage at harvest time in 2020, 2021, and 2022 in Blacksburg and Orange, VA. Bars with different letter designations differ by production systems under both tillage management ($P < 0.05$).

Biomass Yield

Tillage management did not affect ($P \geq 0.7152$) biomass yield in any year. Biomass yield in fiber systems was greater in 2021 and 2022 ($P < 0.001$) when a true fiber variety was tested, but not in 2020 ($P = 0.9434$), when a single, dual purpose cultivar was used for both systems. No interactions ($P \geq 0.2528$) were observed between tillage and production systems or tillage and location in relationship to biomass yield in any growing season (Figure 3-4).

In 2020, the average biomass yield of the hemp cultivar Joey, across tillage management and production systems was 3.2 Mg ha^{-1} ($P = 0.5459$). In 2021, averaging across the two tillage managements, EcoFibre planted for fiber production yielded 18.3 Mg ha^{-1} compared to 3.9 Mg ha^{-1} for Joey, planted for seed production ($P < 0.001$). In 2022, Joey produced similar amounts of biomass (4.8 Mg ha^{-1}) regardless of tillage management and production systems, but this was more than three times less biomass ($P <0.001$) than produced by EcoFibre (18.3 Mg ha^{-1}; Figure 3-4).

Despite variations in plant population density among tillage systems, no differences ($P \geq 0.7152$) in biomass yield were observed between the different tillage systems. This speaks to the adaptive morphology of hemp. Plants with lower population density tended to produce greater biomass by developing more branches and thicker stems. For fiber production systems, achieving a higher plant population density is desirable as it can reduce branching and result in the formation of a great number of higher qualities bast fibers. However, fibers quality was not assessed in this study.

Factors such as the market prices of fiber, seed, and biomass must also be considered when evaluating the suitability of different tillage and production systems. If the goal is only biomass production, both tillage systems have similar potential to produce comparable amounts of dry matter. However, for high-quality fiber production traditional tillage management may remain preferred for such systems.

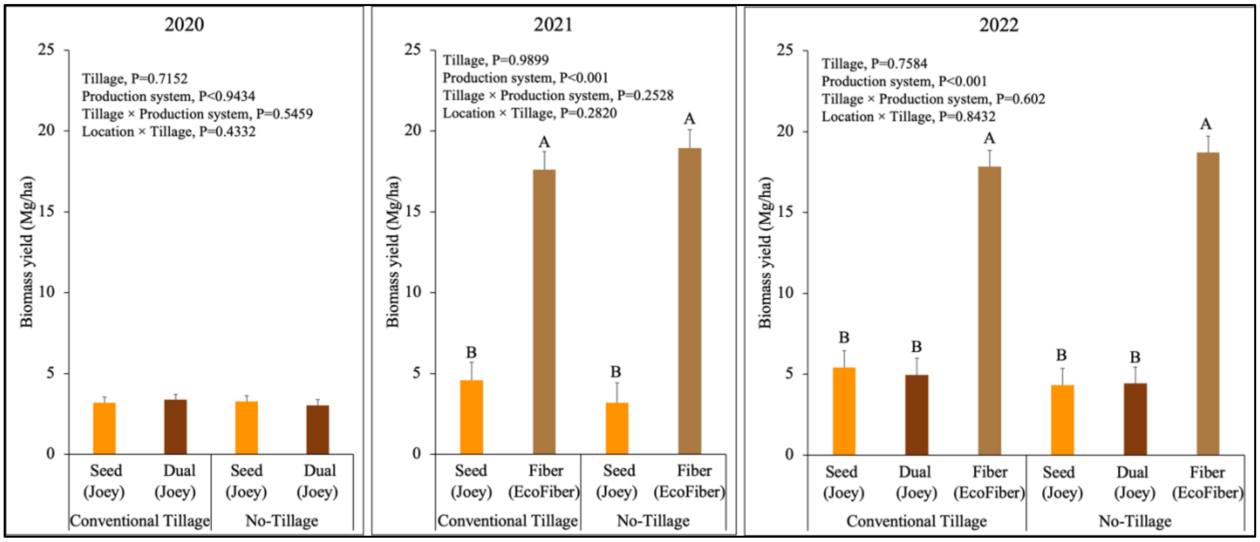


Figure 3 - 4: Mean biomass yield (Mg/ha) of different cultivars of Hemp under conventional tillage and no-tillage at harvest time in 2020, 2021, and 2022 in Blacksburg and Orange, VA. Bars with diff. letters designations differ by production system under both tillage management.

Yield Per Plant

Biomass yield per plant was influenced by production systems in 2020 and 2021 ($P \leq 0.001$; Figure 3-5). In 2020, plants in seed production systems had greater biomass per plant (9.9 g) compared to plants under dual-purpose management (2.6 g). In 2021, fiber systems had greater biomass per plant (70 vs. 10 g plant^{-1}) compared to seed production systems. EcoFibre is a subtropical cultivar and had greater mass per plant than Joey, a Canadian-derived dual-purpose cultivar. However, in 2022, there was no effect (*P = 0.1253*) of production system on biomass yield per plant. In 2021, biomass yield per plant was affected by tillage system (*P = 0.0042*). EcoFibre exhibited greater (*P < 0.0001*) biomass per plant (42 g and 86 g) compared to Joey (9 g and 13 g) under both conventional and no-tillage systems. Both cultivars produced higher (*P = 0.0042*) biomass per plant in the no-tillage system compared to the conventional tillage system, which may be attributed to the lower plant population density, and thus increased branching, and thicker stalks. In 2022, tillage (P = 0.4671) or production system (P = 0.1253) had no significant effects on biomass yield per plant. Biomass yield per plant is highly influenced by plant population density in hemp fields. To facilitate mechanical harvesting and promote slender and less branched plants, however, it is important to maintain a higher plant density in fiber systems regardless of tillage regime (Roseberg et al., 2019).

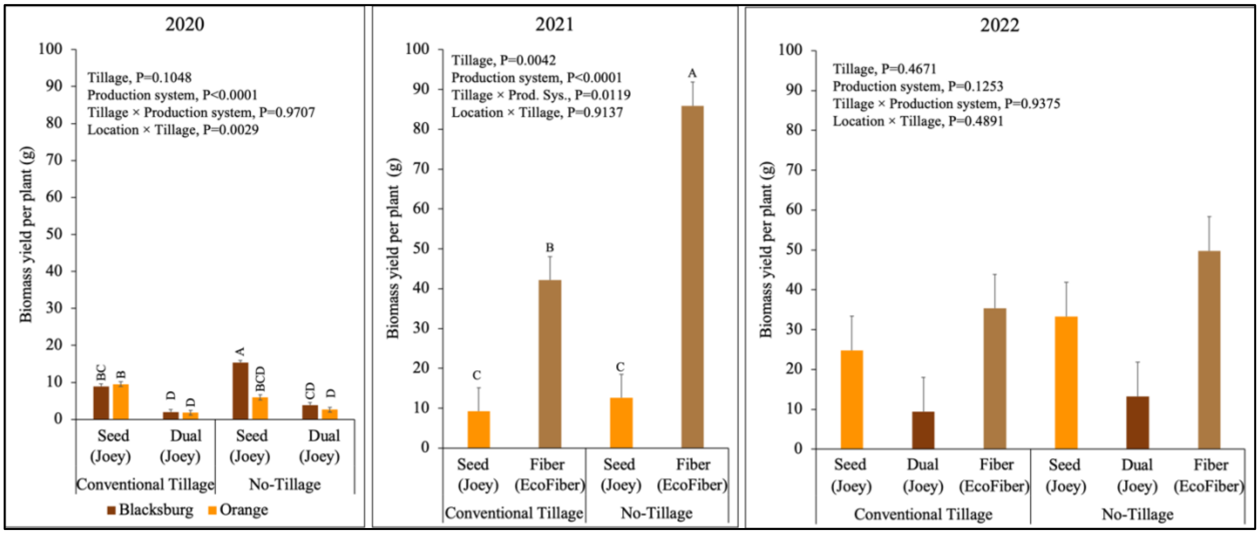


Figure 3 - 5: Mean biomass yield per plant of different cultivars of Hemp under conventional tillage and no-tillage at harvest time in 2020, 2021, and 2022 in Blacksburg and Orange, VA. Bars with different letter designations differ by production system under both tillage management (*P<0.05*).

Seed Yield

No interaction was significant for seed yield by location × tillage ($P \geq 0.0797$), nor by tillage × production system ($P \geq 0.3455$) in any year (Figure 3-6). In 2021, seed yield in the seed production system was greater ($P = 0.0292$) with conventional tillage (1180 kg ha^{-1}) than no tillage (920 kg ha^{-1}). However, tillage management did not affect seed yield in 2020 ($P = 0.0678$) or 2022 ($P = 0.6742$). Seed yields averaged across the two tillage managements and two production systems was 910 kg ha^{-1} in 2020 and 1090 kg ha^{-1} in 2022.

Despite large differences in planting rate, seed yield did not differ ($P \geq 0.1897$) when Joey was grown solely for seed or as a dual-purpose crop (seed or seed + fiber production). Tillage effects were limited, and inconsistent, and generally no-tillage systems may hold promise for seed production.

This may be possible in part because lower plant populations may result in individual plants with greater branching that can produce similar seed yield compared to conventional tillage management that supports greater plant. To facilitate harvesting, slender plant stalks are desired (Roseberg et al., 2019) and optimizing the plant population density at both tillage regimes could be challenging for the adoption of hemp seed production systems in both tillage regimes.

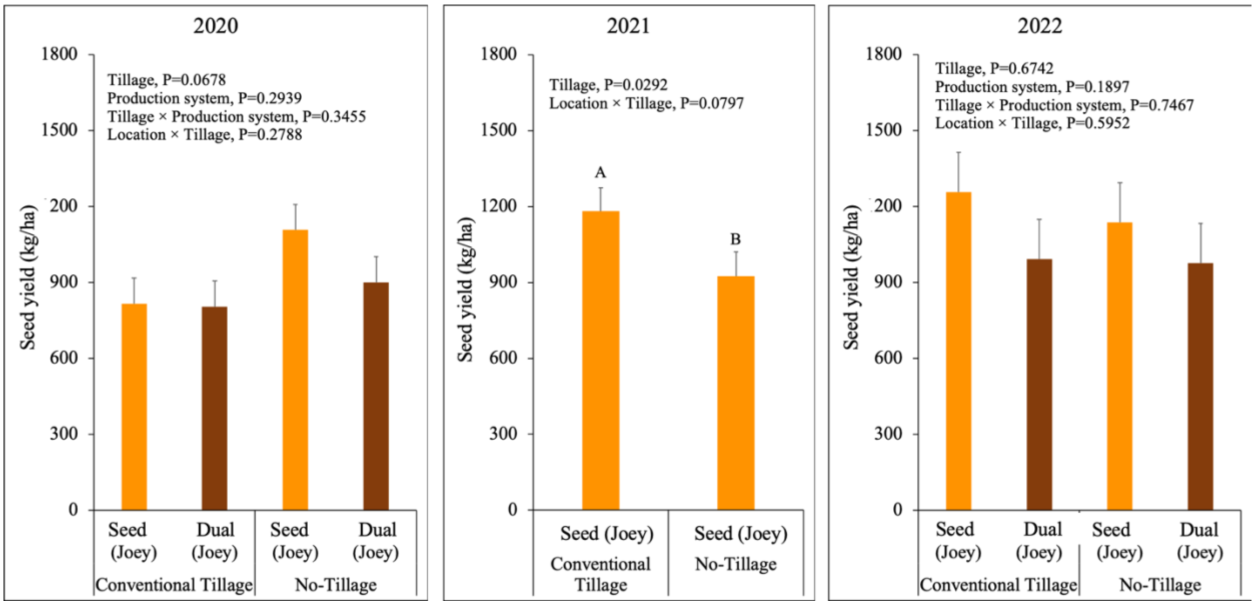


Figure 3 - 6: Mean seed yield of different cultivars of Hemp under conventional tillage and no-tillage at harvest time in 2020, 2021, and 2022 in Blacksburg and Orange, VA. Bars with different letter designations differ by production system under both tillage systems ($P < 0.05$).

Estimating biomass and seed yield

Regression analyses for hemp cultivars EcoFibre and Joey indicated that biomass yield per plant responded in a negative power relationship to increasing plant population (Figure 3-7). Plant mass fell rapidly between about 10 and 40 plants m^{-2} for EcoFibre and between 10 and about 25 plants m^{-2} for Joey. These non-linear models explained a substantial proportion of the data for both hemp types ($R^2 = 0.8255$ for EcoFibre and 0.7243 for Joey). The model of seed yield in relationship to plant population followed a pattern similar to biomass yield for Joey, but with a weaker relationship coefficient of determination ($R^2 = 0.5324$).

Upper limits of production per plant were not established for EcoFibre and Joey, but the maximum mass per plant observed in these studies was about 25 g plant^{-1}. On that basis, and assuming that mass per plant limit would be reached at about 5 plants m^{-2}, hemp could theoretically achieve maximum yield at a planting rate of about 1.5 kg seeds ha^{-1}.

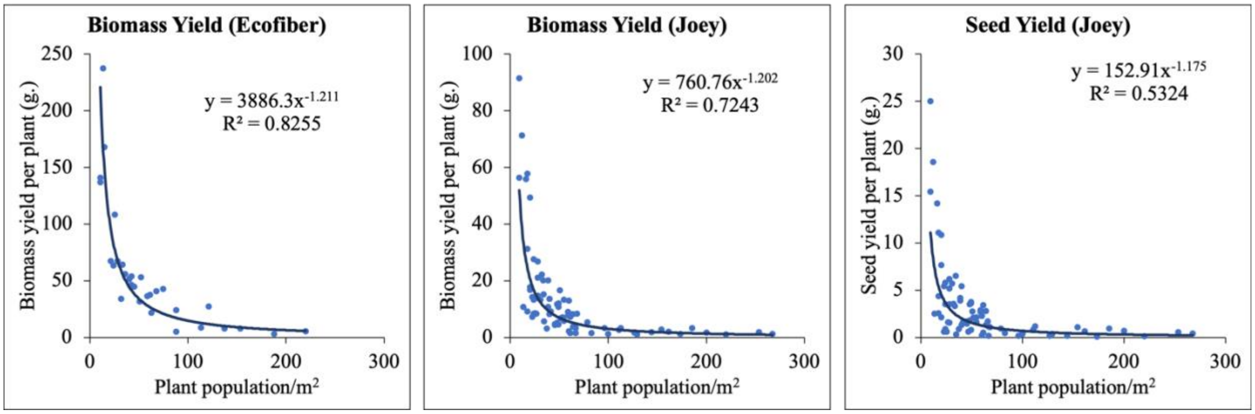


Figure 3 - 7:The biomass yield per plant vs. plant population density for the hemp cultivar EcoFibre and Joey, and seed yield vs. plant density for Joey under conventional tillage and no-tillage 2020, 2021, and 2022 at Virginia Tech's Urban Horticulture Center and Kentland Farm in Blacksburg, VA and the Northern Piedmont Center in Orange, VA.

These results clearly show the importance of plant population to individual yield. Optimizing plant population for a given region will also require understanding differences in growth among cultivars and the interaction of this genetic capacity with environmental factors such as soil type, nutrient availability, latitude and climate.

CONCLUSION

Industrial hemp cultivation is gaining increasing interest due to its versatile applications. Understanding the impact of different agronomic practices on productivity is crucial. This study examined the effects of tillage and production systems on plant height, population density, biomass yield, and seed yield of hemp, which varied across the study years.

Tillage systems had a significant impact on field establishment, with no-tillage systems having 9% to 47% lower plant population in 2020, 37% to 42% lower in 2021, and 21% to 32% lower in 2022 compared to establishments following conventional tillage. Despite these effects, tillage systems generally did not affect hemp plant height, biomass yield, or seed yield.

Fiber-type cultivars originating from low latitudes produced taller plants and greater biomass. Although lower plant population density could lead to greater individual biomass and seed yield, harvest considerations (e.g., ability to combine or ease of chopping) and post-harvest processing needs (i.e., limited branching for longer fibers) suggest higher populations are needed to facilitate mechanical harvest and process quality standards.

Although this study tested the effects of tillage management and production systems, these results clearly show the importance of optimizing the productivity of plant populations relative to individual yield. Further research is needed to gain a greater understanding of differences in growth among cultivars and the interaction of a cultivar’s genetic capacity with environmental with factors to optimize hemp outputs per unit of input.

CHAPTER IV

HEMP SEED YIELD AND QUALITY RESPONSES TO HARVEST TIME

ABSTRACT

Industrial hemp (*Cannabis sativa* L.) seed is of great interest given its unique and healthy nutritional characteristics. However, seed yields are less than other, traditional commodity seeds, in part due to uneven ripening and substantial seed shattering. Information regarding optimal hemp harvest timing that maintains yield and quality for different cultivars is needed. This study aimed to determine the optimum harvest time for seed yield for two hemp cultivars. ‘Joey’, a dual-purpose seed and fiber variety, and ‘Grandi,’ a seed variety, were established in mid-May and early June in the Ridge/Valley (Blacksburg) and Northern Piedmont (Orange) eco-physiographic regions of Virginia in 2021 and 2022. Plants were harvested four times at one-week intervals starting in mid-summer. Seeds were hand-separated from the plants to measure seed yield. Harvest date significantly affected seed yield ($P \leq 0.0005$) *at both sites, Blacksburg and Orange, in both year 2021 and 2022*. Seed yields differed by cultivar × date interaction ($P = 0.001$) in 2022 at the Orange site. In Blacksburg, seed yields were greatest at the second harvest each season (July 22, 2021 and July 25, 2022). In Blacksburg, seed yields were substantially lower in 2022 due to drought (1750 vs. 480 kg ha^{-1}). In the Orange site, planting occurred late in 2021 with harvests also deferred until August 17, 2021 and Seed yields were greatest in that first harvest (1180 kg ha^{-1}). In 2022, yields at the Orange location were greatest for Grandi at the first harvest (July 21; 1510 kg ha^{-1}) and for Joey at the second harvest (July 28; 1280 kg ha^{-1}). Over the subsequent weeks of harvest, yields drastically declined (16 to 41% in 2021 and 27 to 47% in 2022 in Blacksburg; 52% to 91% in 2021 and 28% to 65% in 2022 in Orange, compared to the highest yields). Harvest timing is critical to achieving optimum seed yield, and it varies by cultivar, eco-physiographic location, and weather (e.g., rainfall). Fatty acids (FA) varied by Cultivar, location, and harvest timing, but patterns of response were not consistent across FA. Gamma-linolenic ($P \leq 0.002$) and Oleic ($P \leq 0.023$) acids were generally greater in Joey and Arachidic acids were greater ($P \leq 0.013$) in Grandi. Stearidonic acids declined ($P \leq 0.0034$) with a later harvest date.

INTRODUCTION

Industrial hemp seed has potential for development as a commodity crop given its high nutritional value and consumer acceptability. Depending on the cultivar and production system, hemp may produce over 1100 kg ha^{-1} under dryland conditions, and over 3360 kg ha^{-1} with irrigation (Visković et al., 2023). However, seed shattering can lead to significant losses in seed yield (Ascrizzi et al., 2019 and Amaducci et al., 2008).

Both dioecious female and monoecious hemp plants produce terminal inflorescences. Seeds develop within bracts that bind the seeds to the inflorescences. Typically considered hemp seeds are actually achenes. Ripening seeds mature from the bottom which flowers first to the top of the inflorescence. Because of the uneven ripening, both within and among hemp plants, some seeds may not have begun filling or maybe at the dough stage when the first seeds reach maturity and begin to shatter out.

Seed shattering in hemp can be affected by numerous genetic and environmental factors. Shattering likely is a heritable genetic trait (Liu et al., 2019), thus some cultivars are more likely to shatter than others. In general, hemp seed production systems utilize two types of cultivars:

short-statured seed types, grown specifically for seed, and larger dual-purpose varieties grown both for seed and fiber. Most available seed cultivars also have photoperiod sensitivity which plays a remarkable role in seed development (Jeff and Williams, 2019) and can further complicate variety selection. Thus, optimizing harvesting time for photoperiod sensitive cultivars can be challenging, as harvesting time may follow the specific calendar time frame (based on photoperiod) rather than the number of days after planting. When cultivars originating at northern latitudes are grown at southern latitudes, those plants will begin reproductive development and maturation earlier, leading to earlier shatter. In addition to these factors, flowering and timing of seed maturation may also be influenced by fertility stress, weed pressure, and moisture stress as those stress can trigger earlier flowering in hemp plants (Jeff and Williams, 2019).

The timing of seed formation may also affect seed size. Generally, the early-forming seeds at the bottom of the plants are larger than the later-forming seeds at the top. Harvesting early enough to prevent shattering losses of early-forming seeds may result in lost yield due to the underdevelopment of later-formed seeds. On the other hand, a significant portion of early-formed seeds can be lost due to shattering if harvesting time is delayed in order to capture later-maturing seeds. Thus, maximizing seed yield requires optimizing the time of harvest. Typical recommendations are to harvest seed hemp at 70% of seed maturation (Williams and Mundell, 2016). Although it is challenging to quantify when the plant reaches 70% seed maturity, most of the extension guidelines suggest that early-forming seeds at the bottom of the inflorescence begin shattering (Cherney and Small, 2016; Miller, 1991). However, there is limited information regarding optimum harvesting time frames for the specified seed and dual-purpose hemp cultivars in the mid-Atlantic.

Along with yield, hemp seed quality, particularly fiber, protein and fatty acid concentrations, can be influenced by harvest timing, cultivar, and location (Marzocchi and Caboni, 2020). Hemp seeds are considered potentially more valuable than other commodity seeds because they contain unique fatty acids of value for human nutrition. Hempseed oil contains high concentrations of polyunsaturated fatty acids (PUFAs), which constitute over 80% of its composition. Hemp also has potential value as a source of two essential fatty acids (EFAs), namely linoleic acid (18:2 omega-6) and alpha-linolenic acid (18:3 omega-3; Marzocchi and Caboni, 2020). The omega-6 to omega-3 (n6/n3) ratio in hempseed oil typically falls within the range of 2:1 to 3:1, although ratios as high as 5:1 are reported (Lan et al., 2019). The high omega-6 to omega-3 ratio is widely acknowledged as optimal for human health (Callaway, 2004). Hemp also contains stearidonic acid (18:4n-3) and γ-linolenic acid (18:3n-6), two unique fatty acids found in only a limited number of species (e.g., evening primrose (*Oenothera biennis*) and borage (*Borago officinalis*)) that have limited production potential (Zanetti et al., 2013). Hemp seed oil quality can be impacted by harvest time as saturated fatty acid concentrations are observed to increase with seed maturity (Marzocchi and Caboni, 2020), but there is little exploration of these effects in the mid-Atlantic region.

We hypothesized that harvest timing would have significant effects on seed yield because of uneven ripening and that this would vary by hemp cultivar. Thus, the objective of this study was to determine the effects of harvesting time on seed yield of commercially available hemp cultivars. The objectives also included to evaluate the seed fatty acids as a function of harvest timing.

MATERIALS AND METHODS

Hemp cultivars

Two cultivars representing different growth traits were tested. Commercially available seed (cv ‘Grandi’) and dual-purpose (seed + fiber; cv ‘Joey’) varieties developed in Canada were obtained for this study. Grandi is a dioecious variety grown specifically for seed with food or feed as potential end uses. At 50° latitude, Grandi requires around 105 days to reach maturity and has a plant height of 0.8 to 1.3 m (Verve Seeds Solutions). Joey is a monoecious variety that requires 110 to 120 days to reach maturity at 45° latitude and has a mature height of about 1.58 m (Darby et al., 2021). However, required days to mature in experimental sites in Virginia (37° to 38° latitude) was unknown for both cultivars.

Field design

The experiment was conducted as a randomized complete block design with a repeated measurements arrangement and four replicates. Experiments in the Valley/Ridge province were conducted at Virginia Tech’s Urban Horticulture Center, Blacksburg, VA (37°13’06”N, 80°27’52’’W, 617 m elevation) in 2021 and at the university’s Kentland Farm, Blacksburg, VA (37°11’43”N, 80°34’47’’W, 528 m elevation) in 2022. Soils at the Urban Horticulture Center are Duffield Ernest complex (fine-loamy, mixed, active mesic Ultic Hapludalfs and Fine-loamy, mixed, superactive, mesic Aquic Fragiudults). Soils at Kentland are Unison and Braddock (fine, mixed, semiactive, mesic Typic Hapuldults). Experiments in the Piedmont province were conducted at the Northern Piedmont Center, Orange, VA (38°13’23”N, 78°07’09’’W, 154 m elevation). Soil at Orange were Frederick (fine, mixed, semiactive, mesic Typic Paleudults (Web Soil Survey, 2009). Daily maximum and minimum temperature and rainfall were downloaded from Virginia Tech WeatherSTEM Data Mining Tool (http://vt-arec.weatherstem.com) for the entire study period for 2021 and 2022 (accessed on 13 January 2023).

Field preparation and seeding

Sites were tilled and roto-harrowed to prepare a fine seedbed prior to planting. Seeds were drilling in at 1- to 2-cm soil depth using a Tye Drill. Plots were seeded May 15, 2021 and May 20, 2022 at Blacksburg and on June 2, 2021, and May 18, 2022, at Orange. The experimental sites were fertilized with 112 kg N ha-1 before seeding in 2021 and 2022. Broadleaf and graminaceous weed pressure was significant at the Blacksburg site in 2022 and post-emergence herbicides (Moxy (Octanoic acid ester of bromoxynil* (3,5-dibromo-4-hydroxybenzonitrile)@ 1.4 L/ha) and Poast (2-[1-(ethoxyimino)butyl]-5-[2-(ethylthio)propyl]-3-hydroxy-2-cycloxexen-1-one) (@ 1.4 L/ha) were applied 20 days after seeding. However, herbicides did not adequately control the weeds.

Harvest

First harvest decisions were made based on visual inspection. The first harvests occurred at about eight weeks following establishment and once bract turned brownish color and started opening from the bottom of the inflorescences. Three subsequent harvests occurred at one-week intervals. Hemp was harvested from within three, 0.25-m^2 quadrates (0.75 m^2, total) placed randomly within the plots. Before each harvest, plant heights were recorded. After harvest, male and female plants were counted to determine male:female plant ratio. Inflorescences were then separated from the fibrous stalks and both plant fractions were weighed fresh and again after drying in a greenhouse.

Seed separation

Seed and chaff were separated from the inflorescence by hand once the seed head was adequately dried. Chaff was separated from seeds by vacuum using a homemade seed winnowing chamber attached to a vacuum cleaner.

Biomass, seed yield, harvest index calculation

Dry matter content at harvest, dry biomass and seed yields, and harvest index were calculated from the weights of fresh and dry biomass and seed samples. Harvest index was calculated as (dry seed yield/dry total biomass yield) *100.

Fatty acid analysis

Lipids extraction

The lipid extraction methods employed in this study aimed to determine the fatty acid concentrations in hemp seeds harvested at different time intervals. For each treatment, 2 g of hemp seeds were carefully crushed using a mortar and pestle. Subsequently, 1 g of the crushed seeds were weighed and poured into a screw cap borosilicate tube along with 2 ml of isooctane. The resulting mixture was sonicated for 10 min in an ultrasonic bath operating at a working frequency of 20 kHz and an amplitude of 200 W and maintained 35°C. Following sonication, the tubes were covered and centrifuged for 5 minutes. The top layer of isooctane was then carefully pipetted off and transferred to a new test tube. To ensure preservation of the lipid and isooctane mixture, the tubes were flushed with nitrogen gas, sealed, and subsequently stored in a refrigerator at 4°C until methylation and fatty acid determination were conducted.

Methyl ester preparation

Methyl esters were prepared by combining 1 ml of the extracted lipids (including isooctane) with 1 ml of benzene and 1 ml of 10% BF_3-methanol in a 10-ml screw-cap tube. Samples were then heated in a boiling water bath for 60 min. After cooling to room temperature, 2 ml of H_2O was added to the tube. The top phase was carefully transferred to a new screw-cap tube, and 1 to 2 g of anhydrous sodium sulphate was introduced to absorb any excess water present in the solution. Following a 10-minute incubation period, the top phase was pipetted and transferred to a GC vial for subsequent analysis.

Gas Chromatography–Mass Spectrometry (GC/MS) Analysis

Fatty acid methyl esters (FAMEs) in the extracted oil samples were analyzed using GC/MS instrumentation (GC-2010/MS TQ-8030, Shimadzu, Kyoto, Japan). Separation and identification of FAMEs were achieved using a polar column (Zebron, ZB-Wax plus) with specific dimensions (60-m length, 0.25-mm inner diameter, and 0.25-μm film thickness). Helium (99.999% purity) served as the carrier gas, with a total flow rate of 63.5 mL/min and a column flow rate of 1.22 mL/min. The linear velocity was set at 30.0 cm/s, and a split ratio of 50:1 was used.

The GC inject port temperature was maintained at 250 °C. The initial oven temperature was set at 175 °C and held for 5 minutes. Subsequently, the temperature ramp rate was set at 2.0 °C/min until reaching a final temperature of 225 °C. The final hold time at this temperature was 30 minutes. The ion source temperature and interface temperature were set to 230 °C and 200 °C, respectively. The acquisition mode for MS scans spanned from 40 m/z to 400 m/z, with a scan speed of 1250. The total runtime for the GC/MS analysis was 60 min. Each FAME sample (0.5 μL) was injected for analysis.

The obtained data were processed using equivalent chain length (ECL) calculations, and mass spectra comparisons were performed against a reference library (NIST) for identification purposes.

Data analysis

Data were analyzed with Proc Mixed in SAS Studio (3.8; Cary, NC) using a mixed model ANOVA. A repeated measures analysis was performed to investigate the impact of harvest time on plant height, biomass yield, seed yield, harvest index, and fatty acids profile. The data were analyzed both collectively and separately, first by location and then by year, to observe any potential interactions. The analysis employed a repeated measures design, specifying the variable Plot as the subject and utilizing an unstructured covariance structure to account for the dependencies within the measurements. Data were analyzed separately by year due to the inclusion of additional cultivar and the difference in harvesting times in 2022. Cultivar and harvest date were considered fixed effects while replication was considered a random effect. LS-means and Tukey’s adjusted differences were calculated. Results were considered statistically significant at $P < 0.05$.

RESULTS and DISCUSSION

Weather data

The mean daily temperature for the study site Blacksburg was 21.8 ºC and 22.4 ºC in 2021 and 2022, respectively; for the study site, Orange was 23.39 ºC and 23.92 ºC respectively (Figure 4-1). In 2021 and 2022, from the seeding to the first harvest date, the study site Blacksburg received total precipitation of 81.6 mm and 74.1 mm, respectively; and the study site Orange received total precipitation of 247.1 mm and 290.6 mm, respectively (Figure 4-1). In 2022, the rainfall at the Blacksburg study site was poorly distributed, with most precipitation occurring during the latter half of the study (Figure 4-1).

Visual observation

The hemp cultivar Grandi started flowering in early June, followed by Joey in the second week, in both 2021 and 2022. The plants remained green during the initial harvest but gradually changed to a yellowish color from the first to the fourth harvest (Figure 4-2). During the first harvest, matured seed bracts turned yellow and some opened bract observed though seeds remained attached. In the following weeks as the hemp seeds started to shatter, the presence of birds, particularly dove flocks, became notable in the hemp fields.

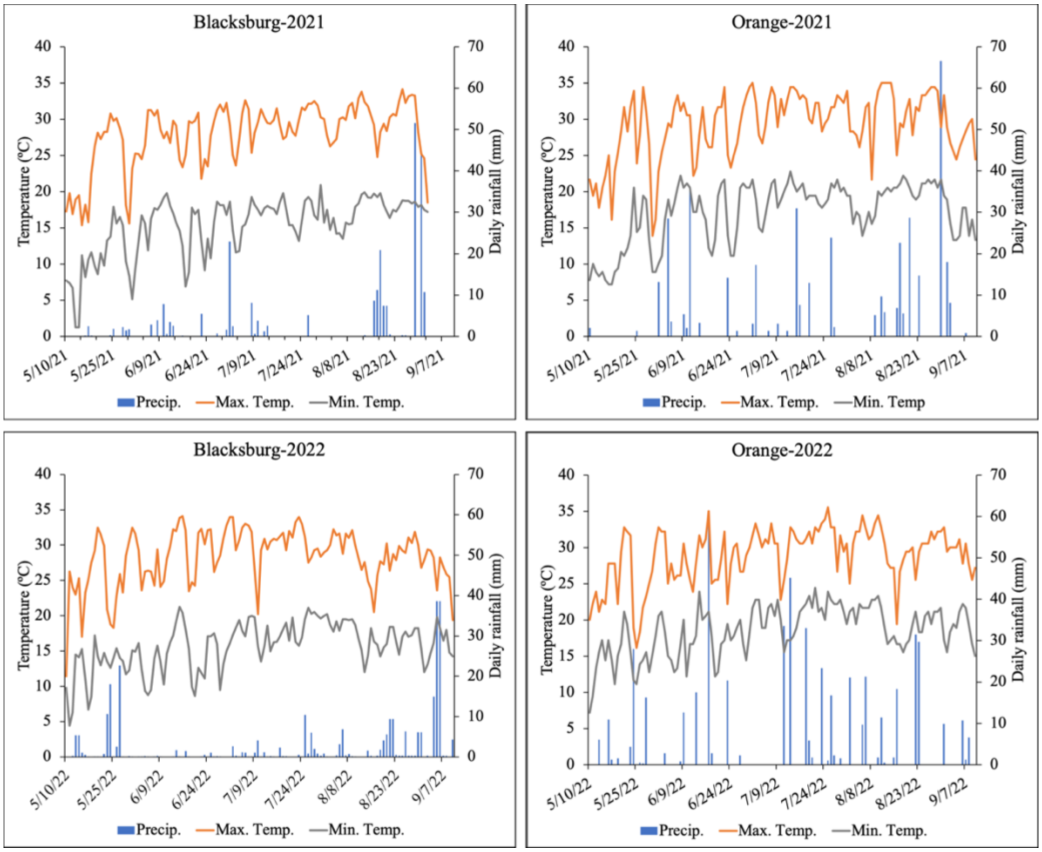


Figure 4 - 1: Daily maximum and minimum temperature and precipitation for the study site at Virginia Tech's Urban Horticulture Center and Kentland Farm in Blacksburg, VA and the Northern Piedmont Center in Orange, VA.

Figure 4 - 2: Hemp plants visual appearance during four harvest times at Blacksburg, VA in 2021.

Plants height

There was no significant interaction observed between harvest time and cultivar on plant height. Plant heights did not differ ($P \leq 0.4175$) among harvest dates in either year in both locations, but were shorter ($P < 0.0001$), for Grandi, the seed cultivar than for Joey, the dual-purpose variety (Figure 4-3). The average heights for Grandi and Joey were 1.03 and 1.41 m at Blacksburg and 0.70 and 1.10 m at Orange in 2021 respectively. In 2022, average heights for Grandi and Joey were 0.47 and 0.54 m, at Blacksburg and 0.75 and 1.24 m, respectively. Shorter heights in 2022 were driven by limited growing-season precipitation in Blacksburg (Site × Year Interaction, $P < 0.0001$).

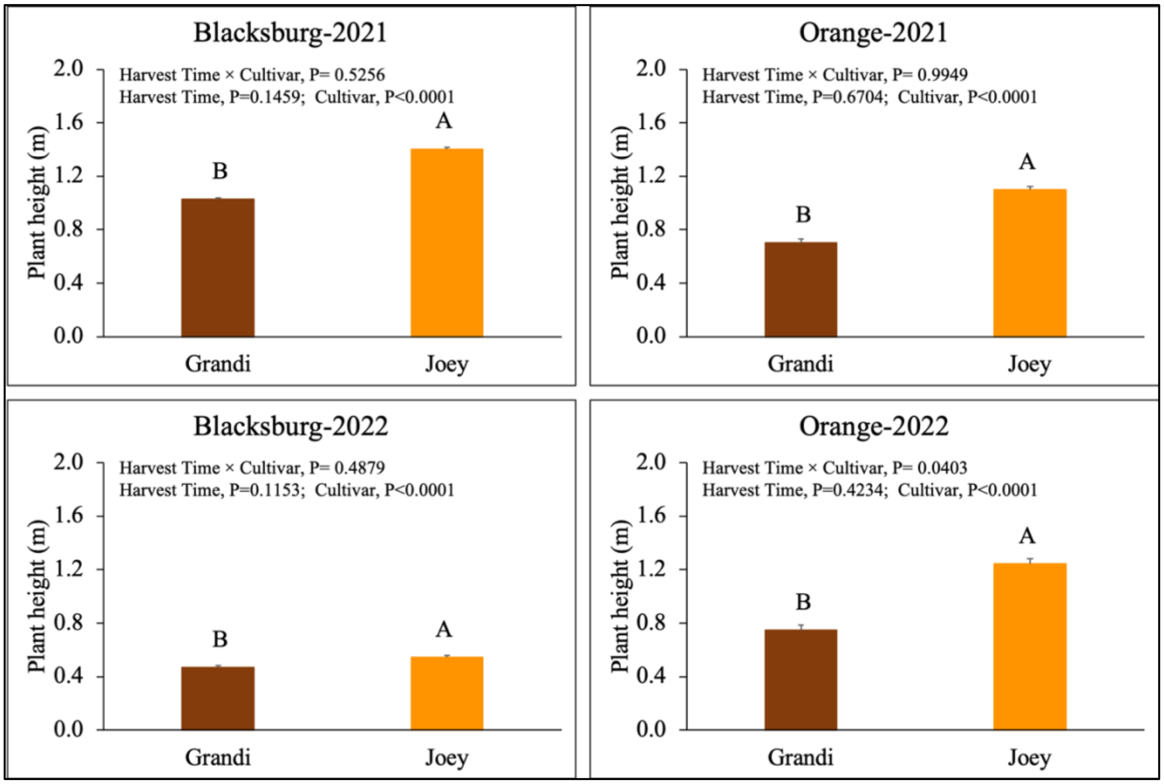


Figure 4 - 3: Mean plant height of two hemp cultivars (Grandi and Joey) at harvest time in 2021, and 2022; average across four different harvest times at two locations at Virginia Tech's Urban

Horticulture Center and Kentland Farm in Blacksburg, VA and the Northern Piedmont Center in Orange, VA. Bars with different letter designations differ by cultivar. (Limited in heights at Blacksburg in 2022 resulted in significant site x year interaction, $P < 0.0001$)

Biomass yield

Biomass yield varied by timing of harvest ($P \leq 0.0221$) and the cultivar ($P \leq 0.0073$), but there was no interaction between these factors. In Blacksburg, harvesting in late July, resulted in the highest biomass yield (Figure 4.4). Peak mean biomass yield was 6479 kg ha^{-1} for Grandi and 8344 kg ha^{-1} for Joey in 2021 and 1243 kg ha^{-1} for Grandi and 1380 kg ha^{-1} for Joey in 2022 at this site. Large year-to-year production variation at this site reflects differences in precipitation patterns from 2021 to 2022. In Orange, biomass yield was greatest at the earliest harvest each year (2750 and 4937 kg ha^{-1} on August 17, 2021 and 4263 and 5000 kg ha^{-1} on July 28, 2022 for Grandi and Joey, respectively), declining at subsequent harvests.

Biomass yield is correlated with plant height, stem diameter, and seed yield. Accumulation of biomass depends on the availability of soil moisture during the growing period, the origin of the cultivar, nutrient availability in the soil, weed pressure, plant population, etc. Plant biomass has limited influence on seed yield as some photoperiod-sensitive cultivars may produce greater biomass and lower seed because of delayed flowering. In this study, greater biomass across cultivars (7412 kg ha^{-1}) was observed at the Blacksburg location at second harvest on July 22 in 2021, which may be supported by better soil and available soil moisture. However, an opposite result-lower plant biomass yield (1312 kg ha^{-1}) was observed at second harvest on July 25 in 2022 because of the dry growing season (Figure 4-1). Declining biomass yield in the later harvest time could be related to the seed shattering and leaves senescence falling especially early matured male plants.

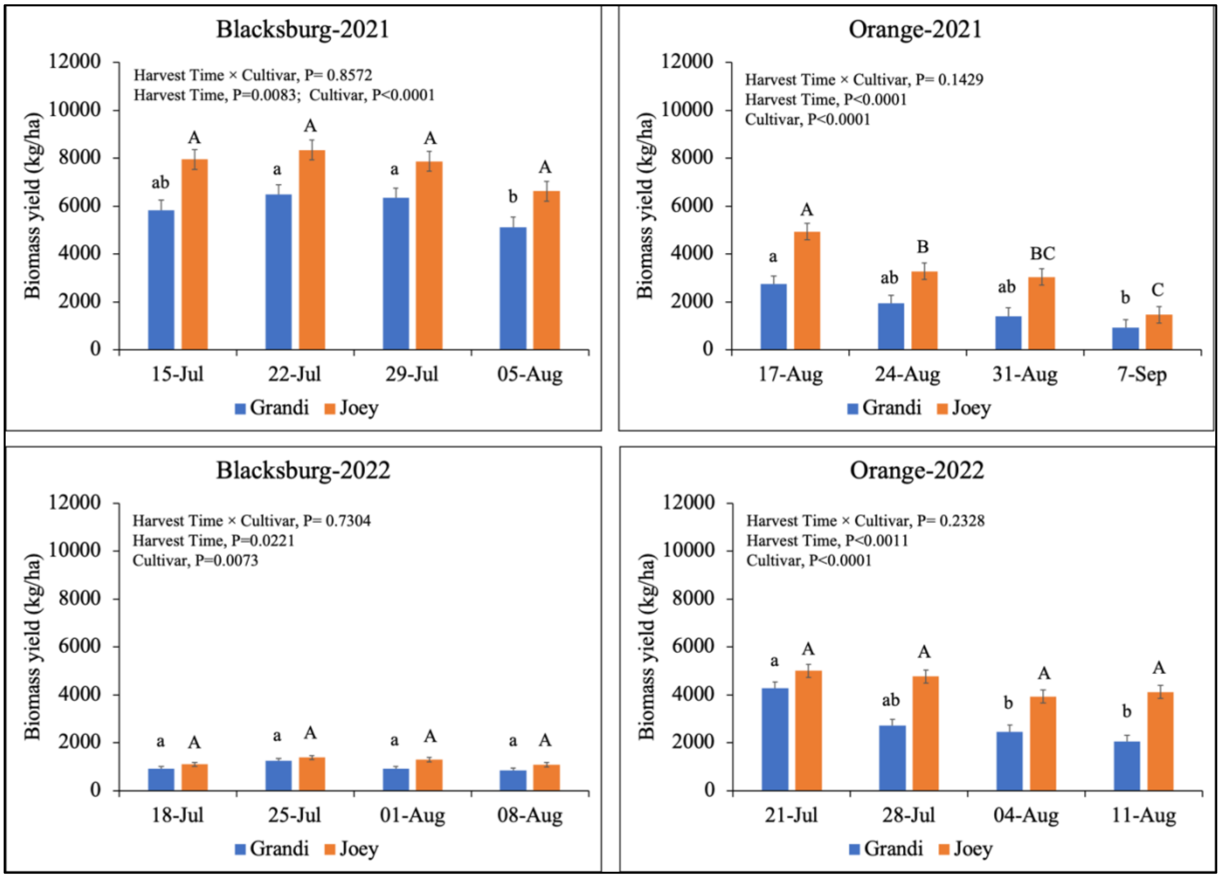


Figure 4 - 4: Mean plant biomass yield of two hemp cultivars (Grandi and Joey) at different harvest time in 2021, and 2022 at Virginia Tech's Urban Horticulture Center and Kentland Farm in Blacksburg, VA and the Northern Piedmont Center in Orange, VA. Bars with different letter designations differ by harvest time, lowercase for Grandi and uppercase for Joey ($P < 0.05$).

Seed yield

As with biomass, harvest date had a significant impact ($P \leq 0.0005$) on seed yield across all experimental locations and years (Figure 4-5). However, at Orange in 2022, cultivar × harvest date interaction (P=0.0010) was observed, as greatest seed yields for Grandi were measured at the earliest harvest and declined at each subsequent harvest time, while for Joey, yields were greatest at the second harvest time (cultivar × harvest date interaction; $P = 0.0010$). (Figure 4-4).

In 2021, greatest seed yields obtained in Blacksburg were 1680 and 1820 kg ha^{-1} for Grandi and Joey, respectively, measured on July 22; in 2022, yields were 460 and 490 kg ha^{-1} for Grandi, and Joey, measured on July 25. At Orange in 2021, the greatest yield, 870 and 1490 kg ha^{-1} for Grandi, and Joey respectively, was achieved at the first harvest on August 17, 2021. In 2022, the greatest seed yield for Grandi (1510 kg ha^{-1}) occurred at the first harvest on July 21, 2022, while for Joey, the greatest yield was 1280 kg ha^{-1}, observed a week later on July 28, 2022.

Later harvests resulted in drastic declines in yield. In Blacksburg, the decrease was 16% to 41% in 2021 and 27% to 47% in 2022. In Orange, the decline ranged from 52% to 91% in 2021 and 28% to 65% in 2022 (Figure 4-5).

Greatest seed yields (1680 kg ha^{-1} for Grandi and 1820 kg ha^{-1} for Joey in 2021) observed at the Blacksburg location likely reflect both soils better suited to hemp production, and greater available soil moisture. Better soil did not compensate for the lack of moisture in 2022, however. On the other hand, in Orange, which has a heavier clay soil that is challenging for hemp production, consistent rainfall across the 2022 growing season resulted in greater overall seed yields (1180 kg ha^{-1}). Lower seed yield at Orange in 2021 likely also reflects delayed planting (June 2, 2021). This reduced time for vegetative growth before flowering, which is generally a fixed time window given that these cultivars are photoperiod sensitive. Although not measured directly, declining seed yield with later harvests is attributed to seed shattering and wildlife predation.

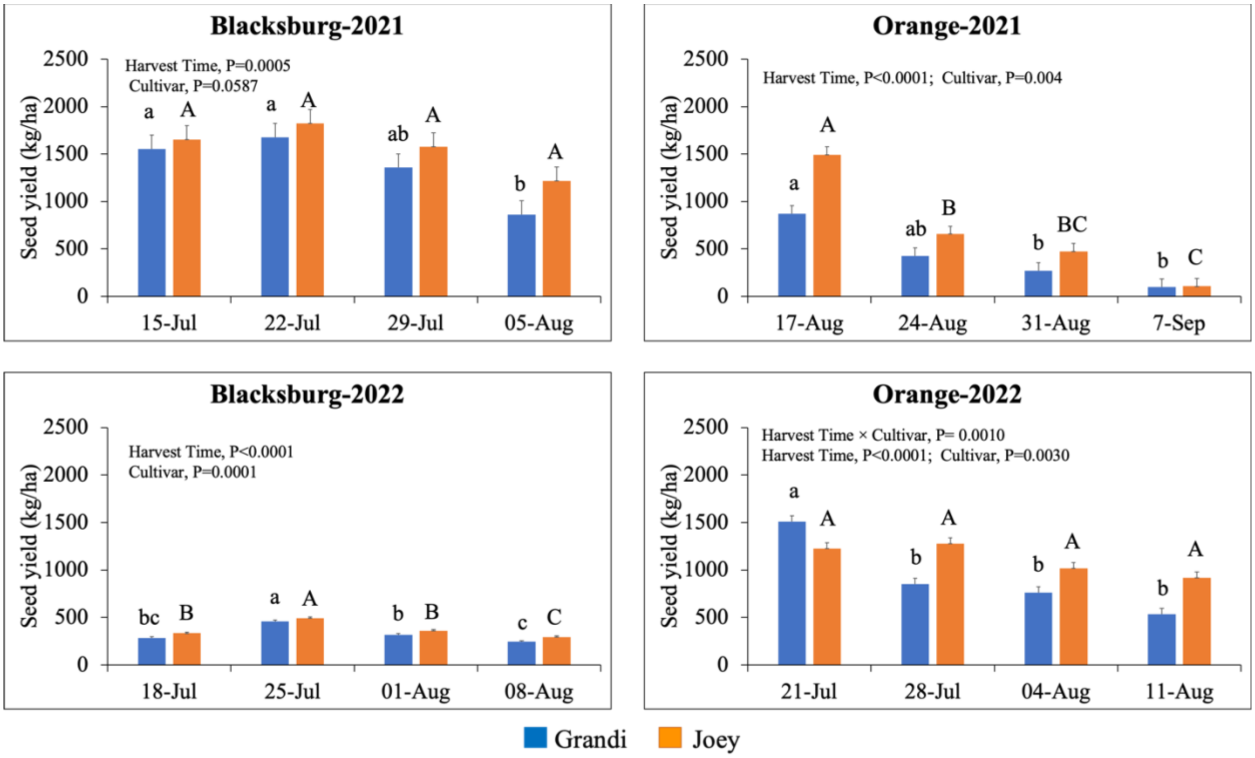


Figure 4 - 5: Mean seed yield of two cultivars Grandi and Joey at four different harvest time at Blacksburg and Orange, VA in 2021 and 2022. Bars with different letter designations differ by harvest time, lowercase for Grandi and uppercase for Joey (P < 0.05).

Harvest index

Hemp harvest index varied depending on the timing of harvest across all experimental locations and years (Figure 4-5). Differences ($P \leq 0.0140$) in harvest index were observed across all sites and years. Harvest index was greater ($P \leq 0.0037$) for Grandi at Orange during both years and at Blacksburg in 2022. Harvest index was also greater for Grandi at Blacksburg in 2021, but only at the first two harvests (cultivar × harvest time interaction; $P = 0.0027$).

Specifically, in Blacksburg, the harvest index was higher for the cultivars Grandi (26) and Joey (22) at the first harvest on July 15, 2021. The harvest index reached a greater value (37) in Blacksburg at the second harvest on July 25, 2022, and for Grandi (36) and Joey (25), at the first harvest in Orange on July 21, 2022. Subsequently, the harvest index declined in Blacksburg after the first harvest in 2021 and after the second harvest in 2022. Similarly, in Orange, the harvest index declined after the first harvest in both years (Figure 4-6).

Harvest index is a good indicator of seed productivity as it positively correlates with seed yield (Sing and Stoskopf 1971), but this metric varies widely depending on crop, cultivar, location, soil type, and growing conditions. Harvest index for many seed crops falls between 0.4 and 0.6 (Hay, 1995) but in this study, the index values generally ranged from the mid 20s to about 30. The greatest harvest index observed over this study (37 in Blacksburg in 2022) occurred when overall biomass production was lower due to water deficit. Harvest index declined at the late harvest time for all four environments, also supporting the idea that seed yield was reduced because of shattering and predation losses. Similar results have been observed for camelina (*Camelina sativa*), another shattering oil seed crop (Sintim et al., 2016).

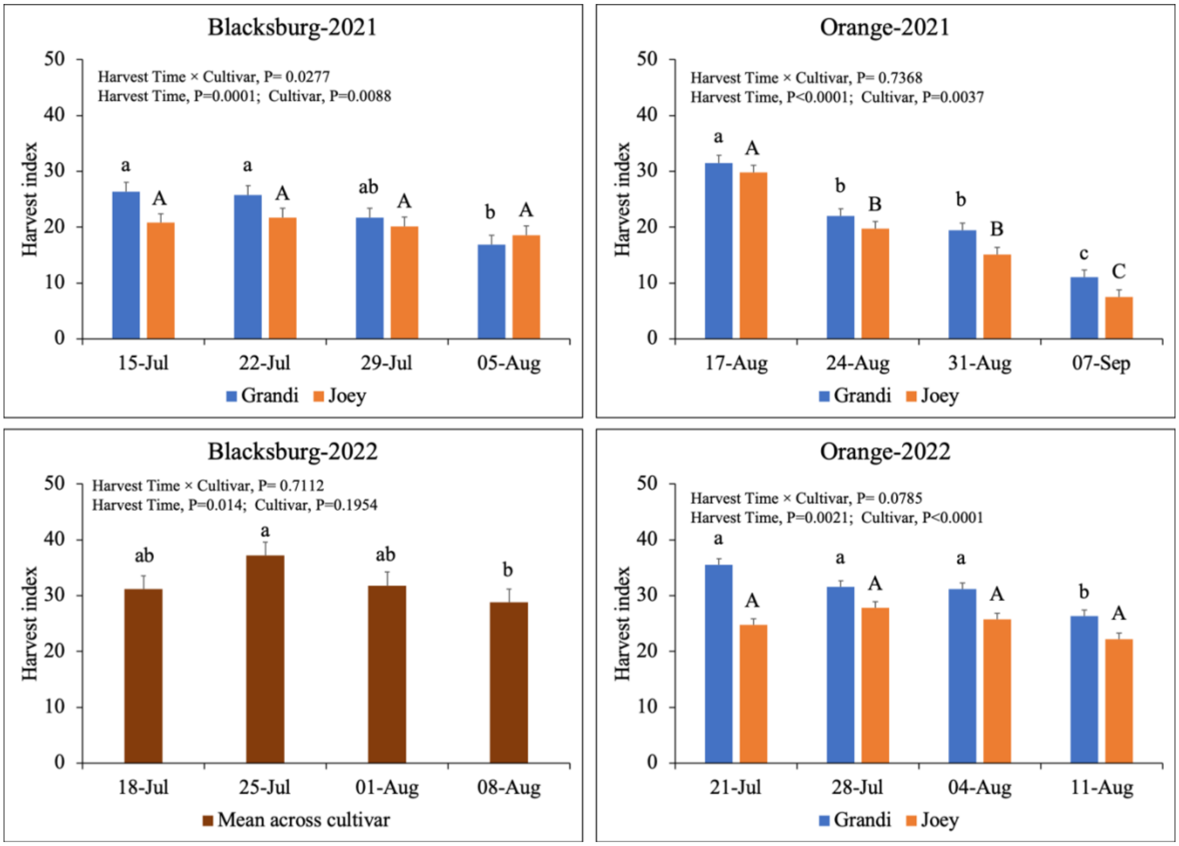


Figure 4 - 6: Mean harvest index of two hemp cultivars (Grandi and Joey) at four different harvest times at Blacksburg and Orange, VA in 2021 and 2022. Bars with different letter designations differ by harvest time, lowercase for Grandi and uppercase for Joey ($P < 0.05$).

Fatty acid profile

Significant location × harvest time interactions ($P \leq 0.05$) were observed for most of the fatty acids analyzed (Table 4-1). Interestingly, there was no discernible influence of harvesting time on the fatty acid profile at the Blacksburg study site, but harvest time effects ($P \leq 0.05$) were common if inconsistent overtime at the Orange study site in 2021 (Table 4-1).

Cultivar effects ($P \leq 0.05$) were observed for several fatty acids at both study sites. Grandi generally had lower concentrations of Oleic acid and gamma linolinc acids and greater concentrations of vaccinnic, alpha linoleic, gondoc, and behenic acids. For hemp harvested in Blacksburg in 2021, Grandi had lower concentrations of oleic (18:1n-9) and γ-linolenic (18:3n-6) fatty acids, with lower concentrations of vaccenic (18:1n-7), α-linolenic (18:3n-3), arachidic (20:0), gondoic (20:1n-9), and behenic (22:0) fatty acids. Lower oleic acid and greater arachidic acids were also observed for Grandi in hemp grown in Orange, but other fatty acids did not follow the same response to cultivar as in Blacksburg. E.g., no cultivar effect was observed for vaccinic, α-linolenic, gondoic or behenic acids at Orange, but Grandi had greater γ-linolenic and lower steardonic acid at that site (Table 4-1)

Hemp seed oil has higher levels of polyunsaturated fatty acids than other oils and is thus considered of superior nutritional value to other food and feed seeds (Small 2015). Linoleic (47.63 to 51.39%) and linolenic (γ+ α) acids (19.68 to 22.8) were the predominant fatty acids found in this study. Our values were similar to observations by Deferne and Pate (1996), and Klir et al. (2019) who reported hemp linoleic acid of 50% and combined linolenic acids of 20%. Although these data are from only a single year of study, they suggest that fatty acid concentrations could be a point of selection, although they also indicate that the differences across fatty acids, particularly those in low concentrations, may be limited.

Table 4 - 1: Fatty acid profile of two hemp cultivars (Grandi and Joey) for the study site Blacksburg (average across harvesting time) and the study site Orange for four different harvesting times in 202

Fatty acid		16:0	16:1n-7	18:0	18:1n-9	18:1n-7	18:2n-6	18:3n-6	18:3n-3	18:4n-3	20:0	20:1n-9	22:0
		Palmitic	Palmitoleic	Stearic	Oleic	Vaccenic	Linoleic	γ-linolenic	α-Linolenic	Stearidonic	Arachidic	Gondoic	Behenic
Cultivar	Date	Blacksburg 2021											
Grandi	Average across date	7.61	0.13	3.15	12.64	1.16	48.94	4.94	17.86	1.45	1.25	0.57	0.28
Joey		7.57	0.14	3.25	14.37	1.07	49.18	5.40	15.80	1.41	1.19	0.49	0.16
P value $_{\text{cultivar}}$		0.5292	0.339	0.254	<0.0001	<0.0001	0.7228	0.0001	<0.0001	0.2608	0.013	0.003	0.452
		Orange 2021											
Grandi	8.17.21	7.85	0.14	3.03	14.18	1.19	48.89	4.38	17.14	1.20	1.19	0.55	0.26
	8.24.21	7.87	0.14	3.11	13.74	1.20	51.39	4.32	15.20	1.08	1.32	0.51	0.11
	8.31.21	8.14	0.14	3.35	14.97	1.27	48.04	4.44	16.00	1.06	1.48	0.65	0.46
	9.07.21	7.43	0.09	3.14	14.56	1.12	51.00	4.33	15.35	1.06	1.23	0.51	0.16
	Mean	7.77		3.11	14.18	1.18	50.35	4.31	15.87	1.08	1.28	0.53	0.25
Joey	8.17.21	7.77	0.16	3.05	14.42	1.16	48.00	5.11	17.13	1.46	1.06	0.47	0.21
	8.24.21	7.76	0.16	3.05	15.22	1.14	49.18	4.86	15.83	1.25	1.12	0.41	0.08
	8.31.21	7.98	0.15	3.29	15.63	1.14	48.15	4.71	15.58	1.24	1.25	0.58	0.30
	9.07.21	7.59	0.14	3.17	15.5	1.14	47.63	4.54	16.93	1.21	1.17	0.54	0.43
	Mean	7.77		3.14	15.19	1.15	48.24	4.81	16.37	1.29	1.15	0.50	0.26
P value $_{\text{cultivar}}$		0.9803	0.060	0.851	0.023	0.088	0.054	0.002	0.065	<0.0001	0.001	0.158	0.883
P value $_{\text{harvest time}}$		0.0182	0.311	0.139	0.162	0.355	0.229	0.413	0.0008	0.0034	0.008	0.060	0.020

CONCLUSION

The study investigated the effects of site and harvest timing in two hemp cultivars (Grandi and Joey) in relationship to plant height, biomass and seed yield, harvest index, and fatty acid profiles. Plant height changed little over the harvesting period for both cultivars. Both greatest and lowest biomass and seed yields were observed in Blacksburg, reflecting contrasting growing conditions from year to year. Harvest timing played a critical role in achieving optimum seed yield. Later season harvests led to substantial declines in plant biomass yield, seed yield, and harvest index, with the strongest effect on seed yield. Generally, late July / early August harvest timing supported the greatest seed yields, but cultivar can affect the optimum harvest timing. Delayed harvests consistently resulted in a drastic reduction in yields, with a decline ranging from 16% to 47% in Blacksburg and 28% to 91% in Orange across different years. The decrease in seed yield during later harvests resulted in low harvest index values. Hemp seed fatty acid profiles were influenced by the cultivar, location, and harvest time. Along with ensuring good agronomic practices, optimizing hemp seed yield will require that several factors be considered, including cultivar selection (particularly a variety’s latitude of origin and the distance from the eco-physiographic regions in which they will be grown) and appropriate harvesting times.

CHAPTER V

HEMP SEED YIELD RESPONSES TO NITROGEN FERTILITY RATES AND YIELD PREDICTION FROM AERIAL IMAGERY

ABSTRACT

Industrial hemp (*Cannabis sativa* L.) is one of the most promising crops for more sustainable economies. Developing nutrient management guidelines for crops and cropping systems is an important agronomic task and precision agricultural-based nutrient management is increasingly being used to ensure farm productivity and improve environmental outcomes. This study aimed to measure hemp plants' response to different rates of N, predict N concentration in plants, and estimate seed yields based on UAV imagery. Growth of two hemp cultivars, 'Joey' and 'Grandi', in response to five N rates (0, 60, 120, 180, 240 kg N ha^{-1}) were tested at Blacksburg, Virginia, in 2020, 2021, 2022. Aerial image acquisition occurred at three different growth stages using dji M 300 drones mounted with multispectral sensors. Red/Blue index (R^2=0.89), NIR band (R^2=0.84) and EVI (R^2=0.81) were better predictors of N content in leaf samples. GNDVI was the best predictor of hemp seed yield, although it had a relatively low coefficient of determination (R^2=0.58). Hemp seed yield was influenced ($P \leq 0.0177$) by N input in all three experimental years, although the pattern of response varied substantially. In 2020, a shorter growing season, N fertility increased seed yield above the control, with peak yield (1640 kg ha^{-1}) occurring with 120 kg N ha^{-1}. However, yield did not differ among N rates based on ANOVA. In 2021, hemp displayed a strong linear response to N inputs, with the highest seed yield (2510 kg ha^{-1}) recorded at 240 kg N ha^{-1}. In 2022, a season characterized by low precipitation and high weed pressure, a weak linear response to N rate was observed, although

highest seed yield (380 kg ha^{-1}) was again observed with 240 kg N ha^{-1}. These findings indicate hemp can be quite responsive to N inputs but that the response to N will be quite sensitive to other factors such as available soil moisture, weed pressure, and growing period.

INTRODUCTION

Industrial hemp is a promising crop for more sustainable economies. With appropriate management, it has high productivity in the sense of high carbon accretion with moderate inputs. It has potential to support greater farm systems diversity in terms of affording several types of harvested products (e.g. seed, fiber, essential oil), and it has potential uses across numerous markets (e.g. food, feed, insulation, absorbents, building materials, medicinal and health care products) (Ely et al. 2022).

Efficient and sustainable crop production is essential to meet the world's growing demands for food and other agricultural products (Spiertz, 2009). In this context, appropriately managing nutrients is one of the important agronomic tasks to meet when establishing economically and ecologically viable cropping systems (Spiertz, 2009). With hemp, nutrient input needs can vary significantly depending on various production systems (i.e., fiber vs. seed vs. flowers production), cultivars, geographic location, and soil conditions (Adesina et al., 2020). Previous cropping history and soil nutrient condition also must be taken into account to avoid over-application of nutrients.

As the nutrient often most limiting in cropping systems (Spiertz, 2009), nitrogen (N) plays a crucial role in optimizing crop yield and quality. However, N fertility management is critical to environmentally healthy and economically sound production systems. Excessive application of N can lead to environmental pollution, and inadequate N can limit yield and cause economic losses. Thus, it is essential to develop precise and accurate methods for assessing plant nutrient status and predicting crop yield to optimize N fertilizer management.

For hemp agronomy, N recommendations typically range from 50 to 100 kg ha^{-1} for fiber production, although positive responses have been observed at rates as high as 240 kg N ha^{-1} observed (Papastylianou et al., 2018). Seed systems, on average, require more nitrogen than fiber production, with recommendations ranging from 100 to 150 kg ha^{-1} (Vera et al., 2004 & 2010). Aubin et al. (2015) observed a positive, linear response to N at rates up to 200 kg ha^{-1} and did not detect a maximum rate for seed. Papastylianou et al. (2018) observed that the application of 240 kg N ha^{-1} resulted in significant improvements in biomass yield, stem dry weight, and inflorescence weight, with respective increments of 37.3%, 48.2%, and 16%.

Research conducted in Québec, Canada, demonstrated that the application of 200 kg N ha^{-1} led to significant increases in biomass and seed yields across different cultivars and environments. Biomass yield rose from 1674 to 4209 kg ha−1, while seed yield increased from 519 to 1340 kg ha^{-1} (Aubin et al., 2015). However, under Mediterranean conditions in central Italy, Campiglia et al. (2017) discovered that higher N fertilization levels (100 kg N ha^{-1}) contributed more to stem yields (28% increase), while inflorescence and seed yields saw smaller improvements of 17% and 4%, respectively. These findings highlight that hemp plants have variable responses to N fertilizer based on sites, cultivars, environments, soil types etc., and indicate that addressing hemp nutritional requirements will require more site-specific research in various soil types and production systems.

In recent years, advances in remote sensing technologies and machine learning algorithms have provided new opportunities for non-destructive and rapid assessment of crop nutrient status and yield prediction. These tools are becoming more widely used and helping to ensure farm productivity for various crops. Although there are established imagery-based nutrient management systems for most of the commodity crops (e.g. wheat, corn, soybean; Fu et al., 2020), imagery-based study for hemp nutrient management is limited. The aims of this study were to measure hemp plants' response to different rates of N fertility and to investigate the potential of using unmanned aerial vehicle (UAV) imagery with multispectral sensors to estimate leaf and stem N content, as well as predict seed yield and biomass yield in hemp plants under varying N fertility rates. If viable, such tools could be utilized in developing precision nutrient management for hemp production systems.

MATERIALS AND METHODS

Study sites

The study was conducted at the Virginia Tech Urban Horticulture Center (37°13'05"N, 80°27'52''W, 616 m elevation) in 2020 and 2021 and the university's Kentland Farm (37°11'43"N, 80°34'47''W, 528 m elevation) in 2022, both located in Blacksburg, Virginia, USA. Soils at the Urban Horticulture Center are Duffield Ernest complex (fine-loamy, mixed, active mesic Ultic Hapludalfs and Fine-loamy, mixed, superactive, mesic Aquic Fragiudults). Soils at Kentland are Unison and Braddock (fine, mixed, semiactive, mesic Typic Hapuldults).

Experimental Design

The experiment was a randomized complete block design with a split-plot treatment arrangement and four replicates. The main plots were the two selected cultivars and the sub-plots were the five N fertilizer treatments (e.g. 0, 60, 120, 180, 240 kg N ha^{-1}).

Hemp Establishment

Prior to establishment, soils were sampled, and P and K fertility were applied as needed based on a recommendation for corn (*Zea mays*) production. Two industrial hemp cultivars (‘Joey’, and ‘Grandi’) of differing morphology were planted for this study. Strips of each cultivar were randomized in space for each replicate (R=4) and planted with a commercial, no-tll planter in all years. Seeds were planted into killed grass sod in 2020 and 2021 and into a tilled fallow field in 2022, with a 1-cm target seeding depth. Row spacing was 30 cm in 2020 and 2021 (four rows per plot) and 19 cm (nine rows per plot) in 2022. Seeding rate for each cultivar was 28 kg ha^{-1}. Seeding events occurred on June 19, 2020, May 13, 2021 and May 17, 2022. Seeding date was delayed in 2020 due to high precipitation and wet soils earlier in the season

Subplot Establishment and Maintenance

To minimize effects of stand variability, locations of subplots within strips were established following hemp germination and initial development. Within each strip, plots (3-m long) of greatest uniformity were selected and randomized to N treatment. In 2020, N (46-0-0) was hand applied between drill rows, two weeks after seeding. A slow-release N source (34-0-0) was applied with the same method in 2021 and 2022 as some leaves had nutrient burn symptoms in 2020.

In 2020 and 2021, hemp plantings faced weed pressure primarily from yellow nutsedge (*Cyperus esculentus*), along with some pigweeds (*Amaranthus* spp.) and bindweed (*Convolvulus arvensis* L.). These were pulled or cut with hand tools to suppress weed pressure. The wider row spacing allowed for these practices to be implemented effectively.

In 2022, weed pressure, primarily from pigweed and foxtail (*Setaria viridis*) was more significant. Post-emergence herbicides (Moxy (Octanoic acid ester of bromoxynil* (3,5-dibromo-4-hydroxybenzonitrile) at 1.4 L/ha) (Winfield Solutions, LLC, MN, USA) and Poast (2-[1-(ethoxyimino)butyl]-5-[2-(ethylthio)propyl]-3-hydroxy-2-cycloxexen-1-one at 1.4 L/ha) (BASF) were applied 20 days after seeding. Unfortunately, these herbicides were not successful in effectively managing the weed population. Additionally, in 2022, the hemp plants faced drought conditions after emergence due to limited precipitation during the growing period. This combination of high plant density, weed pressure, and drought stress posed additional challenges for the hemp plants during the 2022 growing season.

Plant measures

Plant heights were measured before harvests, which occurred when bracts at bottom of the inflorescences began opening, revealing mature seed. Whole plants were harvested from within a 0.25-m^2 quadrate placed randomly at three locations within each plot (0.75-m^2 total area sampled). Hemp plants were harvested on September 10, 2020, July 23, 2021, and July 25, 2022. Total biomass yield and seed yield were measured after drying the collected samples and separating the seeds, respectively.

UAV image acquisition

A dji M 300 RTK drone (DJI, Shenzhen, China) with a multispectral sensor-RedEdge(MicaSense,Inc, Seattle, USA) was flown in 2021 to collect images from the hemp plots. Pix4Dcapture software (Pix4D, Lausanne, Switzerland) was used for the mission planning at an altitude of 25 m above the hemp plants. Images were obtained three times at three different growth stages (Mishchenko et al., 2017) of hemp plants on June 23 (flowering stage-6), July 12 (development of fruits, stage-7), and July 21 (ripening of fruits, stage-8) in 2021 at Urban Horticulture Center, Blacksburg, VA. In 2022, image acquisition was not conducted as plants were shorter because of drought and faced severe weed competition at early vegetative and early flowering stages.

Plant Sampling

After each drone flight, one hemp plant sample was collected from a 0.25-m^2 area within each plot demarcated with a quadrate. Fresh plant parts (leaves, stems, and inflorescence) were separated by hand and then dried (43°C) in a forced-draft oven for a minimum of 48 h. Dried plant samples were ground and prepared for N content analysis using NIR spectroscopy. The scanning was performed using a NIRS DS2500F instrument equipped with ISIScan Nova v. 8.0.6.2 software (Foss North America, Eden Prairie, MN). The 2022 Mixed Hay calibration provided and licensed by the NIRS Forage and Feed Consortium (NIRSC, Berea, KY) was applied to the acquired spectra. These spectra were then used to evaluate the following expert recommendations (McIntosh, personal communication). Accuracy of the predictions was assessed using global and neighborhood statistical tests, ensuring that the entire dataset conformed to the calibration within a limit of fit ($H < 3.0$) (Murray and Cowe, 2004).

Image processing

Generally, the obtained images required some preprocessing before going to formal analysis. Preprocess procedures included radiometric correction, image mosaic, and geometric correction. To start the preprocessing, the images folder was uploaded to pix4DMapper software and following some other modifier procedures, ortho-mosaic images for five different bands (blue, green, red, rededge, and NIR) of the studied plots were obtained. Those five mosaic images with tif format were loaded to ArcMap software to make a composite band image. Using ArcMap software, polygon shape files were created to mark the areas of interest within the experimental plots. Only areas with hemp plants area were selected to avoid the effect of bare soil and weeds on subsequent analysis. The reflectivity data for five bands were extracted from those marked plots using image analysis software ENVI (Exelis Visual Information Solutions, Boulder, CO, USA). The reflectivity data were obtained for each by averaging the reflectivity for whole plots.

Selecting vegetation index

The extracted reflectivity of all five bands for each plot was utilized to generate several commonly-used vegetation indices (Table 5-1).

Table 5 - 1: Vegetation indices used to correlate N content in hemp leaves, stems, inflorescences, and plant biomass and seed yield at harvest.

Description	Index	Formula	References
Normalized difference vegetation index	NDVI	(NIR-R)/(NIR+R)	Rouse et al., 1974, and Taddeo et al., 2019
Green normalized difference vegetation index	GNDVI	(NIR-G)/(NIR+G)	Taddeo et al., 2019; Gitelson 1996
Normalized difference red edge index	NDRE	(NIR-RE)/(NIR+RE)	Gitelson and Merzlyak, 1994
Ration vegetation index	RVI	NIR/R	Pearson and Miller, 1972; Dong et al., 2013
Enhanced vegetation index	EVI	2.5*(NIR-R)/(NIR+6*R-7.5*B+1)	Huete et al., 2002
Difference vegetation index	DVI	NIR-RE	Jordan 1969
Simple Ratio-1	R1	B/G	
Simple Ratio-2	R2	R/NIR	
Simple Ratio-3	R3	R/B	

Statistical analysis

Data were statistically analyzed with ANOVA using Proc Mixed procedures of SAS Studio (version-3.8, Cary, NC). The model was used to test the effects of cultivar and N fertility rates on plant heights, biomass yield, and seed yield. The data were first analyzed with year in the initial model, but data ultimately were analyzed by year given significant ($P < 0.05$) year × N rate interaction. N fertility rates and cultivars were considered fixed effects, while replication was considered a random effect. LS-means and Tukey's adjusted differences were calculated.

Results were considered statistically significant at $P < 0.05$ and considered as trends when $0.05 < P < 0.10$.

Regression analysis was used to determine the biomass yield, seed yield, N content in the leaves and stems. Linear and polynomial regression models were used to determine the response of dependent variables (biomass yield, seed yield, N content in plant leaves and in stems) to N fertilization rate.

Modeling methods and validation

A correlation analysis was conducted to examine the relationships between the different bands, vegetation indices, and the N content in leaves and stems, as well as seed and biomass yields. To visualize the correlations, a correlation matrix table was generated and presented using a heatmap to display the strength and direction of the correlations between the variables. Additionally, the significance levels were indicated using P values, denoted by one, two, or three stars (*) for significance levels of 0.05, 0.01, and 0.001, respectively.

The performance of the Support Vector Regression (SVR) and Random Forest (RF) models in predicting the N content in hemp plant leaves and stems, and seed yield were assessed using a test sample dataset. The evaluation aimed to determine the accuracy and reliability of the models. For each model, correlation plots were generated to compare the predicted values with the observed values of N concentration and seed yield. Error bars were included to visualize the variability of the predictions. The evaluation was conducted with combined sampling dates (two sampling dates for N content and three sampling dates for seed yield) for two hemp cultivars under the five N fertility rates.

SVR model with a kernel setting of 'rbf' and c=10 was utilized to predict seed yield and leaf and stem N concentrations. Grid search was performed to tune the best RF models. For predicting leaf N content, RF models were employed with max_depth=3, max_features=sqrt, min_samples_leaf=1, min_samples_split=2, and n_estimators=5. For predicting stem N content, RF models were employed with max_depth=9, max_features=auto, min_samples_leaf=5, min_samples_split=2, and n_estimators=5. Finally, for predicting seed yield, RF models were employed with max_depth=3, max_features=sqrt, min_samples_leaf=5, min_samples_split=2, and n_estimators=10.

Feature Importance Analysis

Feature importance analysis was performed to understand the significance of different variables in predicting the N content in hemp plant leaves and stems, and seed yield. This analysis helps identify the key factors that contribute most to the predictions.

For the Random Forest (RF) model, feature importance values were calculated. These values indicate the relative importance of each variable in the model's decision-making process. By examining these values, insights were gained into the variables that had the most influence on predicting the N content in hemp plant leaves and stems, and seed yield.

Statistical analysis

To quantitatively assess the performance of the SVR and RF models and compare different bands and vegetation indices, a statistical analysis (RMSE, MAE, and R^2) was conducted. The results were tabulated for comparison and analysis, allowing for the evaluation of the models' predictive performance and the identification of the most effective variables in predicting seed yield and N content in hemp plant leaves and stems.

RESULTS AND DISCUSSION

Weather Data

Growing season averages and totals are provided in Table 3-2 with distribution patterns in Figure 5-1. In general, precipitation was limited in 2022 compare to 2020 and 2021 at Blacksburg, VA.

Table 5 - 2: Growing season averages, maximum and minimum temperature and total precipitation for the study site Blacksburg, VA in 2020, 2021, and 2022.

Year	Site	Seasonal Temp. (°C)			Seasonal Precip. (mm)
		Mean	Max	Min	Total
2020	Blacksburg	22.8	28.5	17.1	710.0
2021	Blacksburg	21.9	28.2	15.5	250.0
2022	Blacksburg	22.6	29.0	16.1	160.0

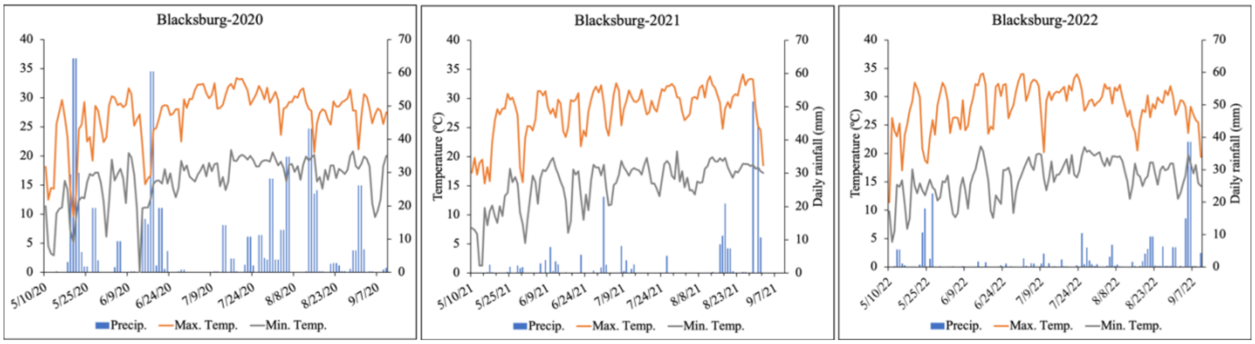


Figure 5 - 1: Daily maximum and minimum temperature and precipitation for the study site Blacksburg, VA, in 2021, and 2022.

Plant population

The plant population density varied across the years, although seeding rate was same. In 2020, the density was 35 plants per square meter, while in 2021, it was 17 plants per square meter. In 2022, a higher plant population density of 90 plants per square meter was maintained. These differences reflect both planting row spacing and conditions in the early phase of establishment.

Plant height

Plant height response to N fertility was limited in 2020 and 2022 but generally increased with increasing application rate in 2021 (rate x year interaction; $P<0.0001$). There was no significant interaction between cultivars and N rate. Plant height generally increased ($P=0.0008$) with increasing N in 2021 but wasn’t influenced by N input in 2020 or 2022. Mean plant heights (averaged across cultivars and N rates) were 1.07 m, 1.17 m, and 0.39 m, in 2020, 2021, and 2022, respectively. In 2021, the tallest plant (1.24 m) observed occurred at a N rate of 240 kg N ha^{-1} (Figure 5-2). Shorter plants in 2022 reflect the combination of limited rainfall, high-density plant, and high weed pressure during the growing season (Figure 5-1).

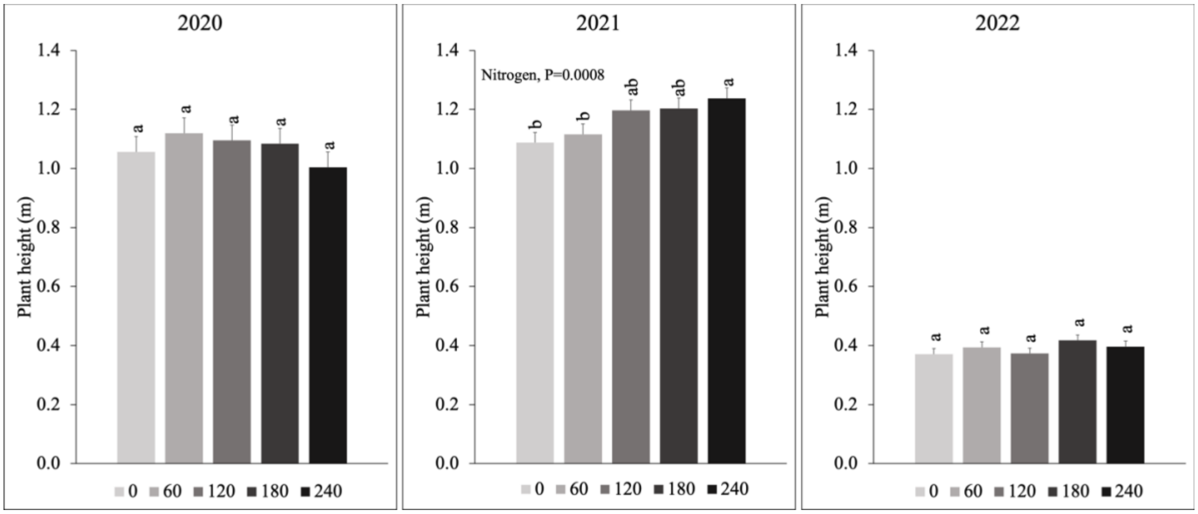


Figure 5 - 2: Mean plant height at harvest time under different rates of N fertilizer application, average across two hemp cultivars (Grandi and Joey) at Virginia Tech's Urban Horticulture Center (2020 and 2021) and Kentland Farm (2022) in Blacksburg, VA.

Biomass yield

Positive response to N fertilization for biomass yield was observed in all three years, although patterns of response differed by season. In 2020, both cultivars had a quadratic yield response to N fertilizer, although the model fit was limited (Grandi: Yield=-0.0000x2 + 0.0153x1 + 2.2634x0, R^2=0.32) and Joey: Yield=-0.0001x2 + 0.0316x1 + 3.3024x0, R^2=0.33). In 2021, response to N was linear for both cultivars (Grandi: Yield=0.0112X+2.50520, R=0.40 and Joey: Yield=0.0259X+3.56745, R^2=0.82), with much stronger model fit for Joey (R^2 =0.82). In 2022, response to N was again linear for both cultivars, although model fit was quite weak (Grandi: Yield=0.0011X + 0.58935, R^2=0.09 and Joey: Yield=0.0035X + 0.7861, R^2=0.22) (Figure 5-3).

In 2020, biomass yield was greater with the first N fertilizer increment applied (60 kg N ha^{-1}), but increasing increments did not increase yield. Applying 240 kg N ha^{-1} produced an intermediate yield, suggesting a detrimental effect of high N to hemp growth. This likely reflects the negative effect of fertilizer burn with urea at the high N rate. In 2021, biomass yield increased incrementally with increasing N rate. Yield increases were about 11.2 and 25.9 kg hemp biomass per kg N applied for Grandi and Joey, respectively, indicating Joey have high potentiality to produce more biomass. The greatest average biomass yield for Grandi 5.2 Mg ha^{-1} and for Joey 9.9 Mg ha^{-1}, was in response to N input of 240 kg N ha^{-1}. In 2022, the biomass yield was compromised by limited rainfall, high plant populations, and particularly by weed pressure. The highest biomass yield for Grandi 3.5 Mg ha^{-1} and for Joey 4.9 Mg ha^{-1} was recorded in response to N input of 180 kg N ha^{-1} (Figure 5-3).

Seed yield

A positive response to N fertilization for seed yield was observed in all three years of the study. In 2020, both Grandi (Yield=-0.0194x2 + 6.6756x1 + 1032.3571x0, R^2=0.27) and Joey (Yield=-0.0381x2 + 10.0512x1 + 1124.7143x0, R^2=0.29), seed yield had quadratic response to N fertilizer, although these models were weak (low R^2). However, in 2021, Grandi (Yield=5.0008X+726.55, R^2=0.48) and Joey (Yield=8.7671X + 863.3, R^2=0.74) seed yield increased linearly with N fertilizer inputs and good fit (R^2=0.74) for Joey. In 2022, seed yield response to N was again linear for Grandi (Yield=0.4550X + 153.35, R^2=0.10) and Joey (Yield=1.1387X + 243.95, R^2=0.26) but coefficients of determination were low (Figure 5-4).

During the 2020 study, the mean seed yield increased for Grandi from 1025 to 1578 kg ha^{-1} in response to 0 to 120 kg N ha^{-1}, and for Joey from 1050 to 1765 kg ha^{-1} in response to 0 to 60 kg N ha^{-1}. However, the seed yield did not increase with the addition of more N fertilizer. This limited response to N fertilizer indicates that other factors might have influenced yield that year. For instance, the late planting of these cultivars, developed in Canada, likely limited opportunities for vegetative growth (and a limited response to N) before they started their reproductive growth phase. Moreover, the plants experienced some nutrient burn issues from the urea fertilizer, which was applied 2 weeks after planting. Fertilizer burn was more evident at the highest N fertility rates (180 and 240 kg N ha^{-1}) (Figure 5-4).

However, a substantial response to N rate was observed in 2021. The mean seed yield for Grandi increased from 837 to 1927 kg ha^{-1} with the applied N rate of 0 to 240 kg N ha^{-1}, and for Joey, the seed yield increased from 1020 to 3087 kg ha^{-1} in response to 0 to 240 kg N ha^{-1} (Figure 5-4). Yield increases were about 4.5 and 8.6 kg hemp seed per kg N applied for Grandi and Joey, indicating both varieties have substantial potential responsiveness to fertility inputs. Our findings from 2021 are consistent with those of Aubin et al. (2015), who reported a positive and linear response to N at rates up to 200 kg/ha. In 2022, a weak and inconsistent response to N rate was observed. Although the greatest seed yield for Grandi increased from 200 to 267 kg ha^{-1} for the N rate of 0 to 180 kg N ha^{-1}, and for Joey, it increased from 260 to 503 kg ha^{-1} for the N rate of 0 to 180 kg N ha^{-1}. Limited rainfall in 2022 led to a lower response to N fertilization. This finding indicates the law of the minimum applies to hemp as well, as the plant could not respond to N inputs without sufficient water.

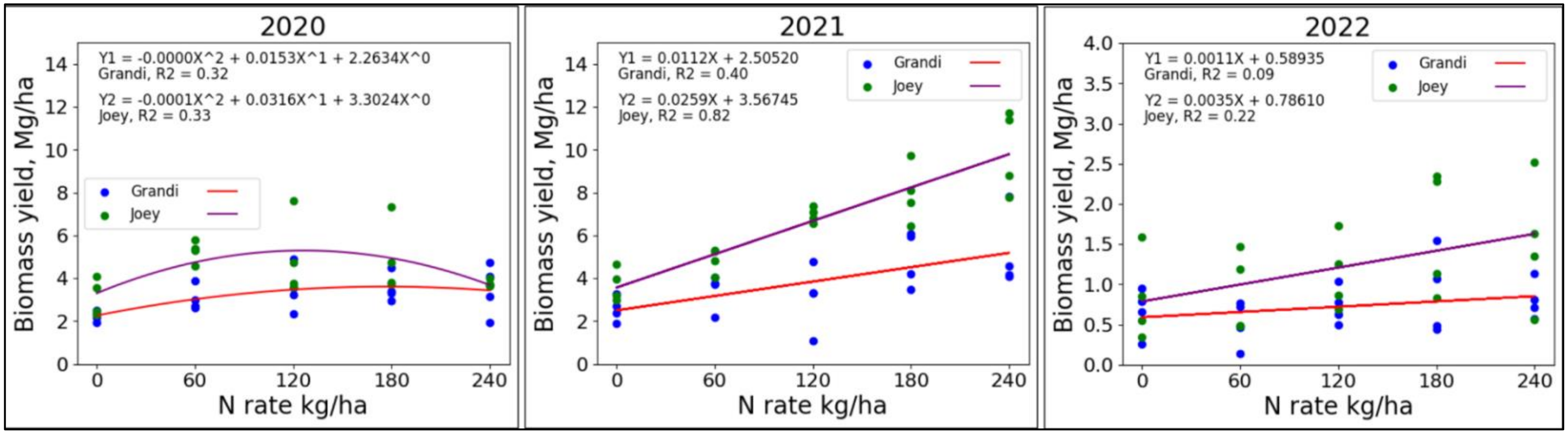


Figure 5 – 3: Regression analysis of seed yield response to nitrogen rate for two hemp cultivars (Grandi and Joey) in Blacksburg, VA, in 2020, 2021, and 2022.

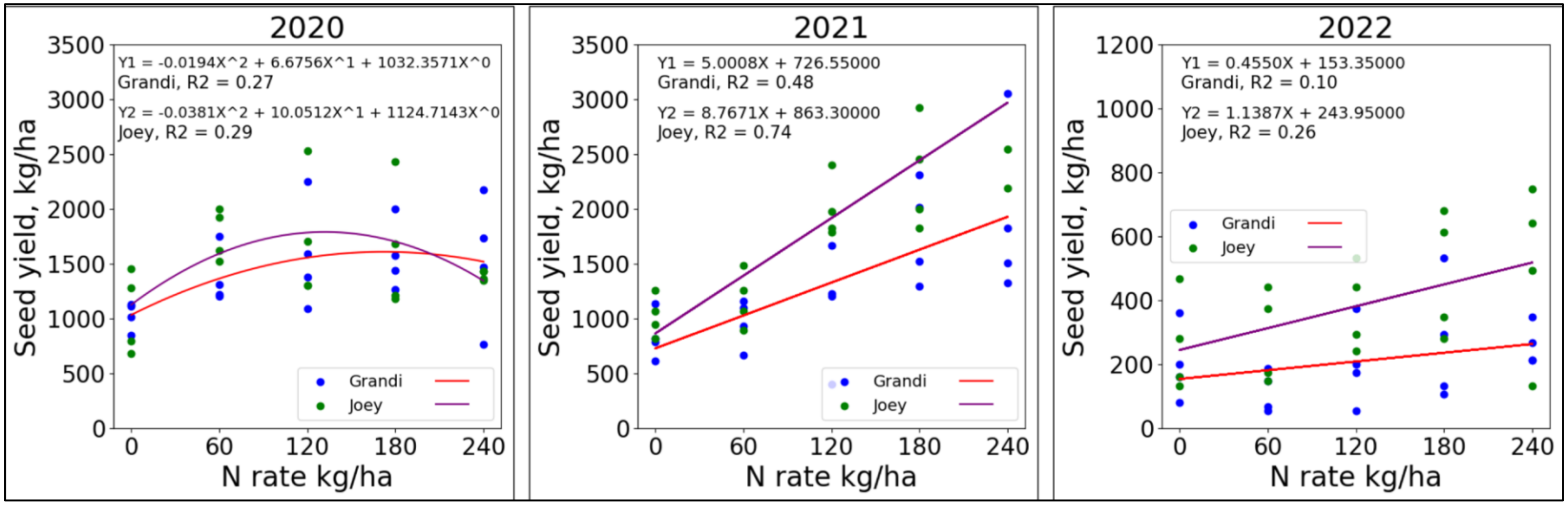


Figure 5 - 4: Regression analysis of biomass yield response to nitrogen rate for two hemp cultivars (Grandi and Joey) in Blacksburg, VA, in 2020, 2021, and 2022.

Plant N concentration

Nitrogen concentrations in plant leaves, and stems were assessed in response to varying rates of N fertilizer application at two dates in 2021 only. For both plant parts, the highest N concentrations were observed when the highest rate of N fertilizer (240 kg N ha^{-1}) was applied.

Both plant parts, leaves and stems, displayed positive linear responses to N fertilizer application. At the June 23 sampling date, the response was weaker (leaves:Y=0.0032X + 4.60379, R^2=0.39; stems: Y=0.0012X + 1.75496, R^2=0.18) than at July 12 (leaves:Y=0.0042X + 2.85441, R^2=0.53; stems: Y=0.0013X + 1.05596, R^2=0.28) (Figure 5-5).

Leaf N concentrations ranged from 3.70% to 5.65% on June 23 and 2.16 4.28 to 4.53% on July 12 (Figure 5-5). The stems had the lowest N concentrations, with values ranging from 1.26% to 2.46% on June 23 and 0.84% to 1.80% on the July 12, 2021 sampling day.

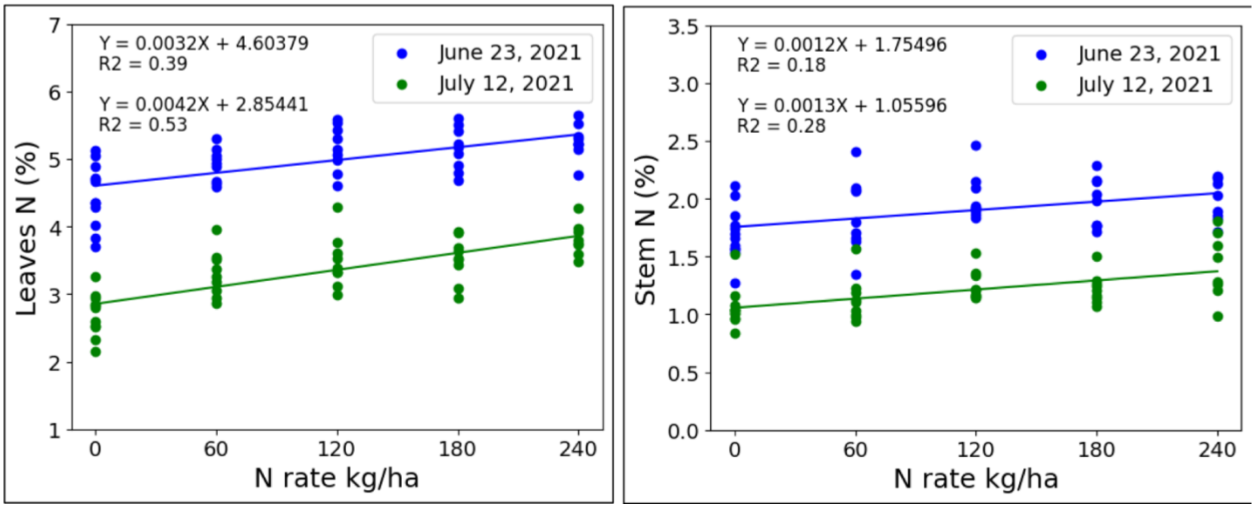
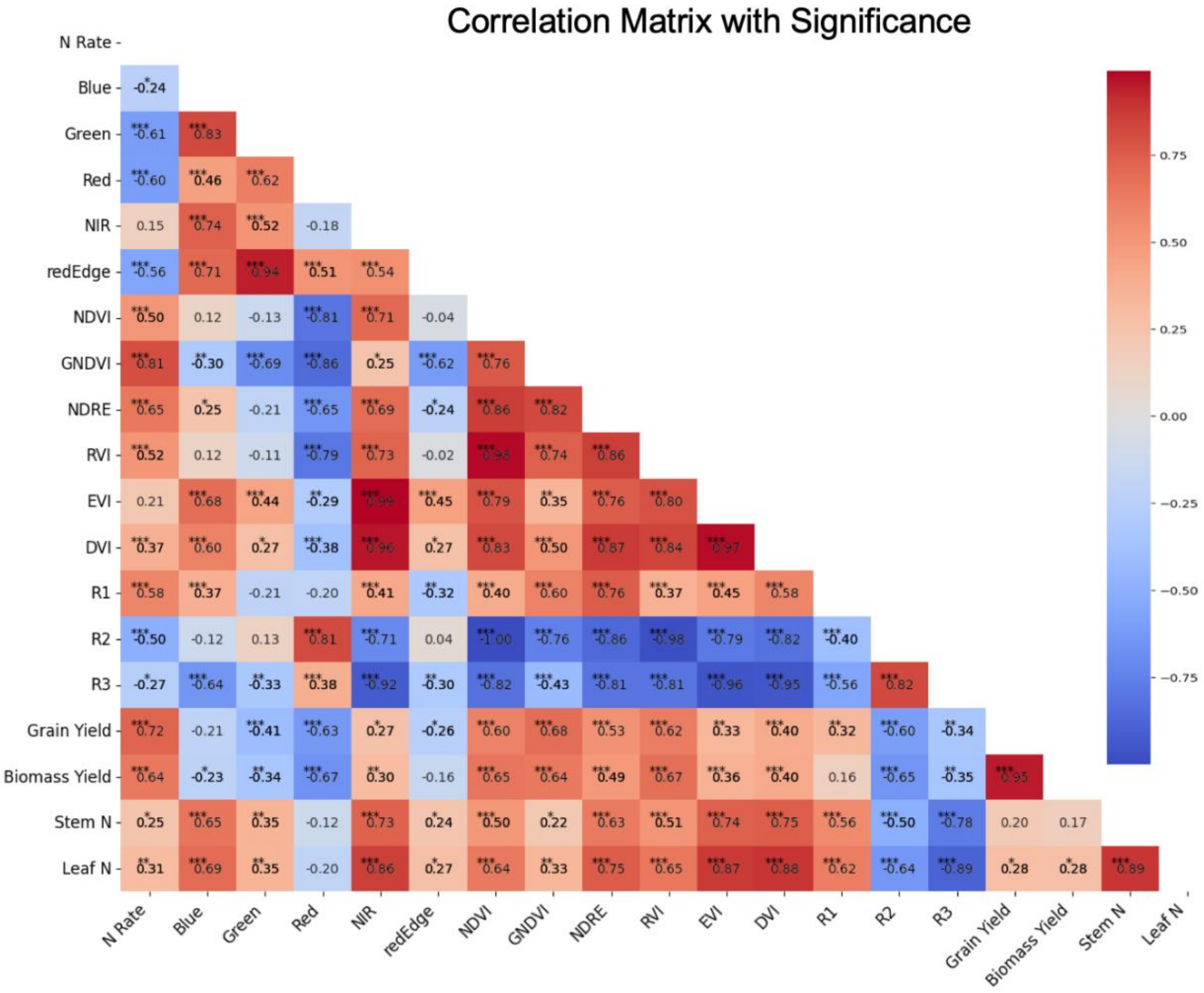


Figure 5 - 5: Regression analysis of leaves and stems N concentration to nitrogen rate for two hemp cultivars (grandi and joey) in Blacksburg, VA, in 2021.

Correlation matrix

N concentrations in leaf, stem, and inflorescence were correlated with reflectance values, various vegetation indices, leaf-stem-inflorescence N and seed and biomass yields. Leaf N concentration was positively correlated with the near-infrared (NIR) band (R^2=0.86), Enhanced Vegetation Index (EVI) (R^2=0.87), and the Difference Vegetation Index (DVI) (R^2=0.88). Leaf N was negatively correlated with R3 (NIR/Red) (R^2= -0.89) (Figure 5-6). Additionally, leaf N concentration exhibited a strong correlation with stem N concentration (R^2=0.89) which indicates measuring leaves N concentration by using non-destructive imaging techniques may be able to predict N concentration in plant stems as well. Stem N concentrations displayed similar positive correlations with the NIR band (R^2=0.73), EVI (R^2=0.74), DVI (R^2=0.75), and R3 (R^2=0.78) (Figure 5-6). High coefficients of determination indicate leaf and stem N concentrations can be effectively estimated using UAV imagery and vegetation indices.

Seed yield at harvest was correlated with the applied N rate (R^2=0.72), and Green Normalized Difference Vegetation Index (GNDVI) (R^2=0.68). These findings indicate uav imagery may be used to develop models to predict biomass and seed yields using GNDVI.



	N Rate	Blue	Green	Red	NIR	redEdge	NDVI	GNDVI	NDRE	RVI	EVI	DVI	R1	R2	R3	Grain Yield	Biomass Yield	Stem N	Leaf N
N Rate																			
Blue	-0.24*																		
Green	-0.61***	0.83***																	
Red	-0.60***	0.46***	0.62***																
NIR	0.15	0.74***	0.52***	-0.18															
redEdge	-0.56***	0.71***	0.94***	0.51***	0.54***														
NDVI	0.50***	0.12	-0.13	-0.81***	0.71***	-0.04													
GNDVI	0.81***	-0.30**	-0.69***	-0.86***	0.25*	-0.62***	0.76***												
NDRE	0.65***	0.25*	-0.21	-0.65***	0.69***	-0.24*	0.86***	0.82***											
RVI	0.52***	0.12	-0.11	-0.79***	0.73***	-0.02	0.98***	0.74***	0.86***										
EVI	0.21	0.68***	0.44***	-0.29**	0.99***	0.45***	0.79***	0.35**	0.76***	0.80***									
DVI	0.37***	0.60***	0.27*	-0.38***	0.96***	0.27*	0.83***	0.50***	0.87***	0.84***	0.97***								
R1	0.58***	0.37***	-0.21	-0.20	0.41***	-0.32**	0.40***	0.60***	0.76***	0.37***	0.45***	0.58***							
R2	-0.50***	-0.12	0.13	0.81***	-0.71***	0.04	-1.00***	-0.76***	-0.86***	-0.98***	-0.79***	-0.82***	-0.40***						
R3	-0.27*	-0.64***	-0.33**	0.38***	-0.92***	-0.30**	-0.82***	-0.43***	-0.81***	-0.81***	-0.96***	-0.95***	-0.56***	0.82***					
Grain Yield	0.72***	-0.21	-0.41***	-0.63***	0.27*	-0.26*	0.60***	0.68***	0.53***	0.62***	0.33**	0.40***	0.32**	-0.60***	-0.34**				
Biomass Yield	0.64***	-0.23*	-0.34**	-0.67***	0.30**	-0.16	0.65***	0.64***	0.49***	0.67***	0.36***	0.40***	0.16	-0.65***	-0.35**	0.95***			
Stem N	0.25*	0.65***	0.35**	-0.12	0.73***	0.24*	0.50***	0.22*	0.63***	0.51***	0.74***	0.75***	0.56***	-0.50***	-0.78***	0.20	0.17		
Leaf N	0.31**	0.69***	0.35**	-0.20	0.86***	0.27*	0.64***	0.33**	0.75***	0.65***	0.87***	0.88***	0.62***	-0.64***	-0.89***	0.28*	0.28*	0.89***	

Figure 5 - 6: The correlation matrix with significance among different spectral bands, vegetation indices, seed and biomass yield, and N content in hemp plants under different rates of N fertilizer inputs. *, **, and *** for significance levels of 0.05, 0.01, and 0.001, respectively

Model evaluation and validation to predict seed yield, and N content in leaves, stems.

For leaf N content prediction, both machine learning models, the Support Vector Regression (SVR) and Random Forest (RF) models exhibited strong correlations with observed values, as reflected in their respective R^2 values of 0.84 and 0.89. The error bars plot (Figure 5-7) demonstrated consistently low error values, indicating the accuracy and reliability of the predictions.

In the case of stem N content, the SVR model showed a robust correlation with an R^2 value of 0.77, while the RF model achieved an R^2 value of 0.69 (Figure 5-8). The error bars closely aligned with observed values, further supporting the effectiveness of these models in predicting stem N content.

However, when it comes to seed yield prediction, the correlations were relatively weaker compared to leaf and stem N content. The SVR model achieved an R^2 value of 0.68, and the RF model had an R^2 value of 0.61 (Figure 5-9). The larger errors observed in seed yield predictions might be attributed to the data being collected at various growth stages, spanning from early reproductive stages up to seed maturation.

Further validation using larger datasets is recommended to enhance the practical applicability of these predictive models. Such efforts will likely improve the models' accuracy and reliability, making them valuable tools for precision agriculture and optimizing hemp crop management.

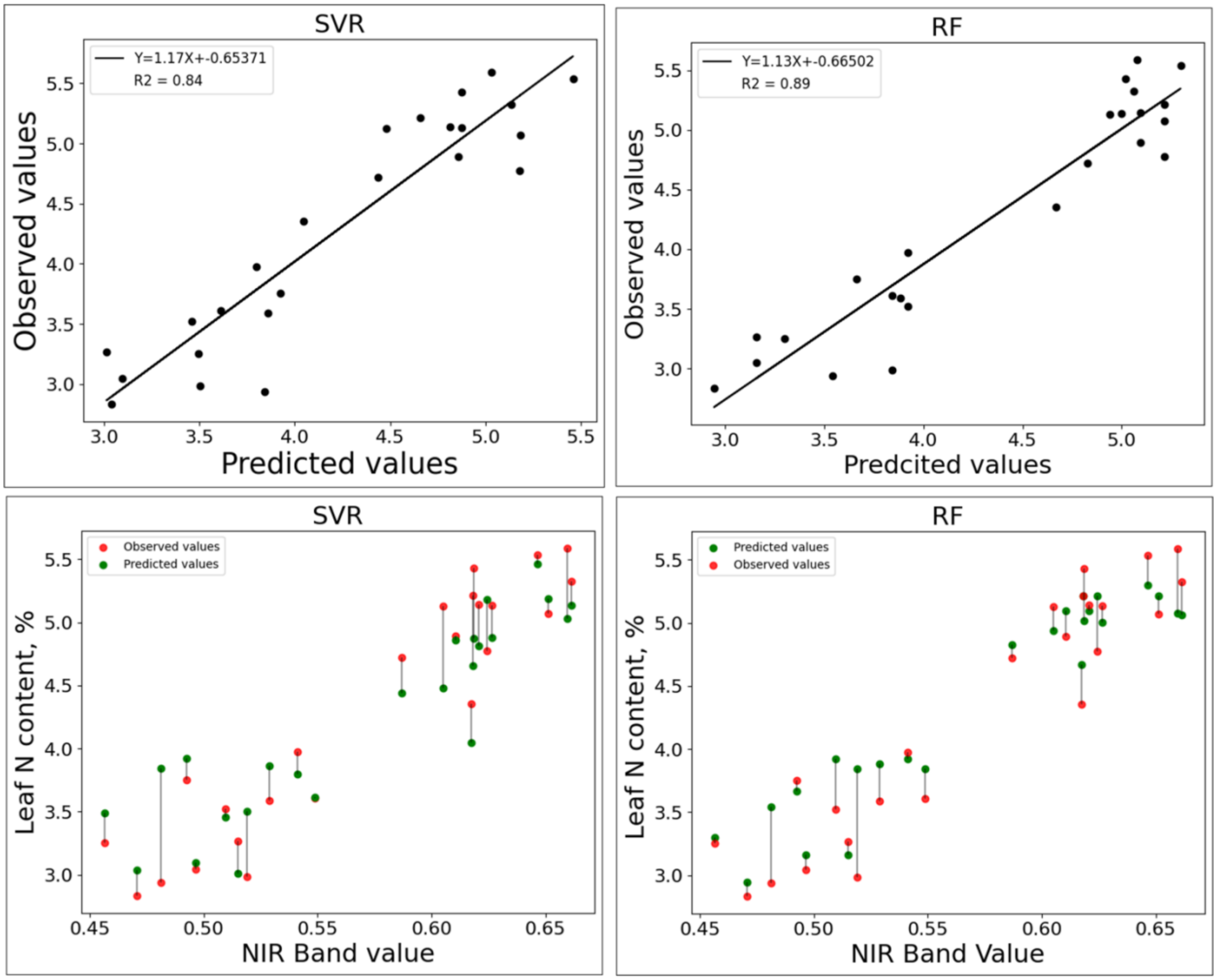


Figure 5 - 7: The correlation plots of predicted and observed values, and error bars of test samples of SVR (left) and RF (right) models for the N content in the leaves of hemp plants at two sampling dates under different rate of N fertilizer inputs.

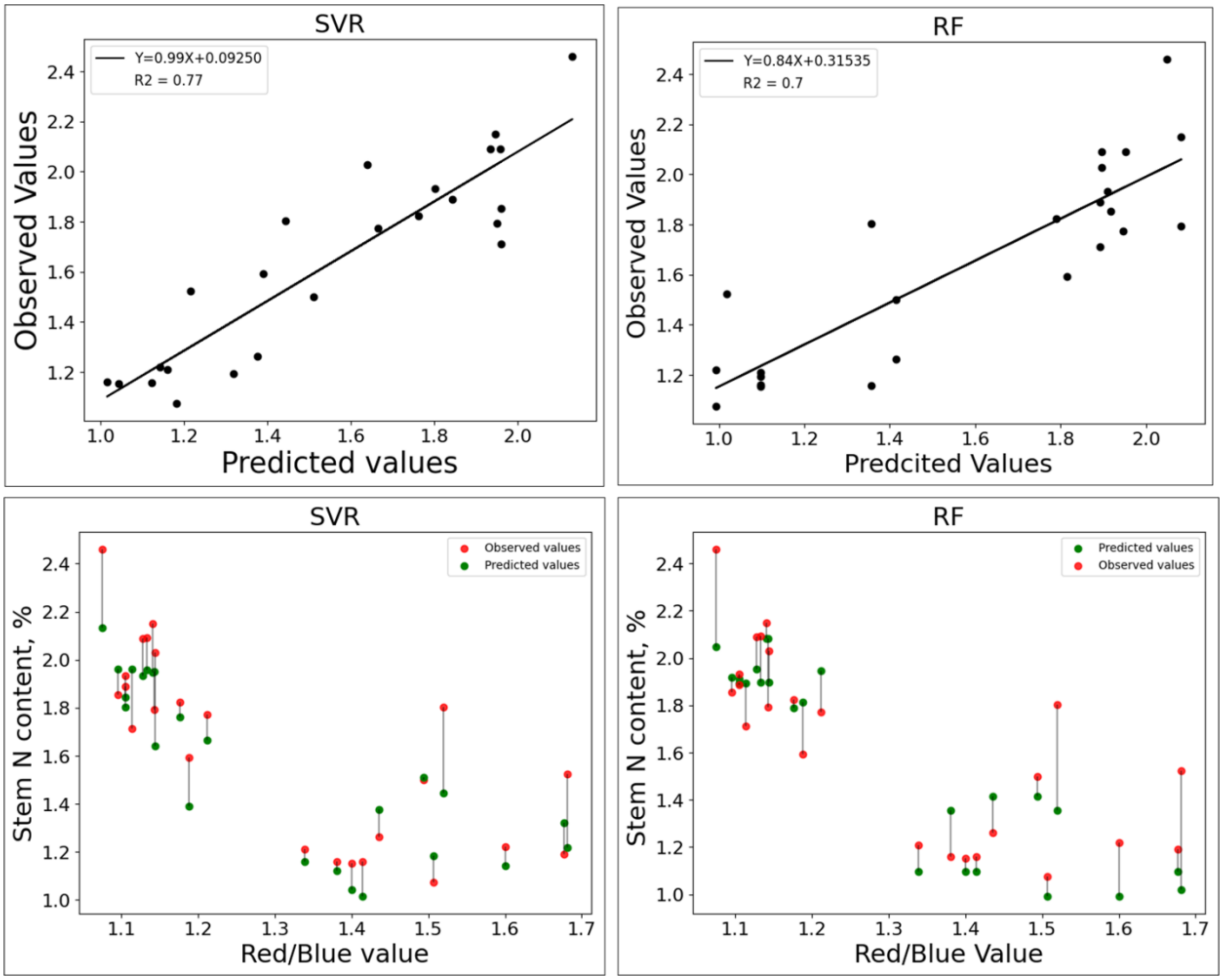


Figure 5 - 8: The correlation plots of predicted and observed values, and error bars of test samples of SVR and RF models for the N content in the stems of hemp plants at two sampling dates under different rate of N fertilizer inputs.

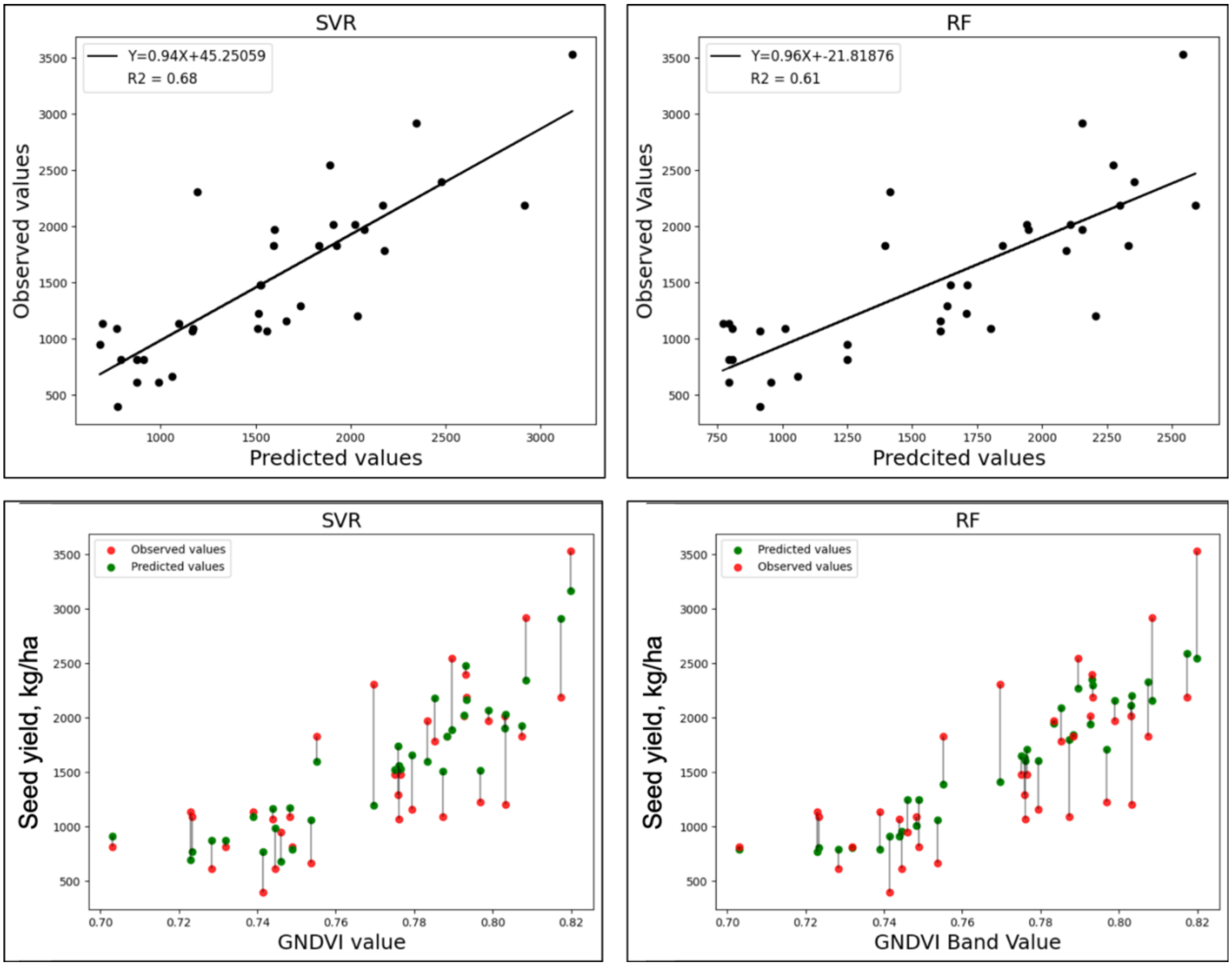


Figure 5 - 9: The correlation plots of predicted and observed values, and error bars of test samples of SVR and RF models to predict hemp seed yield under different rates of N fertilizer inputs.

Feature selection

The EVI and R3 (NIR/Red) indices were the most influential feature in predicting leaf N concentrations with the RF model (Figure 5-10). NDRE band also had high importance but was intermediate to R3 and EVI. Thus, for accurate leaf N estimation using UAV imagery and machine learning, we can consider R3, EVI and NDRE indies. While predicting N content in hemp plant stems R3 index was identified as the most crucial feature. For predicting seed yield with the RF model Red band, R2 (Red/NIR), NDRE, and RVI were the most important features. These indices had a wider influence compared to others in predicting seed yield.

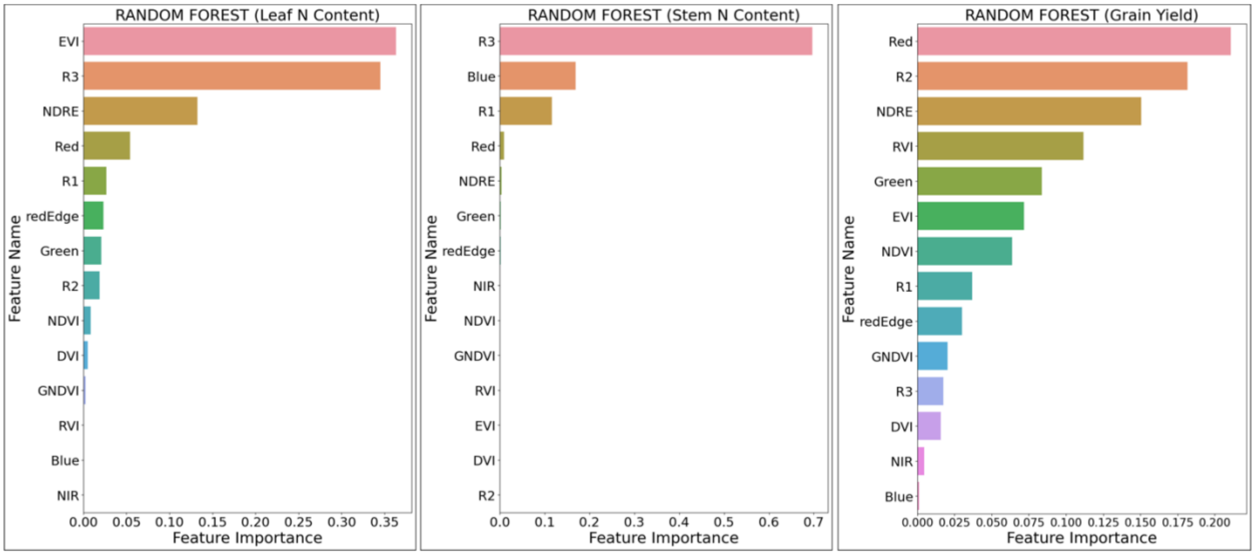


Figure 5 - 10: The feature importance of test samples for Random Forest Model for predicting N content in the hemp plant leaves, stems, and predicting seed yield under different rate of N fertility.

Evaluating different indices for SVR and RF model

The NIR band, EVI, and R3 indices exhibited the most promising performance for both SVR and RF models, with low RMSE (0.207 to 0.484) and MAE (0.186 to 0.369) values and high R^2 (0.70 to 0.89) values in predicting N content in leaves and stems (Table 5-3 and Table 5-4), which indicates effectiveness of those indices in capturing variations in leaf N concentration. When comparing SVR and RF models, the RF algorithm consistently outperformed SVR across most bands and indices regarding predictive accuracy.

However, some indices and bands had poor predictive performance for seed yield. The SVR model performed best with the Red band (R^2=57), while the RF model achieved better results with the GNDVI index R^2=58) (Table 5-5).

UAV images and vegetation indices can accurately predict N content in hemp plants, and the NIR band, EVI, and R3 index are valuable predictors. The RF algorithm generally outperformed SVR in capturing complex relationships between input variables and N content. Nonetheless, some indices and bands may require further refinement or consideration of the growth stage for accurate predictions.

Overall, we can say that UAV images vegetation indices along with different machine learning models, have high potential to build predictive models or tools to monitor real-time plant nutrient status and N recommendation for the hemp plants.

Table 5 - 3: The RMSE, MAE and R^2 values of different band and vegetation indices for the SVR model and Random Forest model to predict leaf N content in hemp plant.

Index	Support Vector Regression			Random Forest		
	RMSE	MAE	*R^2*	RMSE	MAE	*R^2*
Blue	0.572	0.408	0.52	0.486	0.384	0.73
Green	1.129	0.801	-0.53	0.912	0.698	0.06
Red	0.947	0.809	0.12	0.943	0.861	0.00
NIR	0.484	0.356	0.74	0.376	0.328	0.84
redEdge	0.887	0.668	-0.19	1.010	0.821	-0.15
NDVI	0.728	0.563	0.31	0.779	0.692	0.31
GNDVI	0.967	0.794	-0.84	0.923	0.837	0.04
NDRE	0.714	0.609	0.39	0.704	0.578	0.44
RVI	0.764	0.617	0.10	0.736	0.622	0.39
EVI	0.452	0.369	0.76	0.405	0.318	0.81
DVI	0.518	0.389	0.67	0.460	0.383	0.76
R1 (Blue/Green)	0.668	0.539	0.42	0.773	0.681	0.32
R2 (Red/NIR)	0.812	0.623	0.08	0.774	0.657	0.32
R3 (Red/Blue)	0.356	0.305	0.88	0.312	0.248	0.89

Table 5 - 4: The RMSE, MAE and R^2 values of different band and vegetation indices for the SVR model and Random Forest model to predict stem N content in hemp plant.

Index	Support Vector Regression			Random Forest		
	RMSE	MAE	R^2	RMSE	MAE	R^2
Blue	0.315	0.236	0.38	0.242	0.185	0.60
Green	0.460	0.359	-0.28	0.376	0.296	0.04
Red	0.413	0.330	-0.03	0.384	0.341	0.00
NIR	0.237	0.200	0.74	0.225	0.168	0.66
redEdge	0.394	0.315	0.24	0.430	0.352	-0.25
NDVI	0.367	0.309	0.12	0.342	0.305	0.21
GNDVI	0.397	0.348	0.01	0.398	0.331	-0.07
NDRE	0.361	0.251	0.29	0.288	0.221	0.44
RVI	0.403	0.333	0.17	0.324	0.275	0.29
EVI	0.238	0.186	0.70	0.232	0.164	0.64
DVI	0.293	0.225	0.48	0.265	0.215	0.52
R1 (Blue/Green)	0.301	0.227	0.50	0.372	0.300	0.06
R2 (Red/NIR)	0.365	0.295	0.30	0.317	0.255	0.32
R3 (Red/Blue)	0.234	0.187	0.74	0.207	0.147	0.71

Table 5 - 5: The RMSE, MAE and R^2 values of different band and vegetation indices for the SVR model and Random Forest model to predict hemp seed yield.

Index	Support Vector Regression			Random Forest		
	RMSE	MAE	R^2	RMSE	MAE	R^2
Blue	1.981	1.613	0.14	2.195	1.802	0.18
Green	2.545	1.830	0.00	2.136	1.600	0.22
Red	1.694	1.298	0.57	1.729	1.258	0.49
NIR	2.607	1.885	0.00	2.455	1.894	-0.03
redEdge	1.952	1.616	-0.45	2.485	2.067	-0.06
NDVI	1.963	1.486	0.17	2.066	1.500	0.27
GNDVI	1.782	1.385	0.41	1.569	1.265	0.58
NDRE	1.932	1.625	0.27	1.908	1.571	0.38
RVI	2.152	1.587	0.30	2.107	1.529	0.24
EVI	1.970	1.385	0.30	2.139	1.565	0.22
DVI	1.966	1.494	0.24	2.034	1.549	0.29
R1 (Blue/Green)	2.723	2.120	-0.07	2.427	2.074	-0.01
R2 (Red/NIR)	1.894	1.499	0.22	1.947	1.399	0.35
R3 (Red/Blue)	1.573	1.163	0.54	1.766	1.352	0.47

CONCLUSION

Industrial hemp holds great promise for building more sustainable economies. Nutrient management plays a critical role in establishing economically viable cropping systems, and predictive models that use precision agricultural nutrient management techniques offer promise to further improve its environmental potential while supporting farm productivity and economic viability. This study tested the potential to determine hemp N concentrations and seed yield from multispectral images modeled with several vegetation indices in plots treated with N multiple fertility levels. Hemp seed yield was significant though inconsistently influenced by the nitrogen inputs, which reflected differences in precipitation and competition from year to year. In a year with a short growing season (2020), seed yields (1643 kg ha^{-1}) differed little across N rates above control. With adequate rainfall and a full growing season (72 days), yield response to N was linear and reached 2510 kg ha^{-1} at the greatest N rate (240 kg N ha^{-1}). However, limited rainfall and high competition (2022) produced only a weak response to N rate and low seed yields (382 kg N ha^{-1}) and only small differences among N rates. Similar patterns were observed for plant height and biomass response to fertility. These observations emphasize the influence of environmental factors in determining hemp's response to N rate.

Strong positive correlations between predicted and observed N concentrations in leaves and stems were generated with the R3 (R^2=0.88) and the NIR band (R^2=0.84). The GNDVI proved to be a better method of predicting seed yield, although it had a lower coefficient of determination (R^2=0.65). Additionally, the NIR band and DVI (Difference Vegetation Index) showed good predictability for N content in leaf, with an R^2 value of 0.88.

Future research should explore the interaction between N rates and other environmental factors to develop better models for more precise and tailored recommendations for hemp cultivation.

CHAPTER VI

HEMP AS A FORAGE CROP: HARVEST DATE EFFECTS ON YIELD AND NUTRITIVE VALUE

ABSTRACT

Hemp (*Cannabis sativa* L.) has been a valuable species for humans throughout history due to its adaptability and diverse uses. Farmers are interested in hemp as a forage and feedstuff due to its unique nutritional properties and rapid summer production. This study examined yield and nutritive value of three hemp cultivars, ‘Grandi’, ‘Joey’, and ‘EcoFibre’ as potential forage crops when harvested at weekly intervals in Blacksburg, VA, USA, during 2021 and 2022. The greatest biomass and TDN yield across all cultivars were 3.17 Mg ha^{-1} and 2.08 Mg ha$^{-1,}$ respectively, at two months after establishment in 2021. However, biomass and TDN yield were 1.9 Mg ha^{-1} and 1.03 Mg ha^{-1}, respectively at two months after establishment in 2022. The nutritive value in hemp plant biomass harvested at different times varied by cultivar and harvest time ($P < 0.05$). Depending on cultivar and harvest time, hemp plant biomass contained 13 to 32% CP, 22 to 45% NDF, 20 to 38% ADF, 4 to 9% lignin, and 52 to 80% TDN (cultivar × time interaction; $P < 0.05$). Hemp CP and TDN decreased gradually with maturation while ADF, NDF, and lignin increased; however, this decline with maturity did not appear as severe as occurs with many other forages. These preliminary results suggest that hemp has the potential to be used as a forage crop. More research is needed to address hemp management and utilization, including field establishment and production, harvest timing for optimum tonnage and forage quality, and animal intake and performance studies.

INTRODUCTION

Interest in growing hemp in the USA has been increasing since its legalization. One potential avenue for utilizing hemp is as a dedicated forage crop, particularly as a short season summer annual when cool-season forage supply may be limited. Unlike cool-season forages that experience growth slowdown during the summer, hemp grows rapidly during this period, and thus could provide a valuable feed source when other forage resources are scarce. Although there is limited published literature on livestock feeding trials involving hemp, anecdotal evidence and a few previous studies (Ely and Fike 2022) provide valuable insights into its potential as an alternative forage option.

Historically, feeding livestock with cannabis (whether plants, seeds, or by-products) has been a common practice in various regions of the world. For example, early Chinese texts indicate hemp seed served as a source of food and fuel in rural China, with the press cake resulting from oil extraction also being used as animal feed (Whitfield, 1999; Fike, 2019), and similar uses for hemp have been tested in the modern era (Hessle et al., 2008; Karlson et al., 2010). Fresh hemp leaves also have been used as feed for pigs and other livestock (Ely and Fike 2022; Clarke and Diemenstraat, 1995). In Pakistan, water buffalo have been observed grazing on wild *Cannabis sativa* plants (Ahmad and Ahmad, 1990). In Nigeria, growers have also utilized cannabis plants as an appetite stimulant for goats (Bamikole and Ikhatua, 2009). These traditional practices and observations suggest that hemp (in many forms) can be successfully incorporated into livestock diets.

In one of the few available studies that investigated hemp as a forage, Stringer (2018) estimated the nutritive value and digestibility of hemp biomass 42 days after planting. Hemp had an average crude protein (CP) concentration of 17%, comparable to mature alfalfa (*Medicago sativa*) hay or alfalfa meal (NRC, 2007). However, factors such as hemp variety and planting date affected suitability as a forage. Despite the promising results from a nutritional perspective, the economic viability of hemp as a forage option for farmers remains uncertain (Stringer, 2018).

Timing and method of harvest and post-harvest processing may also be important factors in hemp's utility as forage. Pecenka et al. (2007) stated that hemp plants harvested at vegetative stages can produce suitable silage with a CP as high as 24.8%. In Canadian research, hemp silage had CP levels around 19%, comparable to alfalfa silage (Duckworth, 2000). However, further research is required to optimize processing methods and understand their impact on palatability.

Increasing fiber concentrations as plants mature is often considered a primary limitation for forage quality and intake. However, as hemp matures, it produces aromatic terpenes that also may pose challenges to its palatability to and acceptance by livestock. The olfactory perception of livestock plays a crucial role in determining their feed preferences and choices (Rapisarda et al., 2012). Several research studies have documented diverse levels of preference exhibited by lambs towards hemp biomass, encompassing a spectrum that ranges from unrestricted consumption to complete rejection (Parker et al., 2022). Addressing these challenges may involve breeding programs to reduce terpene levels, processing techniques such as pelletizing or blending hemp with other feed materials to dilute the aroma and improve acceptability. Success with these practices also may depend on the target animal species (Coffey et al., 2016)

Currently, hemp is not registered as feed for livestock. However, there is a possibility that the USDA may change the existing rules in the near future once scientific findings support the potentiality of hemp as an alternative feed and forage source. Information regarding hemp crop management as forage is limited. Preliminary research suggests that when harvested at the right stage, hemp can have a high relative feed value (Stringer, 2018). However, the challenge of producing hemp as a forage crop likely comes from balancing the need to produce substantial tonnage with the need for sufficient forage quality. Factors such as cultivar selection, planting date, seeding rate, fertility management, and harvest management will influence the forage quality and overall success of hemp as a livestock feed source.

The primary objective of this study was to assess harvest date effects on productivity and forage nutritive value of two different hemp types. By evaluating the nutritive value, and biomass yield of hemp as a forage crop, this research aims to provide insights into its potential as a feed option for livestock during the summer growing season.

MATERIALS AND METHODS

Study Sites and Soils

This study was conducted at Virginia Tech's Urban Horticulture Center (UHC) in 2021 and at the university's Kentland Farm in 2022. Both sites are located in Blacksburg, VA. Soils at both locations are predominantly loam. Soils at the UHC are Duffield-Ernest complex (fine-loamy, mixed, active mesic Ultic Hapludalfs and Fine-loamy, mixed, superactive, mesic Aquic Fragiudults). Soils at Kentland are Unison and Braddock (fine, mixed, semiactive, mesic Typic Hapuldults). The experiment was a randomized complete block design with repeated measures and four replicates for each of three cultivars.

Weather Data

Daily mean maximum and minimum temperatures, and precipitation data were downloaded from Virginia Tech WeatherSTEM Data Mining Tool (http://vt-arec.weatherstem.com) for the entire study period for 2021 and 2022 (accessed on 13 January 2023).

Seeding

Three hemp cultivars, ‘Grandi’ (a seed variety), ‘Joey’ (a dual-purpose variety), and ‘EcoFibre’ (a fiber variety) were used for the study. Only Grandi and Joey were grown in 2021. EcoFibre was added to the study in 2022. All cultivars were planted with a planned seeding rate of 28 kg ha^{-1} but corrected for germination percentage. The germination percentage of these hemp cultivars was determined in the lab by ragdoll test (Chambliss, 2002) prior to the study. Plots were prepared with conventional tillage and seeds were planted to 1 cm depth with a no-till drill on May 12, 2021 and May 17, 2022. Nitrogen fertilizer (46-0-0) was applied at 112 kg N ha^{-1} within one week after seeding.

Sampling

Harvests at one-week intervals commenced on June 7, 2021, about three weeks following establishment, and continued until July 19, 2021, totaling seven harvests. In 2022, only three harvests were conducted from July 6 to July 19. In 2022, sampling was not done on June 7 to June 28 as plants were shorter and limited biomass were produced because of drought. Before each harvest, plant heights and plant populations were recorded. A 0.25-m quadrat was used to define sampling areas, and one quadrat was harvested per plot. Whole hemp plants were harvested at 10 cm height from the ground surface and a fresh weight was recorded to determine fresh biomass yield. Biomass samples were dried at 43°C in a forced-draft oven for a minimum of 48 h. Sample dry weights were used to estimate biomass yield at harvest.

In 2021, along with the quadrat sampling, an additional six hemp plants were collected each harvest date to partition into different plant components, namely leaf, hurd fiber, bast fiber, and inflorescence. In 2022 biomass partitioning was not performed, as plants didn’t produce enough biomass per plant because of drought.

Sample preparation and analysis

Dried biomass samples of whole plants and the different plant fractions were ground through a 2-mm screen with a Wiley Mill (Arthur H. Thomas Co., Philadelphia, PA, USA) and then through a 1-mm screen with a cyclone mill (Udy Corp, Fort Collins, CO). To ensure consistent moisture levels and reduce variability in predicted results, the prepared samples were placed in a forced air oven at 55°C before scanning on a near-infrared spectrometer (NIRS; McIntosh et al., 2019). The scanning was performed using an NIRS DS2500F instrument equipped with ISIScan Nova v. 8.0.6.2 software (Foss North America, Eden Prairie, MN). Following expert recommendations (McIntosh, University of Tennessee, personal communication), the acquired spectra were then applied to the 2022 Mixed Hay (MH) calibration provided and licensed by the NIRS Forage and Feed Consortium (NIRSC, Berea, KY). Accuracy of the predictions was assessed using global and neighborhood statistical tests, ensuring that the entire dataset conformed to the calibration within a limit of fit ($H < 3.0$; Murray and Cowe, 2004). Nutritive analyses are reported based on measurements at 100% dry matter (DM) across the entire dataset.

Total digestible nutrient concentrations (TDN) were calculated using estimates of neutral and acid detergent fibers (NDF and ADF), CP, and lignin. The TDN equation was based on the formula for legume and legume grass mixes published by Horrocks and Vallentine (1999), where:

$$TDN = 73.5 + 0.62 * \%CP - 0.71 * \%ADF$$

Statistical analysis

Data were analyzed with Proc Mixed in SAS Studio (3.8; Cary, NC) using a mixed model ANOVA. A repeated measures analysis was performed to investigate the impact of harvest time on plant height, biomass yield, and nutritive value. The analysis employed a repeated measures design, specifying the variable Plot as the subject and utilizing an unstructured covariance structure, which was chosen based on lower AIC (Akaike Information Criterion) and BIC (Bayesian Information Criterion) values, to account for the dependencies within the measurements. Data were analyzed separately by year due to the inclusion of an additional cultivar and the difference in harvesting times in 2022. Cultivar and harvest date were considered fixed effects while replicate was considered a random effect. LS-means and Tukey's adjusted differences were calculated. Results were considered statistically significant at $P < 0.05$ while the range of $P \geq 0.05$ to $P \leq 0.1$ was taken into account to consider trends in the data.

RESULTS AND DISCUSSION

Weather

Growing conditions were similar between years, with moderate temperatures and typical rainfall patterns (Figure 6-1). In 2021, the maximum study period temperature was 27.5°C, with a minimum temperature of 14.8°C and an average temperature of 21.6°C with total precipitation of 81.6 mm. In 2022, the maximum temperature increased slightly (28.7°C), while the minimum and average temperatures remained the same or similar at 14.8°C and 21.8°C, respectively. Total precipitation in 2022 was 74.7 mm.

Although both years experienced adequate total rainfall after seeding, there was a notable difference in precipitation distribution. In 2021, rain events were distributed throughout the growing season, ensuring adequate soil moisture for the plants. In contrast, in 2022, initial rains were followed by limited (19.7 mm) precipitation over the rest of the growing season.

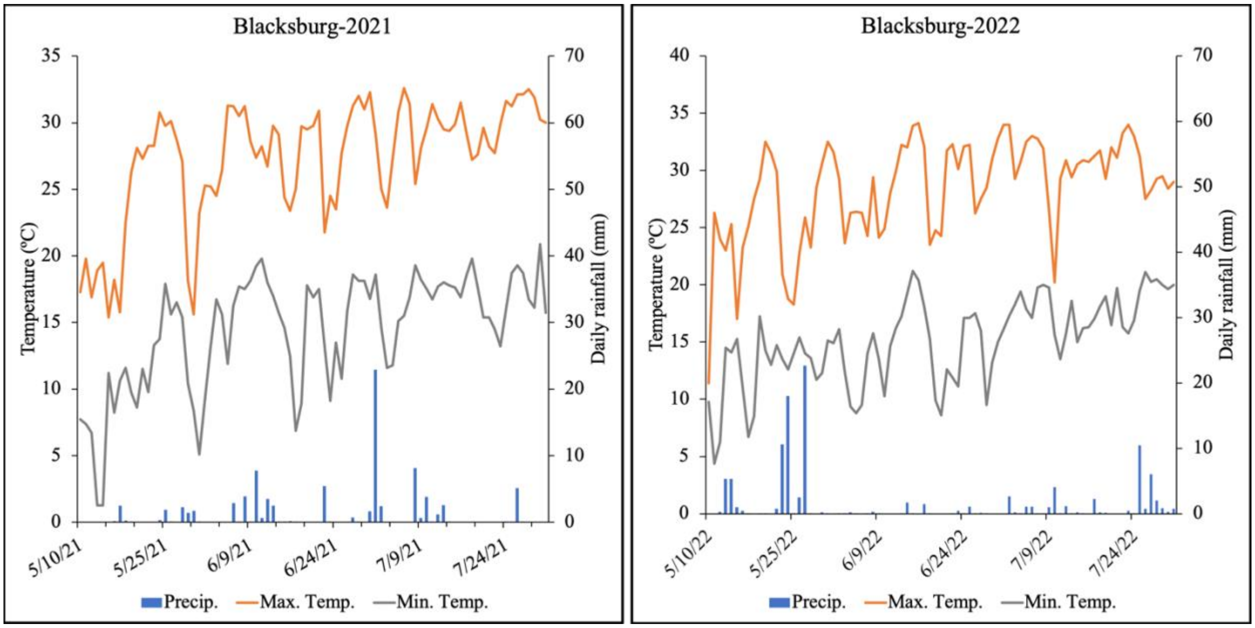


Figure 6 - 1: Daily maximum and minimum temperature and precipitation for the study site Blacksburg, VA, in 2021, and 2022.

Plant height

Plant heights were similar for Grandi and Joey at the first two dates in 2021, but over time Joey continued to grow taller (cultivar × time interaction; $P < 0.0001$). By the fourth harvest, the average height for Joey was about 1.45 m compared to 1.14 m for Grandi. Plant heights remained unchanged for both cultivars at subsequent measurement times (Figure 6-2). Cultivar × time interaction ($P < 0.0001$) was also observed in 2022, but in this case Grandi and Joey had similar plant heights (0.4 m) at all measurement times, whereas EcoFibre continued growing taller, reaching a height of 0.85 m. Continued height growth of EcoFibre was expected, since the vegetative growth of fiber-type hemp continues much later into the summer at this latitude (Fike and Podder, personal observation) until the last harvest, leading to an increase in plant height. Differences in precipitation patterns led to a noticeable difference in plant heights between the two years (Figure 6-1).

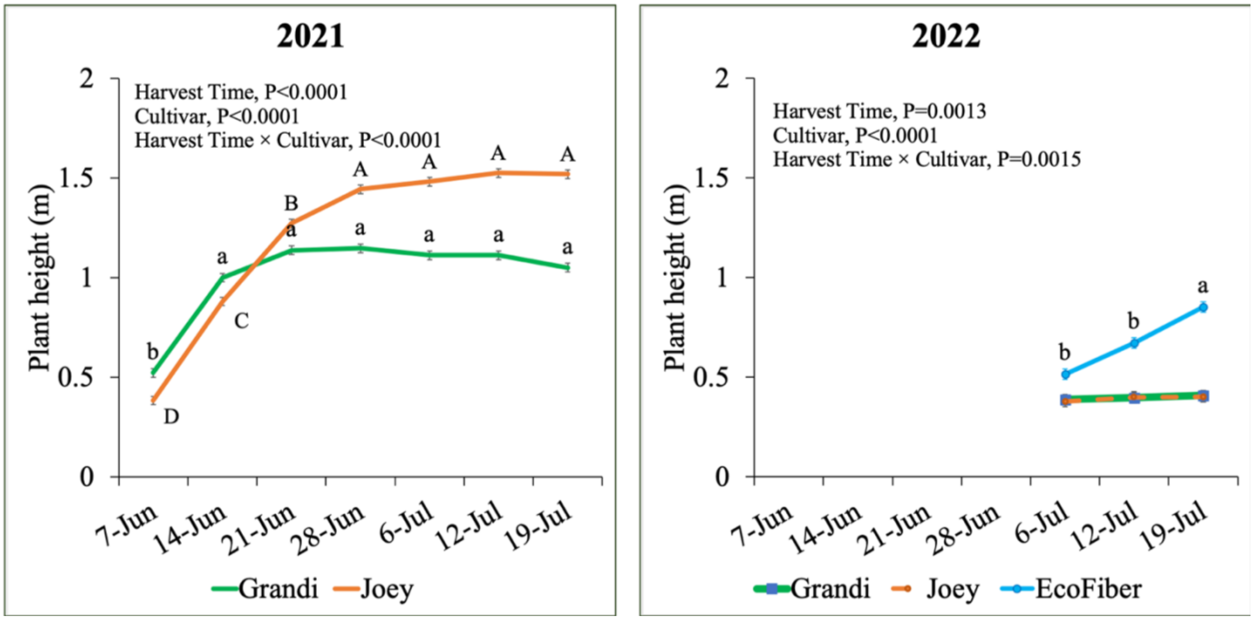


Figure 6 - 2: Mean plant height of two hemp cultivars (Grandi and Joey) at different sampling times in 2021 and three hemp cultivars (Grandi, Joey, and EcoFibre) in 2022 in Blacksburg, Virginia.

Biomass yield

Yields increased with time in each season, although the effect of time was greater in 2021 ($P \leq 0.0001$) than in 2022 ($P = 0.0157$). Additionally, there was a significant variation in biomass yield among cultivars in 2022 ($P = 0.0001$) where EcoFibre produced higher biomass ($P < 0.0001$) compare to Joey and Grandi. However, no significant interaction was observed between harvesting time and cultivar for biomass yield in either of the years (Figure 6-3).

In 2021, biomass yield steadily increased from the first to the seventh harvest. The lowest biomass yield, 0.36 Mg ha^{-1} (average across two cultivar Joey and Grandi), was recorded during the first harvest on June 07, 2021, which occurred three weeks after establishment. The highest biomass yield, 3.16 Mg ha^{-1}(average across two cultivar Joey and Grandi), was observed during the seventh harvest on July 19, 2021, when the plants were nine weeks old (Figure 6-3).

Despite their differences in growing patterns in 2021, Grandi and Joey exhibited similar biomass production. Patterns of growth did not differ between Grandi and Joey in 2022, and the cultivars again displayed comparable biomass production, with peak biomass (1.9 Mg ha^{-1}) at the last harvest on July 19. EcoFibre had the greatest biomass yield in 2022, which was expected as EcoFibre remained in vegetative growth, continuing to accumulate biomass through the last harvest. Biomass yield in 2022 was considerably lower than in 2021due to the limited precipitation during the growing season.

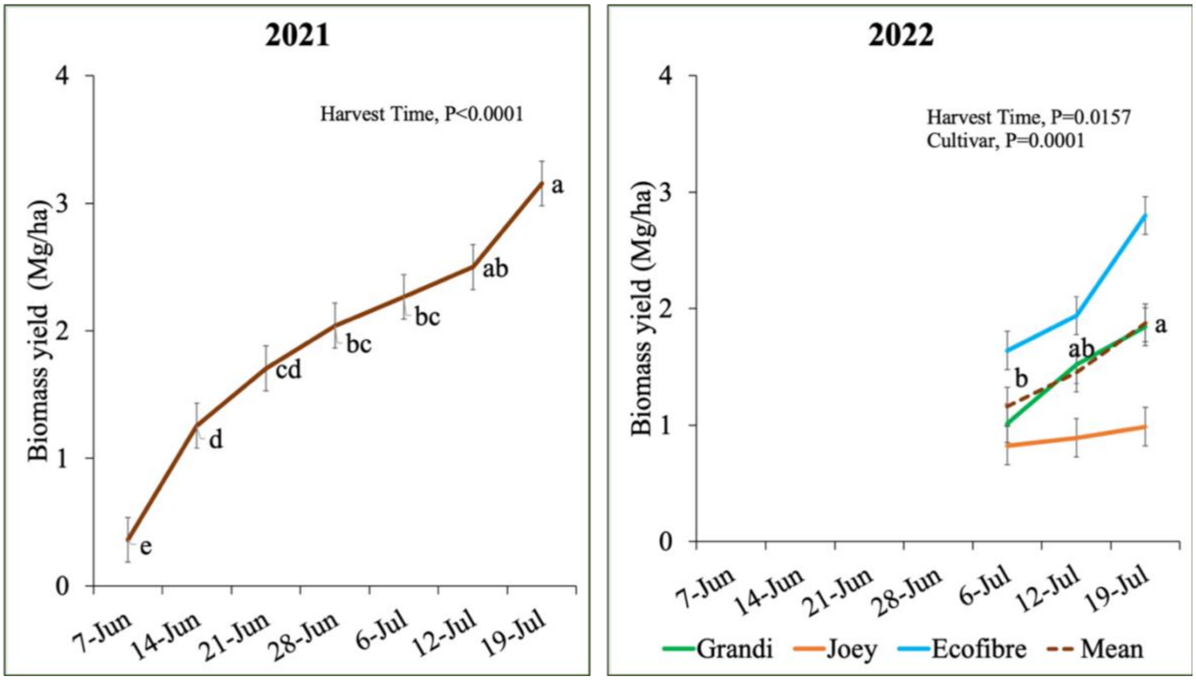


Figure 6 - 3: Mean biomass yield in hemp plants, averaged across two cultivars (Grandi and Joey) for seven different sampling times in 2021, and averaged across three cultivars (Grandi, Joey and EcoFibre) for three different sampling times in 2022, in Blacksburg, VA

Biomass partitioning

The percentage of leaf biomass was greatest during the first harvest on June 7, 2021, ($P < 0.05$) and gradually decreased with subsequent harvests. From first harvest to last, leaf biomass percentages for Grandi decreased from 58.9% to 23.4%. Similar changes were observed for Joey, with leaf percentage dropping from 60.9% to 20% from the first to the last harvest ($P < 0.05$) (Figure 6-4).

Grandi had the highest percentage of hurd fiber biomass (35.7%) during the fourth harvest, with this decreasing to about 31% at subsequent harvests. Joey had its least hurd fiber biomass percentage (32%) at the first harvest, and this fraction's proportion gradually increased over time, reaching its highest hurd fiber percentage (38.7%) at the last harvest.

Bast fiber percentages were lower during the first harvest for both cultivars and essentially doubled from first to last harvest (from 5.6% to 11.0% for Grandi and from, 7.1% to 15.0% for Joey) ($P < 0.05$).

Inflorescence percentage gradually increased for both varieties (from 0% to 33.3% for Grandi and 0% to 25.9% for Joey), although peak inflorescence proportion occurred earlier (July 6) for Grandi. This increase in inflorescence proportion was directly associated with the formation of seeds as the plants began reproductive development.

Determining the biomass partitioning of hemp plants provides important information for evaluating the crops' potential as forage and for the understanding its forage nutritive value. During early harvest events, when hemp was in a vegetative growth stage, a higher percentage of the plant was made up of leaf biomass, but overall biomass yield remained low. As the plant developed, there was an accumulation of hurd and bast fibers that would be needed to support the weight of reproductive structures (Jeff and Williams, 2019). Although such partitioning would generally decrease the digestibility of a forage, this was compensated at later stages of development with hemp seed formation and fill.

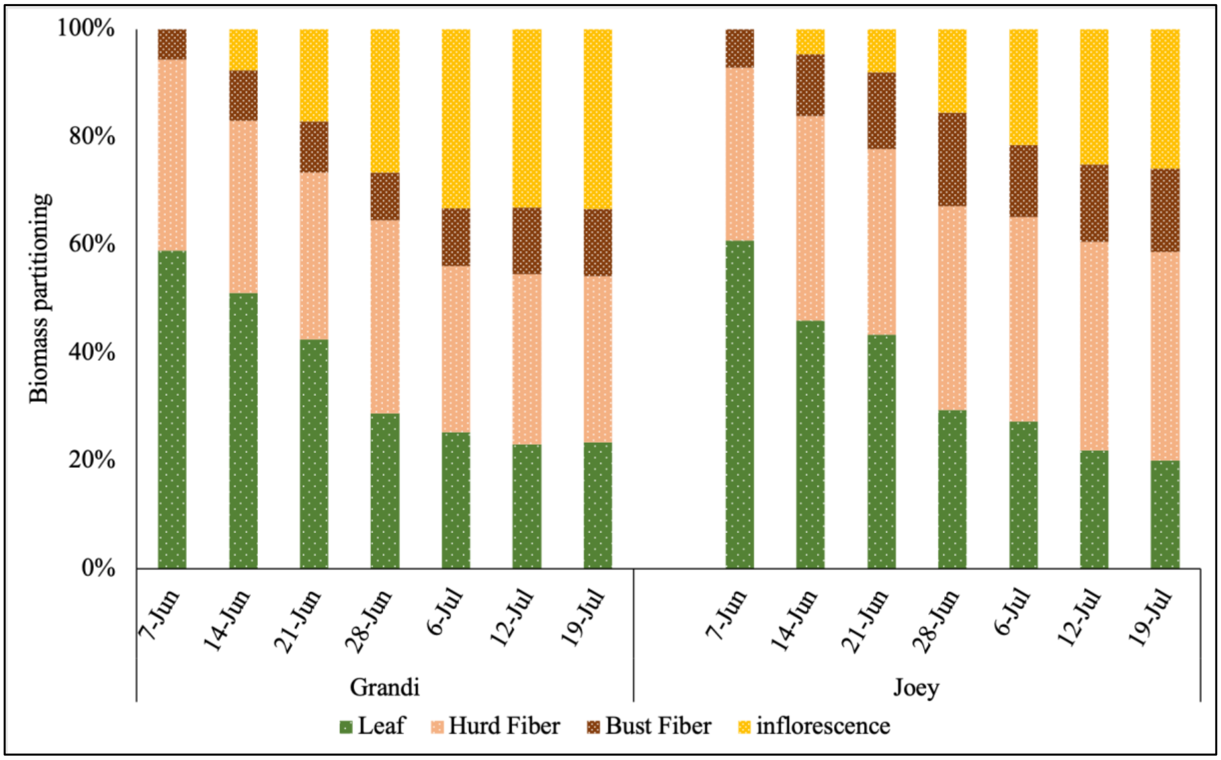


Figure 6 - 4: Biomass partitioning by different parts (e.g. leaf, hurd fiber, bast fiber, inflorescence) in two hemp cultivars (Grandi, and Joey) in Blacksburg, VA in 2021.

Nutritive value in plant parts

Within individual plant fractions (leaves, hurd fiber, inflorescence, and bast fibers) nutritive constituents varied ($P < 0.0001$) with time. Leaf CP concentrations were very high (35.2%) at the first harvest but gradually decreased to about half that (18.6%) six weeks later (seventh harvest). Similarly, CP in hurd fiber declined from a high (22.9%) concentration at the start of the study (June 7), to rather low concentrations (7.7%) by the sixth harvest (July 12, 2021). Bast fiber CP levels were not as high and changes were not as precipitous as with hurd fiber, declining from 11.7% at the first harvest to 7.7% at the sixth harvest. Declines in inflorescence CP concentrations were more moderate, dropping from 31.4% to 26.1% at the final (July 19) harvest (Figure 6-5).

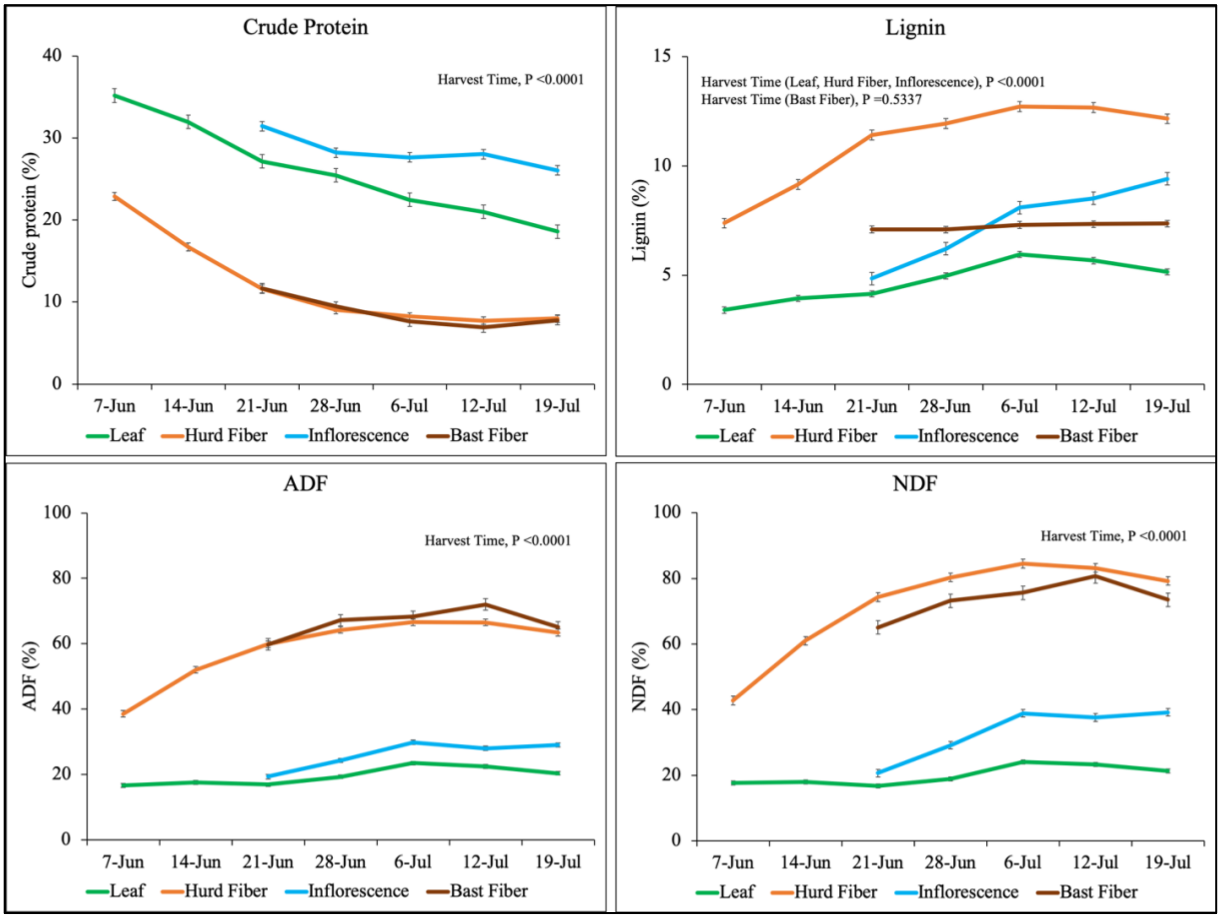


Figure 6 - 5: Mean crude protein, lignin, ADF, NDF content in different plant parts of two hemp cultivars (Grandi and Joey) for different harvest times in Blacksburg, VA in 2021.

Whole-plant nutritive value

Crude protein

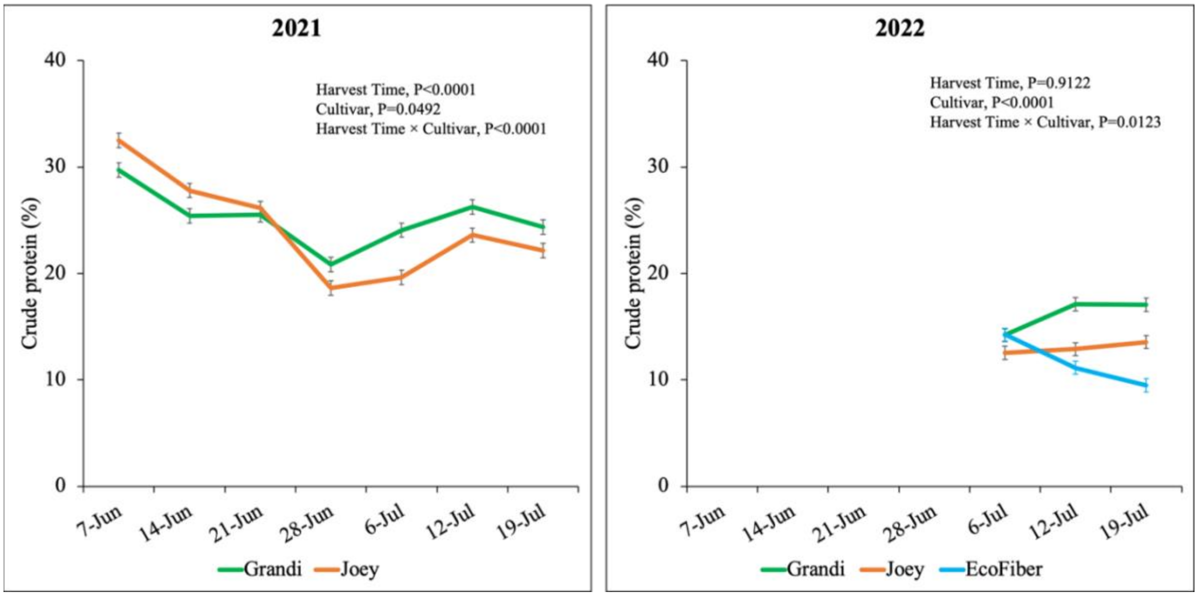
In 2021, hemp CP concentrations initially were lower in Grandi than in Joey (29.7 vs. 32.5%), but CP declined substantially in Joey between the third and fourth harvests, resulting in greater CP in Grandi at later harvest dates (cultivar × time interaction; $P < 0.0001$). In 2022, CP concentrations were relatively consistent for Grandi and Joey and greater in Grandi at all harvests. However, cultivar × time interaction (*P = 0.0123*) was again observed and driven by decreasing CP concentration in EcoFibre over time (Fig. 6-6).

The high initial values for CP were expected due to the greater leaf-to-stem ratio at harvest and the predominantly vegetative growth stage of the plants. As the plants matured and the leaf-to-stem ratio decreased, the CP content gradually decreased. By the fourth harvest on June 28, 2021, CP concentrations were 20.9% and 18.6% for Grandi and Joey, respectively. However, after this time, CP concentration gradually increased again, reaching 26.2% and 23.6% for Grandi and Joey, respectively. This later increase in CP content was attributed to the contribution of CP from hemp seeds, which form during the later stages of plant growth.

In 2022, CP concentrations were not significantly affected by harvest time, as sampling was conducted only during the later stages of growth. From the first to third harvest between July 6 and July 19, hemp CP concentrations increased from 14.2% to 17.1% for Grandi and from 12.5% to 13.6% for Joey, but CP declined from14.3% to 9.5% for EcoFibre (Figure 6-6). The gradual decrease in CP content observed in EcoFibre can be attributed to the increased accumulation of stem biomass (hurd fiber + bust fiber) and the decreasing leaf-to-stem biomass ratio, without any contribution from CP-rich seeds, as EcoFibre remained in the vegetative stages through the last harvest on July 19, 2022.

The higher percentage of CP observed in the early harvests of hemp biomass is indicative of its potential as a nutritionally superior forage option that could support a high level of animal performance (NRC 2007). These findings are consistent with the results stated by Pecenka et al. (2007), who found hemp silage to contain 24% crude protein (CP) during vegetative development stages. Additionally, Duckworth (2000) stated that hemp silage had a CP content of 19%.

The sustained high crude protein (CP) content observed in these hemp seed and dual-purpose cultivars indicates that hemp has the potential to be utilized as a forage option in livestock nutrition. However, additional research is required to validate this potential through feeding trials..



.Figure 6 - 6: Mean crude protein concentrations for different harvest times in Blacksburg, VA in two hemp cultivars (Grandi and Joey) in 2021 and in three hemp cultivars (Grandi, Joey, and EcoFibre) in 2022.

Lignin

Lignin concentrations generally increased ($P \leq 0.0112$) with harvest time during both seasons and were not affected by harvest time ×cultivar interaction ($P \geq 0.1088$). In 2021, lignin concentrations did not vary ($P = 0.2155$) by cultivar, but cultivar effects were observed in 2022 ($P < 0.0001$) (Figure 6-7).

In 2021, lignin content was relatively lower in the early stages of plant growth and steadily increased as the plants matured. Lignin concentrations more than doubled from June 7 (4.2%) to July 19, (9.4%). In 2022, when plants were only harvested as they began going to form seeds, the range of lignin concentrations for Grandi and Joey cultivars (9.2% to 9.4%) were similar to concentrations from the same period of time in 2021. Lignin in EcoFibre increased from 6.8% to 8.1% during the July 6 to July 19 harvest period. The lower lignin content observed in EcoFibre compared to Grandi and Joey likely reflects the fact that EcoFibre remained in vegetative maturity stages through the final harvest in 2022.

Lignin content is an important measure for determining forage quality, as higher lignin content tends to decrease digestibility. The lignin content observed here (ranging from 4.2% to 9.4%) is significantly greater than that reported for warm-season grasses like forage sorghum (ranging from 1.7% to 5.4%; Podder et al., 2020), corn (4.9%), millet (3.5 to 6.3%) (Bhattarai et al., 2019), alfalfa hay 8.2% (Sturgeon et al., 2000). Such concentrations suggest that hemp may have lower digestibility than these warm-season grasses.

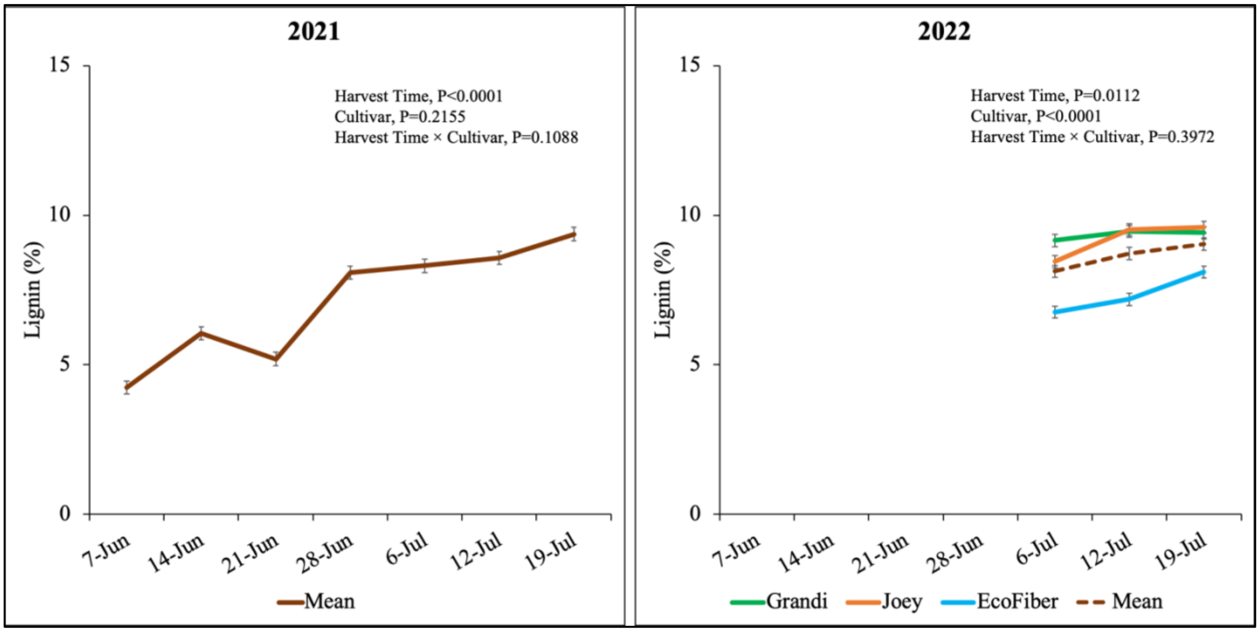


Figure 6 - 7: Mean lignin concentrations for different harvest times in Blacksburg, VA, averaged across two hemp cultivars (Grandi and Joey) in 2021 and three hemp cultivars (Grandi, Joey, and EcoFibre) in 2022.

ADF

Hemp ADF concentrations in 2021 were initially lower in Joey than in Grandi (19.9 vs. 25.0%) but were greater in Joey at the last three harvest dates (cultivar × time interaction; *P = 0.0056*). The highest ADF content was observed on June 28, 2021, after which it gradually decreased. This decrease in ADF content may be associated with the formation of hemp seeds during the later stages of plant growth. In 2022, the ADF concentrations in Grandi and Joey cultivars were relatively stable and similar over the three harvest times, with a range of ADF content from 36.8% to 37.2%. However, the ADF content in EcoFibre was lower (30%) than in Grandi and Joey (36.8%) during the harvest on July 6, 2022, and gradually increased to 38% by the harvest date of July 19, 2022. These varied responses again lead to cultivar × time interaction (*P = 0.0060*) (Figure 6-8).

Although the ADF content in the whole hemp plant during the early growth stages was comparable to that of other forage crops, the levels observed at later harvest stages (ranging from 36% to 38%) were slightly higher than that of other major forage crops, such as forage sorghum (27% to 34%; (Podder et al., 2020)) and again suggest limitations to using these varieties as a forage resource as higher ADF indicates lower digestibility.

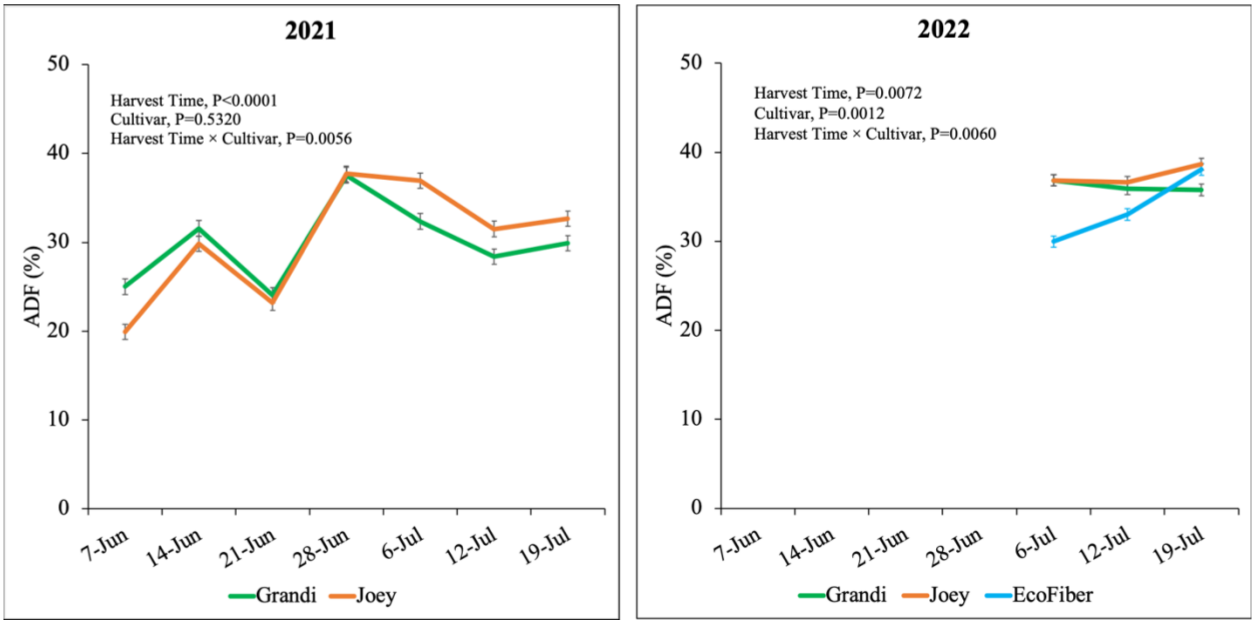


Figure 6 - 8: Mean ADF content for different harvest times in Blacksburg, VA, in two hemp cultivars (Grandi and Joey) in 2021 and in three hemp cultivars (Grandi, Joey, and EcoFibre) in 2022.

NDF

Hemp NDF concentrations followed very similar patterns to ADF (Figure 6-9). Concentrations in 2021 were initially lower in Joey than in Grandi (22.0 vs. 27.8%) but were greater in Joey at the last three harvest dates (cultivar × time interaction; $P = 0.0436$). The greatest concentrations (44%) were measured at the 4th harvest (June 28, 2021), and gradual decreases at the last three harvests were likely associated with the formation of hemp seeds during the later stages of plant growth. In 2022, Grandi and Joey NDF concentrations were relatively stable and similar over the three harvest times, with a range of NDF content from 42.6% to 46.1%, whereas EcoFibre NDF concentration was 33.6% at first harvest and increased to 44.5% at the final harvest (cultivar × time interaction; $P = 0.0091$).

Although the NDF concentration of whole hemp plants during the early growth stages was similar to that of other forage crops, levels were lower than many forage crops during the later harvest events (ranging from 37% to 46%). Greater forage NDF concentrations are generally associated with lower levels of intake (NRC, 2001) and thus hemp may offer promise relative to other forages at least in this regard.

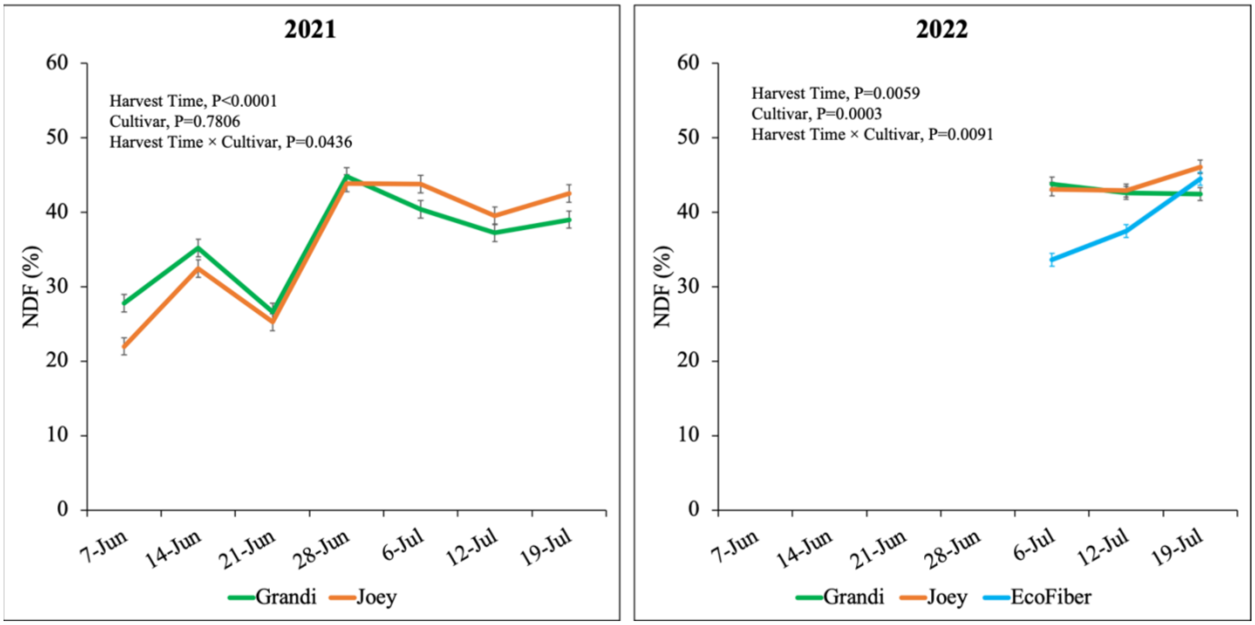


Figure 6 - 9: Mean NDF content for different harvest times in Blacksburg, VA in two hemp cultivars (Grandi and Joey) in 2021 and in three hemp cultivars (Grandi, Joey, and EcoFibre) in 2022.

TDN

In 2021, TDN concentrations generally declined as plants matured, but this response to harvest time was greater in Joey than in Grandi (cultivar × time interaction; *P = 0.0007*). Concentrations were generally high, and at their lowest level (59%) on June 28, 2021, before climbing to about 70% at the latter harvest times (Figure 6-10), would still have been adequate for non-lactating class of cattle (NRC, 2007). In 2022, Grandi and Joey had similar TDN levels lower than EcoFibre, but EcoFibre TDN levels declined as fibrous constituents increased (cultivar × time interaction; *P = 0.0062*).

Notably, TDN concentrations in both years (ranging from 53.4% to 79.5%) consistently exceeded the threshold level of 50% required for maintaining non-lactating cows. This suggests that hemp forage has the potential to serve as a viable alternative source of nutrients for forage-based diets if production levels can be adequate.

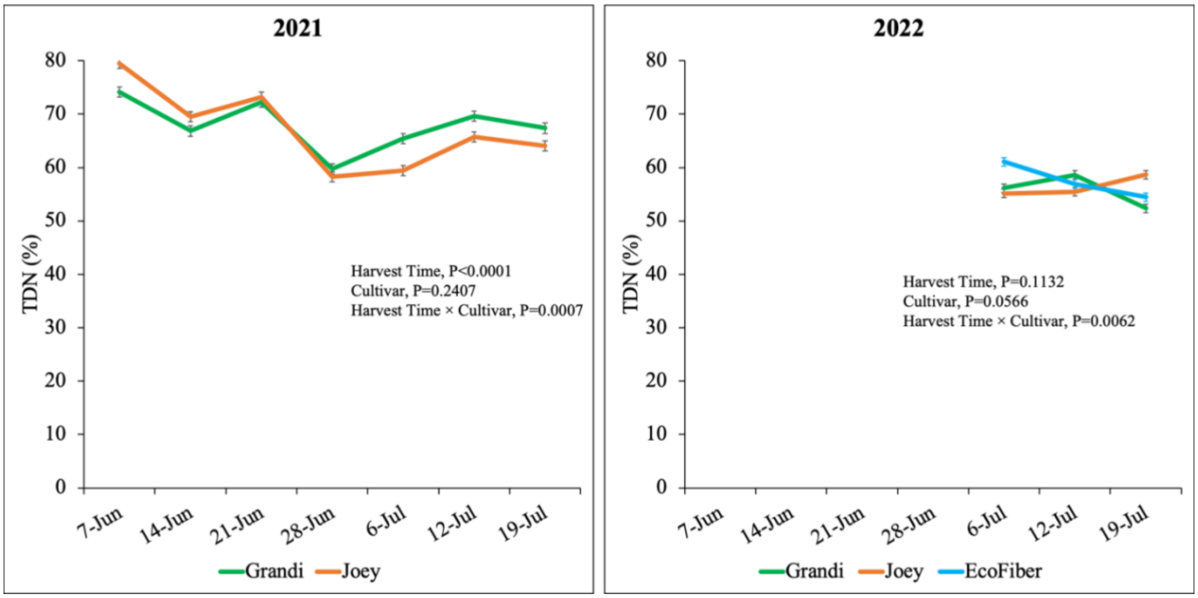


Figure 6 - 10: Mean total digestible nutrients (%) for different harvest time in Blacksburg, VA in two hemp cultivars (Grandi and Joey) in 2021 and in three cultivars (Grandi, Joey, and EcoFibre) in 2022.

TDN yield

Significant yield increases that come with plant maturity often are accompanied with large declines in forage nutritive value, rendering these larger biomass yields of limited value. Thus, we calculated TDN yield (TDN concentration × biomass yield) over the harvest dates in each season to understand this response in hemp. The increase in biomass yield over time had a much stronger effect on TDN yield than the moderate changes in forage nutritive value. In both years, TDN yield increased (*P = 0.0476*) over harvest dates, although this effect was more pronounced in 2021 when responses were measured over a greater number of harvests. TDN yield was the least (0.28 Mg ha-1) at the initial harvest on June 7, 2021, three weeks after establishment. Conversely, the highest TDN yield (2.1 Mg ha^{-1}) was observed during the seventh harvest on July 19, 2021, when the plants were nine weeks old (Figure 6-11).

In 2022, TDN yield varied less over time given the smaller, later-season harvest window, and the peak value (1.03 Mg ha^{-1}) was about half that measured in 2021, again when the plants were nine weeks old. Among the cultivars, Grandi and Joey exhibited similar TDN production in 2022, while EcoFibre displayed a higher TDN yield. This outcome was despite declining TDN percentage with time, but not unexpected as EcoFibre grew rapidly and remained in the vegetative stage until the last harvest, allowing for greater vegetative biomass accumulation.

It is worth noting that the overall TDN yield in 2022 was considerably lower compared to 2021. This may be attributed to limited precipitation during the 2022 growing season, which likely adversely affected the growth and biomass accumulation of the hemp plants. Despite its reputation (seemingly unwarranted) for being able to grow with limited water (Yano and Fu, 2023), hemp uses the C3 photosynthetic pathway (Tang, 2018) which is less water-use efficient than the C4 pathways common to warm-season forage grasses. Thus, the productive value of hemp as a short-season summer crop may be compromised by low yield, particularly in dryland operations experiencing limited moisture.

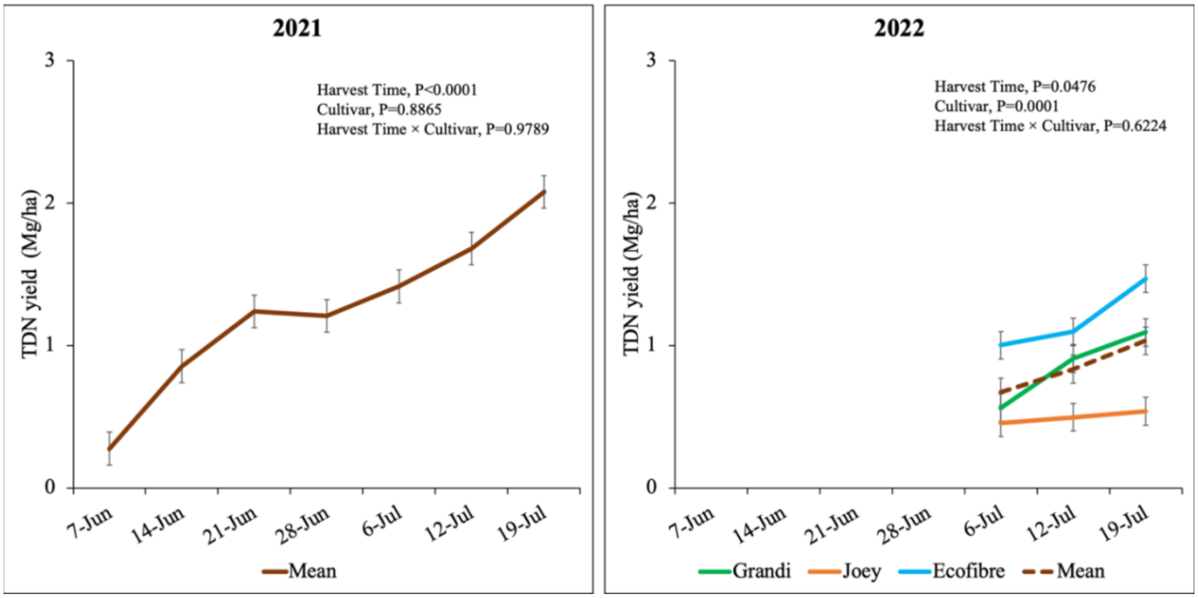


Figure 6 - 11: Mean total digestible nutrients yield (Mg ha-1) in hemp plants for different harvest time in Blacksburg, VA, averaged across two cultivars (Grandi and Joey) in 2021 and three cultivars (Grandi, Joey, and EcoFibre) in 2022.

Findings from this study suggest hemp has potential as a forage crop from a nutritional standpoint. The plant consistently exhibited moderate to high TDN levels that would meet the need of many classes of ruminant livestock. This might be further elevated by altering harvest practices (e.g., by harvesting at greater than a 10-cm cutting height), although that was not explored here. However, any gains in nutritive value at the expense of yield would likely not be justified with the seed and dual-purpose varieties studied here. Both hemp nutritive value and yield may be sensitive to harvest practice. Our yield data were derived by hand harvest and do not reflect the potential in-field and losses that could occur across the spectrum of available harvest practices. We speculate that making silage may be a best practice, given the potential for leaf and seed shatter during curing and collection processes if conserved as hay. To better understand hemp’s potential as a forage crop, further research is needed to explore the variability in TDN content among different cultivars in relationship to harvest times and management practices across growing environments.

Animal nutritional studies are also warranted to determine animal preference, intake, and performance. Such work should also assess whether the crop can be effectively utilized without issues related to cannabinoid transfer to meat and milk. Understanding these factors will be essential for utilizing hemp forage in livestock feeding.

CONCLUSION

This study provides valuable insights into the growth, yield, and nutritive value of hemp biomass, highlighting its potential as a forage crop for livestock nutrition. Harvest time and cultivar selection significantly impacted plant growth and nutritive value parameters with increases in plant yield for seed and dual-purpose varieties more than compensating for declines in hemp nutritive value, which was largely offset by inflorescence development and seed fill. True fiber type cultivars likely will not be as effective in this regard given their stalky nature and delay to reproductive development. The results underscore the importance of considering both harvest time and cultivar selection to optimize plant height, biomass yield, and nutritive value. These findings can contribute to the development of cultivation practices that maximize hemp productivity and enhance its utilization as a viable forage option. However, further research is necessary to investigate the numerous factors related to management practices for improved biomass yield and digestibility and the consequences for animal production.

CHAPTER VII

SUMMARY

The research conducted in this dissertation on industrial hemp production has provided valuable insights into optimizing cultivation practices, improving crop yield and quality, and exploring new applications for this versatile crop. The key findings and take-home messages from each chapter, including some quantitative data, are summarized below.

In the first study investigated the effects of tillage practices on hemp seed and fiber production. Results showed that tillage management significantly influenced plant height, and population density. For instance, in the study year 2020, the seed production system resulted in taller plants compared to the dual production systems. Additionally, the no-tillage systems exhibited lower plant populations for both seed and dual production compared to conventional tillage. Although plant population density varied, seed yields and biomass yields varied less by tillage management and production systems.

The second study focused on determining the harvest timing response on hemp seed yield and quality. Results indicated that harvest timing significantly affected seed yield, with variations observed among cultivars and locations. For example, in Blacksburg, the highest seed yield in the study year 2021 was recorded at the second harvest time, reaching 2,500 kg ha^{-1}. However, subsequent weeks of harvest showed a decline in yields. Fatty acids (FA) varied by cultivar, location, and harvest timing, but response patterns were inconsistent across FA. These findings emphasize the importance of selecting the appropriate harvest window to maximize seed yield and maintain desirable quality attributes.

The third study aimed to optimize nitrogen fertility rates and evaluate the use of aerial imagery for predicting seed yield and N concentration in hemp plants. Results demonstrated that nitrogen input significantly influenced seed yield. In 2021, the highest seed yield recorded in Blacksburg was 2,500 kg ha^{-1} at a nitrogen rate of 240 kg ha^{-1}. However, in 2020 and 2022 hemp plants' response to N wasn't as strong because of shorter growing window and limited rainfall. The findings also indicated the potential of aerial imagery-based prediction models, which showed promise in estimating seed yield and nitrogen content in hemp plants.

The fourth study explored the potential of hemp as a forage crop, focusing on biomass yield and nutritive value. Results showed variations in biomass yield and nutrient content at different growth stages. In 2021, the biomass and total digestible nutrient (TDN) yield at two months after establishment reached 3.17 metric tons and 1.89 metric tons per hectare, respectively. Depending on the cultivar and harvest time, hemp plant biomass contained 13 to 32% CP, 22 to 45% NDF, 20 to 38% ADF, 4 to 9% lignin, and 52 to 80% TDN. These findings highlight the potential of hemp as a forage crops in terms of nutritive value. More research is needed to address hemp management and utilization, and animal intake and performance studies.

In conclusion, this dissertation on industrial hemp production provides valuable insights into optimizing cultivation practices, harvest timing, nitrogen fertility rates, and the potential of hemp as a forage crop. The study reveals the importance of selecting appropriate tillage management to maintain optimal plant population density and establish hemp successfully. Additionally, the research emphasizes the need for careful cultivar selection and precise nitrogen management to maximize seed yield. Harvest timing significantly impacts seed yield, and further investigation is necessary to determine the ideal harvest window for different cultivars and locations. Moreover, hemp shows promise as a nutritive forage crop, but further research is required to fully understand its potential. Overall, both seed and fiber type hemp cultivars hold potential as commodity crops, and genetic improvements and region-specific agronomic management are crucial for enhancing production.

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