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EVALUATING HEMP *(CANNABIS SATIVA)* AS A FORAGE BASED ON YIELD, NUTRITIVE ANALYSIS, AND MORPHOLOGICAL COMPOSITION

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Carol Elizabeth Stringer, Student

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EVALUATING HEMP *(CANNABIS SATIVA)* AS A FORAGE BASED ON YIELD, NUTRITIVE ANALYSIS, AND MORPHOLOGICAL COMPOSITION

THESIS

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in the College of Agriculture, Food and Environment at the University of Kentucky

By Carol Elizabeth Stringer

Lexington, Kentucky

Director: Dr. Ben Goff, Professor of Crop Science

2018

ABSTRACT OF THESIS

EVALUATING HEMP *(CANNABIS SATIVA)* AS A FORAGE BASED ON YIELD, NUTRITIVE ANALYSIS, AND MORPHOLOGICAL COMPOSITION

This experiment examined the forage potential of hemp *(Cannabis sativa)* and kenaf *(Hibiscus cannabinus).* The objectives were to evaluate yield and forage nutritive value (i.e. NDF, ADF, ADL, IVTD, and CP) fluctuations over the course of a growing season based on planting date, morphological composition, and management. Three types of hemp (grain, fiber, and a dual- purpose type) and kenaf were planted on two dates and were sampled approximately every two weeks throughout the growing season at the University of Kentucky (UK) Research Farm in Lexington, KY. Subsamples were separated into morphological components (i.e. leaf, flowers, stem, core fiber, and bast fiber) while the remainder of the sample was ground for laboratory analysis. All samples were scanned in Foss 6500 NIRS and wet chemistry analytical methods were utilized on a subset of samples to develop equations to predict the nutritive value of the remaining samples. Significant interactions for forage type, planting date, and harvest time were observed for yield, % floral components, % bast, and ADL. Significant interactions occurred between planting date and harvest date as well as type and harvest date for NDF, ADF, digestibility, crude protein, % leaf, % core, and % stem. Overall, forage nutritive value declined with increased plant maturity. The later planting date reduced the vegetative growth period, resulting in reduced leaf content, yield, and forage nutritive value. The performance of kenaf in this study indicates that it may be a better alternative forage than hemp due to remaining vegetative longer and having superior nutritive value. Better selection and the development of new hemp varieties with different photoperiod requirements could lengthen the vegetative state and may result in yields and nutritive values that are more competitive with kenaf and other typical forages.

KEYWORDS: hemp, cannabis, forage nutritive value, kenaf, fiber crops, alternative forages

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Chapter 1: INTRODUCTION

Hemp *(Cannabis sativa)* is an annual plant and the only species in family Cannabinaceae (Chabbert, 2013). The term ‘hemp’ is given to *Cannabis sativa* varieties and biotypes containing less than 0.3% delta-9 tetrahydrocannabinol (THC) (a psychoactive compound) as defined by the 2014 US Farm Bill provision (H.R. 2642 sec. 7606 (2)). The distinction is a legal definition rather than a physiological one, as both hemp and ‘marijuana’ (i.e. by the legal definition, any *Cannabis sativa* with a THC content above the 0.3 % threshold) represent intraspecific variations within the *Cannabis sativa* species and readily cross with each other. For the remainder of this thesis, ‘hemp’ will refer to *Cannabis sativa* below the 0.3% limit required for agricultural production and research in the United States and ‘marijuana’ will refer to *Cannabis sativa* above this limit. ‘Cannabis’ will collectively refer to both types when a distinction between the two is not relevant.

Both hemp and kenaf (*Hibiscus cannabinus*) have been cultivated as fiber crops worldwide for centuries. The tough bast fibers, produced from the outer bark, are ideal for cordage, cloth, and paper (Alligret, 2013). While the exact origins of cannabis are unclear, current evidence suggests a point of domestication in Asia. This is reinforced by a long history of production in China where it has been cultivated since 8000 BC (Alligret, 2013; Small and Marcus 2002). After arriving in Europe around 2000 BC, hemp remained an important crop throughout Roman, Medieval, and Renaissance times for sail cloth. Only after the arrival of the cotton gin and the steam engine did European hemp cultivation begin to decline (Alligret, 2013).

There is considerable debate over whether cannabis has been truly domesticated. Cannabis retains a number of qualities atypical of domesticated crops. Specifically, while monoecious lines exist, cannabis retains a dioecious reproductive system, seeds do not fill or ripen simultaneously, and once seeds ripen, they typically shatter rather than remaining on the plant (Small and Marcus, 2002). These features present considerable hurdles to modern agronomic grain production for hemp.

Hemp has a unique historical relationship with the state of Kentucky. Originally introduced by Europeans, it has undergone two major periods of cultivation: colonization to the mid 1800’s and a revival in the 1940’s, which were focused on fiber and rope production for World War II (Hopkins, 1998). Locally adapted cultivars from this time period unfortunately were lost during the prohibition era (1937-2014), which prevented agricultural research and has created a problematic shortage of appropriate varieties for current producers. A lack of regional variants has proved to be only one major hurdle facing widespread adoption of hemp into Kentucky's crop portfolio. Unstable markets for cannabidiol (CBD), as well as a lack of processing infrastructure and harvesting equipment for fiber stalks, have presented their own challenges to the nascent hemp industry in Kentucky.

At this time, renewed interest in hemp production has also led to exploration of a number of alternative applications for this crop, including potential utilization as a forage. With Kentucky firmly established as a major forage state, evaluating the forage potential of any new crop is a logical step. According to literature, other fiber crops, such as kenaf, possess traits desirable for a forage crop under the correct management. These include high levels of crude protein when young, a growth period that corresponds to the period of low growth in cool-season grasses, and high biomass yields. It has been reported that hemp could provide similar levels of nutrition and may be considered as an "emergency forage," (i.e. a crop that is able to produce large quantities of biomass over a short period of time while still providing a suitable level of nutrition for livestock) (Rude et al., 2002).

This experiment evaluated the forage potential of hemp as a forage based on yield, nutritive value, and phenological state. By considering these three facets of hemp forage production, we can better understand the real usefulness of this crop as an emergency feed.

Chapter 2: LITERATURE REVIEW

2.1: Kentucky Forage Systems

Firmly planted in the “transition zone” between areas where cool-season and warm-season grasses are best adapted, Kentucky has long been known as a major forage production state (Ball et al., 2007). In 2017, over 2,150,000 acres of hay and pasture were in production within the state (USDA NASS 2017). The ability to establish both cool-season and warm-season pastures has allowed for more flexibility in management and has helped Kentucky become the biggest beef producing state in the Southeast (USDA NASS 2017). Typical systems in this area are dominated by cool-season perennial grasses such as Kentucky bluegrass *(Poa pratensis),* tall fescue *(Festuca arundinacea*), or orchardgrass *(Dactylis glomerata).* These are often combined with legumes such as red or white clover (*Trifolium pretense,* and *T. repens,* respectively*)* (Ball et al., 2007).

While most ruminants in the eastern U.S. are fed a perennial form of grass or legume, it is not uncommon for an annual crop such as turnip (*Brassica rapa sp. rapa),* corn (*Zea mays),* or sorghum (*Sorghum bicolor)* to be utilized in these systems. Annuals have several advantages over perennials in a livestock system. One advantage is that they are often capable of producing a large quantity of biomass in a short period of time relative to perennials (Teutsch, 2017). Rapid germination, fast growth, and relatively high forage quality can make annuals an attractive alternative during the summer months (Teutsch, 2017). Unfortunately, annuals may be difficult to establish during the variable rainfall of the early summer (Teutsch, 2017).

Annual forage crops may also provide a buffer against the seasonal fluctuations in perennial forage systems (Teutsch, 2017). The period of rapid growth in annuals corresponds to the period of low productivity experienced by perennial cool-season grasses important to pasture production, commonly known as “summer slump.” While warm-season perennial grasses typically have a peak growing season similar to annuals, they often have lower forage quality than cool-season grasses and can be drought sensitive during establishment (Ball et al., 2007). Including fiber crops into these systems to fill this gap may provide a source of feed and permits perennial forages adequate time to rest and restore carbohydrate reserves necessary for fall growth and winter survival.

While there are many factors that influence a producer’s decision of which plant species to include and utilize as part of their forage system, in some situations, it may be beneficial to utilize non- traditional crops as a part of an animal production system. Factors that may limit production, such as climate or soil conditions, may reduce usage of typical species utilized as forages. In these instances, alternative forages may provide a more reliable source of feed than conventional species. For example, in some instances fiber crops could be utilized as alternative forages to provide additional feed in an “emergency” situation, such as drought or stand failure of other forage crops. Utilizing fiber crops as forages may provide an additional market aside from typical textile applications. This section will examine the benefits and drawbacks of utilizing annual fiber crops as forages in a ruminant production system.

2.2 Fiber Crops and Forage Nutritive Value

In addition to yield and environmental suitability of a species for a forage system, the nutritive value should be considered. It is important to understand the underlying components of forage nutritive value, how these are impacted by management, and how these will be different in fiber crops than in traditional grass or legume species.

Plant tissues can be considered in two categories: structural components in the cell wall (hemicellulose, cellulose, and lignin) and non- structural components in the cell contents (protein, sugar, and starch) (Ball et al., 2007). Unlike most animals, ruminants are better able to digest some structural components found in the cell wall. Estimations of digestibility can be made through laboratory testing that breaks down the proportion of cell wall components into neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL). NDF includes hemicellulose, cellulose, and lignin, ADF includes cellulose and lignin, and ADL only measures lignin.

The presence of extensive stem fiber in these crops presents a unique challenge to utilizing fiber crops for forage production. Hemp (*Cannabis sativa*) and kenaf (*Hibsicus cannabinus*) are crops primarily grown for their fibrous stalks which are used for the production of burlap, rope, and other commodities (Smalls and Marcus, 2002). The stalks of these crops produce two types of fibers. The outer layer, referred to as bast fibers, are longer and possess higher tensile strength relative to the inner layer of fibers (i.e. core fibers), which are short and dense (Chabbert et al., 2013). Bast fibers are used for cordage or textiles while the core fibers are often composited with other materials to be used in industrial applications such as the automotive and airline industries (Smalls and Marcus, 2002).

At maturity, fiber crops possess a higher ratio of lignin and cellulose in their dense, bi-layer stem compared to other crops. Ruminants are unable to digest lignin (Ball et al., 2007). For optimal digestibility in livestock, the ratio of cell wall should be reduced while the proportion of cell content should be promoted to improve digestibility and provide a nutritive value suitable for livestock. Typically, higher proportions of cell wall components in the forage are negatively correlated with digestibility (Collins et al., 2003). As fiber crops become more highly lignified throughout the growing season and particularly after flowering (Mediavilla et al., 2000; Amaducci et al., 2008; Struik et al., 1999), it will be important to manage them with frequent cuttings to help suppress lignin accumulation by increasing the proportion of regrowth.

Another component of forage quality is the crude protein content. This is an estimated measure of the "energy" within the plant cells. Not all of this will be available to the animal upon consumption, however, as a nominal amount of protein can be restricted within cell walls (Ball et al., 2007). Crude protein is also negatively correlated with maturity (Ball et al., 2007; Webber, 1993). Considering the significant fiber accumulation in hemp and kenaf throughout the growing season, frequent cuttings should also help reduce the decline in crude protein over time.

2.3 Kenaf as a Forage

Kenaf is a tropical fiber crop traditionally cultivated in Asia to make rope and burlap. Related to cotton and okra (M*alvaceae* family), kenaf is a fast growing, fibrous crop that tolerates saline soil conditions and which has shown promising potential as a forage (Reta-Sanchez, 2010). Kenaf grows well in the humid southeastern United States (Rude et al., 2002). Kenaf is a photoperiod sensitive, short day plant and does not flower until very late in the growing season, if at all, here in the United States (Crane et al., 1946). This delayed flowering causes the crop to remain in a vegetative state, which helps maintain its nutritive value despite increasing maturation. With proper management, kenaf makes a good forage.

In some instances, kenaf could be directly grazed by ruminants in a pasture (Rude et al., 2002). This could be useful during the summer months in the Southeast when grazing options are limited to low protein warm-season grasses (Rude et al., 2002). When used as pasture, kenaf remains vegetative longer, resulting in higher CP (24.5) and lower ADF (11.3) when compared to pearl millet (CP 20.4; ADF 26.4) and warm-season grass pasture mixtures (25% common Bermuda grass (*Cynodon dactylon*) and 75% dallisgrass (*Paspalum dimidiatum*)) (CP 7.2; ADF 33.8) (Rude et al., 2002). Steers in this trial gained weight more rapidly in the kenaf paddocks (.87 kg/d) than either the pearl millet (.8 kg/d) or grass mixture paddocks (.34 kg/d) during the second half of the trial. This could have been due to the decreasing quality of the grass pastures as they matured. However, the authors reported that, based on visual observations, steers in the trial took longer to adapt to eating kenaf in the first year due to their unfamiliarity with the crop.

Kenaf may be managed to produce multiple cuttings from a single stand, as it readily produces regrowth from cut stalks 20 cm or taller (Reta-Sanchez et al., 2015). Multiple harvests resulted in higher leaf to stem ratios (1:0.99 for harvesting every 40 days) compared to single cuttings (1:3.15) (Gonzalez-Valenzuela et al., 2008). Multiple cuttings of kenaf appeared to produce the highest forage quality when grown on narrow rows (Reta- Sanchez et al., 2015). In the same study, it was found that narrow row spacing produced significantly higher yields at first cutting than a typical row spacing. Row spacings of 20 cm (4384 Kg/ha), 38 cm (3849 Kg/ha) and 56 cm (3916 Kg/ha) yielded higher than the 76 cm row spacing (3591 Kg/ha) in 2004; in 2005 only the 20 cm row spacing was significantly different than the 76 cm row spacing (3010a vs 2236 Kg/ha). Kenaf was found to still have lower yields (8196 Kg/ ha) than traditional annuals like forage sorghum (11,107 Kg/ ha) or sorghum x sudangrass (Sorghum bicolor x S. bicolor var. Sudanese) (10,905 Kg/ha) at 90 days after planting (Vinson et al., 1979).

Multiple cuttings of regrowth can also be beneficial for hay production. Urias (1978) found that when the young leaves of kenaf were made into hay they had a nutritional level somewhat comparable to alfalfa hay. These authors reported CP and NDF concentrations of 11% and 52%, respectively, compared to 17.5% and 39% for alfalfa hay. Vinson et al. (1979) also found that kenaf harvested between 45 and 60 days after planting could produce a hay similar in nutritive value to alfalfa hay and superior to other warm-season annuals (sorghum, sorghum x sudangrass, and sudangrass (S. bicolor var. Sudanese).

Kenaf readily ferments into a stable silage (Xiccato, 1997). This study reported crude protein levels of pure kenaf silage to be 35%, and NDF levels to be 31%. However, when kenaf silage was provided to ewes in a feeding trial it had high levels of animal refusal and did not provide sufficient intake for maintenance (Xiccato, 1997). Ultimately, kenaf is promising in terms of nutritive value but may be limited due to palatability. More research is needed to refine the process of producing hay and silage from kenaf to result in more palatable products that retain desirable levels of nutrition.

Despite the promising research on nutritive value of kenaf, it has been found to have high animal refusal as a hay due to odor (Hancock, 1993; Xiccato, 1997) and lower yields (about 7,000 kg/ha) when compared to corn (about 13,000 kg/ha) under similar growing conditions (Reta-Sanchez 2010). Additionally, these authors found that larger yields of kenaf hay were necessary for comparable weight gain in cattle compared to alfalfa hay (Hancock, 1993). This may have been due to reductions in intake due to moldy hay from stems retaining moisture at cutting time (Urias, 1978; Hancock, 1993).

2.4 Hemp as a Livestock Feed

Little research has been conducted on hemp as a forage, but there have been reports that hemp leaves removed from stalks are fed to swine and other livestock in rural China (Clarke, 1995). Much more extensive research has been conducted on the nutritional value of hemp grain as an animal feed, particularly in the European poultry industry (Small and Marcus, 2002). Hens fed a diet consisting of 20% hempseed had statistically higher egg weights (60.5g) compared to the control (60.5g) (56.2g) while hen body weight, total egg production, and feed intake were unaffected (Gakhar et al., 2012). Steers fed diets ranging from 0%, 9%, or 14% hemp seed meal showed no differences in average daily gain, while the proportions of desirable omega-3 fatty acids in the meat increased from 32% to 41% (Gibb, 2005). Similarly, sheep fed various proportions of hemp meal (0%, 25%, 50%, 75% or 100%) also had no significant differences in dry matter intake (Mustafa et al., 1999). Calves fed hempseed cake as a protein source in their diet had higher fiber intake (NDF 1.68 kg) and lower starch intake (1.43 kg) compared to soybean meal (NDF 1.28 kg; 1.55kg), and had no significant difference in weight gain (Hessle et al., 2008). These studies suggest using a grain type hemp for forage or inclusion of a portion of the grain could promote higher levels of crude protein, fiber, and omega-3 fatty acid content.

Research from the European hemp fiber industry has found that the amount of bast fiber in the stem has been finalized by the onset of flowering but the core or secondary fibers still have the potential to increase (Mediavilla et al., 2000; Amaducci et al., 2008; Struik et al., 1999). Core fiber increased from 20% to 45% of total stem content after flowering in fiber hemp (Mediavilla et al., 2000). Traditionally hemp has been harvested at the onset of flowering for optimal fiber yields (Amaducci et al., 2008). However, most forages are harvested prior to flowering and increased lignification after flowering may decrease the potential of hemp as a forage by lowering its nutritive value.

It is possible to produce silage from hemp. It has been reported that when processed into silage, harmful compounds present in the raw plant are reduced (Small and Marcus, 2002). Felina 32 was found to produce silage with a pH of 7.4, crude fiber content of 45.5% DM, and total sugar 5.8% DM (Pecenka et al., 2007). Chop size was evaluated at both 10 mm and 20 mm, with the 20 mm chop size producing 10% more fine pieces than the 10 mm (23% compared to 13%).

Chapter 3: MATERIALS AND METHODS

Establishment

This experiment was conducted at University of Kentucky (UK) Spindletop Research Farm near Lexington, Kentucky during the 2016 and 2017 growing seasons. The experimental area consisted of Lowell-Bluegrass silt loam in 2016 and Bluegrass Maury silt loam in 2017. The sites were prepared using primary and secondary tillage to create a firm seedbed prior to seeding. Nitrogen fertilizer treated with Agrotain (N-(n-butyl) thiophosphoric triamide (NBPT)) was applied at 56 kg/ha for the kenaf and fiber types and 112 kg/ha for the grain and dual- purpose type using a Gandy fertilizer spreader on June 22, 2016 and May 10, 2017 (Table 3.3). Potash was applied at a rate of 186 kg/ha on June 27, 2016, but was not required in 2017.

Two plantings were used to simulate a "typical" (i.e. early May) and "late" (i.e. mid-June) planting date. Seeds were sown at a target depth of .63 cm at rates of 17.9 kg/ha, 22.4 kg/ha, 44.8 kg/ha, 67.2 kg/ha for kenaf ('Whitten'), grain hemp ('Finola'), dual purpose hemp ('Felina') and fiber hemp ('Futura') respectively (Table 3.3). Due to low seed availability of Finola, CRS-1 was substituted as the grain hemp in 2017. The kenaf and fiber hemp were planted on 20 cm rows and 40 cm rows were used for the grain and dual- purpose hemp varieties (Table 3.3), as per UK recommendations (Williams et al., 2016).

Plots were sprayed with Assure II (570 mL/ha) and Prowl (4 L/ha) to reduce grass weed pressure. Plots were also hand weeded until canopy closure to help minimize growth of broadleaf weeds. Permethrin was also applied to the plots at a rate of 292 mL/ha for Japanese beetles (*Popillia japonica*). In addition, Spectracide (Spectrum Brands, Madison, WI) beetle traps were placed around the perimeter of the experimental area and emptied daily.

Harvesting Methods

Plots were harvested approximately 30 days after each planting date and continued every two weeks until the end of the season or until senescence of leaves or grain occurred. There were a total of nine and eight harvests for 2016 and 2017, respectively. During each harvest, samples were collected by harvesting two rows of plants from a random two- meter section of the plot to a residual height of 10 cm. The number of stems were counted from this sample to determine plant populations. This population data was not analyzed due to the differences in seeding rates of kenaf and the three hemp types, but the seasonal average is presented in Table 3.3. Three random plants were selected from the harvested material to determine phenological stage (Table 3.1) and were separated into its botanical components (i.e. leaves, stem, floral components, bast fibers, and core fibers). The rest of the harvested material was used to determine forage nutritive value. All samples were then dried in a forced air dryer for 48 hours at 46C. Following drying, samples were ground to pass through a 4mm screen with a Wylie mill and re-ground to pass through a 1mm screen with Cyclone mill at a 1mm mesh.

Nutritive Analysis

A micro-Kjeldahl procedure utilizing a salicylic acid modification (Bradstreet, 1965; Chaney and Marbach, 1962) was used to determine sample N concentrations with assistance from the Crutchfield lab. Estimates of N were converted to CP by multiplying by 6.25.

Neutral Detergent Fiber (NDF) was determined using the method described by Vogel et al. (1999). Approximately 0.5g of sample was added to pre-weighed fiber bags and heat sealed. Samples were in an ANKOM 200 Fiber Digester (ANKOM Technologies, Macedon, KY) with approximately two liters of neutral detergent solution and four milliliters - amylase. Samples were heated and agitated for 75 minutes before being rinsed with hot water and - amylase. Fiber bags were then gently squeezed and placed in acetone for 5 minutes to remove excess water. Samples were then dried overnight and weighed the following day after 2 hours of drying in an oven at 100 C. Neutral detergent fiber concentrations were determined via weight difference before and after extraction.

Following the determination of NDF, samples were placed in the Ankom Fiber Digester with approximately two liters of Acid Detergent Solution. Samples were then heated and agitated for an hour and fifteen minutes before being flushed with hot water. Fiber bags were then gently squeezed and placed in acetone for 5 minutes to remove excess water, dried overnight, and weighed after 2 hours in the oven at 100 C. Acid detergent fiber concentrations were determined via weight difference before and after extraction.

Following the ADF procedure, samples were placed in a digester jar with 500 ml of 72% H_2SO_4 and placed in the Daisy II Incubator (ANKOM Technology, Macedon, NY) for 3 hours as ambient temperature. Samples were then rinsed with water in the same manner as in the ADF procedure and were corrected for ash content via combustion in an oven at 525C. ADL concentrations were determined by the equation presented by Vogel, et al. (1999).

In Vitro True Digestibility (IVTD) was determined using the method described by Vogel, et al. (1999). Approximately 0.5g of sample was added into a pre-weighed fiber bag and heat sealed (ANKOM Technology, Macedon, NY). A total of 24 bags (i.e. 22 samples, a blank control, and an alfalfa standard) were added to the digester jar. Two buffer solutions were created: Buffer A (KH_2PO_4, $MgSO_47H_2O$, NaCL, $CaCl_22H_2O$, and Urea) and Buffer B (Na_2CO_3, Na_2S9H_2O). The buffer solutions were combined at a 5:1 (A:B) ratio in each digester jar with approximately 400 mL of rumen fluid. Rumen fluid was collected at the UK C. Oran Little Research Center from a cannulated steer being fed a forage- based diet. Following the addition of rumen fluid, each jar was flushed with CO_2 to purge O_2 and was placed in the Daisy II Incubator and heated to 39 degrees C with agitation. Jars were allowed to incubate for 48 hours before the solution was drained and samples were rinsed with hot water. The NDF procedure described previously was then used to remove any foreign material from the sample. The true digestibility of each sample was then determined via weight difference before and after digestion.

Estimating Nutritional Values through Near Infrared Spectroscopy Reflectance Scanning

The reflectance spectrum (400-2500 nm) of each sample was obtained using a Foss NIRSystems 6500 spectrophotometer (Foss NIRSystems Inc, Laurel, MD). The analytical methods described above were used on all of the samples from 2016 and a representative portion (43) of the samples from 2017. These samples were used to develop calibration equations to predict the nutritive value data for the entire study.

Equations were developed using modified partial least squares regressions with two outlier elimination passes and were validated using an internal group cross-validation method (Shenk and Westerhaus, 1991). A 1, 3, 3, 1 math treatment was selected to optimize regression statistics with the critical T and global H outlier values of 2 and 3, respectively. The standard normal variant and detrend option was used to correct for light scatter. The results of each regression are presented in Table 3.2 and were selected based on high R^2 and 1-VR values and low standard errors of calibration (SEC) and cross-validation (SECV).

Data Analysis

The design for this experiment was a RCBD slit-plot design with four replications. Planting dates were used as the main plots and type as the split plot. Data was analyzed in SAS 9.3 using PROC GLIMMIX. Block and years were considered random effects. Harvest was considered a repeated measure using a multivariate approach.

Table 3.1: Hemp phenological stages, adapted from Mediavilla et al., 2000.

Code	Stage	Description
Germination & emergence		
0000	Dry seed	
0001	Radicle apparent	
0002	Emergence of hypocotyl	
0003	Cotyledons unfolded	
Vegetative stage: *Refers to the most advanced leaf pairing on the main stem (where applicable). Leaves are considered unfolded when leaflets are at least 1 cm long.*		
1002	1st leaf pair	Contains 1 leaflet per leaf
1004	2nd leaf pair	Contains 3 leaflet per leaf
1006	3rd leaf pair	Contains 5 leaflet per leaf
1008	4th leaf pair	Contains 7 leaflet per leaf
1010	5th leaf pair	
10xx	nth leaf pair	Code: xx = 2*n
Flowering & seed formation: *Refers to the most advanced flower on the main stem (where applicable). For dioecious varieties, use female plants and ignore male flowering codes)*		
2000	Floral induction (GV Point)	Leaf arrangement changes from opposite to alternate)
2001	Floral initiation	Flowers present, but sex is indistinguishable
2300	Female flower formation	First pistillate flowers present with perigonal bracts but no styles
2301	Beginning of female flowering	First styles visible
2302	Female flowering	50% of bracts formed
2303	Male flower formation	First closed staminate flowers
2304	Male flowering	Most staminate flowers open
2305	Beginning of seed maturity	First seeds hard
2306	Seed maturity	50% seeds hard
2307	End of seed maturity	95% seeds hard or shattered
Senescence		
3001	Leaf desiccation	Leaves are dry
3002	Stem desiccation	Leaves have dropped
3003	Stem decomposition	Bast fibers are beginning to separate from stem

Table 3.2: Results of the NIRS Equations for each Forage Nutritive Value Parameter.

Constituent	**N**	**SEC**	**RSQ**	**SECV**	**1-VR**
NDF	215	2.4931	0.9194	2.6549	0.9081
ADF	219	1.6983	0.9506	1.8228	0.9428
ADL	219	0.9906	0.8301	1.0672	0.8019
IVTD	223	2.3226	0.9392	2.5407	0.927
CP	226	0.9096	0.9739	0.9914	0.9688

N -Number of Samples.
SEC -Standard Error of Calibration.
RSQ -R^2 value.
SECV -Standard Error of Cross Validation.
1-VR -Coefficient of Determination in the Cross Validation.

Table 3.3: Variations in Management and Resulting Average Plant Populations.

Type	**Variety**	**Seeding Rate (kg/ha)**	**Row Spacing (cm)**	**Nitrogen Fertilizer (kg/ha)**	**Avg. Population (plants/ ha)**
Kenaf	Whitten	17.9	20	56	369,706
Fiber	Futura	22.4	20	56	873,151
Dual	Felina	44.8	40	112	707,386
Grain	Finola (2016) CSR1 (2017)	67.2	40	112	549,264

Chapter 4: RESULTS AND DISCUSSION

Maturity

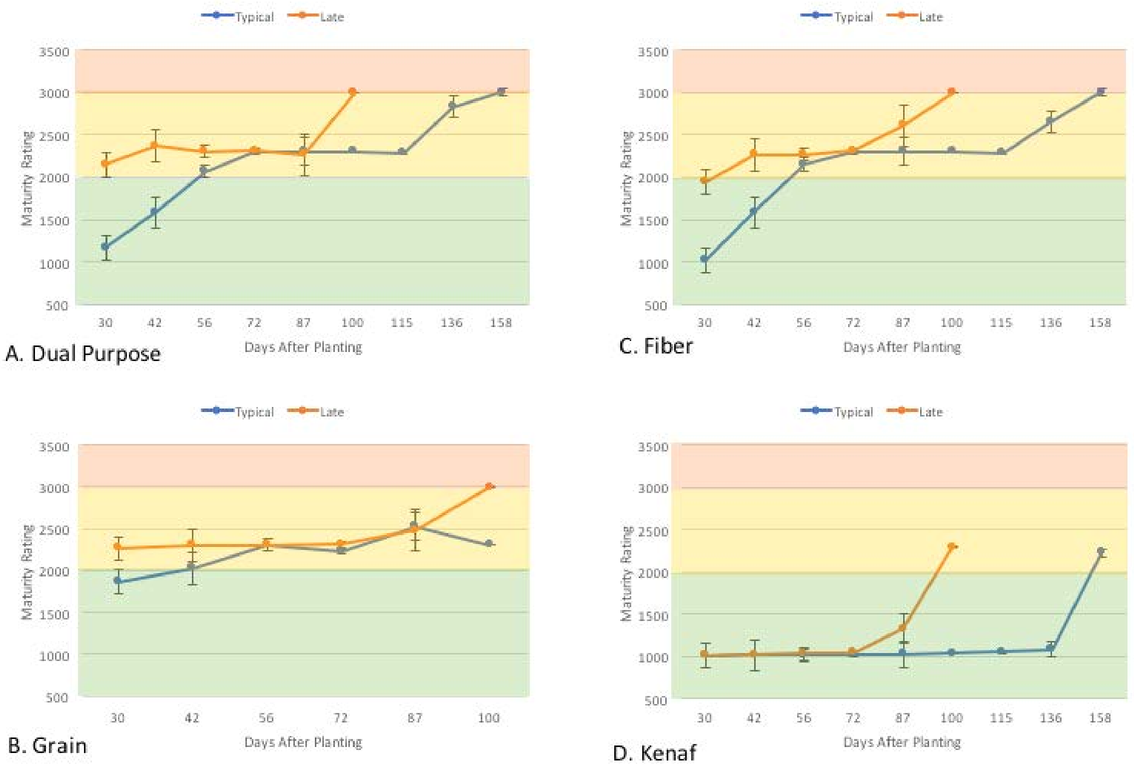

Figure 4.1: Maturity ratings throughout the growing season for dual purpose hemp (A), grain hemp (B), fiber hemp (C), and kenaf (D). Green represents vegetative growth, yellow represents reproductive growth, and red indicates senescence.

Late planting resulted in all three hemp types entering a reproductive state by 30 days after planting (DAP), whereas the early planted hemp remained vegetative for a longer period of time after planting (Fig. 4.1). The earlier planted dual purpose and fiber hemps remained vegetative until approximately 56 DAP (Fig. 4.1 A and C). All three hemps types reached senescence by the end of the season in both planting dates. Kenaf remained vegetative until the final harvest (early: 158 DAP; late: 100 DAP) and did not reach senescence in the time frame evaluated (Fig. 4.1 D). This is because the photoperiod requirements for flowering in kenaf were not met in Kentucky until October. Both hemp and kenaf are photoperiod- sensitive short- day plants (Chabbert, 2013; Crane et al., 1946). However, because kenaf is a tropical crop, it requires fewer hours of daylight to flower than the hemp varieties used in this study, which originated in Northern Europe (i.e. France: Felina 32 and Futura 75; Finland: Finola) (Amaducci et al., 2008; Jankauskiene et al., 2010; Salentjin et al., 2014) or Canada (CRS-1).

Morphological Components

Leaf

The proportion of leaf content was influenced by two interactions: type*harvest (Fig. 4.3) and planting date*harvest (Fig. 4.4). Overall, the leaf content declined with increasing DAP across all four forage types. This is expected as stem accounted for a greater proportion of the plant as they grew and matured (Fig. 4.4). The late planting date had lower leaf content throughout the growing season compared to the typical planting (Fig. 4.3). This was likely due to differences in photoperiod hastening maturity and shortening the vegetative phase for the second planting.

Starting at 87 DAP, differences were visible between kenaf and the three hemp types (Fig.4.4). Kenaf had the highest proportion of leaf at this point and was statistically different from the fiber and dual- purpose hemp. The grain hemp had lower but not significantly different proportions of leaf than kenaf until 100 DAP.

In kenaf, the proportion of leaf tissue plateaued while the hemps proportion continued to decline after 100 DAP. This was likely due to damage from insect defoliation, which damaged the apical meristem of the kenaf plants and increased lateral growth of leafy tissues (Fig 4.3). Throughout both growing seasons, the kenaf plots sustained severe insect defoliation from Japanese beetles *(Popillia japonica)* during July. The hemp plots were unaffected or only suffered negligible damage during this time. This is typical of the life cycle of Japanese beetles, which emerge from the ground in June and have a period of high activity lasting until the end of July (Townsend). Japanese beetles feed in swarms, starting at the top of a plant and working downward, focusing on the leafy tissues (Townsend). For this reason, it is possible that the apical meristems of the plants were damaged and this caused them to produce more lateral growth and resulted in a higher proportion of leafy tissues compared to the hemps (Fig. 4.2).

Figure 4.2: Insect defoliation on kenaf plants, July 2017 ***(authors own photos)***

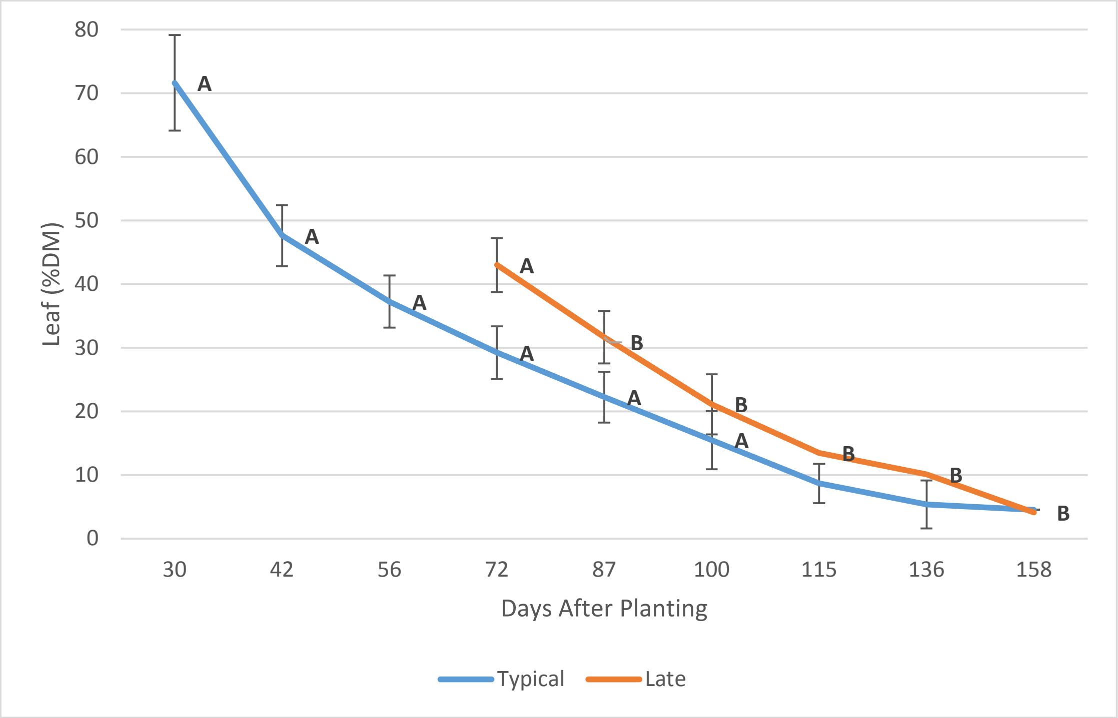

Figure 4.3: Leaf content as a percentage of total dry matter by planting date across harvests. Letters represent significant differences (*P* < 0.05) within a harvest date.

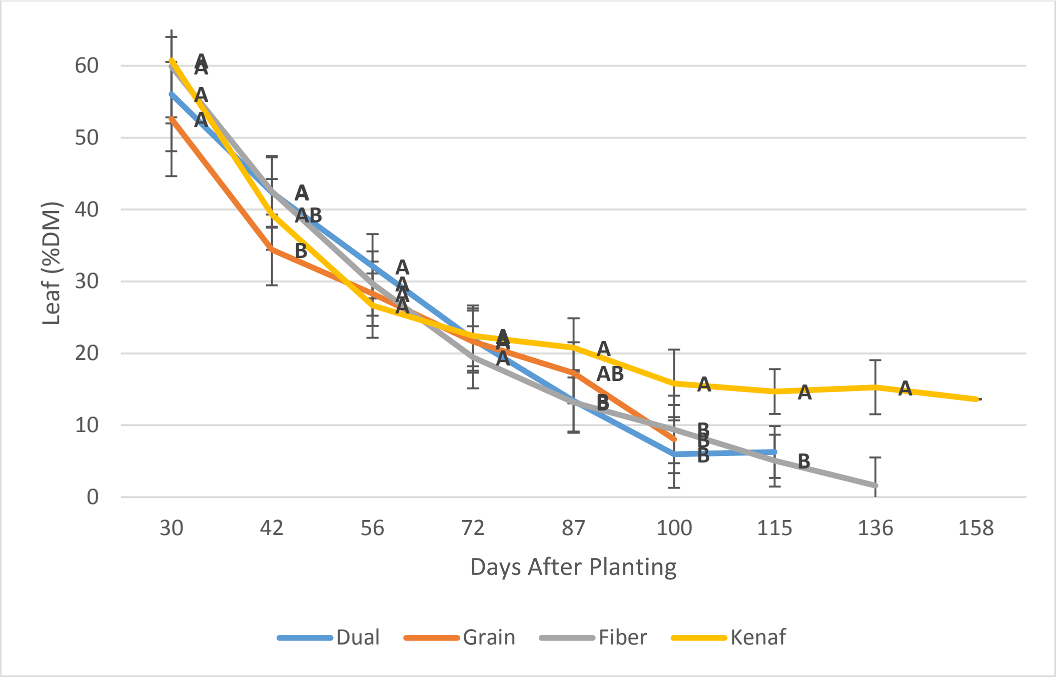

Figure 4.4: Leaf content as a percentage of total dry matter by type across harvests. Letters represent significant differences (*P* < 0.05) within a harvest date.

Stem

The proportion of stem content was influenced by two interactions: type*harvest (Fig. 4.5) and planting date*harvest (Fig. 4.6). Separation in the stem content was observed in the four types by 56 DAP, although grain hemp was significantly lower than the other types by 30 DAP (Fig. 4.5). Kenaf had the highest proportion of stem throughout the growing seasons. While no height data was collected in this study, kenaf has been reported to reach heights of 3 meters or more (Crane, 1946). Its stem content peaked by 100 DAP, which also corresponds to the time of greatest insect damage and the plateau in leaf content (Fig. 4.4). The grain hemp used in this experiment is a short stature variety, which may explain why it had the lowest proportion of stem throughout the growing season and reached maximum stem content by 87 DAP. Both fiber and dual- purpose hemps maintained roughly the same amount of stem (~ 40- 60% DM) after approximately 42 DAP. This may be due to the rapid increase in core fibers after flowering.

The late planting date exhibited a higher proportion of stem content earlier in the growing season (Fig. 4.6). This is most likely due to the shortened vegetative phase of growth associated with this planting date (Fig. 4.1).

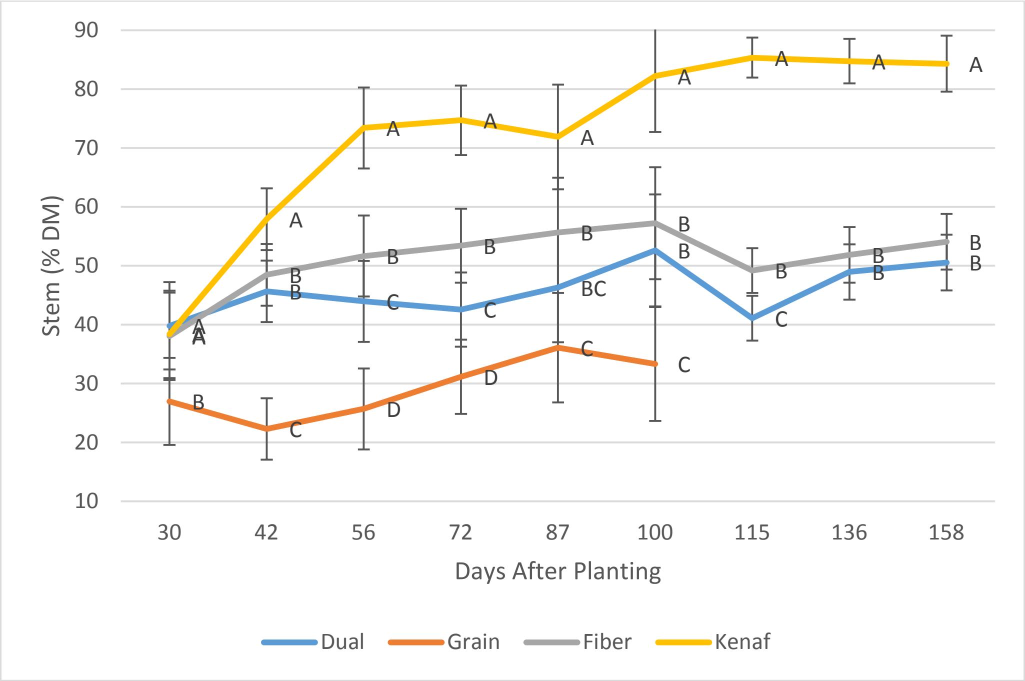

Figure 4.5: Stem content as a percentage of total dry matter by type across harvests. Letters represent significant differences (*P* < 0.05) within a harvest date.

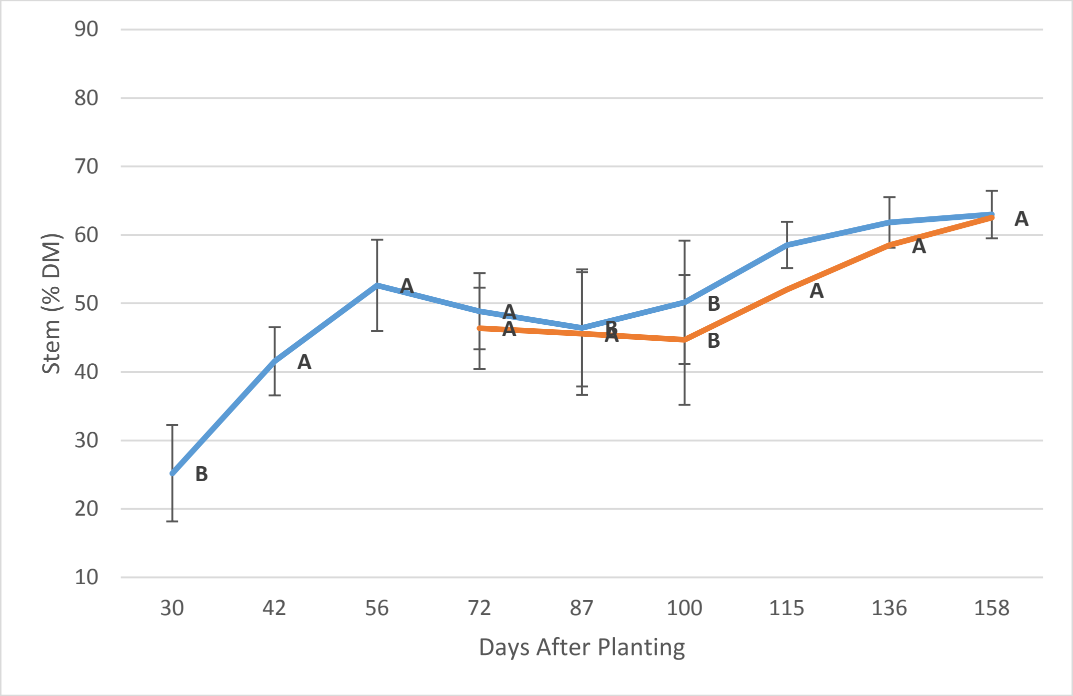

Figure 4.6: Stem content as a percentage of total dry mater by planting date across harvests. Letters represent significant differences (*P* < 0.05) within a harvest date.

Stem: Bast Fiber Content

In fiber crops, the stem is typically divided into two categories of fiber (i.e. bast fibers and core fibers). The proportion of these two fiber types may influence the suitability of these crops as forages. Bast fibers are the long fibers found in the outer bark of the stem, while core fibers are typically short and found in the pith of the stem (Chabbert et al., 2013).

A relationship exists between bast or core fiber production and flowering (Mediavilla et al., 2000). During the vegetative state and until flowering, plant stem growth is typically focused on production of the bast fibers. After flowering, production shifts toward the production of core fiber.

In this study, proportions of bast fiber were influenced by an interaction of type*planting date*harvest. The data is presented in Figure 4.7 and Figure 4.8 by each planting date to better visualize trends. Findings from this study support the claim from Mediavilla, et al. (2000) as it was found that both bast fiber production and floral production were impacted by a three- way interaction between type, harvest date, and planting date. The concentration of core fiber increased steadily throughout the growing season for all four types and both planting dates.

For both planting dates, kenaf had the highest proportion of bast fiber throughout the growing season (Fig. 4.7, Fig. 4.8).For the late- planted dual- purpose and grain hemp (Fig. 4.8), the late planting date started at a higher proportion of bast fiber than the typical planting. The late planting then decreased and was not significantly different than the early planting for dual purpose hemp through 72 DAP. For the fiber hemp, the late planting started at a higher percentage of bast fiber but was similar to the typical planting date by 42 DAP (Fig. 4.7). Late-planted kenaf (Fig. 4.8) had higher percentages of bast fibers until 80 DAP but then declined below the level of the typical planting (Fig. 4.7).

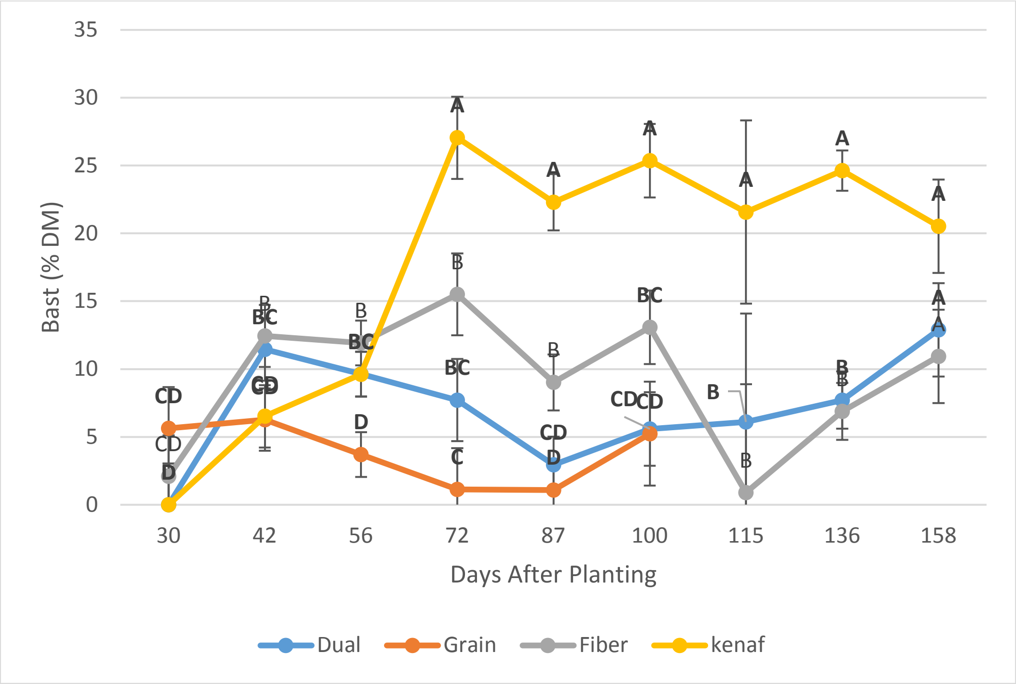

Figure 4.7: Bast fiber content by type across harvests the typical planting. Letters represent significant differences ($P < 0.05$) within a harvest date.

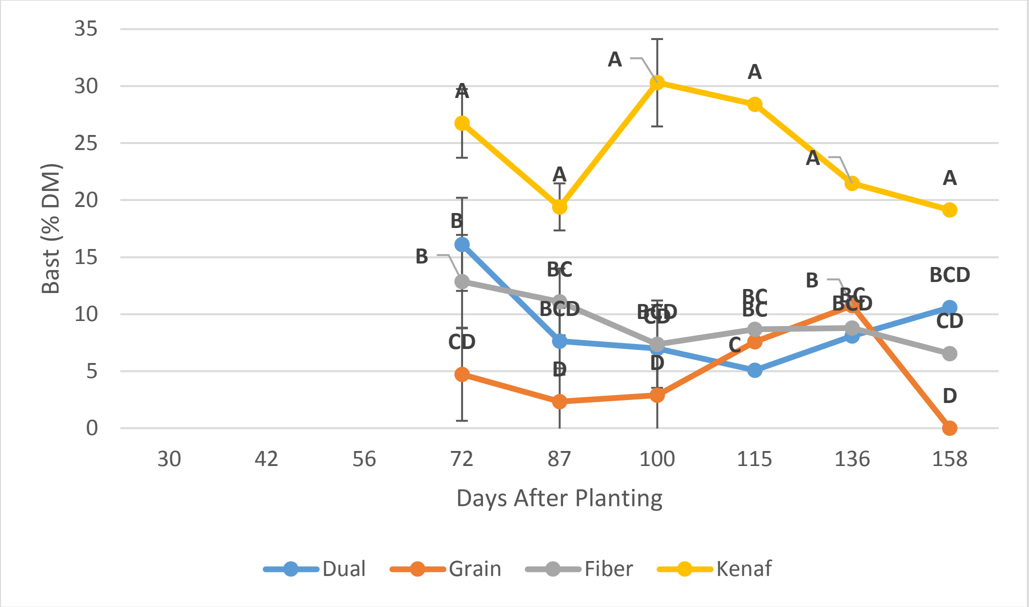

Figure 4.8: Bast fiber content by type across harvests the late planting. Letters represent significant differences ($P < 0.05$) within a harvest date.

Stem: Core Fiber Content

The proportion of core fiber content was influenced by two interactions: type*harvest (Fig. 4.9) and planting date*harvest (Fig. 4.10). Separation in the stem content was observed in the four types by 42 DAP (Fig. 4.9). Kenaf had the highest proportion of core fibers by 42 DAP. Grain hemp was significantly lower in core fiber content by 30 DAP and continued to have the lowest levels throughout the growing season. These differences among types may be attributed to variety height (i.e. grain hemps are typically short- statured) and maturation rate of the crops (Fig. 4.1).

The late planting had more core fibers than the typical planting (Fig. 4.10). As mentioned earlier, Medivilla et al. (2000) identified that a relationship exists between the bast and core fiber production with plant flowering and suggests that more bast fiber is produced prior to flowering where as more core fiber is produced after flowering. All four late planted types entered reproductive stages earlier than the typical planting (Fig. 4.1). This may account for the rapid accumulation of core fibers seen in the late planting after 56 DAP.

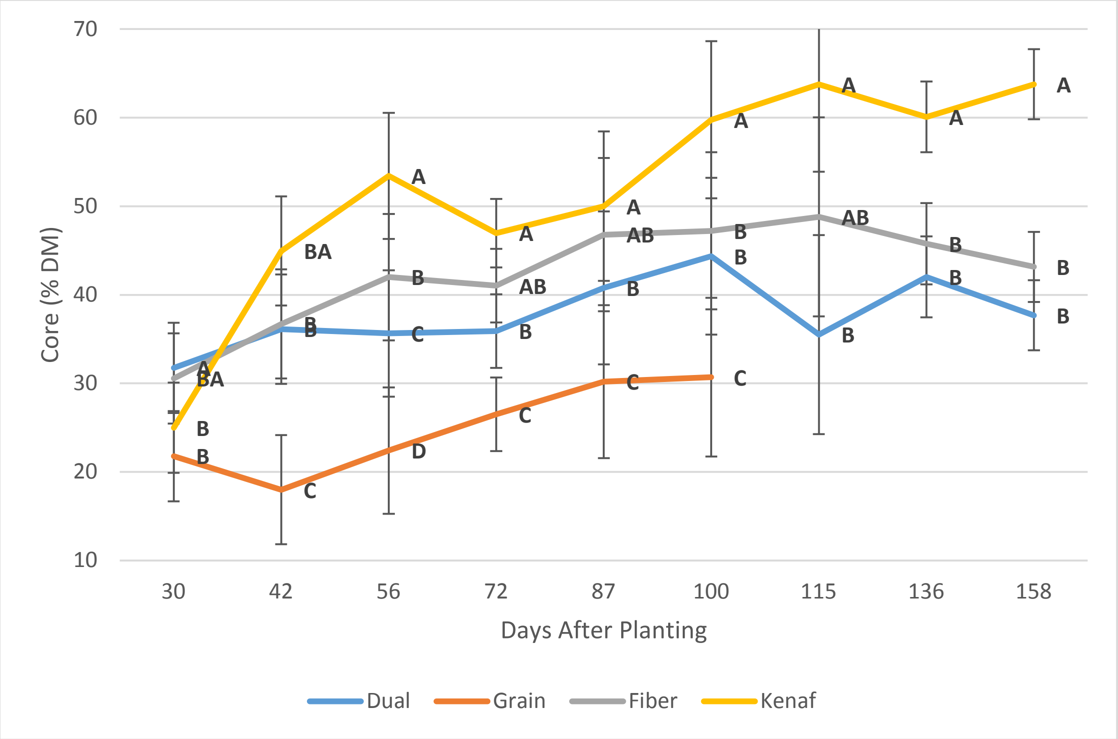

Figure 4.9: Core as a percentage of total dry matter by type across harvests. Letters represent significant differences ($P < 0.05$) within a harvest date.

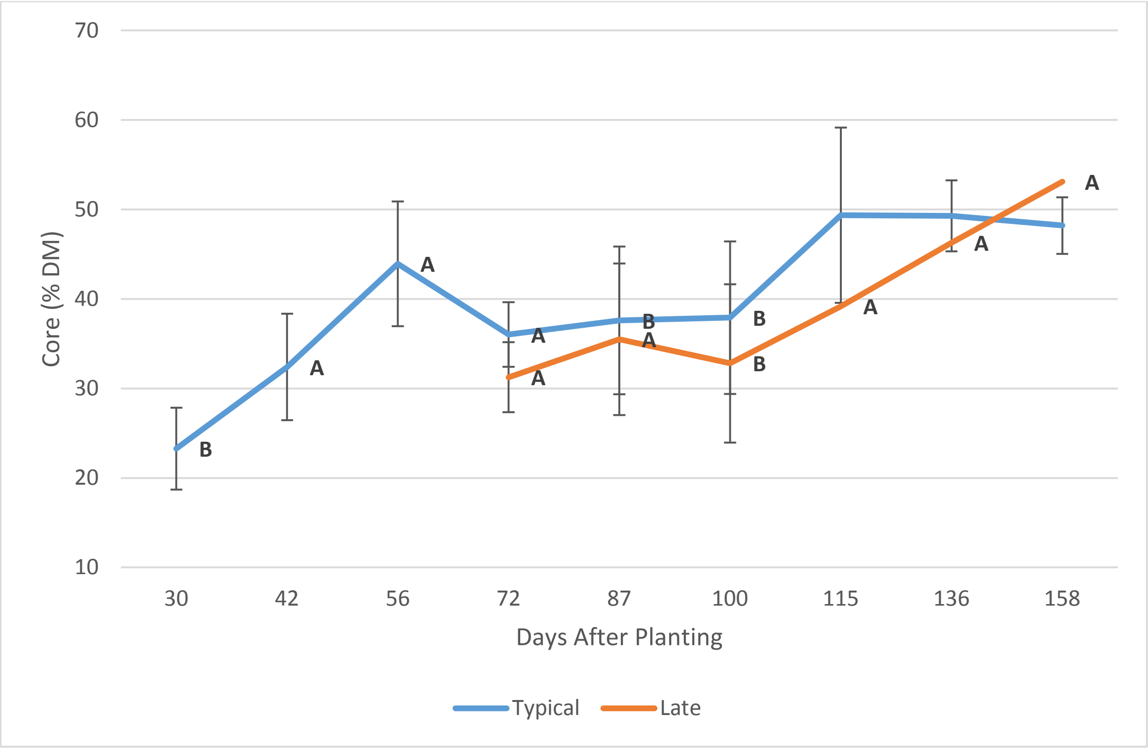

Figure 4.10: Core as a percentage of total dry matter by planting date across harvests. Letters represent significant differences (*P* < 0.05) within a harvest date.

Floral Components

In this study, proportions of floral components were influenced by an interaction of type*planting date*harvest. The data is presented in Figures 4.11 and 4.12 by each planting date to better visualize trends. As previously mentioned, kenaf did not produce sufficient floral components in either year to be considered in the analysis due to its tropical adaptation. The grain hemp typical planting reached over 50% floral tissue by 87 DAP, 28 days sooner than the dual purpose hemp (Fig. 4.11). This is expected due to the grain hemp's lower proportion of stem at 87 DAP (Fig. 4.5). The grain hemp had the highest proportions of floral components for the late planting by 56 DAP. Fiber hemp had the lowest maximum amount of floral components for the typical and late plantings (Figs. 4.11 and 4.12). The high proportion of floral material for all three varieties can in part be explained by declining leaf matter due to senescence later in the season.

Across the three types, the later planting date produced a greater proportion floral components in fewer DAP (Fig. 4.12). Although the late planting had a reduced vegetative state, peak floral content for both plantings occurred simultaneously.

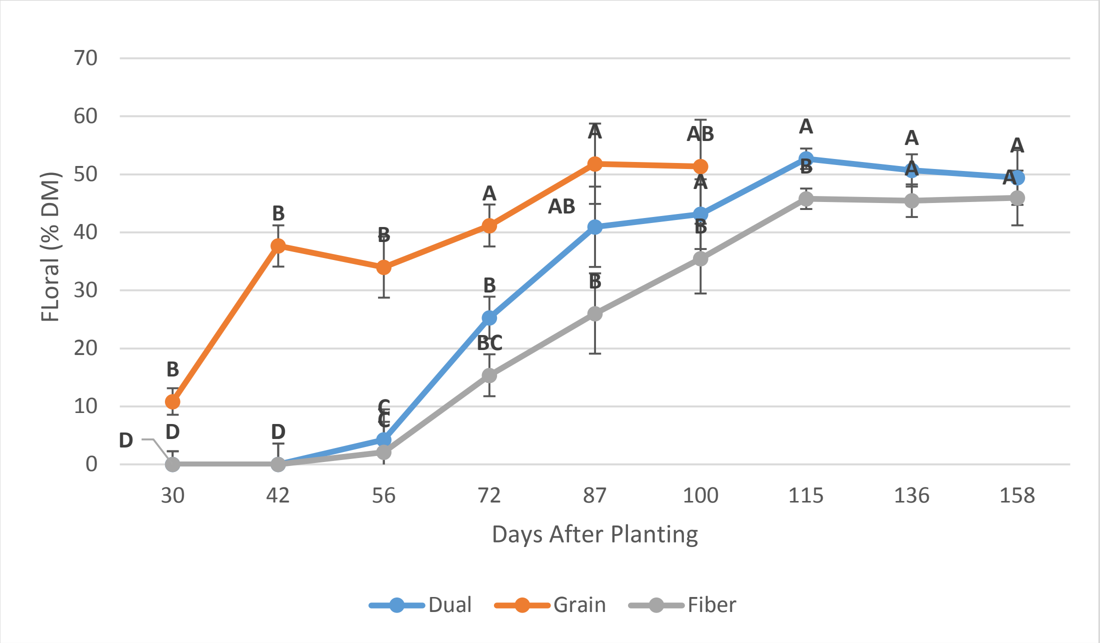

Figure 4.11: Floral content as a percent of total dry matter by planting date across harvests for the typical planting. Letters represent significant differences ($P < 0.05$) within a harvest date.

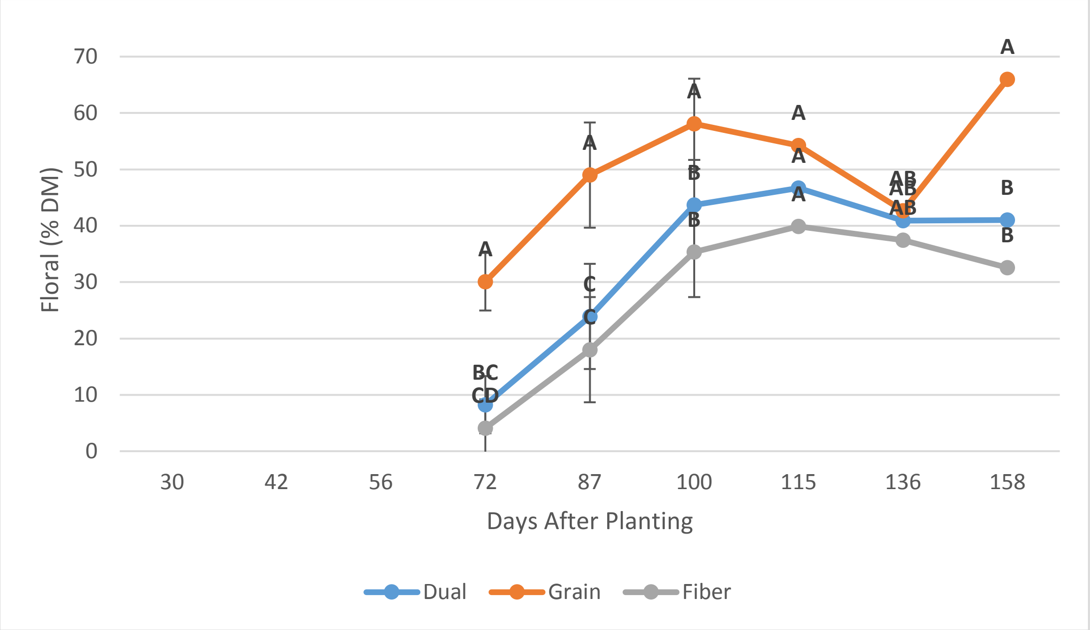

Figure 4.12: Floral content as a percent of total dry matter by planting date across harvests for the late planting. Letters represent significant differences ($P < 0.05$) within a harvest date.

Yield

Yield is perhaps one of the most determining factors in whether a species or variety is utilized for forage. In this experiment, yield was influenced by both type, planting date, and harvest date. The data is presented in Figures 4.13 and 4.14 by planting date to better visualize trends. In general, the typical planting date (Fig. 4.13) resulted in higher biomass due to a longer vegetative phase than the late planting date (Fig. 4.14). Kenaf had the highest yields across both planting dates (Figs. 4.13 and 4.14). Of the three hemp types, the fiber hemp had the highest biomass yields (Figs. 4.13 and 4.14). All three hemps reached their highest yields between 72 and 100 days after planting (Figs. 4.13 and 4.14). Kenaf did not reach maximum yield until 136 days after planting (Figs. 4.13 and 4.14). The fiber and dual- purpose lines had longer vegetative states than the grain hemp considered here (Fig. 4.1). Grain hemp was not harvested past 100 days after planting due to its early maturation and senescence (Fig 4.1 B; Fig. 4.13; Fig. 4.14).

These hemp cultivars are adapted to the longer hours of daylight experienced in more northern latitudes during the summer months. Growing these varieties in Kentucky may have reduced the vegetative portion of their growth habit and led to a decline in biomass production and lower nutritive value. A grain variety with a more suitable photoperiod requirement may increase the length of the vegetative stage and may produce yields more comparable to the dual purpose and fiber type hemp. Jankauskiene et al. (2010) reported biomass yields for Felina 32 in Lithuania that are nearly four times higher than the yields produced in this study (16, 452 kg/ha compared to 4,354 kg/ha).

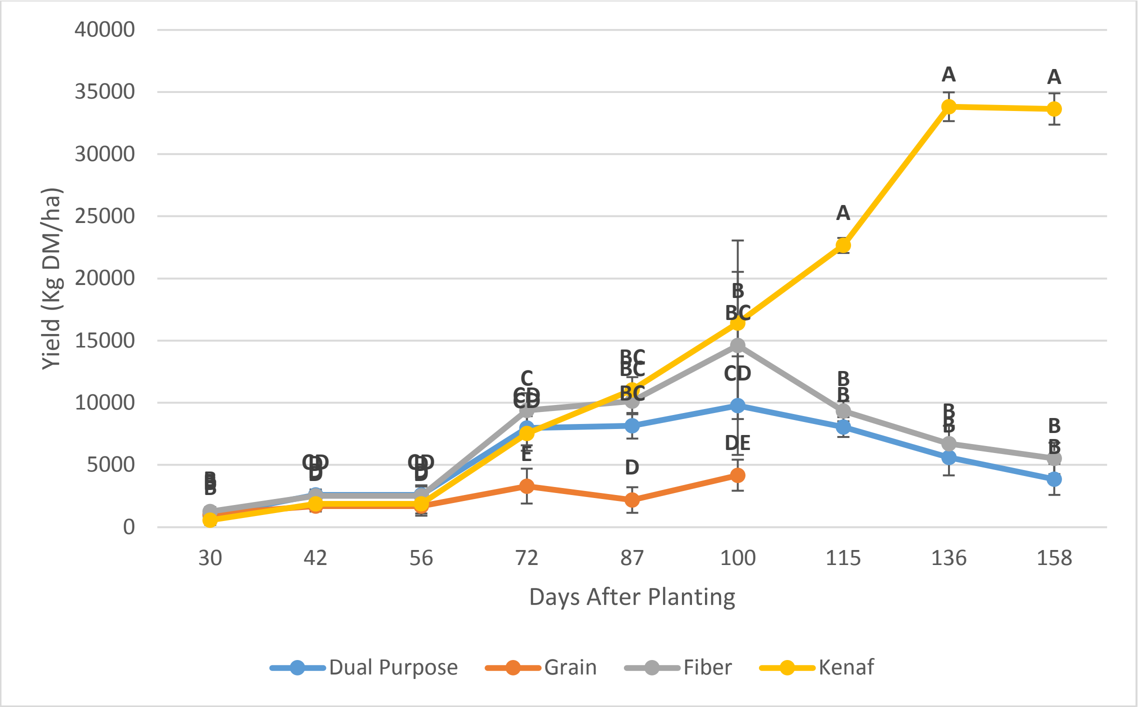

Figure 4.13: Yield by type across harvests for the typical planting. Letters represent significant differences (*P* < 0.05) within a harvest date.

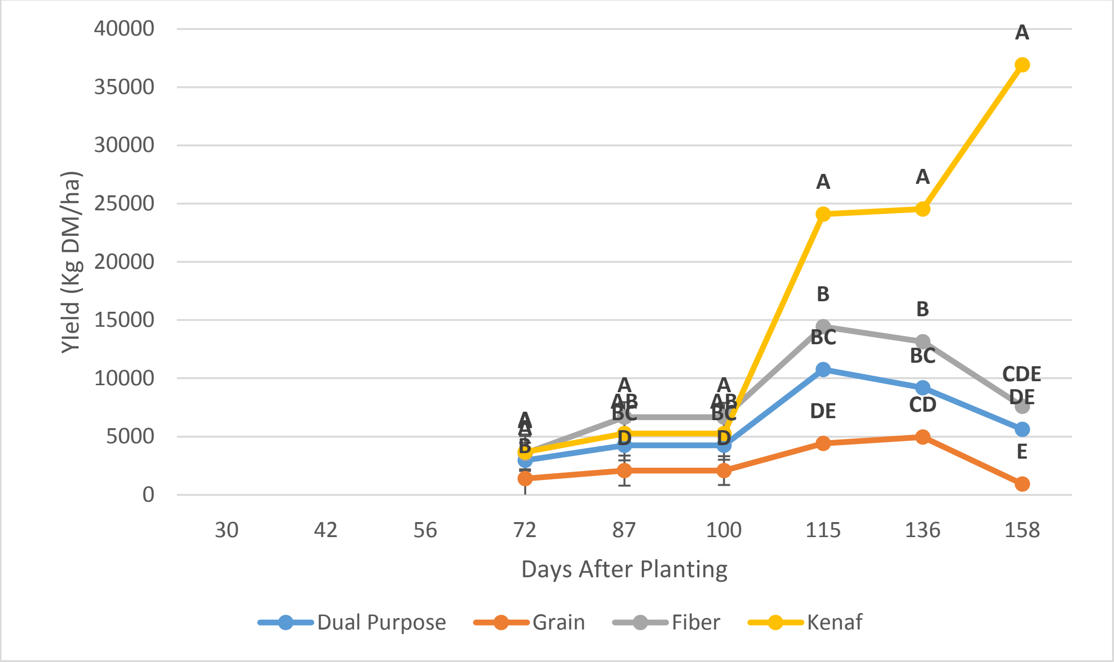

Figure 4.14: Yield by type across harvests for the late planting. Letters represent significant differences (*P* < 0.05) within a harvest date.

Nutritive Value

Crude Protein (CP)

The concentration of CP was influenced by two separate interactions: type*harvest (Fig. 4.15), and planting date*harvest (Fig. 4.16). Grain hemp was consistently higher in CP during the six harvests it was evaluated and is likely due to the higher proportion of grain and leaf material in the samples given their smaller size. Young plants with high proportions of leafy tissues typically have high protein content (Ball et al., 2007). Stem content for grain hemp was the lowest (Fig. 4.5) and floral components the highest at 42 DAP for grain hemp (Fig. 4.11). Crude protein concentrations of approximately 12% DM are typically considered to be the minimum amount required by growing livestock (Fig. 4.15) (NRC, 2000). In this study, all four forages remained above this level until 56 DAP (Fig. 4.15). While grain hemp had higher concentration of CP later into the growing season, both kenaf and dual- purpose hemp had comparable levels of CP to grain hemp at 42 DAP (17% DM). This value is only slightly lower than tall fescue hay cut during early bloom (18% DM) and much higher than mature tall fescue hay (11% DM) (NRC, 2000).

Early and more frequent cuttings could allow for kenaf and dual- purpose hemp to provide higher levels of biomass with similar CP levels as grain hemp. Gonzalez-Valenzuela et al., (2008) found that kenaf regrowth had higher proportions of leaf tissue compared to single cuttings, and Urias (1978) found that hay made from kenaf regrowth had CP levels of 11%. Reta-Sanchez et al. (2015) found that the CP levels were significantly higher for the second cutting for both cutting heights. These levels suggest that management could potentially allow for dual purpose hemp and kenaf to be used as forages. While growing animals require a minimum of 12% CP, maintenance rations for mature animals typically have between 8- 10% CP (National Research Council, 2000). All four types evaluated had CP levels in this range until 87 days after planting. After 100 days, dual purpose and fiber hemp had CP concentrations 12% and 9%, respectively.

Planting date had little overall effect on crude protein levels with no significance difference between planting dates for all harvests except at 42 and 100 days after planting (Fig. 4.16). This was expected, as the leaf content for the two planting dates was similar across all harvests (Fig. 4.3).

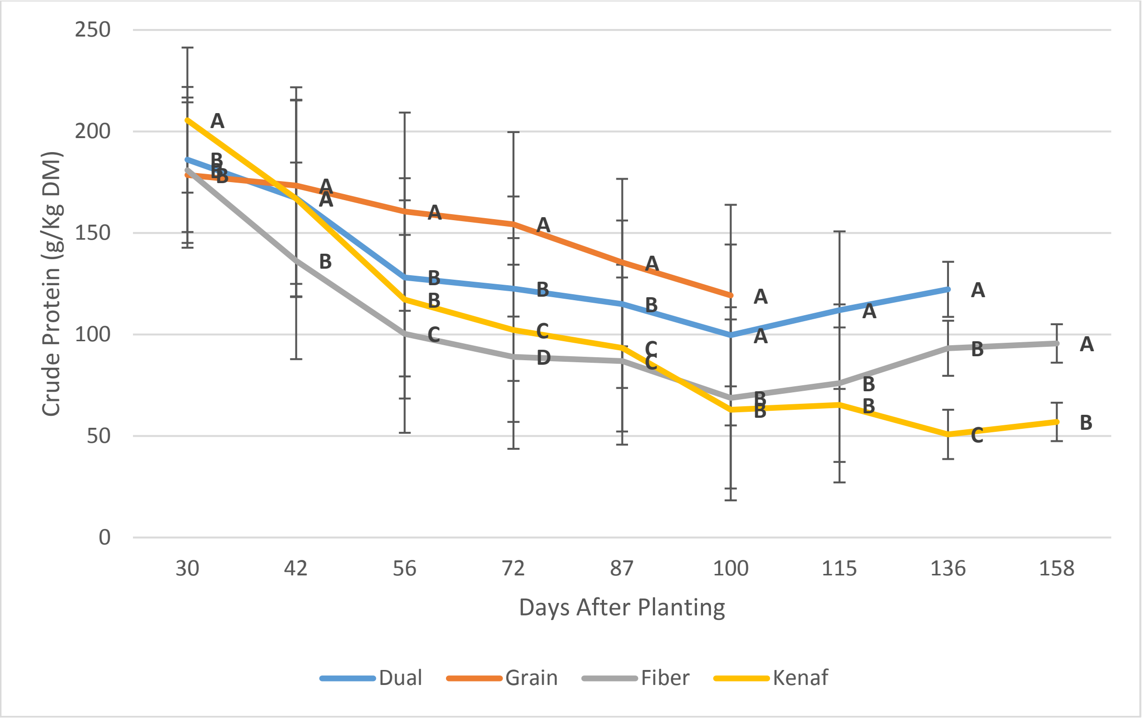

Figure 4.15: Crude protein content by type across harvests. Letters represent significant differences (P < 0.05) within a harvest date.

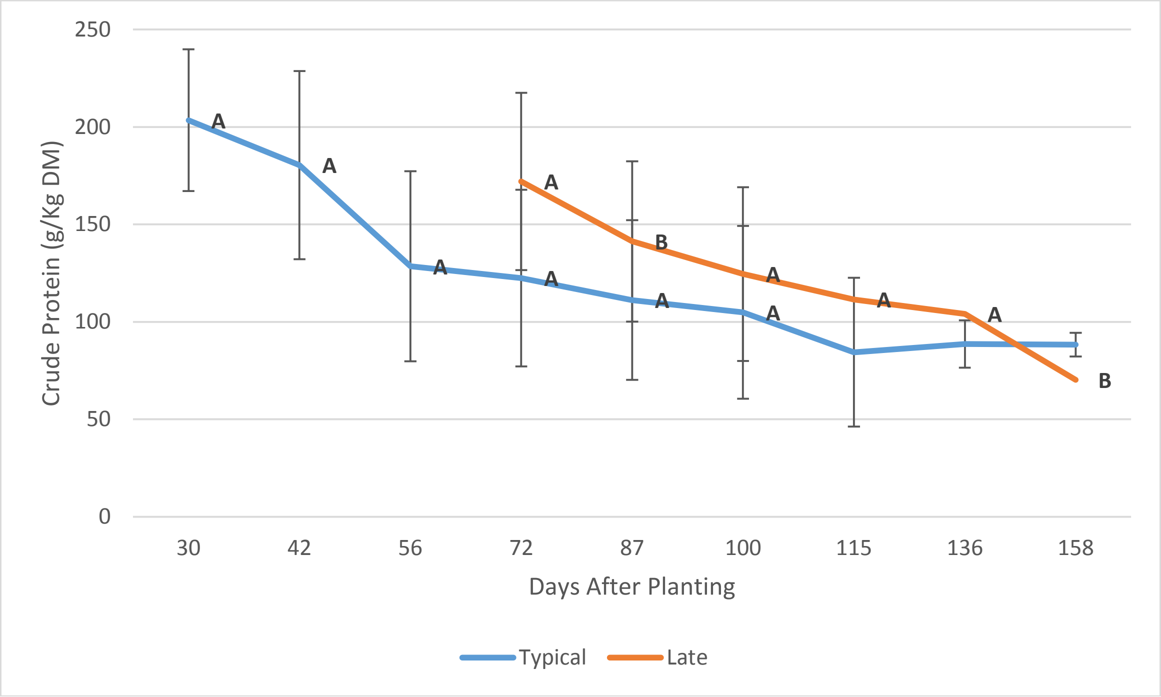

Figure 4.16: Crude Protein content by planting date across harvests. Letters represent significant differences (P < 0.05) within a harvest date.

In Vitro True Digestibility (IVTD)

Similar to CP, the concentrations of IVTD were influenced by two interactions: type*harvest (Fig. 4.17), and planting date*harvest (Fig 4.18). Overall, the four types and two planting dates evaluated followed the general trend of declining digestibility with increasing maturity. All three hemp types followed the same declining trend through 100 days after planting, with grain having the highest digestibility followed by dual purpose and fiber. From 72 days to 136 days the dual- purpose hemp had significantly higher levels of digestibility than fiber hemp. Interestingly, kenaf declined less rapidly after 56 days than the three hemps (Fig. 4.17). This may have occurred due to insect damage from Japanese beetles *(Popillia japonica)* during the summers of both years. The insects appear to have damaged the apical meristem which resulted in increased growth of lateral buds, leaf tissues, and more succulent and less lignified stems (Fig. 4.2).

Plants should have a minimum of 650 g/Kg DM digestibility for ruminants (National Research Council, 1996). Only the fiber hemp dipped below this value before 87 days after planting. 72 DAP could be a tentative time frame to achieve both high yields while maintaining at least minimum digestibility.

While both the early and late planting followed the same declining trend, the late planting had significantly lower digestibility for nearly all harvests (Fig. 4.18). Both hemp and kenaf are photoperiod sensitive plants (Chabbert, 2013; Crane et al., 1946) and a later planting date likely hastened maturity (Fig. 4.1). This would have led to an increased proportion of more lignified stems and reduced leaf tissues in the harvested forage and would contribute to decreased digestibility (Fig. 4.4 and Fig. 4.5).

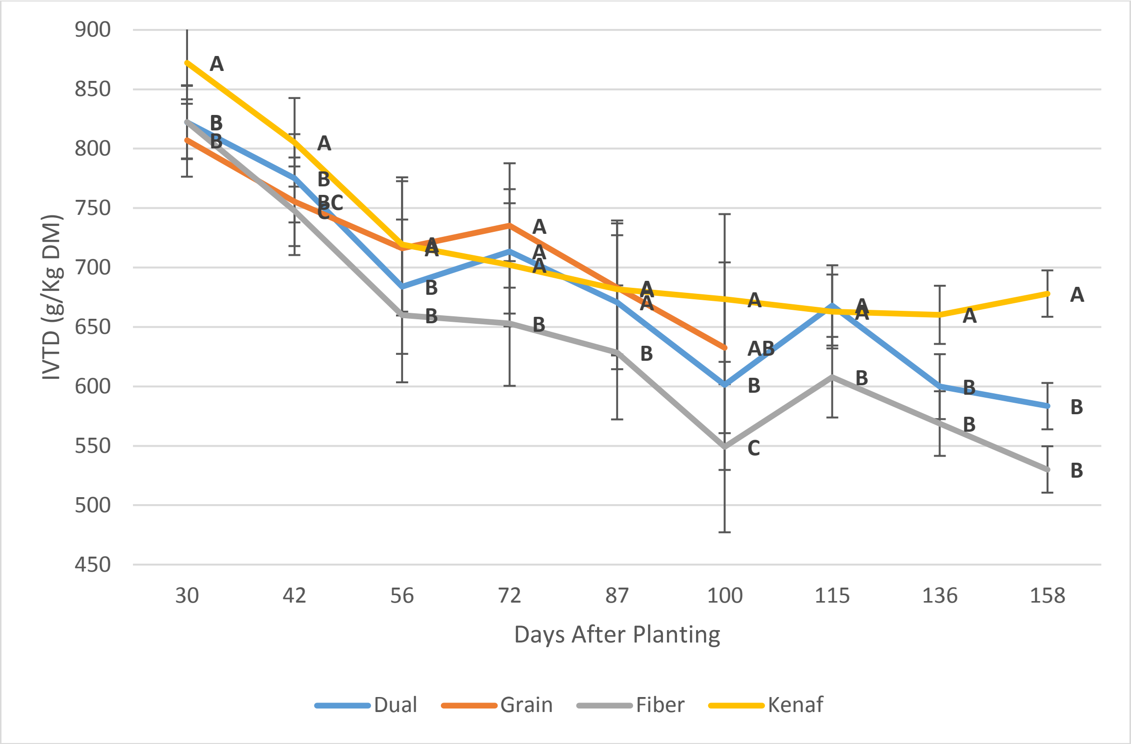

Figure 4.17: In Vitro True Digestibility levels by type across harvests. Letters represent significant differences ($P < 0.05$) within a harvest date.

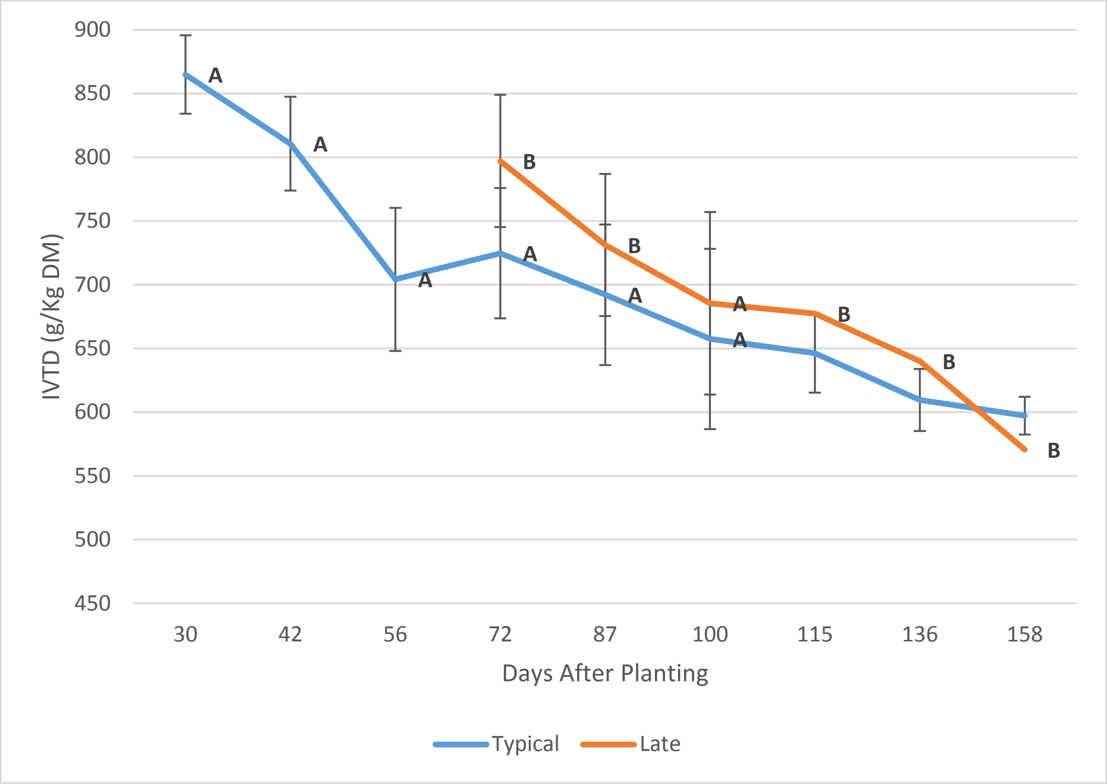

Figure 4.18: In Vitro True Digestibility levels by planting date across harvests. Letters represent significant differences ($P < 0.05$) within a harvest date.

Neutral Detergent Fiber (NDF) and Acid Detergent Fiber (ADF)

Similar to CP and IVTD, the concentrations of NDF and ADF were influenced by two interactions: type*harvest (Fig. 4.19, 4.21), and planting date*harvest (Fig 4.20, 4.22). Because ADF (cellulose and lignin) contains many of the same components as NDF (hemicellulose, cellulose, and lignin), trends for type and planting date are similar across both factors. All four types exhibited trends (Fig. 4.19, 4.21) of increasing levels of NDF and ADF due to the increase in secondary cell wall production as the plants mature. Fiber hemp consistently had the highest levels of NDF (Fig. 4.19) and ADF (fig. 4.21) from 42 DAP until the end of the season. Dual purpose hemp mirrored the fiber hemp trend, but was statistically lower than fiber hemp for NDF and ADF from 72 DAP to 115 DAP. Grain hemp had the lowest levels of NDF and ADF through 87 DAP of the three hemp types and was statistically the same as kenaf from 56 – 87 DAP. Kenaf maintained lower levels of NDF and ADF later in the growing season than the three hemp varieties, reaching a plateau by 56 DAP, likely due to insect damage (Fig 4.19, 4.21).

When both planting dates are considered, (Fig. 4.20, 4.22) the late planting accumulated higher levels of NDF and ADF faster than the typical planting. This, again, is likely due to changes in maturation rate caused by the differences in photoperiod between the two planting dates. Statistically there was no difference between the early and late planting for NDF (Fig. 4.20) or ADF (Fig. 4.22) between 56 – 87 DAP.

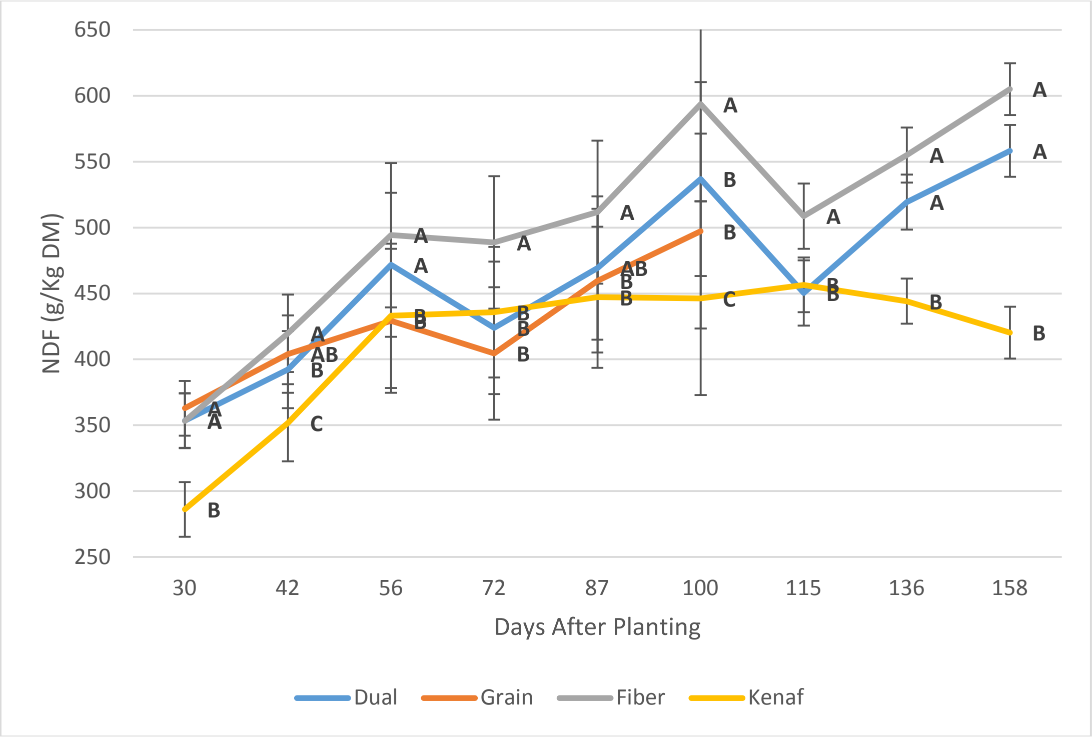

Figure 4.19: Neutral Detergent Fiber levels by type across harvests. Letters represent significant differences ($P < 0.05$) within a harvest date.

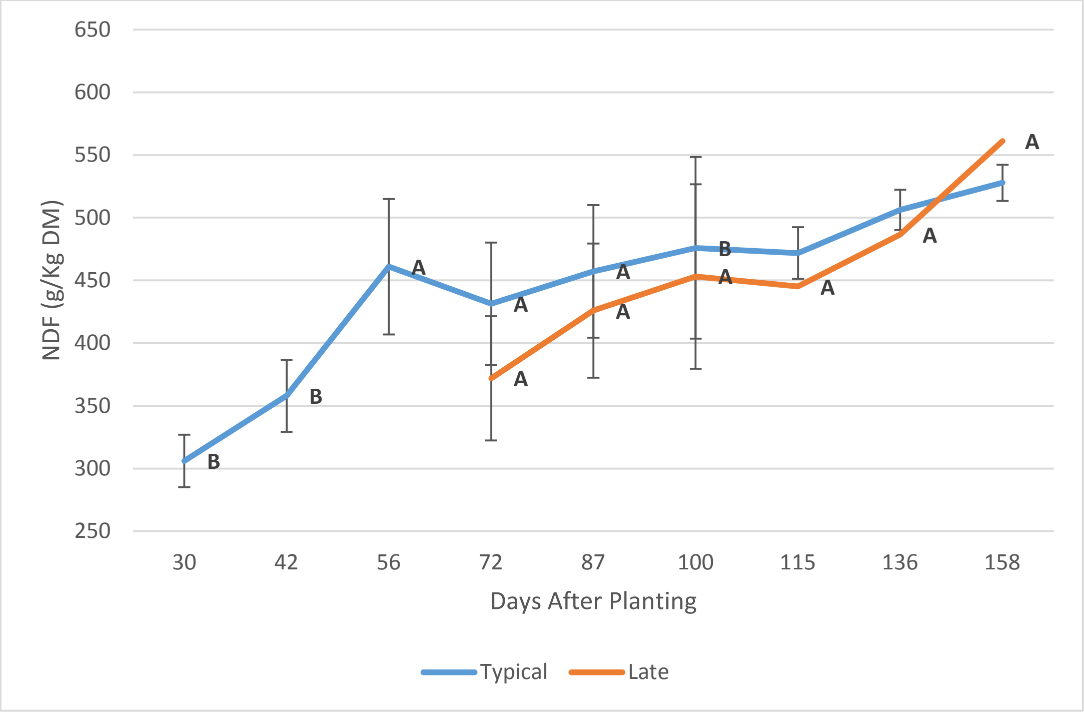

Figure 4.20: Neutral Detergent Fiber levels by planting date across harvests. Letters represent significant differences ($P < 0.05$) within a harvest date.

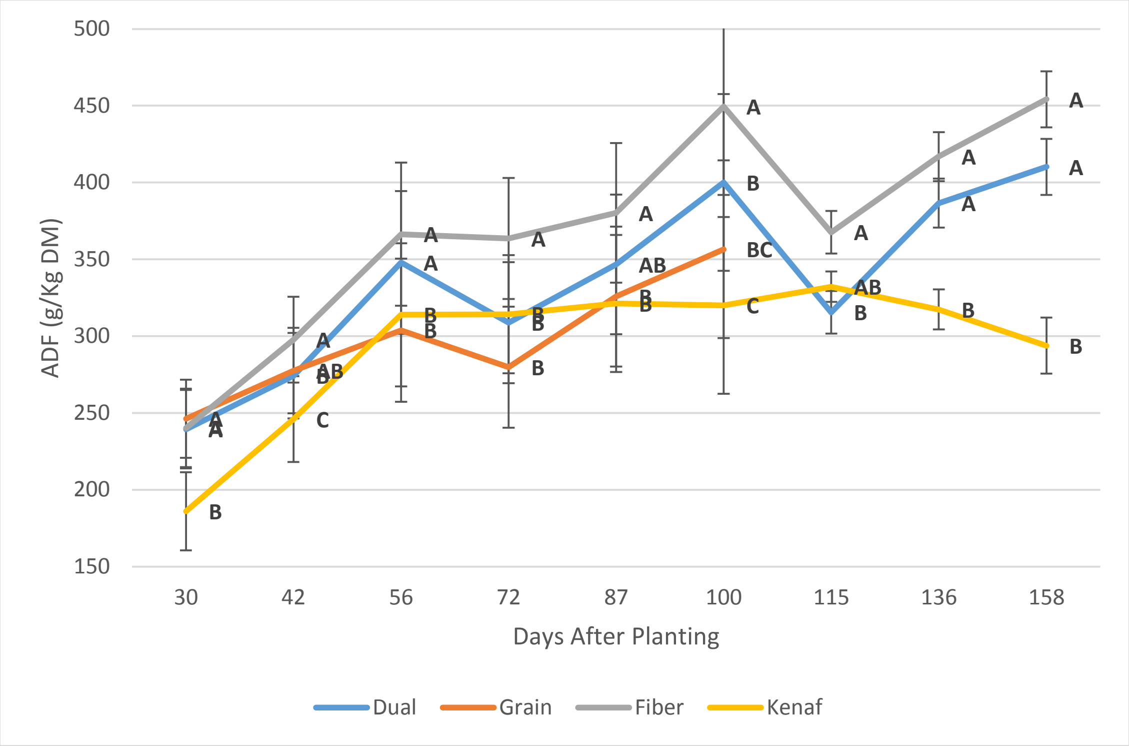

Figure 4.21: Acid detergent fiber (ADF) levels by type across harvests. Letters represent significant differences ($P < 0.05$) within a harvest date.

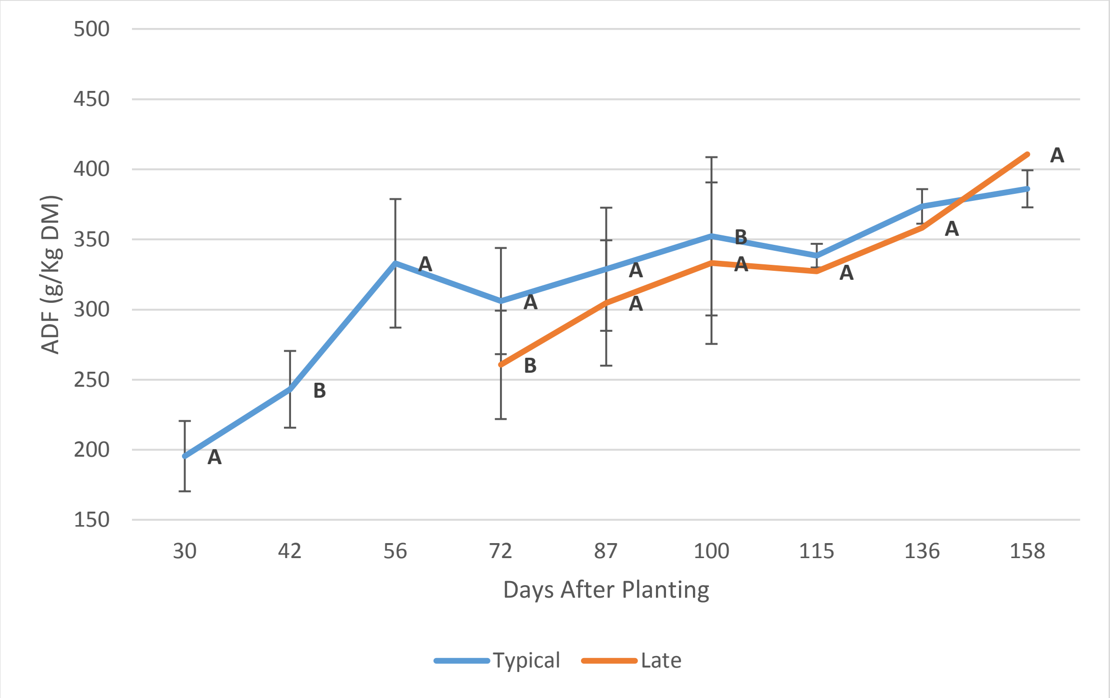

Figure 4.22: Acid detergent fiber (ADF) levels by planting date across harvests. Letters represent significant differences ($P < 0.05$) within a harvest date.

Acid Detergent Lignin (ADL)

In this study, concentrations of ADL were influenced by an interaction of type*planting date*harvest. The data is presented in Figure 4.20 by each type to better visualize trends. The later planting date resulted in higher concentrations of lignin in each of the three hemp types (Fig. 4.20A-C) and increased with maturity. As the late planting had a higher proportion of stem (Fig. 4.6), the increased concentrations of lignin are unsurprising. Concentrations of ADL remained similar for both plantings in all three types across the growing season (Fig. 4.20 D). Kenaf had lower levels of lignin in the late planting and an overall level of lignin that increased slower than the three hemp types and plateaued by 87 DAP for both plantings (Fig. 4.20D). This may have been due to insect damage to the apical meristem, which stimulated an increase in lateral growth and production of leafy tissues.

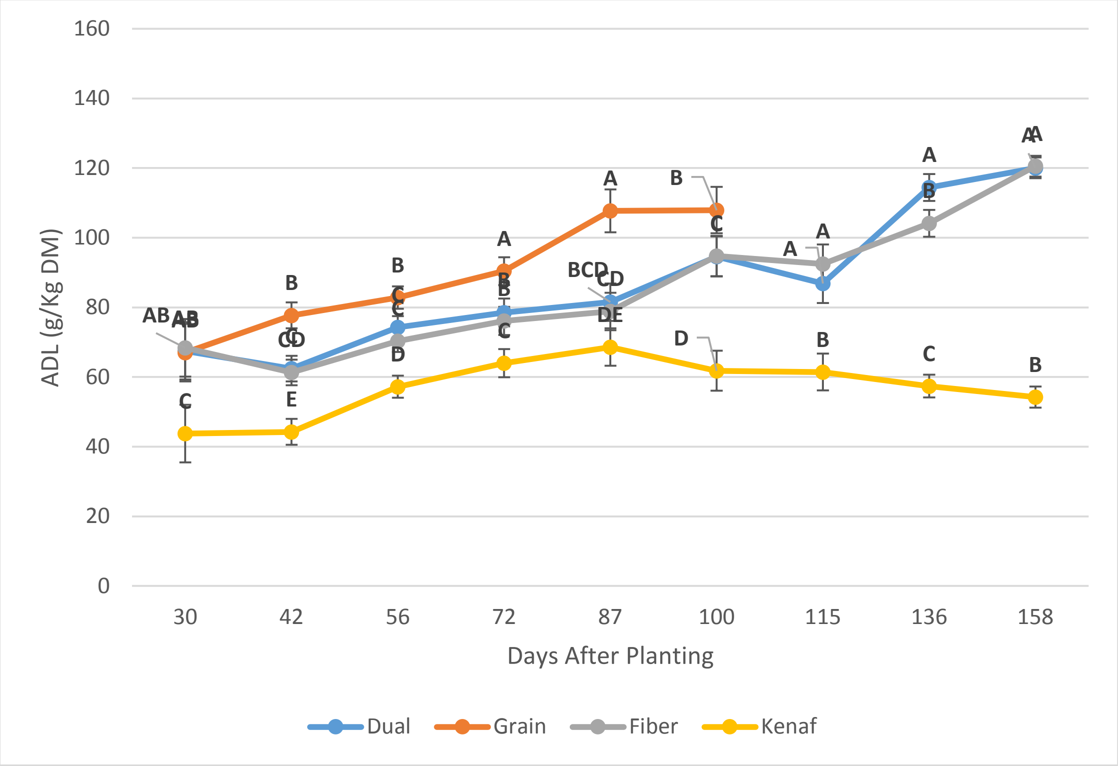

Figure 4.23: Acid Detergent Lignin levels by type across harvests for the typical planting. Letters represent significant differences ($P < 0.05$) within a harvest date.

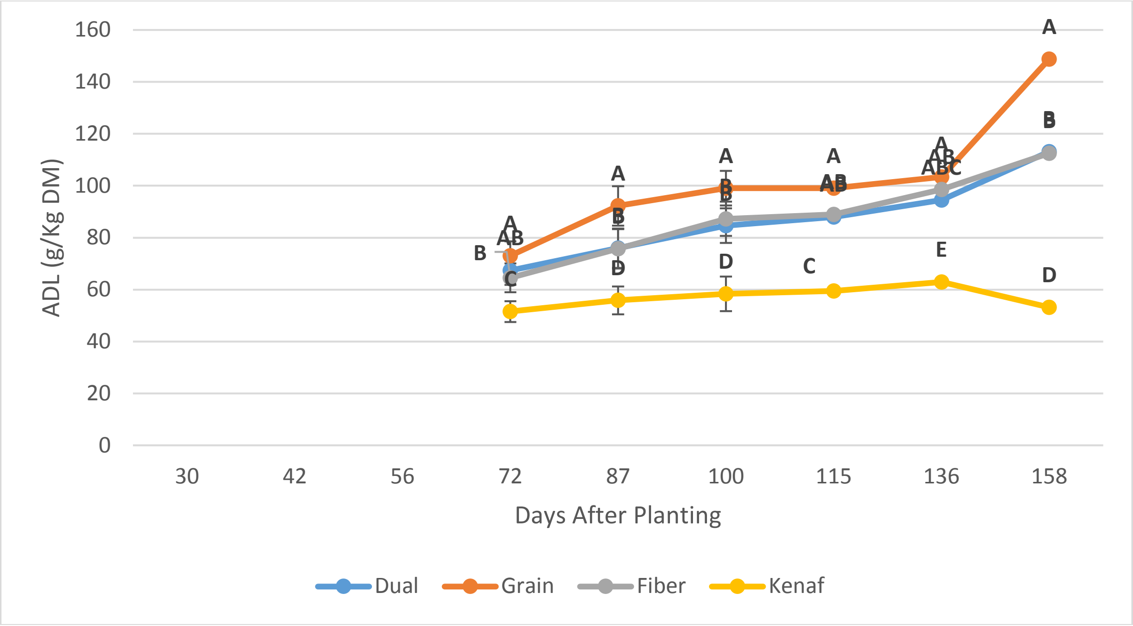

Figure 4.24: Acid Detergent Lignin levels by type across harvests for the late planting. Letters represent significant differences ($P < 0.05$) within a harvest date.

Chapter 5: CONCLUSIONS

The results from this study highlight the interactions of several important factors that influence hemp and kenaf yields and nutritive value: crop type, planting date, and harvest date. As expected, all factors considered in this study interacted with harvest date. This is consistent with most forage species as it is common knowledge that harvest date is one of the most influential factors impacting forage yield and nutritive value.

A later planting date reduced the vegetative phase in all four types relative to the typical planting date. This resulted in a more rapid accumulation of NDF, ADF, core fiber, and a decrease in leaf content. However, the leaf content of both planting dates declined at approximately the same rate. This suggests that the later planting had a shortened vegetative period from an increase in average daily temperatures and increasing day length.

The differences between the kenaf and three hemp types in the various parameters of forage nutritive value (i.e. NDF, ADF, ADL, CP, and IVTD) and morphological composition (i.e. leaf, stem, and floral components) suggest that the phenotypic differences between species/ types may influence their suitability as a forage. The grain hemp had the highest levels of CP and IVTD, but had the lowest levels of NDF, ADF, stem, and core fiber. This suggests the grain hemp has levels of nutritive value and plant composition may make it suitable as an annual species in a forage system. However, yields would need to increase significantly to make hemp attractive to a producer as the yields were consistently lower and may be uneconomical to produce. This study only evaluated one variety of each type, so further research including greater genotypic variation and examination of different agronomic management (i.e. row spacing, seeding rates) may prove vital in improving forage yields of grain hemps. Furthermore, the data from this study suggests a typical planting in mid- May results in a more gradual decline in forage nutritive value and higher forage yields throughout the season compared to the late planting.

While plant population data was recorded, it was not analyzed due to the intentional seeding differences between each type. However, it is well documented that plant density may impact the morphological composition and agronomic attributes of many species. Reta-Sanchez et al (2010) found kenaf yields continued to increase populations of 1 million plants per acre, but also found that yield per plant declined in the same range due to decreasing stem content, height, and leaf content. Thus, it is possible that the differences in yield and nutritive value between type may have been potentially confounded with seeding rate and future research may need to examine this attribute of management.

Cultivars originating from more tropical areas may produce plants in Kentucky that have a longer vegetative state and delayed flowering. For the purpose of increased forage yield and higher forage nutritive value, evaluating more cultivars could be key in producing hemp that rivals kenaf in yields and quality. The importance of locally appropriate cultivars should not be underestimated, as the inclusion of varieties from multiple latitudes can produce widely variable results. Had hemp cultivars from east Asia been available for use in this study, it is reasonable to believe that their results would have been more in line with kenaf in terms of yield and nutritive value due to the latitude and photoperiod of this region being similar to Kentucky. Furthermore, monoecious lines will likely need to be avoided for forage as they mature and senesce more rapidly than the female individuals of the dioecious lines, which may shorten the window in which harvests could be made. Further research will need to examine potential issues with palatability, as secondary metabolites are known to influence preference by livestock, and explore the potential impacts of changes in management on yield and nutritive value.

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