IMPLEMENTING NEW ALFALFA TECHNOLOGIES INTO WATER-LIMITED ENVIRONMENTS

By

ALAYNA GERHARDT

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Thesis Approved:

Dr. Alex Rocateli

Thesis Adviser

Dr. Tyson Ochsner

Dr. Romulo Lollato

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Abstract:

Oklahoma is predicted to experience a shift in rainfall patterns and experience prolonged droughts in future years. With a changing environment, producers will search for new technologies to mitigate forage losses in this area. New technologies that could be incorporated into Oklahoma alfalfa production include reduced lignin alfalfa cultivars and yield prediction models. While reduced lignin alfalfa cultivars are considered beneficial in other regions, their performance is unknown in water-limited environments, such as the central Great Plains (CGP). Also, the development of alfalfa yield predicting models would allow producers to make management decisions that best suit their hay and cattle enterprises. The object of this study was (i) to compare aboveground biomass (AGB), crude protein (CP), neutral detergent fiber (NDF), acid detergent lignin (ADL), and in vitro dry matter digestibility 48 hours (IVTDMD) among reduced lignin (54HVX41) and three reference alfalfas (54VR10, DKA44-16RR, WL 356 HQ.RR) at 28-d, 35-d, and 48-d harvest intervals in two locations (Lahoma and Stillwater, OK), and (ii) to test plant height (PH), grazing height (GH), compressed canopy height (CCH), green canopy cover (GCC), and growing degree days (GDD) as predictive variables of alfalfa aboveground biomass (AGB) to develop a producer-friendly alfalfa prediction model for the CGP. No statistical differences in AGB were observed in either locations across harvest intervals and cultivars. Overall, CP, NDF content, and IVTDMD results varied according to the ADL content changes in cultivars and harvest intervals. In Lahoma, the reduced lignin alfalfa had less ADL content than all reference cultivars. In contrast, no significant differences in ADL content were observed in Stillwater, where drought was observed. We speculate that reference cultivars may accumulate less lignin content, resulting in values similar to the reduced lignin alfalfa during drought conditions. Furthermore, GH and GDD were selected to be the primary and secondary independent AGB predictors; however, sixteen distinct linear models were proposed due to high cultivar × harvest interval variability. Further statistical analysis and site-years data are necessary to incorporate GCC and improve GDD as predicting variables.

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CHAPTER I

INTRODUCTION

Alfalfa Description

Alfalfa (*Medicago sativa L.)* is an ancient crop that has been cultivated for thousands of years, with origins in the Middle East and Asia Minor (Bolton, 1962). Eventually, alfalfa spread throughout the Greek and Roman empires and the European continent. It was not until much later that alfalfa made its way to the Americas. Alfalfa was first documented in the United States in 1736 and was spread throughout many countries in South America at the same time (Gunn, Skrdla, & Spencer, 1978). Alfalfa is often referred to as the “Queen of the Forages” due to its high-quality feed value for livestock production; however, alfalfa’s benefits are not limited to forage production. It has also been proven suitable for crop rotation, wildlife management systems, and human dietary supplement production (USDA-NRCS, 2002).

Alfalfa is a perennial legume ranging from 0.6 to 0.9 m in height, with alternating leaves on the stems (USDA-NRCS, 2002). Alfalfa’s inflorescence is typically purple; however, some subspecies (*Medicago sativa* L. ssp. *falcata* (L.) Arcang.) have yellow flowers (Gunn et al., 1978; Undersander, Vassalotti, & Cosgrove, 1997; USDA-NRCS, 2015). Besides the distinct yellow flowers, *Medicago sativa* L. ssp. *falcata* (L.) Arcang., is better adapted for areas with low annual rainfall and cold temperatures than other alfalfas. Studies have shown that *Medicago sativa* L. ssp. *falcata* (L.) Arcang. experiences slower loss of forage quality, allowing it to be harvested later in maturity (USDA-NRCS, 2015). Alfalfa produces spiral seed pods that contain small, kidney-shaped seeds that turn brown when mature (Undersander et al., 1997). Alfalfa is also known for having a long taproot, which has been found to penetrate the soil up to 6 m deep (Undersander et al., 2000). The deep roots of alfalfa help ameliorate drought, as plants utilize deep roots to obtain a supply of water during water-limited conditions (Khan et al., 2018).

Alfalfa is a widely grown forage across the entire United States, with production reaching 123 million Mg (54.9 million short tons) on a dry basis in 2019 (USDA-NASS, 2018). Alfalfa hay and haylage dry matter production in the United States has decreased by 28% ; in 2009, the United States was producing 53 million Mg of alfalfa on a dry matter basis but in 2019 only produced 38 million Mg (USDA-NASS, 2018) (Figure 1.1.A). Oklahoma alfalfa production has experienced a similar downward trend as United States production (Figure 1.1B). Oklahoma alfalfa production has decreased by 33.8% in hay production on a dry matter basis, and Oklahoma produced 8.4 10^4 Mg ha^{-1}(92,000 short tons $acre^{-1}$) of dry alfalfa hay in 2009. Alfalfa acreage trends are similar to alfalfa hay production. In 10 years, from 2009 to 2019, Oklahoma has seen a reduction in alfalfa harvested area by 46,500 hectares (Figure 1.1C).

The fluctuation in alfalfa hay production is driven by multiple factors such as competition with alternative crops, hay prices, adverse weather conditions, hay exports, ever-changing hay demands in the domestic beef and dairy industries' due to volatile profit margins (Butler, 2005; Hoyt, 2000). In years that California and Idaho had an increase in the number of cows in production, alfalfa price and demand also rose (Hoyt, 2000). Similarly, when beef prices increase, the demand for alfalfa follows (Butler, 2005). Other factors like droughts, floods, or pest infestation can limit the production and acreage of alfalfa (Butler, 2005).

Alfalfa Forage Quality

Producers have generally had to choose between increasing production or maintaining high quality in alfalfa. Forage quality is an important factor to consider in the selection of an alfalfa cultivar. To provide a standard within the industry, the United States Department of Agriculture (USDA) has developed five quality categories (supreme, premium, good, fair, and utility) for alfalfa (USDA: Agricultural Marketing Service). Alfalfa is graded into these categories based on six quality parameters (Table 1.1): acid detergent fibers (ADF), neutral detergent fibers (NDF), relative feed value (RFV), total digestible nutrients (TDN), and crude protein (CP) (USDA: Agricultural Marketing Service; Idaho Hay + Forage Association).

Using a neutral detergent solution (amylase), plant matter is separated into soluble and insoluble components. The insoluble components are the NDF, composed of cell wall contents such as hemicellulose, cellulose, and lignin. Then, the NDF is treated with an acid detergent solution (32% chloridic acid). This last step isolates cellulose and lignin from hemicellulose, allowing ADF determination (Colburn & Evans, 1967; Lacefield, 1988; Van Soest, Robertson, & Lewis, 1991).

Relative feed value estimates forage digestibility and is calculated based on forage digestible dry matter content (DDM) and dry matter intake (DMI) variables. The former variable is solely calculated based on the ADF content of the forage in interest, and the latter is calculated based on other two variables, i.e., the NDF content of the forage in interest and the animal's body weight that will consume it (Jeranyama & Garcia, 2004).

Total digestible nutrients (TDN) factor is the sum of digestible fiber, protein, lipid, and carbohydrate of forage, and it is found by subtracting the energy excreted in animal fecal matter from the total energy from the original feed source (Weiss, Conrad, & Pierre, 1992). However, TDN can be roughly estimated solely based on the forage ADF content, where different equations are available for different forage types (Agri-Food, 2021)

Crude protein is a rough estimation of the total protein content of the forage. The first step in this process is to digest a forage sample with ammonium sulfate (Kjeldahl method), where protein and non-protein N are extracted. Then, the total extracted N content is multiplied by 6.25 to estimate the forage protein content.(Conklin-Brittain, Dierenfeld, Wrangham, Norconk, & Silver, 1999). Crude protein factor tends to overestimate protein content because it accounts for non-protein N in its calculation.

Other parameters that may be included in forage quality but not listed as a requirement for USDA hay standards include acid detergent lignin (ADL) and in vitro dry matter digestibility (IVTDMD). Acid detergent lignin measures the content of insoluble lignin after being treated with acid (Hatfield, Jung, Ralph, Buxton, & Weimer, 1994). Greater lignin content is associated with decreased digestibility, as it interferes and prevents the digestion of digestible nutrients in the cell wall (Moore & Jung, 2001). In vitro dry matter digestibility is a measurement of the amount of digestion that occurs to a feed source within a given time period. After exposing a feed source to ruminal fermentation for 48 hours, the sample is removed, and then pepsin and acid are added for an additional 48 hours (Jung, Mertens, & Payne, 1997). The fraction of the feed that is digested through this procedure is considered the IVDTMD value.

Within quality terms, lignin is not seen as a desired trait, as it reduces fiber digestion by tying up cellulose and hemicellulose (Jung & Vogel, 1986; Undersander et al., 2009). Nevertheless, lignin is an essential component to plant development, as it provides structural support for the plant. Lignin comprises three monomers and is a binding agent for other smaller fibers like cellulose and hemicellulose (Undersander et al., 2009). Lignin binds the smaller, digestible fibers together, creating plant structural integrity and water movement throughout the plant (Undersander et al., 2009). However, as lignin coats cellulose and hemicellulose to aid essential plant functions, it simultaneously limits digestibility (Moore & Jung, 2001; Undersander et al., 2009). Thus, lignin behaves like a physical barrier, limiting the digestibility of cell wall cellulose and hemicellulose in animals and resulting in them being passed through the animal (Moore & Jung, 2001).

Many factors can influence the parameters that determine alfalfa quality. The alfalfa developmental stage at harvest has a significant influence on alfalfa forage quality. As alfalfa progresses from bud to bloom to maturity, quality factors like TDN and CP decrease, while ADF increases. As alfalfa grows and increases in maturity, the plant accumulates fibers and lignin. This cell walls constituents accumulation causes ADF and NDF to increase and TDN to decrease (Lacefield, 1988).

To achieve a premium or supreme grade, alfalfa should be harvested at the bud stage. Alfalfa harvested at the bud stage contains 62% TDN, 19.9% CP, and 31% ADF on a dry matter basis (Council, 1978; Lacefield, 1988). While harvesting alfalfa at the bud stage may optimize quality, it can also result in alfalfa root energy deficits, leading to subsequently low forage yields and a reduction in stand longevity (Marble, 1974; Ta, MacDowall, & Faris, 1990). Ta, MacDowall, and Faris (1990) found that up to 40% of alfalfa root carbon was consumed for supporting plant regrowth within the first 28 days (the bud stage) after harvest (Ta et al., 1990). Thus, harvesting alfalfa at 28-day intervals leads to root energy deprivation, and subsequent regrowth periods will reduce shoot growth and yield. In a study that evaluated alfalfa populations for three years, harvesting prior to the bud stage (21-25 days of regrowth) resulted in 62-71% stand population reduction (Marble, 1974). Marble (1974) found that the optimum harvest interval for stand population longevity was cutting every 33 days during the growing season, resulting in 56% survival after 3 years (Marble, 1974).

While harvesting alfalfa stands at the bud stage can provide the highest quality, it can limit yield. Putnam, Orloff, and Teuber (2005) saw a 4.0 to 7.3 Mg ha^{-1} reduction in total annual alfalfa hay production when harvested 23-24 days rather than every 33 days (Putnam, Orloff, & Teuber, 2005). Maximum alfalfa aboveground biomass occurs once alfalfa stands have reached the mid to full bloom. Yield remains relatively constant after this stage, but quality decreases (Caddel, 2001; Orloff & Putnam, 2007). Alfalfa can reach upwards of 22.4 Mg ha^{-1} (10 short tons per acre) when nutrients, pest management, and weather allow for optimal growth (Undersander et al., 2000); however, the national alfalfa production average for the last five years was 8.41 Mg ha^{-1} (3.756 short tons of dry matter per acre) (Undersander et al., 2000; USDA-NASS, 2018). Yield potential is limited by older stands, low-producing cultivars, insect pests, weeds, water ponding, nutrient deficiencies, adverse weather conditions, and improper harvest scheduling (Putnam et al., 2005; Undersander et al., 2011; Undersander et al., 2000; USDA-NASS, 2018).

One method for achieving increased quality has been through the development of multifoliate alfalfa varieties. Alfalfa varieties are considered multifoliate when leaves consist of more than three leaflets, while varieties with three leaflets per leaf are referred to as trifoliate (Henning, 1991). It was hypothesized that the increased number of leaflets in multifoliate would increase forage quality as the leaf to stem ratio increases; nonetheless, previous research indicated no significant forage quality increase in multifoliate varieties compared to trifoliate ones. (Ball et al., 2001; Henning, 1991).

Alfalfa Cultivars

Selecting an appropriate cultivar for a specific forage system is crucial for maximizing yields and reducing costs. There are many alfalfa variety options for selection, as producers must select one that meets their production needs and performs well in their environment. Cultivars can vary in winter hardiness, level of dormancy, disease, insect resistance, forage production levels, forage quality, resistance to herbicides, and longevity of the stand (Hall, Jennings, & Shewmaker, 2004; Poole, Putnam, & Orloff, 2003; Rocateli, 2019).

Having a vast number of cultivars to choose from is desirable, as the uses and locations of alfalfa can vary widely. As alfalfa is typically produced for livestock consumption, both yield and forage quality potential are important traits to consider during the cultivar selection process. High-quality alfalfa is found when protein and the digestibility of fibers are high and indigestible lignin is low.

To improve alfalfa forage quality potential, reduced lignin alfalfa varieties were developed in 2010 and became available commercially in 2015 (Barros, Temple, & Dixon, 2019). The reduced levels of lignin have been obtained in cultivars in two different ways. Lignin levels can be altered through RNA interference to create an artificial mutation resulting in the downregulation of lignin-producing genes or conventional selection and breeding of alfalfa lines with naturally reduced lignin (Sulc et al., 2016).

The high animal gains, RFV, and TDN values of reduced lignin alfalfas, thanks to their 17-20% ADL reduction, are not the only benefits of this technology (Sulc et al., 2016; Sulc et al., 2017). Reduced lignin alfalfas offer more flexibility in harvest intervals than conventional ones (Sulc et al., 2017) because reduced lignin alfalfas can be harvested 7-10 days later and still maintain high to similar quality to that of conventional alfalfa (Sulc et al., 2016; Sulc et al., 2017). This trait is beneficial to producers as they have more flexibility in maintaining quality in weather delays (Sulc et al., 2016). Also, intentional alfalfa harvest delays allow higher forage accumulation before every cut, resulting in similar quality and total forage production per season with fewer cutting events, eliminating production costs (Grev, Wells, Samac, Martinson, & Sheaffer, 2017; Undersander et al., 2009).

Alfalfa Water Use Efficiency

Campbell et al. (2019) predict that the central Great Plains (CGP) will experience prolonged droughts and more flood events in the following years because the rainfall pattern is shifting from several well-distributed events to a few extreme short-duration, high-intensity ones; thus, soil water will become more scarce. In this context, the producers, who already had taken additional management steps like irrigating the commonly adopted forage crops (Adams et al., 1990), may reconsider their forage options and adopt higher water use efficient forage cultivars.

Alfalfa is frequently referred to as a water spender, a term that is not typically associated with the idea of being efficient in the use of water (Asseng & Hsiao, 2000). Water spenders continue to use water during times of water stress to develop plant structures, like roots, that are beneficial to them in obtaining more water (Jones & Zur, 1984). With the extensive plant growth occurring throughout the season, it is important to recognize that alfalfa water use demand varies according to variety, soil type, or location. As dormancy can vary among varieties, some varieties may begin growth earlier in a calendar year than others. Varieties with greater dormancy ratings may not enter dormancy during winter months and may require additional moisture. Some locations of alfalfa production may also have greater water storage capabilities and have stored moisture from spring and winter precipitation. Stored moisture can support first-cutting growth in some locations like northern California. In many cases, alfalfa begins to experience soil moisture stress in mid-summer (Shewmaker, Allen, & Neibling, 2011). It is understood that alfalfa yield is a function of evapotranspiration, meaning that as regrowth and biomass accumulation occurs, water demands also increase (Shewmaker et al., 2011). This water demand is important to recognize, as alfalfa undergoes multiple regrowth periods each year. Each period of regrowth may differ in water use and water use efficiency (WUE).

Water use efficiency (WUE) varies on a species-by-species basis. Water use can be estimated by measuring the plant's end products (in biomass or grain yield) and the amount of water that the plant transpired (Hubick, Farquhar, & Shorter, 1986). The ratio between these two measurements is WUE (Blum, 2009; Hubick et al., 1986).

Water use efficiency is not a proxy for drought resistance. Instead, WUE is a ratio of plant metabolic processes, photosynthesis, and transpiration related to each other, as influenced by outside factors (Hubick et al., 1986). On the other hand, drought resistance can be selected and bred for by identifying drought resistance mechanisms and related genes (Blum, 2005). Blum (2005) concludes that greater WUE in plants under drought stress is related to a reduction in water use rather than increased plant production. Blum (2005), after evaluating drought resistance, WUE, and yield potential, concluded that WUE is not a highly heritable trait and that selecting plants based on greater WUE or yields in the presence of water deficit stress does not necessarily mean that drought resistance and greater yields will be transferred to offspring. Rather than selecting for greater WUE, Blum (2005) suggests that to obtain increased WUE researchers should select for genetic markers and that positively influences plant characteristics. Blum (2005) encourages selection for smaller plants, decreased leaf area, or short growing periods to improve WUE (Blum, 2005).

As alfalfa is a C3 plant, it experiences carbon dioxide discrimination or preference in carbon isotopes during photosynthesis due to isotope properties. Carbon dioxide discrimination in C3 plants can be used to better understand WUE (Farquhar, Ehleringer, & Hubick, 1989). Carbon dioxide molecules are readily available in the atmosphere to the plant, and their carbons are found in two forms ^{12}C and ^{13}C. ^{12}C is the most readily available carbon molecule in the atmosphere as it makes up 98.9% of atmospheric carbon, while ^{13}C makes up around 1% (Farquhar et al., 1989). These isotopes allow the carbon sinks, like plant biomass, to be evaluated and studied in terms of photosynthesis (Farquhar et al., 1989). When studying photosynthesis and the gas exchanges in plants during water stress, these carbon isotopes provide needed insight into transpiration for C3 plants (Farquhar et al., 1989). The Δ ratio, is found by subtracting 1 from the ratio of the carbon isotope in the air and plant (Farquhar et al., 1989). During times of water or drought stress, photosynthesis is decreased (Jones & Zur, 1984). This causes less carbon dioxide to be taken in by the plant, causing the carbon isotope ratio Δ to increase (Farquhar et al., 1989). This increase is because photosynthesis prefers the ^{12}C isotope and tends to discriminate against ^{13}C (Farquhar et al., 1989; Jones & Zur, 1984).

Low Δ ratios correlate with an increased WUE, while higher Δ tend towards having a lower WUE (Farquhar et al., 1989). It is important to recognize that Δ and WUE can vary due to genetics but can provide meaningful results when these measurements are taken in the same environment (Hubick et al., 1986). Hubick et al. (1986) found that there was a strong correlation between WUE and Δ as a selection method in peanut varieties (Hubick et al., 1986).

C3 plants, such as alfalfa, typically have low WUE during water stress due to photorespiration, as Rubisco can often bind with oxygen instead of carbon dioxide, which limits photosynthesis (Aranjuelo, Molero, Erice, Avice, & Nogués, 2010). However, with increased CO_2 in the atmosphere, there is less competition between oxygen and CO_2, causing more CO_2 to be fixed. These atmospheric changes that increase carbon fixation and photosynthesis efficiency suggest that WUE will also increase (Drake, Gonzalez-Meler, & Long, 1997; Tyree & Alexander, 1993). In Bottomley et al. (1993) and Drake et al. (1997)'s studies, environments with a greater CO_2 concentration have altered the plant-water relationship as transpiration is decreased. Studies that looked at the responses of plants in increased CO_2 environments found that plants had increased water levels in the lower stem/upper taproot area and less wilting /stem shrinkage compared to control groups of typical CO_2 levels (Bottomley, Rogers, & Prior, 1993; Drake et al., 1997). In limited water conditions, C3 plants, like wheat and cotton, benefited from an increase in CO_2, more so than C4 plants, like corn (S. Kang, Zhang, Hu, & Zhang, 2002).

In theory, the increase of CO_2 in the atmosphere looked beneficial to C3 plants, yet recalling that the CGP will experience extreme weather-related events, all aspects of climate impact plant growth and should be evaluated. Despite some studies seeing benefits in elevated CO_2 environments, many forget to incorporate the relationship that CO_2 has with temperature. In a literature review on the impact of air CO_2 and temperature in WUE, Eamus (1991) discussed that C3 plants would decrease photosynthesis rates even though air CO_2 concentration will increase because higher air CO_2 levels will also increase the air temperature by 2-4°C, which is a more influential and detrimental factor in photosynthesis (Eamus, 1991).

Biomass accumulation and transpiration are morphologically interrelated. The stomata dictate both processes. The levels of transpiration (water vapor flux) and photosynthesis (oxygen and carbon dioxide fluxes) increase as stomata open. (Anower et al., 2017). However, in terms of WUE, they are counterproductive to one another (Blum, 2009). Photosynthesis increases biomass accumulation, resulting in greater WUE values. Conversely, transpiration increases plant water demand, resulting in smaller WUE values.

Factors that influence WUE, like water vapor deficit and carbon flux, can change as plant physiology and anatomy are altered through harvest or a period of regrowth. Asseng and Hsiao (2000) found that WUE was the greatest prior to cutting as it was near 10 g CO_2 kg^{-1} of water. Once harvested, the WUE of alfalfa decreased to only 2 g CO_2 kg^{-1} (Asseng & Hsiao, 2000). During regrowth, alfalfa WUE did recover after energy had been spent on accumulating biomass (Asseng & Hsiao, 2000). WUE is decreased after cutting and during the period of regrowth, as the energy balance, transpiration (T), evapotranspiration rate (ET), and the CO_2 assimilation rate per unit of land area are altered (Asseng & Hsiao, 2000). Calculations of WUE are frequently reported in two ways, WUE_T and WUE_{ET}. WUE_T reports the water use through measurements that only account for the transpiration losses of the plant, while WUE_{ET} reports measurements water use of both the plant and the surrounding ground (Asseng & Hsiao, 2000; Eamus, 1991; Shewmaker et al., 2011). The biomass and plant coverage from the vegetative growth of alfalfa is drastically reduced after cuttings, reducing soil coverage. After harvest, greater soil exposure increases the soil heat flux and lowers available energy (Asseng & Hsiao, 2000). With more soil exposed, the energy that would typically be directed towards the plant canopy and transpiration is now redirected towards the newly exposed soil surface (Asseng & Hsiao, 2000). This redirection of energy to the soil surface results in reduced amounts of transpiration. The rate of CO_2 assimilation per land area is similarly reduced as it relies on transpiration (Asseng & Hsiao, 2000). Levels of ET and the rate of CO_2 assimilation per land area both recover to about two-thirds of pre-harvest levels after three weeks of regrowth (Asseng & Hsiao, 2000). Considering these factors, the lowest WUE_{ET} values for alfalfa will be found directly after harvest (Asseng & Hsiao, 2000).

To better understand the response of alfalfa in limited water conditions, a study was conducted to evaluate the impact of limited available water on alfalfa forage yield, quality, water use, and WUE_{ET} (Carter & Sheaffer, 1983). Under four different water supply levels, soil moisture levels were not found to differ until the first harvest in June. Rainfed alfalfa depleted the soil water content to a 1.5 m depth by August, while irrigated alfalfa had not depleted soil water at that time and depth. Forage yield decreased as plants experienced a lower water potential (Carter & Sheaffer, 1983). As water use and yield were both calculated, WUE_{ET} was evaluated for multiple water levels. The treatments with little to no irrigated water (-2.7 to -4.0 MPa) were found to have the lowest level of WUE_{ET}, as the lowest levels of biomass were produced.

Treatments that maintained plant-water potentials around -0.7 to -1.3 MPa were found to have increased levels of WUE. Through the extraction of soil water in this study, Carter and Sheaffer (1983) found that alfalfa with moderately low levels (-2.7 MPa) of irrigation had more than 86% of the yield than stands with high soil moisture requiring 68% less irrigation. These findings indicate that plants that experience some level of soil water depletion may have acceptable forage production while simultaneously conserving water (Carter & Sheaffer, 1983).

Drought Resistance and Strategies

When plants experience drought, they can be classified into two response categories; water spender or saver. A water saver regulates its water balance and maintains turgor reducing transpirational water lost through stomata, which can be done by increasing stomatal resistance or decreasing leaf area (Jones & Zur, 1984). Water spenders are plants that maintain a plant water balance by increasing water uptake by spending energy to develop an extensive root system (Jones & Zur, 1984). Plants can spend or save water to equip four mechanisms of recovering, tolerating, avoiding or escaping drought (Khan et al., 2018). Plants with the ability to continue growth after experiencing drought injury equip drought recovery, while plants that survive times of drought through the alteration of plant losses at stomatal level are identified as drought tolerant (Khan et al., 2018). Plants who's growth period occurs outside of typical drought periods employ drought escape or avoidance (Khan et al., 2018). Alfalfa is predominately thought of as a water spender; however, studies have shown that the WUE of alfalfa can compete with other crops in terms of production and water usage as they practice these mechanisms of surviving periods of drought (Asseng & Hsiao, 2000). Looking at the mechanisms that aid alfalfa in drought resistance, alfalfa can be classified as both a spender and a saver.

One of alfalfa's defining characteristics is its capability to develop roots up to 6 m deep into the soil (Undersander et al., 2000). Increased root growth is a common mechanism to avoid drought conditions and maintain the water balance in plants (Jones & Zur, 1984; Khan et al., 2018). However, this mechanism typically only occurs during mild drought. Alfalfa can limit physiological processes at extreme drought conditions, inhibiting further root development (Khan et al., 2018). Alfalfa's stomata are partially closed to save water, reducing water vapor loss, i.e., transpiration (Y. Kang et al., 2011). By reducing stomatal conductance, the movement of water vapor, oxygen, and carbon dioxide through the stomata are all limited, causing a decrease in transpiration and photosynthesis (Y. Kang et al., 2011). Thus, cell growth is among the first processes negatively affected (Aranjuelo et al., 2010). With the reduction in transpiration and photosynthesis, alfalfa plants decrease shoot growth, while root growth continues to continuously supply the plants with water (Y. Kang et al., 2011). This reduction in transpiration and photosynthesis can quickly be triggered, as alfalfa stands saw a 58% decrease in photosynthesis after withholding water for seven days (Aranjuelo et al., 2010).

In alfalfa cultivars that were watered at four levels that compensate for 100, 75, 50, or 25% transpirational water loss, shoot growth and biomass accumulation were found to be reduced. Some cultivars that were watered at a 25% transpirational water loss showed a 70-73% reduction from their production levels at 100% transpirational water loss levels. (Anower et al., 2017). Interestingly, the cultivar with the lowest biomass production in well-watered conditions showed the least reduction at the 25% watering level. However, this same cultivar was found to have increasing WUE as water stress was increased (Anower et al., 2017). Similarly, as shoot biomass was inhibited, stem elongation also showed a decrease in growth during times of limited water. A decrease in shoot growth was detected for alfalfa plants given 75% of the needed transpirational water. The reduction in stem elongation continued to be reduced at levels of 25 and 50% of needed transpirational water (Anower et al., 2017). As physiological processes are reduced during drought, alfalfa experiences a decrease in yield related to an increase in leaf:stem weight ratios (LSWR) and decreases in plant maturity rates (Peterson, Sheaffer, & Hall, 1992). Despite having a 33% reduction in yield throughout the season, alfalfa still maintained the greatest yield when compared to birdsfoot trefoil (*Lotus corniculatus* L.), cicer milkvetch (*Astragalus cicer* L.), and red clover (*Trifolium pretense* L.) (Peterson et al., 1992).

Depending on the severity, drought can reduce alfalfa growth rate and delay maturity. (Buxton, 1996). This plant response of delayed maturity may be a method of escaping or avoiding drought to increase production when water is not a limiting factor (Khan et al., 2018). Forage quality is also affected by drought-driven photosynthesis suppression, curbing plant growth, which is seen as a contributing factor to why some levels of quality increase (Grant, Kreyling, Dienstbach, Beierkuhnlein, & Jentsch, 2014; Peterson et al., 1992). In some instances, increases in LSWR and decreases in plant maturity resulted in higher forage quality (Peterson et al., 1992). Drought conditions have reduced NDF, ADF, and ADL; meanwhile, CP has inconsistent results. Some studies have found small increases in CP. A slight gain in CP is interesting as some proteins are downregulated during drought, while others have been found to upregulate depending on function (Aranjuelo et al., 2010; Peterson et al., 1992). Proteins associated with energy destination and storage were found to be upregulated, while down-regulated proteins were identified to be closely connected to metabolism, transport, cell structure, and disease defense (Aranjuelo et al., 2010).

Alfalfa is an important crop throughout the world, country, and the state of Oklahoma. With adaptations, like deep penetrating taproots to combat water stress or multifoliate leaves to increase production, alfalfa is an adaptive crop that will continue to meet producers' future demands. Alfalfa has already met the production demands by meeting the desired lower lignin level, creating a more digestible product for livestock; therefore, it is considered a reliable animal feedstock. Simultaneously, reduced lignin alfalfa can be harvested later with minimal forage quality penalties. However, as climate change progresses, the environments that were once ideal for alfalfa production may no longer be. In the CGP, this environmental shift translates into more prominent droughts. Therefore, a focus on alfalfa performance in water-restricted conditions is essential to warrant future recommendations.

CHAPTER II

ASSESSING REDUCED LIGNIN ALFALFA IN WATER-LIMITED ENVIRONMENTS

Abstract

While reduced lignin alfalfa cultivars have been found to be perform well with increased forage quality in other regions, their performance is unknown in water-limited environments, such as the central Great Plains. This study's objective was to compare aboveground biomass (AGB), crude protein (CP), neutral detergent fiber (NDF), acid detergent lignin (ADL), and in vitro dry matter digestibility 48 hours (IVTDMD) between reduced lignin (54HVX41) and three reference alfalfas (54VR10, DKA44-16RR, WL 356 HQ.RR) at 28-d, 35-d, and 48-d harvest intervals in Stillwater and Lahoma, OK. The experimental design was a split-plot arranged in 3×4 factorial with four replications, where harvest interval were the main plots and cultivars were subplots. Results indicated no statistical differences in AGB for either location among harvest intervals or cultivars. Overall, CP, NDF content, and IVTDMD results varied according to the ADL content differences between cultivars and harvest intervals. While the reduced lignin alfalfa had less ADL content than all reference cultivars in Lahoma, no significant differences were observed in Stillwater, where drought stress was observed. We speculate that reference cultivars may accumulated less lignin, resulting in values similar to the reduced lignin alfalfa during drought conditions.

Introduction

Alfalfa (*Medicago sativa* L.) hay and haylage production has decreased by 28% in the U.S. in the last ten years (USDA-NASS, 2018). This reduction in alfalfa hay production was driven by multiple factors such as competition with alternative crops, low hay prices, adverse weather conditions, and ever-changing hay demands in the domestic beef and dairy industries' due to volatile profit margins (Butler, 2005; Hoyt, 2000). Oklahoma's alfalfa hay production are even greater than the national trend, as the state had a 33.8% reduction in alfalfa hay production from 2009 to 2019 (USDA-NASS, 2018). This substantial reduction in Oklahoma alfalfa hay and haylage production might partly due to the lack of testing of new alfalfa technologies and development of specific management recommendations for the state's soil and climate. With the lack of information in Oklahoma's environment, producers may be discouraged from adopting or maintaining alfalfa in their forage systems.

One new alfalfa technology that has not been tested in Oklahoma's environment is reduced lignin alfalfa. Reduced lignin alfalfa varieties were developed in 2010 and became available commercially in 2015 (Barros, Temple, & Dixon, 2019). The reduced levels of lignin have been achieved in cultivars in two different ways. Lignin levels can be altered through RNA interference or conventional selection and breeding (Sulc et al., 2016). Reduced lignin cultivars exhibit high animal gains, RFV, and TDN values, due to their 17-20% ADL reduction, yet the increased quality is not the only benefit of reduced lignin technology (Sulc et al., 2016; Sulc et al., 2017). Reduced lignin is beneficial to producers as they have more flexibility in maintaining quality when weather delays harvest, as reduced lignin alfalfa cultivars can be harvested 7-10 days later and still maintain high to similar quality to conventional alfalfa (Sulc et al., 2016; Sulc et al., 2017). Also, alfalfa harvest delays allow higher forage accumulation before every cut, which in the case of reduced lignin alfalfa, can result in similar quality and total forage production per season with fewer cutting events, reducing production costs (Grev, Wells, Samac, Martinson, & Sheaffer, 2017; Undersander et al., 2009).

The performance of reduced lignin cultivars has been evaluated in a few locations outside of Oklahoma. For instance, one study was conducted in four locations across the state of Minnesota and suggested that the reduced lignin and three commercially available cultivars without the lignin-gene downregulation (reference cultivars) did not differ in total forage dry matter accumulation (Grev et al., 2017). Still, reduced lignin alfalfa averaged 8% less acid detergent lignin (ADL) and 10% more neutral detergent fiber (NDF) than conventional cultivars (Grev et al., 2017). In addition, they concluded that the reduced lignin cultivar harvested at 35-day intervals gained 21% in forage mass and lost only 3% in relative forage quality compared to reference cultivar harvested at 30-day intervals (Grev et al., 2017). In a multi-state study, including California, Kansas, Pennsylvania, Ohio, and Wisconsin, Arnold et al. (2019) concluded that reduced lignin alfalfa had similar yields to conventional cultivars with the same harvest intervals. Moreover, the authors stated that farmers could delay low-lignin alfalfa harvest by 5 to 10 days without forage nutritive value penalties and obtain higher or equivalent forage yields. Furthermore, Getachew et al. (2011) concluded that the reduced lignin alfalfa lines had 5% greater in vitro dry matter digestibility (IVDMD) than conventional alfalfa.

One commonality in the currently available literature evaluating the performance of reduced lignin alfalfa is that all studies were conducted in regions where annual precipitation is greater than the annual potential evapotranspiration (USGS, 2016). In contrast, the Central Great Plains Winter Wheat and Range Region (Land Resource Area H, CGP), where most Oklahoma alfalfa acreage is located, is characterized by a dry subhumid-semiarid transition climate. The CGP annual precipitation ranges from 965 to 381 mm (USDA-NRCS), resulting in a negative annual water balance, which might be restrictive to alfalfa production.

Reduced lignin alfalfa might be an option for locations with limited water conditions due to an improved drought resistance associated with this trait. The down-regulation of the coenzyme HCT (shikimate hydroxycinnamoyl transferase) in alfalfa reduced lignin levels and conferred high drought resistance to the plant (Gallego-Giraldo, Escamilla-Trevino, Jackson, & Dixon, 2011). Down-regulated HCT alfalfa showed fewer symptoms of drought stress after nine days without irrigation in greenhouse conditions, and these plants recovered completely after five days of rehydration. Meanwhile, conventional alfalfa exhibited extensive drought damage and did not survive until the end of the experiment.

Therefore, this study's objective was to compare season-long biomass and quality of reduced lignin with three reference alfalfas at three different harvest intervals in the central Great Plains. This project was carried out under the hypothesis that reduced lignin alfalfa would result in a lower ADL and NDF, increased IVTDMD, while maintaining CP and season-long biomass as detected in past studies. Previous studies in non-water-limited environments have shown that reduced lignin alfalfa can maintain yield while achieving higher quality, yet no reduced lignin alfalfa performance information is available in water-limited environments.

Materials and Methods

Site Description

A study was established in the fall of 2019 and data presented here span one alfalfa season (from April of 2020 to October of 2020) in two locations: Stillwater, OK (36°07’02” N 97°05’53” W), and Lahoma, OK (36°23’23” N 98°06’19” W). Stillwater and Lahoma sites were, respectively, mapped Easpur loam, 0 to 1% slopes, occasionally flooded**,** (fine-loamy, mixed, superactive, thermic Fluventic Haplustolls) and Pond Creek silt loam, 0 to 1% slopes (fine-silty, mixed, superactive, thermic Pachic Argiustolls) (NRCS, 2008). Stillwater was fallow in 2017 and cropped with winter wheat (*Triticum aestivum*) during the fall of 2018, which was mechanically terminated in May of 2018. Lahoma was left fallow for two consecutive years prior to this study (2017 and 2018).

Cultural Practices and Experimental Design

In July 2019, the experimental fields were sprayed with 1.14 kg ha^{-1} glyphosate (Roundup PowerMAX®, Monsanto, St. Louis, MO) and 0.73 kg ha^{-1} 2,4-D (Weedone® LV4 Solventless, Nufarm, Alsip, IL) to terminate standing weeds. Composite soil samples were collected to a depth of 15 cm from each location prior to the experiment establishment in 2019. Nutrient analysis was performed for each location at Oklahoma State University Soil, Water, and Forage Analytical Laboratory (SWFAL) (Zhang & Henderson, 2016). According to Oklahoma Cooperative Extension Service recommendations, soil analysis results indicated adequate pH levels in Stillwater and lower than optimal pH for alfalfa production at Lahoma (Zhang & Henderson, 2016). The average pH in Lahoma was 5.7, and 1.64 Mg ha^{-1} of 100% effective calcium carbonate pelletized lime was applied as advised by OSU extension soil test recommendations in order to reach target pH of 6.0-7.0. The pelletized lime was incorporated using an offset disk (John Deere 225 offset disk, John Deere Manufacturing, Moline, IL). Soil laboratory analyses also determined that macro and micronutrients were sufficient in both locations, and no additional soil amendments were required.

Seedbeds were established in both locations during mid-September of 2019. Both sites were disked with an offset disk (John Deere 225 offset disk, John Deere Manufacturing, Moline, IL) to a depth of 7.5-12 cm followed by a spring tooth harrow (John Deere Manufacturing, Moline, IL) at a 5-7.5 cm depth. A drag harrow was used in both locations to smooth out and firm up the seedbed.

In each experimental replication of both sites, composite soil samples (Table 2.1) were taken before planting to ensure that the lime application corrected the soil pH to the recommended pH levels (Caddel, 2001). Alfalfa plants at replications three and four in the Lahoma site presented nitrogen deficiency symptoms (i.e., yellowing leaves and stunting) in April of 2020 due to poor root nodulation. Although proper amounts of lime were previously applied, the complete lime reaction with the soil was not achieved. As nodulation was limited, 45 kg N ha^{-1} (40 lbs. N/acre) of urea was applied to both replications in April 2020 (Hannaway & Shuler, 1993). Application of nitrogen to alfalfa during establishment can increase yields that are limited by low pH (Hannaway & Shuler, 1993).

The Lahoma experiment site was planted on 19 September 2019. Alfalfa plots were established in Stillwater on 1 October 2019. On the sowing day, a box drill seeder (Brillion SSP-108, Brillion Iron Works, Brillion, WI) was run across the sites to ensure the soil was packed, followed by sowing the same forage box drill seeder. Based on recommendations for Oklahoma alfalfa production, 16.8 kg ha^{-1} pure live seed was planted in both locations to achieve about 450 plants m^2 (Caddel, 2001).

The experimental fields were established in a split-plot design with four replications. Main plots (20-m long and 5-m wide) consisted of three harvest intervals every 28, 35, and 42 days. Main plots were separated into four sub-plots (5-m long and 5-m wide), consisting of four alfalfa cultivars (DKA44-16RR, WL 356HQ.RR, 54VR10, and 54HVX41).

Weeds were controlled in Lahoma and Stillwater 167 and 154 days after planting, using 1.14 kg ha^{-1} glyphosate (Roundup PowerMAX®, Monsanto, St. Louis, MO). Cowpea aphids (*Aphis craccivora*) were controlled with chlorpyrifos (Lorsban®-4E, Corteva™ Agriscience, Wilmington, DE) with 0.52 kg ha^{-1} in Lahoma 188 days after planting. Blister beetles (*Epicauta vittate*) were managed with carbaryl (Sevin® SL Carbaryl, Bayer Environmental Science, Cary, NC) with 1.00 kg ha^{-1} at both 266 and 349 days after planting in Lahoma. The Stillwater experimental site in Stillwater was sprayed for blister beetles at 253 and 328 days after planting.

Harvest Intervals

The three harvest intervals, 28, 35, and 42-days of regrowth, were selected to evaluate the later described cultivars. These harvest intervals were based on the recommendations made for alfalfa production in the southern Great Plains (Caddel, 2001). According to Caddel (2001), the 28-d, 35-d, and 42-d harvest intervals may result in a maximum of six, five, and four cuts per season, respectively. However, alfalfa harvesting was not extended beyond late September, which reduced the 28-d and 35-d harvest intervals to a total of five and four cuts, respectively. During the first production year, it is important to ensure that alfalfa stands regrow for six weeks before the first killing frost, which occurs from late October to early November in most years. This final aftermath will allow plants to enter the winter months with healthy root systems that will withstand the winter (Rocateli, 2020).

Cultivars

The four cultivars selected for this experiment were Roundup Ready®. A brief description of each cultivar is provided below.

'DKA44-16RR' (DEKALB, Bayer Group, St. Louis, MO; Roundup Ready, Monsanto Technology LLC., St. Louis, MO) is known for having high yield, quality potential, and adaptability to most environments. 'DKA44-16RR'is a common cultivar choice in Oklahoma, as it has a fall dormancy of 4.4 and winter hardiness of 1.6. In addition, 'DKA44-16RR' is resistant to several diseases and pests, including multiple wilts, root rots, nematodes, and aphids (Bayer, 2019).

‘WL 356HQ.RR’ (W-L Research, Inc., Madison, WI; Roundup Ready, Monsanto Technology LLC., St. Louis, MO) is a commonly used Oklahoma alfalfa cultivar with high yield potential. Multiple harvest managements ranging from three to five annual cuttings are suitable for this variety and make it ideal for this study. This alfalfa cultivar also has the genetic potential for a high-quality crop and the glyphosate-resistant trait. This cultivar has a fall dormancy and winter-hardiness of 3.8 and 1.6, respectively. ‘WL 356HQ.RR’ provides resistance traits to multiple wilts, root rots, nematodes, and aphids (Monsanto, 2020).

‘54VR10’ (Pioneer, Corteva Agriscience, Johnston, IA; Roundup Ready, Monsanto Technology LLC., St. Louis, MO) contains many traits that make it a successful cultivar throughout much of the US, including Oklahoma. ‘54VR10’ is like the two previous cultivars, as it is also a fall dormancy of 4 and has optimal winter hardiness. ‘54VR10’ is unique in the fact that it survives in both well and poorly-drained soils. Resistance from multiple diseases and insects makes ‘54VR10’ an optimal selection for an alfalfa crop (Pioneer, 2019b).

‘54HVX41’ (Pioneer, Corteva Agriscience, Johnston, IA; Roundup Ready, Monsanto Technology LLC., St. Louis, MO), is a reduced lignin alfalfa cultivar – HarvXtra® technology (Forage Genetics International, LLC, Land O’Lakes, Nampa, ID). This technology allows for harvest delays while maintaining forage quality. This reduced lignin cultivar is genetically similar to the previous three cultivars, except for the down-regulation of the hydroxycinnamoyl CoA: shikimate hydroxycinnamoyl transferase (HCT) gene. It has resistance to pests and diseases, such as aphids and multiple root, stem, and crown diseases. ‘54HVX41’ is a cultivar with optimum relative forage quality, thanks to its low lignin content (Grev, Wells, Samac, Martinson, & Sheaffer, 2017; Pioneer, 2019a).

Data Collection

Weather Data

Daily and long-term average temperatures and precipitation were acquired from the Oklahoma Mesonet website (www.mesonet.org) for stations in Lahoma and Stillwater (McPherson et al., 2007). Both field locations were within one-kilometer of their respective Mesonet stations.

Alfalfa Aboveground Biomass and Nutritive Value Indicators

On scheduled harvest dates (Table 2.2), a 1-m^2 quadrat was placed in the center of each subplot. All alfalfa herbage within the quadrat was clipped at ground level using handheld electric Gardena shears (Gardena 8885-U 3-Inch Cordless Lithium-Ion Grass Shears, Gardena, Ulm, Germany); then, the sampled area was flagged to assure that the same area was re-sampled at every cut according to their assigned harvest intervals. Herbage samples were dried in a forced-air oven at 55°C until constant weight (a minimum of seven days); dry herbage samples were then weighed and converted to aboveground biomass per hectare. Finally, the aboveground biomass values from the repeated cuts in a specific experimental unit were summed to estimate the total seasonal aboveground biomass, which is simply referred to as aboveground biomass (AGB). Afterward, samples were ground to a diameter of 1-mm with a Wiley mill. Samples were analyzed in the Oklahoma State University Ruminant Nutrition Laboratory using near-infrared reflectance spectroscopy (NIRS DS2500 F, FOSS North America, Eden Prairie, MN). The NIRS spectrum data were analyzed using the NIRS Forage and Feed Testing Consortium (Berea, KY) calibration for legume hay. The NIRS Forage and Feed Testing Consortium (Berea, KY) calibration calculated forage nutritive values for crude protein content (CP), neutral detergent fibers (NDF), acid detergent lignin (ADL), and in vitro dry matter digestibility (IVDTMD).

Statistical Analysis

The sum of season-long aboveground biomass was calculated on a subplot basis, while quality factors (CP, NDF, ADL, and IVTDMD) were averaged per subplot across cuttings. Data were analyzed using a linear mixed model in the GLMMIX Procedure of SAS version 9.4 and compared using a two-way ANOVA. Harvest interval, cultivar, and interactions are considered fixed effects, while replication is considered a random effect. As site locations varied in environment, locations were analyzed and reported separately. Pairwise comparisons were made for significant effects. All tests were conducted at the nominal 0.05 level.

Results and Discussion

Weather Conditions

In March of 2020, average daily air temperatures were slightly greater (+0.9 and +1.3°C) than the 14-yr average values in Lahoma and Stillwater, respectively. This modest increase in air temperature might have been the cause of early alfalfa regrowth (breaking dormancy) observations in 2020. Growth chambers studies have shown that alfalfa leaf growth begins once air temperatures reach a threshold of 5^{o}C for most alfalfa cultivars (Sharratt, Sheaffer, & Baker, 1989; Undersander et al., 2011). Temperatures at or above 5°C in the CGP are typically seen in late February to early March, depending on the year (McPherson et al., 2007).

According to Al-Hamdani and Todd (1990), alfalfa growth is optimum at an air temperature of 21±5^{o}C, while alfalfa grown at temperatures >30 °C had less forage mass accumulation and flowered earlier. Higher temperatures tend to increase levels of photorespiration, decreasing photosynthesis and forage production (Al-Hamdani, 1990). In Lahoma, maximum air temperatures greater than 30 °C started to occur from early May and were observed until mid-September, implying that alfalfa stands were at suboptimal growth conditions for several hours on most days of the growing season. In Stillwater, maximum air temperatures greater than 30^{o}C were observed on some days during late March; however, similarly to Lahoma, they became recurring in early May only and persisted until mid-September. Furthermore, monthly average air temperatures in both locations were slightly lower than the 14-y average in all months of the growing season (April–October) except June (Figure 2.1a). The average air temperature in June in both locations was higher than the 14-y average, which may have increased water stress and reduced yields.

In Lahoma, 2020, rainfall was 54, 59, and 60 mm less than the 14-year average for April, May, and June, respectively (Figure 2.1). However, a large amount of rainfall (224 mm) occurred in July, which was 131mm greater than the 14-year average. Although the precipitation was well distributed throughout July, a high-intensity, short-interval rainfall (i.e., 58 mm of rainfall in a day) occurred in the last week of the month, resulting in saturated soils for a few days. Conversely, total rainfall during August (53mm) and September (33 mm) were 34 and 27 mm less than the 14-year average, respectively.

In Stillwater, 2020, experimental fields received 81, 51, and 45 mm less rainfall than the 14-year average for the months of April, May, and June, respectively (Figure 2.1b). Stillwater experienced high precipitation in July (116mm), which was 21mm greater than the 14-y average. Although the precipitation was well distributed throughout July, a high-intensity, short-interval rainfall (i.e., 60 mm of rainfall) occurred in the last week of the month, resulting in a ponded field. High-intensity rainfall, combined with high temperatures in July, resulted in alfalfa scalding. Scalding in alfalfa results in a decrease in stand population and/or delayed regrowth (Summers, 2006). Conversely, total rainfall during August (47mm) and September (58 mm) were 43 and 19mm less than the 14-year average, respectively.

Aboveground Biomass

In Lahoma, 28-d, 35-d, and 42-d harvest intervals had similar cumulative AGB in 2020 (Table 2.3). Although no significant differences were found among harvest intervals, AGB numerically increased when harvest intervals were delayed 7 (35-d) or 14 (42-d) days from the 28-d harvest interval (3% and 7%, respectively). Similar results were observed in Stillwater. No significant differences were found among harvest intervals, yet AGB numerically increased when harvest intervals were delayed 7 (35-d) or 14 (42-d) days from the 28-d harvest interval (7.5% and 19.5%, respectively, (Table 2.4).

This study's forage yield response to various harvest intervals diverts from first-year results from previous studies, where longer harvest intervals tended to statistically increase forage accumulation. For instance, Arnold et al. (2019) found statistical differences between harvest intervals when increasing from 28 to 38-d in the first production year when forage yield was averaged across six locations; however, no significant differences were reported in the second and third-year production. In another similar study, Grev et al. (2017) found statistical differences between harvest intervals that were delayed from 30 to 45-d when evaluated at three separate locations during the second year of production only (harvest intervals were not tested in the first year) (Grev et al., 2017). Putnam et al. (2005) reported increases in forage yield when harvest was delayed from 24-d to 34-d for three years of production. Interestingly, the largest difference between these harvest intervals was in the third year of production (Putnam et al., 2005).

Differences in harvest intervals within the parameters of this study may become more pronounced in future years, as exhibited in two of the above studies.

There were no AGB statistical differences among the tested alfalfa cultivars in Lahoma (Table 2.3). Numerical comparisons indicated that the reduced lignin alfalfa (54HVX41) produced the least AGB accumulation resulting in 11, 10, and 4% less AGB than 54VR10, WL 356HQ.RR, and DKA44-16RR, respectively (Table 2.3). The statistical results in Stillwater were similar to Lahoma: No AGB statistical differences among the tested alfalfa cultivars. However, numerical differences in AGB provided a contrasting narrative to Lahoma's findings. Despite 54HVX41 having the least production in Lahoma, 54VR10 produced the least AGB in Stillwater (Table 2.4). The biomass accumulation of 54VR10 was 16, 13, and 2% less than WL 356HQ.RR, DKA44-16RR, and 54HVX41, respectively (numerical inferences, only).

In one previous study, 54HVX41 produced less AGB than three conventional cultivars in the first production year at three of four locations in Minnesota, while in the second year it was found to only be different from reference cultivars in one location only (Grev et al., 2017). These findings suggest that AGB differences among cultivars might decrease as the age of the alfalfa stands increase. Arnold et al. (2019) also reported reduced lignin alfalfa to produce significantly less AGB in the first year of production when compared to two reference cultivars. Similar to Grev et al. (2017), the multi-location evaluation of Arnold et al. (2019) suggested that AGB's differences are reduced as the stand age increased. In the second year of production, the reduced lignin alfalfa cultivar produced the least biomass and was statistically different from only one cultivar. Yet, in the third final year, no differences in yield were found among cultivars. Based on previous studies, no statistical differences among reduced lignin and reference cultivars are expected in the subsequent production years of this study.

Crude Protein

In Lahoma, the three harvest intervals differed statistically in crude protein (CP), with the greatest CP content in the 28-d harvest interval (Table 2.3). Increasing harvest intervals from 28-d to 35-d and 42-d forfeited 1.8 and 2.9 percent points of CP content, respectively. Similar to Lahoma findings, both Grev et al. (2017) and Arnold et al. (2019) detected a decrease in CP content as days of regrowth increased (Arnold et al., 2019; Grev et al., 2017). In Stillwater, the CP content value at 28-d harvest interval was greater than that for both other harvest intervals, where were similar (Table 2.4). Increasing harvest intervals from 28-d to 35-d or 42-d decreased CP content by 2 percentage points in both cases. In other words, there were no CP content penalties when harvest intervals increased from 35-d to 42-d in Stillwater.

The overall CP results in both locations were as expected – the longer the harvest intervals, the lesser the CP content. Alfalfa forage is mainly composed of leaves and stems, and leaves contain a higher CP concentration. As alfalfa plants mature, leaf senescence occurs, causing leaves to detach from the lower portions of the plants. This phenomenon causes reductions in the leaf-to-stem ratio, which is commonly associated with lower CP contents (Ball et al., 2001). However, leaf loss is not the only factor responsible for reducing CP content in alfalfa in advanced developmental stages. The plant stems become more lignified as alfalfa matures, aggravating CP content reductions in later developmental stages (Ball et al., 2001).

Among the tested cultivars, significant differences in CP were only found in Lahoma, where the CP content of 54HVX41 was similar to 54VR10, and greater than DKA44-16RR and WL 356HQ.RR (Table 2.3). Cultivars DKA44-16RR, WL 356HQ.RR, and 54VR10 had similar CP contents. In Stillwater, no statistical differences in CP content among cultivars were found. (Table 2.4). Different authors have reported contrasting results when comparing CP content among reduced lignin with reference cultivars. Arnold et al. (2019) reported that reduced lignin alfalfa cultivars had the greatest CP content compared to both reference cultivars when averaged across six locations for two years. However, no differences among cultivars were reported in two of three locations in another study including reduced lignin alfalfa (Grev et al., 2017). The authors proposed no explanation or assumptions other than the dilution effect to explain these variations on their results. The CP concentration in reduced lignin alfalfa will increase proportionally to the reduction of the lignin content; therefore, the changes in CP might be explained by the variation in ADL contents among cultivars.

Neutral Detergent Fiber

Neutral detergent fiber (NDF) differed among all three intervals in Lahoma, with 42-d resulted in the greatest and 28-d resulting in the least concentration of NDF (Table 2.3). Neutral detergent fiber content increased by 3.9 when delaying from 28-d to 35-d. Similarly the delay from 28-d to 42-d increased in NDF by 5.8 percent points. In Stillwater, 42-d harvest interval resulted in the greatest NDF concentration, while 28-d harvest interval had the least (Table 2.4). Both 28-d and 42-d harvest intervals were similar to 35-d harvest interval NDF content, but they were statistically different from each other. Increases in NDF content as harvest intervals increased were expected and previously reported. Greater NDF content is due to increased indigestible fibers and components, like cellulose, hemicellulose, and lignin, as days of regrowth increased and the plant matured (Arnold et al., 2019; Putnam et al., 2005; Sulc et al., 2016).

In Lahoma, 54VR10 had the greater NDF content than; WL356HQ.RR and 54HVX41, while the NDF content of DKA44-16RR was similar to all tested cultivars. Although the NDF of 54HVX41 (i.e., reduced lignin alfalfa) was not statically different from DKA44-16RR and WL356HQ.RR, its NDF content was 1.2 and 1.0 percent points less, respectively. In Stillwater, NDF content was not statistically different among cultivars. Even though the reduced lignin alfalfa also had the least numerical NDF content, its difference was negligible, i.e., 0.3 percent points less than all cultivars (Table 2.4).

These contrasting findings also occurred when comparing results from different studies. The reported NDF content in Stillwater was similar to the NDF content results observed by Grev et al. (2017), who reported that lower NDF content among cultivars was uncommon -– in one location out of four. On the other hand, numerical Lahoma trends were similar to the results of Arnold et al. (2019), where the reduced lignin cultivar was statistically lower than the two reference cultivars. The numerical differences in NDF reported by Arnold et al. (2019) were in the same magnitude of those measured in Lahoma; their statistical differences were attributed to larger sample size because the statistical analysis was averaged across 12 site-years replications (i.e., the number of observations).

Acid Detergent Lignin

Acid detergent lignin content was statistically different among all three harvest intervals in Lahoma (Table 2.3): Increasing the 28-d harvest interval by 7 days (35-d harvest interval) resulted in a 0.7 percent point increase in ADL content. Delaying by 14 days (42-d harvest interval) resulted in a 1.3 percent points increase in ADL content. In Stillwater, ADL results followed the same trend as Lahoma. Increasing the 28-d harvest interval by 7 days (35-d harvest interval) resulted in a 0.2 percent point increase in ADL content. Delaying by 14 days (42-d harvest interval) resulted in a 0.8 percent points increase in ADL content. However, in Stillwater, ADL content differences were significantly different between 28 and 42-d harvest intervals only.

The greatest amounts of lignin found in the 42-d harvest intervals were previously reported by Grev et al. (2017) and Arnold et al. (2019). Unsurprisingly, the least amount of ADL was found in the 28-d harvest interval, as it had the least regrowth and time to accumulate lignin in the field. Periods of greater regrowth cumulates greater amounts of lignin. Lignin is a polymer that occurs as plants mature, as it is needed for structural integrity and aids in water movement in plants (Moore & Jung, 2001).

In Lahoma, there were statistical differences in ADL among cultivars. 54HVX41 had significantly less ADL content than all other cultivars, which did not differ statistically from each other. Statistical differences between reduced lignin and reference cultivars were consistent with previous studies (Arnold et al., 2019; Grev et al., 2017). While Grev et al. (2017) and Arnold et al. (2019) found ADL of reduced lignin alfalfa cultivars 6 to 8 percentage points less than reference cultivars, ADL was only reduced by 0.3 to 0.5 percentage points in Lahoma.

Despite the large differences in ADL measured in other studies, there were no statistical differences among cultivars in Stillwater (Table 2.4). Previous studies suggest that conventional cultivars can experience a decrease in lignin accumulation when experiencing drought stress, which might partially explain the results obtained in Stillwater (Vough & Marten, 1971). We note, however, that the effects of drought stress in the ADL accumulation of reduced lignin alfalfa cultivars has not been previously reported yet. Alfalfa drought stress symptoms, such as decreased plant growth and early flowering was observed in Stillwater. Thus, drought might be the reason for all cultivars having lower ADL contents in Stillwater when compared to Lahoma's ADL content values (numerical inferences only). Furthermore, we speculate that drought conditions might have a greater influence in reducing ADL content in reference than in reduced lignin cultivars. This greater drought-induced ADL reduction in reference alfalfas might have leveled the ADL contents to values similar to the reduced lignin alfalfa, which resulted in no ADL significant differences among cultivars in Stillwater. However, our results were limited to the first year of production. Second- and third-year results may reinforce or negate the proposed speculation. Lignin tends to increase with the age of the stand. Grev et al. (2019) reported increases in ADL across all three locations between their first and second years of production

(Grev et al., 2017). Thus, differences among alfalfa cultivars' ADL content may become more prominent in future years of this study. If ADL content of conventional alfalfa cultivars continue to level with the reduced lignin alfalfa values in subsequent years, the benefits of reduced lignin alfalfa in water-limiting environments can be questioned.

In Vitro Dry Matter Digestibility 48 Hours

In vitro dry matter digestibility (IVTDMD) is the measurement of digestibility of a feed source after 48 hours of ruminal fermentation followed by 48 hours of digestion with acid and pepsin (Jung et al., 1997). In vitro dry matter digestibility is negatively related to ADL concentrations, as lignin content determines the digestibility of the forage. This inverse relationship to ADL was observed in IVTDMD results. In Lahoma, IVTDMD values statistically decreased at longer harvest intervals. The IVTDMD at 35 and 48-d harvest intervals were 2.1 and 3.9 percent units lesser than 28-d harvest interval, respectively. In Stillwater, the IVTDMD content at 42-d harvest interval was the greatest and statistically differed from 28 and 35-d harvest intervals. Despite the lack of statistical differences, there was a 0.6 percentage point increase in IVTDMD content from 35 to 42-d harvest intervals.

Differences among cultivars were only found in Lahoma. The cultivar 54HVX41 had the greatest IVTDMD content as compared to all other cultivars, which all had similar IVTDMD contents. The lack of statistical difference in IVTDMD in Stillwater is related to its similar ADL contents among cultivars (Table 2.4).

Conclusion

No statistical differences in AGB were observed in both locations among harvest intervals and cultivars. Overall, CP, NDF content, and IVTDMD results varied according to the ADL content changes in cultivars and harvest intervals. Crude protein tended to increase as ADL content decreased; this correlation was justified by the dilution effect, as crude protein is diluted with cell solutes. Conversely, NDF and IVTDMD tended to decrease as ADL decreased. Acid detergent lignin is a proxy for lignin content, which is a fraction of NDF; consequently, the lesser the ADL content, the lesser the NDF content. Also, lignin is indigestible by livestock, directly reducing IVTDMD values.

Acid detergent lignin content increased as harvest interval length increased in both locations. However, the ADL content changes among cultivars followed different trends between locations. In Lahoma, the reduced lignin alfalfa had less ADL content than all reference cultivars; notwithstanding, no significant differences in ADL content were observed in Stillwater. Previous studies indicated that conventional alfalfa might cumulate less lignin in drought conditions, as experienced in Stillwater during the study. We speculate that reference cultivars may reduce their lignin content to values similar to the reduced lignin alfalfa during drought conditions, which are commonly recurring in the CGP. However, the study must be conducted for subsequent years to validate our speculation.

CHAPTER III

DEVELOPING AN ALFALFA YIELD PREDICTION MODEL FOR CENTRAL OKLAHOMA

Abstract

The development of alfalfa decision-making tools, such as alfalfa yield predicting models, would allow producers to make management decisions that best suit their hay and cattle enterprises. Previous complex process-oriented models used for predicting alfalfa yield have been developed for other geographical regions yet are not producer-friendly. The objectives of this study were (i) to evaluate plant height (PH), grazing height (GH), compressed canopy height (CCH), green canopy cover (GCC), and growing degree days (GDD) as variables of alfalfa aboveground biomass (AGB) accumulation for alfalfa cultivars commonly cultivated in central Oklahoma, and (ii) to develop a producer-friendly alfalfa prediction model based on the best suited evaluated variables. Plant height, GH, CCH were well correlated to AGB. However, only GH was selected as predictive variable because it had the greatest correlation to AGB ($r = 0.89$), and PH and CCH were eliminated. Green canopy cover less than 60% showed fair correlation to AGB, however, greater GCC resulted in no correlation. Although GDD showed a fair correlation with AGB, it was incorporated to measure morphology and harvest timing. However, through statistical analysis, adding GDD as an additional predictor resulted in sixteen distinct models. Improvements in the GDD calculations, such as adding a cap temperature of 30°C, could improve the correlation to AGB, which may simplify and increase model accuracy. Model improvement

attempts will be performed with further analysis of GCC and improved GDD calculations, along with a second site-year dataset.

Introduction

Alfalfa is frequently referred to as a water-spending plant, an undesirable trait in the water-limited environment of the central Great Plains (CGP) (Asseng & Hsiao, 2000). The CGP, which includes central Oklahoma, is predicted to experience a shift in rainfall patterns and experience prolonged droughts (Campbell, Becerra, Middendorf, & Tomlinson, 2019). With a changing environment, producers will search for new technologies to mitigate forage production losses in this area. Alfalfa is a commonly adopted forage crop in central Oklahoma (USDA-NASS, 2018). Hence, the development of alfalfa decision-making tools, such as alfalfa yield predicting models, would allow producers to make management decisions that best suit their hay and cattle enterprises.

Most of the existing alfalfa yield prediction models are complex process-oriented algorithms that have been developed for geographical regions including Canada, Iran, Italy, and Spain (Confalonieri & Bechini, 2004; Jing et al., 2020; Malik, Boote, Hoogenboom, Cavero, & Dechmi, 2018; Soltani et al., 2020). Efforts to adapt and validate these models to predict alfalfa growth in the U.S. were somewhat successful (Feng et al., 2020; Griggs & Stringer, 1988; Zhou et al., 2021). These models have fair accuracy in predicting alfalfa forage yield at large scales, including entire counties and ecoregions. Despite their potential use for yield prediction, these models rely on many complex inputs, including detailed soil physical and chemical parameters, initial soil water content, solar radiation, specific cultivar leaf area dynamics, photosynthesis rate, maximum root depth, nutrient uptake rate, etc. These inputs, while achievable via research, are not typically available to producers. Other alfalfa modeling approaches focusing on commercial use were also developed. These models rely on new technologies, such as UAV and satellite imagery, or demand plant herbage destructive sampling (Feng et al., 2020; Griggs & Stringer, 1988; Zhou et al., 2021). Although those models are more producer-friendly than complex process-oriented algorithms, they demand substantial financial investment and labor use. Thus, few producers might be willing to adopt them in their enterprises.

Predictive models that rely on fast, non-destructive measurements and ordinary weather data may achieve higher producer adoption. Multiple forms of vertical growth, including plant height (PH), grazing height (GH), and compressed canopy height (CCH) demonstrated a positive correlation to alfalfa forage yield and are producer-accessible measurements (Alla, Bakheit, Abo-Elwafa, & El-Nahrawy, 2013). Furthermore, a past study using alfalfa green canopy cover (GCC) values indicated a positive relationship between year-round GCC and forage yield, suggesting GCC may provide valuable contributions to an AGB predicting model (Jáuregui, Delbino, Bonvini, & Berhongaray, 2019). Historically, growing degree days (GDD) are used to estimate the morphological development of alfalfa, not forage yield (Sanderson, Karnezos, & Matches, 1994). However, morphological development is closely related to optimal harvest timing for many producers, as it is a predictor of forage quality (Sanderson et al., 1994).

Therefore, the objectives of this study were (i) to evaluate PH, GH, CCH, GCC, and GDD as predictive variables of alfalfa AGB accumulation for alfalfa cultivars commonly cultivated in central Oklahoma, and (ii) to develop a producer-friendly alfalfa prediction model based on the best suited evaluated variables.

Material and Methods

Site Description

A study was established in the fall of 2019 and conducted for one alfalfa production season (from April to September of 2020) at Oklahoma State University Agronomy Research Station, Stillwater, OK (36°07’02” N 97°05’53” W). The soil was an Easpur loam, 0 to 1% slopes, occasionally flooded (fine-loamy, mixed, superactive, thermic Fluventic Haplustolls) (NRCS, 2008). The site was fallow in 2017 and sown to winter wheat in the fall of 2018, which was terminated in May of 2019.

Cultural Practices and Experimental Design

In July 2019, the experimental field was sprayed with 1.14 kg ha^{-1} glyphosate (Roundup PowerMAX®, Monsanto, St. Louis, MO) and 0.73 kg ha^{-1} 2,4-D (Weedone® LV4 Solventless, Nufarm, Alsip, IL) to terminate standing weeds. Soil samples were collected to a depth of 15 cm from the site location prior to the experiment's establishment in 2019. Initial soil chemical and physical conditions of the field are presented in Table 3.1. Soil analysis results indicated adequate pH levels and appropriate amounts of macro and micro-nutrients; therefore, no soil amendments were required before alfalfa establishment (Zhang & Henderson, 2016).

A seedbed was established in mid-September of 2019. First, the site was disked with an offset disk (John Deere 225 offset disk, John Deere Manufacturing, Moline, IL) to a depth of 12.5 cm, followed by a spring tooth harrow (John Deere Manufacturing, Moline, IL) at a depth of 7.5 cm. Then, a drag harrow was used to smooth out and firm up the seedbed.

Alfalfa plots were established on 1 October 2019. On the planting day, a box drill seeder (Brillion SSP-108, Brillion Iron Works, Brillion, WI) was run across the site to ensure the seedbed was firm to maintain seeds at the top 2 cm of the soil; then, the alfalfa seeds were planted with the same seeder. Based on recommendations for Oklahoma alfalfa production, 16.8 kg ha^{-1} pure live seed was planted to achieve 450 plants m^{-2} (Caddel, 2001). The trial was established in a randomized complete block design with three replications of four alfalfa cultivars (DKA44-16RR, WL 356HQ.RR, 54VR10, and 54HVX41), where each individual plot measured 20 m by 20 m.

To ensure proper alfalfa growth during the spring, weeds were controlled on March 3, 2020 (154 days after planting) with 1.14 kg ha^{-1} glyphosate (Roundup PowerMAX®, Monsanto, St. Louis, MO). All evaluated alfalfa cultivars were Roundup Ready®. In addition, blister beetles (*Epicauta vittata*) were managed with carbaryl (Sevin® SL Carbaryl, Bayer Environmental Science, Cary, NC) at the rate of 1.00 kg ha^{-1} in experimental plots on June 10, 2020 (253 days after planting) and August 24, 2020 (328 days after planting).

Cultivars

The four cultivars chosen for this experiment were Roundup Ready®. A brief description of each cultivar is provided below.

'DKA44-16RR' (DEKALB, Bayer Group, St. Louis, MO; Roundup Ready, Monsanto Technology LLC., St. Louis, MO) is known for having high yield, quality potential, and adaptability to most environments. 'DKA44-16RR'is a common cultivar choice in Oklahoma, as it has a fall dormancy of 4.4 and winter hardiness of 1.6. In addition, 'DKA44-16RR' is resistant to several diseases and pests, including multiple wilts, root rots, nematodes, and aphids (Bayer, 2019).

‘WL 356HQ.RR’ (W-L Research, Inc., Madison, WI; Roundup Ready, Monsanto Technology LLC., St. Louis, MO) is a commonly used Oklahoma alfalfa cultivar with high yield potential. Multiple harvest managements ranging from three to five annual cuttings are suitable for this variety and make it ideal for this study. This alfalfa cultivar also has the genetic potential for a high-quality crop and the glyphosate-resistant trait. This cultivar has a fall dormancy and winter-hardiness of 3.8 and 1.6, respectively. ‘WL 356HQ.RR’ provides resistance traits to multiple wilts, root rots, nematodes, and aphids (Monsanto, 2020).

‘54VR10’ (Pioneer, Corteva Agriscience, Johnston, IA; Roundup Ready, Monsanto Technology LLC., St. Louis, MO) contains many traits that make it a successful cultivar throughout much of the US, including Oklahoma. ‘54VR10’ is like the two previous cultivars, as it is also a fall dormancy of 4 and has optimal winter hardiness. ‘54VR10’ is unique in the fact that it survives in both well and poorly-drained soils. Resistance from multiple diseases and insects makes ‘54VR10’ an optimal selection for an alfalfa crop (Pioneer, 2019b).

‘54HVX41’ (Pioneer, Corteva Agriscience, Johnston, IA; Roundup Ready, Monsanto Technology LLC., St. Louis, MO), is a reduced lignin alfalfa cultivar – HarvXtra® technology (Forage Genetics International, LLC, Land O’Lakes, Nampa, ID). This technology allows harvest delays while maintaining forage quality. This reduced lignin cultivar is genetically similar to the previous three cultivars, except for the down-regulation of the hydroxycinnamoyl CoA: shikimate hydroxycinnamoyl transferase (HCT) gene. It has resistance to pests and diseases. ‘54HVX41’ is a cultivar with optimum relative forage quality, thanks to its low lignin content (Grev et al., 2017; Pioneer, 2019a).

Data Collection

Weather Data

Weather data were recorded by an automated weather station less than 1 km from the study site (www.mesonet.org) (McPherson et al., 2007). Daily maximum and minimum air temperatures were obtained to calculate GDD during the alfalfa growing season (months of March to September). Growing degree days calculation started after five consecutive days above base temperature (5°C). Then, GDD were calculated by using the base temperature of 5°C (Eq. 1) (Sanderson et al., 1994). Any negative GDD values during the early growing season were considered zero. Cumulative GDD were calculated for all 42 days of regrowth and reset after each harvest.

$$\sum_{d=1}^{42} GDD_d = \frac{Tmax+Tmin}{2} - 5°C \quad \text{[Eq. 1]}$$

Where GDD is the cumulative growing degree days, *d* is days of regrowth, T_{max} is the maximum air temperature of a specific *d*, and T_{min} is the minimum air temperature of a specific *d*.

Destructive Aboveground Biomass Measurements

Non-destructive measurements and destructive aboveground biomass (AGB) samples were taken weekly during the growing season of 2020 (from April to September) in five randomly selected areas of 38 cm by 38 cm (0.14 m^2) in each plot. In each select area, a 0.14 m^2 quadrat was randomly placed on the ground, and non-destructive measurements, such as GCC and vertical growing factors (PH, GH, and CCH) were successively recorded in the center of the quadrat, as described in the next section. Then, all the herbage within the quadrat was clipped at ground level using handheld electric Gardena shears (Gardena 8885-U 3-Inch Cordless Lithium-Ion Grass Shears, Gardena, Ulm, Germany). The clipped alfalfa herbage within each quadrat was dried in a forced-air oven at 55°C until constant weight (a minimum of seven days). Dry herbage samples were then weighed and converted to AGB per hectare. The clipped area was flagged to avoid double sampling before alfalfa harvest. Alfalfa herbage was harvested every 42 days with a modified Carter Harvester (Carter Manufacturing Company, Brookston, IN) with an accumulator on the back, allowing forage to be removed from the experimental field. This allowed for regrowth throughout the production season of April to September. Thus, a total of four harvests were performed during the season. The harvest dates were May 18, June 29, August 10, and September 21.

Non-destructive Aboveground Biomass Measurements

Plant height was measured at the highest point of the alfalfa shoot or leaf. Grazing height is defined as the average plant height of all alfalfa shoots within a given area, which was measured when alfalfa shoots form modest resistance when pressed against the hand to assure the hand touched half of the shoots (Rocateli, 2016). Compressed canopy heights (CCH) were measured using a rising plate meter (RPM) (RPM, EC09, Jenquip, Feilding, New Zealand). The RPM was placed vertically at the center of each sampling area, allowing the plate to rest at the height at which the force of the canopy is equal to the force of the plate (Harmoney, Moore, George, Brummer, & Russell, 1997; Jenquip, 2018).

Green canopy cover of each sampling area was estimated by taking a downward-facing (nadir) photograph from a 1.5 m height parallel to the ground using a 12-megapixel camera (iPhone 8, Apple Inc., Cupertino, CA). Pictures were precisely cropped at the edges of the 0.14-m^2 quadrat using Adobe Photoshop CC 2018 (Adobe Inc., San Jose, CA). Then, the green canopy cover of each picture was calculated using the Canopeo Matlab app (https://canopeoapp.com). The Canopeo app measures the percent GCC from a digital photo, using color values in the red-green-blue system to analyze and classify all pixels (Patrignani & Ochsner, 2015). To best evaluate alfalfa GCC, the preset of red to green and blue to green ratios at 0.95 and the minimum excess green set at 20 was used (Patrignani, 2020).

Statistical Analysis

Growing degree days, vertical growing factors (PH, GH, CCH), and GCC were all evaluated for predictive purposes of dry matter using a Tukey HSD pairwise correlation in the GLM Procedure of SAS version 9.4. Through pairwise correlation of all five independent variables, grazing height and growing degree days were selected as the best candidates of covariates to predict yield. Grazing height of subsample replications were averaged weekly for all four cultivars. Measurements of grazing height and growing degree days were analyzed using a linear mixed model for four repeated measurements (identified by harvest) in the GLMMIX Procedure of SAS version 9.4. Harvest, cultivar, and their interactions were considered fixed effects, while week was considered random effects. Pairwise comparisons were made for significant effects. All tests were conducted at the nominal 0.05 level.

Results and Discussion

Independent Variable Selection

Vertical Growth Factors

Grazing height was the vertical growth factor with the greatest correlation (0.86) with AGB (Table 3.2) followed by PH (0.83) and CCH (0.76). Grazing height and PH had the highest correlation with AGB, while CCH was considered followed close behind the other two vertical growth factors (Table 3.2). Thus, GH was selected within the vertical growth factors to develop the alfalfa yield prediction model. Although PH showed 0.83 correlation with AGB, it was not selected to be included in the model. Correlation between the GH and PH variables was 0.90, with this co-linearity precluding the need to evaluate both variables independently (Table 3.2). Consequently, including PH as an additional variable would increase complexity without providing any additional accuracy to the model.

Although CCH is primarily a vertical growth variable, it also accounts for crop density (Haultain, Wigley, & Lee, 2014; Jenquip, 2018). In other words, at a given GH, the greater the number of stems and leaves (crop density), the higher the CCH reading. Thus, GH and CCH were correlated at 0.75 (Table 3.2) when considering the whole 42-day intervals. However, CCH correlation with AGB seems to be high at initial alfalfa regrowth and tend to decrease as regrowth increases as illustrated by (Figure 3.1 C, visual inferences only). This decrease in correlation might be because the adopted RPM was primarily developed to estimate ryegrass and wheat AGB instead of alfalfa (Haultain et al., 2014). Consequently, its plate was too heavy and its shaft too short to provide accurate CCH during the entire alfalfa regrowth periods. Therefore, due to the adopted rise plate meter limitations, it was deemed that CCH readings would not provide additional value to best predict yield throughout the entirety of the regrowth period.

Green Canopy Cover

Green canopy cover and AGB had a correlation value of 0.64 (Table 3.2). We note that GCC values greater than 60% no longer provided a precise estimate of AGB (Figure 3.2 B, visual inferences only). In preliminary analysis via a stepwise regression, GCC was eliminated from the model at the first step ($p > 0.15$), given that other regressors, such as GDD and GH, were more complimentary estimators to predict AGB for the tested cultivars and harvests.

The Canopeo app uses the red-green-blue system to calculate GCC, where the users can preset the thresholds that best suits their target plant (Patrignani & Ochsner, 2015). Pixels are then classified using red/green and blue/green ratios, allowing the percent of green canopy cover to be identified (Patrignani & Ochsner, 2015). Unfortunately, this causes an underestimate of canopy cover at high values of alfalfa AGB, as alfalfa begins to produce purple flowers during reproductive stages, which is not detected as GCC. Thus, an increase in GCC can be seen until alfalfa reaches anthesis, then GCC plateaus or decreases (Figure 3.3). Another weakness in the use of GCC as a proxy for forage yield is that alfalfa ground cover becomes constant in the mid vegetative stage period, while yield and height continuously increase. Alfalfa is commonly referred to as a forage crop that does not compensate for open areas because of its predominantly vertical growth (Volenec, Cherney, & Johnson, 1987).

Growing Degree Days

The correlation coefficient between GDD and AGB was the lowest (0.41) (Table 3.2). This finding is consistent with the fact GDD is mostly used as a predictor of morphological development instead of forage yield in plant growth models. Yet, the inclusion of GDD in alfalfa models could allow the estimation of plant maturity and harvest timing, which were beyond the scope of this study.

Growing degree days is a proxy for solar radiation, which translates into photosynthetic activity and energy production by the plant (Grigorieva, Matzarakis, & De Freitas, 2010). Alfalfa plants invest energy in developing high amounts of stems and leaves during the vegetative stage. Then, the plant shifts its energy use to develop flowers, seeds, and roots. Although flowers and seeds are accounted as forage yield, they have negligible contributions.

Furthermore, alfalfa experiences summer slump, which reduces AGB accumulation due to high temperature and drought (Ottman & Mostafa, 2014; D. H. Putnam & Ottman, 2013). In the central Great Plains, alfalfa is prone to summer slump from mid-July, drastically reducing AGB accumulation at later cuts (Ottman & Mostafa, 2014; D. H. Putnam & Ottman, 2013). The relationship between temperature and alfalfa development that GDD reflects does not account for relevant factors such as photoperiodism and plant-soil water relations (Sharratt, Sheaffer, & Baker, 1989). Without accounting for these factors, GDD may not accurately predict morphological development and AGB accumulation under suboptimal growing conditions. Thus, high AGB accumulation was observed at low GDD values during spring and early summer, when air temperatures and water availability were optimum. Conversely, low AGB accumulation was observed at high GDD values during late summer and early fall, when high air temperatures and low soil water availability restricted alfalfa growth (i.e., alfalfa slump) (Figure 3.4).

Alfalfa Yield Predicting Models

No statistical differences in predictive AGB values were found when harvest effect ($p = 0.29$) was evaluated as a predictive variable for AGB. Conversely, the cultivar effect was significant ($p = 0.04$), indicating that the four tested cultivars needed to be separated into four single predictive models to best predict AGB based on GH. This finding implied that the correlation between GH and AGB is cultivar-dependent. Furthermore, the interaction between both main effects was not significant ($p = 0.75$).

With the addition of GDD as a second predictive variable for each previously proposed model, the harvest × cultivar effect became significant ($p < 0.01$). Finally, for best alfalfa AGB prediction, an equation for every harvest × cultivar effect was proposed. The intercepts and slopes for all equations are listed in Table 3.3. Among all interactions, 9 out of 16 were significant (Table 3.3). Thus, different intercepts and slopes from the full model (i.e., all harvest and cultivars combined) are needed to predict these nine interactions best. An interesting finding was that DKA44-16RR slopes and intercepts for all harvest × cultivar effects were not significant. The reason for this finding is that AGB was less variable among different harvests for this cultivar (data not shown). Thus, DKA44-16RR might be well suited for the adverse conditions during the summer in central Oklahoma because it appears that this cultivar could maintain consistent forage production across a wide range of GDD values. On the other hand, the other cultivars experienced a reduction in AGB accumulation at higher GDD late in the season (i.e., summer forage slump).

Conclusion

Grazing height was the primary independent AGB predictor due, however, GH versus AGB relationship was cultivar dependent which may restrict its widespread use. The other tested vertical growth variables (PH and CCH) showed (0.90 and 0.83, respectively) correlation as well. However, they were not included in the model because they did not improve model accuracy due to their co-linearity with GH. Green canopy cover showed correlation with AGB until 60% value was achieved, then this variable was uncorrelated to AGB. Although this GCC variable was excluded from the final proposed models, further statistical analysis will be performed in the future as an attempt to include this predictor.

In order to incorporate a measurement of morphology and harvest timing, GDD were included in the model. However, through statistical analysis, adding GDD as an additional predictor, resulted in multiple models. Improvements in the GDD calculations, such as the addition of a cap temperature of 30°C, could improve the correlation of GDD with AGB. This improvement might result in a simplified model with higher accuracy. Finally, model improvement attempts will be performed in the future with further GCC analysis and improved GDD calculations along with a second site-year dataset.

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Table 1.1. Alfalfa Quality Parameters adapted from USDA Hay Quality Designation Guidelines

Quality	ADF	NDF	RFV	TDN	CP
Supreme	<27	>34	>185	>62	>22
Premium	27-29	34-36	170-185	60.5-62	20-22
Good	29-32	36-40	150-170	58-60	18-20
Fair	32-35	40-44	130-150	56-58	16-18
Utility	>35	>44	<130	<56	<16

Table 2.1. Soil analysis results after the addition of effective calcium carbonate lime and on day of experiment plot establishment.

Site	**Replication**	**Depth cm**	**pH**	**P (kg/ha)**	**K (kg/ha)**	**OM (%)**	**Sand (%)**	**Silt (%)**	**Clay (%)**
Lahoma	1	0-7.5	6.1	303	1110	2.18	35.0	51.3	13.8
		7.5-15	6.2	252	1165	1.69	35.0	48.8	16.3
	2	0-7.5	6.5	290	961	2.31	35.0	50.0	15.0
		7.5-15	6.7	216	911	1.79	37.5	46.3	16.3
	3	0-7.5	5.8	380	1033	2.03	40.0	45.0	15.0
		7.5-15	5.9	351	1018	1.68	37.5	45.0	17.5
	4	0-7.5	6.0	53	527	1.19	37.5	50.0	12.5
		7.5-15	5.9	27	375	1.06	37.5	47.5	15.0
	Average		6.1	234	888	1.74	36.9	48.0	15.2
Stillwater	1	0-7.5	6.9	177	382	1.33	32.5	47.5	20.0
		7.5-15	7.2	121	326	1.16	28.8	47.5	23.8
	2	0-7.5	7	155	356	1.61	27.5	50.0	22.5
		7.5-15	7.1	68	332	1.25	27.5	47.5	25.0
	3	0-7.5	6.7	177	397	1.52	31.3	46.3	22.5
		7.5-15	6.9	80	332	1.33	30.0	47.5	22.5
	4	0-7.5	6.8	215	396	1.42	35.0	45.0	20.0
		7.5-15	6.8	127	336	1.14	30.0	47.5	22.5
	Average		6.9	140	357	1.35	30.3	47.4	22.4

Table 2.2. 2020 harvest dates for all three harvest intervals (28, 35, 42-days) for both locations.

Location	28 Day Harvest Dates	35 Day Harvest Dates	42 Day Harvest Dates
Stillwater	May 4, 2020	May 11, 2020	May 18, 2020
	June 1, 2020	June 15, 2020	June 29, 2020
	June 29, 2020	July 20, 2020	August 10, 2020
	July 27, 2020	August 24, 2020	September 21, 2020
	August 24, 2020		
Lahoma	May 5, 2020	May 14, 2020	May 19, 2020
	June 2, 2020	June 18, 2020	June 30, 2020
	June 30, 2020	July 23, 2020	August 11, 2020
	July 31, 2020	August 26, 2020	September 23, 2020
	August 26, 2020		

Table 2.3. Cumulative aboveground biomass (AGB), crude protein (CP), neutral detergent fiber (NDF), acid detergent lignin (ADL), in vitro dry matter digestibly 48 hours (IVTDMD) collected in Lahoma, OK in 2020.

	2020				
Lahoma, OK	**AGB**	**CP**	**NDF**	**ADL**	**IVTDMD**
Effects: **Harvest Interval**	Mg ha^{-1}	%	%	%	%
28-days	18.9	22.9a	33.1c	6.3c	84.8a
35-days	19.5	21.1b	37.0b	7.0b	82.7b
42-days	20.3	20.0c	38.9a	7.6a	80.9c
Cultivar					
54HVX41	18.4	21.9a	35.2b	6.7b	84.1a
DKA44-16RR	19.2	20.8b	36.4ab	7.1a	82.3b
54VR10	20.5	21.4ab	37.6a	7.2a	82.1b
WL 356HQ.RR	20.2	21.2b	36.2b	7.0a	82.6b
Type 3 test of Fixed Effects:	**p-value**	**p-value**	**p-value**	**p-value**	**p-value**
Harvest Interval	0.5233	0.0003*	<0.0001*	<0.0001*	0.0002*
Cultivar	0.2671	0.0083*	0.0004*	0.0007*	0.0003*
Harvest Interval x Cultivar	0.7210	0.6175	0.7868	0.7361	0.7615

*significant at 95% confidence interval

[a,b,c,] means within a column and year without a common letter differ at the P-value listed for Harvest Interval and Cultivar effects.

Table 2.4. Cumulative aboveground biomass (AGB), crude protein (CP), neutral detergent fiber (NDF), acid detergent lignin (ADL), in vitro dry matter digestibly 48 hours (IVTDMD) collected in Stillwater, OK in 2020.

	2020				
Stillwater, OK	**AGB**	**CP**	**NDF**	**ADL**	**IVTDMD**
Effects: **Harvest Interval**	Mg ha^{-1}	%	%	%	%
28-days	13.3	24.1a	30.7b	6.1b	84.9a
35-days	14.3	22.1b	32.7ab	6.3b	84.3a
42-days	15.9	22.1b	34.5a	6.9a	82.0b
Cultivar					
54HVX41	13.7	22.9	32.4	6.4	84.3
DKA44-16RR	15.2	22.7	32.7	6.4	83.6
54VR10	13.4	22.6	32.7	6.5	83.6
WL 356HQ.RR	15.5	22.8	32.7	6.4	83.4
Type 3 test of Fixed Effects:	**p-value**	**p-value**	**p-value**	**p-value**	**p-value**
Harvest Interval	0.3169	0.0190*	0.0033*	0.0006*	0.0006*
Cultivar	0.3003	0.4254	0.9708	0.6683	0.1073
Harvest Interval x Cultivar	0.4433	0.2187	0.0916	0.0817	0.1504

*significant at 95% confidence interval

[a,b,c,] means within a column and year without a common letter differ at the P-value listed for Harvest Interval and Cultivar effects.

Table 3.1. Soil test analysis results of samples collected on the day of alfalfa experiment plot establishment at Stillwater, OK.

Site	Replication	Depth cm	pH	P (kg/ha)	K (kg/ha)	OM (%)	Sand (%)	Silt (%)	Clay (%)
Stillwater	1	0-7.5	6.9	177	382	1.33	32.5	47.5	20.0
		7.5-15	7.2	121	326	1.16	28.8	47.5	23.8
	2	0-7.5	7	155	356	1.61	27.5	50.0	22.5
		7.5-15	7.1	68	332	1.25	27.5	47.5	25.0
	3	0-7.5	6.7	177	397	1.52	31.3	46.3	22.5
		7.5-15	6.9	80	332	1.33	30.0	47.5	22.5
	4	0-7.5	6.8	215	396	1.42	35.0	45.0	20.0
		7.5-15	6.8	127	336	1.14	30.0	47.5	22.5
	Average		6.9	140	357	1.35	30.3	47.4	22.4

Table 3.2. Correlation coefficients of growing degree days (GDD), plant height (PH), grazing heights (GH), compressed canopy height (CCH), and green canopy cover (GCC) in relationship to biomass. Correlation among the independent variables in relation to one another is shown. All correlations were significant at $\alpha = 0.05$.

	Correlation Coefficients					
Variable	**AGB**	**GDD**	**PH**	**GH**	**CCH**	**GCC**
AGB	1.00	0.41	0.83	0.86	0.76	0.64
GDD		1.00	0.51	0.34	0.24	0.25
PH		-	1.00	0.90	0.67	0.58
GH		-	-	1.00	0.75	0.64
CCH		-	-	-	1.00	0.58
GCC						1.00

Table 3.3. Aboveground biomass yield prediction intercept and slope estimates for all models for each cultivar-harvest combinations.

Cultivar	Harvest	Intercept	GH Slope	GDD Slope	p-value
DKA44-16RR	1	2,253	473	0.6601	0.3806
DKA44-16RR	2	2,253	-132	0.4146	0.1482
DKA44-16RR	3	2,253	70	0.2894	0.3286
DKA44-16RR	4	2,253	0	0.1719	0.4196
54HVX41	1	1,612	-1144	2.3425	0.0007*
54HVX41	2	1,612	-220	1.0200	0.0009*
54HVX41	3	1,612	175	0.4500	0.1734
54HVX41	4	1,612	0	0.5433	0.0092*
54VR10	1	1,978	-301	1.9672	0.0046*
54VR10	2	1,978	-78	1.3565	<0.0001*
54VR10	3	1,978	703	0.1152	0.7259
54VR10	4	1,978	0	0.7591	0.0004*
WL 356HQ.RR	1	1,995	0	1.4774	0.0512
WL 356HQ.RR	2	1,995	0	1.1431	<0.0001*
WL 356HQ.RR	3	1,995	0	0.7433	0.0082*
WL 356HQ.RR	4	1,995	0	0.5721	<0.0001*

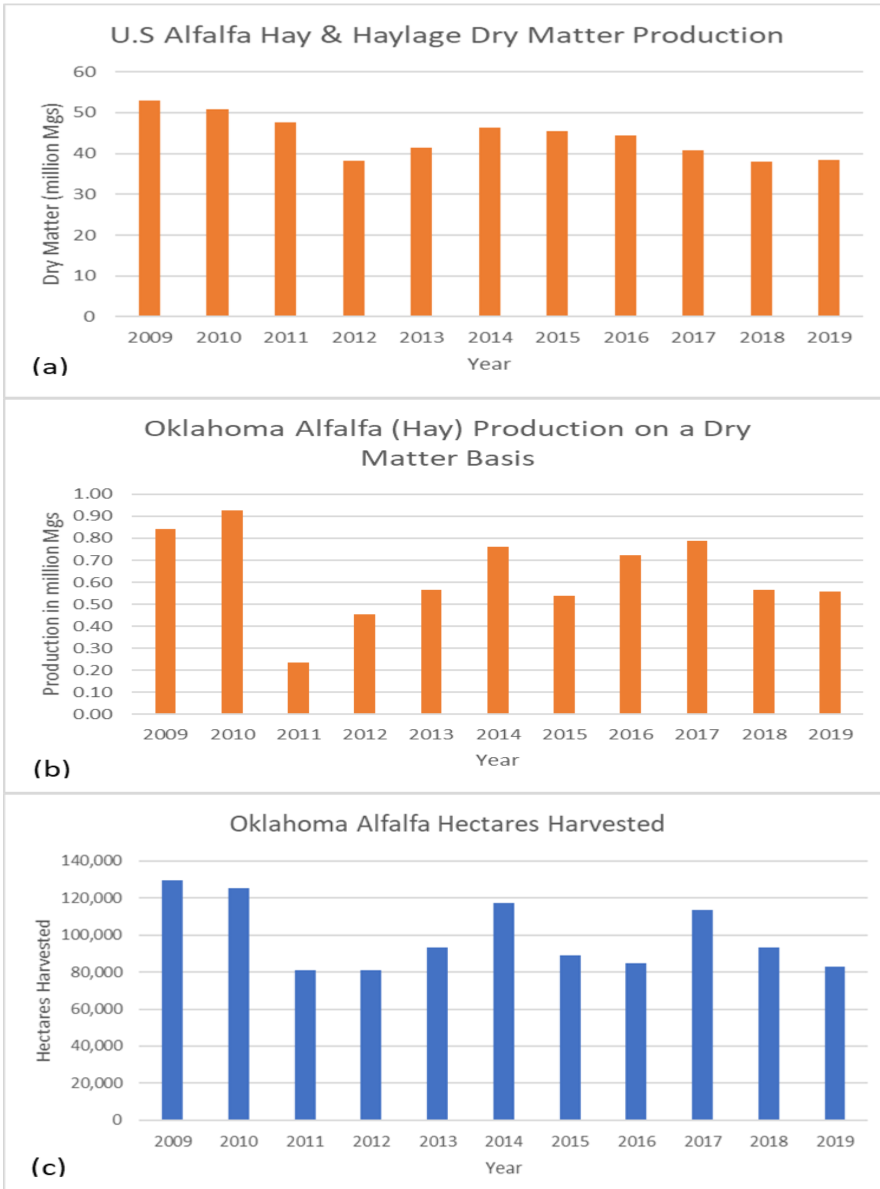


Figure 1.1. (a) U.S. alfalfa hay and haylage dry matter production from 2009-2019, (b) Oklahoma alfalfa hay production on a dry matter basis for the years 2009 to 2019, (c) Oklahoma alfalfa hectares harvested from 2009 to 2019.

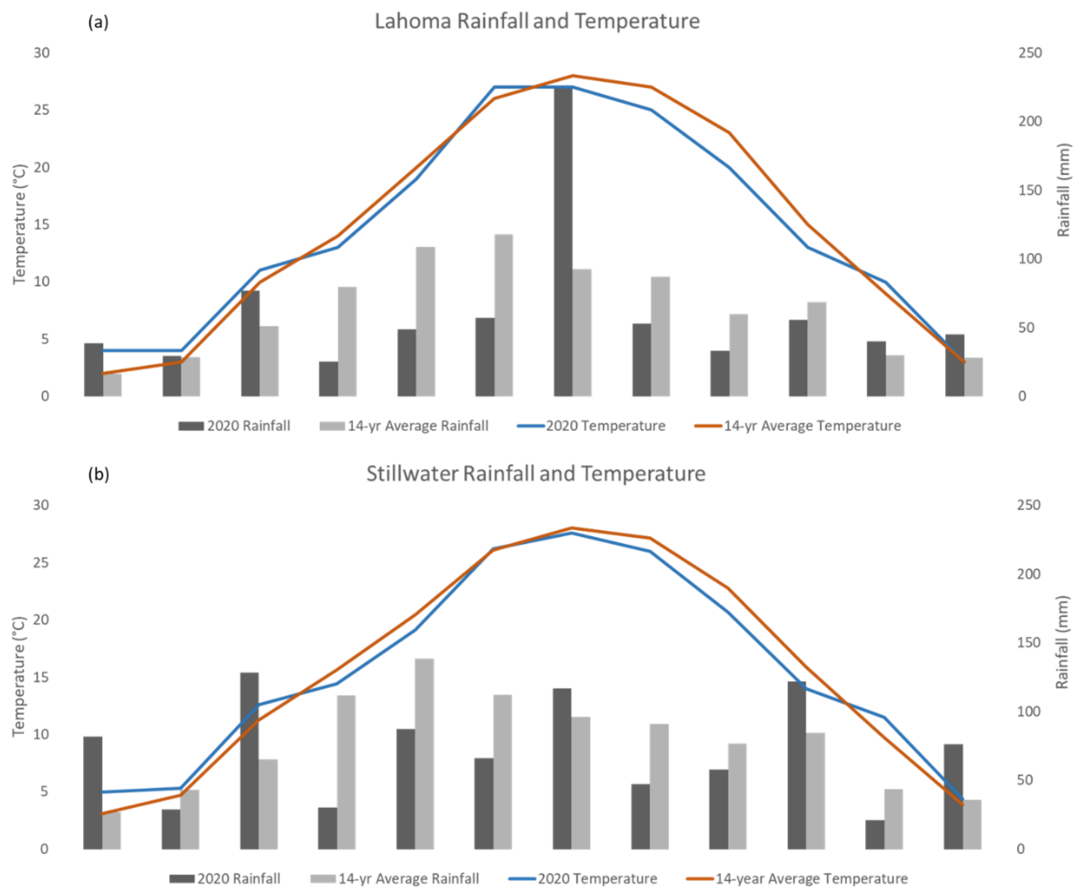


Figure 2.1. (a) Lahoma average monthly rainfall and temperature for both 2020 and 14-year averages, (b) Stillwater average monthly rainfall and temperature for both 2020 and 14-year averages.

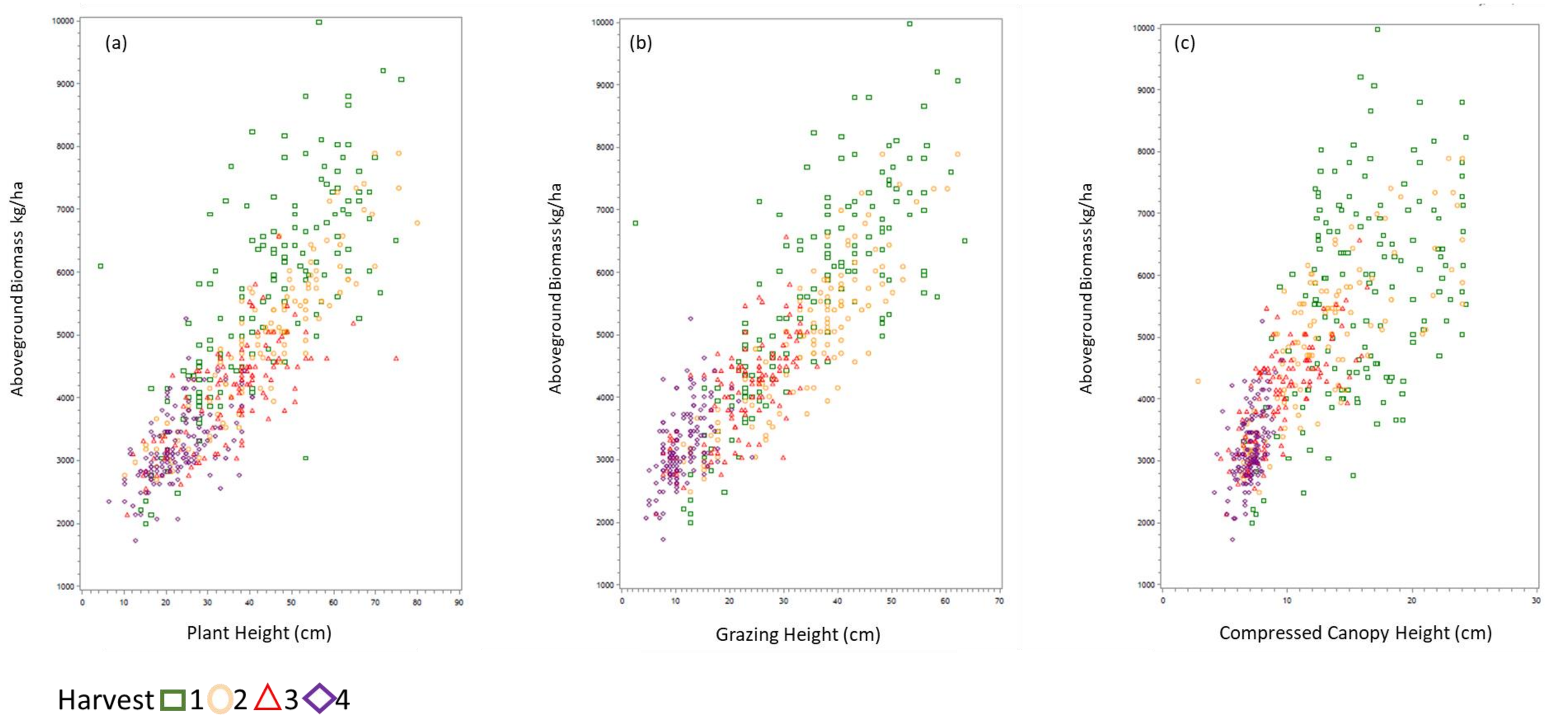


Figure 3.1. Preliminary correlation graphs of vertical growth factors (a) plant height in cm, (b) grazing height in cm, and (c) compressed canopy height in cm identified by each harvest throughout the season in relation to aboveground biomass.

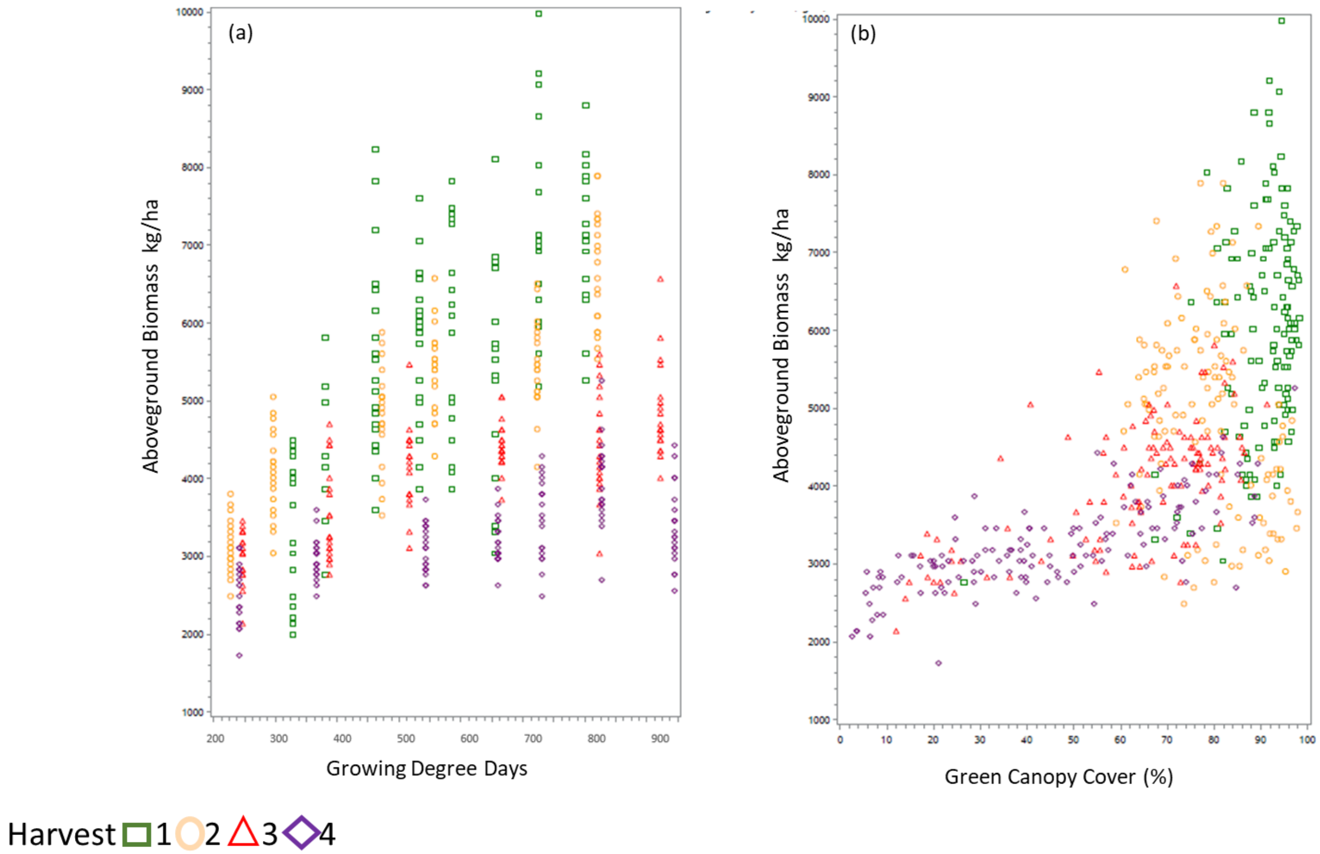


Figure 3.2. Additional independent variable factors, GDD (a) and GCC (b), identified by four cuts throughout the growing season in correlation with aboveground biomass.

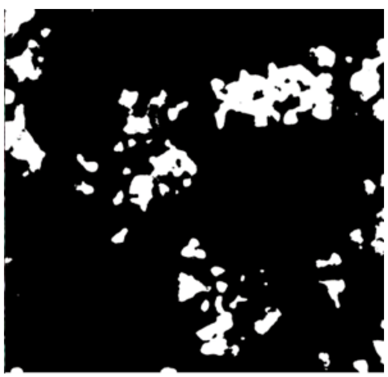
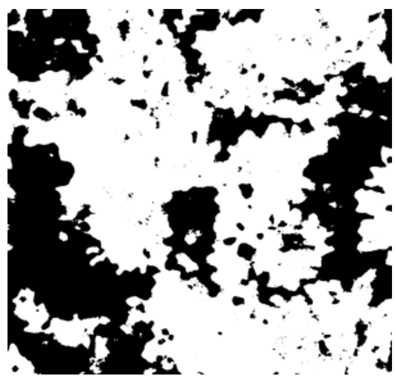
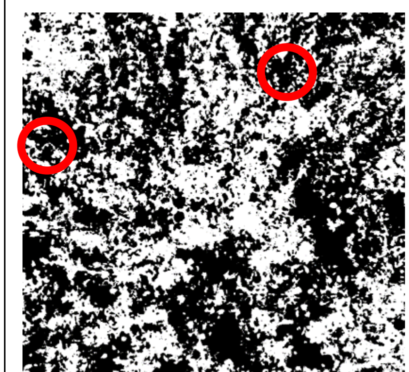
Green Canopy Cover (%)	12%	62%	76%	48%
Weekly Regrowth Images	 1	 2	 3	 4
Canopeo Image				

Figure 3.3. Visual Representation of Green Canopy Cover Progression using Canopeo.

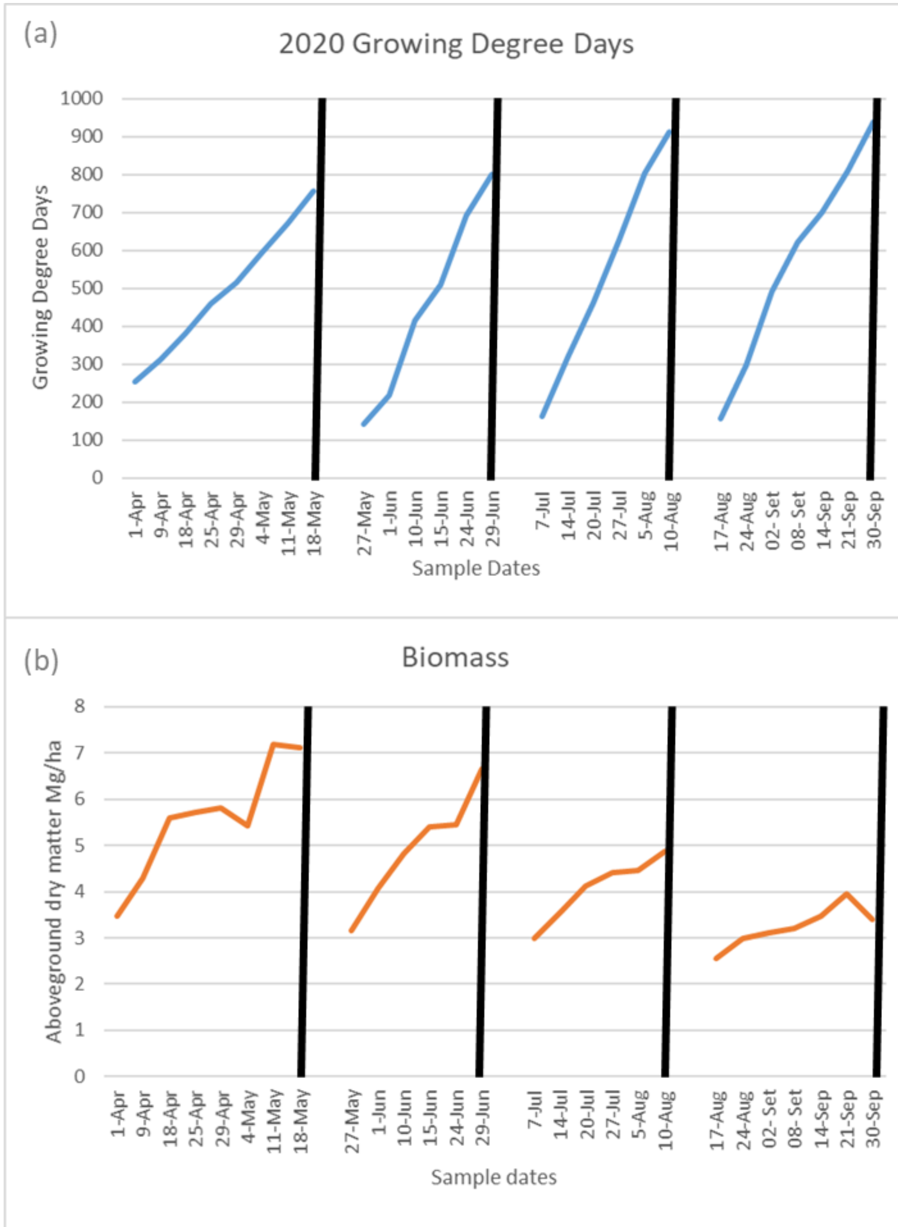


Figure 3.4. Seasonality of alfalfa through (a) growing degree day accumulation by sample date with four harvests and (b) aboveground biomass accumulation by sample day within four harvests.

APPENDICES

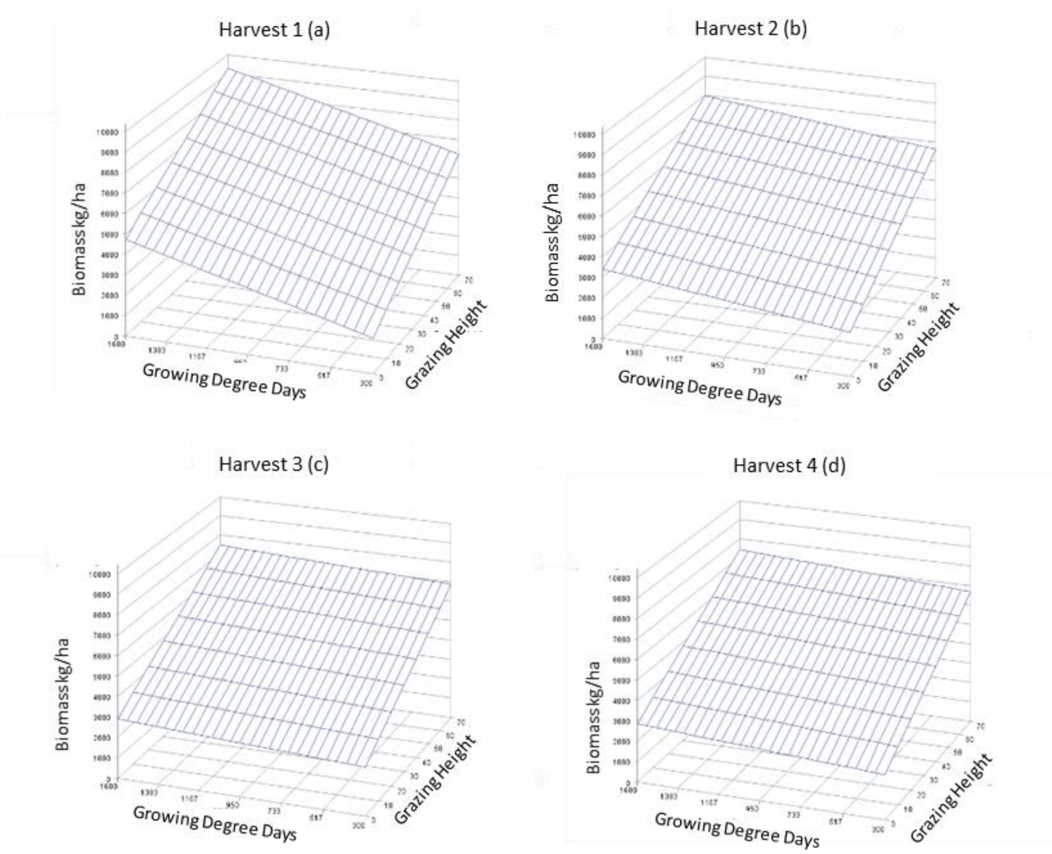


Figure A1. Prediction of cultivar 54HVX41's aboveground biomass using GDD and grazing height across all four harvests.

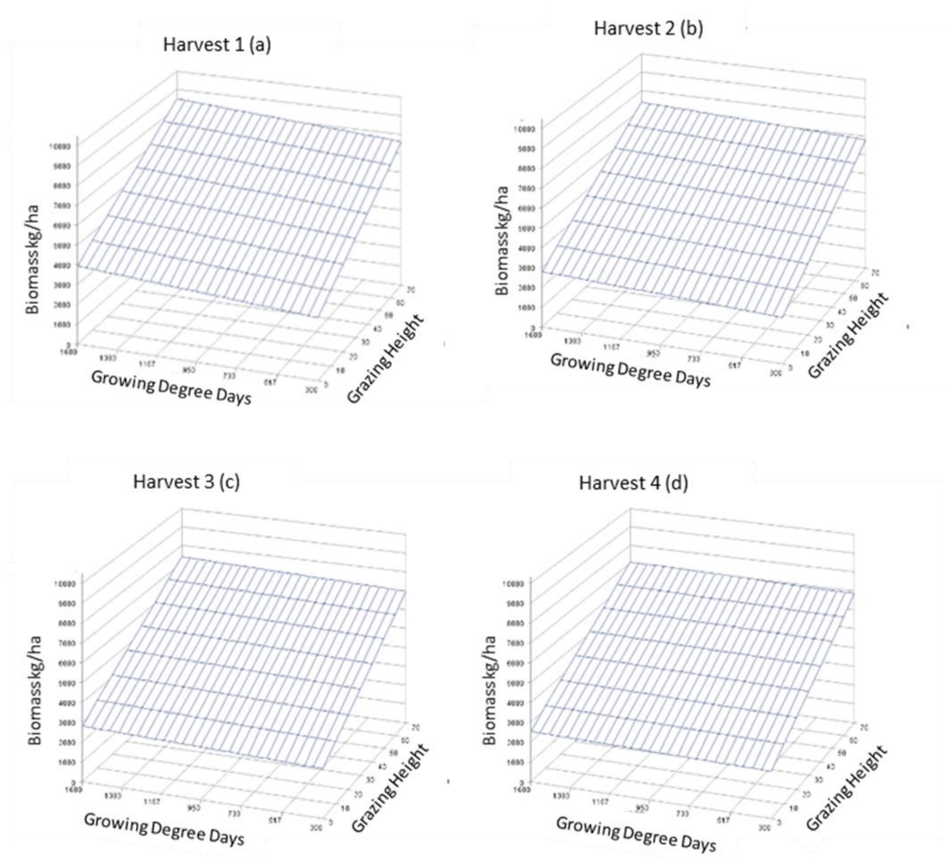


Figure A2. Prediction of cultivar DKA44-16RR's aboveground biomass using GDD and grazing height across all four harvests.

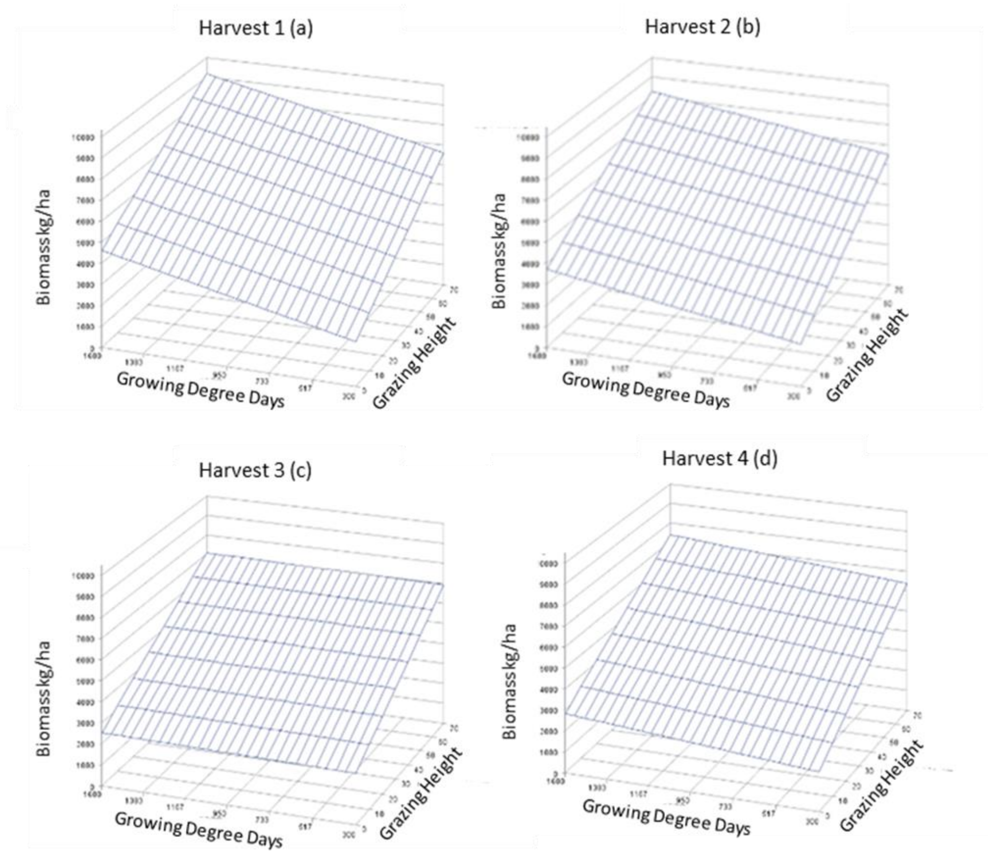


Figure A3. Prediction of cultivar 54VR10's aboveground biomass using GDD and grazing height across all four harvests.

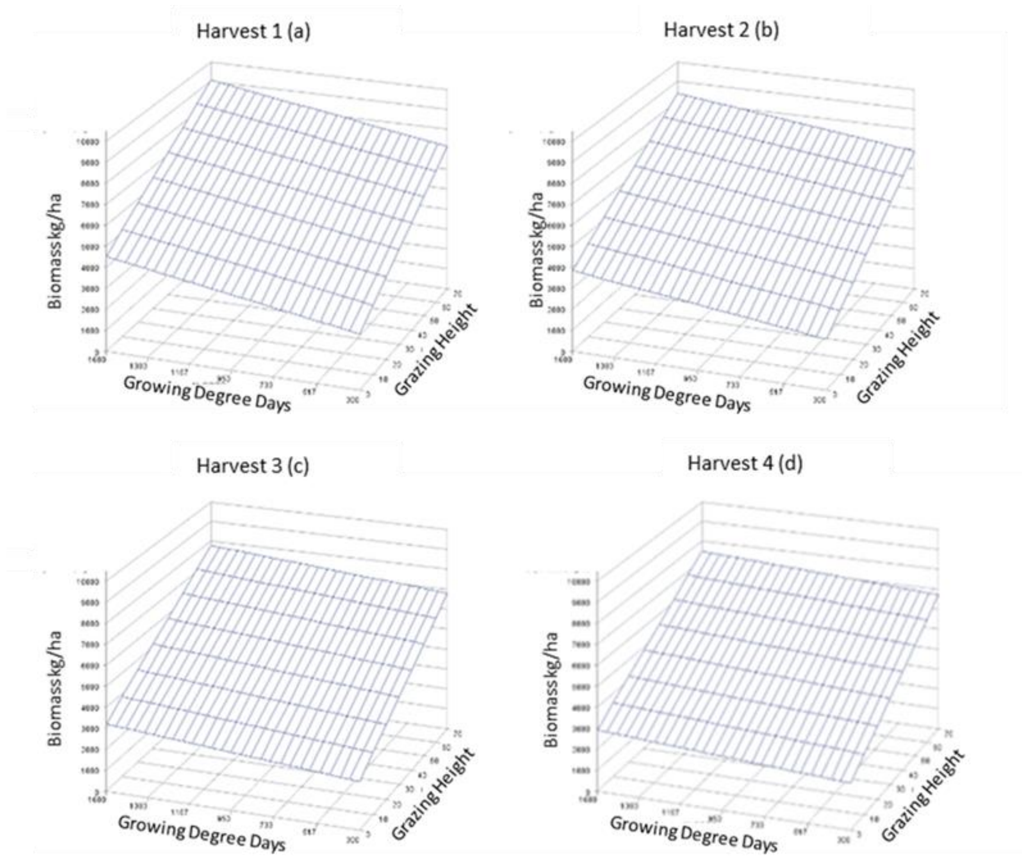


Figure A4. Prediction of cultivar WL 356HQ.RR's aboveground biomass using GDD and grazing height across all four harvests

VITA

Alayna Lynn Gerhardt

Candidate for the Degree of

Master of Science

Thesis: IMPLEMENTING NEW ALFALFA TECHNOLOGIES INTO WATER-LIMITED ENVIRONMENTS

Major Field: Plant and Soil Science

Biographical:

Education:

Completed the requirements for the Master of Science in Plant and Soil Science at Oklahoma State University, Stillwater, Oklahoma in December, 2021.

Completed the requirements for the Bachelor of Science in Plant and Animal Science at Dordt University, Sioux Center, IA, USA in 2019.

Experience: Graduate Research Assistant, Oklahoma State University

Professional Memberships: American Society of Agronomy