Masthead Logo

University of Tennessee, Knoxville
Trace: Tennessee Research and Creative Exchange

Masters Theses

Graduate School

12-2018

Forage mass, nutritive value and persistence of alfalfa and alfalfa mixtures as influenced by forage management

Marcia Pereira da Silva
University of Tennessee

To the Graduate Council:

I am submitting herewith a thesis written by Marcia Pereira da Silva entitled "Forage mass, nutritive value and persistence of alfalfa and alfalfa mixtures as influenced by forage management." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Plant Sciences.

Renata N. Oakes, Major Professor

We have read this thesis and recommend its acceptance:

Gary Bates, Augustin Rius, Carl Sams

Accepted for the Council:
Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

Forage mass, nutritive value and persistence of alfalfa and alfalfa mixtures as influenced by forage management

A Thesis Presented for the
Master of Science
Degree
The University of Tennessee, Knoxville

Márcia Pereira da Silva
December 2018

DEDICATION

To my husband:

Jack Reasoner Quinby III

To my parents:

Eleni Pereira da Silva

Anisio Rodrigues da Silva.

ACKNOWLEDGEMENTS

I would like to thank my graduate advisor and mentor Dr. Renata Nave Oakes for giving me this opportunity and for always being patient and understanding with me throughout this journey. I would like to thank my committee members, Dr. Gary Bates, Dr. Carl Sams, Dr. Mark Sulc and Dr. Agustin Rius for giving me guidance and sharing their experiences, so I could accomplish this degree. I also would like to thank The University of Tennessee's Plant Science Department for always providing me an environment that encourages learning and hard work. Thanks for the director and staff of the Plateau Research and Education Center for their help and friendship, and for all those who were critical during data collection before and after my arrival to The University of Tennessee: Dereck Corbin, Andy Carey, Brent England, J.J. Miller and David McIntosh.

I would like to thank my husband Jack Quinby, for always encouraging me during this journey, as well as my parents Eleni da Silva and Anisio da Silva, that although far way, always send me their love and words of support, and to my siblings Elaine Wilson and Anisio Junior, for always looking after me. I would like to thank Ashley Mayanja and Oluwafemi Oyedeji for their friendship. I also would like to thank Dr. Darrell Thomas, M.D. for providing the resources for my health, which was crucial for the completion of my studies.

Finally, I thank God for being my side, especially during these years and always giving me strength and the possibility of meeting wonderful people that mirror his love.

ABSTRACT

The utilization of alfalfa (*Medicago sativa*) alone or in mixtures with tall fescue (*Lolium arundinaceum* (Schreb.) Darbyish) or bermudagrass (*Cynodon dactylon* (L.) Pers) in the Southeast U.S. must be assessed so better management recommendations can be given. The objective of this first study was to determine the cumulative capacity of alfalfa in monoculture (A) and mixtures with tall fescue (ATF) and bermudagrass (AB), and its indirect improvements on the nutritive (NV). Three species combinations were utilized (A, ATF and AB) and subjected to four harvest frequencies (21, 28, 35 and 42 days) throughout the 2016 and 2017 growing seasons at The University of Tennessee Plateau Research and Education Center (PREC) in Crossville, TN. Samples were collected for analysis of NV and forage mass (FM). Results indicated that on spring of 2016 and 2017, A and ATF showed highest FM values ($P < 0.0001$). In summer 2016, A and AB had higher FM than ATF ($P < 0.0001$), however, in summer of 2017 no differences were observed. The NV increased once alfalfa was incorporated into the mixtures, with higher crude protein (CP) and lower neutral detergent fiber (NDF). In conclusion, harvest frequencies above 28 days are recommended for optimum FM accumulation. Yet, harvest frequencies of 42 days tend to have increased lignification thus decreased NV. The second experiment asses the persistence of the same experiment on the third year. Based on FM, results showed that persistence of A ($P = 0.0042$), AB ($P = 0.0002$), and ATF ($P = 0.0007$) decreased at the third year of growth, and different harvest schedules should be followed for each species combination for increased persistence in the field. For A and AB, harvest frequencies should be 35 days and for ATF, 42 days.

TABLE OF CONTENTS

LIST OF TABLES

Chapter 1_Forage mass and nutritive value of alfalfa and alfalfa mixtures subjected to different harvest intervals

Chapter 2 Persistence of alfalfa and mixtures subjected to different harvest intervals in the southeast USA

LIST OF FIGURES

ABBREVIATIONS AND SYMBOLS

ADF	acid detergent fiber, g ADF kg^{-1} DM
NDF	neutral detergent fiber, g ADF kg^{-1} DM
C_3	cool-season grasses
C_4	warm-season grasses
CP	crude protein, g CP kg^{-1} DM
DM	dry matter, kg ha^{-1}
FM	forage mass, kg DM
NIRS	near-infrared spectroscopy
PREC	Plateau Research and Education Center- Crossville, TN
UT	The University of Tennessee

INTRODUCTION

To ensure abundant production and high-quality forage in grazing-systems, management strategies must be well defined to avoid overgrazing and destruction of valuable forages, such as alfalfa. Alfalfa is a perennial cool-season legume known for its high crude protein (CP) content, producing its highest yields when grown in well-drained soils (Hakl et al., 2016; Jones and Olsen, 1987). Alfalfa requires intensive management to ensure high yields and longevity of the stand (Ball et al., 2007); yet, according to Keuren and Matches (1988), alfalfa exhibits flexible adaptation to different soil types and climatic zones. This adaptability occurs as a result of some strategies that alfalfa can develop in order to sustain its growth in a wide range of environments, such as modification of its leaf area ratio or increasing shoot:root ratio to allow the roots to capture more water during drought (Erice et al., 2010).

The advantage of providing alfalfa to livestock has been known for more than 20 years. Alfalfa has the potential to increase average daily gain (ADG) of ruminants livestock (Nemati et al., 2016; Douglas, 1986; Keuren and Matches, 1988). Htoo (2015) showed that Boer kids with access to creep feeding containing alfalfa had significantly higher ADG and growth performance than kids with creep feeding without alfalfa.

These benefits have also been found in lamb production. McClure et al. (1994) showed that in comparison to orchardgrass and perennial ryegrass, lambs that were fed alfalfa presented higher final body weights (BW) and better carcasses than those fed grasses. In addition, alfalfa is also widely utilized in dairy production for having high energy and protein required for milk production (Higginbotham et al., 2008). Therefore, due to these advances in forage breeding and forage management, producers increased their interest in growing alfalfa in the Southeast U.S. In addition, the rising interest by consumers for food derived from natural pasture-fed production systems (Cangiano et al., 2007) combined with the difficulties of producing hay in the humid conditions of the South (Haby et al., 1999) encouraged producers to switch their operations to grazing systems. Yet, depending on environmental conditions, forage systems have to be well managed and chosen accordingly.

The plant physiology determines its photosynthetic activity and performance in the field. Photosynthetic activity characterizes a plant as C_3 [cool-season], or C_4 [warm-season] (Barbehenm et al., 2004). The difference is that C_3 plants require lower temperatures to produce mass more effectively, because as temperature rises there will be more O_2 incorporated, causing photorespiration instead of photosynthesis, reducing growth. Meanwhile, C_4 plants with their differentiated cell compartmentalization do not allow oxygen to be incorporated in the photosynthetic system at higher temperatures (Griffiths et al., 2013; Wingler et al., 2000; Lee, 2011; Ehleringer, 1978).

The use of C_3 legumes such as alfalfa requires lower average temperatures to provide higher yields and avoid losses through photorespiration (Lee, 2011; Ehleringer, 1978). Therefore, alfalfa production in the Southeast is limited due to not only higher temperatures during summer, but also due to acid soil conditions commonly found in this region. Alfalfa requires soils with high drainage and high pH (Novak et al., 2009), and soils in the Southeast may not possess these characteristics, reducing persistence of the stand.

Tall fescue, a C_3 grass species, produces very well in the Southeast, with limited production during the summer. Also, forage nutritive value of tall fescue is reduced during this period. Meanwhile bermudagrass, a C4 grass species is highly productive under high temperatures, but requires heavy N fertilization to achieve high productivity and high nutritive value.

One way to ensure higher productivity while maintaining nutritive value is to incorporate forage legumes into grass pastures. Therefore, a well-managed mixed pasture can improve forage quality of the whole stand (Ball et al., 2001; Carita et al., 2016), reducing the need for synthetic N fertilization, due to the ability of alfalfa to fix N.

Persistence is an important parameter for producers when considering use of different forages in their system. Alfalfa persistence depends on parameters such as chosen variety, environmental conditions and management on the field (Smith et al., 1992; Brumer and Bouton, 1991; Beck et al., 2016). Harvesting frequency also plays an important role in alfalfa persistence. Alfalfa is a plant with a taproot system, and it relies on its root system to regrow after each harvest. According to Rimi et al. (2014), harvesting alfalfa at early flower allows an increase in its taproot when compared to alfalfa harvested at early bud; and intensive harvesting frequencies decrease the number of plants per m^2, therefore decreasing stand density and persistence of alfalfa.

N_2 fixation and alfalfa mixtures with cool and warm-season grasses

Adding legumes to grass pastures has several benefits, such as increased nutritive value and increased total forage mass of the stand. It allows for continuous forage availability when considering the use of warm and cool-season grasses, while reducing reliability on synthetic N fertilizers.

Alfalfa has the ability of N-fixation, which is a process where N_2 is transformed into ammonia by bacteria that infects the plant roots. These bacteria belong to the *Diazotroph* group and are known as *Rhizobia* (Zehr et al., 2003; Merrick, 2004; Rodrigues et al., 2017; Vymazal, 2007). Nitrogen fixation begins with the formation of nodules on the roots. The rhizobium bacteria colonizes the nodules forming an association with the plant, which provides all the nutrients and energy for the bacteria; and, in exchange, the bacteria provide N for the plant in an efficient way (Bauer, 2003; Atkins et al., 1984).

The outstanding potential of alfalfa for fixing N through its nodules significantly reduces the need for synthetic N fertilizers. It also decreases production costs and reduces environmental concerns, such as nitrate leaching throughout the soil profile or NH_3 volatilization (Patzek, 2004; Crews and Peoples, 2004; Huang, 2009; Massey et al., 2011; Rech et al., 2017).

Mixed grass-legume systems increase forage mass and contribute to a uniform distribution of mass production throughout the season by a complementary effect among species, especially N sharing (Waldron et al., 2017). When root and shoot turnover or bacterial decomposition occurs, N can be available for uptake by non-legume plants that otherwise would not able to access the atmospheric N pool (Pirhofer-Walzl et al., 2012), ensuring the benefits of these mixed systems.

Another potential benefit of growing alfalfa with grasses is that plants will be occupying different niches throughout the soil. By being a deep-rooted plant, alfalfa can better exploit the soil resources when compared to short-rooted grasses. Its roots can reach a depth of 5-6 feet, and up to 20 feet or more depending on the age of the plots

(Weaver, 1926), reducing nutrient competition among species. Grass-legume systems have the potential for success in the Southeast U.S., especially with species commonly grown is this region such as tall fescue and bermudagrass.

Considering that cool and warm-season grasses thrive at different times during the growing season, it is possible to have alfalfa interseeded with tall fescue or bermudagrass to ensure forage availability throughout the whole grazing season. That is because bermudagrass will produce during the summer, and considering its high response to nitrogen, alfalfa could provide this nutrient. Meanwhile, tall fescue will be available earlier in the season and then become dormant as temperature rises in the summer, therefore, alfalfa can provide FM during this period.

Tall fescue is a deep-rooted, cool-season perennial grass (Ball et al., 2003) prevalent in most fields in the U.S. due to its easy establishment and roughness (Hoveland, 1993). In a study conducted by Lauriault et al. (2003) in New Mexico, it was observed that the mixture of tall fescue with alfalfa increased total DM yields compared to tall fescue in monoculture. The higher yield in mixtures was dependent on the legume production, because the percentage of grass in the plots decreased with the presence of alfalfa; therefore, alfalfa-tall fescue mixtures can maintain forage mass for longer periods and decrease the use of fertilizers (Lauriault et al., 2003). The advantages of alfalfa mixed into cool-season grass stands is observed mainly during spring, and during the summer this advantage can be reduced (Mooso and Wedin, 1990) considering their physiological patterns. Therefore, the use of warm-season grasses, such as bermudagrass, in mixtures with alfalfa during summer can fill the gap of production, maintaining yields and extending the growing season (Nelson and Burns, 2006).

Bermudagrass is a warm-season perennial grass used as forage for grazing or hay production (Mitich, 1989), and it demands high N input for optimum productivity (Massey et al., 2011). For this reason, the use of alfalfa as a source of N can be beneficial to this grass. Brown and Byrd (1990) compared the yields of alfalfa and bermudagrass in monoculture and in mixtures subjected to three levels of N fertilization or without N fertilization. Their results suggested that mixing alfalfa with bermudagrass provides similar yields to alfalfa in monoculture and fertilized bermudagrass monocultures (200 kg N ha^{-1}). However, ruminants grazing in grass-legume pastures require caution and management, because a higher percentage of legumes to grasses increase the risk of bloat (Mouriño et al., 2003).

Bloat is a condition that animals can suffer when eating diets based on high concentrations of legume forages such as alfalfa (Hancock et al., 2014). Alfalfa has a high amount of soluble proteins which are rapidly fermented, leading the formation of gases in the rumen and reticulum that are not released during belching. This disorder will affect respiratory and digestive activities and can also lead to death (Cheng et al., 1998; Hancock et al., 2014; Wang et al., 2012). Yet, digestible issues can be diluted with good management in the field, such as incorporating alfalfa into grass pastures, but not allowing the a higher percentage of legumes.

Forage quality as influenced by management

Forage quality can be defined by digestibility, anti-quality factors, intake of forages by the animals and, especially, by its nutritive value (Ball et al., 2001). There are several methods, such as chemical analyses, that can be used to estimate the nutritive value of the feed by segregating the values (such as, CP [crude protein], NDF [neutral detergent fiber], ADF [acid detergent fiber] and lignin).

In mixed systems, it is very important to collect forage samples throughout the entire growing season when estimating forage nutritive value. Changes in botanical composition of the stand will likely occur depending on the management adopted and environmental conditions (Belesky et al., 2002). There is a positive relationship between forage legumes and CP content due to their ability to fix N, while grasses are positively related with NDF content of the mixture (Amiri and Shariff, 2012), so the percentage of each species in the stand will determine overall nutritive value.

If a warm-season grass is utilized in mixtures with alfalfa, it is likely that NDF concentrations will shift during the season towards higher values, because it is expected that during summer the grasses would be more abundant than the alfalfa. For that reason, the management adopted in the field for mixed systems will influence forage quality (Pedreira et al., 2007; Anjos et al., 2016), and a balance between grass and legume should be targeted.

A positive qualitative effect of mixed systems was found by Mooso and Wedin (1990). Their results suggest that the percentage of alfalfa stems might increase throughout the growing season. However, the presence of grasses in the stand can increase the percentage of top leaves instead of stems, compared to legumes in monoculture (Mooso and Wedin, 1990), increasing the nutritive value of the stand.

The nutritive value of the forage is also closely related to harvesting frequencies (Moore and Jung, 2001) regardless of monoculture or mixtures, because forage nutritive value varies according to the stage of physiological maturity of the plant. In longer harvesting frequencies, the DM forage mass will be higher; but since there will be an increase in plant maturity, forage nutritive value will be lower, especially CP content in leaves and stems and decreased digestibility (Buxton et al., 1985; Henderson and Robinson, 1982; Brink et al., 2010; Nave et al., 2014). In addition to harvesting frequencies, seasonality will also determine shoot regrowth (Dhont et al., 2002; Smith et al., 1992; 1989) and, consequently, its ability to compete with grasses, as well as its persistence on the field.

Persistence

Alfalfa relies on its roots' carbohydrates to grow, especially after dormancy. Frequent harvesting does not allow alfalfa to restore its roots' carbohydrates, which affects its regrowth. Also, high temperatures are known to decrease root weight and warm summers are common in the Southeast U.S. (Rice et al., 1989; Smith et al., 1969; Feltner and Massengale, 1965). For this reason, assessing the harvest frequency adopted in the field is important in the Southeast U.S., and this information is crucial in determining how long a stand can persist and what is the best rest period the sward requires to maintain high productivity throughout the years, especially when managing mixed swards.

Marten and Hovin (1980) studied the persistence of four perennial grasses subjected to different harvest intervals, and they observed that infrequent harvest frequencies are detrimental to the plants. Perennial cool-season grass such as tall fescue persisted and produced better when a regime of 4 cuttings per growing season was adopted; therefore, higher harvest frequencies will enable stem elongation due to shading, leaving fewer basal leaves to regrow (Marten and Hovin, 1980).

Smith et al. (1992) studied the persistence of alfalfa and alfalfa-tall fescue mixtures in the Southeast U.S. under continuous grazing. They observed that, although alfalfa-tall fescue mixtures provided higher forage mass than both species in monoculture, persistence was higher when alfalfa is grown as monoculture, because continuous grazing in mixtures made tall fescue overly competitive. Alfalfa has a lower grazing tolerance when compared to tall fescue; therefore, the choice of alfalfa variety is important in grazing systems.

Managing access of animals into pastures will influence persistence of forages in grazing systems; and, according to Beck et al. (2016), the addition of alfalfa into bermudagrass plots can increase alfalfa persistence, especially during the summer where bermudagrass is the predominant growing forage, therefore reducing alfalfa stress during this period.

Recommendations for harvesting intervals of alfalfa alone or in mixtures with the common grasses grown in the Southeast U.S. combined with its influence on field persistence can be far-reaching in determining the best management for optimum productivity and prolonged persistence. Studies considering alfalfa persistence in either monocultures or mixed swards in the Southeast U.S. are limited. Producers are interested to know if, in addition to productivity, a stand can persist for a long period of time, which decreases the costs of production and seed purchase.

Objectives

Considering the importance of forage stage of maturity in nutritive value and ruminant production, and harvest frequencies to forage mass and persistence, the objectives of this study are: (1) to assess the forage mass and nutritive value of alfalfa and alfalfa mixtures subjected to different harvest frequencies. The hypothesis was that adjusting harvesting frequencies would enable an optimum relationship between forage mass and forage nutritive value; (2) to assess the persistence of alfalfa and alfalfa mixtures subjected to different harvest intervals during 3 years. Our hypothesis was that in longer harvest frequencies, the persistence and forage mass is higher, but nutritive value is lower.

CHAPTER 1:

Forage mass and nutritive value of alfalfa and alfalfa mixtures subjected to different harvest intervals

ABSTRACT

In order to utilize alfalfa (*Medicago sativa*) alone or in mixtures to provide sufficient feed for ruminants, management practices must be evaluated to assess its performance in the Southeast US.. The objective of this study was to determine the forage mass (FM) of alfalfa when grown alone and mixtures with tall fescue (*Lolium arundinaceum* (Schreb.) Darbyish) and bermudagrass *(Cynodon dactylon* (L.) Pers), and its indirect improvements on the nutritive value of these grass–legume systems. Three species combinations were utilized [alfalfa (A), alfalfa-tall fescue (ATF) and alfalfa-bermudagrass (AB)] and subjected to four different harvesting frequencies (21, 28, 35 and 42 days) throughout the 2016 and 2017 growing seasons at the University of Tennessee Plateau Research and Education Center (PREC) in Crossville, TN. Samples were collected during this period for analysis of forage nutritive value and forage mass. Results showed that seasons (spring and summer) influence the performance on the field. During spring of 2016 and 2017, A and ATF had higher FM than AB ($p < 0.01$). In summer 2016, A and AB had higher forage mass than ATF ($p < 0.01$); however, in summer of 2017, no differences were observed among species. Nutritive value is considered high once alfalfa is incorporated into the mixtures, in values of crude protein (CP), acid detergent fiber (ADF), neutral detergent fiber (NDF) and lignin. Harvest frequencies above 28 days are ideal for FM accumulation. Yet, harvest frequencies of 42 days or above tend to have increased lignification thus decreased nutritive value.

INTRODUCTION

Alfalfa is a perennial cool-season legume widely known for its high nutritive value and high forage mass (Hakl et al., 2016; Jones and Olsen, 1987). The use of alfalfa in mixed grass swards can provide higher FM and nutritive value, therefore decreasing the need for synthetic N fertilizer applications on pasture-based forage pastures (Beck et al., 2016; Solomon et al., 2011). However, alfalfa production in the Southeast U.S. is still limited, especially when incorporated as a mixture into pastures.

There is a rising interest on expanding alfalfa production in the Southeast, but many producers are still hesitant to grow alfalfa due to the challenging environmental conditions existent in the Southeast. Therefore, growing alfalfa with forages that are commonly cultivated in the South, such as bermudagrass and tall fescue, can encourage producers to adopt this system. For this reason, studies assessing the potential of alfalfa and alfalfa-mixtures grown in the Southeast under different management strategies are necessary. Adequate management recommendations such as harvesting timing can increase the chances of success, resulting in higher productivity and higher quality.

Forage nutritive value is closely related to harvesting frequencies (Moore and Jung, 2001) regardless of monoculture or mixtures, since these parameters are variable according to the stage of physiological maturity of these plants. Longer harvesting intervals will result in higher FM but since there will be an advancement in plant maturity, forage nutritive value will be lower. This advanced maturity will result in loss of leaves and thickening of stems, lowering CP content and digestibility (Buxton et al., 1985; Henderson and Robinson, 1982; Brink et al., 2010; Nave et al., 2014). Also, alfalfa is highly dependent on its roots' carbohydrates to grow, especially after dormancy;

therefore, harvesting frequency and seasonality will determine the shoot regrowth (Dhont et al., 2002) and, consequently, its ability to compete when growing as a mixture with grasses.

Considering that forage nutritive value and FM are dependent on the management adopted in the field, the objective of this study was to determine FM and nutritive value of alfalfa and alfalfa-mixtures subjected to different harvest intervals. Our hypothesis was that adjusting harvesting intervals would identify the optimal relationship between FM and forage nutritive value.

MATERIALS AND METHODS

Site description

This study was conducted at the Plateau Research and Education Center (PREC) in Crossville, Tennessee (36°01′ N, 85°12′ W) from July 2015 to September 2017. The soil conditions on the location were Lily loam (fine-loamy residuum weathered from sandstone, 2-6% slopes, well drained, 21 to 39 inches to lithic bedrock) (NRCS, 2018). Initial soil nutrient levels on the experiment site were pH = 5.5, P = 77 kg ha^{-1}, K = 247.7 kg ha^{-1}, Ca = 1484 kg ha^{-1}, and Mg = 62.8 kg ha^{-1}. The experiment was conducted utilizing 48 experimental units that were 3 x 6 m plots in a split-plot arrangement of a randomized complete block design (whole plot experiment unit: species; subplot experiment unit: harvest).

The treatments consisted of three species combinations, four harvest frequencies and four replications totaling the 48 plots. The species were a grazing tolerant cultivar of alfalfa (cv. Ameristand 403T Plus) (A) grown in monoculture and as a mixture with a perennial cool-season grass novel-endophyte tall fescue (cv. Texoma Max Q II) (ATF) and with a perennial warm-season grass bermudagrass (cv. Vaughn's #1) (AB), and the harvest frequencies were 21 days [H1], 28 days [H2], 35 days [H3] and 42 days [H4] harvest.

In July 16th 2015, the ground was tilled and vegetative bermudagrass was planted via sprigging in the designated area. In September 4th 2015, using a 10' Great Plains No Till Drill, tall fescue and alfalfa were seeded at 11 kg ha^{-1} and 17 kg ha^{-1}, respectively. For establishment of ATF, 67 kg ha^{-1} of N (34-0-0) was applied exclusively on the plots where ATF mixtures were present.

On October 7th 2015, 67 kg ha^{-1} of N was applied to ATF plots to aid establishment of tall fescue. On October 26th 2015, all plots were treated with 7 tons of lime per ha. On February 2nd 2016 and February 24th 2017, boron was applied to all plots at 2 kg ha^{-1}, as this micronutrient is required for alfalfa production. Since this is such a small amount, boron was mixed with sand to allow for better distribution within the whole experimental area. On March 8th 2016, alfalfa was reestablished on the bermudagrass plots at 17 kg ha^{-1} using a hege small tube drill due to the low density of alfalfa in the plots from the previous seeding. On May 25th 2016, 2,4-DB 200 (Agrisolutions, WindField, MN) was applied to all AB plots to control broadleaf at 4.6 L ha^{-1}. Due to the dry conditions in Tennessee in 2016 (Figure 1.1), an irrigation gun on reel system was utilized over the entire experimental area on September 23rd and 24th 2016. On April 6th 2017, Chlorpyrifos (DowAgrosciences, Canada Inc.) was applied to control alfalfa weevil, and on May 9th 2017, potato leafhopper was controlled with the same insecticide as for the alfalfa weevil at 1 L ha^{-1}.

Harvest frequencies were set for each species combination from May to September of 2016 and 2017, H1plots followed a harvest schedule of 21 days, H2 plots followed a harvest schedule of 28 days, H3 plots followed a harvest schedule of 35 days, and H4 plots followed a harvest schedule of 42 days (Table 1.1). On September 13th 2016 and September 19th 2017, all plots were cut to a 4-cm stubble height to prepare for the growing season of the following year.

Measurements

To determine forage mass, a Carter 3' (Carter, Brookston, Indiana) forage harvester with 0.9 x 6 m harvest size was used to collect forage material of each experimental unit from the center of each plot. The harvested material was collected and weighed using a 121 L bucket from each experimental unit. A bulk sample was then collected from the bucket and dried in a forced air dryer at 60°C up to constant weight for determination of total DM forage mass. These bulk samples collected for determination of the DM were then ground in a Wiley Mill Grinder (Thomas-Wiley Laboratory Mill Model 4, Arthur H. Thomas Co., Philadelphia, PA) using a 1-mm screen for nutritive value analyses. Samples were analyzed for CP, ADF, NDF and lignin. These samples were scanned in small ring cups on Near-infrared spectroscopy (NIRS) technology (Unity SpectraStar XL-R, Unity Scientific, Milford, MA). Equations for the forage nutritive analyses were standardized and checked for accuracy with the 2016-2017 Grass Hay and Legume Hay equation developed by the NIRS Forage and Feed Consortium (NIRSC, Hillsboro, WI). The Software used was Infostar version 3.11.3 3 (Unity Scientific, Milford, MA). The Global *H* statistical test compared the samples against the model and other samples within the database for accurate results, where all forage samples fit the equation (*H* < 3.0) and are reported accordingly (Murray and Cowe, 2004).

To determine botanical composition in both ATF and AB mixture plots, pure samples of tall fescue, bermudagrass and alfalfa were collected to develop models to determine the calibration curves in the NIRS. Avoiding the area collected for determination of FM, in 2016 and 2017, a 0.1-m^2 quadrant was placed in each of the mixed plots. The material inside the quadrant was cut (at a 5-cm stubble height) and manually separated into alfalfa, tall fescue, bermudagrass and weeds (if present). In 2016, only one collection was effectuated for separation of the botanical composition, the collection occurred on August 23rd for all species and treatments. In 2017, the H1was collected on May 2nd, July 5th and September 5th 2017. The H2 was collected on May 9th, July 5th and August 30th 2017. The H3 was collected on May 15th, June 20th and August 30th 2017. The H4 was collected on May 23rd, July 5th and August 15th 2017. This material was then ground to 1-mm aid the models developed in the NIRS.

Unstandardized spectra were collected for each ratio from 0 to 100 percent by 10 percent increments for each mixture. The software used to develop this calibration model was UCA (Unity Scientific, Milford, MA). Initial spectra were used to make a calibration model to predict botanical composition of field samples. This initial spectra collection included 115 total spectra, 5 replications per category of grass to legume percentages that were loaded into UCAL. Percentage reference values were associated with the scanned spectra. Extremes of 0 to 10 percent and 90 to 100 percent of the pure material had to have additional material prepared for spectra collection using the 5 duplicate scans per tier. Regression selections were made using the PLS function with maximum factors of 7 used for final model. Outliers and rejections were removed with outer limits of 3.000. Validation predictions were used for spectra with a final STDEV=0.14. Further model expansion was used with 151 field samples manually separated for the actual botanical composition of both ATF and AB mixtures. Re-prediction of the final percentages reported was then performed. The final statistics in the NIRS model were global distance (GD)=0.80, T-value= 0.04, standard error of calibration (SEC) = 1.51 and cross validation (CV)= 0.09, with prediction fitting the allowable H<3.0 (Murray and Cowe, 2004).

Statistical analysis

Mixed model analyses of variance were performed to determine differences in least square means among species composition and harvest intervals on various nutritive value response variables. Models were performed using the GLIMMIX procedure in SAS (Cary, NC, 9.4). The dependent variables tested were DM yield, CP, ADF, NDF and lignin in averages. The fixed effects of species combination, harvest intervals, and their two-way interaction, as well as the random effect of rep within species were included in each model. Years (2016 and 2017) were analyzed separately, as well as seasons (spring and summer), due to the physiological differences of cool and warm season grasses. Harvests carried out in the months of May and June are considered spring; July, August and September harvests were considered summer. Within two of the species combinations (alfalfa-tall fescue and alfalfa-bermudagrass), the effect of harvest intervals were assessed for the dependent variables of legume and grass percentages. The total mass accumulation was analyzed using GLIMMIX procedures in SAS (Cary, NC, 9.4), with species and harvest intervals as fixed effects. All results were evaluated for significance at $P < 0.05$.

RESULTS AND DISCUSSION

Weather

In 2015, May through September temperature was 0.3°C below the 30-yr average. Precipitation in 2015 May through September was 29% above the 30-yr average (770 mm). In 2016, May through September temperature was 0.2°C above the 30-yr average. Precipitation in 2016 May through September was 30% below the 30-yr average. In 2017, May through September temperature was 1.2°C below the 30-yr average. Precipitation in 2017 from May through September was 14% above the 30-yr average (Figure 1.1).

Botanical composition

In spring of 2016 and 2017, species and treatment were significant ($P = 0.004$; Table 1.2). In summer of 2016, there were no treatment or species differences; while in 2017, there was only species difference ($P < 0.01$; Table 1.2).

With the exception of summer 2016, all AB plots showed higher percentage of alfalfa than ATF plots (Table 1.2). This result suggests that most of the time, tall fescue is more competitive with alfalfa than bermudagrass. Similar results were observed by Haby et al. (1999), where alfalfa was competitive when mixed with bermudagrass and had an increased FM as compared to bermudagrass.

The difference between 2016 and 2017 summers might be associated to the drought conditions that occurred in 2016 (Figure 1.1; Table 1.2). These results suggest that in periods of drought, the presence of alfalfa in grasses is interchangeable among bermudagrass and tall fescue; while in 2017 with normal precipitation, AB plots showed higher percentage of alfalfa than ATF plots (Table 1.2). In addition, in summer of 2017, there were higher percentage of weeds in mixed plots, especially ATF plots ($P = 0.0424$; Table 1.2). This pattern suggests that the physiological differences between cool and warm-season grasses played an important role in botanical composition, since tall fescue is a cool-season grass and does not grow as well under high temperatures as compared to bermudagrass (Mitich, 1989; Ball et al., 2007). In addition, as observed by Jung et al. (1996) working with perennial ryegrass in binary mixtures with alfalfa, taller cultivars of grasses are more competitive with alfalfa than shorter cultivars.

In spring 2016, the percentage of alfalfa decreases as harvest frequencies increased, H2 showed the highest legume percentage and did not differ from H1; and H2 also differed from H3 and H4 (Table 1.2), confirming that with an increased harvest frequency decreases the overall presence of alfalfa in the mixture, especially in harvests above or equal 42 days. Also, in situations of water scarcity as occurred in Spring 2016 (Figure 1.1), forages will use most of its energy to increase its root mass instead of leaves (Sheaffer et al., 1988; 2000; Ball et al., 2007). Consequently, with increased harvest frequencies, the percentage of grasses is higher (Table 1.2), suggesting that once alfalfa has its canopy density decreased, grasses have more sunlight to grow and compete for resources.

A different pattern was observed in spring 2017, where an increase in harvest frequency did not always result in a decrease in legume percentage (Table 1.2). The presence of legumes was not different between H1 and H4, and H4 was not different from H2 and H3 (Table 1.2). These results suggest that allowing these mixtures to regrow for a longer period of time can potentially maintain alfalfa percentage more effectively when water is not an issue (Figure 1.1).

Total annual forage mass

There were species and treatment differences in the 2016 growing season (species P <0.01; harvest frequency P <0.01). In 2017, no species differences were observed, only treatment differences (species P = 0.4; harvest frequency P <0.01) (Table 1.3). In 2016, A did not differ from ATF in FM, and both were different from AB (Table 1.3). In AB plots, alfalfa had to be reestablished in March of 2016; and, considering the drought of 2016 (Figure 1.1), our results suggest that alfalfa was not able to recover its carbohydrate reserves to sustain itself during the drought. This is confirmed by observing FM results from 2017, which did not show species differences and was a year with normal precipitation (Table 1.3; Figure 1.1). Although bermudagrass is a warm-season forage and grows better during the summer (Mitich, 1989), it is still not as productive as A because it takes up area where A could be growing alone, suggesting that AB mixtures can be detrimental for the total FM if environmental conditions are extreme.

It is important to keep space between alfalfa and bermudagrass to allow sunlight and water availability to the grass, considering that light is a limiting factor for warm-season plants, and alfalfa competes well for water resources (Haby et al., 2006; 1999).

Stringer et al. (1994) observed that in mixtures, wider row spacing of alfalfa would benefit the grass, reducing shade by alfalfa so that grass growth can be more effective. In our study, since alfalfa was overseeded into bermudagrass plots, that is possibly one reason why bermudagrass did not perform well. According to our results, growing A or ATF are both good strategies for producers when aiming for higher FM.

In both years, H1 resulted in lower FM; as harvest frequency increased, the FM was higher (Table 1.3). However, in periods of drought this increase is not linear. In 2016, H3 and H4 had the highest FM, yet H4 was not different from H2 ($P < 0.01$; Table 1.3). In 2017, H2, H3 and H4 were not significantly different, but all were different from H1 ($P < 0.01$; Table 1.3). These results suggest that after 28 days, longer harvest intervals are not necessarily advantageous when compared to shorter harvest intervals. Longer harvesting frequency (H4) showed a higher rate of leaf losses under drought; therefore, its production was not different than the shorter frequency of H2. Meanwhile, H3 held the productivity higher, due to a longer period for regrowth as compared to H2. Similar results were observed by Fuess and Tesar (1968), where longer harvest frequencies had lower FM because of a decrease in the leaf: stem ratio due to leaf losses, which can also increase disease occurrences that can affect total yield (Fuess and Tesar, 1968; Sheaffer et al., 1988; 2000).

It is also important to understand that a shift in FM throughout the growing season can occur, considering that the studied species have different physiological responses. For this reason, seasonality was also analyzed each year. There were species and treatment effects during spring of 2016 and 2017 (species $P < 0.01$; harvest frequency P

< 0.01; Table 1.4) and summer of 2016 (species $P = 0.0138$; harvest frequency $P < 0.01$; Table 1.4).

In spring of 2016 and 2017, A was similar to ATF and both higher then AB (Table 1.4). Yet in summer of 2016, ATF was not different than A, and A was not different from AB, showing higher FM (Table 1.4). These results suggest that in summer, bermudagrass increased FM during a drought period, while ATF did not follow the same pattern. In summer of 2017, there were no species differences ($P = 0.14$; Table 1.3), which confirms that drought influences FM of cool-season grasses during the summer. In years of ideal precipitation, ATF provided as good FM as AB during the summer. According to our findings, adding alfalfa to bermudagrass plots during a dry summer can be advantageous in maintaining FM; nonetheless, overall productivity in mixtures is attributed to ATF.

In spring of both 2016 and 2017, H3 had higher FM than all the other harvest frequencies (Table 1.4) for all species. This agrees with the results of total annual FM where, with longer harvest frequencies, the leaf losses are higher. In summer of 2016, H1 showed the lowest FM, and there were no differences among H2, H3 and H4 (Table 1.4), which suggests that in periods of scarce precipitation, the growth pattern between species is similar and not totally dependent on harvest frequency. In summer 2017, H4 had the highest FM, followed by H2 and H3, with H3 not different than H1 (Table 1.4).

Considering slower FM production during summer given environmental conditions such as heat, forages will remain vegetative for a longer period, which gives H4 an advantage as a strategy to maintain yields and control weeds. The inconsistency observed in summer 2017, especially among H1, H2 and H3, is attributed to the increased weed populations compared to 2016 (Table 1.2)

There is a lower FM associated with the period of regrowth in short intervals, and this lower FM is also associated with root carbohydrate (RC) reserves. Forage grasses and legumes, such as alfalfa, rely on its RC as well as C and N reserves for regrowth during spring after dormancy; and frequent harvestings do not allow enough time to replenish these carbohydrates, impacting regrowth (Ball et al., 2007; Dhont et al., 2001; Li et al., 1996).

Overall, although AB provided high FM in 2016 summer, ATF or A showed better results throughout the entire growing season.

Average forage mass per harvest

In spring 2016, there were species and treatment effects (species $P < 0.01$; harvest frequency $P < 0.01$), while summer of 2016 and all of 2017 did not show species differences (Table 1.5). In spring 2016, A was not different from ATF, and both were different from AB (Table 1.5) across all treatments, suggesting that ATF can be a good strategy to maintain forage productivity independently of harvest frequency, while reducing the need for N fertilizer application. Considering that alfalfa was overseeded in bermudagrass in March 2016, this could have influenced AB productivity. Although the proportions of alfalfa to bermudagrass plots were higher than tall fescue plots, the cumulative production of FM was lower, therefore FM was lower (Tables 1.2, 1.5). Warm summers and low precipitation can affect plant N uptake and utilization in the field

(Kering et al., 2011), which also explains why bermudagrass showed lower FM in spring of 2016 over 2017 (Table 1.5).

The 2016 lack of precipitation, especially during the months of May and June (Figure 1.1), delayed the bermudagrass growing season. In addition, the shading of alfalfa over bermudagrass could have also inhibited bermudagrass growth and consequent competitiveness during summer, since C_4 plants require light for higher productivity in addition to higher temperatures (Sage et al., 2006; Yamori et al., 2014). These results agree with Stringer et al. (1994), who reported that interseeding alfalfa into bermudagrass plots decreased bermudagrass vigor.

All species (A, ATF and AB) showed similar results when comparing differences in average FM across treatments (Table 1.5). In all instances, H1 presented the lowest FM (Table 1.5). In spring and summer of 2017, H4 showed the highest FM per harvest as a result of a prolonged period for regrowth (Table 1.5). However, in spring and summer of 2016, H4 was not different from H3, results that could have been due to the drought that occurred in 2016 (Figure 1.1). This drought event led to a delay in plant maturity, which could account for the similarities between H3 and H4. These results are in agreement with Peterson et al. (1992), who found that although alfalfa has the highest drought resistance compared to other legumes, there was a delay in maturity and reduced FM (Peterson et al., 1992).

In addition to the demonstrated disadvantages of H1, the effect of defoliation in both legumes and grasses affect forage growth since it limits the acquisition and assimilation of N. Therefore, forages must rely on N from remaining plant parts for regrowth, as excess defoliation decreases these reserves (Belesky and Fedders, 1995). As observed by Teixeira et al. (2007), studying the dynamics of alfalfa yield components in response to defoliation frequency, it was shown that longer harvest intervals provided higher FM per harvest, since more frequent harvest intervals limit growth by decreasing the assimilation of C and N by the plant (Teixeira et al., 2007). Alfalfa relies heavily in its RC for regrowth; and, according to Feltner and Massengale (1965), warm temperatures in addition to frequent harvest decrease RC and affect regrowth.

Considering the physiological differences between cool and warm-season grasses when comparing the two alfalfa-grass mixtures during spring 2016, ATF had higher productivity with FM ranging from 1754.0 to 6411.0 kg ha^{-1} depending on the harvest interval, while AB FM ranged from 218.0 to 1021.6 kg $ha^{-1}$for AB (Table 1.4). These results suggest that regardless of the harvest interval adopted in the field, bermudagrass is not yet active during the spring; therefore, FM is mostly due to alfalfa's presence on the plots (Table 1.2).

It is important to know how much FM is produced per harvest, so producers can make an informed choice between having more frequent harvests per season combined with lower FM/harvest if market is favorable, or higher FM/harvest combined with lower number of harvests/season for practicality.

Forage nutritive value

In spring of 2016, CP content showed no harvest frequencies differences, only species differences (P = 0.0002; Table 1.6). Plots of A had higher CP then ATF and AB, results that are expected due alfalfa's ability to fix N. The presence of grasses in the mixtures will consequently dilute the total amount of CP (Ball et al., 2007). In summer of

2016, there were species and harvest frequencies differences in CP levels (species P <0.01; harvest frequency P< 0.01; Table 1.6). Plots of A once again had the highest CP levels, followed by ATF and then AB (Table 1.6). Although the percentage of legumes was the same for ATF and AB (Table 1.2), and the overall FM was higher for AB (Table 1.3), these results suggest that the presence of bermudagrass in the AB plots is detrimental for CP content. However, considering that the average CP of bermudagrass ranges from 80 to 130 g kg^{-1} (Ball et al., 2007), the CP level observed in our study was still above average when compared to bermudagrass monoculture (from 149 to 181 g kg^{-1}). The same happens for tall fescue that, on average, has 140 g kg^{-1} CP (Mullen et al., 2000) and in our ATF mixtures ranged from 127 to 195 g kg^{-1}.

In spring 2017, there were species and harvest frequencies differences (species *P* <0.01; harvest frequency *P* < 0.01; Table 1.5), with A and AB being higher than ATF. The results suggest that the higher percentage of alfalfa in AB plots (Table 1.2) led to an increase in CP during that period. In summer 2017, no species or harvest frequencies differences were observed (Table 1.6). Although the percentage of alfalfa in AB was higher then ATF (Table 1.2), it is possible that the normal precipitation of 2017 (Figure 1.1) led to a positive response of CP for all species. Meanwhile, the drought of 2016 negatively affected CP, especially since CP content is affected by N availability, and drought can decrease nodulation and N fixation (Kuechenmeister et al., 2013; Ashraf and Iram, 2005).

There were no differences among harvest frequencies in spring 2016, but the harvest frequencies were different in summer 2016 and as harvest frequencies become longer, the CP is lower (Table 1.6), due to advanced maturity. However, in 2017 there was a shift in seasonal response. In 2017, harvest frequencies differences were only found during spring, while summer did not show any differences among harvest frequencies (Table 1.6). These results suggest that, in normal levels of precipitation (Figure 1.1), forages can follow its normal physiological and morphological pattern of losing leaves with maturity and decreasing its cell content, which decreases the CP content (Sheaffer et al., 1988; 2000; Albrecht et al., 1987). In addition, in summer 2017, there were no harvest frequencies differences regarding the percentage of legume in mixtures; while in spring 2017, there were differences (Table 1.2), which explains why summer presented no differences in CP compared to spring 2017.

In spring 2016, no differences in NDF were found between A and AB, with ATF showing highest NDF (Table 1.7). Considering that ATF had a higher percentage of grass than legumes (Table 1.2), NDF levels are consequently higher once that grasses have higher fiber content than legumes (Buxton, 1996). In summer 2016, AB showed higher NDF, followed by ATF, with A having the lowest values (Table 1.7). These results suggest that although the percentage of legume and grasses in the mixtures were equal (Table 1.2), during this period tall fescue remained mostly vegetative; while bermudagrass showed active growth, therefore increasing its fiber content (Buxton, 1996). This increase in fiber can affect overall forage quality, since high fiber content decreases overall digestibility of the feed (Grev et al., 2017; Fustini et al., 2014; Nave et al., 2014).

In spring 2017, a similar pattern was observed (Table 1.7), where A maintained lower NDF, followed by AB and ATF. The higher percentage of alfalfa in AB plots during the spring results in higher forage nutritive value when compared to ATF. Tall fescue shows a rapid growth rate during the spring, with its fiber content increasing rapidly, thus decreasing forage nutritive value (Nave et al., 2013). In summer 2017, A remained lower in NDF, while AB and ATF were not different (Table 1.7). This result suggests that under normal precipitation (Figure 1.1), alfalfa grows better with bermudagrass (Table 1.2) diluting the fiber content of the grass, indicating that AB is promising when precipitation is not an issue.

Harvest frequencies differences were observed in spring and summer of 2016 and spring of 2017 (Table 1.7); and in those instances H4 had higher NDF concentration, confirming that with increased harvest frequencies, the fiber content of forages is higher due to the increase in thickness of cell walls with advanced maturity (Albrecht et al., 1987). No harvest frequencies differences were observed in summer 2017 (Table 1.7); this pattern suggests that when summer shows no abnormal environmental conditions, harvest frequencies are not responsible for forage nutritive value reductions.

During spring of both years, ADF concentration was lower for A and AB plots, with ATF showing highest values (Table 1.8). It is known that legumes have lower ADF than NDF, with a difference of about 100 g kg^{-1}, and for grasses this difference can be as high as 200 g kg^{-1} (Buxton, 1996). Therefore, since percentage of alfalfa was higher in AB than in ATF, ADF concentration is likely to be higher. In summer 2016, ADF concentrations were higher for AB, followed by ATF, with A showing the lowest values (Table 1.8). Warm-season grasses are more productive during this period of the year, with an increase in reproductive stems which have higher ADF. Meanwhile, A and ATF remained mostly vegetative, consequently showing lower ADF content. In summer of

2017, no species differences were observed; and considering the normal precipitation, advanced maturity most likely affected all species to the same extent (Table 1.7).

Harvest frequencies differences in ADF were observed in both seasons of 2016 and spring of 2017 where, as harvest frequencies increased, ADF was higher (Table 1.8). In spring 2016, H2, H3 and H4 did not differ and were all higher than H1 (Table 1.8). Considering that NDF had a similar response (Table 1.7), it is possible to suggest that the total amount of fiber is lower for treatments with higher harvest frequencies, which remains vegetative. In summer 2016, H4 showed the highest ADF, followed by H3, with H1 and H2 having the lowest values (Table 1.8). These results suggest that in dry summers harvest frequencies have a big impact on fiber content, since during warm and dry periods of the year, forages will use energy for root growth rather than shoots (Ball et al., 2007) remaining vegetative longer than in spring.

In spring 2017, with normal environmental conditions (Figure 1.1), harvest frequency responses were the most apparent, with H1 having the lowest ADF, followed by H2, H3 and H4 (Table 1.8), because of the increased fiber content with maturity. In summer 2017, H1was not different than H2 and H3, and H2 and H3 not different than H4, which had higher ADF (Table 1.8). The result suggests that in summer with adequate precipitation (Figure 1.1), harvest frequencies have decreased effects on ADF concentration.

Lignin content showed species and harvest frequencies differences ($P < 0.0001$; Table 1.9) during both years and seasons. In spring 2016, A had higher lignin, followed by ATF, then AB (Table 1.9). These results showed that legumes have higher lignification than grasses, and although alfalfa percentage in AB plots was higher than

ATF (Table 1.2), tall fescue as a cool-season grass has higher lignification during periods of rapid growth when compared to warm-season grasses in the spring (Allison and Osbourn, 1970; Kamstra, 1973). In summer 2016, A had higher lignification followed by AB and ATF (Table 1.9). This occurs because the percentage of legumes in these mixtures was not different (Table 1.2), diluting the amount of lignin in mixed plots, since that legume usually has a higher lignin content than grasses.

In spring 2017, A was not different from AB, and both were higher than ATF (Table 1.9). These results suggest that under adequate precipitation, bermudagrass adds to the overall lignin content of the mixture. In summer 2017, A had higher lignin, followed by AB, then ATF (Table 1.9), suggesting that under adequate precipitation, alfalfa still maintained high lignification. Also, the percentage of alfalfa in AB plots was higher than ATF, increasing lignification of AB as compared to ATF (Table 1.2).

For both years and seasons, lignin increases as harvest frequencies increase ($P<$ 0.01; Table 1.9). In spring 2016, H1 had lower lignin and H4 the highest and not different from H3. In summer 2016 and spring 2017, H1 had the lowest and H4 the highest lignin content; while in summer 2017, H1was the lowest and not different from H2, H3 was intermediate and H4 had the highest lignin content (Table 1.9). This suggests that, seasons, years and environmental conditions play an important role in the overall lignification of forages. In spring with adequate precipitation, the effects of harvest frequencies are more emphasized than in low precipitation, prolonging the vegetative stage. In addition, NDF and ADF values shown in this study confirm these results, indicating that the higher NDF content when forages are kept in longer harvest frequencies are due to an increase in indigestible fiber (cellulose and lignin) rather than hemicellulose.

Lignin is a plant tissue that acts as a barrier to microorganisms in the rumen, which are then incapable of degrading and accessing its content. Once alfalfa reaches maturity, the leaf: stem ratio decreases and considering the higher lignification of stems as compared to leaves, the outcome is higher lignin (Nordkvist et al., 1986; Engels and Jung, 2005; Buxton et al., 1987; Albretch et al., 1987).

Considering the precipitation deficit that occurred during the 2016 growing season, these results suggest that when a drought occurs, nutritive value is affected, especially during summer, once that is combined with higher temperatures. Yet, according to Halim et al. (1989), alfalfa grown under water stress conditions can maintain its nutritive value by the translocation of CP from the leaves that are in senescence to the stems.

Overall, the results suggest that seasonal variation played an important role in nutritive value of forages. Maturity is known for having a negative effect on the overall forage nutritive value (Ball et al., 2007). It is known that as plants mature, the nutritive value of the forage declines due to increased fiber content resulting from thickening of cell walls and lignification, as well as an increased proportion of stems especially in legume forages (Albrecht et al., 1987; Sheaffer et al., 2000).

CONCLUSIONS

Harvest frequencies above 28 days are ideal for FM accumulation. Yet, harvest frequencies of 42 days or above tend to have increased lignification thus decreased nutritive value. Our results suggests that, regardless of the percentage of alfalfa in mixtures with either cool or warm-season grasses, alfalfa contributes to overall nutritive value of the canopy, reducing the need of N fertilizer application.

Once management practices are adopted, alfalfa has the potential to provide sufficient forage mass in the Southeast USA grown either in pure stands or in mixtures with tall fescue. Seasons and environmental challenges, such as drought, play an important role in productivity. In periods of drought, irrigation might be necessary to maintain high FM, especially during the summer. Adding alfalfa to previously established bermudagrass plots could be beneficial especially in summer, as well as to tall fescue fields during the spring and summers where drought does not occur.

APPENDIX A

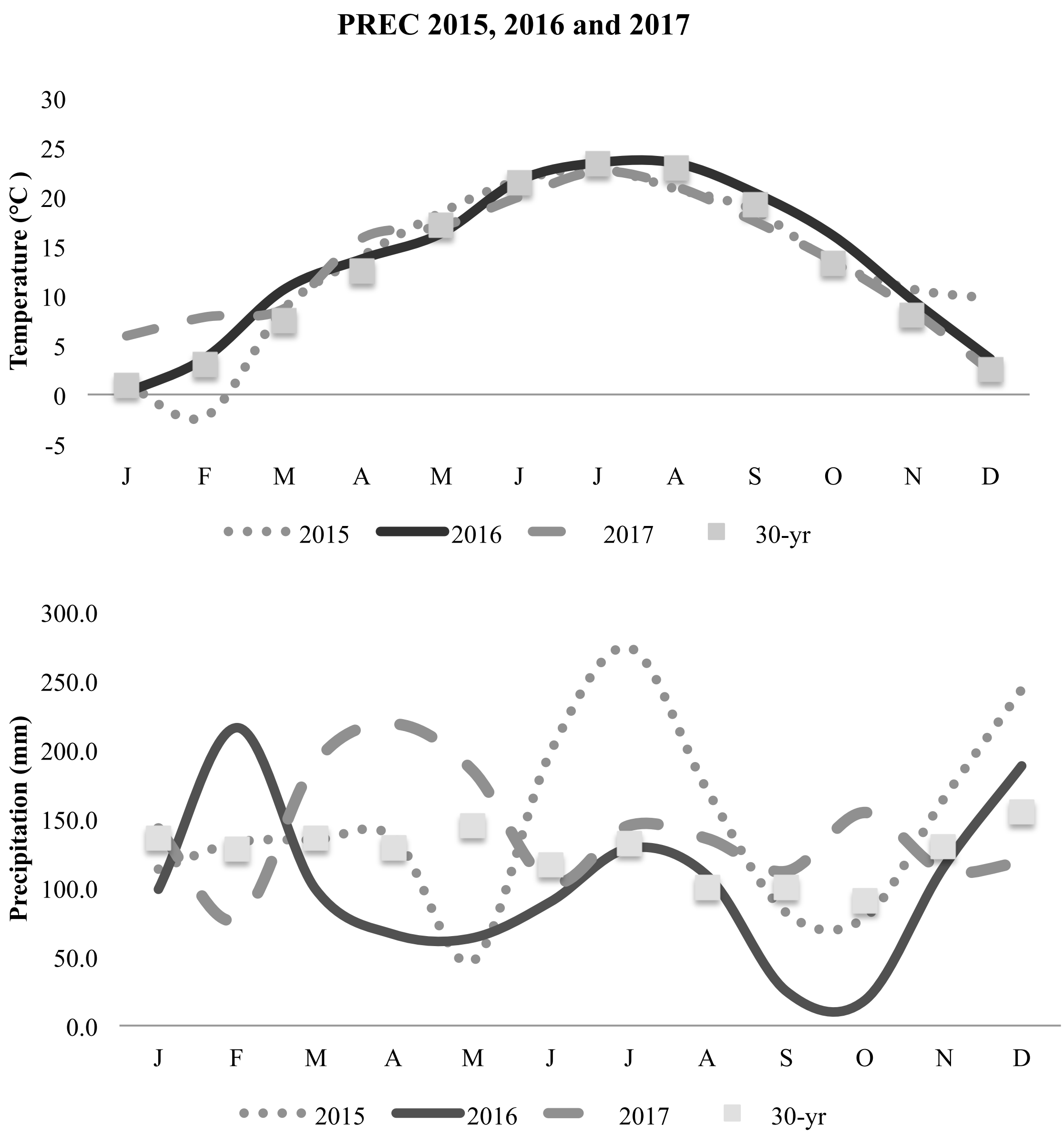

Figure 1.1. Weather for Plateau Research and Education Center, Crossville, TN, 2015-2017 including 30-year average.

Table 1.1 Harvests schedules of each imposed treatment (H1, 21 days harvest interval; H2, 28 days harvest interval; H3, 35 days harvest interval; H4, 42 days harvest interval).

First and last days of harvest of each harvest frequency					
2016					
		Harvest Frequencies			
		H1	H2	H3	H4
Harvests	First	May 3rd	May 10th	May 17th	May 24th
	Last	August 16th	August 30th	August 30th	August 16th
2017					
		Harvest Frequencies			
		H1	H2	H3	H4
Harvests	First	May 2nd	May 9th	May 16th	May 23rd
	Last	September 5th	August 29th	August 29th	August 15th

Table 1.2 Botanical composition of legume and grass in mixtures of alfalfa-tall fescue and alfalfa-bermudagrass (g kg^{-1}) subjected to four different harvesting frequencies, during spring and summer of 2016 and 2017 growing seasons.

		Legume and Grass mixtures (g kg^{-1})			
		‡**H1**	‡**H2**	‡**H3**	‡**H4**
			2016		
			Spring		
Legume	‡AB	717±66.5^{a,AB}	702±63.6^{a,A}	555±51.4^{a,BC}	554±136.7^{a,C}
	‡ATF	463±92.3^{b,AB}	633±93.3^{b,A}	283±89.4^{b,BC}	46±28^{b,C}
Grasses	AB	255±58.3^{b,BC}	276±59.3^{b,C}	414±50.6^{b,AB}	437±129.8^{b,A}
	ATF	494±84^{a,BC}	358±90.9^{a,C}	693±93.5^{a,AB}	948±32.1^{a,A}
Weeds	AB	28±9.4	22±5.6	31±3.5	9±6.1
	ATF	43±8.9	9±2.5	24±5.8	6±4.1
			Summer		
Legume	AB	594±92.3	562±22.4	528±36.3	444±61.2
	ATF	588±55.9	630±33.9	653±18.4	623±41.6
Grasses	AB	401±84	431±21.3	470±34.6	539±58.2
	ATF	403±49.5	362±33.1	343±18.7	365±39.5
Weeds	AB	5±8.9	7±2.2	2±2.6	17±3.9
	ATF	9±5.4	8±3.5	4±2.9	12±4.2
			2017		
			Spring		
Legume	AB	594±70.6^{a,A}	403±43.9^{a,B}	493±32.8^{a,B}	515±44.9^{a,AB}
	ATF	378±82.8^{b,A}	85±41.7^{b,B}	141±52.9^{b,B}	137±44.8^{b,AB}
Grasses	AB	383±66.5^{b,B}	558±41^{b,A}	479±30.8^{b,AB}	453±39.9^{b,AB}
	ATF	601±86.7^{a,B}	886±50.9^{a,A}	834±58.8^{a,AB}	839±49.2^{a,AB}
Weeds	AB	23±4.6	39±4.2	28±2.8	32±5.6
	ATF	21±4.7	29±11.1	25±7.6	24±3.5
			Summer		
Legume	AB	457±66.5^{a}	495±30.8^{a}	639±58.2^{a}	690±84.7^{a}
	ATF	318±42.8^{b}	221±31.6^{b}	69±99.5^{b}	250±99.8^{b}
Grasses	AB	519±69.9^{b}	470±29.6^{b}	346±55.7^{b}	302±88^{b}
	ATF	647±47^{a}	721±37.8^{a}	900±103.6^{a}	737±99.4^{a}
Other	AB	24±10^{b,AB}	35±1.9^{b,A}	15±3.9^{b,AB}	8±12.3^{b,B}
	ATF	35±12^{a,AB}	58±9.6^{a,A}	31±5.4^{a,AB}	13±10.9^{a,B}

(mean ± standard error)

Means within a column for each season without a common superscript letter differ in species effect ($P < 0.05$).

Means within a row for each season without a common upper case letter differ in treatment effect ($P < 0.05$).

‡ H1, 21 days harvest interval; H2, 28 days harvest interval; H3, 35 days harvest interval; H4, 42 days harvest interval; AB, alfalfa-bermudagrass; ATF, alfalfa-tall fescue.

Table 1.3 Total dry matter (DM) forage mass (FM kg ha^{-1}) during 2016 and 2017 growing seasons at Plateau Research and Education Center, Crossville, TN.

	Total ‡DM Forage Mass (kg ha^{-1})			
	‡H1	**‡H2**	**‡H3**	**‡H4**
		2016		
‡A	7255.05±478.7^{,C}	9530.15±726.5^{a,B}	10216.60±1111.7^{a,A}	7792.85±970.1^{a,AB}
‡AB	3503.53±318.8^{,C}	3661.30±535.9^{b,B}	5046.40±799.9^{b,A}	4821.99±669^{b,AB}
‡ATF	6287.46±877.7^{a,C}	7775.29±652.6^{a,B}	10682.45±742.9^{a,A}	9992.38±558.1^{a,AB}
		2017		
A	5604.2±486.7^{B}	7811.41±433.1^{A}	8042.79±1123.6 ^{A}	7167.88 ±439.9^{A}
AB	4866.49±789.2^{B}	7120.62±561.2^{A}	7817.94±765.9 ^{A}	7541.93±642.1^{A}
ATF	4707.64±466.9^{B}	6946.26±720.6^{A}	8277.85±513.6^{A}	8909.98±410.4^{A}

Means within a column for each season without a common superscript letter differ in species effect ($P < 0.05$).
Means within a row for each season without a common upper case letter differ in treatment effect ($P < 0.05$).
‡ DM, dry matter; H1, 21 days harvest interval; H2, 28 days harvest interval; H3, 35 days harvest interval; H4, 42 days harvest interval; A, alfalfa only; AB, alfalfa-bermudagrass; ATF, alfalfa-tall fescue.

Table 1.4 Total annual forage mass (FM kg ha^{-1}) during spring and summer of 2016 and 2017 growing seasons.

	Total annual mass in ‡DM (kg ha^{-1}) per season and year			
	‡H1	**‡H2**	**‡H3**	**‡H4**
		2016		
		Spring		
‡A	5574.10±308.5^{a,B}	5984.62±392.4^{a,B}	7201.85±794.9^{a,A}	4443.6±285.9^{a,B}
‡AB	448.76±59.4^{b,B}	321.09±139.2^{b,B}	1725.27±508.5^{b,A}	1021.6±175.5^{b,B}
‡ATF	5263.50±717.3^{a,B}	5182.42±297.2^{a,B}	7868.18±552.9^{a,A}	6411.6±248.1^{a,B}
		Summer		
A	1680.95±169.5^{ab,B}	3545.53±334^{ab,A}	3014.74±316.7^{ab,A}	3349.2±684.3^{ab,A}
AB	1849.83±190.7^{a,B}	3340.21±396.7^{a,A}	3321.13±291.4^{a,A}	3800.4±493.5^{a,A}
ATF	1023.97±160.4^{b,B}	2592.87±355.4^{b,A}	2814.27±189.9^{b,A}	3580.8±309.9^{b,A}
		2017		
		Spring		
A	4262.09±240.1^{a,C}	5690.12±221.2^{a,B}	6265.15±891.6^{a,A}	4017.2±221.3^{a,B}
AB	3326.58±523.6^{b,C}	4710.74±399.6^{b,B}	5999.56±525.1^{b,A}	3980.6±301.4^{b,B}
ATF	3370.26±232.5^{a,C}	4999.78±518.6^{a,B}	6670.75±341.6^{a,A}	5516.3±54^{a,B}
		Summer		
A	1342.12±246.6^{C}	2121.29±211.9^{B}	1777.63±231.9^{BC}	3150.7±218.6^{A}
AB	1539.91±265.6^{C}	2409.88±161.6^{B}	1818.38±240.8^{BC}	3561.3±340.7^{A}
ATF	1337.38±234.4^{C}	1946.48±202^{B}	1607.09±171.9^{BC}	3393.6±356.3^{A}

Means within a column for each season without a common superscript letter differ in species effect ($P < 0.05$).
Means within a row for each season without a common upper case letter differ in treatment effect ($P < 0.05$).
‡ DM, dry matter; H1, 21 days harvest interval; H2, 28 days harvest interval; H3, 35 days harvest interval; H4, 42 days harvest interval; A, alfalfa only; AB, alfalfa-bermudagrass; ATF, alfalfa-tall fescue.

Table 1.5 Average dry matter (DM) forage mass per harvest (DM kg ha^{-1}) during spring and summer of 2016 and 2017 growing seasons.

	‡DM Forage Mass (kg ha^{-1}) per harvest			
	‡**H1**	‡**H2**	‡**H3**	‡**H4**
		2016		
		Spring		
‡A	1858.0±437.2^{a,C}	2992.3±457.5^{a,BC}	3600.9±298.3^{a,AB}	4443.6±285.8$^{a, A}$
‡AB	218.0±59.5^{b,C}	160.5±72.2^{b,BC}	862.6±148.8^{b,AB}	1021.6±175.5^{b,A}
‡ATF	1754.5±458.8^{a,C}	2591.2±460.5^{a,BC}	3934.1±638^{a,AB}	6411.6±248.1^{a,A}
		Summer		
A	560.3±75.7^{C}	1181.8±93.5^{B}	1507.4±222.4^{A}	1674.6±262.5^{A}
AB	616.6±55^{C}	1113.4±99.4^{B}	1660.6±194.8^{A}	1900.2±170.5^{A}
ATF	341.3±54^{C}	864.3±93.3^{B}	1407.1±226.3^{A}	1790.4±148.3^{A}
		2017		
		Spring		
A	1420.7±191^{D}	2845.1±73.9^{C}	3132.6±330.8^{B}	4017.2±221.4^{A}
AB	1108.9±128.3^{D}	2355.4±151.3^{C}	2999.8±201.8^{B}	3980.6±301.4^{A}
ATF	1123.4±216.2^{D}	2499.9±285.9^{C}	3335.4±477.9^{B}	5516.3±54^{A}
		Summer		
A	335.5±61.7^{C}	707.1±125.1^{B}	888.8±243.8^{B}	1575.4±341.1^{A}
AB	385.0±69.7^{C}	803.3±155.9^{B}	909.2±241.9^{B}	1780.7±413.9^{A}
ATF	334.3±53.6^{C}	648.8±119^{B}	803.5±196^{B}	1696.8±374.3^{A}

(mean ± standard error)
Means within a column for each season without a common superscript lower case letter differ in species effect (P < 0.05).
Means within a row for each season without a common upper case letter differ in treatment effect (P < 0.05)
‡ DM, dry matter; H1, 21 days harvest interval; H2, 28 days harvest interval; H3, 35 days harvest interval; H4, 42 days harvest interval; A, alfalfa only; AB, alfalfa-bermudagrass; ATF, alfalfa-tall fescue.

Table 1.6 Average crude protein (CP) in g kg^{-1} during spring and summer of 2016 and 2017 growing seasons.

	‡CP (g kg^{-1})			
	‡H1	‡H2	‡H3	‡H4
2016				
Spring				
‡A	257.91±10.8^{a}	237.88±11.7^{a}	205.04±8.4^{a}	190.91±6.2^{a}
‡AB	149.35±32.5^{b}	175.20±15.2^{b}	181.53±10.7^{b}	161.80±11.8^{b}
‡ATF	195.94±13.3^{b}	193.35±14^{b}	171.50±16.9^{b}	127.00±5.4^{b}
Summer				
A	273.27±3^{a,A}	256.31±3.9^{a,B}	250.87±4.8^{a,C}	204.04±3.8^{a,D}
AB	208.17±5.4^{c,A}	192.87±3.9^{,B}	181.15±5.8^{c,C}	171.71±3.7^{,D}
ATF	223.57±4^{b,A}	221.93±2.6^{,B}	212.51±1.9^{b,C}	196.33±1.9^{b,D}
2017				
Spring				
A	275.80±9.1^{a,A}	225.04±6.3^{a,B}	216.99±3.2^{a,B}	232.55±3.4^{a,B}
AB	249.18±10.6^{a,A}	214.25±7^{a,B}	219.41±4.4^{a,B}	211.60±5^{a,B}
ATF	213.94±18.2^{b,A}	172.19±16.8^{b,B}	176.20±12.8^{b,B}	155.55±4.3^{b,B}
Summer				
A	169.62±19	202.14±13.6	227.64±9.6	215.35±5.4
AB	184.71±9.4	174.92±15	193.58±11.4	202.03±5
ATF	198.99±6	180.08±6.3	181.84±7	181.61±6.5

(mean ± standard error)
Means within a column for each season without a common superscript letter differ in species effect ($P < 0.05$).
Means within a row for each season without a common upper case letter differ in treatment effect ($P < 0.05$).
‡ DM, dry matter; H1, 21 days harvest interval; H2, 28 days harvest interval; H3, 35 days harvest interval; H4, 42 days harvest interval; A, alfalfa only; AB, alfalfa-bermudagrass; ATF, alfalfa-tall fescue.

Table 1.7 Average neutral detergent fiber (NDF) in g kg^{-1} during spring and summer of 2016 and 2017growing seasons.

	‡NDF (g kg^{-1})			
	‡H1	‡H2	‡H3	‡H4
		2016		
		Spring		
‡A	316.99±12.8^{b,B}	340.06±13.8^{b,AB}	375.63±7.2^{b,A}	418.04±13.7^{b,A}
‡AB	272.51±59.7^{b,B}	415.85±15^{b,AB}	420.09±18.4^{b,A}	446.38±31.4^{b,A}
‡ATF	445.43±15.3^{a,B}	446.50±21.9^{a,AB}	469.53±24.6^{a,A}	543.95±12.4^{a,A}
		Summer		
A	286.18±7.2^{c,C}	299.05±4^{c,C}	340.40±12.6^{c,B}	401.71±5.7^{c,A}
AB	435.18±11.7^{a,C}	435.57±9^{a,C}	481.19±8.4^{a,B}	491.36±11^{a,A}
ATF	428.96±9.7^{b,C}	403.95±8.2^{b,C}	439.43±6.4^{b,B}	443.00±10.9^{b,A}
		2017		
		Spring		
A	284.85±7.6^{c,C}	332.59±5.6^{c,BC}	379.74±7.3^{c,B}	389.18±8.2^{c,A}
AB	439.02±7.8^{b,C}	446.49±4.3^{b,BC}	475.22±4.9^{b,B}	525.90±4.4^{b,A}
ATF	498.29±23.9^{a,C}	525.03±30.9^{a,BC}	535.44±24^{a,B}	611.43±5.2^{a,A}
		Summer		
A	370.71±40^{b}	388.82±19.7^{b}	363.31±15.4^{b}	402.83±11.7^{b}
AB	519.66±14.8^{a}	516.34±22.8^{a}	510.60±9.9^{a}	513.03±12.7^{a}
ATF	523.60±9.1^{a}	536.73±17.1^{a}	579.35±14.9^{a}	559.89±7.9^{a}

(mean ± standard error)
Means within a column for each season without a common superscript letter differ in species effect (*P* < 0.05).
Means within a row for each season without a common upper case letter differ in treatment effect (*P* < 0.05).
‡ DM, dry matter; H1, 21 days harvest interval; H2, 28 days harvest interval; H3, 35 days harvest interval; H4, 42 days harvest interval; A, alfalfa only; AB, alfalfa-bermudagrass; ATF, alfalfa-tall fescue.

Table 1.8 Average acid detergent fiber (ADF) in g kg^{-1} during spring and summer of 2016 and 2017 growing seasons.

	‡ADF (g kg^{-1})			
	‡H1	‡H2	‡H3	‡H4
		2016		
		Spring		
‡A	230.06±9.9^{b,B}	256.07±11^{b,A}	283.55±5.2^{b,A}	309.47±12.7^{b,A}
‡AB	180.33±39.2^{b,B}	287.78±11.5^{b,A}	288.84±12^{b,A}	310.40±11.7^{b,A}
‡ATF	291.56±9.4^{a,B}	309.18±13.6^{a,A}	320.29±13.5^{a,A}	365.08±6.2^{a,A}
		Summer		
A	204.24±4.6^{c,C}	220.09±3.5^{c,C}	251.66±9.8^{c,B}	313.23±5.7^{c,A}
AB	273.2±3.9^{a,C}	278.31±3.9^{a,C}	301.99±5^{a,B}	318.73±5.8^{a,A}
ATF	262.21±4.2^{b,C}	259.58±4.2^{b,C}	280.34±5.7^{b,B}	308.88±6.1^{b,A}
		2017		
		Spring		
A	200.05±5.7^{b,D}	245.96±3.8^{b,C}	293.24±6.4^{b,B}	289.80±6.8^{b,A}
AB	223.71±6^{b,D}	251.09±5.5^{b,C}	273.84±4.9^{b,B}	300.33±3.6^{b,A}
ATF	253.70±14^{a,D}	287.29±15.1^{a,C}	294.05±12.2^{a,B}	350.38±2.9^{a,A}
		Summer		
A	244.24±25.3^{B}	271.62±10.9^{AB}	267.81±9.3^{AB}	312.29±11.2^{A}
AB	264.52±8.2^{B}	275.80±12.8^{AB}	276.23±6.8^{AB}	296.16±5.7^{A}
ATF	263.38±4.2^{B}	282.09±7.3^{AB}	296.95±6.6^{AB}	308.68±2^{A}

(mean ± standard error)
Means within a column for each season without a common superscript letter differ in species effect ($P < 0.05$).
Means within a row for each season without a common upper case letter differ in treatment effect ($P < 0.05$).
‡ DM, dry matter; H1, 21 days harvest interval; H2, 28 days harvest interval; H3, 35 days harvest interval; H4, 42 days harvest interval; A, alfalfa only; AB, alfalfa-bermudagrass; ATF, alfalfa-tall fescue.

Table 1.9 Average lignin in g kg^{-1} during spring and summer of 2016 and 2017growing seasons.

	‡Lignin (g kg^{-1})			
	‡H1	**‡H2**	**‡H3**	**‡H4**
		2016		
		Spring		
‡A	48.84±1.7^{a,C}	55.20±2.6^{a,B}	63.23±1^{a,AB}	69.87±2.3^{a,A}
‡AB	23.43±5^{c,C}	35.40±1.2^{c,B}	37.43±1.8^{c,AB}	41.90±1.1^{c,A}
‡ATF	32.03±0.7^{b,C}	41.75±1.6^{b,B}	43.08±1^{b,AB}	49.05±1^{b,A}
		Summer		
A	44.99±1^{a,D}	47.98±1^{a,C}	55.46±2.5^{a,B}	69.68±1.4^{a,A}
AB	35.14±0.6^{b,D}	38.08±1.2^{b,C}	40.75±1.6^{b,B}	46.68±1.3^{b,A}
ATF	33.48±0.7^{b,D}	37.18±1.2^{b,C}	37.74±1.5^{b,B}	53.03±1.4^{b,A}
		2017		
		Spring		
A	43.44±1.5^{a,D}	51.90±1.1^{a,C}	63.05±1.4^{a,B}	64.00±1.6^{a,A}
AB	45.33±0.9^{a,D}	48.24±1^{a,C}	58.69±1.1^{a,B}	61.93±0.6^{a,A}
ATF	39.50±1^{b,D}	43.63±1.7^{b,C}	49.01±0.9^{b,B}	60.73±1.4^{b,A}
		Summer		
A	51.68±5.3^{a,C}	57.23±2.4^{a,BC}	58.40±1.8^{a,B}	68.59±2.9^{a,A}
AB	45.63±1.1^{b,C}	48.84±2.1^{b,BC}	54.96±1.8^{b,B}	65.38±2.1^{b,A}
ATF	42.21±1.2^{c,C}	43.07±1.5^{c,BC}	46.79±1.6^{c,B}	55.29±3.2^{c,A}

(mean ± standard error)
Means within a column for each season without a common superscript letter differ in species effect ($P < 0.05$).
Means within a row for each season without a common upper case letter differ in treatment effect ($P < 0.05$).
‡ DM, dry matter; H1, 21 days harvest interval; H2, 28 days harvest interval; H3, 35 days harvest interval; H4, 42 days harvest interval; A, alfalfa only; AB, alfalfa-bermudagrass; ATF, alfalfa-tall fescue.

CHAPTER 2:

Persistence of alfalfa and mixtures subjected to different harvest intervals in the Southeast USA

ABSTRACT

Several conditions can influence the persistence of a forage in the field; for this reason, persistence is a parameter of paramount importance when establishing a forage system. Forage management of species such as alfalfa can be challenging, especially in mixtures with grasses. The objective of this study was to determine the persistence of alfalfa when grown alone and in mixtures with tall fescue (*Lolium arundinaceum* (Schreb.) Darbyish) and bermudagrass *(Cynodon dactylon* (L.) Pers). Three species combinations were utilized [alfalfa (A), alfalfa-tall fescue (ATF) and alfalfa-bermudagrass (AB)] and subjected to four different harvesting frequencies (21, 28, 35 and 42 days) throughout the 2016, 2017 and 2018 growing seasons at the University of Tennessee Plateau AgResearch and Education Center (PREC) in Crossville, TN. Samples were collected during this period for analysis of forage mass on the first and last day of harvest schedule to evaluate the fluctuations in nutritive value. Based on FM, results showed that persistence of A (P = 0.0042), AB (P = 0.0002), and ATF (P = 0.0007) decreased at the third year of growth, and different harvest schedules should be followed for each species combination for increased persistence in the field. For A and AB, harvest frequencies should be 35 days and for ATF, 42 days. Mixtures of ATF suppressed alfalfa growth, but the overall productivity was higher than AB mixtures, suggesting that the use of alfalfa in ATF plots is advantageous despite its high percentage in the field; yet, the nutritive value was determined by the amount of alfalfa and percentage of weeds.

INTRODUCTION

The decision of investing in alfalfa seeds by producers is dependent on how long the stand can persist in the area. Ideally, it should be productive for as long as possible after the stand is established. The persistence of alfalfa in the Southeast U.S. will depend on the management practices adopted throughout the years, environmental influences, and if grown in mixtures, by the competitiveness with alfalfa that the companion forages may present.

Forage yield is related to its persistence on the field (Brown, 2005), and legumes are considered very fragile species in these systems (Beuselinck, 1994). It is known that alfalfa does not reseed itself, therefore its density decreases if management practices and fertility are not adequate (Beuselinck, 1994; Wiersma, 1998). According to Beuselinck (1994), the pattern of defoliation in legume forages like alfalfa influences the persistence, and harvest at vegetative stages decreases persistence when compared to stands cut at flowering stage.

Warm summers are commonly experienced in the Southeast USA, and frequent harvesting does not allow alfalfa to restore its roots' carbohydrates which affects its regrowth. Also, high temperatures are known to decrease root weight (Rice et al., 1989; Smith et al., 1969; Feltner and Massengale, 1965). For this reason, assessing the harvest interval adopted in the field is important in the Southeast. These results are crucial in determining how long a stand can persist and what is the optimum rest period the forage requires to maintain its accumulation throughout the years, especially when managing swards of legume-grass mixtures. Stringer et al. (1994) observed that alfalfa in bermudagrass plots decreased bermudagrass vigor due to shading and competitiveness for water. Alfalfa in mixtures with tall fescue can have stand decline depending on the cultivar utilized and management practices adopted (Smith et al., 1992).

Our study assessed alfalfa and alfalfa mixture plots in order to determine its persistence and overall productivity in the field. Considering the importance of maintaining adequate forage mass for as long as possible throughout the growing seasons, the objective of this study was to evaluate these species subjected to different harvest intervals for three consecutive years, hypothesizing that longer harvest intervals would result in longer persistence of alfalfa and mixtures in the field.

MATERIALS AND METHODS

Site description

This study was conducted at the Plateau Research and Education Center (PREC) in Crossville, Tennessee (36°01′ N, 85°12′ W) from July 2015 to September 2018. The soil conditions at the location were Lily loam (fine-loamy residuum weathered from sandstone, 2-6% slopes, well drained, 21 to 39 inches to lithic bedrock) (NRCS, 2018). Initial soil nutrient levels on the experiment site were pH = 5.5, P = 77kg/ha^{-1}, K = 247.7 kg/ha^{-1}, Ca = 1484 kg/ha^{-1}, and Mg = 62.8 kg/ha^{-1}. The experiment was conducted on 48 experimental units that were 3 x 6 m plots in a split-plot arrangement of a randomized complete block design.

The treatments consisted of three species combinations, four harvest frequencies and four replications totaling 48 plots. The species were a grazing tolerant cultivar of alfalfa (cv. Ameristand 403T Plus) (A) grown in monoculture and as a mixture with a perennial cool-season grass novel-endophyte tall fescue (cv. Texoma Max Q II) (ATF) and with a perennial warm-season grass bermudagrass (cv. Vaughn’s #1) (AB), and the harvest frequencies were 21 days [H1], 28 days [H2], 35 days [H3] and 42 days [H4] harvest.

On July 16th 2015, the ground was tilled and vegetative bermudagrass was planted via sprigging in the designated area. On September 4th 2015, using a 10' Great Plains No Till Drill, tall fescue and alfalfa were seeded at 11 kg/ha and 17 kg ha^{-1}, respectively. For establishment of ATF, 67 kg ha^{-1} of N (34-0-0) was applied exclusively on the plots where ATF mixtures were present.

On October 7th 2015, 67 kg/ha of N was applied onto ATF plots to aid establishment of tall fescue. On October 26th 2015, all plots were treated with 7 tons of lime per ha. On February 2nd 2016, February 24th 2017 and February 15th 2018 boron was applied to all plots at 2 kg ha^{-1}, as this micronutrient is required for alfalfa production. Since this is such a small amount, boron was mixed with sand to allow for better distribution within the whole experimental area. On March 8th 2016, alfalfa was reestablished on the bermudagrass plots at 17 kg ha^{-1} using a hege small tube drill due to the low density of alfalfa in the plots from the previous seeding. On May 25th 2016, 2,4-DB 200 (Agrisolutions, WindField, MN) was applied to all AB plots to control broadleaf weeds. Due to the dry conditions in Tennessee in 2016 (Figure 1.1), an irrigation gun on reel system was utilized over the entire experimental area on September 23rd and 24th 2016. On April 6th 2017, Chlorpyrifos (DowAgrosciences, Canada Inc.) was applied to control alfalfa weevil, and on May 9th 2017, potato leafhopper was controlled with the same insecticide as for the alfalfa weevil. Following the soil recommendations of 2018, 200 kg ha^{-1} of K was applied onto the field split into two applications, one on April 11th and the second application on June 13th 2018. Also on June 13th, 2018 Chlorpyrifos

(DowAgrosciences, Canada Inc.) was applied to control alfalfa weevil and alfalfa leafhopper (1L ha^{-1}) and 2-4 DB (4.6 L ha^{-1}) to control broadleaf weeds; Select Max Herbicide (Valent, Walnut Creek, CA) was applied to alfalfa only plots (2.3 L ha^{-1}) to control grasses that were invading the plots.

Harvest frequencies were set for each species combination from May to September of 2016, 2017 an 2018, H1plots followed a harvest schedule of 21 days, H2 plots followed a harvest schedule of 28 days, H3 plots followed a harvest schedule of 35 days, and H4 plots followed a harvest schedule of 42 days (Table 2.1). On September 13th 2016 and September 19th 2017, all plots were cut to a 4-cm stubble height to prepare for the growing season of the following year.

Measurements

To determine forage mass, a Carter 3' (Carter, Brookston, Indiana) forage harvester with 0.9 x 6 m harvest size was used to collect forage material of each experimental unit from the center of each plot. The harvested material was collected and weighed using a 121 L bucket from each experimental unit. A bulk sample was then collected from the bucket and dried in a forced air dryer at 60°C up to constant weight for determination of total DM forage mass. These bulk samples collected for determination of the DM were then ground in a Wiley Mill Grinder (Thomas-Wiley Laboratory Mill Model 4, Arthur H. Thomas Co., Philadelphia, PA) using 1-mm screen for nutritive value analyses. Samples were analyzed for crude protein (CP) and neutral detergent fiber (NDF) at the first and the last sampling day for each treatment and species. These samples were scanned in small ring cups on Near-infrared spectroscopy (NIRS) technology (Unity SpectraStar XL-R, Unity Scientific, Milford, MA). Equations for the forage nutritive analyses were standardized and checked for accuracy with the 2016-2017 Grass Hay and Legume Hay equation developed by the NIRS Forage and Feed Consortium (NIRSC, Hillsboro, WI). The Software used was Infostar version 3.11.3 3 (Unity Scientific, Milford, MA). The Global *H* statistical test compared the samples against the model and other samples within the database for accurate results, where all forage samples fit the equation with the ($H < 3.0$) and are reported accordingly (Murray and Cowe, 2004).

To determine botanical composition in both ATF and AB mixture plots, pure samples of tall fescue, bermudagrass and alfalfa were collected to develop models to determine the calibration curves in the NIRS. Avoiding the area collected for determination of FM in 2016 and 2017, a 0.1-m^2 quadrant was placed in each of the mixed plots. The material inside the quadrant was cut (at a 5-cm stubble height) and manually separated into alfalfa, tall fescue, bermudagrass and weeds (if present). In 2016, only one collection was effectuated for separation of the botanical composition; the collection occurred on August 23rd for all species and treatments. In 2017, the H1was collected on May 2nd, July 5th and September 5th 2017. The H2 was collected on May 9th, July 5th and August 30th 2017. The H3 was collected on May 15th, June 20th and August 30th 2017. The H4 was collected on May 23rd, July 5th and August 15th 2017. This material was then ground to 1-mm aid the models developed in the NIRS.

Unstandardized spectra were collected for each ratio from 0 to 100 percent by 10 percent increments for each mixture. The software used to develop this calibration model was UCA (Unity Scientific, Milford, MA). Initial spectra were used to make a calibration model to predict botanical composition of field samples. This initial spectra collection included 115 total spectra, 5 replications per category of grass to legume percentages that were loaded into UCAL. Percentage reference values were associated with the scanned spectra. Extremes of 0 to 10 percent and 90 to 100 percent of the pure material had to have additional material prepared for spectra collection using the 5 duplicate scans per tier. Regression selections were made using the PLS function with maximum factors of 7 used for the final model. Outliers and rejections were removed with outer limits of 3.000. Validation predictions were used with spectra with a final STDEV=0.14. Further model expansion was used with 151 field samples manually separated for the actual botanical composition of both ATF and AB mixtures. Re-prediction of the final percentages reported was then performed. The final statistics in the NIRS model were global distance (GD)=0.80, T-value= 0.04, standard error of calibration (SEC) = 1.51 and cross validation (CV)= 0.09, with prediction fitting the allowable H<3.0 (Murray and Cowe, 2004).

Due to the increased percentage of weeds in 2018, the models developed in the NIRS were not utilized in 2018 and manually separated samples were utilized to determine the botanical composition. In 2018, the H1was collected on May 1^{st}, July 3^{rd} and September 4^{th} 2018. The H2 was collected on May 8^{th}, July 3^{rd} and August 30^{th} 2018. The H3 was collected on May 15^{th}, July 24^{th} and August 30^{th} 2018. The H4 was collected on May 22^{nd}, July 3^{rd} and August 14^{th} 2018.

Statistical analysis

Mixed model analyses of variance were performed to determine differences in least square means among species composition and harvest. Models were performed using the PROC MIXED procedure in SAS (Cary, NC, 9.4). The dependent variables tested were heights, CP and NDF, with the fixed effects of species combination, harvest intervals, and their two-way interaction. The total FM was analyzed with species, harvest intervals and year as fixed effects as well as the random effect of species within rep included in the model.

Within two of the species combinations (ATF and AB), the dependent variables of legume and grass percentages were analyzed by the first and last sampling schedules and as an average of both data points. All results were evaluated for significance at $P < 0.05$.

RESULTS AND DISCUSSION

Weather

In 2015, May through September temperature was 0.3°C below the 30-yr average. Precipitation in 2015 from May through September was 29% above the 30-yr average (770 mm). In 2016, May through September temperature was 0.2°C above the 30-yr average. Precipitation in 2016 from May through September was 30% below the 30-yr average. In 2017, May through September temperature was 1.2°C below the 30-yr average. Precipitation in 2017 from May through September was 14% above than the 30-yr average. In 2018, May through September temperature was 7.1°C below the 30-yr average. Precipitation in 2018 from May through September was 4% above than the 30-yr average (Figure 2.1).

Tiller counting throughout the growing season

Differences in the amount of tillers were inconsistent (Table 2.2), however, for all differences observed between first and last sampling days within each harvest frequency and species, it was observed that the first sampling day had a higher number of alfalfa tillers than the last sampling day (Table 2.2, upper-case letters), this was observed in the 2017 H1 of A and AB (A: $P < 0.01$; AB: $P < 0.03$), 2018 H2 and H3 of A and AB (A: $P < 0.01$; AB: $P < 0.03$) and 2018 H4 of AB. In addition, all differences observed within a harvest frequency and sampling day showed the decrease in persistence of alfalfa based on tiller count in 2018 (Table 2.2, lower-case letters). This assumption is a reflection of the decrease in tiller counting in 2018 when compared to 2017. This pattern was observed in A plots at the first sampling day of H1 and H4, and on the last sampling day of H2, H3 and H4 (Table 2.2). In AB plots, this pattern was observed in the first sampling day of H1 and on the last sampling day of H3 and H4 (Table 2.2). There were only year differences in ATF treatments ($P < 0.01$); this way 2017 had higher number of tillers than 2018 for all harvest frequencies (Table 2.1).

These results are in agreement with the findings of Pearen and Baron (1996), which observed that as stands aged, alfalfa tillers decrease its density on the field. Also, it is known that young stands are generally denser than older stands (Lafarge and Loiseau 2002). In addition, the number of tillers were higher in the beginning of the season (first harvest), likely due to the rapid mobilization of roots carbohydrates after dormancy, that lead to a increase in plant growth and branching (Li et al., 1996). However, this pattern was observed only in A and AB plots (Table 2.1), which is likely due to the lower presence of alfalfa plants in ATF plots.

The observed differences in harvestings, demonstrate overall advantage of tiller number in longer harvest frequencies. In 2017, alfalfa did not show differences within harvests in the first sampling day, but on the last sampling day H4 ($P = 0.008$) showed higher tiller counting, followed by H2 and H3 ($P = 0.3$), and H1 (P = 0.03; Table 2.2). In 2018, on the first sampling day, differences were only observed on H1 ($P < 0.04$), which had less tillers compared to all the remaining harvests, while on the last sampling day, the differences were more pronounced, with H3 and H4 having more tillers, but not different from each other ($P < 0.01$; Table 2.2).

In 2017, AB mixtures also did not show differences, and in 2018 had the same response as A in the first sampling day (Table 2.2). On the last sampling day, the number of alfalfa tillers was also higher in H3 and H4 harvest frequencies, followed by H2 ($P < 0.05$), then H1 in 2017. In 2018, H1 was lower than H4, probably due to the higher percentage of alfalfa in longer harvests frequencies (Table 2.2).

Alfalfa can shade bermudagrass and decrease its vigor (Stringer et al., 1994), expressing then its grow pattern more effectively. Meanwhile, ATF did not show differences among harvesting frequencies ($P = 0.55$; Table 2.2). The lower number of tillers with shorter harvest frequencies was attributed to the excessive removal of photosynthetic leaves at harvest, which decreases tiller density (Cuomo et al., 1998; Jones and Tracy, 2018; Chatterton et al, 1974). In agreement to the previous studies, an experiment conducted by Cowett and Sprague (1962) observed that harvest alfalfa at more mature stages leads to an increase in the number of stems when compared to young stages.

Although results were inconsistent, it is possible to infer that with increased harvest frequencies, the presence of alfalfa in the field is more consistent, and overall, harvest frequencies of 21 days, tend to have lower tiller counting. Also, when comparing both years within a specific harvest frequency, the second year showed a decrease in the number of tillers, an indicator of decreased persistence for all species and harvest frequencies.

Botanical composition differences during the first and last sampling days of the growing season

Different growth habits throughout the growing season can shift the botanical composition of mixtures; and for this reason, assessing the percentage of legumes and grasses on the first and last day of imposed harvest schedules are important.

I. Alfalfa-bermudagrass mixtures

In 2016, there were no species differences for percentage of legume or grass on the first and last day of harvest, but weed proportions shifted with sampling dates (Table 2.3). Differences between the first and last day of harvest is observed on H1, H2, and H3, with reduced weed proportions on the last sampling day as compared to the first day (P = 0.0009; Table 2.3). At the beginning of the season, the temperature required for growth and germination is as suitable for alfalfa as for weeds (Baskin and Baskin, 1985); therefore, the amount of weeds is higher as compared to the end of the season.

In 2017, species differences were only observed for weed percentage and followed the same pattern observed in 2016, with a higher weed proportion at the beginning of the season (P = 0.0029; Table 2.3). In 2018, there was a higher percentage of legumes on the first day as compared to the last day in H2, H3 and H4 (P = 0.0002; Table 2.3), indicating that shorter harvest frequencies maintained the percentage of alfalfa throughout the growing season. No differences were observed regarding the grass component of AB mixtures, and weeds only showed differences between the first and last sampling day of H2, H3 and H4 (Table 2.3). In 2016 and 2017, the percentage of weeds in AB plots tends to follow the pattern of the percentage of legumes for the third year. For the first and last sampling days of AB, when legume percentage was higher, the weed percentage was lower, indicating that alfalfa plays an important role in weed suppression when in mixtures with bermudagrass. Once persistence starts to fade at the end of the season, and the percentage of grasses drastically decreases compared to the previous years, more resources are available for weed growth (Table 2.3).

In 2016, only weeds at the first sampling day showed treatment differences (*P* = 0.0009; Table 2.3). On the first sampling day, as harvest frequencies increased, the percentage of weeds was lower, with H1being the highest, followed by H2 and H3, and the lowest observed in H4, which is attributed to the increased shading of AB mixtures over weeds, suppressing their growth (Table 2.3). In 2017, the percentage of legume increased as harvest frequencies increased at both the beginning and end of the season (Table 2.3). However, the percentage of grasses followed a different pattern, with differences only at the last sampling day where as harvest frequencies increased, percentage of bermudagrass in AB plots decreased. This occurs because mature alfalfa starts shading bermudagrass causing it to not receive enough light necessary for growth. In addition, the last sampling day of H3 and H4 were August 28th and August 30th, respectively, corresponding to the period where bermudagrass starts to decrease its production, entering dormancy as temperature declines (Figure 2.1).

In 2018, the percentage of legumes increased as harvest frequencies increased (Table 2.3). There were no differences in percentage of grass among mixtures, and the weed percentage decreased (opposite pattern of legumes), which is expected, considering that longer harvest frequencies allow the main species to predominately grow.

It is important to notice at this point that there is a decline in the percentage of legumes and grasses during the third year, indicating the alfalfa persistence starts to decline at this time when mixed with bermudagrass (Table 2.3). Another indicator of decreased persistence of AB mixtures in 2018 is the percentage of weeds, which was considerably higher when compared to 2016 and 2017 (Table 2.3).

II. Alfalfa-tall fescue mixtures

In 2016, species differences were observed for legume, grass and weeds percentages in all harvest frequencies ($P = 0.0009$; Table 2.4). The percentage of legumes in tall fescue mixtures was higher on the last sampling day when compared to the first, and the opposite was true for percentage of grasses, as the first day was higher than the last (Table 2.4). In ATF mixtures, tall fescue can overcome the production of alfalfa (Smith et al., 1992), which was confirmed by our results. However, on the last sampling day, alfalfa showed higher competitiveness with tall fescue as opposed to spring growth, which corresponds to a period of tall fescue rapid growth (Nave et al., 2013).

In 2017, only a few differences were observed in species effect. For H4, the legume percentage in ATF mixture was higher on the first sampling day compared to the last, and the opposite was true for grasses, as the amount of grasses was higher on the last sampling day (P = 0.0124; Table 2.4). Also, these results conflict with the previous year, and since 2016 had abnormal precipitation, it is likely that weather conditions influenced botanical composition.

In 2018, alfalfa seemed to have recovered from the 2017 scarcity, showing a higher percentage within the mixture (Table 2.4). Yet, first and last sampling dates were not different in legume proportions for most treatments, with only H4 showing higher legume percentage on the last day of sampling as compared to the first. These results show a possible decline in alfalfa persistence when ATF mixtures are harvested more frequently. At the same time, grass density decreased on the last sampling day, providing space and resources for weed density to increase as observed (Table 2.4).

As for harvest frequencies differences in 2016, on the first sampling day, percentage of legumes was higher at H2 than all other treatments (Table 2.4), indicating that 28 days would be the threshold for alfalfa to effectively compete with grasses at the beginning of the season and suggesting that at the beginning of the season, harvest frequencies are crucial for increased persistence of alfalfa in tall fescue mixtures. At the end of the season, as H2, H3, and H4 did not differ, any of those harvest frequencies would aid alfalfa growth. However, TN faced a drought in 2016; therefore, the results could have reflected this event. During a drought period, the forages have their maturity delayed, which explains the decreased percentage of grass within mixtures at the end of the season, and alfalfa, being drought tolerant, was able to outcompete effectively

(Peterson et al., 1992; Smith et al., 1992). Percentage of weeds in 2016 was higher for H1than all other treatments on both first and last sampling date, indicating that weed control is extremely dependent on the canopy density of the main species. Meanwhile for H4, the weed proportions were higher at the last sampling day (Table 2.4), probably due to the dry conditions, and also, since the stand was fairly new at this time, the mixture was not strongly established, creating an environment suitable for weed growth and competition which could result in decreased persistence over time (Peterson et al., 1992).

In 2017, differences in harvest frequencies were only observed during the first sampling date for legume and grass proportions ($P = 0.0124$; Table 2.4). Also, these results conflict with the previous year, and since 2016 had abnormal precipitation, it is likely that weather conditions influenced botanical composition. On the last sampling date, alfalfa nearly disappeared from the plots (Table 2.4); yet, alfalfa was still present during mid-season (data not shown).

In 2018, differences in harvest frequencies were observed on the last sampling day for legume, with H4 having the highest legume percentage (Table 2.4). Differences were also observed for the grass component on the first day of sampling, with H1 presenting the lowest grass percentage compared to the other treatments (Table 2.4). Differences regarding the weed component of 2018 ATF mixtures were observed in both sampling days, and as harvest frequencies increase, the weed percentage is lower.

Although weed proportions increased considerably in 2018, longer harvest frequencies appear to aid alfalfa growth while suppressing weed presence in ATF mixtures, indicating that persistence may be possible if management is lenient.

Total forage mass

The total FM of A plots decreased with years (*P* = 0.0042; Table 2.5). In H1, H2 and H3, the decrease was exponential, with 2016 being the most productive, followed by 2017 and 2018 as expected. Meanwhile in H4, the FM of 2016 and 2017 remained equal, decreasing only in 2018 by approximately 35% (Table 2.5). This indicates that the persistence of A starts to decline after three years, and harvest frequencies are important if persistence of the stand is desired.

Total FM in AB plots changed within years (*P* = 0.0002, Table 2.5). For H1, 2016 showed higher FM for AB plots than 2017 and 2018, while for H2 and H3, 2016 and 2018 were lower than 2017. In H4, 2016 showed lower FM, followed by 2018 and then 2017 (Table 2.5). The inconsistency observed for AB mixtures shows the effect of drought and harvest frequency variability within AB. Even though the 2016 and 2018 growing seasons had lower FM than 2017, most of the total mass in 2018 was made of weeds (Table 2.5).

In ATF mixtures, the total FM of ATF mixtures decreased with years (*P* = 0.0007; Table 2.5). In H1 and H3, the decline was exponential, and although in H2 and H4 FM was held constant in the first two years, it also decreased in 2018 (Table 2.5).

For A plots in 2016, H2 and H3 were higher than H1 and H4 (Table 2.5), suggesting that in dry conditions longer harvest frequencies can be as detrimental for forage growth as short harvest frequencies. In these conditions, leaves start to fall, decreasing the leaf: stem ratio and increasing shading on bottom leaves (Marten and Hovin, 1980; Fuess and Tesar, 1968). In 2017, harvest frequencies above 21 days did not differ, and therefore did not impact FM (Table 2.5). However, persistence of alfalfa on A plots declined in 2018, with H1and H2 showing the lowest total FM, suggesting that overall, adequately managing harvest frequencies (more specifically above 28 days) can increase persistence of alfalfa (Table 2.5).

Differences were found among harvest frequencies in AB, with H3 and H4 showing highest total FM for all years (with the exception of 2017 in which H2 also had highest FM), indicating that AB mixtures have a better chance to succeed when managed at higher harvesting frequencies. However, the third year had a considerable amount of weeds, decreasing overall persistence of alfalfa.

In ATF, for all years an increase in harvest frequencies led to an increase in FM; therefore, similar to A plots, for the maintenance of stand persistence as well as FM production, harvests at H4 seemed the most beneficial.

Nutritive value

For this study we decided to focus on CP and NDF since these forage nutritive value parameters are highly relevant to the animal as sources of protein and energy, respectively.

In 2016, A, AB and ATF showed higher CP content on the last day of sampling for each harvest frequency, with the exception of AB mixtures where no differences in CP content were observed in H4 between the first and last sampling dates (A: $P < 0.01$; AB: $P < 0.01$; ATF: $P < 0.01$; Table 2.6). The opposite was true for NDF in A and ATF (A: $P < 0.01$; ATF: $P < 0.01$; Table 2.7), with NDF higher on the first sampling day than the last. This occurs because in the beginning of the season, forages increase their NDF more rapidly during spring regrowth, which also explains the lower CP content at the beginning of the season (Brink et al., 2010). However, the same is not true for AB mixtures (*P* < 0.01; Table 2.7), in which the NDF of H1 and H4 was higher on the first sampling day. For H1, this might be a reflection of the absence of bermudagrass at the beginning of the season, which is to be expected. Meanwhile, the fact that NDF was also higher in the first sampling of H4 is likely due to the presence of alfalfa in later stages of maturity. Summer growth of alfalfa stems is more lignified than spring because of the high temperatures, and considering that in 2016 there was a drought in addition to the high temperature, it could have affected NDF concentration due to advanced maturity (Sanderson and Wedin, 1988).

In 2017, inconsistencies were observed in CP content, because differences within sampling dates were only seen in H1 and H2 for A, and H1, H2 and H3 for AB and ATF, where CP was higher for the first sampling day of A and AB and the last sampling of ATF (A: *P* = 0.0015; AB: *P* = 0.0019; ATF: *P* = 0.0001; Table 2.6). For A and AB plots in 2017, these differences between sampling dates were the opposite of 2016. These results imply that under adequate precipitation, frequent harvests are detrimental to CP content later in the season, while longer harvest frequencies are able to maintain CP throughout the growing season. For ATF the same pattern observed in 2016 occurred in 2017 (Table 2.6). The NDF did not differ in 2017 for ATF, probably due to the lower percentage of alfalfa within ATF mixtures (Table 2.4).

In 2018, A and AB showed differences in H2, H3 and H4, with higher CP on the first sampling day, while ATF showed differences in H1, H2 and H3 with higher CP in the last sampling day, consistent with the previous years (A: *P* < 0.01; AB: *P* = 0.0002; ATF: *P* = 0.0027; Table 2.6). The opposite is true for NDF of A and AB (A: *P* <0.01;

AB: *P* = 0.0044; Table 2.7); however, the pattern expected for ATF did not occur in H1, with the first sampling day showing higher NDF than the last (ATF: P = 0.0010; Table 2.7). This is probably due to the increased percentage of weeds observed in 2018, especially for more frequent harvests, where weeds have more resources to grow fast and reach the reproductive stage, increasing fiber content.

As for harvest frequencies effects in 2016, A showed higher CP in shorter harvest frequencies than longer harvest frequencies in both first and last sampling dates ($P < 0.01$; Table 2.6), which is expected considering that CP decreases with maturity due to a decrease in cell content in plants (Sheaffer et al., 1988; 2000; Albrecht et al., 1987). The AB mixtures showed differences only on the first sampling day ($P < 0.01$; Table 2.6), which was unusual, with increased CP as harvest frequencies increased while NDF remained mostly constant among harvest frequencies (except in H1, where there was not sufficient forage material for sampling) ($P < 0.01$; Table 2.7). This pattern is likely affected by the weed percentage, since as harvest frequencies decreased on the first sampling day, the weed percentage was higher (Table 2.3). In ATF, higher CP for H2 on the first sampling day was attributed to the higher legume percentage observed in this treatment (Table 2.4). However, on the last sampling day, H2 was different than H4, and the remaining harvests were similar to both (Table 2.7), likely due to differences in maturity stage.

In 2017, CP for A and AB on the last sampling day increased as harvest frequencies increased, and the NDF was lower, this pattern is unexpected (A: $P < 0.01$; AB: P < 0.01; Tables 2.6, 2.7), since an increase in maturity is related to an increase in fiber and decrease of CP (Wiersma et al., 1998). These results are likely related to the increased percentage of alfalfa in AB plots (Table 2.3), and considering that the digestibility (which is related to NDF) of perennial grasses declines faster than legume forages, the increased alfalfa in the plots could have influenced the pattern observed (Buxton, 1996). The CP of ATF also followed the pattern of A and AB, except that the difference was observed only on the first sampling day with no differences in NDF (Tables 2.6, 2.7). These results suggest that frequent harvests are detrimental for overall CP of alfalfa and alfalfa mixtures.

In 2018, interestingly, higher CP content was attributed to longer harvest frequencies (Table 2.6). However, NDF remained high for H4 in AB and ATF (Table 2.7). First sampling days of AB and ATF showed high CP and also high NDF at longer harvest frequencies. Considering the increase in the overall weed mass in all plots, it is likely that the presence of weeds interfered with the amount of fiber present in the sample, since weeds tend to grow faster and reach reproductive phase more effectively, which can increase overall fiber. Meanwhile, these weeds were likely present during their vegetative stage with high CP content, maintaining CP of the mixture.

With the results obtained in the third year, it is possible to assume that nutritive value was affected by frequent harvest intervals. The NDF content was, as expected, higher with advanced maturity due to lignification, but CP was very inconsistent due to a significant increase in the percentage of weeds.

CONCLUSION

Alfalfa persistence declined after three years of forage production in the Southeast U.S. For alfalfa mixtures, forage mass decreased, and the percentage of weeds increased during the third year. Mixtures of ATF suppressed alfalfa growth, but the overall productivity was higher than AB mixtures, suggesting that the use of alfalfa in ATF plots is advantageous despite its botanical composition in the field. Yet, the nutritive value was determined by the amount of alfalfa remaining in mixtures and the weed mass. The CP content was closely related to harvest frequencies, which decreased with more frequent harvests. Considering the overall detrimental effect of frequent harvests and considering the goal of maintained persistence of alfalfa plots in the field, it is suggested that harvests occur every 35 days or longer for A and AB mixtures, and every 42 days or longer for ATF. The benefits outweighed the negative aspects of longer harvest frequencies, with constant FM and insignificant decrease in nutritive value.

APPENDIX B

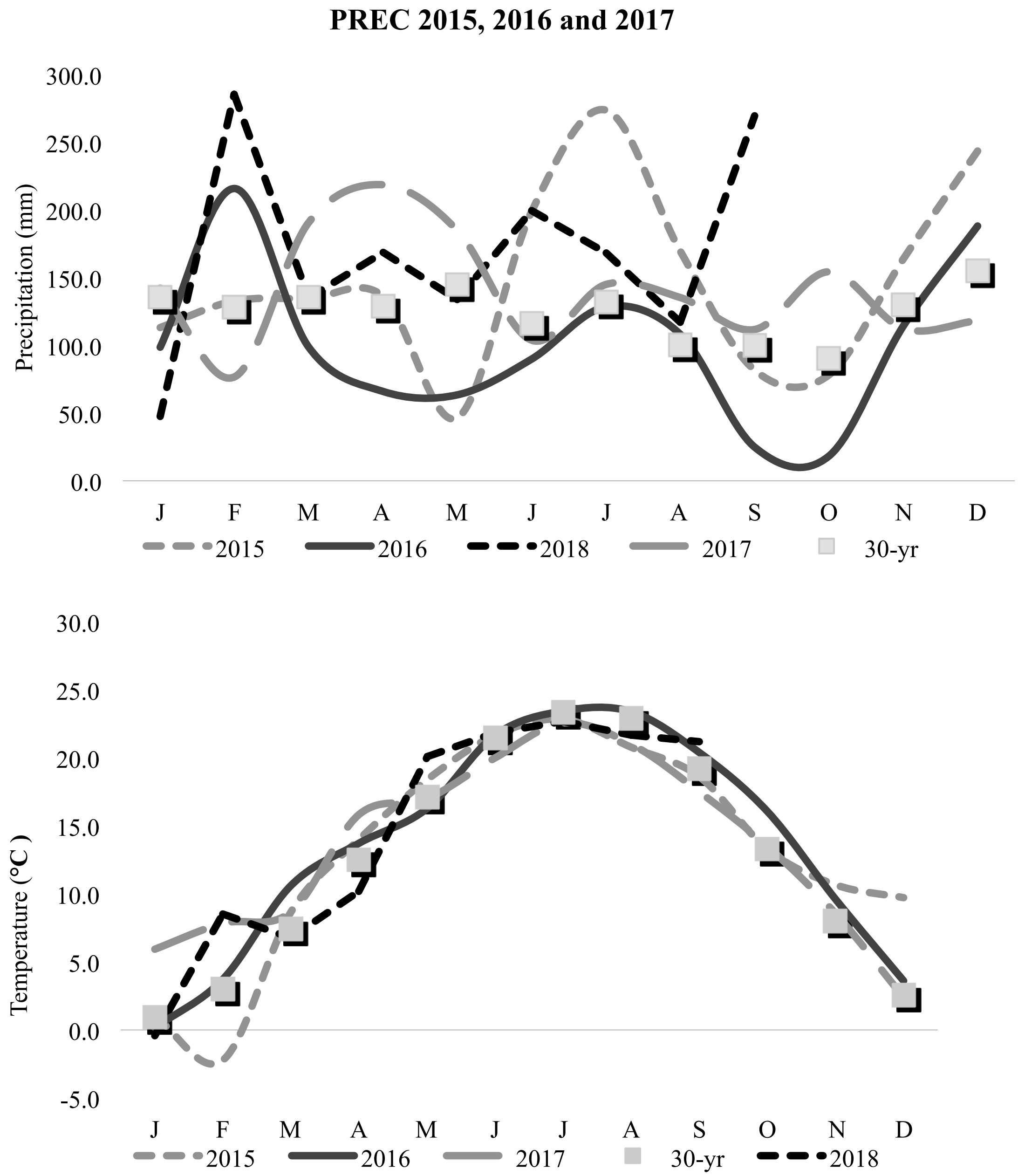

Figure 2.1. Weather for Plateau Research and Education Center, Crossville, TN, 2015-2018 including 30-year average.

Table 2.1 Harvests schedules of each imposed treatment (H1, 21 days harvest interval; H2, 28 days harvest interval; H3, 35 days harvest interval; H4, 42 days harvest interval).

First and last days of harvest of each harvest frequency					
2016					
		Harvest Frequencies			
		H1	H2	H3	H4
Harvests	First	May 3rd	May 10th	May 17th	May 24th
	Last	August 16th	August 30th	August 30th	August 16th
2017					
		Harvest Frequencies			
		H1	H2	H3	H4
Harvests	First	May 2nd	May 9th	May 16th	May 23rd
	Last	September 5th	August 29th	August 29th	August 15th
2018					
		Harvest Frequencies			
		H1	H2	H3	H4
Harvests	First	May 1st	May 8th	May 15th	May 22nd
	Last	September 4th	August 28th	August 28th	August 14th

Table 2.2 Alfalfa tiller counting of alfalfa and alfalfa mixtures subjected to four different harvesting frequencies on the first and last sampling days, during 2017 and 2018 growing seasons.

Number of alfalfa tillers per treatment								
	‡**H1**		‡**H2**		‡**H3**		‡**H4**	
	‡I	‡II	I	II	I	II	I	II
‡A_1	61± 8.6^{a,A}	24±2.3^{B}	68±8.6	48±13.3^{a}	74±10.9	59±10.5^{a}	77±6.7^{a}	89±6.3^{a}
‡A_2	28±10.5^{b}	9±4.3	51±12.7^{A}	12±3.2^{b,B}	55±3.4^{A}	29±7^{b,B}	54±6.3^{b}	43±3.5^{b}
‡AB_1	58±6.3^{a,A}	11±4.4^{B}	44±11.4	32±7.9	55±4.5	52±12.5^{a}	52±7.7	51±11.7^{a}
‡AB_2	13±2.2^{b}	1±0.6	37±7.2^{A}	17±2.8^{B}	49±5^{A}	16±3.3^{b,B}	49±8.1^{A}	25±7.7^{b,B}
‡ATF_1	36±1.7^{a}	29±11.5^{a}	39±4.5^{a}	26±2.8^{a}	36±2.8^{a}	33±5.2^{a}	28±4.5^{a}	43±12.4^{a}
‡ATF_2	6±1.1^{b}	3±2.1^{b}	13±3^{b}	16±4.7^{b}	11±1^{b}	8±4^{b}	21±2.4^{b}	16±1.8^{b}

(mean ± standard error)
Means within a column for each forage category without a common lower-case superscript letter differ in sampling effect (*P* < 0.05).
Means within a row for each forage category without a common upper-case superscript letter differ in treatment effect (*P* < 0.05).
‡ H1, 21 days harvest interval; H2, 28 days harvest interval; H3, 35 days harvest interval; H4, 42 days harvest interval; AB, alfalfa-bermudagrass. A_1, alfalfa 2017; A_2, alfalfa 2018; AB_1, alfalfa-bermudagrass 2017; AB_2, alfalfa-bermudagrass 2018; ATF_1, alfalfa-tall fescue 2017; ATF_2, alfalfa-tall fescue 2017; I, first sampling day; II, last sampling day.

Table 2.3 Botanical composition differences of alfalfa-bermudagrass (g kg^{-1}) subjected to four different harvesting frequencies on the first and last sampling days, during 2016, 2017 and 2018 growing seasons.

		Legume and Grass mixtures (g kg^{-1})			
		‡**H1**	‡**H2**	‡**H3**	‡**H4**
			2016		
Legume	‡AB_1	480±44.4	560±48.7	541±104.8	552±136.7
	‡AB_2	393±45.8	552±36.7	528±50.7	326±57.3
Grasses	AB_1	452±74	405±51	429±102	437±129.8
	AB_2	583±42.3	441±36.1	463±49.4	649±56.4
Weeds	AB_1	$68±10.9^{a,A}$	$35±5^{a,B}$	$30±6.4^{a,B}$	$11±6.1^{C}$
	AB_2	$24±3.8^{b}$	$7±3.7^{b}$	$9±4.4^{b}$	25±3.6
			2017		
Legume	AB_1	$284±29.7^{B}$	$381±54.5^{AB}$	$463±53.4^{AB}$	$515±44.9^{A}$
	AB_2	$189±55.9^{B}$	$251±146.5^{B}$	$583±50.6^{A}$	$649±118.5^{A}$
Grasses	AB_1	676±24	585±50.6	505±49.6	453±39.9
	AB_2	$784±54.1^{A}$	$736±153.9^{A}$	$401±47.5^{B}$	$332±115.1^{B}$
Weeds	AB_1	$40±6.2^{a}$	$34±4.2^{a}$	$32±4.4^{a}$	$32±5.6^{a}$
	AB_2	$27±6.4^{b}$	$13±4.3^{b}$	$16±4.3^{b}$	$19±3.6^{b}$
			2018		
Legume	AB_1	$227±74^{B}$	$704±150^{a,A}$	$740±36.7^{a,A}$	$708±113.8^{a,A}$
	AB_2	$53±27^{B}$	$129±19.2^{b,AB}$	$168±59.7^{b,AB}$	$335±75.9^{b,A}$
Grasses	AB_1	97±54.5	2±1.8	6±3.8	17±9.5
	AB_2	49±29.2	77±45.7	10±5.7	49±24.8
Weeds	AB_1	$676±53.4^{A}$	$294±150.7^{b,B}$	$254±35.9^{b,B}$	$275±110.9^{b,B}$
	AB_2	$898±34.1^{A}$	$794±29.1^{a,AB}$	$822±63.3^{a,A}$	$616±57.3^{a,B}$

(mean ± standard error)
Means within a column for each forage category without a common lower-case superscript letter differ in sampling effect ($P < 0.05$).
Means within a row for each forage category without a common upper-case superscript letter differ in treatment effect ($P < 0.05$).
‡ H1, 21 days harvest interval; H2, 28 days harvest interval; H3, 35 days harvest interval; H4, 42 days harvest interval; AB, alfalfa-bermudagrass.
‡AB_1, first day of sampling; AB_2, last day of sampling.

Table 2.4 Botanical composition differences of alfalfa-tall fescue (g kg^{-1}) subjected to four different harvest frequencies on the first and last sampling days, during 2016, 2017 and 2018 growing seasons.

		Legume and Grass mixtures (g kg^{-1})			
		‡H1	‡H2	‡H3	‡H4
			2016		
Legume	‡ATF_1	$63{\pm}23^{b,B}$	$400{\pm}50^{b,A}$	$70{\pm}43.2^{b,B}$	$46{\pm}28^{b,B}$
	‡ATF_2	$371{\pm}39.7^{a,B}$	$658{\pm}63.1^{a,A}$	$668{\pm}31^{a,A}$	$579{\pm}38.9^{a,A}$
Grasses	ATF_1	$855{\pm}18.2^{a,A}$	$585{\pm}48.9^{a,B}$	$918{\pm}49.2^{a,A}$	$947{\pm}32.1^{a,A}$
	ATF_2	$589{\pm}38.8^{b,A}$	$332{\pm}62.8^{b,B}$	$321{\pm}26.5^{b,B}$	$399{\pm}36.2^{b,B}$
Weeds	ATF_1	$82{\pm}5.9^{a,A}$	$15{\pm}2^{B}$	$12{\pm}8.2^{B}$	$7{\pm}4.1^{b,B}$
	ATF_2	$40{\pm}1.5^{b,A}$	$10{\pm}3.4^{B}$	$11{\pm}4.6^{B}$	$22{\pm}2.8^{a,B}$
			2017		
Legume	ATF_1	0^{B}	0^{B}	$1{\pm}1.2^{B}$	$137{\pm}44.8^{a,A}$
	ATF_2	42±24.2	42±41.6	0	0^{b}
Grasses	ATF_1	1000^{A}	1000^{A}	$989{\pm}11.5^{A}$	$839{\pm}42.4^{b,B}$
	ATF_2	936±30.1	943±57	999±0.8	1000^{a}
Weeds	ATF_1	0	0	10±10.3	24±3.5
	ATF_2	23±9.7	15±15.4	1	0
			2018		
Legume	ATF_1	149±75.8	196±81.4	230±40.9	$175{\pm}46.3^{b}$
	ATF_2	$53{\pm}17.9^{B}$	$126{\pm}47^{B}$	$170{\pm}84.1^{B}$	$442{\pm}74.4^{a,A}$
Grasses	ATF_1	$609{\pm}51.7^{a,B}$	$796{\pm}75.6^{a,A}$	$770{\pm}40.9^{a,A}$	$822{\pm}44.1^{a,A}$
	ATF_2	$245{\pm}23.7^{b}$	$315{\pm}79.9^{b}$	$297{\pm}62.9^{b}$	$351{\pm}51.1^{b}$
Weeds	ATF_1	$242{\pm}53.8^{b,A}$	$8{\pm}8.1^{b,B}$	$0^{b,B}$	$3{\pm}3.4^{b,B}$
	ATF_2	$701{\pm}16.4^{a,A}$	$560{\pm}67.6^{a,B}$	$533{\pm}65.4^{a,B}$	$207{\pm}24.7^{a,C}$

(mean ± standard error)
Means within a column for each forage category without a common superscript lower-case letter differ in sampling effect $P < 0.05$).
Means within a row for each forage category without a common superscript upper-case letter differ in treatment effect ($P < 0.05$).
‡ H1, 21 days harvest interval; H2, 28 days harvest interval; H3, 35 days harvest interval; H4, 42 days harvest interval; ATF, alfalfa-tall fescue.
‡ATF1, first day of the growing season; ATF2, last day of the growing season.

Table 2.5 Total dry matter (DM) forage mass (FM kg ha^{-1}) during 2016, 2017 and 2018 growing seasons.

	Total ‡DM Forage Mass (kg ha^{-1})			
	‡H1	**‡H2**	**‡H3**	**‡H4**
‡A_1	$7255.05 \pm 478.07^{a,B}$	$9530.15 \pm 726.5^{a,A}$	$10216.59 \pm 1111.7^{a,A}$	$7792.84 \pm 970.1^{a,B}$
‡A_2	$5604.21 \pm 486.7^{b,B}$	$7811.41 \pm 433.1^{b,A}$	$8042.78 \pm 1123.6^{,A}$	$7167.88 \pm 439.9^{a,A}$
‡A_3	$2053.04 \pm 645.3^{c,B}$	$2591.49 \pm 502.9^{c,B}$	$3992.78 \pm 668.3^{c,A}$	$4909 \pm 528.6^{b,A}$
‡AB_1	$2503.73 \pm 318.8^{b,C}$	$3661.30 \pm 535.9^{b,B}$	$5046.39 \pm 799.9^{b,A}$	$4821.99 \pm 669^{c,A}$
‡AB_2	$4866.48 \pm 789.2^{a,B}$	$7120.62 \pm 561.2^{a,A}$	$7817.94 \pm 765.9^{a,A}$	$7541.93 \pm 642.1^{a,A}$
‡AB_3	$3782.16 \pm 671.6^{a,B}$	$2936.08 \pm 520.3^{b,C}$	$5182.54 \pm 644.4^{b,A}$	$6092.92 \pm 538.9^{b,A}$
‡ATF_1	$6287.46 \pm 877.7^{a,C}$	$7775.29 \pm 652.6^{a,B}$	$10682.45 \pm 742.9^{a,A}$	$9992.38 \pm 558.1^{a,A}$
‡ATF_2	$4707.64 \pm 466.9^{b,C}$	$6946.26 \pm 720.6^{a,B}$	$8277.85 \pm 513.6^{b,A}$	$8909.98 \pm 410.4^{a,A}$
‡ATF_3	$2754.92 \pm 562.2^{c,B}$	$2620.36 \pm 416.8^{b,B}$	$2930.11 \pm 365.3^{c,B}$	$5255.39 \pm 471.4^{b,A}$

(total production value ± standard error)
Means within a column for each treatment without a common lower-case superscript letter differ in year effect ($P < 0.05$).
Means within a row for each species-year without a common upper-case letter differ in treatment effect ($P < 0.05$).
‡ DM, dry matter; H1, 21 days harvest interval; H2, 28 days harvest interval; H3, 35 days harvest interval; H4, 42 days harvest interval; A^1, alfalfa 2016; A^2, alfalfa 2017; A^3, alfalfa 2018; AB^1, alfalfa-bermudagrass 2016; AB^2, alfalfa-bermudagrass 2017; AB^3, alfalfa-bermudagrass 2018; ATF^1, alfalfa-tall fescue 2016; ATF^2, alfalfa-tall fescue 2017; ATF^3, alfalfa-tall fescue 2018.

Table 2.6 Crude protein (CP) of the first and last sampling of alfalfa, alfalfa-bermudagrass and alfalfa-tall fescue, subjected to four different harvest frequencies during 2016, 2017 and 2018 growing seasons.

	‡CP (g kg^{-1})			
	‡H1	‡H2	‡H3	‡H4
2016				
‡A_1	208.78± 3.4^{b,A}	209.20±8.7^{b,A}	183.07±0.9^{b,B}	190.91±6.6^{b,B}
‡A_2	263.85±3.7^{a,A}	269.48±5.2^{a,A}	262.21±2.8^{a,A}	210.98±2.9^{a,B}
‡AB_1	0.00^{b,C}	135.8±5.5^{b,B}	159.73±14^{b,AB}	161.8±11.8^{A}
‡AB_2	189.28±7.9^{a}	186.55±8^{a}	181.6±9.9^{a}	168.25±5
‡ATF_1	143.43±8^{b,AB}	158.5±8.4^{b,A}	128.23±9.4^{b,B}	127±5.4^{b,B}
‡ATF_2	208.02±3.7^{a,AB}	216.75±4.3^{a,A}	213.17±3.5^{a,AB}	196.05±3.1^{a,B}
2017				
A_1	234.3±1.6^{a}	208.45±1.8^{a}	215.62±4.6	232.55±3.4
A_2	145.5±12.8^{b,B}	167.95±22.8^{b,B}	217.25±18.9^{A}	210.32±10.2^{A}
AB_1	201.55±5.3^{a}	199.6±4.8^{a}	211.82±5.4^{a}	211.6±5.1
AB_2	139.27±5.9^{b,B}	128.62±31^{b,B}	166.52±10.5^{b,AB}	198.2±9.9^{A}
ATF_1	129.25±5.4^{b,B}	129.4±5.5^{b,B}	143.8±7.8^{b,AB}	155.5±4.3^{A}
ATF_2	175.95±5.2^{a}	163.65±5.8^{a}	164.5±2.1^{a}	165.1±3.6
2018				
A_1	150.17±9.3^{C}	209.97±7.4^{a,B}	236.52±3.6^{a,A}	225.5±6.5^{a,AB}
A_2	127.02±3.9^{C}	121.25±5.7^{b,C}	152.9±17.5^{b,B}	188.95±15.1^{b,A}
AB_1	153.2±11.3^{B}	207.9±19.7^{a,A}	228.27±10^{a,A}	200.02±7.9^{a,A}
AB_2	157.4±10.4	139.1±14.6^{b}	154.85±19.2^{b}	164.25±8.3^{b}
ATF_1	106.72±2.3^{b,B}	117.77±2.4^{b,AB}	95.32±3.4^{b,C}	125.37±10.8^{A}
ATF_2	142.37±7.3^{a}	148.32±6.6^{a}	126.05±1.5^{a}	132.85±5.2

(mean ± standard error)
Means within a column for each treatment without a common lower-case superscript letter differ in year effect ($P < 0.05$).
Means within a row for each species year without a common upper-case letter differ in treatment effect ($P < 0.05$).
‡ CP, crude protein; H1, 21 days harvest interval; H2, 28 days harvest interval; H3, 35 days harvest interval; H4, 42 days harvest interval; A^1, 1st sampling alfalfa; A^2, last sampling alfalfa; AB^1, 1st sampling alfalfa-bermudagrass; AB^2, last sampling alfalfa-bermudagrass; ATF^1, 1st sampling alfalfa-tall fescue; ATF^2, last sampling alfalfa-tall fescue.

Table 2.7 Neutral Detergent Fiber (NDF) of the first and last sampling of alfalfa, alfalfa-bermudagrass and alfalfa-tall fescue, subjected to four different harvest frequencies during 2016, 2017 and 2018 growing seasons.

	NDF (g kg^{-1})			
	‡**H1**	‡**H2**	‡**H3**	‡**H4**
		2016		
‡A_1	369.93±11.4^{a,B}	373.96±9.7^{a,B}	389.4±5.32^{a,AB}	418.04±13.7^{A}
‡A_2	209.55±9^{b,B}	210.24±9.1^{b,B}	231.43±7.8^{b,B}	326.49±5^{A}
‡AB_1	0.00^{b,B}	446.98±16.7^{A}	433.4±37^{A}	446.38±31.3^{b,A}
‡AB_2	476.1±14.7^{a}	459.37±14.8	481.77±15	511.72±9.3^{a}
‡ATF_1	498.53±7.8^{a,C}	501.2±10^{a,BC}	530.08±16.6^{a,AB}	543.95±12.4^{a,A}
‡ATF_2	465±9.3^{b,C}	425.72±12.7^{b,B}	431.2±11.3^{b,B}	465.82±5.8^{b,A}
		2017		
A_1	306.2±4.1^{b,B}	340.37±6.1^{b,AB}	373.55±8.7^{A}	389.17±8.2^{A}
A_2	488.2±27.3^{a,A}	451.42±41.9^{a,A}	374.45±31.7^{B}	377.8±14^{B}
AB_1	456.05±4.1^{b,B}	447.32±8.8^{b,B}	480.62±3.2^{b,AB}	525.9±4.4^{A}
AB_2	601.47±11.7^{a,A}	582.47±39.8^{a,A}	531.87±10.7^{a,B}	486.4±15.8^{B}
ATF_1	603.45±8.7	604.57±8.7	597.6±10.9	611.42±5.2
ATF_2	568.1±10.8	607.15±16.3	612.45±9.8	578.5±4.2
		2018		
A_1	396.6±12.3^{b,A}	340.07±11.5^{b,A}	308.8±4.7^{b,B}	360.225±8.7^{AB}
A_2	513.12±18.5 ^{a,AB}	565.45±22.4^{a,A}	473.9^{a}±31.8^{,B}	384.85±36.4^{C}
AB_1	464.35±26.3^{b,AB}	438.67±32.8^{b,B}	437.62±22.3^{b,B}	493.32±15.5^{A}
AB_2	526.7±13.3^{a}	538.1±18.6^{a}	495.95±30.8^{a}	491.7±7.4
ATF_1	510.47±13.5^{b,C}	552.27±9.8^{B}	621.75±10.2^{a,A}	603.75±17.8^{a,A}
ATF_2	548.25±9.3^{a,AB}	522.02±11.8^{B}	574.07±12.6^{b,A}	545.3±21^{b,AB}

(mean ± standard error)
Means within a column for each treatment without a common lower-case superscript letter differ in year effect (P < 0.05).
Means within a row for each species year without a common upper-case letter differ in treatment effect (P < 0.05).
‡ NDF, neutral detergent fiber; H1, 21 days harvest interval; H2, 28 days harvest interval; H3, 35 days harvest interval; H4, 42 days harvest interval; A^1, 1st sampling alfalfa; A^2, last sampling alfalfa; AB^1, 1st sampling alfalfa-bermudagrass; AB^2, last sampling alfalfa-bermudagrass; ATF^1, 1st sampling alfalfa-tall fescue; ATF^2, last sampling alfalfa-tall fescue.

CONCLUSION

Harvest frequencies above 28 days are ideal for FM accumulation. Yet, harvest frequencies of 42 days or above tend to have increased lignification thus decreased nutritive value. Our results suggests that, regardless of the percentage of alfalfa in mixtures with either cool or warm-season grasses, alfalfa contributes to overall nutritive value of the canopy, reducing the need of N fertilizer application.

Alfalfa persistence declined after three years of forage production in the Southeast U.S. For alfalfa mixtures, forage mass decreased, and the percentage of weeds increased during the third year. Mixtures of ATF suppressed alfalfa growth, but the overall productivity was higher than AB mixtures, suggesting that the use of alfalfa in ATF plots is advantageous despite its botanical composition in the field. The CP content was closely related to harvest frequencies, which decreased with more frequent harvests. Considering the overall detrimental effect of frequent harvests and considering the goal of maintained persistence of alfalfa plots in the field, it is suggested that harvests occur every 35 days or longer for A and AB mixtures, and every 42 days or longer for ATF. The benefits outweighed the negative aspects of longer harvest frequencies, with constant FM and insignificant decrease in nutritive value.

REFERENCES

Albrecht, K. A., W. F. Wedin, and D. R. Buxton, 1987. Cell-Wall Composition and Digestibility of Alfalfa Stems and Leaves1. Crop Sci. 27:735-741.

Allinson, D. W, and D. F Osbourn, 1970. The Cellulose-lignin Complex in Forages and Its Relationship to Forage Nutritive Value. The J. of Agric. Sc. 74.1: 23-36.

Amiri, F. and A. R. B. M. Shariff, 2012. Comparison of Nutritive Values of Grasses and Legume Species Using Forage Quality Index. Songklanakarin J. of Sci. and Tech. (SJST) 34 (5): 577–86.

Anjos, A. J., C. A. M. Gomide, K. G. Ribeiro, A. S. Madeiro, M. J. F. Morenz, D. S. C. Paciullo, A. J. Anjos, 2016. Forage Mass and Morphological Composition of Marandu Palisade Grass Pasture under Rest Periods. Ciência E Agrotecnologia 40 (1): 76–86.

Ashraf, M and A., Iram, 2005. Drought stress induced changes in some organic substances in nodules and other plant parts of two potential legumes differing in salt tolerance. Flora, 200(6), pp.535–546.

Atkins, C. A., B. J. Shelp, J. Kuo, M. B. Peoples, and J. S. Pate, 1984. Nitrogen Nutrition and the Development and Senescence of Nodules on Cowpea Seedlings. Planta 162 (4): 316–26.

Ball, D., C. S. Hoveland, and G. D. Lacefield, 2007. Southern forages: Modern concepts for forage crop management (4th ed.). Norcross, Ga.: Potash & Phosphate Institute and the Foundation for Agronomic Research.

Ball, D.M., M. Collins, G. Lacefield, M. Neal, D. Mertens, K. Olson, D. Putnam, D. Undersander, M. Wolf, 2001. Understanding Forage Quality. American Farm Bureau Federation Publication 1-01, Park Ridge, IL.

Ball, D.M., S.P. Schmidt, G.D. Lacefield, C.S. Hoveland, and W.C. Young III, 2003. Tall fescue endophyte concepts. Oregon Tall Fescue Commission. Special Publication 1-03. http://www.aces.edu/pubs/docs/A/ANR-1239/ANR-1239.pdf?PHPSESSID=85ef5ee29a11f16fb8dffb89782a13aa (verified 3 July 2017). Salem, OR.

Ballaré. C. L., and J. J., Casal, 2000. Light signals perceived by crop and weed plants. Field Crops Research, 67(2), pp.149–160.

Barbehenn, R. V., Z. Chen, D. N. Karowe, and A. Spickard, 2004. C3 Grasses Have Higher Nutritional Quality than C4 Grasses under Ambient and Elevated Atmospheric CO2."Global Change Biology 10 (9): 1565–75.

Baskin, J. M., and C. C.Baskin, 1985. The Annual Dormancy Cycle in Buried Weed Seeds: a Continuum. BioScience, vol. 35, 1985, p. 492

Bauer, W., 1981. Infection of Legumes by Rhizobia. Annual Review of Plant Physiology, 32(1), 407-449.

Beck, P., Hess, T., Hubbell, D., Gadberry, M., Jennings, J., & Sims, M., 2016. Replacing synthetic N with clovers or alfalfa in bermudagrass pastures. 2. Herbage nutritive value for growing beef steers. Animal Production Science, 57(3), 547-555.

Belesky, D. and J. Fedders, 1995. Comparative growth analysis of cool- and warm-season grasses in a cool-temperate environment. Agron. J. .87(5), pp.974–980.

Belesky, D. P., J. M. Fedders, J. M. Ruckle, and K.E. Turner, 2002. Bermudagrass–White Clover–Bluegrass Sward Production and Botanical Dynamics. Agron. J. 94 (3): 575–84.

Beuselinck, P.R., J.H. ,Bouton, W.O., Lamp, A.G., Matches, M.H., McCaslin, C.J., Nelson, L.H., Rhodes, C.C., Sheaffer, and J.J., Volenec, 1994. Improving Legume Persistence in Forage Crop Systems. J. of Prod. Agric. 3: 311-22.

Brink, G., M. Hall, G. Shewmaker, D. Undersander, N. Martin, and R. Walgenbach. 2010. Changes in Alfalfa Yield and Nutritive Value within Individual Harvest Periods. Agron. J. 102 (4): 1274–82.

Brown, R. H., and G. T. Byrd, 1990. Yield and Botanical Composition of Alfalfa-Bermudagrass Mixtures. Agron. J. 82 (6): 1074–79.

Brummer, E.C. and J.H. Bouton. 1991. Plant traits associated with grazing-tolerant alfalfa. Agron. J. 83:996-1000.

Buxton, D. R., 1996. Quality-related Characteristics of Forages as Influenced by Plant Environment and Agronomic Factors. Animal Feed Sc. and Tech. 59.1: 37-49.

Buxton, D. R., J. S. Hornstein, W. F. Wedin, and G. C. Marten. 1985. Forage Quality in Stratified Canopies of Alfalfa, Birdsfoot Trefoil, and Red Clover. Crop Scie. 25 (2): 273–79.

Buxton, D.R., Russell, J.R., and Wedin, W.F. 1987. Structural Neutral Sugars in Legume and Grass Stems in Relation to Digestibility. Crop Science: 1279-285.

Cangiano, C.A.; A. R. Castillo, J. Guerrero, D. H. Putnam, 2007. Alfalfa grazing management. In C. G. Summers and D. H. Putnam, eds., Irrigated alfalfa management in Mediterranean and Desert zones. Chapter 18. Oakland: University of California Agriculture and Natural Resources Publication 8304.

Carita, T., N. Simões, J. P. Carneiro, J. M., and A. S. Bagulho, 2016. Forage Yield and Quality of Simple and Complex Grass-Legumes Mixtures under Mediterranean Conditions. Emirates J. of Food and Agric. (EJFA) 28 (7): 501–5.

Chatterton, N. J., G. E. Carlson, R. H. Hart, and W. E. Hungerford, 1974. Tillering, Nonstructural Carbohydrates, and Survival Relationships in Alfalfa1. Crop Sci. 14:783-787.

Cheng, K. J., T. A. McAllister, J. D. Popp, A. N. Hristov, Z. Mir, and H. T. Shin, 1998. A Review of Bloat in Feedlot Cattle. J. of Ani. Scie. 76: 299–308.

Chung, I., and D. A., Miller. 1995. Natural Herbicide Potential of Alfalfa Residue on Selected Weed Species. Agron. J. 87:920-925.

Crews, T.E. and M. B. Peoples, 2004. Legume versus fertilizer sources of nitrogen: ecological tradeoffs and human needs. Agric. Ecos. and Envir., 102(3), pp.279-297.

Cowett, E. R., and M. A. Sprague. 1962. Factors Affecting Tillering in Alfalfa. Agron. J. 54:294-297.

Cuomo, G.J., B.E. Anderson, and L. J. Young, L.J., 1998. Harvest frequency and burning effects on vigor of native grasses. *J. of range management*, (1), pp.32–36.
Cowett, E. R., and M. A. Sprague. 1962. Factors Affecting Tillering in Alfalfa. Agron. J. 54:294-297.
Dhont, C., Castonguay, Y., Nadeau, P., Belanger, G., & Chalifour, F., 2002. Alfalfa root carbohydrates and regrowth potential in response to fall harvests. Crop Science, 42(3), 754-765.

Douglas, J., 1986. The production and utilization of lucerne in New Zealand. Grass and Forage Scie., 41(2), 81-128

Ehleringer, J. R., 1978. Implications of Quantum Yield Differences on the Distributions of C3 and C4 Grasses. Oecologia 31 (3): 255–67.

Engels, and Jung, 2005. Alfalfa Stem Tissues: Impact of Lignification and Cell Length on Ruminal Degradation of Large Particles. An. Feed Sci. and Technology 120: 309-21.

Erice, G., S. Louahlia, J. J. Irigoyen, M. Sanchez-Diaz, and J.-C. Avice, 2010. Biomass Partitioning, Morphology and Water Status of Four Alfalfa Genotypes Submitted to Progressive Drought and Subsequent Recovery. J. of Plant Phys. 167 (2): 114–20.

Feltner, K. C., and M. A. Massengale. 1965. Influence of Temperature and Harvest Management on Growth, Level of Carbohydrates in the Roots, and Survival of Alfalfa (Medicago sativa L.)1. Crop Sci. 5:585-588.

Fuess, F., & Tesar, M., 1968. Photosynthetic Efficiency, Yields, and Leaf Loss in Alfalfa1. Crop Sci., 8(2), 159.

Fustini, M., Canestrari, G., Grilli, E., Formigoni, A., Palmonari, A, Fustini, M, Canestrari, G, Grilli, E, and Formigoni, A., 2014. Influence of Maturity on Alfalfa Hay Nutritional Fractions and Indigestible Fiber Content. J. of Dairy Sci. 97.12: 7729-734.

Grev. A.M., M.S. Wells, D.A. Samac, K.L. Martinson, C.C. Sheaffer, 2017. Forage Accumulation and Nutritive Value of Reduced Lignin and Reference Alfalfa Cultivars. Agronomy Journal. 109.6: 2749-761. Web.

Griffiths, H., G. Weller, L. F. M. Toy, and R. J. Dennis. 2013. You're so Vein: Bundle Sheath Physiology, Phylogeny and Evolution in C3 and C4 Plants. Plant, Cell & Envir. 36 (2): 249–61.

Haby, V. A., J. V. Davis, and A. T. Leonard, 1999. Response of Overseeded Alfalfa and Bermudagrass to Alfalfa Row Spacing and Nitrogen Rate. Agron. J. 91 (6): 902–10.

Haby, V.A., S.A. Stout, F.M. Hons, and A.T. Leonard, 2006. Nitrogen Fixation and Transfer in a Mixed Stand of Alfalfa and Bermudagrass. Agron. Jou. 890-98.

Hakl, J., P. Fuksa, J. Konečná, and J. Šantrůček, 2016. Differences in the Crude Protein Fractions of Lucerne Leaves and Stems under Different Stand Structures. Grass and Forage Sci. 71 (3): 413–23.

Halim, R. A., D. R. Buxton, M. J. Hattendorf, and R. E. Carlson, 1989. Water-stress Effects on Alfalfa Forage Quality after Adjustment for Maturity Differences. Agr. Jou.189-94.

Hancock, K., V. Collette, E. Chapman, K. Hanson, S. Temple, R. Moraga, and J. Caradus, 2014. Progress towards Developing Bloat-Safe Legumes for the Farming Industry. Crop and Pasture Sci. 65 (11): 1107–13.

Henderson, M. S., and D. L. Robinson, 1982. Environmental Influences on Fiber Component Concentrations of Warm-Season Perennial Grasses. Agron. J. 74 (3): 573–79.

Higginbotham, G. E., C L. Stull, N. G. Peterson, A. V. Rodiek, B.A. Reed, J. N. Guerrero, 2008. Alfalfa utilization for livestock. In C. G. Summers and D. H. Putnam, eds., Irrigated alfalfa management in Mediterranean and Desert zones. Chapter 17. Oakland: University of California Agriculture and Natural Resources Publication 8303.

Hoveland, C. S. 1993. Importance and Economic Significance of the Acremonium Endophytes to Performance of Animals and Grass Plant. Agric. Ecos. and Envir. Acremonium/Grass Interac., 44 (1): 3–12.

Htoo, N. N., A. T. Khaing, Y. Abba, N. N. Htin, J. F. F. Abdullah, T. Kyaw, M. A. K. G. Khan, and M. A. M. Lila, 2015. Enhancement of Growth Performance in Pre-Weaning Suckling Boer Kids Supplemented with Creep Feed Containing Alfalfa. Vet. World 8 (6): 718–22.

Huang, W.Y., 2009. Factors contributing to the recent increase in US fertilizer prices, 2002-08. DIANE Publishing.

Jones, J.H., F.J. and Olsen, 1987. Alfalfa establishment and production on soils with different drainage characteristics. Agron. J., (1), 152-154.

Jung, G. A., J. A. Shaffer, and J. R. Everhart. 1996. Harvest Frequency and Cultivar Influence on Yield and Protein of Alfalfa-Ryegrass Mixtures. Agron. J. 88:817-822.

Kamstra, L.D., 1973. Seasonal Changes in Quality of Some Important Range Grasses. Journal of Range Management, 26(4), pp.289–291.

Kering, M., Guretzky, J., Funderburg, E., & Mosali, J., 2011. Effect of Nitrogen Fertilizer Rate and Harvest Season on Forage Yield, Quality, and Macronutrient Concentrations in Midland Bermuda Grass. Communications in Soil Science and Plant Analysis, 42(16), 1958-1971.

Kuechenmeister, K.; Kuechenmeister, F.; Kayser, M.; Kayser, M.; Wrage-Mönnig, N.; Isselstein, J., 2013. Influence of drought stress on nutritive value of perennial forage legumes. Int. J., Plant Prod., 7(4): 693-710

Lauriault, L. M., S. J. Guldan, and C. A. Martin, 2003. Irrigated Tall Fescue–Legume Communities in the Southern Rocky Mountains. Agron. J. 95 (6): 1497–1503.

Lafarge, M. and P. Loiseau, 2002. Tiller density and stand structure of tall fescue swards differing in age and nitrogen level. *European J.l of Agron,*, 17(3), pp.209–219.

Lee, J-S., 2011. Combined Effect of Elevated CO2 and Temperature on the Growth and Phenology of Two Annual C3 and C4 Weedy Species. Agric. Ecos. and Envir. 140 (3–4): 484–91.

Li, Rong, J.J. Volenec, B.C. Joern, S.M. Cunningham, 1996. Seasonal changes in nonstructural carbohydrates, protein, and macronutrients in roots of alfalfa, red clover, sweetclover, and birdsfoot trefoil. Crop Sci., 36(3), 617-623.

Marten, G.C. and A. W. Hovin, 1980. Harvest schedule, persistence, yield, and quality interactions among four perennial grasses [Dactylis glomerata, Festuca arundinacea, Phalaris arundinacea, Bromus inermis]. Agron. Jou. (2), pp.378–387.

Massey, C. G., N. A. Slaton, R. J. Norman, E. E. Gbur, R. E. DeLong, and B. R. Golden. 2011. Bermudagrass Forage Yield and Ammonia Volatilization as Affected by Nitrogen Fertilization. Soil Scie. Soci. of Amer. J. 75 (2): 638–48.

McClure, K. E., R. W. Van Keuren, and P. G. Althouse. 1994. Performance and Carcass Characteristics of Weaned Lambs Either Grazed on Orchardgrass, Ryegrass, or Alfalfa or Fed All-Concentrate Diets in Drylot. J. of An. Scie. 72 (12): 3230–37.

Merrick, M. J. 2004. Regulation of Nitrogen Fixation in Free-Living Diazotrophs. In Genetics and Regulation of Nitrogen Fixation in Free-Living Bacteria, 197–223. Nitrogen Fixation: Origins, Applications, and Research Progress. Springer, Dordrecht.

Mitich, L. W., 1989. Bermudagrass. Weed Tech. 3 (2): 433–35.

Moore, K. J., and H-J. G. Jung, 2001. Lignin and Fiber Digestion. J. of Range Manag.t 54 (4): 420–30.

Mooso, G. D., and W. F. Wedin, 1990. Yield Dynamics of Canopy Components in Alfalfa-Grass Mixtures. Agron. J. 82 (4): 696–701.

Mouriño, F., K. A. Albrecht, D. M. Schaefer, and P. Berzaghi, 2003. Steer Performance on Kura Clover–Grass and Red Clover–Grass Mixed Pastures. Agron. J. 95 (3): 652–59.

Mullen, R., S. Phillips, W. Raun, G. Johnson, W. Thomason, 2000. Forage yield and crude protein of interseeded legume-bermudagrass mixtures as affected by phosphorus fertilizer. J. of Plant Nutr., 23(5), 673-681.

Murray, I., and I. Cowe. 2004. Sample preparation. In: C.A. Roberts et al., editors, Near infrared spectroscopy in agriculture. ASA, CSSA, SSSA, Madison, WI. p. 75–115

Nave, R. L. G., R. M. Sulc, D. J. Barker, and N. St-Pierre. 2014. Changes in Forage Nutritive Value among Vertical Strata of a Cool-Season Grass Canopy. Crop Sci. 54:2837-2845.

Nave, R.L., M.R. Sulc, D.J. Barker. 2013. Relationships of Forage Nutritive Value to Cool-Season Grass Canopy Characteristics. Crop Science. 53(1):341-348.

Nelson, C. J., and J. C. Burns. 2006. Fifty Years of Grassland Science Leading to Change. Crop Scie. 46 (5): 2204–17.

Nemati, M., H. Amanlou, M. Khorvash, M. Mirzaei, B. Moshiri, and M. H. Ghaffari, 2016. Effect of Different Alfalfa Hay Levels on Growth Performance, Rumen Fermentation, and Structural Growth of Holstein Dairy Calves. J. of Ani. Scie. 94 (3): 1141–48.

Nordkvist, E., and P. Åman., 1986. Changes during Growth in Anatomical and Chemical Composition and In-vitro Degradability of Lucerne. Journal of the Science of Food and Agriculture 37.1 (1986): 1-7. Web.

Novak, J. M., W. J. Busscher, D. L. Laird, M. Ahmedna, D. W. Watts, and M. A. S. Niandou. 2009. Impact of Biochar Amendment on Fertility of a Southeastern Coastal Plain Soil. Soil Scie. 174 (2): 105–112.

NRCS. 2018. Custom soil resource report for Cumberland County, Tennessee. NRCS. https://www.nrcs.usda.gov/Internet/FSE_MANUSCRIPTS/tennessee/TN035/0/TNCumberland6_06Web.pdf (accessed 16 January 2018)

Patzek, T. W., 2004. Thermodynamics of the Corn-Ethanol Biofuel Cycle. Critical Rev. in Plant Scie. 23 (6): 519–67.

Pearen, J.R. and V.S., Baron, 1996. Productivity, and composition of smooth and meadow bromegrass mixtures with alfalfa under frequent cutting management. *Can. J. of plant sci.*, 76(4), pp.763–771.

Pedreira, B. C., C. G. S. Pedreira, and S. C. da Silva, 2007. Sward Structure and Herbage Accumulation in Brachiaria Brizantha Cultivar Xaraés in Response to Strategies of Grazing. Pesquisa Agropecuária Brasileira 42 (2): 281–87.

Peterson, P., C. Sheaffer, and M. Hall, 1992. Drought effects on perennial forage legume yield and quality. Agron. Jou. 84(5), pp.774–779.

Pirhofer-Walzl, K., J. Rasmussen, H. Høgh-Jensen, J. Eriksen, K. Søegaard, and J. Rasmussen, 2012. Nitrogen Transfer from Forage Legumes to Nine Neighbouring Plants in a Multi-Species Grassland. Plant and Soil 350 (1–2): 71–84.

Rech, I., J. C. Polidoro, P. S. Pavinato, 2017. Additives Incorporated into Urea to Reduce Nitrogen Losses after Application to the Soil. Pesquisa Agropecuária Brasileira 52 (3): 194–204.

Rice, J. S., V. L. Quinsenberry, and T. A. Nolan. 1989. Alfalfa Persistence and Yield with Irrigation. Agron. J. 81:943-946.

Rimi, F., S. Macolino, B. Leinauer, L. Lauriault, and U. Ziliotto, 2014. Fall Dormancy and Harvest Stage Impact on Alfalfa Persistence in a Subtropical Climate. Agronomy Journal, 106(4), 1258-1266.

Rodrigues, R. R., J. Moon, B. Zhao, and M. A. Williams, 2017. Microbial Communities and Diazotrophic Activity Differ in the Root-Zone of Alamo and Dacotah Switchgrass Feedstocks. GCB Bioenergy 9 (6): 1057–70.

Sage, R. F., and A. D, McKown, 2006. Is C_4 photosynthesis less phenotypically plastic than C_3 photosynthesis? Journal of Experimental Botany, 57(2), 303-317.

Sanderson, M.A., and W. F. Wedin, 1988. Cell Wall Composition of Alfalfa Stems at Similar Morphological Stages and Chronological Age during Spring Growth and Summer Regrowth. *Crop Sci.* 342-47.

Sheaffer, C. C., N. P. Martin, J. F.S. Lamb, G. R. Cuomo, J. G. Jewett, and S. R. Quering. 2000. Leaf and Stem Properties of Alfalfa Entries Joint contribution of the Minnesota Agric. Exp. Stn. and USDA-ARS. Minnesota Agric. Exp. Stn. Journal Series Paper 99-1-13-0127. . Agron. J. 92:733-739.

Sheaffer, C.C., G.D. Lacefield, V.L. Marble 1988. Cutting Schedules and Stands. In: A. A. Hanson, D. K. Barnes, R. R. Hill, editors, Alfalfa and Alfalfa Improvement, Agron. Monogr. 29. ASA, CSSA, SSSA, Madison, WI. p. 411-437.

Smith, D. 1969. Influence of Temperature on the Yield and Chemical Composition of 'Vernal' Alfalfa at First Flower1. Agron. J. 61:470-472.

Smith, S., J. Bouton, and C. S. Hoveland, 1989. Alfalfa persistence and regrowth potential under continuous grazing. Agron. Jou., 81(6), pp.960–965.

Smith, S.R. Jr., J. H. Bouton, and C. S. Hoveland, 1992. Persistence of alfalfa under continuous grazing in pure stands and in mixtures with tall fescue. Crop Sci. (5), 1259-1264.

Solomon, J. K. Q., B. Macoon, D. J. Lang, J. A. Parish, and R. C. Vann. 2011. A Novel Approach to Grass–Legume Management. Crop Sci. 51 (4): 1865–76.

Stringer, W. C., A. Khalilian, D. J. Undersander, G. S. Stapleton, and W. C. Bridges. 1994. Row Spacing and Nitrogen: Effect on Alfalfa-Bermudagrass Yield and Botanical Composition. Agron. J. 86:72-76.

Teixeira, E. I., D. J. Moot, H. E. Brown, A. L. Fletcher, 2007. The dynamics of lucerne (Medicago sativa L.) yield components in response to defoliation frequency. European Journal of Agronomy, 26(4), 394-400.

Tracy, B. F., and G.B. Jones, 2018. Persistence and productivity of orchardgrass and orchardgrass/alfalfa mixtures as affected by cutting height. *Grass and forage science*, 73(2), pp.544–552.

Tracy, B. F., K., Albrecht, J., Flores, M., Hall, A., Islam, G., Jones, W., Lamp, J. W., MacAdam, H., Skinner, and C., Teutsch. 2016. Evaluation of Alfalfa–Tall Fescue Mixtures across Multiple Environments. Crop Sci. 56:2026-2034.

Van Keuren, R. W., A. G. Matches, 1988. Pasture Production and Utilization. In: A. A. Hanson, D. K. Barnes, R. R. Hill, editors, Alfalfa and Alfalfa Improvement, Agron. Monogr. 29. ASA, CSSA, SSSA, Madison, WI. p. 515-538.

Vymazal, J., 2007. Removal of Nutrients in Various Types of Constructed Wetlands. Science of The Total Environment, Contaminants in Natural and Constructed Wetlands: Pollutant Dynamics and Control, 380 (1): 48–65.

Waldron, B. L., M. D. Peel, S.R. Larson, I. W. Mott, and J. E. Creech, 2017. Tall Fescue Forage Mass in a Grass-Legume Mixture: Predicted Efficiency of Indirect Selection. Euphytica 213 (3): 67.

Wang, Y., W. Majak, and T. A. McAllister, 2012. Frothy Bloat in Ruminants: Cause, Occurrence, and Mitigation Strategies. Animal Feed Scie. and Tech., Special Issue: Rumen Health: A 360° Analysis, 172 (1): 103–14.

Weaver, J. 1926. Root development of field crops (1st ed.). New York [etc.: McGraw-Hill book company.

Wingler, A., P. J. Lea, W. P. Quick, and R. C. Leegood, 2000. Photorespiration: Metabolic Pathways and Their Role in Stress Protection. Philosophical Trans. of the Royal Soc. of London B: Biological Sciences 355 (1402): 1517–29.

Wiersma, D. W., R. R. Smith, D. K. Sharpee, M. J. Mlynarek, R. E. Rand, and D. J. Undersander, 1998. Harvest Management Effects on Red Clover Forage Yield, Quality, and Persistence. J. Prod. Agric. 11:309-313.

Yamori, W., K. Hikosaka, and D. Way, 2014. Temperature response of photosynthesis in C_3, C_4, and CAM plants: Temperature acclimation and temperature adaptation. Photosynthesis Research, 119(1-2), 101-17.

Zehr, J. P., B. D. Jenkins, S. M. Short, and G. F. Steward, 2003. Nitrogenase Gene Diversity and Microbial Community Structure: A Cross-System Comparison. Environmental Microbiology 5 (7): 539–54.

VITA

Márcia Pereira da Silva was born in São Paulo, Brazil. She is a 2015 graduate of The São Paulo State University (UNESP) with a Bachelor of Science Degree in Animal Science. Márcia worked in several internships and research projects while pursuing her bachelor's degree, which involved ruminant and non-ruminant nutrition and management, laboratory analysis and forage production. When she decided to begin her Master's Degree at The University of Tennessee in 2017, she moved from Brazil to Knoxville, TN. Following the graduation, Márcia looks forward on starting her Ph.D. to expand her knowledge about forages, so then could incorporate this expertise towards the advancement the scientific community. Márcia enjoys spending time with her husband Jack, learning about the food system and exercising.