Assessing Warm-Season Annual Grasses to Increase Forage Inventory

Andrea Marroquin

University of Massachusetts Amherst

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ASSESSING WARM-SEASON ANNUAL GRASSES TO INCREASE FORAGE INVENTORY

A Thesis Presented
by
ANDREA MARROQUIN

Submitted to the Graduate School of the
University of Massachusetts Amherst in partial fulfillment
of the requirements for the degree of

MASTER OF SCIENCE

September 2022

Plant Biology
Assessing Warm-season Annual Grasses to Increase Forage Inventory

A Thesis Presented

By

ANDREA MARROQUIN

Approved as to style and content by:

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Masoud Hashemi, Chair

_________________________________________________
Michelle DaCosta, Member

_________________________________________________
Sam Hazen, Member

Sam Hazen, Department Head
Plant Biology
ACKNOWLEDGEMENTS

I would like to thank my advisor, Dr. Masoud Hashemi, for giving me this opportunity to complete my masters with him and for his mentorship and support in my graduate career. I am truly appreciative of the lab environment he has formed and the support and new knowledge I was able to learn from him and my lab mates. All my lab mates in Masoud’s lab have been beyond helpful. I especially want to say thank you to Arthur Siller for all their help, guidance, and time in helping me complete this thesis. I would not have been able to do it without their help and am especially very appreciative in all I have learned from them. To Samantha Glaze-Corcoran, thank you for not only broaden my research skills but also being there for support in both everyday life and academia.

I want to also thank my committee members, Dr. Sam Hazen and Dr. Michelle DaCosta. Together, they have made my stay at UMass and my masters a wonderful experience and have prepared me well for my next step in academia through their guidance and teaching. I will always be appreciative of my time in the Plant Biology program.

A special thank you to my family and friends who continually supported me during this project and continue to support me in my life.
ABSTRACT
ASSESSING WARM-SEASON ANNUAL GRASSES TO INCREASE FORAGE INVENTORY
SEPTEMBER 1, 2022
ANDREA MARRQUIN, B.S., NORTHERN KENTUCKY UNIVERSITY
M.S., UNIVERSITY OF MASSACHUSETTS AMHERST
Directed by: Masoud Hashemi

Summers are expected to continue to increase in heat/dryness in the Northeast, causing issues pertaining to forage production during the summer to worsen. Many pastures grow cool season grasses, even during the summer. These grasses enter a dormant period and slowdown in production during the months of July and August, leading to what is referred to as “summer slump”. Some farms grow corn silage during the summer, and while corn silage is a valuable crop, its cultivation often does not support soil biology. This research addresses solutions for both summer slump foraging and more sustainable silage. Summer annuals grow more efficiently during the summer and can produce better quality forage compared to winter grasses. Pearl Millet and Sudangrass were evaluated at seed percentages 0-100%. Biomass of each grass was evaluated by cutting a 2x3 ft section on a bi-weekly basis to establish how the treatments vary over time by seeding ratio and type of warm-season grass. Two separate cuts evaluated yield, quality, and regrowth. Another cut looked at ensiling success and quality of Pearl millet and Sudangrass. Results showed both forage species had similar and comparable quality to cool-season grasses. With how much more Sudangrass produces in yield and the little difference in forage quality compared to Pearl millet, Sudangrass would make a good replacement for cool-season grasses. Pearl millet and Sudangrass can be ensiled successfully and have competitive forage quality compared to corn silage.
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CHAPTER 1

INTRODUCTION

1.1 State of the problem

In the Northeast, future summers are expected to get hotter and dryer. According to the National Integrated Drought Information System for the state of Massachusetts, the summer of 2017 and 2020 have ranged from moderate to extreme drought over the course of June-September, with future summers expected to see similar patterns. In the Northeast, pastures consist of cool-season grasses. The growth of cool-season pastures follows a bimodal growth distribution, with peaks in the spring and fall and dips in the summer and late fall. While cool-season grasses grow successfully in spring and late summer-fall, they enter a dormant period and slowdown in production when temperatures begin to rise. This is referred to as the summer slump and is an issue because the cool-season grasses used in pastures do not perform well enough to meet the livestock’s needs in terms of both quantity and quality during the months of June-August (Darby et al., 2015). The dryer/hotter summers will continue to intensify the summer slump problem in grazing animal operations. Annual warm-season grasses have the advantage of fast germination, rapid growth, and high productivity. Compared to cool-season forages, summer warm-season annuals as C4 plants generally are drought and heat tolerant and produce high biomass during a short period of time in summer which can be grazed and used as stored feed (Darby et al., 2015).

In terms of nutrients, many animals are not having their needs met with cool-season grasses during the summer slump. In some cases, organic producer’s animals must have at least 30% of their dry matter intake from pasture for at least 120 days of the year (Darby et al., 2015). Annual warm-season forage will supply enough yield during the summer slump to help
supplement those 120 days, with the potential of meeting the nutrient requirements as well. These summer forages usually have greater nutritive value compared to their perennial counterpart, with higher crude protein and digestibility (Ball et al., 2001). Many annual warm-season forage has the potential for multiple cuts in one season which could allow for both grazing and ensiling options. Alternative to purchasing or using stored dry hay, summer annual forage can also be used as emergency forage, forming an effective and immediate solution to feed shortage. Plants that make good emergency forage can adapt to adverse climatic conditions in a relatively short period while still providing high yield and quality forage (Young et al., 2015).

1.2 Summer Annual Forage

In the New England area, warm-season grasses such as pearl millet (*Pennisetum americanum*), and Sudangrass (*Sorghum bicolor*), are commonly used as summer annual high producing forages. Both types of grass are drought and heat tolerant. Sudangrass is a tall grass that is known to grow back rapidly after harvest. Pearl millet is a bushy grass that grows rapidly and performs well in poor soil and high heat, providing grazing opportunities 45-60 days after planting. It has been shown to be a high producing dry matter, with high concentrations of crude protein and fatty acids, while having low fibers (NDF and ADF) compared to other grasses (Schmidt et al., 2013). Pearl millet also avoids the potential risk of prussic acid poisoning that can be associated with some summer grasses like sorghum.

Legumes that are commonly used in annual summer legume-grass mixtures in the Northeast include sun hemp, cowpea, soybeans, red clover, and crimson clover. All these legumes have been known to do well in dry conditions. Crimson clover (*Trifolium incarnatum*), and red clover (*Trifolium pratense*), are among the legumes that tend to grow well in both cooler
and drier areas. Crimson clover is a tall legume known to be competitive and produces a decent amount of biomass when grown in a mixture and is one of the most planted annual forage legumes (Brink and Casler, 2015). Additionally, crimson clover has been shown to have a low to no bloat effect on ruminants (Young-Mathews, 2013). Red clover is known to reduce weed pressure. Crimson clover tends to be a taller legume species while red clover is bushier and leafier. While sun hemp, cowpea, and soybeans perform well under warm conditions in the Northeast, they tend to not do as well as crimson clover and red clover in cooler areas like Vermont, Maine, and New Hampshire. All these species have been shown to be successfully grazed in pastures, but much more research is needed to fully understand the potential of grazing annual warm-season forage, especially in different mixtures.

1.3 Ensiling

Preserving forage can allow for a better supply of quality feed when forage inventory is low, or pastures are dormant. Silage is the practice of ensiling crops, where it is fermented anaerobically by lactic acid bacteria present on the crop (Muck and Shinners, 2021). High levels of lactic acid suggest both efficient fermentation and minimal dry matter loss of the product. The pH is the best way to assess silage fermentation, where the lower the pH the better fermented the silage is (Jahanzad et al., 2014). Traditionally, corn silage is used as a high-energy feed in dairy operations and also as low-cost rations for fattening cattle, when a high volume of fresh forage is not accessible. However, corn silage is a full-season crop, and in the Northeast U.S. where the growing season is relatively short, its growth occupies land for the entire season. Additionally, corn requires relatively a large volume of off-farm inputs, including fertilizer and herbicides, thus it does not support the soil biology. Corn also increases the potential for soil erosion when soil conservation practices are not a part of the production system.
Harvesting or grazing, and ensiling summer annual forage can help fill the gaps between the bimodal distribution of cool-season grass growth, implementing forage for the coming late fall and the following summer slump. Intercropped mixtures of legume-grasses are easier to harvest and cure for silage (Baylor, 1974). Previous studies have shown that intercropping mixtures of grasses and legumes can increase protein content and quality of silage (Sadeghpour et al., 2013; Jahanzad et al., 2014).

1.4 Intercropping

Crop diversity can lead to increased overall soil health and crop productivity and improve tolerance and resilience to biotic and abiotic stressors. Intercropping of multiple species of summer forages may result in multiple benefits such as an increase in yield, environmental quality, production security, and great ecosystem services like improving general soil health (Bybee-Finley et al., 2017). A successful cropping system relies on the production of crops, duration of production, and requirements of the land. Monocultures exhaust the land and natural resources, leading to poor agroecosystem performance. Intercropping systems need less energy resources like fertilizers and chemicals, with functional diversity leading to fewer pest-disease incidents (Maitra et al., 2021). Intercrop performance is usually examined by using the Land Equivalent Ratio (LER), which measures the productivity of the intercrop compared to each individual’s monoculture component (Vandermeer, 1989).

\[
LER = \sum (Y_{pi}/Y_{mi})
\]

Where \(Y_{pi}\) is the yield of a crop in intercropping and \(Y_{mi}\) is the crop yield in monoculture system.

Intercropping has been shown to increase overall crop productivity through both resource partitioning and facilitation. This system can lead to more coverage of the ground area by the
canopy of crops, allowing for more transpiration to take place by the foliage, creating a cooler microclimate and minimizing soil temperature. Resource partitioning occurs when intercrops use available resources to the fullest extent compared to the same crops grown as monocultures. Facilitation occurs when one species in the intercrop supplies a limiting resource or function the other species in the intercrop cannot obtain as easily in its monoculture (Bybee-Finley et al., 2016). An example of facilitation is the intercrop mixture of legume and grasses, where the legume makes nitrogen available to the companion grass.

Mixed intercropping is one of several methods of intercropping where two or more crops are grown together by mixing the seeds without any definite row proportion. This type of intercropping is prompted by the USDA for its soil health and conservation benefits (Buck, 2013). A common mixed intercrop example is grass-legume intercropping. Intercropping legumes with grass may lead to increased yield and higher quality of feed (Brink and Casler, 2015). This mixture has also been commonly observed to fill the requirements needed for forage nutrients when the land resource is a limiting factor (Undie et al., 2012). They have also been shown to decrease weed pressure, and as weed biomass decreases, crop biomass tends to increase (Brainard et al., 2008; Bybee-Finley et al., 2017). Legumes can form symbiotic relationships with rhizobacteria that fix nitrogen, and their inclusion in grass-legume intercrop mixtures has been shown to increase yield stability, lower nitrogen immobilization and lead to higher quality crop production than grass monocultures (Brainard et al., 2011). Compared to just using pure legume forage, mixing legumes with grasses can also minimize bloat incident in some animals like cows (Baylor, 1974). While grasses produce high biomass, the inclusion of legumes can increase forage quality by improving crude protein (CP) and neutralizing digestive fiber (NDF) (Sleugh et al., 2000). The intercrop mixture of grass-legume will most likely have a smaller yield
than the grass monoculture component, but the forage quality will be higher than the grass monoculture component and higher than the legume monoculture yield component (Glaze-Corcoran et al., 2020). The benefits of annual intercropping mixtures of grass-legume still need more research. One study looking at summer annual grass-legume mixtures found that resource partitioning, rather than facilitation, could be the suggested mechanism due to the short duration of annual intercropping (Bybee-Finley et al., 2016). There is potential that either yield advantage or yield decline could happen to both species or lead to one overpowering the other. Negative competition affects overall yield due to crop aggressiveness, which can vary by species and population density (Glaze-Corcoran et al., 2020). Different annual warm-season grasses and legumes still need to be observed in different mixtures and varying ratios, specifically mixtures that would perform well in the northeast during June-August.

1.5 Sustainable and Efficient Forage

A continual increase in the human population and a changing climate are expected to continue to happen, with no direct end in sight. This leads to competition for limited resources between humans and livestock, including land to grow and feed both. Forages area necessity to allow humans to continue to consume dairy and meat. To benefit both livestock and humans, forage that is both high in yield and nutrients will help decrease the overuse of land and unwanted additives to animal nutrition and human consumption. Finding an efficient and sustainable mixture to grow instead of cool-season grasses or corn silage is an efficient strategy of increasing forage availability for grazing and/or ensiling to compensate for the feed shortage during the summer months. Little research has looked into summer annuals and different varying seeding ratios of grass-legume mixtures. It is hypothesized that a higher ratio of grass (60:40) and mid ratio of legume (40:60) will have a high yield from the grass and enough nutrients from
the legume to meet the needs of the animals and be higher than cool-season pasture production and corn silage usage.

This study aimed to determine:

1- The right species for intercropping of warm-season annual grasses (C4) and legume (C3) as summer forage

2- The right mixing ratio(s) of the species for the most efficient combinations that maximize yield, provide better quality forage, and nutrient efficiency grown during the summer slump for grazing and silage purposes.

3- The ensiling success and quality of warm-season grass-legume mixtures as silage.
CHAPTER 2

INFLUENCE OF SEEDING RATE OF SUMMER ANNUAL GRASSES ON YIELD, QUALITY AND WEED GROWTH

2.1 Introduction

2.1.1 State of the problem

In the Northeast, future summers are expected to get hotter and dryer. According to the National Integrated Drought Information System for the state of Massachusetts, the summer of 2017 and 2020 have ranged from moderate to extreme drought over the course of June-September, with future summers expected to see similar patterns. In the Northeast, pastures consist of cool-season grasses. The growth of cool-season pastures follows a bimodal growth distribution, with peaks in the spring and fall and dips in the summer and late fall (Figure 1). While cool-season grasses grow successfully in spring and late summer-fall, they enter a dormant period and slowdown in production when temperatures begin to rise. This is referred to as the summer slump and is an issue because the cool-season grasses used in pastures do not perform well enough to meet the livestock’s needs of forage in terms of both quantity and quality during the months of June-August (Darby et al., 2015). The dryer/hotter summers will continue to intensify the summer slump problem in grazing animal operations.

Annual warm-season grasses have the advantage of fast germination, rapid growth, and high productivity. Compared to cool-season forages, summer warm-season annuals are primarily C4 plants which generally are drought and heat tolerant and produce high biomass during a short period of time in summer which can be grazed and/or used as stored feed (Darby et al., 2015). In terms of nutrients, many animals are not having their needs met with cool-season grasses during the summer slump. In some cases, organic producer’s animals must have at least 30% of
their dry matter intake from pasture for at least 120 days of the year (Darby et al., 2015). As seen in figure 1, annual warm-season forage has the potential to supply enough dry matter yield during the summer slump to help supplement those 120 days, with the potential of meeting the nutrient requirements as well. These summer forages usually have greater nutritive value compared to their perennial counterpart, with higher crude protein and digestibility (Ball et al., 2001). Many annual warm-season forage have the potential for multiple cuts in one season which could allow for both grazing and ensiling options. Alternative to purchasing or using stored dry hay, summer annual forage can also be used as emergency forage, forming an effective and immediate solution to feed shortage. Plants that make good emergency forage can adapt to adverse climatic conditions in a relatively short period while still providing high yield and quality forage (Young et al., 2015).

![Relative Forage Yields throughout the Growing Season](image)

Figure 1. Production trend of cool season perennials (green line) and warm-season annuals (red line) throughout the growing season.

### 2.1.2 Summer Annual Forage

In the New England area, warm-season grasses such as Pearl millet (*Pennisetum americanum*) and Sudangrass (*Sorghum bicolor*) are commonly used as summer annual high
producing forage grasses. Both types of grass have been shown to be high-yielding, quick-growing, and growing well in dry conditions (Darby et al., 2019). Sudangrass is a tall grass that is known to grow back rapidly after regrowth harvest. Pearl millet is a bushy grass that grows rapidly and performs well in poor soil and high heat, providing grazing opportunities 45-60 days after planting. Pearl millet is capable of producing high dry matter, with high concentrations of crude protein (CP) and fatty acids, while having lower concentrations of fibers compared to other grasses (Schmidt et al., 2013). Pearl millet also avoids the potential risk of prussic acid poisoning that can be associated with other summer grasses like sorghum and Sudangrass. Both grasses however do present a risk for nitrate poisoning, especially following a prolonged drought condition, since they generally require substantial nitrogen fertilizer to produce high quality forage.

Forage quality is crucial in determining livestock’s performance, whether it's for milk or growth production. Different types of animals have varying gut capacity, so their performance is determined not only by dry matter (DM) intake but also by the nutritive value of the forage. Livestock get all their nutritional need from forage. Therefore, consuming a low quality high fiber forage fills the gut and prevents the animal from more consumption. Therefore, the higher nutrient dense plants are, the less amount that will need to be consumed. A high-quality forage therefore requires less growing space with higher monetary return. Forage genotype, species, maturity stage, season, and management can all affect forage quality (Adesogan et al., 2009). In general, forage quality decreases as plants mature. This is mainly because cell walls become thicker, thus the amount of fiber increases while digestive parts of the cell reduce. Main forage quality parameters include DM, CP, nutrients content, fiber (both acid detergent fiber, ADF, and neutral detergent fiber, NDF).
CP is calculated by measuring N content multiplied by 6.25. The CP is only applicable to ruminants, since these animals have rumen microbes that can convert non-protein nitrogen to microbial protein that the animal can use (Ball, 2007). Reported CP for warm-season grasses range from 5-18%, with the higher the % the better. ADF measures the least digestible fiber portion of forage which includes cellulose, lignin, and silica (Newman et al, 2009). ADF for warm-season grass falls around 50%, with the lower the % the better. In addition to the components measured in ADF, NDF includes the indigestible and slow digesting parts in plant cell walls like hemicellulose, lignin, and ash (Ball et al, 2009). NDF for warm-season grasses can vary, ranging from 50-75%, with the lower the % the better. ADF has been used to predict digestibility and energy while NDF is used as a predictor of forage intake. Results of forage analysis can also include digestible energy, net energy, and total digestible nutrient (TDN). TDN is the sum of CP, fat, non-structural carbohydrates and NDF. TDN is an acceptable measure of nutritive value, which ranges from 45-65% for warm-season grasses. Pearl millet has been shown to be as high as 70% (Newman et al 2009). Non-fibrous carbohydrate (NFC) is also an additional measurement that examines starch content.

Researching on varying seeding percentages grasses as alternative to cool-season grasses during the hot and dry conditions in July and August in Northeast U.S. is not well-documented. We hypothesized that summer annuals could be considered as an efficient strategy to increase forage availability for grazing and/or ensiling thus, compensate the feed shortage during the summer months. In addition, the quality of summer annuals, especially as ensiled forage, compared to traditional corn silage is not reported. Additionally, the weed pressure may change with changes in seeding rate, influencing the yield and quality response of summer annuals to the
seeding rate. It was hypothesized that either of these two summer grasses will produce enough nutrient dense forage to supplement forage slumps through grazing.

This study aimed to determine the right seeding percent of two warm-season annual grasses to maximize yield, optimum forage quality, and nutrient density when grown during the summer slump for grazing and ensiling purposes.

2.2 Material and Methods

2.2.1 Site description

This experiment was conducted at the University of Massachusetts Research Farm in South Deerfield (42° 28’ 30.7524” N and 72°35’10.4892” W) from June 2 to September 2, 2021. The soil at the farm is characterized as Hadley fine sandy loam (super active, non-acidic, mesic type udifluvents). Initial base soil sample for current nutrient level was taken in late May prior to planting, showing a soil pH of 6.7. The soil was disk tilled prior to planting. Planting took place on June 2, 2021, using a custom-made Brillion cone seeder. A week and half after planting, 50 pounds per acre of nitrogen was applied as calcium ammonium nitrate (27%). From late May to early September, the mean precipitation averaged 5.22 mm, while the mean temperature averaged 21°C.

2.2.2 Experimental Design

This experiment examined the yield and quality of two summer annual grasses at varying seeding ratios. AS9301 Sudangrass and Exceed BMR Pearl millet seeds were purchased from King's Agriseeds (Lancaster, PA) and planted at seeding rates that were based on the recommendations of seed supplier (34 kg/ha for Sudangrass, 22 kg/ha for Pearl millet). Seeding ratios for each species included 5 ratios: 0, 25, 50, 75, and 100 percent of the recommended rates.
(Table 1). Seeds were planted into 2 x 6-meter plots and arranged into randomized complete block design with four replications and two plots of each treatment in each block.

Clover species were intercropped with Pearl Millet and Sudangrass at the same ratios during planting time but did not establish during the growing season and were omitted from analysis and experiment.

Table 1. Treatments for monoculture plots: 1) Pearl millet and 2) Sudangrass at the seeding percentages 25-100 for suggested seeding rates.

<table>
<thead>
<tr>
<th>Treatments: 1</th>
<th>25% Seeding Percent</th>
<th>50% Seeding Percent</th>
<th>75% Seeding Percent</th>
<th>100% Seeding Percent</th>
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<tr>
<td></td>
<td>Pearl millet</td>
<td>Pearl millet</td>
<td>Pearl millet</td>
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<tr>
<td></td>
<td>5.5 kg/ha</td>
<td>11 kg/ha</td>
<td>16.5 kg/ha</td>
<td>22 kg/ha</td>
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<td>Sudangrass</td>
<td>Sudangrass</td>
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<td>17 kg/ha</td>
<td>25.5 kg/ha</td>
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2.2.3 Field Measurements

To examine growth over time for the varying ratios of each grass species during the summer, above ground biomass yield was measured by cutting an undisturbed 0.28 m² section from each plot on a bi-weekly basis during the growing season. Biomass yield was collected on four separate harvest dates: July-6, July-13, July-29, and August-10. Grasses were cut at the ground level, collected, and separated from weeds. Collected biomass was then placed in brown paper bags and dried in an oven for over 48 hours until constant weight. The dry weight in grams was then recorded to the first decimal place. The dominant weed species identified as lambsquarter (*Chenopodium berlandieri* Moq.), pig weed (*Amaranthus; L.*), galinsoga (*Galinsoga parviflora*), and fall panicum (*Panicum dichotomiflorum*). There were high population of purslanes (*Portulaca oleracea*) in experimental plots early in the season but by the last harvest their population was no longer significant.
Multiple harvests were made to mimic the grazing and regrowth process which would occur on a typical pasture. The biomass dry matter yields were measured on two different harvest dates to examine yield and quality for grazing purposes. The first cut was taken around eight weeks after germination on July-20, followed by the second cut, five weeks later on September-2 to examine the regrowth of plants after the first cut. Samples were cut 15 cm above the ground from 0.28 square m of undisturbed sections in each plot. Samples of the second harvest were taken from the same spot as the first harvest, after leaving the lower 15 cm. Weeds and grass species were separated, dried, and weighed individually for each plot. Dry matter of each dominant weed was determined individually and reported separately and as total weed biomass. The purpose of weed measurement was to examine how they compete with the Pearl millet and Sudangrass at different seeding percentage after regrowth process following the first harvest.

2.2.4 Forage Analysis

Both grass and weed biomass from harvest July-20 and September-2 were analyzed for forage quality. Biomass for each plot was ground to 1 mm using a Foss Mill. For each harvest, replications were mixed together to create a composite of Sudangrass samples, Pearl millet samples, and weed samples, forming six samples total for analysis. Samples were sent to the Dairy One Forage Lab (Ithaca, NY) where wet chemistry was used to assess the forage quality of each species.

Forage quality components were included crude protein (CP), acid detergent fiber (ADF), neutral detergent fiber (NDF), total digestible nutrients (TDN), net energy for maintenance (NEM), net energy for lactation (NEL), net energy for growth (NEG), relative feed value (RFV), non-fibrous carbohydrate (NFC), and horse digestible energy (HDE). For each plot, analysis
components consist of yield from both grass and weed measurements, calculated, and multiplied
with each forage measurement to get composite plot data points. Overall biomass yield was
calculated as a total of grass + weed, grass, and weed individually.

2.2.5 Statistical analyses

All statistical analyses were performed with R software version 4.1.2 (2021-11-01). The
packages used throughout the analysis were: tidyverse, nlme, lme4. For all analyses, blocking
was treated as a random variable.

To analyze growth over time for the varying ratios of each grass species during the
summer growing season, a linear mixed effect model was used:

\[
lme(g\_yield\sim g\_sp+g\_percent+har\_days+g\_sp:g\_percent+g\_sp:har\_days+g\_percent:har\_days, \\
\quad random=\sim 1|rep, data=summer2)
\]

Analysis of variance (type III sum of squares) was used to
assess main effect and interaction significance at \(P<0.05\). Species was treated as a discrete
binary variable while seeding rate and harvest date were continuous variables. Grass yield was
the response variable. Mean yields for grass species and harvest dates, grass species and seeding
ratio, and grass species, harvest dates, seeding ratio were also calculated.

To analyze the grazing and regrowth process that would occur on farms, a linear mixed
effect model: \(lme(gw\_X\sim g\_sp\_g\_percent*fact\_har\_days, random=\sim 1|rep, data=summer, \\
\quad na.action=na.omit)\) was used on the two different harvest dates, July-20 and September-2. In the
model, \(X\) was replaced with yield and the ten forage components to calculate the effects seed
ratio, harvest date, and grass species had on the response variables of yield and forage quality.
Variance analyses (ANOVA type III) was used to assess main effect and interaction significance
at \(P<0.05\). Species and harvest date were treated as a discrete binary variable while seeding rate
was continuous variables. Mean forage components for grass species and harvest dates, grass species and seeding ratio, and grass species, harvest dates, seeding ratio were calculated. Means for total yield (grass + weed), grass yield, and weed yield were also calculated. Linear regression was used to estimate the effect of seeding rate, grass species, and harvest date on the yield and forage quality.

2.3 Results

2.3.1 Forage Crop and Weed Biomass

Growth over time for the varying ratios of each grass species during the summer growing season was not affected by the interaction between grass species, seed percent, and harvest date. Biomass yield was significantly affected by the interaction between grass species and seed percent, grass species and harvest date and seed percent and harvest date (Table 2).

Table 2. P-value significance at P<0.05 of grass species (GS), seeding ratio (SR), harvest day (HD) and their two way interactions between grass species: seeding ratio (GS:SR), grass species: harvest date (GS: HD), and seeding ratio: harvest date (SR:HD) and their effects on warm-season annual DM yield.

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</table>

Grass species, seed percent, and harvest day also individually affected biomass yield. For all seed percent s (25, 50, 75, 100) and harvest dates (July-6, July-13, July-29, August-10) Sudangrass produced higher yield than Pearl millet grass (Figure 2). Average mean biomass yield was higher for all main effects for Sudangrass, with its mean yield being doubled that of Pearl millet grass mean yield (Table 3a, b, c).
Figure 2. Total yield of Pearl millet (red line) and Sudangrass (blue line) at each seeding percent: 25, 50, 75, 100 for the harvest days July-6, July-13, July-29, August-10. Each harvest day correlates to days after planting.

Table 3. a) Mean yield for Sudangrass and Pearl millet at the seeding percentages: 25, 50, 75, 100 for the harvest days July-6, July-13, July-29, August-10. Each harvest day correlates to days after planting. b) Mean yield for Sudangrass and Pearl millet for each seeding percent. c) Mean yield for Sudangrass and Pearl millet for each harvest day.

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<td>149</td>
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<tr>
<td>Sudan</td>
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<td>71</td>
<td>294</td>
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</table>
When just looking at biomass yield over the growing season, Sudangrass produced the most DM at each harvest date and each seed percent (Figure 3). By 57 days after planting (DAP) Sudangrass had doubled the yield of Pearl millet. In terms of seeding percent, 100% had the highest yield starting at 41 DAP for Sudangrass and 57 DAP for millet. If harvesting is done around 41 DAP and after 69 DAP, 50% and 75% seeding give similar yield for Sudangrass. When looking at harvest data for grazing purposes, there was only a difference between Sudangrass and Pearl millet for harvest 1 and no difference between the two grasses for the regrowth (Figure 4).

Figure 3. Dry Matter yield of Pearl millet and Sudangrass at each harvest day comparing all the seeding percent for both grass species. Blue: 25% Orange: 50% Grey:75% Yellow: 100%
Figure 4. Mean comparison of Sudan grass (Blue) and Pearl millet (Yellow) at harvest 1 and harvest 2.

2.3.2 Forage Quality Assessment

The forage quality for the grazing and regrowth process had varying results for the significance of each main effect and their two-way interactions (Table 4).

With statistical significance of ≤0.001 for each interaction, CP was affected by all main effects and interactions. Between the two harvests, harvest 1 had a higher % of CP compared to harvest 2, with Pearl millet (17.8%) higher than Sudan grass (15.0 %) (Figure 5a; Table 6a). CP was barely higher for Sudan grass (11.1%) compared to Pearl millet (10.8) for harvest 2. Overall, Pearl millet CP stayed relatively the same as seeding percent increased, whereas Sudan grass showed a decreasing trend in quality as seed percent increased (Table 6b).

ADF was affected by all main effects and the two-way interaction between grass species and seed percent. For both harvests, ADF % in Sudan grass was overall lower than Pearl millet (Figure 5b). Sudan grass harvest 1 had the lowest ADF (34.8) and the smallest difference when comparing both harvests (Figure 5a). Overall, as seeding percent increased, ADF did not change significantly among each species but was different when comparing Sudan grass (35%) to Pearl
millet (37%) (Table 6b). For harvest 1, ADF gradually increased as seeding percent increased for both species but stayed similarly the same for harvest 2 (Table 5a, b).

NDF was affected by the main effects i.e., seed percent and harvest day. Overall, NDF increased for both species at both harvests, with NDF % increasing for both species at both harvests as seeding percent increased (Figure 5c, Table 5a, b). Millet (52.8%) at harvest 1 had the lowest NDF between the species and the two harvests, with harvest 1 having the smallest NDF for both species when compared to harvest 2 (Table 6a).

TDN was affected by the main effect, harvest day, and by all two-way interactions. Overall, harvest 1 had higher TDN values compared to harvest 2, with Pearl millet having the highest TDN (58.7%) but only a little difference compared to Sudangrass harvest 1 (57.5) (Figure 5d, Table 6a). Pearl millet stayed consistent as seeding percent increased overall and between the harvest 1 and harvest 2, while just for harvest 1, TDN decreased for Sudangrass as seeding percent increased (Table 5a, b; Table 6b).

NEL, NEG, and NEM were affected by all interactions but grass species (Figure 4). For all three energy components of forage quality, harvest 1 demonstrated the highest values for each component compared to harvest 2, and slightly decreased as seeding percent increased for harvest 1 (Figure 5e, f, g; Table 6a, b). Harvest 1 had the highest % for all three energy indicators, with both species being very similar in value. Harvest 2 stayed consistent for both species as seeding percent increased, but overall, as seeding percent increased, NEL, NEG, and NEM % showed a decreasing trend (Table 6b).

RFV was affected by all main effects and the two-way interactions between grass species and harvest date as well as between seed percent and harvest day. RFV % for Harvest 1 for both species was higher than harvest 2 (Figure 5h, 6a). Overall, RFV decreased as seeding percent
increased, with the biggest difference happening among Sudangrass (Table 6b). For harvest 1, RFV of both species decreased as seeding percent increased, but for harvest 2 Sudangrass dropped slightly as seeding percent increased while Pearl millet had no change in RFV% between the two harvest times (Table 5a, b).

HDE was affected by the main effects including seed percent and harvest day and all two-way interactions. HDE% of Pearl millet for harvest 1 and both harvest times of Sudangrass showed a decreasing trend as seeding percent increased (Figure 5i, Table 5a, b). Overall, HDE of Pearl millet stayed consistent as seeding percent increased (Table 6b). However, overall, the harvest 1 had higher HDE values compared to harvest 2, with Pearl millet having the highest % at first harvest (Table 6a). Pearl millet also had the lowest % at 0.92 in second harvest.

NFC was affected by the main effects of grass species and seed percent as well as the two-way interaction between grass species and harvest date. Overall, both species for both harvests followed a downward trend where NFC % decreased as seed percent increased. However, for harvest 2, Pearl millet had a higher NFC % for 100% seeding rate compared to the other seeding rates (Figure 5j, Table 6b). Sudangrass in second harvest had the highest NFC% at 20.4, while Pearl millet had similar values for both harvests (~18) (Table 6a). Sudangrass, overall, had the highest NFC for both harvesting times (Table 6a).
Table 4. P-value significance at P<0.05 of grass species (GS), seeding ratio (SR), harvest day (HD) and their two way interactions between grass species: seeding ratio (GS:SR), grass species: harvest date (GS: HD), and seeding ratio: harvest date (SR:HD) and their effects on warm-season annual DM yield and forage assessments.

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*0.05  **0.01  ***0.001
Figure 6. Linear regression and mean of forage assessments for Pearl millet and Sudangrass as seeding percent increases at both harvest 1 and 2; Dots: linear regression; Dotted Line: Mean. Red: Pearl Millet H1; Grey: Sudangrass H1; Yellow: Pearl Millet H2; Blue: Sundangrass H2. a) Crude protein b) Acid Detergent Fiber c) Neutral Detergent Fiber d) Total Digestible Nutrients e) Net energy of maintenance f) Net energy of lactation g) Net energy for gain h) Relative feed value i) Horse Digestible energy j) Non-fibrous Carbohydrates
Table 5. a) Mean percentages of forage quality assessments for harvest 1 broken up by each seeding ratio of Pearl millet and Sudangrass. b) Mean percentages of forage quality assessments for harvest 2 broken up by each seeding ratio of Pearl millet and Sudangrass.

a.

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<td>0.95</td>
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<td>0.48</td>
<td>98.4</td>
<td>0.94</td>
<td>18.7</td>
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</table>
Table 6. a) Mean percentages of forage quality assessments for harvest 1 and harvest 2 of Pearl Millet and Sudangrass. b) Mean percentages for forage quality assessments for each seeding ratio of Pearl Millet and Sudangrass. The higher percent the better except for ADF and NDF where the lower the percent the better the value.

### a.

<table>
<thead>
<tr>
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<tbody>
<tr>
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<td>CP</td>
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<tr>
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</tr>
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### b.

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<td>75</td>
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<table>
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<tr>
<td>100</td>
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2.4 Discussion and Conclusion

In this study, Sudangrass (4.1 Mg/ha) produced more yield compared to Pearl millet (2.9 Mg/ha), had less weed growth as its seeding percent increased, while having similar forage quality as Pearl millet. Sudangrass and Pearl millet produced yields comparable to similar other studies in New England (Darby et al., 2010, 2019). Regrowth can affect forage quality negatively, and in this experiment, yield and forage quality did decrease. It begins to decline as the forage regrows due to the accumulation of stems and deposition of lignin in both leaves and stems that cannot be easily digested (Adesogan et al., 2009). Only TDN did not change from 1st harvest to the 2nd. Interestingly, in two similar studies on Sudangrass, CP of the 2nd cut was higher than the CP from the 1st cut (Darby et al., 2010, 2019).

The use of Sudangrass or Pearl millet as grazing material during the summer has potential because warm-season grasses can grow quickly during hotter parts of the year and require less water when compared to the cool-season grasses. Cool-season grasses can produce about 13.5-21.5% CP, 32.6-40.1% ADF, and 51.2-67.5% of NDF through the months of June through September, with a yield of about 2.41 Mg/ha (Tracy et al., 2010; Ritz et al., 2020). While Pearl millet produced less yield than Sudangrass in this study, both still produced more than what would be expected from cool-season grasses.

Pearl millet is known to have a higher CP compared to Sudangrass and this was confirmed in the current study with 17.8% for Pearl millet and 15.01% for Sudangrass. Other studies have shown Pearl millet contained 14.5-22.79% CP and Sudangrass at 15.4-18.9% (Darby et al., 2010, 2019; Schmidt et al., 2013; DeBoer et al., 2017). Both grass species are comparable to the CP reported for various cool-season grasses (Tracy et al., 2010; Ritz et al., 2020).
NDF is a good measurement of total fiber within the cell wall and can help predict dry matter intake, with the lower the percentage the better. However, when comparing cool-season grasses to warm-season grasses, cell wall contents are usually greater for warm-season grasses. For example, 50% of NDF for cool-season grasses may be less digestible than warm-season grasses with the same NDF % (Hancock et al., 2014). In this study, Pearl millet had a lower NDF value (52.8%) compared to Sudangrass (54.6%). When looking at similar studies, both Pearl millet and Sudangrass had better NDF value compared to these studies and similar values when compared to cool-season grasses (Darby et al., 2010, 2019; Tracy et al., 2010; Schmidt et al., 2013; DeBoer et al., 2017; Ritz et al., 2020).

ADF is a good indicator of digestibility of the forage, with the lower the value the better. While Sudangrass had the lowest ADF value (34.8%), it was close to Pearl millet’s ADF (35.6%). The first harvest for both species had the lower ADF value. However, both grasses had similar ADF at harvest 2 (35.4%). ADF measured in current study for both Pearl millet and Sudangrass had similar but overall, lower values compared to other warm-season grass studies and cool-season grasses (Darby et al., 2010, 2019; Tracy et al., 2010; Schmidt et al., 2013; DeBoer et al., 2017; Ritz et al., 2020).

While yield did increase as seeding ratio increased, quality decreased, especially when comparing harvest 1 to harvest 2. For seeding percent 50%-100%, Sudangrass performed better in suppressing weeds, while Pearl millet was never too far ahead of weed presence at any seeding percent. Quality wise, Pearl millet did have better forage assessment values across the board, except for ADF and NFC. However, the differences of all the forage assessment values between Sudangrass and Pearl millet showed very little difference in terms of value, with some cases differing from 0.2 to 1%. The only major difference would be between CP where Pearl
millet contained 17.8% and Sudangrass had 15% CP. Both forage species had similar and comparable quality to cool-season grasses. With how much more Sudangrass produces in yield and the little difference in forage quality compared to Pearl millet, Sudangrass would make a good replacement for cool-season grasses.
CHAPTER 3
ENSILING OF WARM-SEASON ANNUAL GRASSES

3.1 Introduction

3.1.1 State of the problem

In the Northeast, pastures consist of cool-season grasses. The growth of cool-season pastures follows a bimodal growth distribution, with peaks in the spring and fall and dips in the summer and late fall (Figure 1). While cool-season grasses grow successfully in spring and late summer-fall, they enter a dormant period and slowdown in production when temperatures begin to rise. This is referred to as the summer slump and is an issue because the cool-season grasses used in pastures do not perform well enough to meet the livestock’s needs of forage in terms of both quantity and quality during the months of June-August (Darby et al., 2015).

Figure 1. Production trend of cool season perennials (green line) and warm-season annuals (red line) throughout the growing season.

To help supplement these slumps during the grazing periods, preserved forage is grown, fermented, and stored. Currently, corn silage is the main fermented crop grown and sold as stored forage. However, in the Northeast, is very sensitive to temperate change, especially in the late spring frosts and early fall frosts (Bernardes et al., 2018). Corn is warm-season grass that
grows quickly in the summer but requires a long growing season. A typical rotation of corn silage on dairy farms is about 3 to 4 years and grows primarily from late April through September. Corn silage harvests are often delayed to obtain an adequate amount of yield. Due to its long growing season, it can be difficult to pair with spring and fall forages, causing the potential to throw off a season of productivity for the following harvests. With predicted wet springs and additional summer droughts due to climate change conditions, the yield of long-season corn silage and the widespread cool-season grass-legume pastures will be limited (Hristov et al., 2018). Another issue with corn silage is, that while it produces high yield and quality forage, it can have negative environmental consequences (Randall, 2003). Corn silage is not ideal for overall soil heath due to minimal crop residue being left behind after harvest and leaving the soil prone to erosion, and soil carbon to be lost to microbial respiration and leaching (Dolan et al., 2006; Jokela et al., 2009). Planting warm-season annual grasses during the summer for both grazing and ensiling purposes allows for forage conservation which would permit a better supply of quality feed when forage production is in a slump.

Annual warm-season grasses have the advantage of fast germination, rapid growth, and high productivity. Summer warm-season annuals are primarily C4 plants which generally are drought and heat tolerant and produce high biomass during a short period of time in summer which can be grazed and/or used as stored feed (Darby et al., 2015). In terms of fermentation, warm-season grasses have been shown to be similar to cool-season grasses, indicating no concern of potential negative impacts on the dairy cattle diet (Ruh et al., 2018). Warm-season annuals are also more flexible in terms of management and allow for more diverse crop rotations that have the potential to support better soil biology. Unlike corn silage, which is cut only once during the growing season, warm-season annual grasses have the potential for multiple cuts,
depending on time of planting. Alternative to purchasing or using stored dry hay or haylage, summer annual forage can also be used as emergency forage, forming an effective and immediate solution to feed shortages in fall and winter. Plants that make good emergency forage can adapt to adverse climatic conditions in a relatively short period while still providing high yield and quality forage (Young et al., 2015).

3.1.2 Ensiling

Silage is forage that has been fermented and is an old agricultural practice that has been around for more than 3,000 years (Wilkinson et al., 2003). While conserved forage is useful and needed, it rarely matches the nutritive value of fresh forage due to the loss of some sugars, proteins, and fats during the fermentation process. Silage management includes, the selection of forage species, stage of maturity and moisture content at harvest, ensiling methods, level of compacting the ensiling material, type of storage structure, and silage additives (Mahanna and Chase, 2003). The fermentation phase of ensiling is thought to be about 7-45 days (Pahlow et al., 2003). The main way of evaluating silage is by measuring pH and quantifying the production of organic acids and alcohols (Kung et al., 2018). These measurements determine how well the fermentation process occurred. Well-fermented silages should not have strong odors other than a mild odor of vinegar. Another form of ensiling forage is haylage. Haylage, usually composed of grasses sometimes mixed with alfalfa, and wrapped or anaerobically stored forage containing >500 g of dry matter per kg (Müller, 2005; Harris et al., 2017). Haylage is preserved by a combination of drying and airtight storage. Haylage is better quality than hay and is more accessible than corn silage, which can be difficult to transport and market (Ruth and Heinrichs., 2001). However, haylage is lower in nutritive value compared to other ensiled material, has limited intake potential, is very dependent on weather and can lead to high yield losses, and
needs to be stored in dry conditions (Ruth and Heinrichs., 2001). Good silage can be made from warm-season annual grasses but can sometimes be lower in energy when compared to corn silage. Experiments conducted on lactating cows showed the warm-season annual grass species, Sorghum, can completely replace corn silage and not effect milk yield (Oliver et al., 2004; Dann et al., 2008; Colombini et al., 2010, 2012). In another study, researchers found that sorghum silage properly supplemented with corn meal can fully replace corn silage while still maintaining milk yield and feed efficiency (Cattani et al., 2017).

The main goal during the ensiling process is to reduce oxygen and increase acidity quick enough that lactic acid bacteria will grow and stabilize and ferment the forage (Ward and de Ondarza, 2000). The lactic acid goal is 4-7%. While higher lactic acid levels are considered to be better, it can be a problem if it exceeds 10%. Acetic acid, which is another major acid, is found within the fermentation process, and usually ranges at <3% and is the reason for the vinegar smell. It inhibits yeasts and improves aerobic stability of silages (Gerlach et al., 2021). Lactic acid to acetic acid ratio (L/A) can be used as a qualitative indicator of fermentation, with good fermentations having a ratio of 2.5-3.0 (Kung et al., 2018). pH is an important component of accessing silage fermentation because the lower the pH, the better fermented the silage is. The pH comes from lactic acid, and the low pH stabilizes fermentation by preventing the growth of or killing aerobic microorganism which are responsible for silage spoilage (Kung et al., 2018). Butyric acid should measure less than 0.1% and should not be detectable in well-fermented silage. The presence of this acid indicates the metabolic activity from clostridial organisms is present and causes large losses of DM and poor recovery of energy (Pahlow et al., 2003). In general, greater lactic and L/A values are desirable along with lower values for the remaining analyses.
3.1.3 Summer Annual Forage

In the New England area, warm-season grasses such as Pearl millet (*Pennisetum americanum*) and Sudangrass (*Sorghum bicolor*) are commonly used as summer annual high-producing forage grasses. Both types of grass have been shown to be high-yielding, quick-growing, and growing well in dry conditions (Darby et al., 2019). Sudangrass is a tall grass that is known to grow back rapidly after regrowth harvest. Pearl millet is a bushy grass that grows rapidly and performs well in poor soil and high heat, providing grazing opportunities 45-60 days after planting. Pearl millet is capable of producing high dry matter, with high concentrations of crude protein (CP) and fatty acids, while having lower concentrations of fibers compared to other grasses (Schmidt et al., 2013). Pearl millet also avoids the potential risk of prussic acid poisoning that can be associated with other summer grasses like sorghum and Sudangrass, however, ensiling Sudangrass usually eliminates the prussic acid problem (Nleya and Jeranyama, 2005). Both grasses however do present a risk for nitrate poisoning, especially following a prolonged drought condition, since they generally require substantial nitrogen fertilizer to produce high-quality forage.

Forage quality is crucial in determining livestock’s performance, whether it's for milk or growth production. Different types of animals have the varying gut capacity, so their performance is determined not only by dry matter (DM) intake but also by the nutritive value of the forage. Livestock gets all their nutritional need from forage. Therefore, consuming a low-quality high fiber forage fills the gut and prevents the animal from more consumption. Therefore, the higher nutrient-dense plants are the less amount that will need to be consumed. A high-quality forage, therefore, requires less growing space with a higher monetary return. Forage genotype, maturity stage, season, and management can all affect forage quality (Adesogan et al.,}
In general, forage quality decreases as plants mature. This is mainly because cell walls become thicker, thus the amount of fiber increases while digestive parts of the cell reduce. Main forage quality parameters include DM, CP, nutrients content, and fiber (both acid detergent fiber, ADF, and neutral detergent fiber, NDF).

CP is calculated by measuring N content multiplied by 6.25. The CP is only applicable to ruminants since these animals have rumen microbes that can convert non-protein nitrogen to microbial protein that the animal can use (Ball et al, 2007). Reported CP for warm-season grasses ranges from 5-18%, with the higher the % the better. ADF measures the least digestible fiber portion of forage which includes cellulose, lignin, and silica (Newman et al, 2009). ADF for warm-season grass falls around 50%, with the lower the % the better. In addition to the components measured in ADF, NDF includes the indigestible and slow digesting parts in plant cell walls like hemicellulose, lignin, and ash (Ball et al, 2009). NDF for warm-season grasses can vary, ranging from 50-75%, with the lower the % the better. ADF has been used to predict digestibility and energy while NDF is commonly used as a predictor of forage intake. Results of forage analysis can also include digestible energy, net energy, and total digestible nutrient (TDN). TDN is the sum of CP, fat, non-structural carbohydrates and NDF. TDN is an acceptable measure of nutritive value, which ranges from 45-65% for warm-season grasses. Pearl millet has been shown to be as high as 70% (Newman et al 2009). Non-fibrous carbohydrate (NFC) is also an additional measurement that examines starch content.

Quality of warm-season annual grasses ensiled is not well documented. Warm-season annual grasses have the potential to be used as grazing material during the summer period and could have enough yield to allow for a second harvest that can be stored as silage and used during the winter slump. We hypothesized that warm-season annual grasses could be considered
as an efficient strategy to increase forage availability for grazing and/or ensiling thus, compensate the feed shortage during the summer and winter months. In addition, the quality of summer annuals, especially as ensiled forage, compared to traditional corn silage is not reported. It was hypothesized that either of these two summer grasses will produce enough nutrient-dense forage to compensate for forage slumps through grazing or ensiling.

This study aimed to determine the quality and success rate of ensiling two warm-season annual grasses, Pearl millet and Sudangrass.

3.2 Materials and Methods

3.2.1 Site description

This experiment was conducted at the University of Massachusetts Research Farm in South Deerfield (42°28'30.7524" N and 72°35'10.4892" W) from June 2 to September 2, 2021. The soil at the farm is characterized as Hadley fine sandy loam (super active, non-acidic, mesic type udifluvents). Initial base soil sample for current nutrient level was taken in late May prior to planting, showing a soil pH of 6.7. The soil was disk tilled prior to planting. Planting took place on June 2, 2021 using a Brillion cone seeder. Calcium ammonium nitrate (27%) fertilizer was applied 10 days after planting at 50 kg per hectare of nitrogen. From late May to early September, the mean annual precipitation averaged 522 mm, while the mean annual temperature averaged 21°C.

3.2.2 Experimental Design

This experiment examined the forage and silage quality of two summer annual grasses. AS9301 Sudangrass and Exceed BMR Pearl millet seeds were purchased from King’s Agriseeds (Lancaster, PA) and planted at seeding rates that were based on the recommendations of seed supplier (34 kg/ha for Sudangrass, 22 kg/ha for Pearl millet). Seeds were planted into 2 x 6-meter
plots and arranged into a randomized complete block design with four replications and two plots of each treatment in each block.

3.2.3 Field Measurements

A total of one harvest was made to analyze the silage quality of the two grass species and the weeds found within those plots. The harvest was taken around nine weeks after germination on July 28. Samples were cut 15 cm above the ground from 0.28 square m of undisturbed sections in each plot. Weeds and grass species were separated. The dominant weed species identified as lambsquarter (*Chenopodium berlandieri* Moq.), pig weed (*Amaranthus; L.*), galinsoga (*Galinsoga parviflora*), and fall panicum (*Panicum dichotomiflorum*). There was a high population of purslanes (*Portulaca oleracea*) in experimental plots early in the season but by the last harvest their population was no longer significant.

Fresh biomass was chopped into 1.5 cm pieces using an Ohio forage chopper. 800 grams of each forage and weeds was placed into one liter silage containers made of high-density polyethylene plastic. A combination of Sudangrass + weed and Pearl millet + weed was made, each totaling 800 gram with a split of 400 gram for each species. For each rep, a sample of each grass, weed, and grass + weed mixtures were ensiled, for a total of 20 silage containers. Grass + weed combinations were made due to the fact that the weeds present are consumable as high quality forage and identified weeds were harmless to animal consumption in small quantities and could have potential of increasing forage quality which would save money and energy for future weeding. Silage containers were sealed for fermentation on the same day as harvest, July-28, and opened for analysis on December-20. Samples were sent to the Dairy One Forage Lab (Ithaca, NY) where wet chemistry was performed to assess the forage and silage quality of each species.

3.2.4 Forage Analysis
Silage assessment included the forage quality components: crude protein (CP), acid detergent fiber (ADF), neutral detergent fiber (NDF), total digestible nutrients (TDN), net energy for maintenance (NEM), net energy for lactation (NEL), net energy for growth (NEG), relative feed value (RFV), and non-fibrous carbohydrate (NFC). Silage assessment also examined percentages of lactic acid, acetic acid, the ratio of lactic/acetic acid, pH, ammonia, and total nitrogen present in each forage sample. All four reps for each forage product were combined to form average values for lactic acid, acetic acid, lactic/acetic acid, pH, and total nitrogen present, and compared to the goal values that would be expected from ensiling forage. Data for a rep from Sudangrass was taken out during analyses. It was concluded that the jar did not seal properly, altering the fermentation process.

3.2.5 Statistical analyses

All statistical analyses were performed with R software version 4.1.2 (2021-11-01). The packages used throughout the analysis were: tidyverse, nlme, lme4. For all analyses, blocking was treated as a random variable.

To analyze silage quality, a linear regression model: model1 <- lm(X~species, data=summer) was used on collected forage data, where X was replaced with the 16 different forage components. Variance analyses (ANOVA type III) was used to assess the differences between the means of the forage treatments of a significance at $P<0.05$. After ANOVA analyses, mean separations were performed using Tukey honestly significant difference test at a significant difference $P<0.05$.

3.3 Results

For the percent of lactic acid found within each forage product, Sudangrass had the highest value, with the mixture Sudan + weed not being statistically different from the
Sudangrass monoculture product (Table 1). As stated above, greater lactic and L/A values are desirable along with lower values for the remaining analyses. Millet, Millet + weeds, and weeds were all statistically similar to each other and different to Sudangrass and Sudan + weeds. Percent acetic acid found within Sudangrass was not statistically different from Sudan + weeds or weeds product and had the lowest value (1.36) compared to all forage products. All forage products were statistically different from the mixture Millet + weeds, with Millet + weeds having the largest acetic acid percent (7.81). Millet monoculture product was not statistically different from weeds. For the lactic/acetic ratio, results were similar to that of the lactic acid percentages, with Sudangrass monoculture product having the highest value (10.4) and Millet + weeds mixture having the lowest (1.11). The pH was lowest for Sudan at 3.78 and highest for Millet + weeds mixture at 4.57. The pH and % of ammonia results were similar to that of the lactic acid percentages and lactic/ acetic ratios. Sudan had the lowest percent of ammonia (0.48) while Millet + weed mixture had the highest (2.79). For the total % of N found in each forage product, weeds had similar results for all products. Millet and Millet + weeds mixture was statistically different from Sudan and Sudan + weeds mixture.

Millet + weeds mixture had the highest cp % at 16.9, while Sudan monoculture product had the lowest at 8.33%. The Sudan monoculture product was statistically different to all other forage products, with the Millet monoculture product similar to weeds, Millet + weeds mixture,, and Sudan + weeds mixture. For the percentage of ADF, there was no statistical difference among the means for all the forage products. The monocultures for Sudan and Millet were similar in NDF and NEL values and had the highest NDF and lowest NEL percentages. For percent of NFC, Millet had the lowest value and was also statistically different from all other forage products. Sudan monoculture product had the lowest percentages in TDN, NEG, RFV,
and NEM while weeds had the highest percentages for these values. The monocultures Sudan and Millet were similar in mean for TDN, NEG, and RFV percentages.

Table 1. Mean separation of forage assessments of silage treatments. Means followed by the same letter are not significantly different from each other according to the Tukey HSD test (p<0.05).

<table>
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<th></th>
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<th>% Acetic Acid</th>
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<td>7.45 b</td>
<td>3.93 b</td>
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</tr>
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<table>
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<th>% NDF</th>
<th>% TDN</th>
<th>% NFC</th>
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<td>40.90 a</td>
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<td>21.50 a</td>
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<tr>
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<th>% RFV</th>
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<th>% NEM</th>
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<td>0.60 a</td>
<td>0.57 c</td>
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</table>
3.3 Discussion and Conclusion

3.3.1 Examining the Ensiling Process

When looking at the main confirmation to see if fermentation was successful, lactic acid was high in all forage products and butyric acid was 0 for all forage products as well. As seen in figure 2a, the goal value is at 5% with the suggested value being greater than 3. The highest lactic acid percent is Sudangrass at 13.60 while the lowest is 8.23 for Millet and weeds. The amount of lactic acid present for all forage groups lines up to a similar study where Pearl millet was ensiled (Jahanzad et al., 2016). However, in the Jahanzad et al., 2016 study, butyric acid was over 0.1% when the goal is to be below 0.1%. For corn silage, lactic acid has been shown to fall around 3-6% (Ruth and Heinrichs et al., 2001; Kung et al., 2018). Lactic acid determines the pH, with the lower the pH the better. The goal value is below 5 and all forage groups in this study had a pH below five (Figure 2b). Corn silage pH has ranged from 3.7 – 4 and a similar study looking at Pearl millet silage also had a pH of around 3.7 (Ruth and Heinrichs et al., 2001; Jahanzad et al., 2016; Cattani 2017; Kung et al., 2018). pH and lactic acid are strong indicators of a successful fermentation process, and values obtained in the current study suggest that both grass monocultures and their mixtures with the weeds presented in the experimental plots were ensiled successfully.

As stated earlier, greater lactic and L/A values are desirable along with lower values for the remaining analyses. Acetic acid had a goal value of below 3% and only Sudangrass and its mixture with weeds met that criteria (Figure 2c). In the study looking at Pearl Millet ensiling, their value for acetic acid was 3-4%, which is the same for corn silage (Ruth and Heinrichs et al., 2001; Jahanzad et al., 2015; Kung et al., 2018). Too high of acetic acid may cause dry matter intake (DMI) depression in ruminant diets (Gerlach et al., 2021). For this study, Pearl Millet,
while above 3%, was only at 4.4%, falling on the higher side of the range for corn silage. While values for the two grasses in this study do not suggest potential for DMI depression, this should be considered for future work that looks at ensiling grass-legume mixtures, where legume acetic acid ranges from 6-8% (Kung et al., 2018). The goal L/A ratio for good fermentation is about 2.5-3 and anything under 1 is usually an indication of abnormal fermentations (Figure 2d). All forage products met near or above the standards, with Millet + weeds (1.11) being closest to 1. Due to Millet + weeds having the lowest lactic acid % and the highest acetic acid % and Pearl Millet not being too far behind, if choosing Pearl Millet to ensile, weeds should be removed before fermentation.
Figure 2) a. Average Lactic Acid % for each monoculture warm-season annual grasses (Pearl Millet and Sudangrass), their mixture with weeds, and the composite of weeds from all four reps, all compared to the blue line goal value (>3) supplied by Dairy 1 Lab report. b. Average pH for each monoculture warm-season annual grasses (Pearl Millet and Sudangrass), their mixture with weeds, and the composite of weeds from all four reps, all compared to the red line goal value (<5) supplied by Dairy 1 Lab report. c. Average Acetic Acid % for each monoculture warm-season annual grasses (Pearl Millet and Sudangrass), their mixture with weeds, and the composite of weeds from all four reps, all compared to the red line goal value (<3) supplied by Dairy 1 Lab report. d. Average Lactic/Acetic Acid ratio for each monoculture warm-season annual grasses (Pearl Millet and Sudangrass), their mixture with weeds, and the composite of weeds from all four reps, all compared to the blue line goal value (2-3) supplied by Dairy 1 Lab report.
3.3.2 Examining Forage Quality

For this study CP ranged from 8.33-16.90%, with Sudangrass having the lowest, 8.33%, and Millet + weeds having the highest, 16.95%, with Pearl millet and weeds each following at 15% each. These results are similar to the study looking at ensiling Pearl Millet, where they reported a 14% CP (Jahanzad et al., 2016). Corn silage has ranged from 7.7-14 (Ruth and Heinrichs et al., 2001; Cattani 2017; Kung et al., 2018). Ideal forage has high CP and TDN and low NDF and ADF percentages. Pearl Millet and Sudangrass ranged 37-38% ADF and 58-59% NDF. While these values are a little bit higher than the results from the other Pearl millet ensiling study (32% ADF; 50% NDF), ADF and NDF are much lower for corn silage (Jahanzad et al., 2016). ADF for corn silage ranges from 20-29% and 35-48% for NDF (Ruth and Heinrichs et al., 2001; Cattani 2017; Kung et al., 2018). This indicates that Pearl Millet and Sudangrass for this study has more fiber and is not as easily digestible as corn silage. The warm-season grasses for this study did have higher CP than corn silage, especially Pearl millet, which suggests not as much forage would need to be consumed to reach desired nutrient goal. TDN is the total digestive nutrients for forage and for this study Pearl Millet and Sudangrass ranged from 57-58% while corn silage has been shown to fall around 68% (Kung et al., 2018).

This study confirmed that Pearl millet and Sudangrass can be ensiled successfully and have competitive forage quality compared to corn silage. More research would need to be done on the comparisons of these warm-season annual grasses to corn silage to be able to perform economical assessments and see if soil biology does increase with the shorter growing seasons allowing for more diverse crop rotations.
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