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## Genetic Variation and Trait Associations for Forage Yield and Quality among F1 Half-Sib Families of Switchgrass

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# Genetic Variation and Trait Associations for Forage Yield and Quality among F1 Half-Sib Families of Switchgrass

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

Matthew Eric Bobbitt  
May 2014

## **DEDICATION**

I dedicate this to my parents, John and Kathy Bobbitt. This would not have been possible without their continued support.

## **ACKNOWLEDGEMENTS**

I would like to thank my major professor, Dr. Fred Allen, for the opportunity that he has given to me. Without his guidance and willingness to push me in the right direction, this never would have been possible. I would also like to thank Dr. Hem Bhandari, Dr. Dennis West, and Dr. Arnold Saxton for serving on my committee. I would also like to thank my co-workers and the student workers, as well as the crews at the East Tennessee Research and Education Center Plant Science unit and Holston unit, and also David McIntosh for all of their help.

## ABSTRACT

Switchgrass (*Panicum virgatum* L.) is widely accepted as a forage crop throughout the United States. It is known for its performance on field sites that may be marginal for row crop production and as a warm season grass to fill gaps for cool season forages. With the increase of fuel and fertilizer costs, forage producers need higher yields and better quality than ever before. The objectives of this research were to: (i) compare four F<sub>1</sub> [first generation] half-sib populations for their potential of producing superior lines for forage production, (ii) assess the genetic variances for yield, and (iii) evaluate correlations between yield and other agronomic traits for the purpose of indirect selection. The four parental lines were PI 421999 (AR), PI 607837 (TX), Cimarron (OKS), and NSL-2001-1 (OKN). Seed for one hundred and forty F<sub>1</sub> half-sib progeny were produced in a polycross nursery at the East Tennessee Research and Education Center (ETREC), Plant Sciences Unit, Knoxville and planted in 2009. The parents and half-sibs were evaluated at the ETREC, Holston Unit in 2012 and 2013. Data were collected and analyzed on forage yield and nutritive value traits such as protein content, acid detergent fiber (ADF), neutral detergent fiber (NDF), total digestible nutrients (TDN), and relative feed value (RFV). Early-season yields of the F<sub>1</sub> half-sib populations ranged between 1.00 and 1.08 kg plant<sup>-1</sup>[per plant] in 2012 and 1.41 and 1.51 kg plant<sup>-1</sup> in 2013. Genetic variance for yield was not exhibited on a population basis, but was identified in five sub-families in 2012 and ten sub-families in 2013, three sub-families showing genetic variance for yield for both years. The average protein content of populations ranged between 10.3 and 10.8 % [percent] in 2012 and 10.2% for all populations in 2013 for the early-season harvest. The average protein content of the populations for the late-season harvest ranged from 8.1 to 8.6% in 2012 and 9.7 to 10.5% in

2013. ADF ranged from 36.7 to 43.3% and NDF ranged from 73.6 to 79.9% over the two harvests of each year. Moderate correlations were found between yield and: height and canopy regrowth density.

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## **CHAPTER I**

### **Introduction and General Information**

Switchgrass (*Panicum virgatum* L.) has been used as a forage crop, for wildlife and soil conservation, prairie restoration in the USA, and as a biofeedstock because of its superior traits compared to other grasses (Casler et al., 2007). It is a warm season perennial grass with a C4 photosynthetic system and it thrives during the mid-summer when cool season grasses have slowed production because (Vogel, 2004). Switchgrass can be found throughout the United States with approximately 2060 hectares in East Tennessee contributing to the biofuel/forage industry (Johnson, 2012; Bates et al., 2012). Switchgrass yields can range from 4.5-15.7 tonnes per hectare, depending on rainfall, soil type, as well as other environmental conditions.

Research in Tennessee has shown that, if grown exclusively for hay, 9.0-11.2 tonnes per hectare are not uncommon (Bates et al., 2012). Switchgrass forage can be grazed or produce high-quality hay when harvested at the proper time. When grown under management conditions for forage production, switchgrass has been shown to contain around 8-10% protein (Vogel et al., 1984). With the recent push by the government to produce fuel with renewable resources, switchgrass has been a major focus, thus shifting the management practices of farmers who wish to benefit from the additional income of selling switchgrass for biofuel production. This has brought up the dual use crop strategy to produce forage early in the season and utilize the second cut of biomass in the fall for biofuel production (Bates et al., 2012; Guretzky et al., 2011).

## **CHAPTER II**

### **LITERATURE REVIEW**

Switchgrass has been valued for its superior drought tolerance and quick regrowth capabilities during hot, dry summer months. It can be difficult to establish the first year, but after 2 to 3 years, weed competition is not usually a problem in a good stand. Switchgrass has several popular uses such as forage, biomass ethanol production, and erosion control. Switchgrass has a substantial root system that can equal the above ground portion of the plant (Zan et al., 2001). Forage quality in switchgrass is comprised of digestible nutrients, energy, protein, fiber, minerals and vitamins that can be utilized by grazing animals (Waramit et al., 2012; Sanderson and Burns, 2010; Beaty and Engel, 1980). Switchgrass has been used in grazing and hay production practices in the United States for decades due to its adaptation to diverse climate and soils (Anerson et al., 1988; Sanderson, 2008; Koshi et al., 1982). A common practice is to graze switchgrass when it is 45-60 centimeters tall and graze down to 20-25 centimeters (Bates et al., 2012). Beyond 60 centimeters, quality begins to decline as the plant goes into the early boot stage of reproduction. Digestible protein is a very important component of forage. The higher the protein content, the more valuable the forage or hay will be with 16-17 percent crude protein possible with properly managed stands (Bates et al., 2011). Forage yield is a major focus; however, clipping during the first year has shown to reduce yield during the second year by 34 to 60% (Anderson et al., 1989). Yield continues to be a main focus. Increasing yield without decreasing overall quality and environmental adaptability would be ideal. Acid detergent fiber (ADF) and neutral detergent fiber (NDF) are of focus when looking at

forage quality. NDF is a measure of the cell wall fiber and provides a predictor of the voluntary intake of an animal because it provides bulk in the diet (Rasby and Martin, <http://beef.unl.edu/>). Because of this it is better to have a lower NDF. ADF is made up of plant components such as cellulose and lignin which are not as easily digested by animals (Rasby and Martin, <http://beef.unl.edu/>). Lower ADF values are usually better because the higher the ADF, the less digestible the hay becomes, which means that it will contain less potential energy for the animal. In a study by Twidwell et al. (1988) switchgrass had a range of 35.8 to 39.7% ADF and a range of 67.6 to 71.4% NDF. In a study by Vogel et al. (1984) the switchgrass ranged 38.3 to 40.9% ADF and NDF ranged from 68.6 to 77.4%. In that same study by Vogel et al. (1984) the plants ranged from 8.0 to 10.3% protein. Burns et al. (1985) reported on a population of switchgrass with a protein content of 6.9% and ADF and NDF values of 39.8 and 75.3%, respectively. Another population of switchgrass had a protein content of 7.5% and ADF and NDF values of 38.5 and 73.5%, respectively (Burns et al., 1985). Total digestible nutrients (TDN) is calculated based on ADF and is useful for formulating animal feed ratios because it is related to digestible energy (Rasby and Martin, <http://beef.unl.edu/>). A study by Shultz (2013) reported a population of switchgrass with TDN as high as 66% and ranging down to 56%, depending on harvest date. That same study showed a range of NDF of 63-72% depending on harvest date (Shultz, 2013).

Relative feed value (RFV) was developed by the Hay Marketing Task Force of the American Forage Grassland Council and is used to compare forages (Rohweder et al., 1978). These values offer a prediction as to the feeding value of the forage by putting them on an index that is easier to understand and allows for quick comparison between samples (Rasby and Martin,



<http://beef.unl.edu/>). The index ranks forages relative to the digestible dry matter intake of alfalfa at full bloom, assuming that it has 41% ADF and 53% NDF. The RFV index is 100 at this growth stage with values being able to go higher or lower (Jeranyama and Garcia, 2004). A study by Angima et al. (2009) showed three year RFV index averages in switchgrass to range from 88 to 98.

### **Genetic Variance**

Genetic variation within and among crosses is essential for making genetic gain by selection for any trait. Gain from selection depends on genetic variation within or among a population for a given trait, heritability of that trait and the selection intensity used (Hopkins et al., 1993; Falconer, 1981). Newell and Eberhart (1961) identified genetic variation in a population of Nebraska and northern Kansas switchgrass, estimating a narrow sense heritability of up to 0.47. In a study to improve the forage yield using restricted recurrent phenotypic selection which yielded no significant improvement from the base population to the selected population, the lack of gain was attributed to a lack of genetic variation for forage yield in the base population (Hopkins et al., 1993).

### **Heritability**

With breeding, parent plants can be intercrossed that exhibit superior forage production traits to produce populations that will exhibit the yield and forage quality traits of both parents (i.e. high yields and high protein content). This takes advantage of the potential for the trait to pass from parent to progeny and be expressed (i.e. heritability). The degree of heritability of a trait is an important factor to consider when looking at the differences exhibited by a population of

switchgrass, and estimating heritability can help to determine the ratio of genotypic to phenotypic variation within a population. Hopkins et al. (1993) stated that heritability for forage yield of switchgrass is usually low. Newell and Eberhart (1961) estimated narrow-sense heritability of forage yield of switchgrass to be only 0.05. Other studies, however were able to identify slightly higher narrow-sense heritability estimates of 0.20 (Godshalk et al., 1986) and 0.59 (Talbert et al., 1983). Hopkins et al. (1993) estimated narrow-sense heritability to be 0.22 for forage yield of polycross progeny of switchgrass. These results are variable and leave room for further studies.

## **Heterosis**

Heterosis, or hybrid vigor, is defined as when the crossing of two parents gives  $F_1$  hybrids that are superior to the parents. This is a highly desired characteristic to breeders, and has long been used on the development of new  $F_1$  hybrids of maize. While heterosis has been used to describe most agronomic traits, it is most widely studied in relation to yield (Brummer, 1999). Heterosis is described as the  $F_1$  progeny being better than the average of the two parents (mid-parent heterosis) or as being better than the best parent (high-parent heterosis). High-parent heterosis is defined as the positive difference between the mean of the hybrid and the mean of the high-parent with the trait of interest (Lamkey and Edwards, 1999). Heterosis has been found to exist between switchgrass cultivars. This can be used to develop  $F_1$  progeny that are superior to their parents. In a population of Kanlow x Summer and Summer x Kanlow  $F_1$  hybrids, 30 to 38% high parent heterosis was determined for biomass yield (Vogel and Mitchell, 2008). Lamkey and Edwards (1999) suggested that high-parent heterosis is a more useful

measure in self-pollinated crops. In a three year study, Martinez-Reyna and Vogel (2008) found that midparent heterosis existed between Kanlow x Summer hybrids in a space planted field trial.

### **Trait Correlations**

Correlation coefficients measure the degree of association between traits, but provide only limited information because they disregard complex interrelationships among traits; therefore correlations must be used and interpreted with caution (Das et al., 2004). Correlations are useful when comparing traits in order to help in the selection process of breeding. Several studies have evaluated correlations in switchgrass. Das et al. (2004) identified a positive and significant correlation ( $r = 0.45$ ) between height of plants and total plant yield in a small, blue-green switchgrass population. In a medium-tall blue-green population, there was a positive and significant phenotypic correlation of total plant yield with length of leaves and also total plant yield with plant height (Das et al., 2004). Similarly, Talbert et al. (1983) reported a significant positive correlation between plant dry weight and plant height. That same study also identified a significant negative phenotypic association between dry weight and early maturity (Talbert et al., 1983). This is important as maturity strongly influences switchgrass forage quality (Gabrielsen et al., 1990). Hopkins et al. (1995) found that early maturity was often accompanied by low forage yield ( $r = 0.65$ ) and also significant but weak correlation ( $r = -0.12$ ) between the disease rating and forage yield at heading. Redfearn et al. (1997) reported that forage yields were primarily affected by tiller elongation as well as differences in leaf blade length and width, as well as the size and number of tillers.

There were three objectives of this research: (i) compare four  $F_1$  half-sib populations for their potential of producing superior lines for forage production, (ii) assess the genetic variances for yield, and (iii) evaluate correlations between yield and other agronomic traits for the purpose of indirect selection. The results of these objectives will be able to help further advance the breeding work on switchgrass to produce better populations of switchgrass in terms of forage yield and nutritive values.

## CHAPTER III

### Materials and Methods

#### Plant Material

The four parental lines used in this study were PI 421999 (designated as AR), PI 607837 (designated as TX), 'Cimarron' (designated as OKS), and NSL-2001-1 (designated as OKN). Fourteen single plants were chosen from each parent line and planted in a polycross nursery at the East Tennessee Research and Education Center (ETREC) Plant Science Unit in Knoxville, Tennessee (35.53°N 83.57°W) on 1.2m x 1.2m spacing in 2007. A Florida introduction, PI 422016, was also used in the polycross nursery and a half-sib population from the Florida PI was originally included in the study, but was dropped due to poor plant survival, therefore the Florida PI was also a pollen donor to the half-sib families that originated from the other four parental sources. The soil type at this polycross nursery site is classified as Sequatchie loam (fine-loamy, siliceous, semiactive, thermic Humic Hapludults).

In 2009, a F<sub>1</sub> half-sib nursery was planted at the East Tennessee Research and Education Center, Holston Unit (Holston) in Knoxville, Tennessee (35.58°N 83.51°W). The soil type at this site is classified as Huntington silt loam (fine-silty, mixed, active, mesic Fluventic Hapludolls). Within the four F<sub>1</sub> half-sib populations there were 14 sub-families and 10 half-sib progeny plants within each sub-family. Each population was represented by a total of 140 F<sub>1</sub> half-sib progeny plants. The plants were blocked according to parental source. The 10 progeny plants of each half-sib family were planted in a contiguous block in the F<sub>1</sub> half-sib nursery. The half-sib families from each parental source were planted in a block of 14 rows with 10 plants per row. In 2010,

parental clones from the polycross nursery were planted in the nursery by their respective  $F_1$  half-sib population on the border rows of the nursery. The space planted hill plots in the polycross nursery and half-sib nursery were harvested for 1-cut biomass system each fall, 2010 and 2011. Data for this study were collected in 2012 and 2013 on a 2-cut system of spring forage/ fall biomass system. Only the spring forage data are included in this study.

### **Traits Evaluated**

Beginning in May, ratings were taken on the  $F_1$  half-sib and polycross nurseries. Ratings taken include color greenness, leaf angle, canopy score, leaf bloom, and plant height. Color greenness was rated on a 9 point scale of 1 to 5 including half increments where 1 = green and 5 = blue. Leaf angle was scored on a 9 point scale of 1 to 5 where 1 = all leaves are  $\leq 45^\circ$  to a vertical stem, 3 = approximately half of the leaves are  $\approx 45^\circ$  and half are  $> 45^\circ$  at the distal end of the leaf, 5 = all leaves are arched so that from the leaf midpoint to the leaf tip of the leaves are  $> 90^\circ$ . There were half increments to include a gradient between the amount of straight and arched leaves. Canopy type was rated on a 9 point scale of 1 to 5 with half increments where 1 = a very upright plant with greater than 95% of tillers vertical to the ground, 3 = the plant had many tillers that were at a  $45^\circ$  angle to the ground, 5 = tillers were growing  $< 45^\circ$  to the ground. A leaf bloom score was rated on a scale of 1 to 3 with no half increments where 1 = no wax, 2 = moderate wax present, and 3 = high amounts of wax.

The forage cut was taken with a self-propelled Carter Forage Harvester. Each plot was collected on a tarp and weighed on a hanging scale. After weights were recorded, a sub-sample was taken from each plot and a green weight recorded. The sub-samples were then dried in a

Wisconsin Oven for 72 hours at 49° C. Sub-sample dry weights were recorded to determine moisture content at harvest. The dried sub-samples were then ground in a Thomas-Wiley Laboratory Mill Model 4 (Thomas Laboratory, Swedesboro, NJ, USA) until it passed through a 1mm sieve. The ground samples were then scanned with a Model 6500 near-infrared spectrometer for protein, ADF, NDF, estimated TDN, and RFV (FOSS NIRSystems, Inc., Laurel, MD, USA). The forage calibration curves were based on the work of Vogel et al. (2011).

Equations for the forage nutritive analysis were standardized and checked for accuracy using equations developed by the NIRS Forage and Feed Consortium and are reported on a dry matter (DM) basis (Hillsboro, WI). Software used for NIRS analysis was WINSI II supplied by Infrasoft International LLC (State College, PA).

Fifteen days after harvesting the plots, a score was taken on the regrowth of the plants. Plants were rated on a scale of 1 to 5 where 1 = the most dense and full foliage growth in the field, 3 = a medium growth and density, and 5 = no regrowth had occurred. Plant height as well as a maturity stage rating was taken at the time the second harvest would have occurred. The maturity rating was on a scale of 1 to 6 with 1 = fully headed, 2 = mid-heading, 3 = early heading, 4 = late boot, 5 = boot stage, and 6 = early boot. In August, late-season forage yield was predicted for each plot by harvesting five averaged sized tillers and using the average tiller weight multiplied by the number of tillers in each respective plant. These five tillers were also used as a sub-sample from each plot. Harvested tillers were weighed and dried in a Wisconsin Oven for 72 hours at 49° C. After drying, tillers were weighed again to determine moisture. Whole plant weights were predicted by multiplying the average dry tiller weight of each plant by the number of tillers of the plant. The whole plants could not be harvested because they

were needed for a fall biomass study. Twenty plants were randomly selected that were growing with the  $F_1$ 's in this study to test the accuracy of the sampling method. The sampling method was performed on these twenty plants as well as harvesting the whole plant and were then dried using the same procedure as the four populations in this study. The dried samples from the late-season harvest were ground in a Thomas-Wiley Laboratory Mill (1mm sieve) and then scanned with a Foss Model 6500 near-infrared spectrometer just as the early-season samples were processed. Tillers were counted for each  $F_1$  plant in October.

### Statistical Analysis

Population means were tested via t-test allowing unequal variances in Microsoft Excel (2010) to determine differences ( $p \leq 0.05$ ) between two sample means.

### Phenotypic, Genotypic and Environmental Variance Estimates

$$\sigma_{Pijk}^2 = \sigma_{Gijk}^2 + \sigma_{Ei}^2$$

$\sigma_{Pijk}^2$  = phenotypic variance

$\sigma_{Gijk}^2$  = genotypic variance

$\sigma_{Ei}^2$  = Average variance that existed within parental source plants with a bias of parents having genotypic variance among parental clones

Where i = the  $i^{\text{th}}$  population (i = 1, 2, 3, 4)

j = the  $j^{\text{th}}$  sub-family in the  $i^{\text{th}}$  population (j = 1 through 14)

k = the  $k^{\text{th}}$   $F_1$  within the  $j^{\text{th}}$  sub-family within the  $i^{\text{th}}$  population (k = 1 through 10)



**In the case of sub-families:**

$$\sigma_{P_{ijk}}^2 = \frac{\sum X_{ijk}^2 - \frac{(\sum X_{ijk})^2}{n_k}}{n_k - 1}$$

$$\sigma_{G_{ijk}}^2 = \sigma_{P_{ijk}}^2 - \sigma_{E_i}^2$$

### **Phenotypic Variance Among half-sibs (HS)**

The following formula was used in Microsoft Excel (2010) where variances were calculated with the =VAR.S function.

$$\sigma_{HS}^2 = \frac{\sum X_{ijk}^2 - \frac{(\sum X_{ijk})^2}{n}}{n - 1} = \sigma_{P_{ijk}}^2$$

Where i = the i<sup>th</sup> population (i = 1, 2, 3, 4)

j = the j<sup>th</sup> sub-family in the i<sup>th</sup> population (j = 1 through 14)

k = the k<sup>th</sup> F<sub>1</sub> within the j<sup>th</sup> sub-family within the i<sup>th</sup> population (k = 1 through 10)

### **Broad Sense Heritability**

$$H_i^2 = \frac{\sigma_{G_{ijk}}^2}{\sigma_{P_{ijk}}^2}$$

Variance components were estimated on a sub-family and population basis; heritability was estimated on a population as well as across populations basis.

Correlations on yield, plant height, leaf angle, canopy type, color greenness, leaf bloom, regrowth height, regrowth density, protein, ADF, NDF, TDN, RFV, and maturity were performed using SAS statistical software (SAS 9.3 Cary, NC). Boxplots were constructed using ODS Graphics editor in SAS statistical software (SAS 9.3 Cary, NC).

## CHAPTER IV

### Results and Discussion

#### Forage Yield

Average population early-season forage yield of the  $F_1$  half-sibs ranged from 1.00 to 1.08 kg plant<sup>-1</sup> in 2012 and 1.41 to 1.51 kg plant<sup>-1</sup> in 2013 for the early-season harvest (Table 1). The population averages did not differ in 2012 or 2013 ( $p>0.05$ ) (Table 1). The distributions of the 140  $F_1$  half-sibs in each population were similar within each year (Fig. 1); however, the distributions of the boxplots of the 14 sub-families within each population were quite different (Fig. 2). The box plots illustrate that there was greater uniformity among  $F_1$ 's within some sub-families than others (e.g., OKN1-11 vs OKN4-1; Fig. 2a). The sub-family OKN2-13 had a mean over the two years that was above that of the overall population, the highest of all the subfamilies in this study (Fig. 2a). OKN5-12 exhibited a great amount of variation, but had a mean similar to that of the population mean (Fig. 2a). Eight of the 14 sub-families of OKN had high-yielding outliers above 2 kg plant<sup>-1</sup> (Fig. 2a). Similar trends were observed among sub-families of the three other populations. For example, OKS1-4 showed a large amount of variation and had a high mean, with the top quartile of plants yielding above 2 kg plant<sup>-1</sup> (Fig. 2b). The sub-families OKS 2-1, OKS4-7, and OKS5-5 also exhibited considerable variation. Four of the OKS sub-families produced outliers above the 2 kg plant<sup>-1</sup> (Fig. 2b). Six of the TX sub-families had high yielding outliers above 2 kg plant<sup>-1</sup> (Fig. 2c). TX1-10 and TX4-6 had a good portion of the top quartile of plants above 2 kg plant<sup>-1</sup> (Fig. 2c). Only one of the AR sub-families produced a high yielding outlier (AR3-13); however, AR1-1 and AR2-3 had the top quartile of

F<sub>1</sub>'s and about one-half of the second quartile above the 2 kg plant<sup>-1</sup> (Fig. 2d). Variation among F<sub>1</sub>'s of switchgrass was expected since there was heterogeneity within each parent, unlike F<sub>1</sub> hybrids in crops such as corn, soybeans, and wheat where parental lines are homogeneous and thus the F<sub>1</sub> progeny are homogeneous. Because heterogeneity among F<sub>1</sub> half-sibs were expected, estimates were obtained for genetic variance for early-season forage yield for each sub-family within each population as well as the genetic variance among sub-families for each population. In 2012, there were only five such families that exhibited genetic variation for early-season forage yield (OKN2-8, OKN4-1, OKS 4-7, AR1-1, and AR5-4) (Table 2). In 2013, there were ten sub-families among the four populations that exhibited genetic variation for early-season forage yield (OKN2-5, OKS1-4, OKS2-1, OKS3-12, OKS4-7, OKS5-5, TX1-3, TX4-6, AR1-1, and AR5-4) (Table 2). Three of the sub-families (OKS4-7, AR1-1, and AR5-4) had significant genetic variation in both years. The broad sense heritability estimates ranged from 0.14 to 0.47 in 2012 and 0.13 to 0.42 in 2013 (Table 2). These values are in line with the wide range of heritabilities (0.05 to 0.59) reported by earlier researchers (Newell and Eberhart, 1961; Hopkins et al., 1993; Talbert et al., 1983). The genetic variance estimates among sub-families within populations were zero for all four populations in both years (Data not shown).

The entire plant could not be harvested for the late-season harvest due to the same F<sub>1</sub> progeny being included in another study that required a fall biomass harvest; therefore a sampling method of harvesting a set number of tillers was used to predict a late season forage yield. The sampling method used the dry weight of tillers and the tiller number of the plant to predict the yield of the late-season harvest. To test the accuracy of the sampling method intended to be used to estimate the late-season harvest, 20 plants were selected that were growing in the

same nursery as the four populations that were in this study. With the 20 plants, the sampling method was performed, as well as harvesting the entire plant. The predictive method was found to be inaccurate due to substantial overestimating of yield when comparing the tiller method estimations to the actual yield of the 20 subset of plants from the whole-plant harvesting (Fig. 3). The same overestimation was true when the tiller method predictions were compared to the actual fall biomass yields (Fig. 4). Nineteen of the 20 plants were overestimated with an average over-estimation of 123% and a range of 45% to 219% (Fig. 3). The plant that was not overestimated was underestimated by 6%. On the other hand, relative rankings were about the same when comparing the predicted yields of the  $F_1$ 's to the yields obtained from the fall biomass harvest (Fig. 4). This over-prediction may have been the result of a bias towards selecting tillers that were larger to those that were of average size.

## **Heterosis**

Heterosis, or hybrid vigor, was identified in 2012 and 2013. The  $F_1$  populations of OKS and TX showed the highest panmictic mid-parent (MP) heterosis in 2012 of 18.76% and 18.46% above the MP mean, respectively (Table3). Heterosis was calculated for half-sib populations by using the mean yield of the parents. In 2013, AR showed the highest panmictic MP heterosis of 19.3%, compared to the other populations' averages of 12.02-14.83% (Table 3). Panmictic high-parent heterosis was not identified on a population basis (Table 3). Panmictic high-parent heterosis was found within subfamilies in 2012 up to 13% and up to 16% in 2013 (Table 4). These values are lower than the 23-38% identified by Vogel and Mitchell (2008). When looking at the top five plants from each population (20 plants total) in 2012 and 2013, seven plants

were identified as top performers from both years (OKN2-8 (17-6), OKS4-7 (16-17), OKS3-12 (11-18), OKS2-1 (11-20), TX4-6 (17-10), AR1-1 (4-35), AR3-13 (5-32); Table 4). TX4-6 (17-10) and AR1-1 (4-35) had a yield greater than the high parent both years and were the only plants to be greater than the high parent for 2012 (Table 4). OKS2-1 (11-20) was the only top performer from both years to not exhibit panmictic high parent heterosis in either year, but had higher protein content in 2012 and 2013 than that of the high yielding parents (Table 4). In 2013 all but one plant of the top yielding plants had higher protein content than the high-parent from 2012 and 2013 (Table 4). When looking at the other nutritional factors, we can see that the top performing lines had values close to that of the high parent. While the majority of the top performers had better protein content, the top performers had a mix of being slightly better or slightly worse values than the high parent for each respective year. None of the top performers were top performers in every category in either year (Table 4).

### **Forage Nutritional Composition**

**Protein.** Protein content ranged from 10.3% to 10.8% in 2012 with AR population being the highest, but only statistically greater ( $p \leq 0.05$ ) than the OKN population in the early-season harvest (Table 5). The early-season forage harvest protein content averaged 10.2 % for all populations in 2013 (Table 5). The protein content of the late-season forage harvest ranged from 8.1% to 8.6% in 2012 and 9.7% to 10.5% in 2013 (Table 6). The parents ranged from 9.6 to 10.3% protein in 2012 and 9.1 to 10.3% in 2013 for the early-season harvest (Table 5). For the late-season harvest the parents ranged from 6.6 to 6.9% in 2012 and 8.7 to 9.9% protein 2013 (Table 6). This is in line with the protein content values reported by Vogel et al. (1984) of 8.0 to

10.3% and Burns et al. (1985) with a reported finding of 6.9 and 7.5%. There was not much difference for the early-season harvest between the  $F_1$ 's and the parents but for the late-season harvest the  $F_1$ 's outperformed the parents except for OKN in 2013 which was just a little lower (9.7 vs 9.9%) in protein content (Table 5-6). There is not much variation in protein content when looking at the first cut of the four  $F_1$  populations in 2012 or 2013 for the early-season harvest (Fig. 5a). There is more variation in the late-season harvest in 2012 and 2013 between the populations and years (Fig. 5b). On the other hand, the distribution of sub-families from each population shows a great deal of variation in protein for the early-season harvest (Fig. 6). The OKN population had two outliers above 12.5% in OKN2-13 and OKN3-11 despite having means below the population average (Fig. 6a). The boxplots show that some sub-families had more variation than others for protein content (e.g., OKN4-4 vs. OKN5-12, Fig. 6a). The other populations had similar trends for the early-season harvest. OKN5-12 did not have very much variation but all four quartiles were above the population average (Fig. 6a). The OKS population had 6 outliers for protein content with five of them being above 12.5% (Fig. 6b). OKS2-1 and OKS4-2 exhibited a great amount of variation, they had means well below the population average unlike OKS3-12 which exhibited a great amount of variation and had a mean similar to that of the population (Fig. 6b). The TX population had similar amounts of variation in the 14 sub-families and only one high outlier in the TX3-7 sub-family (Fig. 6c). The first three quartiles of TX3-9 are above the population mean and the first quartile is almost completely above 12.5% protein (Fig. 6c). The AR population had several subfamilies with a large amount of variation (AR4-5, AR5-4, AR5-13) and had two high outliers in the AR2-3 and AR3-8 sub-families (Fig. 6d). The first quartile of AR4-5 extends beyond 12.5% protein, up to 15% (Fig. 6d). Like the early

season harvest protein content, the sub-families show a large amount of variation in the late-season harvest as well (Fig 6-7). In the late-season harvest, OKN showed a wide variation in protein content and had 3 high outliers (Fig. 7a). OKN2-13 and OKN5-8 showed the most variation and both had means above the population average (Fig. 7a). OKS had four high outliers between OKS3-12 and OKS4-12 while they both had means less than that of the population (Fig. 7b). TX had 5 high outliers with 4 of them being above 12.5% (Fig. 7c). TX3-9 and TX4-12 exhibited the greatest variation and both had means above that of the population. The AR sub-families did not have any high outliers but several sub-families (AR1-1, AR3-1, and AR4-5) had the first quartile to extend beyond 12.5% and also exhibited the greatest amount of variation in the population (Fig. 7d).

**Acid Detergent Fiber.** A lower ADF is advantageous when evaluating a forage sample. ADF ranged from 41.7% to 41.9% in 2012 and 38.8% to 39.4% in 2013 for early-season harvest (Table 5). In 2012 the late-season harvest ranged from 42.3% to 43.3% and in 2013 it ranged from 36.7% to 37.4% (Table 6). These values are in line with the findings of Twidwell et al. (1988) of 35.8 to 39.7%, Vogel et al. (1984) with findings ranging from 38.3 to 40.9%, and Burns with findings of 38.5 and 39.8% ADF. There was no variation among the populations for ADF in the early-season harvest in 2012 or 2013 (Fig. 8a). Likewise, the late-season harvest did not show much variation in 2012 or 2013, but there was one low outlier in 2012 and two in 2013 for ADF (Fig 8b). The OKN sub-families exhibited a great amount of variation for the early-season harvest ADF (Fig. 9a). OKN5-12 had a mean ADF content well below the population means (Fig. 9a). OKS had several sub-families with a large amount of variation for ADF such as OKS1-4, OKS1-13, OKS2-7, and OKS5-5 that had means above the population average (Fig. 9b).



TX2-12 and TX3-9 had a great amount of variation and had means below the population average (Fig. 9c). AR had one high outlier in the AR4-11 sub-family (Fig. 9d). AR1-14, AR2-6, AR3-8, AR4-5, and AR5-4 exhibited variation and had means lower than the population (Fig. 9d). In comparison to the early-season harvest ADF, the late-season harvest had more variation in the subfamilies but did not have any low outliers in any of the populations (Fig. 10). OKN sub-families had large amounts of variation but only a few had a mean below the population mean (e.g., OKN1-7, OKN2-13, OKN4-1, OKN4-9, OKN5-8; Fig. 10a). This same trend continued through the other three populations. OKS had a few sub-families that had low means and a large amount of variation to include OKS1-4, OKS1-13, OKS2-10, OKS4-2, and OKS5-5 (Fig. 10b). TX had two sub-families with a large amount of variation and low means (TX2-12 and TX5-2; Fig. 10c). Many of the other sub-families in the TX population had means lower than the population average but not as much variation as the OKS sub-families (Fig. 10b-10c). AR did not have many sub-family means that were much lower than the population average (e.g., AR3-1, AR5-4; Fig. 10d).

**Neutral Detergent Fiber.** Similar to ADF, we are looking for a lower NDF than the parents because that allows for a higher intake for animals. NDF ranged from 76.4% to 77.1% in 2012 and 79.0% to 79.9% in 2013 for the early-season harvest (Table 5). The late-season harvest ranged from 76.9% to 77.9% in 2012 and 73.6% to 74.7% in 2013 (Table 6). These values are in line with the findings of Twidwell et al. (1988) with values ranging from 67.6 to 71.4% NDF, Vogel et al. (1984) with findings ranging from 68.6 to 77.4%, Burns et al. (1985) with values ranging from 73.5 to 75.3%, and Shultz (2013) with reported findings of 63 to 72%. NDF did not differ for populations for the first cut of 2012 ( $p>0.05$ ) and in 2013 TX differed from AR

( $p < 0.05$ ). For the second cut in 2012, TX differed ( $p \leq 0.05$ ) from the other populations (76.9 vs 77.5, 77.7, 77.9%) and in 2013 AR had the highest NDF content but was only different from OKS and TX ( $p \leq 0.05$ ). The boxplots for the early-season harvest do not show much variation between the populations for NDF (Fig. 11a). Similarly, the boxplots for late-season harvest NDF do not show much variation but there are a few low outliers in OKN, OKS, and AR in 2012 and one low outlier in 2013 in the AR population (Fig. 11b). The boxplots of the subfamilies really show a better insight into the populations and eight low outliers were present for the OKN population (Fig. 12a). OKN2-13 had a very low outlier that was below 70% and OKN3-11 and OKN4-11 had some very low outliers despite have relatively little variation in comparison to the other subfamilies in the population (Fig. 12a). OKS had several low outliers in the OKS2-10, OKS4-7, and OKS5-5 subfamilies even though those have some of the least variation in the OKS population (Fig. 12b). TX had 4 low outliers for NDF in the early-season harvest (Fig. 12c). TX1-5, TX3-7, and TX3-9 all had relatively large amounts of variation in the population and had means below the population average and had quartiles that extend close to 70% (Fig. 12c). AR had a few low outliers but only two that were close to 70% (Fig. 12d). AR1-14 had a lower mean than the other sub-families in the population but did not show much variation (Fig. 12d). AR3-1 and AR4-5 had lower means than the population and showed a larger amount of variation than most of the other sub-families (Fig. 12d). There was even more variation in the late-season harvest NDF than the early-season harvest (Fig. 13.) OKN had two low outliers, the lowest one which was in the OKN3-11 sub-family (Fig. 13a). Several sub-families exhibited a great amount of variation and had means below that of the population which include OKN1-7, OKN2-5, OKN2-13, OKN4-1, OKN4-9, and OKN5-8 (Fig. 13a). OKS also had several sub-families that had low

means in the late-season harvest and exhibited a lot of variation (e.g., OKS1-4, OKS1-13, OKS2-10, OKS4-2, OKS5-3, OKS5-5; Fig. 13b). OKS also had three outliers from sub-families that did not have low means or a lot of variation (e.g., OKS2-1, OKS2-7, OKS4-7; Fig. 13b). TX had many sub-families with low means and relatively large amounts of variation for NDF to include TX1-3, TX1-10, TX2-12, TX3-7, TX4-3, and TX5-2 (Fig. 13c). TX4-10 had a large amount of variation but had a higher mean than the population (Fig. 13c). TX had two low outliers but only one in the TX2-2 sub-families was close to 70% (Fig. 13c). The fourth quartile of AR3-13 extended down to almost 65% which is the lowest of any sub-family, but it has a mean greater than that of the population (Fig. 13d). AR2-6 had a low outlier that was below 70%, but the sub-family had a higher mean than the population (Fig. 13d).

**Total Digestible Nutrients.** Calculated based on ADF, TDN is used as a quick indicator of the amount of digestible energy in a sample. TDN ranged from 54.7% to 55.0% in 2012 and 57.7% to 58.3% in 2013 for the early-season harvest (Table 5). The late-season harvest ranged from 53.1% to 54.3% in 2012 and 59.9% to 60.8% in 2013 (Table 6). These values are on the low end of the reported values by Shultz (2013) of 56 to 66% TDN. TDN did not differ ( $p>0.05$ ) among the populations in 2012 or 2013 for the early-season harvest. For the late-season harvest TDN did not differ ( $p>0.05$ ) in 2012 nor 2013. OKN had the highest average (54.9%) in 2012 but did not differ ( $p>0.05$ ) from the other populations. Much like the other boxplots of nutritional composition for whole populations, the boxplot for early-season harvest TDN does not show much variation among populations in 2012 or 2013 (54.7-55% and 57.7-58.3, respectively; Fig. 14a). Similarly, the boxplot of late-season harvest TDN does not show much variation in 2012 (53.1-54.3%; Fig. 14b). In 2013 the populations differed with OKS being the top performer

(60.8%) but only greater than OKN and AR (Fig. 14b). There was not much deviation from the mean in the OKN sub-families, but OKN4-1, OKN5-8, and OKN5-12 had higher means than the population and had a moderate amount of variation (Fig. 15a). OKN2-13 exhibited a large amount of variation but had a mean below the population mean while OKN1-7 had a similar amount of variation but had a mean very close to that of the population (Fig. 15a). OKS had three outliers but only one was above the first quartile of the sub-families which was from the OKS3-12 sub-family (Fig. 15b). OKS1-9, OKS3-12, OKS4-7, OKS4-14, and OKS5-11 had a moderate amount of variation and means that were above the population mean (Fig. 15b). A few sub-families had more variation but had means at or below the population mean (e.g., OKS1-13, OKS2-1, OKS2-7, OKS5-3; Fig. 15b). TX1-5 and TX 3-9 had means above the population mean but did not have as much variation as TX1-10 and TX4-12 which had means at or below the population mean for early-season harvest TDN (Fig. 15c). AR did not have any high outliers but three sub-families (AR1-14, AR2-6, AR3-6) had the about half of the third quartile above the population mean (Fig. 15d). There was not as much variation among AR sub-families compared to the other three populations for early-season harvest TDN (Fig. 15d). The late-season harvest TDN showed about the same amount of variation in the sub-families as the early-season harvest (Fig. 16). OKN2-13 and OKN5-8 showed a large amount of variation and had means above the population average (Fig. 16a). Several sub-families (OKN4-1 and OKN4-9) had means higher than the population mean and also had moderate variation (Fig. 16a). OKS2-10, OKS4-2, and OKS5-5 had a large amount of variation and means above the population mean (Fig. 16b). Other subfamilies had means above the population mean but not as much variation (e.g., OKS1-4, OKS1-13, OKS5-11; Fig. 16b). TX sub-families did not vary too much from the population

mean but TX2-12 had a mean above the population mean and a moderate amount of variation but not as much variation as TX4-3 that had a mean below the population mean (Fig. 16c). Like TX, AR sub-families did not deviate much from the population mean but AR3-1 and AR5-4 had means above the population and a moderate amount of variation (Fig. 16d). AR1-1, AR3-13, and AR5-9 had means close to that of the population but had a moderate amount of variation (Fig. 16d).

These values offer a prediction as to the feeding value of the forage by combining the NDF and ADF value and putting them on an index that is easier to understand and allows for quick comparison between samples

**Relative Feed Value.** Based on ADF and NDF, RFV offers a quick way to compare two samples where the higher the value, the better a sample is. RFV ranged from 68.1 to 68.9 in 2012 and 67.9 to 69.2 in 2013 for the early-season harvest (Table 5). The late-season harvest ranged from 66.3 to 67.8 in 2012 and 74.5 to 76.4 in 2013 (Table 6). These values are well below the reported RFV of 88 to 98 by Shultz (2013). For the early-season harvest in 2012, AR had the highest RFV which differed ( $p \leq 0.05$ ) from the other populations but in 2013 AR had the lowest RFV. Given that most other traits studied varied between first cuts in 2012 and 2013, it is worth noting that RFV was very close both years. The boxplots for the four populations' RFV show very little variation in 2012 or 2013 for the early-season harvest (Fig. 17a). On the other hand, the boxplot for the late-season harvest shows more variation in comparison to the early-season, but it is still not very much in either year (Fig. 17b). While the boxplots of the populations did not show much variation, boxplots of subfamilies shows a better view of what

occurred in the populations (Fig. 18). OKN had four high outliers between OKN1-11 and OKN3-11 even though they both had very little variation and had means at or below the population mean (Fig. 18a). OKN1-7, OKN4-1, OKN5-8, and OKN5-12 all had means above the population mean but only OKN1-7 had a large amount of variation of those sub-families (Fig. 18a). OKS had five high outliers among four sub-families (OKS2-10, OKS3-5, OKS4-14, and OKS5-5) while four of them were close to or above a value of 80 (Fig. 18b). TX had one high outlier in the TX3-7 sub-family (Fig. 18c). Several sub-families had a large amount of variation (TX1-3, TX1-10, TX2-12, TX3-3, TX4-3, TX4-12) but only one of them (TX1-5) had a mean above the population mean (Fig. 18c). AR1-14 did not have a large amount of variation for RFV but almost all four quartiles were above the population mean in the early-season harvest (Fig. 18d). AR2-6 also did not have a large amount of variation but most of the first three quartiles are above the population mean (Fig. 18d). AR3-1, AR4-5, AR4-11, AR5-9, and AR5-13 exhibited a relatively large amount of variation but they had means that were very close to that of the population (Fig. 18d). The sub-families exhibited a much larger amount of variation of RFV for the late-season harvest as compared to the early-season harvest (Fig. 19). OKN has several subfamilies with large amounts of variation and mean greater than the population mean (OKN1-7, OKN2-5, OKN2-13, OKN4-1, OKN4-9, OKN5-8) and a few that exhibited a large amount of variation but had means at or below that of the population (e.g., OKN3-4, OKN3-11, OKN5-12; Fig 19a). This same trend continued with the other three populations for RFV in the late-season harvest. OKS1-4, OKS1-13, OKS2-10, OKS4-2, and OKS5-5 had high amounts of variation and had means greater than that of the population mean (Fig. 19b). Other sub-families such as OKS3-5, OKS3-12, OKS4-4, and OKS5-3 exhibited a large amount of variation but had means at or below the mean of the

population (Fig. 19b). The first quartile of TX5-2 extends up to a value of 90 and also has a high mean (Fig. 19c). Other sub-families with large amounts of variation and high means include TX1-3, TX1-10, TX2-12, and TX3-7 (Fig. 19c). TX 4-3 had the most variation of the TX sub-families but had a mean very close to that of the population (Fig. 19c). AR did not have many sub-families with high means but AR3-1 had a high mean and a large amount of variation, but most of the variation was below the population mean (Fig. 19d). AR3-13 had the greatest amount of variation for the AR sub-families but had a mean that was very similar to that of the population (Fig. 19d). Other sub-families with a large amount of variation but means close to the population mean include AR1-14, AR2-3, AR2-6, AR2-6, and AR5-9 (Fig. 19d).

### **Morphological Traits**

The population means for early-season leaf angle scores remained relatively constant between years (2.6-3.4; Fig. 20). The AR population exhibited the most bent leaves with greater than half of the leaves being  $>45^\circ$  at the distal end (Fig. 20).

Similarly, canopy scores remained relatively consistent for populations between the two years, and they all averaged around 2.5 to 2.75 (Fig. 21). That is on average, plants had fairly open canopies that were approximately  $45^\circ$  angles to the ground (Fig. 21).

In 2012, color greenness scores differed among some of the populations ( $p \leq 0.05$ ) (Fig 22). The population AR had a more blue color with a rating average of 3.7 in comparison to the other population averages of about 3.0 (Table 1). In 2013 there was a little more variation with AR having a rating of 3.6 which differed ( $p \leq 0.05$ ) from the other populations (Table 1).

Leaf bloom scores for each population did not differ significantly ( $p>0.05$ ) between years with averages around 2 for both years meaning that a moderate amount of wax was present on the leaves (Fig. 23).

Plant heights for the early-season harvest are similar which means that the populations were harvested at similar stages both years (Fig. 24). Plant regrowth heights varied among the populations in both years (Fig. 25). OKS had the greatest regrowth height (42cm) in 2012 but also had a higher regrowth density score (3.3) meaning that the amount of growth was not as thick and dense as other populations such as OKN which had a regrowth score of 3.2 (Fig. 25-26). In 2013, OKN had the second tallest regrowth height (46cm) and the lowest regrowth density score (3.1) meaning that there was more overall regrowth compared to the other populations (Fig. 25-26). This could be an advantage in commercial growing operations. Plant heights for the late-season harvest differed ( $p\leq 0.05$ ) in 2012 (152-164cm; Fig. 27). A large drop in height for the second harvest was observed in 2013 with averages between 90 and 103cm (Fig. 27). This was attributed to possible reduction in vigor of the plants due to being on a two-cut system, and possible stunting due to the timing of an herbicide application.

Tillers per plant differed among populations in 2012 (184-253) with OKN having the highest average for 2012 as well as 2013 (253 and 175, respectively; Fig. 28). The drop in average number of tillers per population can possibly be explained the same way as the large drop in plant height for the second harvest.

When looking at the maturity stage of the late-season harvest, OKS and OKN tended to mature earlier than TX and AR in 2012 and 2013 (Fig. 29). The late-season harvest in 2013 was also



taken at a slightly more advanced maturity stage for all four populations. This was due to the extra time that was allowed for them to grow to a height closer to that of the previous year.

### **Trait Correlations**

There was a moderate correlation for the early-season harvest between height and yield in 2012 and 2013 ( $r = 0.67$  and  $r = 0.62$ , respectively; Table 7-8). These values are similar to the findings that Das et al. (2004) reported ( $r = 0.45$ ). This means that as height increased, yield also tended to increase. Interestingly, when looking at correlations for the late-late season harvest, there was a low correlation between height and yield in 2012 ( $r = 0.23$ ) while there was a moderate correlation in 2013 ( $r = 0.36$ ) (Table 9-10). The canopy type score was also moderately correlated with yield in 2012 and 2013 ( $r = 0.40$  and  $r = 0.45$ , respectively; Table 7-8). This means that higher yields tended to be associated with more open canopies rather than closed. There was also a moderately negative correlation in 2012 and 2013 ( $r = -0.47$  and  $r = -0.48$ , respectively) between yield of the early-season cut and the density of regrowth after the early-season cut (Table 7-8). This means that higher regrowth densities tended to go along with higher yields. Regrowth density was also correlated with several other traits (Table 7-8). Regrowth height and regrowth density were moderately negatively correlated in 2012 and 2013 ( $r = -0.41$  and  $r = -0.35$ , respectively) meaning plants with taller regrowth and higher densities tended to occur together after the early-season cut (Table 7-8). In 2013 regrowth density and canopy type had a moderate negative correlation ( $r = -0.35$ ) meaning that a more open canopy before the early-season harvest tended to have a higher density on the regrowth (Table 8). Also in 2013, early-season height and regrowth density were moderately negatively correlated ( $r = -0.31$ ) meaning

that taller heights before the first cut tended to go along with greater densities after the first cut (Table 8). Early-season height was also moderately correlated in 2013 ( $r = 0.33$ ) with regrowth height meaning that taller plants before the early-season harvest tended to have the taller regrowth after the early-season cut (Table 8). There was a moderately negative correlation between height and maturity of the late-season harvest in 2012 ( $r = -0.31$ ) and also in 2013 ( $r = -0.58$ ) which means that plants at an earlier maturity stage tended to be taller than plants that were at a later maturity stage at the time of the late-season harvest (Table 9-10).

## CHAPTER V

### Conclusions

The objectives of this research were to: (i) evaluate four  $F_1$  half-sib populations for their potential of producing superior lines for forage production, (ii) assess the genetic variances for yield, and (iii) evaluate correlations between yield and other agronomic traits for the purpose of indirect selection. Forage yield ranged from 1.00 to 1.08 kg plant<sup>-1</sup> in 2012 and 1.41 to 1.51 kg plant<sup>-1</sup> in 2013 for the early season harvest. While the populations' means did not differ in 2012 or 2013, there was a considerable amount of variation in the sub-families both years. The variation can be seen in the boxplots when comparing sub-families and also by the outliers that were found. This variation was expected due to the heterogeneity of the parents due to the self-incompatibility of switchgrass. Because heterogeneity was expected among the half-sib progeny from the crossing of heterogeneous germplasm, genetic variances were estimated for each population as well as sub-family. None of the four populations exhibited genetic variance for yield for either year, but there were five sub-families in 2012 and 10 sub-families in 2013 to exhibit genetic variance. Three sub-families, two from AR and one from OKS, exhibited genetic variance for yield both years. Broad-sense heritability for the sub-families ranged from 0.14 to 0.47 in 2012 and 0.13 to 0.42 in 2013.

It was determined that harvesting a set amount of tillers was an inaccurate way of predicting late-season forage yields, but ranking was found to stay fairly consistent between the tiller method and harvesting the whole plant. The yields were over estimated by 123% on average using the tiller prediction method.

Heterosis was observed in 2012 and 2013. Mid-parent heterosis was identified in all four populations with TX and OKS having the highest amount in 2012 (18.5 and 18.8%, respectively). Mid-parent heterosis values were in the same range in 2013; however, AR showed the highest mid-parent heterosis with a value of 19.3% compared to 12.0-14.8% by the other three populations. High-parent heterosis was not identified for the populations, but some of the sub-families had plants to exhibit high-parent heterosis. When looking at the top five yielding F<sub>1</sub> plants from each family for both years, seven plants were identified as top performers. These seven plants had high-parent heterosis values up to 13% in 2012 and 16% in 2013.

Protein content ranged between 10.3 and 10.8% in 2012 and all four populations had a protein content of 10.2% in 2013 for the early-season harvest. The late-season harvest had lower protein content ranging from 8.1 to 8.6% in 2012 and 9.7 to 10.5% in 2013. There was not much variation in the early season harvests, but OKS, TX, and AR populations had higher protein content than their respective maternal sources.

Acid detergent fiber was found to range between 41.7 and 41.9% in 2012 and 38.8 to 39.4% in 2013 for the early-season harvest. ADF ranged from 42.3 to 43.3% in 2012 and 36.7 to 37.4% in 2013 for the late-season harvest. There was not a lot of variation in ADF for both harvests in both years within and among sub-families in all four populations.

Neutral detergent fiber ranged between 76.4 and 77.1% in 2012 and 79.0 to 79.9% in 2013 for the early-season harvest. For late-season harvests, NDF ranged from 76.9 to 77.9% in 2012 and 73.6 to 74.7% in 2013. There was not a lot of variation in the populations but many of the sub-families exhibited high NDF levels in the late-season harvest which is desirable in forage.

Total digestible nutrients for the early-season harvest were found to range between 54.7 and 55.0% in 2012 and 57.7 to 58.3% in 2013. TDN ranged from 53.1 to 54.3% in 2012 and 59.9 to 60.8% in 2013 for the late-season harvest. Again there was variation for TDN within and among sub-families in each of the populations.

For the early-season harvest, relative feed values indices ranged between 68.1 and 68.9 points in 2012 and 67.9 to 69.2 points in 2013. RFV ranged from 66.3 to 67.8 points in 2012 and 74.5 to 76.4 points in 2013 for the late-season harvest. While the values found in this study were lower than other reported values, there was still some variation among the populations. AR had the highest RFV in 2012 and the lowest in 2013. The early-season values were consistent from year to year, but there was overall large increase in RFV in 2013 vs 2012 in the late-season harvest.

For the morphological traits, the leaf angle scores, canopy scores, and leaf bloom remained relatively constant between years. Color greenness differed between some of the populations with AR having the bluest color in comparison to the other three populations. Plant heights were consistent within the early-season harvest and within the late-season harvest. The stage of maturity was similar for the early-season harvests, but the maturity was more advanced for the late-season harvest in 2013 versus 2012. Plant regrowth after the early-season harvest varied among populations in both years. OKS had the tallest regrowth at 15 days, but one of the least dense regrowth amounts in 2012. In 2013 OKN had the tallest regrowth and the densest regrowth of all the families meaning that it had the superior regrowth of all the families at 15 days after the early-season harvest. Tiller numbers varied among populations in 2012 with OKN

having the highest average for both years. There was a drop in tiller number from 2012 to 2013 which can possibly be explained by the reduction of nutrients available to the plants.

Yields were correlated with plant height, as well as canopy type, so focusing on taller plants with more open canopies and selecting for these could result in higher yielding progeny. There was also a correlation between yield and regrowth density after the early-season harvest, meaning that higher yielding plants tended to have the greater amount of regrowth. There was also a correlation between regrowth density and regrowth height, meaning plants that have taller regrowth also tended to have the densest regrowth as well.

These results from this study are particularly encouraging in that there appears to be sufficient non-additive genetic variance (i.e., heterosis) among these four lowland parental sources to warrant further investigation into bi-parental combination that might give high specific combining ability for forage yield. The outcome could affect the types of varieties (i.e., hybrids versus synthetics) that might be the target in switchgrass breeding programs. Furthermore, the results indicate that the parental sources are productive and genetically different enough that genetic gains could be made for from recurrent selection methods (additive genetic variance) for forage yield, and nutritional value as well as associated morphological traits. Lastly, several different polycross nurseries could be set up for high forage yield and other targeted traits using selected  $F_1$ s from all four populations.

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## APPENDICES

## Appendix A

Table 1. Means for early-season forage yield and morphological traits for F<sub>1</sub> half-sib populations of NSL-2001-1 (OKN), Cimarron (OKS), PI607837 (TX), and PI421999 (AR) evaluated at the East Tennessee Research and Education Center, Holston Unit, Knoxville in 2012 and 2013.

Year		Early Season Yield	Height	Regrowth Height †	Scores				
					Leaf Angle	Canopy Type	Color Greenness	Leaf Bloom	Regrowth Density
2012	<b>Population</b>	kg plant <sup>-1</sup>	cm	cm	1-5	1-5	1-5	1-3	1-5
	NSL-2001-1 (OKN)	1.02a‡	128a	41a	2.7c	2.6c	3.1b	2a	3.2b
	Cimarron (OKS)	1.08a	129a	42a	2.8b	2.7b	3b	2a	3.3ab
	PI 607837 (TX)	1.07a	126a	41a	2.6d	2.9a	3b	2a	3.2ab
	PI 421999 (AR)	1.00a	131a	40a	3.4a	2.7b	3.7a	2.1a	3.3a
	<b>Parent</b>								
	NSL-2001-1 (OKN)	1.08	94	37	2.3	1.9	2.1	1.6	2.5
	Cimarron (OKS)	0.82	106	40	2.7	2.2	2.6	2.0	3.1
	PI 607837 (TX)	0.87	103	40	2.6	2.3	2.2	1.7	2.7
	PI 421999 (AR)	0.86	109	37	3.3	2.3	2.9	1.8	3.1
2013	<b>Population</b>								
	NSL-2001-1 (OKN)	1.41a	122b	46a	2.8c	2.4c	3.1b	2a	3.1b
	Cimarron (OKS)	1.42a	122b	46a	2.9b	2.5b	3b	2a	3.3a
	PI 607837 (TX)	1.45a	119b	44a	2.6d	2.9a	2.9c	2a	3.2ab
	PI 421999 (AR)	1.51a	130a	45a	3.3a	2.6b	3.6a	2.1a	3.1b
	<b>Parent</b>								
	NSL-2001-1 (OKN)	1.18	45	22	2.9	2.2	3.1	2.0	3.1
	Cimarron (OKS)	1.24	43	21	2.9	2.3	3.0	2.0	3.1
	PI 607837 (TX)	1.16	42	20	3.1	3.0	2.7	2.0	3.0
	PI 421999 (AR)	1.48	49	21	2.9	2.5	3.4	2.0	2.9

† Regrowth height was taken 15 days after the early season harvest. Leaf Angle is the relation of the leaf angle to the stem where 1= all leaves are ≤ 45° to a vertical stem, 3= approximately half of the leaves are ≈45° and half are >45° at the distal end of the leaf, 5= all leaves are arched so that from the leaf midpoint to the leaf tip of the leaves are >90°. Canopy type was rated on a scale of 1 to 5 with 1= a very upright plant with greater than 95% of tillers vertical to the ground, 3=the plant has many tillers that were at 45° to the ground, 5= tillers were growing completely spread out with tillers growing < 45° to the ground. Color was rated on a scale of 1 to 5 with 1= green and 5 =blue. Bloom was taken with 1= no wax, 2= moderate wax present, 3= high amounts of wax. Density was taken 15 days after the early-season harvest on the regrowth and rated with 1=most dense and full foliage, 3= medium growth and density, and 5= no regrowth had occurred.

‡Means followed by a common letter within a column and year and among populations are not significantly different at p≤0.05.

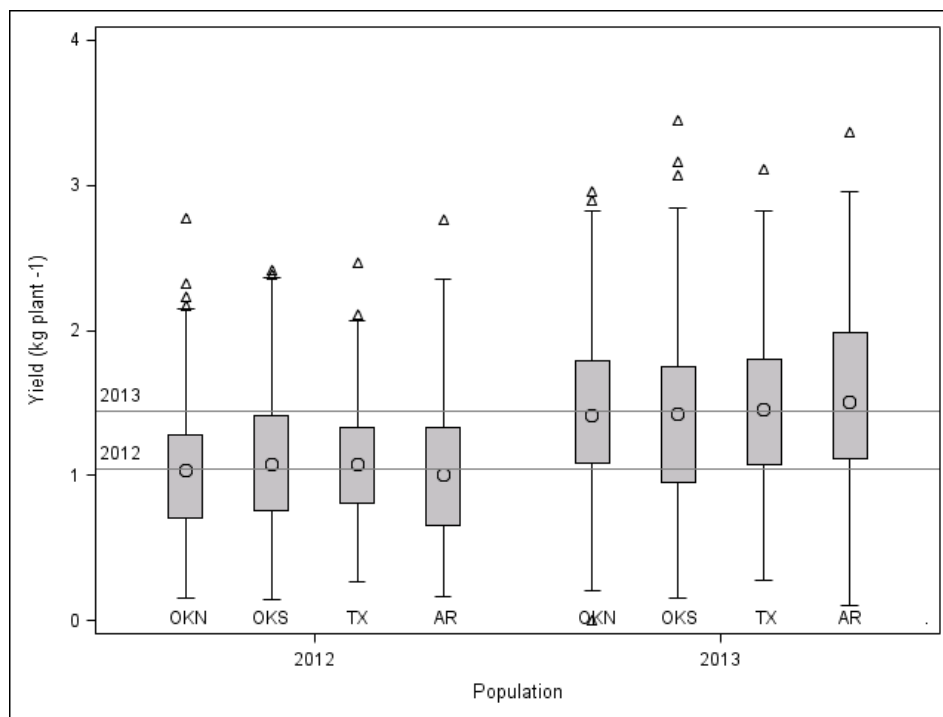


Fig. 1. Boxplots of early-season forage yields of  $F_1$  half-sib populations of NSL-2001-1 (OKN), Cimarron (OKS), PI607837 (TX), and PI421999 (AR) evaluated at the East Tennessee Research and Education Center, Holston Unit, Knoxville in 2012 and 2013. The horizontal black lines are the overall populations mean for 2012 and 2013. Triangles represent high or low outliers.

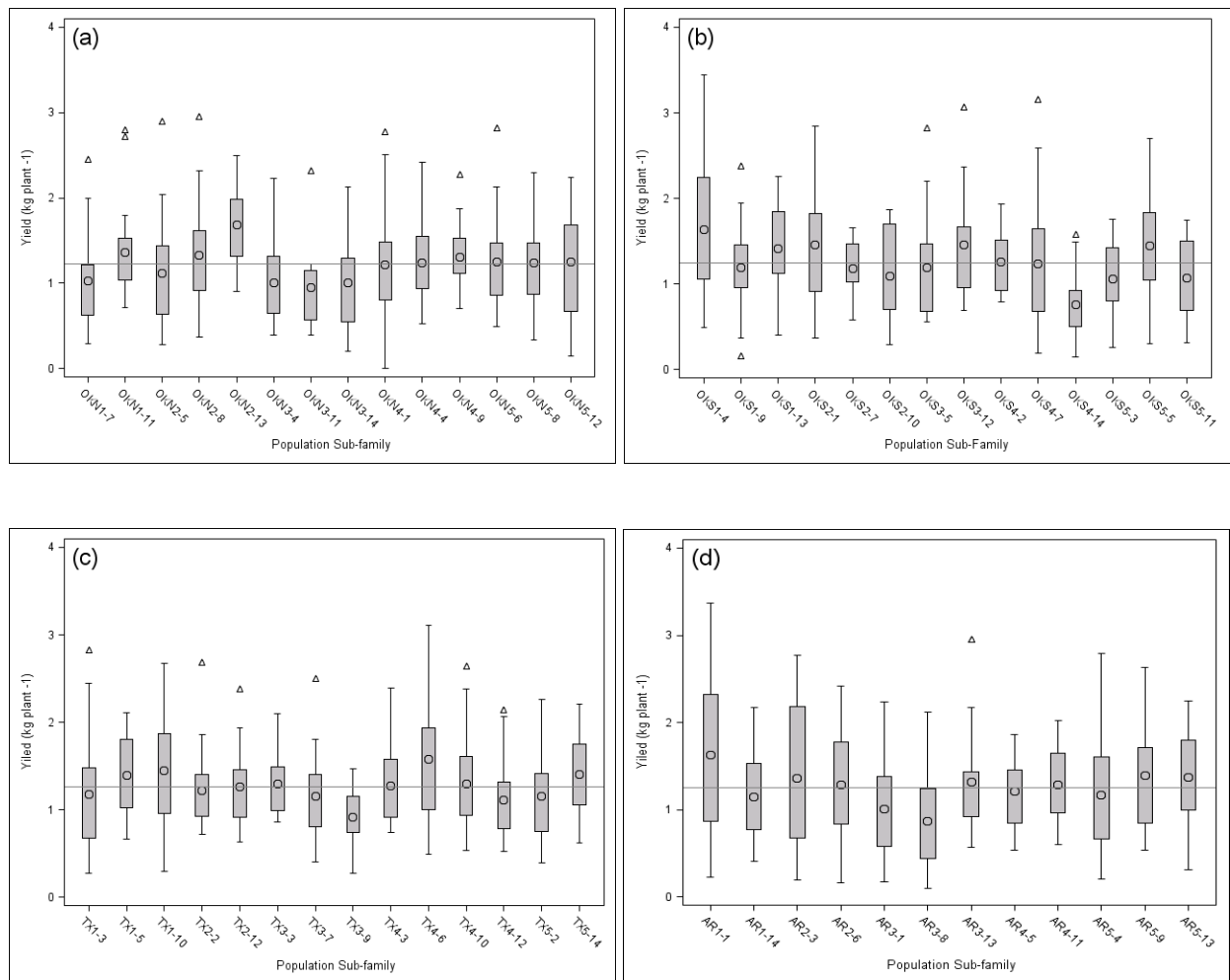


Fig. 2. Boxplots of early-season forage yields of F<sub>1</sub> half-sib populations of NSL-2001-1 (OKN)(a), Cimarron (OKS)(b), PI607837 (TX)(c), and PI421999 (AR)(d) evaluated at the East Tennessee Research and Education Center, Holston Unit, Knoxville in 2012 and 2013. The horizontal line signifies the two year average of each population. Triangles represent high or low outliers.

Table 2. Genetic variance and heritability for forage yield (kg plant<sup>-1</sup>) among F<sub>1</sub> half-sib populations of NSL-2001-1 (OKN), Cimarron (OKS), PI607837 (TX), and PI421999 (AR) evaluated at the East Tennessee Research and Education Center, Holston Unit, Knoxville in 2012 and 2013.

Year	Sub-Family	Genetic Variance	Broad-Sense Heritability
2012	OKN4-1	0.06	0.15
	OKN2-8	0.06	0.14
	OKS4-7	0.08	0.18
	AR5-4	0.09	0.20
	AR1-1	0.32	0.47
2013	OKN2-5	0.07	0.13
	TX4-6	0.21	0.30
	TX1-3	0.11	0.18
	OKS5-5	0.07	0.13
	OKS4-7	0.35	0.42
	OKS3-12	0.08	0.14
	OKS2-1	0.10	0.17
	OKS1-4	0.33	0.40
	AR5-4	0.36	0.42
	AR1-1	0.25	0.34

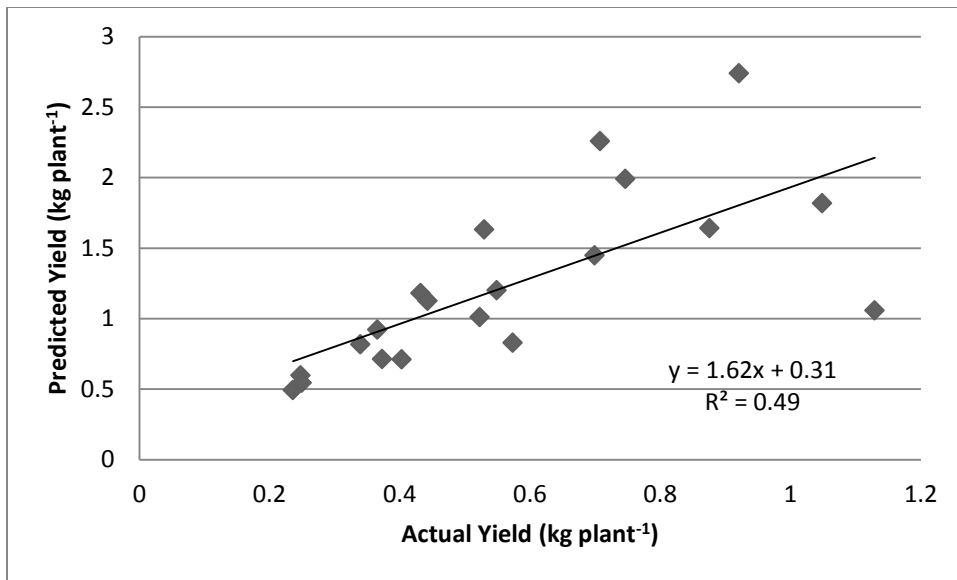


Fig. 3. Predicted yield of twenty switchgrass plants based on harvesting a set number of tillers compared to actual yield of the whole plant. Plants were evaluated at the East Tennessee Research and Education Center, Holston Unit, Knoxville in 2013.



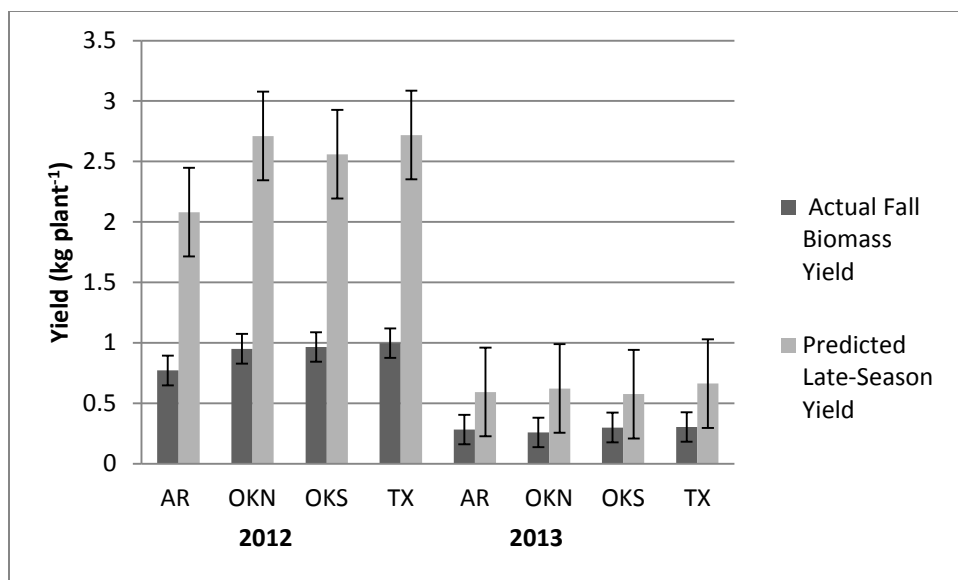


Fig. 4. Yields predicted for the late-season harvest based on tillers compared to the actual fall biomass harvest among  $F_1$  half-sib populations of NSL-2001-1 (OKN), Cimarron (OKS), PI607837 (TX), and PI421999 (AR) evaluated at the East Tennessee Research and Education Center, Holston Unit, Knoxville in 2012 and 2013.

Table 3. Heterosis (mid-parent is expressed as percent above the mean and high-parent is expressed as percent above the high parent) of F<sub>1</sub> half-sib populations of NSL-2001-1 (OKN), Cimarron (OKS), PI607837 (TX), and PI421999 (AR) evaluated at the East Tennessee Research and Education Center, Holston Unit, Knoxville in 2012 and 2013.

Population	Mid-Parent Heterosis		High-Parent Heterosis	
	2012	2013	2012	2013
OKN	13.98	12.02	-44.45	-51.00
OKS	18.76	12.29	-42.12	-50.88
TX	18.46	14.83	-42.27	-49.77
AR	10.63	19.30	-46.08	-47.82

Table 4. Top yielding individual F<sub>1</sub> half-sibs of NSL-2001-1 (OKN), Cimarron (OKS), PI607837 (TX), and PI421999 (AR) evaluated at the East Tennessee Research and Education Center, Holston Unit, Knoxville in 2012 and 2013.

Year	Population	Half-Sib	Yield		Height		Protein		ADF†		NDF		TDN		RFV	
			2012	2013	2012	2013	2012	2013	2012	2013	2012	2013	2012	2013	2012	2013
			— kg —		— cm —		— %DM —		— %DM —		— %DM —		— index —			
2012	NSL-2001-1 (OKN)	High-Parent	2.46	0.28	91	99	10.34	9.03	41.43	38.07	75.71	82.53	55.31	59.15	69.58	66.77
2013	PI 421999 (AR)	High-Parent	1.72	2.89	132	124	8.21	8.11	43.79	40.52	78.15	82.57	52.63	56.35	65.22	64.59
<b>2012 &amp; 2013</b>																
	NSL-2001-1 (OKN)	OKN2-8 (17-6)‡	2.32	2.96	160	147	11.61	10.58	38.82	39.50	73.80	81.29	58.29	57.51	73.94	66.52
	Cimarron <sup>1</sup> (OKS)	OKS4-7 (16-17)	2.42	3.16	142	147	12.97	7.72	37.67	43.45	70.28	82.34	59.60	53.01	78.82	62.19
		OKS3-12 (11-18)	2.37	3.07	142	152	7.49	9.64	46.78	41.27	83.34	80.77	49.21	55.50	58.56	65.36
		OKS2-1 (11-20)	2.19	2.84	165	152	11.25	10.89	42.03	41.05	76.67	81.51	54.63	55.75	68.14	64.96
	PI 607837 (TX)	TX4-6 (17-10)	2.47	3.11	170	155	8.48	10.14	42.91	37.84	78.15	78.65	53.63	59.41	66.03	70.29
	PI 421999 (AR)	AR1-1 (4-35)	2.77	3.36	157	147	8.50	10.85	45.26	37.86	80.73	79.76	50.95	59.38	61.82	69.28
		AR3-13 (5-32)	2.17	2.95	168	150	10.03	9.64	42.26	41.15	78.20	81.53	54.37	55.63	66.59	64.85

† ADF= Acid detergent fiber, NDF= Neutral detergent fiber, TDN= Total digestible Nutrients, RFV= Relative feed value

‡ Location in F<sub>1</sub> nursery, i.e. (row-range)

Table 5. Means for measured nutritional composition values from early-season harvest of F<sub>1</sub> half-sib populations of NSL-2001-1 (OKN), Cimarron (OKS), PI607837 (TX), and PI421999 (AR) evaluated at the East Tennessee Research and Education Center, Holston Unit, Knoxville in 2012 and 2013.

Year	Population	Protein	ADF <sup>†</sup>	NDF	TDN	RFV
2012	<b>Population</b>	-----	%DM	-----		Index
	NSL-2001-1 (OKN)	10.3b‡	41.8a	77a	54.9a	68.3a
	Cimarron (OKS)	10.5ab	41.9a	77.1a	54.7a	68.1a
	PI 607837 (TX)	10.5ab	41.7a	76.5a	55a	68.7a
	PI 421999 (AR)	10.8a	41.7a	76.4a	55a	68.9a
	<b>Parent</b>					
	NSL-2001-1 (OKN)	10.3	41.2	76.5	55.6	69.2
	Cimarron (OKS)	9.6	42.2	77.0	54.4	67.8
	PI 607837 (TX)	10.0	41.7	76.3	55.1	69.0
	PI 421999 (AR)	10.1	42.5	77.9	54.1	66.8
2013	<b>Population</b>					
	NSL-2001-1 (OKN)	10.2a	38.8a	79.3ab	58.3a	69ab
	Cimarron (OKS)	10.2a	39.3a	79.4ab	57.8a	68.4ab
	PI 607837 (TX)	10.2a	38.8a	79b	58.3a	69.2a
	PI 421999 (AR)	10.2a	39.4a	79.9a	57.7a	67.9b
	<b>Parent</b>					
	NSL-2001-1 (OKN)	9.6	36.6	80.5	57.4	67.1
	Cimarron (OKS)	10.3	38.8	79.8	58.3	68.4
	PI 607837 (TX)	9.1	40.7	81.5	56.2	65.3
	PI 421999 (AR)	10.2	39.5	80.3	57.5	67.5

† ADF= Acid detergent fiber, NDF= Neutral detergent fiber, TDN= Total digestible Nutrients, RFV= Relative feed value

‡Means followed by a common letter within a column and year and among populations are not significantly different at p≤0.05.

Table 6. Means for tiller weight and number, predicted late-season forage yield, morphological traits and measured nutritional compositional values among F<sub>1</sub> half-sib populations of NSL-2001-1 (OKN), Cimarron (OKS), PI607837 (TX), and PI421999 (AR) and parents evaluated at the East Tennessee Research and Education Center, Holston Unit, Knoxville in 2012 and 2013.

Year		Tiller weight	Tillers	Predicted Late Season Yield	Protein	ADF <sup>†</sup>	NDF	TDN	RFV	Height	Maturity
2012	<b>Population</b>	g	no.	kg plant <sup>-1</sup>	-----%DM-----				index	cm	(1-6)
	NSL-2001-1 (OKN)	10.7a‡	253a	2.71a	8.1b	42.7b	77.9a	53.8a	66.5b	152c	3.3a
	Cimarron (OKS)	11.5b	222c	2.56a	8.6a	42.5b	77.7a	54.1a	66.9b	158b	3.1b
	PI 607837 (TX)	11.4b	239ab	2.72a	8.6a	42.3b	76.9b	54.3a	67.8a	153bc	3.2ab
	PI 421999 (AR)	11.3c	184d	2.08b	8.1b	43.3a	77.5a	53.1b	66.3b	164a	2.2c
	<b>Parent</b>										
	NSL-2001-1 (OKN)	12.7	358	4.54	6.9	43.5	76.9	53.0	66.6	79	3.0
	Cimarron (OKS)	12.2	256	3.13	6.7	44.8	77.9	51.4	64.5	64	3.5
	PI 607837 (TX)	12.2	265	3.24	7.5	42.7	76.8	53.9	67.5	66	3.0
	PI 421999 (AR)	13.3	235	3.13	6.6	44.7	79.1	51.6	63.6	77	2.5
2013	<b>Population</b>										
	NSL-2001-1 (OKN)	3.5c	175a	0.62a	9.7b	37.2ab	74.1ab	60.2bc	75.5ab	94bc	4a
	Cimarron (OKS)	3.8a	154a	0.58a	10.5a	36.7c	73.6b	60.8a	76.4a	98ab	3.8b
	PI 607837 (TX)	3.9a	170a	0.66a	10.2a	36.8bc	73.6b	60.6ab	76.3a	90c	4.1a
	PI 421999 (AR)	3.7b	158a	0.59a	10.4a	37.4a	74.7a	59.9c	74.5b	103a	3.2c
	<b>Parent</b>										
	NSL-2001-1 (OKN)	6.7	207	1.39	9.9	38.0	77.9	59.3	71.1	130	4.0
	Cimarron (OKS)	4.8	151	0.72	9.5	39.5	76.4	57.6	70.8	109	2.5
	PI 607837 (TX)	4.9	150	0.73	9.4	38.1	77.8	59.2	70.9	119	4.0
	PI 421999 (AR)	4.2	186	0.79	8.7	40.3	80.4	56.6	66.5	127	2.5

† ADF= Acid detergent fiber, NDF= Neutral detergent fiber, TDN= Total digestible Nutrients, RFV= Relative feed value, Panicle maturity was taken on a scale of 1 to 6 with 1= fully headed, 2= mid-heading, 3= early heading, 4= late boot, 5= boot stage, and 6= early boot

‡Means followed by a common letter within a column and year and among populations are not significantly different at p≤0.05.

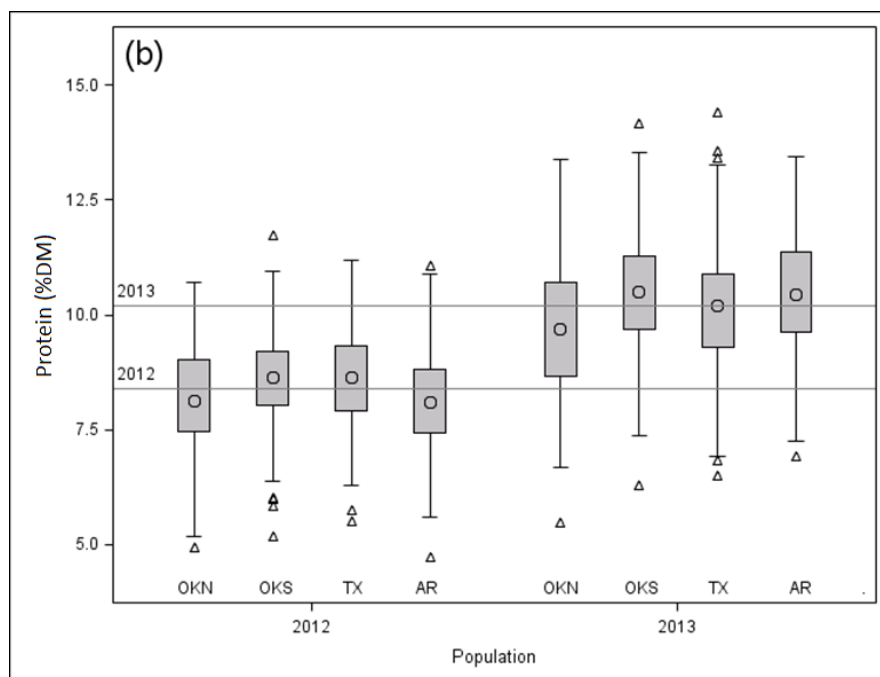
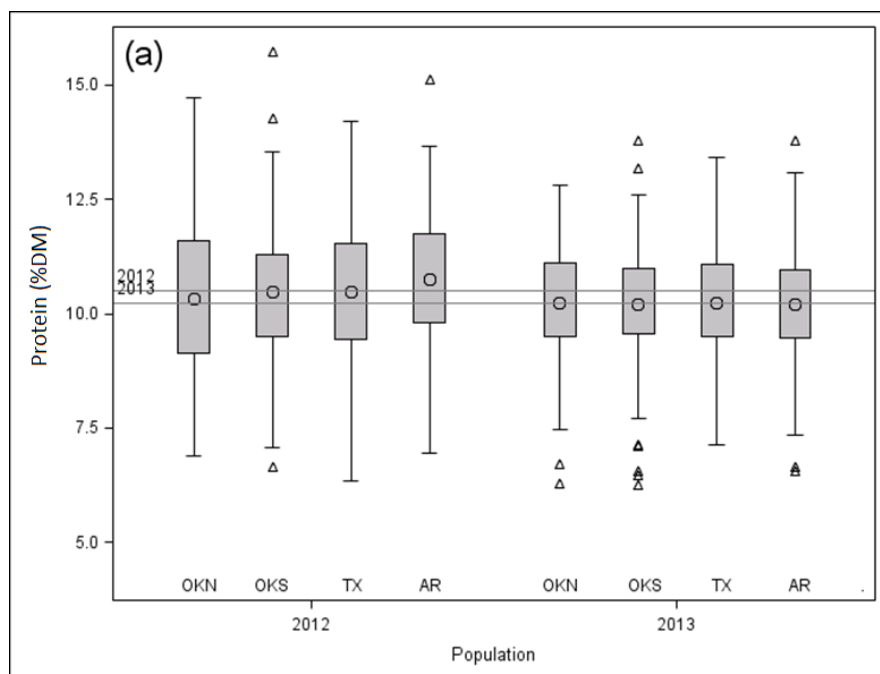


Fig. 5. Boxplots of early-season (a) and late-season (b) protein content of  $F_1$  half-sib populations of NSL-2001-1 (OKN), Cimarron (OKS), PI607837 (TX), and PI421999 (AR) evaluated at the East Tennessee Research and Education Center, Holston Unit, Knoxville in 2012 and 2013. The horizontal black lines are the overall population mean for 2012 and 2013. Triangles represent high or low outliers.

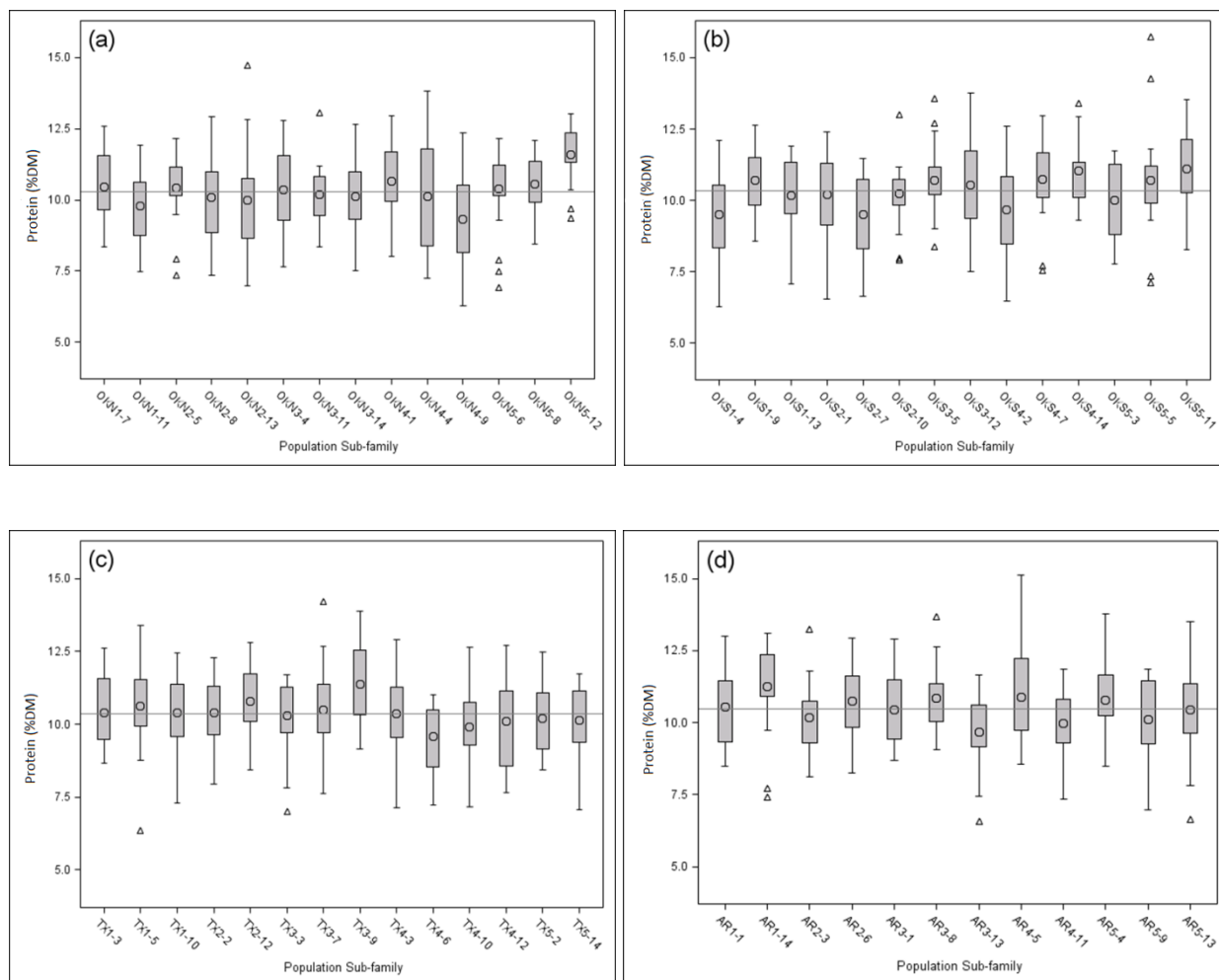


Fig. 6. Boxplots of early-season protein content of F<sub>1</sub> half-sib populations of NSL-2001-1 (OKN)(a), Cimarron (OKS)(b), PI607837 (TX)(c), and PI421999 (AR)(d) evaluated at the East Tennessee Research and Education Center, Holston Unit, Knoxville in 2012 and 2013. The horizontal line signifies the two year average of each population. Triangles represent high or low outliers.

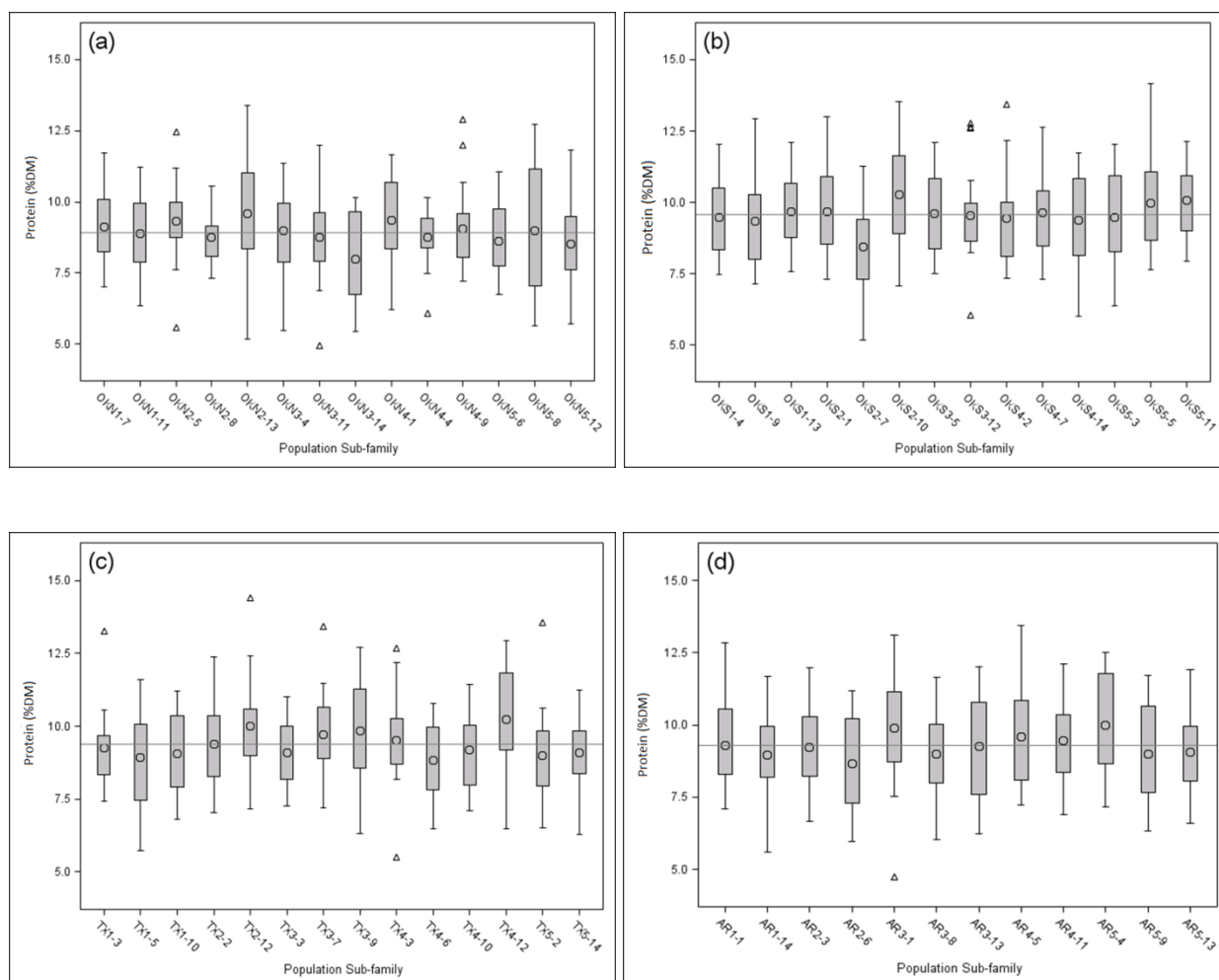


Fig. 7. Boxplots of late-season protein content of F<sub>1</sub> half-sib populations of NSL-2001-1 (OKN)(a), Cimarron (OKS)(b), PI607837 (TX)(c), and PI421999 (AR)(d) evaluated at the East Tennessee Research and Education Center, Holston Unit, Knoxville in 2012 and 2013. The horizontal line signifies the two year average of each population. Triangles represent high or low outliers.



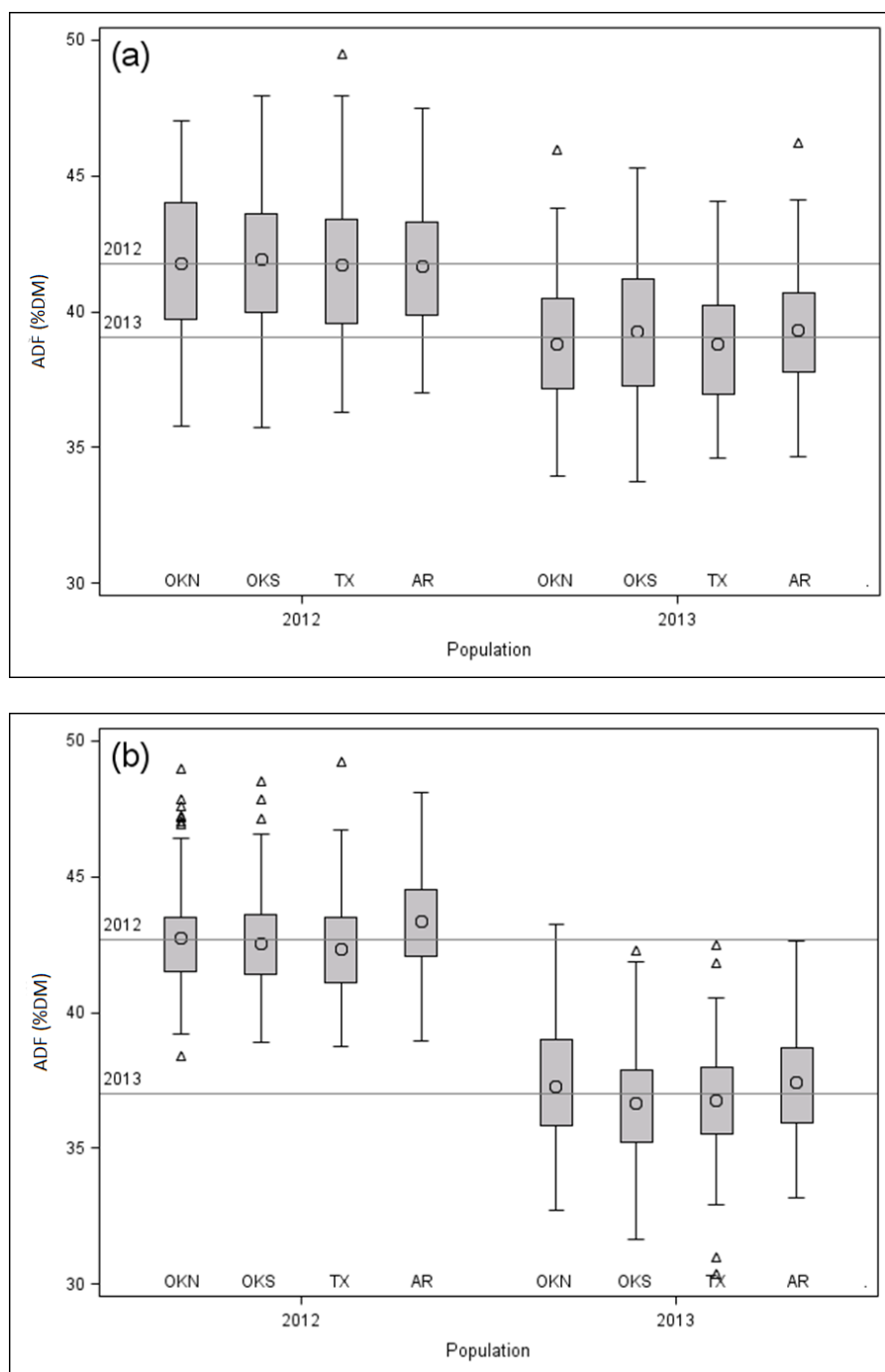


Fig. 8. Boxplots of early-season (a) and late-season (b) acid detergent fiber (ADF) content of  $F_1$  half-sib populations of NSL-2001-1 (OKN), Cimarron (OKS), PI607837 (TX), and PI421999 (AR) evaluated at the East Tennessee Research and Education Center, Holston Unit, Knoxville in 2012 and 2013. The horizontal black lines are the overall populations mean for 2012 and 2013. Triangles represent high or low outliers.

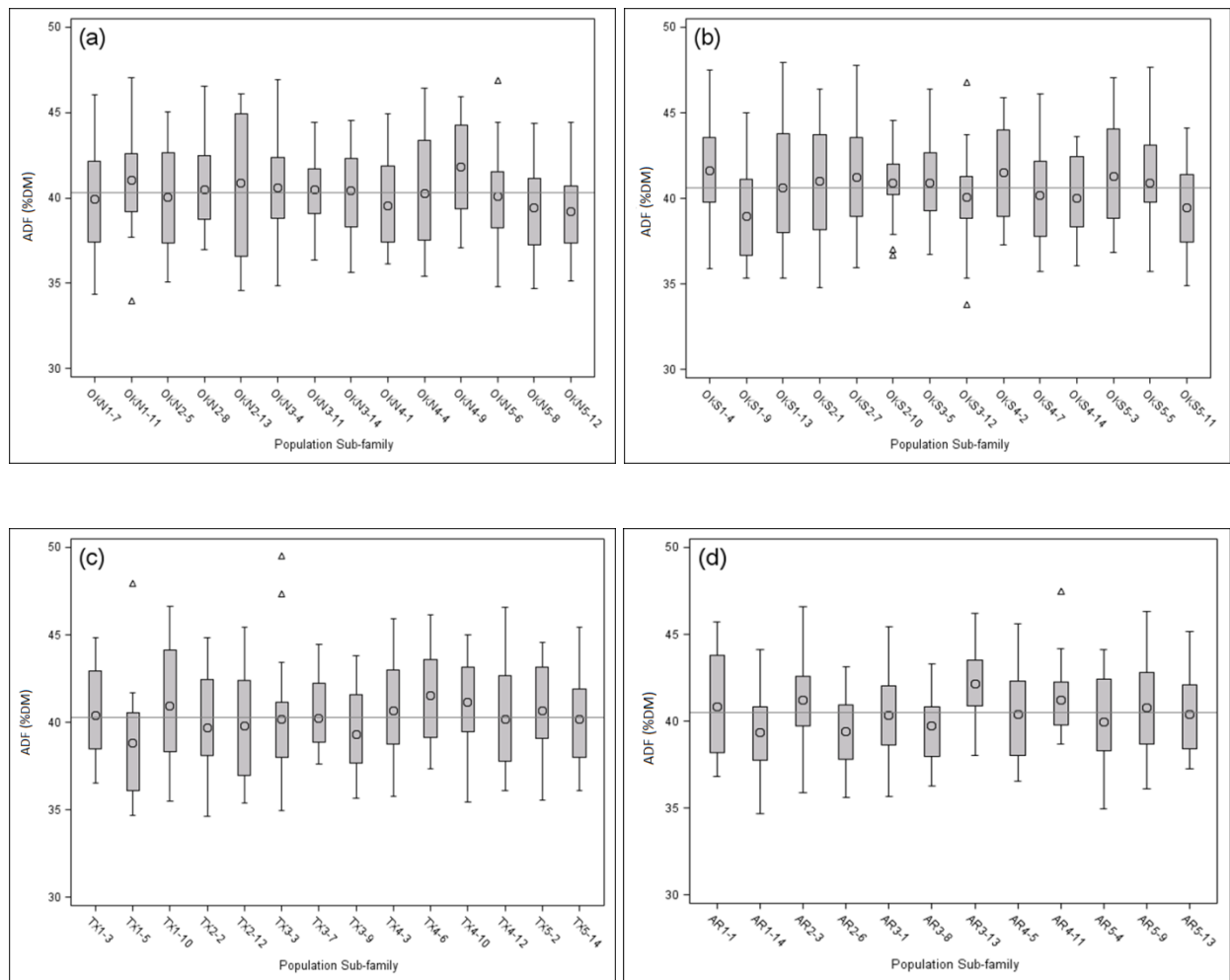


Fig. 9. Boxplots of early-season acid detergent fiber (ADF) content of F<sub>1</sub> half-sib populations of NSL-2001-1 (OKN)(a), Cimarron (OKS)(b), PI607837 (TX)(c), and PI421999 (AR)(d) evaluated at the East Tennessee Research and Education Center, Holston Unit, Knoxville in 2012 and 2013. The horizontal line signifies the two year average of each population. Triangles represent high or low outliers.

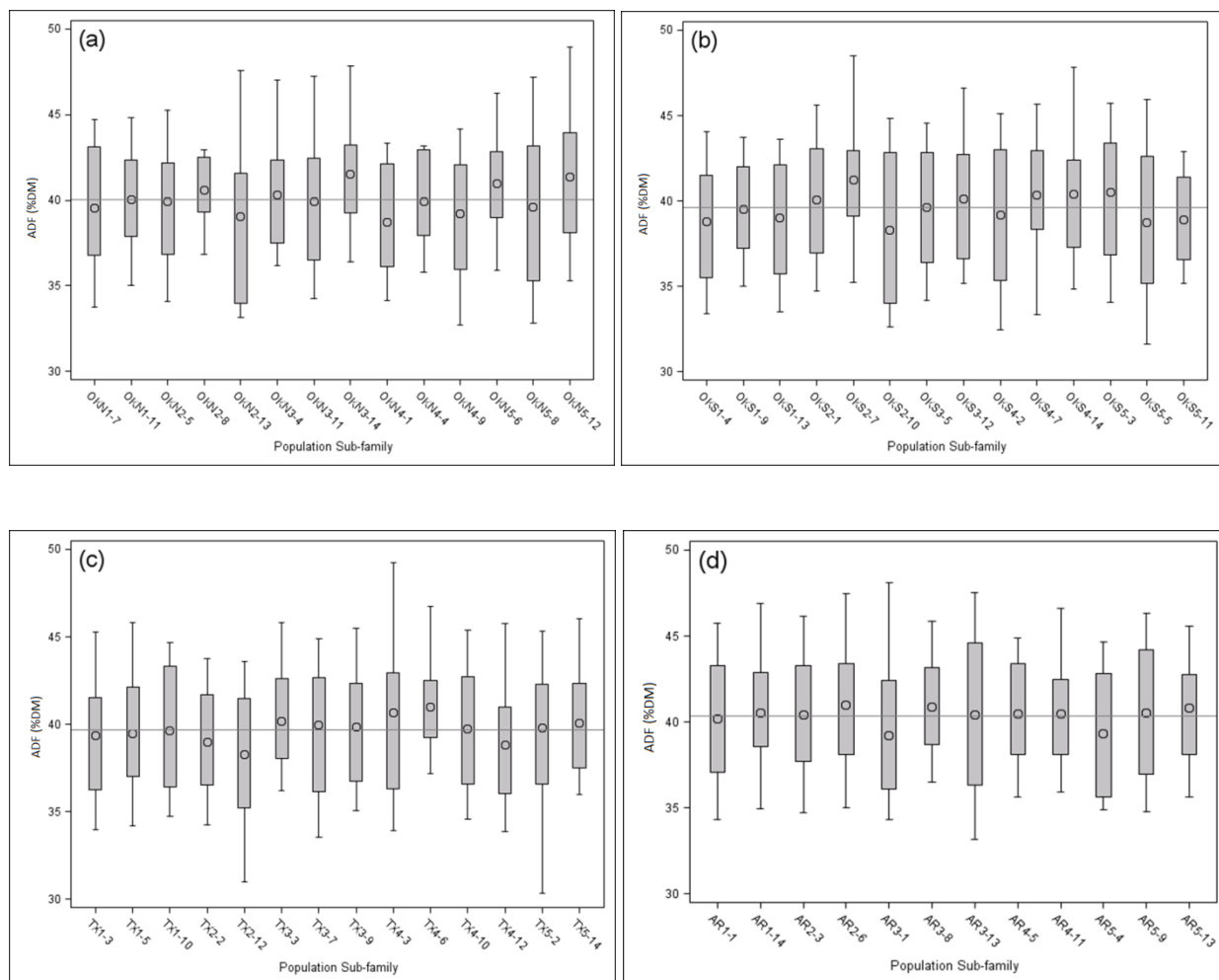


Fig. 10. Boxplots of late-season acid detergent fiber (ADF) content of  $F_1$  half-sib populations of NSL-2001-1 (OKN)(a), Cimarron (OKS)(b), PI607837 (TX)(c), and PI421999 (AR)(d) evaluated at the East Tennessee Research and Education Center, Holston Unit, Knoxville in 2012 and 2013. The black line signifies the two year average of each population.

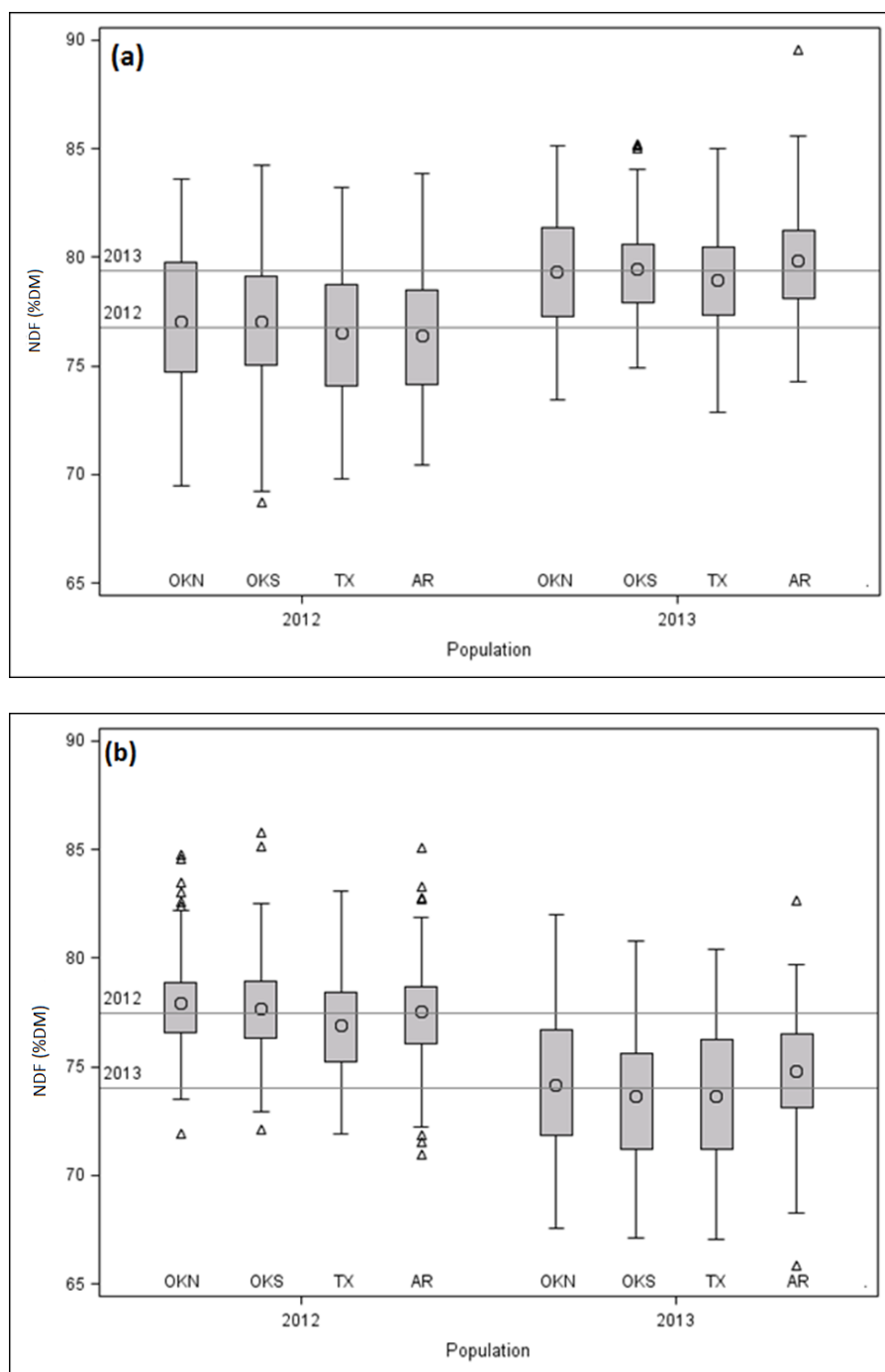


Fig. 11. Boxplots of early-season (a) and late-season (b) neutral detergent fiber (NDF) content of  $F_1$  half-sib populations of NSL-2001-1 (OKN), Cimarron (OKS), PI607837 (TX), and PI421999 (AR) evaluated at the East Tennessee Research and Education Center, Holston Unit, Knoxville in 2012 and 2013. The horizontal black lines are the overall population mean for 2012 and 2013. Triangles represent high or low outliers.

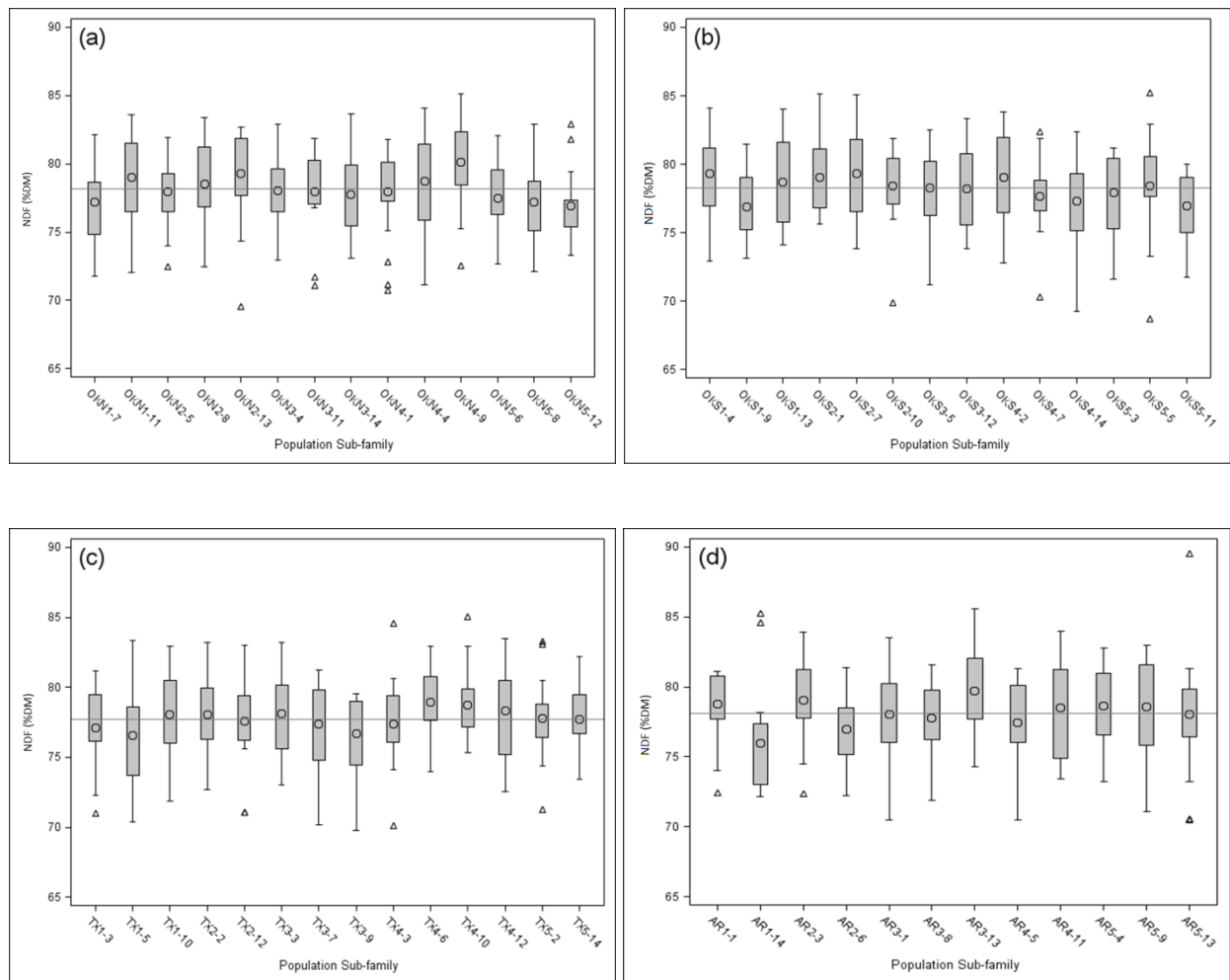


Fig. 12. Boxplots of early-season neutral detergent fiber (NDF) content of F<sub>1</sub> half-sib populations of NSL-2001-1 (OKN)(a), Cimarron (OKS)(b), PI607837 (TX)(c), and PI421999 (AR)(d) evaluated at the East Tennessee Research and Education Center, Holston Unit, Knoxville in 2012 and 2013. The horizontal line signifies the two year average of each population. Triangles represent high or low outliers.

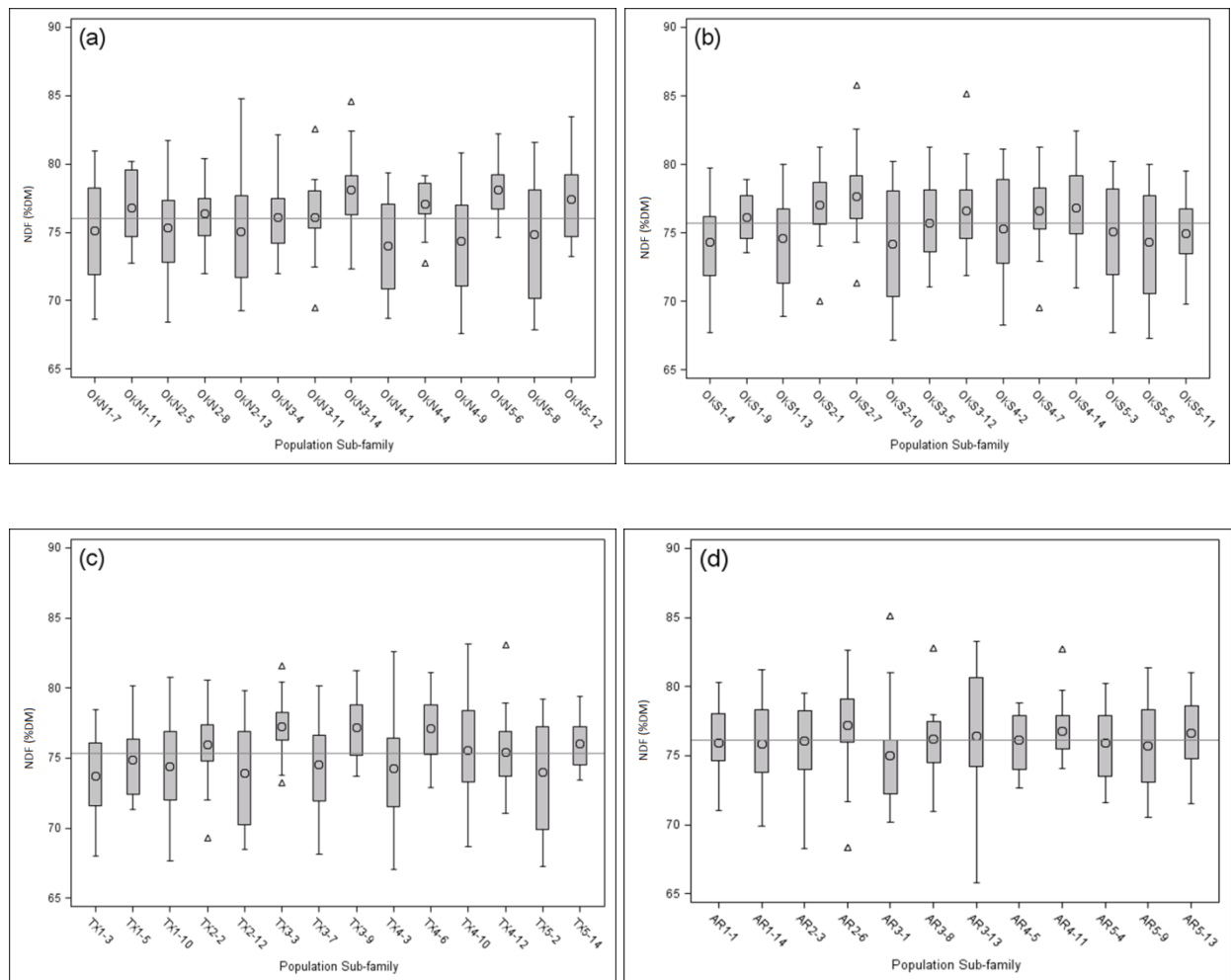


Fig. 13. Boxplots of late-season neutral detergent fiber (NDF) content of  $F_1$  half-sib populations of NSL-2001-1 (OKN)(a), Cimarron (OKS)(b), PI607837 (TX)(c), and PI421999 (AR)(d) evaluated at the East Tennessee Research and Education Center, Holston Unit, Knoxville in 2012 and 2013. The horizontal line signifies the two year average of each population. Triangles represent high or low outliers.

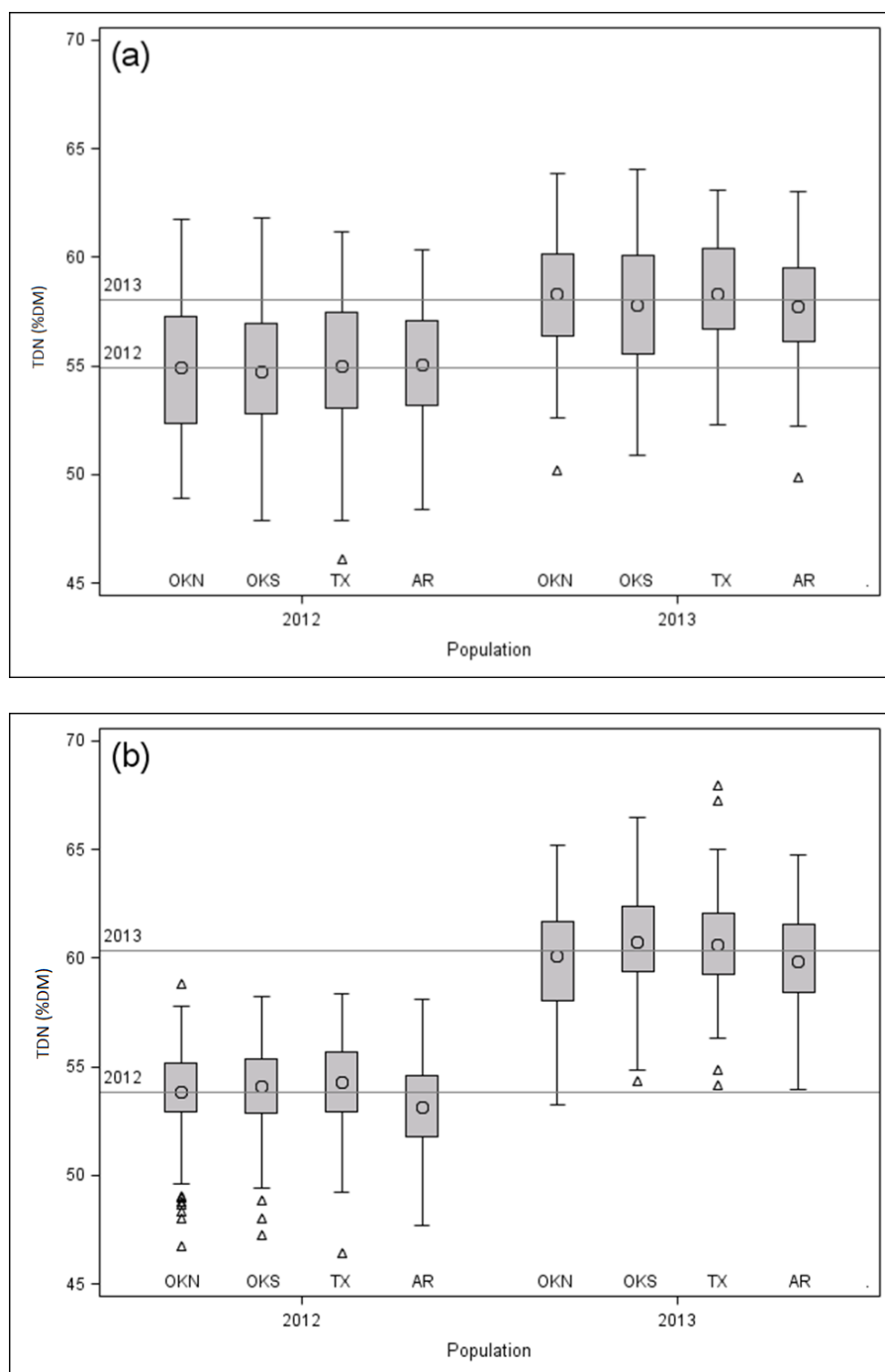


Fig. 14. Boxplots of early-season (a) and late-season (b) total digestible nutrients (TDN) of  $F_1$  half-sib populations of NSL-2001-1 (OKN), Cimarron (OKS), PI607837 (TX), and PI421999 (AR) evaluated at the East Tennessee Research and Education Center, Holston Unit, Knoxville in 2012 and 2013. The horizontal black lines are the overall populations mean for 2012 and 2013. Triangles represent high or low outliers.

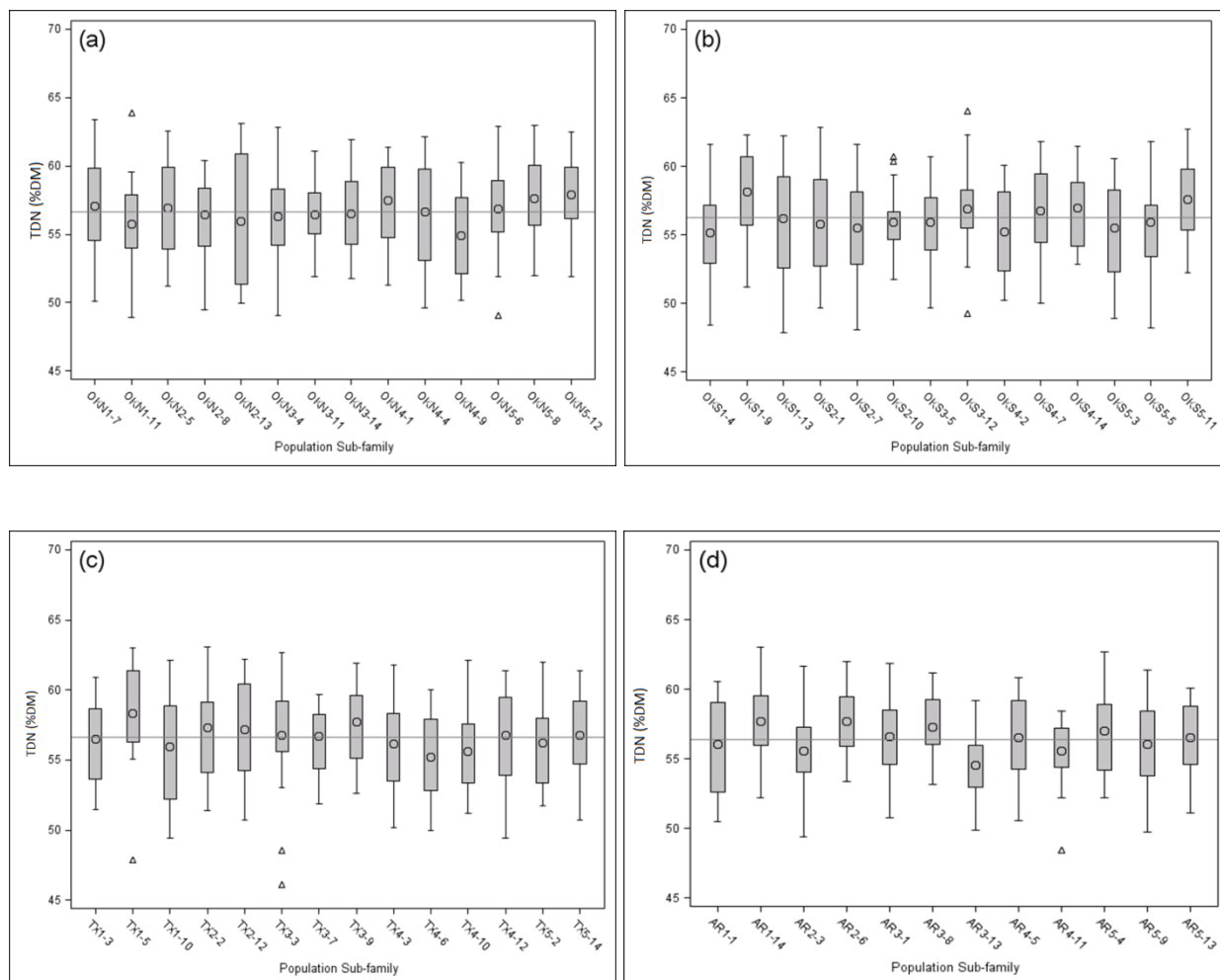


Fig. 15. Boxplots of early-season total digestible nutrients (TDN) of F<sub>1</sub> half-sib populations of NSL-2001-1 (OKN)(a), Cimarron (OKS)(b), PI607837 (TX)(c), and PI421999 (AR)(d) evaluated at the East Tennessee Research and Education Center, Holston Unit, Knoxville in 2012 and 2013. The horizontal line signifies the two year average of each population. Triangles represent high or low outliers.



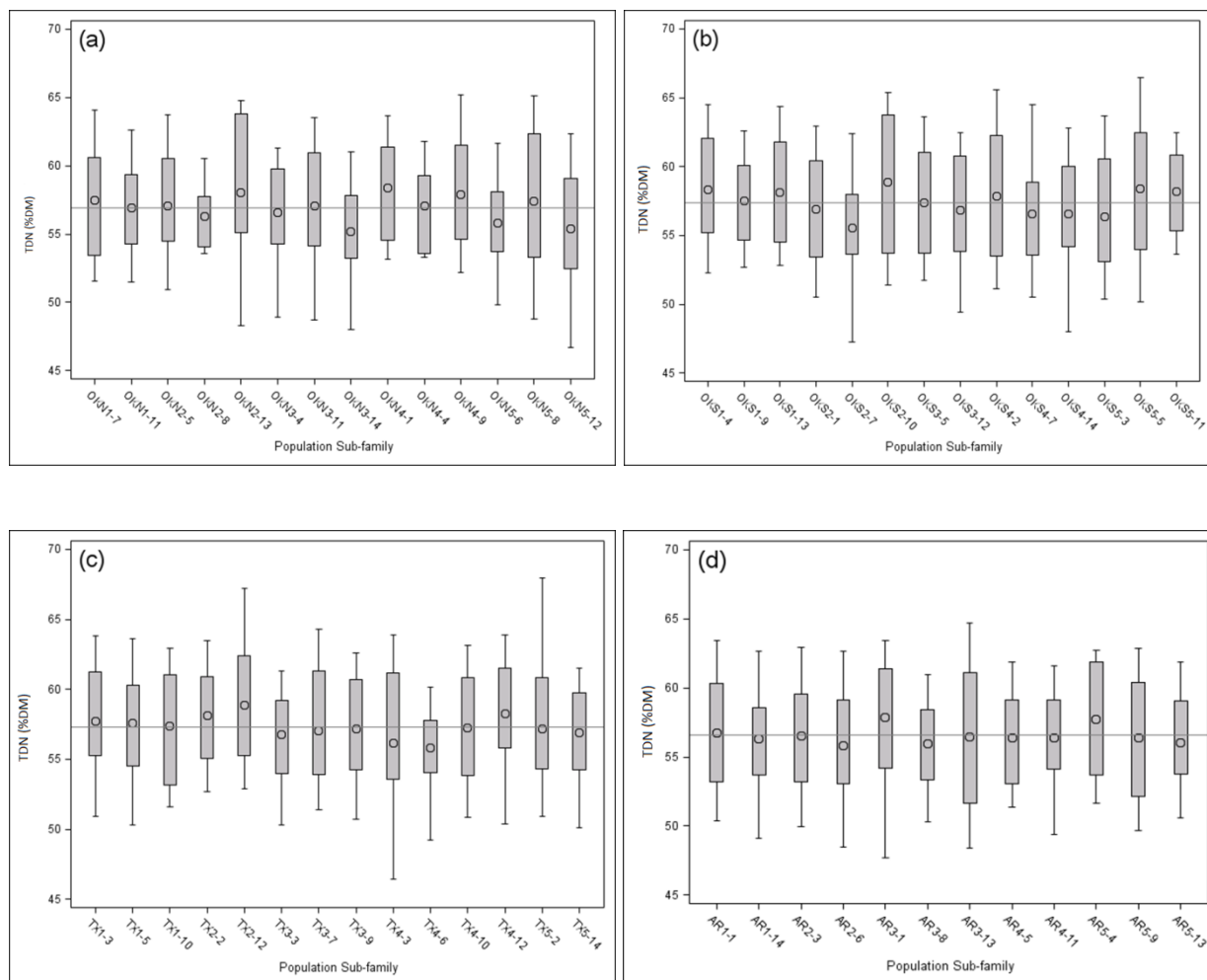


Fig. 16. Boxplots of late-season total digestible nutrients (TDN) of F<sub>1</sub> half-sib populations of NSL-2001-1 (OKN)(a), Cimarron (OKS)(b), PI607837 (TX)(c), and PI421999 (AR)(d) evaluated at the East Tennessee Research and Education Center, Holston Unit, Knoxville in 2012 and 2013. The horizontal line signifies the two year average of each population. Triangles represent high or low outliers.

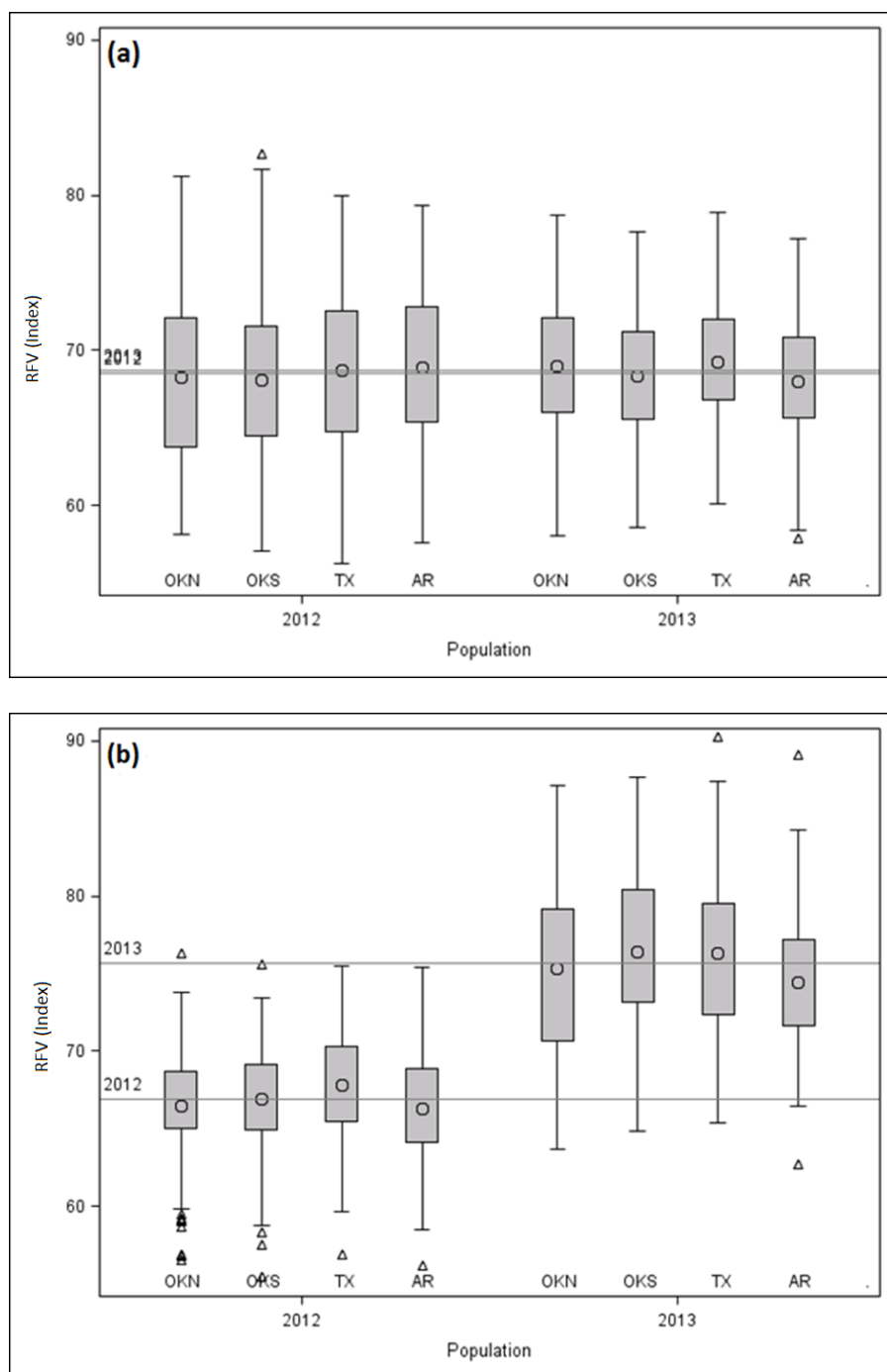


Fig. 17. Boxplots of early-season (a) and late-season (b) relative feed value (RFV) of  $F_1$  half-sib populations of NSL-2001-1 (OKN), Cimarron (OKS), PI607837 (TX), and PI421999 (AR) evaluated at the East Tennessee Research and Education Center, Holston Unit, Knoxville in 2012 and 2013. The horizontal black lines are the overall populations mean for 2012 and 2013. Triangles represent high or low outliers.

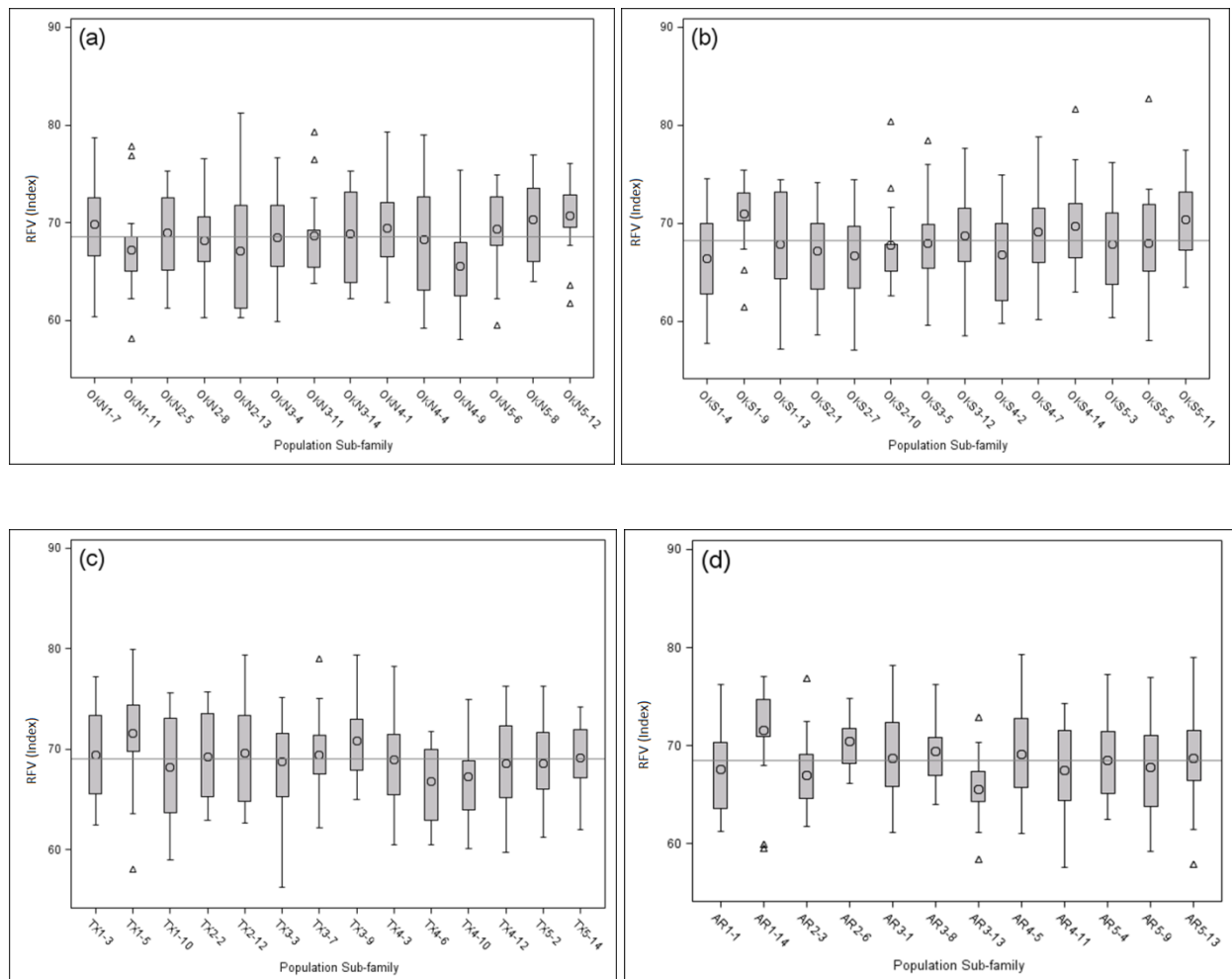


Fig. 18. Boxplots of early-season relative feed value (RFV) of  $F_1$  half-sib populations of NSL-2001-1 (OKN)(a), Cimarron (OKS)(b), PI607837 (TX)(c), and PI421999 (AR)(d) evaluated at the East Tennessee Research and Education Center, Holston Unit, Knoxville in 2012 and 2013. The horizontal line signifies the two year average of each population. Triangles represent high or low outliers.

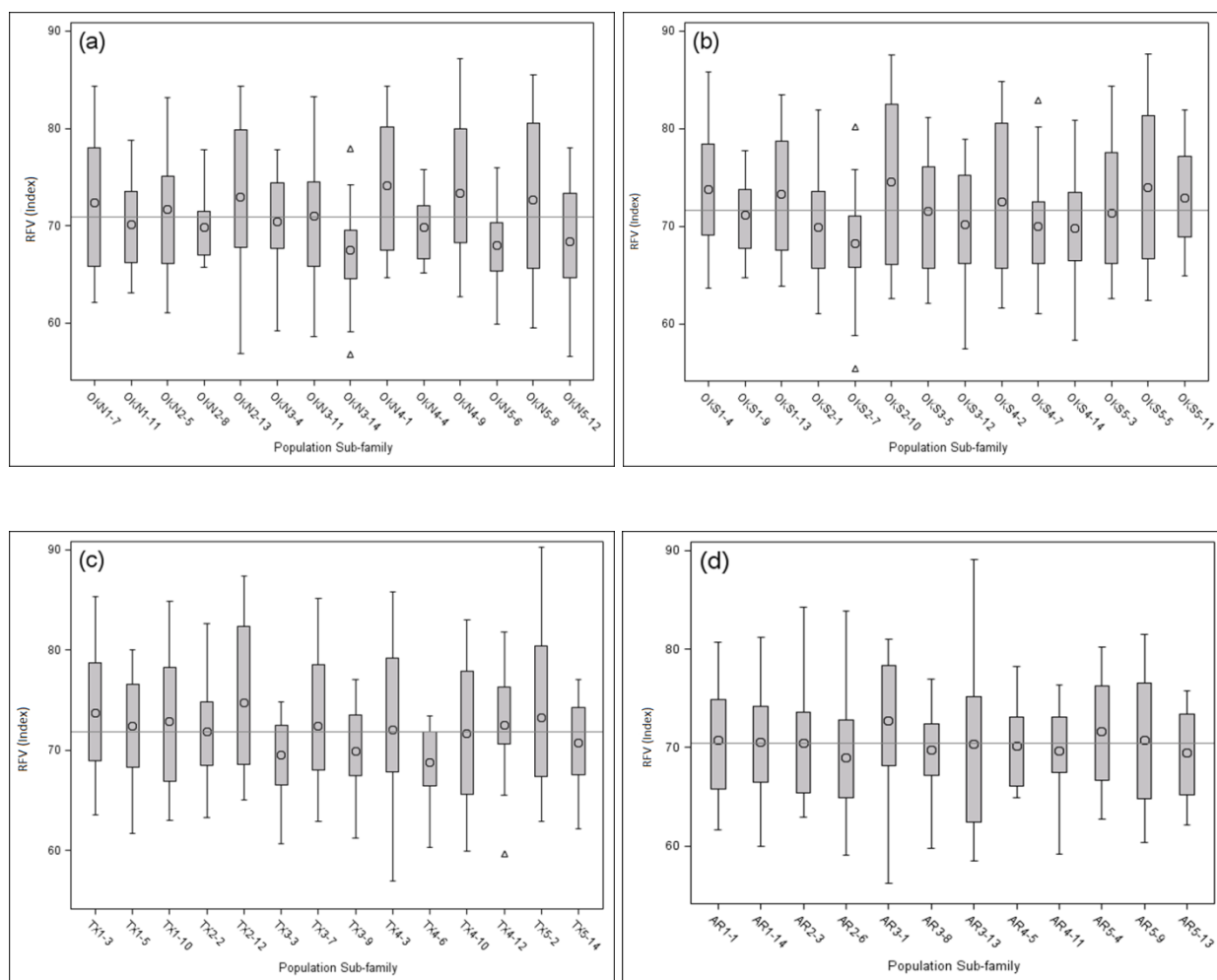


Fig. 19. Boxplots of late-season relative feed value (RFV) of  $F_1$  half-sib populations of NSL-2001-1 (OKN)(a), Cimarron (OKS)(b), PI607837 (TX)(c), and PI421999 (AR)(d) evaluated at the East Tennessee Research and Education Center, Holston Unit, Knoxville in 2012 and 2013. The horizontal line signifies the two year average of each population. Triangles represent high or low outliers.

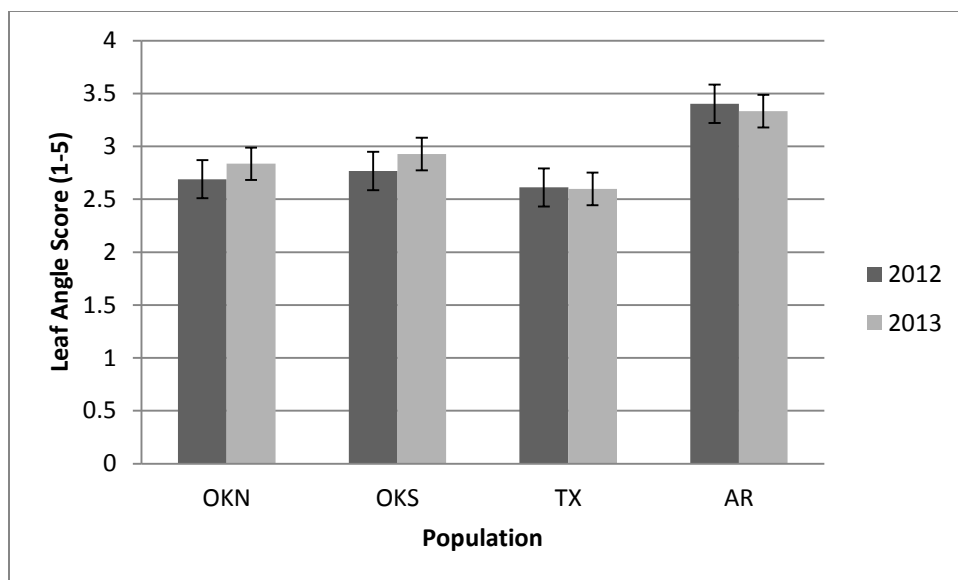


Fig. 20. Early-season leaf rating of  $F_1$  half-sib populations of NSL-2001-1 (OKN), Cimarron (OKS), PI607837 (TX), and PI421999 (AR) evaluated at the East Tennessee Research and Education Center, Holston Unit, Knoxville in 2012 and 2013. Leaf rating is angle of the leaf to stem with 1 = all leaves are  $\leq 45^\circ$  to the vertical stem, 3 = approximately half of the leaves are  $\approx 45^\circ$  and half are  $> 45^\circ$  at the distal end of the leaf, 5 = all leaves are arched so that from the leaf midpoint to the leaf tip of the leaves are  $> 90^\circ$ .

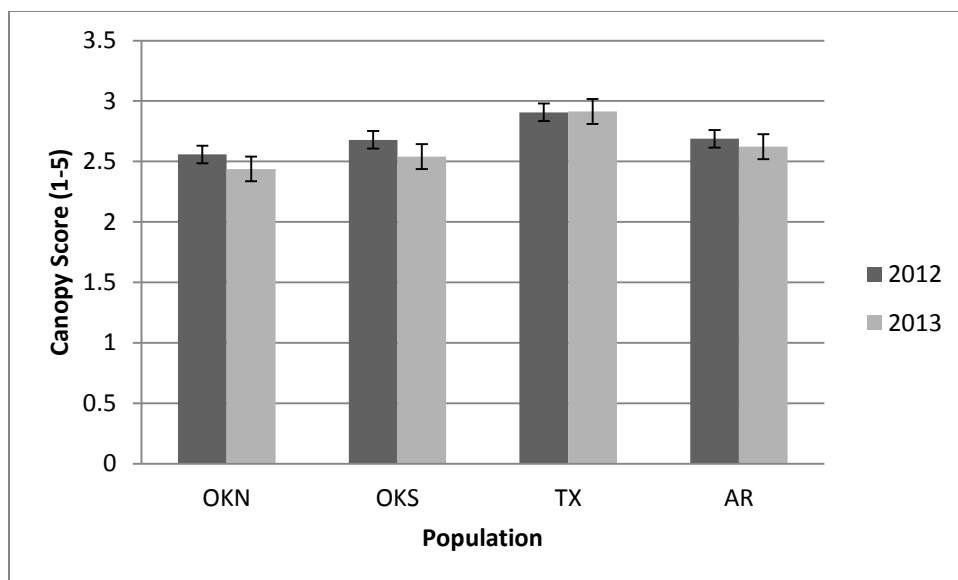


Fig. 21. Early-season canopy score of  $F_1$  half-sib populations of NSL-2001-1 (OKN), Cimarron (OKS), PI607837 (TX), and PI421999 (AR) evaluated at the East Tennessee Research and Education Center, Holston Unit, Knoxville in 2012 and 2013. Canopy type was rated on a scale of 1 to 5 with half increments where 1 = a very upright plant with greater than 95% of tillers vertical to the ground, 3 = the plant has many tillers that were at a 45° to the ground, 5 = tillers were growing completely spread out with tillers growing < 45° to the ground.

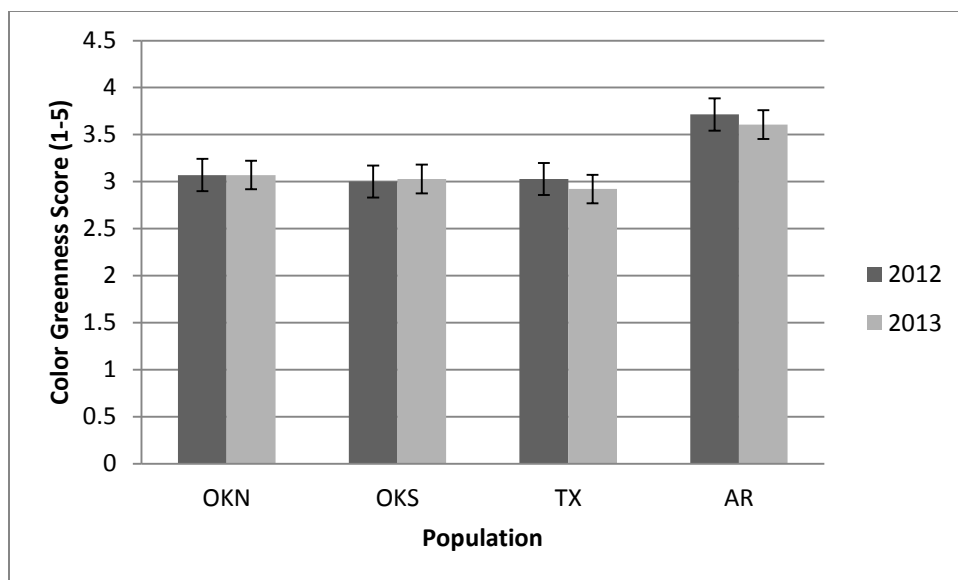


Fig. 22. Early-season color score of F<sub>1</sub> half-sib populations of NSL-2001-1 (OKN), Cimarron (OKS), PI607837 (TX), and PI421999 (AR) evaluated at the East Tennessee Research and Education Center, Holston Unit, Knoxville in 2012 and 2013. Color was rated on a scale of 1 to 5 with 1 = green, 5 = blue and there were half increments in between.

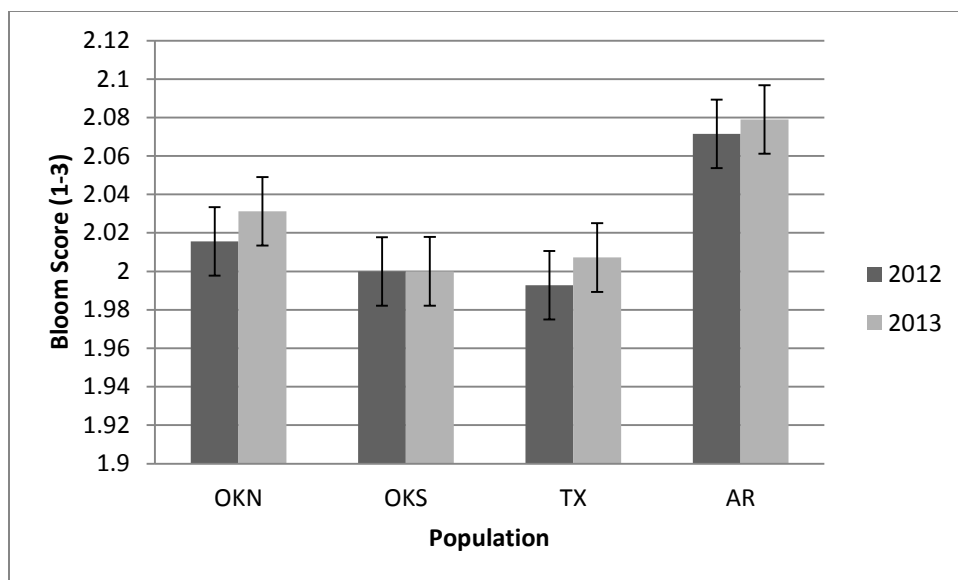


Fig. 23. Early-season bloom score of F<sub>1</sub> half-sib populations of NSL-2001-1 (OKN), Cimarron (OKS), PI607837 (TX), and PI421999 (AR) evaluated at the East Tennessee Research and Education Center, Holston Unit, Knoxville in 2012 and 2013. A leaf bloom score was rated on a scale of 1 to 3 with no half increments where 1= no wax, 2 = moderate wax present, and 3 = high amounts of wax.



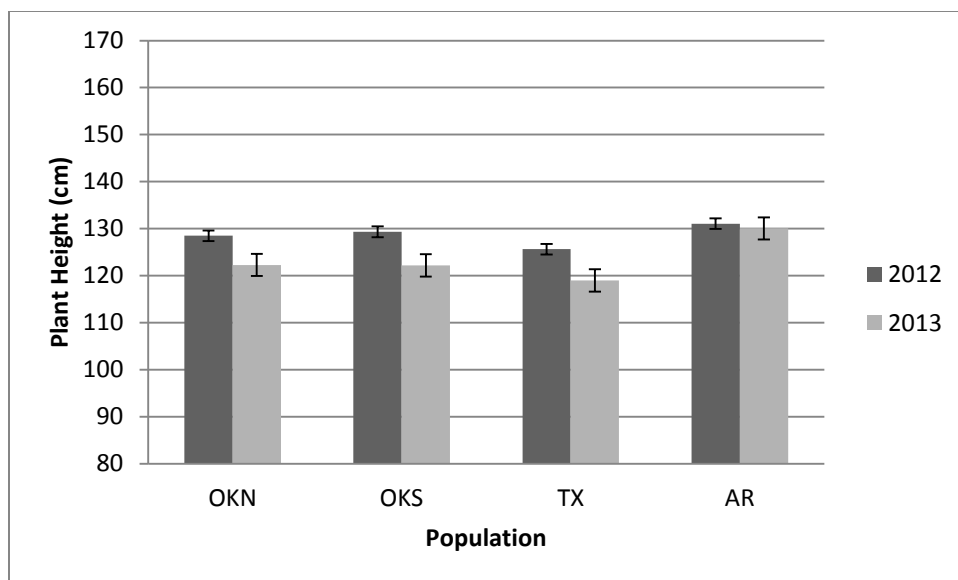


Fig. 24. Early-season heights of F<sub>1</sub> half-sib populations of NSL-2001-1 (OKN), Cimarron (OKS), PI607837 (TX), and PI421999 (AR) evaluated at the East Tennessee Research and Education Center, Holston Unit, Knoxville in 2012 and 2013.

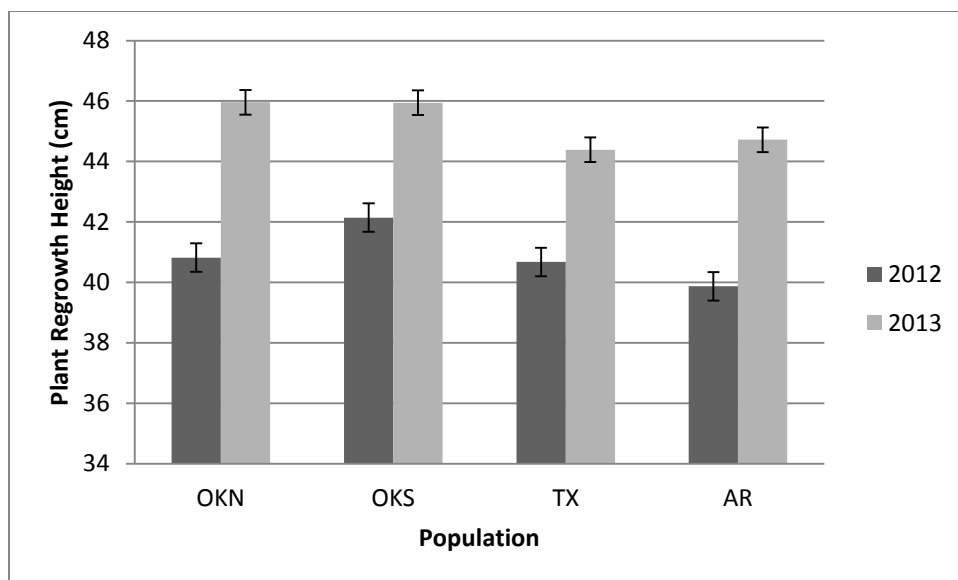


Fig. 25. Early-season regrowth height of  $F_1$  half-sib populations of NSL-2001-1 (OKN), Cimarron (OKS), PI607837 (TX), and PI421999 (AR) evaluated at the East Tennessee Research and Education Center, Holston Unit, Knoxville in 2012 and 2013. Regrowth height was measured 15 days after the early-season harvest.

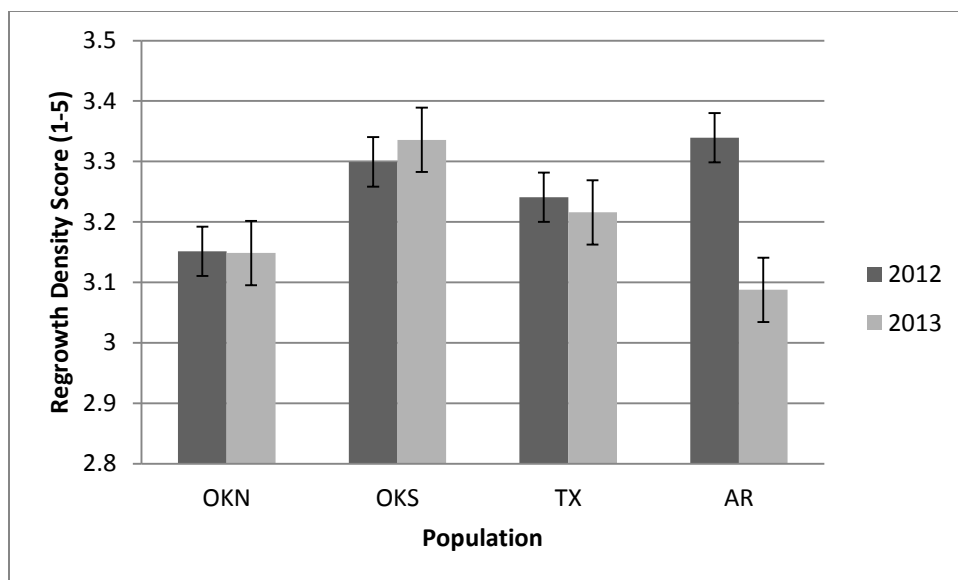


Fig. 26. Early-season regrowth density of  $F_1$  half-sib populations of NSL-2001-1 (OKN), Cimarron (OKS), PI607837 (TX), and PI421999 (AR) evaluated at the East Tennessee Research and Education Center, Holston Unit, Knoxville in 2012 and 2013. Regrowth density was rated 15 days after the early-season harvest. Plants were rated on a scale of 1 to 5 with 1 = among the most dense and full foliage growth in the field, 3 = a medium growth and density, and 5 = no regrowth had occurred.

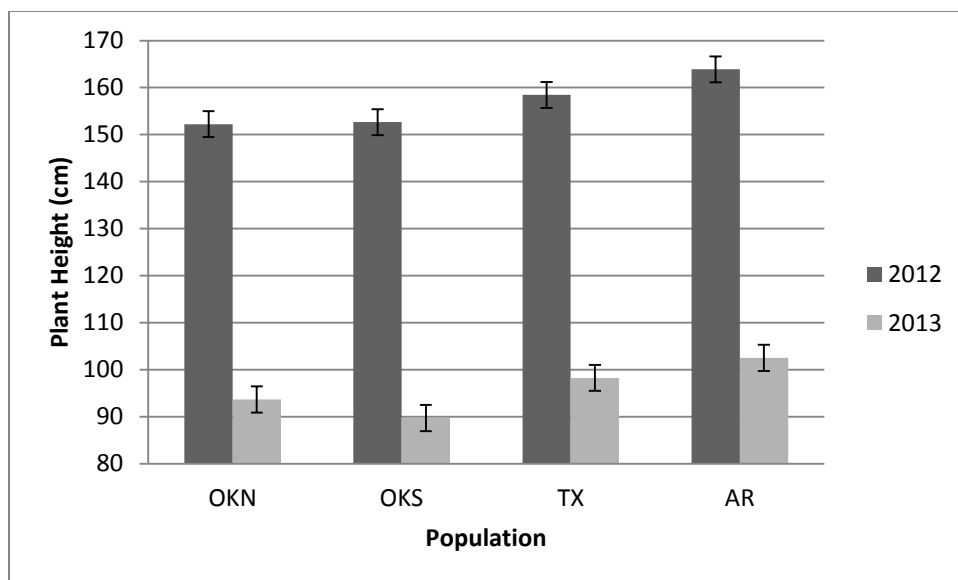


Fig. 27. Late-season heights of F<sub>1</sub> half-sib populations of NSL-2001-1 (OKN), Cimarron (OKS), PI607837 (TX), and PI421999 (AR) evaluated at the East Tennessee Research and Education Center, Holston Unit, Knoxville in 2012 and 2013.

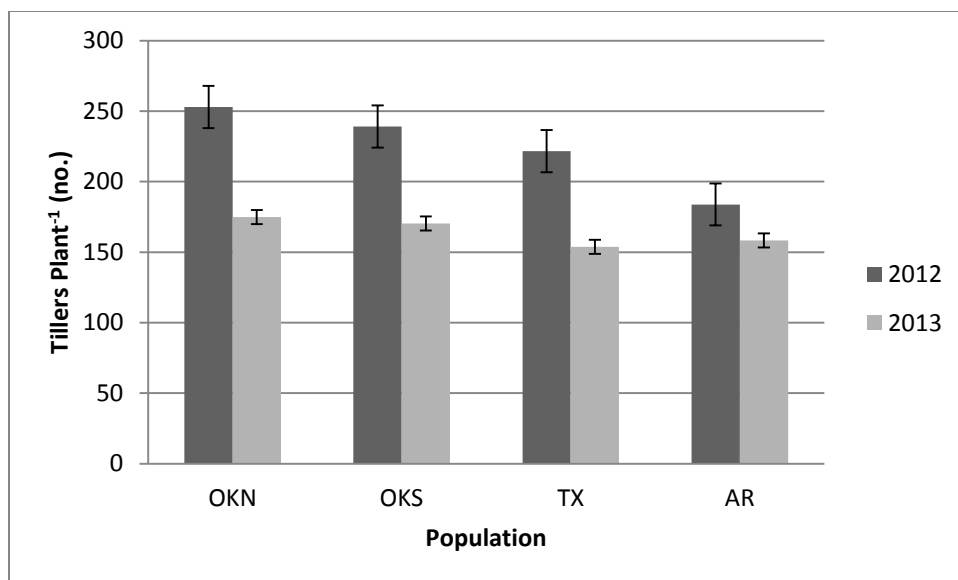


Fig. 28. Late-season tiller number of F<sub>1</sub> half-sib populations of NSL-2001-1 (OKN), Cimarron (OKS), PI607837 (TX), and PI421999 (AR) evaluated at the East Tennessee Research and Education Center, Holston Unit, Knoxville in 2012 and 2013.

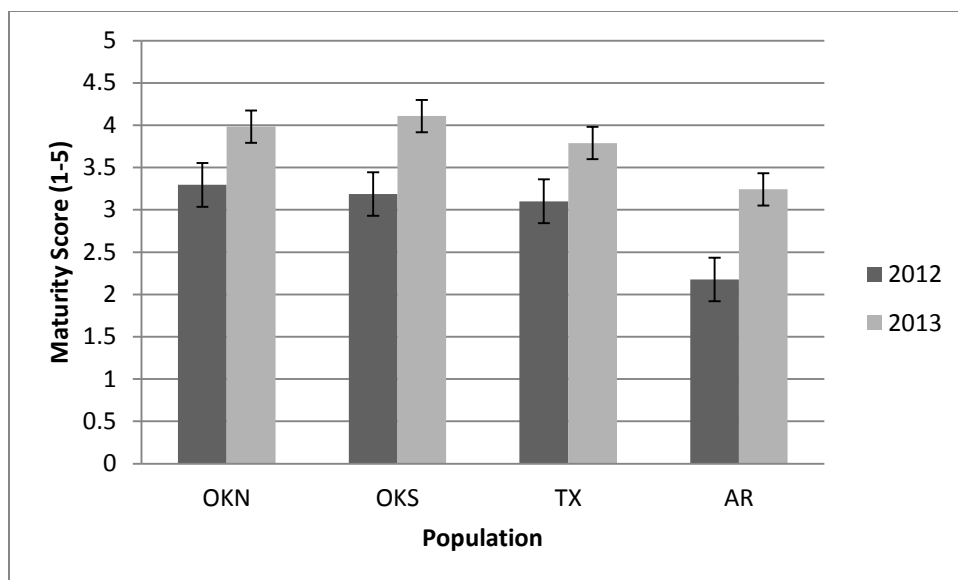


Fig. 29. Late-season maturity rating of  $F_1$  half-sib populations of NSL-2001-1 (OKN), Cimarron (OKS), PI607837 (TX), and PI421999 (AR) evaluated at the East Tennessee Research and Education Center, Holston Unit, Knoxville in 2012 and 2013. Panicle ratings were taken before the late-harvest to determine maturity. The maturity rating was on a scale of 1 to 6 with 1 = fully headed, 2 = mid-heading, 3 = early heading, 4 = late boot, 5 = boot stage, and 6 = early boot.

Table 7. Early-season harvest correlations of yield and morphological traits of F<sub>1</sub> half-sib populations of NSL-2001-1 (OKN), Cimarron (OKS), PI607837 (TX), and PI421999 (AR) evaluated at the East Tennessee Research and Education Center, Holston Unit, Knoxville in 2012.

Pearson Correlation Coefficients							
Number of Observations							
	Height	Yield	Leaf Angle	Canopy Type	Color Greenness	Leaf Bloom	Regrowth Height
Yield	0.67*** 515						
Leaf Angle	0.07 521	-0.2*** 516					
Canopy Type	0.13** 521	0.4*** 516	-0.18*** 522				
Color Greenness	0.07 517	0.04 516	0.21*** 518	-0.06 518			
Leaf Bloom	0.07 517	0.04 516	0.09* 518	-0.08 518	0.27*** 518		
Regrowth Height	0.18*** 519	0.26*** 516	0 520	0.2*** 520	-0.06 518	-0.02 518	
Regrowth Density	-0.3*** 519	-0.47*** 516	0.18*** 520	-0.27*** 520	0.05 518	0.05 518	-0.41*** 520
*, **, ***- P ≤ 0.05, 0.01, and 0.001 respectively. No asterisk- non-significant at P ≤ 0.05.							

Table 8. Early-season harvest correlations of morphological traits of F<sub>1</sub> half-sib populations of NSL-2001-1 (OKN), Cimarron (OKS), PI607837 (TX), and PI421999 (AR) evaluated at the East Tennessee Research and Education Center, Holston Unit, Knoxville in 2013.

Pearson Correlation Coefficients							
Number of Observations							
	Height	Yield	Leaf Angle	Canopy Type	Color Greenness	Leaf Bloom	Regrowth Height
Yield	0.62*** 512						
Leaf Angle	0.22*** 518	-0.15*** 512					
Canopy Type	0.04 518	0.45*** 512	-0.21*** 518				
Color Greenness	0.16*** 518	0.06 512	0.3*** 518	-0.08 518			
Leaf Bloom	0.06 518	0.06 512	0.04 518	-0.01 518	0.28*** 518		
Regrowth Height	0.33*** 518	0.27*** 512	0.04 518	0.13** 518	0.02 518	-0.1*	
Regrowth Density	-0.31*** 518	-0.48*** 512	0.14** 518	-0.35*** 518	0.00 518	-0.06 518	-0.35*** 519
*, **, ***- P ≤ 0.05, 0.01, and 0.001 respectively. No asterisk- non-significant at P ≤ 0.05.							



Table 9. Late-season harvest correlations of forage nutritional composition of F<sub>1</sub> half-sib populations of NSL-2001-1 (OKN), Cimarron (OKS), PI607837 (TX), and PI421999 (AR) evaluated at the East Tennessee Research and Education Center, Holston Unit, Knoxville in 2012.

Pearson Correlation Coefficients							
Number of Observations							
	Height	Yield	Protein	ADF	NDF	TDN	RFV
Yield	0.23*** 519						
Protein	0.04 500	-0.03 500					
ADF	-0.02 500	-0.05 500	-0.78*** 500				
NDF	0.01 500	-0.02 500	-0.72*** 500	0.82*** 500			
TDN	0.02 500	0.05 500	0.78*** 500	-1*** 500	-0.83*** 500		
RFV	0.00 500	0.03 500	0.78*** 500	-0.95*** 500	-0.96*** 500	0.95*** 500	
Maturity	-0.31*** 521	0.07 500	-0.03 500	-0.10* 500	0.08 500	0.09* 500	0.00 500
*, **, ***- P ≤ 0.05, 0.01, and 0.001 respectively. No asterisk- non-significant at P ≤ 0.05.							

Table 10. Late-season harvest correlations of forage nutritional composition of F<sub>1</sub> half-sib populations of NSL-2001-1 (OKN), Cimarron (OKS), PI607837 (TX), and PI421999 (AR) evaluated at the East Tennessee Research and Education Center, Holston Unit, Knoxville in 2013.

Pearson Correlation Coefficients							
	Number of Observations						
	Height	Yield	Protein	ADF	NDF	TDN	RFV
Yield	0.36*** 489						
Protein	0.01 491	0.04 485					
ADF	0.09 491	0.01 485	-0.81*** 491				
NDF	0.21*** 491	0.12** 485	-0.52*** 491	0.76*** 491			
TDN	-0.09 491	-0.01 485	0.81*** 491	-1*** 491	-0.76*** 491		
RFV	-0.18*** 491	-0.09 485	0.66*** 491	-0.90*** 491	-0.96*** 491	0.90*** 491	
Maturity	-0.58*** 520	-0.29*** 489	0.06 491	-0.18*** 491	-0.16*** 491	0.18*** 491	0.18*** 491
*, **, ***- P ≤ 0.05, 0.01, and 0.001 respectively. No asterisk- non-significant at P ≤ 0.05.							

## Appendix B

Chart of possible parental crosses that make up the F<sub>1</sub> populations that were evaluated at the East Tennessee Research and Education Center, Holston Unit, Knoxville in 2012 and 2013.

Population	Female Parent	Contributing Male Parents
1	Exp. NSL-2001-1 (OKN)	Exp. NSL-2001-1 (OKN) PI 607837 (TX) PI 421999 (AR) Cimarron (OKS)  PI 422016 (FL)
2	PI 607837 (TX)	Exp. NSL-2001-1 (OKN) PI 607837 (TX) PI 421999 (AR) Cimarron (OKS)  PI 422016 (FL)
3	PI 421999 (AR)	Exp. NSL-2001-1 (OKN) PI 607837 (TX) PI 421999 (AR) Cimarron (OKS)  PI 422016 (FL)
4	Cimarron (OKS)	Exp. NSL-2001-1 (OKN) PI 607837 (TX) PI 421999 (AR) Cimarron (OKS)  PI 422016 (FL)

Chart of traits that were evaluated on switchgrass and the rating scale used for each trait.

<b>Trait</b>	<b>Rating Scale</b>
Canopy Color Greenness	1 to 5, 1 = Green, 5 = Blue
Leaf Angle Rating	1 to 5, 1 = straight, stiff, 5 = bent, floppy
Canopy Type	1 to 5, 1 = vertical, 5 = open, parallel to ground
Leaf Bloom Score	1 to 3, 1 = absent, 2 = present, 3 = abundant
Regrowth Density	1 to 5, 1 = most dense, 3 = medium growth, 5 = no growth
Maturity Stage Rating	1 to 6, 1 = fully headed, 6 = early boot
Height	Measured in inches
Forage Quality Traits (Protein, ADF & NDF)	Measured as percentage of dry matter
Forage Yields (Dry Matter)	Measured by weight
Tiller Number	Counted

## **VITA**

Matthew Eric Bobbitt was born October 12<sup>th</sup>, 1989, in Jackson, TN and grew up in Dyer, TN. He graduated from Peabody High School in 2008. He earned a Bachelor of Science degree in Agricultural Business- Farm and Ranch Management from the University of Tennessee- Martin in December 2011. After working for a local landscape nursery and completing an internship with the University of Tennessee- Extension, he entered the Plant Science program at the University of Tennessee, where he worked as a graduate research assistant for Dr. Fred Allen in the State Agronomic Crop Variety Testing Program. He is currently a candidate for a Master of Science degree in the department of Plant Sciences with a concentration in Plant Breeding. He will graduate in May 2014.