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Gas Exchange And Leaf Anatomy Of Andropogon Gerardii Ecotypes Over A Climatic Gradient Of The Great Plains

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GAS EXCHANGE AND LEAF ANATOMY OF *ANDROPOGON GERARDII* ECOTYPES OVER A CLIMATIC GRADIENT OF THE GREAT PLAINS

being

A Thesis Presented to the Graduate Faculty
of Fort Hays State University in
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the Degree of Master of Science

by

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the Master of Science degree

by

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ABSTRACT

The phenotype of two *Andropogon gerardii* subspecies, big bluestem and sand bluestem, varies broadly throughout the Great Plains of North America, giving rise to ecotypes within the species. This study sought to discriminate between genetic and environmental variation of big bluestem and sand bluestem by examining gas exchange and leaf anatomy in common gardens across a climatic gradient of the Great Plains. Thirteen populations of big bluestem and one population of sand bluestem, constituting five ecotypes, were planted in community plots and a single plant plots in a common garden at each of four sites ranging from western Kansas to southern Illinois. Photosynthesis, stomatal conductance, intercellular CO_2, transpiration, and intrinsic water use efficiency were measured three times in the 2010 growing season. In addition, leaf thickness, midrib thickness, bulliform cells, interveinal distance, and vein size were assessed by light microscopy.

Abundant phenotypic variation exists among ecotypes within community plots. At all planting sites, big bluestem ecotypes from xeric environments had higher photosynthesis, stomatal conductance, and transpiration compared to mesic ecotypes. Single plant plots also had abundant phenotypic variation; ecotypes native to xeric environments also had higher photosynthesis, stomatal conductance, and transpiration, but differences were more distinct. In addition, sand bluestem, which was only planted in single plant plots, had similar photosynthesis, stomatal conductance, and transpiration to the big bluestem ecotype native to the most xeric environment. Sand bluestem also had higher water use efficiency and lower intercellular CO_2 than any big bluestem ecotype. Leaf anatomy assessments indicated xeric ecotypes of *A. gerardii* had thicker leaves and fewer bulliform cells. Environmental variation was as important as genetic variation for gas exchange and leaf anatomy in both community and single plant plots. Compared to xeric sites, mesic sites had higher photosynthesis, stomatal conductance, and water use efficiency and lower intercellular CO_2 and transpiration in community and single plant plots. Leaves from mesic sites also had thicker midribs, larger veins, and a greater proportion of bulliform cells.

Ecotypes of *A. gerardii* across the Great Plains are adapted to water availability. Drought-adapted ecotypes of *A. gerardii* were shorter in stature and had smaller, thicker, narrower leaves, which reduced the evaporative surface area of these plants. Evidently, *A. gerardii* controls water loss by reducing evaporative surface area more than it does by increasing the proportion of bulliform cells. This allows drought-adapted ecotypes to have higher photosynthetic rates, stomatal conductances, and transpiration rates in both mesic and xeric environments compared to ecotypes native to mesic environments.

This study brings to light potential responses of big bluestem ecotypes to climate change. This study also indicates the phenotypic variation among big bluestem could prove useful in the restoration of native prairies.

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PREFACE

Chapter One is written in the style of Ecology. Chapter Two is written in the style of New Phytologist. Chapter Three is written in the style of Annals of Botany. These are peer-reviewed journals to which a manuscript of each chapter will be submitted.

CHAPTER 1: GAS EXCHANGE AMONG FOUR ECOTYPES OF *ANDROPOGON GERARDII* ALONG A CLIMATIC GRADIENT

INTRODUCTION

Compared to C_3 plants, C_4 plants are more efficient users of light, water, and nitrogen under high temperatures (Williams, 1974; Ripley et al., 2010). C_4 plants are adapted to hot environments (Boutton et al., 1980) where the majority of yearly rainfall comes during the growing season (Nippert et al., 2006). For example, the Great Plains of North America is dominated by C_4 plants (Epstein et al., 1997).

Under water stress, photosynthetic rates (A) of C_3 species decrease primarily due to stomatal limitations (Flexas and Medrano, 2002; Frole, 2008; Ghannoum, 2009). In C_3 plants, stomates close, decreasing stomatal conductance (g_s) and causing the internal (intercellular) CO_2 concentration (C_i) to decrease as A draws down CO_2. Stomata of C_4 plants are also sensitive to drought (Morrison and Gifford, 1983), but C_4 plants do not decrease A until g_s and C_i become very low. This is possible because the CO_2 concentrating mechanism of C_4 plants saturates Rubisco, even when C_i is low (Dai et al., 1993; Ghannoum et al., 2003). Under water stress, C_4 plants generally experience non-stomatal (e.g., metabolic) limitations rather than stomatal limitations (Ghannoum et al., 2003; Ripley et al., 2007; Frole, 2008). Metabolic limitations are common in C_4 plants; before the plant is under sufficient water stress to lower g_s and C_i enough to affect A, metabolic pathways have been inhibited (Ghannoum, 2003).

Non-stomatal limitations on C_4 photosynthesis can result from decreased enzymatic activities of Rubisco (Crafts-Bradner and Salvucci, 2002), PEP carboxylase (Soares-Cordeiro et al., 2009), and pyruvate P_i dikinase (Du et al., 1996). Other non-stomatal limitations of A include photodamage to the light-harvesting system (Melis, 1999) and a decrease in nitrogen content in stems and newly expanded leaves (Heckathorn and DeLucia, 1994), which limits the production of proteins and chlorophyll necessary for photosynthesis. Stomatal limitations of A can be discriminated from non-stomatal limitations by gas exchange measures. Stomatal limitation in environmentally stressed plants is indicated by decreased C_i compared to non-stressed plants, whereas C_i will remain constant in stressed and non-stressed plants experiencing non-stomatal limitations (Farquhar and Sharkey, 1982).

Transpiration (E) has a linear relationship with g_s when there is sufficient air flowing over the leaf (Jarvis and McNaughton, 1986). Guard cells control g_s and are governed by many environmental factors, including light, CO_2, hormones (Schroeder et al., 2001), temperature, humidity (Lange et al., 1971; Freeden and Sage, 1999), wind (Campbell-Clause, 1998), and soil moisture (Bano et al., 1993). Therefore, measures of g_s, A, and E can be useful for assessing climatic effects on plants, especially drought.

Andropogon gerardii Vitman (big bluestem) is a dominant C_4 grass in tallgrass and mixed grass prairies (Gustafson et al., 2004) and has high phenotypic variation across the precipitation gradient of the Great Plains, giving rise to many ecotypes within the species (McMillan, 1959). To assess genetic differences in *A. gerardii* versus environmental effects, a common garden experiment was arranged along the west-east precipitation gradient of the Great Plains. Previous common garden experiments have demonstrated many functional and developmental differences between ecotypes of plants (e.g., Clausen et al., 1939; Etterson, 2004; Oyarzabal, 2008). Etterson (2004) planted three ecotypes of *Chamaecrista fasciculata* (Michx.) Greene in common gardens across the climatic gradient of the Great Plains. Local ecotypes produced more seed than non-local ecotypes, supporting the idea of local adaptation of ecotypes. Similarly, restoration projects within the Great Plains using non-local *A. gerardii* seeds observed shorter plants that were more susceptible to insect herbivory, produced fewer culms and inflorescences, and were phenologically behind the local *A. gerardii* ecotypes (Gustafson et al., 2001; Gustafson et al, 2005). In the present study, common gardens were established in four locations (Table 1.1) and at each location, three ecotypes of *A. gerardii* were planted. I sought to compare responses of *A. gerardii* ecotypes to decreasing moisture availability across sites.

Climatic gradients generally involve abiotic stress ranging from more intense to less intense (Normand et al., 2009). In the case of the Great Plains, a strong precipitation gradient exists from drier conditions in the west to wetter conditions in the east. In the present study, the climatic gradient compared responses of *A. gerardii* to differential precipitation across common garden sites. Based on the preceding, I expected local adaptation of gas exchange in *A. gerardii* with respect to precipitation, where ecotypes have higher gas exchange rates in their native environment.

Response of *A. gerardii* ecotypes to drought might be highly varied based on adaptation to differing amounts of annual precipitation across the Great Plains (Lei et al., 2006). I hypothesized (1) local ecotypes would have higher A, E, and g_s in their local environment, and (2) the Central KS ecotype, collected from a xeric environment in the west, would maintain higher WUE, g_s and A compared to ecotypes from more mesic environments in the east under water stress. (3) Sites with greater moisture availability were expected to have higher A. These hypotheses were evaluated in ecotypes of *A. gerardii* based on measures of A, g_s, E, and C_i.

MATERIALS AND METHODS

Seed Collection Sites

Andropogon gerardii seed was hand collected in autumn 2008 from three climatically distinct regions along a precipitation gradient and on at least three dates from KS to IL. Plants from each region were considered an ecotype and each ecotype contained four populations. Populations originated from native prairies within an 80 km radius of the reciprocal garden planting site. *Andropogon gerardii* seed was analyzed for seed filling, germination, and live seed determination by the KS Seed Crop Improvement Center, Manhattan, KS. Through this analysis, percent live seed was determined and used to calculate the amount of seed of each ecotype to be planted. The four populations within each ecotype were mixed in equal quantities for the final seed mix used in plot establishment.

Reciprocal Common Garden Design and Plot Establishment

Three big bluestem ecotypes were used for this experiment. The Central KS ecotype was collected near Hays, KS, the Eastern KS ecotype was collected near Manhattan, KS and the Illinois ecotype was collected near Carbondale, IL. Common gardens were established at Colby, KS (most xeric), Hays, KS, Manhattan, KS, and Carbondale, IL (most mesic), with mean annual precipitation ranging from 400 to 1200 mm yr^{-1} (Table 1.1). Although we did not have a Colby, KS ecotype, the Colby location was included to test the tolerance of all three ecotypes to a more xeric environment, similar to what *A. gerardii* might experience under climate change if rainfall decreases.

The design of the experiment included reciprocally transplanted randomized plots with four 4 × 4 m plots of each ecotype per site. Seeds were planted in June 2009, using 70:30 ratio of live C_4 grass to C_3 grass and forb seed. *Andropogon gerardii* was planted at a density of 270 live seeds m^{-2}. Seeds were mixed with damp sand to aid in homogenous dispersal. Sand and seed were hand-broadcast into experimental plots and raked into the soil. Total seed density for each plot was 600 seeds m^{-2}, as recommended for prairie restoration (Packard and Mutel, 1997). To simulate a natural prairie environment, the co-dominant C_4 grass, *Sorghastrum nutans* (Indiangrass) was collected in the same native prairies as big bluestem and was seeded at a density of 90 live seeds m^{-2} and eight commercially purchased sub-dominant species that occur in all regions were each seeded at 30 live seeds m^{-2}. The sub-dominant species include the C_3 grass, *Elymus canadensis,* and seven C_3 forb species, *Ruellia humilis*, *Penstemon digitalis*, *Monarda fistulosa*, *Asclepias tuberosa*, *Solidago rigida*, *Dalea purpurea*, and *Chamaecrista fasciculata*. Big bluestem and Indiangrass were the only hand collected seeds, all others were commercially purchased. The 2-m border areas between plots were planted with commercially purchased *Bouteloua curtipendula* and *Schizachyrium scoparium*.

Data collection

Four EC-20 soil moisture probes, 20 cm in length (Decagon Devices, Inc., Pullman, WA, USA), monitored volumetric water content of soil (%) in representative plots arranged diagonally across each site, with one probe per block. Probes were positioned vertically at the beginning of the growing season (early May), and allowed to settle before soil water content was measured twice weekly. Precipitation data were gathered from local research stations.

Gas Exchange Measures

Gas exchange measurements of *A. gerardii* were taken with three LI-6400 (Li-Cor Biosciences Inc., Lincoln, NE, USA) instruments, which were tested against a gas standard and accurate within 2.8%. The youngest, fully-expanded one or two leaves were placed in the LI-6400 leaf chamber. Measurements were taken with CO_2 levels at 385 ppm, humidity and temperature at ambient levels, and photosynthetically active radiation (PAR) at 1500 µmol photons m^{-2} s^{-1}. Gas flow through the chamber was 400 µmol s^{-1}.

Gas exchange measurements were taken early in the growing season (26% to 43% GDD), mid-season (48% - 67% GDD), and late season (70% - 89% GDD). Measurements were made on sunny days between 10:00 h and 15:00 h to minimize adjustment time of leaves to leaf chamber conditions. Measurements were made when photosynthesis and conductance had stabilized, usually requiring 1-2 minutes. Gas exchange measurements included photosynthesis (A), stomatal conductance to water vapor (g_s), internal (intercellular) CO_2 concentration (C_i), transpiration (E), and intrinsic water use efficiency (WUE), calculated as A/g_s.

Within each plot at each site, duplicate individuals were measured at random during the first measuring period. Plants were chosen randomly by facing away from plots and throwing a flag overhead into the plot. The nearest *A. gerardii* plant to the flag was measured. Flags remained in the plots to mark plants for subsequent measures and all plants were averaged for a plot mean.

Statistical Analysis

All data were analyzed with JMP 9.0.2 (SAS Institute Inc., Cary, NC, USA). Replicate measures were determined as the mean of six duplicate measurements per plot. A MANOVA was used; factors were sites, ecotypes, and measurement periods. All interactions were analyzed. This was followed by univariate tests and Tukey's honest significant difference (HSD) tests to reveal significant differences between means (Appendices 1 – 5). All analyses were run at $\alpha=0.05$ to determine statistically significant differences.

RESULTS

Photosynthesis (*A*)

A ranged from 6.5 to 21.9 μmol CO_2 m^{-2} s^{-1} across sites, months, and ecotypes (Figs. 1.1-1.4). *A* was higher early in the growing season, compared to the middle and late measurement periods (F=7.77, df=2, 108, p<0.001), which were not different from each other. *A* was not different between Carbondale, IL and Hays, KS, but both maintained higher *A* than Manhattan, KS and Colby, KS sites (F=51.45, df=3, 108, p<0.001), which were not different from each other. No differences were detected among ecotypes (F=38.12, df=2, 108, p=0.205), but there was a month by site interaction (F=1.61, df=6, 108, p<0.001) (Table 1.2, Appendix 1.1).

Stomatal Conductance (g_s)

g_s ranged from 0.063 to 0.232 mol m^{-2} s^{-1} across sites, months, and ecotypes (Figs. 1.1-1.4). During the early season, g_s was higher than in the late growing season, which was higher than mid-growing season (F=8.97, df=2, 108, p<0.001). Across sites, g_s at Hays, KS and Carbondale, IL was not different, but both were higher than Manhattan, KS and Colby, KS (F=66.40, df=3, 108, ANOVA, p<0.001), which were not different from each other. An ecotype effect was detected for g_s (F=27.38, df=2, 108, p=0.015); the Central KS ecotype had higher g_s than the Eastern KS and Illinois ecotypes, which were not different from each other. There was a month by site interaction (F=4.31, df=6, 108, p<0.001) (Table 1.2, Appendix 1.2).

Intercellular CO_2 (C_i)

C_i ranged from 99 to 303 ppm across sites, months, and ecotypes (Figs. 1.1-1.4). C_i was higher late season than during early season, which was greater than mid-season (F=9.22, df=2, 108, $p<0.001$). Colby, KS had the highest C_i, which was higher than Hays, KS and Manhattan, KS, which were not different from each other, but were both higher than the Carbondale, IL (F=45.66, df=3, 108, $p<0.001$). No ecotype effect was detected (F=32.16, df=2, 108, p=0.692), but there was a month by site interaction (F=0.37, df=6, 108, $p<0.001$) (Table 1.2, Appendix 1.3).

Transpiration (E)

E ranged from 2.47 to 7.30 mmol m^{-2} s^{-1} across sites, months, and ecotypes (Figs. 1.1-1.4). E was greater early in the growing season compared to mid-season and late season (F=14.93, df=2, 108, $p<0.001$), which were not different from each other. Hays, KS had higher E compared to all other sites (F=14.60, df=3, 108, $p<0.001$). The Central KS ecotype was not different from the Eastern KS ecotype, but was higher than the Illinois ecotype (F=31.37, df=2, 108, p=0.039). The Eastern KS and Illinois ecotypes were not different from each other. There was a month by site interaction (F=3.34, 108, df=6, 108, $p<0.001$) (Table 1.2, Appendix 1.4).

Intrinsic Water Use Efficiency (WUE)

The intrinsic water use efficiency was calculated as A/g_s. WUE ranged from 34.2 to 162.2 μmol CO_2 mol^{-1} H_2O across sites, months, and ecotypes (Figs. 1.1-1.4). WUE was higher mid-growing season compared to early season, which was higher than late season WUE (F=13.20, df=2, 108, p<0.001). At Carbondale, IL, the WUE was highest, followed by Manhattan, KS and Hays, KS, which were not different from each other, followed by Colby, KS with the lowest WUE (F=52.13, df=3, 108, p<0.001). No ecotype effects were detected for WUE (F=29.43, df=2, 108, p=0.674), but there was a month by site interaction (F=0.40, df=6, 108, p<0.001) (Table 1.2, Appendix 1.5).

DISCUSSION

Many studies of *A. gerardii*, documenting differences among population, have examined ploidy levels (Norrmann et al., 1997; Keeler and Davis, 1999; Keeler, 2004), while other studies have quantified differences in genetic diversity (Gustafson et al., 1999) and Gustafson et al. (2004) observed local ecotypes were larger than non-local ecotypes. Previously, no experiments have studied the effects of decreasing moisture availability on ecotypes of *A. gerardii* using multiple common gardens, particularly in community plots. The response of *A. gerardii* (a dominant C_4 species in the Great Plains) to reduced rainfall could indicate potential responses of other C_4 prairie species to predicted climate change. Adaptations to xeric climates can explain much of the variation between ecotypes of *A. gerardii*.

Drought Adaptation

Previous studies on ecotypes have documented that local ecotypes produced more seeds (Etterson, 2004), were taller and more resistant to insect herbivory, produced more culms, and were phenologically advanced compared to nonlocal populations (Gustafson et al., 2001; Gustafson et al., 2005). Similarly, drought adapted ecotypes of tree species have higher photosynthetic rates (A) and stomatal conductance (g_s) and experience fewer non-stomatal limitation of A than their mesic counterparts (Abrams et al., 1992; Kubiske and Abrams, 1992). Knight et al. (2006) observed *Boechera holboellii* from dry sites had higher instantaneous water use efficiencies than plants from wetter sites because of reduced rates of transpiration. By contrast, Rice et al. (1992) found few differences in gas exchange among xeric and mesic populations of cheatgrass (*Bromus tectorum* L.), but saw obvious differences in phenology and biomass allocation of these populations. In the present study, I observed large phenotypic differences among ecotypes of *A. gerardii*. Among ecotypes, there were no differences in A, internal (intercellular) CO_2 (C_i), or water use efficiency (WUE), but there were significant differences in g_s and transpiration (E; Table 1.2).

Drought tolerance in *A. gerardii* ecotypes is indicated by an ability to maintain high g_s in dry conditions. g_s was higher for the Central KS ecotype than the Eastern KS and Illinois ecotypes. Being native to a more xeric environment, the Central KS ecotype likely has adaptations that help it maintain higher g_s and E during drought compared to the other ecotypes. Other adaptations could include smaller, thicker leaves (Abrams et al., 1990), increased pigmentation (El-Tayeb, 2006), and the ability to generate more negative water potentials (Kolb and Sperry, 1999). Generation of lower water potentials could allow higher g_s under drought. Consequently, E would be higher in the Central KS plants and data indicate E was higher for the Central KS ecotype over the Illinois ecotype. Higher E is generally viewed as a liability in xeric climates. However, increased E under droughted circumstances might lead to more evaporation and increased cooling of leaves, which could reduce damage to photosynthetic proteins and pigments during hot summer months.

The high g_s of the Central KS ecotype could be advantageous if it allows A to remain high under drought, especially during times when stomates of the Eastern KS and

Illinois ecotypes would be closed enough to reduce C_i and *A*. An ability to maintain high g_s under drought appears to be an adaptation in the most drought tolerant ecotype of *A. gerardii*. This was somewhat unexpected in *A. gerardii*, as high affinity of C_4 plants for inorganic carbon means g_s and C_i can remain low and are not typically sensitive to drought (Ghannoum et al., 2003). Stomatal closure in the Eastern KS and Illinois ecotypes at dry sites indicates an increased sensitivity to drought in mesic ecotypes compared to xeric ecotypes.

Hypotheses regarding drought tolerance were investigated further by planting all ecotypes at a dry site in Colby, KS, which is beyond the range where *A. gerardii* commonly occurs. The drought tolerance of the Central KS ecotype was evident at Colby, where soil moisture was low for the majority of the season. *A* was not different among ecotypes during the early season at Colby, but differences began to emerge during the middle and late growing season. During mid-season, mean Central KS *A* was 74% and 49% higher than means of the Eastern KS and Illinois ecotypes, respectively. Late in the growing season, Central KS *A* was 165% and 138% higher than the Eastern KS and Illinois ecotypes, respectively. Although these were not statistically significant differences, a larger sample size would likely result in significant differences in *A* as drought increases. The Central KS ecotype was typified by smaller plants with shorter leaves compared to the other ecotypes (Johnson and Tetreault, in prep). Instead of growing large shoots and leaves, Central KS plants might use more energy creating pigmentation and moving nutrients to roots for storage in anticipation of a longer period of dormancy, which are other potential drought adaptations. Similarly, the Central KS ecotype intrinsic WUE was not different compared to the other ecotypes, but as soil moisture decreased, its mean WUE was 48% and 97% higher than the mean WUE of the Illinois ecotype during mid-season and late season, respectively. This was not significant but greater replication would likely indicate a significant difference. g_s was significantly higher for the Central KS ecotype during mid and late season measurements. Mid-season and late season measurements at Colby were taken during periods of very dry soil (volumetric water content <5%); the higher E and g_s of the Central KS ecotype support the hypothesis it is more drought adapted compared to the more mesic ecotypes of *A. gerardii.* Drought adaptations of the Central KS ecotype do not reduce A in a mesic environment as evident by measures in Manhattan and Carbondale, where A was not different among ecotypes.

A is a good indication of plant performance in an environment. In the present study, A decreased from early season to late season across Kansas sites. *Andropogon gerardii* begins to reallocate leaf nitrogen (N) to roots for storage as the season progresses (Hayes, 1985). Reallocation of leaf N, coupled with metabolic limitations that decrease N production in leaves (Heckathorn and DeLucia, 1994), likely causes a reduction in the quantity of photosynthetic proteins and chlorophyll, leading to a reduction in A. In addition, high mid-season and late season temperatures can reduce A by limiting Rubisco (Crafts-Brandner and Salvucci, 2000; Cui et al., 2006), PEP carboxylase (Soares-Cordeiro et al., 2009), and pyruvate P_i dikinase activities (Du et al., 1996). Exact mechanisms that decrease A in *A. gerardii* during the growing season and how they might differ between ecotypes, remain an interesting area for future research.

The mesic ecotypes of Illinois and Eastern KS were poorly adapted to drought as seen by lower g_s in Colby, KS. Illinois g_s was not different from the other ecotypes in the mesic environment of Carbondale, IL, where soil moistures were consistently high (volumetric water content >19%). Larger leaves and taller plants of the Illinois ecotype create a disadvantage in dry climates based on total evaporative surface area compared to the smaller surface area of the Central KS ecotype (Abrams et al. 1990). However, the Illinois ecotype appears to recover quickly when large rainfall events happen. At mid-season when the soil was dry at Hays, KS, g_s of the Central KS and Eastern KS ecotypes were 11% and 16% higher than the Illinois ecotype, respectively. After a late season rainfall event, the Illinois ecotype was 17% and 14% higher than the Central KS and Eastern KS ecotypes, respectively. The increase in g_s was matched by a similar increase in E. The Illinois ecotype seems to be well adapted to wet soils and quickly begins to shut down when soil moisture is low.

Few differences in gas exchange indicate local adaptation of *A. gerardii* ecotypes as indicated by the lack of site × ecotype interaction; rather, most indicate the presence or absence of drought adaptation among each ecotype. The Central KS ecotype, native to a semi-arid environment, is more adapted to drought compared to the other ecotypes as indicated by higher E and g_s. The Eastern KS and Illinois ecotypes, from areas of greater rainfall, are less adapted to drought than the Central KS ecotype.

Site Differences

Site differences are particularly meaningful for comparisons of *A. gerardii* ecotypes. Differences were detected in all gas exchange measurements across sites. There was little variation in mean temperatures along the east-west climatic gradient, differing by 3°C across sites. Soil moistures varied greatly across sites, averaging from 20% to over 30% at Carbondale, IL and in stark contrast to the dry soil at Colby, KS, which averaged 1% to nearly 30% for brief periods after rainfall events. Soil moisture appears to be the driving force behind the differences among sites along the climatic gradient.

Throughout the growing season, Manhattan, KS had unexpectedly low g_s, *E*, and *A*. The soil at the Manhattan site contained fewer nutrients and had a smaller microbial biomass than did the soil at other sites (Mendola et al., in prep.). Despite the high moisture availability, plants were limited by their ability to take up nutrients. In addition, plants at Manhattan were severely impacted by leaf rust (*Puccinia andropogonis*). Leaf rust is a fungal pathogen that reduces leaf chlorophyll content, g_s, *E*, and *A* of many crops (Zhao et al., 2011). At Colby, KS, *A* and g_s were low, which correlated with low soil moisture throughout the season.

Many of the gas exchange parameters followed predictable patterns based on available soil moisture. *A* and g_s at Carbondale, IL were high, as expected considering the high soil moisture at the site. Interestingly, *A* and g_s at Hays, KS were not different from Carbondale early in the growing season, despite the lower soil moisture at Hays. This is likely because of the greater soil moisture availability in Hays early in the growing season. Later in the growing season, differences in *A* and g_s emerged between sites when there were larger differences in soil moisture.

At Carbondale, IL, C_i was significantly lower than at the other sites, particularly during mid-season. Despite the consistently high soil moisture at Carbondale, there was a large decrease in C_i and *A* from early to mid-growing season. The decrease in C_i and a 63% decrease in g_s indicate there were stomatal limitations to *A*, which is unusual for C_4 plants. Soil moisture was around 20% during mid-season, which was high compared to the other sites. The planting sites in KS maintained higher C_i compared to Carbondale throughout the growing season, indicating limitations in *A* were non-stomatal (Ghannoum et al., 2003).

Conclusion

The Central KS ecotype is better adapted to drought through its ability to maintain higher g_s and *E* under drought compared to its mesic counterparts from Eastern KS and Illinois. Ecotypes differed greatly in phenotype, but little evidence of local adaptation was seen in gas exchange. The advantage of the Central KS ecotype in its local environment was not nearly as substantial as it was at the more xeric Colby, KS site. Thus, differences among ecotypes likely correspond to their respective abilities to endure drought. Although climate change is predicted to decrease summer and winter precipitation for the Great Plains region (Weltzin et al., 2003), rising CO_2 levels might alleviate the effects of drought (Markelz et al., 2011; Morgan et al., 2011). The Central KS ecotype is evidence *A. gerardii* can survive and adapt to conditions that are more xeric and will continue to be an important and dominant plant throughout much of this region.

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Table 1.1. The location and environmental conditions of the reciprocal garden planting sites during 2010.

Environmental Conditions	Reciprocal Garden Planting Site			
	Colby, KS	Hays, KS	Manhattan, KS	Carbondale, IL
	KSU Ag. Res Center	KSU Ag. Res Center	USDA Plant Material Center	SIU Agronomy Center
	(Thomas Co.)	(Ellis Co.)	(Riley Co.)	(Jackson Co.)
Location	39° 23’N 101° 04’W	38° 51’ N 99° 19’ W	39° 08’ N 96° 38’ W	37°73’ N 89°22’ W
Mean annual precipitation in 2010 (cm)	44.57	50.11	67.82	66.95
Growing Season ppt. (cm). Mar 15-aug 31, 2010	38.14	45.72	60.96	53.34
Mean annual precipitation, since 1961 (cm)	50.47	58.22	87.15	116.73
Ppt. of driest year (cm, yr)	28.37 (1967)	36.27 (1988)	39.16 (1966)	67.38 (1963)
Std dev of MAP (cm)	11.77	13.13	20.04	24.76
Average growing degree days (GDD) *	3167	3799	4156	4087
GDD (2010)	3461	4193	4105	4474
Potential Evapotranspiration (PET) (cm)	144	139	127	99
Aridity index (moisture deficit=PET – ppt.)	97	81	41	-18
Soil Type	Silt-loam	Roxbury Silt-loam	Sandy-loam	Stoy silt-loam

* GDD = Atmx+Tmin/2-50; if GDD is less than zero set to zero

Table 1.2. Results of the MANOVA comparing photosynthesis (*A*), stomatal conductance (g_s), internal CO_2 (C_i), transpiration (*E*), and intrinsic water use efficiency (WUE) of *A. gerardii* ecotypes at 1500 µmol photons m^{-2} s^{-1} (df error = 108).

	site			ecotype			measurement period			period*site			period* ecotype			site*ecotype			period*site* ecotype		
	df	F	P	df	F	P	df	F	P	df	F	P	df	F	P	df	F	P	df	F	P
A	3	51.45	**<0.001**	2	38.12	0.204	2	7.77	**<0.001**	6	1.61	**<0.001**	4	0.64	0.638	6	1.47	0.196	12	1.09	0.379
g_s	3	66.40	**<0.001**	2	27.38	**0.015**	2	8.97	**<0.001**	6	4.31	**<0.001**	4	1.12	0.348	6	0.85	0.536	12	1.42	0.166
C_i	3	45.66	**<0.001**	2	32.16	0.692	2	9.22	**<0.001**	6	0.37	**<0.001**	4	1.12	0.348	6	1.98	0.074	12	0.66	0.789
E	3	14.60	**<0.001**	2	31.37	**0.039**	2	14.93	**<0.001**	6	3.34	**<0.001**	4	0.64	0.638	6	0.24	0.962	12	0.83	0.628
WUE	3	52.13	**<0.001**	2	29.43	0.674	2	13.20	**<0.001**	6	0.40	**<0.001**	4	1.15	0.339	6	1.93	0.082	12	0.68	0.763

Degrees of freedom (df), F-scores (F), and P-values (P) from statistical analysis of variance are presented. Significant values ($P<0.05$) are bold.

Table 1.2. The location and environmental conditions of the reciprocal garden planting sites during 2010.

Environmental Conditions	Reciprocal Garden Planting Site			
	Colby, KS KSU Ag. Res Center (Thomas Co.)	Hays, KS KSU Ag. Res Center (Ellis Co.)	Manhattan, KS USDA Plant Material Center (Riley Co.)	Carbondale, IL SIU Agronomy Center (Jackson Co.)
Location	39° 23’N 101° 04’W	38° 51’ N 99° 19’ W	39° 08’ N 96° 38’ W	37°73’ N 89°22’ W
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Growing Season ppt. (cm). Mar 15-aug 31, 2010	38.14	45.72	60.96	53.34
Mean annual precipitation, since 1961 (cm)	50.47	58.22	87.15	116.73
Ppt. of driest year (cm, yr)	28.37 (1967)	36.27 (1988)	39.16 (1966)	67.38 (1963)
Std dev of MAP (cm)	11.77	13.13	20.04	24.76
Average growing degree days (GDD) *	3167	3799	4156	4087
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Potential Evapotranspiration (PET) (cm)	144	139	127	99
Aridity index (moisture deficit=PET – ppt.)	97	81	41	-18
Soil Type	Silt-loam	Roxbury Silt-loam	Sandy-loam	Stoy silt-loam

* GDD = Atmx+Tmin/2-50; if GDD is less than zero set to zero

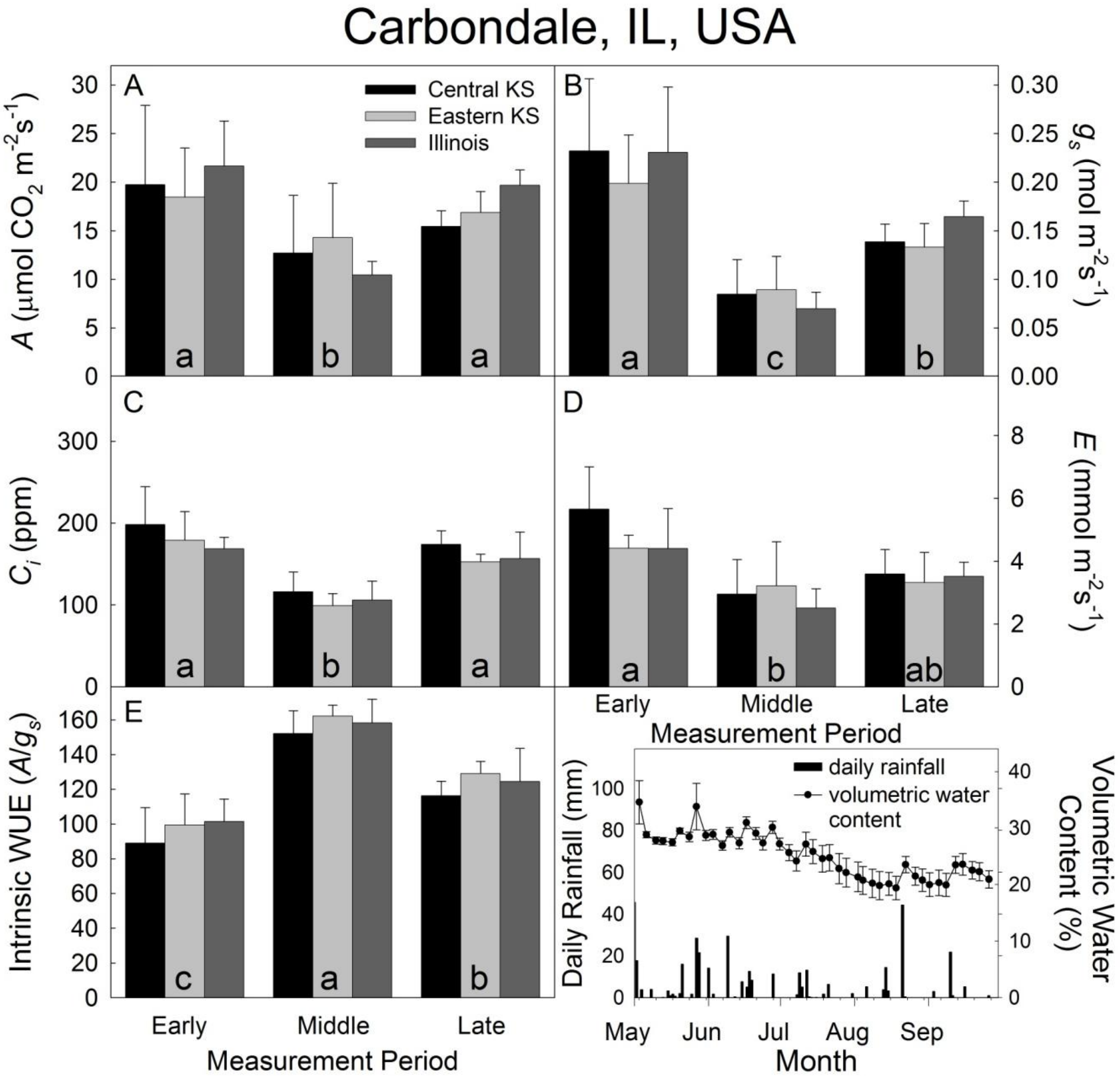

Figure 1.1. (A) Photosynthesis rates (A), (B) stomatal conductance (g_s), (C) internal CO_2 (C_i), (D) transpiration (E), and (E) intrinsic water use efficiency (WUE) of *A. gerardii* ecotypes at the Carbondale, IL site. All measures were made at 1500 μmol photons m^{-2} s^{-1}. Bars are means of four replicate plots ± SE during three measurement periods. (Bottom-right) Daily rainfall at Carbondale (left axis, in mm) is represented by histogram bars and volumetric water content (right axis, in %) is represented by circles. Lower case letters indicate statistical significance between measurement periods; groups of bars containing the same letter are not different. Tukey's HSD comparisons are presented in Appendices 1.1-1.5.

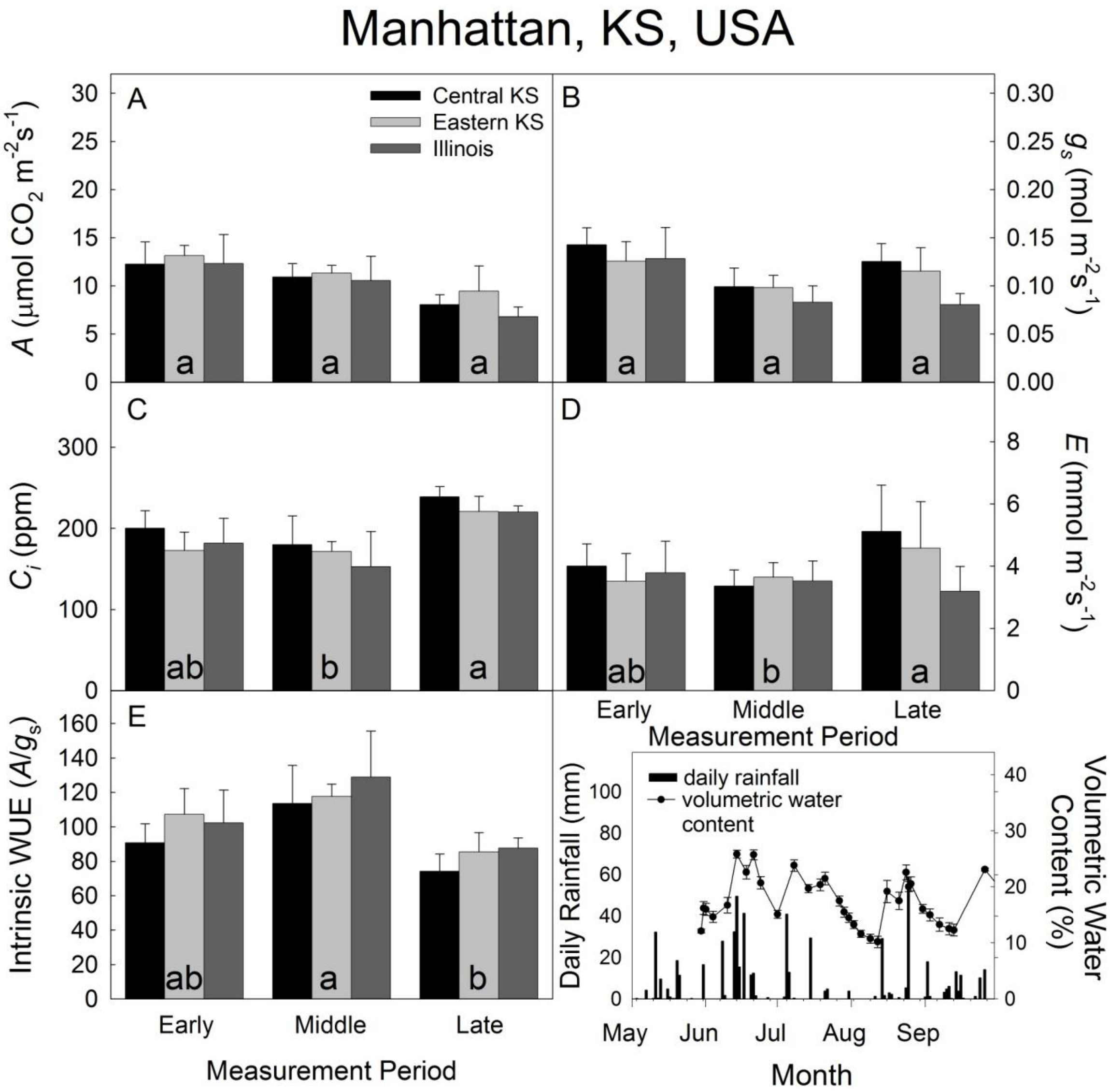

Figure 1.2. (A-E) *A, g_s, C_i, E,* and WUE of *A. gerardii* ecotypes at the Manhattan, KS site. Panels and abbreviations are as in Fig. 1.1. Bars are means of four replicate plots ± SE during three measurement periods. (Bottom-right) Daily rainfall at Manhattan (left axis, in mm) is represented by histogram bars and volumetric water content (right axis, in %) is represented by circles. Tukey's HSD comparisons are presented in Appendices 1.1-1.5.

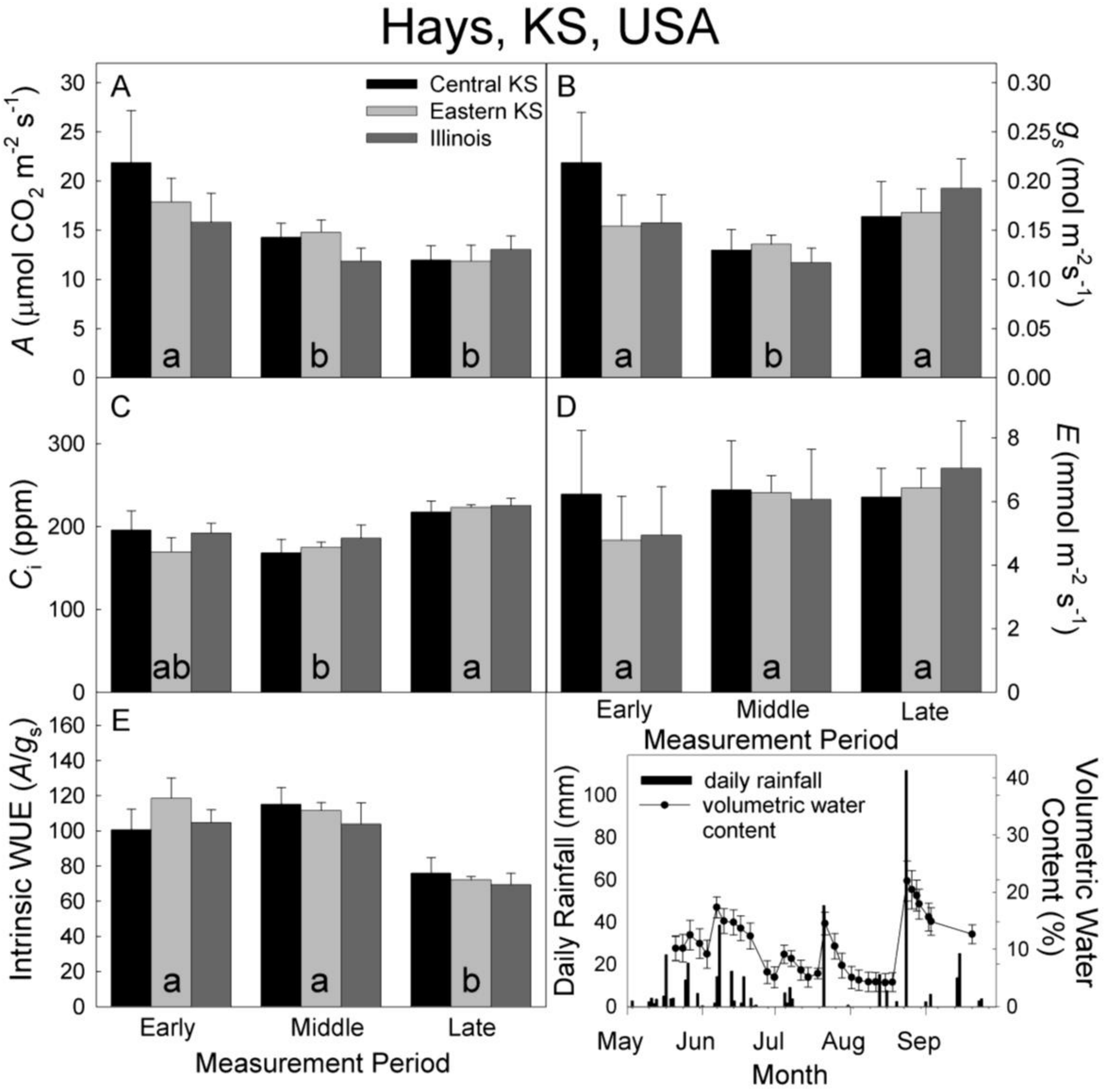

Figure 1.3. (A-E) *A, g_s, C_i, E,* and WUE of *A. gerardii* ecotypes at the Hays, KS site. Panels and abbreviations are as in Fig. 1.1. Bars are means of four replicate plots ± SE during three measurement periods. (Bottom-right) Daily rainfall at Hays (left axis, in mm) is represented by histogram bars and volumetric water content (right axis, in %) is represented by circles. Tukey's HSD comparisons are presented in Appendices 1.1-1.5.

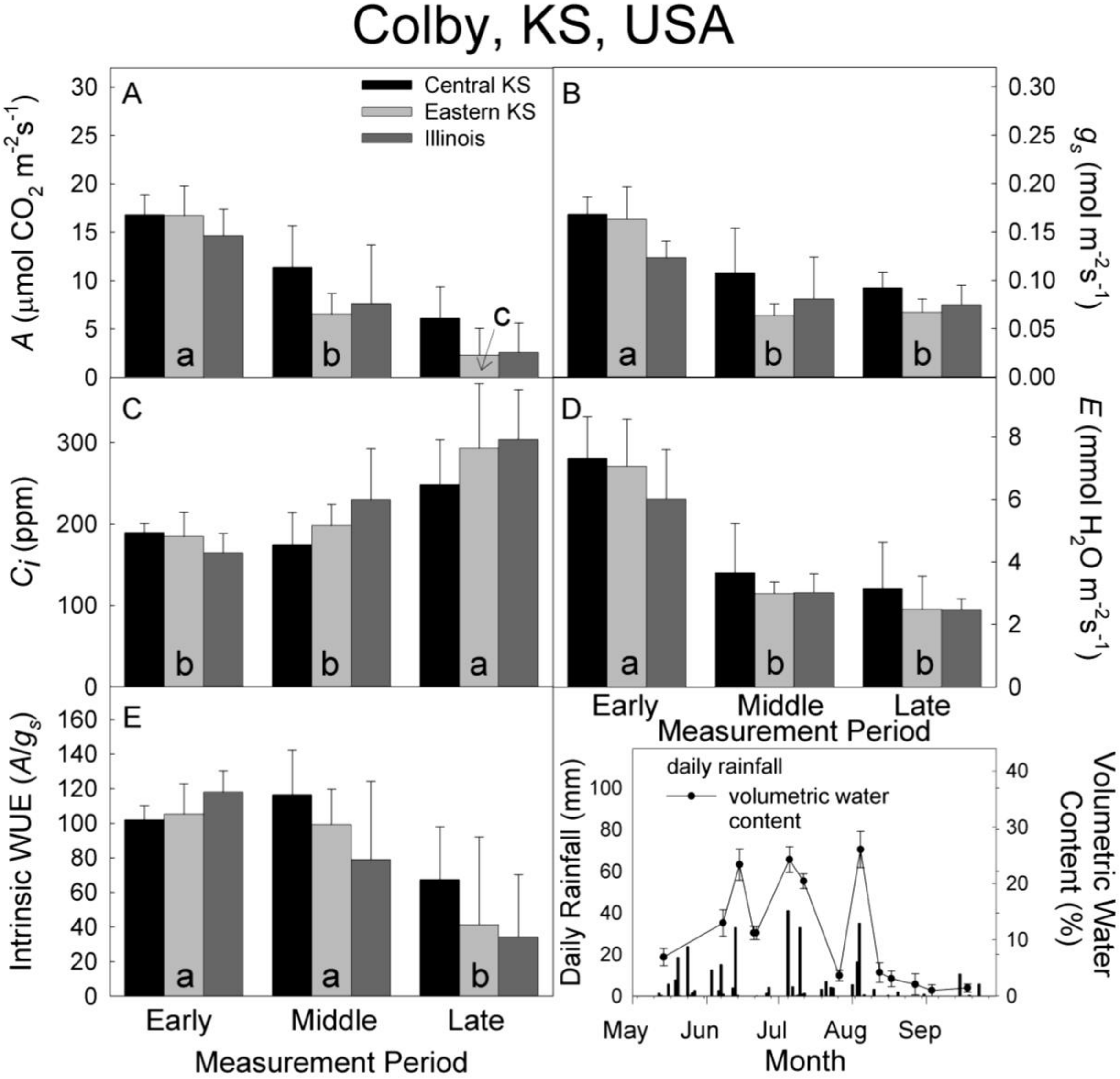

Figure 1.4. (A-E) *A, g_s, C_i, E,* and WUE of *A. gerardii* ecotypes at the Colby, KS site. Panels and abbreviations are as in Fig. 1.1. Bars are means of four replicate plots ± SE during three measurement periods. (Bottom-right) Daily rainfall at Colby (left axis, in mm) is represented by histogram bars and volumetric water content (right axis, in %) is represented by circles. Tukey's HSD comparisons are presented in Appendices 1.1-1.5.

Appendix 1.1. Tukey's HSD results for photosynthesis.

Site results of Tukey's HSD for photosynthesis (μmol CO_2 m^{-2} s^{-1}). Sites not connected by the same letter are significantly different.

Site			**Least Squares Mean**
Carbondale, IL	A		16.58
Hays, KS	A		14.81
Manhattan, KS		B	10.52
Colby, KS		B	9.41

Period × site results of Tukey's HSD for photosynthesis (μmol CO_2 m^{-2} s^{-1}). Levels not connected by the same letter are significantly different.

Level							**Least Squares Mean**
Early, Carbondale	A						19.95
Early, Hays	A						18.52
Late, Carbondale	A	B					17.33
Early, Colby	A	B	C				16.06
Middle, Hays		B	C	D			13.63
Early, Manhattan			C	D	E		12.57
Middle, Carbondale			C	D	E		12.47
Late, Hays			C	D	E		12.28
Middle, Manhattan				D	E		10.92
Middle, Colby					E		8.51
Late, Manhattan					E	F	8.10
Late, Colby						F	3.66

Appendix 1.2. Tukey's HSD results for stomatal conductance.

Site results of Tukey's HSD for stomatal conductance (mol m^{-2} s^{-1}). Sites not connected by the same letter are significantly different.

Site			**Least Squares Mean**
Hays, KS	A		0.160
Carbondale, IL	A		0.149
Manhattan, KS		B	0.111
Colby, KS		B	0.104

Ecotype results of Tukey's HSD for stomatal conductance (mol m^{-2} s^{-1}). Ecotypes not connected by the same letter are significantly different.

Ecotype			**Least Squares Mean**
Central KS	A		0.142
Eastern KS		B	0.126
Illinois		B	0.125

Period × site results of Tukey's HSD for stomatal conductance (mol m^{-2} s^{-1}). Levels not connected by the same letter are significantly different.

Level								**Least Squares Mean**
Early, Carbondale	A							19.95
Early, Hays		B						18.52
Late, Hays		B	C					17.33
Early, Colby		B	C	D				16.06
Late, Carbondale		B	C	D	E			13.63
Early, Manhattan			C	D	E	F		12.57
Middle, Hays				D	E	F		12.47
Late, Manhattan					E	F	G	12.28
Middle, Manhattan						F	G	10.92
Middle, Colby							G	8.51
Middle, Carbondale							G	8.10
Late, Colby							G	3.66

Appendix 1.3. Tukey’s HSD results for intercellular CO_2.

Site results of Tukey’s HSD for intercellular CO_2 (ppm). Sites not connected by the same letter are significantly different.

Site				**Least Squares Mean**
Colby, KS	A			220
Hays, KS		B		195
Manhattan, KS		B		193
Carbondale, IL			C	150

Period × site results of Tukey’s HSD for intercellular CO_2 (ppm). Levels not connected by the same letter are significantly different.

Level						**Least Squares Mean**
Late, Colby	A					282
Late, Manhattan		B				227
Late, Hays		B	C			222
Middle, Colby		B	C	D		200
Early, Hays		B	C	D		186
Early, Manhattan		B	C	D		185
Early, Carbondale			C	D		182
Early, Colby				D		179
Middle, Hays				D		176
Middle, Manhattan				D		169
Late, Carbondale				D		161
Middle, Colby					E	107

Appendix 1.4. Tukey’s HSD results for transpiration.

Site results of Tukey’s HSD for transpiration (mmol H_2O m^{-2} s^{-1}).
Sites not connected by the same letter are significantly different.

Site			**Least Squares Mean**
Hays, KS	A		6.03
Colby, KS		B	4.24
Manhattan, KS		B	3.85
Carbondale, IL		B	3.73

Ecotype results of Tukey’s HSD for transpiration (mmol H_2O m^{-2} s^{-1}).
Ecotypes not connected by the same letter are significantly different.

Ecotype			**Least Squares Mean**
Central KS	A		4.79
Eastern KS	A	B	4.39
Illinois		B	4.20

Period × site results of Tukey’s HSD for transpiration (mmol H_2O m^{-2} s^{-1}).
Levels not connected by the same letter are significantly different.

Level							**Least Squares Mean**
Early, Colby	A						6.79
Late, Hays	A						6.53
Middle, Hays	A	B					6.24
Early, Hays	A	B	C				5.32
Early, Carbondale		B	C	D			4.82
Late, Manhattan			C	D	E		4.29
Early, Manhattan			C	D	E	F	3.76
Middle, Manhattan				D	E	F	3.51
Late, Carbondale				D	E	F	3.49
Middle, Colby					E	F	3.21
Middle, Carbondale					E	F	2.89
Late, Colby						F	2.70

Appendix 1.5. Tukey's HSD results for intrinsic water-use efficiency.

Site results of Tukey's HSD for intrinsic water-use efficiency (A/g_s). Sites not connected by the same letter are significantly different.

Site				**Least Squares Mean**
Carbondale, IL	A			125.8
Manhattan, KS		B		100.9
Hays, KS		B		96.9
Colby, KS			C	84.7

Period × site results of Tukey's HSD for intrinsic water-use efficiency (A/g_s). Levels not connected by the same letter are significantly different.

Level							**Least Squares Mean**
Middle, Carbondale	A						157.4
Late, Carbondale		B					123.2
Middle, Manhattan		B	C				120.0
Middle, Hays		B	C				110.2
Early, Colby		B	C	D			108.4
Early, Hays		B	C	D			108.0
Early, Manhattan		B	C	D			100.1
Middle, Colby		B	C	D	E		98.2
Early, Carbondale			C	D	E		96.6
Late, Manhattan				D	E		82.4
Late, Hays					E	F	72.5
Late, Colby						F	47.6

CHAPTER 2: GAS EXCHANGE IN BIG BLUESTEM (*ANDROPOGON GERARDII*) ECOTYPES AND SAND BLUESTEM (*A. GERARDII* VAR. *HALLII*) IN A RECIPROCAL COMMON GARDEN EXPERIMENT

INTRODUCTION

North American grasslands cover nearly 6.8 million km^2 (White et al. 2000). Productivity is strongly correlated with growing season precipitation in these grasslands (Sala 1988); productivity is higher in eastern tallgrass prairies compared to western shortgrass prairies (Wang et al. 2003). In tallgrass prairies, precipitation comes primarily during the summer when temperatures are warm (Lauenroth et al. 1999), allowing C_4 grasses and forbs to dominate (Ehleringer 1978, Epstein et al. 1997).

When considering productivity in grasslands, photosynthesis rates of plants are of clear importance. This is especially true when considering potential limitations from precipitation. Photosynthesis (A) in C_4 plants generally is not limited by internal (intercellular) CO_2 (C_i) as typically seen in C_3 plants during stomatal closure (Farquhar and Sharkey 1982). Rather, A in C_4 plants is restricted by non-stomatal limitations during times of water stress (Ripley et al. 2007). Non-stomatal limitations are often a result of reduced nitrogen uptake or a reallocation of nitrogen to roots (Heckathorn et al. 1994). Consequences of reduced nitrogen in leaves include decreased ATP synthesis and RuBP regeneration, reduced enzymatic activities of Rubisco (Crafts-Brandner and Salvucci 2002), PEP carboxylase (Soares-Cordeiro et al. 2009), pyruvate P_i dikinase, and NADP-ME (Du et al. 1996). Decreased nitrogen also reduces the number of light-absorbing pigments in leaves (Ripley et al. 2007), leading to photodamage of photosystem II (Melis 1999).

Although stomatal limitations do not generally affect *A* in C_4 plants, stomata are the primary regulators of transpiration (*E*) when there is sufficient air flow over the leaf (Vasquez-Robinet et al. 2008). Jarvis and McNaughton (1986) noted g_s is linearly related to *E*. Similarly, Morison and Gifford (1983) observed g_s decreased linearly as vapor pressure deficit increased. At a constant temperature, leaf-air vapor pressure deficit increases as an environment becomes more arid (Campbell and Norman 1998), thereby increasing potential plant *E*. This indicates xeric environments have a greater potential for *E*. In xeric environments, plants often reduce total *E* by reduced leaf surface area, which additionally increases sensible heat loss and prevents overheating (Smith 1978, Maricle et al. 2007). Reduced leaf temperatures decrease the rate of *E* and increase water use efficiency (WUE).

Andropogon gerardii (big bluestem) is a dominant C_4 grass in tallgrass prairies (Gustafson et al. 2004) and can comprise nearly 80% of biomass in some areas (Knapp et al. 1998). The ecology of *A. gerardii* has been studied intensively, but little attention has been given to phenotypic variation among *A. gerardii* populations across the climatic gradient of the Great Plains (McMillan 1959). *Andropogon gerardii* var. *hallii* (sand bluestem) is a subspecies of *A. gerardii* that is commonly found in sandy soils (Barnes 1985). Barnes (1985) observed sand bluestem was able to maintain higher mid-day water potential than big bluestem, thereby avoiding desiccation. Gustafson et al. (2004) planted local and nonlocal populations of big bluestem in greenhouse and field experiments. Local populations were generally larger than nonlocal populations, suggesting local adaptation to environment occurs in *A. gerardii* and variation among populations is a mixture of genetics and environment (Gustafson et al. 2004).

Common garden experiments are frequently used to isolate genetic characteristics from environmental characteristics of plants along climatic gradients (Clausen et al. 1939, Etterson 2004, Oyarzabal et al. 2008). Through a common garden experiment, Oyarzabal et al. (2008) observed phenotypes of grass species changed over a precipitation gradient. This gradient explained 30-85% of variation, depending on the species. McMillan (1965) transplanted multiple populations of four dominant prairie grasses from across the United States, including *A. gerardii*, to indicate ecotypic variation. Similarly, Etterson (2004) used common gardens across a climatic gradient of the Great Plains to study three populations of *Chamaecrista fasciculata* (Michx.) Greene and found strong evidence of local adaptation. Local populations produced more seeds than nonlocal populations, indicating fitness was greater in local populations. Other common garden experiments along climatic gradients have been carried out within grasslands (Leger and Rice 2007, Oyarzabal et al. 2008), across the Great Plains of North America (Etterson 2004), and even within *A. gerardii* (McMillan 1965, Barnes 1985, Gustafson et al. 2004).

The response of a dominant C_4 grass such as *A. gerardii* to drought might indicate the response of prairie communities to drought (Arnone et al. 2011). In the present study, five ecotypes of *A. gerardii*, consisting of 14 populations, were planted in single plant common gardens across the east-west precipitation gradient of the Great Plains. Gas exchange measures of A, E, g_s, and C_i were used to evaluate responses of *A. gerardii* ecotypes to decreased moisture availability and the extent to which local adaptation has occurred in each ecotype. However, no previous studies have investigated ecotypes of *A. gerardii* at the scope of this experiment, both in terms of genetic variation and geographic extent. By using common gardens over a gradient of decreasing annual precipitation, the extent to which local adaptation has taken place and the extent to which ecotypes are adapted to drought was identified.

The response of ecotypes and populations of *A. gerardii* to drought might be highly varied based on differing amounts of annual precipitation across the Great Plains and local adaptations of each population to its native environment. In light of this, I addressed the following questions. (1) Are *A. gerardii* ecotypes locally adapted to their native environments? (2) Are ecotypes from xeric environments better adapted to drought than ecotypes from mesic environments? (3) To what degree are characteristics of gas exchange are environmentally controlled or the result of adaptation?

It was hypothesized (1) *A. gerardii* ecotypes would have higher photosynthetic rates in their local environment compared to non-local ecotypes and (2) plants collected from xeric environments in the west would be less sensitive to drought than plants from mesic environments in the east as evident by gas exchange rates. (3) A and g_s were expected to decrease in xeric sites due to non-stomatal limitations on photosynthesis. Similarly, plants at more xeric sites were expected to have higher intrinsic WUE. (4) Photosynthetic rates at sites with high rainfall were expected to be higher than at sites with low rainfall. Questions were assessed by gas exchange measures of A, E, g_s, and C_i.

MATERIALS AND METHODS

Seed Collecting and Planting

Seeds of *A. gerardii* ecotypes were hand collected within an 80 km radius of Hays, KS (38°51'N, 99°19'W); Manhattan, KS (39°08'N, 96°38'W); and Carbondale, IL (37°41'N, 89°14'W) in autumn 2008 (Table 2.1). Plants from each region were considered an ecotype and each ecotype contained four populations. These ecotypes follow a west-east precipitation gradient with the most xeric ecotype originating from Hays, KS and the most mesic from Carbondale, IL. In addition to three collected ecotypes, seeds of *A. gerardii* 'Kaw' (big bluestem, accession 421276) and *A. gerardii* var. *hallii* 'Garden' (sand bluestem, accession 421277), two cultivars of *A. gerardii*, were obtained from the USDA Manhattan Plant Materials Center (Manhattan, KS). Each cultivar was considered an ecotype, totaling five ecotypes and 14 populations.

In autumn 2008, seeds from each population of *A. gerardii* were planted in 10 cm x 10 cm pots in a greenhouse at Kansas State University in Metromix 510 potting mix (Scotts Company, Marysville, OH, USA). In August 2009, *A. gerardii* plants were transplanted from pots into common experimental gardens at Colby, KS; Hays, KS; Manhattan, KS; and Carbondale, IL (Table 2.2). Colby, KS was included to test the performance of *A. gerardii* ecotypes to an environment beyond the normal distribution of *A. gerardii*. At each planting site, 10 replicate plants of each of the 14 *A. gerardii* populations were transplanted, totaling 140 plants at each planting site. At Colby, KS, no individuals of the Carnahan population were planted due to limited seed supply. At each site, plants were arranged in 10 rows, 50 cm apart, with one plant of each population occurring in each row. Dewitt Sunbelt landscape fabric (Dewitt Co., Sikeston, MO, USA) was laid down around plants to inhibit growth of unwanted plants . In addition, plots were weeded regularly to remove unwanted species.

Data Collection

An EC-20 soil moisture probe, 20 cm in length (Decagon Devices, Inc., Pullman, WA, USA), monitored volumetric water content of soil (%) in each garden. Probes were placed in the ground under shade cloth at mid-growing season and soil water content was recorded twice weekly. In addition, four EC-20 soil moisture probes were positioned prior to the field season at each site in ground not covered by shade cloth. Measurements were made twice weekly. Rainfall data were obtained from local weather stations within 4.5 km of each site.

Gas Exchange Measures

Photosynthetic measurements were taken with three LI-6400 instruments (Li-Cor Biosciences Inc., Lincoln, NE, USA), one at each institution. Each LI-6400 was tested against a CO_2 gas standard and found to be accurate within 2.8% of actual values. The youngest, fully-expanded one or two leaves were placed inside the LI-6400 leaf chamber. Measurements were taken with CO_2 at 385 ppm, humidity and temperature were ambient, gas flow was 400 μmol s^{-1}, and photosynthetically active radiation (PAR) was 1500 μmol photons m^{-2} s^{-1}.

Gas exchange measurements of each plant were taken early in the 2010 growing season (23% - 36% GDD), mid-season (47% - 57% GDD), and late in the growing season (70% - 91% GDD). Measurements were taken on sunny days between 10:00 h and 15:00 h to maximize photosynthetically active radiation and minimize time required by leaves to adjust to leaf chamber conditions. Measurements were taken when photosynthesis and stomatal conductance had stabilized, typically requiring 1-2 min. Gas exchange measurements included photosynthesis (A), stomatal conductance to water vapor (g_s), internal (intercellular) CO_2 concentration (C_i), transpiration (E), and intrinsic water use efficiency (WUE), calculated as A/g_s.

Statistical Analysis

All data were analyzed with JMP 9.0.2 (SAS Institute Inc., Cary, NC, USA). Each plant was a replicate measure. A MANOVA was used; factors analyzed were sites, ecotypes, and measurement periods, including all interactions. This was followed with univariate tests and Tukey's honest significant difference (HSD) tests to reveal significant differences between means (Appendices 2.1-2.5). To determine statistically significant differences, analyses were performed at $\alpha=0.05$.

RESULTS

Differences were evident in all factors of each measurement, and in nearly every interaction at ecotype and population levels (Tables 2.2, 2.3). No differences were detected among populations within a single ecotype (Fig. 2.1). All four populations of each collected ecotype were similar in all measurements of gas exchange at sites and across measurement periods.

Carbondale, IL experienced drought during 2010 and total precipitation (80.3 cm) was nearly 40 cm lower than the annual mean (116.6 cm) and similar to precipitation in Manhattan, KS (84.7 cm). Growing season precipitation (April through September) in Carbondale was 47.0 cm, which was slightly less than the 48.8 cm received in Hays, KS but more than Colby, KS (Table 2.2). Volumetric water content of soil was consistently higher in Carbondale compared to the Kansas sites and among Kansas sites Manhattan maintained higher volumetric water content compared to Hays and Colby (Figs. 1.1-1.4 in Chapter 1).

Photosynthesis **(A)**

Central KS and Sand bluestem, the xeric ecotypes, generally had the highest *A* across sites and measurement periods. Mean leaf-level photosynthesis ranged from 10.3 to 35.4 μmol CO_2 m^{-2} s^{-1} across sites, measurement periods, and ecotypes (Figs. 2.2-2.5). Among ecotypes, sand bluestem and Central KS were not different, but were higher than Eastern KS, Illinois, and Kaw ($F=42.13$, $df=4, 1498$, $p<0.001$), which were not different from each other. Mean *A* at Carbondale, IL was 26.7 μmol CO_2 m^{-2} s^{-1} and higher than all three KS sites ($F=137.52$, $df=3, 1498$, $p<0.001$), which were not different from each other. Mean *A* at the KS sites ranged from 17.2 to 17.6 µmol CO_2 m^{-2} s^{-1}, or 9 µmol CO_2 m^{-2} s^{-1} lower than at Carbondale, IL. *A* was higher early in the growing season compared to mid-season, and *A* was higher mid-season compared to late season (F=201.44, df=2, 1498, p<0.001). All interactions except site × ecotype × measurement period were significant (Table 2.2, Appendix 2.1).

Stomatal conductance (g_s)

The drought-adapted ecotypes of *A. gerardii*, sand bluestem and Central KS, had the highest g_s and higher g_s was measured at mesic planting sites compared to xeric sites. Mean stomatal conductance ranged from 0.107 to 0.356 mol m^{-2} s^{-1} across sites, measurement periods, and ecotypes (Figs. 2.2-2.5). Sand bluestem and the Central KS ecotype were not different, but had higher g_s compared to the Manhattan and Illinois ecotypes (F=18.15, df=4, 1498, p<0.001). The Kaw ecotype had lower g_s compared to Central KS, but was not different from sand bluestem, Eastern KS, or Illinois. Carbondale, IL had higher g_s compared to the KS planting sites (F=70.38, df=3, 1498, p<0.001). Among the KS sites, g_s at Hays was higher compared to Manhattan, while Colby was not different from Hays or Manhattan. Early growing season g_s was higher compared to mid-season g_s, and mid-season g_s was higher than late season g_s (F=65.55, df=2, 1498, p<0.001). All interactions except site × ecotype × measurement period were significant (Table 2.2, Appendix 2.2).

Intercellular CO_2 (C_i)

C_i was higher in sand bluestem compared to big bluestem and higher at xeric sites compared to mesic sites. Mean internal (intercellular) CO_2 ranged from 93 to 247 ppm across sites, measurement periods, and ecotypes (Figs. 2.2-2.5). C_i in sand bluestem was lower than in the four big bluestem ecotypes (F=12.57, df=4, 1498, p<0.001), which did not differ from each other. C_i at Colby, KS was not different from Hays, KS, but both were higher compared to Manhattan, KS and C_i at Manhattan was higher compared to Carbondale, IL (F=44.51, df=3, 1498, p<0.001). Early season and mid-season measurements of C_i were not different from each other, but both were lower than late season measurements (F=41.49, df=2, 1498, p<0.001). Site × ecotype and site × measurement period interactions were significant (Table 2.2, Appendix 2.3).

***Transpiration* (E)**

The highest *E* was measured in sand bluestem and the Central KS ecotype at xeric sites. Mean *E* ranged from 2.3 to 9.4 mmol m^{-2} s^{-1} across sites, measurement periods, and ecotypes (Figs. 2.2-2.5). *E* in sand bluestem did not differ from Central KS, but both were higher than Eastern KS, Illinois, and Kaw (F=22.32, df=4, 1498, p<0.001), which were not different from each other. *E* at Colby, KS and Hays, KS was not different, but both sites had higher *E* than Carbondale, IL, and Carbondale had higher *E* than Manhattan, KS (F=48.85, df=3, 1498, p<0.001). *E* during the early and middle growing seasons was not different, but during both periods *E* was higher than late growing season (F=140.57, df=2, 1498, p<0.001). All interactions except site × ecotype × measurement period were significant (Table 2.2, Appendix 2.4).

Intrinsic Water Use Efficiency (WUE)

The highest intrinsic WUE was measured in sand bluestem and at mesic sites. Mean WUE (A/g_s) ranged from 60.6 to 168.7 µmol CO_2 mol^{-1} H_2O across sites, measurement periods, and ecotypes (Figs. 2.2-2.5). Sand bluestem had a higher WUE than Central KS, Eastern KS, and Illinois (F=8.82, df=4, 1498, p<0.001), which were not different from each other; Kaw was not different from any ecotype. Carbondale, IL had a higher WUE than Manhattan, KS and Manhattan had a higher WUE than Hays, KS and Colby, KS (F=27.37, df=3, 1498, p<0.001); WUE at Hays and Colby was not different. Early and middle growing season WUE were not different from each other, but both were higher than late season WUE (F=34.19, df=2, 1498, p<0.001). Site × ecotype and site × measurement period interactions were significant (Table 2.2, Appendix 2.5).

DISCUSSION

Populations of *Andropogon gerardii* have highly variable phenotypes across the Great Plains of North America (McMillan 1959). Variation in leaf length, width, and thickness are evident across ecotypes (Barnes 1985, Ravenek et al. in review, Johnson and Tetreault in prep., Chapter 3). In addition, variation in fitness (Gustafson et al. 2004), phenology, plant height (Tetreault et al. in prep.), and net primary productivity (Goad et al. unpublished data) are apparent. In the present study, four common gardens oriented across the west-east climatic gradient of the Great Plains were used to distinguish between environmentally controlled and genetically controlled characteristics of *A. gerardii* ecotypes. Adaptation to water availability explains much of the morphological and functional variation among ecotypes and populations of *A. gerardii* across the Great Plains.

Adaptation to Xeric Climates

Ecotype × site interactions were detected in all gas exchange measures in the present study. Under the influence of natural selection and in the absence of other evolutionary forces, it is thought local plants evolve an advantage over nonlocal plants by adapting to the local environment (Kawecki and Ebert 2004). Local adaptation is widespread and commonly seen among plant species (Joshi et al. 2001, Etterson 2004) and in *Andropogon gerardii* (Gustafson et al. 2004). However, genetic drift and gene flow often confound local adaptation (Kawecki and Ebert 2004), allowing nonlocal populations to outperform local populations. In my study, local adaptation was not observed, rather xeric ecotypes developed adaptations to xeric climates that improved gas exchange rates at all sites compared to mesic populations.

When comparing *A. gerardii* at the population level rather than the ecotype level, it is intriguing to note there were no significant differences in gas exchange measurements among populations within a single ecotype (Fig. 2.1). Each collected ecotype (Central KS, Eastern KS, and Illinois) contained four populations, collected within an 80 km radius of each other. Because of the close proximity of populations within an ecotype, gene flow likely occurs, causing closely oriented populations to be more similar compared to distant populations.

Among ecotypes, sand bluestem and Central KS maintained higher photosynthetic rates (A) in xeric climates than did the Eastern KS, Illinois, or Kaw ecotypes. In a greenhouse study, Ravenek et al. (in review) found no difference in A between sand bluestem and big bluestem. In the present study, stomatal conductance (g_s) correlated well with A, indicating an ability to maintain high g_s was related with high A. Barnes (1985) measured lower g_s in sand bluestem than in big bluestem in the field, whereas Ravenek et al. (in review) measured no difference in stomatal conductance between sand bluestem and big bluestem in field experiments. Sand bluestem in the present study had g_s similar to the xeric ecotype of big bluestem. Further, both sand bluestem and the xeric ecotype of big bluestem maintained higher g_s than the more mesic ecotypes of big bluestem. Like most drought-tolerant grasses (Redmann 1983), stomata do not close in *A. gerardii* until plants are sufficiently stressed. An ability to maintain high g_s under low water potentials appears to be an adaptation to drought (Maricle and Adler 2011), although C_4 plants can maintain high photosynthetic rates even when g_s is low through their CO_2 concentrating mechanism (Farquhar and Sharkey 1982, Dai et al. 1993, Ghannoum et al. 2003). As a result, non-stomatal limitations typically reduce A in C_4 plants (Ripley et al. 2010). Low g_s paired with unchanged C_i in *A. gerardii* is an indicator non-stomatal limitations are decreasing A (Ghannoum et al. 2003). Despite higher A in sand bluestem and Central KS, these ecotypes were generally the shortest plants and had the smallest leaves (Johnson and Tetreualt in prep.). Ravenek et al. (in review) state sand bluestem has a slower growth rate than big bluestem. Sand bluestem and Central KS plants might allocate a larger proportion of resources toward increasing below ground production of roots and storage material (Skinner et al. 2006). This might be an adaptation of xeric ecotypes of *A. gerardii* to xeric environments, helping them endure prolonged drought.

In xeric climates, transpiration rates (E) can be very high, particularly if a constant water source is available (Rahman and Batanouny 1965). High E in xeric climates can result in loss of turgor if water supply is limited, but this not commonly found because stomates typically close before turgor loss occurs (Redmann 1983). Sand bluestem and Central KS had higher E than Eastern KS, Illinois, and Kaw, largely due to their inherently high g_s. Comparable to high E, these plants had higher gas exchange rates at all four planting sites. Short, narrow leaves reduced total leaf surface area and total transpiration (Abrams et al. 1990) of sand bluestem (Ravenek et al. in review) and the Central KS ecotype (Tetreault et al. in prep., Chapter 3), giving them an advantage over their more mesic counterparts in xeric climates. The Eastern KS and Illinois ecotypes commonly had long, wide leaves with large surface areas compared to xeric ecotypes (Tetreault et al. in prep., Chapter 3), resulting in high total transpiration despite lower transpiration rates per unit area. In addition, high *E* might be an advantage in hot environments by increasing evaporative leaf cooling (Abrams 1990, Campbell and Norman 1998) and nutrient transport to leaves (Cintrón et al. 1978). This could result in reduced damage to enzymes and photosynthetic proteins of drought-stressed plants on hot, dry days (Ludlow and Björkman 1984). Furthermore, drought-adapted big bluestem populations might also have greater quantities of epicuticular wax than mesic populations. Barnes (1985) A greater quantity of epicuticular wax has been observed in sand bluestem compared to big bluestem (Barnes 1985, Shelton et al. in prep) and Ravenek et al. (in review) measured up to five times greater epicuticular wax in sand bluestem than big bluestem, offering another advantage of sand bluestem to reduced water loss.

It can be expected maintaining high g_s will result in low intrinsic water use efficiency (WUE), unless *A* is sufficiently high. Sand bluestem had high g_s and high *A*, yet still maintained the highest WUE of any ecotype. However, the Central KS ecotype had high g_s and high *A*, but a relatively low WUE, similar to that observed in other big bluestem ecotypes. Ravenek et al. (in review) also observed WUE in sand bluestem was higher than in big bluestem. Sand bluestem appears to be better adapted to drought through its high *A*, WUE, epicuticular wax load, and small leaf area compared to big bluestem ecotypes. Among big bluestem ecotypes, Central KS holds an advantage over Eastern KS, Illinois, and Kaw in regards to gas exchange.

Adaptations of *A. gerardii* to hot, xeric environments might also include other common physiological factors such as increased pigmentation in leaves (El-Tayeb 2006), an ability to generate low water potentials (Kolb and Sperry 1999), and bulliform cells to facilitate leaf rolling (Maricle et al. 2009, Chapter 3). Increased pigmentation absorbs more energy from the sun and reduces damage to photosynthetic pigments by excess radiation (Flint et al. 1985). An ability of xeric ecotypes to maintain net photosynthesis during prolonged periods of drought by generating low plant water potentials compared to mesic ecotypes is likely an adaptation to xeric environments (Odening et al. 1974, Abrams 1990).

Through drought adaptations, sand bluestem and Central KS were able to maintain higher A in xeric climates compared to Eastern KS, Illinois, and Kaw (Appendix 2.1). Interestingly, these same adaptations were beneficial at the mesic Manhattan, KS and Carbondale, IL sites. At Manhattan and Carbondale, the same pattern measured at xeric sites was evident, with sand bluestem and Central KS maintaining higher A than Eastern KS, Illinois, or Kaw. An ability to maintain high g_s is evidently beneficial in both mesic and xeric environments for *A. gerardii*. Because sand bluestem and Central KS are generally shorter and small-leafed (Tetreault et al. in prep.), they might be out-competed by taller, large-leaved mesic ecotypes when competition is primarily above ground. The shorter, small-leaved sand bluestem and Central KS ecotypes are well suited for below ground competition (Burke et al. 1998). However, in our single plant plots, sand bluestem and Central KS maintained more advantageous gas exchange rates at xeric and mesic sites.

Plant photosynthesis declined as the growing season progressed. Among *A. gerardii* ecotypes, A decreased during each measurement period at each site with the most pronounced decreases coming between midseason and late season measurements. Early season measurements of A were consistently higher than midseason or late season measurements. Hayes (1985) observed *A. gerardii* reallocates leaf nitrogen and amino acids to roots late in the growing season as a preparation for dormancy and as an adaptation to drought. Nitrogen removal and increased amino acid concentrations in leaves are the result of leaf proteins being broken down into amino acids, thereby reducing *A* (Hayes 1985). In addition, as the growing season progresses, light can damage photosynthetic pigments and further reduce *A* (Flint et al. 1985). Both nitrogen reallocation and light damage likely contribute to decreased photosynthesis in *A. gerardii* as it senesces.

Environmental Differences

Although common garden experiments are often used to separate genetic differences from environmental differences, there is not always an overriding genetic or environmental control. In the case of *A. gerardii*, all gas exchange measures exhibited significant differences at the ecotype (genetic) and site (environmental) levels, including site × ecotype interactions, indicating both factors and how they interact are important for gas exchange.

Soil at the Manhattan site contained significantly fewer nutrients and microbes than soils at other sites (Mendola et al. in prep). This correlates well with the generally low photosynthetic rates at Manhattan, KS, despite the high soil moisture there (Figures 1.1-1.4 in Chapter 1). No difference in *A* was seen between Manhattan, KS and Hays, KS or Colby, KS. In addition, plants at the Manhattan site were infected prominently by leaf rust (*Puccinia andropogonis*). Leaf rust is often responsible for crop losses and is known to reduce *A* in affected leaves (Robert et al. 2005). *A* in rust-affected plants is reduced primarily by decreased chlorophyll content and lower g_s (Zhao et al. 2011). Leaf rust was present at other planting sites, but in small enough quantities to have negligible effects on *A. gerardii* at these sites. Soil moisture content was higher at eastern mesic sites and lower at western xeric sites. Although Carbondale, IL had lower than typical rainfall, it consistently had the highest soil moisture content, and Colby, KS consistently had the lowest soil moisture content. High soil moisture at Carbondale might be a result of stored ground water from previous years or hydraulic redistribution of moisture by plants coupled with a high water table. The high soil moisture at Carbondale corresponded well with the high photosynthetic rates (A) and stomatal conductance (g_s) measured there. Barnes (1985) noted A and g_s in sand bluestem and big bluestem remained high when soil moisture was high and decreased as soil moisture decreased. Mean A at Carbondale was 50% higher than mean A at each of the KS planting sites. The primary factor limiting A at Colby and Hays was most likely low soil moisture.

Intrinsic water use efficiency increased in Carbondale, IL, but decreased at all other sites as the season progressed. At Carbondale, early season and midseason g_s was 0.32 and 0.28 mol m^{-2} s^{-1}, respectively. From midseason to late season, g_s dropped 57% to 0.12 mol m^{-2} s^{-1}. From midseason to late season, A only dropped by 40%, causing intrinsic WUE to increase. Because soil moisture remained high throughout the growing season at Carbondale, A was not limited by water availability. Instead, A had stomatal limitations late in the season. C_i dropped from 142 to 118 ppm between midseason and late season. Low C_i indicates stomatal limitations are present (Ghannoum et al. 2003), despite high soil moisture. Field (1987) noted g_s decreased sharply as leaves began to senesce, explaining the large drop in g_s at Carbondale late in the growing season. Kansas sites did not have a sharp drop in g_s during the late season because g_s at these sites was already limited by low soil moisture. C_i at Kansas sites increased as the season progressed due to a decrease in A.

Conclusion

Sand bluestem and big bluestem are subspecies of *A. gerardii*, but sand bluestem is better adapted to xeric climates compared to big bluestem ecotypes used in this study with the exception of the Central KS ecotype. The drought tolerant ecotypes sand bluestem and Central KS had the highest A, g_s, and E and sand bluestem had a higher intrinsic WUE than all big bluestem ecotypes. Other drought tolerant traits of sand bluestem and Central KS were also typified by shorter plants and shorter, narrower leaves when compared to Eastern KS, Illinois, or Kaw ecotypes. When below ground competition is high, as seen in xeric environments (Burke et al. 1998), these characteristics are beneficial; however, the short stature of sand bluestem and Central KS could be a liability under mesic environments where above ground competition is generally high (Burke et al. 1998) and short plants are quickly shaded by taller plants. Local adaptation of gas exchange was not observed among populations within the Central KS, Eastern KS, or Illinois ecotypes; rather performance of ecotypes corresponds well with adaptations to xeric environments where more the more drought adapted ecotypes of Central KS and sand bluestem had higher gas exchange rates. Strong environmental influences were also observed, where sites with higher precipitation had higher photosynthesis rates. Within the Great Plains, temperatures are expected to rise and summer precipitation is expected to decline (Weltzin et al. 2003), threatening to change plant distribution. *Andropogon gerardii* has demonstrated an ability to adapt to reduced rainfall and it will likely remain a dominant grass of the prairies.

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Table 2.1. Collection sites for *A. gerardii* populations that constitute each ecotype.

Ecotype	Seed collection site of populations	Latitude (N)	Longitude (W)
Central KS*	Relict Prairie	38° 51’	99° 22’
	Webster Reservoir	39° 24’	99° 32’
	Saline Experimental Range	39° 02’	99° 14’
	Cedar Bluff Reservoir	38° 45’	99° 46’
Eastern KS*	Carnahan Cove at Tuttle Creek Reservoir	39° 20’	96° 38’
	Konza Prairie Natural Area	39° 05’	96° 36’
	Tallgrass Prairie National Park	38° 25’	96° 33’
	Top of the World Park	39° 13’	96° 37’
Illinois*	(Desoto) Faulkner-Franke Pioneer Railroad Prairie Natural Preserve	37°51’	89° 14’
	Twelve Mile Prairie	38°46'	88° 50'
	Fults Hill Prairie State Natural Area	37°58’	89° 48’
	Walters Prairie	38°59'	88° 09'
Kaw†	Manhattan Plant Material Center	39°14'	96° 63’
Sand Bluestem†	Manhattan Plant Material Center	39°14'	96° 63’

*Indicates ecotype was hand collected.

†Indicates ecotype is a cultivar.

Table 2.2. The location and environmental conditions of the reciprocal garden planting sites during 2010.

Environmental Conditions	Reciprocal Garden Planting Site			
	Colby, KS KSU Ag. Res Center (Thomas Co.)	Hays, KS KSU Ag. Res Center (Ellis Co.)	Manhattan, KS USDA Plant Material Center (Riley Co.)	Carbondale, IL SIU Agronomy Center (Jackson Co.)
Location	39° 23’N 101° 04’W	38° 51’ N 99° 19’ W	39° 08’ N 96° 38’ W	37°73’ N 89°22’ W
Mean annual precipitation in 2010 (cm)	44.57	50.11	67.82	66.95
Growing Season ppt. (cm). Mar 15-aug 31, 2010	38.14	45.72	60.96	53.34
Mean annual precipitation, since 1961 (cm)	50.47	58.22	87.15	116.73
Ppt. of driest year (cm, yr)	28.37 (1967)	36.27 (1988)	39.16 (1966)	67.38 (1963)
Std dev of MAP (cm)	11.77	13.13	20.04	24.76
Average growing degree days (GDD) *	3167	3799	4156	4087
GDD (2010)	3461	4193	4105	4474
Potential Evapotranspiration (PET) (cm)	144	139	127	99
Aridity index (moisture deficit=PET – ppt.)	97	81	41	-18
Soil Type	Silt-loam	Roxbury Silt-loam	Sandy-loam	Stoy silt-loam

* GDD = Atmx+Tmin/2-50; if GDD is less than zero set to zero

Table 2.3. Ecotype level results of the MANOVA comparing photosynthesis (*A*), stomatal conductance (g_s), internal CO_2 (C_i), transpiration (*E*), and intrinsic water use efficiency (WUE) of *A. gerardii* ecotypes at 1500 µmol photons m^{-2} s^{-1} (df error=1498).

	site			ecotype			measurement period			period*site			period*ecotype			site*ecotype			period*site*ecotype		
	df	F	P	df	F	P	df	F	P	df	F	P	df	F	P	df	F	P	df	F	P
A	3	137.52	**<0.001**	4	42.13	**<0.001**	2	201.44	**<0.001**	6	19.97	**<0.001**	8	3.66	**<0.001**	12	2.34	**0.006**	24	1.19	0.244
g_s	3	70.38	**<0.001**	4	18.15	**<0.001**	2	65.55	**<0.001**	6	42.85	**<0.001**	8	18.52	**0.018**	12	2.73	**0.001**	24	1.24	0.195
C_i	3	44.51	**<0.001**	4	12.57	**<0.001**	2	41.49	**<0.001**	6	29.15	**<0.001**	8	1.76	0.082	12	2.38	**0.005**	24	0.87	0.648
E	3	48.85	**<0.001**	4	22.32	**<0.001**	2	140.57	**<0.001**	6	14.02	**<0.001**	8	2.11	**0.033**	12	5.05	**<0.001**	24	1.05	0.400
WUE	3	27.37	**<0.001**	4	8.82	**<0.001**	2	34.19	**<0.001**	6	70.00	**<0.001**	8	1.61	0.119	12	2.36	**0.005**	24	1.05	0.390

Degrees of freedom (df), F-scores (F), and P-values (P) from statistical analysis of variance are presented. Significant values ($P<0.05$) are bold.

Table 2.4. Population level results of the MANOVA comparing photosynthesis (A), stomatal conductance (g_s), internal CO_2 (C_i), transpiration (E), and intrinsic water use efficiency (WUE) of *A. gerardii* populations at 1500 µmol photons m^{-2} s^{-1} (df error=1327).

	site			population			measurement period			period*site			period*population			site*population			period*site* population		
	df	F	P	df	F	P	df	F	P	df	F	P	df	F	P	df	F	P	df	F	P
A	3	197.54	**<0.001**	12	14.85	**<0.001**	2	285.56	**<0.001**	6	25.26	**<0.001**	24	1.97	**0.003**	36	1.51	**0.028**	72	0.99	0.496
g_s	3	97.57	**<0.001**	12	7.02	**<0.001**	2	87.60	**<0.001**	6	60.62	**<0.001**	24	1.46	*0.070*	36	1.45	**0.042**	72	1.16	0.170
C_i	3	70.87	**<0.001**	12	4.82	**<0.001**	2	77.83	**<0.001**	6	38.46	**<0.001**	24	1.23	0.208	36	1.39	*0.063*	72	0.85	0.807
E	3	57.81	**<0.001**	12	7.73	**<0.001**	2	185.01	**<0.001**	6	19.57	**<0.001**	24	1.06	0.382	36	1.87	**0.002**	72	0.72	0.962
WUE	3	43.62	**<0.001**	12	3.51	**<0.001**	2	68.77	**<0.001**	6	80.00	**<0.001**	24	1.12	0.318	36	1.41	*0.056*	72	0.92	0.676

Degrees of freedom (df), F-scores (F), and P-values (P) from statistical analysis of variance are presented. Significant values ($P<0.05$) are bold. Values near significance are italicized.

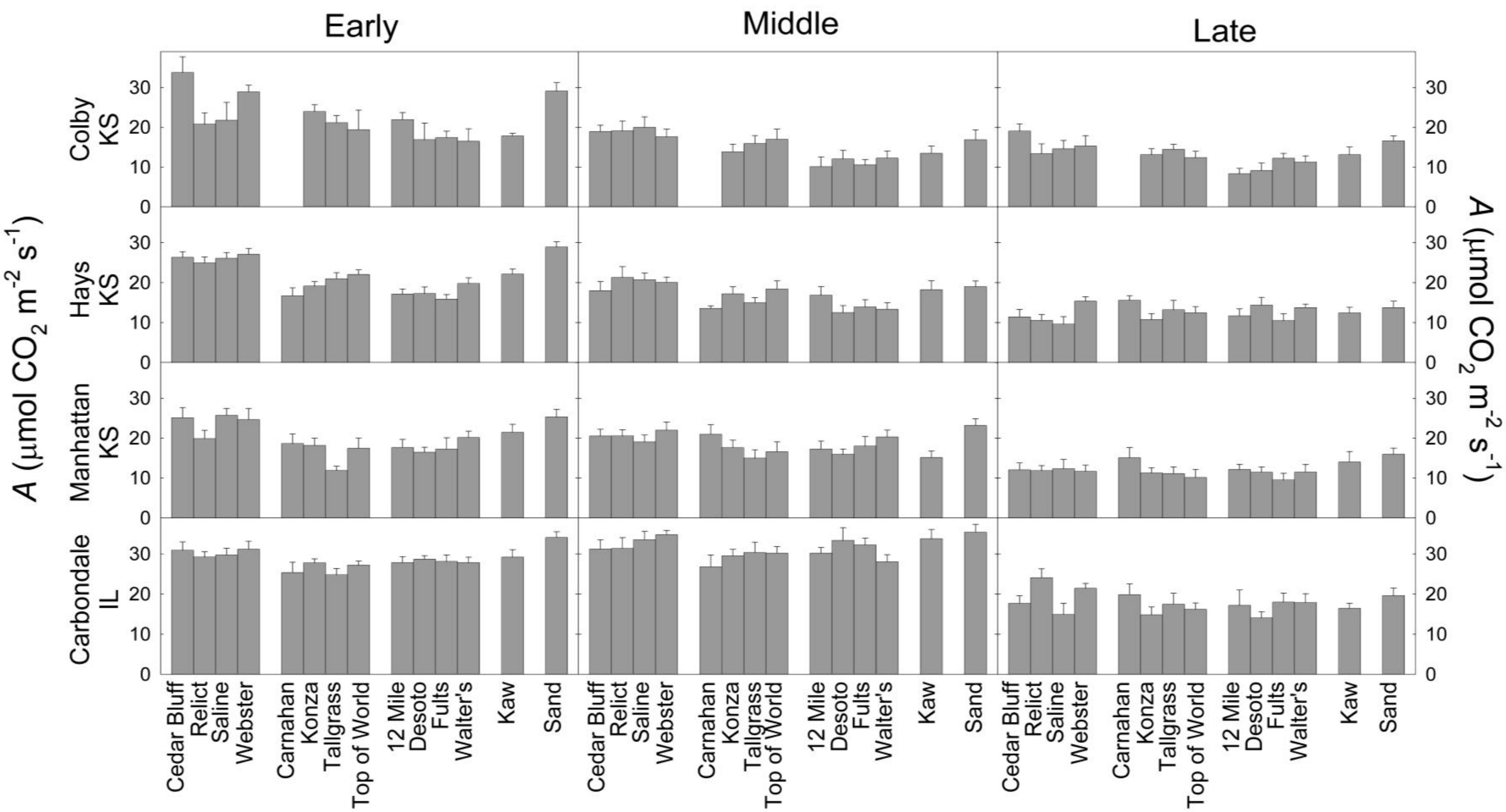

Figure 2.1. Photosynthesis rates (*A*) of *A. gerardii* populations at each site (rows) and across measurement periods (columns). Hand collected populations are grouped into ecotypes. No significant differences were detected among populations within an ecotype. All measures were made at 1500 µmol photons m^{-2} s^{-1}. Bars are means of 10 replicate plots ± SE.

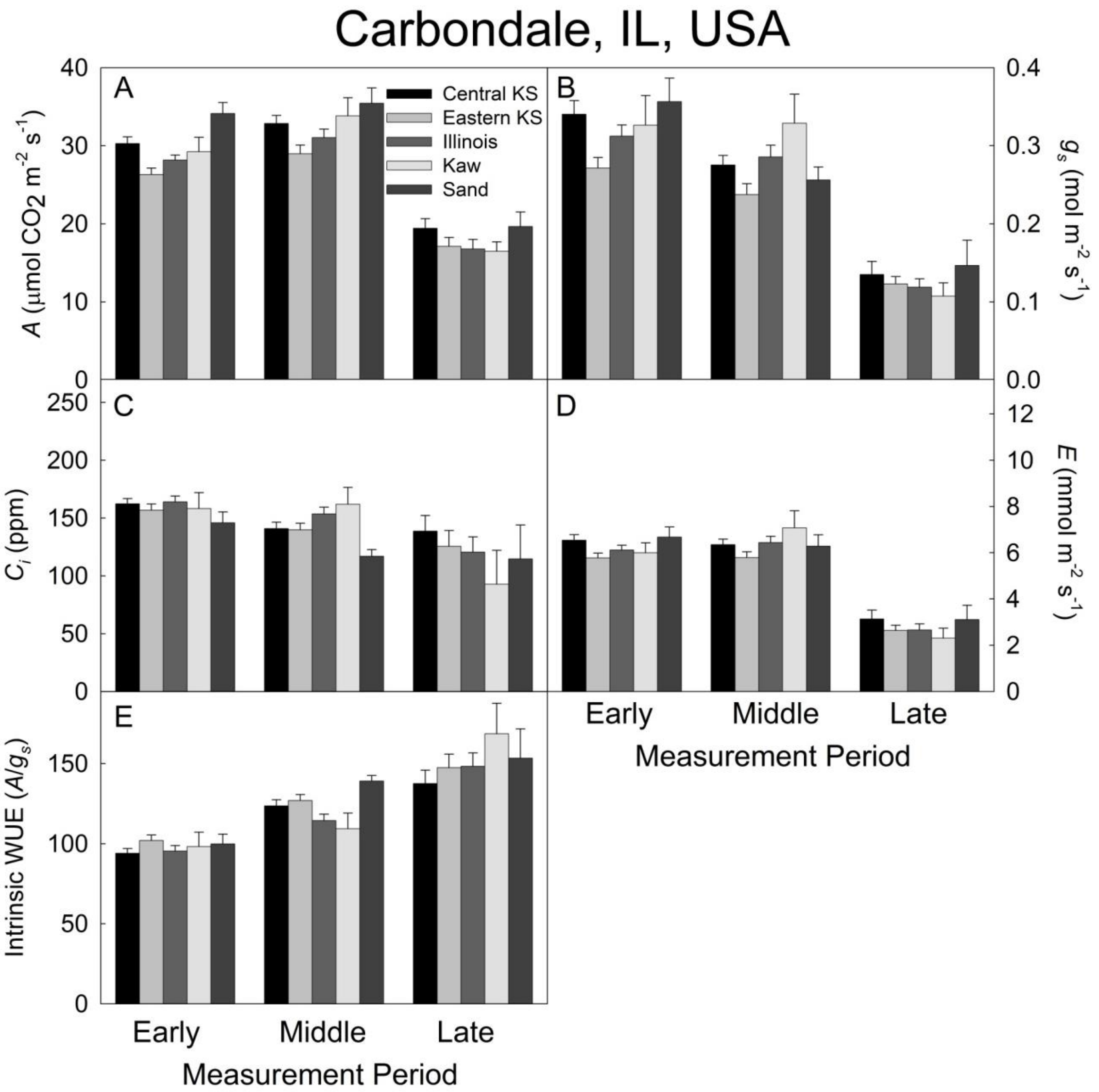

Figure 2.2. (A) Photosynthesis rates (*A*), (B) stomatal conductance (g_s), (C) internal CO_2 (C_i), (D) transpiration (*E*), and (E) intrinsic water use efficiency (WUE) of *A. gerardii* ecotypes at the Carbondale, IL site. Central KS, Eastern KS, and Illinois bars are averages of their four respective populations (see Table 2.1). All measures were made at 1500 µmol photons m^{-2} s^{-1}. Bars are means of 10 replicate plots ± SE during each of the three measurement periods. Tukey's HSD comparisons are presented in Appendices 2.1-2.5.

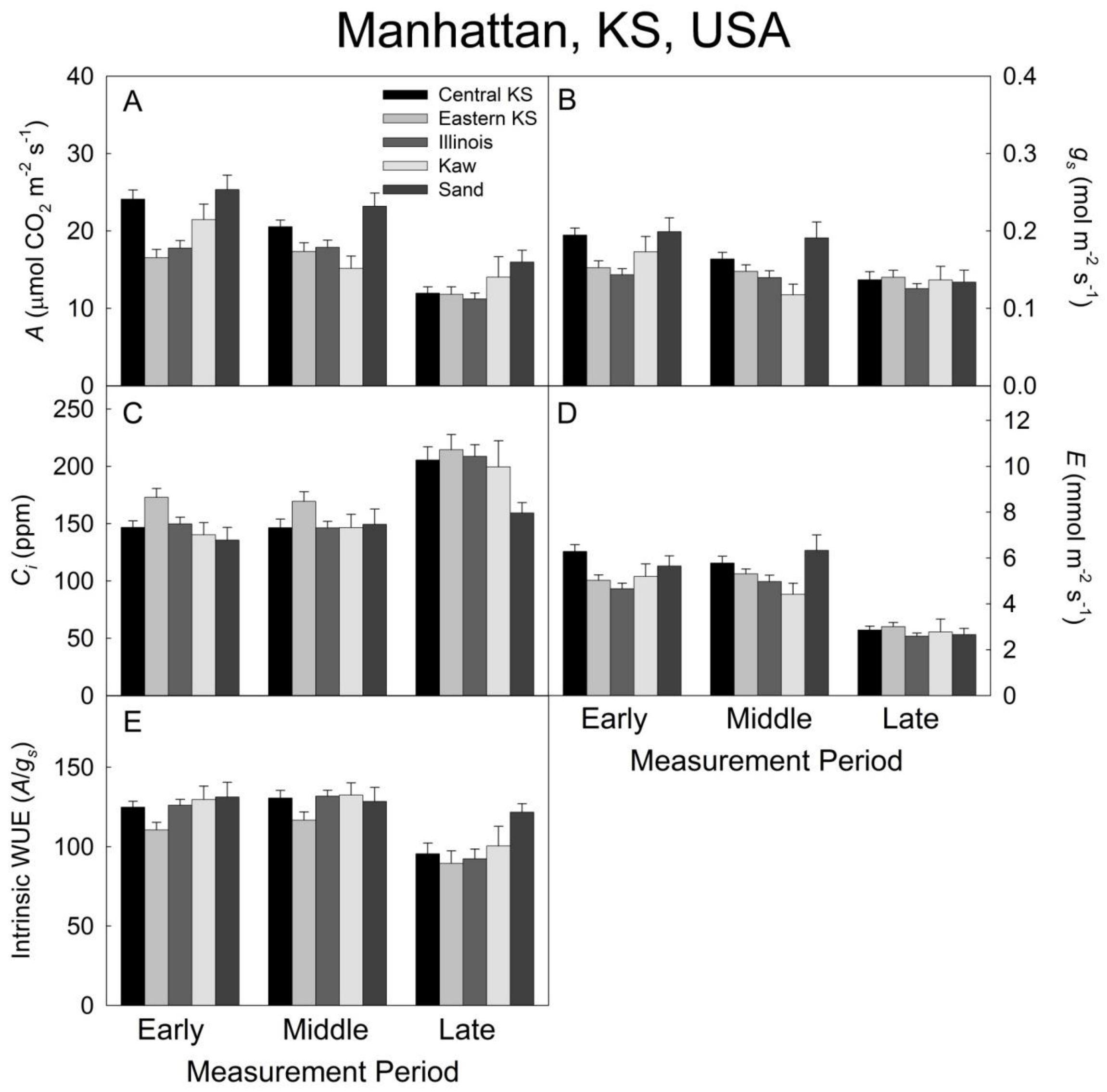

Figure 2.3. (A-E) *A, g_s, C_i, E,* and WUE of *A. gerardii* ecotypes at the Manhattan, KS site. Panels and abbreviations are as in Fig. 2.2. Bars are means of 10 replicate plots ± SE during three measurement periods. Tukey's HSD comparisons are presented in Appendices 2.1-2.5.

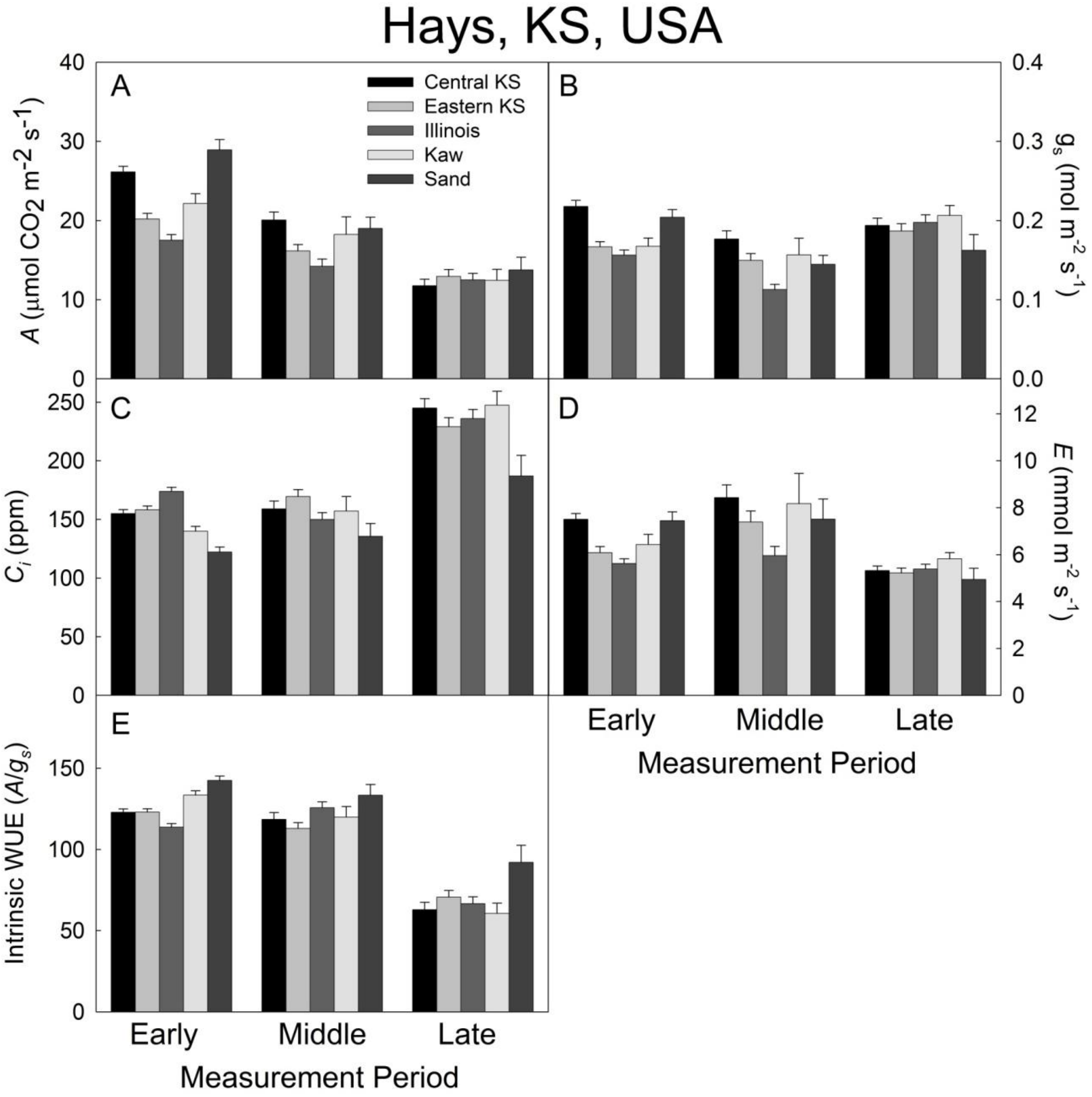

Figure 2.4. (A-E) *A, g_s, C_i, E,* and WUE of *A. gerardii* ecotypes at the Hays, KS site. Panels and abbreviations are as in Fig. 2.2. Bars are means of 10 replicate plots ± SE during three measurement periods. Tukey's HSD comparisons are presented in Appendices 2.1-2.5.

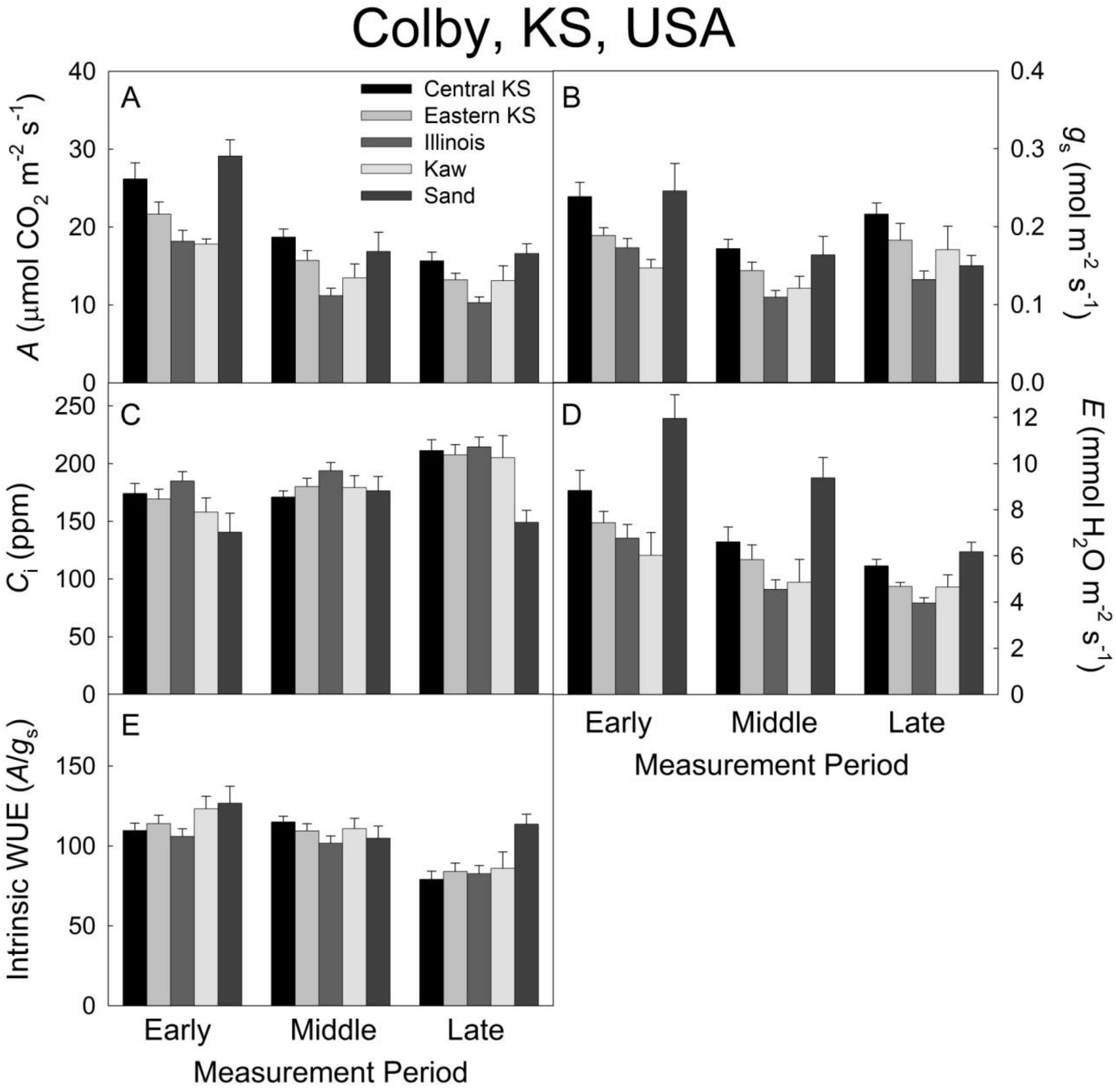

Figure 2.5. (A-E) *A, g_s, C_i, E,* and WUE of *A. gerardii* ecotypes at the Colby, KS site. Panels and abbreviations are as in Fig. 2.2. Bars are means of 10 replicate plots ± SE during three measurement periods. There were no individuals of the Carnahan population at Colby, KS. Tukey's HSD comparisons are presented in Appendices 2.1-2.5.

Appendix 2.1. Tukey's HSD results for photosynthesis.

Site results of Tukey's HSD for photosynthesis (µmol CO_2 m^{-2} s^{-1}).
Sites not connected by the same letter are significantly different.

Site			Least Squares Mean
Carbondale, IL	A		26.69
Hays, KS		B	17.74
Manhattan, KS		B	17.62
Colby, KS		B	17.19

Ecotype results of Tukey's HSD for photosynthesis (µmol CO_2 m^{-2} s^{-1}).
Ecotypes not connected by the same letter are significantly different.

Ecotype			Least Squares Mean
Sand	A		23.16
Central KS	A		21.54
Kaw		B	18.95
Eastern KS		B	18.16
Illinois		B	17.23

Measurement period results of Tukey's HSD for photosynthesis (µmol CO_2 m^{-2} s^{-1}).
Measurement period not connected by the same letter are significantly different.

Measurement Period				Least Squares Mean
Early	A			24.06
Middle		B		20.99
Late			C	14.37

Appendix 2.2. Tukey's HSD results for stomatal conductance.

Site results of Tukey's HSD for stomatal conductance (mol m^{-2} s^{-1}).
Sites not connected by the same letter are significantly different.

Site				**Least Squares Mean**
Carbondale, IL	A			0.242
Hays, KS		B		0.173
Colby, KS		B		0.170
Manhattan, KS			C	0.153

Ecotype results of Tukey's HSD for stomatal conductance (mol m^{-2} s^{-1}).
Ecotypes not connected by the same letter are significantly different.

Ecotype				**Least Squares Mean**
Central KS	A			0.206
Sand	A	B		0.196
Kaw		B	C	0.179
Eastern KS			C	0.174
Illinois			C	0.167

Measurement period results of Tukey's HSD for stomatal conductance (mol m^{-2} s^{-1}).
Measurement period not connected by the same letter are significantly different.

Measurement Period				**Least Squares Mean**
Early	A			0.218
Middle		B		0.179
Late			C	0.155

Appendix 2.3. Tukey's HSD results for intercellular CO_2.

Site results of Tukey's HSD for intercellular CO_2 (ppm).
Sites not connected by the same letter are significantly different.

Site				**Least Squares Mean**
Colby, KS	A			181
Hays, KS	A			177
Manhattan, KS		B		166
Carbondale, IL			C	138

Ecotype results of Tukey's HSD for intercellular CO_2 (ppm).
Ecotypes not connected by the same letter are significantly different.

Ecotype			**Least Squares Mean**
Illinois	A		174
Eastern KS	A		174
Central KS	A		170
Kaw	A		165
Sand		B	144

Measurement period results of Tukey's HSD for intercellular CO_2 (ppm).
Measurement period not connected by the same letter are significantly different.

Measurement Period			**Least Squares Mean**
Late	A		185
Middle		B	157
Early		B	155

Appendix 2.4. Tukey's HSD results for transpiration.

Site results of Tukey's HSD for transpiration (mmol H_2O m^{-2} s^{-1}).
Sites not connected by the same letter are significantly different.

Site				**Least Squares Mean**
Colby, KS	A			6.49
Hays, KS	A			6.48
Carbondale, IL		B		5.14
Manhattan, KS			C	4.50

Ecotype results of Tukey's HSD for transpiration (mmol H_2O m^{-2} s^{-1}).
Ecotypes not connected by the same letter are significantly different.

Ecotype			**Least Squares Mean**
Sand	A		6.51
Central KS	A		6.12
Eastern KS		B	5.35
Kaw		B	5.31
Illinois		B	4.97

Measurement period results of Tukey's HSD for transpiration (mmol H_2O m^{-2} s^{-1}).
Measurement period not connected by the same letter are significantly different.

Measurement Period			**Least Squares Mean**
Early	A		6.60
Middle	A		6.37
Late		B	3.98

Appendix 2.5. Tukey's HSD results for intrinsic water-use efficiency.

Site results of Tukey's HSD for intrinsic water-use efficiency (A/g_s).
Sites not connected by the same letter are significantly different.

Site				Least Squares Mean
Carbondale, IL	A			124.2
Manhattan, KS		B		117.4
Hays, KS			C	106.6
Colby, KS			C	104.3

Ecotype results of Tukey's HSD for intrinsic water-use efficiency (A/g_s).
Ecotypes not connected by the same letter are significantly different.

Ecotype			Least Squares Mean
Sand	A		123.9
Kaw	A	B	114.3
Central KS		B	109.9
Eastern KS		B	108.9
Illinois		B	108.7

Measurement period results of Tukey's HSD for intrinsic water-use efficiency (A/g_s).
Measurement period not connected by the same letter are significantly different.

Measurement Period			Least Squares Mean
Middle	A		120.3
Early	A		116.3
Late		B	102.9

CHAPTER 3: LEAF ANATOMY VARIATION AMONG POPULATIONS OF *ANDROPOGON GERARDII* ALONG A PRECIPITATION GRADIENT

INTRODUCTION

Many plant species show clinal variation of phenotype across latitudinal, longitudinal, or altitudinal gradients (Clausen et al., 1939;Banks and Whitecross, 1971; Chapin and Chapin, 1981; Ehleringer and Cooper, 1988; Hogan et al., 1994; Etterson, 2004; Ingvarsson et al., 2006). The Great Plains of North America has a west-east climatic gradient, where annual precipitation ranges from 30 cm in the west to more than 120 cm in the east (Sala et al., 1988; Epstein et al., 1998). Much of the phenotypic variation among plants across this gradient is environmentally caused (Scheiner and Goodnight, 1984).

Grasses, which are abundant across the Great Plains, can have either the C_3 or C_4 photosynthetic pathway. Conditions in the Great Plains favor C_4 species (Ehleringer, 1978; Epstein et al., 1997), as most precipitation comes during the growing season when temperatures are warm (Adler and HilleRisLambers, 2008). There are prominent structural differences between C_3 and C_4 leaves. C_4 grasses can be differentiated from C_3 grasses by Kranz anatomy in leaves, characterized by enlarged bundle sheath cells containing chloroplasts rich in starch (Dengler et al., 1994; Muhaidat et al., 2007). In addition, few mesophyll cells separate the closely arranged vascular bundles of C_4 grasses compared to the wide spacing and many mesophyll cells between C_3 vascular bundles (Sudderth et al., 2007).

Many grasses are well adapted to xeric environments. Narrow leaves and leaf rolling are common in drought tolerant grasses (Redmann, 1985). Narrow leaves increase the efficiency of heat exchange, can decrease intercepted irradiance (Parkhurst and Loucks, 1972) by half, and increase conductance to heat loss by a factor of three (Redmann, 1983). Specialized epidermal cells, called bulliform cells, facilitate leaf rolling or folding by decreased turgor pressure as plant water potential decreases (O'Toole and Cruz, 1980; Barnes, 1985, 1986; Maricle et al., 2009).

Drought is a prominent environmental stress that influences leaf structure (Smith and Nobel, 1978; Carmo-silva et al, 2009). Decreased soil water potential can decrease leaf length and growth rate (Smith and Nobel, 1978). Carmo-Silva et al. (2009) found specific leaf area and the relative water content of leaves decreased with soil water content. They also measured an increase in the mesophyll to bundle sheath ratio in droughted leaves. Anatomical variation is expected within and among ecotypes in response to water availability.

Common garden experiments are frequently used to determine whether characteristics of a population are influenced by genetic or environmental factors. Leaf anatomy has been studied in common gardens over climatic gradients (Cordell et al., 1998; Hovenden and Schoor, 2004) and variation has generally been attributed to environmental factors (Dickison, 2000). For example, leaf form and function can differ greatly under high and low light conditions. High light can lead to larger and thicker leaves, large and abundant mesophyll cells, increased photosynthetic rates, and higher stomatal density (Nobel et al., 1975; Lichtenthaler et al., 1981). Altitudinal variation in temperature, partial pressure of CO_2, and irradiation affects leaf size (Williams and Black, 1993), stomatal density, and leaf thickness (Körner et al., 1986; Cordell et al., 1998). Increased salinity leads to decreased stomatal pore area and thinner epidermal and mesophyll tissue (Parida et al., 2004). Much published work indicates environment plays the primary role in shaping leaf anatomy of populations within a species (Smith and Nobel, 1978; Hovenden and Schoor, 2004; Carmo-Silva et al., 2009), showing the plasticity of leaf anatomy, but natural selection could shape leaf structure in populations adapted to dry or wet climates.

General leaf anatomy between many grass species has been documented (Brown, 1958; Brown et al., 1985; Garnier and Laurent, 1994; Garnier et al., 1999; Maricle et al., 2009), while anatomical variation and adaptations of leaves among populations of a single species has received relatively little attention. Stace (1991) suggested anatomical features in grasses might be more important than morphological features because anatomical features are less susceptible to environmental change. Among populations, differences have been described in chloroplast shape (Zheng et al., 2000), vascular bundle area, midrib succulence, and stomatal density (Hameed et al., 2009). Ferris et al. (1996) observed leaves of *Lolium perenne* L. and determined stomatal density, mesophyll area, and epidermal cell length varied with CO_2 and temperatures.

Andropogon gerardii Vitman (big bluestem) is dominant in tallgrass and mixed grass prairies (Gustafson et al., 2004) and *A. gerardii* has high variation of phenotypes across the climatic gradient of the Great Plains (McMillan, 1959). The present study investigated variation in leaf anatomy to differentiate environmental and genotypic characters in *A. gerardii*. Leaves of five populations of *A. gerardii*, including sand bluestem (*A. gerardii* var. *hallii* = *A. hallii* Hack.), were sampled from four common gardens across a climatic gradient of the Great Plains and evaluated by light microscopy. Because of conspicuous phenotypic differences, populations were expected to have genetic differences in leaf thickness and internal structure, in addition to environmentally-controlled phenotypic plasticity.

Anatomical and morphological differences have been documented among populations of a single grass species grown at a common site (McWhorter, 1971; Barnes, 1986) and following this, I expected leaf anatomy of *A. gerardii* populations to vary when grown at common sites. I hypothesized (1) xeric populations, sand bluestem and Central KS, would be more drought adapted than mesic populations by containing a higher percentage of bulliform cells for leaf rolling or folding. (2) All populations would have a higher percentage of bulliform cells when grown at dry sites compared to wet sites and (3) plants grown at mesic sites would have larger veins and a greater proportion of the leaf would consist of veins than at xeric sites. (4) Leaves of sand bluestem and Central KS, the xeric populations, would be thicker and based on previous research (5) environment would influence leaf anatomy of populations more than genetics.

MATERIALS AND METHODS

Seed Collection and Planting of Common Gardens

Seeds of five populations of *A. gerardii* were planted in four common gardens along the west-east precipitation gradient of the Great Plains as part of a large reciprocal common garden experiment. Seeds for three *A. gerardii* populations were hand collected in 2008 from Cedar Bluff Reservoir in western Kansas (38°45'N, 99°46'W), Konza Prairie in eastern Kansas (39°05'N, 96°36'W), and Twelve Mile Prairie in southern Illinois (38°46'N, 88°50'W). Seeds of two widely used cultivars, *A. gerardii* 'Kaw' (big bluestem, accession 421276) and *A. gerardii* var. *hallii* 'Garden' (sand bluestem, accession 421277), were obtained from the USDA Manhattan Plant Materials Center in Manhattan, KS.

In autumn 2008, seeds from each population of *A. gerardii* were planted in Metromix 510 potting mix (Scotts Company, Marysville, OH, USA) in 10 cm x 10 cm pots in a greenhouse at Kansas State University. In August 2009, *A. gerardii* plants were transplanted from pots into four common experimental gardens arranged from west to east in Colby, KS (39°24'N 101°04'W), Hays, KS (38°52'N, 99°20'W), Manhattan, KS (39°12'N, 96°35'W), and Carbondale, IL (37°44'N, 89°10'W), following a precipitation gradient ranging from 50 cm annually in Colby, KS, to 120 cm annually in Carbondale, IL. Plants were arranged 50 cm apart and were surrounded by Dewitt Sunbelt landscape fabric (Dewitt Co., Sikeston, MO, USA) to decrease competition from unwanted plants. Common gardens were arranged in 10 rows with one plant of each population in each row, totaling 10 plants of each population at each common garden site. Plants grew under ambient conditions at each site.

Collecting and Fixing of Leaf Tissue

In the middle of the growing season, one leaf from each of three randomly-selected plants of each population was sampled at each common garden site, totaling 60 leaves. The middle portion of the youngest, fully-expanded leaf was taken from these plants. Leaves were fixed in FAA (50% ethanol, 5% glacial acetic acid, 10% formaldehyde, 35% dH_2O), stored at 4°C, and were washed for one hour with 50% ethanol prior to embedding.

Dehydration and Embedding

Leaves were dehydrated and infiltrated with paraffin over a series of ethanol and tert-butyl alcohol solutions after Ruzin (1999) with a minimum of 30 minutes per solution. A final step was added with 33% tert-butyl alcohol and 67% paraffin oil. Leaves were embedded in Carowax (Carolina Biological Supply Co., Burlington, NC, USA) by melting the wax at 58°C and immersing leaves into melted wax for a minimum of 12 hours.

Sectioning, Mounting, and Staining

Embedded leaves were sectioned at thicknesses from 10 - 20 μm with a Spencer Model 815 Microtome (American Optical Co., Buffalo, NY, USA) to find the optimal thickness for each leaf. Glass slides were prepared by smearing a drop of Haupt’s adhesive uniformly over the surface of the slide and then flooding the surface with 4% aqueous formaldehyde. Microtome ribbons were placed on the flooded slide and the slide was placed on a warming tray at 37°C for a minimum of 10 minutes, then removed and stored horizontally overnight. Leaf sections underwent a xylene-alcohol series to dewax and rehydrate. Sections were stained with 1% Safranin and 0.05% Toluidine blue. Dehydration followed with an alcohol-xylene series.

A drop of Kleermount (Carolina Biological Supply Co., Burlington, NC, USA) was placed on the processed slides and a coverslip was placed on the slide. Slides were stored horizontally overnight.

Measurements

An Olympus BX51 microscope (Olympus Corporation, Center Valley, PA, USA) with an attached Olympus DP71 digital camera was used to take brightfield images of leaf sections. Images were analyzed with Image J (National Institute of Health, Bethesda, MD, USA). Measurements on each section included maximum leaf thickness at the midrib, maximum and minimum leaf thickness at a non-midrib area, interveinal distance, minor and major vein area, percent bulliform cells, and percent vascular tissue. Interveinal distance was measured as the minimum distance between the two nearest bundle sheath cells of adjacent vascular bundles. Maximum and minimum leaf thicknesses at a non-midrib area were measured at a minimum of 200 µm from the midrib, but at least 200 µm from the leaf edge. Minor and major vein areas include the vascular bundle and bundle sheath. Measurements for each leaf are the mean of six duplicate veins to account for small variations in vein size.

Statistical Analysis

All data were analyzed with JMP 9.0.2 (SAS Institute Inc., Cary, NC, USA). Replicate measures were from three plants of the same population at each site. A

MANOVA was used; factors were site, population and the site × population interaction. This was followed with univariate tests and Tukey's honest significant difference tests to determine significant differences between means (Appendix 1). To determine statistically significant differences, analyses were performed at $\alpha=0.05$.

RESULTS

Vascular Tissue

Leaves from all populations and sites had Kranz anatomy (Dengler and Nelson, 1999) with two distinct types of photosynthetic cells and closely spaced veins (Fig. 3.1). Photosynthetic tissue was comprised of a single layer of large chlorenchymatous bundle sheath cells encircled by one layer of chlorenchymatous mesophyll cells. Each mesophyll cell was in direct contact with a bundle sheath cell. In some cases, colorless parenchyma cells separated mesophyll cells from those of neighboring veins. Bundle sheath cells were large and contained centrifugal chloroplasts. Frequently the bundle sheath of major veins was connected to the epidermis by a bundle sheath extension. Bundle sheath extensions were especially notable in the sand bluestem population (Fig. 3.1). In all leaves, major veins contained an inner mestome sheath of sclerenchymatous cells. Mestome sheaths surrounded the phloem, but not the entire vascular bundle, similar to observations by Brown (1975). In all populations except sand bluestem, bundle sheath cells on the xylem side of major veins had thick secondary walls. The most heavily lignified bundle sheath cells contained no chloroplasts. Additionally, the abaxial side of major and minor veins and the adaxial side of major veins were protected by sclerenchymatous fiber bundles, which were particularly conspicuous in the sand bluestem population. Large bulliform cells were aligned with the adaxial side of minor veins. Interveinal distances ranged from 21 μm to 53 μm across sites and populations (Fig. 3.2D). In some leaves, interveinal distances were short because mesophyll cells of neighboring vascular bundles were directly contacting each other. In other leaves, colorless parenchyma cells separated mesophyll cells of neighboring vascular bundles, increasing the interveinal distance.

Interveinal distance varied from leaf to leaf (Table 3.1) and was not a site (F=0.96, df=3, p=0.422) or population (F=1.67, df=4, p=0.176) difference. No interactions were detected (F=0.70, df=12, p=0.736).

Cross-sectional areas of major veins ranged from 4,925 to 23,925 μm^2 per vein across sites and populations (Fig. 3.3A). Vein sizes differed by site because of differences in phloem and xylem cross-sectional area, often with larger sieve tubes and vessel elements at the mesic Carbondale site compared to other sites. At Carbondale, IL, major veins were 47% and 49% larger than at Manhattan, KS and Hays, KS, respectively (F=5.18, df=3, p=0.004). At Colby, KS, major veins were not different from other sites. No differences were detected among populations (F=1.22, df=4, p=0.316) and no interactions were detected (F=1.04, df=12, p=0.428).

Minor veins were surrounded by large chlorenchymatous bundle sheath cells with no lignification and no mestome sheath similar to observations of Brown (1975). The cross-sectional area of minor veins ranged from 1,184 to 3,688 μm^2 per vein across sites and populations (Fig. 3.3B). No differences were detected across sites (F=0.88, df=3, p=0.462), or among populations (F=1.29, df=4, p=0.291), and no interactions were detected (F=0.78, df=12, p=0.670).

The percentage of leaves comprised of vascular bundles ranged from 15% to 33% across sites and populations (Fig. 3.3D). Variation arose from abundances of bulliform cells, colorless parenchyma cells, and mesophyll cells. No differences in percentage of vascular bundles were detected across sites (F=1.71, df=3, p=0.181). Among populations, the vascular bundle areas of 12 Mile and Kaw were 15% and 16% greater than in Cedar

Bluff (F=2.41, df=4, p=0.065), whereas sand bluestem and Konza were not different from other populations. No interaction was detected (F=0.86, df=12, p=0.593).

Bulliform Cells

Bulliform cells occurred in groups of two to eight adjacent cells, and occupied most of the epidermis adaxial to minor veins (Fig. 3.1). Bulliform cells were not present on the abaxial side of leaves. Bulliform cells were the largest cells in leaves, but differed considerably in size among populations and sites (Table 3.1), with larger bulliform cells at the mesic Carbondale, IL site and smaller bulliform cells at xeric sites. The percentage of the leaf comprised of bulliform cells ranged from 15% to 35% across sites and populations (Fig. 3.3C). Across sites, Colby, KS had a 27% higher percentage of bulliform cells compared to plants at Manhattan, KS (F=5.23, df=3, p=0.004). At Hays, KS and Carbondale, IL, percentage of bulliform cells did not differ from other sites. Among populations, 12 Mile and Konza had a 27% and 33% greater proportion of bulliform cells than did sand bluestem (F=4.73, df=4, p=0.003), while Cedar Bluff and Kaw were not different from other populations. No interaction was detected (F=0.70, df=12, p=0.739).

Midribs

Midribs consisted of large, colorless parenchyma cells on the adaxial side of leaves and chlorenchyma and vascular tissue on the abaxial side, with a large abaxial bundle of sclerenchymatous fibers protecting the midvein (Fig. 3.4). Midveins typically had a prominent mestome sheath. In some cases, mechanical cells lined the adaxial side of the midrib. Leaf midrib thickness ranged from 198 µm to 740 µm across sites and populations (Fig. 3.2A). Variation in midrib size was predominantly due to the number and size of colorless parenchyma cells. The abundance of colorless parenchyma cells in each midrib cross-section ranged from under 20 to well over 100 cells. Variation in the size of vascular bundles and thickness of abaxial fiber bundles were minor contributions to midrib thickness. Midribs were 41% and 67% thicker in Carbondale, IL than in Manhattan, KS or Hays, KS, respectively (F=6.89, df=3, p<0.001). Midrib thicknesses at Manhattan and Hays were not different from each other, and midrib thicknesses at Colby, KS were not different from other sites. There were no differences among populations (F=0.57, df=4, p=0.687) and there were no interactions (F=0.35, df=12, p=0.972).

Leaf Thickness (non-midrib)

Leaf blades retained a consistent thickness at distances from 200 µm outside the midrib to 200 µm inside the leaf edge (Figs. 3.1, 3.4). The majority of leaf volume consisted of vascular bundles, chlorenchyma, epidermal cells, and bulliform cells, with minor contributions from intercellular spaces and colorless parenchyma cells. Leaf thickness varied primarily due to sizes of bulliform cells and vascular bundles. Maximum leaf thickness ranged from 104 µm to 243 µm across sites and populations (Fig. 3.2B). Maximum leaf thickness did not differ across planting sites (F=2.05, df=3, p=0.122). Among populations, sand bluestem leaves were not different from Konza leaves, but were 20%, 28%, and 42% thicker than Cedar Bluff, 12 Mile, and Kaw, respectively (F=8.56, df=4, p<0.001). Konza was not different from Cedar Bluff or 12 Mile, but was 23% thicker than Kaw. Cedar Bluff, 12 Mile, and Kaw were not different from each other. No interaction was detected (F=1.27, df=12, p=0.276).

Minimum leaf thickness ranged from 85 to 208 µm across sites and populations (Fig. 3.2C). Minimum leaf thickness was not different across sites (F=1.94, df=3, p=0.138). Sand bluestem and Konza were not different, but had minimum thicknesses 28% and 23% thicker than Kaw, respectively (F=3.58, df=4, p=0.014), whereas Cedar Bluff and 12 Mile were not different from any other populations. No interactions were detected (F=0.54, df=12, p=0.875).

DISCUSSION

General Leaf Anatomy of **Andropogon gerardii**

Studies of grass leaf anatomy have included variation across seasons (Ferris et al., 1996), between perennials and annuals (Garnier and Laurent, 1994), between C_3 and C_4 species (Dengler et al., 1994), within C_4 subtypes (Carmo-Silva et al., 2009), across C_4 species (Magai et al., 1994), between species of a single genus (Maricle et al., 2009), and adaptations of species to water stress (Maricle et al., 2007; Hameed et al., 2009). Other studies have compared variation among multiple species along a precipitation gradient (Cunningham et al., 1999), but few studies have addressed variation in leaf anatomy of a single species over a precipitation gradient (Vasellati et al., 2001). In my study, five populations of *A. gerardii* were planted in common gardens across a precipitation gradient to investigate which anatomical characteristics of leaves are environmentally controlled, and which are genetically controlled. All populations of *A. gerardii* from all sites exhibited Kranz anatomy in the "classical" NADP-ME type, with one layer of large bundle sheath cells containing centrifugally-arranged chloroplasts, similar to observations by Dengler and Nelson (1999). Variation in leaf anatomy was due to a combination of drought adaptation of populations and plasticity of anatomical characteristics.

Drought Adaptations

Many grasses are well adapted to xeric environments; typical adaptations are narrow leaves (Redmann, 1985) and specialized epidermal cells called bulliform cells. Bulliform cells facilitate leaf folding and rolling, decreasing the surface area of leaves and reducing water loss (O'Toole and Cruz, 1980; Maricle et al., 2009). Bulliform cells decrease in volume as turgor pressure and plant water potential decrease, leading to the rolling or folding action of the leaf. *Andropogon gerardii* has abundant bulliform cells (Magai et al., 1994) and the percentage of the leaf that consists of bulliform cells varied among populations and sites. Although bulliform cells were under considerable environmental control, there was also a significant genetic component. Sand bluestem had a lower percentage of bulliform cells compared to big bluestem populations. Previous observations indicate sand bluestem rolls leaves to a greater degree than big bluestem (Barnes, 1985, 1986). While bulliform cells are involved in leaf rolling, perhaps proportion of bulliform cells in leaf does not directly relate to leaf rolling ability. Placement of bulliform cells in a leaf might be more important than size. The mesic big bluestem populations generally had greater proportions of bulliform cells than did the xeric population. These findings were contrary to our expectation that the most xeric population, Cedar Bluff, would have the greatest proportion of bulliform cells. A large proportion of bulliform cells was anticipated to be a drought adaptation to enhance leaf rolling and folding. While having larger and more abundant bulliform cells might allow a leaf to roll more under drought, it requires more water to produce large bulliform cells and maintain their turgor pressure. Furthermore, leaf rolling and folding in grasses might have a limited role in water loss, as stomata typically close prior to leaf rolling or folding during times of water stress (Redmann, 1983). Perhaps more important were prominent differences in leaf size between populations of *A. gerardii*. Plants of mesic populations had longer and wider leaves compared to xeric populations (Tetreault et al. in prep.). The smaller leaf size of xeric populations reduces evaporative surface area and might reduce the need for water conservation by other strategies like leaf rolling. In xeric environments, narrower and shorter leaves must be more beneficial than large bulliform cells for conserving water.

Studies have indicated leaf thickness increases as annual precipitation decreases (Abrams, 1990), indicating increased thickness of leaves is an adaptation to drought. Hovenden and Schoor (2004) reported leaf thickness of southern beech was dependent on population origin rather than the planting site. Similarly, we found maximum and minimum thicknesses of *A. gerardii* leaves to be influenced by the population of origin and not the planting site. Sand bluestem had leaves 28% thicker than the Kaw leaves, supporting sand bluestem as the most drought-adapted population. This is consistent with the work of Barnes (1986) and Ravenek et al. (in review), who measured thicker leaves in sand bluestem compared to big bluestem. Sand bluestem generally grows in sandy soils that have poor water retention (Barnes, 1985). Having a thicker leaf decreases the surface area to volume ratio, decreasing the evaporative surface of the leaf. Thicker leaves often have greater quantities of mesophyll, increasing photosynthetic capabilities at a rate of transpiration similar to an equally-sized, thin leaf (Nobel et al., 1975; Sims and Pearcy, 1992). Moreover, in the present study, all plants were grown in bright sunlight and leaves subjected to bright sunlight are expected to be thicker than shaded leaves. Our measures were consistent with sun leaf measures in *A. gerardii* by Knapp and Gilliam (1985).

Big bluestem populations have a greater density of leaf trichomes compared to sand bluestem (Caudle and Maricle, unpublished data), consistent with descriptions by Rydberg (1932). Leaves of sand bluestem can also have trichomes (McGregor et al., 1986), but instead typically have glaucous leaves (Rydberg, 1932; McGregor et al., 1986). Sand bluestem has five times more epicuticular wax than big bluestem (Ravenek et al., in review), likely an alternative strategy for water conservation in sand bluestem (Barnes, 1986). Consistent with our observations of bulliform cells, drought adaptation in *A. gerardii* leaves might be primarily based on area of the evaporative surface, and less dependent on factors like leaf rolling (Redmann, 1983).

C_4 plants have closely-spaced veins, maintaining short diffusive distances for metabolite transfer (McKown and Dengler, 2007). In *A. gerardii*, the percentage of leaf area consisting of vascular tissue was lower in populations from xeric regions compared to the mesic populations. The area of vascular tissue often decreases as soil moisture decreases (Barss, 1930; Penfound, 1931). As soils become drier, less water is available to pass through plants and leaves, reducing the need for vascular tissue to transport water. Contrary to this, Oppenheimer et al. (1960) stated an increase in vascular area is a drought adaptation and Jacobsen et al. (2007) discovered C_3 species under high water stress generally had a greater density of xylem. Proportion of vascular tissue in C_4 leaves is dependent on the size of veins and interveinal distance. It is unlikely C_4 plants will change proportion of vascular tissue as a response to drought because veins are already closely spaced. Within C_4 grasses, vascular bundles are closely arranged and there is likely no advantage to decreasing this already short distance. In addition, no differences were detected between populations or sites for interveinal distances or the size of minor veins. Drought-adapted populations had greater leaf thickness; when paired with equally sized veins and interveinal distances, thicker leaves resulted in a lower proportion of cross-sectional area of vasculature.

Percentage of vascular tissue is an adaptation among populations but was not influenced by drought in this study. By contrast, major vein size was environmentally influenced, but did not differ among populations. Consequently, various features of vasculature are important in drought responses of *A. gerardii*, either from genetic or environmental influences.

Plastic Characteristics

Size of leaf major veins in *A. gerardii* was controlled by water availability, as the drier site had smaller veins compared to mesic sites. Major veins decreased in size as soil moisture decreased. Sizes of major veins in this study were similar to measures by Knapp and Gilliam (1985), who demonstrated vein cross-sectional area in *A. gerardii* increased with bright sunlight. The results of the present study indicate veins can also become larger with increasing moisture availability. The largest veins for all populations were in Carbondale, IL, where soil moisture was consistently high. Similarly, Villar-Salvador et al. (1997) noted maximum size of xylem vessels of many *Quercus* species increased in size with annual precipitation. An increase in area of major veins would allow passage of greater volumes of water as it becomes available. However, Barnes (1986) measured the reverse pattern, where size of major veins increased in bluestems grown in sand dunes compared to those grown in meadows. This suggests vascular development in *A. gerardii* leaves might depend on more than soil water content. Increased vein size might be an adaptation to sandy soils, allowing sand bluestem to take up water quickly when available, because sandy soils have poor water retention (Campbell and Norman, 1998). Interactions between soil water potential, soil type, and the resulting plant development could be an interesting area for future research.

Leaf midrib thickness increased across sites as soil moisture increased. Midribs primarily consisted of large, colorless parenchyma cells that were highly vacuolated and likely functioned to store water. Carmo-Silva et al. (2009) noted leaves of *Paspalum dilatum* had prominent midribs, causing folding of leaves rather than rolling. Barnes (1985, 1986) noted big bluestem leaves generally fold, whereas sand bluestem leaves roll into cylinders. Thick midribs of the mesic big bluestem populations might induce leaf folding rather than rolling. Leaves at the Carbondale site had larger colorless parenchyma cells compared to leaves at xeric sites. Small cells are adapted to maintain lower water potentials compared to large cells (Cutler et al., 1977), enabling the plant to retain turgor pressure in drier soils. As soil moisture increased, more water was available for cell and leaf expansion. *Andropogon gerardii* grown in mesic environments evidently has an increased ability to fold leaves at the midrib. Nevertheless, how leaf thickness in *A. gerardii* is affected by soil type and moisture remains an open question. In a field experiment, Barnes (1986) measured increased leaf thickness in bluestems grown in sand dunes compared to those grown in meadows. By contrast, a greenhouse experiment by Ravenek et al. (in review) measured lower specific leaf area (thicker leaves) in big bluestem and sand bluestem grown in prairie soil compared to sand. Measurements of Barnes (1986) and Ravenek et al. (in review) are likely different due to soil type, water availability, and environmental variation between field and greenhouse such as radiation, wind, and humidity during leaf development of *A. gerardii*.

Size of bulliform cells was influenced by the environment (Table 3.1). At Manhattan, KS, where rainfall was abundant, bulliform cells were 26% larger than at Colby, KS where conditions were dry; however, Hays, KS and Carbondale, IL were not different from Colby and Manhattan. The 2010 growing season in Carbondale was drier than average, which contributed to the smaller-than-expected area of bulliform cells in leaves at Carbondale. In addition, the large and abundant colorless parenchyma cells in leaf midribs at Carbondale might offset the benefit of large bulliform cells adaxial to minor veins. Leaf folding potentially reduces the need for large bulliform cells. These data support leaf rolling or folding, facilitated by bulliform cells and midrib parenchyma cells, as less beneficial than narrower, shorter leaves in xeric environments.

Conclusion

Whereas environmental factors are highly influential on leaf anatomy, a genetic predisposition for certain characteristics plays an equal role. Within *A. gerardii*, clear differences in characteristics were evident between drought-adapted and mesic populations. Genetic characteristics in drought-adapted leaves of *A. gerardii* included a smaller proportion of bulliform cells, a smaller proportion of vascular tissue, and thicker leaves with a reduced evaporative surface area compared to leaves of *A. gerardii* from mesic sites. Plastic characteristics of drought-adapted leaves included decreased midrib thickness, smaller major veins, and a smaller proportion of bulliform cells within each leaf. Leaves at xeric sites responded to smaller volumes of water by having smaller major veins and a decreased surface area, thereby reducing transpiration.

Leaves from mesic environments genetically had more and larger bulliform cells, more vascular tissue, and thinner, longer, and wider leaves. As precipitation in an environment increased, leaf midribs became thicker and major veins and bulliform cells became larger. Leaves at mesic sites had larger major veins to increase water transport and thick midribs with abundant colorless parenchyma (water storage) cells. In addition, large bulliform cells were available to increase leaf rolling, potentially reducing water loss when conditions became dry.

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Table 3.1. Main and interactive effects of the population of *A. gerardii* and the planting site on leaf anatomy (df error = 40)

	Population			Site			Population*Site		
	df	F	P	df	F	P	df	F	P
Midrib thickness	4	0.57	0.687	3	6.89	**<0.001**	12	0.35	0.972
Maximum thickness (non-midrib)	4	8.56	**<0.001**	3	2.05	0.122	12	1.27	0.276
Minimum thickness	4	3.58	**0.014**	3	1.94	0.138	12	0.54	0.875
Interveinal distance	4	1.67	0.176	3	0.96	0.422	12	0.70	0.736
Major veins (area)	4	1.22	0.316	3	5.18	**0.004**	12	1.04	0.428
Minor veins (area)	4	1.29	0.291	3	0.88	0.462	12	0.78	0.670
% Bulliform cells	4	4.73	**0.003**	3	5.23	**0.004**	12	0.70	0.739
% Vascular bundles	4	2.41	*0.065*	3	1.71	0.181	12	0.86	0.593

Degrees of freedom (df), F-scores (F), and P-values (P) from statistical analysis of variance are presented. Significant values (P<0.05) are bold and values close to significance are italicized.

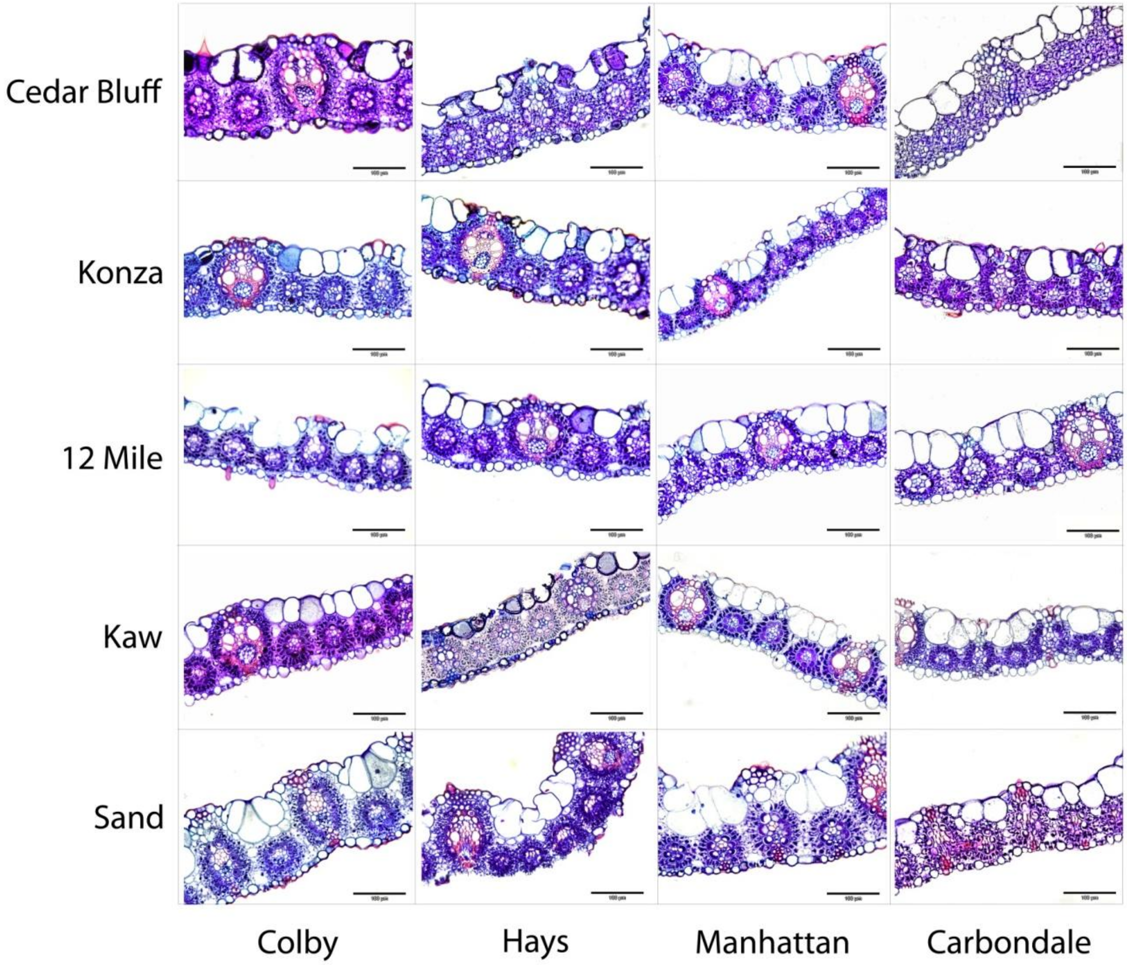

Figure 3.1. Leaf cross-sections of five populations of *A. gerardii* leaves (rows) at each planting site (columns), arranged from most xeric site on the left to most mesic site on the right. Scale bars, 100 µm.

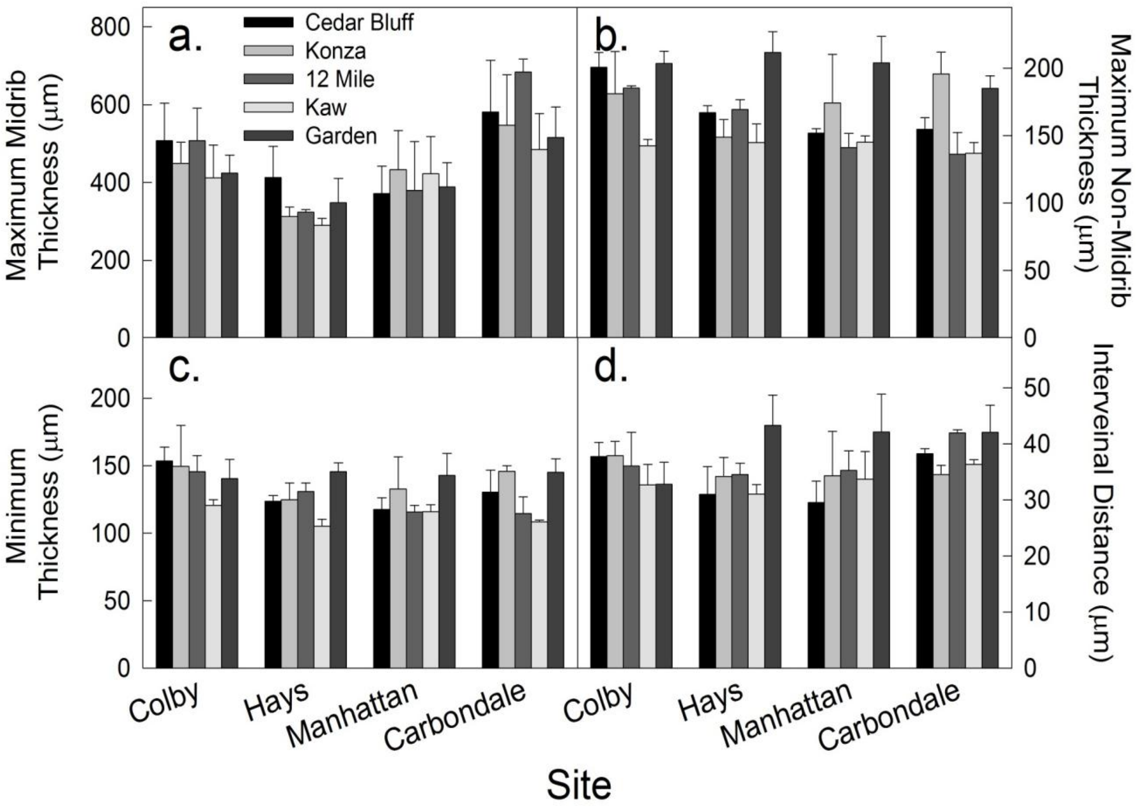

Figure 3.2. (a) Maximum midrib thickness, (b) maximum leaf thickness outside of the midrib, (c) minimum leaf thickness, (d) interveinal distance, in five *A. gerardii* populations at the four planting sites. Bars are means from three replicate leaves ± SE. Tukey's HSD comparisons are presented in Appendix 3.1.

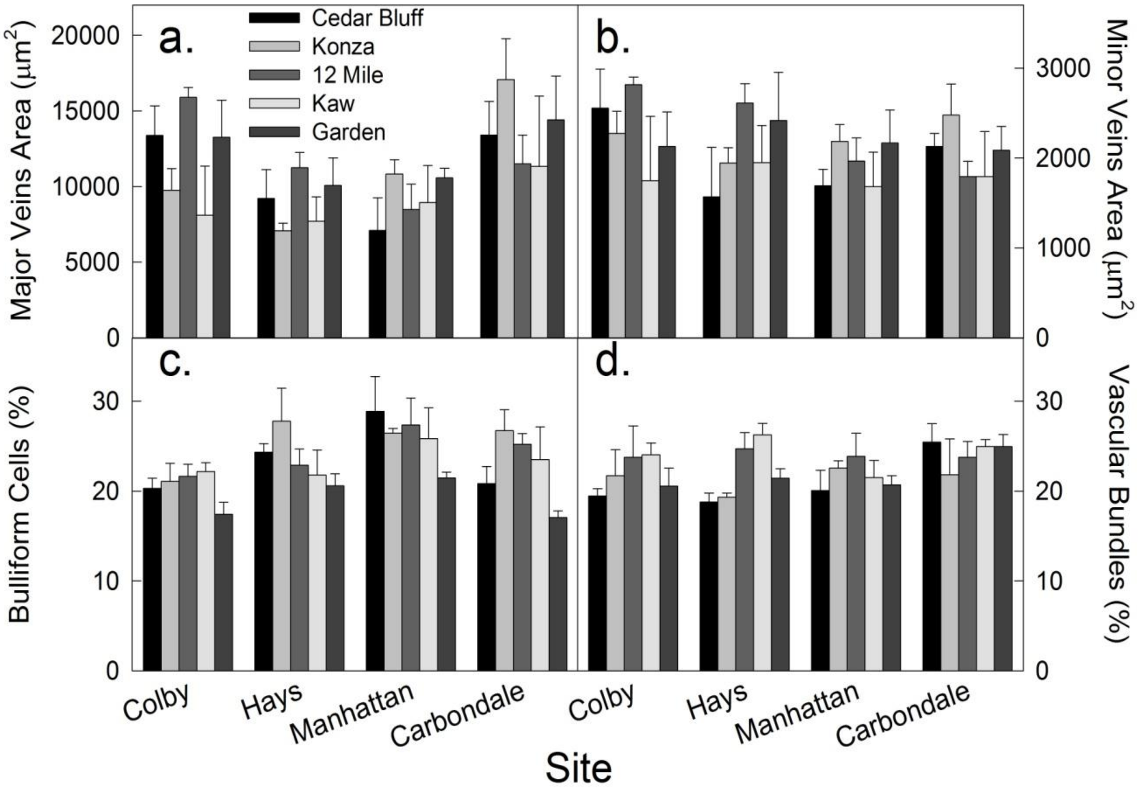

Figure 3.3. (a) Cross-sectional area of major veins, (b) cross-sectional area of minor veins, (c) cross-sectional area of leaf consisting of bulliform cells, and (d) cross-sectional area of leaf consisting of vascular bundles in five *A. gerardii* populations at the four planting sites. Bars are means from three replicate leaves ± SE. Tukey's HSD comparisons are presented in Appendix 3.1.

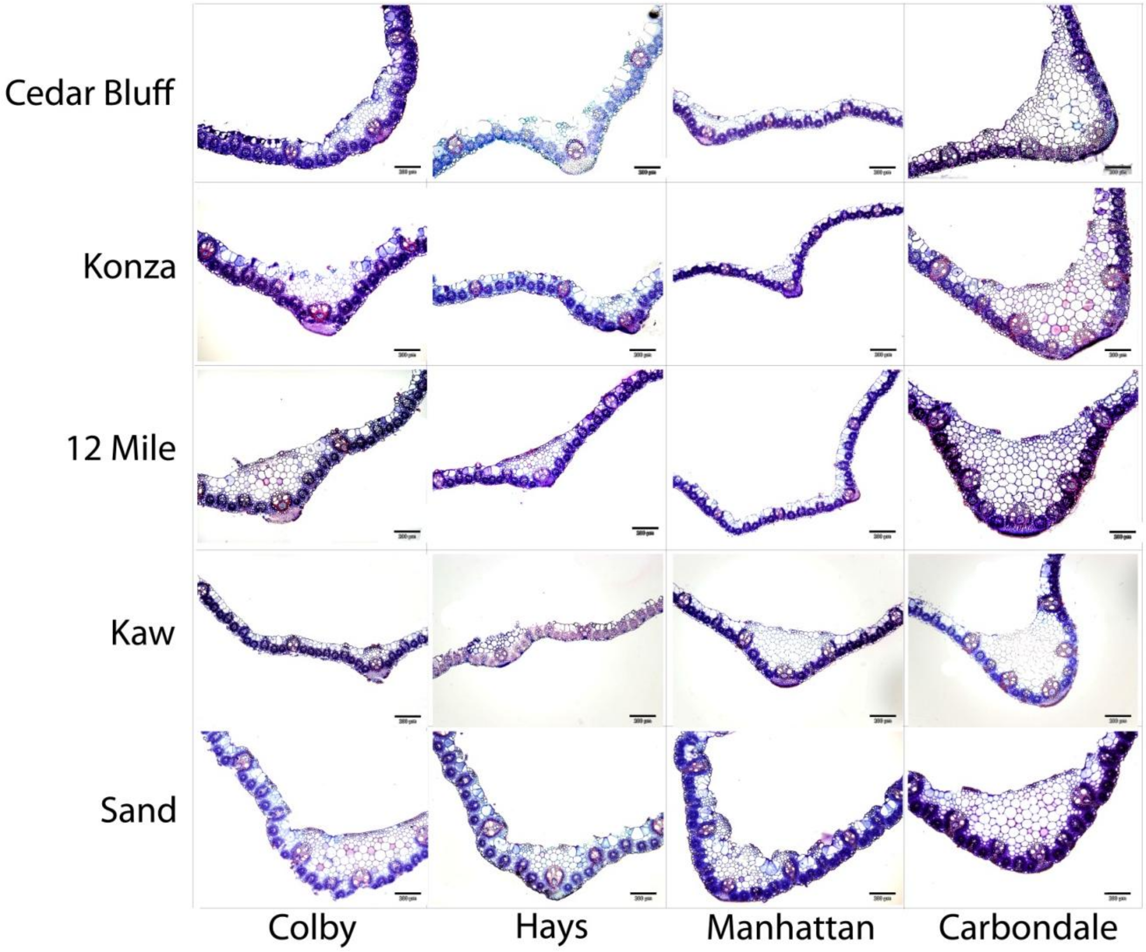

Figure 3.4. Leaf cross-sections of *A. gerardii* leaves showing midribs of each population (rows) at each planting site (columns), arranged from most xeric site on the left to most mesic site on the right. Scale bars, 200 µm.

Appendix 3.1. Tukey's HSD results for leaf anatomy characteristics.

Site results of Tukey's HSD for maximum midrib thickness (μm). Sites not connected by the same letter are significantly different.

Site			**Least Squares Mean**
Carbondale, IL	A		562.5
Colby, KS	A	B	459.9
Manhattan, KS		B	398.8
Hays, KS		B	337.1

Populations results of Tukey's HSD for maximum leaf thickness (μm) at a non-midrib area. Populations not connected by the same letter are significantly different.

Ecotype				**Least Squares Mean**
Sand	A			201.0
Konza	A	B		174.9
Cedar Bluff		B	C	168.5
12 Mile		B	C	157.9
Kaw			C	142.3

Populations results of Tukey's HSD for minimum leaf thickness (μm) at a non-midrib area. Populations not connected by the same letter are significantly different.

Ecotype			**Least Squares Mean**
Sand	A		143.4
Konza	A		138.2
Cedar Bluff	A	B	131.2
12 Mile	A	B	126.6
Kaw		B	112.5

Site results of Tukey's HSD for maximum midrib thickness (μm^2). Sites not connected by the same letter are significantly different.

Site			Least Squares Mean
Carbondale, IL	A		13541
Colby, KS	A	B	12082
Manhattan, KS		B	9188
Hays, KS		B	9062

Populations results of Tukey's HSD for proportion of leaf consisting of bulliform cells. Populations not connected by the same letter are significantly different.

Ecotype			Least Squares Mean
Konza	A		0.255
12 Mile	A		0.243
Cedar Bluff	A	B	0.236
Kaw	A	B	0.233
Sand		B	0.191

Site results of Tukey's HSD for proportion of leaf consisting of bulliform cells. Sites not connected by the same letter are significantly different.

Site			Least Squares Mean
Manhattan, KS	A		0.260
Hays, KS	A	B	0.235
Carbondale, IL	A	B	0.227
Colby, KS		B	0.205