PLANT STRESS AND DRIVERS IN ECOSYSTEM

SUSTAINABILITY

By

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CHAPTER I

INTRODUCTION

Sustainability

With increased awareness of humanity's impact on the planet, the idea of

sustainability weighs heavy in plans to manage our natural resources. Sustainability

allows the use of a resource without exhausting it. Rather than draining resources,

sustainable practices maintain them through careful regulation of consumption and waste

production (Goodland 1995). Sustainable agriculture has been a topic of debate among

environmentalists, with definitions changing so that emphasis of protection ranges from

farmers and their lifestyle to the environment taking first priority. Environmental

historian John Opie summed up the underlying goal behind sustainability well when he

said "sustainability gives priority to preservation and improvement of fertile soils and

expansion of supplies of clean water and protection and regeneration of a satisfying

quality of life in the work force," and defined sustainable agriculture as "development

that meets the needs of the present without compromising the ability of future generations

to meet their own needs" (Hurt 2001). The earth is host to myriad life forms, many of

which we know little about. As human beings our first concern regarding the

environment often relates to the planet's ability to sustain human life, with other species taking second place. However, we cannot effectively determine which species are integral to maintaining human life, so it is essential to minimize human impact on the entire ecosystem (Goodland 1995).

Focusing on human needs may not be the best way to approach our handling of the environment because as the population grows, humans create a greater demand for food, housing, and recreation which in turn places a greater strain on natural resources and increases waste production.

Agriculture now occupies 38% of the earth's terrestrial surface, with about 75% of that devoted to raising animals (feed production, grazing, and pasture). Production of common crops like cereals, fruits, and vegetables increased by 47% between 1985 and 2005. The intensification of agriculture has led to water quality degradation and pollution, contributing about 30-35% of the world's greenhouse gas emissions (Foley et al. 2011). With greater tracts of land being turned to supporting human life, the ability of the planet to support the same variety of life forms is challenged, increasing the likelihood of a catastrophic correction event which would shift the population back below a sustainable carrying capacity. Biodiversity is a developing concern, and both federal and private rangeland will be affected by changing regulations intended to preserve it. A loose definition of biodiversity is "the variety of life and its processes," including, but not limited to, variations in living organisms and their genetic material, the habitats and ecosystems in which they live, and the interaction of biotic and abiotic factors within these environments (West 1993).

One of the predominant biomes in the United States—and also a biome at risk--is the Great Plains. As with any plan regarding land preservation, dissent is inevitable. There are many environmentalists who believe the only way to ensure the well-being of the land is to de-privatize property and make land a public commodity (Hurt 2001). De-privatizing land is not only impractical, but would also be met with great resistance from citizens. One of the ways a compromise can be reached is through the Conservation Reserve Program (CRP), which encourages private landowners to preserve their land for the future, in essence making privately held land part of a publicly held trust.

The CRP has been a major instigator in replanting grassland in the Great Plains. As the prairie is gradually restored by such programs, many changes occur in ecosystem structure which are poorly understood, especially in former farmland that is being returned to native range. According to Camill et al. (2004), studies of grassland restoration indicate that biodiversity gradually decreases over time in restored habitats and despite the efforts of reserve programs the tallgrass prairie will not regain its rich diversity in the near future, if ever. The CRP can best be described to perform "functional restoration" as the former diversity of the Great Plains is not being fully reclaimed (Camill et al. 2004).

Great Plains

The Great Plains is a challenging ecosystem for agricultural production, with extreme temperature fluctuations, variable precipitation, and soil that demands careful management to prevent erosion (Duram 1995). Land that is not currently being farmed in the Great Plains such as the tallgrass prairie fall under the category of rangelands.

Roughly 43 percent of the United States can be classified as rangeland, and about 70 percent of total landmass in the world. Rangelands are often defined as lands that are not farmed but are capable of supporting life with the native vegetation (Vavra 1995).

The productivity of tallgrass prairies worked against them, as most of the native habitat was destroyed after European settlement due to non-sustainable row crop agriculture (Camill et al. 2004). In the 1930's, a devastating drought and dust bowl were the result of farming soils that should not have been tilled along with poor farming practices in the western Great Plains. After 1934, rangeland practices were bent toward sustainable livestock production, but problems such as soil loss and invasive weeds prevented recovery from previous damage. The founding principles of restoration and reclamation ecology stemmed from the attempt to repair the damaged rangelands in the Great Plains (Vavra 1995).

One of the most direct ways human beings impact ecosystems is through land use practices, many of which dramatically change the native vegetation. Cropping alters the magnitude and direction of the carbon flux between the plant-soil system and the atmosphere. In temperate grasslands cropping can temporarily increase aboveground productivity while decreasing belowground productivity and decreasing soil organic carbon quantity and quality (Bradford et al. 2005).

Ecology

Ecology is the study of organisms and their interaction with each other and the abiotic components of their surroundings. Without understanding ecology, we cannot properly develop a plan for sustainability. To correctly plan how to manage our

resources, we must first understand the complex relationship between living things and their environment. In particular, plants are an integral component of ecosystems and a key player in preserving ecosystem sustainability. Understanding plant stress and how environmental conditions affect plants' ability to tolerate stress may facilitate exploring drivers in ecosystem function and sustainability. In addition to stress, studying how plants interact with soil microbes and their combined effect on the environment is key to understanding ecosystems. For example, rising CO_2 is an important factor to consider when attempting to predict future ecosystems. A consequence of elevated CO_2 is a larger C/N ratio in the soil largely due to increased root biomass, turnover, and exudation, and nutrients may eventually have a reduced flow in sustained high CO_2 environments. Studies have shown that under elevated CO_2 non-mycorrhizal plants increase substrate release into the soil, allowing soil microflora to increase mineral sequestration and thus limit nutrient availability to plants. (Diaz 1995). Another factor to consider is the availability of nitrogen and how organisms affect availability. Nitrifiers, denitrifiers, and N_2 fixers are some of the microbial groups responsible for maintaining the nitrogen cycle (Patra et al. 2005). In stressful environments such as salt marshes, deserts, and disturbed areas plants can facilitate the growth of their neighbors by improving the harsh conditions of their habitat (Hacker and Bertness 1995). The environment also exerts selective pressure on plants, removing those plants which lack traits that allow them to grow and reproduce under specific conditions (Diaz et al. 1998).

Switchgrass

Switchgrass is a native warm-season C4 perennial grass found growing in the undisturbed tallgrass prairie with a broad native range spanning from northern Mexico to southern Canada and most of the land east of the Rocky Mountains (Barney et al. 2009; Sanderson and Reed 2000). Along with forage, it is useful for a hay crop and can be used for soil or water conservation (Xu et al. 2010). Its ecology depends on grazing and fire. Switchgrass is adapted for a broad range of environmental conditions due to the diverse genotypic variation in the species (Parrish and Fike 2005). There are two ecotypes; upland, which is found in drier sites, and lowland, which prefers wetter habitats (Stroup et al. 2003). Switchgrass is a crop of interest regarding carbon sequestration and biofuel production and has been shown to sequester more carbon than most row crops (Ugarte et al. 2010). Switchgrass is used extensively in the CRP to control erosion. It has also been shown to facilitate the breakdown of herbicides, trinitrotoluene, polychlorinated biphenyls, polynuclear aromatic hydrocarbons, chromium, and radionuclides (Parrish and Fike 2005).

Biotic Interactions

Plant-Microbe Interactions. Soil microbes are staggeringly diverse, with possibly several thousand genomes per gram of soil. Plant-microbe interactions are relatively understudied, largely due to the difficulties in such an endeavor. Many studies exclude microbes because of use of artificial soils, soil treatments that deplete the levels of microbes, or the view of microbes as an extension of the plant and not an integral and separate factor in ecosystem function (Reynolds 2003).

It is apparent that the interactions between plants and microbes play a very important role in ecosystems. Microbes decompose matter and make it available to plant use, while plants supply much of the organic material for microbe use; however, plants and soil microbes also compete for nutrients (Reynolds et al. 2003). By reducing organic matter to its mineral components, microbes increase nutrient availability for plants and enhance productivity. Microbes may also convert nitrogen into its gaseous form and thus decrease availability for plants. Through their effect on plants, microbes impact herbivores by altering the nutrient availability of soils (Hines et al. 2006). Other soil microbes protect plants from pathogens and help plants take up nutrients (Reynolds et al. 2003). Soil resources are more affected by plant presence than plant species (Robles et al. 1997).

Plant-Herbivore Interactions. Large herbivores directly and indirectly impact plant community and structure, creating feedbacks, affecting nutrient cycling, energy flux, and are themselves a disturbing force. Plant and soil microenvironments are also sensitive to ungulate activity. If palatable plants are not severely damaged from grazing they may become dominant over other palatable, less tolerant plants. Plants intolerant to grazing-or those that are entirely consumed by herbivores- become less abundant than unpalatable plants, creating a shift in the population (Augustine et al. 1998).

Abiotic Interactions

Stress. Exposure to environmental stressors can decrease plant resistance to other threats. While under duress defensive compounds in leaves may decrease while nutrient levels increase, but this is not the case for all species. While originally believed to be less

resistant to herbivores, some plants may also become less edible (Louda et al. 1992). For example, while under water stress turgor pressure and water availability decreases and allelochemicals may increase (Huberty et al. 2004). Stress can also induce changes in morphology and local extinctions (Levine et al. 2004). The physiological changes a plant undergoes under less than optimum conditions depends on the type of stress present. Stress tolerance and gradients of abiotic stresses are also thought to play an important role in plant community structure, with plants exhibiting tolerance plasticity (the ability to grow and reproduce at reduced size in unfavorable conditions) or compensatory plasticity (the ability to alter allocation, resulting in equal performance in alternative conditions) in addition to stress affecting interspecies interactions (Jurjavcic et al. 2002).

Types of Plant Stress. Since plants are non-motile, they adopted complex internal reactions to cope with their environment (Yang et al. 2002). A plant may be subject to chronic or acute stress, from biotic or abiotic factors. Biotic stresses include insect predation, ungulate grazing, pathogens, and competition with soil microbes. Abiotic stresses include heat, water, cold, and oxidative challenges. Plants may employ methods to either tolerate or avoid stress by changing their phenology, morphology, and physiology through gene expression (Zhang et al. 2000).

Heat Stress. Acute heat stress primarily affects proteins and membranes. Under elevated temperatures cell membranes integrity is impaired, causing leakiness of solutes and ions (Basra, 2001). Both heat and oxidative stress invoke pathways that result in the accumulation or expression of heat shock proteins, but evidence suggests that heat stress results in oxidative stress (Panchuck et al. 2002). Chronic heat stress causes abortion of seed development, accelerated developmental stages for the whole plant, and affects

photosynthesis, energy metabolism, and translocation of assimilates (Basra, 2001).

Proteins are sensitive to heat stress, particularly enzymes. Plant enzymes have been

shown to operate within a thermal kinetic window (TKW), which varies among species.

High temperatures may degrade existing proteins, interfere with the production of new

proteins, and inhibit enzyme activity. This further interferes with membrane effectiveness

by disrupting integral enzymes necessary for organelle function (Basra, 2001). Heat

shock proteins (HSPs) are believed to enable the repair or recycling of damaged proteins

during recovery from stress, or to prevent damage to proteins and membranes during heat

stress. Chloroplast HSPs may limit damage to photosystem II (PSII) during heat stress,

among other benefits. There is a wide variety in the amount, array, and kinetics of heat

shock proteins among closely related plant species, and even within a plant depending on

cell type and developmental stage. HSP production is resource-limited, particularly with

respect to nitrogen. Small variations in nitrogen levels were shown to significantly affect

HSP production (Heckathorn et al. 1996a). Heat stress appears to cause photoinhibition,

although because high photon flux density (PFD) often occurs with high temperatures the

reduction in photosynthetic capacity is often attributed to high irradiance (Gamon and

Pearcy 1989).

Oxidative Stress. Plants are often exposed to light in excess of that needed to

capture and reduce CO_2. Plants must be able to rapidly dissipate the excess electrons

generated in the thylakoids, as well as excess photon energy, to avoid photoinhibition and

avoid producing reactive oxygen species (ROS) (Asada 2000). The principle sites for

oxidative damage are chloroplasts and mitochondria (Basra 2001). Plants in high light

have a greater potential to accumulate high levels of hydrogen peroxide and superoxide

due to O_2 being reduced at high rates (Robinson et al. 2000). Reactive oxygen species are part of the plant's signal transduction cascade. An example of an ROS is hydrogen peroxide, which appears to either increase damage to tissues, or under times of stress signals the plant's defenses (Yang et al. 2002). Antioxidants are viewed to be important in plants' ability to adapt to stresses such as heat and excessive light due to their ability to control ROS levels (Basra 2001). For example catalase, a principal enzyme in degrading H_2O_2 in plants, plays an important role in regulating the levels of H_2O_2 and thus greatly influences senescence, aging, and plant defenses (Yang et al. 2002). Anthocyanins may act as radiation attenuators and antioxidants in light-stressed plants protecting against photooxidative damage, and have been shown to scavenge H_2O_2, O_2^-, and $ONOO^-$ along with possibly interfering with other ROS (Hughes et al. 2005).

Water Stress. In severely water-stressed tissue, certain phytoalexins accumulate and inhibit stomatal opening. Proline and glutamine accumulate in large amounts in water-stressed leaves, probably acting as osmotic adjusters because they do not affect stomatal closure (Plumbe et al. 1986). Under water stress, protein metabolism and amino acid synthesis are impaired, thus raising levels of free amino acids for insect consumption, while at the same time plants produce osmoprotectants which are high in nitrogen, a limiting factor for herbivorous insects. While historical reports indicated that plant water stress directly contributed to outbreaks of herbivorous insects, not all herbivorous insects benefit from drought (Huberty et al. 2004)**.** Through direct and indirect effects, drought can interfere with mutually beneficial relationships and leave plants more vulnerable to destructive relationships (Levine et al. 2004). During times of osmotic stress plants accumulate osmolytes such as proline, trehalose, mannitol,

glycinebetaine, and fructan, although their role in stress tolerance is not completely understood (Zhang 2000).

Goals and Objectives

The health of a plant and its supporting microbial community often hinges on the plant's ability to withstand stressful conditions. The overall goal of this multidisciplinary project is to determine interactions between plant and soil microbe activities and in doing so acquire a better understanding of the conditions necessary to promote a sustainable rangeland ecosystem. In parallel with our study, a team of soil scientists is conducting research on soil microbial activity related to our sampling sites. We compared stress reactions of switchgrass (*Panicum virgatum* L.) in an old field plot and land enrolled in the CRP by assessing protein concentration, DPPH radical scavenging capacity, and chlorophyll content. In addition to the field study, a growth chamber experiment was conducted by testing drought-stressed switchgrass grown in soils from both types of land management. Our hypothesis was that plants from CRP plots are more resistant to stress than plants from the old field.

CHAPTER II

MATERIALS AND METHODS

Field Experiment

     Weather. Data for the months of collection were collected from Mesonet (www.mesonet.org). Rainfall, maximum and minimum temperatures, soil temperatures, and solar intensity were assessed and included in the results.

     Sites. Plant samples were taken from land enrolled in the CRP, and old field plots near Medford in Grant County, Oklahoma. Although cultivation records for the old field plots were not available, they have not been cultivated in several decades. Two locations, each with adjacent CRP and old field plots were selected for their matching soil type, Kirkland silt loam (fine, mixed, superactive, thermic udertic paleustoll). The CRP plots were planted with a mix of species [*Schizachyrium scoparium* (Michx.) Nash , *Andropogon gerardii* Vitman , *Sorghastrum nutans* (L.) Nash, *Panicum virgatum* L. , *Bouteloua curtipendula* (Michx.) Torr. , *Desmanthus illinoensis* (Michx.) MacMill. ex B.L. Rob. & Fernald , *Chamaecrista fasciculata* (Michx.) Greene, *Helianthus maximiliani* Schrad., and *Bouteloua gracilis* (Willd. ex Kunth) Lag. ex Griffiths]. Plant voids in one of the old field plots resulted in an unbalanced design, so only one old field

plot was used. The old field plot had about one half as many plant species as the CRP plots (S. Deng, unpublished data). One of the CRP plots is burned periodically, the last time being spring of 2009. The other CRP plot has never been burned. In the spring of 2010, the old field plot was burned.

      Plant Collection. Samples were collected twice a year from two different locations for the CRP and one location for the old field for two consecutive years. At each location samples were taken from two different ecosystems, with five replications within each ecosystem except for the old field plot at the location with plant voids. A total of 480 samples were collected and processed each year. Sampling was performed in the second week of June in 2009 and the first week of June in 2010, as well as the last week of July in 2009 and first week of August of 2010 to collect from pre-stress and post-stress plants. The initial sampling of each year was designated "early" (pre-stress) and the second sampling was designated "late" (post-stress). Plant tissue was collected in biovials with friction caps. Vials were labeled and had a moistened filter-paper disc inserted to prevent the tissue samples from drying out. Tissues were collected from the middle of the grass blades to reduce variability related to position. Eight vials per plant were collected, four subsamples for buffer extraction (protein determination) and four subsamples for ethanol extraction (reducing power and chlorophyll measurements). At both CRP plots and the old field plot five replicate subplots were randomly selected and sampled. A total of 120 biovials were collected for each sampling trip. Samples were stored on ice in the field, then transferred to a -80°C freezer. The first plant samples from June 2009 were frozen immediately in a -80°C freezer and sorted later, but subsequent

samples were held in a refrigerator for 1-2 days until sorting was complete to reduce freeze-thaw cycles.

Growth Chamber Experiment

Plant Culture. Soil was collected from both systems for a growth chamber experiment. Samples from all five replicate plots in each system were pooled, mixed, and sieved through 7 mm mesh. Approximately 600 g of the prepared soil was added to each conetainer (25 x 6.8 cm) with filter paper on the bottom to retain soil. Six seeds of Blackwell switchgrass (upland cultivar) were planted in each conetainer and thinned to four seedlings after emergence. Additional conetainers were prepared as described without seedlings. Conetainers were maintained in a growth chamber (model LT-105, Percival Scientific, Inc., Perry, IA) at $27.1 \pm 0.2 / 22.9 \pm 0.3°C$ (mean $\pm$ standard deviation) day/night temperatures with a 14 hour photoperiod. Light intensity at canopy height was approximately 400 µE m^2 s at wavelengths from 400 to 750 nm. Relative humidity was 47 ± 6 %. Plants were watered as needed during establishment (12 weeks) and no fertilizer was added.

After establishment, randomly selected conetainers were assigned one of two treatments; watered to field capacity or water withheld for up to 17 days. Conetainers were weighed daily to establish water loss gravimetrically. Changes in plant mass were assumed to be relatively small compared to changes in mass due to water loss. Each treatment was sampled initially, at the midpoint of the water stress period, or at the endpoint of 17 days. The sampling times were initially planned based on gravimetric targets (100, 87.5, and 80% of initial conetainer weight for initial, midpoint, and endpoint,

respectively), but differences in water retention properties between soils from the two systems necessitated an endpoint based on time for both systems. Conetainers with soil from the CRP system asymptotically approached an average mass 85% of the initial mass, but containers with soil from native/abandoned cropland reached an average mass 79% of the initial mass 17 days after water was withheld.

Samples were collected from three conetainers at each collection time. Four biovials were collected from each conetainer (two for MES (2-(*N*-morpholino)ethanesulfonic acid) extraction and two for EtOH extraction), with a total of three reps and two subsamples per extraction per rep for each soil/water combination. After results were obtained from the tissue analyses as described below, the data for protein concentration and tissue midpoint for DPPH reduction was normalized by dividing by the percent relative water content. Chlorophyll was expressed as mg per gram protein.

Tissue Analyses

Protein. Protein levels were assessed using the Bradford assay (Bradford 1976) with ovalbumin as a standard. The reagent dye binds to arginine residues, making protein quantifiable by reading at a wavelength of 595 nm with a spectrophotometer. Approximately 50 mg of leaf tissue were cut into small sections with a razor blade, weighed, and added to 15 mL MES buffer at a pH of 6.5. Tissue was homogenized using a polytron (Brinkmann Instruments, Westbury, NY) at settings of 7-9 in six 40-second bursts, with the test tube submerged in ice water to prevent heat build-up. After the tissue was homogenized, the polytron probe was rinsed in 5 ml MES 6.5 buffer, and the rinse

was added to the extract to maximize tissue collection. Plant extract was centrifuged using a Beckman J2-21 centrifuge (Beckman Coulter, Brea, CA) for 20 min at 16k x g at 10°C. Three polystyrene cuvettes had 400 uL of supernatant pipetted into them along with 3 mL of Bradford reagent.

Ovalbumin standards were prepared from frozen stock solutions by diluting 1 mL 5x ovalbumin in 4 mL MES buffer at a pH of 6.5. The protein was added to 5 mL polystyrene cuvettes in 10, 20, 30, and 40 uL amounts, with 0 µL as the control. Buffer was added to bring each volume to 400 uL, then 3 mL of Bradford reagent were added to each cuvette. Each concentration was assayed in triplicate. Protein levels were measured within 15 minutes of adding the reagent. The absorption at 595 nm was measured with a Beckman DU 640B spectrophotometer. The reagent without protein was used as a blank.

Reducing potential. Radical reducing potential was determined using 2,2-Diphenyl-1-picrylhydrazyl (DPPH), a relatively stable free radical (Masuda et al. 1999). The reagent was prepared by adding 0.031 g of DPPH to 20 ml ethanol (EtOH) and vortexing for 5-8 minutes. Approximately 100 mg of leaf tissue was sectioned, weighed, and added to a test tube containing 15 ml EtOH. The tissue was homogenized with a polytron using speeds of 6-7 in six 40-second bursts, with the test tube submerged in ice water. The polytron tip was rinsed in 5 ml EtOH and the rinse was added to the extract. The extract was centrifuged at 16k x g for 20 minutes at 10°C. After centrifugation, the supernatant was immediately pipetted into test tubes to prevent pellet fragments from becoming re-suspended in the solution. The supernatant was subsequently pipetted into cuvettes in 20, 80, 200, 320, 400, 500, and 600 ul volumes, with each cuvette being brought to 970 ul with EtOH. These dilutions were carried out in triplicate. Each cuvette

had 30 ul of DPPH solution added (final concentration of 2.5 mM) except for those used for blanking the spectrophotometer. Ethanol was substituted for DPPH in those cuvettes to keep tissue concentration consistent. DPPH levels were measured after 30 minutes using a spectrophotometer measuring absorption at a wavelength of 517 nm (Masuda et al. 1999). Controls were used as blanks. Nonlinear regression was used to determine the tissue concentration at which half of the DPPH had been reduced. Plants with greater reducing power required less tissue to reach the midpoint of the sigmoidal response curve.

Chlorophyll. Three cuvettes had 1 ml of supernatant from the ethanol extract prepared for the DPPH assay pipetted into them. Chlorophyll concentration was determined by measuring extract absorbance at wavelengths of 664.5 nm and 647 nm and applying extinction coefficients for ethanol reported by Ritchie (2006).

Data Analysis

Data assessing the amount of protein, chlorophyll, and reducing capacity per gram fresh weight of tissue were ascertained from the assays performed. For the growth chamber, sample weight was adjusted with the relative water content obtained from the soil measurements to reduce the bias caused by protein concentrating in dehydrated tissues. Data were analyzed by ANOVA using the GLM procedure (SAS Institute Inc., Cary, NC). Following convention, effects were considered significant at $P \leq 0.05$ and highly significant at $P \leq 0.01$. PROC NLIN was used to determine the tissue concentration that reduced the absorbance value by one half in the DPPH assays (Abe et al 1998). Data from both years were analyzed separately..

CHAPTER III

RESULTS

Field Experiment

      Weather. There was little difference in the average temperatures from the

sampling months of June and July in 2009. The highest temperature recorded in July

2009 in Medford was 45°C, but during the two weeks preceding sampling the highest

temperature was 37.2°C, with the highest average temperature reaching 29.7. The

average temperature for the entire month was 26.8°C. For the two weeks before sampling

in June of 2009, the highest temperature reached was 36.1°C, and the highest average for

the two weeks was 28.6. The average for the entire month was 26.7°C. In June, 1.6 cm of

rain fell two weeks before sampling, and in July 4 cm fell. The total rainfall for each

month was 5.9 cm for June and 6.8 cm for July. In 2010, the two weeks preceeding the

June sampling had a high of 35.5°C with the highest average reaching 29.1°C. The

average temperature for the entire month was 27.2°C. For the two weeks preceding the

August sampling, the maximum temperature was 41.7°C with the highest average

reaching 33.7°C. The average temperature for the entire month of July could not be

accurately assessed due to missing data. Rainfall for the two weeks preceding the June 2010 sampling was 2.4 cm, and 0.46 cm for the two weeks preceding August (Figure 1).

Protein. In 2009 mean protein concentration was not significantly different across systems for early samples. Late samples increased in protein concentration for both systems. Plants from the CRP had significantly higher protein concentration than those from the old field in late season samples. In 2010 there was no significant difference between early and late samples for the old field. Early season CRP was similar to both early and late old field samples. Plants collected from the CRP in the late season had significantly higher protein concentrations than those in the early sampling. The late CRP had significantly higher protein concentration than that of the old field.

DPPH. In 2009 there was no significant difference between systems for tissue midpoint for DPPH reduction. Tissue midpoints decreased from early to late collection times in both systems. Early samples taken in 2010 were significantly different with the old field having a higher tissue midpoint than CRP. Later samples were not significantly different between systems.

Chlorophyll. In 2009 the early samples taken from both systems were not significantly different. Chlorophyll concentration in the late samples from the old field decreased significantly, but not those from the CRP. The samples from the CRP were not significantly different from early to late season.   In 2010, early samples from the old field had significantly higher chlorophyll concentration than the early CRP samples. Late samples from the old field had significantly lower chlorophyll concentration than that of the CRP. Both systems experienced a significant decrease in chlorophyll concentration from early to late sampling.
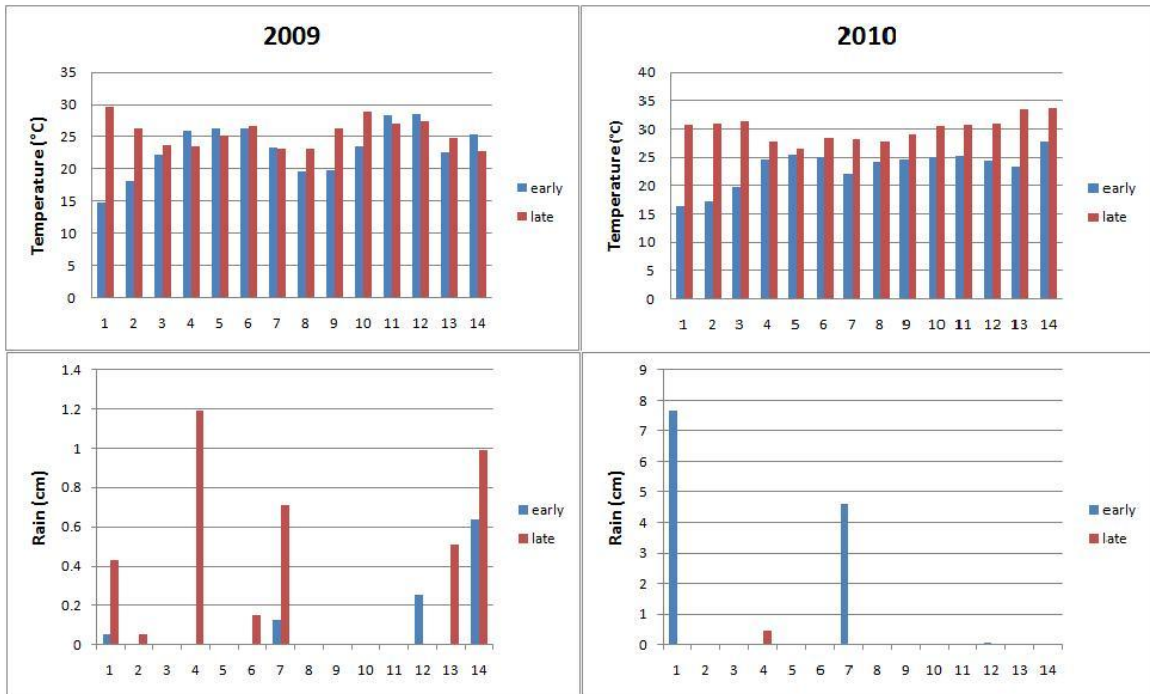
Figure 1. Weather data for 14 days prior to each sampling. Monthly rainfall in centimeters and average monthly temperatures in Celsius in 2009 and 2010 for Medford, Oklahoma. Data obtained from Mesonet.
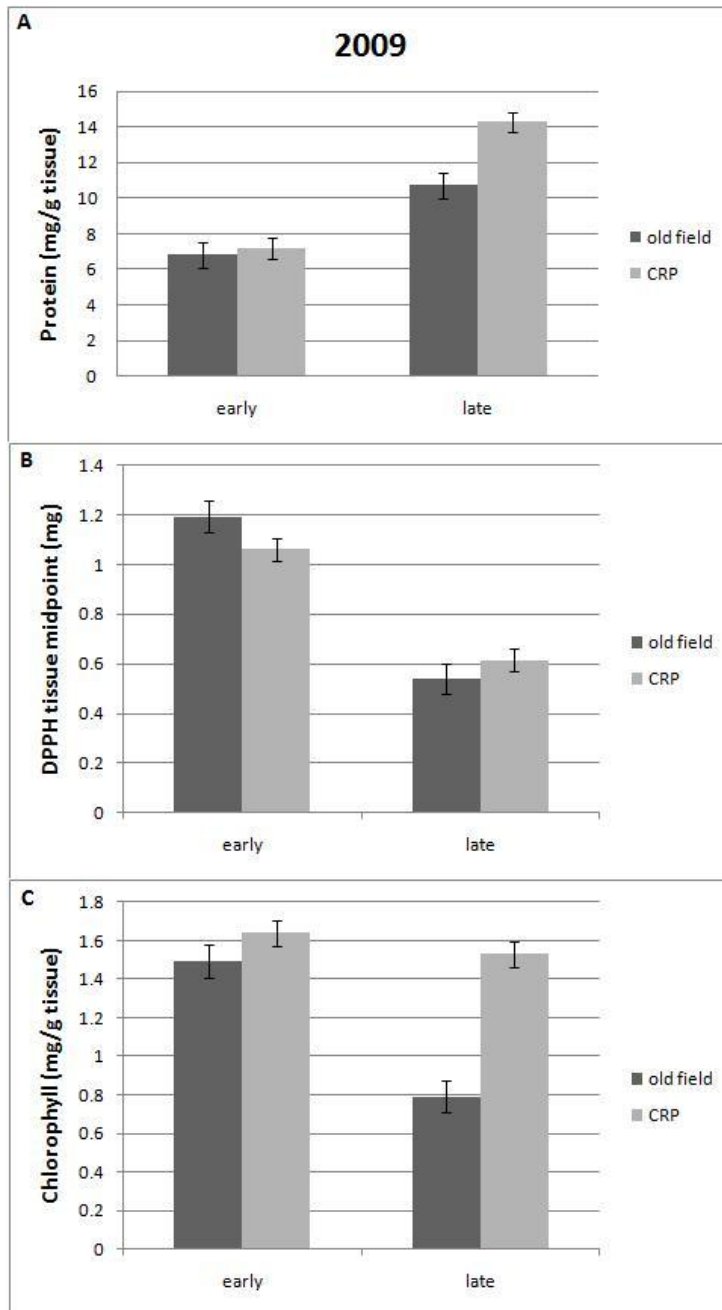
Figure 2. Comparison of A) Soluble protein concentration (mg/g tissue), B) tissue midpoint for DPPH reduction, and C) chlorophyll concentration from switchgrass leaves sampled early or late in the season during the field study in the year 2009. Error bars represent a standard error. For old field, n=80, and for CRP n=160, with both CRP plots merged to establish repetition.
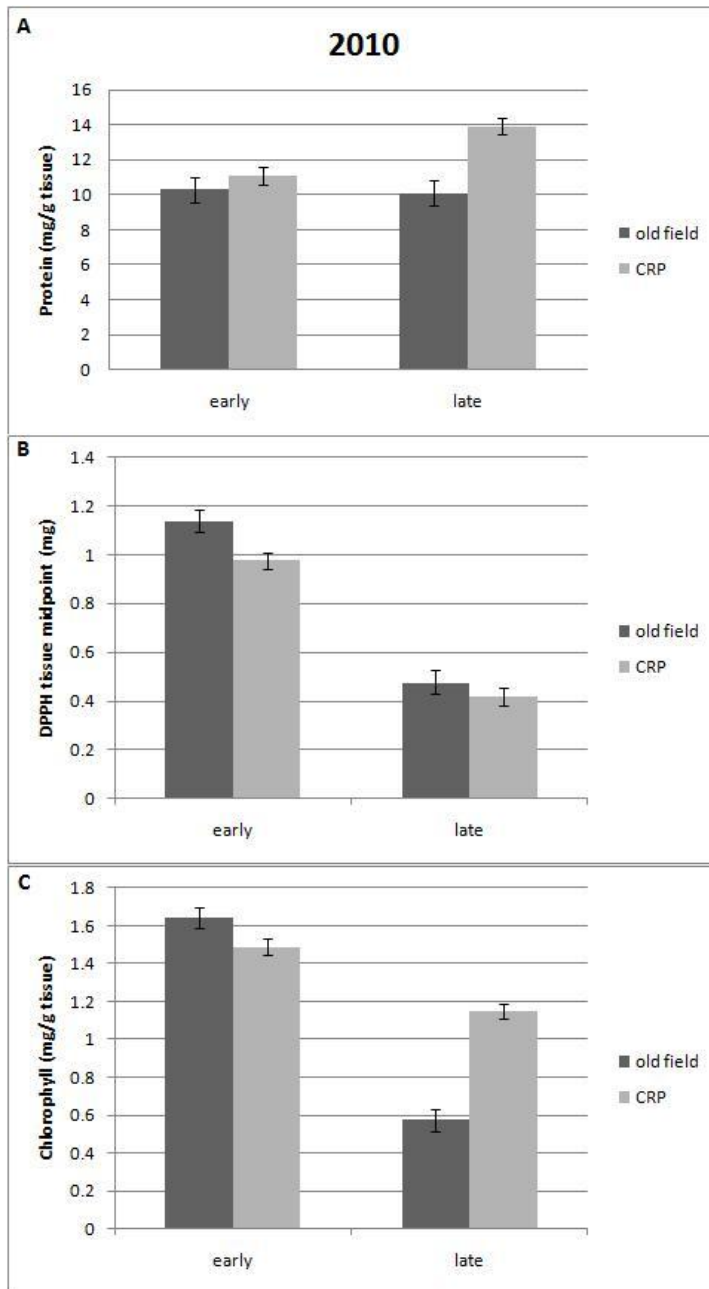
Figure 3. Comparison of A) Soluble protein concentration (mg/g tissue), B) tissue midpoint for DPPH reduction, and C) chlorophyll concentration from switchgrass leaves sampled early or late in the season during the field study in the year 2010. Error bars represent a standard error. For old field, *n*=80, and for CRP *n*=160, with both CRP plots merged to establish repetition.

Growth Chamber Experiment

     Soil and Plant Water Contents. While both systems had lost 12% of water mass at the middle collection date, conetainers with old field soil averaged 79% of their initial weight and containers with CRP soil averaged 86% of their initial weight by the end of the experiment. For plants growing in old field soils, leaf relative water content was 98.3% for the initial measurement. The middle measurement varied little from the beginning. End measurements yielded 97.5% for the control and 52.3% for samples with water withheld. Plants grown in soils collected from the CRP locations (CRP) had similar relative water contents for the initial and middle control measurements but plants in conetainers that had water withheld had an average relative water content of 71.2%. For the end measurement, the control averaged 97.6% and the water withheld treatment averaged 32%. At day 10, plants grown in old field soil showed little visible difference between watered and water-stressed. Plants grown in the CRP soil under water stress showed some chlorosis and leaf-rolling along with green leaves. At the end, stressed plants grown in old field soil showed several tan and brown leaves, but still had green leaves. Plants in CRP soil had very few green leaves (Figure 4).

     Protein. There was a highly significant soil by treatment by date interaction (Figure 3). In both soils there was a significant protein decrease from the middle collection date to the end of the 17 day period in water stressed plants. While plants in both soil types showed a significant response to water stress, those in CRP soil had a significantly lower soluble protein concentration than those in old field soil at the final sampling. There was no soil effect between watered plants, but there was between those that were not watered.

DPPH. There was a significant soil by treatment by date interaction (Figure 3). There was a significant soil effect between plants that were not watered by the end of the experiment, with plants grown in CRP soil having higher tissue midpoints (less reducing power) than those in old field soil. There was a significant decrease in the average tissue midpoint for the old field soil control from the beginning of the experiment to the middle, but no change from the middle to the end. The values for the other soil/treatment combinations were significantly higher at the middle of the experiment, with similar averages. Of the remaining three soil/water combinations, both of the water-stressed soils had plants with significantly higher end averages than those recorded for the middle, with CRP soil having higher averages than old field soil.

Chlorophyll. In old field soil there was no significant difference in mean chlorophyll concentration between control and water-stressed plants (Table 1). The same was also true for CRP soil. Both control and water-stressed plants grown in CRP soil had significantly lower mean chlorophyll concentration at the end of the experiment than at the beginning. There was no significant difference between the soils for the water-stressed treatments at the end of the experiment. Water-stressed plants grown in both soils decreased in chlorophyll concentration by the end of the experiment, but the difference was not statistically significant despite plants grown in CRP soil appearing more yellow than those grown in old field soil (Figure 4).

Table 1. Slice effect for growth chamber experiment. Pr>F indicates significance (P=0.05 significant, P=0.001 highly significant). Differences between soil, date, and treatment were compared to determine significance of interaction. Sampling dates are indicated as beginning (beg), middle (mid), and end (end) Blank spaces in the columns indicate which effect is being tested. For each soil/treatment/date combination $n=6$ ($n=120$ total samples tested).

Table 1

| Effect	Date	Soil	Trt	Pr > F		
				Protein	DPPH	Chlorophyll
Soil*Trt*Date		old field	water	0.1599	0.0369	0.6218
Soil*Trt*Date		old field	no water	0.002	0.0562	0.1141
Soil*Trt*Date		CRP	water	0.0411	0.4985	0.0485
Soil*Trt*Date		CRP	no water	0.0001	0.0253	0.0493
Soil*Trt*Date	Beg	old field		*	*	*
Soil*Trt*Date	Mid	old field		0.95	0.0492	0.9726
Soil*Trt*Date	End	old field		0.0042	0.0226	0.111
Soil*Trt*Date	Beg	CRP		*	*	*
Soil*Trt*Date	Mid	CRP		0.1373	0.6034	0.6224
Soil*Trt*Date	End	CRP		0.0001	0.0012	0.2359
Soil*Trt*Date	Beg		water	0.9282	0.9996	0.3248
Soil*Trt*Date	Mid		water	0.6835	0.072	0.5242
Soil*Trt*Date	End		water	0.5791	0.3323	0.5332
Soil*Trt*Date	Mid		no water	0.0726	0.7317	0.8569
Soil*Trt*Date	End		no water	0.0121	0.044	0.8499
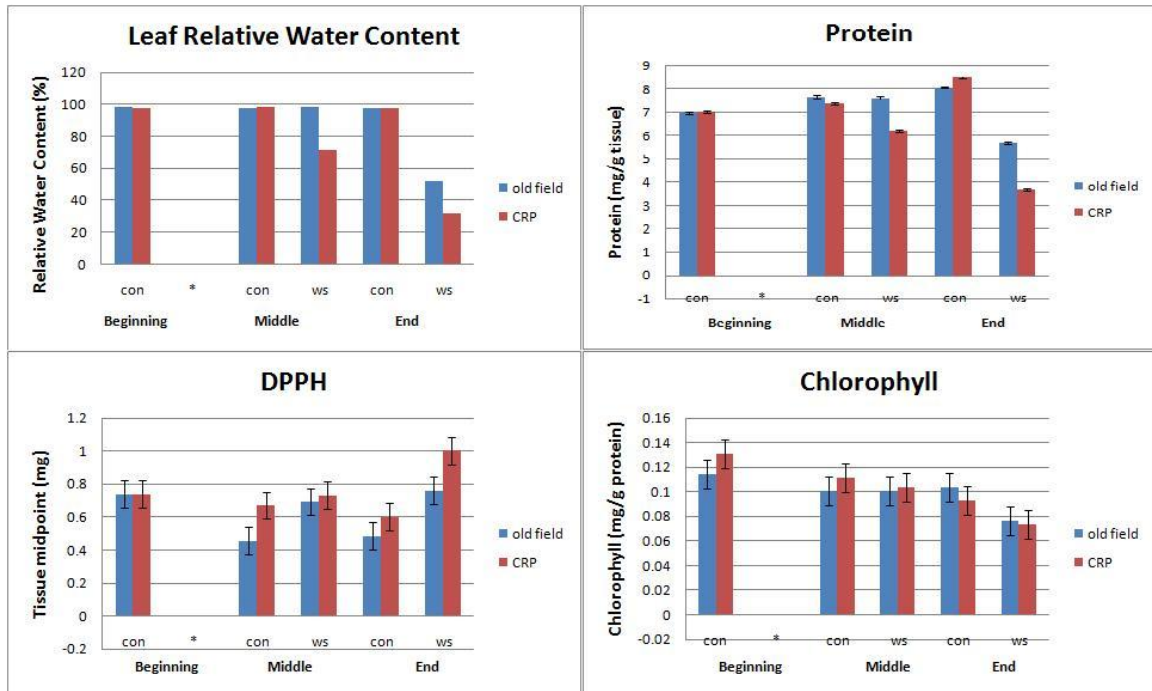
Figure 4. Least squared means and standard error values for switchgrass responses to stress in a growth chamber experiment. Plants were grown in soils collected from the field study locations. Soil was collected from old field and CRP. Measurements were taken 12 weeks after germination (beginning), when the gravimetric potential of the soil reached 88% (middle) and 17 days after water was withheld (end). In the beginning of the experiment no samples had water withheld. Protein concentration was expressed as mg protein per gram of fresh weight tissue after normalizing with RWC. DPPH reduction was measured by determining the tissue concentration at which half of the DPPH had been reduced. Chlorophyll was expressed as mg per gram protein. At the beginning of the experiment, none of the plants had been subjected to water stress, thus this portion of the graphs are marked with an (*).For each soil/treatment/date combination *n*=6 (*n*=120 total samples tested).

Figure 5. Switchgrass grown under watered and drought conditions in the growth
chamber. A) Day 10 (mid) Plants grown in old field under water stress appeared same as
controls. Plants in CRP soil showed some chlorosis and leaf rolling B) Old field controls
and water stressed on left, CRP controls and water stressed on right..

CHAPTER IV

DISCUSSION

Field experiment

      Weather. Despite the high temperatures, the short duration of hot periods probably did not contribute to chronic heat stress. The high temperatures also did not approach the record high temperature in Oklahoma of 48.9°C, which occurred in July of 1936. While it is unlikely the plants collected in the field experienced significant heat stress, a difference in soluble protein and chlorophyll were evident between the two different soil types under field conditions. No significant difference in soil moisture was observed between the plots (S. Deng, unpublished data).

      Protein. Mean protein concentration increased between early and late sampling times for old field and CRP in 2009. The CRP had significantly higher protein than the old field that year. In 2010, the year the old field was burned, only the CRP showed a significant increase in chlorophyll concentration from early to late. While it is impossible to be certain that the difference between years was due to burning without a separate study, there may have been some effect. Late samples taken from CRP had a higher

protein concentration than those taken from old field in both years. Since it is unlikely the plants experienced significant chronic heat stress, plants grown in CRP plots may be better able to utilize resources than those grown in old field plots. Burning in the old field may have released nutrients into the soil that allowed increased microbial growth leading to competition for nutrients and thus the decrease in protein for the burned year. Also to be considered are the differences in soil properties affecting relative water content rather than a greater ability to produce heat shock proteins. Soil properties in the CRP plots are different than those in the old field, despite being the same soil type (S. Deng, unpublished data). In the growth chamber portion of the study, the soil from the old field plots had lower relative water content at the middle and final sampling. However, during both summers the area sampled did not experience significant drought so this was probably not a major factor.

DPPH. Tissue midpoints for DPPH reduction decreased from early to late sampling times in both years, but there was no significant difference between systems in 2009. In 2010 the early samples were significantly different with the old field having higher tissue midpoints for DPPH reduction, but that difference was not observed with the late samples. If drought were a factor, one might assume that plants from the CRP have lower reducing capacity than those from the old field plot and that this effect has been hidden by the difference in water retention between CRP and old field soils. However, the lack of difference in soil moisture indicates this is not the case. It is possible that the effect was from increased tissue concentration from reduced water content in the leaves, but during both years the difference in rainfall between sampling dates was not significant.

Chlorophyll. Average chlorophyll concentrations were higher for all systems for the early sampling time with no significant difference between systems in 2009. Later samples, however, yielded a lower average chlorophyll concentration for old field. There was no significant reduction in chlorophyll for CRP. In 2010 the chlorophyll concentration started off higher in the old field plot but the later samples revealed a much lower chlorophyll concentration than samples taken from the CRP. The initial higher chlorophyll concentration in the old field may have been the result of greater access to nutrients released by the burning, but the effect did not persist through the growing season. Since both systems received the same amount of sunlight, plants from CRP devoted more resources to harvesting light, indicating better access to resources than those in the old field plot through the growing season.

Growth chamber experiment

Soil and Plant Water Contents. Despite being the same soil type, differences in the soils may have impacted the stress tolerance of the plants. Soil taken from the old field site had higher organic matter and larger aggregate size (S. Deng, unpublished data). Xu et al. (2006) compared the water stress tolerance of seedlings for switchgrass, foxtail millet, and Old World bluestem in a growth chamber study. After watering was stopped, the soil gravimetric moisture content for switchgrass decreased the slowest, and switchgrass had the highest leaf RWC. During the drought treatment, switchgrass had a higher photosynthetic rate, but was shown to have the lowest shoot and whole-plant water use efficiency. Switchgrass also has the highest root/shoot ratio of the three species under both drought and well-watered conditions. While switchgrass did not have the highest

drought tolerance, this high root/shoot ratio gives it an advantage over other grasses in drought conditions once the seedlings have been established. In a study by Barney et al. (2009), both upland and lowland ecotype switchgrass suffered severe reductions in biomass yield, tiller production, and leaf area under water stress conditions of -4 MPa, but lowland switchgrass thrived under flooded conditions, meaning it is probably a facultative wetland species.

Protein. Plants grown in both soil types had reduced average soluble protein concentration from the middle collection date to the end date, with plants grown in CRP soil having a lower average soluble protein content than those grown in old field. Plants that were watered showed no significant difference by soil or sampling date. A positive correlation between soluble protein and specific leaf weight, and by extrapolation transpiration efficiency, in switchgrass was noted in a study by Byrd and May (2000).

DPPH. Water-stressed plants grown in CRP appeared to have less reducing power than those grown in old field by the end of the growth chamber experiment. Cultivars of Kentucky bluegrass under drought stress were shown to have an increase in malondialdehyde, a reactive chemical that indicates oxidative stress, indicating increased reactive oxygen species-mediated lipid peroxidation. These results were also supported by increased electrolyte leakage and decreased activity of superoxide dismutase and catalase in the plants under drought stress (Xu et al. 2011). A study by Ali et al. (2010) showed reduced DPPH scavenging capacity in seed oil of maize under drought conditions. The study also showed lower phenolics and carotenoids, but higher flavonoids and tocopherols when compared to well watered conditions, indicating that DPPH scavenging capacity is affected by phenolic and carotenoid levels. Given the

results of this study and those cited, it is apparent that the plants grown in CRP soil experienced greater oxidative stress as a result of drought as compared to old field.

Chlorophyll. By the end of the growth chamber experiment, no significant difference between soils was observed for average chlorophyll concentration. Plants grown in both soils decreased in average chlorophyll concentration by the end of the experiment, although neither system was determined to be significantly different. In the field, a decrease from early to late sampling was noted, although CRP had a higher concentration of chlorophyll than old field. The lack of significant difference between soils in the growth chamber compared to the field experiment despite the differing soil properties points to a more complicated mechanism for controlling chlorophyll production than water stress alone.

The decrease in chlorophyll and proteins is consistent with a previous study. Creeping bentgrass (*Agrostis stolonifera* L.) grown under polyethylene glycol (PEG) induced water stress experienced a decrease in proteins involved in all three phases of the dark reaction, specifically Rubisco, Rubisco activase, chloroplastic aldolase, and chloroplastic GADPH. In addition to these, proteins responsible for respiration and metabolism, amino acid metabolism also decreased, while proteins involved in antioxidant metabolism decreased (Xu and Huang 2010).

Although drought and heat stress often occur simultaneously in the field, little is known about their combined effect on plants, although studies in tobacco show that the molecular response of the combined stresses is distinct from the responses to individual stresses. Based on a study by Rizhsky et al. (2004), a combination of heat and drought stress enhances respiration, suppresses photosynthesis, elicits a complex pattern of stress

response transcripts, and causes the accumulation of sucrose and other sugars in both *Arabidopsis* and tobacco.

Bermudagrass, a warm-season perennial C_4 grass showed reduced photochemical activities under heat stress, likely due to the vulnerability of Photosystem II to heat stress (Du et al. 2011). In a study by Zhou and Abaraha (2007), exposure to high temperatures and drought resulted in a 2-4 fold decrease in chlorophyll content for two bermudagrass cultivars at 26° and 38° C. The same study showed no significant changes in soluble protein content under heat and water stress, although Western blot showed increased production of HSPs in both cultivars (Zhou and Abaraha 2007).

Different metabolites accumulate under heat stress that may play a role in stress tolerance. Some abiotic stresses have been shown to lead to protein degradation and ammonium accumulation, which in turn lead to increased production of amides such as asparagine to combat toxicity. Du et al. (2011) found asparagine increases in bermudagrass under drought stress. Their study also found an increase in methionine, a direct precursor for SAM, which is the main biological methyl donor in transmethylation reactions. Proline, a stress related amino acid, was also found to increase.

Land use

The burning of the old field plot appears to have had little effect except for protein concentration, so it may still be appropriate to consider as one treatment. Burning does not always change things. Although the plant community was different from the one we observed, a study comparing plant species composition in Minnesota by Knops (2006) showed no significant changes between burned and unburned plots. Since the

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author noted that fire response is largely dependent on ecosystem, their results may not be directly comparable to the system we examined. If species composition is unchanged, perhaps in some ecosystems plants do not experience a change in the ability to tolerate heat stress as well. Fire is not always a key factor determining prairie vegetation (Knops 2006).

Models run by Parton et al. (2005) indicate that different cultivation practices of grassland leads to loss of soil carbon and increased N mineralization, with the most dramatic changes happening about 20-30 years after cultivation begins. It has been observed that increasing nutrients in the soil leads to decreased plant diversity due to increased shading and competition for light, leading to an inverse relationship between diversity and productivity (Baer et al. 2003). Given that CRP plots are replanted with a diverse assortment of plants and are maintained by burning, it is possible that CRP plots have higher diversity and thus lower nutrients than the old field plot observed. One of the key limiting nutrients is nitrogen. Heckathorn et al. (1996b) grew corn (*Zea maize* L.) with different N levels and then subjected the corn to heat stress. Plants raised with higher N had higher levels of Hsp60 and Hsp24 than those raised with low N, indicating that decreased resource availability reduces heat stress tolerance. Also, at low light, net CO_2 assimilation was 30% lower in low-N plants than high-N plants, although decreases in PSII efficiency (F_v/F_m) were smaller for low-N plants. Heckathorn and DeLucia (1994) also observed reduced leaf N in several C_4 perennial grasses under drought stress due to translocation to the rhizome. Some of this translocated N was derived from photosynthetic proteins, leading to the assumption that N availability plays an important role in photosynthesis under water-stressed conditions.

Conclusion

While one might expect plants in CRP land to have better responses to stress due to greater diversity in soil microbes, this may not be the case if the growth chamber experiment is an indicator of the differences in soils, particularly if the increase in diversity means lower nutrients. Since stress tolerance is linked to nutrient availability, it makes sense that plants grown in CRP soil did not fare as well as those grown in soil from the old field plot. However, field conditions differ from controlled growth chamber studies so the conditions present in the growth chamber may not accurately reflect those found in the field.

The plants growing in CRP plots in the field generally did better than those in the old field plot, having higher protein concentration and higher chlorophyll concentration during late sampling. CRP land may produce healthier plants, but under extreme stress the lower water retention in the soil may counteract the benefits, as well as the lower organic matter and smaller aggregate size. Since heat stress was not a significant factor, it may be hard to claim that one land management practice is better than the other under extreme conditions. It is important to understand relationships between resource availability, productivity, and diversity for management and restoration (Baer et al. 2003). Switchgrass has been shown to successfully germinate, establish stands, and reproduce under drought and flooded conditions. Its ability to tolerate drought, rapid growth rate, and adaptability make it an ideal forage grass for rangeland (Barney et al. 2009). Switchgrass is also adept at recovering from drought stress. In the growth chamber experiment, stressed plants in both soils were alive and generating new leaves a week after watering was resumed, with plants grown in old field soil making a faster recovery

than those grown in CRP soil (data not presented). Sanderson and Reed (2000) found that at low plant densities switchgrass is more limited by N than water, making soil nutrients more of a factor in switchgrass survival than moisture.

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VITA

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Scope and Method of Study:

Switchgrass (*Panicum virgatum* L.) was sampled from one old field and two CRP plots
near Medford, OK during the summers of 2009 and 2010 and evaluated for differences in
stress response between land management systems by assessing soluble protein
concentration, DPPH radical reduction, and chlorophyll concentration.

Findings and Conclusions:

Switchgrass in the CRP plots appeared to have a higher soluble protein concentration
later in the summer than in the old field plot during 2009 and 2010. There was no
difference in DPPH reduction between systems for either year, but there was a difference
between sample times, with later samples having higher reducing power. Mean
chlorophyll concentration in old field was significantly lower than the CRP later in the
summer. In a companion growth chamber study under water stressed conditions, plants
grown in soil collected from the CRP site had lower protein concentration and lower
reducing power than those grown in soil from the old field site, but no difference was
observed in chlorophyll concentration between the sites. Overall, plants grown in CRP
soil did better in the field and worse in the growth chamber, probably due to a lack of
heat stress under field conditions.

ADVISER'S APPROVAL: Dr. Jeffrey Anderson