THESIS

# YIELD AND QUALITY OF COOL-SEASON PERENNIAL GRASSES FOR FORAGE AND BIOMASS FEEDSTOCKS IN NORTHEAST COLORADO

Submitted by

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In partial fulfillment of the requirements

For the Degree of Master of Science

Colorado State University

Fort Collins, Colorado

Spring 2011

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ABSTRACT

YIELD AND QUALITY OF COOL-SEASON PERENNIAL GRASSES FOR FORAGE AND BIOMASS FEEDSTOCKS IN NORTHEAST COLORADO

The burning of fossil fuels has led to an increase of the greenhouse gas $CO_2$, which traps heat and increases temperatures of the global climate. The increases of the greenhouse gases, CO2, CH4 and N2O, have been attributed as the cause of the climatic warming during the industrial era. One identified GHG mitigation strategy is the use of long term perennial grass production for bio-fuel use and rangeland restoration. It is estimated that biofuels could offset 30% of current fossil fuel use. Switchgrass, a $C_4$ grass species, was chosen by the DOE as the model crop for cellulosic biofuel because of the plant's perennial nature, high water use efficiency, wide range of exploitable genetics, and its ability to be grown in diverse regions. Yield potentials are often lower for cool-season grasses, but for warm-season grasses like switchgrass difficult establishment and winter stand loss from extreme conditions can be a problem for production. There are many $C_3$ species utilized in the Northeastern and the Western of the United States for rangeland and pasture cattle production. Production difficulties are less likely in some of the hardier $C_3$ grasses in cooler environments. $C_3$ grasses have been historically utilized for animal forage because of superior digestibility and high feed values. The high digestibility is directly correlated to reduced lignin content. Lignin is a primary barrier to

the bioconversion process to make ethanol. Increasing the polysaccharide to lignin ratio is one identified route to increasing bio-fuel feedstock quality. These qualities produced in $C_3$ grasses could create a dual feedstock for both animal and bio-fuel production. This may even decrease competition for land resources between livestock producers and bio-energy crop production. Relying on a diversity of bio-energy crops in ecologically different regions will allow for greater stability, resistance, and resilience to climatic and environmental variability. The goals of this study are to compare forage quality analyses of $C_3$ grasses, seasonal partitioning of dry matter (DM), crude protein content (CP), and neutral detergent fiber (NDF) and acid detergent fiber (ADF). Fifteen cool-season grasses were selected based on potential productivity under limited irrigation typical to Eastern Colorado. Two spring harvest dates were selected based on important production phases of the plant. The June 1, 2009 harvest (H1) corresponded to the boot to early heading stage. The average for the species statistically grouped the highest yield was 4500 kg/ha. The second spring harvest (H2) was on June 22, 2009 and corresponded to the mid to late heading stage and average yields for this harvest was 6390 kg/ha. These are high yields for the Eastern Plains of Colorado, but it is important to point out at that 2009 had an exceptionally wet spring and summer for the region. Tall, intermediate, crested and western wheatgrass were species that performed the best for the delayed harvest in terms of biofuels quality because they had the greatest increases in yield and structural carbohydrates, measured in NDF and ADF analysis. These species also had the greatest decrease in CP content. However, in general all species retained fairly high CP levels of over 10%, which is too high for biofuel quality standards, and an even further delay in harvest timing is recommended to decrease CP levels. This may be easily attained since forages evaluated decreased in quality at a rapid rate after seed head emergence. There are two major hurdles for the use of these forages as biofuels: 1) competition from feedstock and livestock feeders and 2) reducing the CP levels of the forages.

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# CHAPTER ONE

# LITERATURE REVIEW

The goal of this review is to demonstrate the potential utilization and integration of perennial cool-season forages into biomass energy production. The review starts with biofuel production and an overview of how anthropogenic activities are related to global climate change. A general discussion then follows about the potential for cellulosic ethanol as a renewable fuel, with switchgrass as a model perennial grass crop, as well as factors affecting sustainability, conversion efficiencies and carbon mitigation strategies. The case is then presented to evaluate cool-season grasses forages as perennial biofuel crops for the semi-arid conditions of Northeast Colorado. The review will then conclude with brief summaries of highly researched animal forage quality analyses in order to evaluate certain growth characteristics and nutritional values of $C_3$ grasses. This may give insight for the bio-energy sector when selecting grass species for efficient bio-fuel production. Cool-season grasses are highly utilized for animal forage and by using existing forage crops a dual purpose system could be created supporting the needs of both cattle and bioenergy industries.

## 1.1 Global Climate Change and Fossil Fuels

An increase in greenhouse gas (GHG) emissions has amplified solar radiative forcings in the atmosphere. One of the leading causes of elevated levels of carbon dioxide ($CO_2$) in the atmosphere is from the burning of fossil fuels. These increased levels can cause disruptions to the earth's meteorological patterns. When released, $CO_2$, a known GHG, traps heat and increases global temperatures (Johnson et al., 2007). Elevated levels of $CO_2$, methane ($CH_4$) and nitrous oxide ($N_2O$) have been attributed to the increase of global warming during the industrial era (Salinger, 2006). Most of these elevated emission levels have been attributed to anthropogenic activities. Current fossil fuel energy systems are being scrutinized for serious environmental, economical, and political reasons (Demirbas, 2006).

U.S. fossil fuel use for transportation alone accounts for 56% of all U.S. air emissions, with $CO_2$ emissions accounting for 32%; at an equivalent annual release rate of 19 billion tons (Morrow et al., 2006). The United Nations Framework Convention for Climate Change called for stabilization of GHG emissions in order to prevent dangerous climatic changes from anthropogenic sources (Kheshgi et al., 2000). Immediate action is needed in order to stabilize future $CO_2$ levels, which are currently at two or three times the pre-anthropogenic level (Kheshgi et al., 2000).

With the advancement of "Green Technology", agriculture will play a key role in moving the transportation sector towards a renewable energy source by producing feedstock that are economically, socially and environmentally sustainable. The United States has begun to implement research towards reducing the reliance of fossil fuel through alternative renewable energies. Of priority is finding alternative fuels for the transportation sector through the conversion of biomass into cellulosic ethanol. Cellulose and lignin are the two most abundant biopolymers on earth (Boerjan et al., 2003) and have been identified as potential contributors to energy demands. The 2007 Energy

Independence Act of the United States called for 36 billion gallons of biofuels, with 21 billion gallons coming from advanced biofuels (Costello et al., 2009).

**1.2 Cellulosic Ethanol and Sustainability**

Anthropogenic activities have also been linked to a decrease in land-based carbon sink capacity, which includes an increase in severity and extent of soil degradation and desertification (Lal, 2009). The historic loss of SOC through periodic tillage has lead to an overall depletion of carbon (C) in agricultural soils and thereby has enabled agricultural soils to become potential $CO_2$ sinks (Paustian, 1997). There is a strong link between desertification and global warming (Sivakumar, 2007), and it is feared that desertification will likely to be intensified with the predicted climate change (Meadows and Hoffman, 2003). Agriculture has a strong influence on the local weather and climate by influencing the transfer of heat from the land surface to the overlying air (Raddatz, 2007). There is no generally accepted definition of sustainable agriculture (Sivakumar et al., 2000), but a reoccurring theme among the definitions is that agricultural systems must remain productive over time (Senanayake, 1991). By converting energy reliance to land-based fuels, sustainability will be of primary concern in order to maintain the integral quality of land, air and water.

Biomass production and rangeland restoration have been identified as key GHG mitigation strategies (Lal, 2001; McLaughlin et al., 2005; Tilman et al., 2006; Adler, 2006; Schmer et al., 2008; Williams et al., 2009; Lal, 2009). It is estimated that biofuels could offset 30% of current $CO_2$ emissions, with irrigation and other agronomic inputs being major components of successful grassland restoration (Lal, 2001; Perlack et al., 2005). Rangeland and prairies are intrinsically sensitive natural habitats and are ecologically sensitive as potential regions for biofuel production. Historically, conversion from native prairies and forest to intensive agriculture has increased anthropogenic $CO_2$ (Johnson et al., 2007). Grasslands contain a naturally high level of soil organic matter (SOM), containing an estimated 12% of the earth's SOM (Sere et al., 1995), which makes proper management crucial to sustainability (Conant et al., 2001). In the United States alone intensive cultivation of these areas has resulted in the historical release of 993Tg of SOM into the atmosphere as $CO_2$ (Kern, 1994). Agricultural tillage can cause disturbance deep in the soil profile and break macro-aggregates. This disturbance causes microbes to oxidize soil organic matter, releasing otherwise deep sequestered C in the soil profile. The SOC reservoir is so large that it is greater than the entire amount of terrestrial vegetation (Paustian et al., 1997). It is assumed, that under good agricultural management these historic losses can be reversed through atmospheric carbon sequestration. Some of these practices include permanent pastures and the sowing of perennial forage grasses and legumes (Conant et al., 2001). Perennial grasses increase SOC annually by allocating nutrients back into the root system as storage for the next year's growing season. Since perennial grass stands can have potentially high rates of C sequestration, restoration of grassland areas has become an even more important topic due to the potential mitigation of atmospheric $CO_2$ as a means to negate the effects of global climate change (Lal, 2009).

Switchgrass (*Panicum vergatum L.*), a $C_4$ grass species, was chosen by the United States Department of Energy (USDOE) as the model crop for cellulosic biofuel because of the plant's perennial nature; it is a native species to North America with high water use efficiency (WUE), high nitrogen use efficiency (NUE), a wide range of exploitable genetics, and an ability to be grown in diverse regions (Sanderson et al., 2008). The specific goal for cellulosic ethanol is to make it cost competitive with grain ethanol as a transportation fuel blend by 2012 (Advance Energy Initiative, 2006).

Manufacturing cellulosic ethanol is an advance technology that has not yet been fully realized due to high production costs. There are three basic steps in utilizing biomass for biochemical conversion to cellulosic ethanol. These are: 1) physical size reduction and thermochemical pretreatment of the biofeedstock; 2) enzymatic hydrolysis of cell wall polysaccharides from a polymer to a monomer and 3) fermentation (Lorenz et al. 2009). Recently, an effective method of pretreatments using phosphoric acid and acetone have been used, this method separates the lignocellulosic components from the biomass and recycles both solvents (Zhang et al., 2007). This allows for the final two steps of the biochemical process to be combined into a single operation called simultaneous saccharification and fermentation (SSF) and “avoids end product inhibition of hydrolytic enzymes” (Lorenz et al., 2009). Manufacturing biofuels is a cutting edge technology and experts have evaluated this process as the most energy efficient method to keep production costs low.

A means to calculate the overall net energy gains of harvesting a crop and investments necessary to create the biofuel is by a net energy balance (NEB) equation. This calculates the net energy gained by converting from conventional gasoline to a biomass based fuel.  This equation measures inputs and outputs of energy used in the production process of bio-fuel synthesis (Hill et al., 2006). Many fossil fuels need very little refining once harvested from geological areas, but have high rates of GHG emissions once combusted. In a NEB equation for the production of cellulosic ethanol from perennial grasses, the manufacturing of biofuels would have high energy costs, but this would be set off by soil C sequestration rates of the plant during annual growth. Bioenergy crops represent a cycle of $CO_2$ and thus have reduced impacts on atmospheric GHG's compared to conventional transportation fuels. Cellulosic ethanol from perennial switchgrass was modeled to produce 500-700% more energy than used in production (Schmer et al., 2008).The C cost or inputs of a NEB equation also include distribution, transportation, and conversion efficiencies at the ethanol refinery plant. Distribution and transporting are important to the NEB equation because of the long distances from rural farms where the crop is grown to urban areas where it is in demand (McLaughlin et al., 2005).

As mentioned previously, a large part of the higher efficiencies for perennial biofuels are from stored C in the soil. C sequestration can be 20-30x's higher in perennial crops than in row crops because they provide continuous ground cover and develop deep rooting systems (Williams et al., 2009). Switchgrass studies have estimated the reductions in GHG emissions to be as great as the amount of harvestable biomass (Kim and Dale, 2005). Estimates determining the amount of GHGs displaced have used the amount of harvested biomass plus the amount of carbon sequestered into the soil profile (Schmer et al., 2008). C sequestration rates can be much higher in perennial versus annual row crops.

In high-diversity grasslands there were increasingly higher bioenergy yields that were up to 238% greater than monoculture yields after a decade (Tilman et al., 2006). Low input, high diversity (LIHD) grasslands for biofuels can be produced on agriculturally degraded lands and will thus neither displace food production nor cause loss of biodiversity through habitat destruction (Tilman et al., 2006). Some dedicated energy crops that are intensely managed as monocultures may require much higher pesticide and nitrogen (N) fertilizer. Research suggests that a bioenergy feedstock could be managed more efficiently as polycultures and could have 10 times more C sequestration potential (Williams et al., 2009). Added ecological benefits from polycultures are increased plant diversity and functional groups, which have also been demonstrated to increase soil fertility (Dybzinski et al., 2008), and retain a higher prevalence of N fixers (Tilman et al., 2001). However, during the manufacturing of the biofuel, there needs to be consistency in the biomass composition for increased ethanol recovery and processing efficiency.

Efficiencies at the ethanol production plant are other critical factors when evaluating the NEB in biofuels because of the intrinsically high valued byproducts that can be recovered and used for other societal benefits. A contributing factor to corn grain ethanol efficiency is the high value animal feed which is generated as a byproduct. In cellulosic ethanol, the byproduct lignin is used to power the refinery through the combustion of the ezymatically indigestible polymer. This polymer byproduct has the potential to generate more energy than is needed to run a production plant and can be reverted back to the electric grid (Schmer et al., 2008). Corn ethanol plants are run off of coal because the byproducts go to high value animal feed and are not used for power (Schell et al., 2008). It is estimated that if 10% of the coal fired plant were replaced by power from co-firing biomass that 2.3 million tons of $CO_2$ could be displaced from energy production (Schmer et al., 2008). Replacing non-renewable energy with lignin byproducts and carbon sequestration are two of the major carbon offsets and benefits of cellulosic ethanol produced from perennial plant species (Schmer et al., 2008).

Cellulosic ethanol from switchgrass has been modeled to have significantly fewer environmental consequences compared to corn grain ethanol due to decreased soil erosion and N leaching (Costello et al., 2009). When irrigation is applied to perennial bioenergy crops there could be less risk of denitrification because of reduced management inputs (Williams et al., 2009). N fertilizer synthesis is directly correlated to large amounts of fossil fuel consumption and prohibits crop production from moving away from fossil fuel based practices. The N requirements for a biofuel crop should be a key point when assessing the benefits of a biofuel feedstock (Boehmel et al., 2008). N fertilizer is one of the most costly energy inputs and the N requirements of a crop are a good indicator of how "green" the feedstock is because of associated GHG emissions (Sanderson and Reed, 1999). N fertilizer can be a source of air and stream pollution from agricultural use in crop production (McLaughlin et al., 2005) and most $N_2O$ pollution is attributed to anthropogenic sources (Del Grosso et al., 2005). When plant materials are combusted for bioenergy uses, the nitrogen compounds in the biomass are released into the atmosphere as $NO_x$ emission. These compounds are more than 300 times more effective as a GHG then $CO_2$ (Schell et al., 2008). There are integrated management practices that could reduce crop N requirements for a biomass feedstock such as the sowing of legumes, but it is not completely clear how these will affect conversion efficiencies (Tillman et al., 2001).

Cellulosic technology has the potential for greater utilization of biomass material generated from diverse plants and parts of plants (Sarath et al., 2008). Cleaner and safer transportation fuels and energy resources can reduce serious public health and environmental concerns caused by air pollution and water contamination (Louime, 2008).

Not only will human health benefits be seen from this energy source, but benefits from reduced military expenditures, a decrease in subsidies and the avoidance of economic disruptions due to fluctuating fossil fuel prices could also be had (McLaughlin et al., 2002). Projected savings from cellulosic derived energy are estimated at $1.5-11.5 billion; with $1.3-7.7 billion from increased farm revenue, $1.2-5.7 billion from reduced government subsidies, and $0.54-3.2 billion from reduced carbon emissions (McLaughlin et al., 2002).

**1.3 Cool-Season Grasses for Biofuels**

While switchgrass is a good model crop as a biomass source, there are several reason why cool-season perennial grasses may also be important sources for biomass energy. These reasons include that cool-season grasses are well established in many parts of the world, have promising compositional make-up, may help balance the annual distribution of biomass availability, may be more adapt to some semi-arid regions and allow for greater versatility in dual forage/biomass systems.

Cool-season or $C_3$ species are major contributors to forage production in the United States (Burns and Fischer, 2010) and have been historically utilized for animal forage because of superior digestibility and high feed value. In relation to cellulosic biofuel, the high digestibility of these forages is directly correlated to reduced lignin content. Lignin is a primary barrier to the bioconversion process that impedes hydrolyses of cellulose and hemi-cellulose for fermentation (Anderson and Akin, 2008), and plant tissues with reduced amounts will have greater conversion efficiencies (Sarath et al., 2008). Lignin reduction has been a major focus in breeding programs for switchgrass (Pedersen and Vogel, 2005). Increasing the polysaccharide to lignin ratio is one identified route to increasing biofuel feedstock quality (Lorenz et al., 2009) and $C_3$ grass species utilized for forage have inherently high polysaccharide to lignin ratios.

There are also reported difficulties in switchgrass stand establishment (Schmer et al., 2008) and certain strands are subject to stand loss during harsh winters (Nielsen, 1947). Establishment in some of the hardier $C_3$ grasses can be easier to achieve due to larger seed size in certain species. Many $C_3$ grasses were initially selected for characteristics related to establishment and production, not quality (Hein, 1955). For example, smooth bromegrass (*Bromus inermis* Leyss.) was one of the few cool-season forage grasses to survive droughts, which lead to an increase in demand in the Great Plains area(Casler and Carlson, 1995).This coincided with the widespread value and popularity of smooth bromegrass for re-vegetation of drought-damaged grasslands and marginal croplands throughout the Great Plains and Midwestern regions (Jensen, et al. 2006). There has also been an increase of interest in meadow bromegrass (*Bromus biebersteinii* Roem. & Schult.) as an alternative source of forage on less productive land; these areas are often associated with periods of reduced irrigation, soil salinity, and low fertility, (Jensen et al., 2006). Intermediate wheatgrass (*Thinopyrum intermedium* (Host) Barkworth & D.R. Dewey) cultivars were released due to increased winter hardiness (Karn et al., 2006). Tall fescue (*Festuca arundinaceous)* has moderate salt and drought tolerance, but is subject to stand loss if conditions are prolonged (Kirksey et al.1993). Crested wheatgrass (*Agropyron cristatum* (L.) Gaertn) and Russian wildrye (*Psathyrostachys juncea* (Fisch.) Nevski) are the most common grasses for reseeding in the Northern Great Plains and research has shown that these species are well adapted for pasture use and reducing the number of hectares (ha) by producing high yields and animal intake (Smoliak and Slen, 1974). Crested wheatgrass is also well known for its drought tolerance (Hull and Klomp, 1966).

In a study by Waldron et al. (2002) a comparison of eight perennial cool-season grass species at five irrigation levels were conducted and concluded that tall fescue and meadow bromegrass would be best suited in limited irrigation environments near Logan, UT. Of those tested, meadow bromegrass was the only species with the root plasticity to avoid stress (Alderson and Sharp, 1995) and the ability to recover quickly once the stress was removed (Beuselinck et al., 1994; Duncan and Carrow, 1998).

In a similar trial, in which irrigation was only applied once to promote germination, Russian wildrye was the only species to persist after the first production season and still had 55% stand after the fourth year. It was also observed that Russian wildrye was the first to green up after precipitation. This might be an indication of this species' ability to make use of small precipitation events compared with other grasses that are slower to reactivate growth (Laurialt et al., 2005). Hendrickson and Berdahl (2002) concluded that Russian wildrye had higher tiller numbers than intermediate wheatgrass after grazing because auxiliary buds were more readily activated in Russian wildrye. However, Russian wildrye stand improvement may not be observed with the resumption of irrigation like that of meadow bromegrass (from previous Waldron et al., 2002), because Russian wildrye is a crown forming bunch grass. Drought tolerant clone forming species such as intermediate wheatgrass, smooth bromegrass, and western wheat grass were also tested and it was concluded that recovery could occur if irrigation was initiated (Laurialt et al., 2005). With the exception of Russian wildrye, the other crown forming bunch grasses, tall fescue and tall wheatgrass would also not adequately recover after stand loss due to drought (Schuster and Garcia, 1973). Russian wildrye, tall fescue, tall wheatgrass and western wheatgrass maintained uniform ground cover across soil moisture treatments and thus have value for stabilization of marginal lands, including poorly drained saline/sodic soils (Lauriault et al. 2005). Smooth bromegrass was slower to establish than other species, but eventually achieved a nearly 100% ground cover under typical irrigation (April –October).

Understanding the temporal niches between cool-season and warm-season species that determine seasonal net plant productivity is important with $C_3$ species having primary productivity during the cool spring from winter and spring precipitation and the $C_4$ species responding to precipitation during the warmer summer months (Niu et al., 2005). Cool-season forage production is highly correlated to stored pre-growing season soil water (Frank and Bauer, 1991). Ranchers use these differences to increase seasonal forage supplies through sophisticated grazing strategies of warm- and cool-season grasses. This same strategy could be incorporated into biomass cropping systems which would allow for a more and longer supply of biomass. Using seasonal biomass supplies would also reduce storage costs and help retain feedstock integrity that is often compromised with prolonged storage.

There is extreme pressure from milk and beef production on the world's grasslands, which provide 25% of forage production for cattle industries (Sere et al., 1995). Livestock producers could be pushed further onto marginal lands through increased biomass production (Sanderson et al., 2008). Livestock is an important part of the economy in agricultural communities and it is for these reasons that $C_3$ forage grasses should be considered as a dual feedstock for forage and biomass production. $C_3$ grasses have been historically utilized for animal forage because of superior digestibility and high feed value; because of these attributes these forages could act as a dual feedstock for animal and biofuel production. A dual acting feedstock could decrease competition, decrease stakeholder risk and enhance participation in bioenergy programs. Also, relying on a diversity of bioenergy crops in ecologically different regions will allow for greater stability, resistance, and resilience to climatic and environmental variability (Jordan et al., 2007).

**1.4 Animal Forage Quality Analysis**

One of the goals of this review is to demonstrate the potential utilization and integration of perennial $C_3$ grasses into biomass production by researching seasonal partitioning of dry matter (DM) yields, crude protein content (CP), and neutral detergent fiber (NDF) and acid detergent fiber (ADF) content of $C_3$ grasses. There is already sufficient literature on $C_3$ grasses as forages, so it will be useful to review these studies and find how this information can be translated into knowledge for the biofuel industry. In animal nutrition, NDF, ADF and acid detergent lignin (ADL) analyses are used to estimate the cellulose, hemi-cellulose and lignin content of forages. The difference between ADF and ADL are used as estimates of cellulose content, while the difference between NDF and ADF is used as an estimate for hemi-cellulose content (Jung, 1997). Cellulose and hemi-cellulose are the major constituents used in cellulosic ethanol production.

These values are important from an animal forage perspective because it gives a measure of quality for feed. CP is a measure of N compounds and is an indicator of how much energy the forage contains for animal growth and weight gain. CP is highest when the plant is young and decreases with stand age. Cellulose and hemi-cellulose increase with stand age and as these values go up the animal forage quality decreases. Total fiber is contained in the NDF and can be used to estimate dry matter intake (DMI) of livestock; as NDF increases there is reduced consumption. The percent of ADF is correlated to the total digestible nutrient (TDN) and net energy of the forage. Forage feeding values are negatively correlated to fiber since the less digestible portion is contained in the cell wall. These measures are different for the animal forage feeding values and biomass quality, because biomass quality and yield increase as NDF and ADF values get higher.

These documented analyses of animal forage quality can be of great use to the biomass energy industry when evaluating different species for utilization. Seasonal timings of harvest affect not only yield, but also biofuel quality (Weimer et al., 2005) by altering the carbon composition of the feedstock which is a major determinant of the feedstock energy density (Boateng et al., 2006). Ash content has been a major hurdle for the biofuel industry in relation to conversion and NEBs. Ash content in the plant decreases with stand age, but is lower in concentration with accumulated biomass, leading to increased biofuel quality (Vogel et al., 2006). The plant reallocates N to the roots as it completes its annual life cycle, and uses the allocated nutrient for the following year's spring regrowth. Evaluating N for biomass energy quality is essential because ash greatly limits optimal performance at the refinery and causes corrosion problems during the conversion process (Capablo et al., 2009). In addition to this, increased N requirements for a crop ultimately lead to greater emissions of $N_2O$ during crop production as well as $NO_x$ emissions during biomass combustion. The following summaries will be given in order to evaluate the seasonal production trends of cool-season species used for animal forage, and how these trends pertain to biofuel quality.

Crested wheat grass and Russian wildrye are the most commonly used forages in the northern Great Plains, however these two species have different periods of peak nutrient quality; crested wheatgrass in the spring and Russian wildrye in the fall (Smoliak and Slen, 1974). For continuous fall grazing, Russian wildrye has been cited to have superior live weight gain for cattle compared to crested wheatgrass and has benefits in regards to late summer and early fall grazing due to high nutrient contents (Smoliak and Slen, 1974). Grazing Russian wildrye during the late summer and fall also increased cattle weight gain because of more nutritious feed compared to that of native rangeland species. During a 6 year study, yearlings had an average weight gain of six times greater while continuously grazed on Russian wildrye than those on the rotation of rangeland or free choice systems (Smoliak and Sydney, 1974).

Crested wheat grass is a long-lived perennial bunch grass that can tolerate heavy grazing and is widely used in rangeland improvement in western regions of the United States. However, there has been little research in developing nutritive quality traits despite its wide spread use (Ray et al., 1996). This cool-season grass is high in digestible nutrients during the spring and loses the high productivity during fall regrowth. Reportedly crested wheatgrass demonstrated significant differences in nutritive quality among cultivars (Coulman and Knowles, 1974; Lamb et al., 1984). It was concluded that there were significant variations for genotype by year for CP, IVDMD, NDF, and ADF and that cultivars should be evaluated over multiple years.

Western wheatgrass (*Pascopyrum smithii* (Rydb.) A. Löve) is the major diet of cattle in south eastern Wyoming (WY) (Samuel and Howard, 1982) and presumably in similar rangelands areas. Studies have found that herbage production is greatly reduced in western wheatgrass by clipping (Hart and Balla, 1980). Conversely, Bedel (1973) reported that by clipping crested wheatgrass during or before anthesis herbage yield production would not depress below that of grass cut after maturity, but forage regrowth was greatly depressed more and more as cutting was delayed after the 3-leaf stage.

Smart (et al., 2006) measured yield and forage quality of smooth bromegrass and intermediate wheatgrass in Lincoln, Nebraska. On average, smooth bromegrass yielded 750 kg/ha, which was 270 kg/ha more than intermediate wheatgrass. Mean forage yield was significantly greater for smooth brome grass during a low precipitation year. However, intermediate wheatgrass outperformed smooth bromegrass during a year with a high precipitation average. Smooth bromegrass had greater leaf blade yields than intermediate wheatgrass during the two year study and higher leaf /stem ratios than intermediate wheatgrass in May, but quality was similar for both in June. Smooth bromegrass had greater CP than intermediate wheatgrass until late May but intermediate wheatgrass had greater CP in late June. Smooth bromegrass develops morphologically earlier then intermediate wheatgrass and it is safe to assume that smooth bromegrass favors the growing conditions of early spring more than intermediate wheatgrass. The greater DM accumulation in smooth bromegrass in the spring is due to rapid stem growth, and this rapid maturation can make it difficult to manage for optimum forage use compared to intermediate wheatgrass (Smart et al., 2006). This makes the timing of harvest critical in order to maintain high feed quality due to rapid degradation of the plant as maturity increases.

Karn et al. (2006) conducted a study related to changes in quality through the different stages of development. Smooth bromegrass had the highest IVDMD and the lowest leaf NDF of western wheatgrass, crested wheatgrass, and intermediate wheatgrass varieties, but with significant interactions involving species, maturity and year. In stem tissue WWG had the greatest CP yield and the lowest NDF, with a strong species by stage of maturity interaction which indicated that stem CP declined more between heading and anthesis than the other three species. Hycrest crested wheatgrass had significantly higher NDF concentrations than its comparison variety Nordan. On average there was a 20% decline in leaf tissue at the anthesis + 10 day stage. Leaf NDF increased as maturity advanced from vegetative to anthesis + 10 day stage and the whole plant tissue IVDMD declined from heading to anthesis. Whole plant NDF was lowest at heading and highest at anthesis. For all species there were lower correlation coefficients for leaf CP vs. IVDMD and leaf CP vs. NDF than for IVDMD vs. NDF. All species had a high negative correlation between IVDMD and NDF. All species in this study met CP requirements for steer and lactating beef cattle in leaf tissue, but were deficient in stem tissue, except for western wheat grass. The plant changes significantly during development and leaves have higher CP then the stems (Fohner, 2002). Smooth bromegrass leaves in this study had the highest IVDMD and CP, but western wheatgrass had the highest whole plant IVDMD.

**Conclusion**

There is a great degree of variability in cool-season grasses in terms of forage quality and DM partitioning, but the extensive research on these high value forages will aid in selecting species for biofuel production. The trend among the research indicated high spring nutritional values for brome and wheatgrass speices, but with brome species developing earlier in the season. RWR was one species with exceptionally high fall CP levels. The high CP levels will be one hurdle that will be difficult to overcome with cool-season forages; CP decreases but remains high even after biomass production has peaked. N requirements and content for a crop are good indicators of how “green” a biofuel crop really is due to the associated $NO_x$ emissions. However, this should not eliminate these forages as potential energy crops because it could simply mean that different harvest schedules address this concern.

Quick establishment, increased seasonal supply of biomass, high yields in harsh conditions, diverse range of growing environments, and wide popularity by forage producers make cool-season forages a highly potential biomass crop. Further delays in harvest timing, far beyond what any forage producer would find suitable for a harvest, will likely be need if cool-season grasses are to be utilized for advanced cellulosic ethanol. Many cool-season forages are being used for rangeland and pasture due to the suitability of environmental conditions in many cooler areas of the United States. It will be essential to evaluate sustainability in general when researching land-based biofuel crops because of the need to maintain and sustain edaphic conditions, water resources and air quality.

The NEB of any biofuel is dynamic and complex, and even different according to the specific longitude and latitude. Correct species selection for each area should be closely evaluated. The United States is evaluating biofuel as a means to national energy security. Biofuels could create more pressure for land based resources and cattle producers and farmers are a part of this struggle. While switchgrass is a native species to the US, by converting to high value cattle producing pastures to a biofuel crop could create a great deal  of competition between ranchers and the energy industry. Because the US produces a great deal of its own cattle, this may become a question of national food security as well. This too however has already been brought to fore front of this ongoing debated of the give and take of natural resources and whether it is right to use land resources for fuel needs when there are millions of food insecure countries globally. Weighing and evaluating the intricate web of sustainability will be of great need in order to ensure all factors are receiving proper consideration.

# CHAPTER TWO

## 2.1 Introduction

An increase in greenhouse gas (GHG) emissions has amplified solar radiative forcings in the atmosphere, altering the global energy balance (U.S. DOE, 2005). The burning of fossil fuels has led to an increase in $CO_2$ in the atmosphere (Adler et al., 2007), and when released this GHG traps heat and increases temperatures of the global climate (Johnson et al., 2007). The increase of the GHG's, carbon ciosxide ($CO_2$), methane ($CH_4$) and nitrogen oxides ($NO_x$), have been attributed as the cause of the climatic warming during the industrial era (Salinger, 2006). Current fossil fuel energy systems could threaten food security due to interference with meteorological patterns (Salinger, 2006). An increase in anthropogenic activities has been attributed to the cause of a decrease in land-based on carbon sink capacity, which increases the severity and extent of soil degradation and desertification (Lal, 2009). Since there is a strong link between desertification and global warming (Sivakumar, 2007), it is feared that risks of desertification will likely be intensified with the predicted climate change (Meadows and Hoffman, 2003).

One identified GHG mitigation strategy that will contribute to climate change scenarios is the use of long term perennial grass production for bio-fuel use and rangeland restoration (McLaughlin and Kszos, 2005; Lal, 2001; Tilman et al., 2006; Schmer et al., 2008; Williams et al., 2009). It is estimated that biofuels could offset 30%

of current fossil fuel use, but irrigation and other agronomic inputs are major components of successful grassland restoration (Lal, 2001; Perlack et al., 2005). Switchgrass, a $C_4$ grass species, was chosen by the USDOE as the model crop for cellulosic bio-fuel because of the plant's perennial nature, high water use efficiency (WUE), wide range of exploitable genetics, and its ability to be grown in diverse regions (Sanderson et al., 2008). Biofuels have reduced emissions due to the $CO_2$ cycle that occurs during the photosynthetic assimilation of carbon. Cellulosic ethanol from switchgrass has been modeled to have significantly fewer environmental consequences compared to corn grain ethanol due to decreased soil erosion and N leaching (Costello et al., 2009). Irrigation for perennial bio-energy crops have less risk of denitrification and $N_2$ leaching compared to when conventionally grown corn is irrigated because of fewer applications (Williams et al., 2009). Also, carbon (C) sequestration can be 20-30 times higher than row crops because they provide continuous ground cover and develop deep root systems (Williams et al., 2009).

The net energy balance (NEB) for bio-fuels measures the overall energetic inputs versus outputs used to manufacture bio-fuel products, and cellulosic ethanol from perennial switchgrass was modeled to produce over 700% more energy than used in production; whereas corn grain ethanol has a lower efficiency rate of 25% (Schmer et al., 2008; Farrell et al., 2006). The specific goal for cellulosic ethanol is to make it cost competitive with grain ethanol as a transportation fuel blend by 2012 (Advance Energy Initiative, 2006).

In order to utilize biomass for cellulosic ethanol there are three basic steps in the biochemical conversion process: 1) physical size reduction and thermochemical pretreatment of the bio-feedstock; 2) enzymatic hydrolysis of cell wall polysaccharides; and 3) fermentation (Lorenz et al., 2009). The final two steps of the biochemical process, enzymatic hydrolysis and fermentation, can be combined into a single operation called simultaneous saccharification and fermentation (SSF), the bonding of these two steps "restricts end product inhibition of hydrolytic enzymes and eliminates the need for separate hydrolysis and fermentation reactors" (Lorenz et al., 2009).

Once cellulosic technology is fully realized, this bio-energy will allow for greater utilization of biomass material generated from diverse plants and different parts of plants (Sarath et al., 2008). It is therefore crucial to evaluate existing crop production systems and find innovative ways of incorporating them into new and upcoming technologies. Livestock production is an important part of the economy in agricultural communities and cool-season species are major contributors to forage production in the United States (Burns and Fischer, 2010). $C_3$ grasses have been historically utilized for animal forage because of superior digestibility and feed value; because of these attributes these forages could act as a feedstock for both animal and bio-fuel production. Livestock producers could be pushed further onto marginal lands through increased biomass production (Sanderson et al., 2008) and a dual acting feedstock could decrease competition. A dual acting feedstock could also decrease stakeholder risk and enhance participation in bio-energy programs. Also, relying on a diversity of bio-energy crops in ecologically different regions will allow for greater stability, resistance, and resilience to climatic and environmental variability (Jordan et al., 2007).

In relation to cellulosic bio-fuel, the high digestibility of $C_3$ forages is directly correlated to reduced lignin content. Lignin is a primary barrier to the bioconversion process that impedes hydrolyses of cellulose and hemi-cellulose for fermentation (Anderson and Adkin, 2008), and plant tissues with reduced amounts will have greater conversion efficiencies (Sarath et al., 2008). Lignin reduction has been a major focus in breeding programs for switchgrass (Pedersen and Vogel, 2005). Increasing the polysaccharide to lignin ratio is one identified route to increasing bio-fuel feedstock quality (Lorenz et al., 2009) and $C_3$ grass species utilized for forage already have inherently high sugar to lignin ratios.

High lignin content with increased yield, difficulty in stand establishment (Schmer et al., 2008) and variable yields under unfavorable growing conditions are the primary concerns regarding switchgrass as a bio-energy crop. It is worth mentioning that during this study, switchgrass stands were not successfully established even under full irrigation and high spring precipitation. Establishment in some of the hardier $C_3$ grasses is much easier to achieve due to larger seed coats and resistance against winterkill. For example smooth bromegrass (SBG) was one of the few cool-season forage grasses to survive droughts which lead to an increase in demand (Casler et al., 1995), and coincided with the widespread value and popularity of smooth bromegrass for revegetation of drought-damaged grasslands and marginal croplands throughout the Great Plains (Casler et al., 2000; Jensen et al., 2006). There has also been interest in meadow bromegrass for utilization of marginal agricultural land, which is often associated with periods of reduced irrigation, soil salinity, and low fertility and is cited for early season forage production and rapid regrowth after defoliation (Jensen et al., 2006).

Other cool-season grasses used for forage include intermediate wheatgrass (IWG) cultivars which were released due to increased winter hardiness. Tall fescue is one of the most salt and drought tolerant cool-season grass forages (Kirksey et al., 1993). Crested wheatgrass is known for its high drought tolerance (Hull and Klomp, 1966); this species along with Russian wild rye are the most common species used for reseeding in the northern Great Plains and research has shown that these grasses are well adapted for pasture use. Crested wheatgrass and Russian wildrye is also efficient at reducing of the number of hectares under production due to high yields and animal intake (Smoliak and Slen, 1974).

Understanding the temporal niches between cool season and warm-season species that determine seasonal net plant productivity is important; $C_3$ species have primary productivity during the cool spring from winter precipitation and $C_4$ species respond to precipitation during the warmer summer months (Niut et al., 2005). Cool-season forage production is also highly correlated to stored pre-growing season soil water (Frank and Bauer, 1991). Sophisticated grazing strategies require knowledge of seasonal partitioning throughout the growing season for different species (Smart et al., 2006). Identifying areas of optimal production for bio-fuels is critical in order to increase successful implementation, and cool-season grasses have already been highly produced in many of the cooler areas of the United States.

One of the goals of this study is to evaluate animal forage quality analyses of $C_3$ grasses, such as seasonal partitioning of dry matter (DM) yields, crude protein content (CP), and Neutral Detergent Fiber (NDF) and Acid Detergent Fiber (ADF), and relate the results to potential biomass use for bio-energy. NDF and ADF analyses are correlated to cellulose, hemi-cellulose and lignin content. The difference between NDF and ADF is used as an estimate for hemicellulose content (Jung, 1997). Total fiber is contained in the

NDF and can be used to estimate dry matter intake (DMI) and as NDF increases the animal consumption is reduced. The percent of ADF is correlated to the total digestible nutrients (TDN)s and net energy of the forage. Forage feeding values are negatively correlated to fiber since the less digestible portion is contained in the cell wall.

Harvest timing is an important management factor for forage quality and is also is important to the bio-energy sector because seasonal timings of harvest effects not only yield, but also bio-fuel quality (Weimer et al., 2005; Adler et al., 2009) by altering the C composition of the feedstock; this is a major determinant of the feedstock energy density (Boateng et al., 2007). Cool-season grasses reach the peak development by the beginning of the summer and harvest dates after this time could decrease biomass yields because the plant starts senescing. Ash content decreases as stand age increases leading to increased bio-fuel quality and potentially decreased N requirements (Vogel et al., 2002); ash also limits optimal refinery performance and is corrosive during the conversion process (Monti et al., 2008). Cellulose and hemicelluloses are the structural $C_6$ and $C_5$ sugars that are hydrolyzed and fermented into ethanol. Carbohydrate concentration measurements of glucan, xylan and are related to cell wall concentration, which ultimately correlates to the total ethanol yield (Lorenz et al., 2009). Near infrared (NIR) spectroscopy shows high correlation between cellulose and hemicellulose estimates using detergent fiber techniques, but are largely driven by total extractives measured by ADF and NDF (Wolfrum et al., 2009).

It is therefore the efforts of this research to analyze the potential utilization and integration of perennial $C_3$ grasses into biomass production by evaluating seasonal partitioning of yields, CP, and neutral detergent fiber NDF and ADF. The study compares yield and quality of 15 different species of $C_3$ forages. These species were evaluated for performance under three different irrigation treatments and two different harvest dates. Belowground biomass samples were also taken to evaluate C content and give rough estimates to potential C sequestration.

**2.2 Materials and Methods**

The research site was located near Iliff, CO (latitude 40.7678, longitude 103.45, elevation 3822). The topography is characteristic of the Platte river drainage basin. This study's focus was on 15 cool-season grasses that were selected based on potential productivity under limited irrigation (Table1). The grasses were planted during the spring of 2007 with a no till drill (Model 3P605NT, Great Plains Mfg., Inc., Salina, KN) with 17 centimeter (cm) row spacing and 7.3 kilograms/ hectare (kg/ha) of herbicide were applied after seeding. Plots were reseeded in the spring of 2008 at the same seeding rate due to a thinly established first stand. Thus the research was started on a fully established stand in the summer of 2009. The soils in the regions are classified as Dix-Eckle-Chappell that are gently sloping, well drained to excessively drained, forming a gravely alluvium with upland ridges and alluvial fans. The soil was classified as clay loam, with medium to high levels of lime, 3.3% organic matter and a pH of 7.3. Total precipitation accumulation for 2009 was 43 cm, which was 116% of the average (Prisms, 2010).

The experiment consisted of randomized complete block with main irrigation treatments plots, grass species as subplots, and harvest timing as sub-sub plots, with three replications. Irrigation main plots measured 18.3 meters (m) by 68.6 m, and harvest timing subplots measured 4.6 m by 18.3 m. The three main plot irrigation treatments set at full season (FS) irrigation, spring and fall (SP/FA) and spring only (SO) irrigation

(Figure 1). Irrigation water was applied using a linear irrigation system, and the irrigation schedule determined based on local calculations of evapotranspiration (ET) and irrigation requirements for forages. Irrigation started on April 23, 2009 and FS irrigation was received through October 5, 2009, with a total application of 40 cm. Irrigation stopped for the SP/FA and SO irrigation treatment on May 20, 2009 and the SP/FA irrigation was resumed on August 24, 2009. The total seasonal application of 23 cm was applied to SP/FA and the SO irrigation received a total of 7.6 cm of irrigation water. Nitrogen fertilizer was applied uniformly to all main plots during the spring of each growing season at a rate of 14.6 kg/ha.

Plots were subdivided by two harvest timings. The first harvest was taken on June 1, 2009 (H1) and the later harvest was taken three weeks later on June 22, 2009 (H2). The two dates that were selected for the harvest times corresponded to important developmental stages of the grass. H1 tried to capture the developmental boot stage of all the species; this was difficult because species varied in the early heading phase. H2 corresponded to the late heading stage, where the entire seed head was exposed. Again the same was true for the second harvest because of varying degrees of development for each species. While this was a split plot design the side of the plot the each harvest was taken on was not random due to the complexity of performing a harvest with this many variables and species. A third harvest date was selected on October 17, 2009 and was done separately for the sides of the plots corresponding to H1and H2. The October harvest date is thus labeled RG1 and RG2, corresponding to H1 and H2, respectively.

Harvests were performed using a Lacerator Green Chopper (Gruett Potter, WI) with an attached weigh bin to collect forage from 1.5 m by 16.5 m area. Plots were harvested at a 10 cm cutting height. A subsample of approximately 400 g was collected from each plot as harvested materials entered the weigh bin using a net to capture a random sample along the entire plot length. Subsamples weights were taken to the laboratory. The samples were dried at $55^{o}$ C for a minimum of 72 hours in a forced-air oven to determine dry matter (DM) content. Samples were put through a course grind of 2.0 millimeter (mm) using a Wiley Mill. A subsample was taken of the coarsely ground materials and put through a second fine grind using a cyclone mill (Udy Analyzer Co., Boulder CO) with a 0.1 mm screen, which was used for analyses. The dried, ground forage was stored in plastic cups at room temperature.

The two spring cuttings and each corresponding re-growth were analyzed for Crude Protein (CP) content and ADF and NDF content. For CP analyses, each sample contained approximately 0.1 gram (g) which was loaded into foil for determination of total N using a LECO CHN analyzer (LECO CHN-2000, St Joseph, MI). Crude protein concentration was calculated as by % N x 6.25.

The detergent fiber analyses were determined using the ANKOM filter bag procedures (http://ANKOM.com/09prcedures/procedures.html, ANKOM technology, Macedon, NY). Approximately 0.5 g of sample was weighed out and placed in filter bags, which were heat sealed. NDF and ADF were determined sequentially with the ANKOM-200 Fiber Analyzer. Samples were extracted with neutral detergent solution (Van Soest, 1994), and the residue was dried at $100^{o}$ C for a period of 24 hours and re-weighed to determine percent NDF. The NDF residue was then extracted with acid detergent solution (Van Soest, 1994). The samples were then washed with acetone and dried again for another 24 hour time period and reweighed to determine percent ADF.

Soil and root samples were taken on Oct 22, 2009 for six species, CWG, EMB, ESB, TFF, PWG and RWR, which represented the range of species and yields in H1. A 7.6 cm diameter core was taken for three depths field soil characteristics and soil moisture for three depths 0-25 cm, 25-50 cm, and 50-75 cm. Soils were oven dried at $100^{o}$ C for a period of 72 hours and dry weights were recorded. The samples were grouped together by irrigation treatment and repetition and mixed using approximately 1.0 g from each bag sample.

A 4.2 cm diameter core was used for root sampling and was divided into three depths of the top 0-.25 cm, 25-50 cm and 50-75cm. Roots were measured in per hectare basis. The soil root samples were taken the USDA Hydrology Laboratory in Fort Collins, CO. and extracted using a soil hydrology washer. Once roots were fully exposed, they were loaded into vials with DI water and transported to the CSU Plant and Soil Science laboratories where two more washes were performed to dispose of any residual soil on the fine root hairs. Once fully cleaned, roots were placed in the drier at $100^{o}$ C for 72 hours and dry weights were taken for each depth. Since there was so little root material the depths were then combined and ground through
2 mm screen of a Wiley Mill in preparation for total C and N analysis using a LECO CHN analyzer (LECO Corp., St Joseph, MI) for seasonal plant C and N allocation into the roots.

*Statistical analysis*

Statistical analyses were conducted to compare yield, NDF and ADF content and CP of all 15 species on a per harvest basis. Analysis of variance using least significant difference (LSD) was performed for irrigation, harvest timing and species using PROC

MIXED (SAS Institute, 2008) and mean differences were considered significant at $\alpha$=0.10. A log transformation was performed on yields, but all other data tested normal. In order to compare the first two harvest dates, H1 and H2 were analyzed together for significant differences between yield and quality analyses; the two re-growths harvests were also analytically compared with these given tests. Main irrigation effects were also analyzed for each re-growth due to irrigation by species interactions; this was not performed for the first two harvests because irrigation treatments were not significant.

**2.3 Results and Discussion**

The objective of this study was to evaluate 15 cool-season grass species under varying irrigation treatments and harvest timings through yield, digestible fiber and CP content. These measures of animal forage quality such as cellulose, hemicellulose and CP content can also be related to biofuel quality. The novelty of this research comes in that the 15 species are being evaluating at a later harvesting date, one that is not typical for high quality animal forage, but allows for maximum biomass yield. This will give insight to how these forages accumulated structural carbohydrates later in the season. Also, the late harvest would not eliminate this harvest from being used as a forage crop if the biofuel market was down. Many species retained high enough quality that it could still be used as lower quality feed.

### 2.3.1 Harvest 1 and Harvest 2 Analyses

*H1 and H2 Yields.*

Two 2009 spring harvest timings were taken in 2009. The goal of the first cutting (H1) was to optimize forage quality for animal feed and to compare the trade off value of compromised yield. The goal of the second harvest (H2) was to maximize biomass yield and evaluate the lower quality bio-feedstock as an energy crop. The first harvest taken on June 1, 2009 (H1) corresponded to the boot to early heading stage of development; this is the optimal time to harvest for animal feed. The analysis will start by evaluating the first harvest H1.

Since there was no statistical significance due to irrigation, H1 and H2 yields were averaged across treatments (Figure 2). There were statistical differences among species (P=0.0001) for H1 yields. The highest yielding species, ESB, EMB, PWG, CWG, and IWG, averaged 4500 kg/ha and were statistically different than RWR and the two tall fescue varieties which had yields of 2730 kg/ha  and 3190kg/ha, respectively. Russian wildrye was the lowest yielding species for the H1 and was the only species which was significantly different than HB, MB1, MB2, SWG, TWG and WWG. Disregarding the low yielding species, TFF, TFQ and RWR, all other species had very similar yield averages. The brome species matured earlier compared to most species and had the highest H1 yields.

The second spring harvest (H2) was on June 22, 2009 and corresponded to the mid to late heading stage. Except for tall wheatgrass which was still in the boot, all other species had reached the growth peak of forage production. There were statistical differences among species (P=0.0001) for H2 yields (Figure 2). The highest yielding species in the second harvest were CWG, IWG, PWG and TWG with a yield range of 6650-8650 kg/ha. This group of wheatgrasses had yield ranges of 4850-5825 kg/ha and were significantly different from TFQ, RWR, EMB, MB1 and MB2. It is clear that the brome species in harvest H1 had reached the growth peak during early spring, due to suppressed yield in H2 harvest. The wheatgrasses in H2 outperformed the yields of the brome species, which indicates later development and different production peaks. TFF had the lowest yield of 4535 kg/ha and was the only species that was significantly different from the mid-range yielding species, ESB, HB, HWG, SWG, and WWG.

Most species followed an expected yield range, all except TFF. It was surprising to see the low yields for the two fescue varieties since both are later developing species as well. Tall fescue is reportedly a late producer and maintains low vegetation until the fall compared to other cool-season forages (Collins et al., 1981). Unlike this study, Pearson (2004) cited tall fescue as being a high yielding species in western Colorado. It is not fully understood what prohibited higher yield in this study. Statically there was no difference between the endophyte free species and the novelty endophyte tall fescue variety TFQ. However in H2, TFQ was not statistically different than the highest yielding species and TFF did vary significantly.

Comparing H1 and H2 yield there was a significant interaction between species and harvest date (P=0.0001). H1 had a yield average of 4135 kg/ha and H2 had a yield average of 6390 kg/ha. By delaying harvest for three weeks there was an average increase of 37% in yield. All species had a significant increase in yield with delayed harvest, but the degree of increase varied among species. It is typical that when a plant reaches boot stage, that it has produced approximately one half of yield potential that occurs at full maturity (Fohner, 2002), thus explaining the significant increases in yield for delayed harvest. TWG, PWG and IWG had the largest increases of 43% in yield. The TFF, EMB and MB2 had the least significant yield increases.

*H1 and H2 Neutral Detergent Fiber.*

There was a strong species effect (P=0.0001) in NDF for the H1 harvest (Table 2). CWG, EMB, ESB, HWG, MB1 and MB2 had the highest NDF concentration with an average of 60%, and were statistically different from the lowest group of species PWG, RWR, TFQ and TWG, which averaged 57.5%. It was surprising that the higher quality spring forages such as, CWG and ESB, had such high NDF content in H1 harvest. This demonstrates earlier development of the forage species CWG, EMB, ESB, HWG, MB1 and MB2, compared to some of the wheatgrasses and fescues. The early developing species had already begun to decline in forage quality by the June 1 harvest and were likely in their peak mid May. This may be an indication of which species have early and late seasonal development because, PWG, RWR, TFQ and TWG, all had lower NDF values and better forage quality for the H1 harvest. The overall H1 species NDF average was 59.5%.

In H2 NDF analysis there was no significant difference among species (P=0.1034) and the NDF average for this harvest interval was 63.5% (Table 2). This lack of difference between species for NDF is likely due to the late harvest date where most species had fully reached the growth peak and were going into dormancy for the summer. It may also reflect the peak NDF accumulation that the cool-season grass species can achieve during the late growing season. Peak NDF content is important to consider when evaluating species for biofuel quality because this is ultimately a measure of fermentable structural carbohydrates for cellulosic ethanol.

Comparing H1 and H2 NDF values, there was a significant species by harvest effect (P=0.0001), indicating the differences in species capacity to accumulate structural carbohydrates between the two cutting dates. This confirmed the difference in forage quality between the two cutting dates, which was expected. There was an overall 5.5 percentage point increase in NDF from H1 to H2. CWG, IWG, PWG, SWG, TWG, and WWG had the greatest decline in animal forage quality and had an increased in NDF by 10%. A study by Karn et al. (2006) reported smooth brome grass to have the highest invitro dry matter digestibility (IVDMD) and the lowest leaf NDF of WWG, CWG, and IWG varieties; there was however significant interactions involving species, maturity and year.

*H1 and H2 Acid Detergent Fiber.*

There were statistical differences among species (P=0.0668) for H1 in ADF content (Table 3). EMB had the highest ADF value of 36.7%, which was statistically different than ESB and PWG which averaged only 30.5% in ADF content. No other species in H1 had statistically different ADF values; which is likely due to the early harvest timing, but fully demonstrates the difference in development between meadow brome and smooth brome species. Most species, especially ESB, PWG, HB, RWR, WWG, and TFF, were highly digestible animal forages. It was interesting that in H1 harvest ADF had less of a species effect between species compared to the H1 NDF species effect. It seems that cool-season grasses do not develop ADF structural carbohydrates as readily, even with a delayed harvest.

Analysis for H2 harvest ADF content there was also a strong species effect (P=0.0085) and verifies a significant difference between species for ADF (Table 3). EMB had the highest ADF value of 42%, which was only statistically different than HB, TFF and TFQ. HB, TFF and TFQ had the lowest ADF values for H2 harvest maintained good feed value probably because maximum production had not been reached.

There was a statistically significant species by harvest interaction (P=0.0074) between H1 and H2 for ADF percentages. The ADF average for H1 was 32.5% compared to the 37.5% average of H2. On average ADF values increased by 13% by delaying the harvest timing from H1 to H2, and coincide with other research that NDF and ADF increase with stand age (Karn et al., 2006; Smart et al., 2006). There was a significant increase in ADF of 14% for the species CWG, ESB, IWG, SWG and RWR. While these were among the highest, all species had a significant change in percent ADF by delaying harvest for three weeks. It is clear that many species have very high quality for animal consumption in the early spring. Even with delayed harvest however these species still maintained a fair to average quality for cool-season grasses. Since these species are so highly digestible, especially in the early spring, it will be interesting to see how the high non-structural sugars will affect bio-fuel yield. It was found that the non-structural carbohydrates that make up a large percentage of these grasses do have an effect on correlation models when evaluated through near infrared spectroscopy (Wolfrum et al., 2008).

Interestingly this study was also similar to a study by Karn (2006) performed on smooth bromegrass which cited the species for its difficulty in management due to high spring nutritive values and a fast rate of decline in quality after boot stage. For this study of dual purpose biomass crops, the varietal species ESB underwent a 25% increase in ADF. Given the strong association between ADF and nutrition this species would be excellent for animal feed in the early spring, but also a potentially good cellulosic biofuel grass because ADF is a measure of hemicellulose. In this research, ESB had some of the most dramatic changes in quality than any other species, going from the most palatable in early spring to one of the lowest quality species within a three week time frame. Smooth bromegrass has a fast increase in stem growth, dry matter accumulation and fast decline in forage quality (Smart et al., 2006).

There were many similarities between ESB and CWG for this research in regards to growth and development patterns. CWG had one of the lowest fiber percentages in the early spring and quickly increased in cellulose and hemicellulose content in the later harvest date. However, CWG yield continued to increase significantly unlike the ESB. Other studies have cited crested wheat grass as a long lived perennial bunch grass that can tolerate heavy grazing and is widely used in rangeland improvement in western regions of the United States (Ray et al., 1996). There has however been little research in developing nutritive quality traits despite its wide spread use (Ray et al., 1996). This cool-season grass species is high in digestible nutrients during the vegetative and early reproductive stage, but declines rapidly as it reaches later maturity stages (Maryland et al., 1992; Newell and Moline, 1978). Reportedly crested wheatgrass demonstrated significant difference in nutritive quality among cultivars (Coulman and Knowles, 1974; Lamb et al., 1984). Ray (et al., 1996) also concluded that there was a significant genotype by year variance components for CP, invitro dry matter digestibility (IVDMD), NDF and ADF and should be evaluated over multiple years.

*H1 and H2 Crude Protein.*

There was no significant difference between species for crude protein (CP) in H1 and the overall harvest average was 17.5% (Table 4), which is high quality for animal forage. This research is similar to other studies which reported crested wheatgrass and smooth bromegrass as having superior CP content for spring grazing (Smoliak and Slen, 1974; Karn et al., 2006). High CP is a major hindrance for the biofuel industry because of corrosion during biofuel synthesis and $NO_x$ emissions during combustion. These are major factors when evaluating a feedstock for biofuel.

In H2 there was a significant species effect (P=0.055) for CP content (Table 4). There was a significant difference between RWR and other species. RWR had the highest average CP content in H2 of 14.7%. The ESB, TFQ, TWG and WWG species had the lowest average CP of 11.7%. TWG was also one of the highest yielding species in H2, and coupled with a fast decline of CP, making this one of better suited species for biofuel cropping in this research because of the benefits related to conversion and life cycles analyses. TWG also has high feed quality in the early spring making it a good species for a dual forage/ biofuel crop. In the later harvest RWR had significantly higher CP than other species, proving why this species is considered a late seasonal developer, as well as why it is a highly utilized as a cattle grazing forage. However since H2 was the harvest interval to evaluate maximum quality for biomass, RWR would be a very poor species because it had some the lowest yields for H2 and even with delayed harvest retained a high CP content. In general, all species retained CP content of over 10%, which may be too high for a biofuel feedstock.

Comparing the two harvest dates for CP there was a significant species by harvest date interaction (P=0.0001). CP concentration had the largest net change of 29% increase from H1 to H2. On average H1 CP concentrations ranged from17%, where as H2 CP content had an average of 12% CP among species. It is the combined effects that the plant undergoes during maturity that changes the proportion of leaves and stems, which decreases nutritional quality of the whole plant (Fohner, 2002). The plant CP is highest in the leaves and the whole plant during early developmental stages (Fohner, 2002), which in this case corresponds to H1. ESB and RWR were two species with significant changes in CP. However, even with the large decrease in CP from H1 to H2, these CP values are still too high for good biomass energy quality.

In other studies comparing CP levels of cool-season grasses Karn (et al., 2006) demonstrated that smooth bromegrass (SBG) stem CP decreased to a lower level then IWG and CWG. Smart (et al., 2006) showed that IWG matures 1 to 2 weeks later than smooth bromegrass, and is vegetative later in the growing season. The greater DM accumulation in smooth bromegrass in the spring is due to rapid stem growth, and this rapid maturation can make SBG difficult to manage for optimum forage use compared to other cool-season forages (Smart et al., 2006).In stem tissue, WWG had the greatest CP yield and the lowest NDF, with a strong species by stage of maturity interaction which indicated that stem CP declined more between heading and anthesis than the other three species.  For all species there were lower correlation coefficients for leaf CP vs. IVDMD and leaf CP vs. NDF than for IVDMD vs. NDF. All species in this study met CP requirements for steer and lactating beef cattle in leaf tissue, but were deficient in stem tissue, except for WWG.

### 2.3.2 Re-Growth Analyses

*RG1 and RG2 Yield.*

There was only one harvest date for the fall re-growth of H1 and H2, but the sides of the plot corresponding to each spring harvest were measured separately to compare the effects of the prior harvest timings. Hence RG1 correlates to H1 regrowth and RG2 correlates to H2 regrowth. The fall regrowths were not expected to have very high yields and were considered valuable only as culmed fall grazing for livestock. Good green color is an indicator of feed quality and many of the species in the re-growth trials were lacking in color.

For RG1 yield analysis there was a strong species effect (P=0.0001). The highest yielding species for RG1 were TWG, TFQ TFF and HB which had average yields of 2730 kg/ha (Figure 3). It was not until the fall regrowth harvests did tall fescue outperformed other species in terms of yield. One of the reasons for tall fescue's popularity is its ability to adapt to a wide range of soil, climatic and management conditions (Asay et al., 2001). Tall fescue has also been cited as having superior fall re-growth and nutritional value compared to smooth bromegrass. The second lowest yielding species were MB2, IWG and CWG with average yield of 1500 kg/ha and the native species SWG had the lowest yield of 650 kg/ha. In fact none of the native species, SWG, PWG and IWG, did not performed as well in either of the fall regrowth harvests. It was interesting that TWG, PWG, and IWG followed very similar production patterns up until the fall, where TWG out yielded both IWP and PWG by nearly 1200 kg/ha. Collins and Balasko (1980) reported similar results for TWG, in that this species has late seasonal maturity. TWG has also been cited as a cool-season grass species with good resilience to limited irrigation (Schuster and Garcia, 1973). Other reported characteristics of TWG are reduced resilience due to frequent clipping (Schuster and Garcia, 1973) and lower NUE than other cool-season grasses (Cooper and Hyder, 1959).

In RG2 there was also a significant species effect (P=0.0064) for yield. The species with the highest yields again were HB, TWG, TFF and TFQ, with an average of 1110 kg/ha. This is nearly half the yield of RG1 and is because of the shortened regrowth period. Species with the lowest yields, PWG, SWG, CWG, WWG, EMB and MB2, had a group average of 600 kg/ha. It is interesting that MB2 had lower yields compared to other brome species, MB1and that EMB. In fact, HB and MB1 were the highest yielding brome species in the RG2 harvest. This yield performance may be marking an important difference between MB1 and MB2 cultivars regrowth abilities during the fall. Again in this harvest TWG had the highest yields and may have an advantage over other cool-season grass species due to it relatively late maturity date (Cooper and Hyder, 1959). IWG was also cited as having later maturity dates then ESB and CWG (Smart et al., 2006). While this was true for H2 harvest interval, IWG was one of the lowest yield species even under FS irrigation for RG2.

Hybrid bromegrass performed the best out of the brome species in fall regrowth. Hybrid bromegrass is a cross between meadow bromegrass and smooth bromegrass. HB was not only the highest yielding brome species, but one of the top yielding species in . Meadow bromegrass has been utilized in non-irrigated and irrigated pastures due to a high tolerance to grazing, which can extend the grazing season as well as increase total forage production (Jensen, 2002). Comparing forage yields between meadow brome and smooth brome, meadow brome has higher yields, recovers from grazing much more rapidly, and has superior fall re-growth (Jensen, 2002). It is safe to assume that hybrid bromes receives its late fall development characteristics from meadow bromegrass.

Comparing yields for RG1 and RG2 there was also a statistically significant species by harvest date effect (p=0.0003). The average yield for RG1 was 2730kg/ha, which was 49% greater than the RG2 yield average of 725 kg/ha, across irrigation treatments. The higher yield for RG1 corresponds to a 20 day longer re-growth period that followed the earlier spring harvest H1. This also documents the dramatic changes that these species can undergo in a very short period of time. Evaluating total annual biomass production (H1+RG1 and H2+RG2), H1+RG1 averaged over species was 5535 kg/ha and H2+RG2 averaged 7030 kg/ha (Figure 6), which is nearly a 1500 kg/ha increase in annual forage production. This marked yield increase with delayed makes the later spring harvest date the best interval for a biofuels production system. Both of these harvest intervals fully demonstrate the high seasonal yielding ability of these 15 $C_3$ grasses. Considering total annual biomass production, the regrowth was nearly 30% RG1, while the regrowth only produced 10% of the total yield for RG2. RG2 yields were extremely low and maybe an indication of these cool-season grass species reliance on tillers for re-growth. Bedell (1973) reported that by clipping CWG during or before anthesis, it did not depress total herbage yield below that of grass cut after maturity, but forage regrowth was greatly depressed more and more as cutting was delayed after the 3-leaf stage. This may be an overall pattern for cool-season grasses productivity since RG2 regrowth yields was greatly depressed by the delayed H2 harvest.

There was a species by irrigation treatment interaction for yield in both RG1 (P=0.0756) and RG2 (P=0.016) (Figures 4 and 5). Full season irrigation greatly influenced fall re-growth yield and is an indication of species relative potential yield (Schuster and Garcia, 1973). TFF, TFQ, TWG and HB were the species with the most significant responses to FS irrigation during the fall in comparison to other species. Of the high yielding species for fall regrowth, TWG and TFF had the smallest difference in yield between FS and the limited irrigation treatments, which may be an indication of these two species higher drought tolerance. In RG1, FS irrigation treatment for TWG and TFF was only 700 kg/ha more than the limited irrigation treatments. Because $C_3$ grasses have a second production phase in the fall, it was interesting that there were no effects for the fall irrigation treatment. There may be evidence of the benefits of these irrigation treatments in the next years (2010) DM yields due to extra stored soil moisture. The lowest yielding species for fall regrowths were SWG, MB2, and EMB and there was very little difference between FS and the limited irrigation treatments. Further, there was virtually no difference between FS and limited irrigation for ESB, which demonstrates that this species early seasonal development is not followed by high fall productivity.

Other studies have also compared yield performances for cool-season grasses under varying levels of irrigation. Waldron et al. (2002) compared eight perennial cool-season grass species, at five irrigation levels, concluding that tall fescue (TF) and meadow bromegrass (MBG) would be best suited for limited irrigation environments near Logan, UT. Of species tested, meadow bromegrass has the only root system plasticity to avoid stress (Alderson and Sharp, 1994) and can recover quickly once the stress is removed (Beuselinck et al., 1994; Duncan and Carrow, 1998). It was also observed, before complete stand loss, that RWR was the first to green up after precipitation, which might be an indication of this species' ability to make use of small precipitation events compared with other grasses that are slower to reactivate growth (Lauriault et al., 2005). Hendrickson and Berdahl (2002) concluded that RWR had higher tiller numbers than IWG after grazing because auxiliary buds were more readily activated in RWR. However, RWR stand improvement may not be observed with the resumption of irrigation due to the fact that RWR is a bunch-grass. As a drought tolerance clone formers (Beauslick et al., 1994) intermediate wheatgrass, smooth bromegrass and western wheatgrass could possibly improve stand if irrigation was reinitiated. With the exception of RWR, tall fescues and TWG would not be adequate in recovery after stand loss due to drought if any plant survived (Schuster and Garcia, 1973). RWR, TFF, TWG and WWG have value for stabilization of marginal land, including poorly drained saline/sodic soils (Lauriault et al., 2005).

Smeal (et al., 2005) measured yield responses in eight cool-season grasses to varying rates of irrigation on the western plateau of Colorado, and found that meadow bromegrass and tall fescue produced greater DM yields than CWG, MBG, IWG and PWG at the highest rate of irrigation 700mm applied water. IWG produced the most DM under limited irrigation of less than 600mm, but only produced half of the DM yields compared to the highest yielding species under full irrigation treatments. Tall fescue produced the greatest amount of forage under all irrigation treatments. There was strong linear correlation between total seasonal DM yield and the amount of water applied. The intermediate and pubescent wheatgrass and tall fescue out produced smooth bromegrass, while the crested wheatgrass had the lowest rate of yield increase with applied irrigation.

*RG1 and RG2: Neutral Detergent Fiber.*

The NDF analysis for RG1 (Table 2) shows a significant difference between species (P=0.0001). SWG, TWG, TFF, TFQ and WWG were the species with the highest NDF values with an average of 59%. While TWG, TFF and TFQ were among species with the highest yield averages, SWG and WWG had very low yields. CWG, EMB, HB, HWG, IWG, MB1, MB2, PWG, and RWR had the lowest NDF with an average of 55%. HB had an average NDF value of 53% and was one of the highest yielding species in the trial under full irrigation, making it excellent for fall grazing. This is not to say that the other high yielding species with higher NDF values would not be appropriate fall grazing species, because even though the NDF values were higher than others, they are still within the range of a good NDF feed value.

The NDF analysis of RG2 (Table 2) there was a significant species effect (P=0.0001). In RG2, SWG, TFF and WWG had the highest NDF values of 57%, and were significantly different than CWG, ESB, HB, MB1, MB2, and TWG which had the lowest average NDF value of 52%. The high feed quality for CWR, RWR and the meadow bromes, once again reiterates why these forages are highly utilized in the cattle industry. However CWG and most brome species excluding HB are limited by fall yield potential. The high yields and palatability for TFF, TWG and HB also make them the most suitable fall forages in this study.

Comparing RG1 and RG2 there was a species by harvest interaction (P=0.0016) between the re-growths. RG1 had the higher average of 57% and RG2 had an average of 55%, which was equivalent to a 2.6%. For both regrowths there were only a few species that had high regrowth and the bromes, except for HB, remained low in productivity.

However the brome species did maintain lower NDF values in RG1, which had the longer growing period. Of the two regrowths, RG2 had slightly better quality in forage, but because of the very low yields this higher feed quality may not be a good trade off. It was not surprising that both culmed vegetative regrowths were high in forage quality, and that RG1 is better suited for fall grazing because of higher DM yields.

*RG1 and RG2: Acid Detergent Fiber.*

In the RG1 ADF analysis there was a significant difference (P=0.0150) among species. SWG had the highest average ADF value of 34.4%, which was statistically different from CWG, HB, MB1 and TFQ which had the lowest group average of 30.5% (Table 3). Since TFQ and HB were among species with the highest yields and they retained high feed value, they would be well suited as fall grazing forages. In RG2, there was also a significant species effect (P=0.0008) for ADF analysis with SWG, IWG, and WWG the highest averages of 33.7%. These species were statistically different from the lowest group ESB, MB1, MB2, RWR, and TFF, which had an average ADF value of 29.5%. In regards to livestock feed, species with lower ADF values would be better suited for fall grazing due higher nutrient content.

The RG1 and RG2 had a significant species by harvest date interaction (P=0.0144) for ADF. There was an overall difference of 2.3% between RG1 and RG2, with RG1 having the lower quality due to the longer re-growth. Since the quality between the two re-growths was fairly minor it can be assumed that the culmed fall growth will be well suited for animal forage, but yield will be the most limiting factor.

*RG1 and RG2: Crude Protein.*

Almost all species for both re-growths had very high crude protein and would be well suited for fall grazing. There was strong species effect (P=0.0001) for CP yield for the RG1 harvest (Table 4). The species with the highest CP content were EMB, ESB, HB and MB1 with averages of 17.5%. These species were significantly different than HWG, SWG, TWG and WWG which had the lowest average CP content of 13.6%. While it was a little disappointing that TWG had low CP, because this was one of the most drought tolerant and highest yielding species for the fall, but in general all species retained fairly high CP content for this harvest.

In the RG2 fall harvest there was a significant difference (p=0.0018) between species for CP content as well (Table 4). The species with the highest CP content for RG2 were CWG, EMB, HB, MB1, MB2 and RWR which had averages of 19%, and were statistically different than IWG, SWG, TFF and WWG which had the lowest CP averages of 15.7%. In RG1 the two fescues were in the intermediate group for CP among species, while in RG2 TFF was in the lowest group. Even though the two fescue species had lower CP levels than other species, the CP was still at a high enough level and yields were amongst the highest for both fall harvests. This is a better representation of why these forages have been cited for superior quality and yield in other studies.

There was a species by harvest interaction (P=0.0593) between RG1 and RG2 CP content. On average RG1 had a CP concentration of 15.7% and RG2 had a higher average of 17.6%. Overall, there was an 11% change in CP content between the two re-growths, which is from RG2 having less growth time due to the late spring H2 harvest. The species with the most dramatic change was TWG which had a CP content of 12.7%

in RG1, and 18.3% in RG2, which is a 30% net change between the two cuttings. High yield and high CP concentration in culmed vegetative re-growth makes TWG an excellent species for fall grazing. However, due to the high CP content in some of the species it should be investigated further for whether crude proteins are available or unavailable.

The meadow brome species as well as HB, CWG and RWR had the highest CP content species for fall re-growth which is compatible with other related literature. Finding pastures to finish cattle on during the fall is important in order to extend grazing the season. CWG and RWR are the most commonly used forages in the northern Great Plains (Smoliak and Slen, 1974). However, these two species have different periods of peak nutrient quality; crested wheatgrass in the spring and RWR in the fall (Smoliak and Slen, 1974). Cattle that were continuously grazed during the fall on RWR had superior live weight gain for cattle versus crested wheatgrass (Smoliak and Slen, 1974). RWR also has reported benefits of late summer and early fall grazing due to high nutrient contents. During a 6 year study yearlings gained significantly more live weight on continuously grazed RWR than any free choice rangeland systems (Smoliak and Slen, 1974).

### 2.3.3 Root Biomass, Crude Protein, and Carbon Content

Root biomass was sampled on October 22, 2009 at three separate depths: 0-25, 25-50 and 50-75cm. Only the first spring harvest (H1) and the full season (FS) and spring only (S) irrigation treatments were sampled. CWG, EMB, ESB, TFF, PWG and RWR were the species selected for root biomass evaluation. It was the intent of these sampling methods to capture the highest to lowest above ground biomass yield range and each group of species. There was no statistical significance in root biomass among species or irrigation treatments in the top 0-25 cm depth (Table 5). The second sampling depth, 26-50cm, had a significant species effect (P=0.075) for root biomass. The species with the lowest root biomass in depth 2 were CWG and ESB, with 1,300 kg/ha and 1,047 kg/ha when averaged over irrigation treatment. The other species, EMB, PWG, RWR and TFF had significantly more root biomass, with a group average of 1,950 kg/ha. This is especially interesting since TFF and RWR had the lowest above ground biomass yields in the spring and some of the highest root biomass. Extensive root systems may be an indication of increased drought tolerance in these species. In the third sampling depth, 51-75 cm, there was a significant irrigation effect, where the spring only (S) irrigation treatment had significantly more root biomass than the FS irrigation treatment across all species. Spring only irrigation average 939 kg/ha and full season irrigation had an average of 433 kg/ha. This may be an indication that plants allocated more carbohydrates to below ground biomass in order to find water deeper in the soil profile.

Root crude protein and carbon content was determined (Table 6). There were no significant differences among species or treatments. On average crude protein for root biomass was 10.9 % and C content average 37.2%.

**Conclusion**

Utilizing diverse feedstocks for the bio-fuel industry is critical if the United States is to meet renewable energies goals. Identifying biomass resources that are already being produced could aid in the supply and demand for biofuel feedstocks, as well as support other industries such as livestock production. Therefore it was the objective of this study to evaluate 15 different $C_3$ grasses for yield and quality in relation to harvest date and irrigation. The ultimate goal and future products of this work is to eventually select several of these species for further evaluation with near infared spectroscopy analyses based on the yield and forage quality data conducted during this research. The selected species will then be harvested on a larger scale and sent to the biofuel refining plant to be fermented on a microscale level into cellulosic ethanol. This research was the first important step towards the end goal and will aid in selecting and correlating cool-season grass forage quality data to net ethanol production.

There was a clear and significant response in the 15 cool-season grasses tested in regards to species, harvest timing and irrigation and how these relate to quality and DM partitioning. The first harvest (H1) was extremely high in palatability and quality and steadily declined as harvest timing was delayed to the later harvest (H2). In each of the harvests there were several distinct groups among species in terms of yield and forage quality. The brome species had the highest yields and overall quality in H1, with the exception of CWG which was also a top performer in terms of animal forage for H1. In H2 the brome species dropped out of the high yields and the wheatgrass species had significantly higher DM.  It was apparent that there were different peak production and quality trends between the bromegrass and wheatgrass species in this study. It was also interesting that CWG was similar to a brome species in regards to yield and forage quality in H1, but in H2 maintained high yields later in the season much like the wheatgrasses. IWG, PWG and TWG also followed similar production patterns in the spring harvests, but drastically changed in terms of yields for fall regrowth.

In terms of animal forage quality, NDF, ADF, and CP for both fall regrowths were extremely high in feed value, making them good fall grazing forages. Both RG1 and RG2 also followed a similar trend of decreased animal forage quality as stand age increased. The RG2 culmed harvest had extremely high CP content and several species including CWG, EMB, HB, MB1 and TWG had higher values than the early spring harvest H1. The largest drawback for RG2 was the greatly depressed yields, which would not be sufficient for a single source of fall grazing. While RG2 had higher CP than RG1, RG1 had nearly twice the yields and was still high in animal quality. However neither of these two fall regrowths had high enough yields to mechanically harvest and would be best suited for animal grazing.

It is clear that H1 was the best harvest interval for animal feed because of high nutrition and digestibility. All species performed exceptionally well in terms of animal feed quality. It is unclear however, how this high forage quality will affect biofuel conversions because of the high amount of extractives and decreased fiber content. Cellulose and hemicelluloses are the polysaccharides fermented in cellulosic ethanol, and these two compounds increase with stand age. Because the early spring harvest is high in non-structural carbohydrates, the simple sugars may go straight into fermentable compounds during refining. Even if this is the true about the sugars, the high CP content may be one of the most obstructing barriers for this harvest timing in terms of biofuel utilization. High CP content causes corrosion at the refinery and increased GHG emissions during combustion.

Because of how structural carbohydrates are formed in the cell wall and accumulate over time, this research speculates that the H2 harvest interval would be best suited for a biomass feedstock system. The high yields and lower CP content in H2 is the best suited harvest interval for biofuels. However the major short coming to this system is that CP are still not low enough and that fall regrowth have very low yields, which would provide little grazing. If harvest was delayed even further into the summer to decrease CP for biofuels, the fall regrowth yield would be even lower. This would eliminate the forages being used as both biofuels and animal forage in a single year. However the advantages of using cool-season grasses to create a dual acting feedstock still exists because the acres are not being converted to lower value forages crops, and since these are perennial species they can be utilized for whatever market is the most profitable any given year. Ultimately this may give the producer more options as to which market the crop is to be sold too.

Yield potentials when comparing $C_3$ versus $C_4$ in general is a production constraint for $C_3$ grasses, but since difficult establishment and stand loss is less likely in many of the $C_3$ grass this could end up being a fair trade off in some of the cooler environments of the United States. There are two major hurdles the biofuel industry is going to have to face in general, if high quality forages are to be utilized for biofuels. The first is high feedstock gate prices; this is a critical consideration in order to compete with high quality animal forage. Animal feedstock prices are very high due to elevated demand, making it questionable whether producers would sell at lower bio-energy feedstock prices. The only way producers would sell at lower prices is if the higher yield would make up the difference of this loss. The second is the high CP levels. These will have to be reduced in order to better fit biofuels industry standards, but since these forages quickly degrade in terms of animal forage quality, there is still a high potential for them to fit into the biofuels market. In general $C_3$ perennial grasses should be explored as a biofuel feedstock because of the high polysaccharide to lignin ratio, which has been cited to increase biofuel conversions.

These species are also considered high yielding for the arid state of Colorado, but this is partly due because these species growth patterns often closely follow the area's precipitation pattern; this being wet springs from snow precipitation and hot dry summers when cool-season grasses are dormant. However it is important to remember that for this particular year, 2009, there exceptionally high amounts of spring and summer precipitation. This region can be subject to drought as well. Regardless, the cooler areas of the US are more limited to what type of biofuels can be grown due to the niche climate; many of the current warm season biofuels would not be as productive in these areas. Also, in order to evenly distribute biofuel production within the US, or even globally, a diversity of feedstocks for land based biofuels should be evaluated.

## Tables and Figures

Table 1. Common, variety and scientific names of cool-season grass species evaluated for forage yield and quality at Iliff, CO. in 2009. Abbreviations (Abrv.) are given for how the species were cited in text and the rate of pure live seed (PLS) planted in study.

| | Common Name | Variety | Abrev | Scientific Name | Seeding Rate (PLS kg/ha) |
|---|---|---|---|---|---|
| **1** | Smooth brome | Experimental | ESB | *Bromusinermis*Leyss. | 16 |
| **2** | Intermediate wheatgrass | Beefmaker | IWG | *Thinopyrum intermedium* (Host) Barkworth& D.R. Dewey | 15 |
| **3** | Pubescent wheatgrass | Manska | PWG | *Thinopyrum intermedium* | 15 |
| **4** | Tall wheatgrass | Jose | TWG | *Thinopyrum ponticum* | 20 |
| **5** | Hybrid wheatgrass | Newhy | HWG | *Elytrigiarepens* (L.) *nevski x Pseudoroegneriaspicata (*PURSH*) A.* Love | 14 |
| **6** | Slender wheatgrass | San Luis | SWG | *Elymustrachycaulus ssp. trachycalaus* | 12 |
| **7** | Western wheatgrass | Rosana | WWG | *Pascopyrum smithii* | 15 |
| **8** | Crested wheatgrass | Hycrest | CWG | *Agropyron cristatum x desorturum* | 10 |
| **9** | Russian wildrye | Bozoisky-select | RWR | *Psathyrostachys junceus* | 10 |
| **10** | Meadow brome | Cache | MB1 | *Bromus biebersteinii* Roem. &Schult | 24 |
| **11** | Meadow brome | Montana | MB2 | *Bromus biebersteinii* Roem. &Schult | 24 |
| **12** | Meadow brome | Experimental | EMB | *Bromus biebersteinii* Roem. &Schult | 16 |
| **13** | Tall fescue | Fawn-endophyte free | TFQ | *Festuca arundinacea* | 8 |
| **14** | Tall fescue | MaxQ | TFF | *Festuca arundinacea* | 8 |
| **15** | Hybrid brome | Newhy | HB | *Bromus inermsi x B. biebersteinii* | 20 |

Table2. Neutral detergent fiber (NDF) for harvests on June 1, 2009 (H1), June 22, 2009 (H2) and each corresponding regrowth (RG1) and (RG2) of 15 cool-season grass species. Letters indicate least significant difference (LSD, P <0.10) is given for each harvest.

| | H1 | H2 | RG1 | RG2 |
|---|---|---|---|---|
| **CWG** | 61.0(a) | 66.4 | 56.1(b) | 53.3(b) |
| **EMB** | 61.2(a) | 63.0 | 53.7(b) | 54.4(b) |
| **ESB** | 62.6(a) | 59.3 | 60.5(ab) | 54.6(ab) |
| **HB** | 60.6(a) | 63.4 | 53.8(b) | 53.4(b) |
| **HWG** | 60.1(a) | 66.3 | 56.9(b) | 55.9(ab) |
| **IWG** | 56.4(b) | 64.2 | 55.2(b) | 55.9(ab) |
| **MB1** | 60.5(a) | 63.7 | 53.0(b) | 50.3(b) |
| **MB2** | 61.2(a) | 63.5 | 52.1(b) | 51.2(b) |
| **PWG** | 56.2(b) | 63.5 | 55.7(b) | 58.8(ab) |
| **RWR** | 57.7(b) | 63.5 | 57.7(ab) | 55.4(ab) |
| **SWG** | 60.5(a) | 68.5 | 61.6(ab) | 60.5(a) |
| **TFF** | 59.0(ab) | 63.5 | 58.9(ab) | 59.1(a) |
| **TFQ** | 57.6(b) | 63.5 | 57.7(ab) | 56.1(ab) |
| **TWG** | 57.7(b) | 63.5 | 60.3(ab) | 52.3(b) |
| **WWG** | 59.6(ab) | 63.5 | 62.4(a) | 61.3(a) |
| **Average** | 59.5 | 63.9 | 57.1 | 55.4 |
| **LSD** | 1.2 | 3.9 | 2.5 | 2.3 |

Table3. Acid detergent fiber (ADF) for harvest on June 1 (H1), June 22 (H2) and each corresponding regrowth (RG1) and (RG2) of 15 cool-season grass species. Letters indicate least significant difference (LSD, P < 0.10) are given for each harvest.

| | H1 | H2 | RG1 | RG2 |
|---|---|---|---|---|
| **CWG** | 34.4(ab) | 40.1(ab) | 29.6(b) | 30.9(ab) |
| **EMB** | 37.1(a) | 36.0(b) | 32.3(ab) | 31.3(ab) |
| **ESB** | 30.9(b) | 44.2(a) | 32.3(ab) | 29.9(b) |
| **HB** | 34.5(ab) | 36.8(b) | 30.9(b) | 31.0(ab) |
| **HWG** | 32.9(ab) | 38.7(ab) | 32.2(ab) | 32.5(ab) |
| **IWG** | 32.6(ab) | 37.5(ab) | 31.1(ab) | 32.9(a) |
| **MB1** | 33.1(ab) | 36.9(ab) | 30.8(b) | 29.3(b) |
| **MB2** | 35.4(ab) | 37.8(ab) | 31.3(b) | 29.4(b) |
| **PWG** | 30.3(b) | 37.2(ab) | 30.3(ab) | 32.9(a) |
| **RWR** | 31.2(b) | 33.9(b) | 32.5(ab) | 29.5(b) |
| **SWG** | 32.3(ab) | 39.5(ab) | 34.4(a) | 34.4(a) |
| **TFF** | 32.7(ab) | 35.3(b) | 31.3(ab) | 29.8(b) |
| **TFQ** | 31.8(ab) | 35.1(b) | 30.9(b) | 32.3(ab) |
| **TWG** | 32.8(ab) | 36.8(ab) | 33.9(ab) | 30.1(ab) |
| **WWG** | 31.4(b) | 37.4(ab) | 32.4(ab) | 33.7(a) |
| **Average** | 32.9 | 37.6 | 32.0 | 31.3 |
| **LSD** | 2.7 | 3.2 | 1.7 | 2.8 |

Table 4. Crude protein (CP) levels of 15 cool-season grass species for harvests taken on June 1 (H1), June 22 (H2) and each corresponding regrowth (RG1) and (RG2). Letters indicate least significant difference (LSD, P < 0.10) for each harvest.

| | H1 | H2 | RG1 | RG2 |
|---|---|---|---|---|
| **CWG** | 17 | 12.1(ab) | 16.4(ab) | 19.1(a) |
| **EMB** | 16.3 | 12.5(ab) | 17.1(a) | 19.9(a) |
| **ESB** | 24.9 | 11.1(b) | 17.9(a) | 17.2(ab) |
| **HB** | 15.8 | 12.1(b) | 17.1(a) | 19.3(a) |
| **HWG** | 16.4 | 11.4(ab) | 14.2(b) | 16.7(b) |
| **IWG** | 19.0 | 12.8(ab) | 15.3(ab) | 15.8(b) |
| **MB1** | 15.9 | 12.4(ab) | 17.7(a) | 18.5(a) |
| **MB2** | 16.7 | 12.6(ab) | 16.7(ab) | 19.9(a) |
| **PWG** | 17.3 | 13.1(ab) | 16.6(ab) | 16.5(ab) |
| **RWR** | 20.4 | 14.7(a) | 16.4(ab) | 17.9(a) |
| **SWG** | 17.8 | 12.8(ab) | 14.0(b) | 15.5(b) |
| **TFF** | 15.8 | 12.4(ab) | 15.1(ab) | 16.0(b) |
| **TFQ** | 16.8 | 12.0(b) | 15.3(ab) | 17.8(ab) |
| **TWG** | 16.6 | 11.8(b) | 12.7(b) | 18.3(ab) |
| **WWG** | 16.6 | 11.7(b) | 13.5(b) | 16.0(b) |
| **Average** | 17.5 | 12.4 | 17.6 | 15.7 |
| **LSD** | 4.1 | 1.3 | 1.5 | 1.8 |

Table 5. Root biomass by depth, 0-25cm, 26-50 cm, 51-75 cm, and total over depths for 6 cool-season grass species under full season (FS) and spring only (S) irrigation treatments sampled on October 22, 2009.

| Species | Irrigation | ------------------------Root biomass (kg/ha) ------------------- | | | |
|---|---|---|---|---|---|
| | | 0-25 cm | 26-50 cm | 51-75 cm | Total |
| CWG | FS | 9894 | 1300 | 289 | 11482 |
| EMB | FS | 11410 | 2239 | 289 | 13938 |
| ESB | FS | 4766 | 1805 | 722 | 7294 |
| PWG | FS | 13721 | 1517 | 289 | 15526 |
| RWR | FS | 12132 | 3972 | 578 | 16682 |
| TFF | FS | 9027 | 2022 | 578 | 11627 |
| CWG | S | 9171 | 1300 | 433 | 10905 |
| EMB | S | 11771 | 1878 | 2022 | 15671 |
| ESB | S | 9460 | 289 | 361 | 10110 |
| PWG | S | 8666 | 1589 | 939 | 11193 |
| RWR | S | 9171 | 1444 | 867 | 11482 |
| TFF | S | 10110 | 1228 | 1228 | 12566 |
| Significance of Main Effects | | | | | |
| Species | | N.S. | * | N.S. | |
| Irrigation | | N.S. | N.S. | * | |

N.S. = not significant at p=0.10, * p<0.10

Table 6. Root crude protein (CP) and carbon content of 6 cool-season grass species for full season irrigation (FS) and spring only irrigation sampled on October 22, 2009. Values were determined on composite samples over the three sampling depths, 0-25 cm, 25-50 cm, and 50-75 cm.

| Species | Treatment | Carbon | CP |
|---|---|---|---|
| | | --------- % ---------- | |
| CWG | FS | 37.3 | 11.5 |
| CWG | S | 37.2 | 8.8 |
| EMB | FS | 37.1 | 11.6 |
| EMB | S | 36.1 | 12.6 |
| ESB | S | 37.3 | 10.3 |
| ESB | FS | 37.7 | 10.2 |
| PWG | S | 37.8 | 10.5 |
| PWG | FS | 40.0 | 9.7 |
| RWR | FS | 37.5 | 10.1 |
| RWR | S | 36.2 | 8.8 |
| TFF | S | 41.4 | 9.9 |
| TFF | FS | 32.5 | 11.5 |
| Significance of Main Effects | | | |
| Species | | N.S. | N.S. |
| Irrigation | | N.S. | N.S. |

N.S. = not significant at p=0.10

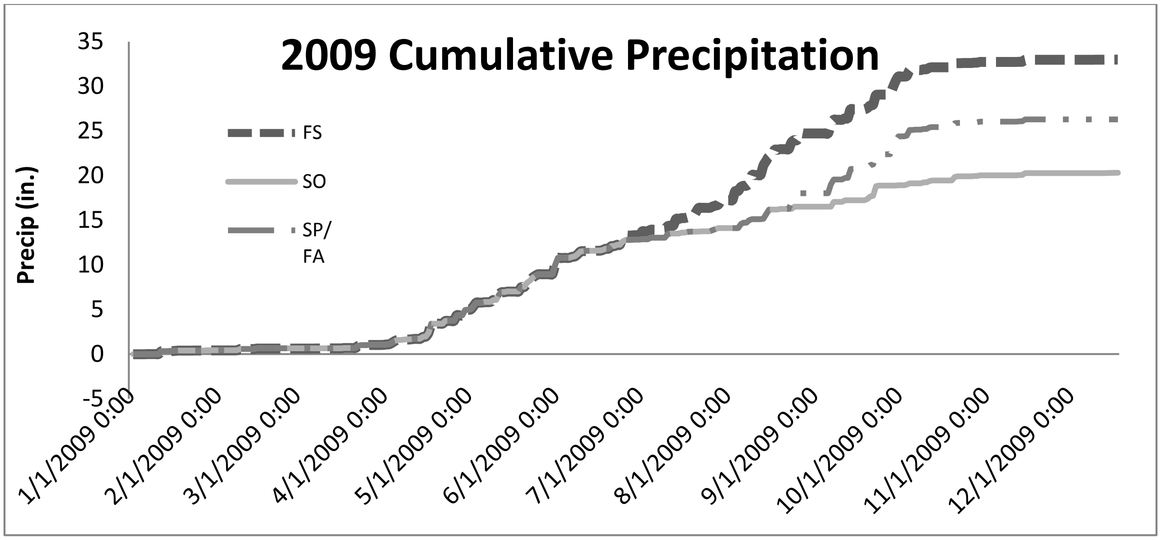


Figure 1. Cumulative precipitation and irrigation for full season (FS), spring only (SO), and spring and fall (SP/FA) irrigation treatments for 15 cool-season grasses harvested for forage and biomass at Iliff, CO 2009 research plots.

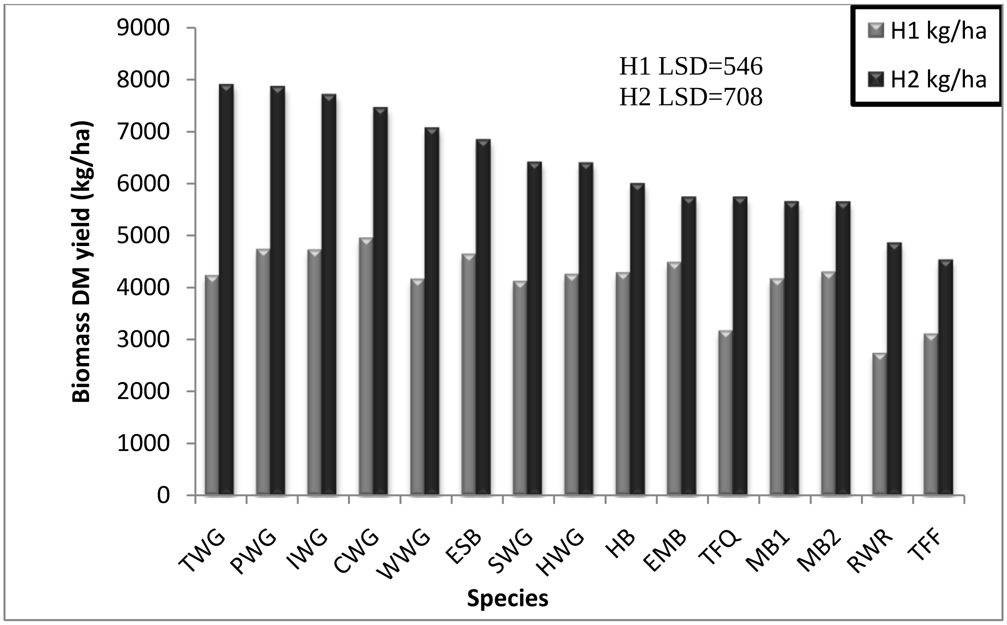


Figure 2. Dry mater (DM) yields for harvest dates June 1 (H1) and June 22 (H2) of 15 cool-season grasses averaged across three different irrigation treatments. Species are listed in order from highest to lowest yields in the H2 biomass harvest and the least significant difference (LSD, $P < 0.10$) is given for each harvest.

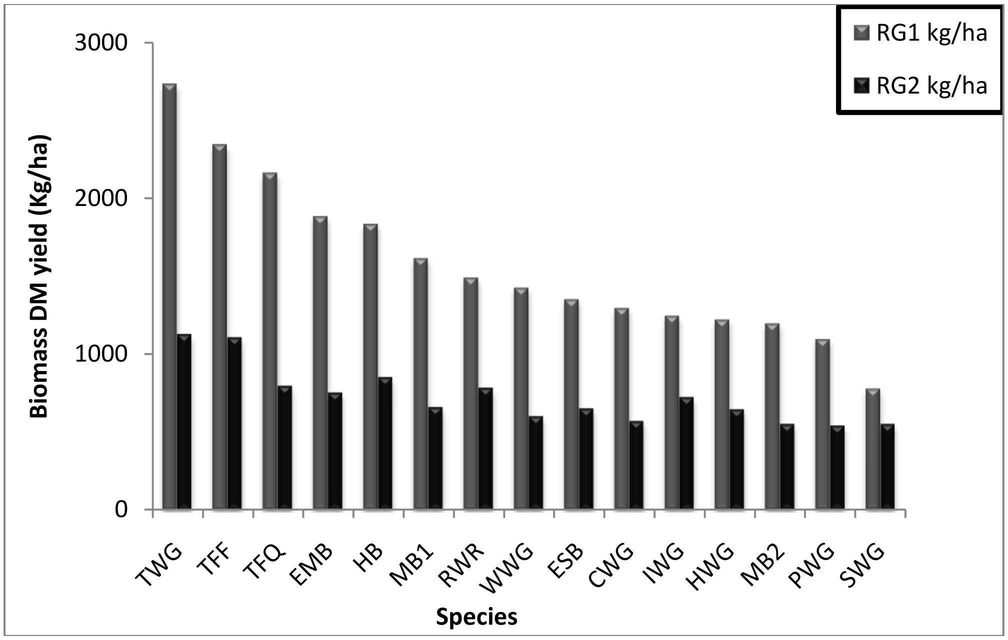


Figure 3. Dry matter (DM) yields for fall regrowths dates June 1, 2009 (RG1) and June 22, 2009 (RG2) of 15 cool-season grasses averaged across three different irrigation treatments. Species are listed in order from highest to lowest yields in the H2 biomass harvest and the least significant difference (LSD, $P < 0.10$) is given for each harvest.

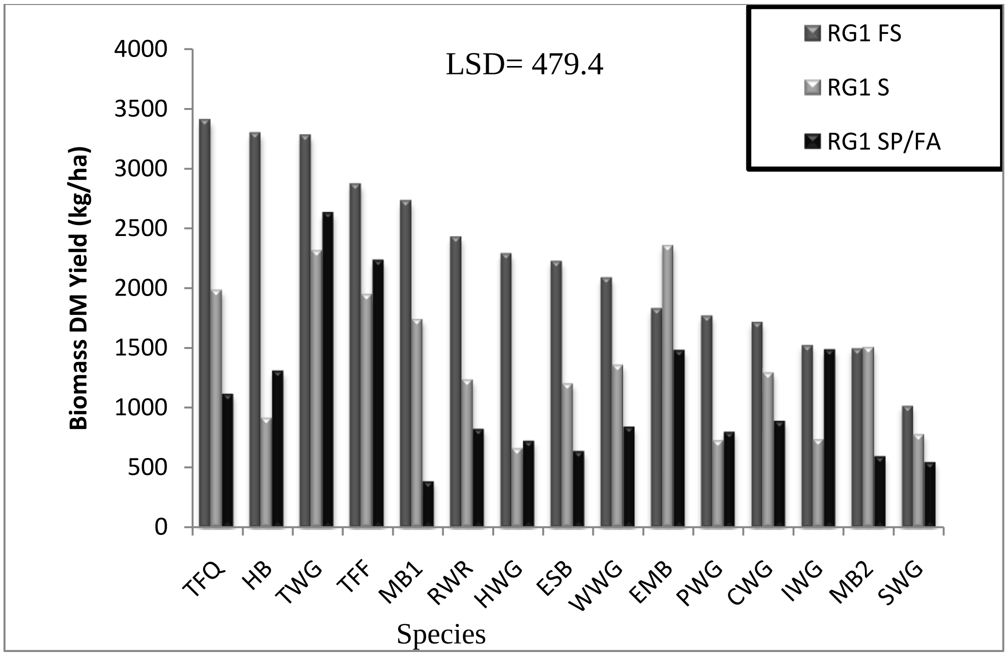


Figure 4. Dry matter (DM) yields for October 17, 2009 fall regrowth (RG1) of 15 cool-season species with three irrigation treatments: full season (FS), spring only (SO), and spring and fall (SP/FA).

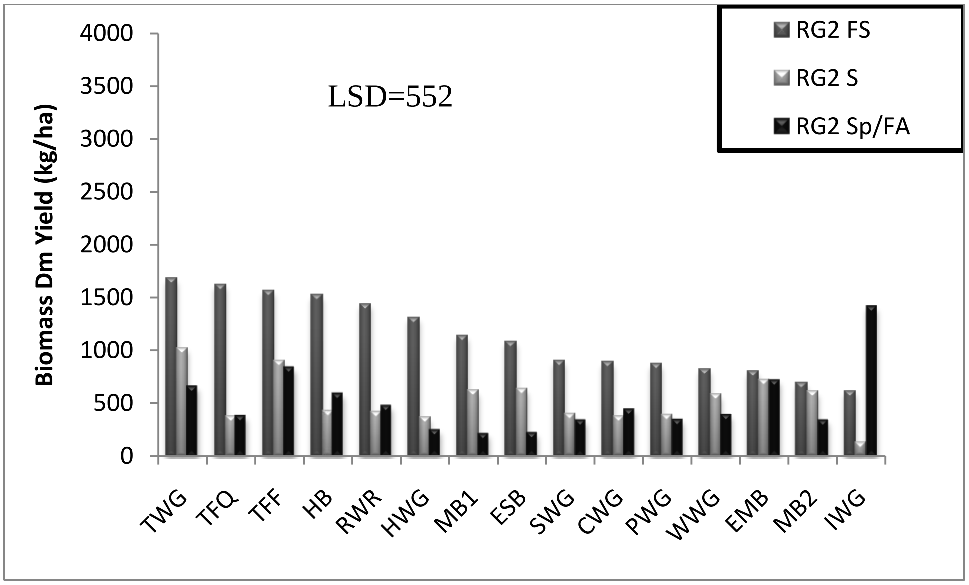


Figure 5. Dry matter (DM) yields for October 17, 2009 fall regrowth (RG2) of 15 cool-season species with three irrigation treatments: full season (FS), spring only (SO), and spring and fall (SP/FA).

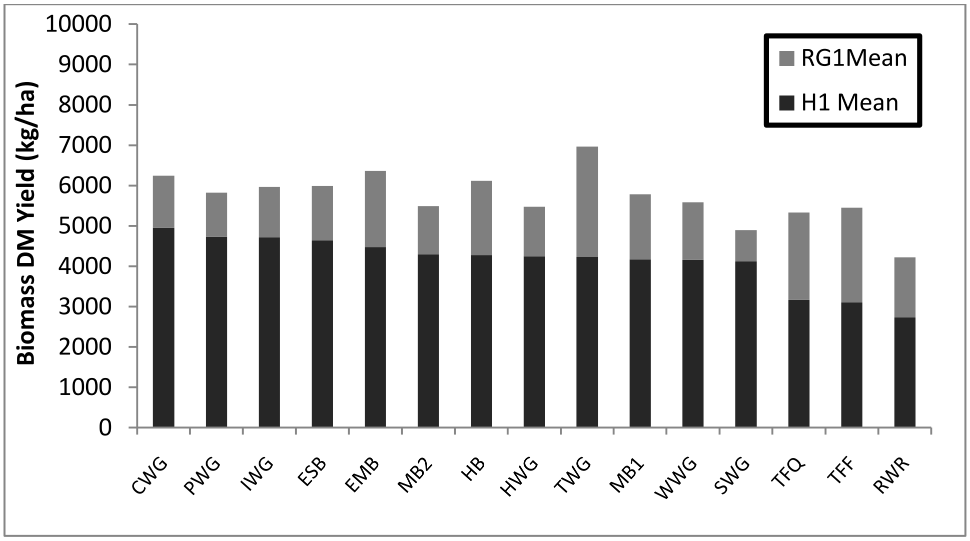


Figure 6. Total annual dry matter yields of 15 cool-season grass species for the June 1 harvest and fall regrowth (H1+RG1) sorted from highest to lowest yields averaged across three irrigation treatments.

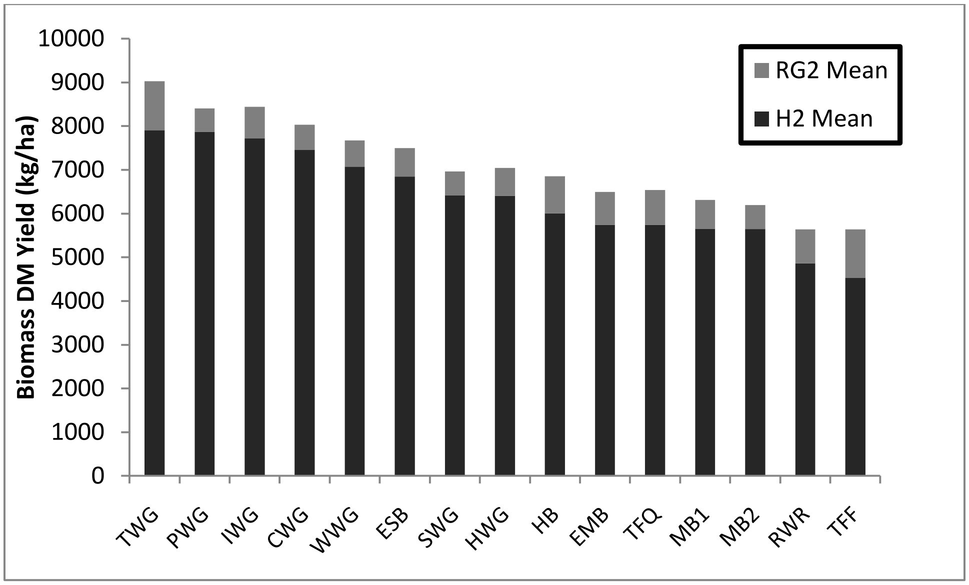


Figure 7. Total annual dry matter yields of 15 cool-season grass species for the second harvest June 22 and regrowth (H2+RG2) sorted from highest to lowest averaged across three irrigation treatments.

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