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The Effects of Harvest Regime, Irrigation, and Salinity on Stem Lignocellulosic Concentrations in Alfalfa (Medicago sativa L.)

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The Effects of Harvest Regime, Irrigation, and Salinity on Stem Lignocellulosic Concentrations in Alfalfa (*Medicago sativa* L.)

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ABSTRACT

The effects of harvest regime, irrigation, and salinity on stem lignocellulosic concentrations in alfalfa (*Medicago sativa* L.)

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Rapid consumption of crude oil reserves has made it necessary to find methods of processing a renewable and sustainable feedstock for conversion into ethanol. Lignocellulosic feedstocks are promising because they are typically environmentally friendly and can meet the high-yield potential necessary for ethanol production. Alfalfa (*Medicago sativa* L.) has promise as a feedstock for ethanol production because of its high biomass yields, perennial-habit, relationship with nitrogen-fixing bacteria, and other co-products. This study focused on the effects of harvest regime, irrigation, and salinity on stem lignocellulosic concentrations in alfalfa for ethanol production during the 2010 and 2011 growing seasons in southern Minnesota. Stem cellulose, hemicellulose, lignin (lignocellulosic) concentrations, and theoretical ethanol yields were examined in eight alfalfa varieties with full bud and 50% flower harvest regimes, irrigation, and salinity as applied treatments. Plants received weekly applications of (1) 1.27 cm of well water (0.75 dS m^{-1}), (2) 1.27 cm of saline (NaCl) water (5.0 dS m^{-1}) or (3) ambient precipitation.

Holocellulose concentrations were greatest during the full bud (2010) and 50% flower (2011) harvest regimes with concentrations averaging 45.50 and 45.23%, respectively. Holocellulose to lignin ratios increased from 2010 to 2011 and averaged

2.3 to 3.1. Theoretical ethanol yields were generally higher for the 50% flower harvest regime, suggesting the longer growth period increased holocellulose concentrations while not being hindered by the increased lignin typical with increased growth periods of alfalfa.

Alfalfa plants that received saline treatments in 2010 had 3.2 and 3.5% more holocellulose than plants that were irrigated or received ambient precipitation (control), respectively. Holocellulose concentrations between the control and irrigated treatments were not different in 2010, which was a wet year and irrigation added no supplementary benefit. However, in 2011 plants growing in saline treatments had 1.3 and 6.1% more holocellulose than irrigated and control treatments, respectively. Lignin concentrations across all treatments were almost 23% lower during the second year of growth. Interestingly, plants growing under saline treatments had higher holocellulose to lignin ratios (and higher theoretical ethanol yields) during both field seasons suggesting that moderate levels of salt may stimulate holocellulose concentrations.

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INTRODUCTION

World crude oil reserves are predicted to be depleted in approximately 40 years at the current rate of consumption, and it has become essential to find methods of processing renewable and sustainable raw materials for conversion into fuel (Maheshwari, 2008). Increasing global population has further amplified this necessity. Shifting society's reliance away from petroleum to renewable biomass resources is viewed as an important contributor to the development of a sustainable industrial society and for effective management of greenhouse gas emissions (Rugauska et al., 2006). Biofuels have been heralded as a renewable, cost-effective alternative to petroleum-based liquid fuels. The starch-based ethanol industry has grown very rapidly in the United States, however, most experts see the need for the development of a lignocellulosic-based biofuels industry to meet the current Federal biofuels mandate for displacing 30% of petroleum consumption by 2030 (McCaslin and Miller, 2007).

A major source for biofuel comes from polysaccharides created by the photosynthetic process. These polysaccharides can be divided into two major groups: starch, a storage polymer, consisting of glucose monomers with α (1→4) and α (1→6) glycosidic linkages, and cellulose, a structural polymer, consisting of glucose monomers with β (1→4) glycosidic linkages. In addition to cellulose, plant secondary cell walls also contain lignin. Lignin is a complex phenolic polymer that is closely linked to polysaccharides in the cell wall and hinders the degradation of these polysaccharides to simple sugars, which is required for fermentation to ethanol (Chapple et al., 2007). The most common measures of fiber content in plant cell walls are the neutral detergent fiber

(NDF) and the acid detergent fiber (ADF) methods (Van Soest et al., 1991). The NDF method provides a close estimate of the total fiber constituents of feedstocks because it measures cellulose, hemicellulose, and lignin. The ADF method measures the fraction of un-digestible plant material in forage, usually cellulose fibers coated with lignin. These methods were created for useful measures of feedstock digestibility and energy values but can also be used for determination of fiber values for lignocellulosic ethanol production.

Ethanol production from plant-produced polysaccharides has been commercialized using starch from corn grains. The starch in corn kernels is much easier to break down than cellulose and hemicelluloses (collectively holocellulose) in the cell wall of biomass material. Corn starch is converted to glucose and fermented to produce ethanol. However, there are several economic problems associated with the production of ethanol from corn grain. The increased demand for corn is depleting the world's food stock and driving up the prices of corn-based products. In addition, the large amounts of fossil fuels used to process starch-based ethanol are expensive and release greenhouse gases. The vision of a future bio-based industry includes the simultaneous production of biofuels, bioelectricity, and bioproducts using not only corn grain and soybean oil, but also a host of renewable lignocellulosic feedstocks (Walsh et al., 2007). Lignocellulosic ethanol is particularly promising because it can take advantage of biotechnology to dramatically reduce costs, is derived from low-cost and abundant feedstocks, can achieve high yields, and is typically environmentally friendly (Wyman, 2007). However, there are problems with commercializing lignocellulosic ethanol due to the high initial capital

cost. Separating cellulose from lignin during processing is costly and produces potentially harmful by-products.

Corn stover, corn cobs and wheat straw are obvious annual crop residue feedstocks for lignocellulosic ethanol production. Switchgrass (*Panicum virgatum)*, a native C4 perennial forage grass, is often mentioned as a leading perennial energy crop candidate. Drought tolerance, low fertility requirements, and the ability to grow on marginal soils will likely make switchgrass an important component in a biofuel cropping system in some regions (McCaslin and Miller, 2007). Ultimately, identifying plants that have high holocellulose to lignin ratio is an essential step when determining what species are best suited for ethanol production. This study will focus on the use of alfalfa (*Medicago sativa*) as a potential crop in a biofuels production system.

Alfalfa has promise as a feedstock for production of ethanol and other industrial materials because of its high biomass yields, perennial-habit, relationship with nitrogen-fixing bacteria and other valuable co-products (Jung and Engels, 2002). Alfalfa is a widely-grown traditional crop that fits well into a typical crop rotation. It is grown on a variety of soil types with well-drained soils ideal for maximum productivity. Soils with a pH level of 6.5-7.0 and adequate levels of phosphorous (60-100 kg ha^{-1}) and potassium (180-250 kg ha^{-1}) are optimal for subsequent years of production (McKenzie, 2005). Selection of alfalfa varieties is typically based upon the winter survival index (WSI), fall dormancy (FD), and disease and pest resistance (DRI). Winter survival index is the ability to withstand severe winters. Alfalfa with lower WSI ratings will have the ability to survive potentially harsh winters. Fall dormancy is the measure of how tall a alfalfa

plant grows after the last cutting and before going dormant for the season. Alfalfa with a lower number goes dormant earlier in the fall. The DRI is based upon selecting varieties with superior disease resistance to ensure a long productive stand. The WSI rating is very important in Minnesota due to potentially harsh winters. A variety that can withstand severe low temperatures is crucial when selecting alfalfa.

Alfalfa can be harvested for biomass in the year of planting and provides nitrogen to the soil for use by subsequent cereal crops in rotation (Sheaffer et at., 2000). The growth stages of alfalfa are well-known and harvest schedules for leaf protein are determined upon them for ruminant livestock feed. Typical harvest schedules produce three to four cutting per growing season. An advantage of using alfalfa for lignocellulosic biofuel production, compared to other crops, is the ability to easily separate leaves and stems to produce co-products (Samac et al., 2006). Alfalfa leaves typically have two to three times the crude protein of the stems while stems typically have two to three times the crude fiber of the leaves (Shinners et al., 2007). The high protein leaf portion could be utilized as an animal feed, while the high lignocellulosic stem portion could be used as a biofuel feedstock (McCaslin and Miller, 2007).

Using alfalfa for a biofuels system would require research to determine the optimal holocellulose to lignin ratio for ethanol production. Recommended harvest schedules for modern alfalfa cultivars in a lignocellulosic biofuel system are unknown because the comparative value of leaf and stem components is likely to vary with energy consumption and livestock feed prices (Sheaffer et al., 2000). Based on previous research (Lamb et al., 2007), mature alfalfa stems had higher concentrations of

lignocellulosic material on a seasonal yield adjusted basis under the biomass management system than the hay system. Typically as alfalfa ages, the stems become more lignified and have lower cellulose concentrations (Sanderson and Wedin, 1988). Previous research has focused on plant density along with harvest intervals. We strictly focused on harvest intervals across the same plant density. Determining the optimal harvest schedule for protein and lignocellulosic concentrations will be a vital step for the future of alfalfa as a biofuel feedstock. However, to achieve maximum biomass yields of alfalfa, irrigation may be needed in some portions of the country.

Crop yield depends on the amount of irrigation water and its distribution (Montazar and Sadeghi, 2008). Alfalfa has a high water requirement compared to other commonly grown crops because it has a long growing season, a deep root system, and high biomass yields. (Krogman and Hobbs, 1965; Bauder et al., 1992). Drought stress on alfalfa can inhibit cell elongation, reduce photosynthesis, interfere with nutrient uptake, and alter plant hormone levels (Saeed and El-Nadi, 1997). Saeed and El-Nadi (1997) have shown that alfalfa stem density, stem height and leaf size decreased when soil water deficits developed.

A typical problem of irrigated agricultural land is the gradual buildup of salts in the root zone (Vaughan et al., 2002) and salinity is a major factor limiting plant growth and productivity (Allakhverdiev et al., 2000). According to Munns et al. (2006), plant growth response to salinity involves two phases. In the first phase, the presence of salt in the soil solution decreases the ability of the plant to take up water, which results in slower growth. Growth rate is presumably regulated by hormonal signals released by roots in

response to the osmotic or water-deficit effect of salinity (Emam et al., 2009; Munns, 2002). The toxic effects of salt inside the plant make up the second phase. This is due to salts accumulating in transpiring leaves to excessive levels beyond the ability of the cells to compartmentalize salts in the vacuole (Munns, 2002). In some cases, these phases may occur sequentially (Munns et al., 2006). The ability of plants to tolerate salt is determined by multiple biochemical pathways that facilitate retention and/or acquisition of water, protect chloroplast functions, and maintain ion homeostasis (Parida and Das, 2005). There are two main types of mechanisms for salt tolerance in plants. There are plants that are able to minimize the entry of salt into the plant and those minimizing the concentration of salt in the cytoplasm (Munns, 2002). Root and shoot growth in alfalfa is restricted significantly by increased salinity (Esechie et al., 2002). For long-term productivity, perennial crops such as alfalfa must be able to adapt to increasing heterogeneous root zone salinity (Vaughan et al., 2002). The relationship between alfalfa growth and water utilization under an irrigated system is very important in determining the effects of salinity on stem lignocellulosic concentrations.

The purpose of this study was to examine the effects of harvest regime, irrigation, and salinity on stem lignocellulosic concentrations in alfalfa. Irrigation and salinity are both factors that affect plant growth and there is little data on how they affect stem lignocellulosic concentrations. In combination with a harvest schedule these two factors provided valuable information for alfalfa's potential as a biofuel feedstock.

MATERIALS AND METHODS

PLOT ESTABLISHMENT AND VARIETY SELECTION

The field experiment was conducted over three growing seasons (2009 - 2011) on an agricultural field located 2.5 miles west of Geneva, Minnesota (43°81'N × 93°32'W). The soil at this location is a Webster Clay Loam-113 (Carlson et al., 1980) and has a pH of 6.5. Phosphorous and potassium concentrations averaged ≥ 65 and ≥ 190 kg ha^{-1}, respectively (data not shown). Precipitation was collected by a rain gauge at the field site and is presented in Table 1.

We chose eight varieties of alfalfa based upon the winter survival index (WSI), fall dormancy (FD), and disease and pest resistance (DRI). The eight varieties used in this study included: 1-WL 363HQ: *Waterman-Loomis Seed Company* (WSI-1.6 and FD-4.8), 2-Viking 357: *Viking Seeds* (WSI-2.5 and FD-3.4), 3-L447HD: *Wolf River Valley Seeds* (WSI-2.0 and FD-3.7), 4-Enforcer: *Allied Seed* (WSI-2.2 and FD-3.5), 5-Viking 3100: *Viking Seeds* (WSI-2.6 and FD-3.0), 6-Fontanelle Hybrid – Ovation 2: *Fontanelle Hybrids* (WSI-2.3 and FD-3.4), 7-Gold Country 24/7: *Gold Country Seed* (WSI-2.5 and FD-3.8), and 8-Iroquois: *Iroquois Seed* (WSI and FD-unknown). All varieties had sufficient disease and pest resistance ratings. Varieties 1-5 were obtained from Albert Lea Seed in Albert Lea, Minnesota. Varieties 6-8 were obtained from a local dairy farmer. These varieties are all well established in Minnesota and are suitable for this research.

We used a complete block in a split-plot arrangement with two or three harvest regimes as whole plots and eight alfalfa cultivars, irrigation, and salinity treatments as

subplots. There were two replicates at the experimental location (Figure 1). Plots were 3.66 by 5.49 m (cultivar) and subplots were 1.83 by 3.66 m (treatment). A seeding rate of 14.6 kg ha^{-1} resulted in stand densities for all plots ≈ 450 plants/m^{-2}. Weeds were controlled by using a post-emergence application of 292 ml ha^{-1} of ammonium salt of imazethapyr (Pursuit) {(±)-2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1*H*-imidazol-2-yl]-5-ethyl-3-pyridinecarboxylic acid}. Plots were sprayed as needed with S-Cyano (Mustang Max) [(3-phenoxyphenyl) methyl (+) cis/trans 3-(2,2-dichloroethenyl)-2,2 dimethylcyclopropane carboxylate] for potato leafhopper [*Empoasca fabae* (Harris)] control.

IRRIGATION AND SALINE APPLICATIONS

The irrigation applications were made using a 492 L water tank located in the back of a pickup truck. The applications were applied with an 18.9 L per minute pump attached by rubber hoses to a hand held sprinkler. Each subplot had 5 rain gauges (one in each corner and one in the center of the plot) to ensure accurate treatment applications. Each irrigation subplot received a 1.27 cm (83.28 L) application of well water (0.75 dS m^{-1}) every 7 to 10 days, depending on local weather patterns. Salinity applications were performed in a similar fashion on the same day with each saline subplot receiving a 1.27-cm (83.28 L) application of saline (NaCl) water (5.0 dS m^{-1}).

FORAGE SAMPLING AND SAMPLE PREPARATION

Forage was harvested in the establishment year (2009) prior to treatment application. Subplots were harvested in the second and third years of production (2010

and 2011) when alfalfa reached full-bud (>50% of stems having one or more buds) and 50%-flower (66-100% of stems having one or more flower). The full-bud regime was harvested three times per season and the 50%-flower regime was harvested twice per season. Each subplot had ten samples collected at each growth stage.

Herbage yields were determined by harvesting a 0.91-by-3.66 m strip of forage to a 5-cm height from the center of each plot with a hand operated sickle bar mower. At harvest, ten random subsamples were collected for analysis. Samples were placed in labeled paper bags and oven dried at 60°C. The remaining non-sampled plants were cut at 5-cm above ground level with a hay-bine then bailed and removed from the plots. Each subsample was manually separated into leaf and stem fractions. The remaining portions of the stems were ground with a Wiley mill through a 1-mm screen in preparation for constituent analysis (see below).

CONSTITUENT ANALYSIS

A fiber analyzer (model A200; ANKOM Technology, Macedon NY, USA) was used to estimate concentrations of cellulose, hemicellulose and lignin in dried samples. Dried samples (0.5 g) were placed into pre-weighed filter bags and analyzed with Acid Detergent Fiber (ADF) solution (20 g cetyl trimethylammonium bromide to 1 L 1.00N H_2SO_4) at 100°C for 60 min. Samples were rinsed with hot dH_2O and acetone, dried, and placed in a drying oven (102°C) overnight. Samples were then cooled, weighed and %ADF (cellulose + lignin) was calculated.

The second sub-sample for each treatment was then used to estimate Neutral Detergent Fiber (NDF). Dried samples (0.5 g) were placed into pre-weighed filter bags and analyzed with NDF solution (sodium lauryl sulfate, ethylendiamine-tetraacetic disodium salt dihydrate, sodium tetraborate decahydrate, sodium phosphate dibasic, anhydrous and triethylene glycol). Heat-stable bacterial alpha amylase and sodium sulfite was added to the analyzer and samples were incubated at 100°C for 75 min. Samples were then rinsed twice with alpha amylase solution, then once in acetone and dried overnight (102°C). Samples were then cooled, weighed and %NDF (cellulose, hemicellulose + lignin) was calculated.

Acid Detergent Lignin (ADL) was estimated on samples used for ADF analysis. Samples were immersed in 72% H_2SO_4 for 3 h and agitated every 30 min. Samples were then rinsed in dH_2O and acetone, dried overnight (102°C) and weighed. Samples were then ashed in a muffle furnace (525°C) for 3 h, cooled and weighed. Cellulose concentrations were calculated as %ADF - %ADL, and hemicellulose concentration were calculated as %NDF - %ADF.

Theoretical ethanol yields were determined using assumed cellulose and hemicellulose conversion and fermentation efficiencies following Badger (2002). Fermentation assumptions were based on 1000 kg of dried biomass. Ethanol yields from glucose were calculated for alfalfa stems using the average cellulose concentrations and ethanol yields from xylose were calculated for alfalfa stems using the average hemicellulose concentrations.

STATISTICAL ANALYSIS

The general linear model procedure was used with a one-way analysis of variance (ANOVA; SigmaPlot, 2008) to examine differences in cellulose, hemicellulose, lignin, and theoretical ethanol yields between harvest regimes and treatments (control, saline, and irrigated) during the 2010 and 2011 growing seasons. The least significant difference (LSD) post-hoc test was then used to compare individual means. A two-way ANOVA was used to analyze differences in cellulose, hemicellulose, lignin, and theoretical ethanol yields between the harvest regime + treatment (control, saline, and irrigated) and the variety differences during the 2010 and 2011 growing seasons, followed by post-hoc LSD test (SigmaPlot, 2008). Differences were considered significant at the $P < 0.05$ level unless otherwise noted.

RESULTS

PRECIPITATION PATTERNS

During the 2010 growing season the field site received 2.5 cm more precipitation than the historical average from 2001-2009 (Table 1). The months of June and September received 60.0 and 57.3% more precipitation than the historical averages for those months, respectively (Figure 2). During the 2011 growing season the field site received 3.6 cm less precipitation than the historical average from 2001-2009 (Table 1). Precipitation during the months of August, September, and October was 84.0, 88.4, and 87.9% less precipitation than the historical averages from 2001-2009 for those months, respectively (Figure 2).

CELLULOSE CONCENTRATIONS

Cellulose concentrations averaged 36.9 and 36.5% across all treatments in 2010 for plants sampled at the full-bud and 50%-flower harvest regimes, respectively (Table 2). There was no significant difference between harvest regimes for the 2010 growing season. In 2011, cellulose concentrations averaged 35.2 and 37.2% across all treatments for plants sampled at the full-bud and 50%-flower harvest regimes, respectively (Table 2). Plants sampled at the 50%-flower harvest regime had 5.4% more cellulose than plants sampled at the full-bud harvest regime in 2011 ($P < 0.01$; Figure 3A). Cellulose concentrations for plants sampled at the full-bud harvest regime decreased 4.6% from 2010 to 2011 ($P < 0.01$; Figure 3A). However, cellulose concentrations for plants sampled at the 50%-flower harvest regime increased 1.6% from 2010 to 2011 ($P < 0.05$; Figure 3A).

Cellulose concentrations averaged 36.6, 37.4, and 36.1% in 2010 for plants growing under the control, saline, and irrigated treatments in 2010, respectively (Table 3). Plants growing under saline treatments had 2.1 and 3.5% increased cellulose concentrations over the plants growing in the control and irrigated treatments in 2010, respectively ($P < 0.05$; Figure 4A). Plants growing in the control and irrigated treatments were not significantly different during the 2010 growing season. In 2011, cellulose concentrations averaged 35.5, 37.0, and 36.1% for plants growing under the control, saline, and irrigated treatments, respectively (Table 3). Following a similar trend to 2010, plants growing in the saline treatments had 4.1 and 2.4% increased cellulose concentrations over plants in the control and irrigated treatments in 2011, respectively (*P*

< 0.01; Figure 4A). Plants growing under the saline and irrigated treatments both had significantly greater cellulose concentrations than plants in the control in 2011 ($P < 0.01$; Figure 4A). Cellulose concentrations decreased 3.0 and 1.1% for plants growing under the control and saline treatments from the 2010 to the 2011 growing season, while plants in the irrigated treatment had the same cellulose concentration for both growing seasons (Figure 4A).

Cellulose concentrations averaged 37.0, 37.3, and 36.3% for plants growing under the full-bud control, saline, and irrigated treatments in 2010, respectively (Table 4). Plants in the full-bud irrigated treatments had lower cellulose concentrations than plants in the full-bud saline treatment ($P < 0.05$; Figure 5A). Plants in the full-bud control treatment were not significantly different than the plants in the full-bud saline or irrigated treatments. Plants growing in the 50%-flower harvest regime in 2010 had average cellulose concentrations of 36.2, 37.6, and 35.9% for the control, saline, and irrigated treatments, respectively (Table 4). Plants in the 50%-flower saline treatment had significantly higher cellulose concentrations (2.9 and 3.8%, respectively) than plants in the control and irrigated treatments ($P < 0.01$; Figure 5A). In 2011, plants in the full-bud harvest regime had average cellulose concentrations of 34.3, 36.4, and 35.0% for the control, saline, and irrigated treatments, respectively (Table 5). Plants growing in the full-bud saline treatment had significantly higher cellulose concentrations (5.8 and 3.8%, respectively) than plants in the control and irrigated treatments ($P < 0.01$; Figure 5A). Plants growing in the 50%-flower harvest regime in 2011 had average cellulose concentrations of 36.7, 37.7, and 37.2% for the control, saline, and irrigated treatments,

respectively (Table 5). In both growing seasons (2010 and 2011) and harvest regimes (full-bud and 50%-flower) plants growing under the saline treatments generally had the highest cellulose concentrations. Plants in the full-bud control, full-bud saline, full-bud irrigated, and 50%-flower irrigated treatments were significantly different from the 2010 to the 2011 growing season ($P < 0.05$; Figure 5A).

The average cellulose concentrations of the eight varieties during the 2010 growing season were as follows: Fontanelle, 36.8%; Gold Country, 38.0%; Iroquois, 37.3%; Viking 3100, 37.7%; Viking 357, 36.6%; L447HD, 35.9%; WL363HQ, 35.1%; and Enforcer, 35.2% (Table 6). Gold Country, Iroquois, and Viking 3100 had the highest cellulose concentrations during the 2010 growing season with averages of 38.0, 37.3, and 37.7%, respectively ($P < 0.05$; Figure 6A). There was a 7.6% increase in cellulose concentration between the lowest variety (WL363HQ) and the highest variety (Gold Country) in 2010. In 2011, the average cellulose concentrations of the eight varieties were as follows: Fontanelle, 37.1%; Gold Country, 37.2%; Iroquois, 35.5%; Viking 3100, 36.4%; Viking 357, 37.0%; L447HD, 36.5%; WL363HQ, 36.8%; and Enforcer, 35.6% (Table 7). Fontanelle, Gold Country, L447HD, and WL363HQ had the highest cellulose concentrations during the 2011 growing season with averages of 37.1, 37.2, 37.0, 36.5, and 36.8%, respectively ($P < 0.05$; Figure 6A). There was a 4.6% increase in cellulose concentration between the lowest variety (Iroquois) and the highest variety (Gold Country) in 2011. Gold Country, Iroquois, Viking 3100, and WL363HQ had significantly different cellulose concentrations between the 2010 and 2011 growing

seasons ($P < 0.05$; Figure 6A). However, the Gold Country variety had the highest cellulose concentrations in both years.

HEMICELLULOSE CONCENTRATIONS

Hemicellulose concentrations averaged 8.6 and 8.1% across all treatments during the 2010 growing seasons for plants sampled at the full-bud and 50%-flower harvest regimes, respectively (Table 2). Plants sampled at the full-bud harvest regime had 5.8% more hemicelluloses than plants sampled at the 50%-flower harvest regime in 2010 ($P < 0.05$; Figure 3B). In 2011, hemicellulose concentrations averaged 8.4 and 8.0% across all treatments for plants sampled at the full-bud and 50%-flower harvest regimes, respectively (Table 2). Following the same trend as the 2010 growing season, plants sampled at the full-bud harvest regime had 4.8% more hemicelluloses than plants sampled at the 50%-flower harvest regime ($P < 0.05$; Figure 3B). Hemicellulose concentrations were slightly decreased from the 2010 to the 2011 growing season, but the results were not significant ($P < 0.15$; Figure 3B).

Hemicellulose concentrations averaged 7.9, 8.7, and 8.6% in 2010 for plants growing under the control, saline, and irrigated treatments, respectively (Table 3). Plants growing under the saline and irrigated treatments had increased hemicellulose concentrations of 9.2 and 8.1%, respectively, over plants in the control treatment in 2010, ($P < 0.05$; Figure 4B). Plants in the saline and irrigated treatments were not significantly different during the 2010 growing season. In 2011, hemicellulose concentrations averaged 7.3, 8.5, and 8.9% for plants growing under the control, saline, and irrigated

treatments, respectively (Table 3). As in the 2010 growing season, plants growing in the saline and irrigated treatments had increased hemicellulose concentrations of 14.1 and 18.0% in 2011, respectively ($P < 0.01$; Figure 4B).

Hemicellulose concentrations averaged 7.3, 9.4, and 9.2% for plants growing under the full-bud control, saline, and irrigated treatments in 2010, respectively (Table 4). Plants in the full-bud saline and irrigated treatments had significantly higher hemicellulose concentrations than plants in the control treatment ($P < 0.01$; Figure 5B). Plants in the full-bud saline and irrigated treatments were not significantly different. Plants growing under the 50%-flower harvest regime in 2010 had average hemicellulose concentrations of 8.6, 8.0, and 7.9% for the control, saline, and irrigated treatments, respectively (Table 4). Plants growing in the 50%-flower control treatment had 7.0 and 8.1% more hemicelluloses than plants growing in the saline and irrigated treatments in 2010. In 2011, plants growing under the full-bud harvest regime had hemicellulose concentrations of 7.2, 8.6, and 9.3% for the control, saline, and irrigated treatments, respectively (Table 5). Similar to the 2010 growing season, plants in the saline and irrigated treatments had significantly more hemicelluloses than plants in the control treatment in the full-bud harvest regime in 2011 ($P < 0.01$; Figure 5B). Plants growing under the 50%-flower harvest regime in 2011 had average hemicellulose concentrations of 7.3, 8.4, and 8.4% for the control, saline, and irrigated treatments, respectively (Table 5). Plants in the saline and irrigated treatments for the 50%-flower harvest regime in 2011 had significantly higher hemicellulose concentrations (13.1%) than plants in the control treatment ($P < 0.01$; Figure 5B). Plants growing in the full-bud saline, 50%-

flower control, and 50%-flower irrigated treatments were significantly different from the 2010 to the 2011 growing season ($P < 0.05$; Figure 5B).

The average hemicellulose concentrations of the eight varieties during the 2010 growing season were as follows: Fontanelle, 7.8%; Gold Country, 8.1%; Iroquois, 9.1%; Viking 3100, 8.1%; Viking 357, 8.1%; L447HD, 8.4%; WL363HQ, 9.2%; and Enforcer, 8.5% (Table 6). Iroquois and WL363HQ had the highest hemicellulose concentrations during the 2010 growing season with averages of 9.1 and 9.2%, respectively ($P < 0.05$; Figure 6B). There was a 15.2% increase in hemicelluloses between the lowest variety (Fontanelle) and the highest variety (WL363HQ) in 2010. In 2011, the average hemicellulose concentrations of the eight varieties were as follows: Fontanelle, 7.8%; Gold Country, 7.9%; Iroquois, 8.3%; Viking 3100, 8.1%; Viking 357, 8.6%; L447HD, 8.4%; WL363HQ, 8.5%; and Enforcer, 8.1% (Table 7). Iroquois, Viking 357, L447HD, and WL363HQ had the highest hemicellulose concentrations during the 2011 growing season with averages of 8.3, 8.6, 8.4, and 8.5%, respectively ($P < 0.05$; Figure 6B). There was a 9.3% increase in hemicelluloses between the lowest variety (Fontanelle) and the highest variety (Viking 357) in 2011. Iroquois, Viking 357, and WL363HQ had significantly different hemicellulose concentrations between the 2010 and 2011 growing seasons ($P < 0.05$; Figure 6B).

LIGNIN CONCENTRATIONS

Lignin concentrations averaged 20.2 and 17.4% across all treatments in 2010 for plants sampled at the full-bud and 50%-flower harvest regimes, respectively (Table 2).

Plants sampled at the full-bud harvest regime had 13.9% more lignin than plants sampled at the 50%-flower harvest regime during the 2010 growing season ($P < 0.01$; Figure 3C). In 2011, lignin concentrations averaged 14.4 and 14.7% across all treatments for plants sampled at the full-bud and the 50%-flower harvest regimes, respectively (Table 2). There was no significant difference for plants sampled between harvest regimes during the 2011 growing season. Lignin concentrations decreased 28.7 and 15.5% for plants sampled at the full-bud and 50%-flower harvest regimes, respectively, from 2010 to the 2011 growing season (Figure 3C).

Lignin concentrations averaged 19.0, 18.8, and 18.7% in 2010 for plants growing under the control, saline, and irrigated treatments, respectively (Table 3). There were no significant differences between treatments during the 2010 growing season. In 2011, lignin concentrations averaged 14.8, 14.1, and 14.8% for plants growing under the control, saline, and irrigated treatments, respectively (Table 3). Plants in the saline treatment had 2.3% less lignin than plants in the control and irrigated treatments in 2011 ($P < 0.05$; Figure 4C). Lignin concentrations in plants growing under all treatments (control, saline, and irrigated) significantly decreased from the 2010 to the 2011 growing season ($P < 0.01$; Figure 4C).

Lignin concentrations averaged 21.4, 18.3, and 20.1% for plants growing in the full-bud control, saline, and irrigated treatments in 2010, respectively (Table 4). Plants in the full-bud saline treatment had 14.5 and 9.0% decreased lignin concentrations from plants in the control and irrigated treatments, respectively ($P < 0.01$; Figure 5C). Plants in the 50%-flower harvest regime in 2010 had average lignin concentrations of 16.7,

19.2, and 16.4% for the control, saline, and irrigated treatments, respectively (Table 4). Contrary to the full-bud harvest regime, plants in the 50%-flower saline treatment had increased lignin concentrations of 13.0 and 14.6% over plants the control and irrigated treatments, respectively ($P < 0.01$; Figure 5C). In 2011, plants in the full-bud harvest regime had lignin concentrations of 14.6, 14.0, and 14.8% for the control, saline, and irrigated treatments, respectively (Table 5). Similar to the trend in 2010, plants growing in the full-bud saline treatment had significantly decreased lignin concentrations (4.1 and 5.4%, respectively) from plants in the control and irrigated treatments ($P < 0.05$; Figure 5C). Plants in the 50%-flower harvest regime in 2011 had average lignin concentrations of 15.0, 14.2, and 14.8% for the control, saline, and irrigated treatments, respectively (Table 5). Plants in the 50%-flower harvest regime in 2011 followed the same tendency as the 2010 and 2011 full-bud harvest regimes where the saline treatment decreased lignin concentrations, 5.3 and 4.1%, respectively, from the control and irrigated treatments ($P < 0.05$; Figure 5C). With the exception of plants in the 50%-flower harvest regime in 2010, the saline treatments decreased lignin concentrations for both growing seasons and harvest regimes. Lignin concentrations were significantly greater for both harvest regimes (full-bud and 50%-flower) and all treatments (control, saline, irrigated) in 2010 compared to 2011 ($P < 0.05$; Figure 5C).

The average lignin concentrations of the eight varieties during the 2010 growing season were as follows: Fontanelle, 17.6%; Gold Country, 17.9%; Iroquois, 18.4%; Viking 3100, 16.7%; Viking 357, 18.0%; L447HD, 17.6%; WL363HQ, 17.4%; and Enforcer, 17.2% (Table 6). Viking 3100, WL363HQ, and Enforcer had the lowest lignin

concentrations during the 2010 growing season with averages of 16.7, 17.4, and 17.2%, respectively ($P < 0.05$; Figure 6C). There was a 9.2% decrease in lignin concentration between the highest variety (Iroquois) and the lowest variety (Viking 3100) in 2010. In 2011, the average lignin concentrations of the eight varieties were as follows: Fontanelle, 14.6%; Gold Country, 14.6%; Iroquois, 14.4%; Viking 3100, 14.4%; Viking 357, 14.4%; L447HD, 14.4%; WL363HQ, 14.7%; and Enforcer, 14.0% (Table 7). Enforcer had the lowest lignin concentration with an average of 14.0% ($P < 0.05$; Figure 6C). There was a 4.8% decrease in lignin concentration between the highest variety (WL363HQ) and the lowest variety (Enforcer) in 2011. All eight varieties had significantly decreased lignin concentrations from the 2010 to the 2011 growing season ($P < 0.01$; Figure 6C).

HOLOCELLULOSE CONCENTRATIONS

Holocellulose concentrations averaged 45.5 and 44.7% across all treatments in 2010 for plants sampled in the full-bud and 50%-flower harvest regimes, respectively (Figure 7B). Plants sampled at the full-bud harvest regime had 1.8% more holocellulose than plants sampled at the 50%-flower harvest regime for the 2010 growing season ($P < 0.05$; Figure 7B). In 2011, holocellulose concentrations averaged 43.6 and 45.2% across all treatments for plants sampled at the full-bud and 50%-flower harvest regimes, respectively (Figure 7B). Plants sampled at the 50%-flower harvest regime had 3.5% more holocellulose than the full-bud harvest regime in 2011 ($P < 0.01$; Figure 7B). Holocellulose concentrations for plants sampled at the full-bud harvest regime significantly decreased by 4.7% from the 2010 to the 2011 growing season ($P < 0.01$; Figure 7B). Although not a significant difference, holocellulose concentrations increased

for plants sampled at the 50%-flower harvest regime from the 2010 to the 2011 growing season ($P = 0.10$; Figure 7B).

Holocellulose concentrations averaged 44.5, 46.1, and 44.7% in 2010 for plants growing under the control, saline, and irrigated treatments, respectively (Figure 8B). Plants growing in the saline treatments had increased holocellulose concentrations of 3.5 and 3.0% over plants in the control and irrigated treatments in 2010, respectively ($P < 0.01$; Figure 8B). Plants in the control and irrigated treatments were not significantly different during the 2010 growing season. In 2011, holocellulose concentrations averaged 42.7, 45.5, and 44.9% for plants growing under the control, saline, and irrigated treatments, respectively (Figure 8B). Similar to the 2010 growing season, plants growing in the saline treatments had increased holocellulose concentrations of 6.2 and 1.3% over plants in the control and irrigated treatments in 2011, respectively ($P < 0.05$; Figure 8B). Plants in the saline and irrigated treatments were both significantly greater than plants in the control in 2011 ($P < 0.05$; Figure 8B).

Holocellulose concentrations averaged 44.2, 46.7, and 45.6% for plants in the full-bud control, saline, and irrigated treatments in 2010, respectively (Figure 9B). Plants in the full-bud saline treatment significantly had increased holocellulose concentrations of 5.4 and 2.4% over plants in the control and irrigated treatments in 2010, respectively ($P < 0.05$; Figure 9B). Plants in the irrigated treatment had 3.1% more holocellulose than plants in the control treatment in 2010 ($P < 0.05$; Figure 9B). Plants in the 50%-flower harvest regime in 2010 had average holocellulose concentrations of 44.8, 45.6, and 43.8% for the control, saline, and irrigated treatments, respectively (Figure 9B). Plants in

the saline and control treatments were not significantly different for the 50%-flower harvest regime in 2010. Plants in the saline treatment had 3.9% more holocellulose than plants in the irrigated treatment in 2010 ($P < 0.01$; Figure 9B). In 2011, plants in the full-bud harvest regime had holocellulose concentrations of 41.5, 45.0, and 44.2% for the control, saline, and irrigated treatments, respectively (Figure 9B). As in 2010, plants in the full-bud saline treatment had significant increases of holocellulose, 7.8 and 1.8%, respectively, over plants in the control and irrigated treatments in 2011 ($P < 0.05$; Figure 9B). Plants in the 50%-flower harvest regime in 2011 had average holocellulose concentrations of 44.0, 46.1, and 45.6% for the control, saline and irrigated treatments, respectively (Figure 9B). Plants in the saline and irrigated treatments had 4.6 and 3.5%, respectively, more holocellulose than plants in the control treatment for the 50%-flower harvest regime in 2011 ($P < 0.05$; Figure 9B). In both growing seasons (2010 and 2011) and harvest regimes (full-bud and 50%-flower) plants growing under the saline treatments generally had the highest percentages of holocellulose concentrations. . Plants in the full-bud control, full-bud saline, full-bud irrigated, and 50%-flower irrigated treatments were significantly different from the 2010 to the 2011 growing season ($P < 0.05$; Figure 9B).

The average holocellulose concentrations of the eight varieties during the 2010 growing season were as follows: Fontanelle, 44.6%; Gold Country, 46.0%; Iroquois, 46.4%; Viking 3100, 45.8%; Viking 357, 44.7%; L447HD, 44.3%; WL363HQ, 44.3%; and Enforcer, 43.6% (Figure 10B). Gold Country, Iroquois, and Viking 3100 had the highest holocellulose concentrations during the 2010 growing season with averages of

46.0, 46.4, and 45.8%, respectively ($P < 0.05$; Figure 10B). There was a 6.0% increase in holocellulose concentration between the lowest variety (Enforcer) and the highest variety (Iroquois) during the 2010 growing season. In 2011, the average holocellulose concentrations of the eight varieties were as follows: Fontanelle, 44.9%; Gold Country, 45.1%; Iroquois, 43.4%; Viking 3100, 44.5%; Viking 357, 45.6%; L447HD, 44.9%; WL363HQ, 45.3%; and Enforcer, 43.6% (Figure 10B). Viking 357, WL363HQ, and Gold Country had the highest holocellulose concentrations during the 2011 growing season with averages of 45.6, 45.3, and 45.1%, respectively ($P < 0.05$; Figure 10B). There was a 4.8% increase in holocellulose concentration between the lowest variety (Iroquois) and the highest variety (Viking 357) during the 2011 growing season. Gold Country, Iroquois, Viking 3100, and Viking 357 had significantly different holocellulose concentrations between the 2010 and 2011 growing season ($P < 0.05$; Figure 10B).

HOLOCELLULOSE TO LIGNIN RATIOS

The holocellulose to lignin ratio (holocellulose: lignin) averaged 2.31 and 2.65 across all treatments in 2010 for plants sampled at the full-bud and 50%-flower harvest regimes, respectively (Figure 7A). Plants sampled at the 50%-flower harvest regime had a 12.8% increase of the holocellulose: lignin over plants sampled the full-bud harvest regime in 2010 ($P < 0.01$; Figure 7A). In 2011, the holocellulose: lignin averaged 3.07 and 3.10 across all treatments for plants sampled at the full-bud and 50%-flower harvest regimes, respectively (Figure 7A). There was no significant difference for the holocellulose: lignin between plants sampled at the full-bud and 50%-flower harvest regimes in 2011. There was a 24.8 and 14.5% increase for plants sampled at the full-bud

and 50%-flower harvest regimes, respectively, from the 2010 to the 2011 growing season ($P < 0.01$; Figure 7A).

The holocellulose: lignin averaged 2.44, 2.55, and 2.47 in 2010 for plants growing under the control, saline, and irrigated treatments, respectively (Figure 8A). Plants in the saline treatment had an increased the holocellulose: lignin by 4.3 and 3.1% over plants in the control and irrigated treatments in 2010, respectively ($P < 0.05$; Figure 8A). Plants in the saline treatment had a significantly greater holocellulose: lignin than the control in 2010 ($P < 0.05$). Although not significant ($P = 0.09$), plants in the saline treatment had an increased holocellulose: lignin over plants in the irrigated treatment in 2010. In 2011, the holocellulose: lignin averaged 2.92, 3.27, and 3.07 for plants growing under the control, saline, and irrigated treatments, respectively (Figure 8A). Plants in the saline treatment had an increased holocellulose: lignin by 10.7 and 6.1% over plants in the control and irrigated treatments in 2011, respectively ($P < 0.01$; Figure 8A). In both growing seasons (2010 and 2011) plants in the saline treatments had an increased holocellulose: lignin over plants in the control and irrigated treatments.

The holocellulose: lignin averaged 2.11, 2.61, and 2.22 for plants in the full-bud control, saline, and irrigated treatments in 2010, respectively (Figure 9A). Plants in the full-bud saline treatment had a significantly greater holocellulose: lignin (19.2 and 14.9%, respectively) over plants in the control and irrigated treatments in 2010 ($P < 0.01$; Figure 9A). Plants in the full-bud irrigated and control treatments were not significantly different. Plants in the 50%-flower harvest regime in 2010 had average holocellulose: lignin of 2.77, 2.50, and 2.73 for the control, saline, and irrigated treatments, respectively

(Figure 9A). Contrasting to the plants sampled at the full-bud harvest regime in 2010, plants in the 50%-flower saline treatment had a significant decreased holocellulose: lignin of 9.7 and 8.4% from plants in the control and irrigated treatments in 2010, respectively ($P < 0.01$; Figure 9A). Plants in the 50%-flower control and irrigated treatments were not significantly different in 2010. In 2011, the holocellulose: lignin averaged 2.89, 3.29, and 3.03 for plants in the full-bud control, saline, and irrigated treatments, respectively (Figure 9A). Similar to the 2010 growing season, plants in the full-bud saline treatment had a significantly increased holocellulose: lignin of 12.2 and 7.9% over plants in the control and irrigated treatments in 2011, respectively ($P < 0.01$; Figure 9A). Plants in the full-bud irrigated treatment also had a significantly greater holocellulose: lignin over plants in the control treatment in 2011 ($P < 0.05$; Figure 9A). Plants in the 50%-flower had average holocellulose: lignin of 2.94, 3.26, and 3.10 for the control, saline, and irrigated treatments in 2011, respectively (Figure 9A). Following the same pattern as the 2010 and 2011 full-bud harvest regimes, plants in the 50%-flower saline treatment had significant increases of 9.8 and 4.9% over plants in the control and irrigated treatments in 2011, respectively ($P < 0.01$; Figure 9A). The holocellulose: lignin was significantly greater for both harvest regimes (full-bud and 50%-flower) and all treatments (control, saline, irrigated) for plants in 2011 compared to 2010 ($P < 0.05$; Figure 9A).

The average holocellulose: lignin of the eight varieties during the 2010 growing season were as follows: Fontanelle, 2.64; Gold Country, 2.78; Iroquois, 2.66; Viking 3100, 2.85; Viking 357, 2.60; L447HD, 2.62; WL363HQ, 2.70; and Enforcer, 2.65

(Figure 10A). Gold Country, Viking 3100 and WL363HQ had the highest holocellulose: lignin during the 2010 growing seasons with averages of 2.78, 2.85, and 2.70, respectively ($P < 0.05$; Figure 10A). There was an 8.8% increase for the holocellulose: lignin between the lowest variety (Viking 357) and the highest variety (Viking 3100) in 2010. In 2011 the average holocellulose: lignin of the eight varieties were as follows: Fontanelle, 3.14; Gold Country, 3.14; Iroquois, 3.10; Viking 3100, 3.13; Viking 357, 3.24; L447HD, 3.19; WL363HQ, 3.13; and Enforcer, 3.20 (Figure 10A). Viking 357 had the highest holocellulose: lignin with an average of 3.24 ($P < 0.05$; Figure 10A). There was a 4.3% increase for the holocellulose: lignin between the lowest variety (Iroquois) and the highest variety (Viking 357) in 2011. The holocellulose: lignin increased for all eight varieties from the 2010 to the 2011 growing season ($P < 0.01$; Figure 10A).

THEORETICAL ETHANOL YIELDS

Theoretical ethanol yields averaged 146.3 and 144.0 liters per 1000 kilograms of dry weight (L / 1000kg DW) across all treatments in 2010 for plants sampled at the full-bud and 50%-flower harvest regimes, respectively (Figure 11). The cellulosic ethanol yield comprised 84.4 and 85.0% of the total ethanol yield for plants sampled the full-bud and 50%-flower harvest regimes in 2010, respectively (data not shown). In 2011, theoretical ethanol yields averaged 140.0 and 145.0 L / 1000kg DW across all treatments for plants sampled the full-bud and 50%-flower harvest regimes, respectively (Figure 11). The cellulosic ethanol yield comprised 84.2 and 85.5% of the total ethanol yield for plants sampled at the full-bud and 50%-flower harvest regimes in 2011, respectively

(data not shown). Total theoretical ethanol yields decreased 4.3% for plants sampled at the full-bud harvest regime from the 2010 to the 2011 growing season ($P < 0.01$; Figure 11). However, total theoretical ethanol yields increased 1.2% for plants sampled the 50%-flower harvest regime from 2010 to 2011 ($P < 0.05$; Figure 11).

Theoretical ethanol yields averaged 149.0, 154.4 and 149.5 L / 1000kg DW for plants growing under the control, saline, and irrigated treatments in 2010, respectively (Figure 12). The cellulosic ethanol yield comprised 82.3, 81.2, and 80.1% of the total ethanol yield for plants growing under the control, saline, and irrigated treatments in 2010, respectively (data not shown). Plants growing in the saline treatments had increased theoretical ethanol yields of 3.5 and 3.2% over plants in the control and irrigated treatments in 2010, respectively ($P < 0.01$; Figure 12). In 2011, theoretical ethanol yields averaged 143.1, 152.5, and 150.4 L / 1000kg DW for plants growing under the control, saline, and irrigated treatments, respectively (Figure 12). The cellulosic ethanol yield comprised 83.0, 81.3, and 80.4% of the total ethanol yield for plants in the control, saline, and irrigated treatments in 2011, respectively (data not shown). Following a similar trend to the 2010 growing season, plants in the saline treatments in 2011 had increased theoretical ethanol yields of 6.2 and 1.8% over plants in the control and irrigated treatments, respectively ($P < 0.05$; Figure 12).

Theoretical ethanol yields averaged 143.0, 149.8, and 146.0 L / 1000kg DW for plants in the full-bud control, saline, and irrigated treatments in 2010, respectively (Table 8). Plants in the 50%-flower harvest regime in 2010 had average theoretical ethanol yields of 143.9, 147.0, and 140.9 L / 1000kg DW for the control, saline, and irrigated

treatments, respectively (Table 8). Plants in the full-bud saline treatment had the highest theoretical ethanol yield in 2010, averaging 149.8 L / 1000kg DW, with the cellulosic ethanol yield comprising 83.4% of the total ethanol yield ($P < 0.05$; Table 8). In 2011, plants in the full-bud harvest regime had average theoretical ethanol yields of 133.8, 144.5, and 141.6 L / 1000kg DW for the control, saline, and irrigated treatments, respectively (Table 9). Plants in the 50%-flower harvest regime in 2011 had average theoretical ethanol yields of 142.2, 148.4, and 146.9 L / 1000kg DW for the control, saline, and irrigated treatments, respectively (Table 9). Plants in the 50%-flower saline treatment had the highest theoretical yield in 2011, averaging 148.4 L / 1000kg DW, with the cellulosic ethanol yield comprising 85.0% of the total ethanol yield ($P < 0.05$; Table 9).

During the 2010 growing season the Iroquois variety had the highest theoretical ethanol yield with an average of 149.1 L / 1000kg DW ($P < 0.05$; Table 10). Gold country was not significantly different with an average of 148.5 L / 1000kg DW in 2010. Interestingly, the Gold Country variety had the highest cellulosic ethanol yield in 2010 with an average of 127.2 L / 1000kg DW ($P < 0.05$; Table 10). In 2011, the Viking 357 variety had the highest theoretical ethanol yield with an average of 146.7 L / 1000kg DW ($P < 0.05$; Table 11). Although not a significant difference, the Viking 357 variety also had the highest hemicellulosic ethanol yield in 2011 with an average of 22.8 L / 1000kg DW ($P < 0.13$; Table 11).

DISCUSSION

Growing season, harvest regime, irrigation, and salinity all affected stem lignocellulosic concentrations in alfalfa. Harvest regime affected stem lignocellulosic concentrations but results varied significantly between growing seasons. Precipitation patterns had a major influence on the effects of irrigation and salinity on stem lignocellulosic concentrations. Plants under supplemental irrigation had higher lignocellulosic concentrations and this effect was more pronounced during the dry period (August-October) in the 2011 growing season (Table 1). However, over the duration of this study there were never any signs or symptoms of drought stress.

Harvest regime did not significantly affect cellulose concentrations during the 2010 growing season (Figure 3A). However, during the 2011 growing there was a 5.4% increase in cellulose concentration between the plants sampled at full-bud and 50%-flower harvest regimes ($P < 0.01$; Figure 3A). Plants harvested during the 2010 and 2011 growing seasons followed similar trends for hemicellulose concentrations. Hemicellulose concentrations of the plants sampled at the 50%-flower harvest regime in both growing seasons decreased by 5.8 and 4.8%, respectively, from the full-harvest regime ($P < 0.05$; Figure 3B). Plants harvested at the full-bud harvest regime had significantly higher lignin concentrations than those harvested at the 50%-flower harvest regime in 2010 ($P <0.01$; Figure 3C). However, there were no significant differences in lignin concentrations in plants sampled during the 2011 growing season (Figure 3C). While maturity is the single most important factor impacting stem lignocellulosic concentrations in alfalfa, growth environment causes some additional shifts in stem lignocellulosic

allocation concentrations. Unfortunately these environmental impacts are complex and their effects are difficult to predict (Samac et al., 2006). Sanderson and Wedin (1988) found substantially higher lignocellulosic concentrations in alfalfa stems during one year, however, the same plots harvested at the same growth stage the following year showed a small difference in lignocellulosic concentrations. In this study temperature and moisture were not independently evaluated. Studies that evaluated temperature and moisture separately found moisture stress alone affected the amount of cell wall accumulated by alfalfa plants but did not change cell wall composition (Samac et al., 2006). We found similar results in the holocellulose to lignin ratios between harvest regimes and growing seasons. During the 2010 growing season, there was a 12.8% increase in the holocellulose to lignin ratio between the full-bud to the 50%-flower harvest regimes ($P < 0.01$; Figure 7A). However, in 2011, there was no significant difference between harvest regimes, but the holocellulose: lignin ratio increased 24.8 and 14.5% for plants sampled at the full-bud and 50%-flower harvest regimes, respectively, from the 2010 to the 2011 growing season (Figure 7A). Total theoretical ethanol yields also varied by harvest regime and growing season. Lamb et al. (2007) found that alfalfa grown under a biomass-type management system (50%-flower harvest regime) increased lignocellulosic concentrations by 4% and could increase theoretical ethanol yields by 6.5%. During the 2011 growing season the plants sampled at the 50%-flower harvest regime had a 3.2% increased lignocellulosic concentrations over the full-bud harvest regime which increased theoretical ethanol yields by 4.0% ($P < 0.01$; Figure 11). However, during the 2010 growing season there was a decrease in theoretical ethanol yield from the plants sampled

at the full-bud to the 50%-flower harvest regime ($P < 0.05$; Figure 11). Rock et al. (2009) observed similar patterns where stem lignocellulosic concentrations in alfalfa exhibited year by harvest interactions with no clear pattern and concluded that industries that wish to utilize alfalfa for lignocellulosic ethanol production must be prepared to deal with significant feedstock quality variation due to macro-environment fluctuations.

This study was conducted in a natural field setting with the control plots receiving ambient amounts of precipitation. During the 2010 growing season the field site received an average 2.5 cm more precipitation per month than the historical average (Table 1). The irrigated and saline treatments received an additional 5.0 cm of well water per harvest regime depending on local precipitation patterns. Irrigation did not seem to have a significant effect on stem lignocellulosic concentrations during the 2010 growing season although the hemicellulose concentrations showed a significant increase compared to the control ($P < 0.05$; Figure 4B). However, plants irrigated with salt had significantly higher holocellulose concentrations (cellulose and hemicellulose), holocellulose to lignin ratios, and the theoretical ethanol yields ($P < 0.05$; Figures 4A, 4B, 4C, 8A and 12). In 2011, which was a drier year (3.6 cm less precipitation per month than the historical monthly average), irrigation and salinity appear to have contributed to plants with higher holocellulose concentrations (cellulose and hemicellulose) over the control treatment ($P < 0.05$; Figures 4A, 4B, 4C, 8A and 12). Deetz et al. (1994) found that alfalfa plants that grew under water-deficit conditions had reduced stem lignocellulosic concentrations. The reduction in stem lignocellulosic concentrations was most likely the result of delayed maturity and decreased cell wall accumulation (Deetz et al., 1994). Interestingly, alfalfa

growing under saline treatments had higher holocellulose to lignin ratios (and higher theoretical yields) during both growing season suggesting that moderate levels of salt may stimulate holocellulose concentrations. Alfalfa growing under saline treatments had higher holocellulose concentrations but lower lignin concentrations during the 2011 growing season ($P < 0.05$; Figure 4C). These findings could be significant because selecting species with high holocellulose to lignin ratios will be an important characteristic when selecting feedstocks for ethanol production.

The alfalfa variety Gold Country had the highest percent cellulose concentrations with averages of 38.0 and 37.2% during the 2010 and 2011 growing season, respectively (Table 6 and 7). Gold Country was high yielding with WSI and FD rankings of 2.5 and 3.8, respectively. Although having the highest percent cellulose concentrations during both growing seasons, Gold Country also had some of the highest lignin concentrations. This resulted in lower holocellulose to lignin ratios compared to other varieties ($P < 0.05$; Figure 10A). Iroquois had the highest total theoretical ethanol yield during the 2010 growing season (Table 10). Iroquois was obtained from a local farmer and the WSI and FD rankings were unknown. Typically, Iroquois is a non-genetically modified variety with average yields (Manske and Goetz, 1982). During the 2011 growing season Iroquois had the lowest total theoretical ethanol yield (Table 11). Gold Country also had very high total theoretical ethanol yields during both growing seasons (Table 10 and 11). Variety selection did not seem to have a pronounced effect on stem lignocellulosic concentrations in alfalfa. High yielding varieties (Gold Country) did not have a significantly greater holocellulose to lignin ratio or total theoretical ethanol yields

compared to typical non-genetically modified alfalfa (Iroquois). Research on biomass yields and forage nutrition quality could prove beneficial for variety selection in the future if alfalfa is used as a feedstock for lignocellulosic ethanol production.

In conclusion, alfalfa shows great potential as a biomass feedstock for lignocellulosic ethanol production. Benefits such as a reduced requirement for nitrogen fertilizer, increased environmental protection and a well-known cropping system give it an advantage over other comparable feedstocks. In many scenarios alfalfa leaves and stems would be separated for lignocellulosic ethanol production. Generally, management systems have emphasized harvesting alfalfa forage at immature growth stages to maximize the leaf component and crude protein concentrations; although a biomass production system would make the stem component as valuable as the leaf yield (Lamb et al., 2003). Separating the leaves from the stems in the field would create a much more viable system than separation facilities that have been proposed by other researchers (Arinze et al., 2003; Downing et al., 2005). Another improvement on the alfalfa cropping system would be to seed the alfalfa in the fall after the current crop has been harvested. This has the potential to greatly increase the first year alfalfa yields. Genetic improvements could also increase alfalfa's value as biomass feedstock. Genetically decreasing the concentration of lignin in alfalfa stems would decrease fermentation costs and in turn increase ethanol yields.

Crops such as *Miscanthus spp.*, *Populus spp.*, and switchgrass (*Panicum virgatum*) could be used as a feedstock for lignocellulosic ethanol production. Unlike alfalfa, many of the proposed crops do not have well-established cropping systems and

farmers may be reluctant to invest in these systems. If lignocellulosic ethanol production reaches the scale of corn grain ethanol production there may be government incentives and subsidies for farmers to grow specific crops. Production of any system will be highly dependent on a variety of factors, including the ability and need to produce a given volume of ethanol, protection of environmental quality and natural resources, the promotion of rural economic growth and stability, and current and future farm production strategies and goals (Vadas et al., 2008).

LITERATURE CITED

Allakhverdiev, S. I., A. Sakamota, Y. Nishiyama, M. Inaba, and N. Murata. 2000. Ionic and osmotic effects of NaCl-induced inactivation of photosystems I and II in *Synechococcus sp. Plant Physiology*. 123:1047-1056.

Arinze, E. A., G. J. Schoenau, S. Sokhansanj, and P. Adapa. 2003. Aerodynamic separation and fractional drying of alfalfa leaves and stems – A review and new concept. *Drying Technology*. 21:1669-1698.

Badger, P. C. 2002 Ethanol from cellulose: a general review. *In Trends in New Crops and New Uses.* J. Janick and A. Whipkey (eds.). ASHS Press, Alexandria VA. 17-21.

Ball, S. T. 1998. Alfalfa growth stages. *New Mexico State University, Cooperative Extension Service.*

Bauder, J. W., J. S. Jacobsen, and W. T. Lanier. 1992. Alfalfa emergence and survival response to irrigation water quality and soil series. *Soil Science Society American Journal.* 56: 890-896.

Carlson, C. R., J. F. Cummins, M. P. Diers, D. R. Dykhuizen, G. Poch, R. E. Rolling, C. T. Saari, and O. J. Whitaker. 1980. Soil Survey of Freeborn County, Minnesota. *United States Department of Agriculture Soil Conservation Service in cooperation with the Minnesota Agricultural Experiment Station.* 30-31.

Chapple, C., M. Ladisch, R. Meilan. 2007. Loosening lignin's grip on biofuel production. *Natrue Biotechnology.* 25: 746-748.

Deetz, D. A., H. J. Jung, and D. R. Buxton. 1994. Water-deficit effects on cell-wall composition and in-vitro degradability of structural polysaccharides in alfalfa stems. *Crop Science.* 36:383-388.

Dien, B. S., H. J. Jung, K. P. Vogel, M. D. Casler, F. S. Lamb, L. Iten, R. B. Mitchell, and G. Sarath. 2006. Chemical composition and response to dilute-acid pretreatment and enzymatic saccharification of alfalfa, reed canarygrass, and switchgrass. *Biomass and Bioenergy.* 30:880-891.

Downing, M., T. A. Volk, and D. A. Schmidt. 2004. Development of new generation cooperatives in agriculture for renewable energy research, development, and demonstration projects. *Biomass and Bioenergy*. 28:425-434.

Emam, Y., E. Bijanzadeh, R. Naderi, and M. Edalat. 2009. Effects of salt stress on Vegetative growth and ion accumulation of two alfalfa (*Medicago sativa* L.) cultivars. *Desert.* 14: 163-169.

Esechie, H. A., B. Al-Barhi, S. Al-Gheity, and S. Al-Khanjari. 2002. Root and shoot growth in salinity stressed alfalfa in response to nitrogen source. *Journal of Plant Nutrition*. 25:2559-2569.

Jung, H.G., and F. M. Engels. 2002. Alfalfa stem tissues: cell wall deposition, composition, and degradability. *Crop Science*. 42:524-534.

Krogman, K. K., and E. H. Hobbs. 1965. Evapotranspiration of irrigated alfalfa as related to season and growth stage. *Canadian Journal of Plant Science.* 45:310-313.

Lamb, F. S., C. C. Sheaffer, and D. A. Samac. 2003. Population density and harvest maturity effects on leaf and stem yield in alfalfa. *Agronomy Journal.* 95:635-641.

Lamb, F. S., H. G. Jung, G. C. Sheaffer, and D. A. Samac. 2007. Alfalfa leaf protein and stem cell wall polysaccharide yields under hay and biomass management systems. *Crop Science*. 47:1407-1415.

Maheshwari, S. 2008. The science behing the biofuels controversy. *Current Science*. 95:594-602.

Manske L. and H. Goetz. 1982. Alfalfa variety trial. <www.ag.ndsu.edu/archive/research/1982/alalfa/variety/trial.pdf>

McCaslin, M., D. Miller. 2007. The Future of Alfalfa as a Biofuels Feedstock. *California Alfalfa & Forage Symposium.*

McKenzie, R. H. 2005. Soil and Nutrient Management of Alfalfa. *Agri-Facts*. Agdex 121/531-5.

Montazar, A., and M. Sadeghi. 2008. Effects of applied water and sprinkler irrigation uniformity on alfalfa growth and hay yield. *Agricultural Water management*. 95:1279-1287.

Munns, R. 2002. Comparative physiology of salt and water stress. *Plant, Cell and Environment*. 25: 239-250.

Munns, R., A. J. James, and A. Lauchli. 2006. Approaches to increasing the salt tolerance of wheat and other cereals. *Journal of Experimental Botany*. 57: 1025-1043.

Parida, A. K., and A. B. Das. 2005. Salt tolerance and salinity effects on plants: a review. *Ecotoxicology and Environmental Safety*. 60:324-349.

Ragauska, A. J., C. K. Williams, B. H. Davison, G. Britovsek, J. Caimey, C. A. Eckert, W. J. Frederick, J. P. Hallett, D. J. Leak, C. L. Liotta, J. R. Mielenz, R. Murphy, R. Templer, and T. Tschaplinski. 2006. The path forward for biofuels and biomaterials. *Science*. 311:484-489.

Rock, K. P., R. T. Thelemann, H. J. G. Jung, U. W. Tschirner, C. C. Sheaffer and G. A. Johnson. 2009 Variation due to growth environment alfalfa yield, cellulosic ethanol traits, and paper pulp characteristics. *Bioenergy Research*. 2:79-89.

Saeed, I. A. M., A. H. El-Nadi. 1997. Irrigation effects on the growth, yield, and water use efficiency of alfalfa. *Irrigation Science*. 17:63-68.

Samac, D.A., H.J.G. Jung, and J.F.S. Lamb. 2006. Development of Alfalfa (*Medicago sativa* L.) as a feedstock for production of ethanol and other bioproducts. . *In Minteer, S., editor. Alcoholic Fuels.* Boca Raton, FL: CRC Press. p. 79-98

Sanderson, M. A., and W. F. Wedin. 1988. Cell wall composition of alfalfa stems at similar morphological stages and chronological age during spring growth and summer regrowth. *Crop Science*. 28:342-347.

Sheaffer, C. C., N. P. Martin, F. S. Lamb, G. R. Cuomo, J. G. Jewett, and S. R. Querting. 2000. Leaf and stem properties of alfalfa entries. *Agronomy Journal*. 92:733-739.

Shinners, K.J., M.E. Herzmann, B.N. Binversie, and M.F. Digman. 2007. Harvest fractionation of alfalfa. *Trans. ASABE*. 50:713-718.

Tayfur, G., K. K. Tanji, B. House, F. Robinson, L. Teuber, and G. Kruse. 1995. Modeling deficit irrigation in alfalfa production. *Journal of Irrigation and Drainage Engineering.* 121:442-151.

Undersander, D., P. Vassalotti, D. Cosgrove. 1997. Alfalfa germination and growth. *University of Wisconsin Extension*.

Vadas, P. A., K. H. Barnett, D. J. Undersander. 2008. Economics and Energy of Ethanol Production from Alfalfa, Corn, and Switchgrass in the Upper Midwest, USA. *Bioenergy Research*. 1:44-55.

Van Soest, P. J., J. B. Robertson, and B. A. Lewis. 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *Journal of Dairy Science*. 74: 3583-3597.

Vaughan, L. V., J. W. MacAdams, S. E. Smith, and L. M. Dudley. 2002. Growth and yield of differing alfalfa rooting populations under increasing salinity and zero leaching. *Crop Science.* 42:2064-2071.

Walsh, M. E., D. G. De La Torre Ugarte, B. C. English, K. Jensen, C. Hellwinckel, R. Jamey Menard, and R. G. Nelson. 2007. Agricultural Impacts of Biofuels Production. *Journal of Agricultural and Applied Economics*. 39:365-372.

Wyman, C. E. 2007. What is (and is not) vital to advancing cellulosic ethanol. *Trends in Biotechnology*. 25:153-157.

FIGURE LEGENDS

Figure 1. Experimental design displaying the numbered varieties, plot dimensions, and the corresponding treatments and harvest regimes. 1-WL363HQ, 2-Viking 357, 3-L447HD, 4-Enforcer, 5-Viking 3100, 6-Fontanelle Hybrid – Ovation 2, 7-Gold Country 24/7, 8-Iroquois

Figure 2. The average monthly precipitation for the 2010 and 2011 growing seasons and the historical monthly average precipitation (2001-2009) at the research site, 2.5 miles west of Geneva, Minnesota.

Figure 3. The percent cellulose (A), hemicellulose (B), and lignin (C) concentrations for plants sampled at the full-bud and 50%-flower harvest regimes during the 2010 and 2011 growing seasons. Values are means of harvest regimes during each growing season (n=240, 2010 and n=480, 2011). Vertical error bars represent ± 1SE. Letters (a-c) denote significant difference between harvest regimes ($P < 0.05$).

Figure 4. The percent cellulose (A), hemicellulose (B), and lignin (C) concentrations for plants growing under the control, saline, and irrigated treatments during the 2010 and 2011 growing seasons. Values are means of the treatments during each growing season (n=160, 2010 and n=320, 2011). Vertical error bars represent ± 1SE. Letters (a-e) denote significant differences among treatments ($P < 0.05$).

Figure 5. The percent cellulose (A), hemicellulose (B), and lignin (C) concentrations for plants sampled at the full-bud and 50%-flower regimes with the corresponding treatment (control, saline, or irrigated) during the 2010 and 2011 growing seasons. Values are means of the harvest regime and the corresponding treatment during the 2010 (n=80, white bars) and 2011 (n=160, grey bars) growing seasons. Vertical error bars represent ± 1SE. Letters (a+b, 2010) and (r-u, 2011) denote significant differences among treatments and a * signifies differences between years ($P < 0.05$).

Figure 6. The percent cellulose (A), hemicellulose (B), and lignin (C) concentrations for each variety during the 2010 and 2011 growing seasons. Values are means of each variety during the 2010 (n=90, white bars) and 2011 (n=140, grey bars) growing seasons. Vertical error bars represent ± 1SE. Letters (a-d, 2010) and (r-t, 2011) denote significant differences among varieties and a * signifies differences between years ($P < 0.05$).

Figure 7. The holocellulose to lignin ratio (A) and the percent holocellulose concentration (B) for plants sampled at the full-bud and 50%-flower harvest regimes during the 2010 and 2011 growing seasons. Values are means of harvest regimes during each growing season (n=240, 2010 and n=480, 2011). Vertical error bars represent ± 1SE. Letters (a-c) denote significant difference between harvest regimes ($P < 0.05$).

Figure 8. The holocellulose to lignin ratio (A) and the percent holocellulose concentration (B) for plants growing under the control, saline, and irrigated treatments during the 2010 and 2011 growing seasons. Values are means of the treatments during each growing season (n=160, 2010 and n=320, 2011). Vertical error bars represent ± 1SE. Letters (a-e) denote significant differences among treatments ($P < 0.05$).

Figure 9. The holocellulose to lignin ratio (A) and the percent holocellulose concentration (B) for plants sampled at the full-bud and 50%-flower regimes with the corresponding treatment (control, saline, or irrigated) during the 2010 and 2011 growing seasons. Values are means of the harvest regime and the corresponding treatment during the 2010 (n=80, white bars) and 2011 (n=160, grey bars) growing seasons. Vertical error bars represent ± 1SE. Letters (a-d, 2010) and (r-t, 2011) denote significant differences among treatments and a * signifies differences between years ($P < 0.05$).

Figure 10. The holocellulose to lignin ratio (A) and the percent holocellulose concentration (B) for each variety during the 2010 and 2011 growing seasons. Values are means of each variety during the 2010 (n=90, white bars) and 2011 (n=140, grey bars) growing seasons. Vertical error bars represent ± 1SE. Letters (a-d, 2010) and (r+s, 2011) denote significant differences among varieties and a * signifies differences between years ($P < 0.05$).

Figure 11. The theoretical ethanol yield (L/1000kg DW) for the full-bud and 50%-flower harvest regimes during the 2010 and 2011 growing seasons. Values are means of harvest regimes during each growing season (n=240, 2010 and n=480, 2011). Vertical error bars represent ± 1SE. Letters (a-c) denote significant difference between harvest regimes ($P < 0.05$).

Figure 12. The theoretical ethanol yield (L/1000kg DW) for the control, saline, and irrigated treatments during the 2010 and 2011 growing seasons. Values are means of the treatments during each growing season (n=160, 2010 and n=320, 2011). Vertical error bars represent ± 1SE. Letters (a-d) denote significant differences among treatments ($P < 0.05$).

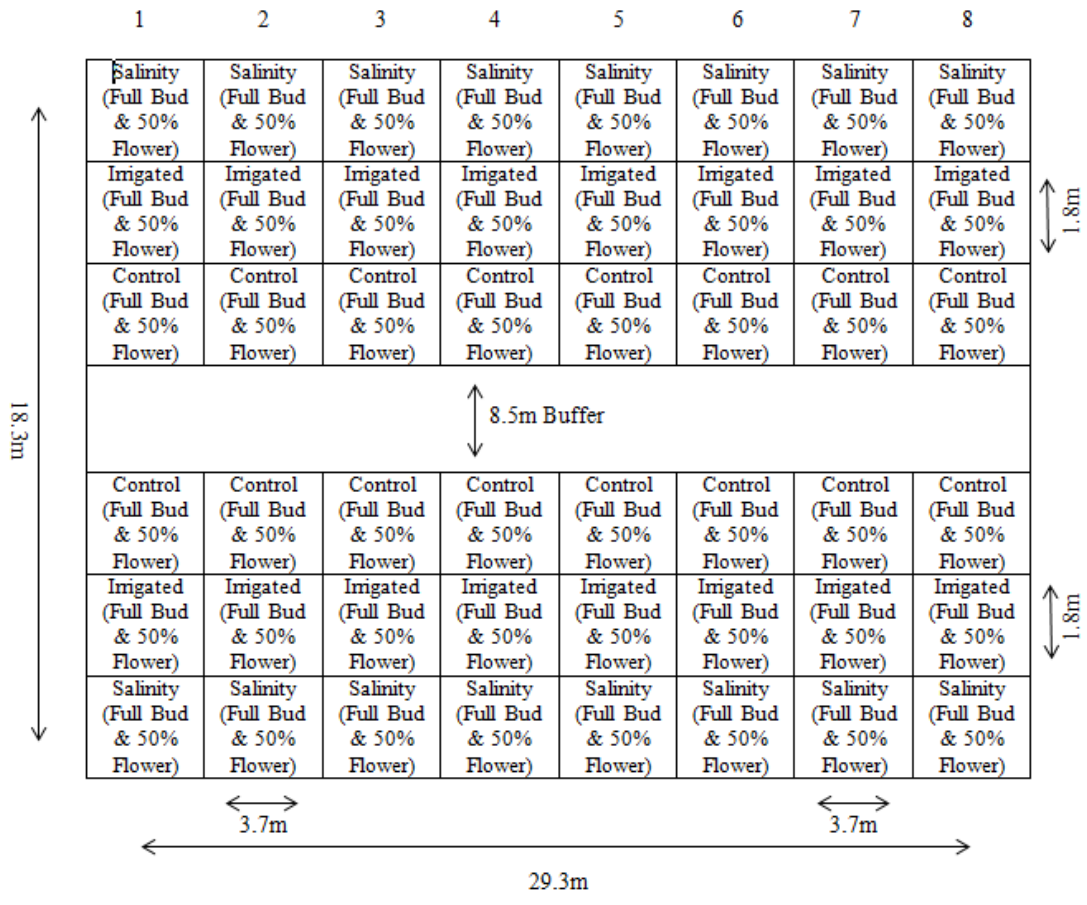
1
2
3
4
5
6
7
8
Salinity (Full Bud & 50% Flower)
Irrigated (Full Bud & 50% Flower)
Control (Full Bud & 50% Flower)
8.5m Buffer
18.3m
1.8m
1.8m
3.7m
3.7m
29.3m

Figure 1

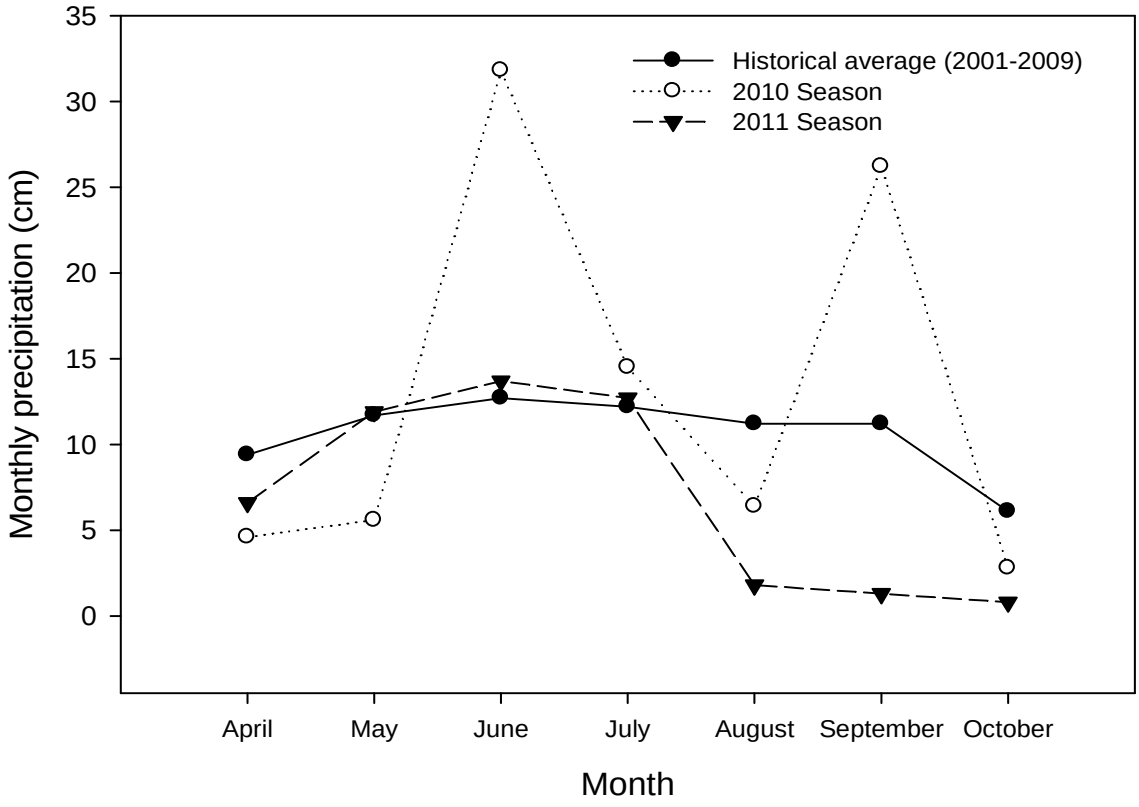
35
30
25
20
15
10
5
0
Monthly precipitation (cm)
Historical average (2001-2009)
2010 Season
2011 Season
April
May
June
July
August
September
October
Month

Figure 2

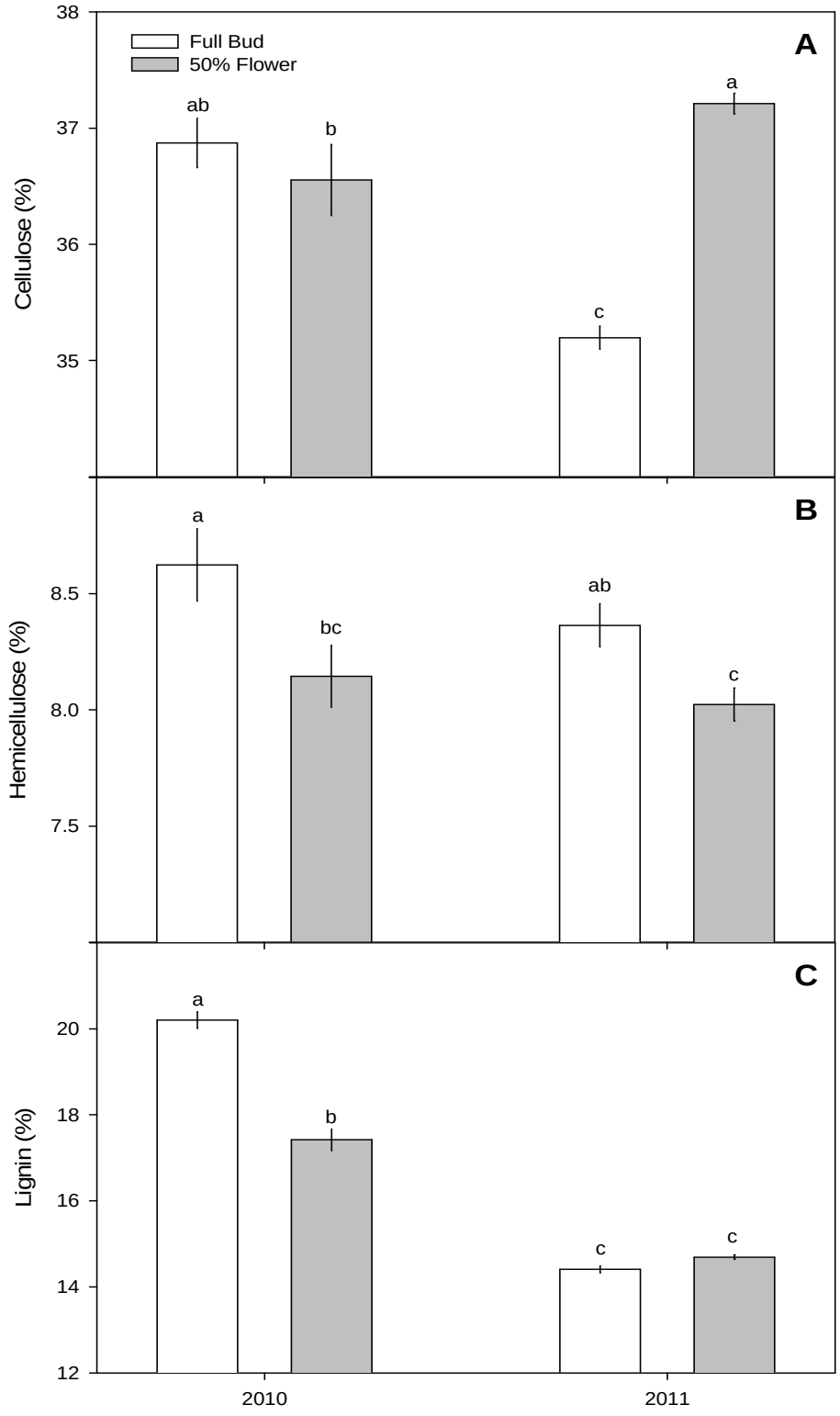
Full Bud
50% Flower
A
B
C
Cellulose (%)
Hemicellulose (%)
Lignin (%)
38
37
36
35
8.5
8.0
7.5
20
18
16
14
12
ab
b
c
a
a
bc
ab
c
a
b
c
c
2010
2011

Figure 3

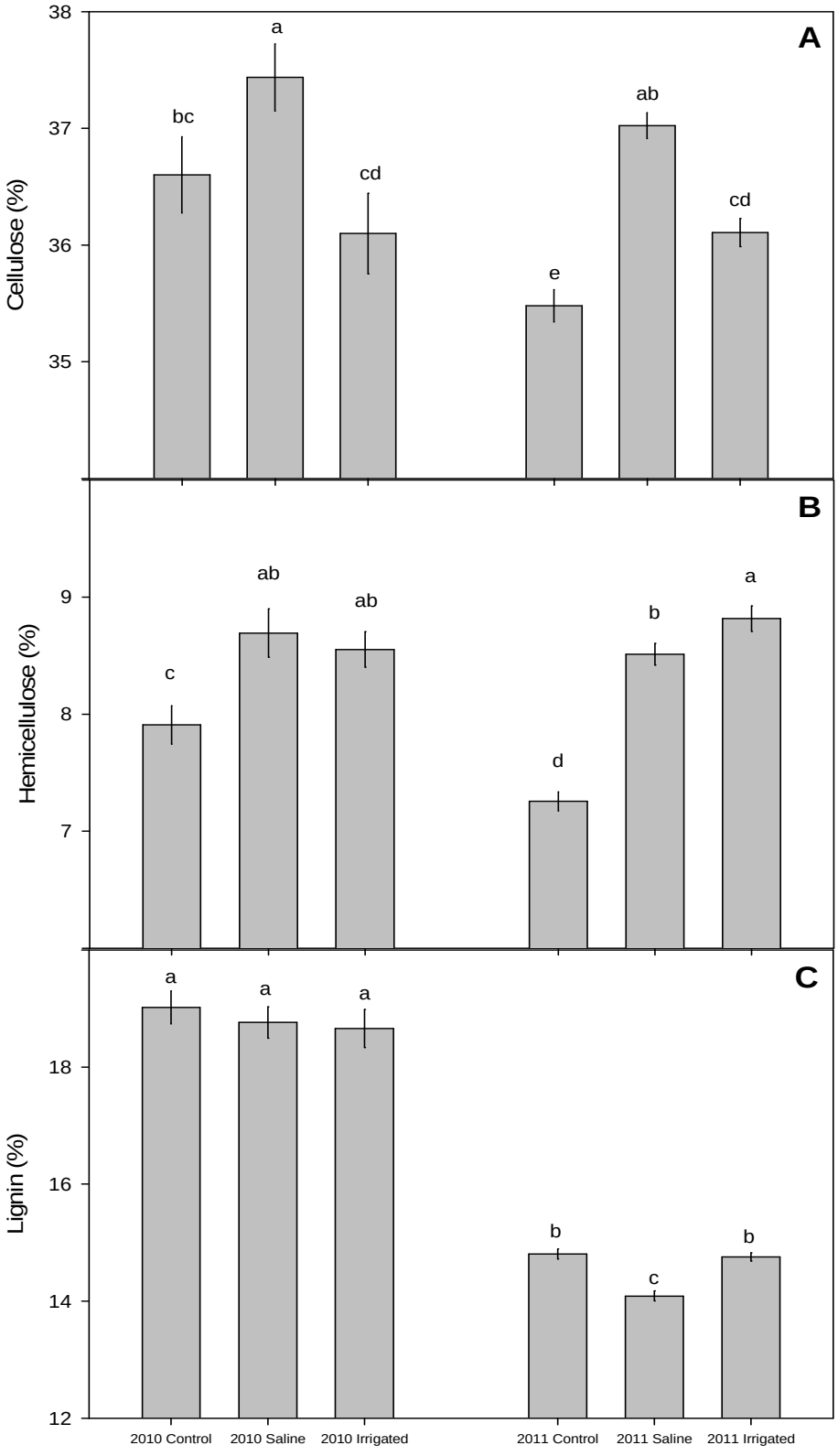
A
B
C
Cellulose (%)
Hemicellulose (%)
Lignin (%)
38
37
36
35
9
8
7
18
16
14
12
bc
a
cd
e
ab
cd
c
ab
ab
d
b
a
a
a
a
b
c
b
2010 Control
2010 Saline
2010 Irrigated
2011 Control
2011 Saline
2011 Irrigated

Figure 4

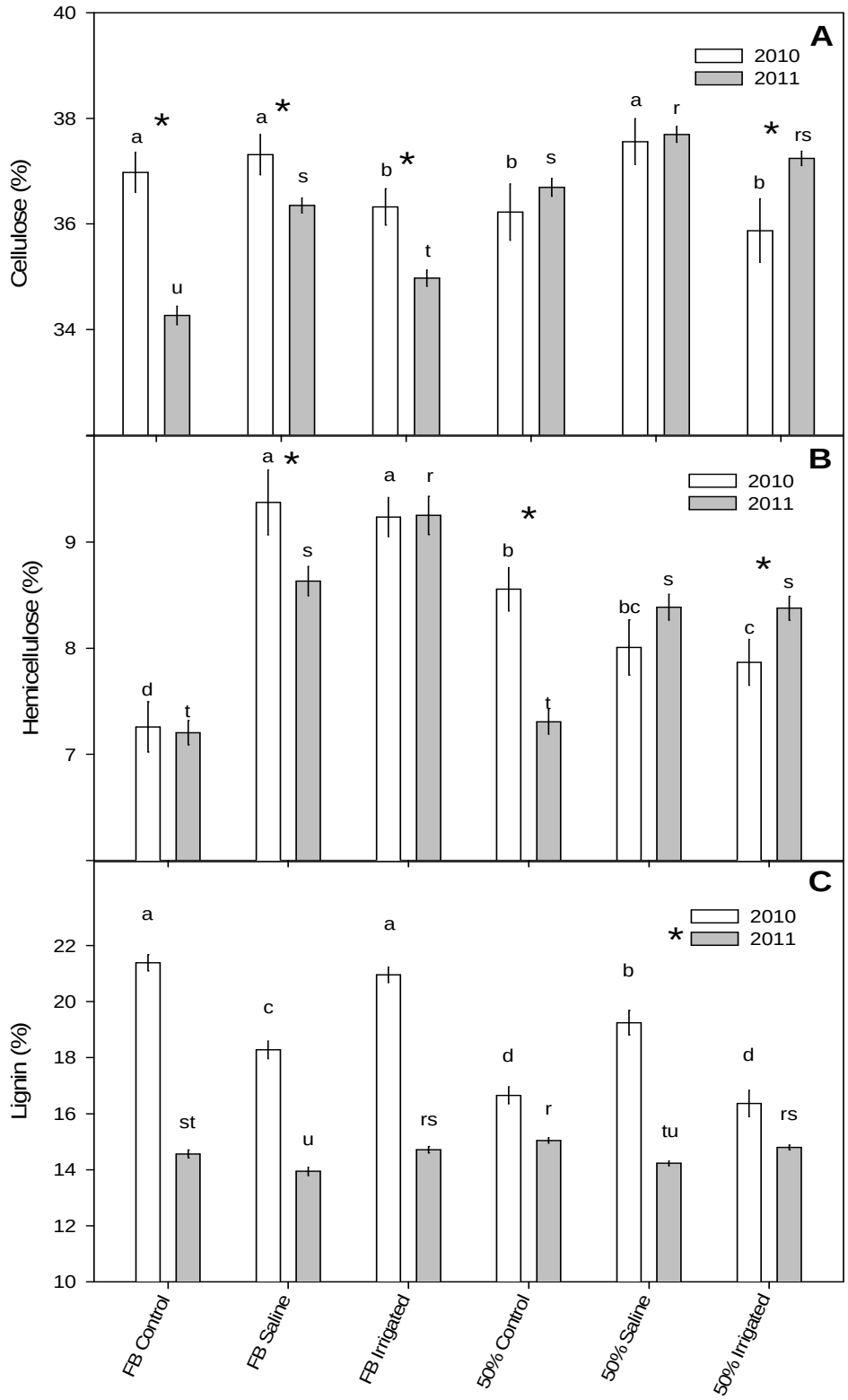
A
B
C
2010
2011
Cellulose (%)
Hemicellulose (%)
Lignin (%)
40
38
36
34
9
8
7
22
20
18
16
14
12
10
a
u
a
s
b
t
b
s
a
r
b
rs
d
t
a
s
a
r
b
s
bc
s
c
s
a
st
c
u
a
rs
d
r
b
tu
d
rs
FB Control
FB Saline
FB Irrigated
50% Control
50% Saline
50% Irrigated

Figure 5

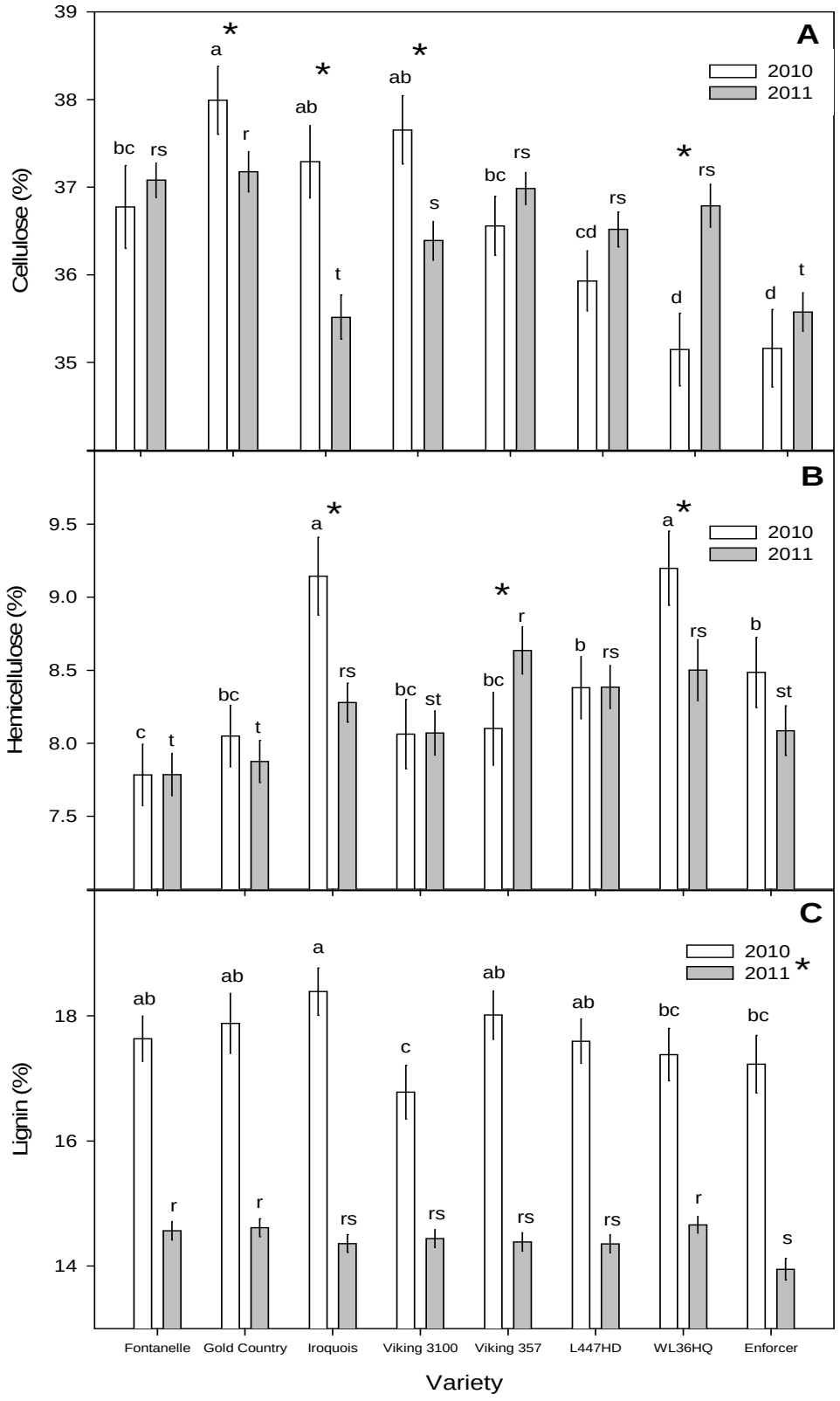
A
B
C
2010
2011
Cellulose (%)
Hemicellulose (%)
Lignin (%)
Variety
Fontanelle
Gold Country
Iroquois
Viking 3100
Viking 357
L447HD
WL36HQ
Enforcer

Figure 6

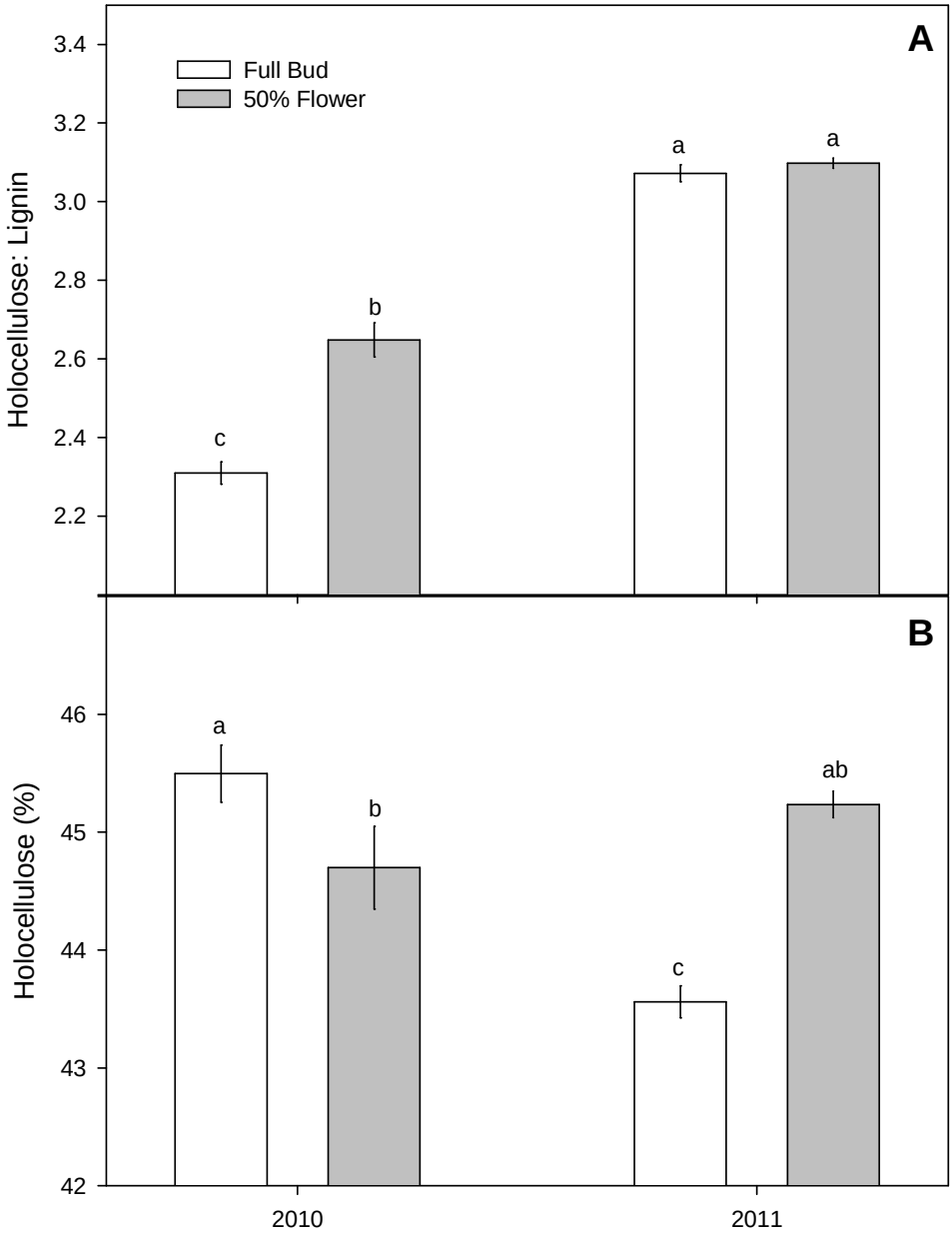
A
Full Bud
50% Flower
Holocellulose: Lignin
3.4
3.2
3.0
2.8
2.6
2.4
2.2
a
a
b
c
B
Holocellulose (%)
46
45
44
43
42
a
b
c
ab
2010
2011

Figure 7

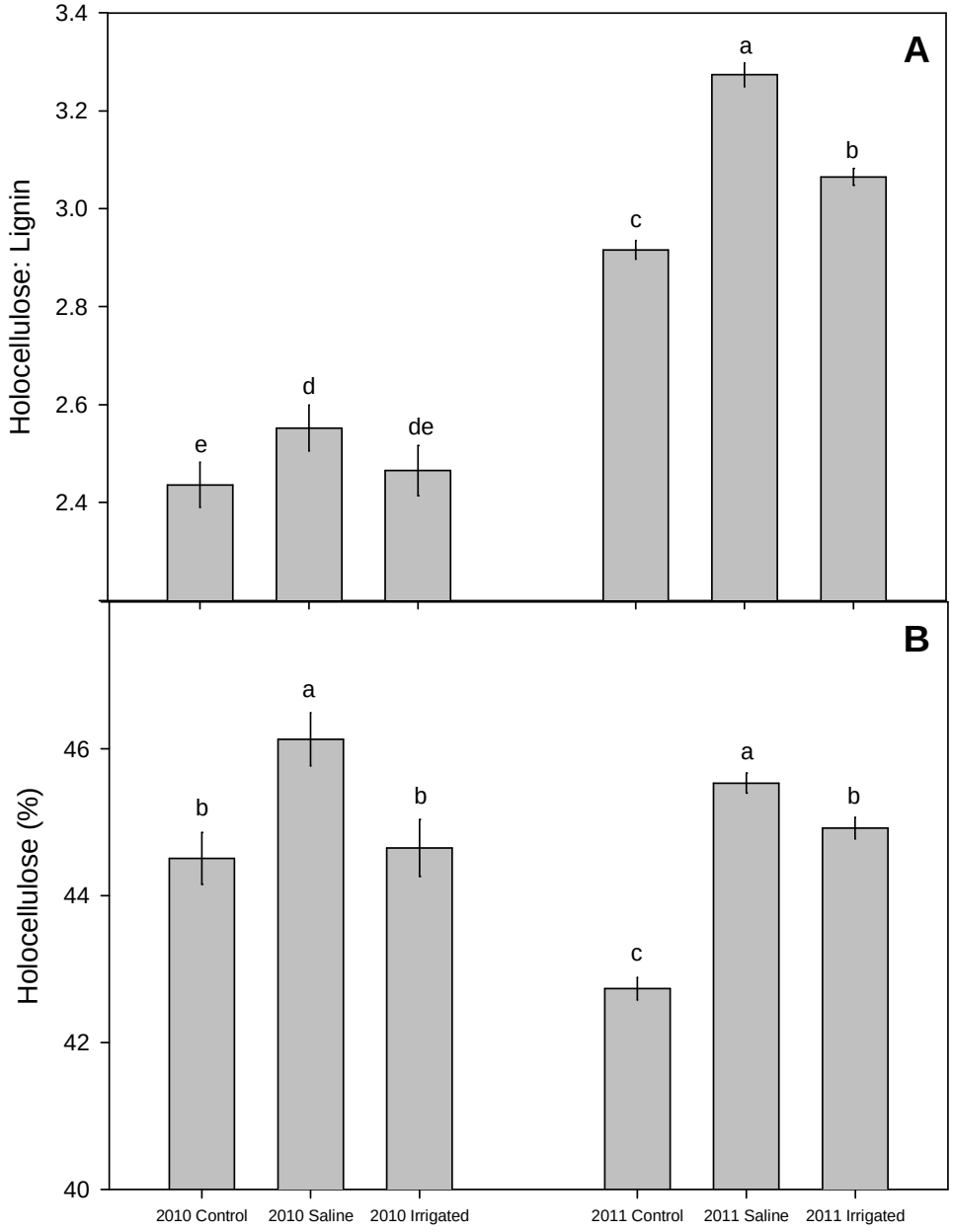
A
B
Holocellulose: Lignin
Holocellulose (%)
3.4
3.2
3.0
2.8
2.6
2.4
46
44
42
40
e
d
de
c
a
b
b
a
b
c
a
b
2010 Control
2010 Saline
2010 Irrigated
2011 Control
2011 Saline
2011 Irrigated

Figure 8

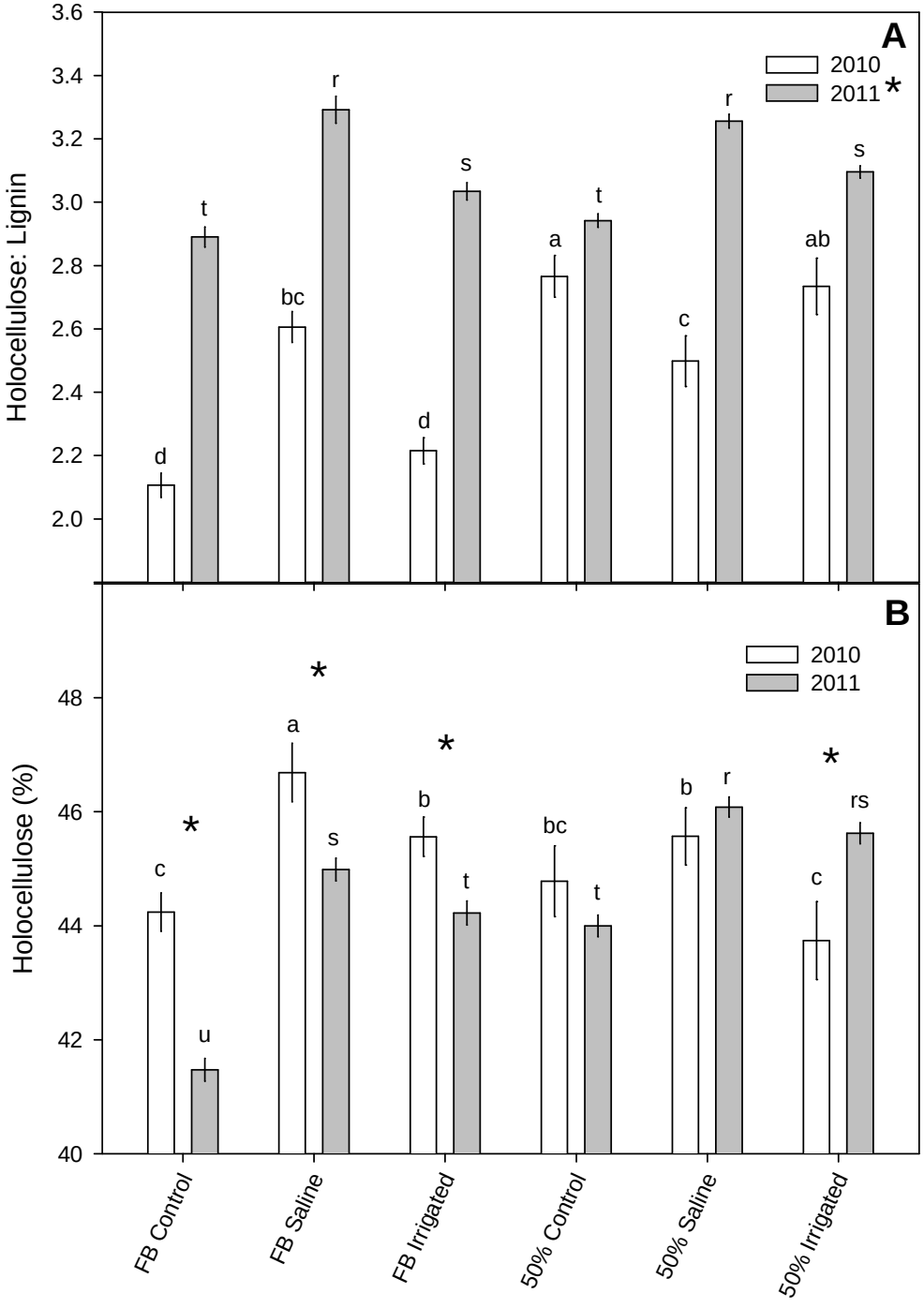
A
2010
2011
*
Holocellulose: Lignin
3.6
3.4
3.2
3.0
2.8
2.6
2.4
2.2
2.0
d
t
bc
r
d
s
a
t
c
r
ab
s
B
2010
2011
*
*
*
Holocellulose (%)
48
46
44
42
40
c
u
a
s
b
t
bc
t
b
r
c
rs
FB Control
FB Saline
FB Irrigated
50% Control
50% Saline
50% Irrigated

Figure 9

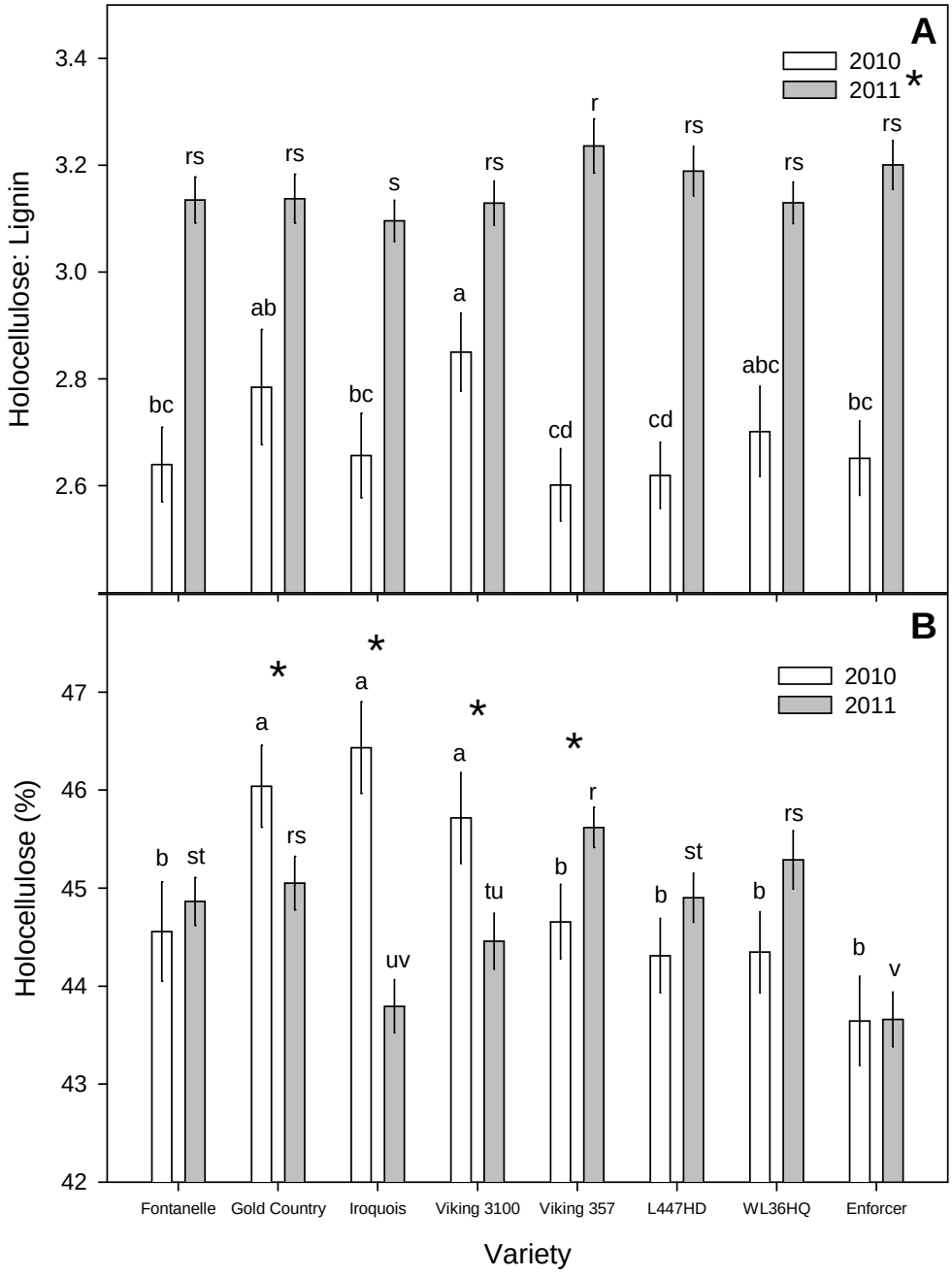
A
2010
2011
*
Holocellulose: Lignin
3.4
3.2
3.0
2.8
2.6
bc
rs
rs
ab
rs
bc
s
a
rs
r
cd
rs
cd
abc
rs
bc
rs
B
2010
2011
Holocellulose (%)
47
46
45
44
43
42
b
st
*
a
rs
*
a
uv
*
a
tu
*
b
r
b
st
b
rs
b
v
Fontanelle
Gold Country
Iroquois
Viking 3100
Viking 357
L447HD
WL36HQ
Enforcer
Variety

Figure 10

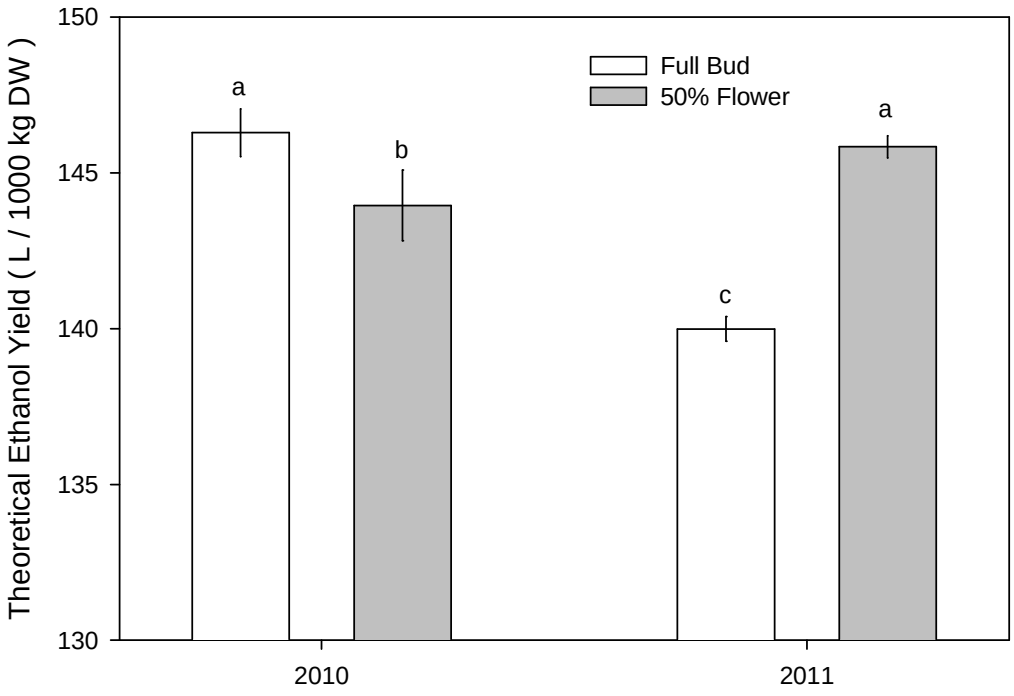
150
145
140
135
130
Theoretical Ethanol Yield (L / 1000 kg DW)
Full Bud
50% Flower
a
b
c
a
2010
2011

Figure 11

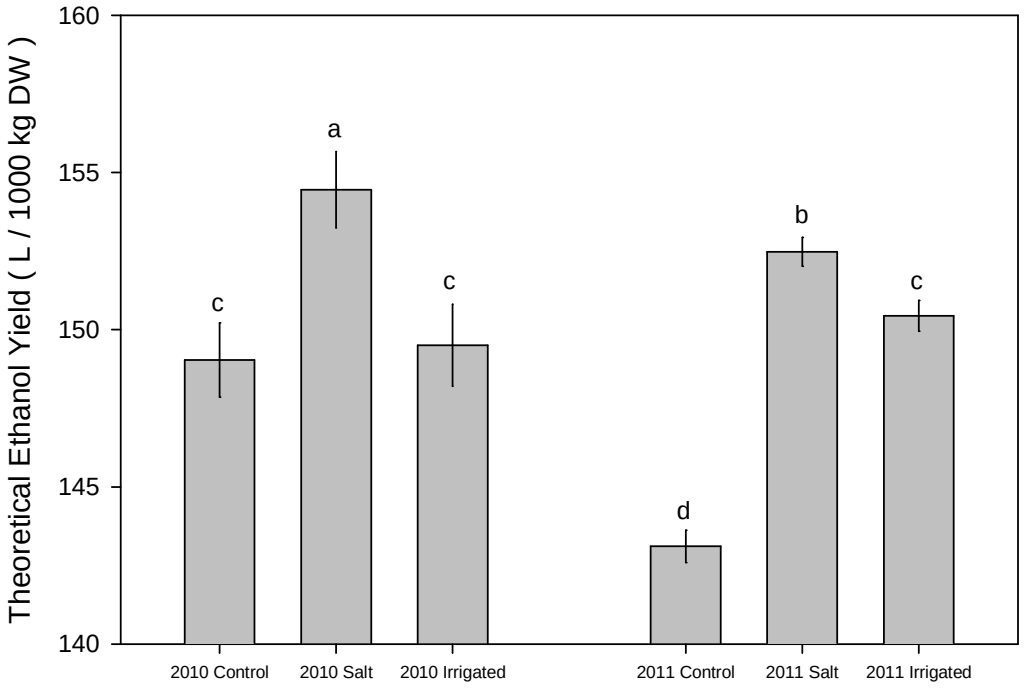
Theoretical Ethanol Yield (L / 1000 kg DW)
160
155
150
145
140
a
b
c
c
c
d
2010 Control
2010 Salt
2010 Irrigated
2011 Control
2011 Salt
2011 Irrigated

Figure 12

TABLE CAPTIONS

Table 1. The average monthly precipitation for the 2010 and 2011 growing seasons and the historical monthly average precipitation (2001-2009) at the research site, 2.5 miles west of Geneva, Minnesota.

Table 2. The percent cellulose, hemicellulose, and lignin concentrations for plants sampled at the full-bud and 50%-flower harvest regimes during the 2010 and 2011 growing seasons. Values are means of harvest regimes during each growing season (n=240, 2010 and n=480, 2011).

Table 3. . The percent cellulose, hemicellulose, and lignin concentrations for plants growing under the control, saline, and irrigated treatments during the 2010 and 2011 growing seasons. Values are means of the treatments during each growing season (n=160, 2010 and n=320, 2011).

Table 4. The percent cellulose, hemicellulose, and lignin concentrations for plants sampled at the full-bud and 50%-flower regimes with the corresponding treatment (control, saline, or irrigated) during the 2010 growing season. Values are means of the harvest regime and the corresponding treatment (n=80).

Table 5. The percent cellulose, hemicellulose, and lignin concentrations for plants sampled at the full-bud and 50%-flower regimes with the corresponding treatment (control, saline, or irrigated) during the 2011 growing season. Values are means of the harvest regime and the corresponding treatment (n=160).

Table 6. The percent cellulose, hemicellulose, and lignin concentrations for each variety during the 2010 growing season. Values are means of each variety (n=90).

Table 7. The percent cellulose, hemicellulose, and lignin concentrations for each variety during the 2011 growing season. Values are means of each variety (n=180).

Table 8. Cellulosic, hemicellulosic, and total theoretical ethanol yields following Boyer (2002) for the full-bud control, saline, and irrigated treatments and the 50%-flower control, saline, and irrigated treatments for the 2010 growing season. Values represent treatment means (n=80) and * indicates a significant difference ($P < 0.05$).

Table 9. Cellulosic, hemicellulosic, and total theoretical ethanol yields following Boyer (2002) for the full-bud control, saline, and irrigated treatments and the 50%-flower control, saline, and irrigated treatments for the 2011 growing season. Values represent treatment means (n=160) and * indicates a significant difference ($P < 0.05$).

Table 10. Cellulosic, hemicellulosic, and total theoretical ethanol yields following Boyer (2002) for the Fontanelle, Gold Country, Iroquois, Viking 3100, Viking 357, L447HD, WL36HQ, and Enforcer varieties during the 2010 growing season. Values represent treatment means (n=90) and * indicates a significant difference ($P < 0.05$).

Table 11. Cellulosic, hemicellulosic, and total theoretical ethanol yields following Boyer (2002) for the Fontanelle, Gold Country, Iroquois, Viking 3100, Viking 357, L447HD, WL363HQ, and Enforcer varieties during the 2011 growing season. Values represent treatment means (n=140) and * indicates a significant difference ($P < 0.05$).

Table 1

Month	2010 Season – Precipitation (cm)	2011 Season – Precipitation (cm)	Historical average (2001-2009) (cm)
April	4.6	6.6	9.4
May	5.6	11.9	11.7
June	31.8	13.7	12.7
July	14.5	12.7	12.2
August	6.4	1.8	11.2
September	26.2	1.3	11.2
October	2.8	0.8	6.1
Average	13.1	7.0	10.6

Table 2

Growing Season and Growth Stage	Cellulose (%)	Hemicellulose (%)	Lignin (%)
2010 Full-Bud	36.9	8.6	20.2
2010 50%-Flower	36.5	8.1	17.4
2011 Full-Bud	35.2	8.4	14.4
2011 50%-Flower	37.2	8.0	14.7

Table 3

Growing Season and Treatments	Cellulose (%)	Hemicellulose (%)	Lignin (%)
2010 Control	36.6	7.9	19.0
2010 Saline	37.4	8.7	18.8
2010 Irrigated	36.1	8.6	18.7
2011 Control	35.5	7.3	14.8
2011 Saline	37.0	8.5	14.1
2011 Irrigated	36.1	8.9	14.8

Table 4

Growth Stage and Treatment	Cellulose (%)	Hemicellulose (%)	Lignin (%)
Full-bud Control	37.0	7.3	21.4
Full-bud Saline	37.3	9.4	18.3
Full-bud Irrigated	36.3	9.2	20.1
50%-flower Control	36.2	8.6	16.7
50%-flower Saline	37.6	8.0	19.2
50%-flower Irrigated	35.9	7.9	16.4

Table 5

Growth Stage and Treatment	Cellulose (%)	Hemicellulose (%)	Lignin (%)
Full-bud Control	34.3	7.2	14.6
Full-bud Saline	36.4	8.6	14.0
Full-bud Irrigated	35.0	9.3	14.8
50%-flower Control	36.7	7.3	15.0
50%-flower Saline	37.7	8.4	14.2
50%-flower Irrigated	37.2	8.4	14.8

Table 6

Variety	Cellulose (%)	Hemicellulose (%)	Lignin (%)
Fontanelle	36.8	7.8	17.6
Gold Country	38.0	8.1	17.9
Iroquois	37.3	9.1	18.4
Viking 3100	37.7	8.1	16.7
Viking 357	36.6	8.1	18.0
L447HD	35.9	8.4	17.6
WL363HQ	35.1	9.2	17.4
Enforcer	35.2	8.5	17.2

Table 7

Variety	Cellulose (%)	Hemicellulose (%)	Lignin (%)
Fontanelle	37.1	7.8	14.6
Gold Country	37.2	7.9	14.6
Iroquois	35.5	8.3	14.4
Viking 3100	36.4	8.1	14.4
Viking 357	37.0	8.6	14.4
L447HD	36.5	8.4	14.4
WL363HQ	36.8	8.5	14.7
Enforcer	35.6	8.1	14.0

Table 8

Growth Stage and Treatment	Cellulosic EtOH Yield[1]	Hemicellulosic EtOH Yield[1]	Total Theoretical EtOH Yield[1]
Full-bud Control	123.8	19.2	143.0
Full-bud Saline	125.0	24.8*	149.8*
Full-bud Irrigated	121.6	24.4	146.0
50%-flower Control	121.3	22.6	143.9
50%-flower Saline	125.8*	21.2	147.0
50%-flower Irrigated	120.1	20.8	140.9

[1]Ethanol yields are expressed in liters of ethanol per 1000 kg of dried biomass.

Table 9

Growth Stage and Treatment	Cellulosic EtOH Yield[1]	Hemicellulosic EtOH Yield[1]	Total Theoretical EtOH Yield[1]
Full-bud Control	114.8	19.0	133.8
Full-bud Saline	121.7	22.8	144.5
Full-bud Irrigated	117.1	24.5*	141.6
50%-flower Control	122.9	19.3	142.2
50%-flower Saline	126.2*	22.2	148.4*
50%-flower Irrigated	124.7	22.2	146.9

[1]Ethanol yields are expressed in liters of ethanol per 1000 kg of dried biomass.

Table 10

Variety	Cellulosic EtOH Yield[1]	Hemicellulosic EtOH Yield[1]	Total Theoretical EtOH Yield[1]
Fontanelle	123.2	20.6	143.8
Gold Country	127.2*	21.3	148.5
Iroquois	124.9	24.2	149.1*
Viking 3100	126.1	21.3	147.4
Viking 357	122.4	21.4	143.8
L447HD	120.3	22.2	142.5
WL363HQ	117.7	24.3*	142.0
Enforcer	117.8	22.4	140.2

[1]Ethanol yields are expressed in liters of ethanol per 1000 kg of dried biomass.

Table 11

Variety	Cellulosic EtOH Yield[1]	Hemicellulosic EtOH Yield[1]	Total Theoretical EtOH Yield[1]
Fontanelle	124.2	20.6	144.8
Gold Country	124.5*	20.8	145.3
Iroquois	118.9	21.9	140.8
Viking 3100	121.9	21.3	143.2
Viking 357	123.9	22.8	146.7*
L447HD	122.3	22.2	144.5
WL363HQ	123.2	22.5	145.7
Enforcer	119.1	21.4	140.5

[1]Ethanol yields are expressed in liters of ethanol per 1000 kg of dried biomass.