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

Adam H. Warnke, Christopher T. Ruhland*

Department of Biological Sciences, Minnesota State University, Mankato, MN 56001, USA

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ABSTRACT

Alfalfa (*Medicago sativa* L.) is a potential candidate for cellulosic ethanol production due to its high biomass, perennial-habit, relationship with nitrogen-fixing bacteria, and other co-products. We examined the effects of harvest regime, irrigation, and salinity on stem lignocellulose concentrations in alfalfa during the 2010 and 2011 growing seasons in Southern Minnesota. Stem cellulose, hemicellulose and lignin concentrations, and theoretical ethanol yields were examined in eight alfalfa cultivars 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 ("fresh water;" 0.75 dS m^{-1}), (2) 1.27 cm of saline water (NaCl; "brackish water"; 5.0 dS m^{-1}) or (3) ambient precipitation ("rainfed"). Holocellulose concentrations reached the highest values during the full bud (2010) and 50% flower (2011) harvest regimes with concentrations averaging 45%. 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 more lignin in older stems. Alfalfa growing under brackish-water treatments had 1.3–6.1% more holocellulose than those receiving irrigation or ambient precipitation over two growing seasons. Lignin concentrations across all treatments were almost 23% lower during the second growing season. Interestingly, plants growing under brackish-water 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.

1. Introduction

At the current rate of consumption, world crude oil reserves are predicted to deplete in approximately 40 years, hence, 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 magnified this necessity. Shifting society's reliance away from petroleum to renewable biomass resources is viewed as an important contribution to the development of a sustainable industrial society and to an effective management of greenhouse gas emissions (Rugagauska et al., 2006). Biofuels have been recognized 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 lignocellulose-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 $(1 \rightarrow 4)$ and α $(1 \rightarrow 6)$ glycoside linkages, and cellulose, a structural polymer, consisting of glucose monomers with β $(1 \rightarrow 4)$ glycoside linkages. In addition to cellulose, plant secondary cell walls also contain appreciable amounts of 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).

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 ("holocellulose") in the cell wall of biomass material. In this sense corn starch is converted to glucose and fermented

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Abbreviations: ADF, acid detergent fiber; ADL, acid detergent lignin; ANOVA, analysis of variance; DRI, disease and pest resistance index; FD, fall dormancy; NDF, neutral detergent fiber; WSI, winter survival index.

* Corresponding author at: Department of Biological Sciences, TS-242 Trafton Sciences Center, Minnesota State University, Mankato, MN 56001, USA.
E-mail address: christopher.ruhland@mnsu.edu (C.T. Ruhland).

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to produce ethanol. However, there are several economic problems associated with the production of ethanol from corn grain. The increased demand for corn may impact the world's food stock and possibly drive 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 lignocellulose-based feedstock (Walsh et al., 2007). Lignocellulose-based 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 lignocellulose-based ethanol due to the high initial capital cost. Separating cellulose from lignin during processing is costly and produces potentially harmful bi-products.

Corn stover and cobs, as well as wheat straw are obvious annual crop residue feedstocks for lignocellulose 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 Engel, 2002). It grows on soils with a pH level of 6.5–7.0 and adequate levels of P (60–100 kg ha^{-1}) and K (180–250 kg ha^{-1}) are optimal for subsequent years of production (McKenzie, 2005). Varietal selection of alfalfa is typically based upon the winter survival index (WSI), fall dormancy (FD), and disease and pest resistance index (DRI). Alfalfa cultivars with lower WSI ratings will have the ability to survive potentially harsh winters. Fall dormancy is the measure of how tall an alfalfa plant grows after the last cutting and before going dormant for the season. The DRI is based upon selecting cultivars with superior disease resistance to ensure a long productive stand. The WSI rating is very important in Minnesota due to potentially harsh winters. A cultivar that can withstand severe low temperatures is crucial when selecting alfalfa.

Alfalfa can be harvested for biomass in the year of planting and provides N to the soil for use by subsequent cereal crops in rotation (Sheaffer et al., 2000). The growth stages of alfalfa are well known in this sense, 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 lignocellulose 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 lignocellulose stem portion could be used as a biofuel feedstock (McCaslin and Miller, 2007).

Using alfalfa for biofuels would require research to determine the optimal holocellulose to lignin ratio for ethanol production. Recommended harvest schedules for modern alfalfa cultivars in a lignocellulose 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 lignocellulose 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. In this study, we focused on harvest intervals across the same plant density. Determining the optimal harvest schedule for protein and lignocellulose 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 several productive regions of the world.

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 regulators (Saeed and El-Nadi, 1997). Saeed and El-Nadi (1997) observed that alfalfa stem density, stem height and leaf size decreased when soil water deficits occurred.

Root and shoot growth in alfalfa is restricted by increased salinity (Esechie et al., 2002), presumably due to changes in water uptake (Munns et al., 2006) and salt toxicity (Munns, 2002) resulting in reductions in growth and yield (Allakhverdiev et al., 2000). 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 use, under an irrigated system, is very important in determining the effects of salinity on stem lignocellulose concentrations.

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

2. Materials and methods

2.1. Plot establishment and cultivar selection

The field experiment was conducted during 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 $\geq$65 and $\geq$190 kg ha^{-1}, respectively (data not shown). Precipitation was collected by a rain gauge at the field site. The site was tilled using a 3.05-m field cultivator at a depth of 0.10–0.15 m. Alfalfa cultivars were planted using a 2.0-m Tye drill with 0.2-m row spacing.

Eight cultivars, two harvest regimes (full-bud and 50%-flower), and irrigation/salinity regimes (irrigated with fresh water, brackish water and rainfed) were compared. We selected cultivars of alfalfa based upon the winter survival index (WSI), fall dormancy (FD), and disease and pest resistance (DRI). The eight cultivars 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 cultivars had sufficient disease and