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Organization: Minnesota State University, Mankato
Award ID: DE-FG36-08GO88156
Final Report for Period: 7/31/2008 to 8/31/2012

Principal Investigator: Ruhland, Christopher T.
Title: Alfalfa variety selection for maximum fiber content, protein and nitrogen fixation.
Part of: Cellulosic ethanol, optimization of bio-fuels in engines, and course development for renewable energy technicians. U.S. Department of Energy. (PIs: {J Knox, S Ward, V Agarwal, J Frey} D Carrow, B Jones, J Rife and **CT Ruhland**)

Project Participants

Senior Personnel

Name: Ruhland, Christopher T
Contribution to Project: Supervised overall project. Supervised field experimental design and data analysis at MNSU. Supervised graduate and undergraduate students and prepared manuscripts for publication. Support from academic year was from MNSU, while this grant provided some summer support.

Graduate Students

Name: Warnke, Adam H
Contribution to Project: Is a graduate student on the project advised by Christopher Ruhland. Has been on the project since the beginning, first as an undergraduate then as a graduate student (January 2010). Responsible for field establishment and maintenance of alfalfa plots in Geneva, MN as well as sample harvesting and overseeing undergraduate research assistant's extractions of cell-wall constituents. Due to defend Master's Thesis in Spring 2013. Received financial support for two summers.

Name: Wozniak, Breann M
Contribution to Project: Joined the project in August 2009. Participated until December 2010. Her time on the project consisted of assistance with sample preparation for constituent analyses and sampling of total organic carbon and nitrogen in stem, leaf and soil samples. She quit the project to pursue a MS in Education and go back to teaching at a high-school setting. Received partial support for one summer.

Undergraduate Students

Names: Janet Wood (Senior, Biology); Alex Cahlander-Mooers (Senior, Biology); Adam Warnke (Junior & Senior, Biology); Kayla Kiecker (Senior, Biology); Michael Euerle (Senior, Biology); Josephine Hartung (Senior, Environmental Science); Jared Tibbetts (Senior, Biology); April Pressler (Senior, Biology); Ky McCracken (Senior; Biotechnology); Brock Bermel (Junior, Plant Biology); Amanda Schuman (Senior; Biotechnology); Brandon Bohks (Senior; Ecology); Courtney Johnson (Senior; Ecology); Brooks Kennedy (Senior; Environmental Science); Bradley Clyne (Senior; Biology); Jacob Neubauer (Senior, Biology)

Contribution to Project: Sample preparation and analysis of constituent analyses of both aspects of this project. J. Hartung and M. Euerle received partial support for one summer.

Other Partners

Warnke Research Services, Geneva, Minnesota

We contracted with Dr. John Warnke of Warnke Research Services to rent field plots for the alfalfa portion of this research. The field plots are located on a research farm in Geneva, MN (43°81'N × 93°32'W). Warnke Research Services currently contracts with companies like Monsanto and Pioneer Hi-Bred to conduct field trials and collect preliminary data for different varieties of corn, soybean, canola etc. being considered for commercial introductions.

Plant Biotechnology Course (BIOL 451/551) at Minnesota State University

Plant Biotechnology is a course that meets during the Fall Semester of the academic year. Course enrollment is typically eight to twelve students which are comprised of both upperclassmen and graduate students. Students have been involved in sample collection, processing and constituent analyses for both aspects of this project. In addition, three students were authors on a peer-reviewed publication (See Ruhland et al. 2011).

Dr. Thomas A. Day, Arizona State University

Professor Day is a plant ecophysiologist at ASU and is currently studying the effects of ultraviolet radiation on photodegradation in the Sonoran Desert. During the early initial phases of the project we collaborated with Dr. Day in examining the chemical constituents of plant litter exposed to different levels of visible and ultraviolet radiation. We honed our technique for chemical analysis of cellulose, hemicelluloses and lignin using these samples provided by Dr. Day in the Fall of 2009. Results from this collaboration resulted in a publication that was submitted to the journal *Ecosystems* (Day and Ruhland, in revision). The techniques we learned from this study partially form the basis for three submitted proposals to the National Science Foundation (2010; 2011 & 2012).

Research Activities and Findings

Background

There are increasing concerns about the availability and long-term reserves of crude oil on a global scale. Current reserves of world crude oil are predicted to deplete in approximately 40 years (Maheshwari 2008) and focus has been on discovering new sources of energy that are not only renewable but are also effective at mitigating greenhouse gas emissions (Rugauska et al. 2006). Biofuels have been heralded as a renewable, cost-effective alternative to petroleum-based liquid fuels. Although a corn-based ethanol industry has grown very rapidly in the United States, most experts see the need for the development of a cellulosic-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 production comes from hydrolysis of polysaccharides ultimately created by photosynthesis. These polysaccharides are typically divided into two major groups: storage polymers such as starch that can be stored in various parts of the plant and structural polymers such as cellulose that is a major component of the plant cell wall. Starch (amylose & amlyopectin) consists of glucose monomers with α $(1\rightarrow4)$ and α $(1\rightarrow6)$ glycosidic linkages, while cellulose (and associated hemicelluloses) consists of mostly five and six-carbon carbohydrate monomers with high degrees of β $(1\rightarrow4)$ glycosidic linkages. It has long been recognized that these complex polysaccharides can be hydrolyzed and the resulting simpler sugar monomers can be fermented to ethanol and used as a potential source of fuel.

Commercialization of ethanol production for the sole purpose of biofuel has traditionally relied upon corn. Corn kernels are >70% starch by dry weight and hydrolyzed sugars are easily converted to ethanol using a variety of industrial methods. According to the Renewable Fuels Association, there are currently 211 ethanol distilleries in operation or currently under construction in the United States and 96% of these plants are currently using corn as a feedstock for ethanol production (http://www.ethanolrfa.org/ bio-refinery-locations/). However, there are economic and ethical concerns about the availability and use of corn as a feedstock for ethanol production. The demand for corn for both food and ethanol is driving up the prices of corn-based products. Tribulations with corn grain ethanol production include the large amounts of fossil fuels being used in the process to make the finished product. The vision of a future biobased industry includes the simultaneous production of biofuels, bioelectricity, and bioproducts that uses not only corn grain and soybean oil, but also a host of cellulose feedstocks (Walsh et al., 2007). An alternative method to produce renewable fuels is of growing concern and urgency.

Currently there are only two plants in the United States (Firebright LLC, Blairstown IA & Abengoa Bioenergy Corporation, Hugoton KS) utilizing cellulose as source for ethanol. The apparent discrepancy between starch- and cellulose-based feedstock utilization on a commercial scale seems to rely upon the ease of isolation of the polysaccharide. While starch stored in the endosperm is easily separated from corn kernels; cellulose is somewhat recalcitrant and is imbedded in a lignin matrix in the secondary cell wall of plants. Lignin is a phenolic polymer that hinders the degradation of cell wall polysaccharides to simple sugars destined for fermentation to ethanol (Chapple et al., 2007). The separation of cellulose from lignin is costly, labor intensive and produces large amounts of waste. However, cellulosic ethanol is particularly promising because it can capitalize on the power of biotechnology to dramatically reduce costs, is derived from low cost and plentiful feedstocks, can achieve the high yields vital to success, has high octane and other desirable fuel properties and is environmentally friendly (Wyman, 2007). *Ultimately, identifying potential feedstock that has high cellulose and low lignin content is a crucial step when determining whether a plant species is suitable for ethanol production.*

Rationale and Research

The fertile soils of Southern Minnesota are highly productive from both a natural and agricultural standpoint. We chose to examine potential feedstock in both of these settings as a focus for our study. In part one of this report we detail our findings examining potential feedstock in prairie and wetlands in the Mankato, MN area in 2009. We were able to familiarize ourselves with techniques used to isolate and quantify concentrations of cellulose, hemicellulose and lignin in both a classroom and laboratory setting. These techniques were then used for our experiments that examined the effects of harvest regime, irrigation and salinity on eight potential-feedstock varieties of alfalfa (*Medicago sativa*).

Part I: Cellulose and lignin concentrations in prairie and wetland species: implications for feedstock in cellulosic ethanol production.

Natural communities with high net primary productivity have recently been proposed as sources of plant material for cellulosic ethanol production. In particular, prairies (McLaughlin et al. 2002, Palmer 2006, Tilman et al. 2006) and constructed wetlands designed for phytoremediation (Suda et al. 2009, Zhang et al. 2010) have gained considerable attention due to their species composition that consist of members that are relatively high in structural polymer concentrations. These "feedstocks" for cellulosic ethanol are high in lignocellulose (lignin + holocellulose) content and concentrations of these compounds may vary between species. The purpose of this study was to examine and compare concentrations of cellulose, hemicellulose and lignin of plant species found in naturally growing wetlands and in prairies of Southern Minnesota. We hypothesized there would be subtle differences in concentrations of these compounds that could potentially play a role in the selection of feedstocks for the production of cellulosic ethanol. In addition, understanding differences in cellulose and lignin concentrations in naturally-growing wetland species may help aid in species selection for construction of wetlands for the sole purpose of phytoremediation.

We examined Big Bluestem (*Andropogon gerardii*), Indian Grass (*Sorghastrum mutans*), Little Bluestem (*Schizachyrium scoparium*), Reed Canary Grass (*Phalaris arundinacea*) and Switchgrass (*Panicum virgatum*) from the Kasota Prairie (44^{o}16'N x 93^{o}59'W; Kasota, MN) and Cattails (*Typha angustifolia*), Reed Canary Grass and Bulrush (*Scirpus sp.*) in Rasmussen Woods Nature Area wetlands (44^{o}15' N x 94^{o} 01' W; Mankato, MN; Image 1).

We used a fiber analyzer (A200, ANKOM Technology, Macedon, NY) to assess concentrations of cellulose, hemicellulose and lignin in prairie and wetland plants. The fiber analyzer extracts and quantifies concentrations of these constituents using acid and neutral detergents with amylase enzymes in combination with acid hydrolysis. Theoretical ethanol yields were determined following Badger (2002) using cellulose and hemicellulose concentrations and fermentation assumptions based on 1000 kg of dried biomass. Ethanol yields from each community were calculated based on average cellulose and hemicellulose species content, species density and dried aboveground biomass per unit area in the prairie and wetland areas sampled.

Image 1: Plant Biotechnology students harvesting wetland plants from Rasmussen Woods (September 2009).

Findings and Significance

Holocellulose, cellulose and lignin concentrations were generally higher for species found growing in prairie than in wetland communities. Hemicellulose concentrations also tended to be greater in prairies than in wetlands (Figure 1). All prairie species had higher concentrations of holocellulose and cellulose than wetland plants, with the exception of Reed Canary Grass growing in the prairie. On a species basis, it appears that Switchgrass and Big Bluestem would be good candidates for cellulosic ethanol production based on their relatively high cellulose content combined with greater hemicellulose concentrations, which has a higher conversion efficiency than that of cellulose.

The cellulose to hemicellulose ratio was not different between prairie and wetland species (Figure 2). The holocellulose to lignin ratio was higher in wetland than in prairie species (Figure 2). The total calculated theoretical ethanol yield on a species basis was higher in prairie plants than in wetland plants, averaging 150 L and 136 L per 1000 kg dried plant material, respectively (Figure 2C). With the exception of Reed Canary Grass growing in the prairie community, total theoretical ethanol yields ranged from 155 - 157 L per 1000 kg of dried plant material among prairie plants. This was mainly due to prairie species having a higher cellulose concentration than the wetland species (Figure 1).

We also measured total aboveground biomass in our prairie and wetland communities we sampled for constituent analyses. In the tall-grass prairie community, total aboveground biomass production (± SE) averaged 364 (± 46) g m^{-2}. We used average cellulose and hemicelluloses concentrations of the prairie grasses to calculate theoretical ethanol yield on an area basis. Based on standing aboveground biomass and using the conversion efficiency equations of Boyer (2002), we estimate total theoretical ethanol production to average 54.7 (± 0.66) ml m^{-2} in the tall-grass prairie. Total aboveground biomass production averaged 935 (± 114), 1408 (± 238) and 642 (± 175) in the Cattail, Reed Canary Grass and Bulrush wetland communities, respectively (data not shown). Based upon these aboveground biomass numbers, we estimate total theoretical ethanol production to average 126.4 (± 1.70), 184.9 (± 0.94) and 91.6 (± 2.54) ml m^{-2} in the Cattail, Reed Canary Grass and Bulrush communities, respectively (data not shown). It appears that wetland species may be good candidates for cellulosic ethanol production based on species density and aboveground biomass production, rather than solely on holocellulose content.

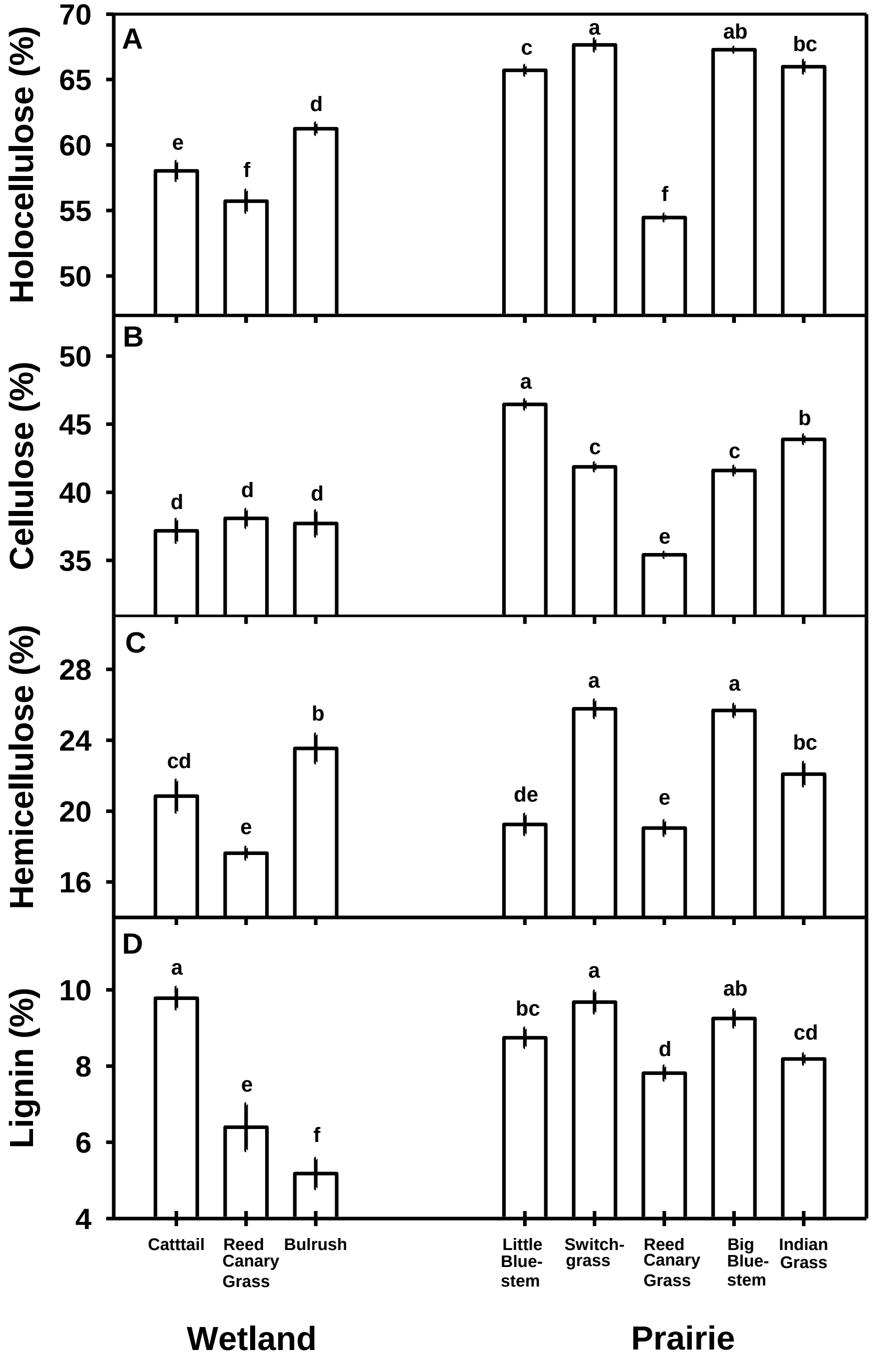


Figure 1. Mean concentrations of (A) holocellulose, (B) cellulose, (C) hemicelluloses, and (D) lignin on a dry-mass basis of wetland plants found in Rasmussen Woods Nature Area and prairie plants found in the Kasota Prairiein Southern Minnesota. Values are means of individual species (n=8). Vertical error bars represent 1 SE. Mean values with the same letter designation are not significantly different (LSD comparison P>0.05)

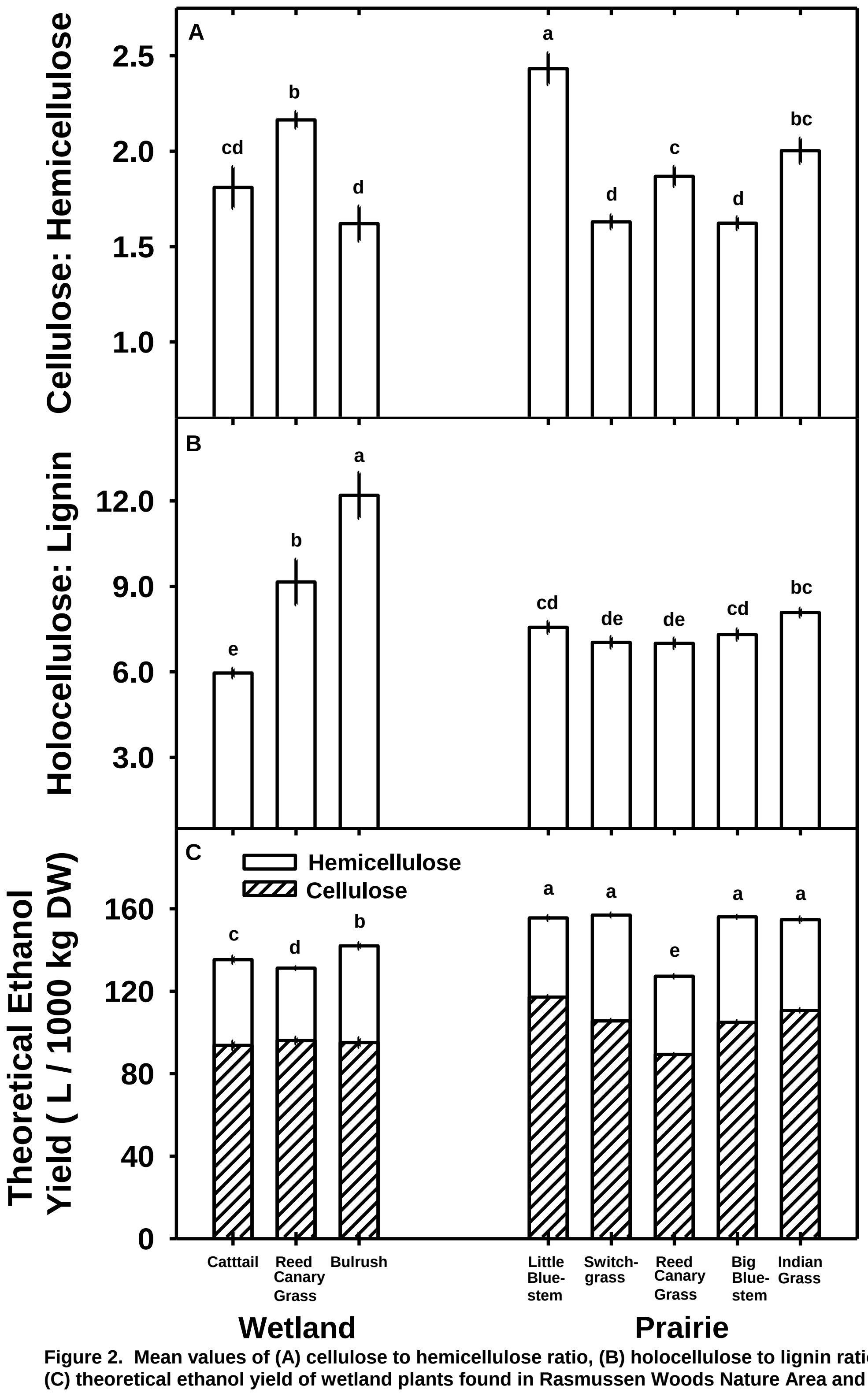


Figure 2. Mean values of (A) cellulose to hemicellulose ratio, (B) holocellulose to lignin ratio, and (C) theoretical ethanol yield of wetland plants found in Rasmussen Woods Nature Area and prairie plants found in the Kasota Prairiein Southern Minnesota. Values are means of individual species (n=8). Vertical error bars represent 1 SE. Mean values with the same letter designation are not significantly different (LSD comparison P>0.05)

Part II: The effects of harvest regime, irrigation and salinity on stem cellulose and lignin concentrations in alfalfa (Medicago sativa L.)

Identification of important feedstock for cellulosic ethanol is of paramount importance if the Federal Biofuels Mandate for displacing 30% of petroleum consumption by 2030 is to be met (McCaslin and Miller, 2007). Several feedstocks have been proffered as potential candidates for cellulosic ethanol production. Corn stover, corn cobs and wheat straw are obvious annual crop residue feedstocks that may hold promise. Switchgrass, a native C4 perennial forage grass, is also 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 biofuels cropping systems in some regions of the United States (McCaslin and Miller, 2007). Like switchgrass, additional candidates for feedstock should ideally have minimal purchased inputs in order to be effective for cellulosic ethanol production.

We propose that alfalfa has considerable potential as a feedstock for production of ethanol and other industrial materials because of its high biomass production, perennial nature, ability for nitrogen fixation and valuable co-products (Jung and Engels, 2002). Alfalfa is a widely grown traditional crop that fits well into a typical crop rotation. It 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 al., 2000). Using alfalfa for a biofuels system would require research to determine the optimal cellulose-to-lignin ratio for ethanol production. The growth stages of alfalfa are well classified and harvest schedules are determined based upon them. Recommended harvest schedules for modern alfalfa cultivars in a biofuel system are unknown because the relative value of leaf and stem components is expected to vary with energy and livestock feed prices (Sheaffer et al., 2000).

An advantage of using alfalfa for 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 than that of the stems, while the stems typically have two to three times the crude fiber than that of the leaves (Shinners et al., 2007). The high protein leaf portion could be utilized as an animal feed, while the high ligno-cellulose stem portion could be used as a biofuel feedstock (McCaslin and Miller, 2007). Determining the optimal harvest schedule for protein and ligno-cellulose concentrations will be a vital step for the future of alfalfa as a biofuel feedstock. To achieve maximum biomass yields of alfalfa, irrigation may be needed in some portions of the country.

Data on water use of potential biomass crops grown throughout the country are crucial for optimum irrigation management strategies. Alfalfa has a high water requirement compared to other commonly grown crops (Krogman and Hobbs, 1965). Studies have shown that alfalfa stem density, stem height and leaf size decreased when soil water deficits developed (Saeed and El-Nadi, 1997). Irrigated agricultural land is commonly afflicted by soil salinization and waterlogging (Tayfur et al., 1995) and a typical problem of irrigated agricultural land is the gradual buildup of salts in the root zone (Vaughan et al., 2002). Salinity is a major factor limiting plant growth and productivity (Allakhverdiev et al., 2000). The relationship between alfalfa growth and water utilization, under an irrigation system, is very important in determining the effects of salinity on protein and lingo-cellulose concentrations.

Image 2: Establishment of field experiment in Summer 2009 at Warnke Research Services Farm in Geneva, MN (left) and tornado damage that occurred on 17 June 2010 (right). Irrigation equipment was housed in this shed.

A field experiment was conducted over three growing seasons (2009, 2010 and 2011) on an agricultural field located 2.5 miles west of Geneva, Minnesota on Highway 35 (43°81'N × 93°32'W). The soil at this location is Webster Clay Loam-113 (Carlson et al., 1980) with a pH of 6.5, P greater than 45 kg ha^{-1}, and K greater than 190 kg ha^{-1}. Eight alfalfa cultivars (WL 363 HQ, Viking 357, L447 HD, Viking 3100, Fontanelle Hybrid – Ovation 2, Gold Country 24/7, and Iroquois) were planted on 10 May 2009 (Image 2). These varieties are commonly grown in Southern Minnesota and have various forage qualities and winter hardiness. The field plot design was 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. 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 approximately 450 plants/m^2.

Irrigation was affected by using a 130 gallon water tank located in the back of a pickup truck. Rubber hoses attached to a 5 gallon per minute pump were used to force the water out of a hand held sprinkler. Each subplot had 5 rain gauges (1 in each corner and one in the center of the plot) to ensure accurate applications. Each cultivars 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 done in the same method (on the same day) with each subplot receiving a 1.27 cm (83.28 L) application of saline (NaCl) water (4.0 dS m^{1}).

Forage was harvested in the establishment year (2009) but no treatments were applied. Subplots were harvested in the second and third years of production (2010 and 2011) when alfalfa reached full-bud (>50% of stems having on or more buds) and late flower (66-100% of stems having one or more flower). The full-bud harvest regime was harvested three times per season and the late flower harvest regime was harvested twice per season. The subplots had ten samples collected at each harvest regime.

The fiber analyzer was again used for constituent analysis (see Part I). Cellulose, hemicelluloses and lignin were measured using the Acid Detergent Fiber, Neutral Detergent Fiber and Acid Detergent Lignin methods following Ruhland et al. (2011). Crude protein was estimated on the leaves and stems using a Total Organic Carbon and Nitrogen Flash Combustion Analyzer (Flash EA 1112 Elemental Analyzer, Thermo Finnigan). Dried samples (12.0 mg) were loaded in pre made tin discs and placed in the analyzer. Crude protein was estimated as N × 6.25 following Dien (2006).

Findings and Significance

Initial field season:

During the first field season (2009), we planted eight different varieties of alfalfa in our field plot. We only harvested at the end of the growing season (October) as alfalfa is not commonly cut until the end of the first year of establishment. The harvest occurred when all varieties had reached the 50% flowering stage in October 2009. Stem concentrations of holocellulose (cellulose + hemicelluloses) ranged from 42 to 45% and did not vary between variety (P>0.05; Table 1). However, we did detect subtle yet significant, differences in how stem holocellulose was partitioned between varieties which would influence theoretical ethanol yields due to differences in conversion efficiencies between cellulose and hemicelluloses. For example, stem cellulose concentration was lowest in the Iroquois variety (29%) and highest in the Gold Country variety (34%), while hemicelluloses were highest in Iroquois (13%) and lowest in the Viking 3100 variety (9.9%) Due to these subtle differences in carbohydrate constituents, theoretical ethanol yield after the first field season was highest in the Gold Country variety and lowest in the Iroquois variety (Table 1) mainly due to this varieties' high cellulose content after one season of growth. The Gold Country variety is widely adapted for a broad range of soil conditions and is resistant to most major alfalfa diseases in Southern Minnesota. Currently, this variety is commonly used in a 3 to 4-cut per season harvest system and initially appears to be a good candidate for feedstock in cellulosic ethanol production.

Table 1: Stem constituent analyses and total theoretical ethanol yield following Boyer (2002) at the end of the first field season (establishment) and harvested in October 2009. Values represent variety means (n=10) and * indicates a significant difference (P<0.10).

Variety	Holo-cellulose (%)	Cellulose (%)	Hemi-cellulose (%)	Lignin (%)	Holocellul-ose:Lignin	Cellulose: Hemi-cellulose	Theoretical EtOH Yield[1]
Fontanele	42.7	32.2	10.5	19.3	2.4	3.2	117.7
Gold Contry	44.7	34.0*	10.7	18.8	2.4	3.4	123.5*
Iroquois	41.9	29.0	13.0*	17.6	2.5	2.3	114.0
Viking 3100	42.5	32.6	9.9	18.0	2.5	3.7*	117.5
Viking 357	42.9	31.0	11.8	19.6	2.3	2.8	117.4
L447HD	43.0	31.2	11.8	16.4*	2.7	2.7	117.8
WL36HQ	42.4	30.0	12.3	18.2	2.4	2.5	115.6
Enforcer	42.0	30.3	11.7	17.5	2.5	2.7	115.0

[1]Theoretical yield is expressed in liters of ethanol per 1000 Kg of dried biomass.

Second field season:

We performed five harvests over the course of the 2010 growing season. The initial harvest occurred on 24 May 2010. Surprisingly, early season stem concentrations of holocellulose were highest in the Iroquois (46.2%) and Gold Country (45.4%) and lowest in the Enforcer variety (38.3%; $P<0.01$; data not shown). Again, the primary reason for these high holocellulose concentrations in stems of the Iroquois and Gold Country varieties were the result of higher concentrations of cellulose. In addition, we measured concentrations of organic nitrogen and carbon in our stems using a gas chromatograph. Total organic nitrogen in stems averaged 3.2% and was highest in the Viking 3100 variety ($P<0.05$). Total organic carbon averaged 40.7% and Carbon: Nitrogen averaged 14.4 and did not differ between varieties (data not shown).

Irrigation and salinity treatments were first applied to the alfalfa plants on 01 June 2010. However, a large tornado hit the Warnke Research Farm on 17 June 2010 and destroyed multiple buildings and caused severe damage to the watering truck and irrigation equipment (Image 2). Due to this unfortunate natural disaster and the inability to effectively apply our treatments, irrigation and salinity regimes were not affected until 21 July 2010. In addition, monthly rainfall totals were unusually high for the month of June. Total rainfall averaged 31.6 cm (12.45 inches) for June 2010 and would have most likely overshadowed our previous irrigation and salinity treatments. Our treatments resumed on 21 July 2010 until the final harvest on 08 September 2010.

In addition to our regular cuttings at the full bud and 50% flower stages we conducted measurements of stem water potential and leaf chlorophyll fluorescence between our treatments towards the end of the field season. There were few differences observed between treatments or alfalfa varieties in stem water potential or chlorophyll fluorescence over the course of the experiment. Stem water potentials typically averaged between 0.5 to 0.8 MegaPascals while the quantum yield of photosynthesis typically averaged between 0.4 to 0.6 and the variable to maximal ratio of chlorophyll fluorescence typically averaged between 0.6 to 0.8 (data not shown).

Because irrigation and salt treatments did not start until later in the season, we are only presenting results from our final harvest in September 2010 on alfalfa plants at the 50% flower developmental stage. Concentrations of holocellulose after the final cutting of the 2010 field season ranged from 42.1 to 48.3% (Figure 3). There were no consistent effects of irrigation or salinity across varieties with some varieties increasing concentrations of constituents in response to water and or salt while other varieties had the opposite response. Holocellulose concentrations were greatest once again in the Gold Country variety, averaging 47.7% across all three treatments which was a response to a greater cellulose (39.5%) and hemicellulose (8.2%) concentrations (Figure 3) which should relate to higher theoretical ethanol yields. We are in the process of calculating these yields across all treatments and will present our findings in a future publication. In addition, lignin concentrations were highest in the Gold Country variety with concentrations averaging 19.4% across all three treatments (Figure 3).

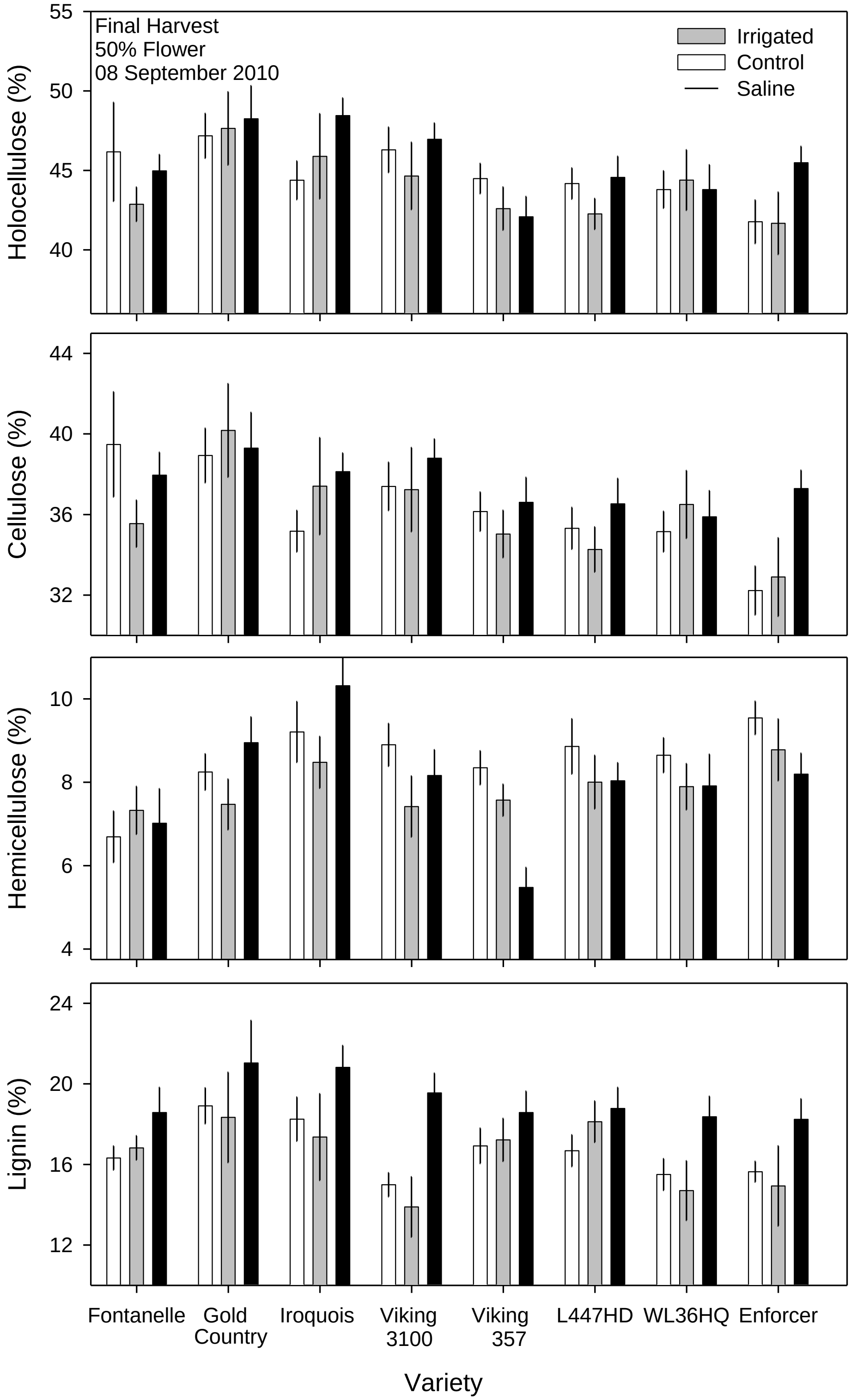


Figure 3: Final constituent analyses for eight varieties of alfalfa after the final harvest of the second field season (2010). Treatments: Open bars were control (no supplemental irrigation), gray bars received supplemental water and black bars received supplemental saline water (see text for more details). Values are means +/- 1SE (n=10)

Third Field Season:

We conducted our first harvest on 06 June 2011at the full bud stage. Concentrations of holocellulose were highest in the WL36HQ variety and values ranged from 42.5 to approximately 47% (Figure 4). The high concentration of holocellulose was once again attributed mainly to differences in cellulose concentrations rather than hemicelluloses (Figure4). This was reflected in the highest theoretical ethanol yield of the WL36HQ variety which averaged 114.4 liters per 1000 Kg of dry weight of biomass (Figure 5). Initial theoretical ethanol yields averaged between 103.2 to 114.4 liters per 1000 Kg dry weight of biomass across all eight varieties (Figure 5). Initial lignin concentrations were highest in the Fontanelle variety (14.2%) and lowest in the WL36HQ variety (Figure 4). There were no observed differences in initial cellulose to hemicellulose ratios or holocellulose to lignin ratios (Figure 5).

We conducted four additional cuttings during the 2011 field season. Our irrigation and salinity treatments started after the second cutting which occurred at the 50% flower developmental stage on 09 July 2011. We continued to harvest samples at full bud and 50% flower developmental stages from all eight varieties over the next 4.5 months and conducted our final cutting on 24 October 2011. We spent the winter and following spring and summer of 2012 performing constituent analyses and are now performing final statistical analyses to determine the effects of harvest date, irrigation and salinity treatments. There were subtle differences in constituent concentrations based on harvest date. In general, constituent concentrations tended to be higher at the later 50% flower developmental stage than at the full bud developmental stage (Figure 6). The effects of irrigation and salinity were variety specific, with irrigation and salinity increasing holocellulose concentrations in several varieties. Further statistical analysis will be conducted to tease apart of the effects of supplemental irrigation and salinity on potential ethanol yield in alfalfa.

In some alfalfa varieties like Enforcer, the increase in holocellulose concentrations under irrigation and salinity treatments was mainly due to increases in both cellulose and hemicelluloses over the course of the growing season (Figure 6). In other alfalfa varieties like Viking 357, there were few effects on holocellulose concentrations (Figure 7). Our preliminary findings suggest that supplemental irrigation and salinity increase holocellulose concentrations in the Viking 3100, L447HD and Enforcer varieties. Irrigation and salinity effects were more subtle in the Fontanelle and Iroquois varieties and in general did not affect the Viking 357 and Gold Country varieties (data not shown). It appears that all eight varieties of alfalfa that we tested are tolerant of moderate salt concentrations and holocellulose concentrations can be improved with the addition of modest irrigation regimes.

Concentrations of holocellulose increased in all eight varieties over three growing seasons. Based on concentrations of holocellulose and tolerance of salinity, it appears that the Gold Country variety has high levels of structural carbohydrates and would make an excellent choice as feedstock for cellulosic ethanol production.

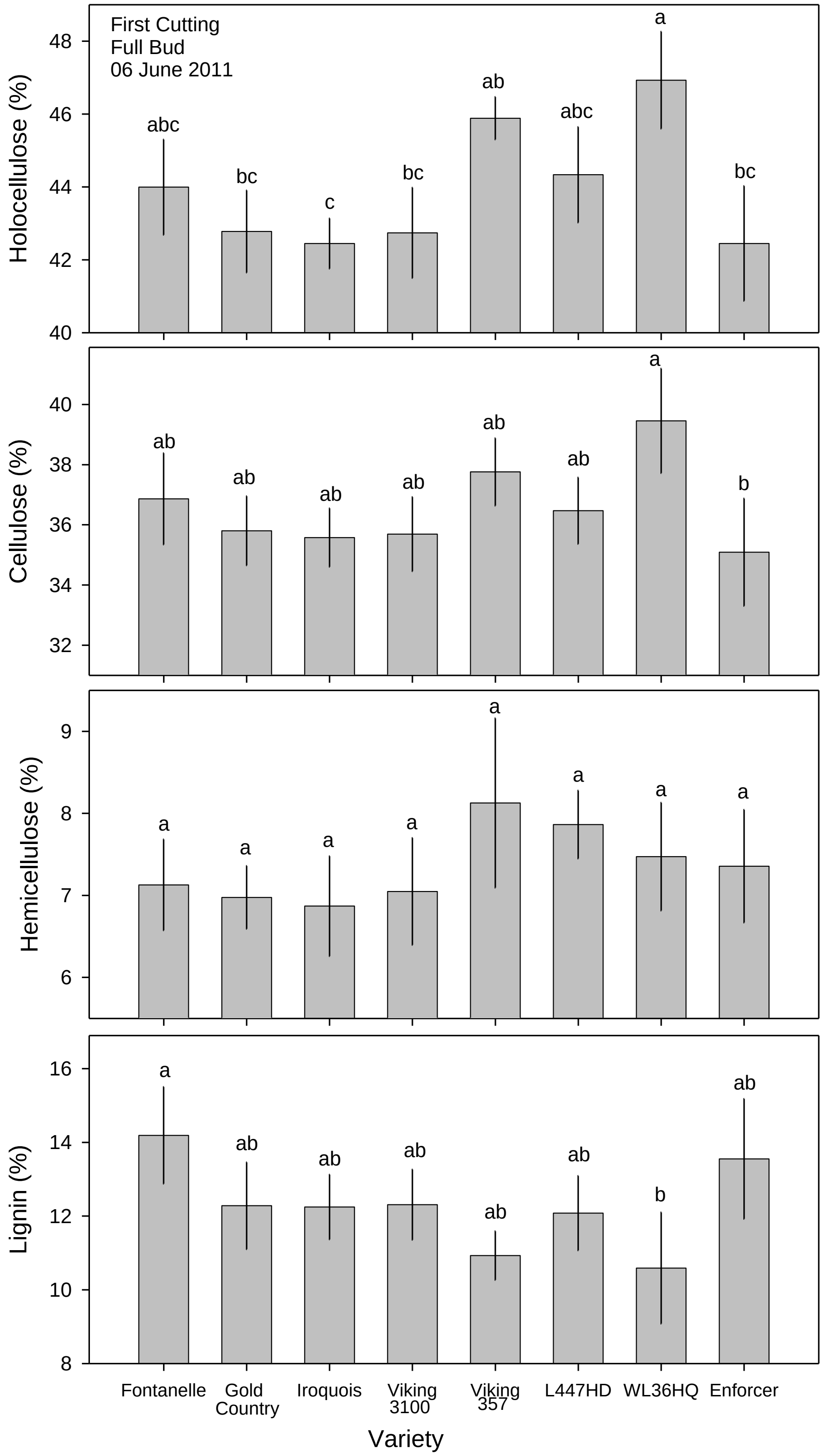


Figure 4. The holocellulose, cellulose, hemicellulose and lignin concentrations from eight different varieties of alfalfa from the initial cutting (Full bud) on 06 June 2011. Values are means +/1 1SE (n=10).

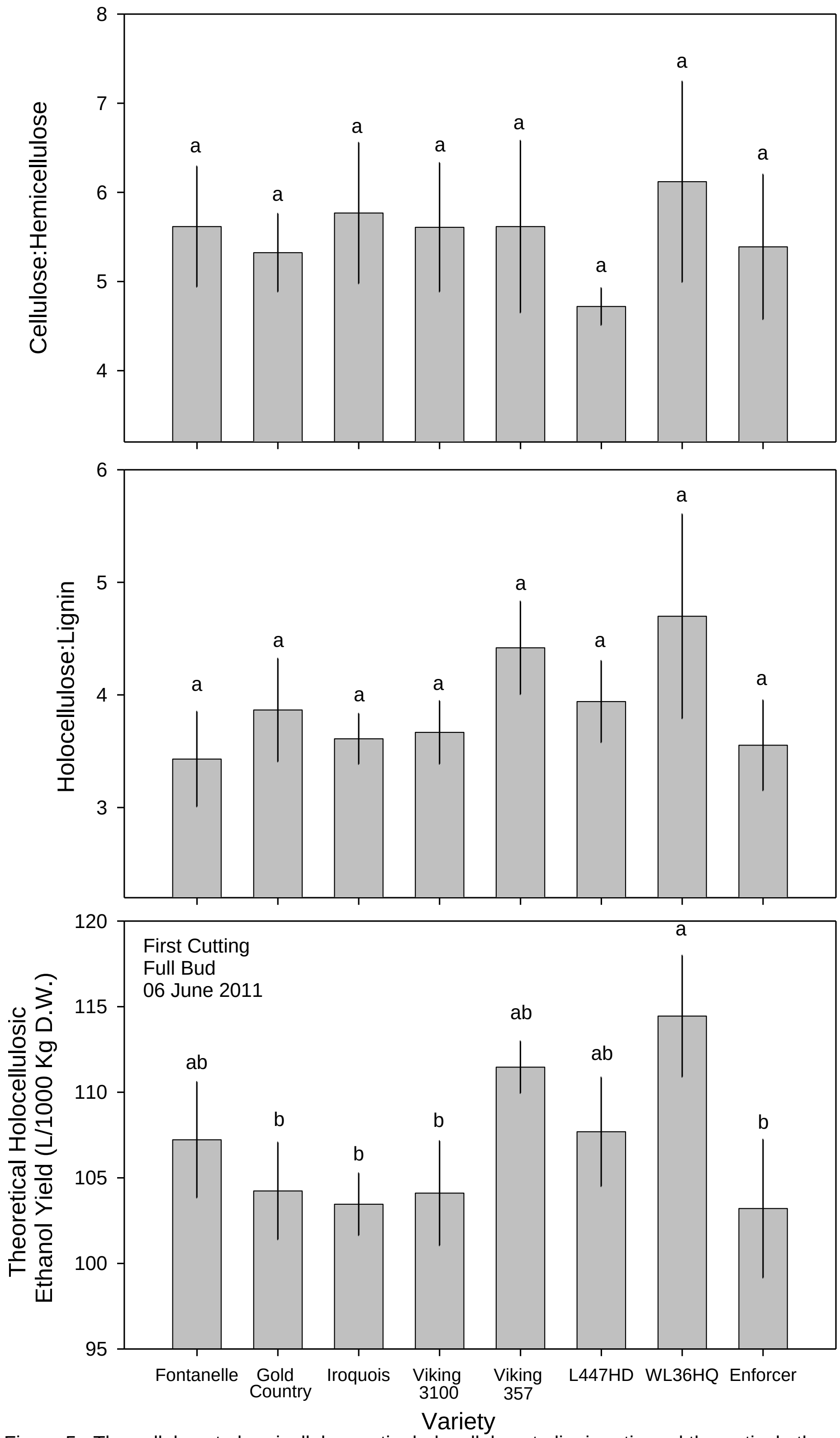


Figure 5. The cellulose to hemicellulose ratio, holocellulose to lignin ratio and theoretical ethanol yields from eight different varieties of alfalfa from the initial cutting (Full bud) on 06 June 2011. Values are means +/- 1SE (n=10). Theoretical ethanol yields are calculated following Boyer (2002).

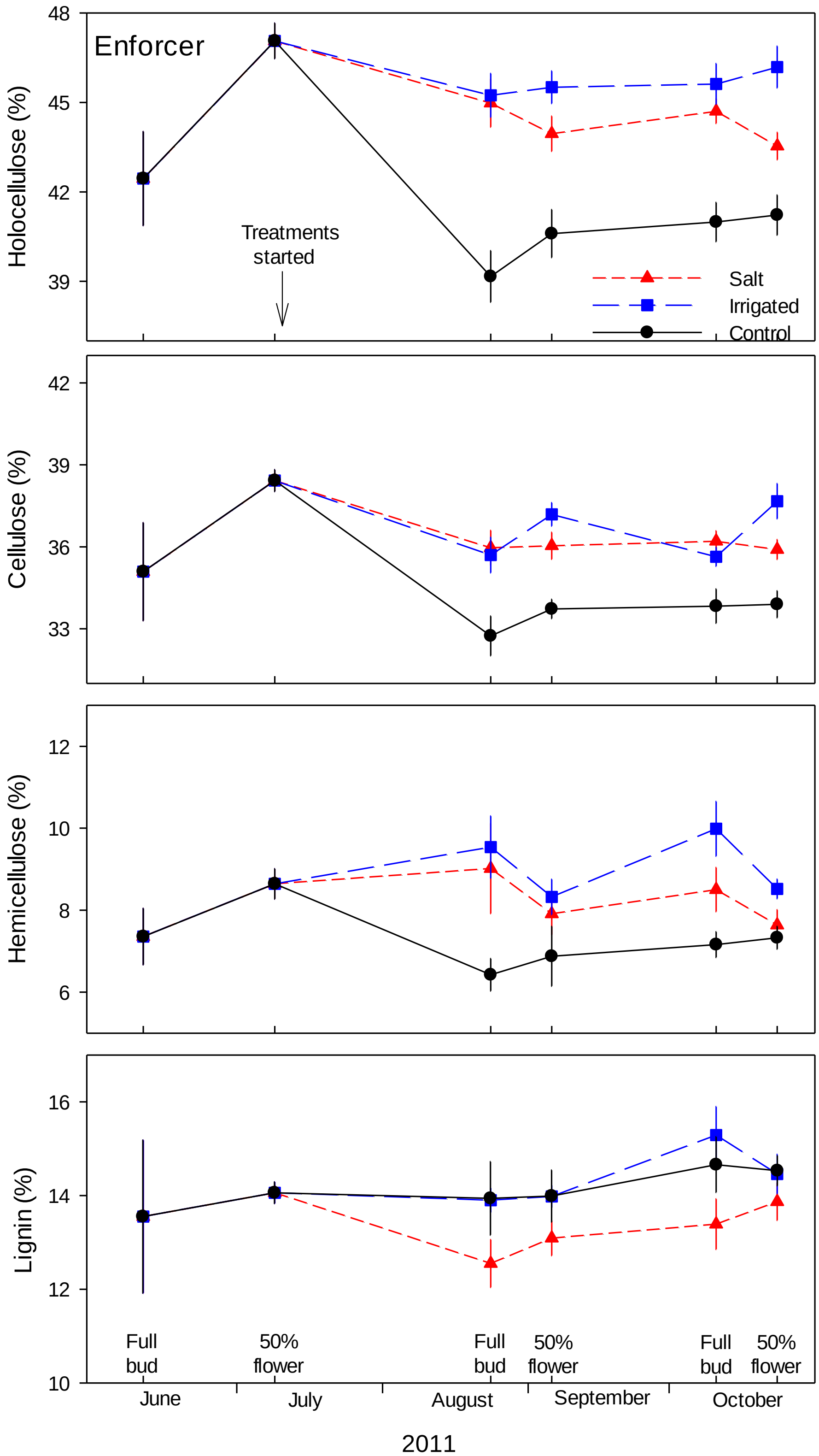


Figure 6. Constituent analyses of the Enforcer variety of alfalfa over the 2011 growing season. Circles represent the control treatment (no supplemental water), squares are irrigated treatments and triangles are salinity treatments. Values are means +/- 1 SE.

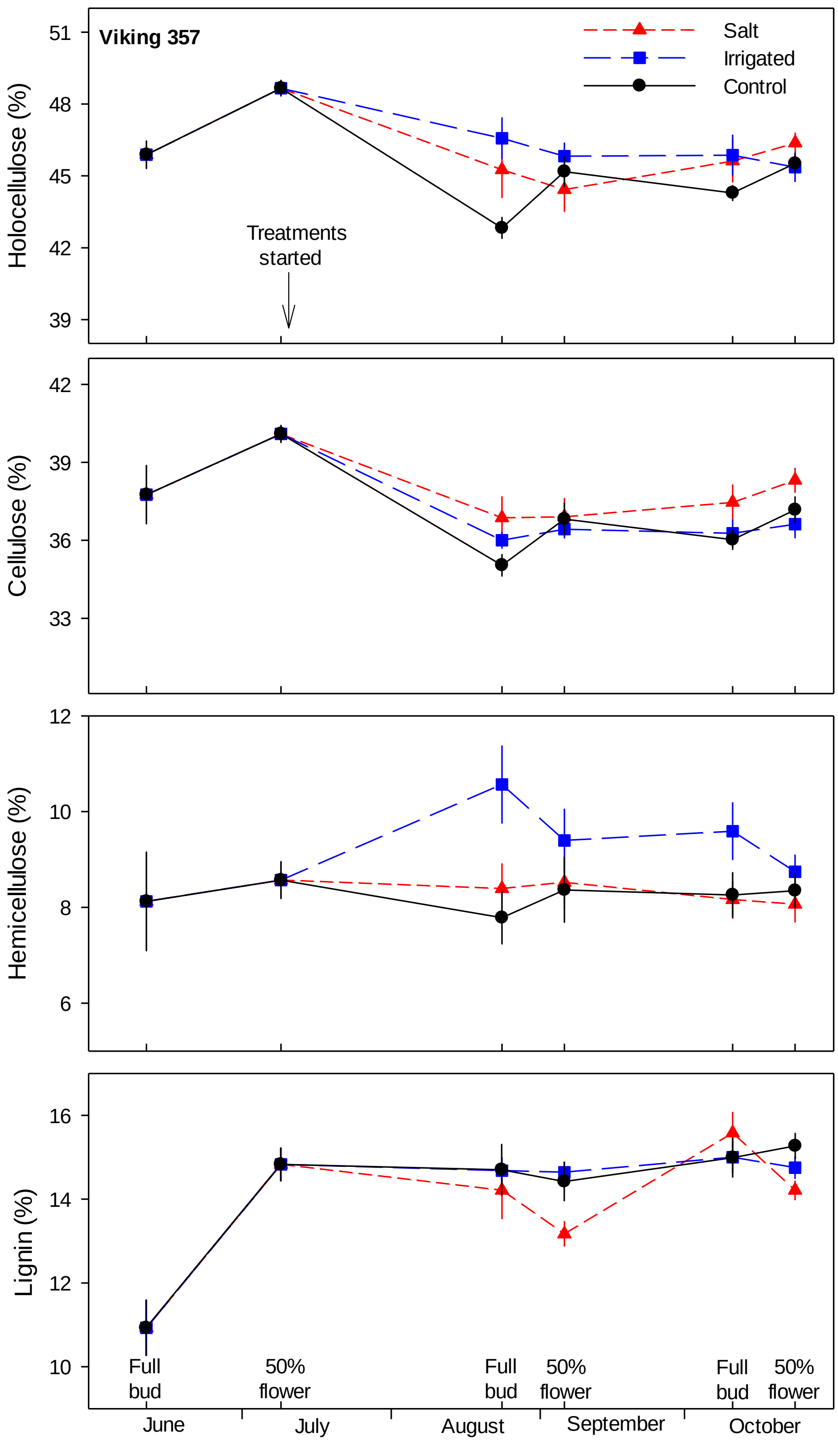


Figure 7. Constituent analyses of the Viking 357 variety of alfalfa over the 2011 growing season. Circles represent the control treatment (no supplemental water), squares are irrigated treatments and triangles are salinity treatments. Values are means +/- 1 SE.

Journal Publications

Ruhland CT and AH Warnke. The influence of irrigation and salinity on water potential and photosynthesis in *Medicago sativa.* In preparation.

Warnke AH and CT Ruhland. The effects of variety selection, irrigation and harvest date on feedstock for cellulosic ethanol production in alfalfa. In preparation for submission to ***Biomass and Bioenergy***.

Day TA and CT Ruhland. The relative effectiveness of different wavebands of solar radiation on the decomposition of contrasting litter in the Sonoran Desert Submitted to ***Ecosystems*** (In revision).

Ruhland CT, Dylsin MJ and JD Krenz. Big Wyoming Sagebrush screens ultraviolet radiation more effectively at higher elevations. ***Journal of Arid Climates*** (submitted).

Ruhland CT, Warnke AH, Cahlander-Mooers A, Wood J and BM Wozniak. (2011) Cellulose and lignin concentrations in prairie and wetland species: implications for feedstock in cellulosic ethanol production. ***Proceedings of the North American Prairie Conference*** 22:176-180.

Presentations

Warnke AH. The effects of variety selection, irrigation and harvest date on feedstock for cellulosic ethanol production in alfalfa. Expected Thesis Defense: Spring 2013.

Tibbetts J. Near-infrared Reflectance Spectroscopy and Chemometrics: Rapid Determination of the Major Cell Wall Constituents of foliage. Department of Biological Sciences, Minnesota State University (Thesis Proposal Seminar). April 2012.

Warnke AH. The effects of harvest regime, irrigation and salinity on stem and cellulose and lignin concentrations in alfalfa (*Medicago sativa* L.) Department of Biological Sciences, Minnesota State University (Thesis Proposal Seminar). December 2010.

Cahlander-Mooers A., Wood J. and C.T. Ruhland. Comparing the potential cellulosic ethanol production of six prairie grasses. 23rd National Conference on Undergraduate Research. La Crosse, WI. April 2009.

Cahlander-Mooers A., Wood J. and C.T. Ruhland. Comparing the potential cellulosic ethanol production of six prairie grasses. Undergraduate Research Conference. Minnesota State University. April 2009

Warnke A.H., Cahlanders-Mooers A., Wood J., Wozniak B.M. and C.T. Ruhland. Cellulose and lignin concentrations in prairie and wetland species: Implications for ethanol production. The 22nd North American Prairie Conference. The University of Northern Iowa. Cedar Falls, IA. August 2010.

New Proposals

2012 Collaborative Research: Photodegradation in deserts: litter optical and structural considerations. National Science Foundation, Office of Ecosystem Science. Principal Investigators: Drs. Thomas A. Day, Ferran Garcia-Pichel and Christopher T. Ruhland. $1,320,061 (in review)

2011 Collaborative Research: Photodegradation in deserts: litter optical and structural considerations. National Science Foundation, Office of Ecosystem Science. Principal Investigators: Drs. Thomas A. Day and Christopher T. Ruhland. $1,013,459. Final NSF-panel ranking: **Outstanding (Top 14%; Not funded)**

2010 Collaborative Research: Photodegradation in desert decomposition: Litter optical and structural considerations. National Science Foundation, Office of Ecosystem Science. Principal Investigators: Drs. Thomas A. Day and Christopher T. Ruhland. $721,377. Final NSF-panel ranking: **Meritorious (Top 20%; Not funded)**

References

1. 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.
2. 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.
3. 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.
4. Chapple, C., M. Ladisch, R. Meilan. 2007. Loosening lignin's grip on biofuel production. *Nature Biotechnology*. 25: 746-748.
5. 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.
6. Jung, H.G., and F. M. Engels. 2002. Alfalfa stem tissues: cell wall deposition, composition, and degradability. *Crop Science*. 42:524-534.
7. 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.
8. Maheshwari, S. 2008. The science behind the biofuels controversy. *Current Science*. 95:594-602.
9. McCaslin, M., D. Miller. 2007. The Future of Alfalfa as a Biofuels Feedstock. *California Alfalfa & Forage Symposium.*
10. McLaughlin, S. B., D. G. de la Torre Ugarte, C. T. Garten, L. R. Lynd, M. A. Sanderson, V. R. Tolbert, and D. D. Wolf. 2002. High-renewable energy from prairie grasses. Environmental Science and Technology 36: 2122-2129.
11. Palmer M. W. 2006. Harvesting our meadows for biofuel? Science 312: 1745.
12. 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.
13. Ruhland CT, Warnke AH, Cahlander-Mooers A, Wood J and BM Wozniak. (2011) Cellulose and lignin concentrations in prairie and wetland species: implications for feedstock in cellulosic ethanol production. *Proceedings of the North American Prairie Conference* 22:176-180.
14. 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.

15. 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
16. 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.
17. Shinners, K.J., M.E. Herzmann, B.N. Binversie, and M.F. Digman. 2007. Harvest fractionation of alfalfa. *Trans. ASABE*. 50:713-718.
18. Suda, K., A. Shahbazi, and Y. Li. 2009. The feasibility of using cattails from constructed wetlands to produce bioethanol. Pages 9-15. *In* G.A. Uzochukwu (ed.). Proceedings of the 2007 National Conference on Environmental Science and Technology. Springer, New York.
19. 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.
20. Tilman, D., J. Hill, and C. Lehman. 2006. Carbon-negative biofuels from low input high-diversity grassland biomass. Science 314: 1598-1600.
21. 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.
22. 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.
23. Wyman, C. E. 2007. What is (and is not) vital to advancing cellulosic ethanol. *Trends in Biotechnology*. 25:153-157.
24. Zhang, B., A. Shahbazi, and L. Wang. 2010. Alkali pre-treatment an enzymatic hydrolysis of cattails from constructed wetlands. American Journal of Engineering and Applied Sciences 3: 328-322.

Organization: Minnesota State University, Mankato
Award ID: DE-FG36-08GO88156
Final Report for Period: 7/31/2008 to 8/31/2012

Principal Investigator: Rife, James E.
Title: Investigation of Cattails and Fall Leaves as Potential Biomass Feedstock for the production of Cellulosic Ethanol.
Part of: Cellulosic ethanol, optimization of bio-fuels in engines, and course development for renewable energy technicians. U.S. Department of Energy. (PIs: {J Knox, S Ward, V Agarwal, J Frey} D Carrow, B Jones, **J Rife** and CT Ruhland)

Project Participants

Senior Personnel

Name: Rife, James E.
Contribution to Project: Supervised overall project. Supervised method development, experimental design, laboratory experiments and data analysis at MNSU. Supervised undergraduate students and prepared manuscripts for publication. Support from academic year was from MNSU, while this grant provided some summer support.

Graduate Students (Biological Sciences)

Name: Wozniak, Breeann M.
Contribution to Project: Conducted characterization of structural polysaccharides and lignin in cattail leaves of species *Typha angustifolia*.

Name: Lyimo, Omary
Contribution to Project: Conducted characterization of structural polysaccharides and lignin in cattail leaves of species *Typha x glauca*. Compared efficiency of different pretreatment methods. Conducted fermentation studies on cattail hydrolysate. Mr. Lyimo completed his Master's Thesis in the Spring of 2012. The thesis was based on work initiated with the DOE grant.

Undergraduate Students

Name: Lama, Tenzing
Contribution to Project: Developed initial HPLC method for analysis of monosaccharides obtained from hydrolysis of cattail leaves. Prepared hydrolysis samples.

Name: Burum, Justin
Contribution to Project: Performed initial analysis of monosaccharides obtained from hydrolysis fall leaf litter.

Name: Krahmer, Kristen
Contribution to Project: Developed and used a Glucose Oxidase assay to compare the pretreatment methods of cattail leaves.

Name: Wreh, Elijah
Contribution to Project: Developed and used a Glucose Oxidase assay to evaluate the pretreatment methods.

Name: Bhetawal, Sarita
Contribution to Project: Initiated characterization of structural polysaccharides and lignin in cattail leaves.

Name: Menne, Nick
Contribution to Project: Developed the HPLC method for analysis of monosaccharides that became the standard method for the project. Performed the determination of the structural polysaccharides and lignin of fall leaf litter.

Name: Sanchez, Daniel J.
Contribution to Project: Investigated effect of enzyme load on hydrolysis of cattail leaves. Prepared ethanol from cattail hydrolysate.

Name: Amusan, Korede
Contribution to Project: Compared efficiency of cellulase and beta-glucanase in the hydrolysis of pre-treated fall leaf litter.

Research Activities and Findings

Background

This project has focused on exploring the use of cattails and autumn leaves as feedstock for the production of cellulosic ethanol. Development of cattails as a biofuel resource could provide an incentive for farmers to return marginal agricultural land to wetlands. The use of any feedstock for the production of cellulosic ethanol requires the expenditure of fuel to harvest and transport the feedstock. It may also be necessary to expend energy to plant and cultivate the feedstock. In the case of fall leaves, we already expend energy to collect and transport them. However, they are treated then as rubbish to be disposed. It would be more efficient to tap into this energy reserve.

The project has involved two phases. The first phase involved the characterization of the lignocellulosic composition of these two biomass sources. The second phase involved testing methods for converting the cellulose in these two sources into the fermentable sugar glucose.

Methods

Characterization of Structural Polysaccharides and Lignin

Biomass from cattails and autumn leaves was characterized using the NREL Laboratory Analytical Procedure, Determination of Structural Polysaccharides and Lignin in Biomass, prepared by A. Sluiter in 2006. A 0.300 g sample of biomass was combined with 3.0 mL of 72% sulfuric acid and incubated for 60 minutes at 30°C. After adding 84.0 g of water, the sample was autoclaved at 121°C for 60 minutes. The sample was filtered through a dried filter crucible. The ultraviolet absorbance of the filtrate was measured at 240 nm and 320 nm to determine the amount of acid soluble lignin. The solid residue in the crucible filter was thoroughly washed with water. The crucible and solid residue were dried at 105°C to constant mass to determine acid insoluble lignin and ash. The crucible and solid residue were then heated at 575°C until a constant mass was obtained to determine the ash. The filtrate was neutralized to pH 6 using calcium carbonate. Monosaccharides in the neutralized filtrate were determined by High Performance Liquid Chromatography

Carbohydrate Analysis

Monosaccharides were analyzed by High Performance Liquid Chromatography (HPLC). A Shimadzu HPLC was used. A Kromasil-NH_2 column that was 250 mm by 4.6 mm from Grace was used. The column temperature was maintained at 30°C. Samples were eluted isocractically with 75% acetonitrile and 25% water at a flow rate of 1.0 mL per minute. Run times were 15 minutes. A refractive index detector was used. Glucose, galactose, mannose, arabinose, xylose and cellobiose standards with concentrations between 0.100 and 10.000 mg/mL were used to generate standard curves.

Ethanol Analysis

Ethanol was analyzed by HPLC using the Shimadzu system. A 150 mm by 7.8 mm Fermentation Monitoring column from BioRad was used. The column temperature was maintained at 50°C. Samples were eluted isocractically with 0.005 M sulfuric acid in water at a flow rate of 1.0 mL per minute. A refractive index detector was used.

Pretreatment and Enzymatic Hydrolysis of Fall Leaves

A 5.0 g sample of powdered leaves was combined with 50 mL of water and autoclaved for 60 minutes at 121°C. 10X Sodium Citrate buffer was added to yield a final concentration of 50 mM and pH 4.8. 1250 units of □-glucanase (Sigma G4423) were added to the pretreated sample. Samples were incubated with gently swirling at 37.5°C. Aliquots for glucose analysis were removed at 0, 24, 48, 72 and 144 hours after addition of enzyme. Glucose was measured by HPLC as described in Carbohydrate Analysis.

Fermentation

After enzymatic hydrolysis samples were filtered to remove the solid residue. 10X Yeast Extract and Peptone were added to each sample to yield final concentrations of 0.01 g/mL of Bacto Yeast Extract and 0.02 g/mL of HiVeg Peptone. After autoclaving for 25 minutes, the samples were inoculated with *Saccharomyces cerevisiae* D$_5$A. The samples were incubated anaerobically at 35°C with shaking in flasks fitted with a gas traps. Fermentation was typically complete within 60 hours. Ethanol was analyzed by HPLC as describe in Ethanol Analysis. Ethanol yields were corrected with a blank containing only Yeast Extract and Peptone.

Findings and Significance

Characterization of Structural Carbohydrates and Lignin

In the first phase of this project, we completed an analysis of the structural carbohydrates and lignin in cattails and fall leaves using the procedure published by NREL.

Cattails

One objective of this project was to compare the structural carbohydrate and lignin composition of different cattail species. We have completed characterizations of *Typha angustifolia* (regarded as an invasive species) and *Typha x glauca* (a cross between *T. angustifolia* and the native *T. latifolia*). The results of this analysis are summarized in Table One and Table Two.

Table One
Structural Carbohydrate and Lignin Composition of
Typha x glauca

	October 2009 Analysis (per cent)	June 2007 Analysis (per cent)
Acid Soluble Lignin	7.5 +/- 0.2	3.7 +/- 0.1
Acid Insoluble Lignin	21.9 +/- 0.1	*
Acid Insoluble Ash	0.45 +/- 0.07	*
Cellulose	32.0 +/- 0.5	37.4 +/- 4.4
Arabinan	2.5 +/- 0.5	11.5 +/- 5.3
Xylan	8.32 +/- 0.08	24.5 +/- 7.0

- Acid insoluble ash and lignin were not separately determined in the June 2007 study. However, the combined percentage of acid insoluble ash and lignin were 22.1 +/- 0.3 in that study.

There is good agreement in these two characterizations. The studies confirm that the biomass of the hybrid cattail runs around one third cellulose, the component that can most be converted into the easily fermentable sugar glucose. The observation that the arabinan and xylan components observed in October 2009 are significantly lower than the levels observed in June 2007 is disappointing. We have more confidence in the more recent study. While arabinan and xylan yield sugars that are less easily fermented, they still have the potential to contribute to the overall ethanol yield.

Table Two
Comparison of Structural Carbohydrate and Lignin Composition of
Typha x glauca and Typha angustifolia

	Typha x glauca October 2009 (per cent)	*Typha angustifolia* June 2010 (per cent)
Acid Soluble Lignin	7.5 +/- 0.2	1.20 +/- 0.09
Acid Insoluble Lignin and Ash	22.4 +/- 0.1	23.9 +/- 0.3
Cellulose	32.0 +/- 0.5	31.2 +/- 0.8
Arabinan	2.5 +/- 0.5	1.6 +/- 0.2
Xylan	8.32 +/- 0.08	5.3 +/- 0.2

The two species have similar amounts of cellulose. Typha angustifolia appears to have less hemicellulose as is indicated by the lower levels of arabinan and xylan. There also appears to be less acid soluble lignin in T. angustifolia.

Findings and Significance

Fall Leaves

We have completed two analyses of autumn leaf litter. The results are summarized in Table Three.

Table Three
Structural Carbohydrate and Lignin Composition of Autumn Leaves

	November 2009 Analysis (per cent)	March 2008 Analysis (per cent)
Acid Soluble Lignin	14.0 +/- 1.0	22.3 +/- 1.0
Acid Insoluble Lignin	28.7 +/- 0.2	27.6
Acid Insoluble Ash	1.6 +/- 0.03	2.6
Cellulose	16.2 +/- 0.9	23.8 +/- 1.6
Arabinan	2.7 +/- 0.9	7.9 +/- 0.8
Xylan	2.2 +/- 0.3	7.4 +/- 0.7

There is good agreement in these two characterizations. The studies indicate that the autumn leaf litter contains about 20% cellulose and levels of arabinan and xylan between 2% and 7%.

Comparison of Pretreatment Methods

An essential step in the generation of cellulosic ethanol is pretreatment of the biomass to render the structural polysaccharide accessible to the hydrolytic enzymes. Considerable research has been expended in the optimization of pretreatment steps. We compared some simple pretreatment that involved autoclaving the powdered biomass with 2% sulfuric acid, 0.4 M phosphoric acid, 0.04 M acetate buffer, 0.05 M citrate buffer and water. The results are compared in Table Four.

Table Four
Percent Conversion of Available Cellulose to Glucose Under Various Pretreatment Conditions

Pretreatment Conditions	Fall Leaves After Pretreatment	Fall Leaves After Enzyme Hydrolysis	Cattails After Pretreatment	Cattails After Enzyme Hydrolysis
Sulfuric Acid	15.8 %	62.0 %	12.4 %	28.3 %
Phosphoric Acid	14.6 %	44.0 %	10.5 %	27.8 %
Acetate Buffer	4.9 %	39.3 %	2.7 %	19.3 %
Citrate Buffer	15.9 %	47.6 %	6.0 %	22.0 %
Water	8.3 %	61.5 %	12.6 %	26.8 %

This project yielded two significant results. First, the total recovery of glucose after enzyme hydrolysis in the samples simply pretreated by autoclaving the biomass in water was essentially as high as other pretreatment conditions or higher. This combines glucose liberated after pretreatment and glucose released by enzymatic hydrolysis. This trend is observed both with fall leaf biomass and cattail biomass.

The second observation is the percent recovery after enzyme hydrolysis is significantly higher with the fall leaf biomass than it is with the cattail biomass. Even though cattail biomass contains approximately twice the cellulose found in fall leaf biomass (previous report), the actual mass of glucose recovered after enzyme hydrolysis was essentially equal for cattails and leaves. In the case of water hydrolysis, the total amount of glucose recovered was actually a little higher for leaves than for cattails. The higher efficiency of enzyme hydrolysis with leaves compared to cattails suggests that cellulose is more accessible in leaves than it is in cattails. Leaf biomass appears to be more fragile than the cattail biomass.

Optimization of Enzyme Load for Hydrolysis

One objective of this project was to determine optimum levels of hydrolytic enzymes for the conversion of cellulose in the pretreated biomass to glucose. Two problems were observed in these experiments. First there was little production of glucose after 24 hours of hydrolysis and we were getting glucose yields of over 100%.

To resolve these problems, the effect of the biomass load on glucose production with the two separate enzymes was examined. Duplicate biomass samples of 1.0, 2.5 or 5.0 g were combined with 50 mL of water and autoclaved for 60 minutes. Sodium Citrate buffer was added to yield a final concentration of 50 mM and pH 4.8. Either 1250 units of Cellulase (Sigma C118) or 1250 units of □-glucanase (Sigma G4423) were added to each set of samples. Final volumes for each sample were 57.6 mL. Samples were incubated with gently swirling at 37.5 degrees C. Aliquots for glucose analysis were removed at 0, 24, 48, 72 and 144 hours after addition of enzyme. Again glucose was measured by HPLC. Figures One and Two present the results of this experiment.

In Figure One, we see the results with the cellulase enzyme. We see a very high level of glucose at time zero and we see little glucose production after 24 hours. Also we do not see an increase in glucose production as we go from 2.5 g of biomass to 5.0 g of biomass. Apparently 2.5 g of biomass is saturating the enzyme. In Figure Two, we see a very different picture with the □-glucanase enzyme. First the level of glucose at time zero is over 4 times lower. Much less glucose is being introduced with this enzyme. Secondly, the enzyme continues to hydrolyze cellulose and produce glucose past 24 hours. It appears to still be actively working after 144 hours. Moreover, this enzyme was not saturated by the biomass load. Actually, this enzyme shows good kinetic behavior as determined by a Lineweaver-Burk Graph (Figure Three). Under these conditions, the enzyme has a Km of 0.99 g of biomass.

Figure One

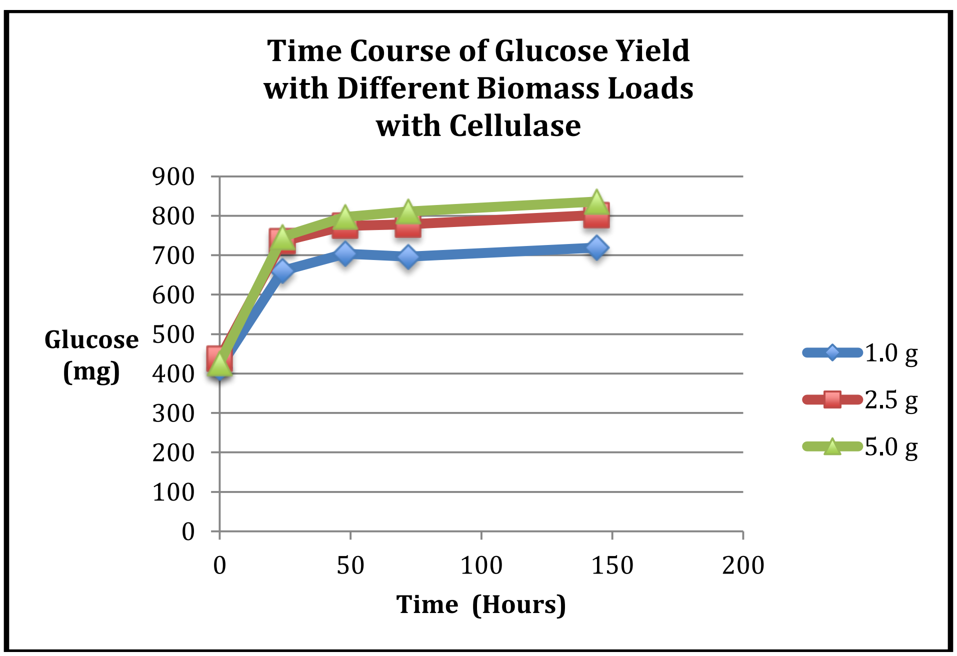


Figure Two

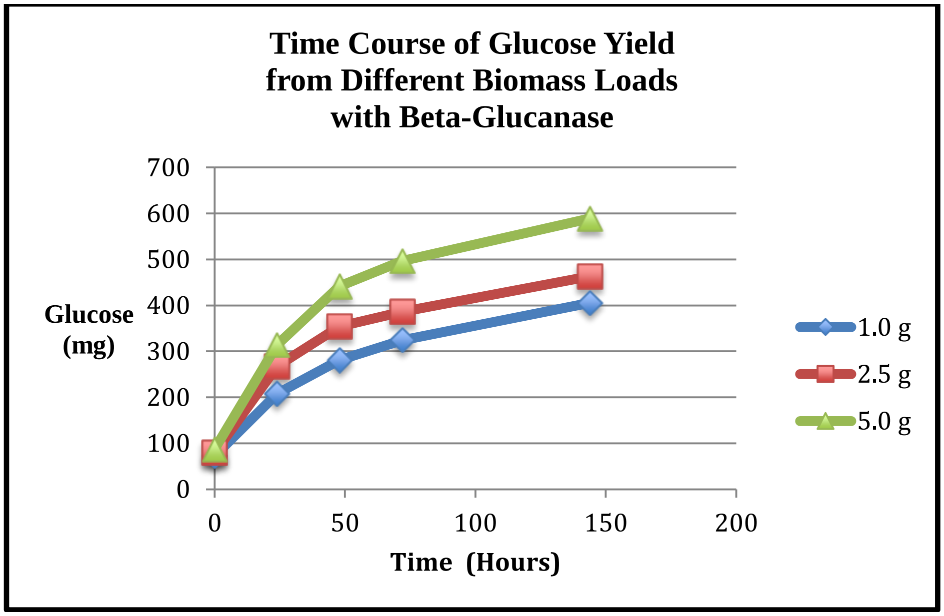
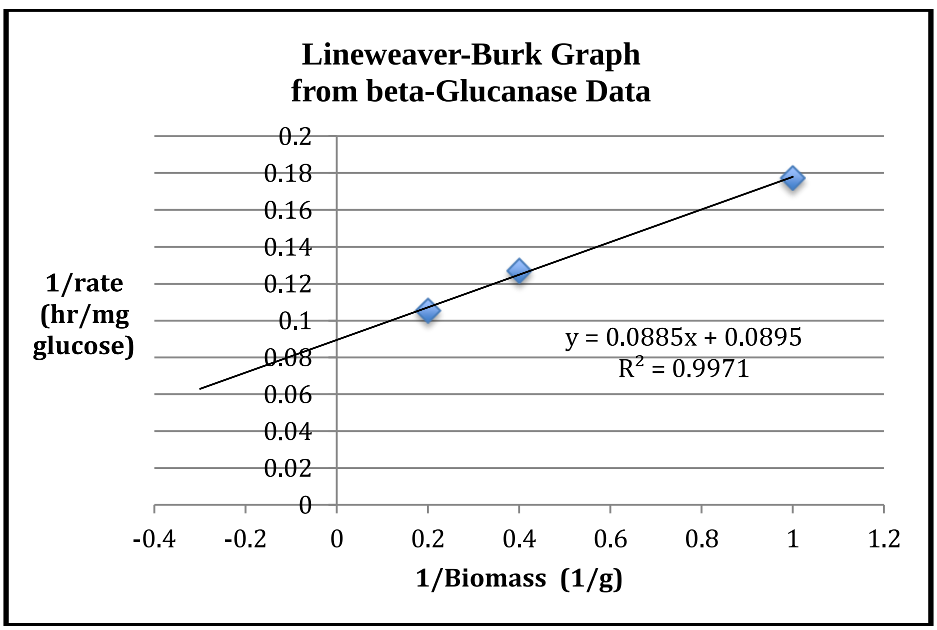


Figure Three



In preliminary studies we have scaled up the amount of biomass to 50 grams using the □-glucanase in the hydrolytic step. The enzyme appears to be active well past 144 hours and yields the expected amount of glucose with minimal introduction of extraneous glucose.

Fermentation

Samples recovered from enzymatic hydrolysis of cattail leaves study were carried on to the fermentation stage. Table Five shows the results of the fermentation. Ethanol yields have been corrected for a blank containing only Yeast Extract and Peptone. The results indicated that cellulosic ethanol can be indeed be made from cattail biomass.

Table Five
Ethanol Yield

Sample	Starting Glucose (mg)	Theoretical Ethanol (mg)	Ethanol Obtained (mg)	Per Cent Yield
1	200	102	90.6	62
2	347	177	162.8	76

Conclusions

Findings and Significance

This project has shown that cattails and autumn leaf litter can serve as feedstocks for the production of cellulosic ethanol. While fall leaves have significantly lower levels of cellulose and hemicellulose than other feedstocks for cellulosic ethanol, they deserve further investigation since they do not remove agricultural land from food production and mechanisms are already in place to harvest and transport them. Secondly, they can be pretreated under much milder conditions than other feedstocks.

The comparison of pretreatments suggest that simple autoclaving in water is comparable to other pretreatments. This pretreatment is considerable less expensive and has less environmental impact then more elaborate pretreatment methods.

The studies on the optimization of the enzymatic hydrolysis step revealed the importance of the enzymes used. The cellulase enzyme that was used in our early studies was not only ineffective; it was introducing extraneous glucose to our samples. It also lost activity after 24 hours. The □-glucanase from *Trichoderma longibriatum* is more effective and introduces much less extraneous glucose. Consequently we will be using this enzyme in subsequent studies.

Journal Publications

Burum, Justin (James E. Rife, Mentor), "Characterization of Fall Leaves for Ethanol Production," Journal ***of Undergraduate Research***, Volume 8 (2008).

Presentations

Burum, Justin. "Characterization of Fall Leaves for Ethanol Production," Undergraduate Research Conference (2008).

Menne, Nick. "Are Fall Leaves a Viable Feedstock for the Production of Cellulosic Ethanol," Undergraduate Research Conference (2010).

Sanchez, Daniel J. "Cellulosic Ethanol from Cattail Leaves,"
Undergraduate Research Conference (2011).

Organization: Minnesota State University, Mankato
Award ID: DE-FG36-08GO88156
Final Report for Period: 7/31/2008 to 8/31/2012

Principal Investigator: Jones, Bruce E.
Title: Efficiency Consumption of Ethanol-Blended Fuels in Engines.
Part of: Cellulosic ethanol, optimization of bio-fuels in engines, and course development for renewable energy technicians. U.S. Department of Energy. (PIs: {J Knox, S Ward, V Agarwal, J Frey} D Carrow, **B Jones**, J Rife and CT Ruhland)

Project Participants

Senior Personnel

Name: Jones, Bruce E. (faculty)
Name: Mead, Gary (faculty)
Name: Steevens, Paul A. (staff)
Contribution to Project: Investigated, researched, selected and obtained emissions research equipment. Researched analytical exhaust system technologies and selected a system. Purchased, installed and commissioned system. Developed testing procedures using the new equipment along with a quality control plan for ensuring accurate data measurement and system operations.

Graduate Students

Name: Haliburton, Ross
Name: Brandt, Dylan
Name: Neubauer, Mark
Name: Smith, Calvin

Undergraduate Students

Names: Justin Kunz, Courtney Roberts, Jonathan Kay, Zach Prestegard, Chris Reek, Jon Olmstead, & Kurtis Mercil, Marshall Lam, Ryan Olson, Justin Randall, Cody Schrupp, Stafford Taillon, Takeru Yamamoto, Tony Carlson, Peter Krahn, Birendra Chaudhary, Siva Yalauarthi, Max Anderson.

Other Partners

California Analytical Instruments, Inc.
1312 W. Grove Ave
Orange, CA 92865
714. 974. 5560 Office
714. 921. 2531 Fax

Environmental Testing Corporation
2022 Helena St.
Aurora, CO 80011
303.344.5470

Research Activities and Findings

Background

The project scope has one distinct goal; to determine and obtain the equipment required to test the fuel consumption and emission characteristics of internal combustion engines operating on a variety of renewable fuels following US EPA protocol. Specific equipment was identified along with the development of testing procedures that will be used in the future to measure the efficiency and emission characteristics of engines for the renewable energy lab at Minnesota State University, Mankato (MSUM) that will allow research to be conducted on renewable fuel in internal combustion engines.

This grant was part of a much larger initiative at MSUM to design and construct a new research facility to support research activities in the area of alternative and renewable fuels. Figure 1 shows the architect's rendering of the building in 2006.

Figure 1 - Center of Renewable Energy (C.O.R.E.)

A major feature of the building was to be able to perform research that would yield data that would be recognized by the US Environmental Protection Agency (EPA). In order to be able to meet specific EPA procedures outlined in the US Code of Federal Regulations (CFR) Title 40, Part 86 specific test equipment and procedures must be utilized. The CFR served as the main document used to develop the equipment list for the lab. A detailed floor plan of the building can be found in Figure 2. The building was designed to conduct 3 specific types of testing: Vehicle Chassis Testing; Engine Testing; and Evaporative Emissions testing. All 3 test areas are controlled by equipment located in one centralized Control Room.

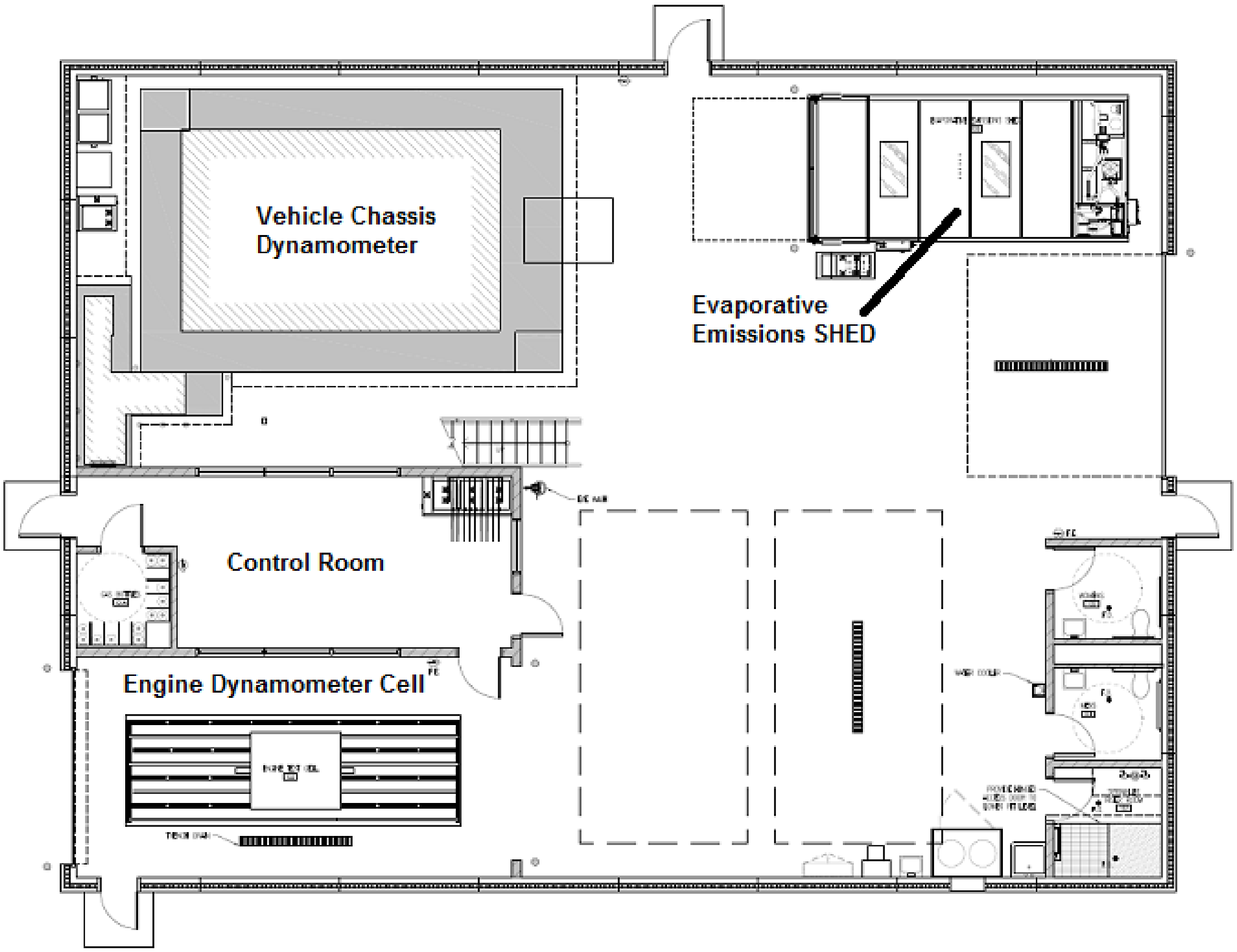


Figure 2 - Floor Plan of Lab Facility

The first area required was the Vehicle Chassis Dynamometer cell. A chassis dynamometer is a device that allows a vehicle to be "driven" on a set of rollers that "simulates" driving on the road. Figure 3 shows the dynamometer purchased as part of this grant installed in the facility. The dynamometer purchased is a Burke Porter electric dynamometer capable of meeting the most recent EPA specifications. The dynamometer has the capability to test full time 4 wheel drive vehicles in addition to 2 wheel drive vehicles. Currently we have raised enough money for ½ of the unit. Figure 4 shows a portion of the system that allows testing of 2 wheel drive vehicles. The second portion of the dynamometer will be purchased at a later date.

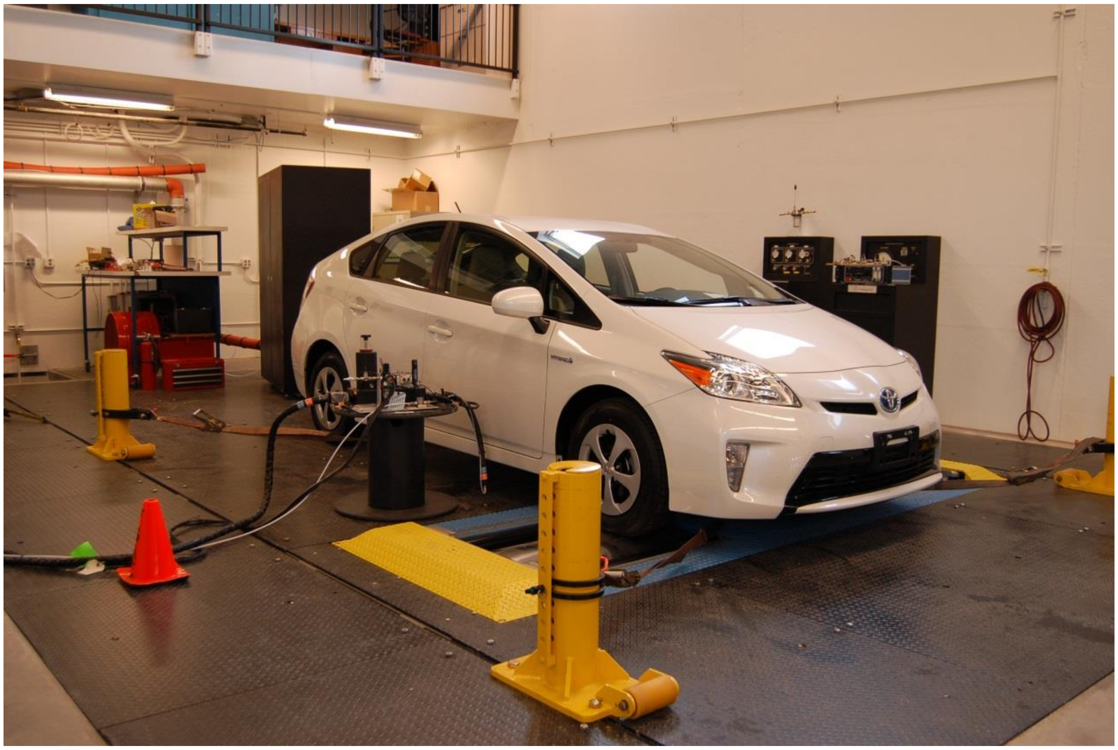


Figure 3 - Vehicle Chassis Dynamometer

The dynamometer ensures that every vehicle is driven under the same conditions each time the test is run to eliminate variability. The drive cycle is called the Federal Test Procedure 75 LA4 (FTP LA4) and simulates a driving cycle on US highway #4 in LA, California. Figure 4 is the trace the vehicle must follow when conducting the test. The “X” axis is time in seconds and the “Y” axis is vehicle speed in MPH.

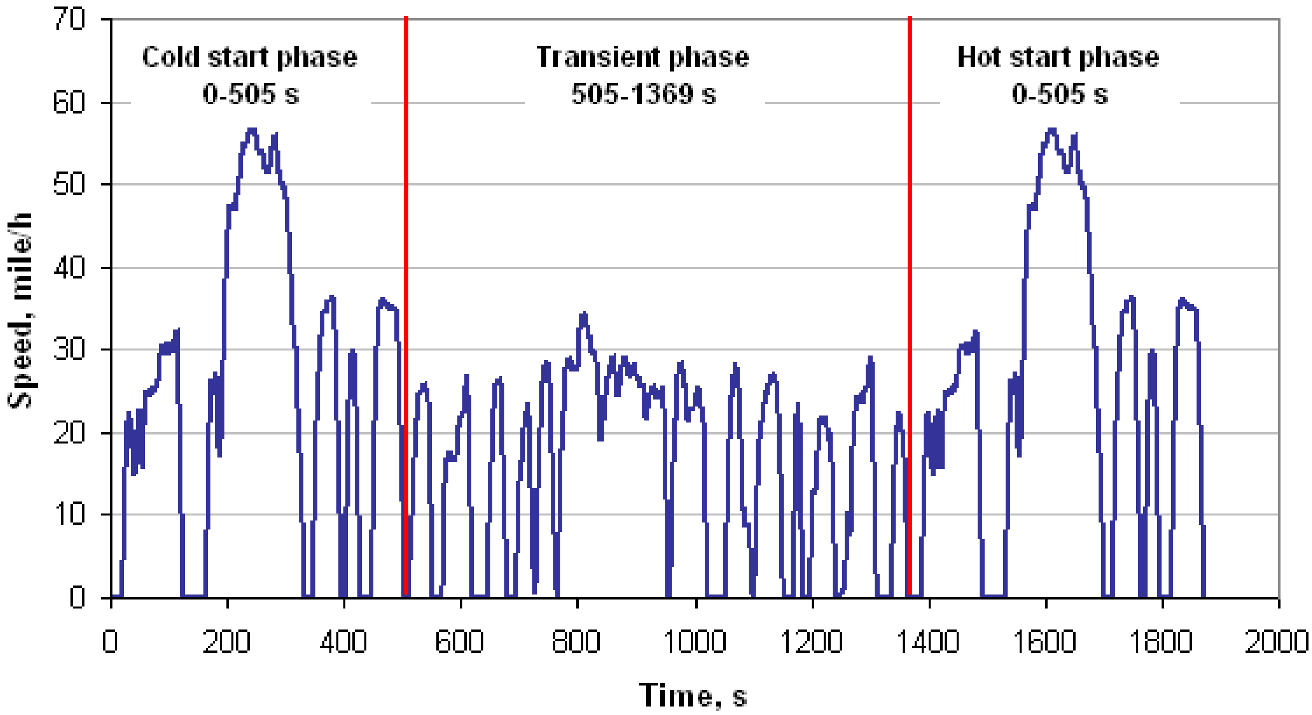


Figure 4 - FTP LA4 Drive Trace

The second major test area is the Engine Dynamometer Cell shown in Figure 5. This area and equipment are used to test engines that are not in a vehicle. Engines tested in this type of area include everything from small lawn mower engines all the way up to large Diesel generators used to generate electricity.

Figure 5 – Engine Dynamometer Cell

The third test area of testing is the Sealed Housing Evaporative Determination test (SHED). It involves placing the vehicle in a sealed enclosure, precisely heating the vehicle and measuring the hydrocarbon emissions evaporating from the vehicle. This is a significant contributor to pollution. The EPA recently implemented new requirements for certification and the test has been changed from a 1 hour test to a 3-day test where the temperature is cycled up and down simulating the vehicles sitting outside in ambient temperatures. Figure 6 shows a Variable Volume/Variable Temperature SHED capable of meeting the new EPA requirements.

Figure 6 – Evaporative Emissions SHED

The final area of the lab is the Control Room. The control room houses the analytical emissions measurement equipment that is used to measure the emission characteristics of vehicles in the Chassis Dynamometer Cell or stationary engines in the Engine Dynamometer Cell. Placing the control room between both cells allowed us to develop a more efficient lab at a lower cost by eliminating duplication. In Figure 7 you can see the exhaust analyzer "rack". This one rack has all of the analyzers for the measurement of HC, CO, CO_2, NO_x and O_2 in it. If you look closely at the photo you can see 3 columns of analyzers. Each column is a set of analyzers for specific types of testing including: the tailpipe of the vehicle, before the catalytic converter, and raw emissions out of a stationary engine.

Figure 7 – Control Room in the MnCAR Lab. Showing equipment purchased with DOE funds.

Finally, Figure 8 is a photo from the mezzanine of the facility that shows the Chassis Dynamometer Cell, Control Room, Evaporative Emissions SHED and Vehicle Preparation area.

Figure 8 – Lab Overview Photo

Presentations

SAE Small Engine Technology Conference, Student Poster Presentation, October 16-18, 2012, Monona Terrace Convention Center, Madison, Wisconsin.

References

1. "Daily Fuel Gauge Report." *AAA*. N.p., 12/16/2011.Web. 16 Dec 2011. <http://fuelgaugereport.aaa.com/?redirectto=http://fuelgaugereport.opisnet.com/index.asp>.

2. Jiang, Li, Hakan Yilmaz, Mark Christie, Hyung-hoAhn, and Anna Stefanopoulou. "Optimally Controlled Flexible Fuel Vehicle." n. page. Print.

3. Kunz, Justin. Roberts, Courtney. Kay, Jonathan. “E85 Ethanol Efficiency in an Automotive Engine. Utilization of Direct Injection Using General Motors Ecotec Engine.” (2011). Web. 16 Nov. 2011.

4. Pulkrabek, Willard W. *Engineering Fundamentals of the Internal Combustion Engine.* 2nd ed. Upper Saddle River: Pearson Prentice-Hall, 2004. Print.

5. Szybist, James, Matthew Foster, Wayne R. Moore, Keith Confer, Adam Youngquist, and Robert Wagner. "Investigation of Knock Limited Compression Ratio of Ethanol Gasoline Blends." *SAE International*. (2010). Web. 16 Nov. 2011.

6. United States. U.S. Department of Energy. *Fuel Economy*. 2012. Web. <www.fueleconomy.gov>.

Active Contracts

Minnesota Corn Research and Promotion Council, Mead, G and Jones, B.
Minnesota Corn Growers Association. Contract: $90,874

Seasonal Storage Issues of Intermediate Ethanol Blends, Mead, G and Jones, B.
Minnesota Corn Growers Association. Contract: $78,230

Enhanced Handheld Engine Ethanol Test Plan V2, Mead, G and Jones, B.
Minnesota Corn Growers Association. Contract: $116,873

Briggs & Stratton 5.25 Redo, Mead, G and Jones, B.
Minnesota Corn Growers Association. Contract: $29,997

Arctic Cat Vehicle Research and Development Testing, Jones, B. and Mead, G.
Artic Cat, Inc. Contract: $54,000

Organization: Minnesota West Community and Technical College
Award ID: DE-FG36-08GO88156
Final Report for Period: 7/31/2008 to 8/31/2012

Principal Investigator: Carrow, Duane
Title: NovaTech Ethanol Training Simulator (NETS).
Part of: Cellulosic ethanol, optimization of bio-fuels in engines, and course development for renewable energy technicians. U.S. Department of Energy. (PIs: {J Knox, S Ward, V Agarwal, J Frey} **D Carrow**, B Jones, J Rife and CT Ruhland)

Project Participants

Senior Personnel

Name: Carrow, Duane
Contribution to Project: Supervised overall project. Develop generic ethanol Process Flow Diagram. Supervised project consultants and reviewed publication of Training Manual and Workbooks. This grant provided some support during development of the workbooks.

Other Partners

NovaTech Process Solutions, LLC

Provided software engineers to write simulation program as per Process Flow Diagram. Developed interactive screen graphics for ethanol process.

Mike Bauman

We contracted industry consultant Mike Bauman, Shift Supervisor, Granite Falls Ethanol, to write and proof the Standard Operation Procedures for the NETS training manual and workbooks.

Kayla Westra, Westra Communications

We contracted with Westra Communication to provide technical writing services for development and design of the training manual and workbooks.

Research Activities and Findings

Background

MN West Community and Technical College completed the NovaTech Ethanol Training Simulator (NETS) project. Development of NETS, supported in part by DOE funds, was a cooperative effort between industry and education to develop the first ever ethanol process training simulator. NETS trainees now have an opportunity to experience simulated startup, shutdown, and steady state operations of an ethanol process facility without risk to personnel or damage to equipment.

Equipment Purchased

A total of $30,000 of DOE funds were used to support this project through the purchase of simulator hardware including 1 instructor station, 8 complete computer stations, 2 servers, 2 PCM4100 Controllers and all connecting hardware

Courses Impacted

P&ID and PFD Reading Online Course (RNEW1125) at Minnesota West Community & Technical College

This course will cover the symbols and diagrams commonly used on Piping and Instrumentation Diagrams (P&ID) and Process Flow Diagrams (PFD). Focus will be on identifying the types of diagrams, identifying instrument symbols and line symbols used on P & ID's, understanding the types of information typically found on a legend, using a P & ID to locate the components of a system, and reading a PFD to trace the flow paths of a system.

Process Optimization Lab Course (RNEW1155) at Minnesota West Community & Technical College

This course is designed to pull together all of the concepts explored in the previous three semesters and apply them in real-life case studies. Participation in class is critical. The concept that decisions made by the process operator have immediate impacts on the bottom-line of a company will be an important theme running through this course. Emphasis will be placed on report generating and interpreting using real-life examples. Prerequisite: RNEW 1135.

Top Left
Biofuels student contemplates his next action on the ethanol simulator as part of the Process Optimization course.

Bottom Left
Instructor Rose Patzer works with Biofuels student reviewing the Plumbing and Instrumentation Diagram on the Ethanol Training Simulator.

Principal Investigator: Carrow, Duane
Title: Wind Energy Training Tower
Part of: Cellulosic ethanol, optimization of bio-fuels in engines, and course development for renewable energy technicians. U.S. Department of Energy. (PIs: {J Knox, S Ward, V Agarwal, J Frey} **D Carrow**, B Jones, J Rife and CT Ruhland)

Project Participants

Senior Personnel

Name: Carrow, Duane
Contribution to Project: Supervised overall project.

Name: Olsen, Gary
Contribution to Project: Gary Olsen, as the lead Wind Energy faculty member, used his industry connections to garner support for the completion of this project. Gary acted as the liaison between supporting industry groups to coordinate transportation and construction activities.

Other Partners

SMI & Hydraulics, INC

Contracted to construct customized top section for placement on top of the climb tube. In addition to painting the tube and logo, SMI also installed multiple climb ladders and a mechanical lift inside the tube.

American Engineering and Testing

Contracted to develop an engineering plan for tower construction site including soil boring, steel reinforcement and concrete requirements. American Engineering also conducted final specification testing and certification.

CEEC, INC.

CEEC, INC. was contracted to provide transportation and erection of the climb tube.

Research Activities and Findings

Background

A wind turbine tube section was donated to MN West Community and Technical College for the purpose of constructing a climb training tower on the MWCTC Canby Campus. DOE funds supported completion of the Wind Energy Climbing Tube. This 110 foot tall climb tube features a mechanical lift and two separate ladder systems (rail and cable). In addition to climb training on both ladder types the multiple ladders allow for supervised rescue training and industry certification.

Equipment Purchased

$14,000 of DOE Funds was used to support this project through the purchase of equipment and supplies used in or on the climb tower.

Courses Impacted

Environmental, Health, & Safety Wind Energy online course (ELWT1160) at Minnesota West Community & Technical College

The SAFETY of the wind industry focuses on avoiding, minimizing, and controlling (Environmental, Health, and Safety) issues during the construction and operation of a project or facilities. Some of the topics to be addressed will include the safety of proper climbing techniques and certification, working at heights, working in a confined space, working with rotating machinery and addressing falling objects. Community health, safety, environmental issues will also be addressed.

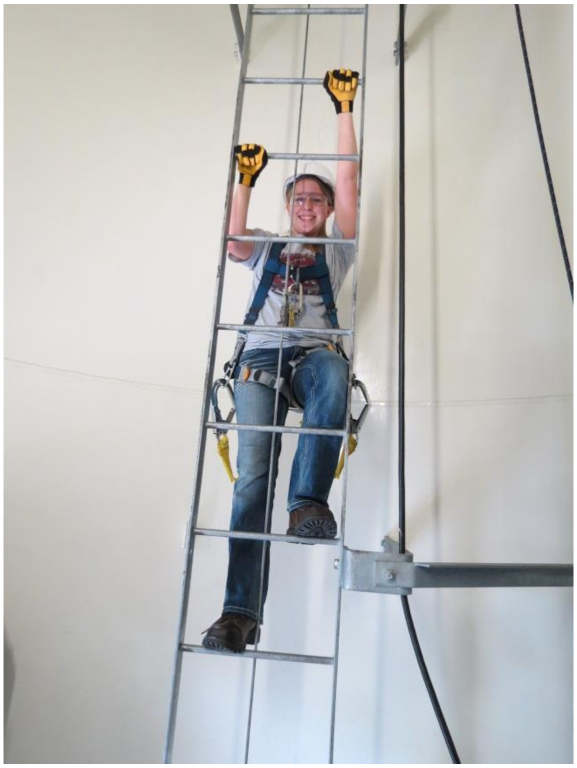
Wind Energy OSHA Standards Climb Lab Course (ELWT1170) at Minnesota West Community & Technical College

This course provides students with information regarding basic safety principles in the Wind Energy Industry. A brief overview of the Occupational Safety and Health Administration (OSHA) will be discussed. The primary focus will be on OSHA regulations and standards that pertain to the construction and maintenance of wind turbines and the energy industry. This class will also include the proper and safe way to climb in a wind turbine.

Left
Construction begins on the Wind Energy Training Tower.

Right
The newly constructed Wind Energy Training Tower located on the Canby campus of Minnesota West Community College will be utilized by wind energy students as they prepare to enter the wind industry.

The tower will also be utilized by local wind energy companies to conduct employee OSHA climb certification.



Left
Wind Energy student demonstrates safe climbing technique on the cable ladder system in the Wind Energy Training Tower.

Below
Wind energy students take a break from their rigorous climb safety and conditioning training to pose for a picture in front of the Wind Energy Training Tower.

Principal Investigator: Carrow, Duane
Title: Wind Energy Mechanic Training on the White Earth Reservation
Part of: Cellulosic ethanol, optimization of bio-fuels in engines, and course development for renewable energy technicians. U.S. Department of Energy. (PIs: {J Knox, S Ward, V Agarwal, J Frey} **D Carrow**, B Jones, J Rife and CT Ruhland)

Project Participants

Senior Personnel

Name: Carrow, Duane
Contribution to Project: Supervised overall project.

Other Partners

Minnesota State Community and Technical College

Served as a collaborative partner in identifying training locations and adjunct faculty to teach some of the course work on the reservation.

White Earth Band of Ojibwe Tribal Council

Through a collaborative effort between the two MnSCU institutions and tribal members the White Earth Band received a DOL Pathway out of Poverty grant which included offering Wind Energy Mechanic Training on the reservation.

Minnesota West Community and Technical College Faculty

The following MWCTC faculty members contributed at multiple levels sharing their expertise to support this project: Gary Olsen, Brad Bolluyt, Laceson Town and Keith Hagen.

Wind Energy Advisory Committee

The Wind Energy Advisory committee is made up of individuals representing wind industry organizations, secondary and post-secondary education, and wind industry construction and maintenance companies. This committee plays a key role in the development of course content, access and methodology. Following is a list of committee members and the organization they represent:

Name	**Company/address**
Rory Artig	Self-employed Formerly with the MN Department of Commerce
Steve Scott	Outland Renewable Energy 302 1st Street East Canby, MN 56220
Jeremy Krug	Outland Renewable Energy 302 1st Street East Canby, MN 56220

Deb VanDerostyne	Outland Renewable Energy 302 1st Street East Canby, MN 56220
Jim Nichols	Independent Owner 1577 County Rd. 101 Lake Benton, MN 56149
John Dunlop	American Wind Energy Assn. Great Plains Regional Office 448 Morgan Ave S, Suite 300 Minneapolis, MN 55405-2030
Dan Juhl	Danmar & Associates 520 Fifth Ave. SE Pipestone, MN 56164
Gerald D. Toland	SMSU Dean of Distance Learning BA 268, Office of Dis. Learning 1501 State Street Marshall, MN 56258
Aaron Thomsen	GE Wind Energy PO Box 323 Lake Benton, MN 56149
Mike DeVries	GE Wind Energy PO Box 323 Lake Benton, MN 56149
Gary Stoks	SMI Hydraulics - President 401 Lone Tree Street Porter, MN 56280
Dallas Drietz	Trico Wind PO Box 722 Litchfield, MN 55355
Steve Mikels	Suzlon Rotor Company 620 Third Ave Pipestone, MN 56164
Loren Hacker	Superintendent Canby HS 106 Ring Ave North Canby, MN 56220
Joe Kolbach	Energy Maintenance Service PO Box 158 Gary, SD 57237
Deb Full	Energy Maintenance Service PO Box 158 Gary, SD 57237
Jeff Peters	Missouri River Energy PO Box 88920 Sioux Falls, SD 57109-8920

Dennis Hampel	Dean of Technical Programs PO Box 269 Jackson, MN 56143
Gary Olsen	Wind Energy Instructor-MN West 1011 First Street West Canby, MN 56220
Rebecca Weber	Canby Campus Manager 1011 First Street West Canby, MN 56220

Research Activities and Findings

Background

In 2009 the White Earth Reservation Tribal Council and Minnesota West Community and Technical College, Canby Campus, formed a formal partnership to deliver Wind Energy Mechanic training to participants located on the White Earth Reservation. The partnership began when members of the Ojibwe Tribal Council contacted two MnSCU Community and Technical colleges, Minnesota State and Minnesota West with a request for collaboration on an application for U.S. Department of Labor "Pathways out of Poverty Grant". The proposal was forged with the intent to have Minnesota State deliver Green Manufacturing and Minnesota West to deliver Wind Energy Mechanic training on the White Earth Reservation to a select and underserved group of trainees. The collaborative proposal titled, **"Pathways to a Greener Future,"** was awarded in the fall of 2009.

The partnership between the White Earth Tribal Council and Minnesota West Community and Technical College was designed to focus on training participants to enter the high job growth wind industry. Curriculum designed for this training focused on job skills required to become a successful technician in the maintenance of wind turbines. Areas of study included courses in electricity, hydraulics, and wind energy fundamentals, mechanical fundamentals, climbing, wind power generation / transmission / distribution, OSHA and environmental safety. Location of the training, Ogema, Waubun, and Naytahwaush, was a mutual benefit to all constituents. With the exception of climb training, where students traveled to the Minnesota West Canby campus, all courses were conducted on the White Earth Reservation. Minnesota West transported its training equipment to classroom facilities provided by the Ojibwe tribe. Conducting the training on the reservation was an efficient way to utilize resources while keeping the cost down for individual trainees.

Purchase of equipment with DOE dollars allows Minnesota West Community and Technical College to increase capacity on the Canby campus and offer Wind Energy Mechanic Training on the White Earth Reservation. The collaborative training effort between multiple Minnesota State College and Universities (MnSCU) and the White Earth Band of Ojibwe resulted in a 2012 Academic and Student Affairs award for Innovative Partnering and Collaboration.

Equipment Purchased

Over $200,000 of DOE funds were used to support this project through the purchase of equipment that is used for training on the reservation and on the Canby campus of MWCTC. Most of the equipment can be transported between multiple training sites resulting in a substantial increase in training capacity. Some of the virtual equipment purchased allows online access to the NIDA Lab (electrical trainers)

Courses Impacted

Wind Energy Fundamental Online Course (ELWT1100) at Minnesota West Community & Technical College

This course introduces the student to turbine designs, types and development, as well as their current status. The evolution of current models and sizes offered by existing companies will be traced from earlier examples. The operational experience, track record, and number of turbines in operation will be evaluated. The students will also discover the economic, environmental and political issues associated with Wind Energy.

Mechanical Systems Lab Course (ELWT1110) at Minnesota West Community & Technical College

In this course we will discuss the different gearbox options for wind turbines as well as the types of gears found in a gearbox system. The Mechanical Systems course includes research projects on gearboxes as well as servicing procedures. This course will also focus on investigative techniques, inspection reporting, Non-Destructive testing, performing a failure analysis and corrective actions for failed gearbox systems. Each student will be required to participate in the inspection, disassembly, re-inspection, cleaning, measuring, documenting and re-assembly of a gearbox. Students will learn to use special measuring and inspection tools (micrometer, dial indicator, bore gages, bore scope and dial calipers). Students will always demonstrate proper safety procedures at all times.

Basic Hydraulics Online Course (FLPW1103) at Minnesota West Community & Technical College

This course introduces students to basic concepts, formulas and applications of hydraulic system components. Studies the use of directional, flow and pressure control of circuits as it applies to the wind turbine. Also provides students with the knowledge and understanding of the operation, function, and application of hydraulic pumps, continuous rotation motors and limited rotation motors.

White Earth Reservation Project Summary

An innovative partnership formed between the White Earth Band of Ojibwe Tribal Council and Minnesota West Community and Technical College was designed to focus on training participants to enter the high job growth wind industry. Curriculum, delivered by Minnesota West on the White Earth Reservation, focused on job skills required to become a successful technician in the maintenance of wind turbines. Areas of study included courses in electricity, hydraulics, wind energy fundamentals, mechanical fundamentals, climbing, wind power generation/transmission/distribution, OSHA and environmental safety.

White Earth Reservation students attend climb training on the Canby Campus