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RESEARCH

Alfalfa Leaf Protein and Stem Cell Wall Polysaccharide Yields under Hay and Biomass Management Systems

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ABSTRACT

Alfalfa (*Medicago sativa* L.) has been proposed as a biofuel feedstock in which the stems would be processed to produce ethanol and the leaves sold separately as a livestock feed. Our objectives were to evaluate the effects of management strategy on leaf crude protein (CP), and stem carbohydrate concentrations and yields of alfalfa germplasms differing in genetic background. Two hay-type and two biomass-type alfalfas were established at 450 plants m^{-2} and harvested at early bud (hay management system) and at 180 plants m^{-2} and harvested at green pod (biomass management system) in three environments. The biomass-type alfalfas under the biomass management had lower leaf CP, higher stem cell wall polysaccharide, and higher stem lignin concentrations, comparable leaf CP yield, and 37% greater stem cell wall polysaccharide yields compared to the hay-type alfalfas under the hay management treatment. The impact of altered stem cell wall composition and increased stem dry matter yield of a biomass-type alfalfa under the biomass system compared to a hay-type alfalfa under the hay system increased the theoretical potential ethanol yield by 99%.

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Abbreviations: CP, crude protein; DM, dry matter; IVDMD, in vitro dry matter digestibility; NIRS, near infrared reflectance spectroscopy.

Proposals for U.S. renewable energy sources have included several biomass feedstocks from agricultural crops including alfalfa (*Medicago sativa* L.). In an alfalfa biomass energy production system, the forage could be fractionated into stems and leaves. The stems could be processed to generate electricity or biofuel (ethanol), and the leaves could be sold as a supplemental protein feed for livestock (Delong et al., 1995). One of the advantages of using alfalfa to produce biomass energy compared to other crops is the secondary income stream from selling the leaves as a higher-value animal feed. Therefore, the key traits of interest for an alfalfa germplasm that would be used in biofuel production systems include concentrations and seasonal yields of leaf protein and stem cell wall polysaccharides. In addition, the degree

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to which biomass is lignified is known to affect pretreatment requirements and ethanol yield (Dien et al., 2006); therefore, alfalfa grown for biomass should contain as little lignin as possible.

A two-cut harvest regime in which alfalfa is harvested at the late flower to early pod stage of maturity and population density is reduced to 60% of traditional planting density has been proposed for an alfalfa biomass energy production system to maximize stem yield, enhance wildlife habitat, and reduce harvest costs (Lamb et al., 2003; Sheaffer et al., 2000). In comparison to the traditional management scheme of higher population density and three to four harvests per growing season at the early bud maturity stage, the alfalfa biomass management scheme had greater stem yield and comparable leaf yield per growing season (Lamb et al., 2003). However, numerous researchers have reported decreases in whole plant forage quality when plants are harvested at later maturity stages (Albrecht et al.,1987; Buxton et al.,1985; Fick and Holthausen, 1975; Griffin et al., 1994; Juan et al., 1993; Kalu and Fick, 1983; Kilcher and Heinrichs, 1974; Sanderson et al., 1989; Sanderson and Wedin, 1988; Sheaffer et al., 1998, 2000). It is possible that harvesting alfalfa forage at late flower or later maturity stages may affect the forage quality of the leaves, leading to a less valuable secondary product. Several researchers have reported that alfalfa leaves are higher in crude protein (CP) and lower in cell wall concentration than stems (Bourquin and Fahey, 1994; Hintz and Albrecht, 1991), and that digestibility of the material declines and cell wall concentration on a dry matter (DM) basis increases at much slower rates in leaves compared to stems as maturity advances (Albrecht et al., 1987; Buxton et al., 1985; Kilcher and Heinrichs, 1974).

Several studies have documented alfalfa population density effects on stem, leaf, and total forage yield and quality. Sund and Barrington (1976) found no association between alfalfa population density and whole plant forage yield, while Bolger and Meyer (1983), Hansen and Krueger (1973), and Volenec et al. (1987) reported increased whole plant forage yield as alfalfa population densities increased. However, CP concentration (Bolger and Meyer, 1983; Sund and Barrington, 1976) and acid detergent fiber concentration (Bolger and Meyer, 1983; Hansen and Krueger; 1973) were unaffected by plant density, and the authors stated that increasing seeding rates would not improve forage quality. Volenec et al. (1987) stated that in vitro DM digestibility (IVDMD) of the herbage did not increase with plant population even though stem diameter and stem lignin concentration decreased.

Variation for leaf and/or stem yield and forage quality is evident in different genetic sources of alfalfa (Barnes et al., 1977; Buxton et al., 1987; Sheaffer et al., 2000). Marquez-Ortiz et al. (1999) reported that individual stem diameter was heritable and controlled by additive genetic effects, and suggested that selection for larger stems in alfalfa was feasible. Volenec et al. (1987) found that selection for high yield per stem was an effective method to increase forage yield, but plants may have less digestible, larger stems. Buxton et al. (1987) reported variation among alfalfa cultivars for stem IVDMD and CP. Flemish germplasms from southern Europe are a genetic source for large stem size and resistance to foliar diseases but display early maturity, lack winterhardiness, and are susceptible to root and crown diseases (Barnes et al., 1977). Multifoliolate alfalfa types produce four or more leaflets per leaf compared to three leaflets for the normal trifoliolate alfalfa leaf. Several studies have reported greater leaf-to-stem ratios in alfalfa cultivars with the multifoliolate trait compared to the normal trifoliolate cultivars (Ferguson and Murphy, 1973; Juan et al., 1993; Volenec and Cherney, 1990).

The effects of the proposed biomass production management scheme on nutritive value of the leaf and stem components of alfalfa are unknown. The objectives of our study were to evaluate the effects and interactions of environment and biomass management strategy compared to traditional hay production practices on leaf CP, stem cell wall Klason lignin, and polysaccharide concentrations and component yields of alfalfa germplasms differing in genetic background.

MATERIALS AND METHODS

Plant Materials

Four alfalfa germplasms differing in genetic background, fall dormancy, and leaf-to-stem ratio were evaluated. MP2000 is a commercial multileaf alfalfa cultivar selected for greater leaf-to-stem ratio. MWNC-4 (UMN 3041) is an experimental population selected for resistance to Phytophthora (*Phytophthora medicaginis* Hansen and Maxwell) and Aphanomyces (*Aphanomyces euteiches* Drechs.) root rots and root-lesion nematode [*Pratylenchus penetrans* (Cobb) Fillipjev and Schurmans-Stekhoven] and is adapted to the upper Midwest region. These two germplasms have fall dormancy ratings between 2 and 3 and were considered to be hay-type alfalfas. New Europa is a southern European alfalfa cultivar of Flemish origin (Barnes et al., 1977). ORCA-WTS (UMN 3040) is an experimental population selected from the Flemish cultivar ORCA for large, nonlodging, woody stems at the late-flower maturity stage. Both of these Flemish germplasms have dormancy ratings of 5 and were designated as biomass-type alfalfas.

Experimental Design

The original experimental design was a randomized complete block with four replicates in a split plot factorial arrangement of the subplot treatments, in which four population densities (16, 50, 180, and 450 plants m^{-2}) were the whole plots, and all combinations of the four germplasms and two plant maturities (early bud and green pod) were the subplots. Data for total DM yield were reported by Lamb et al. (2003). In the current study two management treatments from the original study were compared: a conventional hay management treatment in which

the population density was 450 plants m^{-2} and plots were harvested three to four times each growing season (depending on the environment) at the early bud maturity stage, and a biomass management treatment in which the population density was 180 plants m^{-2} with harvests twice per growing season at the green pod maturity stage. The 180 plants m^{-2} density had 7.5 cm between plants in 0.45- × 0.45-m plots. The 180 plants m^{-2} population density treatment was seeded by hand with two to three seeds per hole and thinned to one plant per hole 15 to 20 d after seeding. The 450 plants m^{-2} population density was mechanically seeded using a Plotman plot planter (Wintersteiger, Inc., Salt Lake City, UT), at a rate of 11 kg ha^{-1} in 1.8-m × 2.0-m plots with 10 rows drilled 12 cm apart at an approximate population density of 450 plants m^{-2}. A 0.30-m × 0.45-m area was harvested for forage yield. No changes occurred for target population densities of the 180 and 450 plants m^{-2} for all four alfalfa entries for the 2 yr of this study.

The experiment was planted at the Sand Plains Research Farm, Becker, MN (Hubbard loamy sand; sandy, mixed, Udorthentic Haploborall) on 20 Aug. 1996 and at the Minnesota Agricultural Experiment Station at Rosemount, MN (Tallula silt loam; coarse silty, mixed, mesic, Typic Hapludoll) on 19 May 1997. Soil pH and P and K levels were adjusted to those recommended for alfalfa production (Rhem and Schmitt, 1989). Weeds were controlled by hand weeding. All plots were sprayed periodically with Pounce 25 WP [active ingredient: permethrin (3-phenoxyphenyl)methyl 3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropaneccarboxylate] to control potato leafhopper [*Empoasca fabae* (Harris)]. The conventional hay management plots were harvested at early bud when 10 to 33% of the stems in the plot had flower buds, and the biomass management plots were harvested at green pod when more the 10% of the stems had green seedpods, but less than 10% of the stems had mature brown pods. Hay management plots were hand harvested at a stubble height of 5 cm at the early bud stage either three or four times per season; on 12 June, 7 July, and 6 Aug. 1997 and on 28 May, 1 and 23 July, and 21 Aug. 1998 at Becker, MN, and at the Rosemount site on 21 May, 26 June, 22 July, and 25 Aug. 1998. Biomass management plots were harvested at the green pod stage of maturity twice per season on 30 June and 8 Aug. 1997, and on 30 June and 4 Aug. 1998 at Becker, MN, and on 23 June and 10 Aug. 1998 at Rosemount, MN. To assess leaf and stem quality under both the hay and biomass management systems, each plot was subsampled by randomly selecting 25 stems at the target maturity. These subsamples were dried at 55°C in forced-air ovens, weighed, and then stems were hand separated from leaves (all floral components remained with the leaf portion). The leaf and stem portions were reweighed, and leaf and stem DM concentrations were estimated for each plot. All leaf and stem samples were ground through a 1-mm screen in preparation for forage quality analysis.

Forage Quality Analysis

Crude protein, Klason lignin, and cell wall polysaccharide component sugars (glucose, xylose, galactose, mannose, rhamnose, fucose, and uronic acids) were estimated by near infrared reflectance spectroscopy (NIRS) analysis using NIRS prediction equations developed in Minnesota. All dried and ground leaf and stem samples were scanned (model 6500, NIRSystems, Silver Springs, MD). The NIRS prediction equations were developed using the software program Calibrate (NIRS 3 version 4.0, Infrasoft International, Port Matilda, PA) with the modified partial least-squares regression option (Shenk and Westerhaus, 1991a, 1991b). Prediction equations for alfalfa stem cell wall concentration and composition were developed by updating a previous set of calibrations that contained 144 alfalfa stem samples (Jung and Lamb, 2003). The Select software program identified 56 stem samples from the current study that had spectra that were not represented in the existing NIRS calibration set. These samples were analyzed for cell wall components as described below, and the resultant data were added to the calibrations. Prediction equations for alfalfa leaf CP were developed by updating a previous set of calibrations developed at the University of Minnesota for leaf, stem, and whole herbage CP. The Select software program identified 14 leaf samples from the current study that had spectra that were not represented in the existing NIRS calibration set for CP. These samples were subjected to the Kjeldahl procedure to determine N concentration from which CP concentration was calculated (Kjeldahl N × 6.25), and these results were added to the calibrations. Statistics for the prediction equations are shown in Table 1.

Cell wall concentration and composition of the calibration samples were determined using the Uppsala dietary fiber method (Theander et al., 1995). After acid hydrolysis, the neutral sugar components (glucose, xylose, arabinose, galactose, mannose, rhamnose, and fucose) of the cell wall polysaccharides were quantified by gas chromatography as alditol acetate derivatives. The acidic sugars (glucuronic, galacturonic, and 4-*O*-methylglucuronic acids) were measured as total uronic acids by the colorimetric method of Ahmed and Labavitch (1977) using galacturonic acid as the reference standard. Klason lignin was estimated as the ash-free, acid-insoluble residue remaining after hydrolysis. All cell wall neutral sugar data were converted to an anhydro basis to reflect their incorporation in cell wall polysaccharides and corrected to a 100°C DM basis. Total cell wall polysaccharides were calculated as the sum of glucose, xylose, arabinose, galactose, mannose, rhamnose, fucose, and uronic acids. Cell wall concentration was calculated as the sum of total cell wall polysaccharides plus Klason lignin.

Concentrations of CP, cell wall total polysaccharides, and component monosaccharides were calculated on a DM basis

Table 1. Calibration statistics from NIRS for leaf CP, stem Klason lignin and stem polysaccharide concentrations.

Prediction equations	Mean	Range	SE†	R^2
	g kg^{-1} dry matter			
Leaf crude protein	186	59–351	4.4	0.99
Stem Klason lignin	150	104–197	7.8	0.75
Stem glucose	292	225–359	4.8	0.95
Stem xylose	86	61–111	3.7	0.81
Stem galactose	18	14–22	0.7	0.69
Stem mannose	18	12–24	1.5	0.36
Stem arabinose	18	11–24	1.0	0.79
Stem rhamnose	7	5–7	0.2	0.78
Stem fucose	1.4	0.6–2.1	0.2	0.56
Stem uronic acids	89	74–104	4.5	0.21

†SE, Standard error of calibration for the prediction equations.

for the entire growing season by weighting individual harvest concentrations for the proportion of seasonal yield. The seasonal yields of these components were also calculated. Cell wall composition was determined by calculation of Klason lignin and cell wall polysaccharide component concentrations, on a seasonal adjusted basis, as a proportion of total cell wall.

Statistical Analyses

Data was analyzed as a randomized complete block with four replicates in a split plot arrangement of the subplot treatments, where management treatments were the whole plots, and the four germplasms were the subplots. In the analysis of variance, each harvest year at a location was considered as an environment. Forage quality data were subjected to analysis of variance to determine the effects of environment, management treatment, and alfalfa germplasms on whole-season weighted averages (PROC GLM; SAS Institute, 1998). Environments were considered random and management treatments and alfalfa germplasms were considered fixed. Means for all traits evaluated for main effects and interactions of the three environments, two management treatments, and four alfalfa germplasms were compared (PROC GLM, LSMEANS; SAS Institute, 1998). Significance was declared at $p < 0.05$ unless otherwise indicated.

RESULTS AND DISCUSSION

Environment, management treatment, and germplasm as well as interactions among these main effects impacted stem cell wall composition (Table 2), stem cell wall and leaf CP concentrations (DM basis) (Table 3), and leaf CP and stem cell wall polysaccharide yields (Table 4). Environment × management treatment interactions for all of the stem cell wall compositions traits (except glucose and Klason lignin), and for stem cell wall galactose, mannose, xylose, and arabinose and leaf CP concentrations (DM basis) were caused by differences in magnitude, not direction. For these traits, the management treatments ranked in the same order, but the magnitude of difference between the means varied depending on the environment. An environment × germplasm interaction was found for xylose concentration on both a cell wall and DM basis. The two biomass-type germplasms (New Europa and ORCA-WTS) were consistently lower in xylose concentrations compared to the hay-type germplasms (MP2000 and MWNC-4), and for two of the environments, ORCA-WTS had higher xylose concentrations than New Europa, while for the third environment the reverse occurred. All stem carbohydrates, Klason lignin, and leaf CP concentrations and yields were consistently lower at Becker in 1997 compared to Rosemount or Becker in 1998 (data not shown). Differences between the management treatments and among the germplasms were also smaller at Becker 1997 compared to the other two environments, causing most of the environmental interactions in our study. The environment × management treatment interaction for leaf CP yield was more complicated and will be discussed in more detail below.

Stem Cell Wall Carbohydrates and Klason Lignin

As expected for more mature alfalfa, stems had higher concentrations of cell wall material on a seasonal, yield-adjusted basis under the biomass management system than the hay system (Table 5). Cell wall composition was impacted by harvest management with increased Klason lignin and xylose concentrations under biomass management compared to hay management, whereas cell wall glucose concentration was similar between the two management treatments and concentrations of the other cell wall polysaccharide components (galactose, mannose, arabinose, rhamnose, fucose, and uronic acids) declined under biomass management (Table 5). On a DM basis, alfalfa stem concentrations of total cell wall polysaccharides, glucose, mannose, and xylose increased with biomass management compared

Table 2. Analysis of variance for stem Klason lignin and component monosaccharide concentrations on a cell wall basis.

Source	df	Mean squares								
		Klason lignin	Glucose	Galactose	Mannose	Xylose	Arabinose	Rhamnose	Fucose	Uronic acids
Environment (E)	2	2041***	1430***	25.8**	31.0***	353***	121.5***	13.37***	0.709***	1550***
Rep (E)	9	55	68	0.2	0.3	6	0.5	0.03	0.003	1
Management treatment (MT)	1	3920***	1	98.4***	25.1***	627***	70.4**	3.89*	1.977***	3604***
E × MT	2	67	85	16.1**	1.6*	158***	22.4*	2.19*	0.280**	165*
Error A	9	30	66	1.5	0.2	6	4.3	0.40	0.032	40
Germplasm (G)	3	17	363***	5.7***	0.4**	6	13.7***	1.12***	0.127***	150***
MT × G	3	21	41	0.7	0.2	4	2.5*	0.11	0.013*	17
E × G	6	11	16	0.4	0.1	8*	0.7	0.04	0.009	8
E × MT × G	6	23	37	0.1	0.2	5	0.3	0.03	0.006	5
Error B	54	20	24	0.2	0.1	3	0.7	0.04	0.005	5

*Significant at the 0.05 probability level.

**Significant at the 0.01 probability level.

***Significant at the 0.001 probability level.

Table 3. Analysis of variance for stem total cell wall, total polysaccharides, and component monosaccharide concentrations on a dry matter basis.

Source	df	Mean squares							
		Cell wall	Total polysaccharides	Glucose	Galactose	Mannose	Xylose	Arabinose	Leaf crude protein
Environment (E)	2	16655***	5241***	6493***	0.09	0.13	62	14.9***	12767***
Rep (E)	9	14	41	43	0.05	0.11	2	0.2	365
Management treatment (MT)	1	42553***	14545***	7659***	1.39**	5.12***	2010***	0.1	69091***
E × MT	2	661	286	257	3.14***	1.19*	125**	4.7*	6714***
Error A	9	474	237	206	0.10	0.20	13	0.9	187
Germplasm (G)	3	1902***	1127***	968***	0.28***	0.94***	45***	2.0***	413***
MT × G	3	123	69	62	0.04	0.05	7	0.4	33
E × G	6	95	47	31	0.06	0.03	9*	0.2	69
E × MT × G	6	54	37	34	0.02	0.07	5	0.1	76
Error B	54	59	30	26	0.04	0.05	3	0.2	55

*Significant at the 0.05 probability level.

**Significant at the 0.01 probability level.

***Significant at the 0.001 probability level.

Table 4. Analysis of variance for yields of stem total polysaccharides, hexose and pentose monosaccharides, and leaf crude protein.

Source	df	Mean squares						
		Total polysaccharides	Glucose	Galactose	Mannose	Xylose	Arabinose	Leaf crude protein
Environment (E)	2	22625863***	8151485**	20822**	21841**	600828**	14441**	2124664**
Rep (E)	9	1477911	445312	1606	1945	43987	1528	272121
Management treatment (MT)	1	67948225***	22262027***	58418***	76021***	2557675***	62079***	807475
E × MT	2	3193401	899028	4252	4527	95496	4158	11511769*
Error A	9	1570625	503680	1473	1809	47145	1371	220110
Germplasm (G)	3	1491088*	581011*	876	1632*	45758*	534	92610
MT × G	3	443236	145136	383	450	12628	402	31075
E × G	6	1004409	316171	918	1192	33459	878	177946
E × MT × G	6	338490	102362	330	411	11865	316	103507
Error B	54	463746	144860	457	540	14292	460	85041

*Significant at the 0.05 probability level.

**Significant at the 0.01 probability level.

***Significant at the 0.001 probability level.

Table 5. Yield-adjusted mean cell wall concentration and composition of alfalfa stems as influenced by germplasm source and harvest management system.

Trait	Germplasm					Management system		
	MP2000	MWNC-4	New Europa	ORCA-WTS	$LSD_{0.05}$	Hay	Biomass	$LSD_{0.05}$
Concentration	———	———	———	g kg^{-1} dry matter	———	———	———	———
Cell wall	667	677	682	687	4	657	699	10
Composition	———	———	———	g kg^{-1} cell wall	———	———	———	———
Klason lignin	214	215	216	214	NS†	208	221	3
Glucose	424	428	429	433	3	429	428	NS
Galactose	26.4	26.0	25.7	25.3	0.3	26.9	24.8	0.06
Mannose	27.3	27.3	27.3	27.1	0.2	27.8	26.7	0.2
Xylose	136	136	136	136	NS	134	139	1
Arabinose	26.7	26.2	25.6	25.0	0.5	26.8	25.0	1.0
Rhamnose	9.5	9.3	9.1	9.0	0.1	9.4	9.0	0.3
Fucose	1.90	1.83	1.76	1.75	0.04	1.95	1.66	0.08
Uronic acids	133	131	128	128	1	136	124	3

†NS, nonsignificant at the 0.05 probability level.

to hay management, but galactose declined and arabinose did not change in concentration (Table 6).

These shifts in cell wall composition were similar to the pattern of changes observed during maturation of an individual alfalfa stem internode (Jung and Engels, 2002). Maturation of alfalfa stems results in accumulation of xylem tissues that are rich in cellulose, xylans, and lignin (Engels and Jung, 1998; Jung and Engels, 2002). The nonlignified tissues, which are rich in pectin, do not proliferate during alfalfa stem maturation; therefore, cell wall concentrations of arabinose, galactose, and rhamnose declined with maturity.

The alfalfa germplasms differed in cell wall concentration and composition across management systems (Tables 5 and 6). A management system × germplasm interaction in magnitude rather than direction was found only for arabinose concentration on a cell wall basis (Table 2). The four germplasms ranked in the same order in both management treatments, but the differences among the germplasm means were greater under the hay management system compared to the biomass management system. MP2000 was lowest and ORCA-WTS was highest for total stem cell wall and cell wall glucose concentrations; however, ORCA-WTS was lower than MP2000 for cell wall concentrations of galactose, mannose, arabinose, rhamnose, fucose, and uronic acids (Table 5). In contrast, cell wall concentrations of Klason lignin and xylose did not differ among germplasms. The MWNC-4 and New Europa germplasms were intermediate compared to the more extreme germplasm sources, although for most traits MWNC-4 was more similar to MP2000 and New Europa was more similar to ORCA-WTS. Dry matter concentrations of total cell wall polysaccharides, and the glucose, mannose, and xylose cell wall polysaccharide components were greater for ORCA-WTS than MP2000 (Table 6). Galactose and arabinose concentrations were lower for ORCA-WTS than MP2000. The MWNC-4 and New Europa germplasms were again intermediate for concentrations of cell wall polysaccharides on a DM basis.

Increasing Klason lignin concentration has been shown to be negatively correlated with glucose release by dilute acid pretreatment and enzymatic saccharification for several biomass crops, including alfalfa stems (Dien et al., 2006). Our data suggest that conversion efficiency should not differ among the four alfalfa germplasms we examined because they did not differ for lignin concentration in the cell wall (Table 5). However, alfalfa stems from the biomass management system may be more difficult to saccharify because of the increased lignification of the cell wall under this management system.

Management treatment and germplasm effects impacted yields of total cell wall polysaccharides and the component monosaccharides (Table 4). Total cell wall polysaccharide, glucose, galactose, mannose, xylose, and arabinose yields increased substantially under the biomass management system compared to the hay management system (Table 7). ORCA-WTS was the highest and MP2000 was the lowest for total polysaccharides, glucose, mannose, and xylose yields, with New Europa and MWNC-4 yields intermediate between the other two germplasms. Galactose and arabinose yields were similar for all four germplasm sources.

Potential Biofuel Yield

Based on the DM concentrations of hexose (glucose, galactose, and mannose) and pentose (xylose and arabinose) cell wall polysaccharide components of alfalfa stems (Table 6), the National Renewable Energy Laboratory's on-line theoretical ethanol yield calculator (www1.eere.energy.gov/biomass/ethanol_yield_calculator.html) predicted that ORCA-WTS stem material could be converted to 323 L of ethanol Mg^{-1} of biomass compared to 310 L Mg^{-1} from MP2000 stems, averaged across both management systems. This represents a 4.2% difference in theoretical ethanol yield between these two germplasms. Alfalfa stems harvested from the biomass management system could theoretically yield 6.5% more ethanol than the hay management system (326 vs. 306 L Mg^{-1}), across all germplasm sources. Of course, actual ethanol yield would depend on conversion process efficiency and response to differences in lignin concentration of alfalfa from the different management systems.

Table 6. Yield-adjusted mean concentrations of total cell wall polysaccharide and the hexose and pentose monosaccharide components of alfalfa stems as influenced by germplasm source and harvest management system.

Trait	Germplasm					Management system		
	MP2000	MWNC-4	New Europa	ORCA-WTS	$LSD_{0.05}$	Hay	Biomass	$LSD_{0.05}$
	g kg^{-1} dry matter							
Total polysaccharide	524	531	535	539	3	520	544	7
Glucose	284	290	294	299	3	282	300	7
Galactose	17.5	17.5	17.4	17.3	0.1	17.6	17.4	0.1
Mannose	18.2	18.5	18.6	18.6	0.1	18.2	18.7	0.2
Xylose	91	92	94	94	1	88	97	2
Arabinose	17.7	17.6	17.4	17.1	0.2	17.5	17.5	NS†

†NS, nonsignificant at the 0.05 probability level.

Use of an upper Midwest alfalfa variety developed for feeding livestock (MP2000) grown under a typical immature, frequent harvest hay management system resulted in yields of 1600 kg of hexose (glucose, galactose, and mannose) and 534 kg of pentose (xylose and arabinose) cell wall polysaccharide component monosaccharides ha^{-1} yr^{-1}. In contrast, the ORCA-WTS germplasm grown under the more mature, reduced harvest frequency biomass management system yielded 3079 kg hexose and 1030 kg pentose polysaccharide components ha^{-1} yr^{-1}. Both hexose and pentose yields were nearly doubled with the combined superior germplasm and biomass management system. Clearly, selection of appropriate germplasm and harvest management system will impact overall productivity and economic viability of a biofuels alfalfa production system. However, maximum efficiency of an alfalfa-based biofuels production system would be attained if the 669 kg ha^{-1} yr^{-1} of other monosaccharides (rhamnose, fucose, and uronic acids) in the stem cell wall polysaccharides could be fermented to ethanol as these carbohydrates represent 14% of the total cell wall polysaccharide yield. These other cell wall polysaccharide components are predominately components of pectin, found in nonlignified alfalfa stem tissues, which are rapidly and almost completely degraded by rumen bacteria (Hatfield and Weimer, 1995; Jung and Engels, 2001). If an ethanol-producing microorganism were available that could ferment rhamnose, fucose, and uronic acids, then these polysaccharide components should be efficiently converted to ethanol.

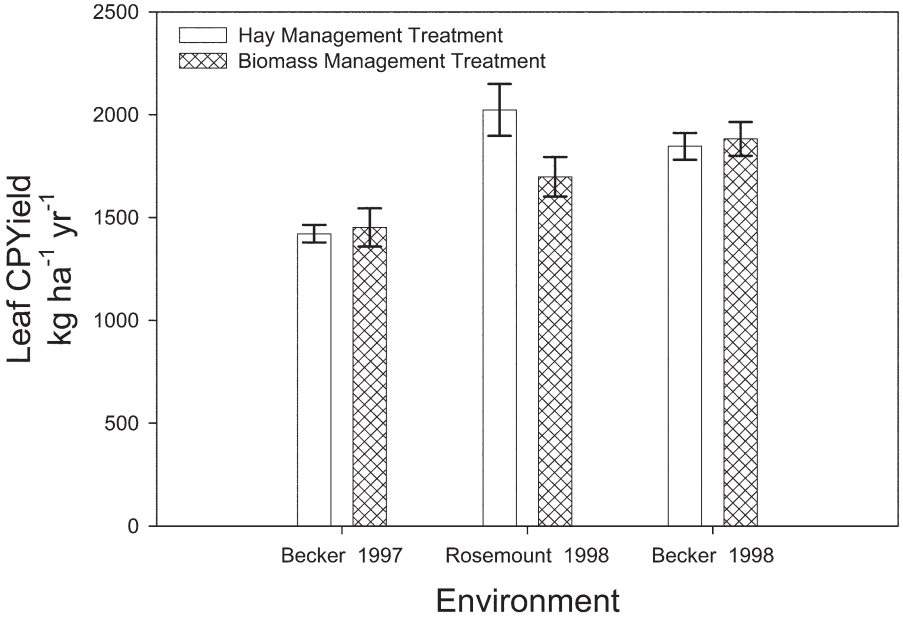

Figure 1. Leaf CP yield as affected by environment × management treatment interaction.

Theoretical ethanol yield from the combination of biomass alfalfa germplasm, ORCA-WTS, and biomass management system was 3048 L ha^{-1} yr^{-1}. This ethanol yield was 99% greater than the yield from the conventional alfalfa variety MP2000 (1533 L ha^{-1} yr^{-1}) harvested with a typical hay management system. The differences in stem cell wall concentration and composition for the ORCA-WTS germplasm grown under the biomass management system compared to MP2000 under the hay management system increased theoretical ethanol yield 11.4%. Therefore, the increased total DM (Lamb et al., 2003) and resultant cell wall polysaccharide yield under the biomass management system (Table 7), rather than altered biomass composition, was the driving force that accounted for the majority of enhanced ethanol yield observed for the combined biomass-type germplasm source and biomass management system.

Leaf CP

Leaf CP concentration was higher under the hay management treatment (294 ± 3 g kg^{-1}) compared to the biomass treatment (240 ± 4 g kg^{-1}). Because the biomass treatment was harvested at green pod and the hay treatment was harvested at early bud, our results concur with several previous reports that CP concentration of alfalfa forage declines with advancing maturity (Albrecht et al., 1987; Buxton et al., 1985; Fick and Holthausen, 1975; Griffin et al., 1994;

Table 7. Seasonal total cell wall polysaccharide and component monosaccharide yields of alfalfa stems as influenced by the germplasm source and harvest management.

Trait	Germplasm					Management system		
	MP2000	MWNC-4	New Europa	ORCA-WTS	$LSD_{0.05}$	Hay	Biomass	$LSD_{0.05}$
	kg ha^{-1} yr^{-1}							
Total polysaccharide	3492	3751	3919	3996	395	2941	4635	579
Glucose	1901	2055	2160	2220	211	1605	2561	328
Galactose	116	123	127	127	NS†	99	148	18
Mannose	121	130	136	137	14	103	159	20
Xylose	608	652	686	694	69	497	823	100
Arabinose	116	124	125	125	NS	97	148	17

†NS, nonsignificant at the 0.05 probability level.

Kalu and Fick, 1983; Kilcher and Heinrichs, 1974; Sheaffer et al., 1998, 2000). Yield-adjusted average leaf CP concentration was greater for MP2000 compared to the other three germplasm sources (272, 267, 265, and 263 g kg^{-1} [$LSD_{0.05}$ = 4] for MP2000, MWNC-4, New Europa and ORCA-WTS, respectively). Leaf CP yield was similar for all four germplasm sources (data not shown).

An environment × management treatment interaction impacted leaf CP yield (Table 4). Leaf CP yield was higher under the hay management system at Rosemount in 1998, and similar for both management treatments at Becker in 1997 and 1998 (Fig. 1). The main effect of management treatment for leaf CP yield was not significant in the analysis, implying that on average over the environments tested, leaf CP yields were similar between the two management treatments. However, this environment × management treatment interaction for leaf CP yield suggests environmental variability impacted both leaf yield and CP concentration at each harvest regardless of the management treatment. We speculate that the biomass management treatment may not produce the highest leaf CP yield in every environment but could produce comparable leaf CP yields to the hay management treatment in most environments. The biomass management treatment produced comparable leaf CP yields in two harvests compared to three or four harvests under the hay management treatment.

CONCLUSIONS

Management treatments had the largest impact on the concentration and yield of stem cell wall polysaccharides. The biomass management treatment had a 4% increase in total stem polysaccharide concentration and a 37% increase in total stem polysaccharide yield averaged over all germplasms compared to the hay management system. Cell wall material from the biomass management system was more lignified and contained more xylose, similar glucose, and less of the other polysaccharide components compared to material from the hay management system. Leaf CP concentration was lower under the biomass management, but leaf CP yield varied between the two harvest management treatments, depending on growth environment, but was not consistently lower for the biomass management system. Stem lignin composition and leaf CP yields were similar for all four germplasms, but stem glucose, mannose, and xylose DM concentrations and yields were greater in the biomass-type alfalfas than the hay-type alfalfas. The impact of altered stem cell wall composition and increased stem DM yield of a biomass-type alfalfa under a biomass system compared to a hay-type alfalfa under a hay system doubled the theoretical potential ethanol yield. Equivalent leaf protein yield, reduced harvesting costs, and potentially longer stand life under the less frequently harvested biomass management system would contribute additional economic efficiencies.

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