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Growth environment, harvest management and germplasm impacts on potential ethanol and crude protein yield in alfalfa

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ABSTRACT

An alfalfa (*Medicago sativa* L) biomass energy production system would produce two products. Leaves would be separated from stems to produce a protein feed for livestock while stems would be processed to produce ethanol. Therefore, maximum yields of both leaves and stems are essential for profitability of this biomass production system. Our objective was to evaluate the impact of growth environment (locations, years and plant density) and harvest maturity stage (early bud (4 annual cuts) and late flower (3 annual cuts)) on leaf crude protein and potential ethanol yields for four alfalfa germplasms, two with high forage quality, and two non-lodging biomass types. Potential ethanol yield was greater at late flower compared to early bud, while leaf crude protein concentration was similar at the two harvest maturity stages at both locations. Leaf crude protein yield was greater at the Minnesota (MN) site compared to Wisconsin (WI) site. The two non-lodging biomass germplasms had greater potential ethanol yield compared to the high forage quality cultivars in WI, but no differences among the alfalfa germplasms were found for ethanol yield at MN. In WI, no differences were found among the germplasms for leaf crude protein yield, but the high quality cultivars had greater leaf crude protein yield than the non-lodging germplasms in MN. While germplasm differences were found for leaf crude protein and potential ethanol yields, the environmental influences of harvest date and locations had the greatest impact on these two alfalfa biomass energy products.

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1. Introduction

A biomass energy production system using alfalfa (*Medicago sativa* L.) would fractionate the herbage into leaves and stems. The stems would be processed to generate biofuel (ethanol), and the leaves would be sold as a protein feed for livestock [1]. 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 for bioenergy production

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include maximizing seasonal yields of stems and leaves and increased concentrations of leaf protein and stem cell wall polysaccharides. In addition, the degree to which biomass is lignified is known to affect ethanol processing efficiency [2] therefore, alfalfa grown for biomass should contain as little lignin as possible.

Increasing stem yield in alfalfa can be as simple as harvesting alfalfa at later maturity stages than early bud, which is what is typically used in hay production [3–5]. Sheaffer et al. [3] showed that at the bud stage, alfalfa herbage as approximately half leaf and half stem. As plant maturity advanced, leaf-to-stem ratio or leaf concentration of the forage declined, and stem yield increased, but the changes in leaf yield per se at advanced maturity stages were not discussed. The decrease in whole plant forage crude protein and increased lignin concentration on a dry matter basis when plants are harvested at later maturity stages is well documented [3,6–14].

Increased alfalfa population density has been reported to increase whole plant forage yield [15–17]. Plant density has also been reported to influence stem quality characteristics in alfalfa [18]. Volenec et al. [17] reported alfalfa at higher plant densities had less lignin and was more digestible than alfalfa grown at lower plant densities. Lamb et al. [5] evaluated the same density treatments used in the current study and reported that decreased plant density in combination with delayed harvest maturity (maturity × density interaction) increased cell wall polysaccharide concentrations in alfalfa.

Variation for leaf and/or stem yield and forage quality for different genetic sources of alfalfa is well documented [3,19,20]. Marquez-Ortiz et al. [21] reported that individual stem diameter was heritable and controlled by additive genetic effects, and suggested that selection for larger stems in alfalfa was feasible. Flemish germplasms from southern Europe are a genetic source for large stem size [19]. Multifoliolate alfalfa types produce four or more leaflets per leaf rather than the normal three leaflet (trifoliolate) alfalfa leaf and have been reported to have greater leaf-to-stem ratios than trifoliate alfalfa cultivars [10,22,23]. Commercial alfalfa breeding companies have recently released non-lodging alfalfa cultivars with the objective of widening the harvest window (number of days) for growers to better manage the crop for maximum yield and hay quality. The USDA-ARS alfalfa breeding project at St. Paul, MN has recently developed an experimental alfalfa germplasm with large, erect, non-lodging stems with the intention of having a more productive alfalfa to use in a biofuel production system [23].

Our objective was to assess the impact of growth environment (locations, years, planting density) and harvest maturity stage (early bud and late flower) on the variability of herbage, stem, leaf, leaf crude protein and potential ethanol yields, and leaf-to-stem, stem pentose-to-hexose polysaccharide ratios, and leaf crude protein, stem Klason lignin, stem cell wall and potential ethanol concentrations between non-lodging biomass alfalfas and high forage quality alfalfas. We also investigated the interrelationships among these traits at each location over harvest maturity stages and alfalfa germplasms.

2. Materials and methods

2.1. Plant materials

Four alfalfa germplasms were selected to evaluate leaf crude protein and potential ethanol yield. Two cultivars, 4A421 and 6415, were chosen because they had been selected for improved forage quality. The cultivar 54H11 was included because it is the resistant standard check for Standability Expression (non-lodging) in alfalfa [24]. An experimental germplasm MN Bio I_{C3} created as a bioenergy alfalfa by three cycles for selection for large, erect non-lodging stems when the alfalfa was in bloom was also included.

2.2. Experimental design

The experimental design was a randomized complete block with three replicates in a split-split-split plot arrangement of the treatments, in which the whole plots were the two harvest maturity stages (early bud and late flower), the subplots were the two plant population densities (180 and 450 m^{-2}), and the four germplasms were the sub-subplots. The 180 m^{-2} plant density had 7.5 cm between plants in 0.9 × 3.0 m plots, and was seeded by hand with 2–3 seeds per hole and thinned to one plant per hole 15–20 d after seeding. The 450 m^{-2} plant density was mechanically seeded using a Plotman plot planter (Wintersteiger, Inc. Salt Lake City, Utah) at a rate of 11 kg ha^{-1} in 0.9 × 3.0 m plots with 5 rows drilled 12 cm apart. No changes occurred for target population densities of 180 and 450 m^{-2} for all four alfalfa germplasms over the three years of this study.

The experiment was planted at the Sand Plains Research Farm, Becker, MN (Latitude = 45.37, Longitude = 93.87); Hubbard loamy sand; sandy, mixed, Udorthentic Haploborall) on 25–26 May 2004 and at the University of Wisconsin Agricultural Experiment Station, Arlington WI (Latitude = 43.38, Longitude = 89.38); Plano silt loam fine-silty, mixed, superactive, mesic typic argiudolls) on 4–5 August 2004. The Arlington, WI site was rain fed, while the Becker, MN site was irrigated to meet plant moisture needs using the checkbook method [25]. Soil pH, P and K levels were adjusted to levels recommended for alfalfa production [26]. 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*).

2.3. Sampling procedure

The early bud stage plots were harvested four times in each growing season when 10–33% of the stems in the plot had flower buds and the late flower stage plots were harvested three times per season when there were at least two nodes per stem with open flowers. Sub-samples for forage quality analysis were taken by hand harvesting at least four grab samples per plot at a stubble height of 5 cm before harvesting the entire plot with a forage flail harvester. Early bud samples were harvested on 26 May, 26 June, 19 July, and 12 August 2005