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Forage Accumulation and Nutritive Value of Reduced Lignin and Reference Alfalfa Cultivars

Amanda M. Grev, M. Scott Wells,* Deborah A. Samac, Krishona L. Martinson, and Craig C. Sheaffer

ABSTRACT

Reduced lignin alfalfa (*Medicago sativa* L.) cultivars have the potential to increase the feeding value of alfalfa for livestock by improving forage fiber digestibility and to increase harvest management flexibility. The objectives were to compare the forage accumulation and nutritive value of reduced lignin and reference alfalfa cultivars when subject to diverse cutting treatments in the establishment and first production year. Research was established in 2015 at four locations in Minnesota. Reference alfalfa cultivars 54R02, DKA43–22RR, WL 355.RR, and the reduced lignin cultivar 54HVX41 were subject to cutting treatments with variable intervals between harvests. Cultivar by cutting treatment interactions were not significant ($P > 0.05$), but cultivar and cutting treatment effects were significant. Cultivars did not consistently differ in forage accumulation. Establishment year forage accumulation was greater when a fall harvest was taken, and first production year forage accumulation was generally greatest when alfalfa was harvested on a 40-d cutting schedule. Compared to reference alfalfa cultivars, 54HVX41 had an average of 8% less acid detergent lignin (ADL) and 10% greater neutral detergent fiber digestibility (NDFD) but was similar in crude protein (CP) and neutral detergent fiber (NDF) concentrations. Cutting treatments with shorter harvest intervals increased forage CP and NDFD and decreased NDF and ADL concentrations. With a 5-d harvest delay, 54HVX41 harvested on a 35-d harvest interval had a 21% gain in forage mass and a 3% reduction in relative forage quality (RFQ) compared to reference cultivars harvested on a 30-d harvest interval, which could allow for increased management flexibility.

Core Ideas

- Reduced lignin and reference cultivars did not differ in forage accumulation.
- Forage accumulation was greater with a fall harvest or a 40-d cutting schedule.
- Reduced lignin alfalfa averaged 8% less acid detergent lignin and 10% greater neutral detergent fiber digestibility.
- Cutting treatments with shorter harvest intervals increased forage nutritive value.
- Delaying reduced lignin alfalfa harvest increased forage mass and maintained quality.

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Alfalfa is widely used as forage for livestock due to its high nutrient content (Marita et al., 2003; Yu et al., 2003). However, the digestibility and utilization of alfalfa by these animals is hampered by its lignin content (Sewalt et al., 1997; Casler et al., 2002). Lignin is a complex structural polymer that is the second most abundant component of secondary plant cells walls (Li et al., 2015b), providing the strength and rigidity necessary for the plant to stand upright (Inoue et al., 1998; Guo et al., 2001a). As a plant matures, lignin concentration increases, filling the space between cellulose, hemicellulose, and pectin molecules and forming cross-linkages with hemicellulose (Albrecht et al., 1987; Jung et al., 1997b; Inoue et al., 1998; Casler and Vogel, 1999).

While it is essential for normal plant growth, the deposition of lignin into plant cell walls can reduce the feeding value of alfalfa by negatively affecting rumen microbial degradation and the digestion of feed by intestinal enzymes (Buxton and Hornstein, 1986; Liu and Yu, 2011). Lignification has been reported to be the major factor limiting both the in vitro digestibility of plant cell-wall polysaccharides (Morrison, 1979; Albrecht et al., 1987; Jung et al., 2012) and the in vitro dry matter digestibility (DMD) of whole plant forage (Casler, 1986, 1987; Reddy et al., 2005). These negative effects have primarily been associated with lignin concentration, as numerous studies have found strong negative correlations between lignin concentrations and forage digestibility (Albrecht et al., 1987; Casler, 1987; Jung et al., 1997a, 1997b; Reddy et al., 2005).

With such a strong influence on forage digestibility, small decreases in the lignin concentration of forages can be expected to improve the fiber digestibility at any plant maturity stage (Casler, 1987; Undersander et al., 2009). Predictions by Casler (1987) estimated that a single unit decrease ($g\ kg^{-1}$) in the concentration of ADL of smooth bromegrass (*Bromus inermis* L.) would result in a 7.0 unit increase in in vitro DMD. Feeding and grazing studies have shown that small changes in forage digestibility can significantly impact animal performance. For

A.M. Grev and K.L. Martinson, Univ. of Minnesota, Dep. of Animal Science, 1364 Eckles Ave, St. Paul, MN 55108; M.S. Wells and C.C. Sheaffer, Univ. of Minnesota, Dep. of Agronomy and Plant Genetics, 1991 Buford Circle, St. Paul, MN 55108; D.A. Samac, USDA-ARS, Plant Science Research Unit and Univ. of Minnesota, Dep. of Plant Pathology, 1991 Buford Circle, St. Paul, MN 55108. Received 26 Apr. 2017. Accepted 8 Aug. 2017. *Corresponding author (mswells@umn.edu).

Abbreviations: ADF, acid detergent fiber; ADL, acid detergent lignin; CCOMT, caffeoyl CoA 3-O-methyltransferase; COMT, caffeic acid 3-O-methyltransferase; CP, crude protein; DM, dry matter; DMD, dry matter digestibility; GDD, growing degree days; MSC, mean stage by count; NDF, neutral detergent fiber; NDFD, neutral detergent fiber digestibility; NIRS, near infrared reflectance spectroscopy; RFQ, relative forage quality.

a number of grass cultivars, Casler and Vogel (1999) reported a positive relationship between in vitro DMD improvement and animal daily gains, with a 1% increase in in vitro DMD resulting in a 3.2% increase in daily weight gains for beef cattle.

Several experimental lines of alfalfa have been developed with downregulation of the caffeic acid 3-O-methyltransferase (COMT) and caffeoyl CoA 3-O-methyltransferase (CCOMT) lignin biosynthetic genes (Inoue et al., 1998; Guo et al., 2001a; Marita et al., 2003; Getachew et al., 2011). Experimental populations of COMT and/or CCOMT downregulated alfalfa have shown a 4 to 29% decrease in stem lignin concentration and a 1 to 24% decrease in herbage lignin concentration compared to reference alfalfa cultivars (Guo et al., 2001a; 2001b; Marita et al., 2003; Reddy et al., 2005; Undersander et al., 2009; Getachew et al., 2011). The wide variation in lignin reduction reported could be due to the specific downregulated gene (Guo et al., 2001a, 2001b; Marita et al., 2003; Undersander et al., 2009; Getachew et al., 2011), the methods used for lignin analysis (Guo et al., 2001a; 2001b; Jung et al., 2012), or the plant growing conditions (Baucher et al., 1999).

Populations of reduced lignin alfalfa have shown an increase in in vitro DMD, in situ rumen digestibility, and in vitro NDFD (Guo et al., 2001b; Reddy et al., 2005; Mertens and McCaslin, 2008; Weakley et al., 2008; Undersander et al., 2009; Getachew et al., 2011). Reddy et al. (2005) reported a strong negative linear relationship between in situ digestibility and ADL levels across all reduced lignin lines. In addition to increased digestibility, reduced lignin alfalfa populations have also shown reduced NDF concentrations and greater non-fiber carbohydrate concentrations compared to control lines (Guo et al., 2001b; Reddy et al., 2005; Getachew et al., 2011; Li et al., 2015a), while CP concentrations remained similar for reduced lignin and reference alfalfa lines (Getachew et al., 2011; Li et al., 2015a).

Recently released reduced lignin alfalfa cultivars have potential to increase the digestibility of alfalfa forage compared to reference cultivars (Guo et al., 2001a, 2001b; Marita et al., 2003; Reddy et al., 2005; Getachew et al., 2011). These improvements in alfalfa forage nutritive value may lengthen the time period when alfalfa has a forage nutritive value suitable for high-producing livestock. This could allow for a wider optimal harvest window, making it possible for alfalfa growers to achieve greater forage accumulation by delaying alfalfa harvest while still maintaining acceptable forage nutritive value (Undersander et al., 2009).

Research with experimental populations of reduced lignin alfalfa has shown their potential to improve forage digestibility. However, field evaluations under diverse conditions are needed to determine the performance of new commercial alfalfa cultivars containing the reduced lignin trait, especially with regard to forage accumulation and nutritive value under different harvest frequencies. The objectives of this study were to evaluate forage accumulation and nutritive values of reduced lignin and reference alfalfa cultivars when subject to diverse cutting treatments during the establishment and first production year.

MATERIALS AND METHODS

Research was conducted at the University of Minnesota Agricultural Experiment Stations at St. Paul, Becker, Rosemount, and Rochester, MN, in 2015, and continued at St. Paul, Becker, and Rosemount, MN, in 2016. Similar establishment year results across locations combined with a lack of resources resulted in the exclusion of the Rochester location from the 2016 experiment. The soil was a Waukegan silt loam (fine-silty over sandy or sandy-skeletal, mixed, superactive, mesic Typic Hapludoll) at St. Paul (44°59′14″ N, 93°10′24″ W, elevation 291 m), a Hubbard–Mosford complex (sandy, mixed, frigid Entic Hapludoll) at Becker, MN (45°23′13″ N, 93°53′18″ W, elevation 290 m), a Port Byron silt loam (fine-silty, mixed, superactive, mesic Typic Hapludoll) at Rosemount, MN (44°41′16″ N, 93°04′21″ W, elevation 288 m), and a Marshan silt loam (fine-loamy over sandy or sandy-skeletal, mixed, superactive, mesic Typic Endoaquoll) at Rochester, MN (44°00′46″ N, 92°25′02″ W, elevation 317 m).

Monthly mean air temperature and precipitation data were collected for each location and year. Mean daily air temperature for the 2015 and 2016 growing seasons (May through October) was similar to the 30-yr average except for September and October, which tended to be warmer than normal (Fig. 1A–1B). Total rainfall during the 2015 and 2016 growing seasons (May through October) fell between 56 and 78 cm and was greater compared to the 30-yr average, which ranged from 51 to 62 cm across locations (Fig. 1A–1B). Seasonal rainfall was not evenly distributed and varied greatly across location, month, and year.

All sites were planted between 27 and 30 Apr 2015. Inoculated seed was seeded into a prepared seedbed at a rate of 18.8 kg ha^{-1} in plots measuring 0.9 by 6.1 m. Soil fertility at each site was amended to meet recommendations for alfalfa hay production according to University of Minnesota fertility guidelines (Kaiser et al., 2011). In the establishment year, weeds were controlled using a single application of glyphosate [N-(phosphonomethyl)glycine] applied at a rate of 2.34 L a.i. ha^{-1}; additional weed control was not required during the first production year. Potato leafhopper (*Empoasca fabae*) was controlled using Arctic 3.2 EC [(m-Phenoxybenzyl)-*cis*,trans-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate] as needed when potato leafhopper exceeded threshold populations, which generally occurred each July (Cancelado and Radcliffe, 1979; Chasen et al., 2015).

At all sites, the experimental design was a randomized complete block with five replicates and a split-plot arrangement of treatments. Whole plots were four cutting treatments with varying harvest frequencies. In 2015, cutting treatments began a minimum of 60 d following seeding and included "Standard" (60d + 30d + 30d), "Standard + Fall" (60d + 30d + 30d + Fall), "Standard + Delay" (60d + 37d + 37d), and "Delay + Fall" (67d + 45d + Fall). A first harvest 60 d following seeding is a recommended practice to promote the establishment of alfalfa (Sheaffer et al., 1988). A 30-d cutting interval was chosen to represent the standard for high quality alfalfa production in the northern Midwest, while the 37- and 45-d cutting intervals were chosen to represent alfalfa production for maximum forage accumulation and persistence (Undersander et al., 2011), as well as to test the effects of reduced lignin alfalfa grown under a delayed harvest schedule. A fall cut refers to an alfalfa harvest taken around the first week of October in the northern Midwest (Undersander et al., 2011). Fall cuts are a common method used to increase seasonal forage accumulation in the northern Midwest; however, a fall cut will often reduce stand persistence

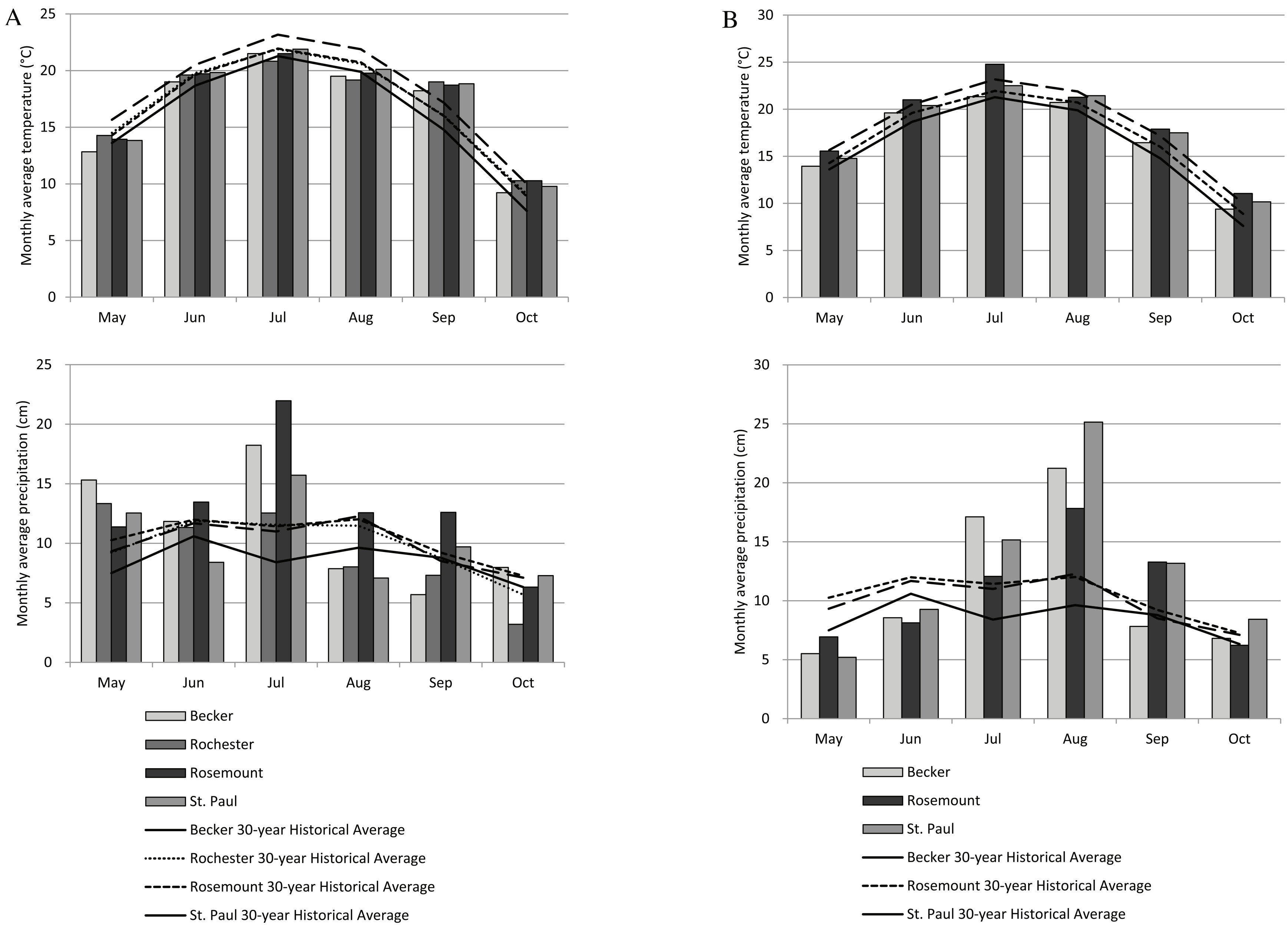

Fig. 1. Monthly air temperature (°C), precipitation (cm), and 30-yr historical average for Becker, Rochester, Rosemount, and St. Paul, MN, during the (A) 2015 and (B) 2016 growing season. Weather data was obtained from http://mrcc.isws.illinois.edu/.

and forage mass the following spring (Undersander et al., 2011; Wells et al., 2014). For these reasons, a fall cut was included for two of the 2015 cutting treatments to test the effects of a fall harvest on reduced lignin alfalfa cultivars. In 2016, the first cutting occurred on the same day for all treatments to determine the effect of establishment year cutting treatments on forage mass and to asses any potential winter injury. Results from the 2016 first cut showed a slight reduction in forage mass for the more intensive Standard + Fall establishment year cutting treatment at some locations (see forage accumulation results for further details). As a result, the plots in the 2015 Standard + Fall cutting treatment continued to be harvested under a more intensive 30-d cutting schedule in 2016 to investigate the potential effects of a more intense harvest schedule. No differences in forage mass were found among the rest of the 2015 cutting treatments; therefore, the remaining 2016 cutting treatments were randomly assigned to plots. Cutting treatments in 2016 included "30-d" (30d + 30d + 30d + Fall), "35-d" (35d + 35d + 35d + Fall), "40-d" (40d + 40d + Fall), and "45-d" (45d + 45d + Fall). Similar to the establishment year, a range of cutting intervals was chosen to test the effects of reduced lignin alfalfa when grown for a variety of production goals. Harvest dates for cutting treatments within each location and year are shown in Table 1. Subplots were four alfalfa cultivars, which included the reference alfalfa cultivars 54R02, DKA43-22RR, WL 355.RR, and the reduced lignin cultivar 54HVX41. All alfalfa cultivars were marketed as Round-up Ready [*N*-(phosphonomethyl)glycine] and were rated as Fall Dormancy 4 cultivars.

To determine plant maturity and forage nutritive value, random duplicate samples were hand-harvested from non-border rows within each plot to a stubble height of 5 cm. Samples were weighed to determine wet weight. One sample from each plot was used to evaluate alfalfa maturity using the mean stage by count (MSC) method developed by Kalu and Fick (1981), where vegetative growth included stages 0 through 2, budding plants included stages 3 and 4, and flowering plants included stages 5 and 6. The other sample from each plot was dried in forced-air ovens for 48 h at 60°C and weighed for dry matter (DM) determination. Dried samples were ground through a 6-mm screen in a Wiley mill (Thomas Scientific, Swedesboro, NJ) followed by a 1-mm screen in a Cyclotec (Foss, Hillerød, Denmark). Samples were mixed thoroughly and scanned under near infrared reflectance spectroscopy (NIRS) using a Perten NIRS (Model DA 7200; Perten Instruments, Springfield, IL) with calibration equations developed in Minnesota to estimate forage nutritive value for CP, NDF, ADL, and NDFD. The standard error of cross validation was 0.98, 1.98, 1.52, and 2.64, respectively, for CP, ADL, NDF, and NDFD, while the R^2 was 0.98, 0.80, 0.86, and 0.87, respectively. Wet chemistry procedures were as follows: CP (N × 6.25; AOAC, 2010); NDF and ADL (Goering and Van Soest, 1970; Van Soest et al., 1991); and NDFD (Hoffman et al., 1993). Forage nutritive value parameters from the NIRS analysis were used to calculate RFQ using the equations provided by Moore and Undersander (2002) to provide a relative measure of forage quality.

Following the hand-sampling, alfalfa plot forage masses were determined by mechanically harvesting a 0.9 by 5.2 m strip using a flail harvester (Carter Manufacturing Company Inc., Brookston, IN) set to leave a 5-cm stubble. Mechanically harvested samples were weighed, and hand-sample wet weights were added to calculate a total plot wet weight for DM forage mass determination. After each harvest was complete, stand density was assessed via stem counts, which were measured as the number of green stems (≥2.5 cm in length) along a 0.3-m section in non-border rows in two locations within each harvested plot (Smith et al., 1989). In the fall of each year, plant densities were measured in two locations within each plot using a frequency grid (Vogel and Masters, 2001).

Data were analyzed using the MIXED procedure of SAS (version 9.4; SAS Institute Inc., Cary, NC). Individual plots comprised the experimental unit, and statistical significance was set at $P \leq 0.05$. Due to management differences (i.e., cutting treatments), analysis of the establishment year (2015) and first production year (2016) was completed separately. Differences among environments resulted in significant interactions between location and cutting treatment; therefore, locations were analyzed and reported separately. Replicate was considered a random effect; cutting treatment and cultivar were designated as fixed effects. Within each year and location, forage masses are reported as seasonal cumulative forage accumulation, and forage nutritive values are reported for the mid-season harvests (excluding the first and fall cut). The first harvest of each year was excluded because harvest for all plots occurred on the same date and initiated the different cutting treatments. The fall cut was excluded because it did not correspond to a specific harvest frequency or follow cutting treatment schedules. Means separations were performed on significant effects using Tukey's HSD test. Variables analyzed included maturity (MSC), forage accumulation, CP, NDF, ADL, and NDFD. To assess the relationship between plant maturity and forage nutritive value, Pearson correlation coefficients were calculated between MSC and the forage nutritive

Table 1. Alfalfa harvest schedule for 2015 and 2016 growing seasons in Becker, Rochester, Rosemount, and St. Paul, MN.

Cutting treatment	Cutting interval	Cut 1	Cut 2	Cut 3	Cut 4	Fall cut
			2015 Harvest dates			
Standard	60, 30, 30	25 June–2 July	23 July–30 July	18 Aug–27 Aug.	–	–
Standard + Fall	60, 30, 30, Fall	25 June–2 July	23 July–30 July	18 Aug.–27 Aug.	–	2 Oct.–7 Oct.
Standard + Delay	60, 37, 37	25 June–2 July	31 July–5 Aug.	8 Sept.–11 Sept.	–	–
Delay + Fall	67, 45, Fall	1 July–10 July	14 Aug. –27 Aug.	–	–	2 Oct.–7 Oct.
			2016 Harvest dates			
30-d	30, 30, 30, Fall	23 May-26 May	20 June–23 June	18 July–20 July	15 Aug–17 Aug	30 Sept.–6 Oct.
35-d	35, 35, 35, Fall	23 May–26 May	27 June–30 June	1 Aug.–3 Aug.	7 Sept.–8 Sept.	30 Sept.–6 Oct.
40-d	40, 40, Fall	23 May–26 May	5 July–7 July	15 Aug.–17 Aug.	–	30 Sept.–6 Oct.
45-d	45, 45, Fall	23 May–26 May	8 July–14 July	22 Aug.–30 Aug.	–	30 Sept.–6 Oct.

values for CP, NDF, ADL, and NDFD using the CORR procedure of SAS (version 9.4; SAS Institute Inc., Cary, NC).

Cumulative growing degree days (GDD, $T_{base} = 5.6°C$) were tracked and reset between each cutting interval for all sites during the 2016 growing season. The number of GDD accumulated at harvest in 2016 averaged across cuttings and locations were 832, 1035, and 1248 GDD for the 30-, 35-, and 40-d cutting treatments, respectively. Due to the large decrease in RFQ for the 45-d cutting treatment, the 45-d cutting treatment was excluded to better reflect high quality alfalfa production management. Cumulative GDD were utilized to further explore the relationships between alfalfa harvest frequency (i.e., cutting treatments) and forage mass, as well as between alfalfa harvest frequency and RFQ. The REG procedure in SAS and the Akaike's Information Criterion (AIC) were used to aid in model selection comparing linear and quadratic models that best predict forage mass and RFQ (Burnham and Anderson, 2002; version 9.4; SAS Institute Inc., Cary, NC). Quadratic models with cumulative GDD as the predictor variable were selected to best predict forage mass. Forage nutritive value parameters from the NIRS analysis were applied to the RFQ equation and the RFQ estimates were regressed on cumulative GDD. The quadratic model with MSC as a covariate was determined to best predict RFQ.

RESULTS AND DISCUSSION

For maturity, forage accumulation, and forage nutritive values, statistical analysis indicated no significant interactions ($P > 0.05$) between cutting treatment and cultivar; therefore, only the main effects of cutting treatment and cultivar were reported. Data for cutting treatments were averaged across cultivars, and data for cultivars were averaged across cutting treatments.

Maturity

Cultivar Response

Maturities differed among cultivars (Table 2), although differences were minimal and inconsistent. In 2015, there were no differences in maturity among cultivars at Rosemount. At Becker, Rochester, and St. Paul, 54HVX41 was among the least mature (MSC ≤ 2.6), while reference cultivars were among the most mature. In 2016, 54HVX41 was less mature (MSC = 3.5) compared to DKA43-22RR (MSC = 3.9) at Rosemount. At Becker and St. Paul, cultivars had similar maturities. Maturities for all cultivars were within normal ranges, ranging from the late vegetative to early bud stage in 2015, and from the early bud to early flower stage in 2016 (Kalu and Fick, 1981).

Cutting Treatment Response

Maturities differed among cutting treatments (Table 2). In 2015, plants subject to the Standard and Standard + Fall cutting treatments were the least mature (MSC ≤ 2.1), while plants in the Delay + Fall cutting treatment were the most mature (MSC ≥ 3.1). In 2016, plants in the 30-d cutting treatment were the least mature (MSC ≤ 3.2), while plants in the 40- and 45-d cutting treatments were the most mature (MSC ≥ 4.4). The increase in maturity with increasingly delayed cutting treatments was expected, as a wider harvest interval allows for further growth and maturation.

Forage Accumulation

Cultivar Response

Forage accumulation differed among alfalfa cultivars but was not consistent across locations (Table 3). Forage accumulation differences were more pronounced during the establishment year (2015) than during the first production year (2016). In 2015

Table 2. Average stage of maturity across multiple cuts for alfalfa grown in Becker (BE), Rochester (ROC), Rosemount (ROS), and St. Paul (SP), MN in 2015 and 2016 as determined by cultivar and cutting treatment.

Treatment	2015				2016		
	BE	ROC	ROS	SP	BE	ROS	SP
	Maturity index†						
	Alfalfa cultivar						
54HVX41	2.5b‡	1.7b	1.9	2.6b	4.0	3.5b	4.6
54R02	2.9a	2.1a	2.1	2.8ab	4.0	3.6ab	4.5
DKA43-22RR	2.7ab	2.1a	2.1	3.0a	4.0	3.9a	5.0
WL 355.RR	2.9a	2.2a	2.1	2.8ab	4.0	3.7ab	4.9
SE	0.09	0.09	0.12	0.08	0.10	0.12	0.15
	2015 Cutting treatment						
Standard§	2.1c	1.5c	1.4c	2.0c	–	–	–
Standard + Fall	2.1c	1.5c	1.4c	2.0c	–	–	–
Standard + Delay	2.7b	2.0b	1.9b	2.8b	–	–	–
Delay + Fall	4.1a	3.1a	3.5a	4.3a	–	–	–
SE	0.09	0.09	0.12	0.08	–	–	–
	2016 Cutting treatment						
30-d	–	–	–	–	2.8c	2.3d	3.2d
35-d	–	–	–	–	3.5b	3.0c	4.4c
40-d	–	–	–	–	4.7a	4.4b	5.4b
45-d	–	–	–	–	4.9a	5.1a	6.0a
SE	–	–	–	–	0.10	0.12	0.15

† Numerical index referring to stage of alfalfa development (Kalu and Fick, 1981). Vegetative growth includes Stages 0 through 2, budding plants includes Stages 3 and 4, and flowering plants includes Stages 5 and 6.

‡ Within column and section, means without a common letter differ based on a Tukey's HSD test ($P \leq 0.05$).

§ 2015 cutting treatments included Standard (60d + 30d + 30d), Standard + Fall (60d + 30d + 30d + Fall), Standard + Delay (60d + 37d + 37d), and Delay + Fall (67d + 45d + Fall).

at Becker and Rosemount, DKA43-22RR and WL 355.RR were among the cultivars with the greatest forage accumulation (≥7.3 Mg ha^{-1}), while 54HVX41 was among those with the least forage accumulation (≤7.0 Mg ha^{-1}). At Rochester, 54HVX41 had decreased forage accumulation (5.8 Mg ha^{-1}) compared to all reference cultivars (≥6.6 Mg ha^{-1}). At St. Paul, forage accumulation was similar for all alfalfa cultivars. In 2016, only the Becker location resulted in seasonal cumulative forage accumulation differences. At Becker, 54HVX41 had a decreased forage accumulation (15.0 Mg ha^{-1}) compared to all reference cultivars (15.9 Mg ha^{-1}).

To the best of our knowledge, this is the first public study comparing forage accumulation between reduced lignin and reference alfalfa cultivars under diverse cutting schedules. Forage accumulation for 54HVX41 tended to be decreased compared to reference cultivars at some locations in the establishment year. However, fewer differences in forage accumulation were observed during the first production year, indicating that forage accumulation differences between 54HVX41 and reference alfalfa cultivars could be minimal following establishment. Forage accumulations ranged from 5.8 to 9.3 Mg ha^{-1} in the establishment year and from 15.0 to 20.8 Mg ha^{-1} in the first production year and are comparable to previously reported alfalfa forage accumulations. In Wisconsin, establishment year forage accumulations for University variety trials ranged from 2.7 to 16.1 Mg ha^{-1} for alfalfa planted between 2013 and 2015 (Undersander, 2016). First production year forage accumulations ranged from 8.5 to 23.8 Mg ha^{-1} and from 11.2 to 25.6 Mg ha^{-1} in University variety trials planted between 2013 and 2015 in Minnesota and Wisconsin, respectively (Sheaffer et al., 2016; Undersander, 2016).

Cutting Treatment Response

Forage accumulation differed among cutting treatments (Table 3). In 2015, the Standard + Fall and Delay + Fall cutting treatments were among those with the greatest forage accumulation (≥6.7 Mg ha^{-1}), while the Standard cutting treatment was among those with the least forage accumulation. In 2016, the 40-d cutting treatment was among those with the greatest forage accumulation (≥16.3 Mg ha^{-1}), while the 30- and 45-d cutting treatments were among those with the least forage accumulation. These results demonstrate that alfalfa has high forage accumulation potential in the both the establishment and first production year, and that forage accumulations were affected by harvest scheduling.

During the establishment year, forage accumulations were improved when a fall cut was added. During the first production year, alfalfa forage accumulations were greater with the 40-d cutting treatment compared to the 30-d cutting treatment. These results suggest that adding a fall harvest or using a cutting schedule with a longer interval between harvests can result in greater forage accumulation compared to a traditional 30-d cutting system or one without a fall harvest. Compared to the traditional 30-d cutting system, a 40-d harvest interval also offered the advantage of reducing the number of cuts per season from five to four while still producing greater forage accumulation.

Previous research has also concluded that as the interval between harvests increased, annual DM forage accumulation increased (Brink and Marten, 1989; Kallenbach et al., 2002; Putnam et al., 2005; Probst and Smith, 2011; Min, 2016). In Missouri, Kallenbach et al. (2002) reported that alfalfa harvested four times per year produced 7 and 28% more than when harvested five or six times per year, respectively. Similarly, Min (2016) reported increasing alfalfa forage accumulation as cutting intervals increased from 28 to 42 d in Kansas. However, there is a point

Table 3. Seasonal cumulative forage accumulation for alfalfa grown in Becker (BE), Rochester (ROC), Rosemount (ROS), and St. Paul (SP), MN, in 2015 and 2016 as determined by cultivar and cutting treatment.

Treatment	2015 BE	2015 ROC	2015 ROS	2015 SP	2016 BE	2016 ROS	2016 SP
	Mg ha^{-1}						
Alfalfa cultivar							
54HVX41	7.0b†	5.8b	6.9b	8.6	15.0b	16.2	20.1
54R02	7.2b	6.8a	7.2ab	8.7	15.9a	16.8	20.5
DKA43-22RR	7.6ab	6.9a	7.4a	9.3	15.9a	16.5	20.8
WL 355.RR	7.9a	6.6a	7.3ab	8.8	15.9a	16.5	20.6
SE	0.31	0.29	0.44	0.34	0.31	0.30	0.33
2015 Cutting treatment							
Standard‡	6.5b	5.8b	6.3b	7.6b	–	–	–
Standard + Fall	7.7a	7.1a	7.1ab	9.7a	–	–	–
Standard + Delay	7.3ab	6.5ab	7.2ab	8.2b	–	–	–
Delay + Fall	8.2a	6.7a	8.1a	9.8a	–	–	–
SE	0.35	0.32	0.56	0.34	–	–	–
2016 Cutting treatment							
30-d	–	–	–	–	15.1b	15.5b	19.6b
35-d	–	–	–	–	16.6a	16.7ab	20.5b
40-d	–	–	–	–	16.3a	17.5a	21.9a
45-d	–	–	–	–	14.7b	16.2ab	20.1b
SE	–	–	–	–	0.31	0.40	0.33

† Within column and section, means without a common letter differ based on a Tukey's HSD test ($P \leq 0.05$).

‡ 2015 cutting treatments included Standard (60d + 30d + 30d), Standard + Fall (60d + 30d + 30d + Fall), Standard + Delay (60d + 37d + 37d), and Delay + Fall (67d + 45d + Fall).

where increasing the interval between harvests no longer results in increased forage accumulation. Delayed harvests beyond early flowering can reduce forage accumulation as a result of leaf loss from lower portions of the canopy (Sheaffer et al., 1988). In the present study, the 45-d cutting treatment had the longest harvest interval but was among the cutting treatments with the least forage accumulation. Min (2016) also observed that alfalfa DM forage accumulation decreased by approximately 16% when alfalfa was harvested every 49 d compared to every 42 d.

To clarify the effects of establishment year (2015) cutting treatments on first cut forage masses the following spring (2016), a uniform harvest date was applied over all cutting treatments for the first cut in 2016. Cultivar forage masses for the first cut in 2016 did not differ based on establishment year (2015) cutting treatments (data not shown). However, establishment year (2015) cutting treatments had minor effects on first cut forage masses within the first production year (2016; data not shown). At St. Paul, there was no effect of 2015 cutting treatments on first cut forage masses in 2016. At Becker, 2016 first cut forage masses were reduced following the Standard + Fall (4.1 Mg ha^{-1}) cutting treatment compared to the Standard cutting treatment (4.6 Mg ha^{-1}). At Rosemount, 2016 first cut forage masses were decreased following the Standard + Fall cutting treatment (5.0 Mg ha^{-1}) compared to all other cutting treatments (≥5.4 Mg ha^{-1}). These results suggest that the more intensive Standard + Fall establishment year cutting treatment may have had negative impacts on first cut forage masses in 2016; however, results were inconsistent across locations and further research is needed for definite conclusions to be drawn.

Forage Nutritive Value

Crude Protein

Cultivar Response. Crude protein concentrations differed among alfalfa cultivars only at the Becker location (Table 4). In 2015, CP concentrations were greater for 54HVX41 (230 g kg^{-1}) compared to 54R02 and DKA43-22RR (≤224 g kg^{-1}). In 2016, CP concentrations were greater for 54HVX41 (187 g kg^{-1}) compared to WL 355.RR (178 g kg^{-1}).

Across locations and years, CP concentrations for all cultivars ranged from 175 to 234 g kg^{-1} and are comparable to previously reported values for alfalfa (Hall et al., 2000; Kallenbach et al., 2002; Palmonari et al., 2014; Min, 2016). Previous studies examining reduced lignin alfalfa experimental lines also found similar CP concentrations for reduced lignin alfalfa compared to reference alfalfa cultivars (Weakley et al., 2008; Getachew et al., 2011; Li et al., 2015a).

Cutting Treatment Response. Crude protein concentrations differed among cutting treatments at all locations (Table 5). In 2015, CP concentrations were greatest for the Standard and Standard + Fall cutting treatments (≥230 g kg^{-1}) and least for the Standard + Delay and Delay + Fall cutting treatments (≤226 g kg^{-1}). In 2016, CP concentrations were greatest for the 30-d cutting treatment (≥215 g kg^{-1}) and least for the 40- and 45-d cutting treatments (≤163 g kg^{-1}).

More frequent cutting schedules with shorter intervals between harvests resulted in greater CP concentrations. This agrees with findings from previous studies examining the CP content of alfalfa under various harvest frequencies. It has

Table 4. Crude protein, neutral detergent fiber, acid detergent lignin, and neutral detergent fiber digestibility for alfalfa cultivars grown in Becker (BE), Rochester (ROC), Rosemount (ROS), and St. Paul (SP), MN, in 2015 and 2016.

Treatment	2015 BE	2015 ROC	2015 ROS	2015 SP	2016 BE	2016 ROS	2016 SP
	g kg^{-1}						
				Crude protein			
54HVX41	230a†	218	226	234	187a	186	182
54R02	220b	211	221	226	180ab	182	178
DKA43-22RR	224b	212	224	228	179ab	186	175
WL 355.RR	225ab	215	227	230	178b	184	178
SE	0.16	0.27	0.30	0.32	0.24	0.34	0.25
				Neutral detergent fiber			
54HVX41	387c	388	351	351	441b	403	416
54R02	408a	403	359	364	456ab	414	424
DKA43-22RR	401ab	399	357	363	458a	405	430
WL 355.RR	396bc	389	346	356	452ab	407	419
SE	0.31	0.48	0.62	0.67	0.44	0.68	0.57
				Acid detergent lignin			
54HVX41	74c	74b	67b	65b	81b	79b	77b
54R02	86a	83a	73a	74a	87a	85a	81a
DKA43-22RR	84ab	83a	71a	73a	88a	84a	83a
WL 355.RR	83b	81a	71a	73a	88a	85a	82a
SE	0.08	0.10	0.12	0.11	0.08	0.14	0.12
				Neutral detergent fiber digestibility			
54HVX41	443a	391a	447	453a	333a	333	339a
54R02	397b	353b	429	419b	288b	300	306b
DKA43-22RR	404b	359b	428	418b	282b	305	305b
WL 355.RR	402b	365b	436	419b	278b	301	306b
SE	0.68	0.61	0.79	0.41	0.69	1.05	0.81

† Within column and section, means without a common letter differ based on a Tukey's HSD test ($P \leq 0.05$).

been well-documented that CP content declines as harvest intervals are lengthened (Weir et al., 1960; Nordkvist and Åman, 1986; Hall et al., 2000; Kallenbach et al., 2002; Yu et al., 2003; Palmonari et al., 2014; Min, 2016). In Kansas, Min (2016) reported that delaying a 28-d harvest by 5 or 18 d reduced CP concentrations by 4 and 6%, respectively. In Missouri, Kallenbach et al. (2002) found that CP levels averaged 250 g kg^{-1} when alfalfa was harvested six times per year compared to 227 and 195 g kg^{-1} when harvested five or four times, respectively. Hall et al. (2000) and Yu et al. (2003) both also reported declines in CP concentrations with advancing morphological development across multiple harvests.

In the present study, plant maturity (MSC) was negatively associated with CP concentrations at all locations in both the establishment (2015) and first production (2016) year. In the establishment year, correlation coefficients were −0.77, −0.77, −0.82, and −0.52 for Becker, Rochester, Rosemount, and St. Paul, respectively. In the first production year, correlation coefficients were −0.65, −0.78, and −0.68 for Becker, Rosemount, and St. Paul, respectively. This decline in CP concentration with progressing plant maturity can be attributed to the associated effects of increasing stem proportions and decreasing leaf proportions on forage nutritive value as the plant matures (Kalu and Fick, 1983; Nordkvist and Åman, 1986; Albrecht et al., 1987; Sanderson and Wedin, 1988; Sheaffer et al., 2000). As plants mature, defoliation increases as leaf senescence and abscission occurs in the lower, shaded portions of the plant, further contributing to the loss in CP (Albrecht et al., 1987; Sheaffer et al., 1988; Undersander et al., 2011). Although leaf loss was not measured in the present experiment, the research team did observe some leaf loss from the lower portions of alfalfa stems under the 40- and 45-d cutting treatments.

Neutral Detergent Fiber

Cultivar Response. Concentrations of NDF differed among alfalfa cultivars only at the Becker location (Table 4). In 2015, NDF concentrations were less for 54HVX41 (387 g kg^{-1}) compared to 54R02 and DKA43-22RR (≥401 g kg^{-1}). In 2016, NDF concentrations were less for 54HVX41 (441 g kg^{-1}) compared to DKA43-22RR (458 g kg^{-1}).

Across locations and years, NDF concentrations for all cultivars ranged from 346 to 458 g kg^{-1} and are comparable to reports from previous studies (Hall et al., 2000; Kallenbach et al., 2002; Palmonari et al., 2014; Min, 2016). Studies investigating reduced lignin alfalfa experimental lines have also found similar or slightly reduced NDF concentrations for reduced lignin alfalfa compared to reference cultivars (Guo et al., 2001b; Getachew et al., 2011). Similarly, preliminary results evaluating reduced lignin alfalfa hay found either no change (Mertens and McCaslin, 2008) or decreased (Li et al., 2015a) NDF concentrations for reduced lignin cultivars compared to controls.

Table 5. Crude protein, acid detergent fiber, neutral detergent fiber, acid detergent lignin, and neutral detergent fiber digestibility for alfalfa grown in Becker (BE), Rochester (ROC), Rosemount (ROS), and St. Paul (SP), MN, under various cutting treatments in 2015 and 2016.

	2015				2016			
Treatment	BE	ROC	ROS	SP	Treatment	BE	ROS	SP
	g kg^{-1}					g kg^{-1}		
	Crude protein					Crude protein		
Standard†	242a‡	230a	239a	253a	30-d	215a	221a	215a
Standard + Fall	242a	238a	236a	248a	35-d	188b	199b	187b
Standard + Delay	219b	215b	226b	209b	40-d	163c	161c	157c
Delay + Fall	195c	173c	197c	208b	45-d	160c	158c	153c
SE	0.16	0.30	0.30	0.32	SE	0.24	0.34	0.27
	Neutral detergent fiber					Neutral detergent fiber		
Standard	367c	369bc	321c	331b	30-d	391d	343d	366c
Standard + Fall	371c	352c	324c	336b	35-d	426c	368c	408b
Standard + Delay	390b	373b	359b	373a	40-d	483b	444b	446a
Delay + Fall	464a	486a	408a	395a	45-d	507a	473a	469a
SE	0.31	0.48	0.61	0.69	SE	0.44	0.68	0.65
	Acid detergent lignin					Acid detergent lignin		
Standard	80b	77b	64c	68c	30-d	79b	76b	75b
Standard + Fall	78b	76b	65c	69bc	35-d	82b	73b	77b
Standard + Delay	80b	71c	71b	72ab	40-d	92a	90a	83a
Delay + Fall	90a	95a	84a	76a	45-d	93a	94a	87a
SE	0.08	0.11	0.14	0.12	SE	0.08	0.14	0.15
	Neutral detergent fiber digestibility					Neutral detergent fiber digestibility		
Standard	436a	396ab	476a	454a	30-d	362a	389a	373a
Standard + Fall	440a	424a	470a	451a	35-d	322b	367a	347b
Standard + Delay	414b	380b	440b	418b	40-d	257c	242b	262c
Delay + Fall	357c	267c	355c	385c	45-d	241c	241b	275c
SE	0.68	0.72	0.78	0.54	SE	0.69	1.04	0.81

† 2015 cutting treatments included Standard (60d + 30d + 30d), Standard + Fall (60d + 30d + 30d + Fall), Standard + Delay (60d + 37d + 37d), and Delay + Fall (67d + 45d + Fall).

‡ Within column and section, means without a common letter differ based on a Tukey's HSD test ($P \leq 0.05$).

Cutting Treatment Response. Concentrations of NDF differed among cutting treatments at all locations (Table 5). In 2015, the Standard and Standard + Fall cutting treatments were among those with the least NDF concentrations (≤371 g kg^{-1}), while the Delay + Fall cutting treatment was among those with the greatest NDF concentrations (≥395 g kg^{-1}). In 2016, NDF concentrations were least for the 30-d cutting treatment (≤391 g kg^{-1}) and greatest for the 40- and 45-d cutting treatments (≥444 g kg^{-1}).

A more frequent cutting schedule with shorter intervals between harvests resulted in decreased NDF concentrations. These results were expected, and agree with findings from previous studies demonstrating an increase in NDF concentrations as harvest intervals are lengthened (Weir et al., 1960; Hall et al., 2000; Kallenbach et al., 2002; Brink et al., 2010; Min, 2016). As the cutting interval was increased from 28 to 49 d, Min (2016) reported increasing NDF concentrations from 277 to 455 g kg^{-1}. Kallenbach et al. (2002) found that NDF concentrations were approximately 46 g kg^{-1} greater when alfalfa was harvested four times per year compared to five, and 28 g kg^{-1} greater when harvested five times per year compared to six. Hall et al. (2000) also reported increasing NDF concentrations across multiple alfalfa harvests. Similar to CP, plant maturity is likely the main attributing factor affecting NDF concentrations, with NDF concentrations increasing as cutting intervals lengthened. In the present study, plant maturity (MSC) was positively associated with NDF concentrations at all locations in both the establishment (2015) and first production (2016) year. In the establishment year, correlation coefficients were 0.80, 0.75, 0.79, and 0.49 for Becker, Rochester, Rosemount, and St. Paul, respectively. In the first production year, correlation coefficients were 0.71, 0.71, and 0.54 for Becker, Rosemount, and St. Paul, respectively. As plants mature, leaf proportions decrease, stem proportions increase, stem cell wall concentrations increase, and whole plant nutritive value decreases (Kalu and Fick, 1983; Nordkvist and Åman, 1986; Albrecht et al., 1987; Sanderson and Wedin, 1988; Sheaffer et al., 2000).

Acid Detergent Lignin

Cultivar Response. Across all years and locations, 54HVX41 contained less ADL compared to reference alfalfa cultivars (Table 4). Acid detergent lignin concentrations for reference cultivars ranged from 71 to 88 g kg^{-1}, while ADL concentrations for 54HVX41 ranged from 65 to 81 g kg^{-1}.

Compared to reference alfalfa cultivars, 54HVX41 demonstrated a 7 to 12% reduction in ADL during the establishment year (2015) and a 6 to 8% reduction in ADL during the first production year (2016). Acid detergent lignin concentrations for reference cultivars are comparable to previous reports (Jung et al., 1997a; Palmonari et al., 2014). Previous studies investigating reduced lignin alfalfa experimental lines have shown a 4 to 29% decrease in stem lignin concentration (Guo et al., 2001b; Marita et al., 2003; Reddy et al., 2005) and a 1 to 24% decrease in whole plant lignin concentration (Guo et al., 2001a, 2001b; Getachew et al., 2011) compared to control lines. Preliminary results evaluating reduced lignin alfalfa hay also reported decreased lignin concentrations ranging from 4 to 12% compared to control cultivars (Mertens and McCaslin, 2008; Undersander et al., 2009).

The reduced lignin concentration for 54HVX41 compared to other cultivars could be due to a number of reasons, including a greater leaf/stem ratio, a reduction in stem lignin, or a decreased plant maturity. In the present study, the leaf/stem and the stem lignin content of the plants were not measured, but 54HVX41 did demonstrate a slight reduction in maturity at some of the locations. However, maturity differences among cultivars were minimal and inconsistent across locations and years, while the reduction in lignin was present across all locations and years, which suggests that the maturity differences had little impact on lignin concentrations. Further research is needed to pinpoint the cause of lignin reduction for 54HVX41.

Cutting Treatment Response. Concentrations of ADL differed among cutting treatments at all locations (Table 5). In 2015, the Delay + Fall cutting treatment contained greater ADL concentrations (≥84 g kg^{-1}) compared to all other cutting treatments (≤80 g kg^{-1}) at Becker, Rochester, and Rosemount. At St. Paul, the Delay + Fall cutting treatment contained greater ADL concentrations (76 g kg^{-1}) compared to the Standard and Standard + Fall cutting treatments (≤69 g kg^{-1}). In 2016, the 30- and 35-d cutting treatments contained the least ADL (≤82 g kg^{-1}), while the 40- and 45-d cutting treatments contained the most ADL (≥83 g kg^{-1}) across all locations.

A more frequent cutting schedule with shorter intervals between harvests generally resulted in decreased ADL concentrations. Findings from previous studies have also shown that lignin concentrations increase as harvest intervals are lengthened (Weir et al., 1960; Nordkvist and Åman, 1986; Palmonari et al., 2014). As harvest intervals increased from 21 to 35 d, Palmonari et al. (2014) reported an increase in lignin concentrations from 63 to 73 g kg^{-1}. Similarly, Nordkvist and Åman (1986) reported lignin contents increasing from 43 to 147 g kg^{-1} across harvest intervals encompassing a range of alfalfa maturities. Increasing lignin concentrations with widening harvest intervals are a function of increasing plant maturity and the growth of secondary plant cell walls (Albrecht et al., 1987; Sanderson and Wedin, 1988). In the present study, plant maturity (MSC) was positively associated with ADL concentrations at all locations in both the establishment (2015) and first production (2016) year. In the establishment year, correlation coefficients were 0.58, 0.62, 0.77, and 0.43 for Becker, Rochester, Rosemount, and St. Paul, respectively. In the first production year, correlation coefficients were 0.55, 0.58, and 0.62 for Becker, Rosemount, and St. Paul, respectively. As a plant grows, the deposition of lignin is necessary to provide the strength and rigidity for a plant to stand upright (Inoue et al., 1998; Guo et al., 2001a).

Neutral Detergent Fiber Digestibility

Cultivar Response. With the exception of Rosemount, NDFD for 54HVX41 were greater compared to all reference alfalfa cultivars (Table 4). Increases in NDFD ranged from 8 to 10% in the establishment year (2015) and 11 to 18% in the first production year (2016). There were no differences in NDFD among alfalfa cultivars at Rosemount in either 2015 or 2016. The lack of differences detected among alfalfa cultivars at Rosemount could be related to several factors, including but not limited to a larger amount of variation among cultivars (indicated by greater standard errors) and a lower plant maturity at this particular location. Average plant maturities for alfalfa cultivars at Becker and St. Paul were ≥2.5 in 2015 and ≥4.0 in 2016, while maturity averages at Rosemount were ≤2.1 in 2015 and ≤3.9 in 2016. Alfalfa grown in Rosemount generally had less NDF and more NDFD compared to other locations; this, coupled with a lower maturity, could be indicative of a more digestible forage and might have masked some cultivar differences.

Previous studies investigating reduced lignin alfalfa experimental lines found similar results, reporting increases in DMD (Reddy et al., 2005; Getachew et al., 2011), in situ rumen digestibility (Guo et al., 2001b; Reddy et al., 2005), and NDFD (Guo et al., 2001b) for reduced lignin alfalfa compared to control cultivars. Preliminary results evaluating reduced lignin alfalfa hay also showed greater DMD and NDFD, with a 3 to 5% increase in DMD (Mertens and McCaslin, 2008) and a 3 to 26% increase in NDFD (Mertens and McCaslin, 2008; Weakley et al., 2008; Undersander et al., 2009; Li et al., 2015a) for reduced lignin alfalfa. Increases in NDFD for 54HVX41 can likely be attributed to reduced lignin concentrations, as the deposition of lignin into plant cell walls can negatively affect rumen microbial degradation and the digestion of feed by intestinal enzymes (Buxton and Hornstein, 1986; Liu and Yu, 2011). These results have potential biological significance, as feeding and grazing studies have shown that small changes in forage digestibility can impact animal performance. Casler and Vogel (1999) reported that a 1% increase in in vitro DMD resulted in a 3.2% increase in daily animal weight gains. Similarly, a one-unit increase in NDFD has been associated with a 0.17-kg increase in dry matter intake and a 0.25-kg increase in 4% fat-corrected milk for dairy cows (Oba and Allen, 1999).

Cutting Treatment Response. Neutral detergent fiber digestibility differed among cutting treatments at all locations (Table 5). In 2015, the Standard and Standard + Fall cutting treatments had the greatest NDFD (≥396 g kg^{-1}), while the Delay + Fall cutting treatment had the least (≤385 g kg^{-1}). In 2016, the 30- and 35-d cutting treatments contained the greatest NDFD (≥322 g kg^{-1}), while the 40- and 45-d cutting treatments contained the least (≤275 g kg^{-1}).

A more frequent cutting schedule with shorter intervals between harvests resulted in increased NDFD. Previous studies have made similar conclusions, showing decreasing fiber or DMD digestibility as harvest intervals increased (Weir et al., 1960; Nordkvist and Åman, 1986; Hall et al., 2000; Brink et al., 2010; Palmonari et al., 2014). Hall et al. (2000) reported a drop in DMD by 43 g kg^{-1} across four weekly sampling periods, and Palmonari et al. (2014) reported a reduction in NDFD levels from 440 to 340 g kg^{-1} as the harvest interval increased from 21 to 35 d. Similar to the other forage nutritive value components, the decrease in NDFD with increasing harvest intervals can be attributed to advancing plant maturity. In the present study, plant maturity (MSC) was negatively associated with NDFD at all locations in both the establishment (2015) and first production (2016) year. In the establishment year, correlation coefficients were −0.76, −0.76, −0.84, and −0.63 for Becker, Rochester, Rosemount, and St. Paul, respectively. In the first production year, correlation coefficients were −0.55, −0.75, and −0.64 for Becker, Rosemount, and St. Paul, respectively. Previous research has shown that increased maturity negatively influences the fiber digestibility of alfalfa through decreased leaf/stem ratios and increased lignification of the stem portion of the plant (Weir et al., 1960; Mowat et al., 1965; Albrecht et al., 1987; Yu et al., 2003).

Alfalfa Stand Density

Minor differences in alfalfa population densities and stem counts were detected across cutting treatments and cultivars (data not shown); however, differences were negligible and inconsistent. Compared to initial plant populations, plant densities were ≥88% at the end of the establishment year and ≥79% at the end of the first production year. Stem densities ranged from 743 to 942 stems m^{-2} during the establishment year and from 520 to 795 stems m^{-2} during the first production year and fall within the normal range suggested to maximize alfalfa forage accumulation potential (Undersander et al., 2011).

Differences in alfalfa population measurements were expected to be minor, as the present study contains only establishment and first production year data. Previous research has shown that along with increasing forage accumulation, a delayed cutting schedule could also benefit stand longevity. Frequent, repeated harvests of immature alfalfa have been shown to reduce stand persistence, vigor, and forage accumulation (Brink and Marten, 1989; Sheaffer and Marten, 1990; Probst and Smith, 2011). Continuation of this study is required to further evaluate the effects of cutting treatment and alfalfa cultivar on plant persistence over time.

Relationships in Forage Mass and Relative Forage Quality

To examine the effect of cutting treatment on alfalfa forage mass and RFQ from the first production year (2016), values were regressed across GDD for 54HVX41 and the average of the reference cultivars (Fig. 2 and 3). The number of GDD accumulated at harvest in 2016 averaged across cuttings and locations were 832, 1035, and 1248 GDD for the 30-, 35-, and 40-d cutting treatments, respectively (Fig. 2 and 3).

As expected, alfalfa forage masses increased across GDD for both 54HVX41 and reference cultivars (Fig. 2). No differences in forage mass were observed between 54HVX41 and the reference cultivars across GDD ranging from 700 to 1400. These results support the previously stated forage accumulation comparisons, which showed very minimal differences in forage accumulation among alfalfa cultivars during the first production year. With a 5-d harvest delay (e.g., delayed from 30 to 35 d), all cultivars produced an average of 0.83 kg DM ha^{-1} in additional forage mass. Delaying the alfalfa harvest by 10 d (e.g., delayed from 30 to 40 d) resulted in a 1.3 kg DM ha^{-1} increase in cultivar forage masses. Under these circumstances (i.e., 10-d delay), a delayed harvest can result in as much as a 28% increase in DM production; however, this increase in DM forage mass will traditionally be coupled with a reduction in alfalfa forage quality.

The relationship between alfalfa forage mass and quality is widely recognized (Kalu and Fick, 1983). As expected, alfalfa RFQ decreased with increasing GDD for both 54HVX41 and reference cultivars (Fig. 3). Although a 5-d delay in harvest improved forage mass, RFQ for 54HVX41 and reference cultivars was reduced by 11 and 13%, respectively, with increasing GDD (Fig. 3). While there were no differences between 54HVX41 and reference cultivars in forage mass, RFQ for

54HVX41 was 9, 10, and 12% greater compared to reference cultivars when cut under the same 30-, 35-, and 40-d treatment schedule, respectively (Fig. 3). A significantly greater RFQ (i.e., non-overlapping 95% confidence intervals) for 54HVX41 compared to reference cultivars was observed from 772 to 1248 GDD and represented a range of cutting intervals from 28 to 40 d (Fig. 3). This finding illustrates that the reduced lignin cultivar 54HVX41 has increased RFQ relative to reference cultivars under cutting schedules with increasing harvest intervals.

The greater RFQ observed for 54HVX41 across a wide range of GDD when compared to reference cultivars can offer flexibility for producers by reducing the forage digestibility penalty associated with a lengthened harvest window. Both 54HVX41 and reference cultivars produced a 22% gain in forage mass with a delayed harvest from 30 to 35 d. In addition, both showed similar reductions in RFQ across the same period. However, the value of the reduced lignin technology is most apparent when making cross comparisons. Reference cultivars cut at 30-d produced 3.74 kg DM ha^{-1} with a RFQ of 144, whereas 54HVX41 cut at 35-d produced 4.53 kg DM ha^{-1} with a RFQ of 139. This represents a 21% gain in forage mass with only a 3% reduction in RFQ. According to these results, a producer could successfully maintain RFQ by growing 54HVX41 instead of reference cultivars. Depending on production goals, this could allow for a wider optimal harvest window, making it possible for alfalfa growers to increase forage mass by delaying alfalfa harvest while still maintaining greater forage digestibility. A delayed harvest may also provide additional benefits in the form of increased plant persistence over time. Although outside the scope of this study, future research should investigate the long- term effects of harvest delay for reduced lignin alfalfa cultivars.

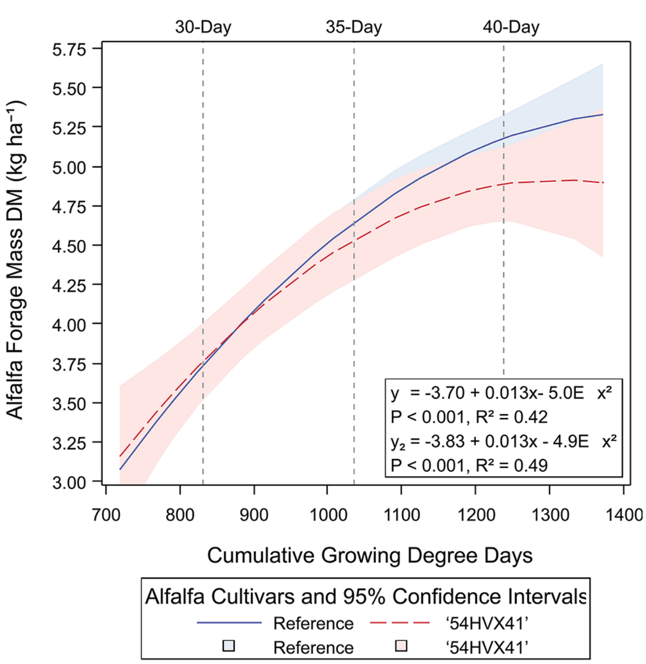

Fig. 2. Predicted alfalfa forage dry matter mass and 95% confidence intervals (shaded area) for reduced lignin cultivar 54HVX41 (y_1) and reference alfalfa cultivars (y_2) in response to average cumulative growing degree days. Vertical dashed lines correspond to treatment prescribed cutting intervals and their direct relationship to cumulative growing degree days during the 2016 growing season.

SUMMARY AND CONCLUSIONS

Forage accumulation differences among alfalfa cultivars were more pronounced during the establishment year than during the first production year. During the establishment year, forage accumulations for 54HVX41 alfalfa were decreased compared to reference cultivars at some locations. During the first production year, forage accumulation differences were minimal, indicating that forage accumulation differences between 54HVX41 and reference alfalfa cultivars could be minimal following establishment. Forage accumulations were also affected by cutting treatment. During the establishment year, alfalfa forage accumulations were increased when a fall cut was added. During the first production year, alfalfa forage accumulations were greater with the 40-d cutting treatment compared to the 30-d cutting treatment. These results suggest that during a normal production year, adding a fall harvest or using a cutting schedule with a longer interval between harvests can maximize forage accumulation, and that a longer harvest interval can reduce the number of cuts per season while still producing greater forage accumulation. Alfalfa cultivars in this study were tolerant of a diversity of cutting treatments and maintained adequate population densities throughout the establishment and first production year.

Compared to reference alfalfa cultivars, 54HVX41 generally had reduced ADL concentrations, increased NDFD, and similar CP and NDF concentrations. Cutting treatments with shorter harvest intervals had decreased plant maturities and generally resulted in greater forage nutritive value, including increased CP concentration, decreased NDF and ADL concentrations, and increased NDFD.

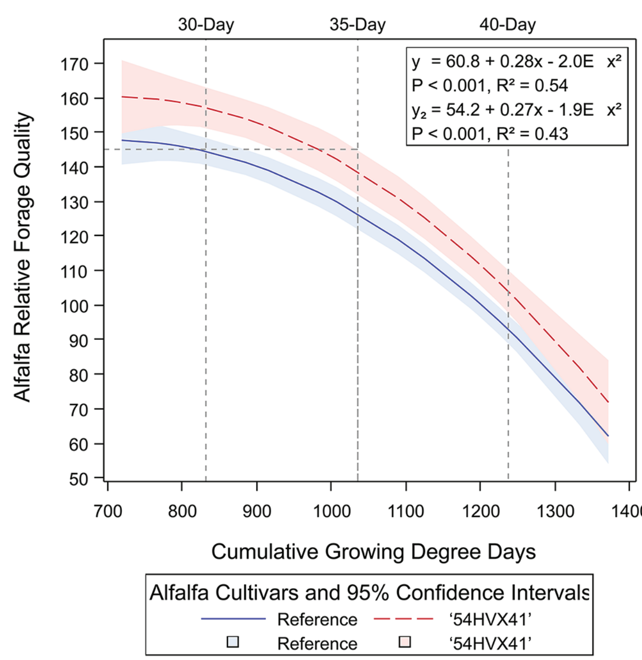

Fig. 3. Predicted alfalfa relative forage quality and 95% confidence intervals (shaded area) for reduced lignin cultivar 54HVX41 (y_1) and reference alfalfa cultivars (y_2) in response to average cumulative growing degree days. Vertical dashed lines correspond to treatment prescribed cutting intervals and their direct relationship to cumulative growing degree days during the 2016 growing season. Horizontal dashed lines correspond to the RFQ for reference alfalfa cultivars harvested under a 30-d cutting interval.

When alfalfa forage mass and RFQ were regressed across GDD in the first production year, forage masses for 54HVX41 and reference cultivars were similar, but RFQ for 54HVX41 was greater compared to reference cultivars from 772 to 1248 GDD. This increase in RFQ observed for 54HVX41 across a wide range of GDD can offer increased flexibility for producers. If 54HVX41 were harvested at the same time as reference cultivars, it would allow producers to obtain a higher quality and more digestible forage. However, if 54HVX41 were harvested under a delayed cutting schedule, it would provide producers with an option to reduce the quality penalty associated with a lengthened harvest window. With a 5-d harvest delay, 54HVX41 harvested on a 35-d harvest interval showed a 21% gain in forage mass and a 3% reduction in RFQ compared to reference cultivars harvested on a 30-d harvest interval. This could allow for a wider optimal harvest window, making it possible for alfalfa growers to achieve greater forage mass by delaying alfalfa harvest while still maintaining higher forage nutritive value.

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