LOW LIGNIN ALFALFA: WIDE AREA FIELD TEST RESULTS

R. Mark Sulc, Angela Parker, Kenneth Albrecht, Kim Cassida, Marvin Hall, Doo-Hong Min, Steve Orloff, Dan Undersander, and Xuan Xu[1]

ABSTRACT

Alfalfa (*Medicago sativa*) growers are faced with the recurring dilemma of having to balance yield and forage quality when harvesting their alfalfa crop. Yield increases while digestibility decreases as the plant matures, primarily because of increasing lignin content in the stems. A consortium of scientists at Forage Genetics International, The Samuel Robert Noble Foundation and U.S. Dairy Forage Research Center collaborated to alter the lignin content in alfalfa through genetic modification, resulting in the recent commercial release of the HarvXtra® alfalfa brand. A different approach was taken by breeders at Alforex Seeds who used conventional breeding to select for reduced whole plant lignin content in alfalfa, resulting in the recent release of the Hi-Gest® brand of alfalfa. Reducing the lignin content in alfalfa should provide a longer time period when forage with high nutritive value can be harvested. Field trials were established in six states (KS, MI, OH, PA, CA, WI) in spring 2015 to evaluate yield and nutritive value over time of the transgenic HarvXtra-008 alfalfa compared with conventional varieties. Forage samples were collected over time during different growth cycles and analyzed for nutritive value. Forage yield and nutritive value were also evaluated under 28-day, 33-day, and 38-day cutting intervals. Across all six states in the seeding year, HarvXtra-008 forage had consistently lower neutral detergent fiber (-3 to -3.8 units of NDF), lower acid detergent lignin (-1 unit of ADL), and higher NDF digestibility (+4.5 to +5.5 units of NDFD) compared with conventional alfalfa. This represents about a 10-day advantage in nutritive value for HarvXtra-008. When cut on the 38-day schedule, HarvXtra-008 yielded similarly or more and with higher nutritive value than the other varieties cut more frequently on 33-day or 28-day schedules. Hi-Gest 360 (included in CA and PA trials) was not significantly different in nutritive value than a conventional variety selected for high forage quality. Results with HarvXtra-008 are promising for alfalfa growers who want to maintain high forage nutritive value while increasing forage yields with less frequent harvests. More years of data will show how harvest interval affects nutritive value, yield, stand persistence, and profitability of alfalfa with the reduced lignin transgenic trait.

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[1]R. Mark Sulc (sulc.2@osu.edu) Professor and Extension Forage Specialist, The Ohio State University, Columbus, OH 43210. Angela Parker (parker. parker.773@buckeyemail.osu.edu), Graduate Research Assistant, The Ohio State University, Columbus, OH 43210. Kenneth Albrecht (kaalbrec@wisc.edu), Professor, University of Wisconsin, Madison, WI 53706. Kim Cassida cassida@msu.edu), Forage Extension Specialist, Michigan State University, East Lansing, MI 48824. Marvin Hall (mhh2@psu.edu), Professor, Pennsylvania State University, University Park, PA 16802. Doo-Hong Min (dmin@ksu.edu), Assistant Professor, Kansas State University, Manhattan, KS 66506. Steve Orloff (sborloff@ucanr.edu), UCCE Farm Advisor, Siskiyou County, Yreka, CA96097. Dan Undersander (djunders@wisc.edu), Professor and Forage Agronomist, University of Wisconsin, Madison, WI 53706. Xuan Xu (xuanxu@ksu.edu), Graduate Research Assistant, Kansas State University, Manhattan, KS 66506.

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DEVELOPMENT OF LOW-LIGNIN ALFALFA

Forage quality is important for animal health and productivity, but maintaining adequate nutritional quality to meet livestock requirements can be difficult due to accumulation of indigestible plant constituents. Alfalfa growers are faced with a recurrent dilemma of having to balance yield and quality when harvesting their alfalfa crop. Morphological and physiological changes occur in the plant as it matures that increase yield but decrease nutritional quality of the forage.

Leaves contribute significantly to the nutritive value of alfalfa while stems contain hiher concentrations of compounds that are highly indigestible by ruminant animals. The most important indigestible constituent in stems is lignin, which is located in the cell walls (Albrecht et al. 1987). Highly lignified plant tissue passes through the animal's digestive system and is not utilized for animal growth and development. Therefore, lignin limits ruminant digestibility, feed intake potential, and energy availability, all of which ultimately result in limiting animal production and performance. In order to significantly alter the potential forage quality of alfalfa, the nutritive value of stems must be improved, because that is where most of the lignin is found.

For the past decade, breeders and geneticists have focused on reducing the overall lignin content in alfalfa forage. A consortium of scientists at Forage Genetics International, The Samuel Robert Noble Foundation and U.S. Dairy Forage Research Center collaborated to alter the lignin content in alfalfa through genetic modification, resulting in the recent commercial release of the HarvXtra® alfalfa brand. Reduced lignin concentration in the plant was achieved by genetic modification using RNA interference to down regulate the Caffeoyl coenzyme A O-methyltransferase (CCoAOMT), a technique that essentially suppressed genes that code for specific enzymes in the lignin biosynthesis pathway in alfalfa (McCaslin et al., 2014). A different approach was taken by breeders at Alforex Seeds who used conventional breeding methods in an attempt to select for reduced whole plant lignin and leaf content in alfalfa, resulting in the recent release of the Hi-Gest® brand of alfalfa. There is a need to evaluate these products in field trials across the alfalfa production areas.

A reduction in lignin content in alfalfa and the associated improvement in digestibility should enable growers to lengthen the time period when alfalfa has acceptable forage quality for high producing animals. Thus, growers would have a wider 'optimal' harvest window, and it may be possible to achieve higher yields by harvesting alfalfa later while also maintaining acceptable forage quality. Field evaluations are needed to determine the field performance of alfalfa with this new trait, especially with regard to yield and quality under different harvest timings and frequencies. One question in particular is whether a reduced lignin content makes it possible to harvest later, with less frequency, in order to obtain higher forage yield with similar forage quality as conventional varieties that are harvested earlier and more frequently. Collaborative field evaluations among six universities were initiated in 2015 to address those management questions. The specific objectives were: 1) to determine if the change in quality over time of HarvXtra alfalfa differs from conventional alfalfa varieties, and 2) to provide information that will help alfalfa growers determine appropriate harvest schedules for reduced lignin alfalfa that maximizes yield while maintaining adequate forage quality for the class of livestock being fed.

FIELD RESEARCH APPROACH

Three alfalfa varieties ('HarvXtra-008' with the reduced lignin trait, '54R02' with high yield, and 'WL 355RR' selected for high forage quality), were sown at 18 lbs/acre of pure live seed in spring 2015 in six states (CA, KS, WI, MI, OH, PA). In CA and PA the variety 'Hi-Gest 360' (selected for reduced lignin via conventional breeding) was also included. Fertilizer applications were made at each location according to state recommendations based on soil test results. Herbicide, insecticide and fungicide treatments were applied as needed to control weeds, insects and foliar diseases, respectively. Two experiments were established using a randomized complete-block design with a split plot restriction on treatment randomization, with four replications.

Experiment 1

The first experiment was designed to focus on the change in forage nutritive value over time within a growth cycle for the different varieties planted. Plots in experiment I were arranged so that a given growth cycle was the main plot factor and alfalfa varieties were the subplot factor. The first growth of the seeding year was clipped off and discarded to avoid differences in development during establishment. Beginning with the second growth cycle in the seeding year and continuing into the first production year (2016), one main plot (containing all varieties) in each replication was sampled by hand clipping to 2-inch stubble on day 20, 23, 27, 30, 34, and 37 of regrowth from the previous harvest. For the spring 2016 growth cycle, samples were collected on the same intervals beginning when alfalfa reached a height of 22 inches. A different whole plot, not sampled previously, was used in each growth cycle to avoid any variation in alfalfa regrowth caused by variable clipping dates within previously sampled plots. The forage samples were dried in a force air oven, ground, and analyzed for nutritive value using calibrated NIRS equations. The following nutritive value traits are reported here: acid detergent lignin (ADL), neutral detergent fiber (NDF), NDF digestibility (NDFD), relative forage quality (RFQ), and crude protein (CP) concentrations.

Experiment 2

The second experiment evaluated harvest schedule effects on yield and nutritive value of the alfalfa varieties. Plots were arranged so that harvest schedule was the main plot factor and alfalfa variety was the subplot factor. Before each harvest, a 300 to 400 gram sample was hand clipped from plots to be harvested and the fresh weight was recorded. The samples were dried and weighed to determine dry matter percentage, then ground and analyzed for nutritive value using calibrated NIRS equations. As in the first

experiment, the first growth of the seeding year was clipped off and no data collected. For the second and third growth cyles in the seeding year (2015), three different cutting schedules (28-, 33-, and 38-day intervals) were imposed randomly to a whole plot in each replication (each with the variety subplots). Plots were clipped to a 2-inch stubble and dry matter yields were calculated. In first full production year (2016), Experiment 2 was continued with the same three harvest schedules as in the establishment year. However, instead of harvest intervals beginning at the same time as in the first measured growth cycle of the seeding year, the harvest schedules were staggered at 5-day intervals beginning with the 28-day schedule, which was harvested when the alfalfa reached a 28-inch height in the spring. Subsequent harvests were taken at the specific cutting interval for the remainder of the season, until the last harvest in September.

MULTI-LOCATION RESULTS

The low lignin variety HarvXtra-008 was consistently higher in forage nutritive value (lower ADL and NDF, higher NDFD, RFQ, and CP) than the other two varieties tested across all states and both growth cycles measured in 2015 (Table 1). HarvXtra-008 had about 20% less ADL and 12% higher NDFD compared with the two other varieties.

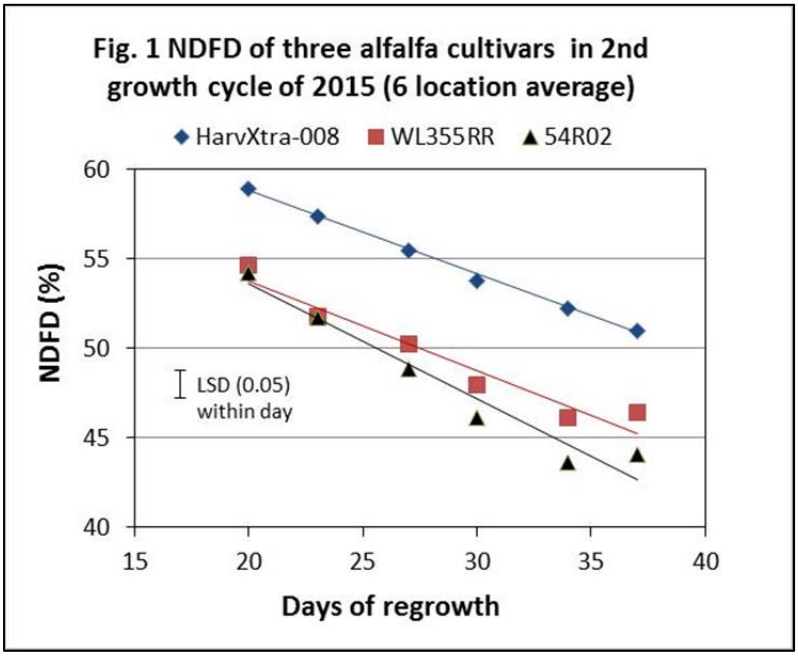
Table 1. Forage nutritive value of three alfalfa varieties averaged over six sampling dates during each of two growth cycles in the 2015 seeding year (6-state average).

Variety	ADL %	NDFD %	NDF %	RFQ	CP %
HarvXtra-008	4.0 b	55.5 a	26.7 c	297 a	26.4 a
WL 355 RR	4.9 a	51.0 b	28.7 b	262 b	25.8 b
54R02	5.0 a	50.1 b	30.5 a	243 c	25.0 c

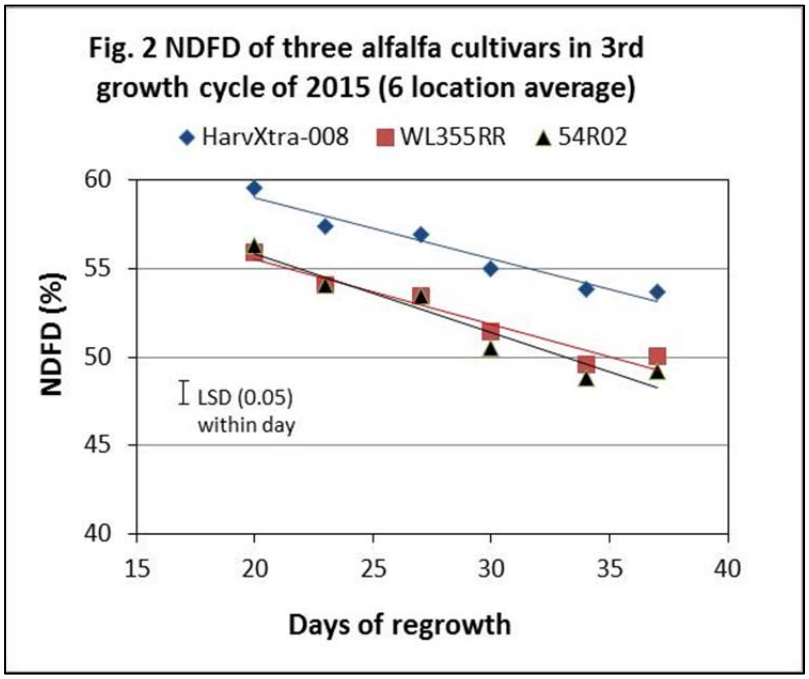
Values followed by different letters are significantly different at *P*=0.05.

As expected, nutritive value declined for all varieties during regrowth (Fig. 1). Nutritive value differences among varieties in the second growth cycle in 2015 were relatively consistent over time (Fig. 1).

HarvXtra-008 maintained about a 10-day advantage in nutritive value compared with the other varieties. In other words, HarvXtra-008 harvested with 37 days of regrowth had the same NDFD level as the other varieties harvested 10-days earlier, on day 27 of regrowth.



A 10-day spread was also found in the third growth cycle when comparing NDFD of HarvXtra-008 to the other two varieties (Fig. 2).



The Hi-Gest 360 variety was planted in the two experiments in CA and PA. Hi-Gest 360 was not significantly different in nutritive value compared with WL 355 RR, a variety selected for overall high forage quality (Table 2). The lignin concentration in Hi-Gest 360 was reduced by 4.2% compared with WL 355 RR and 54R02. Lignin content in HarvXtra-008 was 12.5% less than for Hi-Gest 360 and 17.3% lower than the other two varieties (Table 2). The varietal rankings were consistent across all sampling dates.

Table 2. Forage nutritive value of three alfalfa varieties averaged over six sampling dates during each of two growth cycles in California and Pennsylvania in the 2015 seeding year.

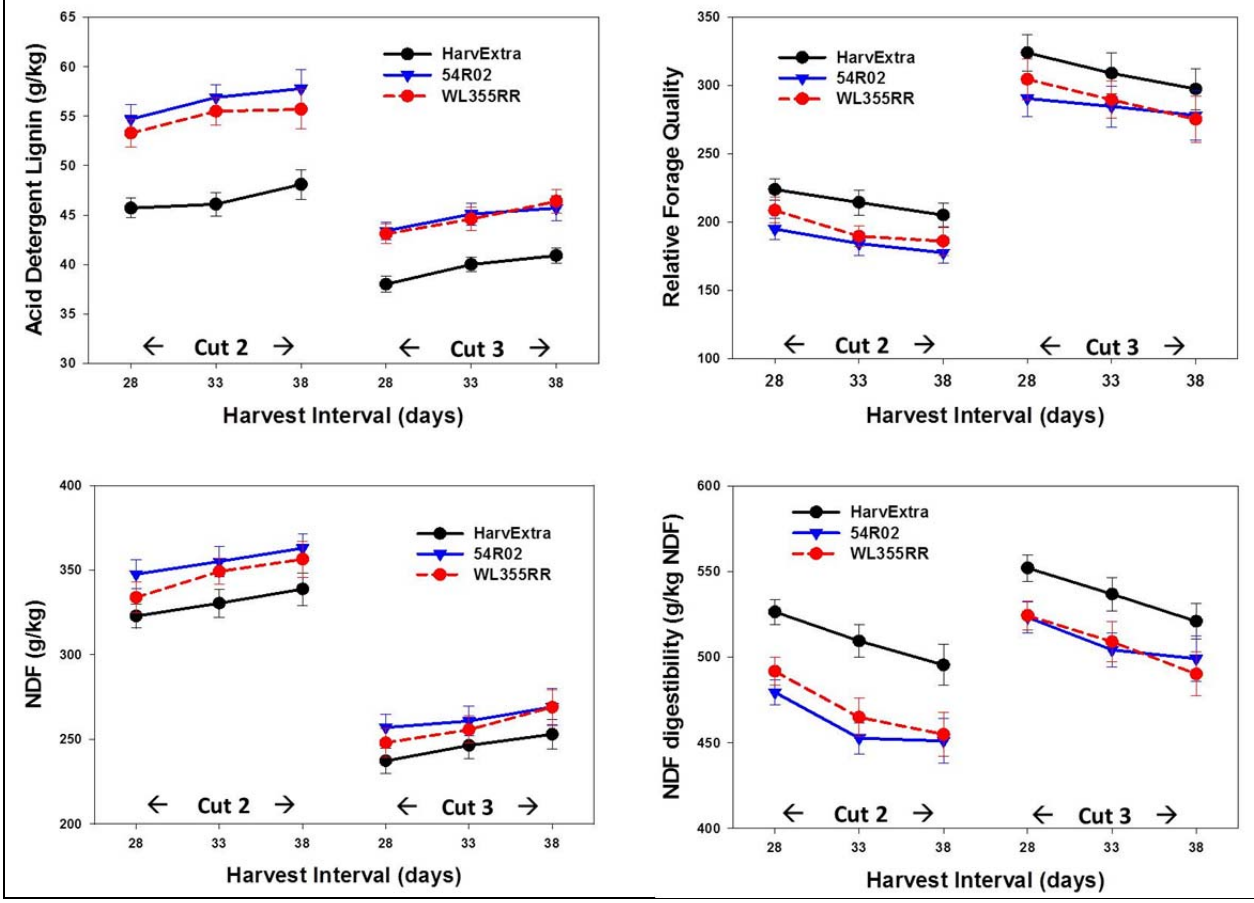
Variety	ADL %	NDFD %	NDF %	RFQ	CP %
HarvXtra-008	4.2 b	56.2 a	27.3 c	284 a	26.2 a
Hi-Gest 360	4.8 a	52.5 a	28.3 bc	265 ab	26.0 a
WL 355 RR	5.0 a	51.5 a	29.1 b	254 bc	25.8 a
54R02	5.1 a	50.7 b	30.7 a	237 c	24.9 a

Values followed by different letters are significantly different at P=0.05.

Results from the harvest schedule study (Fig. 3) confirmed the conclusions from Experiment 1. Although the varieties did not differ significantly for NDF concentration ($P > 0.05$) in Experiment 2, HarvXtra-008 contained less ADL than 54R02 and WL 355 RR across sites, harvest intervals, and cuttings ($P < 0.05$, Fig. 3). Consequently, NDFD and RFQ were greater for HarvXtra-008 than for the other varieties ($P < 0.05$, Fig. 3). Third cut alfalfa was greater in nutritive value than second cut alfalfa for all varieties ($P < 0.05$, Fig. 3), but variety differences were generally less pronounced in the third cutting. Across cuttings, values of ADL, RFQ, NDF, and NDFD in HarvXtra-008 cut on a 38-day interval were equivalent to or better than values for

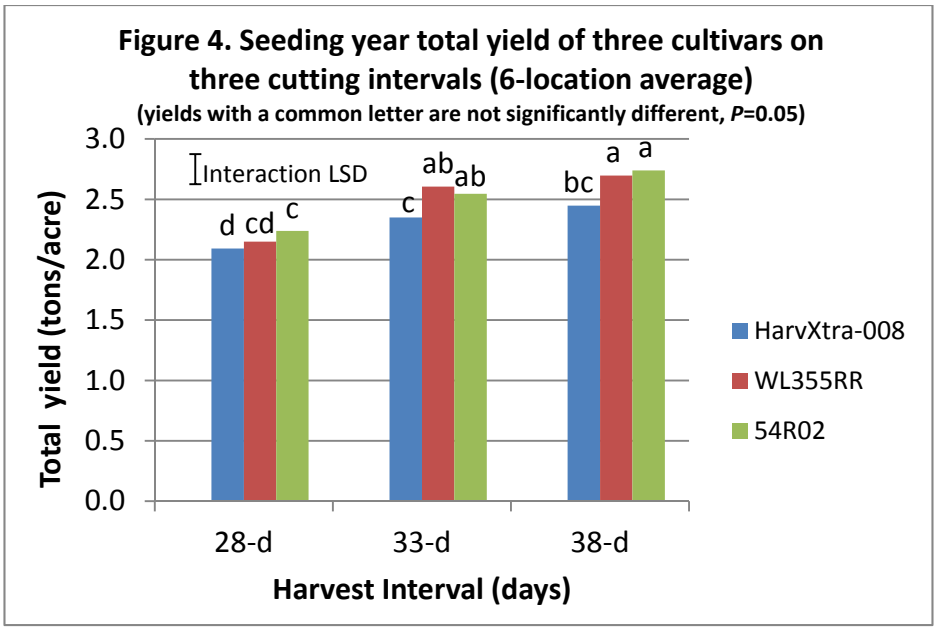
the other varieties cut on a 28-day interval ($P < 0.05$, Fig. 3). These results support of the idea that HarvXtra-008 has a longer harvest window for achieving excellent forage quality.

Figure 3. Average ADL, NDF, NDFD, and RFQ of three alfalfa cultivars grown in six locations and harvested twice in the seeding year at 28- 33-, and 38-day intervals. (Note, % values for ADL, NDF, and NDFD are obtained by dividing values by 10).



When HarvXtra-008 was compared with the average of 54R02 and WL 355 RR across all sites and cuttings in the seeding year harvest schedule study, it averaged 14% less ADL (4.3 vs. 5.0 percentage units, respectively, $P < 0.05$), 7% greater NDFD (52.4 vs. 48.7 percentage units, $P < 0.05$), and 10% greater RFQ (262 vs. 239 units, $P < 0.05$).

Across sites and cuttings, total alfalfa yield in the seeding year general increased with harvest interval for all varieties, as expected (Figure 4). HarvXtra-008 yielded slightly less than 54R02 on the 28-day schedule and slightly less than both 54R02 and WL 355 RR on the 33-day and 38-day cutting schedules (timing*variety interaction, $P < 0.05$). However, HarvXtra-008 cut on the 38-day schedule yielded similarly or more than the other two varieties cut on the 33-day or 28-day schedules.



SUMMARY

The transgenic reduced-lignin alfalfa variety HarvXtra-008 maintained lower lignin content and greater NDFD and RFQ than conventionally bred varieties during the seeding year. The conventionally bred Hi-Gest 360 variety was similar in nutritive value traits to WL 355 RR, which bred for overall high forage quality. The transgenic HarvXtra-008 reduced lignin variety maintained high nutritive value for up to 10 days longer than conventional high quality alfalfa varieties, and produced greater yields when compared at similar nutritive value levels.

The results with HarvXtra-008 are promising for alfalfa growers who want to increase forage yields but maintain high forage nutritive value when harvesting less frequently. The results are also very promising for those who want to achieve higher forage nutritive value while harvesting on their normal harvest frequency, because HarvXtra-008 was consistently higher in nutritive value on any given harvest date. The studies reported here were continued in 2016 (results are being processed and analyzed). More years of data from these studies will demonstrate how harvest interval affects nutritive value, yield, stand persistence, and profitability of alfalfa with the reduced lignin transgenic trait, and will clarify optimal harvest strategies for alfalfa growers.

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