Agronomic and Nutritional Attributes of Reduced Lignin Alfalfa

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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 number of alfalfa varieties having the reduced lignin HarvXtra® trait are being marketed. Reducing the lignin content in alfalfa should extend the time interval when forage can be harvested and still maintain adequate nutritive value for ruminants with high nutritive requirements. Field trials were established in 6 states (KS, MI, OH, PA, CA, and WI) in spring 2015 to evaluate yield and nutritive value over time of the transgenic HarvXtra-008 alfalfa variety compared with 2 other varieties (one selected for high quality and one for high yield). Forage samples were collected over time during 2 growth cycles in 2015 and analyzed for nutritive value. Forage yield and nutritive value were also evaluated under 28, 33, and 38-day cutting intervals in 2015. Across all 6 states in the seeding year, HarvXtra-008 forage had consistently lower neutral detergent fiber (-2 to -3.8 units of **NDF**), lower acid detergent lignin (-1 unit of **ADL**), and higher NDF digestibility (+4.2 to +5.4 units of **NDFD**) compared with the other alfalfa varieties. This represents a 7 to 10 day advantage in nutritive value for HarvXtra-008. When cut on the 38-day schedule, HarvXtra-008 yielded similarly or more and often had higher nutritive value than the other varieties cut more frequently on 33- or 28-day schedules. Results with HarvXtra-008 from the first year 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.

Introduction

Alfalfa is a high-yielding forage legume with nutritional attributes that complement those of corn (*Zea mays L.*) silage when used in dairy rations. Morphological and physiological changes occur in the plant as it matures that increase yield of DM per acre but decrease the nutritional value of the forage. As alfalfa approaches the ideal time for harvest, its nutritional value declines on a daily basis due to the accumulation of indigestible plant constituents in the cell walls. The yield

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increase versus nutritional value decrease is generally greater in the spring and early summer growth cycles than in late summer in humid environments (Brink et al., 2010), corresponding with the growth cycles that produce the largest proportion of the annual DM yield per acre. As a consequence, alfalfa growers are faced with the recurrent dilemma of having to balance forage yield and quality when harvesting their alfalfa crop. Weather conditions in humid environments can delay harvesting of alfalfa so the optimal window of time is often missed, resulting in high forage yield with less than ideal nutritive value for animals with high nutritional requirements. This factor has limited the use of alfalfa on many dairy farms, resulting in corn silage use being more strongly favored due to the ease of one harvest with a more consistent nutritive value content.

Leaves contribute significantly to the nutritive value of alfalfa, while stems contain higher concentrations of compounds that are highly indigestible by ruminant animals. The most important indigestible constituent in stems is lignin, which occurs in association with the thickening of secondary cell walls during the maturation process (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 decades, breeders and geneticists have focused particularly on reducing the overall lignin content in alfalfa forage as a means of improving its nutritive value as the plant matures. 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 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 animals with high nutritive requirements. Thus, growers would have a wider 'optimal' harvest window of opportunity, making it possible to possibly achieve higher yields by harvesting alfalfa later, while also maintaining acceptable forage nutritive value. One question in particular is whether a reduced lignin content will make it possible to harvest later, with less frequency, in order to obtain higher forage yield with similar forage quality as standard varieties that must be harvested earlier and more frequently to maintain adequate nutritive value. Collaborative field evaluations among 6 universities were initiated in 2015 to address those management questions. The specific objectives were: 1) to determine if the change in nutritive value 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 and maintains adequate forage quality for the class of livestock being fed.

Experimental Approach

Three alfalfa varieties ('HarvXtra-008' with the reduced lignin trait, '54R02' selected for high yield, and 'WL 355 RR' selected for high forage quality), were sown at 18 lb/acre of pure live seed in spring 2015 in 6 states (CA, KS, WI, MI, OH, and PA). 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 4 replications.

The first experiment was designed to focus on the change in forage nutritive value over time within a growth cycle for the 3 varieties. 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, one main plot (containing all varieties) in each replication was sampled by hand clipping forage samples to 2-inch stubble on day 20, 23, 27, 30, 34, and 37 of regrowth from the previous date of cutting. A different whole plot, not sampled previously, was used in the third growth cycle of the seeding year 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 near infrared reflectance spectroscopy (**NIRS**) equations. The following nutritive value traits are reported here: ADL, NDF, NDFD, relative forage quality (**RFQ**), and crude protein (**CP**) concentration.

The second experiment evaluated harvest schedule effects on yield and nutritive value of the 3 alfalfa varieties. As in the first experiment, the first growth of the seeding year was clipped off and no data were collected. For the second and third growth cycles in the seeding year (2015), plots were arranged so that harvest schedules (28-, 33-, and 38-day intervals) were the main plots and alfalfa varieties were the subplots. Before each harvest, a 0.6 to 1.0 lb sample was hand clipped from plots to be harvested and the fresh weight was recorded. The samples were dried and weighed to determine DM percentage, then ground and analyzed for nutritive value using calibrated NIRS equations. Plots were clipped to a 2-inch stubble and DM yields were calculated. A forage plot harvester was used to cut and weigh plot fresh weights that were converted to dry weights for determination of DM yield.

Results

The reduced lignin variety HarvXtra-008 was consistently higher in forage nutritive value (lower ADL and NDF; higher NDFD, RFQ, and CP) than the other 2 varieties 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 2 other varieties.

As expected, nutritive value declined for all varieties during regrowth in both growth cycles sampled (Figure 1). Differences among varieties for NDFD were relatively consistent over the periods sampled. HarvXtra-008 maintained about a 7 to 10 day advantage in NDFD compared with the 2 other varieties. In other words, HarvXtra-008 harvested with 37 days of regrowth had the same NDFD level as the other varieties harvested on day 27 to 30 of regrowth.

Nutritive value data from the harvest schedule study (Figure 2) confirmed what had been observed in Experiment 1. HarvXtra-008 contained lower ADL and NDF concentrations than 54R02 and WL 355 RR averaged across sites, harvest intervals, and cuttings ($P < 0.05$, Figure 2). Consequently, NDFD was greater for HarvXtra-008 than for the other varieties ($P < 0.05$). Average ADL, NDF, and NDFD of 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 ($P < 0.05$, Figure 2). These results support the idea that HarvXtra-008 has a longer harvest window for achieving excellent forage quality.

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 15% less ADL (4.3 vs. 5.1 percentage units, respectively, $P < 0.05$), 9% greater NDFD (52.4 vs. 48.2 percentage units, $P < 0.05$), and 5% lower NDF (29.6 vs. 31.5 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 3). HarvXtra-008 yielded about 8% less ($P < 0.05$) than 54R02 and WL 355 RR when averaged across all harvest schedules, but the difference was most apparent for the 33 and 38-day schedules. HarvXtra-008 cut on the 38-day schedule yielded similarly to the other 2 varieties cut on the 33-day and more than the 2 other varieties cut on the 28-day schedules.

Summary

The transgenic reduced-lignin alfalfa variety HarvXtra-008 maintained lower lignin and NDF contents and greater NDFD than the 2 other varieties during the seeding year. The transgenic HarvXtra-008 reduced lignin variety maintained high nutritive value for 7 to 10 days longer than the other 2 alfalfa varieties. This trait represents a significant new tool for alfalfa growers. The results with HarvXtra-008 are very promising for alfalfa growers who want to maintain adequate forage nutritive value when harvesting less frequently, or when weather systems delay harvest. 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 than the other varieties (one of which was characterized as a "high quality" variety). The studies reported here were continued in 2016 (results are being processed and analyzed). The reduced lignin trait and forage yield levels will likely improve with continued breeding progress. More years of data from these and similar studies along with on-farm evaluations 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 using this new tool.

References

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Table 1. Forage nutritive value of 3 alfalfa varieties averaged over 6 sampling dates during 2 growth cycles in the 2015 seeding year (average of 6 locations).[1]

Variety	ADL, %	NDFD, %	NDF, %	RFQ	CP, %
HarvXtra-008	4.0[b]	55.5[a]	26.7[c]	297[a]	26.4[a]
WL355 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]

[abc]Values followed by different letters are significantly different at P = 0.05.
[1]ADL = Acid detergent lignin, NDF = neutral detergent fiber, NDFD = NDF digestibility, RFQ = relative forage quality, and CP = crude protein.

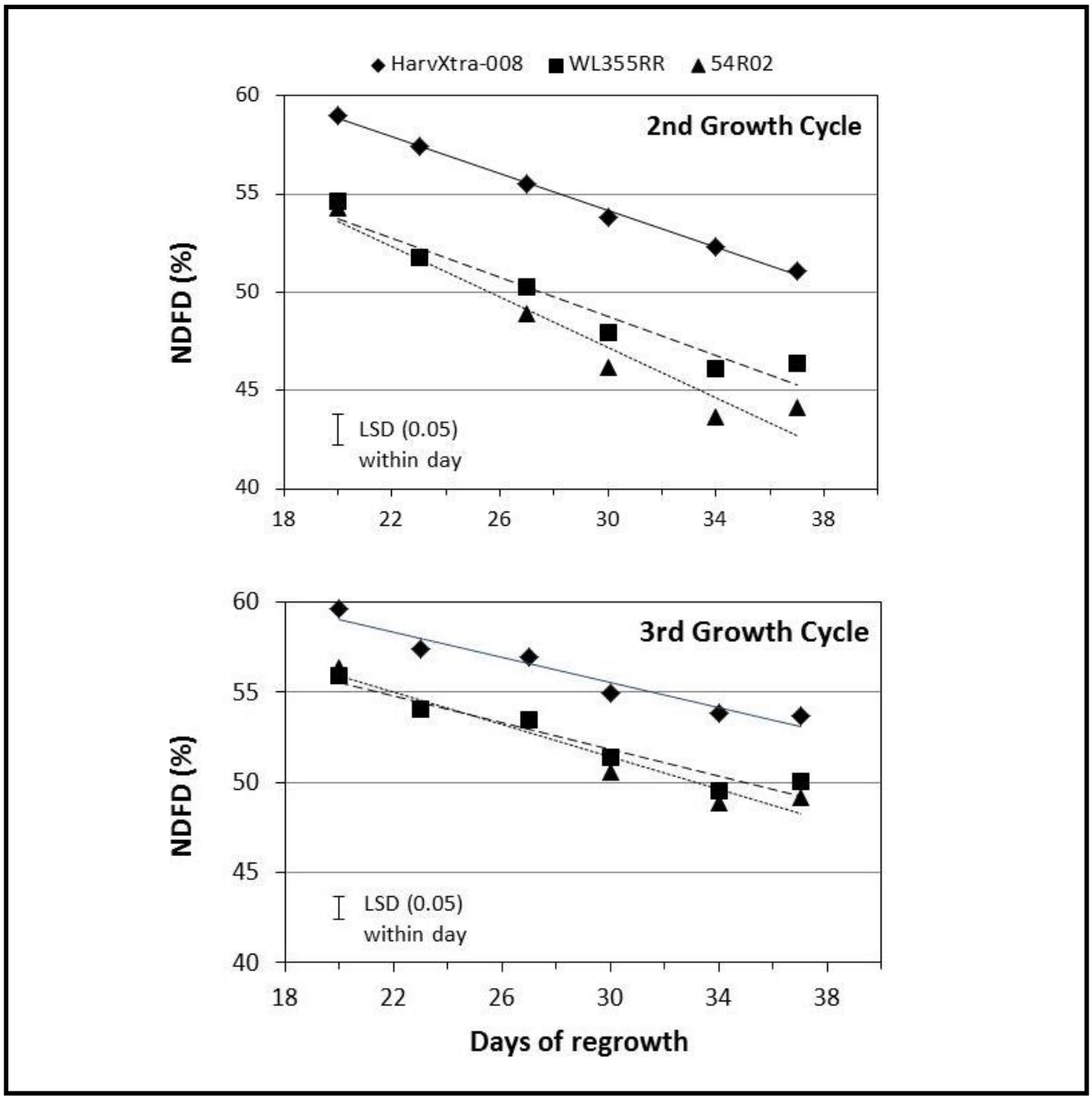

Figure 1. Neutral detergent fiber digestibility (**NDFD**) of 3 alfalfa cultivars during the second and third growth cycles in the 2015 seeding year (averaged over 6 locations).

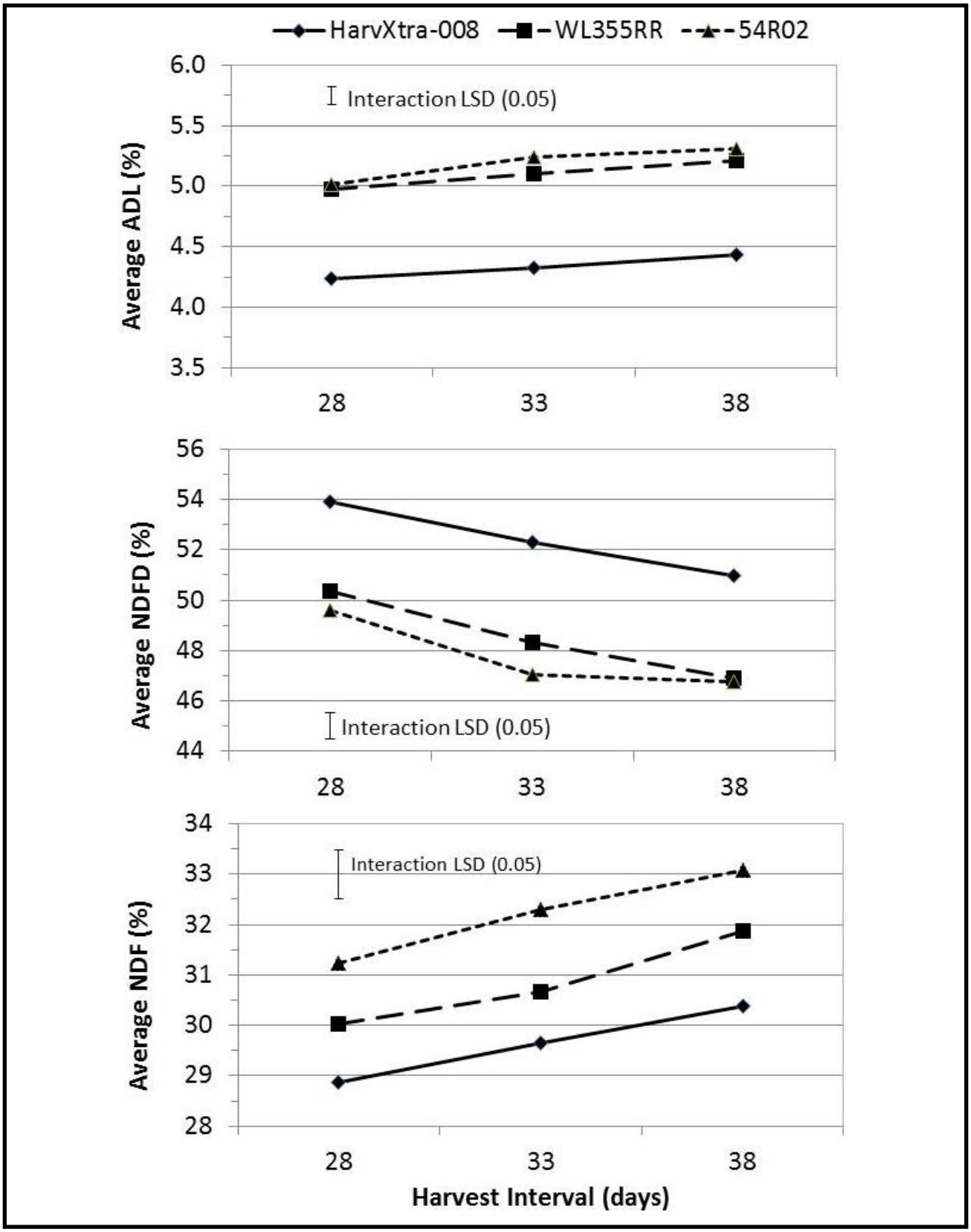

Figure 2. Average acid detergent lignin (**ADL**) and NDF concentrations and NDF digestibility (**NDFD**) of 3 alfalfa varieties harvested on 28, 33, and 38 day intervals in the 2015 seeding year (averaged over 2 harvests and 6 locations).

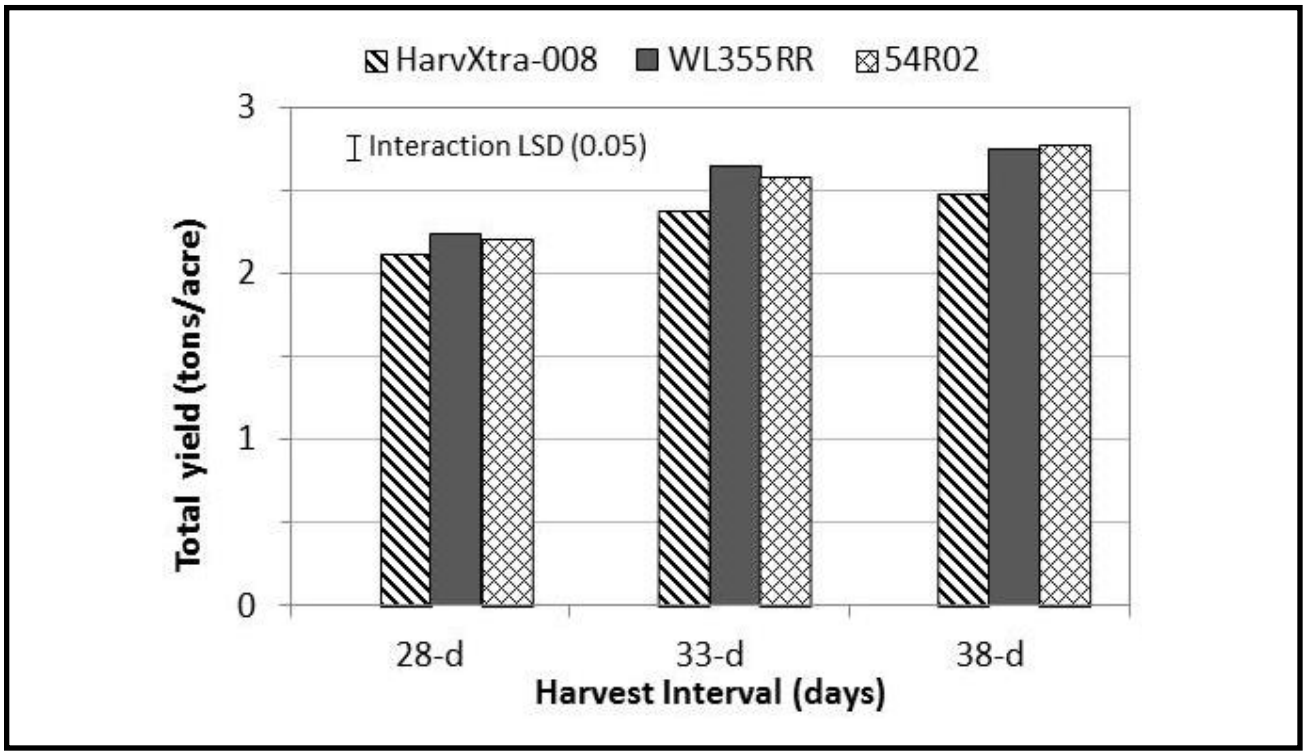

Figure 3. Total 2015 seeding year yield of 3 alfalfa varieties harvested on 28, 33, and 38 day intervals (total of 2 harvests, averaged over 6 locations).