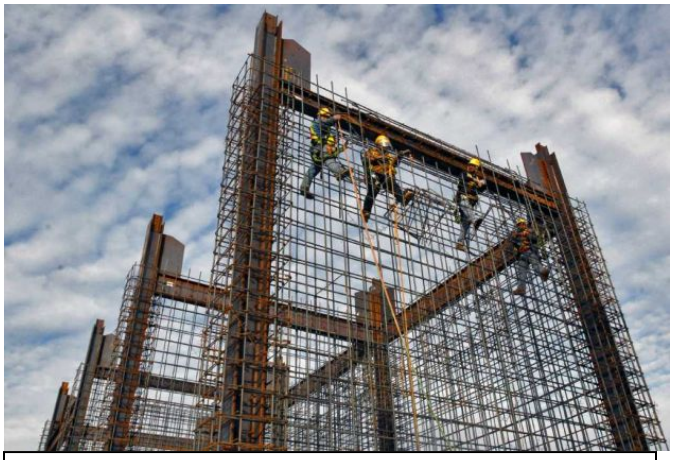
UC Davis Field Day, 11 May, 2017

EXPERIMENTS WITH REDUCED LIGNIN ALFALFA

D. Putnam, Chris DeBen, Brenda Chavez, Steve Orloff, UC Davis

The Concept: Lignin is important for plant structure (holding the plants erect), but not so good for animal growth, milk production or utilization. Plant cell walls are made up of cellulose, hemicellulose and lignin, about 35-45% of the Dry Matter. Since lignin is essentially not-at-all digestible by ruminants, it is one of the most important limiting factors for quality. It also ties up cellulose and hemicellulose in a complex so it can't be broken down by microorganisms. Lignin can greatly reduce 'digestibility' of an alfalfa plant, usually predicted using in-vitro NDFD (Neutral Detergent Fiber Digestibility). The USDA-ARS estimates that an increase in 10% of fiber digestibility would increase milk/beef by $350 million/year, and decrease manure by 2.8 million t/year, due to more efficient utilization by ruminants.



Lignin is the 'structural carbohydrate' in cell walls which gives it rigidity and strength, much like the re-bar in cement construction.

New Varieties. In 2014, a Genetically-Engineered (GE) trait called 'HarvXtra' was developed in cooperation with Nobel Inst. (Ardmore, Oklahoma) and USDA Dairy Forage Center (Madison, WI) and commercialized by Forage Genetics International (Land-O-Lakes). This GE construct reported to reduce the lignin content in alfalfa by about 15%. About the same time, Alforex (Dow AgroSciences) introduced non-GE varieties called 'Hi-Gest' which claimed also to have reduced lignin characteristics. Currently, both are being marketed.

IS THIS A QUALITY TRAIT OR A YIELD TRAIT?

The key quandary is the tradeoff between yield and quality with alfalfa. Very high digestibility alfalfa is typically obtainable (with any variety) when harvested very early. But yields are typically so low that it is not worthwhile. Growers routinely cut early to attain quality, but they compromise significant yield, and if they cut too late, they don't produce 'quality' hay, and take a significant price hit. In Yolo County, yield maximizes at about 2 tons/acre per cutting at about 35-40 days, but is approximately ½ ton/acre when harvested at about 21 days (Figure 1)! Conversely, quality is lowest at the late cutting schedule and highest at a short cutting schedule. Typically, growers compromise between yield and quality, cutting at about 28 days – unfortunately this often misses 'dairy quality' designation, resulting in both lower yields and lower quality and price.

The Low-Lignin Concept may change this. If field data holds true, 'dairy quality' hay may be produced when the crop is significantly delayed in harvest scheduling. Thus this trait could either:

- Improve Quality and Digestibility at the same harvest schedule
- Improve yields when hay is harvested 'late' – producing forage equal in quality to hay harvested at an earlier time.

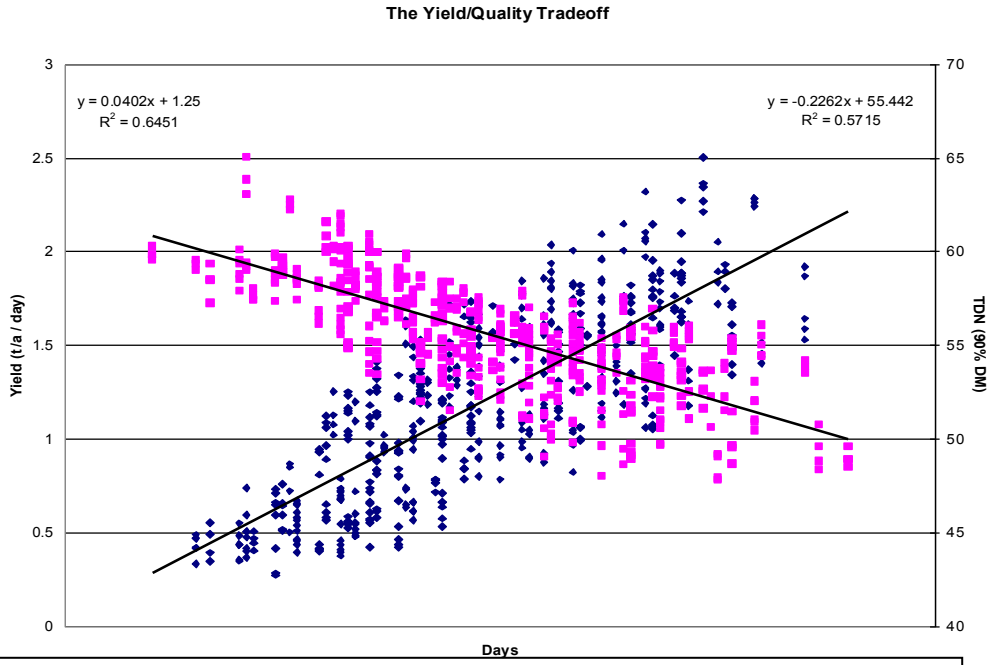


Figure 1. Relationship between yield and quality-Yolo County. Maximum quality rarely occurs at maximum yield, and maximizing yields reduces quality.

WHAT DOES THE DATA SAY?

While extensive field trials are still ongoing, here are some preliminary results. . In 2016, Marc Sulc from Ohio State University presented a national dataset from 2014-2016 on this concept. In that dataset, three alfalfa varieties ('HarvXtra-008' with the reduced lignin trait, '54R02' with high yield, and 'WL 355RR' selected for high forage quality), were sown 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. These were all Fall Dormancy 4-type varieties. A cutting schedule factor (maturity at harvest) to test the yield-quality concept (above) (see Sulc et al., 2016 for complete report).

We have conducted our own trials in 2012-2014, and are continuing today. In that two-year study, 4 HarvXtra preliminary lines were compared with 4 controls at 2 cutting schedules on the Davis campus and in Tulelake

PRELIMINARY CONCLUSIONS

- The HarvXtra varieties tested maintained lower lignin content and exhibited higher digestibility (NDFD) and higher RFQ than conventionally-bred varieties in a 6 location nationwide dataset and in a 4-location/year California study.

- In the datasets so far, Hi-Gest 360 had nutritive values similar to other conventionally-bred high quality lines, and differed from HarvXtra, which had higher quality than conventional lines.
- The HarvXtra reduced lignin line maintained high nutritive value for up to 10 days longer than conventional high quality alfalfa varieties, confirming the possibility to improve yields while maintaining quality
- There was some indication of slightly lower yields vs. controls with HarvXtra in some trials, but yields of harvXtra cut late were superior to conventional lines cut early

FURTHER QUESTIONS and RESEARCH

- **Does this Hold up?** Do the promising yield and quality results for HarvXtra hold up over multiple locations/years?
- **Non-Dormant Varieties.** How does the trait work with non-dormant varieties commonly grown in the Southwestern US? Current research has been primarily on dormant lines.
- **Comparing Company Claims.** How do the different 'low lignin' lines compare in reality and over multiple tests, fall dormancies and locations?
- **Seasonal Effects.** What are the effects of excessive heat in summer harvests – can we improve quality of these summer harvests which are typically low quality vs. fall or spring which are usually high quality anyway?
- **Markets must recognize different methods of measurement** – will markets respect 'digestibility' estimates (e.g. NDFD, other in-vitro methods) compared with the current method of simply looking at ADF or NDF (which determines TDN and RFV)? UC, USDA, and other universities have long recommended that digestibility be considered when pricing forage crops.
- **GMO Acceptance of Markets** – Will sensitive markets, particularly export and organic accept the HarvXtra GE trait? (the answer is currently no, but that may change).
- **Cost-Benefit.** Can the additional cost of the seed be justified by hay growers through either higher quality or higher yield harvests or more flexibility of harvest timings?

REFERENCES

Sulc, M.R., A. Parker, K. Albrecht, K. Cassida, M. Hall, D. Min, S. Orloff, D. Undersander, X Xu. 2016. Low Lignin Alfalfa: Wide Area Field Test Results. **In**: Proceedings, 2016 California Alfalfa and Forage Symposium, Reno, NV, Nov 29-Dec 1, 2016. UC Cooperative Extension, Plant Sciences Department, University of California, Davis, CA 95616. (See http://alfalfa.ucdavis.edu for this and other alfalfa conference Proceedings.) For 2016 proceedings: http://alfalfa.ucdavis.edu/+symposium/2016/index.aspx

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).

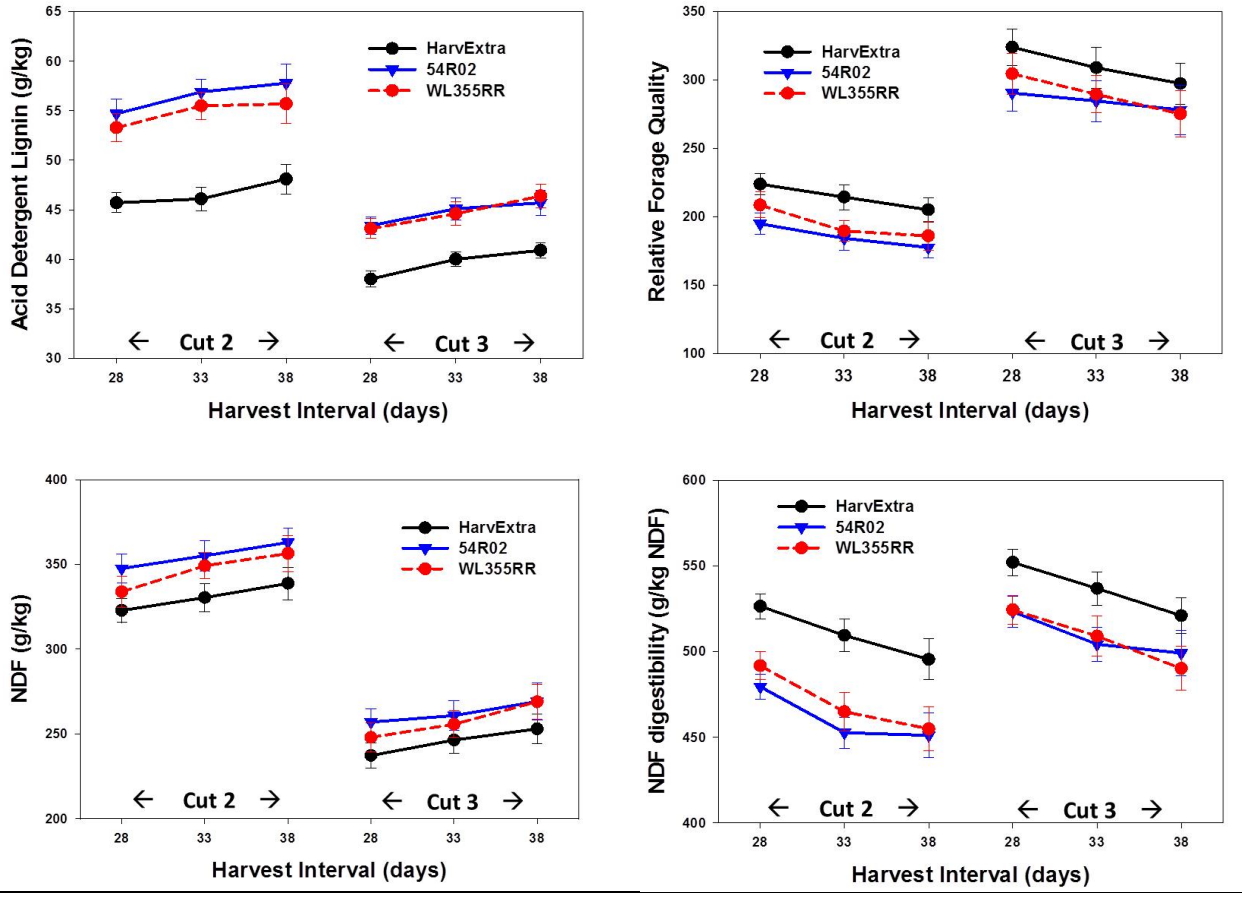


Table 2. Forage nutritive value of three alfalfa varieties averaged over six sampling dates during each of two growth cycles in California and Pennsylvania in the 2015 seeding year. (Sulc et al., 2016

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.

Table 3. Effect of LL (HarvXtra) alfalfa (pre-release lines) compared with control varieties on yield, average over cuts, Davis and Tulelake, CA (2013-2014)

	Tulelake					Davis				
	2013		2014		Mean	2013		2014		Mean
					Harvest Schedule					Harvest Schedule
	Early	Late	Early	Late	2013-14	Early	Late	Early	Late	2013-14
Harvests	4	3	4	3		5	4	7	5	
Yield										
HarvXtra	9.10	7.70	8.39	8.32	**8.37**	5.77	6.54	7.39	8.16	**7.08**
Control	8.87	7.67	8.40	8.24	**8.29**	6.50	6.81	7.43	8.50	**7.40**
Mean	8.32		8.34			6.4		7.87		
CV%	4.07		6.95			5.44		8.09		
LSD (p=0.05)	0.17		0.29			0.2		0.31		
F-Test										
Type	ns		ns			***		ns		
Cutting Schedule	***		ns			***		***		

Table 4. Data from Tulelake comparison of HarvXtra low lignin experimental varieties vs. controls (2014 data) from two different cutting schedules, early and late.

Table of NDFD						
	Variety	**2nd Cutting**	**3rd Cutting**	**4th Cutting**	**Aftermath**	**Avg. by Variety**
Early	12RRL -1	51.38	48.44	53.59	52.94	*51.59*
	12RRL-2	52.75	49.97	53.06	50.93	*51.68*
	12RRL-3	51.30	49.10	53.70	52.98	*51.77*
	12RRL-4	51.59	48.85	53.20	52.38	*51.50*
	54R01	47.61	44.95	50.35	48.08	*47.75*
	Liberator	48.22	45.91	49.02	48.82	*47.99*
	WL 355.HQ.RR	47.69	44.26	49.62	51.53	*48.27*
	Ameristand 405TRR	46.15	45.77	47.86	52.32	*48.02*
	Avg. by Cutting	*49.59*	*47.16*	*51.30*	*51.25*	
Late	12RRL -1	48.14	50.02		51.52	*49.89*
	12RRL-2	47.95	47.21		50.93	*48.70*
	12RRL-3	50.29	47.42		50.45	*49.38*
	12RRL-4	49.37	48.83		50.58	*49.60*
	54R01	45.37	45.63		50.11	*47.04*
	Liberator	43.93	45.61		49.91	*46.48*
	WL 355.HQ.RR	44.19	46.16		52.97	*47.77*
	Ameristand 405TRR	42.63	45.33		49.65	*45.87*
	Avg. by Cutting	*46.48*	*47.03*		*50.76*	

Table 5. Data from Davis comparisons of HarvXtra low lignin experimental varieties vs. controls (2014 data) from two different cutting schedules, early and late (2014 data).

Early schedule (28 day) forage quality (NIRS results) by variety. 7 harvests Davis, CA											
Variety	ADF	Ash	CP	ADL	IVTDM	IVdOM	NDF	NDFD	TDN1	RFQ	NFC
54R01 (control)	23.8	7.2	26.0	4.6	87.4	79.5	27.6	51.9	72	271	39
Ameristand 405TRR (control)	23.3	6.8	25.8	4.5	87.5	80.1	27.1	51.9	72	279	40
Liberator (control)	23.2	7.0	26.1	4.5	87.7	79.9	27.1	52.0	72	276	39
WL 355RR (control)	23.1	6.8	25.9	4.5	87.8	80.3	26.7	52.0	72	285	40
12RRL-1 (Lot RRL-1281-F)	22.2	7.0	26.1	4.0	89.3	81.9	25.8	54.9	73	299	41
12RRL-2 (Lot RRL-1284-F)	21.8	7.1	26.3	3.9	89.7	82.2	25.5	55.1	73	306	41
12RRL-3 (Lot RRL-1290-F)	22.3	7.1	26.3	3.9	89.2	81.7	26.2	54.8	73	294	40
12RRL-4 (Lot-1291-F)	22.1	7.1	26.1	3.9	89.4	81.9	25.9	55.0	73	298	40
Mean	**22.7**	**7.0**	**26.1**	**4.2**	**88.5**	**80.9**	**26.5**	**53.4**	**72.6**	**288.6**	**39.9**
variety	***	***	ns	***	***	***	***	***	***	***	***
cut	***	***	***	***	***	***	***	***	***	***	***
variety*cut	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

Table 5. Late schedule (35 day) forage quality of 4 experimental LL varieties and 4 released varieties, 5 harvests/year Davis, CA, average of 2 years											
Variety	ADF	Ash	CP	ADL	IVTDM	IVdOM	NDF	NDFD	TDN1	RFQ	NFC
54R01 (control)	26.2	6.6	23.4	5.0	84.4	77.4	30.8	48.8	70	233	39
Ameristand 405TRR (control)	27.9	6.6	22.8	5.3	82.9	76.3	32.6	47.3	68	218	37
Liberator (control)	26.8	6.7	23.0	5.1	83.9	76.9	31.5	48.3	69	230	38
WL 355RR (control)	27.5	6.7	22.8	5.3	83.1	76.4	32.2	47.4	69	220	38
12RRL-1 (Lot RRL-1281-F)	25.6	6.8	22.9	4.5	85.6	79.0	30.1	51.3	71	246	40
12RRL-2 (Lot RRL-1284-F)	26.3	6.8	23.0	4.5	85.2	78.7	31.2	51.3	70	236	39
12RRL-3 (Lot RRL-1290-F)	25.4	6.8	23.2	4.4	85.8	78.9	30.3	51.8	71	247	39
12RRL-4 (Lot-1291-F)	26.0	6.8	22.9	4.6	85.2	78.5	31.0	51.1	70	238	39
Mean	**26.5**	**6.7**	**23.0**	**4.8**	**84.5**	**77.8**	**31.2**	**49.7**	**69.8**	**233.4**	**38.6**
variety	***	ns	ns	***	***	***	**	***	***	**	**
cut	***	***	***	***	***	***	***	***	***	***	***
variety*cut	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns