

Progress Report on Reduced-Lignin Alfalfa: Part I, Plant Modifications

by Heathcliffe Riday and Ron Hatfield, USDA-ARS, U.S. Dairy Forage Research Center

Alfalfa is the most important forage legume in U.S. dairy cow diets because it has a high protein content, it increases feed intake and milk production, and it is an excellent complement to non-forage components of dairy cow diets. It is also an excellent source of effective fiber which is essential for maintaining proper rumen function and cow health.

With alfalfa, as with other plants, contents inside the cell wall (i.e., protein, soluble sugars, and starch) are completely digestible. Sometimes, however, intact cell walls keep cell contents from being “released” during digestion, making these nutrients unavailable to the cow. Alfalfa would be a better energy source if more carbohydrates within cells were available.

Alfalfa cell walls make up the fiber portion of the plant, and alfalfa DM contains between 30-80% fiber. Fiber content is usually dependent on maturity. However, only about 50% of alfalfa fiber is digested by dairy cows. For many years it has been postulated that if alfalfa digestibility were improved (i.e., more cell walls were broken down), cows would capture more energy stored in the cell wall carbohydrates and produce more milk and less manure. In fact, the U.S. Dairy Forage Research Center estimates that a 10% increase in cell wall digestibility (measured as neutral detergent fiber digestibility or NDFD) would increase milk and beef production by \$350 million/year and decrease manure production by 3 million tons/year. There would also be a savings of reduced grain supplements (2 million tons) mostly from corn grain.

Why Reduce Lignin?

Researchers have been studying cell walls for many years in an attempt to find ways for cows to more completely digest this fiber. (The same concept applies to other industries, such as wood pulp or cellulosic ethanol production.) Alfalfa hay cell walls consist of: pectin, hemicellulose, cellulose and lignin.

The pectin portion is almost completely digested. The non-pectin cell wall fraction (hemicellulose, cellulose and lignin) is also known as Neutral Detergent Fiber (NDF); ideally, alfalfa would contain 40% NDF on a DM basis. The lignin portion of cell walls is completely indigestible; it remains intact during digestion. But enzymes and microbes eat away at the cell wall carbohydrates which break up and become available to the cow; this process also weakens the foundation around the lignin, allowing the cell wall to break open so that cell content nutrients can be utilized by the cow.

Lignin is the biggest impediment to more complete fiber digestion. However, it is also essential to the plant; it provides structure and strength, and it is critical for water transport in the plant. Because lignin cannot be eliminated, one research goal has been to decrease the percentage of it in the cell wall by increasing the more digestible celluloses and pectins. Also, lignin is more crucial in some cells than others, so another research goal has been to selectively decrease lignin in some cells and leave it intact in others (such as in the vascular tissue which transports water).

Past research has determined the key enzymes and genes controlling these enzymes in the monolignol pathway (i.e., the plant biochemical pathway that determines how and what type of lignin is produced in the plant). By combining this knowledge with transgenic gene regulation technology, it has become possible to engineer this pathway to reduce lignin. Such engineered plants have been created by downregulating 5 different enzymes. Plant material collected from such greenhouse-grown transgenic alfalfa plants showed reduced lignin and increased *in vitro* (test tube) DM digestibility.

Current research is working towards creating commercially available reduced-lignin varieties for producers. Concurrently, reduced-lignin alfalfa is being tested for agronomic field performance and digestibility in ruminants.

Agronomic Performance

In general, the question has been raised as to whether reducing cell wall constituents would lead to poorer agronomic performance, especially since lignin provides structural strength to plant stems and decreases plant lodging. Past studies in other forage crops have shown both agronomically negative and positive responses. However, with the advent of alfalfa plants transgenically downregulated in different monolignol pathway enzymes, specific agronomic effects can be associated with the degree of downregulation of specific lignin enzymes. Furthermore, determining the consistency of these correlated responses in diverse environments becomes feasible. Such an understanding is a first step to creating commercially viable lignin-downregulated alfalfa cultivars.

Using transgenic alfalfa plants produced by Forage Genetics International and the Samuel Roberts Noble Foundation, researchers at the U.S. Dairy Forage Research Center, in collaboration with the Consortium for Alfalfa Improvement*, conducted a study to evaluate two key lignin biosynthetic enzymes (referred to as COMT and CCOMT) in alfalfa plants to determine the agronomic effects of downregulating these enzymes in a field setting. Plants were grown in 3 field environments (Prairie du Sac, WI; West Salem, WI; and Ardmore, OK) and harvested 3-4 times/year at each location during 2005 and 2006.



Reduced-lignin alfalfa plants being established in Wisconsin for agronomic performance testing.

Measured traits focused on stem characteristics and basic agronomic plant characteristics including: acid detergent lignin (ADL), neutral detergent fiber digestibility (NDFD), DM yield, plant height, maturity, plant lodging, stem pliability, fall dormancy, DM content, regrowth at 1 week after cutting, and extent of leafhopper damage.

Both COMT and CCOMT downregulated plants had reduced ADL in most test environments compared to the control plants (non-downregulated alfalfa). COMT downregulated plants, in general, displayed greater agronomic differences than the CCOMT downregulated plants relative to the control plants. However, the agronomic differences between downregulated and control plants grown in different environments generally were not extreme. Furthermore, among reduced-lignin plants, transgenic events with no agronomically negative effects were observed, suggesting that normal event selection and plant breeding can be used to avoid any agronomically negative effects while still capturing the significant improvements in forage quality. This suggests that agronomically useful lignin-modified cultivars are commercially feasible.



Reduced-lignin alfalfa was grown in Oklahoma to study agronomic performance in varying environments.

An original target of reducing lignin was to increase fiber digestibility. Both the COMT and CCOMT reduced-lignin lines had increased alfalfa stem neutral detergent fiber digestibility (NDFD – used to predict fiber digestibility in dairy cattle rations). Averaged across all locations and harvests, the results were: COMT - 41% NDFD, CCOMT - 36% NDFD, Control alfalfa - 30% NDFD.

The greatly increased fiber digestibility of the lignin-reduced alfalfas, compared to the control, was observed at all locations tested during every harvest taken. To determine if this increase in fiber digestibility translates to improved animal performance, the U.S. Dairy Forage Research Center is currently conducting feeding trials with ruminants. Preliminary results of those studies will be in the next issue of Forage Focus.

Summary

- Transgenic alfalfa plants have been generated that show decreased lignin content and increased fiber digestibility.
- Early studies show that agronomically useful lignin-modified cultivars are commercially feasible because, wherever the plants are grown, the reduced lignin trait will be expressed without negatively impacting alfalfa stand performance.
- Future research will focus on additional/alternative ways to decrease lignin's impact on digestibility (i.e., understanding how lignin cross-links to other wall components).
- Although this project is now on a commercial track, it will be 5-7 years before commercialization is possible.

**Much of the research mentioned here has been conducted by the organizations in the Consortium for Alfalfa Improvement: The Noble Foundation; Forage Genetics International; and the U.S. Dairy Forage Research Center. The Consortium is also collaborating on studies to improve the protein utilization of alfalfa.*