Novel Forages and Novel Research

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Greener Horizons for Crops, Cows, and Communities
This talk will report...

- U. S. Dairy Forage Research Center
- Production of perennial forage crops
- Redesigning alfalfa for livestock diets – CAI
- Potential of alfalfa as cellulosic ethanol feedstock
- Forage legume breeding - USDFRC
- Forage grass breeding - USDFRC
Unique Mission

- One of about 100 units in the USDA-ARS.
- Only ARS unit with the mission of improving forage use by dairy cattle.
- **Mission:** *To develop knowledge and tools to enhance sustainable and competitive dairy forage systems that protect the environment, promote animal health, and ensure a safe, healthy food supply.*
Unique Approach

multidisciplinary

How do changes in plants/crops react in the cow/diet?

What is the cow telling us about changes that could be made in plants/crops?

How do crop management, harvest, and storage methods affect the environment?

How do changes in plants or diets affect manure and emissions, and consequently the environment?
3 Locations

Prairie du Sac, WI – research farm; 2,006 acres, 325 cows, since 1980.

Marshfield, WI – Institute for Environmentally Integrated Dairy Management – laboratory (south), since 2009; and research farm (north), soon to be completed.

Madison, WI – main office and laboratories for 12 scientists, since 1981.
Alfalfa Hay Trends . . .

U. S. Alfalfa Hay Production

U. S. Alfalfa Hay Acres
Forage Trends . . .

2008 U.S. Alfalfa Production

• Hay
  – 69.6 million tons
  – 21.0 million acre
  – $10.8 billion
  – 4th following corn, soybeans & wheat

• Forage
  – 80.7 million tons
  – 23.1 million acres
  – ~$13.9 billion
Leading Alfalfa Forage States, 1,000 tons, 2008

Top 10 States
- 61% of U.S. tons
- 60% of acres
- 7 states NC
- 3 states West
- 5 Lead Dairy
Forage trends . . .

Least aggressive in yield increases

Yield/acre for all hay

Year

Tons per acre


Yield/acre for 4 major crops

Year

Bushels per acre


All wheat
Soybean
Corn
Leading States in Corn Silage production 1,000 tons, 2008

Top 10 States
- 64 % of U. S. acres
- 64 % of tonnage
- 6 NC
- 2 West
- 2 states NE
- 7 Lead Dairy
Forage trends . . .

Corn silage production in the U.S., 1986-2006

Corn silage production in 5 leading dairy states, 1986-2006

Dec 8, 2009 U.S. Dairy Forage Research Center, USDA-Agricultural Research Service
Leading Dairy States, million pounds of milk, 2008

Top 10 States
- 73 % of U. S. prod
- 71 % of cows
- 5 west
- 3 NC
- 2 NE
Forage Trends . . .

<table>
<thead>
<tr>
<th>Category</th>
<th>Acres (million)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cropland harvested</td>
<td>406,424,909</td>
</tr>
<tr>
<td>Cropland pastured</td>
<td>35,771,154</td>
</tr>
<tr>
<td>Woodland pastured</td>
<td>370,297</td>
</tr>
<tr>
<td>Permanent pasture &amp; rangeland</td>
<td>408,832,116</td>
</tr>
</tbody>
</table>

Between 2007 & 2002 acreage harvested for hay and haylage declined by 4%
Acreage grazed declined by 28%

2007 U.S. Census of Agriculture
CA Hay Production Per Dairy Cow (lbs alfalfa/cow/day)

\[ y = 0.0071x^2 - 1.0507x + 52.834 \]

\[ R^2 = 0.9432 \]

Source: Dan Putnam, 2005 Consortium for Alfalfa Improvement
Trends . . .

- Hay acreage remains unchanged
- Dairy cattle feeding – declining amounts
Barriers to increasing alfalfa usage . . .

- Increasing use of corn silage
- Forage quality of alfalfa haylage, alfalfa hay and corn silage
  - Low fiber
  - Excessive crude protein resulting in excessive ruminal degradable protein
  - Less consistent quality of hay and haylage
- Relative to corn, alfalfa yields have lagged
We don’t want to see reduced perennial forage crops in rotation because . . .

- Perennial forage crops are good for environment
- Good for cow health
Forage & Livestock Industries Need

Perennial Legumes and Grasses

• Increased yield and persistence
  – Improved forage digestibility of legumes
  – Enhanced protein utilization of alfalfa
Redesigning Alfalfa

Two goals:

1. **Reduce the amount of protein degraded in silage and in the rumen.**

2. **Increase the availability of carbohydrates in the plant cells.**
Redesign Alfalfa for Dairy Cattle

Consortium for Alfalfa Improvement

• Noble Foundation
• Forage Genetics International
• Plant Science Research Unit, USDA-ARS
• US Dairy Forage Research Center, USDA-ARS
Research Challenge/Opportunity

... fiber digestion
Redesigning alfalfa . . .

First: A lesson on cell walls

Cell contents are completely digestible.

But sometimes intact cell walls keep them from being available to the cow.
Redesigning Alfalfa

Cell walls contain carbohydrates such as:
- celluloses
- pectins
- xylans
that are partially available to the cow.

Cows cannot digest lignin.
Redesigning Alfalfa

During digestion, lignin remains intact. But enzymes and microbes eat away at the other carbohydrates which break up and become available to the cow.

This 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.
Can’t get rid of lignin . . . So research goal is to decrease the percentage of it in the cell wall by increasing celluloses and pectins.
Lignin biosynthesis can be reduced by “silencing” genes for one or more key enzymes.

Use genetic engineering to knockout/silence “lignin gene(s)” in alfalfa.
Lignin is more crucial in some cells than others . . . So research goal is to selectively decrease lignin in some cells and leave it intact in others.
Two key genes in the lignin biosynthetic pathway were identified

- Phenylalanine (PAL)
- Cinnamate (C4H)
- 4-Coumarate (4CL)
- 4-Coumaroyl CoA (CCR)
- 4-Coumaraldehyde (CAD)
- 4-Coumaryl alcohol
- 4-Coumaroyl shikimate (COMT)
- 4-Hydroxyconiferaldehyde (F5H)
- Sinapaldehyde (COMT)
- 5-Hydroxyconiferyl alcohol (F5H)
- Sinapyl alcohol (COMT)

Measure affect on lignin content and composition

Noble Foundation gene knockouts

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Transgenic plants have been generated that show decreased lignin content and increased fiber digestibility.

Source: McCaslin et. al., 2002

Consortium for Alfalfa Improvement
Lactating Cow Response

<table>
<thead>
<tr>
<th>Alfalfa hay in diet!</th>
<th>CP (%DM)</th>
<th>NDF (%)</th>
<th>NDFD</th>
<th>Milk lb/day</th>
</tr>
</thead>
<tbody>
<tr>
<td>COMT Inactive</td>
<td>18.1</td>
<td>31.1</td>
<td>53.5**</td>
<td>84.7*</td>
</tr>
<tr>
<td>COMT Active (Control)</td>
<td>18.4</td>
<td>29.3</td>
<td>42.5</td>
<td>82.1</td>
</tr>
<tr>
<td>CCOMT Inactive</td>
<td>18.1</td>
<td>31.2</td>
<td>48.6**</td>
<td>84.5</td>
</tr>
<tr>
<td>CCOMT Active (Control)</td>
<td>18.3</td>
<td>31.1</td>
<td>44.5</td>
<td>86.7</td>
</tr>
</tbody>
</table>

! TMR diets - 50 % alfalfa hay, 10 % corn silage, 40 % concentrate
*Significant, P < 0.10; ** significant P <0.01

# Digestibility in Lambs

<table>
<thead>
<tr>
<th>100% Alfalfa Hay Diet</th>
<th>aNDF</th>
<th>ADL</th>
<th>NDFD</th>
<th>DMD</th>
</tr>
</thead>
<tbody>
<tr>
<td>COMT Inactive</td>
<td>38.2</td>
<td>5.3</td>
<td>57.5*</td>
<td>67.5*</td>
</tr>
<tr>
<td>COMT Active (Control)</td>
<td>39.0</td>
<td>5.8</td>
<td>49.1</td>
<td>64.5</td>
</tr>
<tr>
<td>CCOMT Inactive</td>
<td>39.4</td>
<td>5.2</td>
<td>50.1*</td>
<td>65.3*</td>
</tr>
<tr>
<td>CCOMT (Control)</td>
<td>39.4</td>
<td>5.9</td>
<td>46.4</td>
<td>63.7</td>
</tr>
</tbody>
</table>

*Significant, P < 0.05

**SOURCE:** Mertens et al. 2008. J. Dairy Sci. Supple. 1
# Lignin Composition - Analysis

<table>
<thead>
<tr>
<th>Test</th>
<th>Null</th>
<th>COMT</th>
<th>CCOMT</th>
</tr>
</thead>
<tbody>
<tr>
<td>NIRS breeder equation ---------------</td>
<td>----------</td>
<td>----------</td>
<td>----------</td>
</tr>
<tr>
<td>ADL, DM</td>
<td>12.5b</td>
<td>11.2a</td>
<td>10.9a</td>
</tr>
<tr>
<td>NDFD, NDF</td>
<td>31.8a</td>
<td>35.8a</td>
<td>35.3a</td>
</tr>
<tr>
<td>Chemical analysis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ADL, DM</td>
<td>10.2c</td>
<td>9.6b</td>
<td>7.9a</td>
</tr>
<tr>
<td>T. Thioacidolysis, DM</td>
<td>6.1c</td>
<td>3.8a</td>
<td>5.0b</td>
</tr>
<tr>
<td>S-lignin, DM</td>
<td>2.7b</td>
<td>0.7a</td>
<td>2.7b</td>
</tr>
<tr>
<td>G-lignin, DM</td>
<td>3.3b</td>
<td>3.1b</td>
<td>2.2a</td>
</tr>
<tr>
<td>H-lignin, DM</td>
<td>0.7a</td>
<td>0.7a</td>
<td>0.9b</td>
</tr>
<tr>
<td>NDFD, NDF</td>
<td>30.6c</td>
<td>41.0a</td>
<td>36.2b</td>
</tr>
</tbody>
</table>

Values within row differ significantly, *P* < 0.05

Milk Yield from Alfalfa Silage and Hay Diets

- Fish meal is beneficial in alfalfa silage diets, but not alfalfa hay diets.
- Bottom line: alfalfa silage nitrogen is not efficiently used by the cow

Source: Vagnoni and Broderick, 1997
Feed Storage Problems

- However in alfalfa, our primary forage:
1. The Goal

Reduce protein degradation with silage

Two game plans

Add PPOs and \( o \)-diphenols

Add tannins
Oct. 28, 2009

(PPO is an enzyme, found in many plants, that causes browning and loss of quality.)
Redesigning Alfalfa

Goal #1: Reduce protein degradation

Save protein

Unlike alfalfa, red clover contains PPOs (polyphenol oxidase) and o-diphenol.

The PPO acts on the o-diphenols to produce o-quinones.
Redesigning Alfalfa

Goal #1: Reduce protein degradation

The highly reactive o-quinones bind with protein.

Proteases, which want to degrade protein, cannot do this when o-quinones are bound to the protein.
Redesigning Alfalfa

Goal #1: Reduce protein degradation

Therefore, red clover, compared to alfalfa, loses much less protein when ensiled.

Can we take what works in red clover and transfer it to alfalfa?
Redesigning Alfalfa
Redesigning Alfalfa

PPO genes occur naturally in some variety of alfalfa? NO.

Insert red clover gene into alfalfa? YES. DFRC has successfully done this.
Redesigning Alfalfa

Add plant material that contains o-diphenols at the time of ensiling?

Currently being researched.

Introduce genes for the enzymes needed to produce o-diphenol?

Currently being researched.
Redesigning Alfalfa

Chart shows how much less protein is degraded in alfalfa silage when alfalfa has PPO gene and o-diphenol* is added at time of ensiling.

*caffeic acid in these studies
Tannin is found naturally in some forages such as birdsfoot trefoil.

Tannins bind to protein and protect it during ensiling and in the cow’s rumen.
Redesigning Alfalfa

Research efforts:

- Can we insert a tannin gene into alfalfa?
- Can we grow and ensile birdsfoot trefoil with alfalfa and have an effect?
Redesigning Alfalfa

Milk yield (lbs/day)-alfalfa and birdsfoot trefoil silages

Hymes-Fecht et al., 2005
Redesigning Alfalfa

Potential high value alfalfa

1. Allow us to feed lower protein diets
2. Allow for digestion of complex carbohydrates – new feeding approach
3. Reduce the number of cuttings per season
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Corn Is Ethanol’s Present

Cellulose Is Ethanol’s Future
Ethanol from corn grain is expected to provide only a portion of a biofuels solution. Forestry and process residues, perennial crops, crop residues, and other designated energy crops will provide the bulk of a biofuels solution longer term.
Cellulosic Ethanol: A Dairy Farm System

Several value-added products

CO₂

Stems

Leaves

N₂

Manure

Water quality

Soil

Ash

Several value-added products
Cellulosic Ethanol: Feedstocks - Alfalfa

Biomass-Type Alfalfa

Developed by USDA-ARS

Traits Incorporated:
- Large, lodging resistant stems
- Maintenance of leaf yield
- Winter hardiness
- Disease/pest resistance
  - Root rot
  - Leaf hopper
Cellulosic Ethanol: Feedstocks - Alfalfa

Alfalfa Biomass Production Practices

Modify production practices to maximize both leaf and stem yield.

1. **Bigger Plants**: Decrease stand density to give plants more room to grow.

<table>
<thead>
<tr>
<th>First Production Year Stand</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Traditional</strong></td>
</tr>
<tr>
<td>42 plants/ft²</td>
</tr>
</tbody>
</table>
2. More Stem Biomass: Delay harvest from early bud stage to late flower/green pod stage to get longer stems. Lodging increases at later maturities.
Cellulosic Ethanol: Feedstocks - Alfalfa

Biomass-Type Alfalfa + Biomass Management Doubles Ethanol Yield

Cellulosic Ethanol: Feedstocks-Alfalfa

Research to design equipment to separate alfalfa leaves and stems in-field; ongoing at the University of Wisconsin

Kevin Shinners and Matt Digman
Cellulosic Ethanol: Feedstocks - Alfalfa

Efficient separation of stem and leaf material is technically feasible

Stripped Leaves
- Leaf Fraction: 60%*
- Purity: 90% leaves
- Protein: 27%
- Fiber: 20%

Remaining Stems
- Stem Fraction: 40%*
- Purity: 90% stems
- Protein: 13%
- Fiber: 50%

*Ratio of leaves to stems can be adjusted by changing rotor speed
Bio-based products from alfalfa

Products from fermentation of fiber from wet fractionation, or of stems from dry fractionation
- Ethanol
- Butanol
- Adhesives
- Methane from anaerobic digestion

Products from transgenic alfalfa
- Enzymes (phytase, a-amylase)
- Plastics (poly-b-hydroxybutryate)
Cellulosic Ethanol: Sustainability

ARS Scientist:
Peter Vadas
Madison, WI

Comparison of three systems

**continuous corn**

1

2

3

4

**switch grass**

1

2

3

4

**alfalfa/corn rotation**

1 - establish

2

3

4
Cellulosic Ethanol: Sustainability

Nitrogen loss for three crop systems with normal and high yields (lbs. nitrogen per acre)

- Continuous corn
- Switchgrass
- Corn/alfalfa rotation

Yield
N = normal yield, H = high yield

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Cellulosic Ethanol: Sustainability

Potential farmer profit for the three crop systems with normal and high yields plus low, medium and high commodity prices

- **continuous corn**
  - low price: N, H
  - med. price: N, H
  - high price: N, H

- **alfalfa/corn rotation**
  - low price: N, H
  - med. price: N, H
  - high price: N, H

- **switch grass**
  - low price: N, H
  - med. price: N, H

N = normal yield
H = high yield
ARS and bioenergy research:

Focus on feedstock

- development
- production
- conversion
Redesigning forages: Grasses

While genetic engineering is used with alfalfa, it is not used with other legumes and grasses. Why?

- Small market and very small profit margins with each individual grass species makes it difficult for company to recoup R&D costs.
- The traits that are most desired – better yield and persistence – are not easy traits to genetically engineer by 1 gene.
- A lot of producers who want to grow grasses are philosophically opposed to genetic engineering.
Red Clover Breeding

Red Clover
• Establishment and Management Versatility
• Less Persistent

Breeding Targets
• Increased Persistence
• Increased Yield
• Plant Vigor
50+ years of breeding at USDFRC has dramatically increased red clover persistence

Hay Management
(Smith, 2000)

Rotationally Grazed in Grass Pasture (Riday, 2009)
Grazing Red Clover

- Newer red clover more persistent
  - 100% red clover ground cover at 9 or more plants per sq. ft
  - Not all new red clover varieties are equally persistent

\[
\text{LSD}_{0.05} = 0.8
\]
Kura Clover Breeding

Kura Clover
• Very Persistent
• Difficult to Establish

Breeding Targets
• Seedling Establishment
• Seed Production and Yield
• Plant Vigor

Prairie du Sac, WI – Breeding Nursery
Birdsfoot Trefoil Breeding

Birdsfoot Trefoil - Non Bloating Legume

New Cultivar 'WITT'

- Commercial License Granted to Allied Seed
- Seed to Market in 2-3 Years

'WITT' had Superior Persistence Across 16 MN & WI Variety Trials
Redesigning forages: Grasses

Recent pasture/grazing research
Geoff Brink, DFRC Agronomist

Agronomic potential of meadow fescue

Response to N rate and defoliation management at grazing maturity by:
Bronc orchardgrass
Barolex soft-leaf tall fescue
Azov, Bartura, and Hidden Valley meadow fescue.
Redesigning forages: Grasses

- Tall fescue and orchardgrass yielded 500-1000 lb DM/acre/year more than meadow fescue.

- NDFD of meadow fescue > tall fescue and orchardgrass throughout growing season at similar maturity.
Redesigning forages: Grasses

Animal response to meadow fescue

Compare intake, digestibility, and behavior of bred dairy heifers grazing meadow fescue, orchardgrass, reed canarygrass, and quackgrass (grasses that differ in sward structure and quality).

Collaborative with Dr. Kathy Soder, USDA-ARS Pastures Systems and Watershed Management Research Unit.
Redesigning forages: Grasses

- Meadow fescue had greater *in situ* digestibility than tall fescue or orchardgrass, but greater intake in fall only.
Redesigning forages: Grasses

Targets for Grass Breeding

• **The past:** hay/silage production
  – The focus of grass breeding since its beginning.
  – Many excellent, well-adapted varieties exist.
Redesigning forages: Grasses

• **The present:** management-intensive grazing & pastures
  – Virtually no grass breeding efforts until 1990.
  – Most breeding programs have shifted toward this goal.
  – The best hay types are not necessarily the best pasture types and vice versa.
Recent grass breeding activities
(Mike Casler, ARS-U.S. Dairy Forage Research Center, Madison, WI)

- Timothy and bromegrass:
  - Breeding grazing-tolerant varieties
- Reed Canarygrass:
  - New cultivar with improved establishment by selection and breeding
  - Determining the mechanism for improved establishment

Original germplasm taken from natural, undisturbed locations such as cemeteries and ditches.
Redesigning forages: Grasses

- ‘Spring Green’ Festulolium
  - Meadow fescue x perennial ryegrass hybrid
  - Quality & establishment similar to ryegrass
  - Drought tolerance similar to fescue
  - Selected for winter survival on-farm
Redesigning forages: Grasses

• ‘Spring Green’ Festulolium
  – Tested in 8 states from Minnesota and Iowa to New York and Virginia . . .
  – . . . and compared to previous fescue x rye varieties, Tandem and Kemal
  – Spring Green showed a 31% increase in survival (52 vs. 40%). . .
  – . . . and a 2% increase in tons/acre (3.98 vs. 3.91)
Redesigning forages: Grasses

- **Meadow Fescue:**
  - New cultivar, ‘Hidden Valley,’ selected from Charles Opitz farm, WI
  - Drought tolerance and highly palatable

Opitz Farm, Wisconsin
Redesigning forages: Grasses

• Non-heading Orchardgrass
  – A management technique, not breeding
  – Designed to simplify grazing management in the spring
  – Orchardgrass flowering genes turned off in cold weather, but not in warm weather
  – Take seeds produced in warm Oregon and plant them in hardiness zones 3, 4, and 5 where winters are cold.
Redesigning forages: Grasses

Grass and legume breeding summary:

• We have changed the focus of our grass and legume breeding program from hay harvesting to grazing.
• We are developing new varieties with unique traits that will simplify and enhance the grazing operation.
• There is a growing interest and market for these varieties.
Team Plant Modification - targeted

- Use conventional and molecular breeding where appropriate
- Target breeding at dairy cattle intake, nutrient digestion and nutrient metabolism
- Use multi-disciplinary approach within unit and with partners
- Benefit of high risk research with partners in alfalfa is dependent on acceptance genetic modification by customers