U.S. Dairy Forage Research Center

USDA, Agricultural Research Service

Biotechnology & Alfalfa: Future Implications for Alfalfa Producers

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Biotechnology & Alfalfa: Future Implications for Alfalfa Producers

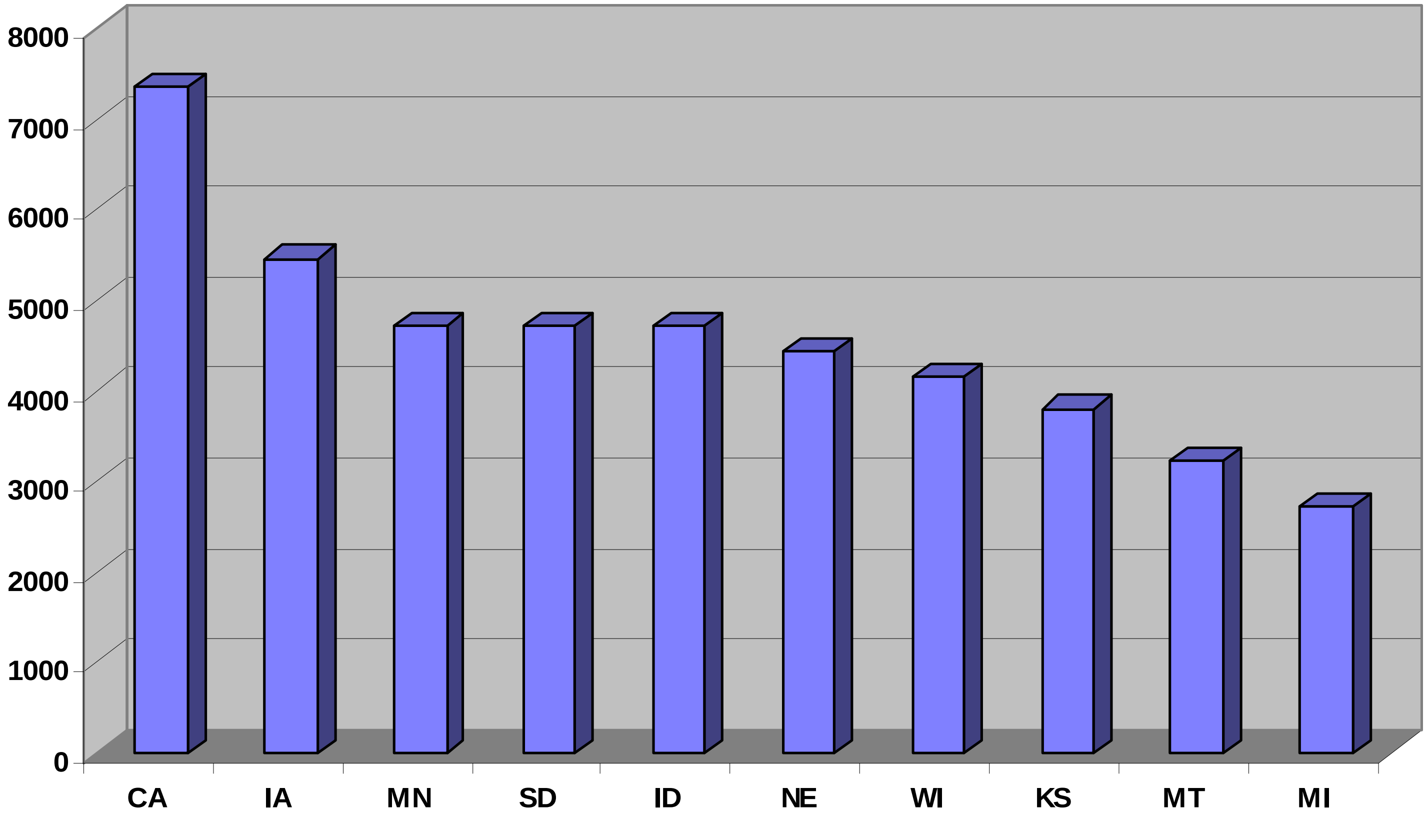
- Alfalfa production: trends and uses
- Biotechnology in alfalfa
- Advantages of alfalfa for dairy operations
  - Outstanding dairy forage
  - Crop rotations
  - Can be a nitrate scavenger
- The perfect alfalfa plant on dairy farms
- Future innovations needed to maintain or expand alfalfa acreage

2004 U S Alfalfa Production

- **Hay**
  - 75.4 million tons
  - 21.7 million acre
  - $ 7.0 billion
  - 3rd following corn and soybeans

- **Forage**
  - 83.9 million tons
  - 24.7 million acres
  - ~$8.2 billion
  - 3rd following corn and soybeans

Leading Alfalfa Hay Production States, 1,000 tons, 2004



- **Top 10 States**
  - 60 % of U. S.
  - 61 % of Acre
  - 7 states NC
  - 3 states West
  - 6 Lead Dairy

Acreage – 21.7 mil
Production – 75 mil

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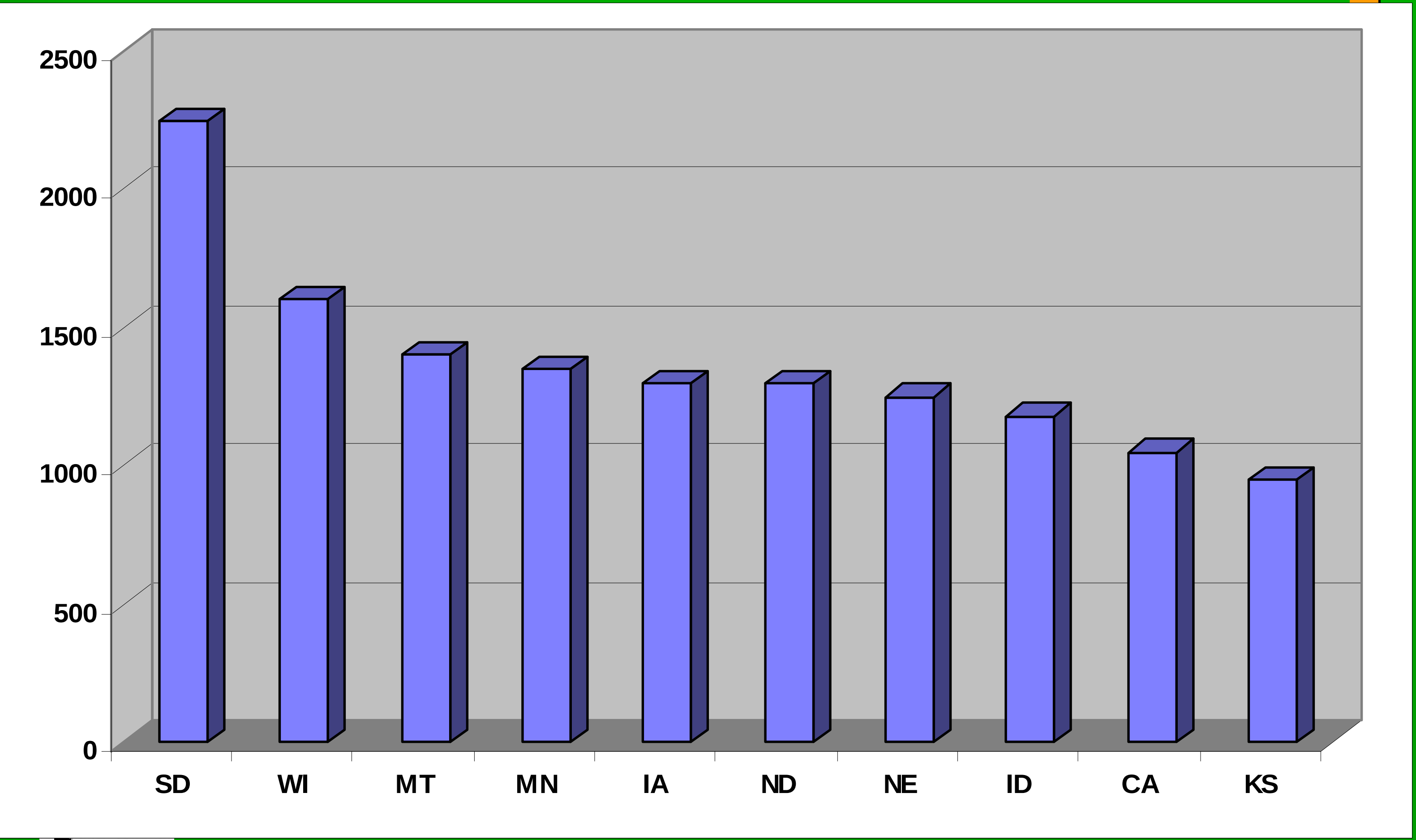
Leading Alfalfa Hay States, 1,000 acres, 2004



- Top 10 States
  - 63 % of U. S.
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  - 7 states NC
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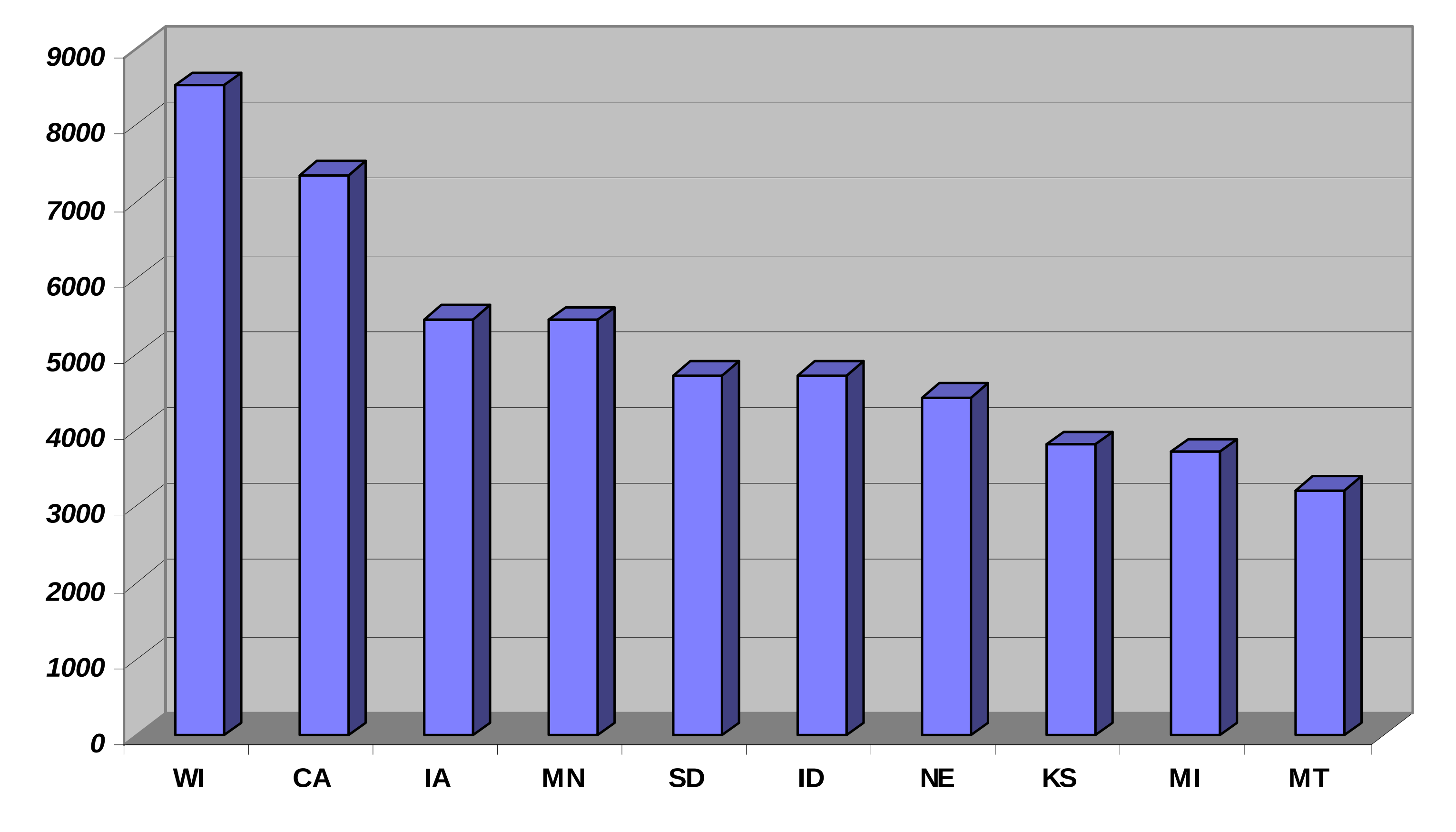
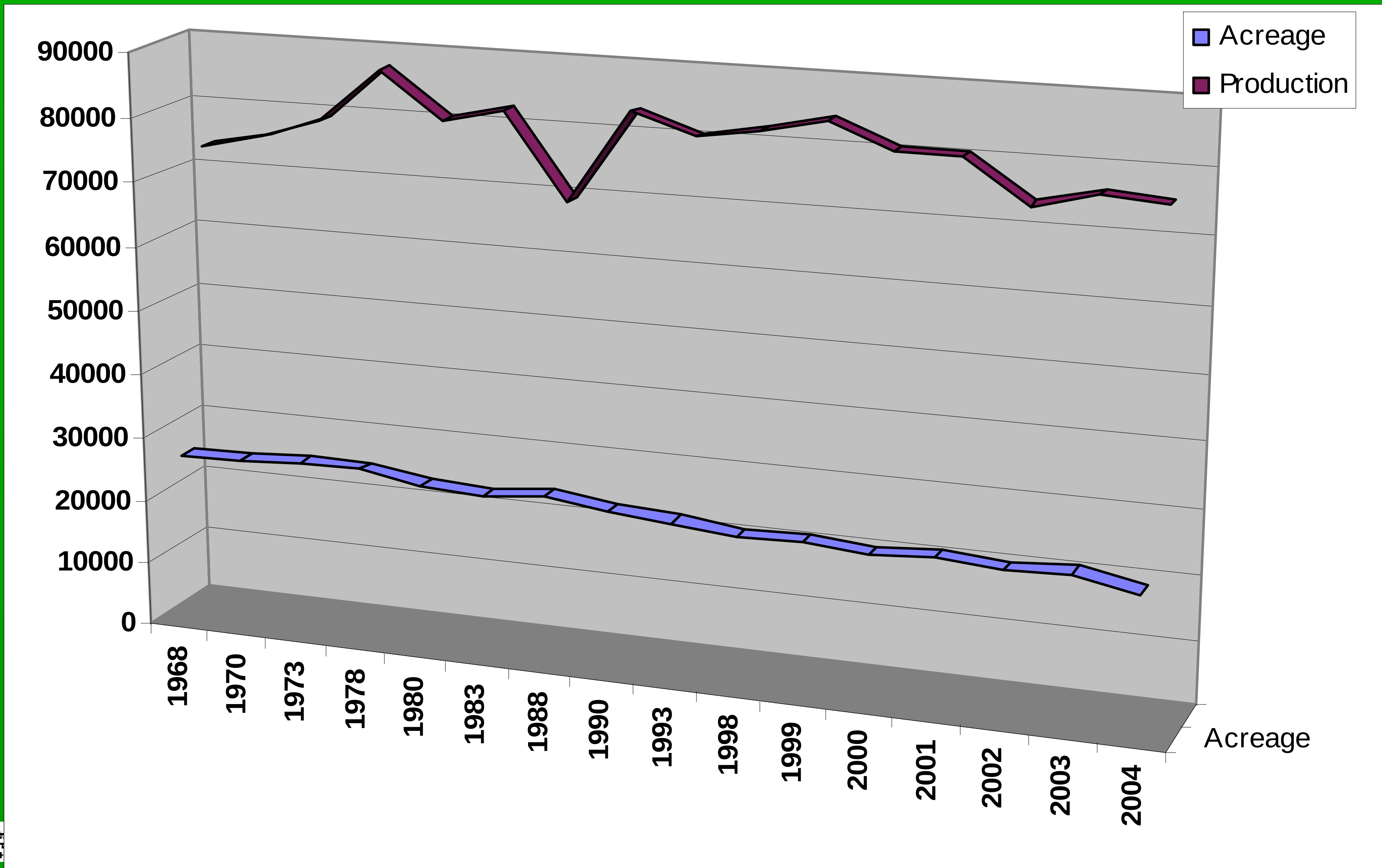
Leading Alfalfa Forage Production States, 1,000 tons, 2004



- Top 10 States
  - 61% of U. S.
  - 61 % of Acre
  - 7 states NC
  - 1 state NE
  - 3 states West
  - 5 Lead Dairy

Acreage – 23.3 mil
Production – 84 mil

U. S. Alfalfa Hay in 1,000 tons

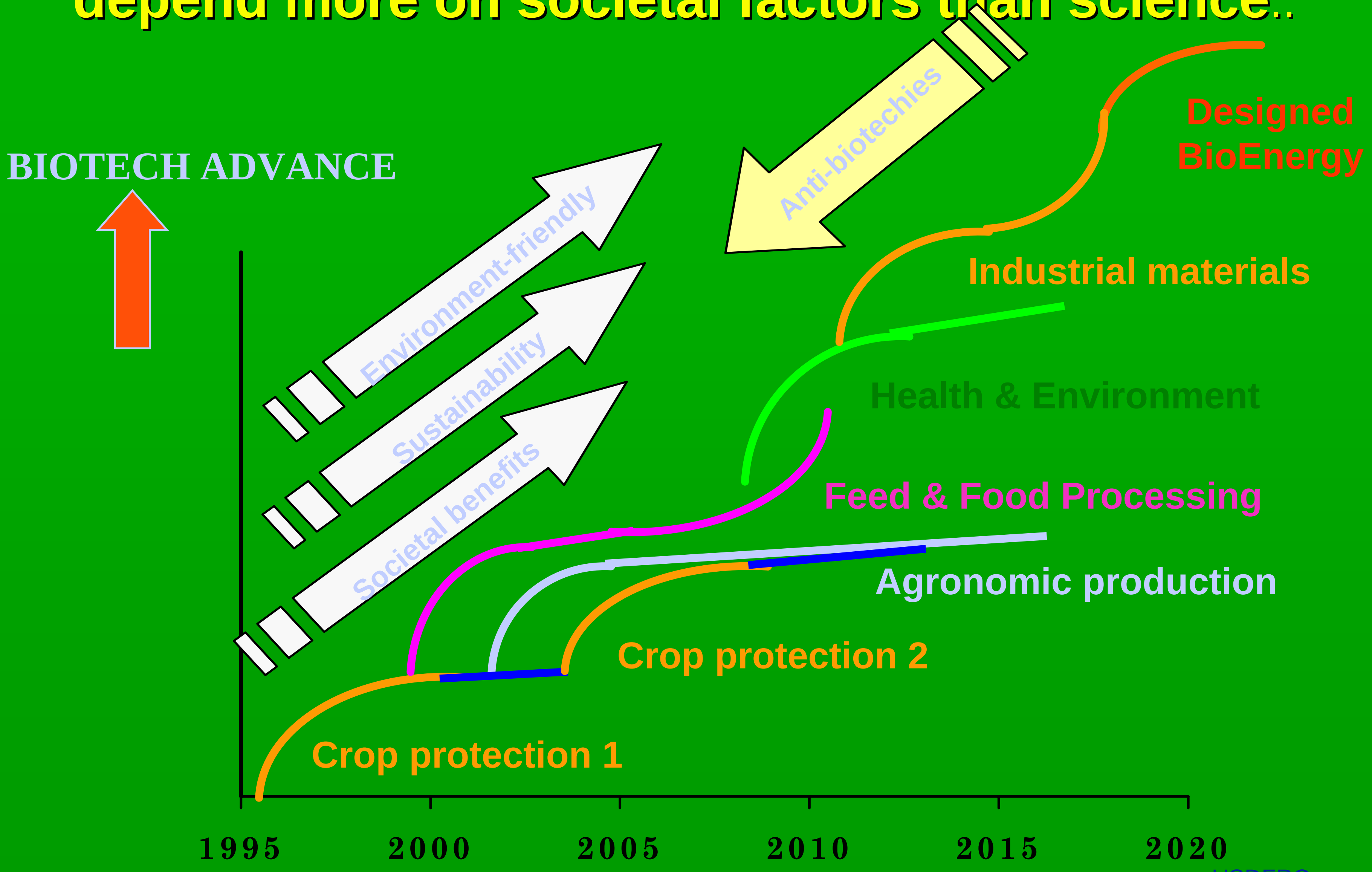


Alfalfa Hay and Silage Usage

- Dairy Industry
- Beef Industry
- Horse Industry
- Export
- New uses

“Biotech creates a new platform for advance of agricultural science, which will allow for the development of higher standards for generation of products that will enhance Agriculture” James S. McLaren

Biotech will deliver many benefits but the timing will depend more on societal factors than science..



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Biotechnology in Alfalfa

- Time line for Biotechnology
  - 1970 – Talk
  - 1980 – Vision
  - 1990 – Laboratory
  - 1998 - Field

Alfalfa: One of Easiest Crops to Use Biotech

- **Why only a few traits ready to enter market?**
  - Area relatively new – limited # cloned genes for important traits
  - Best promoters may not be in hand
  - Both genes and promoters may be patented
  - Lingering concerns about GMO's
  - Some regulations regarding GMO's still in place especially for perennials with weedy relatives

SOURCE: Edwin T. Bingham. AFGC-NAAIC Conference. July 17, 2000. P 385-387.

Alfalfa Biotechnology Research

- Over-expression of salt tolerance
- Commercialization of Roundup Ready gene and down regulation of lignin genes to increase digestibility
- Identifying alfalfa genes controlling yield and winter-hardiness
- Identifying genes controlling salt and drought tolerance
- Cloning genes for vegetative storage proteins

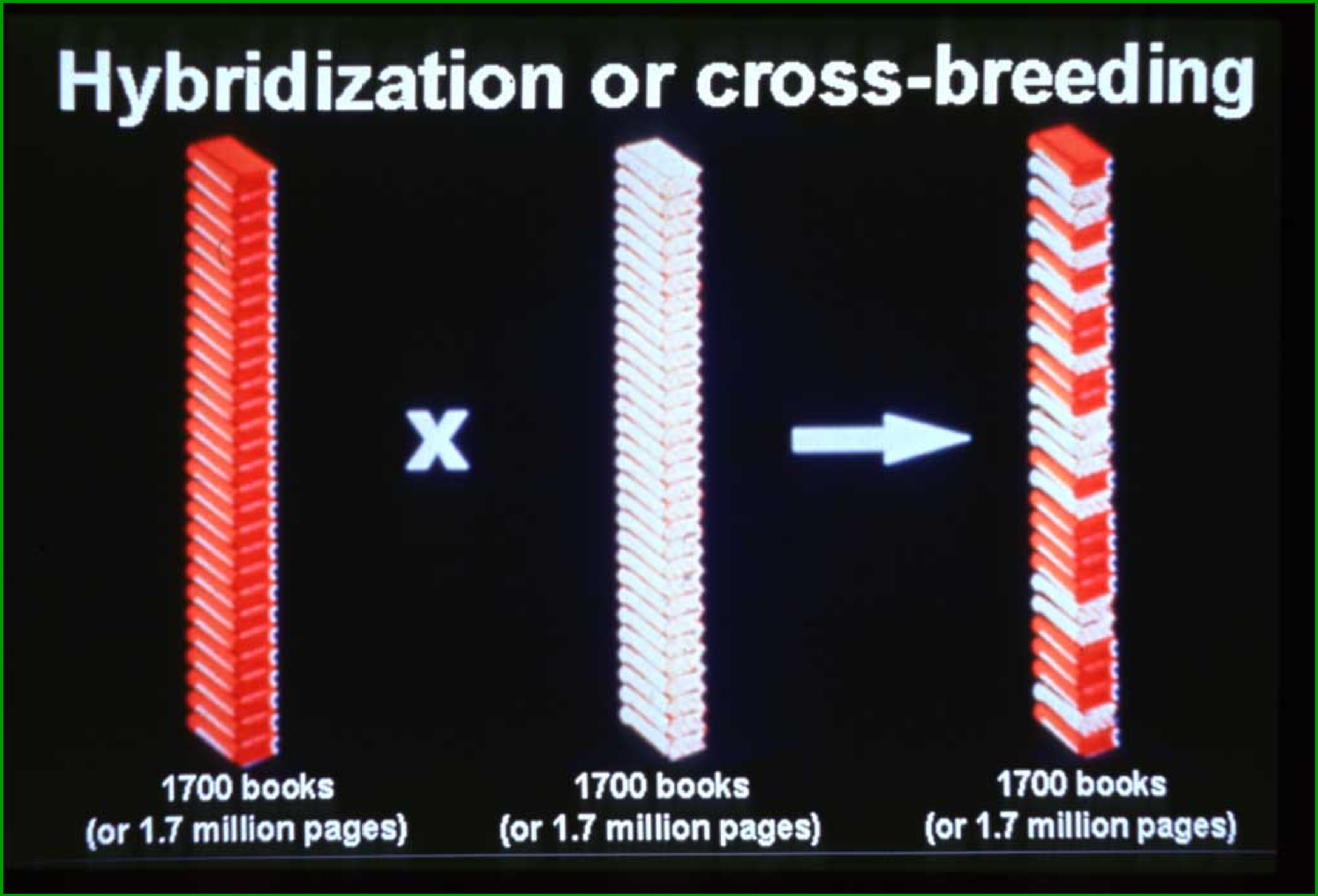
SOURCE: Brummer et al., 2004

Alfalfa Biotechnology Research

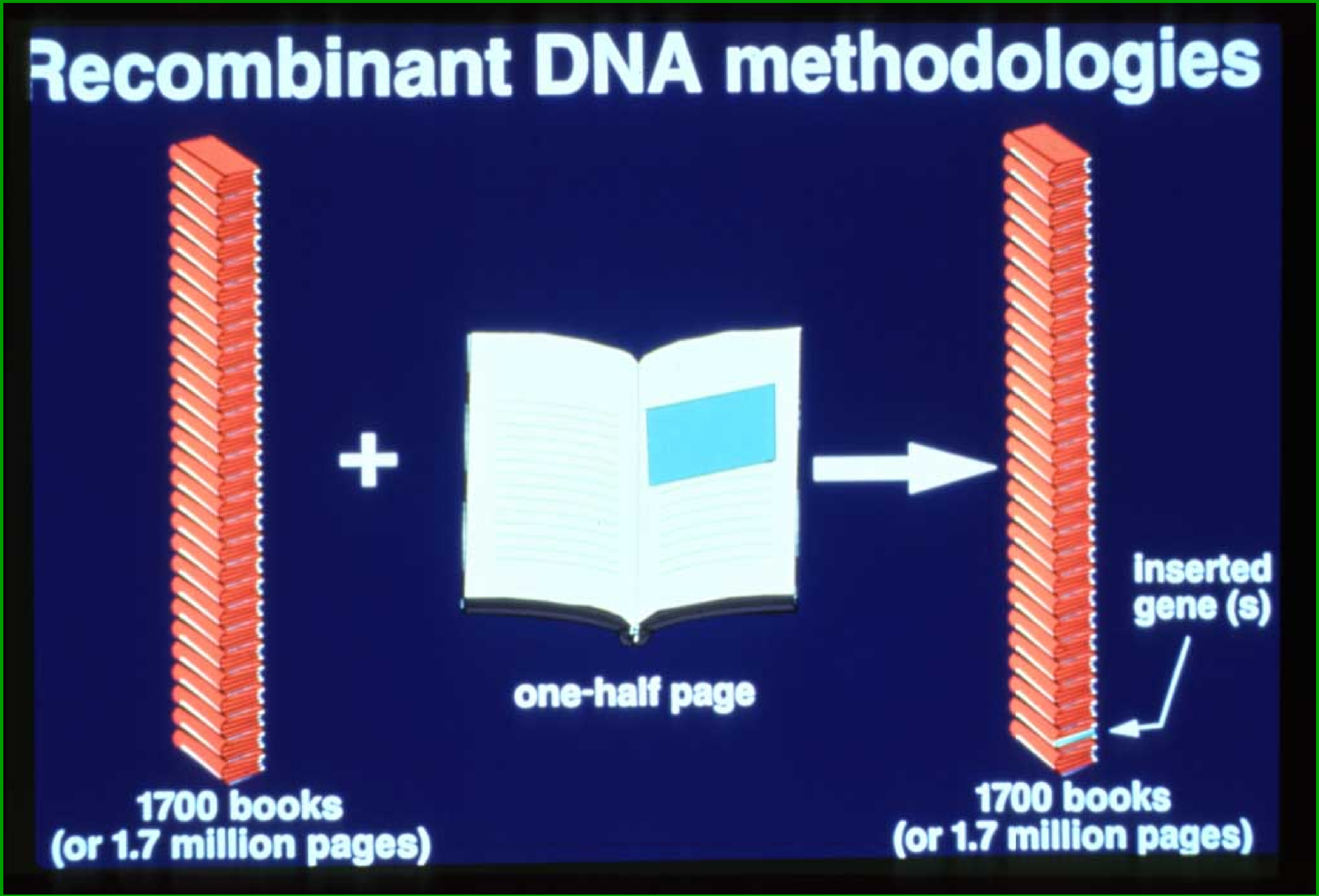
- Developing molecular markers, studying down regulation of lignin genes, insertion of genes for condensed tannins, identifying and introgression of drought and aluminum tolerance genes
- Developing molecular markers and using markers for identifying genes for yield and winter survival
- Characterize genes controlling post-harvest proteolysis
- Insertion of genes to allow remediation of atrazine and genes to control pectin in cell walls

Agricultural Research Service

SOURCE: Brummer et al., 2004 USDFRC



Hybridization or cross-breeding
x
1700 books
(or 1.7 million pages)
1700 books
(or 1.7 million pages)
1700 books
(or 1.7 million pages)


Recombinant DNA methodologies
+
one-half page
inserted gene (s)
1700 books
(or 1.7 million pages)
1700 books
(or 1.7 million pages)

Use Biotechnology in Alfalfa

- Enhance yield
- Forage quality improvements
- Environmental enhancements
- New products

Alfalfa Hay and Silage Usage

- Dairy Industry
- Beef Industry
- Horse Industry
- Export
- New uses

Changes in U. S. Dairy Since 1935

Year	Dairy Farms	Milk Cows	Total Milk
	Thousands	1,000 hd	Billion lbs
1935	>4,100	24,187	100
1965	1,108	14,953	124
2002	92	9,141	170

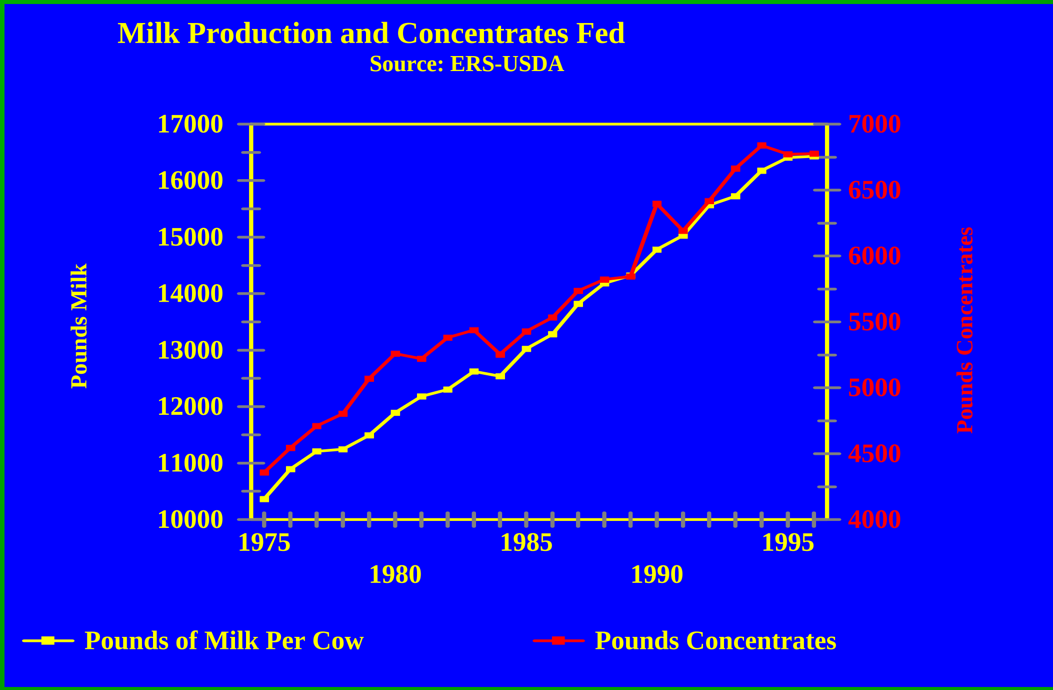
Alfalfa – Outstanding Forage for Dairy

- ✓ *High nutrient content*
  - *Protein, Minerals*
- ✓ *Good fiber digestibility*
- ✓ *Rapidly digested*
- ✓ *Supports high DM intakes*
- ✓ *Supports high milk production*
- ✓ *Cows like it*

SOURCE: Jim Linn, University of Minnesota

Less alfalfa being fed in dairy rations

- Lower yield of alfalfa than other crops
- Increased use of corn silage
- Minimized forage in ration
  - Cheap grain
  - Greater quality consistency of grain
  - Inability to accurately estimate energy of forage



Impact of Harvest Management on Forage Quality

Description	CP	EE	Ash	Starch	Pectin	aNDF	ADF	ADL
ALFALFA HAY								
Exceptional	25.4	2.7	10.4	3.1	14.2	30.0	24.0	4.53
Very high	24.0	2.6	9.9	2.9	13.2	34.1	27.0	5.38
High quality	22.5	2.5	9.5	2.7	12.3	38.2	30.0	6.23
Good quality	21.0	2.4	9.1	2.5	11.4	42.2	33.0	7.08
Fair quality	19.5	2.2	8.7	2.3	10.5	46.3	36.0	7.93
CORN SILAGE								
V. high grain	8.3	3.2	4.1	31.1	1.7	36.0	21.0	1.57
High grain	8.6	3.1	4.6	27.2	1.6	40.5	24.0	1.91
Normal	8.8	3.0	5.1	23.2	1.5	45.0	27.0	2.25
Low grain	9.0	2.8	5.7	19.2	1.4	49.5	30.0	2.59
Very low grain	9.3	2.7	6.2	15.3	1.3	54.0	33.0	2.93

High Alfalfa Haylage Diet

Item	Control	Protein	Fat
DM intake, lb	48.4[b]	55.9[a]	49.5[b]
BW gain, lb	50.6	48.4	33.0
3.5 % FCM, lb	63.4[c]	75.0[a]	67.5[bc]
Milk protein, lb	1.89[b]	2.29[a]	1.94[b]

[abc] Means in same row with different superscripts differ ($p<0.01$)

SOURCE: Dhiman and Satter, 1993.

Protein Use of Alfalfa

Item	silage	hay	silage +FM[1]	hay+FM[1]
CP,% of DM	17.1	15.4	18.6	17.0
	pounds DM per day per cow			
DM intake	49.2[c]	52.9[a]	51.4[b]	53.4[a]
BW change	-0.86[c]	0.99[a]	0.18[b]	1.08[a]
Milk	77.8[c]	79.6[b]	82.5[a]	81.4[a]
Fat	2.65[b]	2.60[b]	2.82[a]	2.69[b]
Protein	2.29[c]	2.43[b]	2.51[a]	2.49[a]
SNF	6.64[c]	6.81[b]	7.05[a]	7.01[a]

[abc] Means in same row with different superscripts differ ($p<0.05$)

[1] Diets supplemented with 3 % (DM basis) low-soluble fish meal.

SOURCE: Broderick, 1995.

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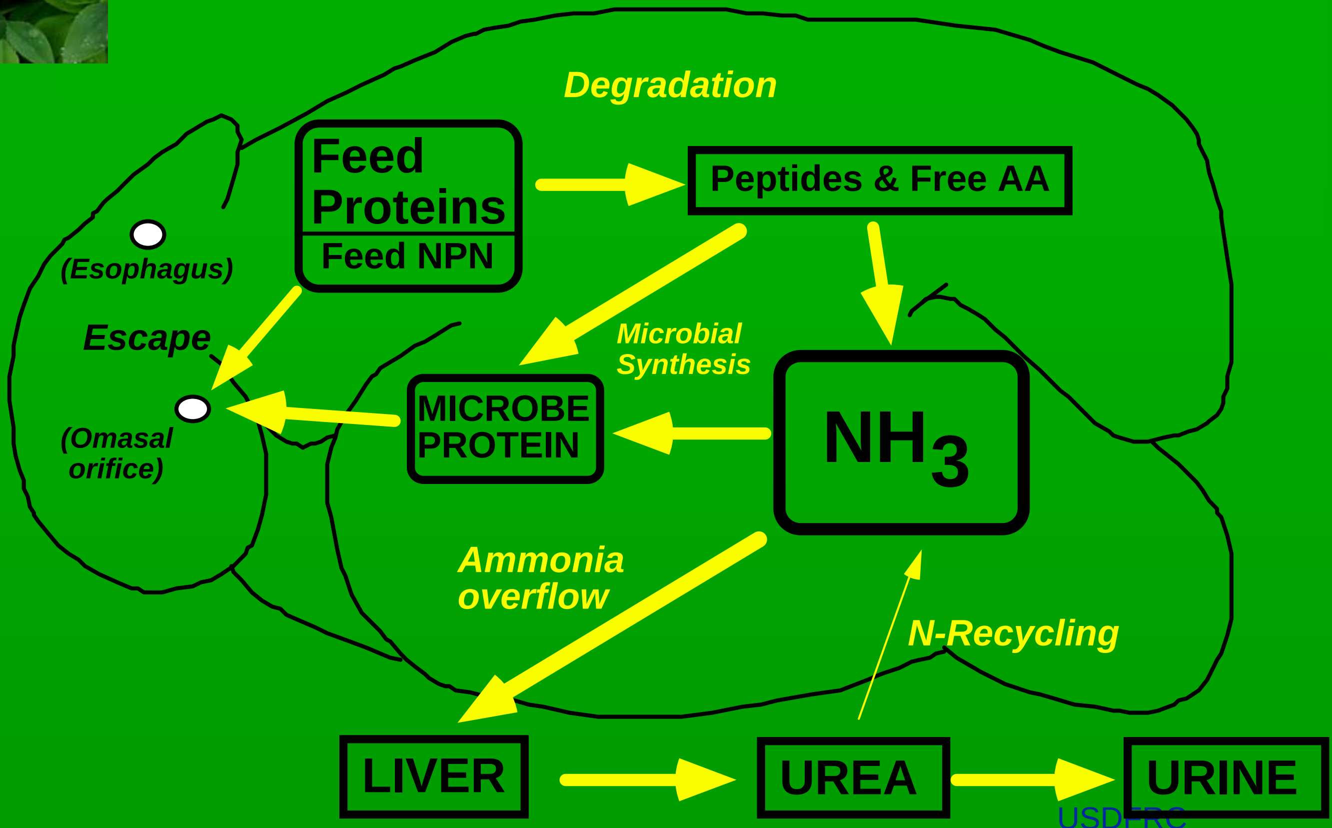
Alfalfa protein is wasted

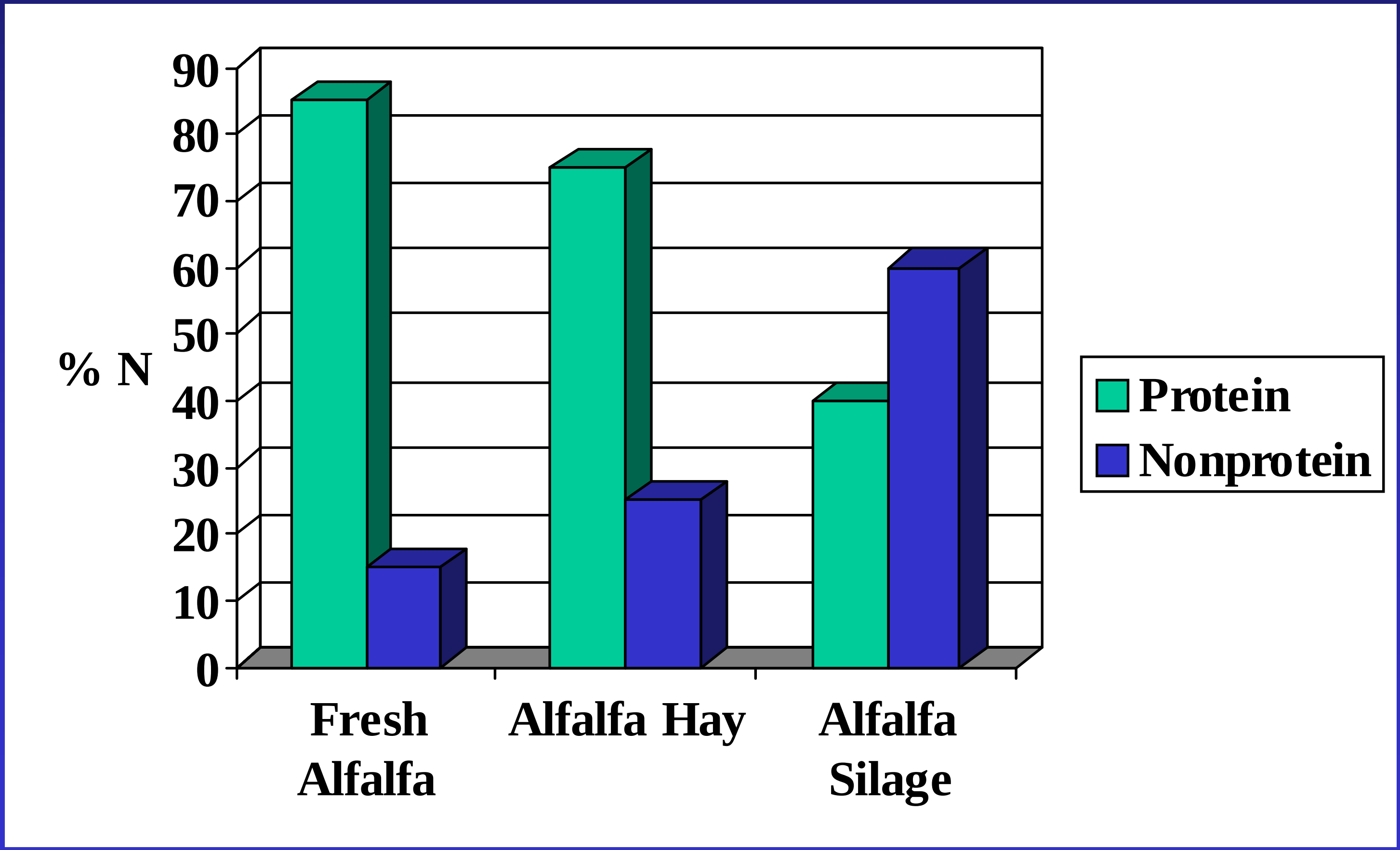
20+% protein in the field

5% protein exits the rumen

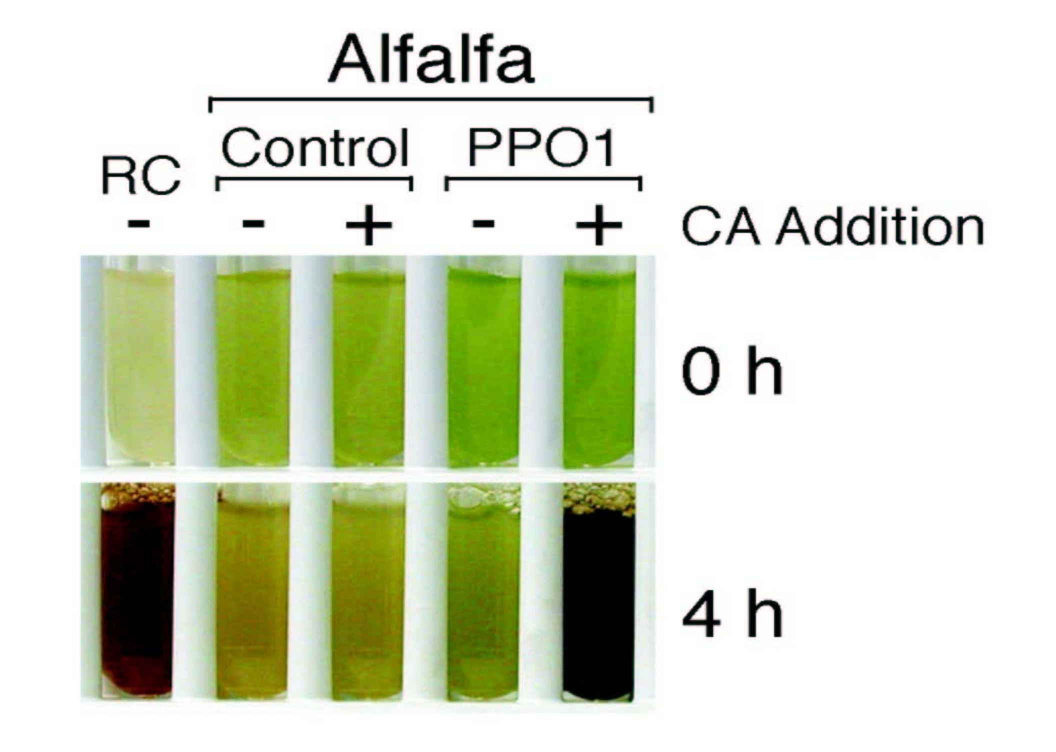


Feed Storage Problems

- However in alfalfa, our primary forage:



Expression of red clover PPO1 in transgenic alfalfa



In alfalfa, browning is dependent on:

- A PPO transgene
- Exogenous o-diphenol, e.g. caffeic acid

SOURCE: Sullivan, Michael L. and Ron D. Hatfield. 2003 DFRC Research Report

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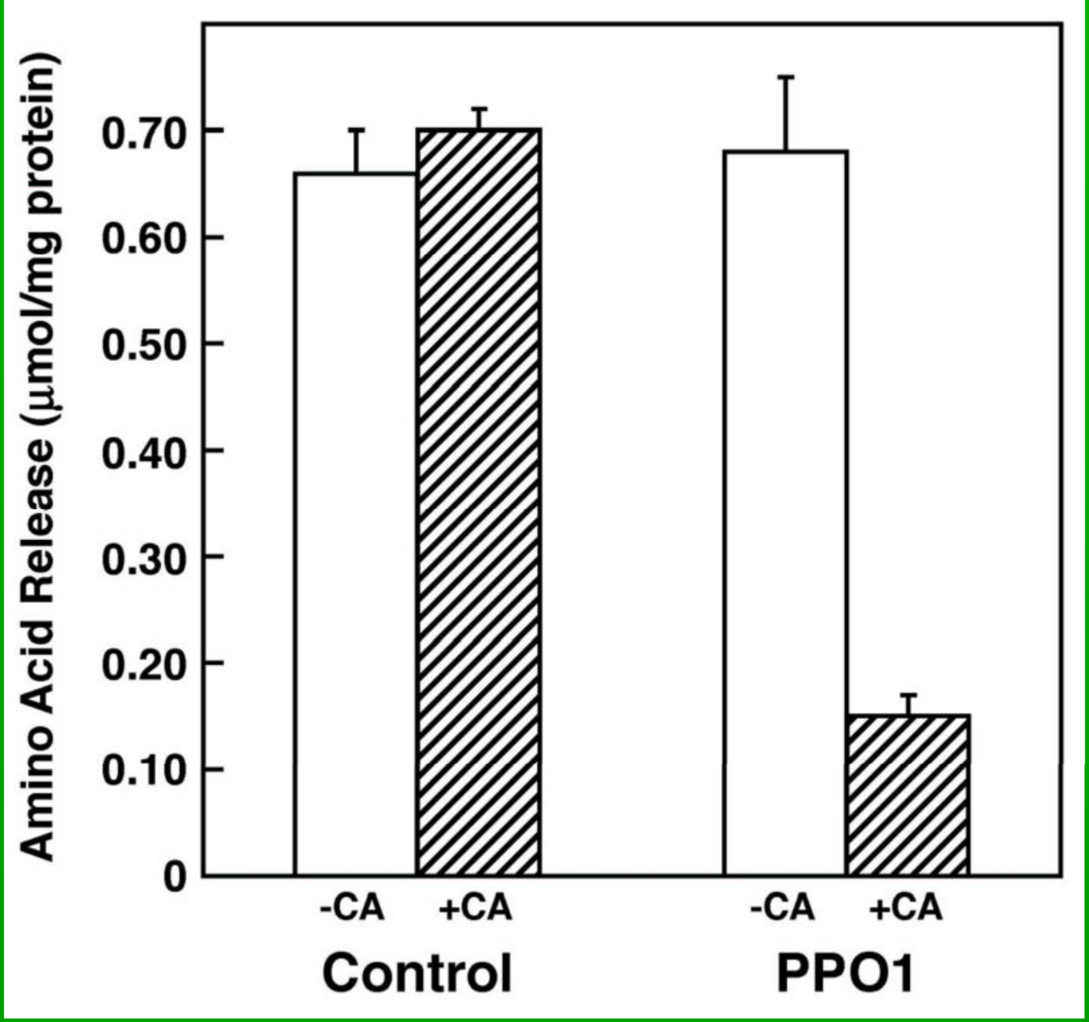
PPO Inhibits Postharvest Proteolysis



SOURCE: Sullivan et al., 2004

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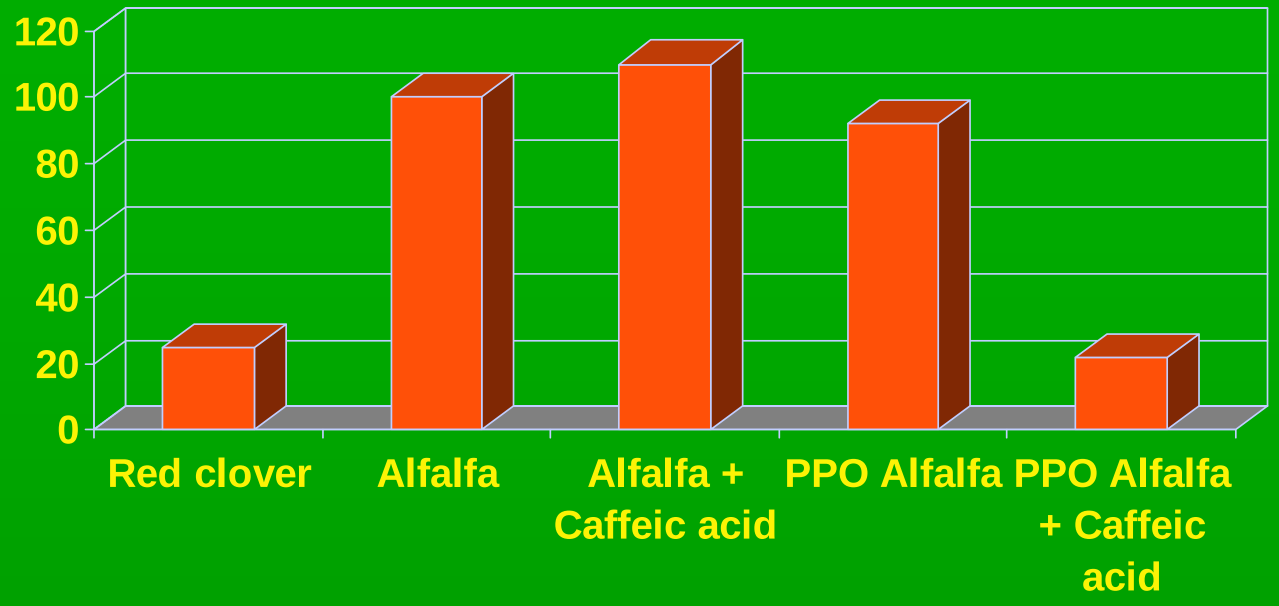
Red Clover vs. Alfalfa Silage

Protein breakdown (% of alfalfa)



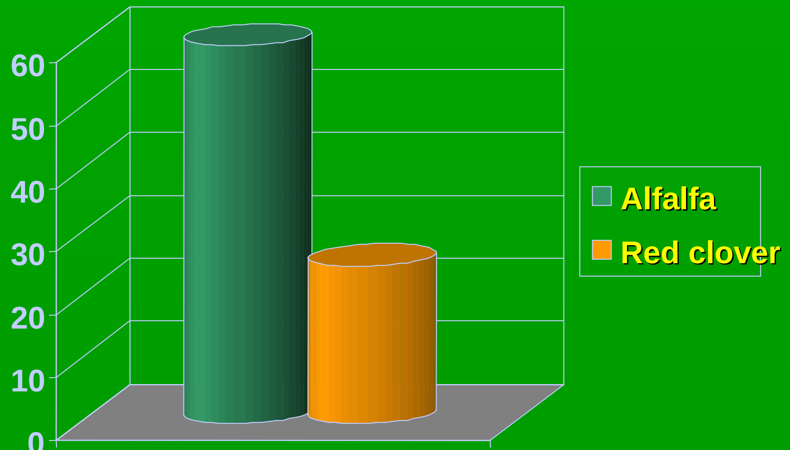
Alfalfa can be used as a model to study the inhibition of protein breakdown in silages.

PPO = Polyphenol Oxidase gene from red clover

Post Harvest Proteolysis in Alfalfa
Impact on dairy production

- Increased NPN decreases the efficiency of protein utilization in ruminants
  - Inefficient utilization of alfalfa protein requires the feeding of supplemental protein with high RUP to maximize milk production.
  - Inefficient utilization of alfalfa protein also results in the excretion of excess rumen NH3, leading to increased N losses to the environment.

Typical NPN content of silage



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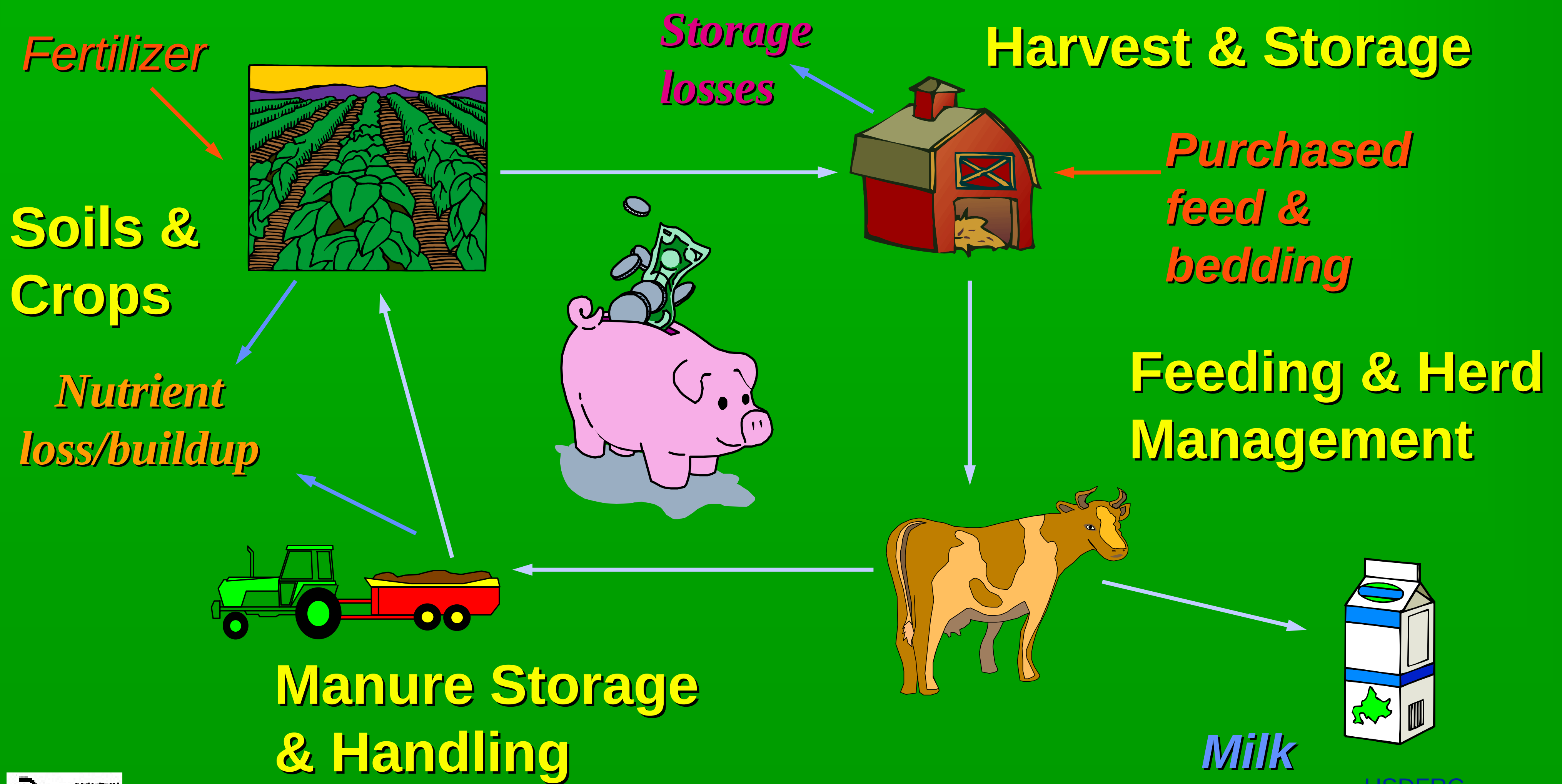
Evaluated the potential impact of tannins on dairy farms with DAFOSYM



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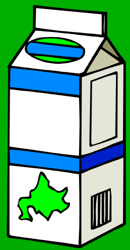
Tannins improve protein utilization

- Condensed tannins are polyphenolic compounds that bind to protein in the pH range 3.5 to 7, potentially protecting protein in the silo, rumen, & soil
- Protein-tannin complexes dissociate at pH <3.5 and >8.5, permitting digestion in the gastrointestinal tract of cattle
- Livestock given tannin-containing feeds need less protein supplementation and excrete less urea
- Tannins slow nitrogen release from crop residues and manure
- Major U.S. feedstuffs, including alfalfa, have inadequate tannin levels to protect protein (< 0.2% DM). Probably about 2% tannin is needed.

Milk production (lb per cow)



	Alfalfa silage 70%	Alfalfa silage 50%	Alfalfa silage 30%
Normal alfalfa	27,160	27,460	27,800
Tannin alfalfa	27,620	27,830	28,000
Tannin impact	+ 460	+ 370	+ 200

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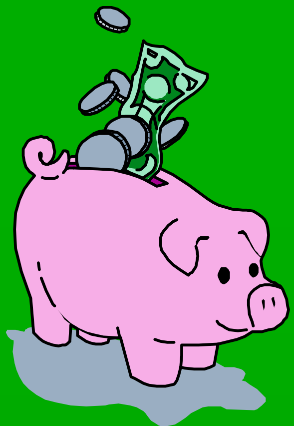
Added value of forage with tannin (per ton dry matter)



Alfalfa silage	$ 23
Alfalfa hay	$ 11

Rumen undegradable protein (RUP)

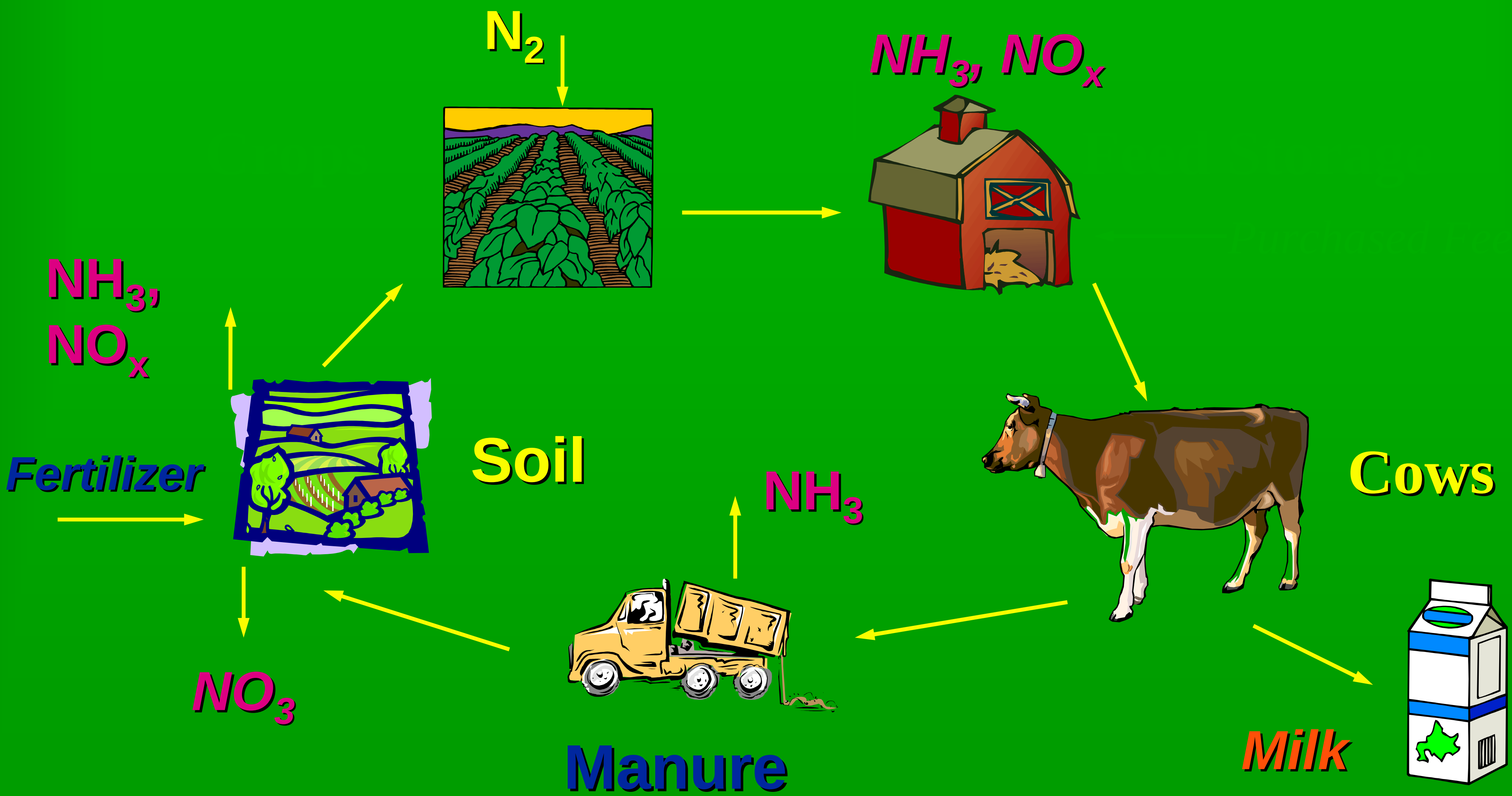
	Conventional	Macerated
	--% of total crude protein--	
Alfalfa	20.1	24.9
Low tannin trefoil	22.7	32.0
High tannin trefoil	28.8	40.6
Red clover with o-diphenols and PPO	32.8	27.9

Forage X Conditioning (P < 0.01)

Ongoing work: Assess gastrointestinal degradability of RUP, especially in red clover

Uptake and loss of manure and forage residue N by subsequent annual crops

Nitrogen Cycle on Dairy Farms



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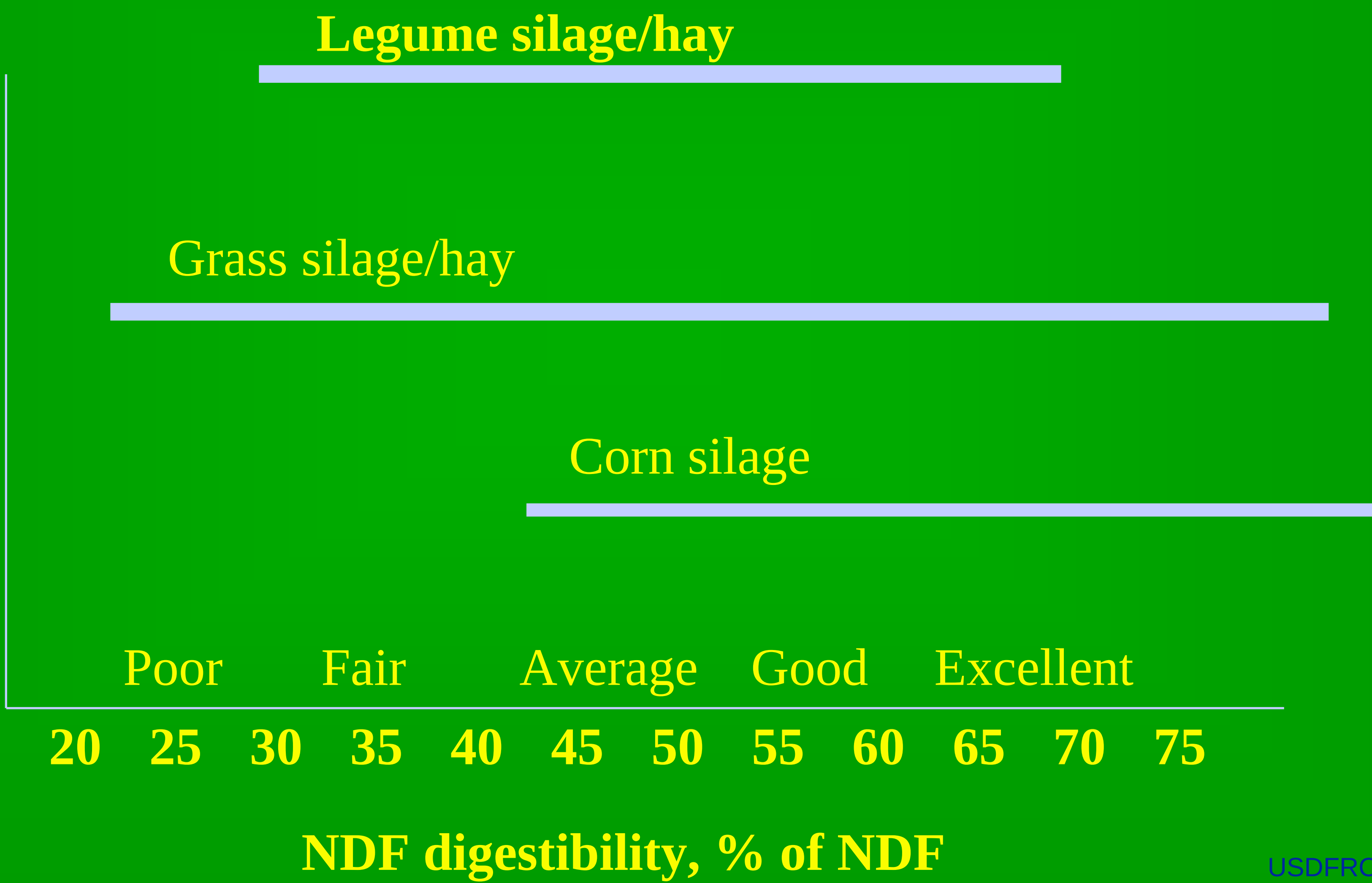
NDF Digestibility of Forages



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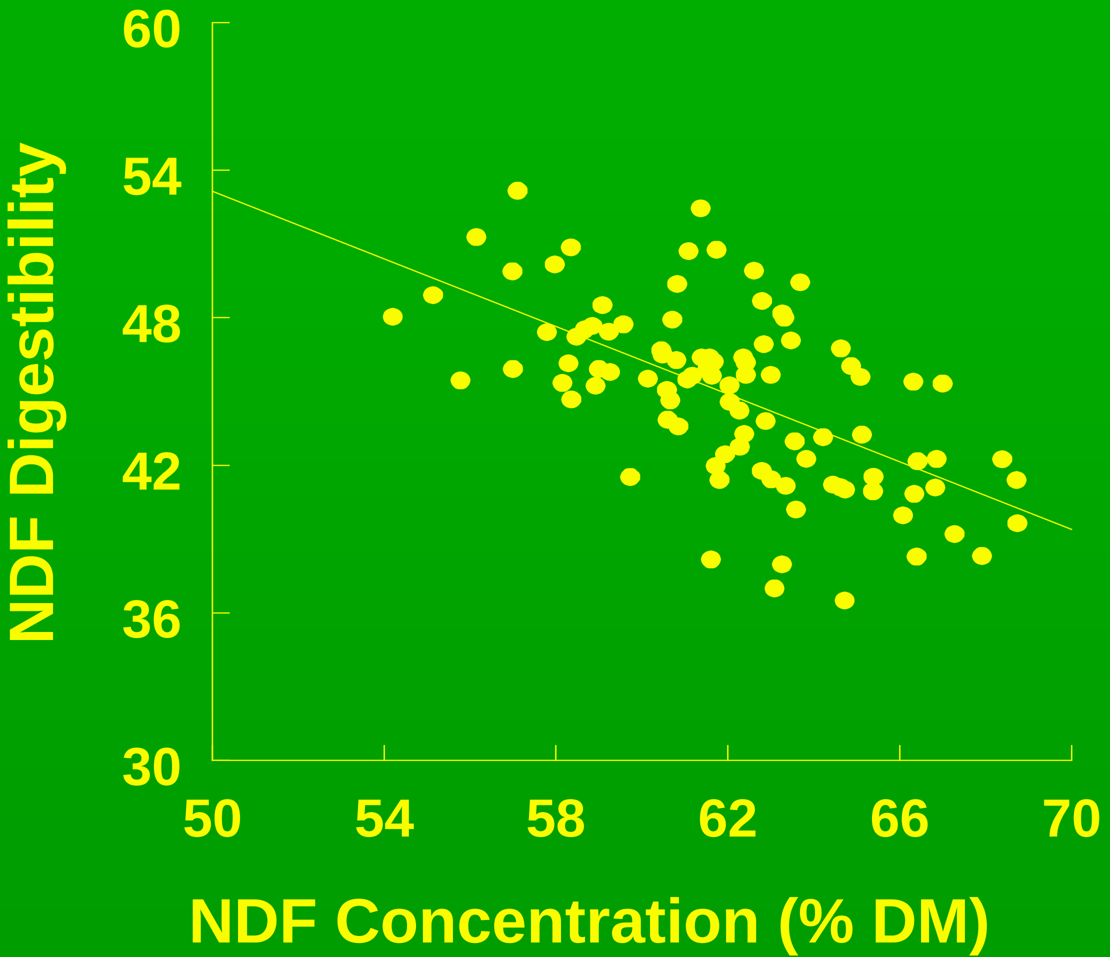
NDF Digestibility of Alfalfa Stems



SOURCE: Jung and Lamb, 2002. Unpublished USDA-ARS. St. Paul, MN

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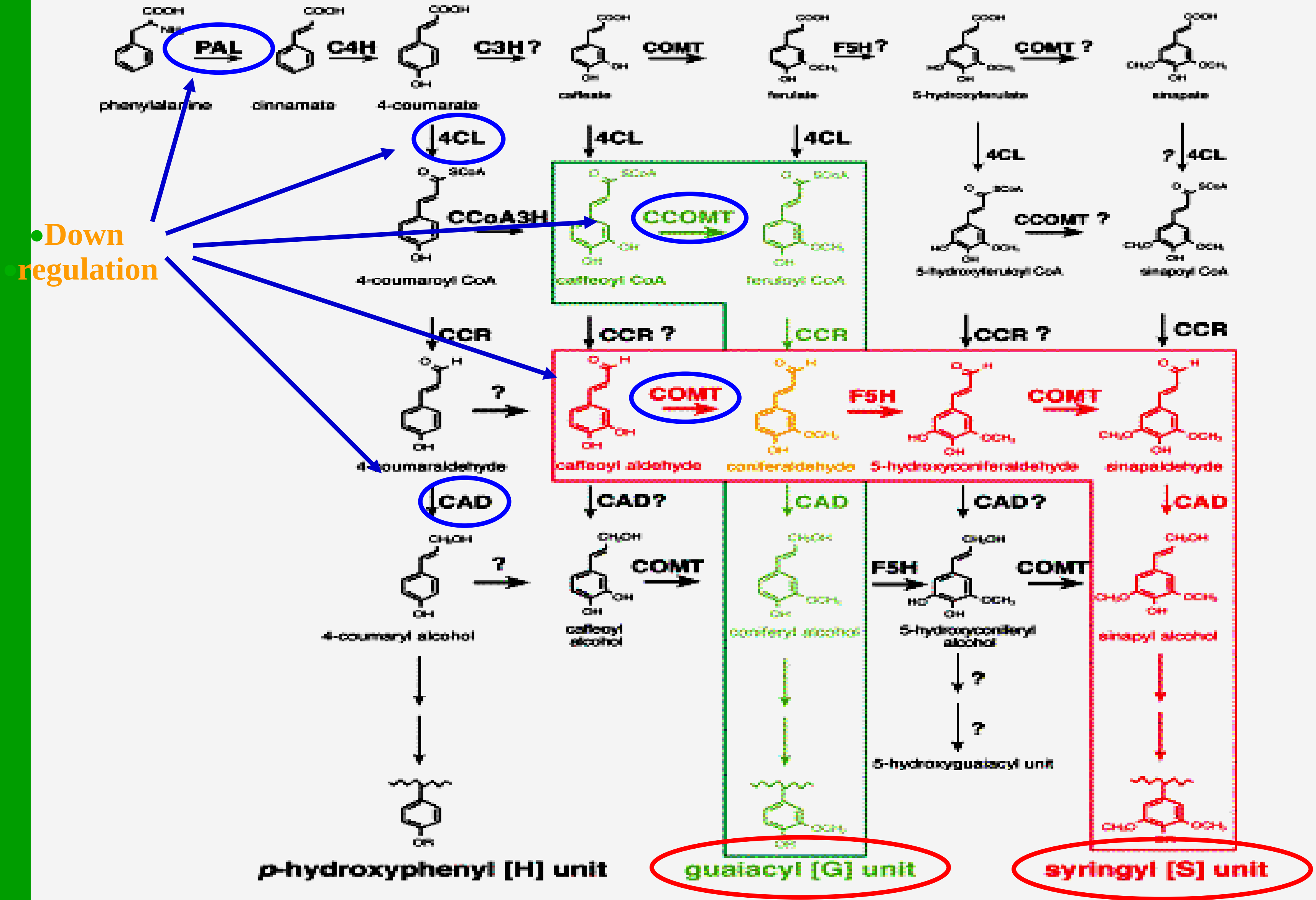


Engineering the lignin biosynthetic pathway in alfalfa

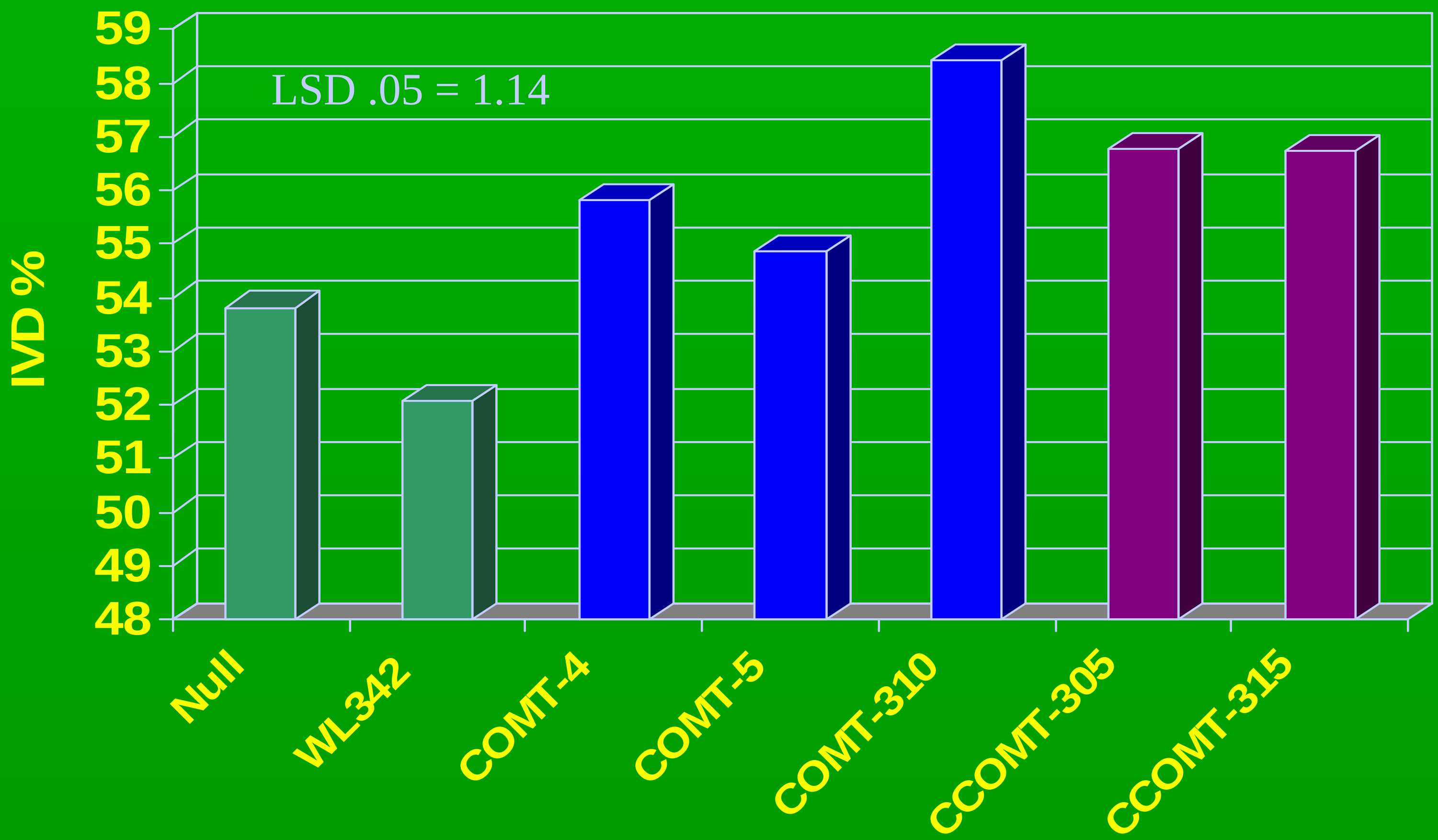
Genetic engineering for improved forage quality in alfalfa

- **Altered lignin content/composition in alfalfa**
  - Low lignin transgenic alfalfa produced based on "knockouts" of enzymes involved in lignin biosynthesis.

	COMT pkat/mg	CCOMT pkat/mg	Klason Lignin %	S/G ratio
Control	6.55	23.77	**17.91**	.47
COMT-	1.24	22.26	**12.46**	.04
CCOMT-	14.39	0.78	**14.58**	1.05
Dual-	0.78	5.59	**14.72**	.23

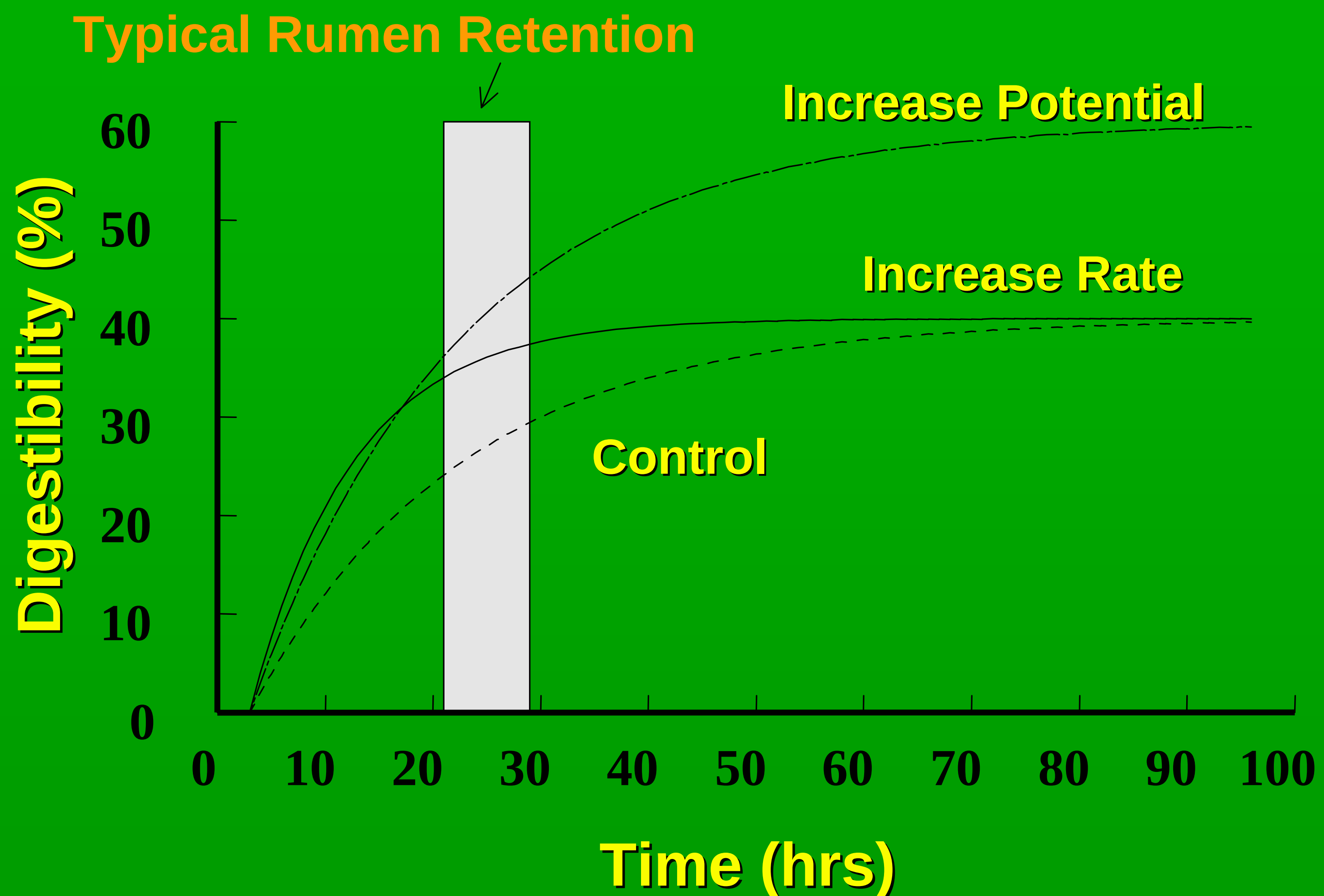
Dixon et. al., 2000

Lower Stem IVD – 2001 summary



McCaslin et al. 2002. Unpublished. Forage Genetics

NDF Digestion Profiles



The Perfect Alfalfa Plant

- Yield of individual cuttings high enough to reduce number of cuts per year (2 or 3)
- Maturation that is not strongly tied to quality
- Minimal leaf loss during growth and harvest
- Total protein available to the animal, 16-18 %, of that 30-35 % ruminal undegradable
- Cell wall digestibility ~ 80 % (20-30 % rapidly fermented pectin)
- Protein loss during ensiling no greater than 10-15 %

Attributes of Idea Alfalfa Plant

- Better balance of protein and rapidly fermentable carbohydrate
- At optimum aNDF of 40 %
  - 18 % crude protein
  - Less ash
  - 30 % nonfiber carbohydrate (NFC)
- Improve current balance of amino acids with slower rate of degradation in ensiling
- Increase fat to 4 %
- Improve the extent of digestion of fiber
- Removal or suppression of bloat causing properties

Why Plant Genetic Engineering

- Introduce traits not available in alfalfa or alfalfa relatives.
  - Roundup Ready (bacterial gene)
  - PPO (red clover gene)
  - Phytase (fungal gene)
- Knock out existing genes that negatively affect crop performance/crop quality
  - Improved digestibility (lignin knockout)

1999 Results - Flexibility in timing of Roundup application

Roundup application 18 days after emergence

- Good tolerance at all stages of plant development
- Good tolerance during all times of the growing season

Strategies for decreasing post-harvest proteolysis in alfalfa silage

- Some compounds bind with alfalfa protein to decrease rate of post-harvest proteolysis. Transgenic alfalfa will be produced that contain these compounds.
  - Tannins – altered expression of genes for alfalfa tannin biosynthesis
  - Polyphenol oxidase (PPO) – gene isolated from red clover (USDA)

Consortium for Alfalfa Improvement

- Share expertise of Noble Foundation, Forage Genetics, Plant Science Unit and USDFRC to redesign alfalfa for dairy cattle
  - November 2000 at Noble Foundation
  - September 2002 at Forage Genetics
  - February 2003 at Noble Foundation
  - January 2004 at Noble Foundation
  - August 2004 at USDFRC

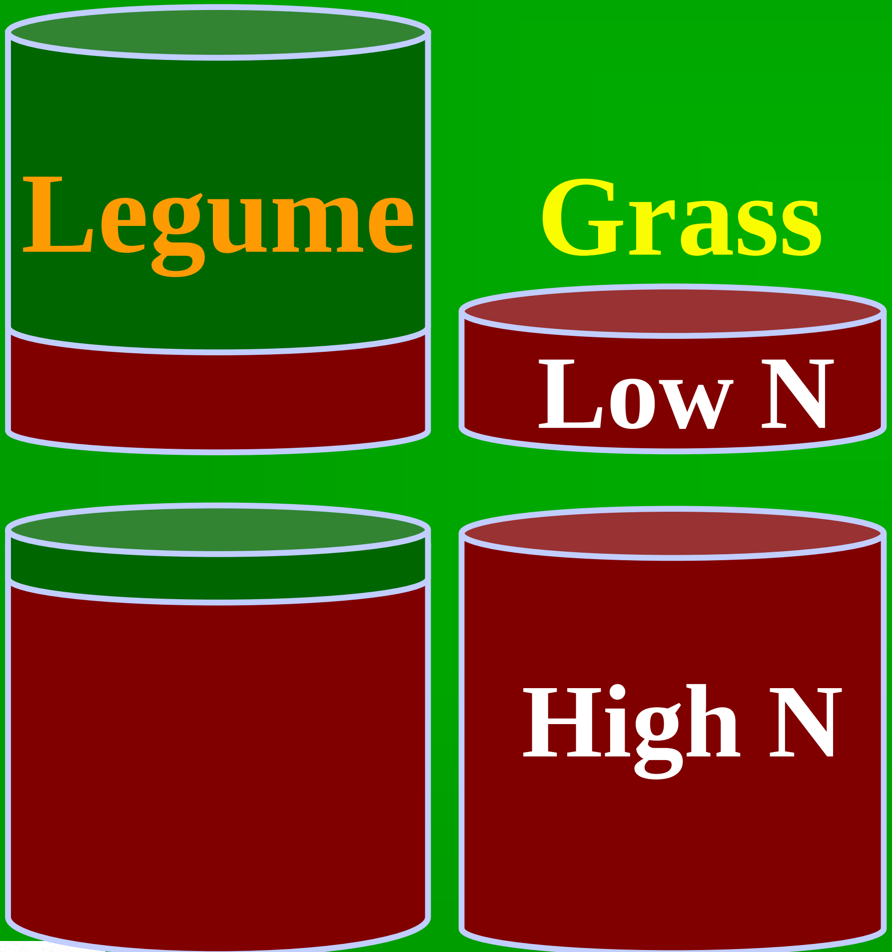
Alfalfa in Crop Rotations:

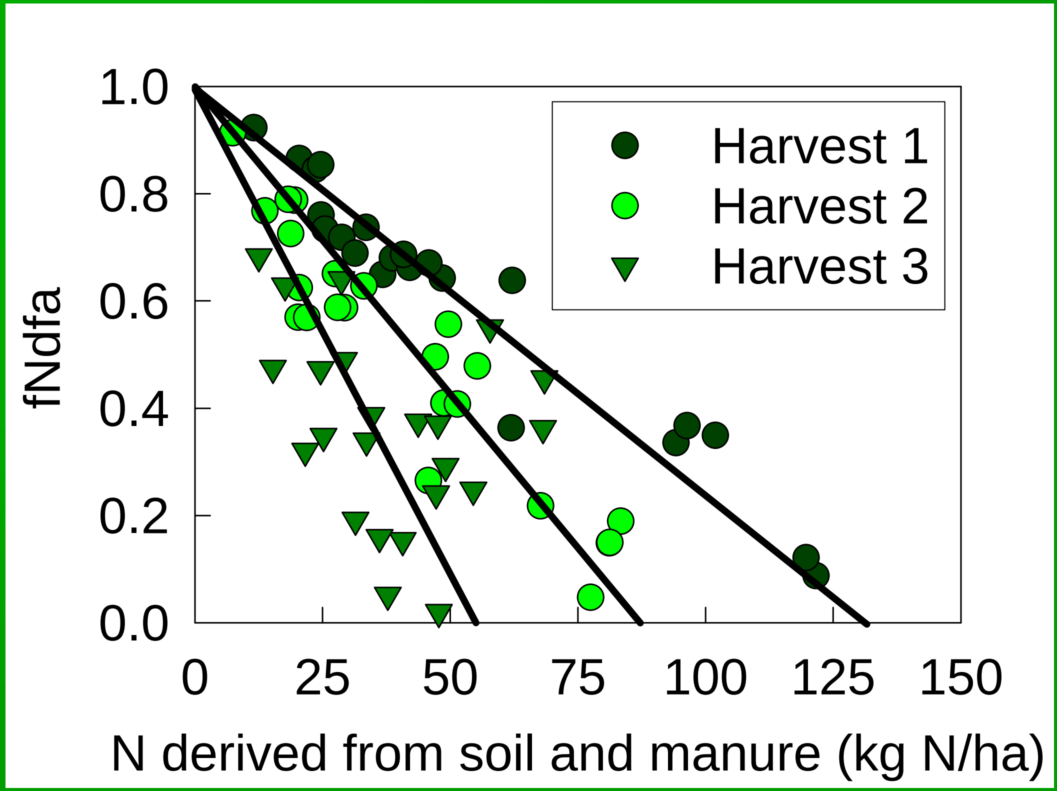
- Adds nitrogen via biological fixation
- Improves water infiltration and soil quality
- Reduces soil erosion from wind and water
- Improves yield of subsequent crop
- Reduces N fertilizer demands of subsequent crops

Alfalfa in Crop Rotations:

- Helps protect surface and ground water
- Acts as waste-water recycler

Legumes serve as N buffers





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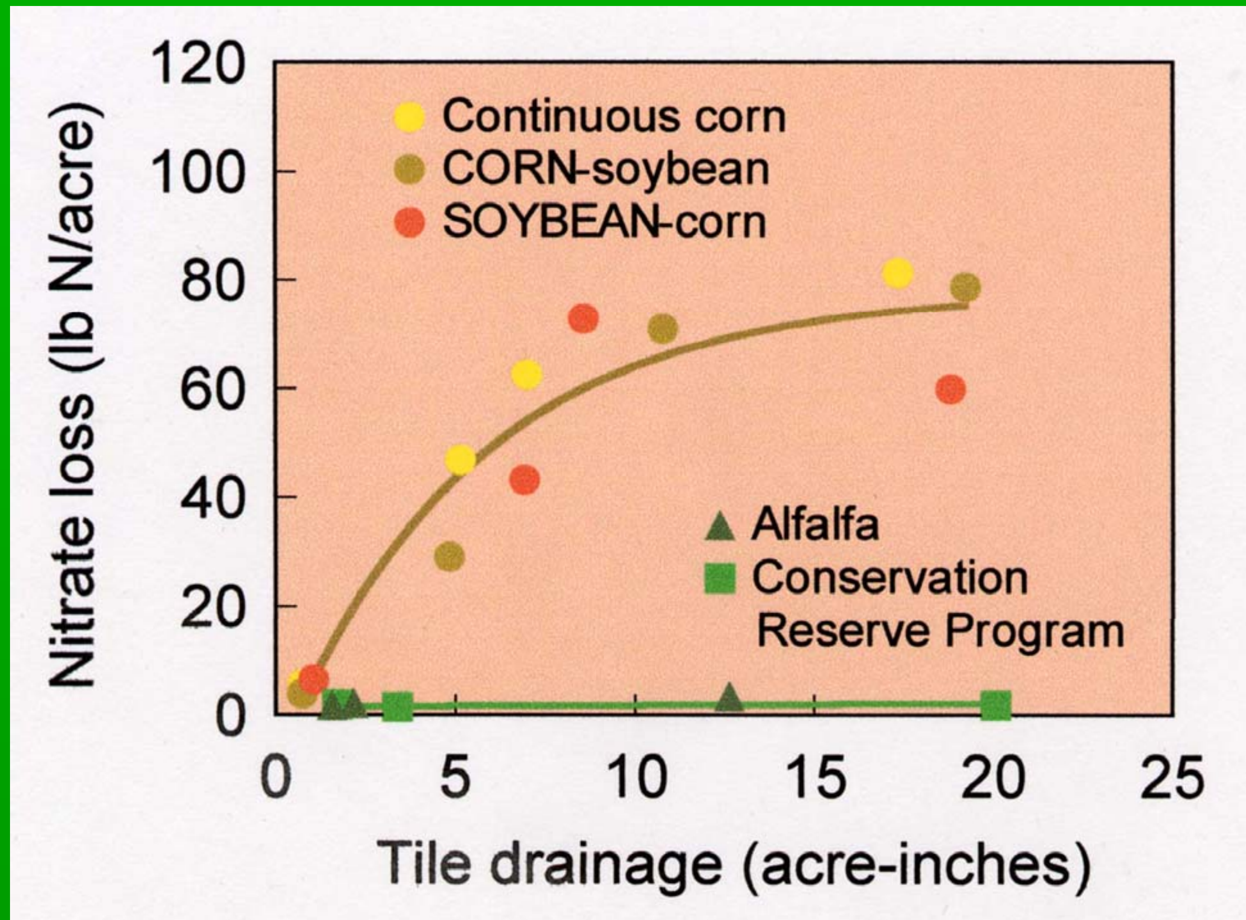
Russelle et al., 2002

Alfalfa and grass CRP effectively filter tile drain water



>40 million acres are tile drained in the Upper Midwest

Randall, Huggins, Russelle et al., 1997

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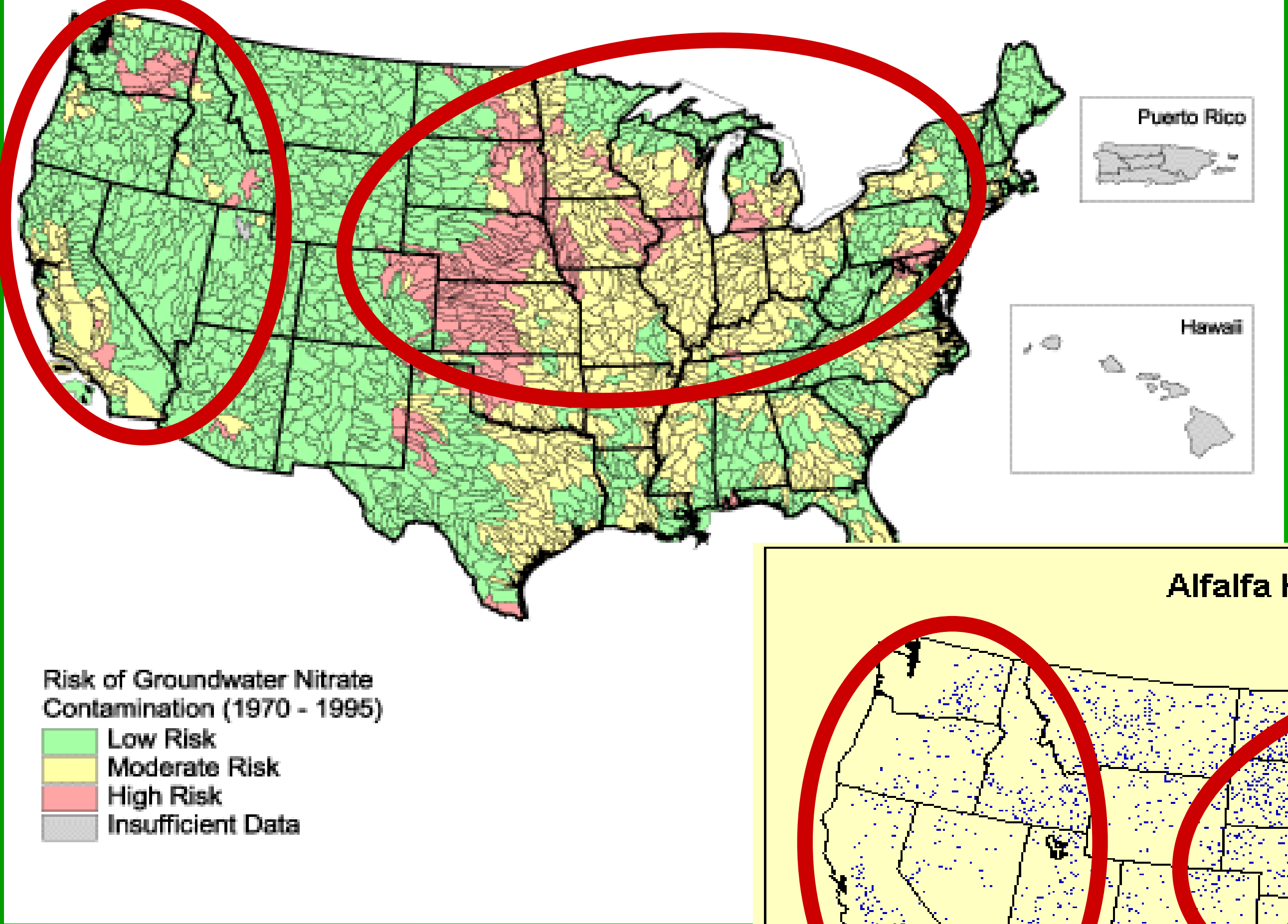
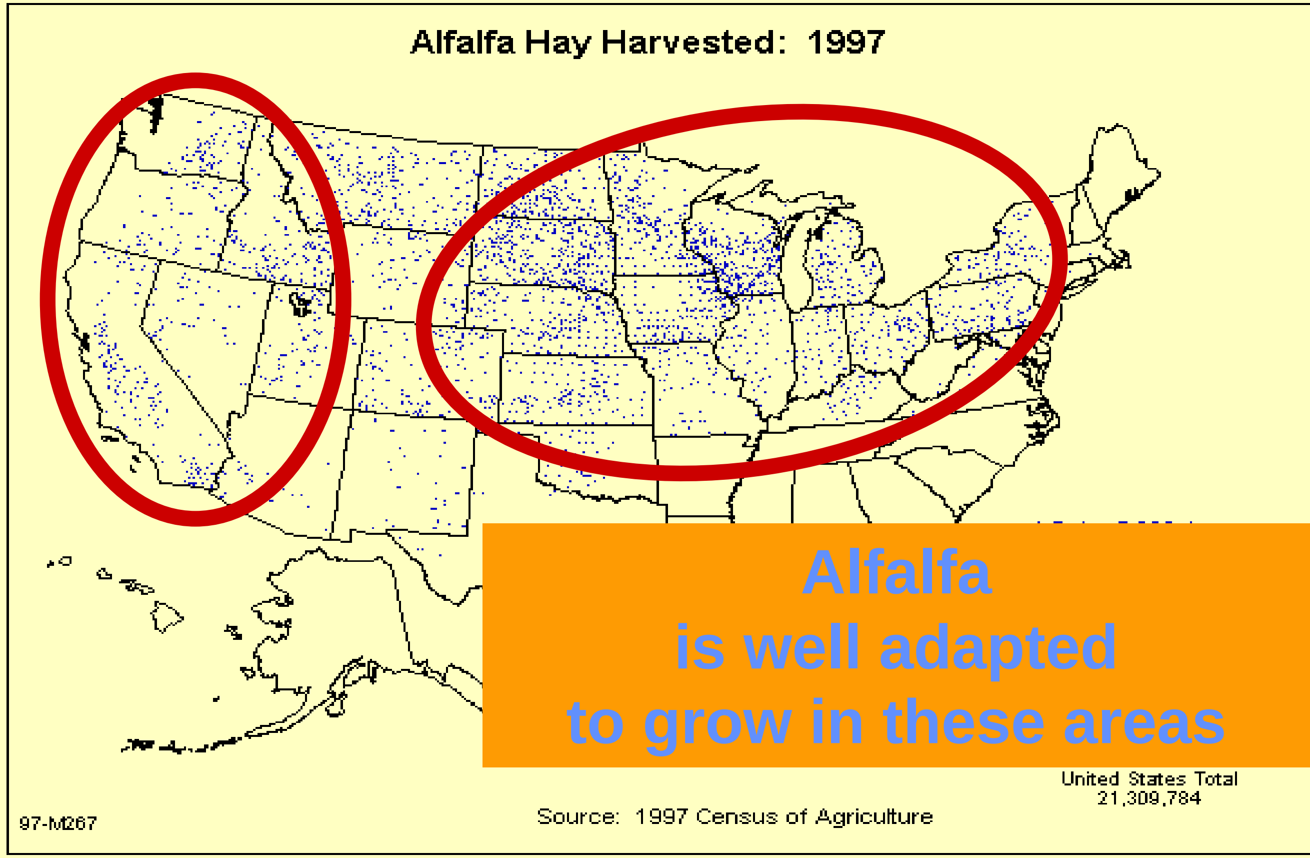
Risk of ground water nitrate contamination

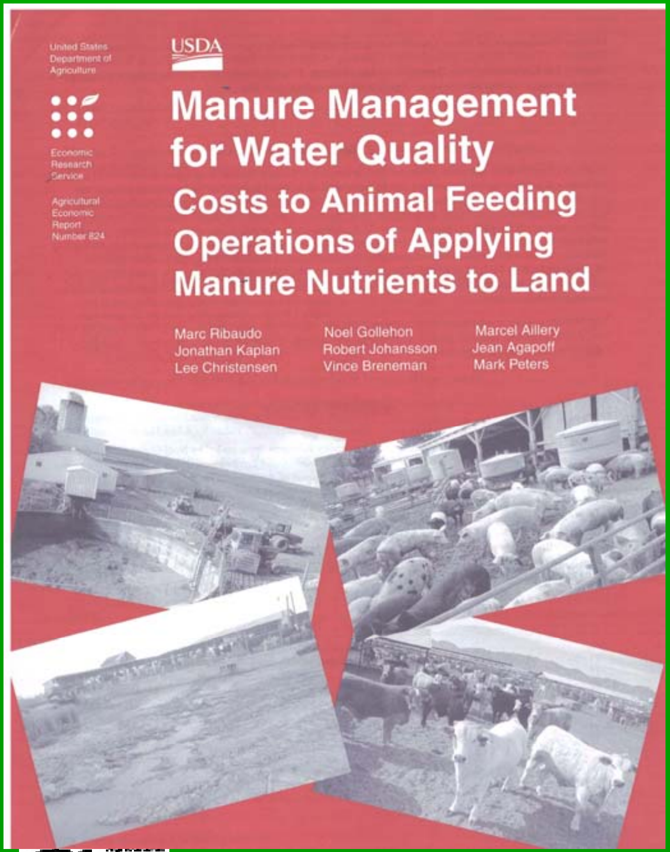


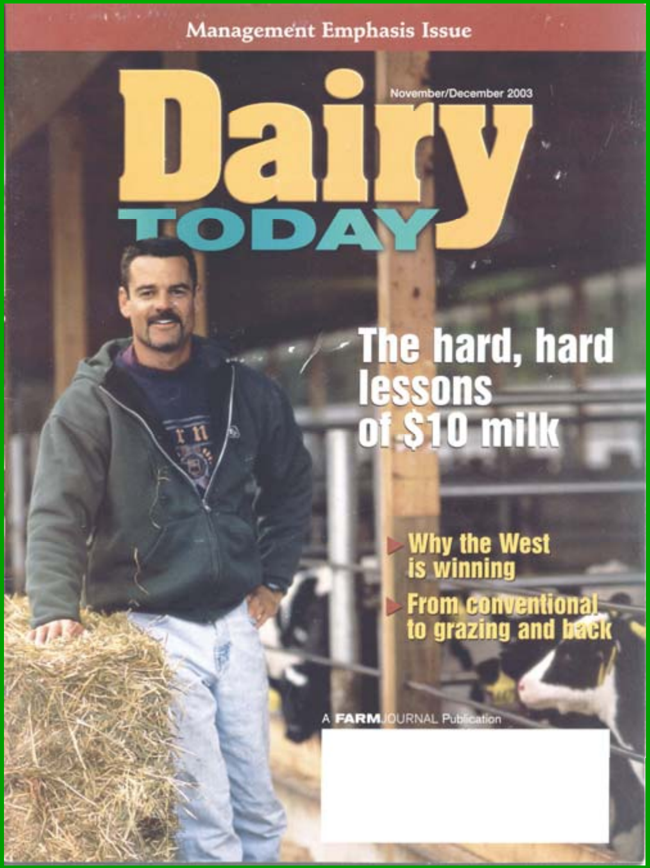


Alfalfa
is well adapted
to grow in these areas

Using Biotech To Keep Alfalfa Competitive

- **Solutions to major challenges in agriculture**





Nutrient necessities

Nutrient management planning for 1,300 cows and 1,000 acres takes planning, bucks and persistence

By Kim Bower-Spence

Expanding a dairy from 315 to 1,325 mature cows with a land base of just under 1,000 owned and rented acres, Phil Kulp knew nutrient management would drive decision making from the get-go.

Kulp Family Dairy LLC's two-step expansion began in 1999 with a leap to 600 milking cows. The family added another barn, for a total of 1,100 in 2002. Their 1999 nutrient management plan took into account the second-phase cows. Their five-year concentrated animal feeding operation (CAFO) permit, issued in May 2001, also counted their final total.

The Martinsburg, Pa., operation includes Phil and his wife, Becky; his parents, Larry and Mary Ann; and about 30 employees.

Plethora of permits. Bill Fink, agronomist with the Ephrata, Pa.–based consulting firm Team Ag, devised Kulp's nutrient management plan. He helped them wade through the myriad regulations concerning animal equivalent units, concentrated animal operation (CAO) status, local watershed designation, manure storage needs and township requirements.

The Kulps hit the trigger for both state and federal regulations. Having more than 1,000 animal equivalent units brought them under federal CAFO rules. Since they had more than two animal units per acre, they also needed to meet Pennsylvania standards for CAOs. Kulp Family Dairy also lies within a special protection watershed.

Besides their nutrient management and conservation plans, the Kulps needed a Water Quality Management Part II permit to construct manure storage. Engineering plans by Team Ag included an engineering survey, site map, manure storage and transfer system design, erosion and sediment control plans, and storm water management plans.

Phil Kulp estimates that the operation paid about $25,000 total in permitting costs for everything from storm water management to septic and CAFO. "We got into it early enough that maybe it wasn't as costly," he says.

Fink says permitting can run $5 to $6 an acre for nutrient management, plus $1,000 for state permit applications. CAFO plans range from $1,000 up to $15,000, depending on engineering. And the process takes six to eight months.

Permitting challenges aside, Kulp cites four steps to successful nutrient management.

1. Plan for land. "You have to maintain an adequate land base, and we do that by cooperating with our neighbors," Kulp notes. "As we were talking about building and expanding, I was already talking with other landowners."

Those conversations yielded three 5-year feed contracts that set price and include import-export agreements for manure. "We buy all the forage from those acres, and we also return the manure in accordance with the nutrient management plan."

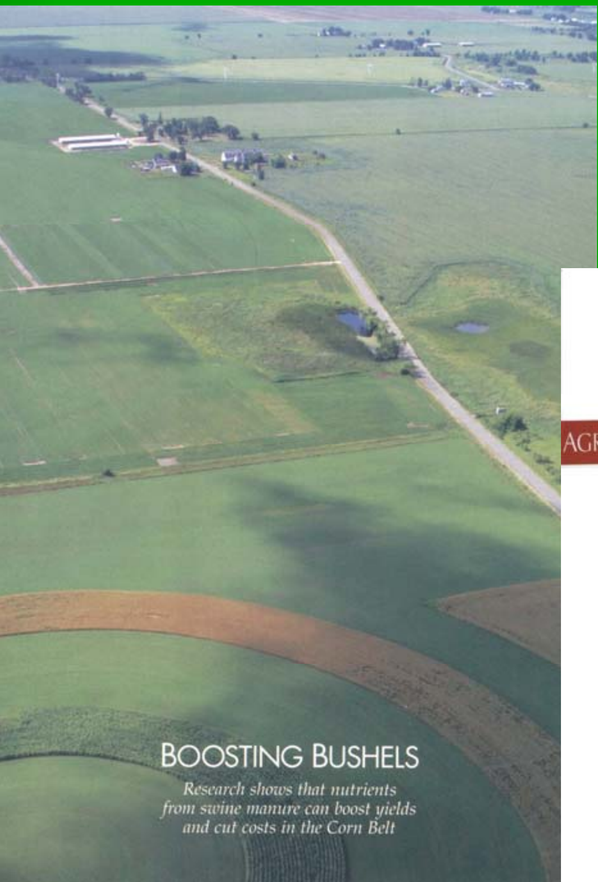
Word apparently spread that it's a ▶

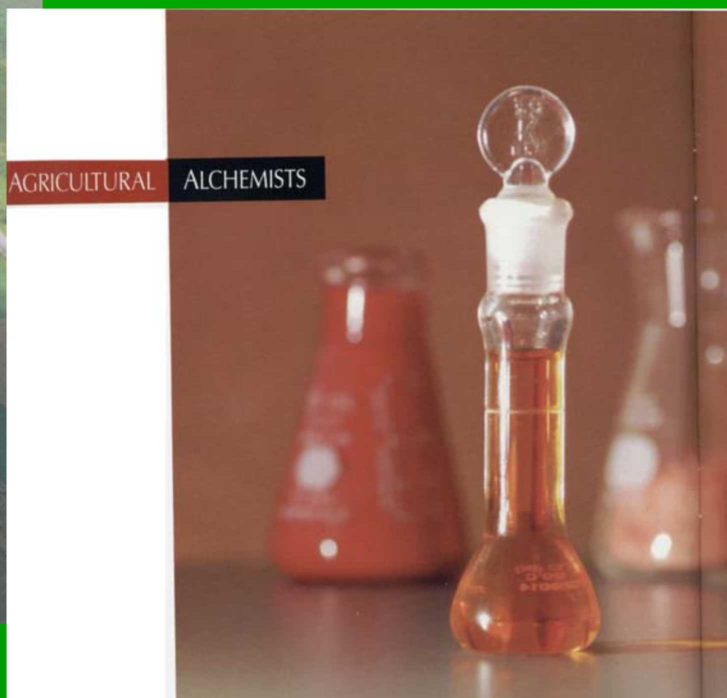
Phil Kulp, Martinsburg, Pa., wrote five-year contracts with other local farmers to buy their forages and apply manure from Kulp Family Dairy on their fields.

10 Elite Producer November/December 2003

Using Biotech To Keep Alfalfa Competitive

- Solutions to major challenges in agriculture





"We visualized this as a way to expand uses for corn," she says. "It also was a way to lessen our dependence on foreign oil. And PLA made from corn is more environmentally friendly, since it is completely biodegradable."

PLA is beginning to pay off for corn growers. Cargill Dow built a plant in Blair, Neb., to produce NatureWorks PLA. The facility, one of the world's largest biorefineries, uses about as much corn each year as a 40-million-gallon ethanol plant.

NatureWorks PLA is used in consumer products marketed by well-known U.S. companies like Coca-Cola and Pacific Coast Feather Company.

Many consumers don't realize that today's food wrap and plastic packaging, as well as the majority of synthetic fibers, originally comes from oil. That's according to Randy Howard, president and CEO of Cargill Dow. "We're using the tools and resources Mother Nature provided us as the foundation for a new industrial revolution," he says. "We are manufacturing products that will minimize the impact on our environment."

One of those revolutionary products made from corn-produced PLA is Ingeo fiber. This new textile has been incorporated into the Spring Planting line of modular carpeting tile from InterfaceFlor, Inc. These

Above: Carpet tiles made from a corn-based fiber offer beauty and style while being easily recycled.

Above right: Massive Cargill Dow plant in Blair, Neb., turns out NatureWorks PLA used in making plastics.

19.5-square-inch tiles can be applied over almost any surface, and the company will take used tiles back for recycling.

Ingeo fiber also is being used in a line of bedding from Generations Bedding Company. Lofty, lasting hypoallergenic fiberfill is used in pillows, comforters, and other bedding products. In its store displays, the company highlights the fact that a renewable resource, corn, is used in the manufacture of its products.

Not to be outdone, the nation's soybean growers also are involved in the textile industry. The soybean checkoff and the United Soybean Board have helped fund the development of a soy-based polyol (SoyOyl) that is a major component of a new carpet-backing system. SoyOyl replaces a portion of the petroleum-based components used to make polyurethane backing.

Ultimate alchemy. While today's crop of products developed by ag alchemists is impressive, the potential for future products is staggering. Scientists are just scratching the surface when it comes to using biotechnology to manipulate the very genes that can do such things as turn ordinary crops into pharmaceutical factories.

As director of Iowa State University's Plant Transformation Facility, researcher Kan Wang oversees the ultimate ag alchemy laboratory. Born in Shanghai, she received her doctorate in plant science from the University of Ghent in Belgium. There she worked with early pioneers of plant genetic engineering who learned how to use a common soil organism, a strain of *Agrobacterium*, to transfer desired genes into plants.

Wang and Iowa State colleague Bronwyn Frame have refined this technique for use at the Plant Transformation Facility. It is the only gene-transfer facility open to public-sector researchers and has quickly become the world's largest public operation for the production of transgenic corn. And Wang oversees experiments in genetic transformation of corn and soybeans for a number of other researchers around the world.

Researcher Kan Wang studies gene-transfer techniques that could lead to life-saving vaccines from farm crops.

Wang is aware that her work, like that of the early alchemists, may seem mysterious and sometimes scary to the rest of society. "Biotechnology has the potential for being a powerful tool to advance society," she says. "It also has a potential negative side. But then, electricity can be dangerous, and so can most any other technology used by humans."

She sees potential for such things as pharma crops that could literally grow life-saving vaccines in the field. "We are just in the infancy of knowledge in this area," she says. "Putting a stop to such technology would be unfortunate. There are so many good things yet to come."