# Transforming Forage Plants To Increase Nitrogen Utilization In Dairy Systems: What Are The Possibilities?

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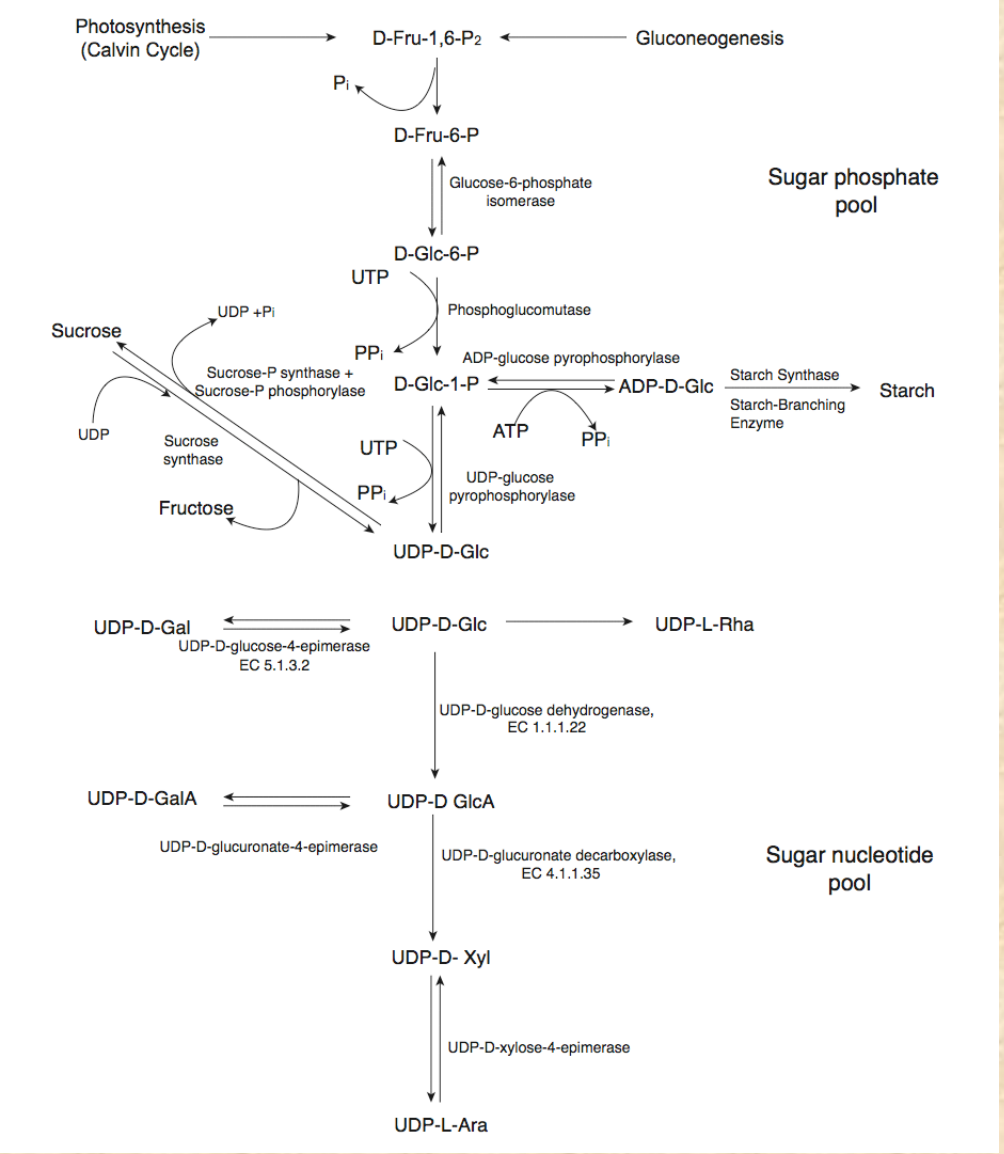
# Strategies For Altering Plants To Decrease N Losses

- Increase availability of structural carbohydrates
- Decrease storage losses
- Decrease rumen losses

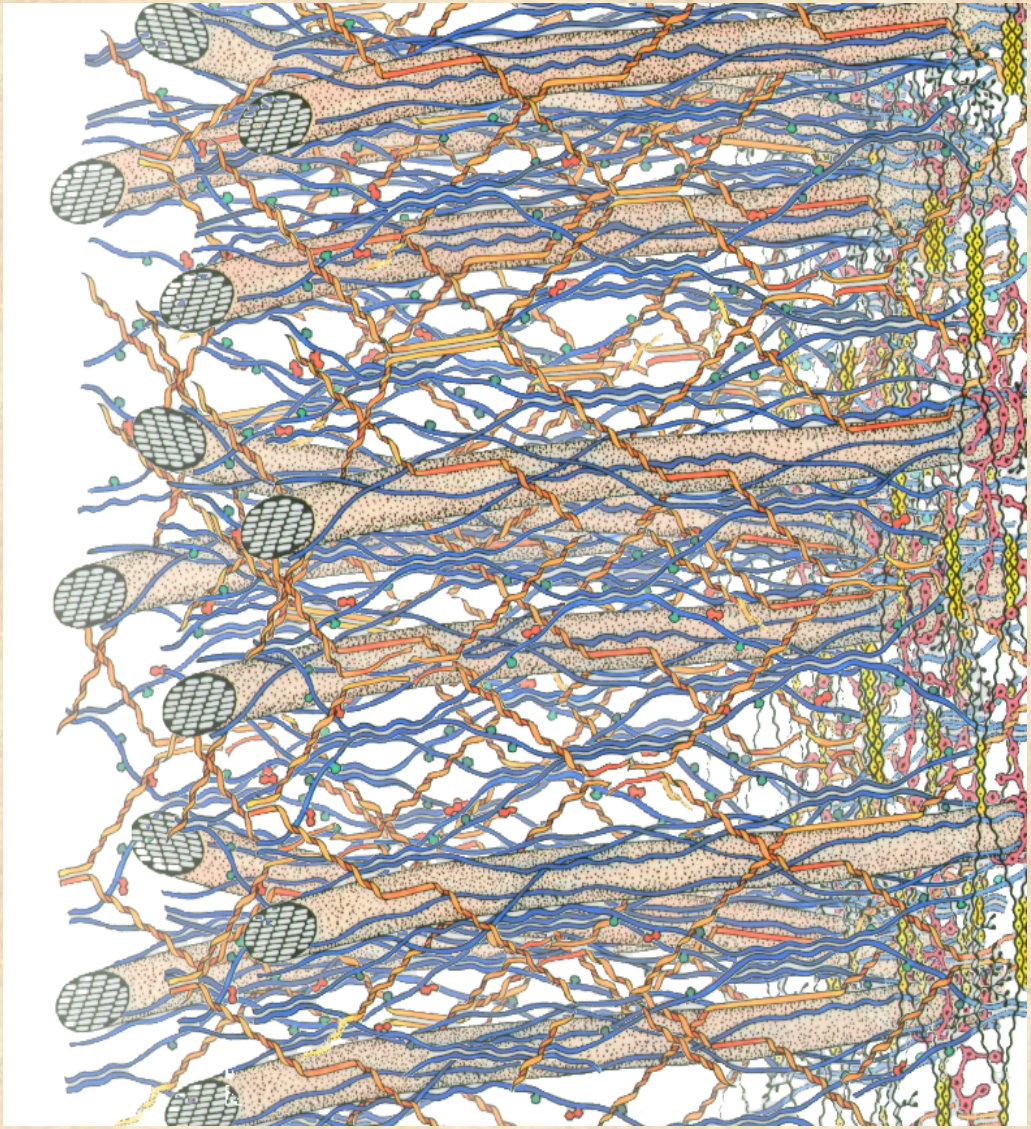
# Carbohydrate Pathways

- Soluble carbohydrate fraction
- Structural carbohydrate fraction



# Cell Wall Model

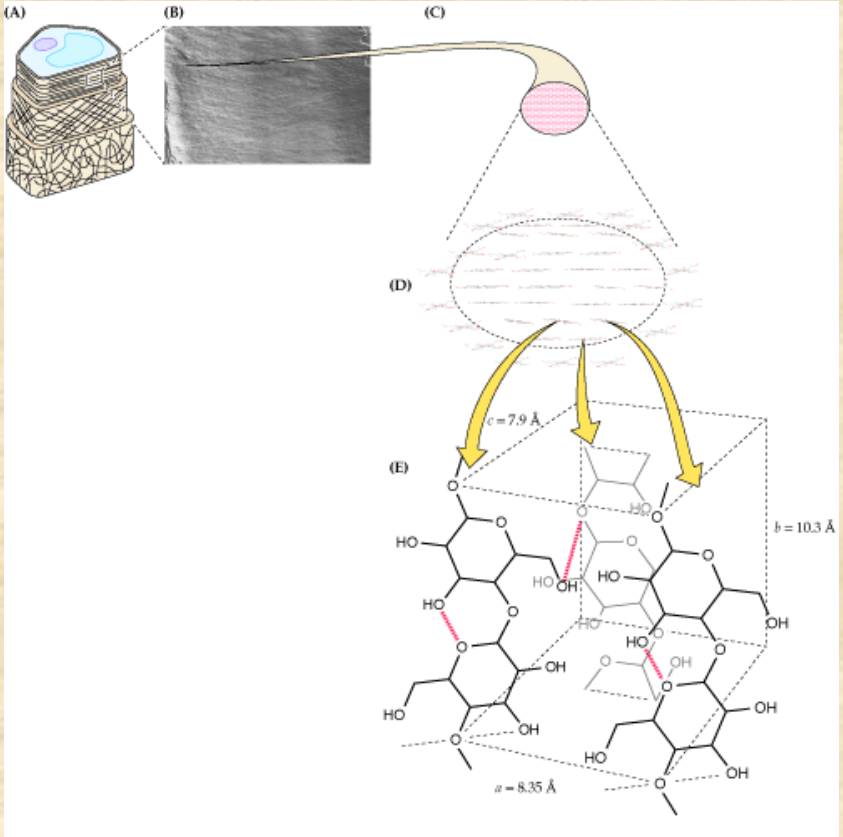
- Complex matrix of polysaccharides
- Cellulose synthase genes have been identified



Carpita and Gibeaut, Plant J. 1993

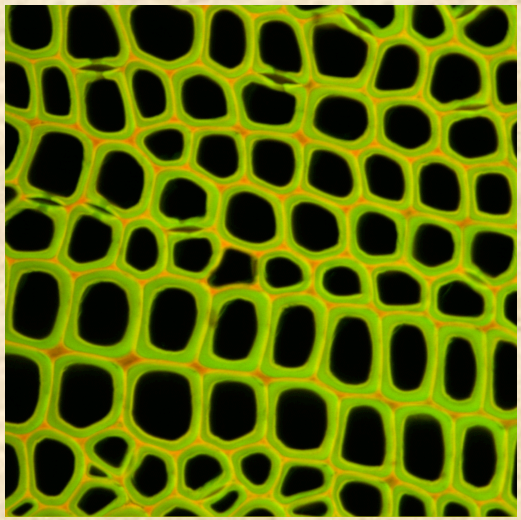
# Cellulose Synthesis

- Family of CesA genes in plants
- Two groups of CesA genes
  - One for primary wall formation
  - One for secondary wall formation

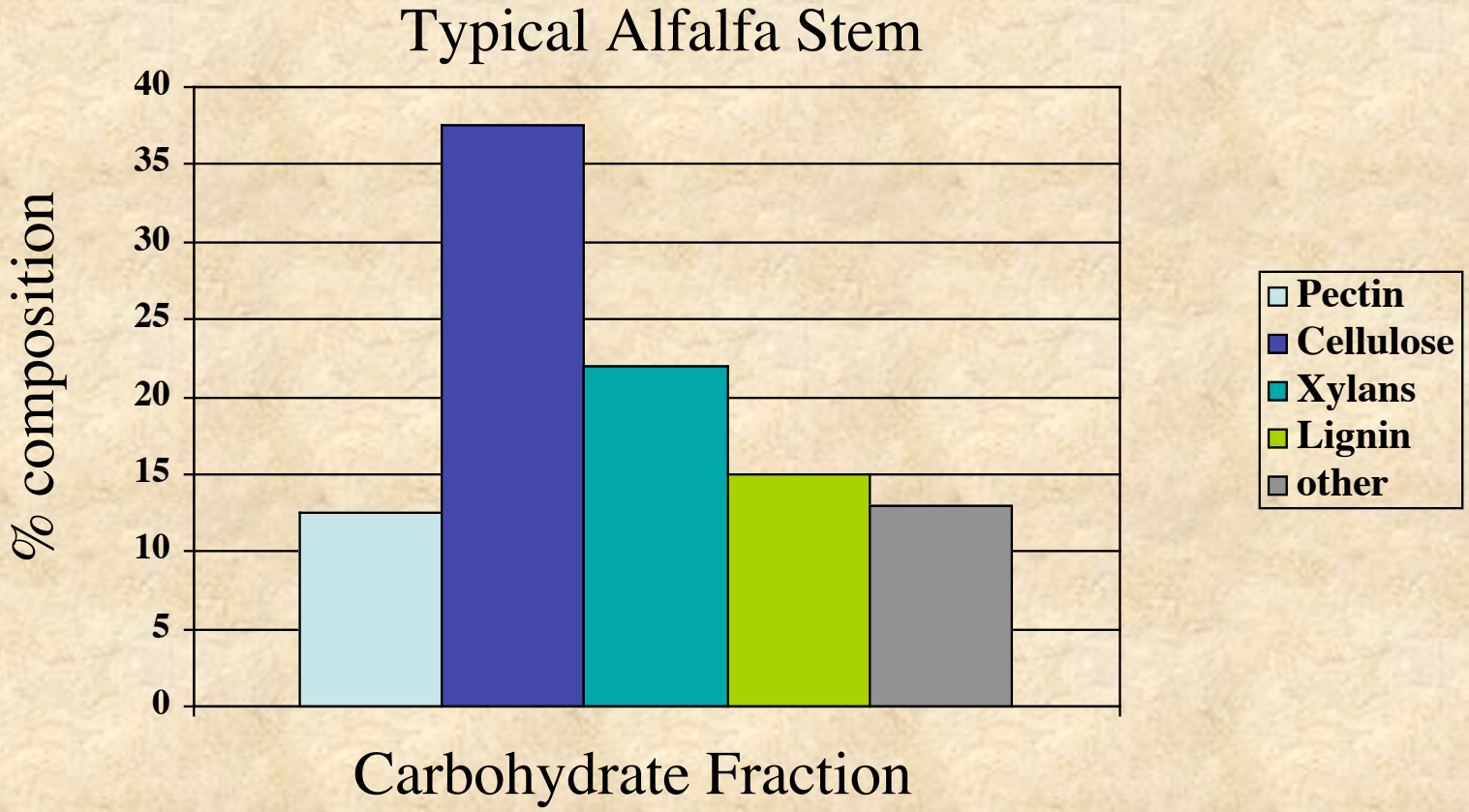


# Increased Cellulose Increase Stem Digestion

- Increase in cellulose should provide more degradable cell wall
- Increase in cellulose should not decrease stem strength

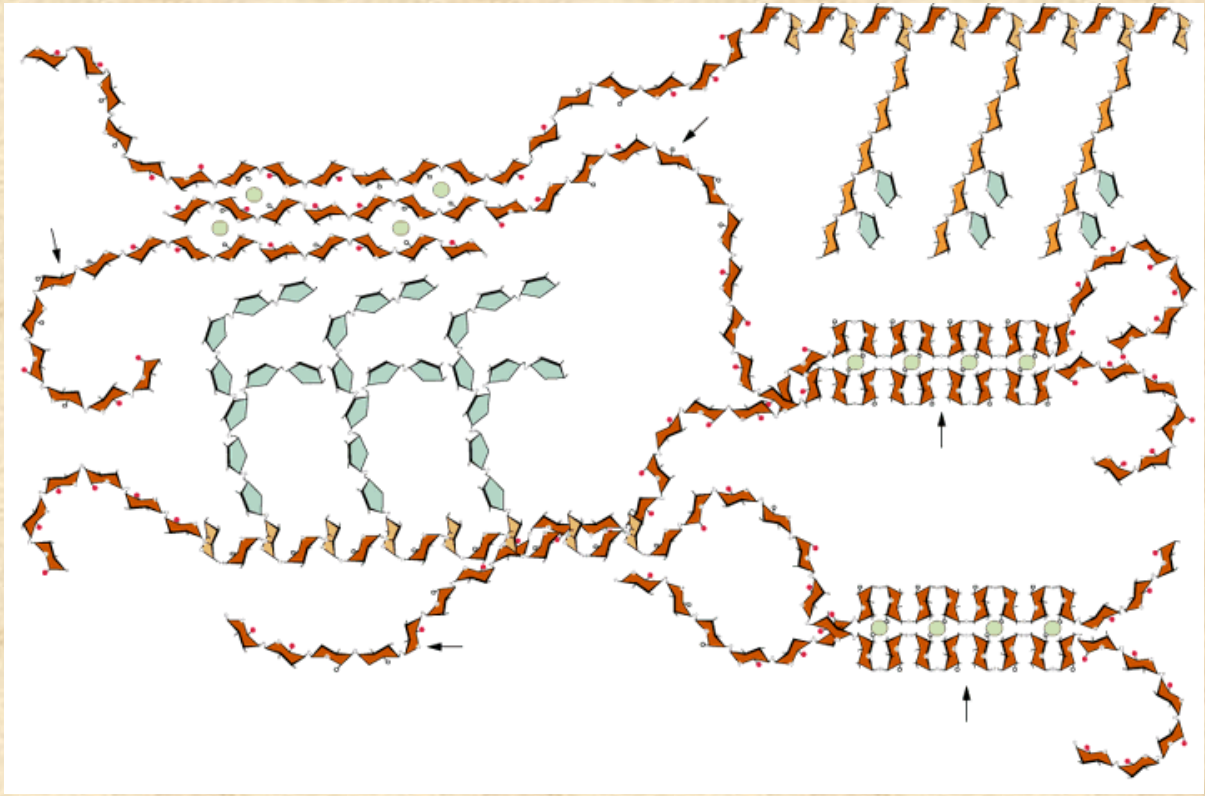


# Potential For Other Polysaccharides

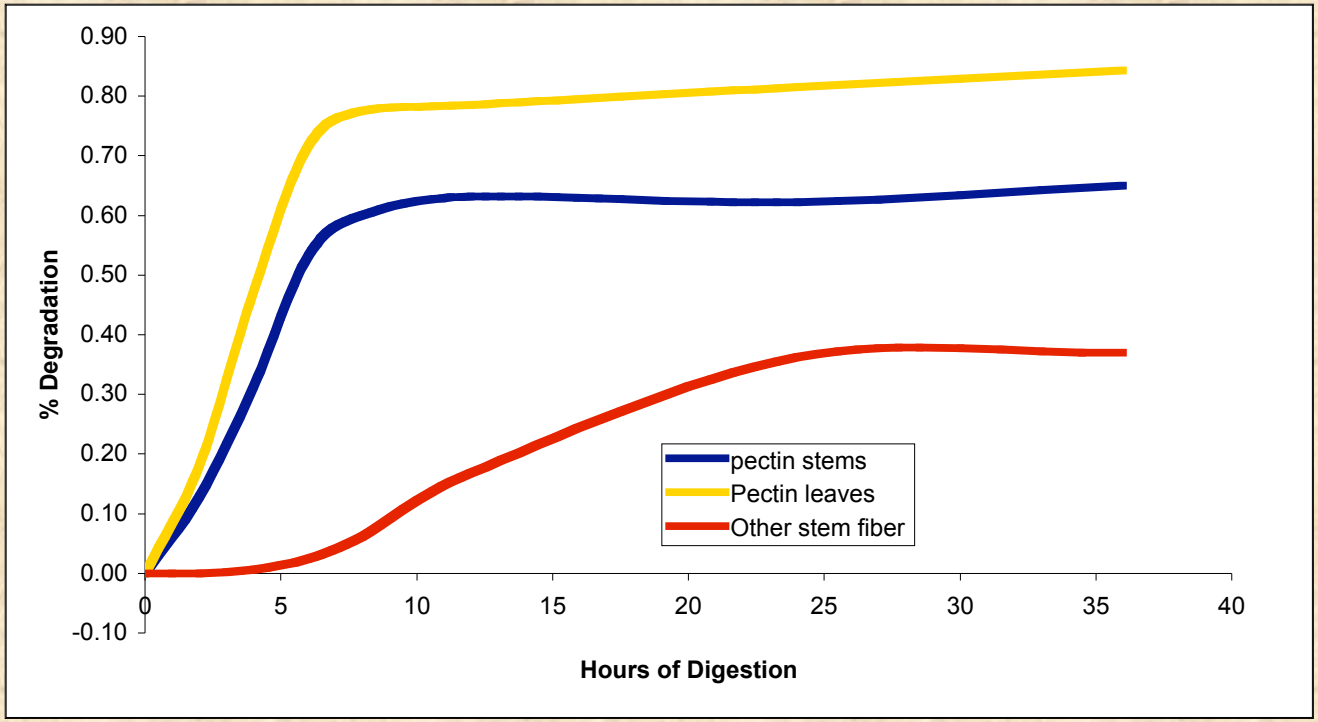


# Pectins

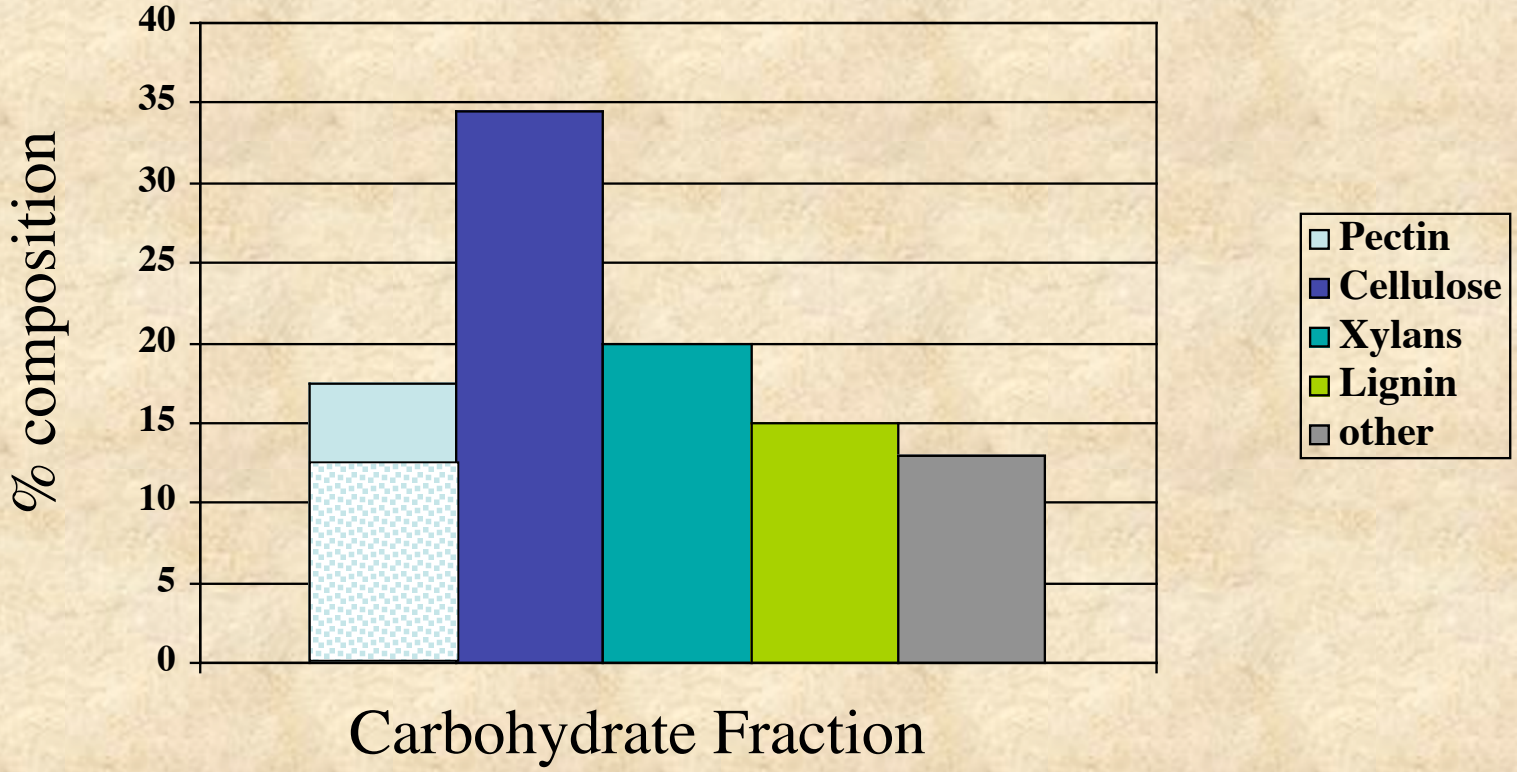
- Complex polysaccharide, highly branched, highly soluble



# Degradation Pattern of Pectins



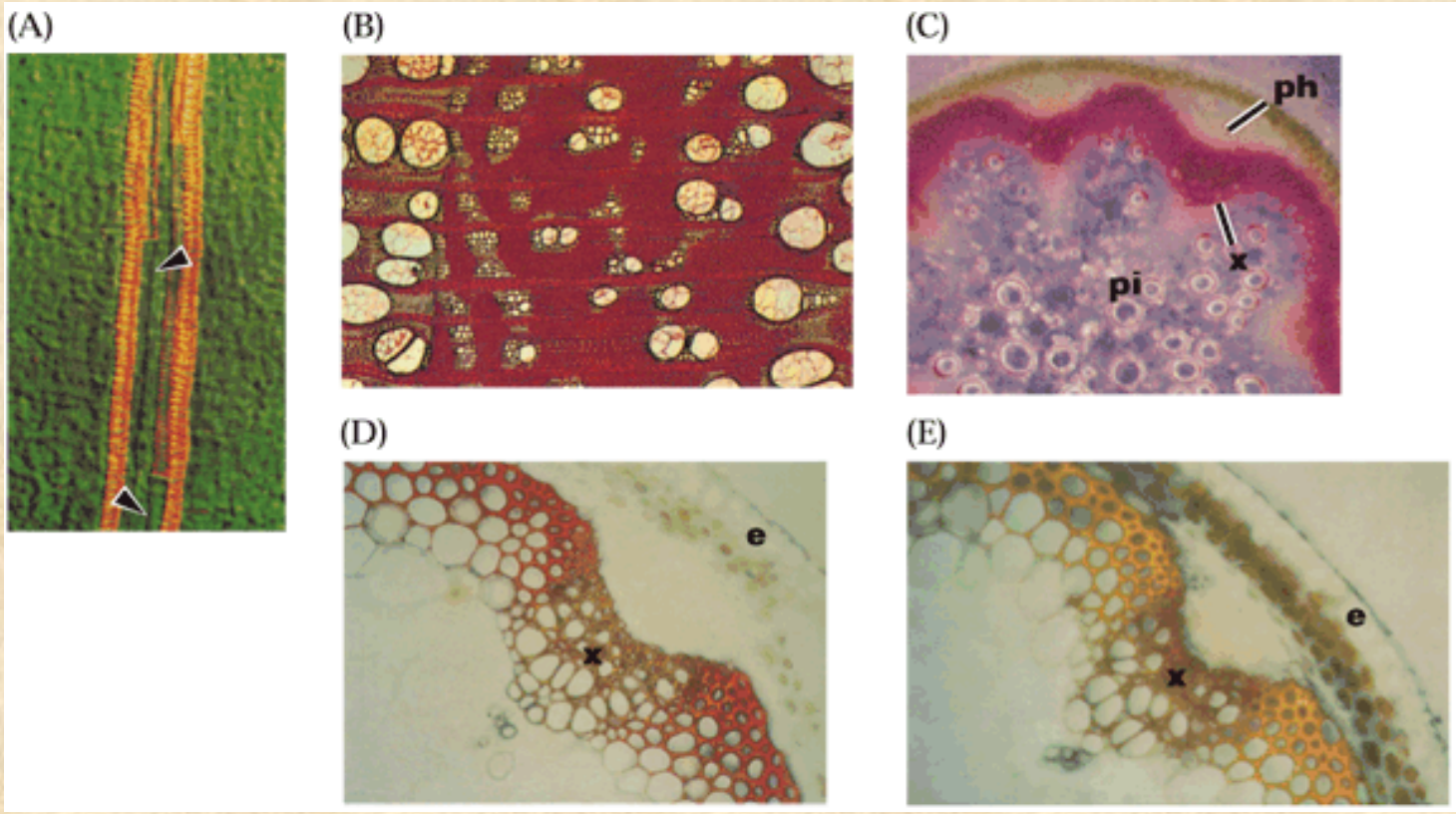
# Increase in stem pectin



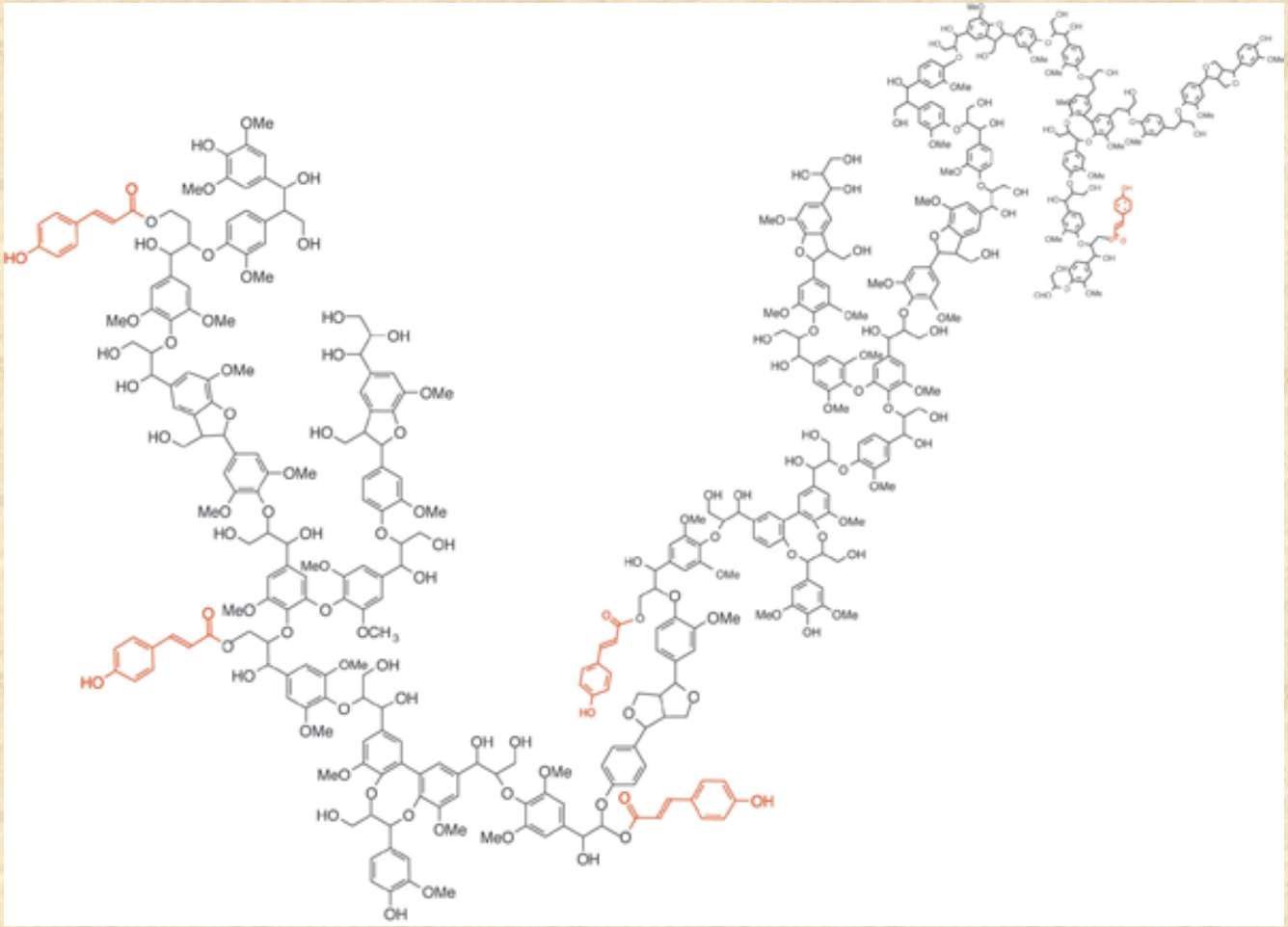
# Genetic Selection For Increased Stem Pectins

- Complex compositional and structural makeup
- Requires a complex array of synthases
- Requires a complex coordination of synthase genes

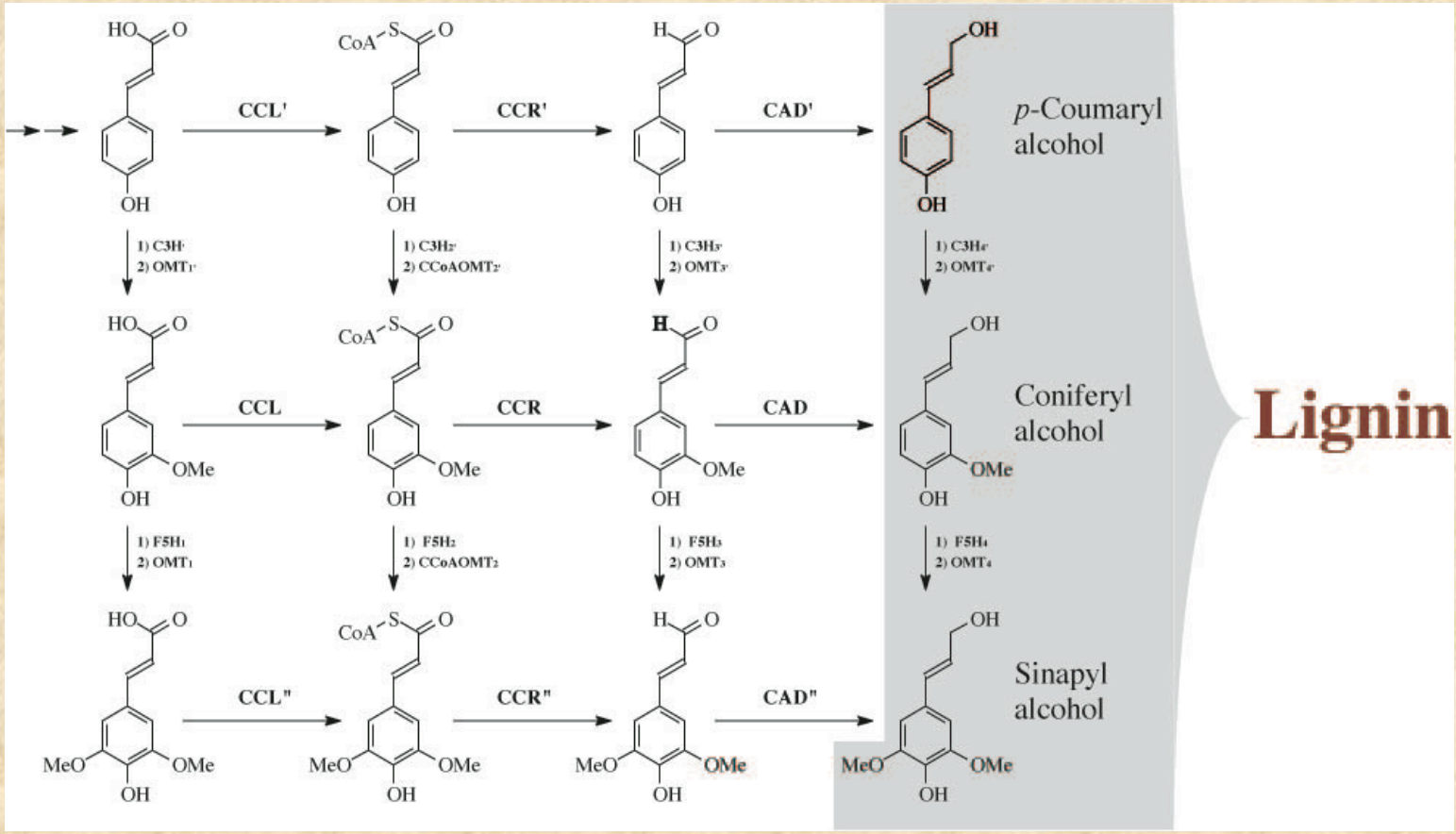
# Altering Lignin



# Indigestible Polymer



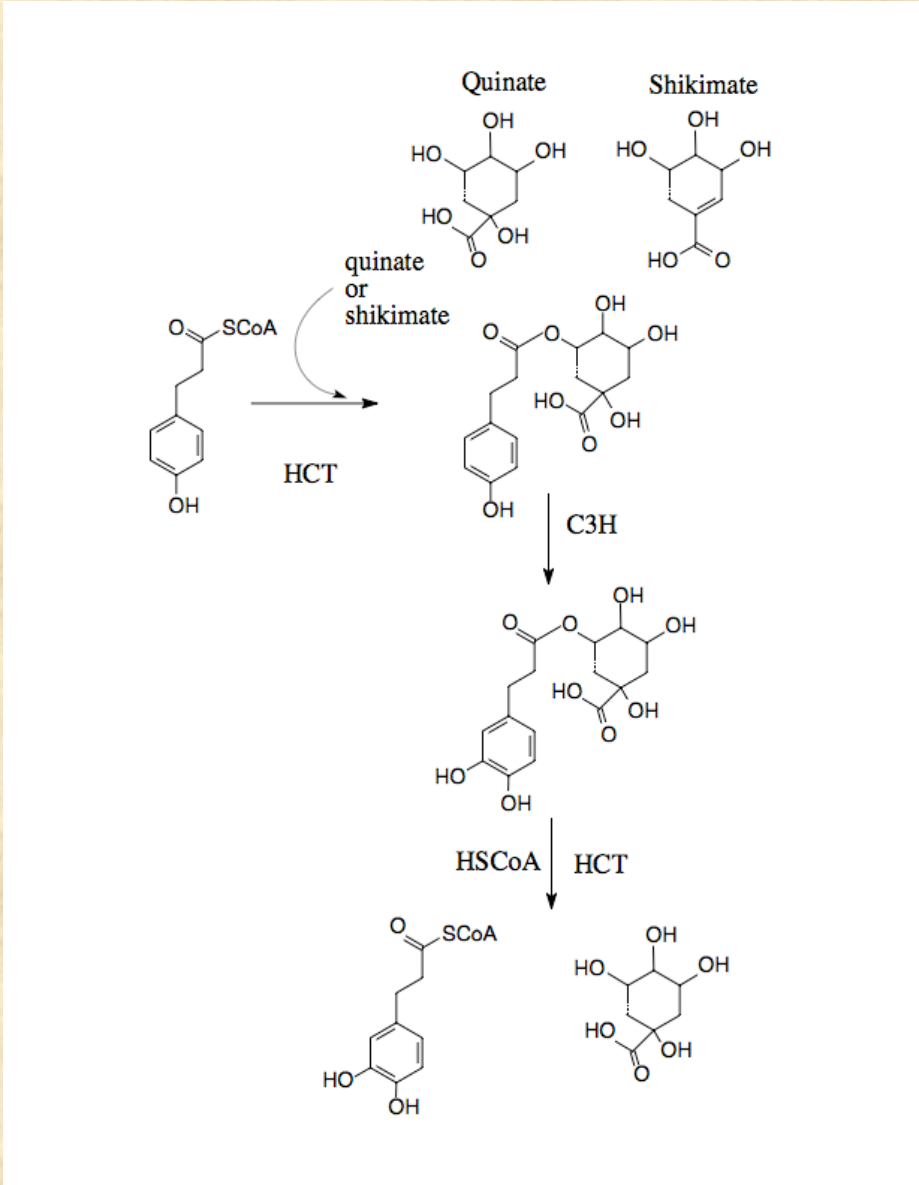
# Lignin Monomer Biosynthesis



# Understanding of Genes

Developing a clear understanding of the genes involved in lignin monomer synthesis

Lack fundamental knowledge of factors controlling lignification

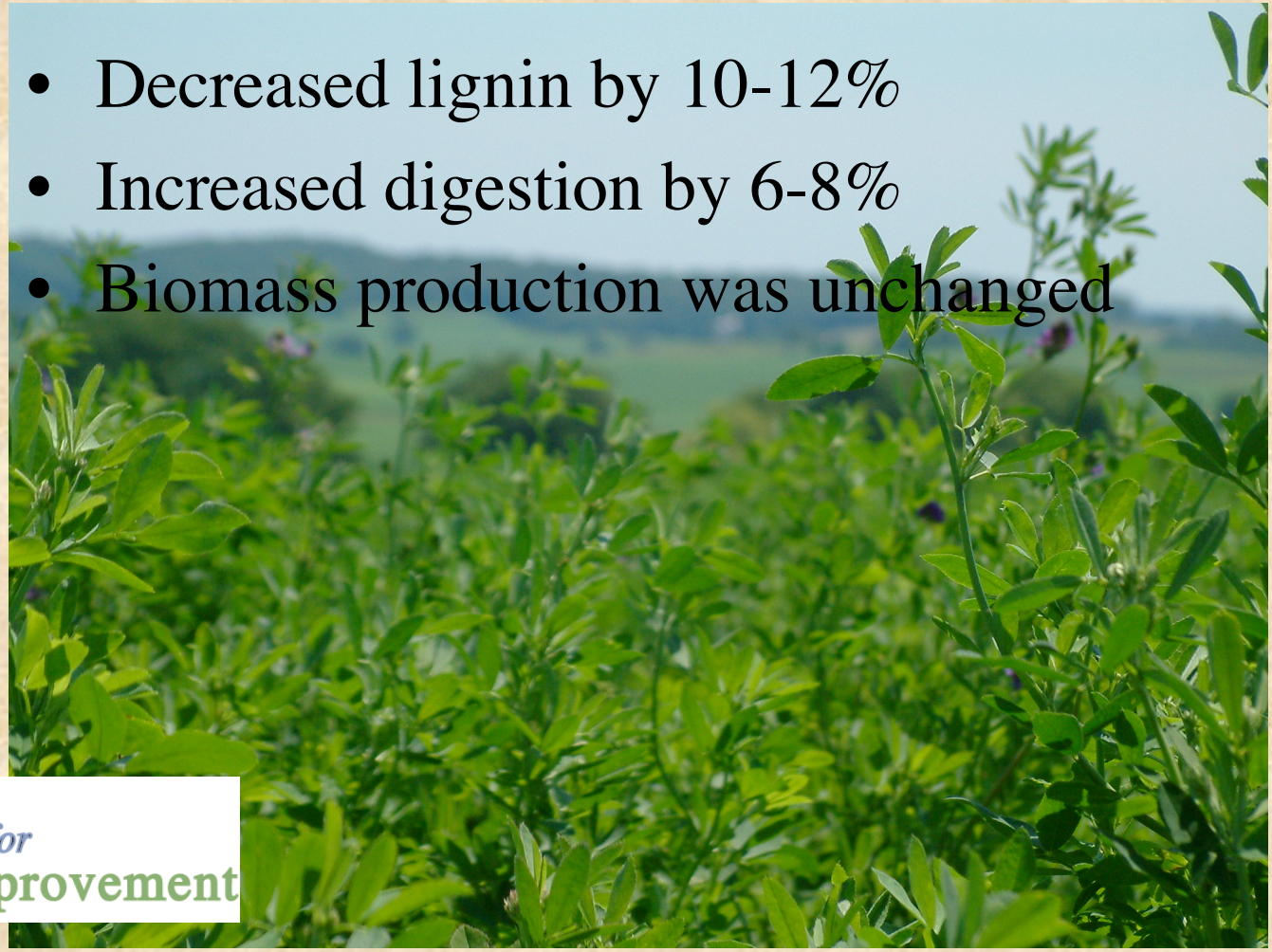


# Down regulation of COMT and CCoAOMT in Alfalfa

- Challenges of lignin modification
  - Decrease lignin
  - Do not decrease biomass production
  - Increase digestion

Consortium *for* Alfalfa Improvement

# Decreasing Lignin In Alfalfa



- Decreased lignin by 10-12%
- Increased digestion by 6-8%
- Biomass production was unchanged

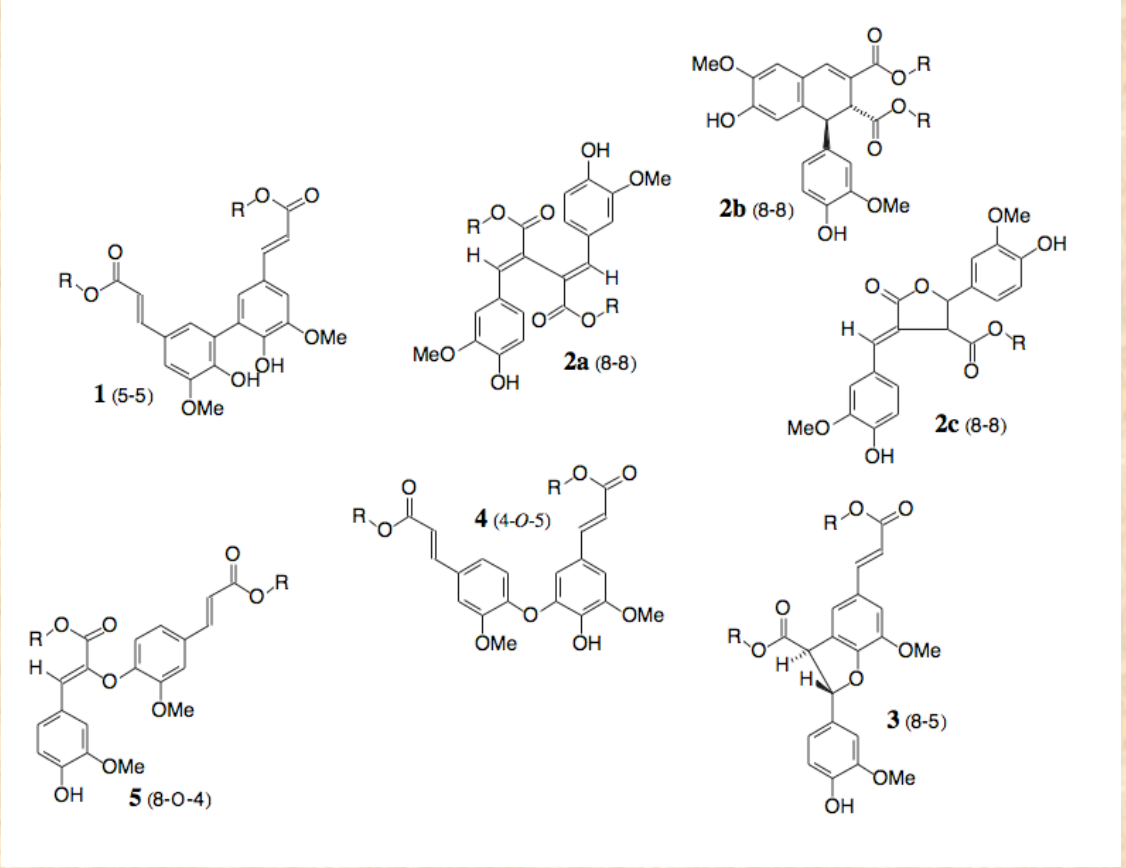
Collaborative effort with private and public institutions and ARS, Forage Genetics, Noble Foundation, Plant Research Unit St. Paul, MN, and USDFRC

Consortium *for* Alfalfa Improvement

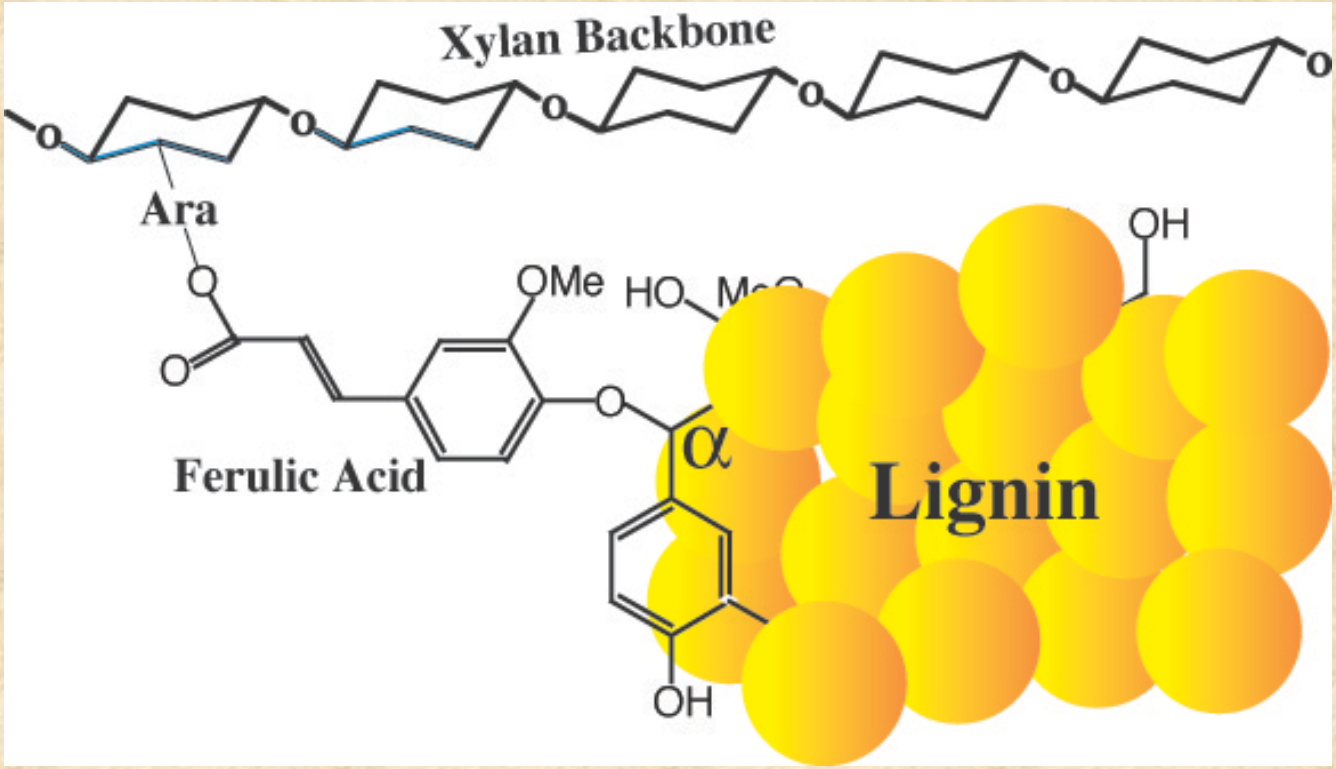
*U.S. Dairy Forage Research Center* *USDA-ARS-MWA*

# Ferulate Dimers

Arabinoxylans of grasses are crossed linked through the formation of ferulate dimers.



# Ferulate Cross-Link To Lignin



# Impact of Ferulate Cross-Linking

- Decrease rate and extent of cell wall degradation
- Modify plants to produce less ferulate cross-linking
  - Identify the feruoyl CoA transferase
  - Isolate transferase gene
  - Down regulation

# Strategies For Altering Plants To Decrease N Losses

- Increase availability of structural carbohydrates
- Decrease storage losses
- Decrease rumen losses

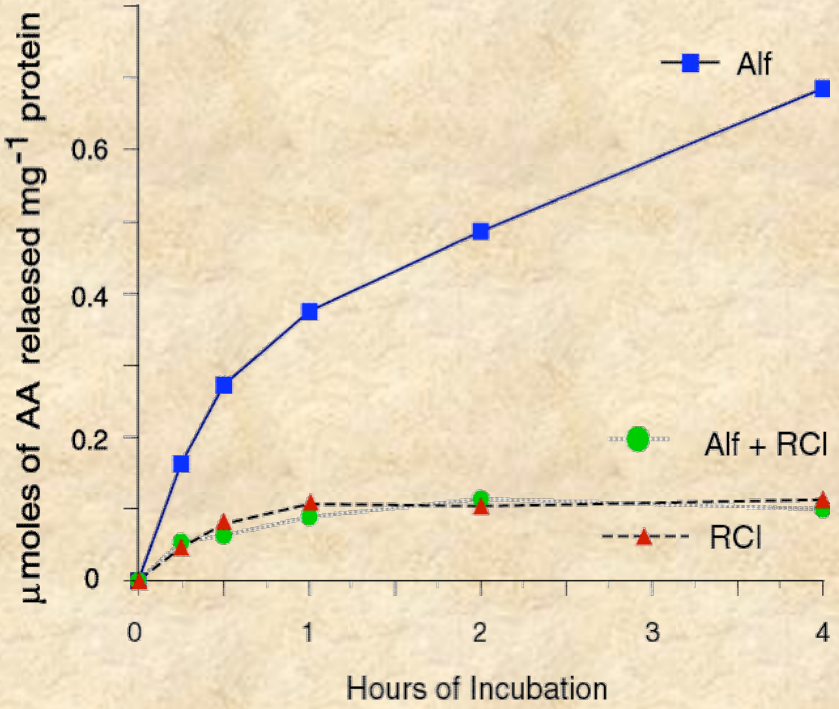
# Problem With Silage

- Proteolysis during ensiling results in losses of up to $70 per ha
- Increase in cost of silage additives/treatments
- Net losses can be as high: ***$94 million*** per year across the U.S.
- Large negative environmental impact
  - Poor utilization of nitrogen
  - Increased nitrogen excretion

# The Beauty of Red Clover

# Why are we interested in polyphenol oxidase (PPO)?

- Red Clover story
  - 90% less proteolysis during ensiling
  - Contains polyphenol oxidase and *o*-diphenols



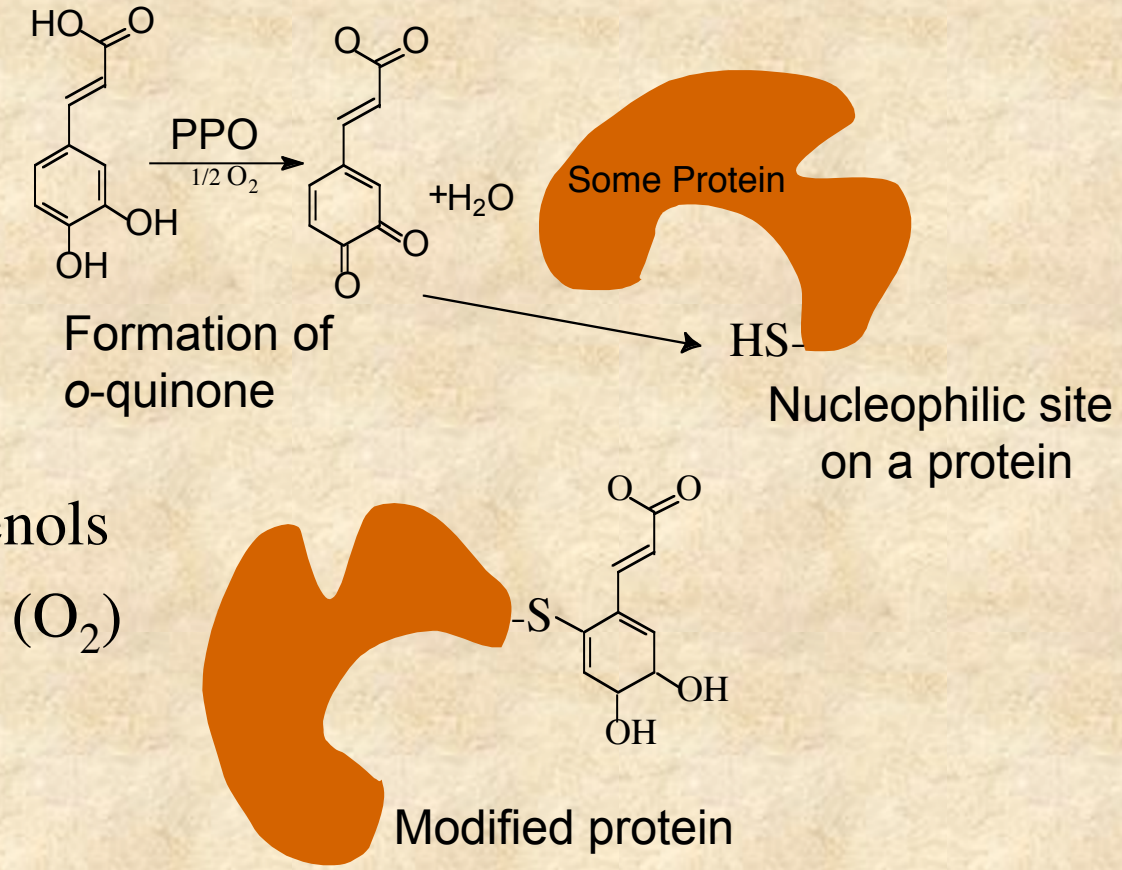
# What is PPO?

- Enzyme found in numerous plants
- Causes browning of fruits/loss of quality

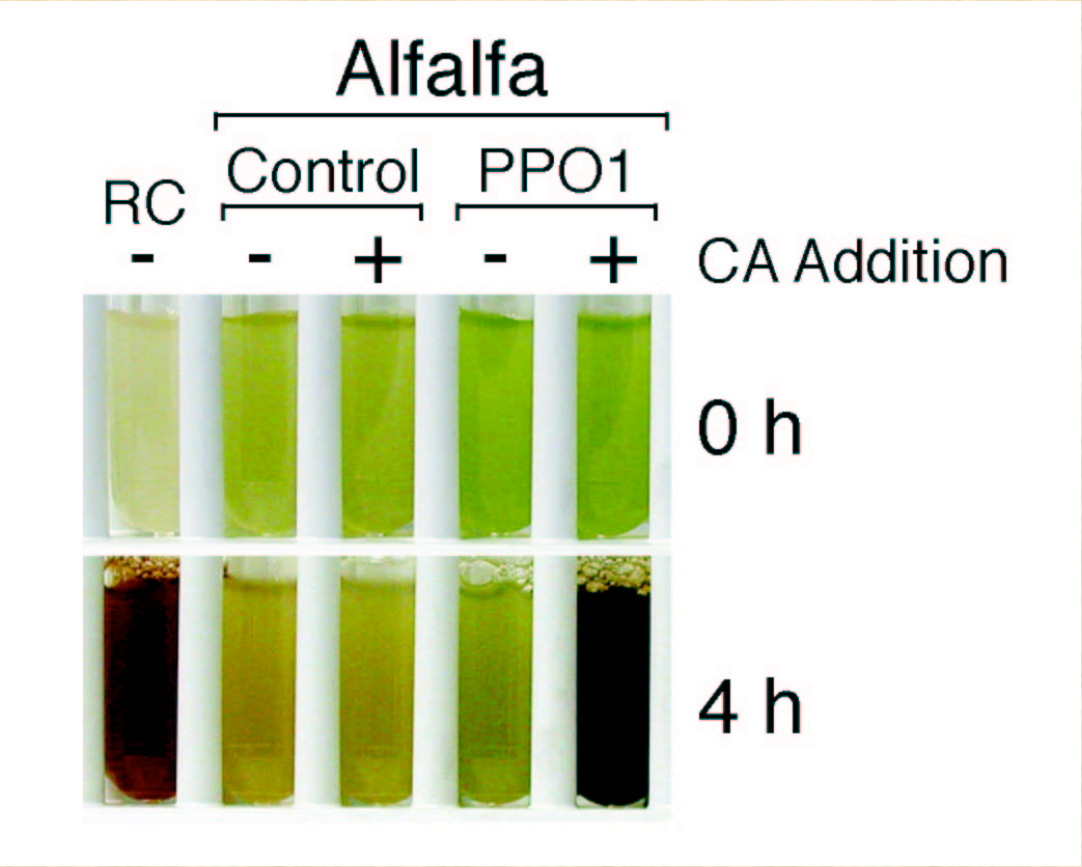
*U.S. Dairy*

# What is polyphenol oxidase?

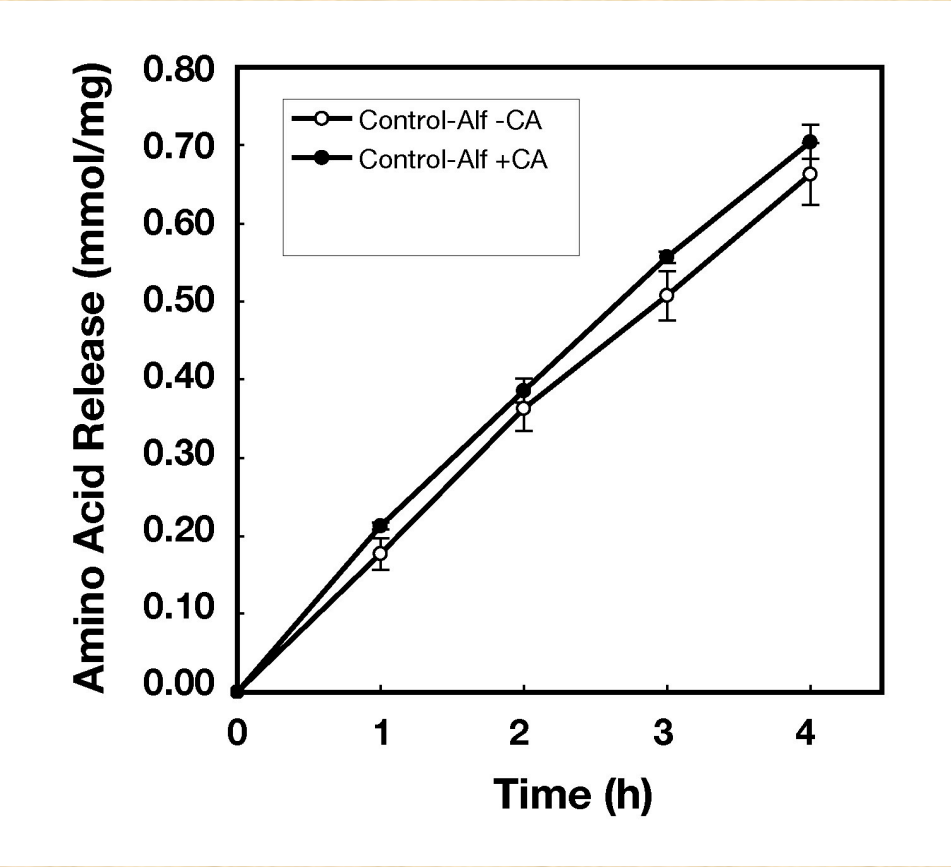
- Oxidase type enzyme
  - Requires *o*-diphenols
  - Requires oxygen ($O_2$)



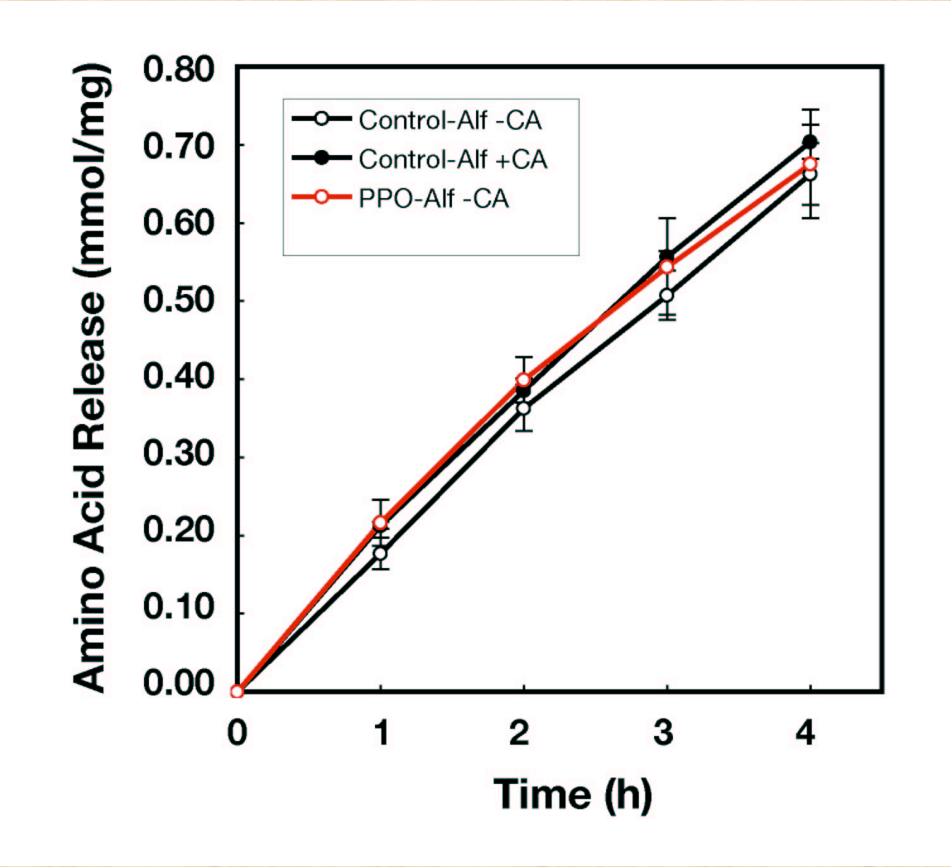
# Expression of red clover PPO1 in transgenic alfalfa



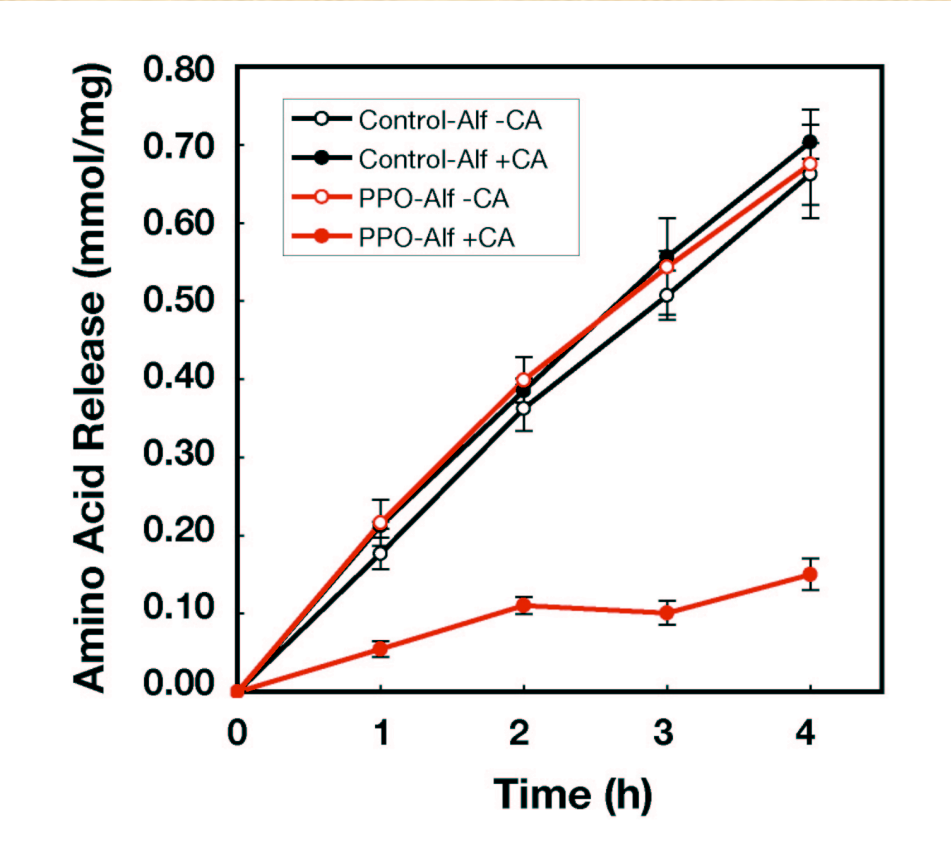
# Can PPO inhibit proteolysis in alfalfa?



# Can PPO inhibit proteolysis in alfalfa?



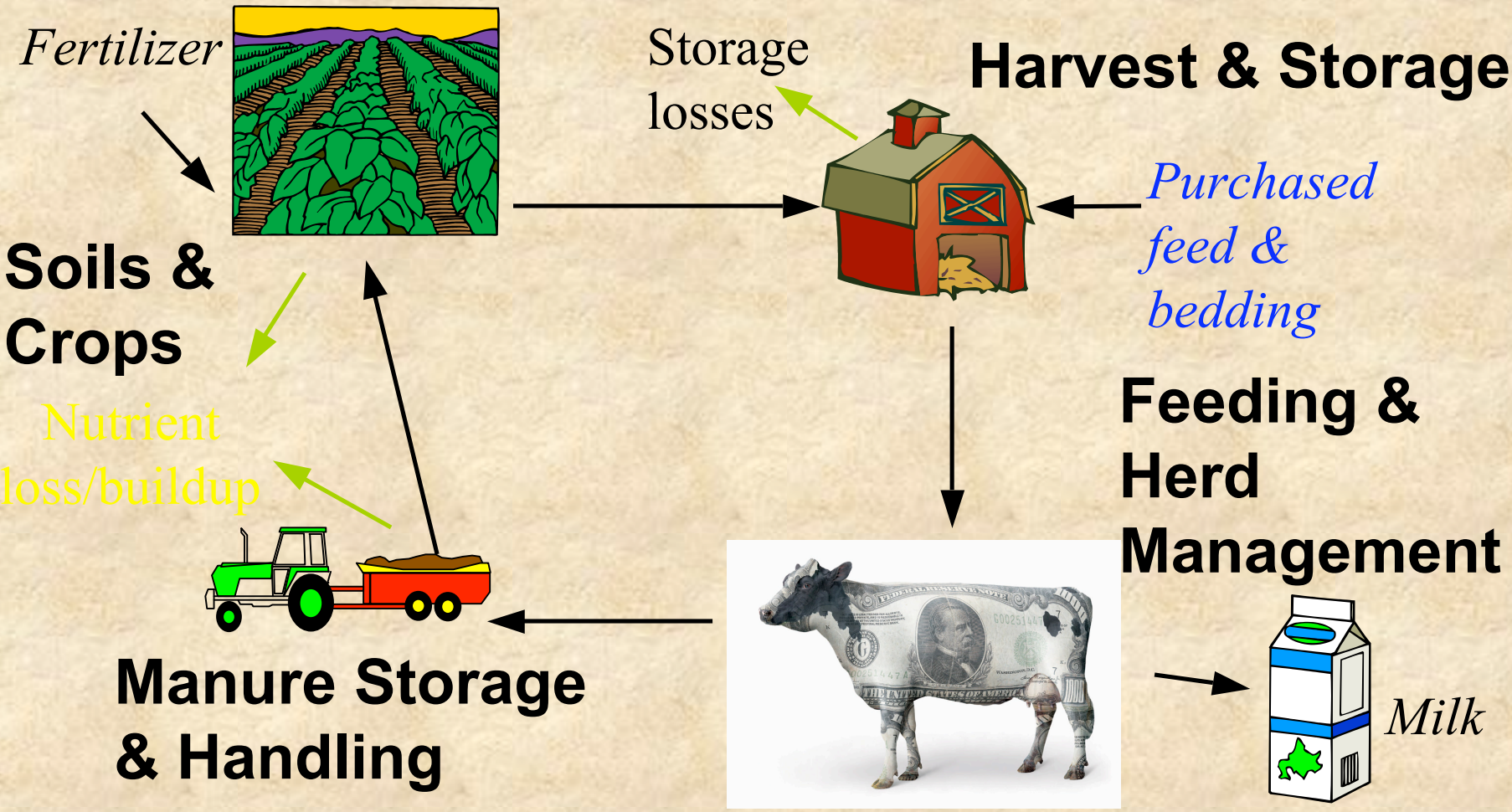
# PPO Alfalfa Inhibits Proteolysis



# Strategies For Altering Plants To Decrease N Losses

- Increase availability of structural carbohydrates
- Decrease storage losses
- Decrease rumen losses

# Model the impact of tannin-containing alfalfa on a 100-cow dairy farm (DAFOSYM)

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*USDA-ARS-MWA*

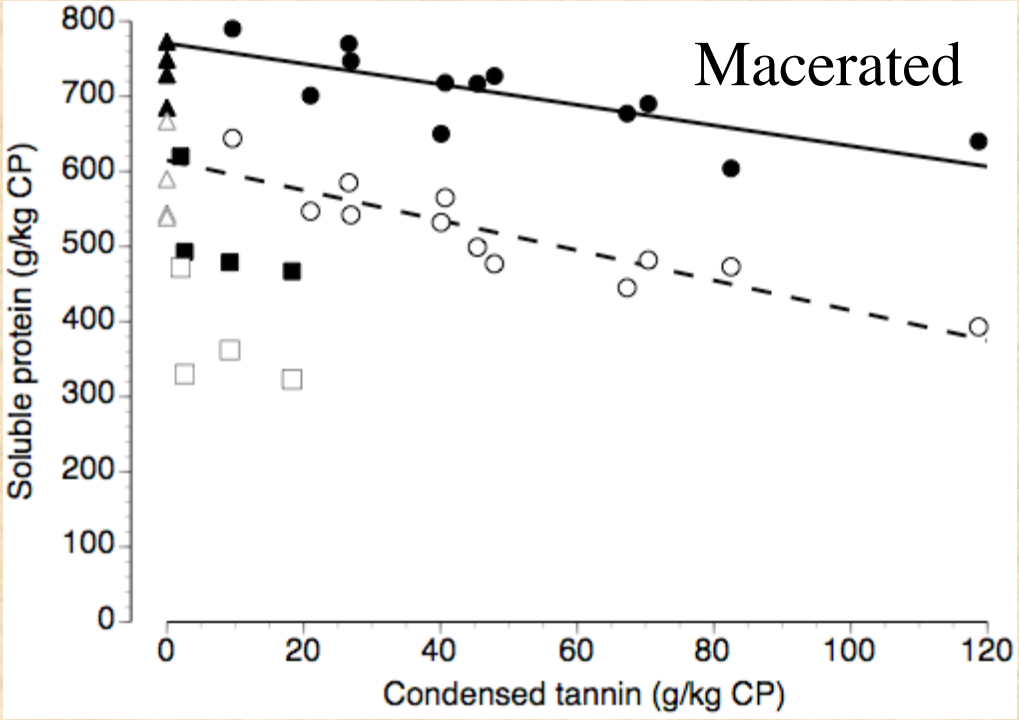
"© Cash cow image contributed by WMMB, 2004"

# Predicted annual farm performance if alfalfa is fed as ~ 45% of the diet

| | Net return $/cow | Milk yield kg/cow | Soybean fed kg/cow | Maize fed kg/cow | Total N loss kg/cow |
|---|---|---|---|---|---|
| Normal alfalfa | 1,145 | 12,330 | 1026 | 2433 | 157 |
| Tannin alfalfa | 1,270 | 12,540 | 436 | 3060 | 118 |
| Tannin impact | +125 | +210 | - 590 | +627 | -39 |

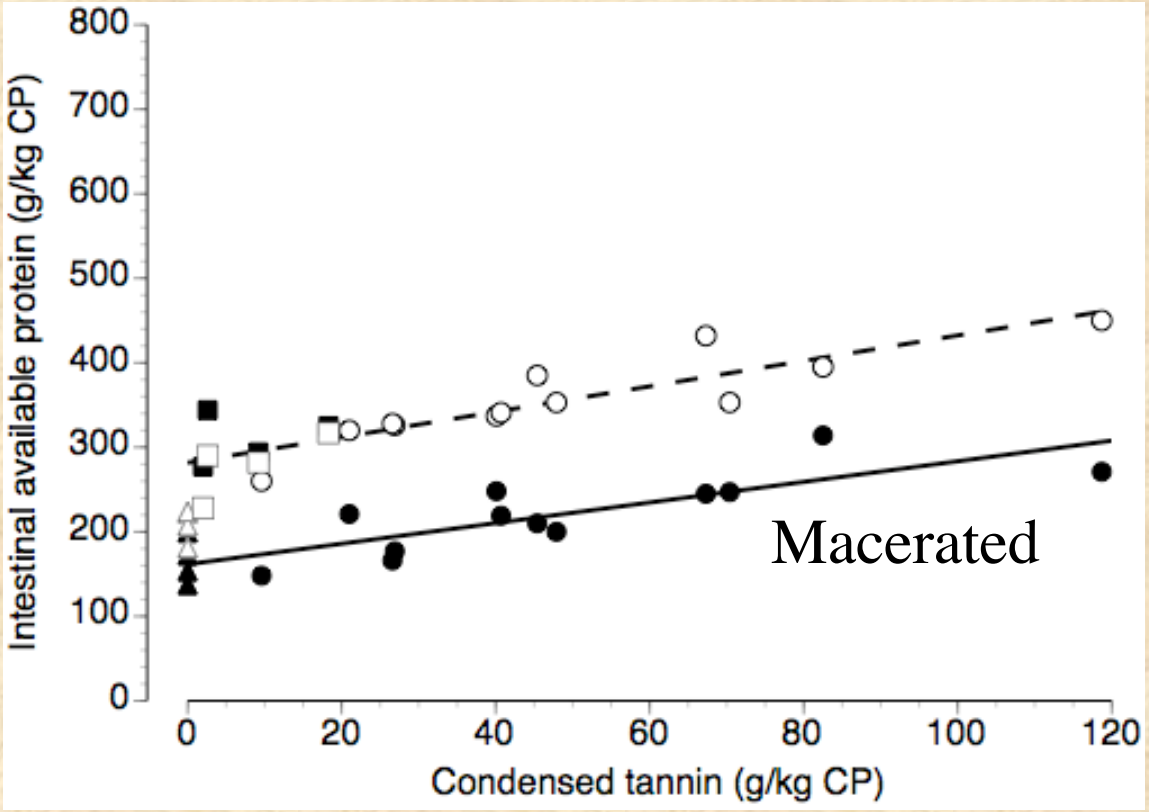
# Impact of tannins on soluble protein in silage

- Increasing amounts of tannin decreases the amount of soluble protein.



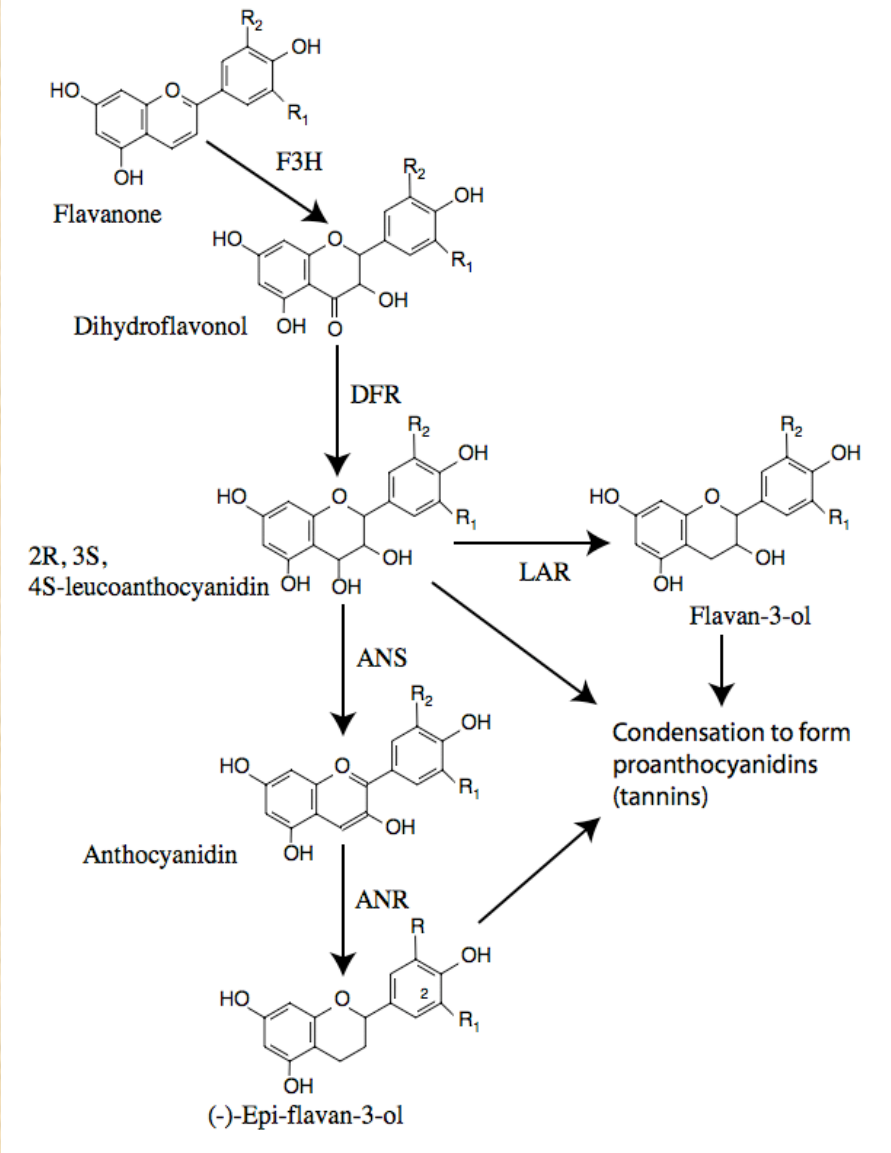
# Impact of Tannins on Availability of Proteins

- Availability of proteins in the intestinal tract of dairy cows.



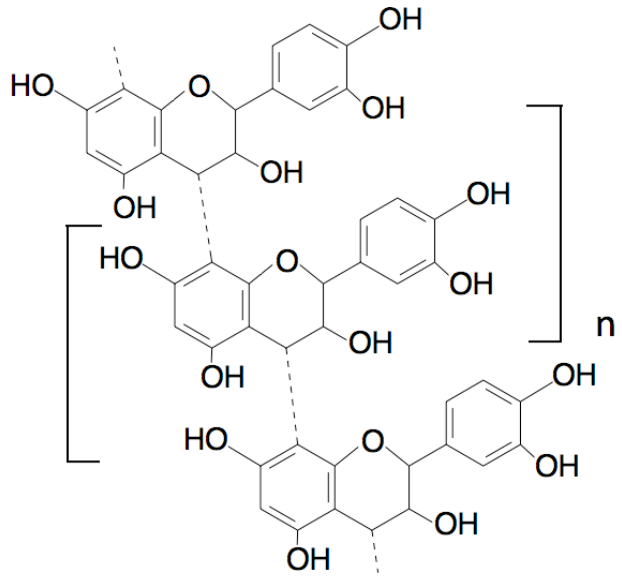
# Tannin Pathway

- Key enzymes
  - F3H, flavanone 3-hydroxylase
  - DFR, dihydroflavonol reductase
  - LAR, leucoanthocyanidin reductase
  - ANS, anthocyanidin synthase
  - ANR, anthocyanidin reductase



# Engineering Alfalfa to Produce Tannins

- Proanthocyanidins are found in alfalfa seed coats
- Typically for alfalfa it is an catechin subunit where n=5



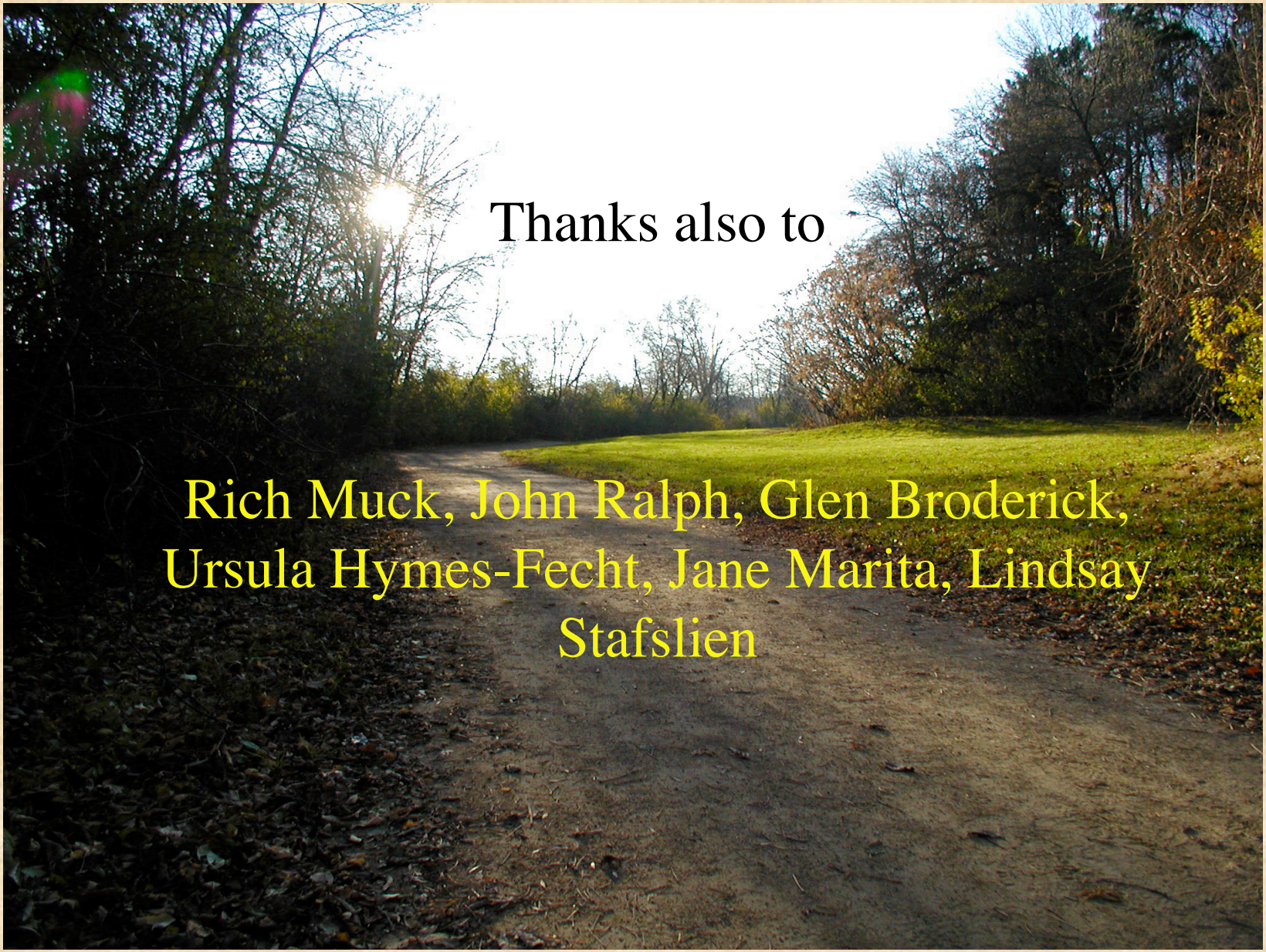

Catechin based proanthocyanidin

# Other Approaches

- Alter plant protein composition
  - Probably not effective (50-60% is RuBisCo)
- Alter proteolytic activity in plant
  - Probably not effective (numerous types, poorly understood

There are numerous opportunities if we look for them!

Thank you!!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