Genetic Engineering in Alfalfa

A tool for improving forage quality and nutrient management on dairy farms

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Why Alfalfa?

- High quality forage crop
  - High protein content, high fiber digestibility
  - High forage yield, long-lived perennial legume
- Rotational benefits
  - Nitrogen credit (~100 lb N)
  - Improved yield of succeeding corn crop (~20 bu/A)
  - Break in disease/insect/weed pest cycle
  - Improved soil tilth

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Why Alfalfa?

- Environmental benefits
  - Decreased soil erosion on sloping land
  - Low pesticide use
  - Lessens need for N fertilizer on succeeding crop

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Why Plant Genetic Engineering

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

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Genetic engineering for improved forage quality in alfalfa

- Desired improvements in alfalfa quality
  - Better source of fiber
    - Improved fiber digestibility
      - Higher energy content in forage
      - Higher milk production potential
      - Decreased manure generation
  - Better source of protein
    - Improved efficiency of protein utilization
      - Increased rumen bypass
      - Improved protein stability in silage
      - Higher milk production potential
      - Decreased N excretion

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Alfalfa Fiber Quality
Impact on dairy production

- Alfalfa is a primary source of fiber in most dairy diets
- Lignin cross-linking with cell wall polysaccharides limits both rate and extent of fiber fermentation in rumen
- Impact of rate and extent of fiber digestibility
  - Energy content of forage (energy is common limiting factor in diet for high producing dairy cows)
  - Non-digestible fiber is primary manure solids component

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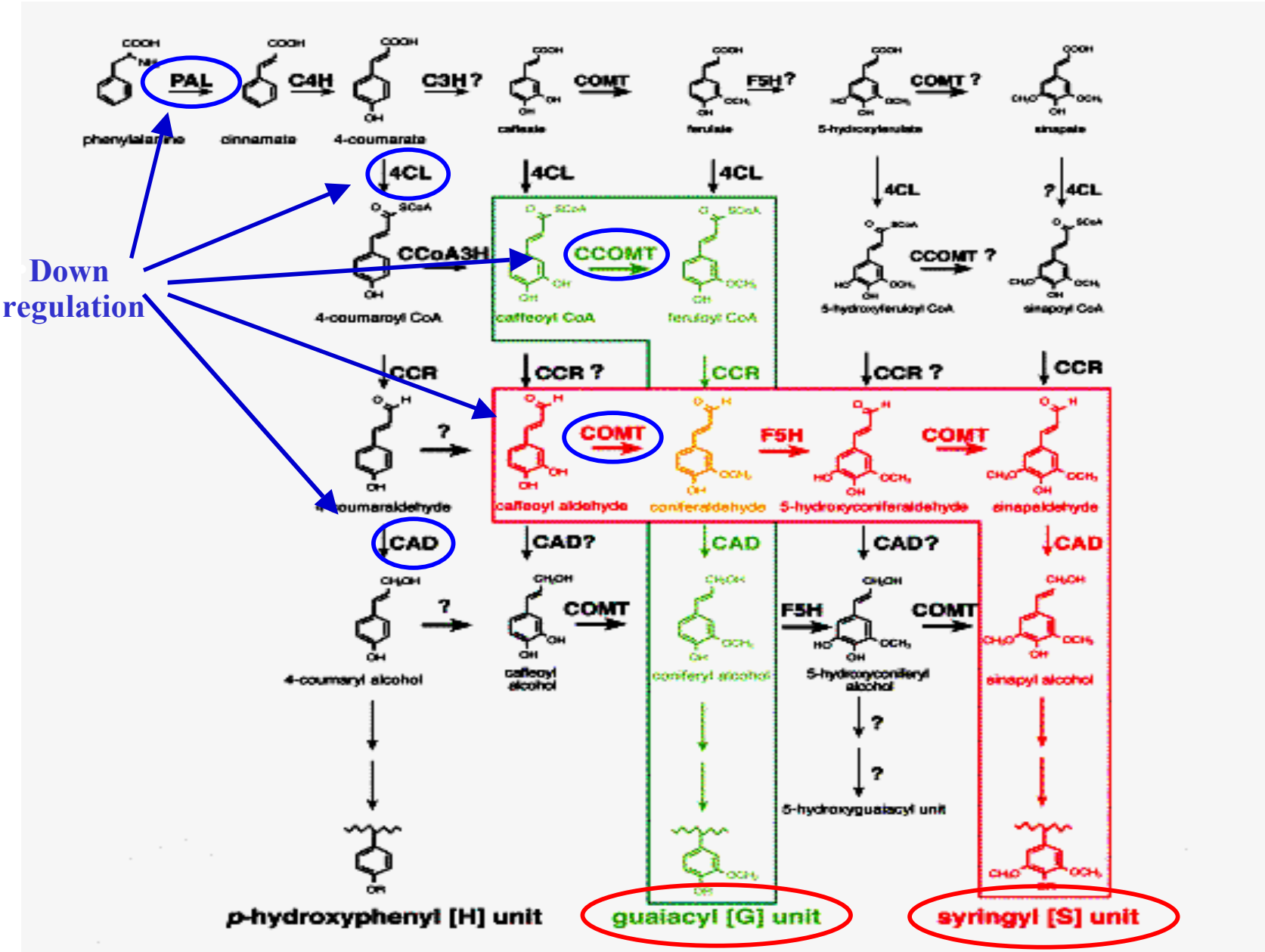

Engineering the lignin biosynthetic pathway in alfalfa

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

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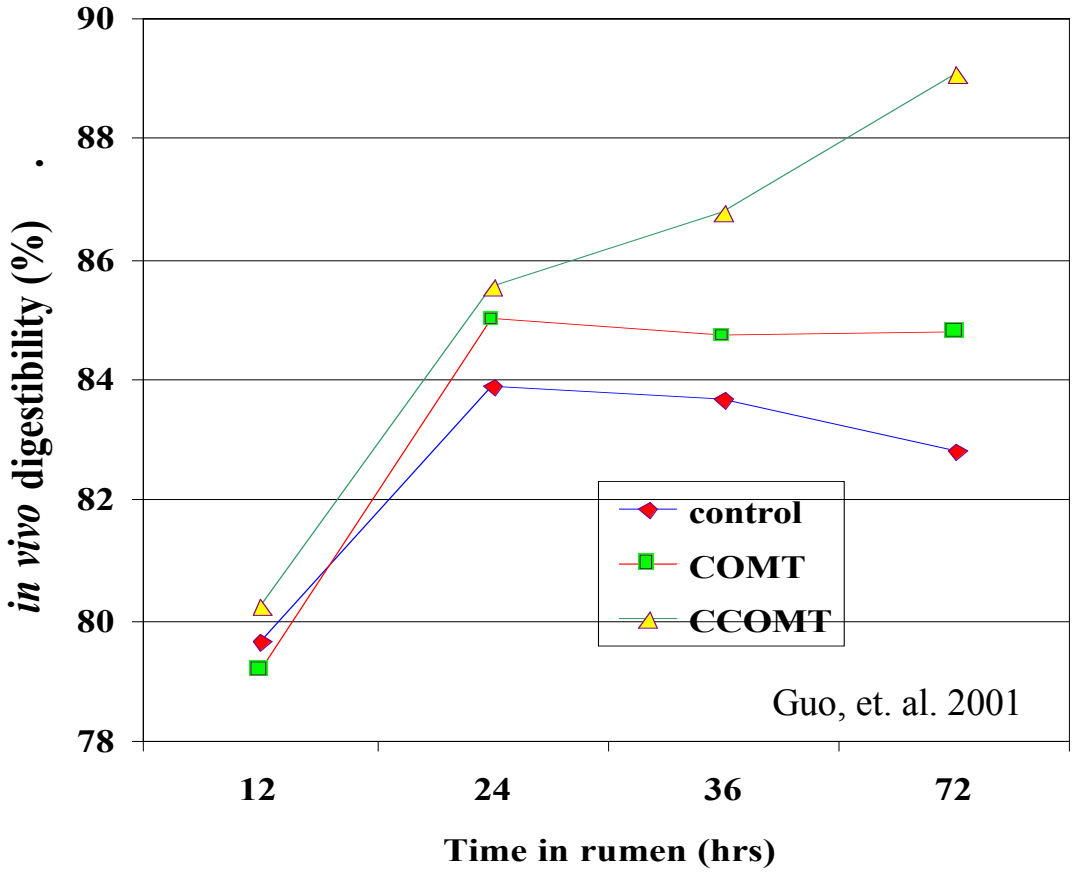
Effect of gene knockout on *in vivo* digestibility in alfalfa



Altered lignin content and composition resulted in increased rate and extent of rumen digestion

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Potential benefits of transgenic alfalfa with increased cell wall digestibility

- Increased energy from forage
  - Dietary fiber is required for rumen health, increased digestibility of this fiber will result in more energy for milk/beef production.
- Increased milk/beef production potential
  - USDFRC estimates that a 10% increase in fiber digestibility would result in an annual $350 million increase in milk/beef production.
- Decreased generation of manure
  - USDFRC estimates that a 10% increase in fiber digestibility = 2.8 million tons decrease in manure solids produced each year.

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Alfalfa Protein Quality
Impact on dairy production

- Alfalfa is a primary source of protein in most dairy diets
- Alfalfa protein is rapidly degraded in the rumen (low RUP)
- This rapid degradation of alfalfa protein limits the efficiency of protein utilization in ruminant animals
  - 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 increased N losses to soil/water/air.

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Strategies for improving efficiency of alfalfa protein utilization in the rumen

- Transgenic alfalfa expressing a novel protein (high RUP)
  - Novel leaf storage proteins (LSP) can be designed that largely bypass rumen fermentation, yet be fully digested in the gut.
  - Such novel LSPs have now been expressed in alfalfa
- Transgenic alfalfa with leaf tannins
  - Tannins bind with plant proteins, slowing rate of rumen degredation
  - Forage species with leaf tannins:
    - Have higher RUP than those species without leaf tannins
    - Do not cause bloat when grazed

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Impact of improving efficiency of alfalfa protein utilization in the rumen

- Transgenic alfalfa expressing a novel protein (high RUP)
  - Decrease/eliminate requirement for supplemental feed protein
    - Decrease in $ and N input required
- Transgenic alfalfa with leaf tannins
  - Decrease/eliminate requirement for supplemental feed protein
    - Decrease in $ and N input required
  - Decreased rate of protein fermentation = more efficient use of alfalfa protein (decreased N content of excretia).
    - Decreased N losses likely

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Post Harvest Proteolysis in Alfalfa
Impact on dairy production

- Post harvest degradation of alfalfa protein begins at mowing/conditioning and continues until limited by moisture (hay) or pH (haylage).
- Post harvest proteolysis results in increased non-protein nitrogen (NPN) in the forage. NPN is less efficiently utilized in the rumen than intact protein.
  - Nitrogen in alfalfa hay is typically 20% NPN
  - Nitrogen in alfalfa silage is typically 60-75% NPN
  - Trend is for more alfalfa silage, less alfalfa hay

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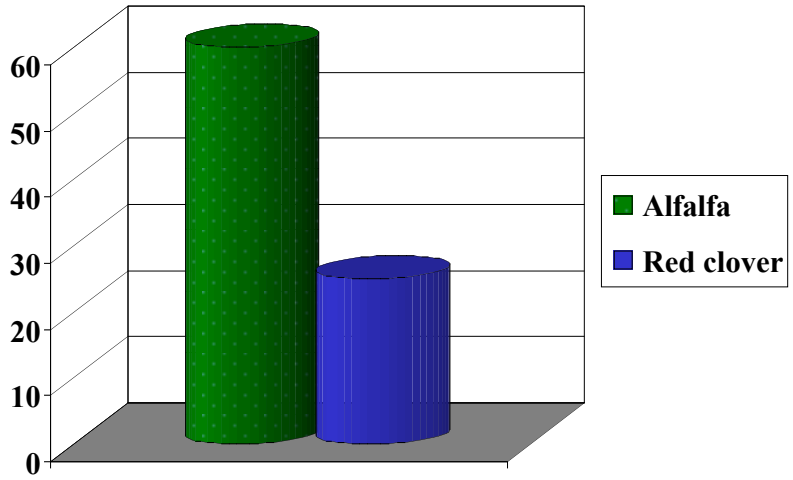
Post Harvest Proteolysis in Alfalfa
Impact on dairy production

- 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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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)
- Potential "knockout" of key plant enzymes important in post harvest proteolysis.
  - PPO
  - Protease inhibitors

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Impact of improving efficiency of alfalfa protein utilization in the rumen

- Transgenic alfalfa with decreased post-harvest proteolysis
  - Much higher protein content of alfalfa silage
  - Increased N use efficiency of alfalfa silage
    - Decrease requirement for supplemental feed protein
    - Decrease in $ and N input required
  - More efficient use of alfalfa protein (decreased N content of excretia).
    - Decreased N losses to air/soil/water

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Genetic engineering for improved forage quality in alfalfa

- Genetic engineering for improved forage quality in alfalfa should offer added value for alfalfa growers and dairy producers.
  - Increased cell wall digestibility = more energy from alfalfa = increased milk production.
  - Increased alfalfa protein stability in the rumen and silo will increase efficiency of N utilization
    - Decreased cost of protein supplementation
      - Current cost ~$.70/cow/day

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Genetic engineering for improved forage quality in alfalfa

- Genetic engineering for improved forage quality in alfalfa should offer new tools to for better nutrient management on dairy farms.
  - Increased cell wall digestibility=decreased manure production
  - Increased alfalfa protein stability in the rumen and silo will increase efficiency of N utilization
    - Decreased N losses to water/air/soil
    - Decreased N input from protein supplement

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A multidisciplinary collaboration of public and private scientists

- Dairy Nutrition (USDFRC)
- Biochemistry (Noble Foundation and USDFRC)
- Molecular/cell biology (Noble, FGI and DowAgro)
- Agronomy
- Plant breeding (FGI)

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