# Reduced Lignin Alfalfa

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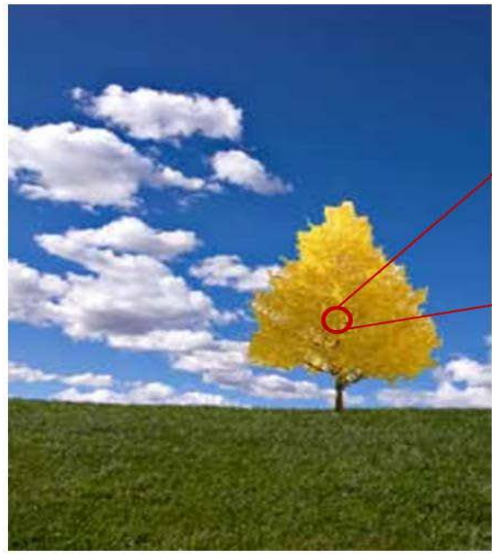
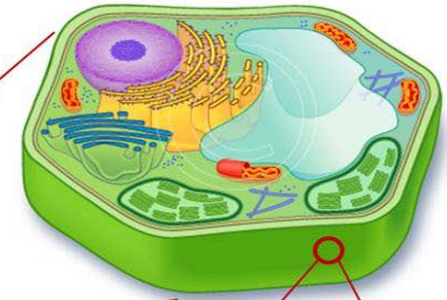
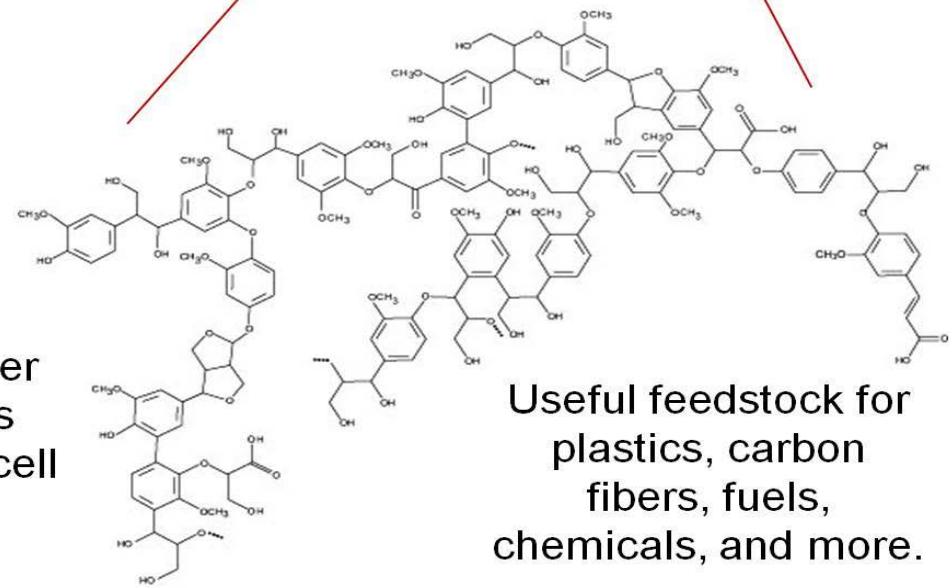
University of Wisconsin





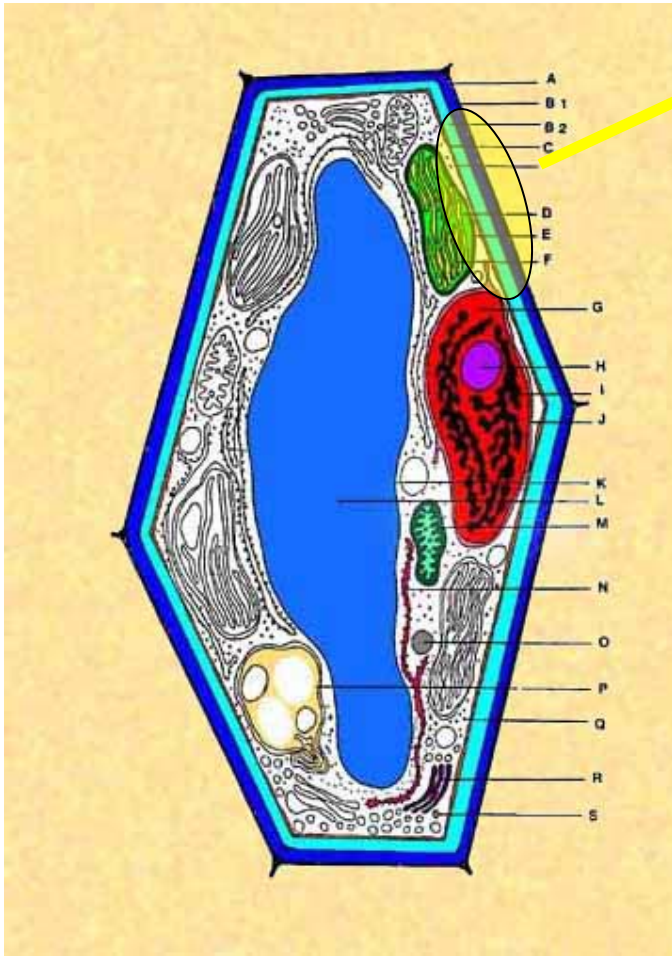
## Lignin

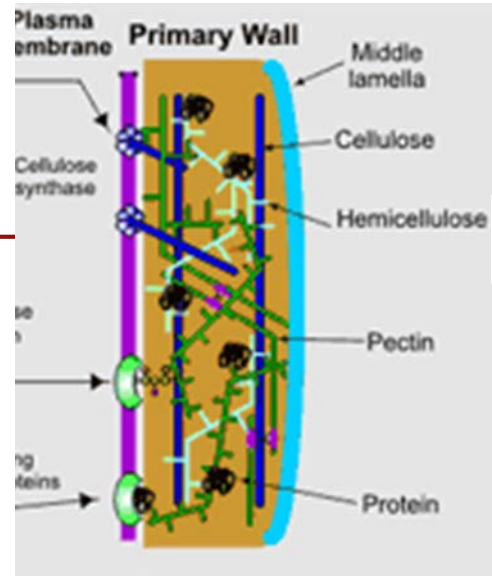


Most abundant aromatic biopolymer on earth; interacts strongly with other cell wall polymers.

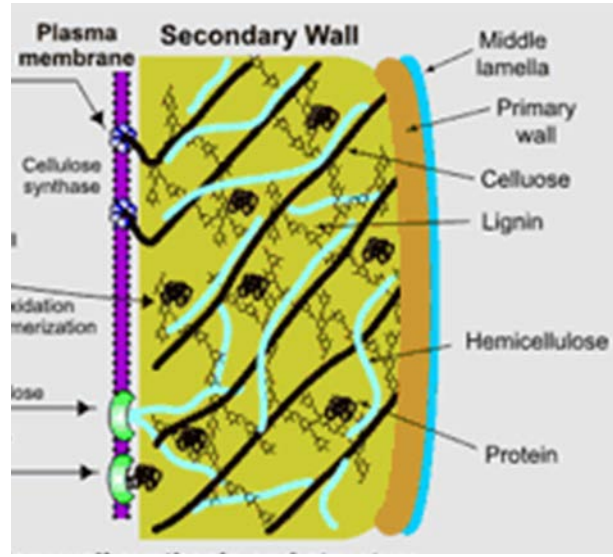
Useful feedstock for plastics, carbon fibers, fuels, chemicals, and more.

# Cell Wall



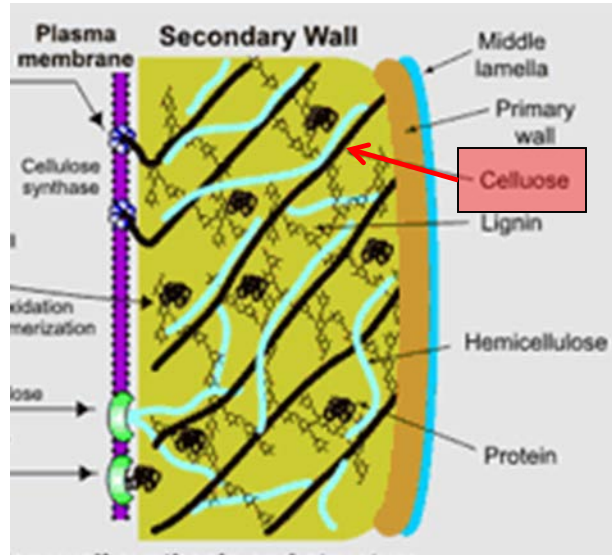

Developing cell wall



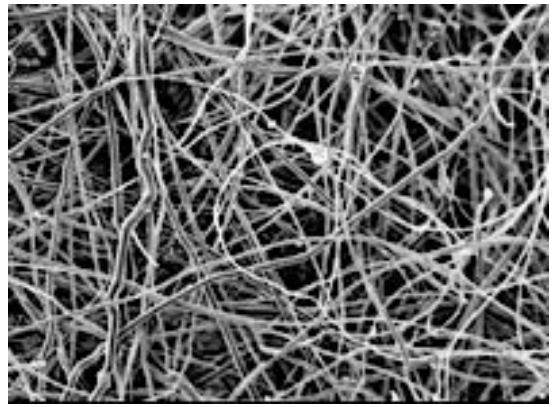

Mature cell wall

# Cellulose

- Consists of D-glucose units
- is a straight chain polymer: unlike starch, no coiling or branching occurs,
- the molecule adopts an extended and stiff rod-like shape

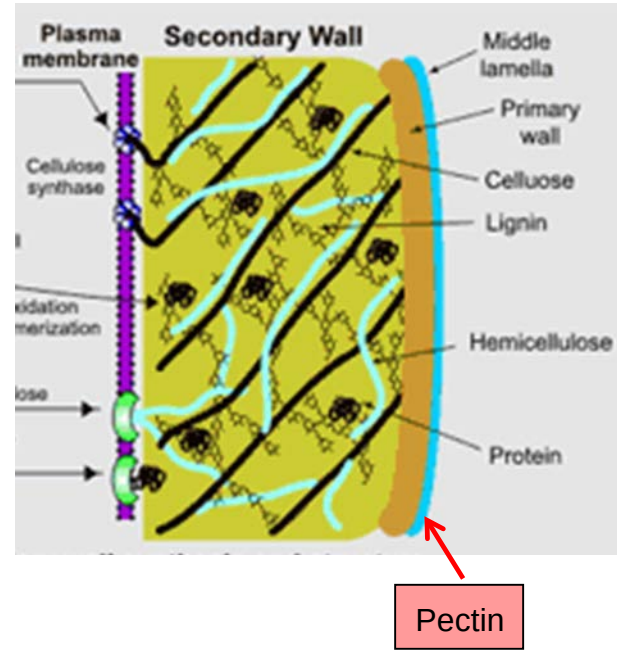



Pure cellulose fibers



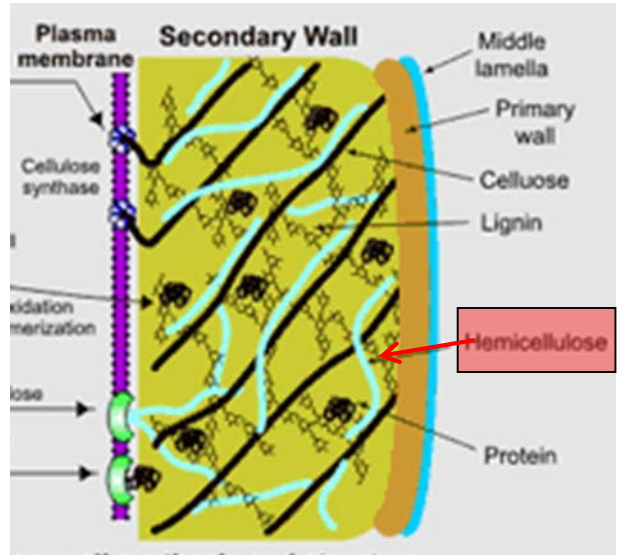
# Pectin – to congeal

- Polymer of sugar acids
- Mainly composed of pectinic acid,
- Is water soluble,
- Is able to form gels in the presence of acid and sugar
- A major component of the middle lamella, where it helps to bind cells together,



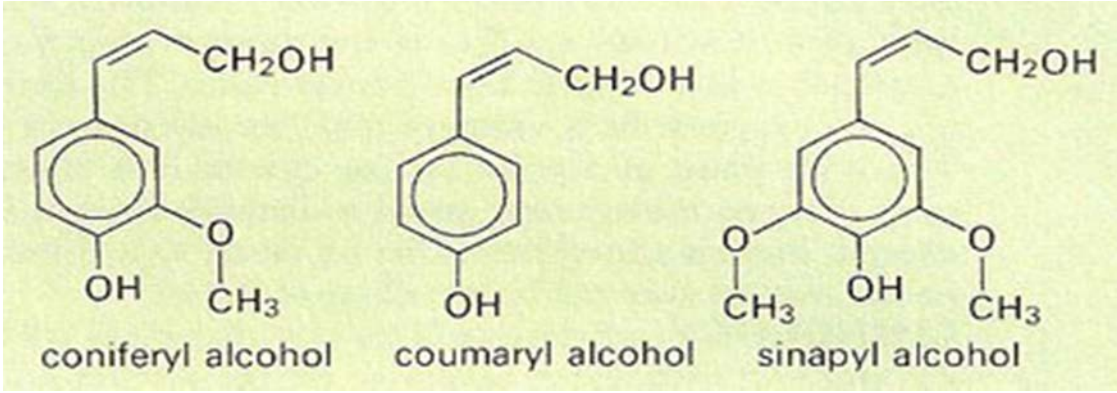
# Hemicellulose

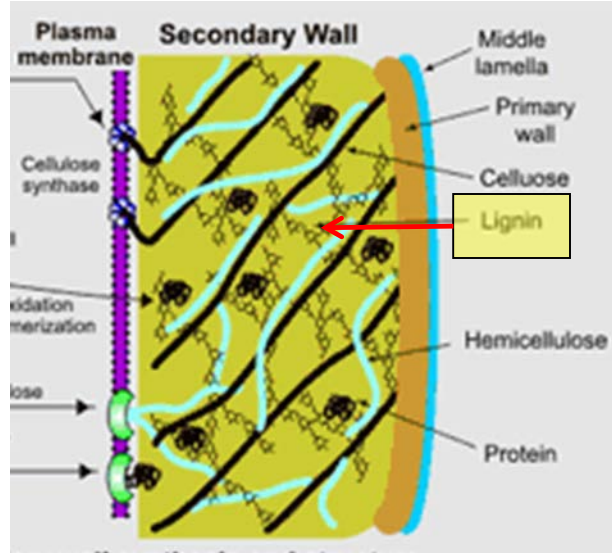
- Is a polymer of several sugars
- Besides glucose, sugars can include xylose, mannose, galactose, rhamnose, and arabinose.
- Has a random, amorphous, branched structure with little strength.
- Is easily hydrolyzed by dilute acid or base as well as hemicellulase enzymes.



# Lignin

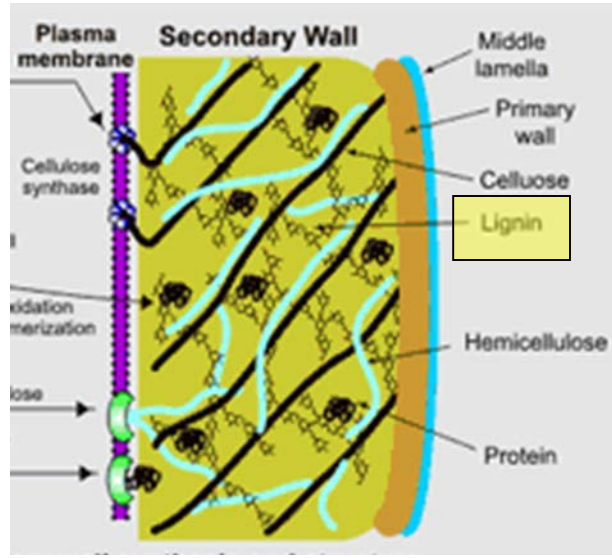
- Is a polymer of aromatic alcohols



# Importance of Lignin

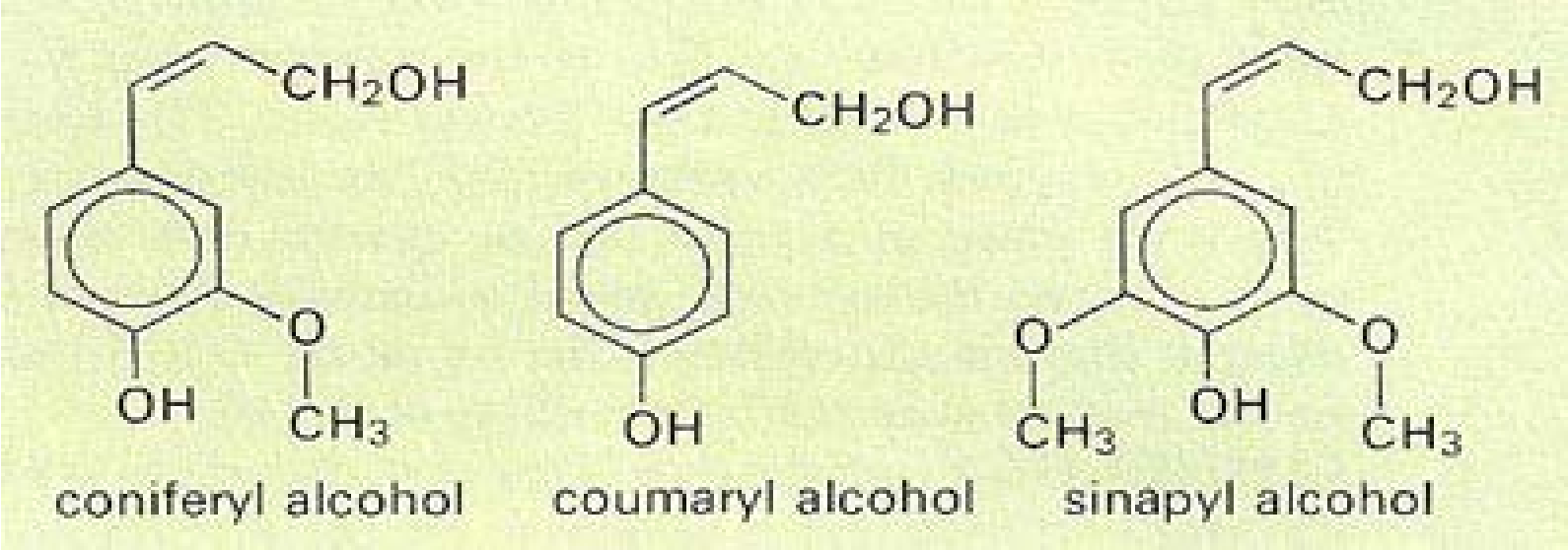
- Lignin is second most abundant organic compound on earth
- Lignin constitutes 30% of non-fossil organic carbon
- Lignin fills spaces in the cell wall between cellulose, hemicellulose, and pectin molecules
- It is linked to hemicellulose



# Importance of Lignin in Alfalfa

- ✓ Provides strength to plants
- ✓ Allows the plant vascular system to transport water in the plant without leakage.
- ✓ Sequesters atmospheric carbon into vegetation
- ✓ Is one of the most slowly decomposing components of dead vegetation, contributing a major fraction of soil organic matter.

# Lignin is a polymer of phenyl propane units



# Plant lignins can be broadly divided into three classes

- 1) softwood (gymnosperm) composed principally of coniferyl alcohol units

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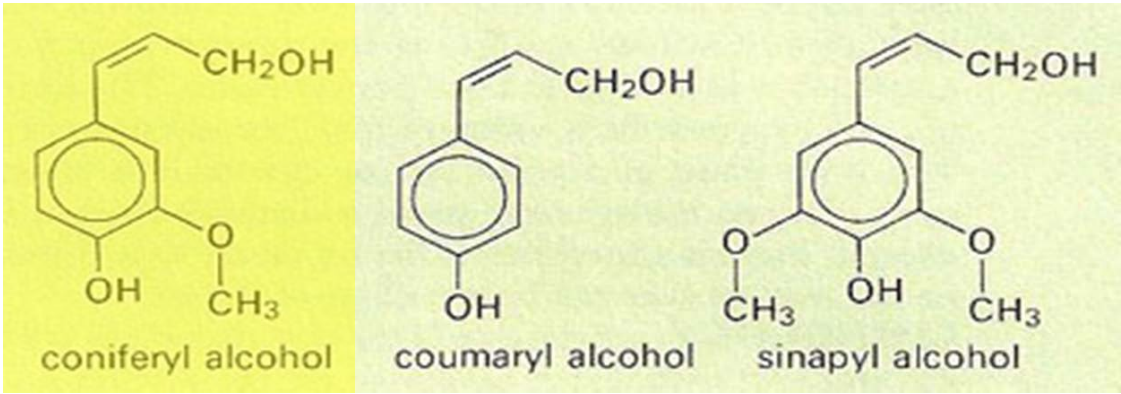
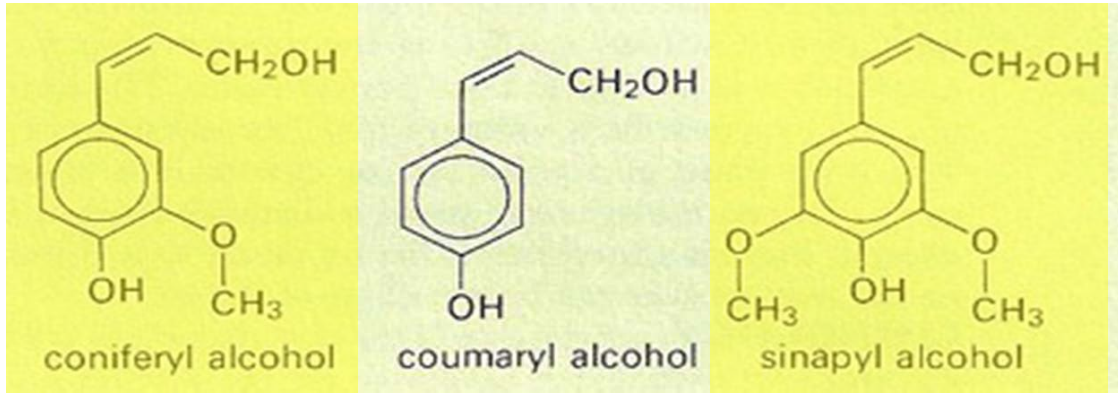


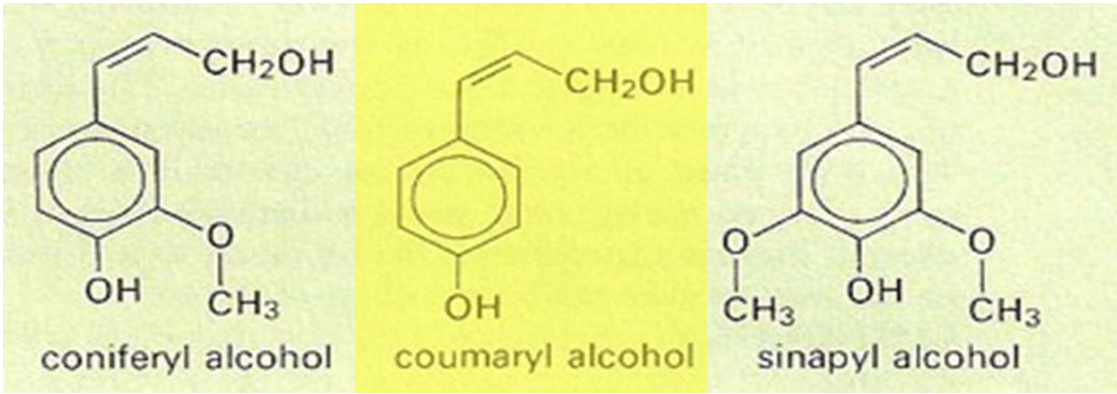
# Plant lignins can be broadly divided into three classes

- 1) softwood (gymnosperm) composed principally of coniferyl alcohol units
- 2) hardwood (angiosperm) composed of coniferyl and sinapyl alcohol units.



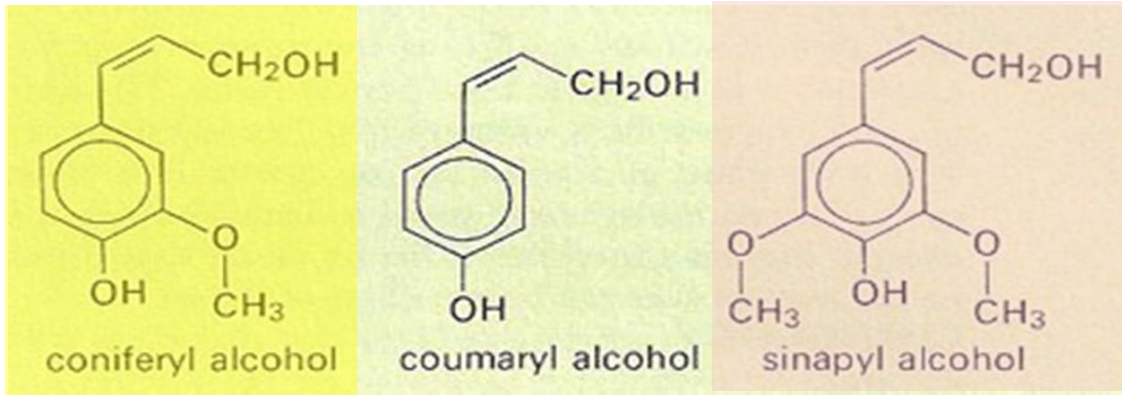
# Plant lignins can be broadly divided into three classes

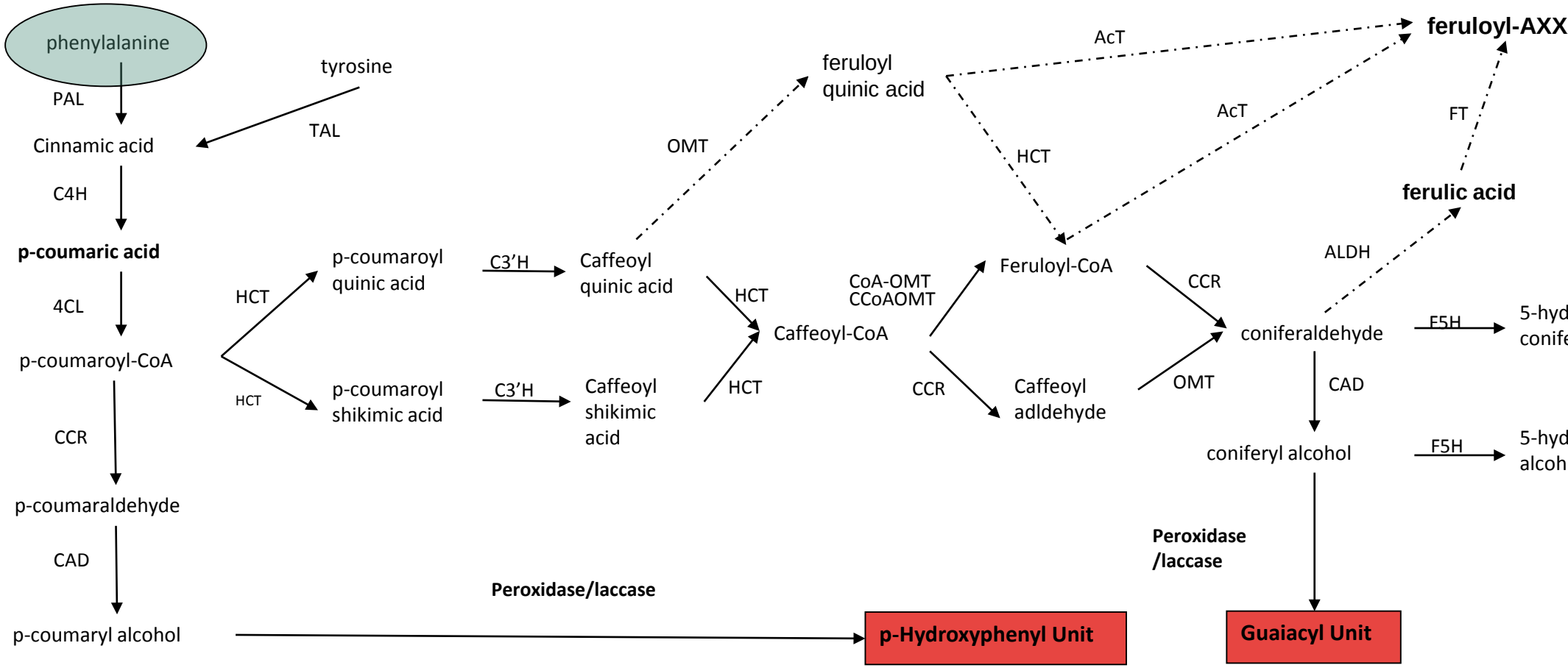
- 1) softwood (gymnosperm) composed principally of coniferyl alcohol units
- 2) hardwood (angiosperm) composed of coniferyl and sinapyl alcohol units.
- 3) grass or annual plant (graminaceous) lignin composed mainly of p-coumaryl alcohol units.

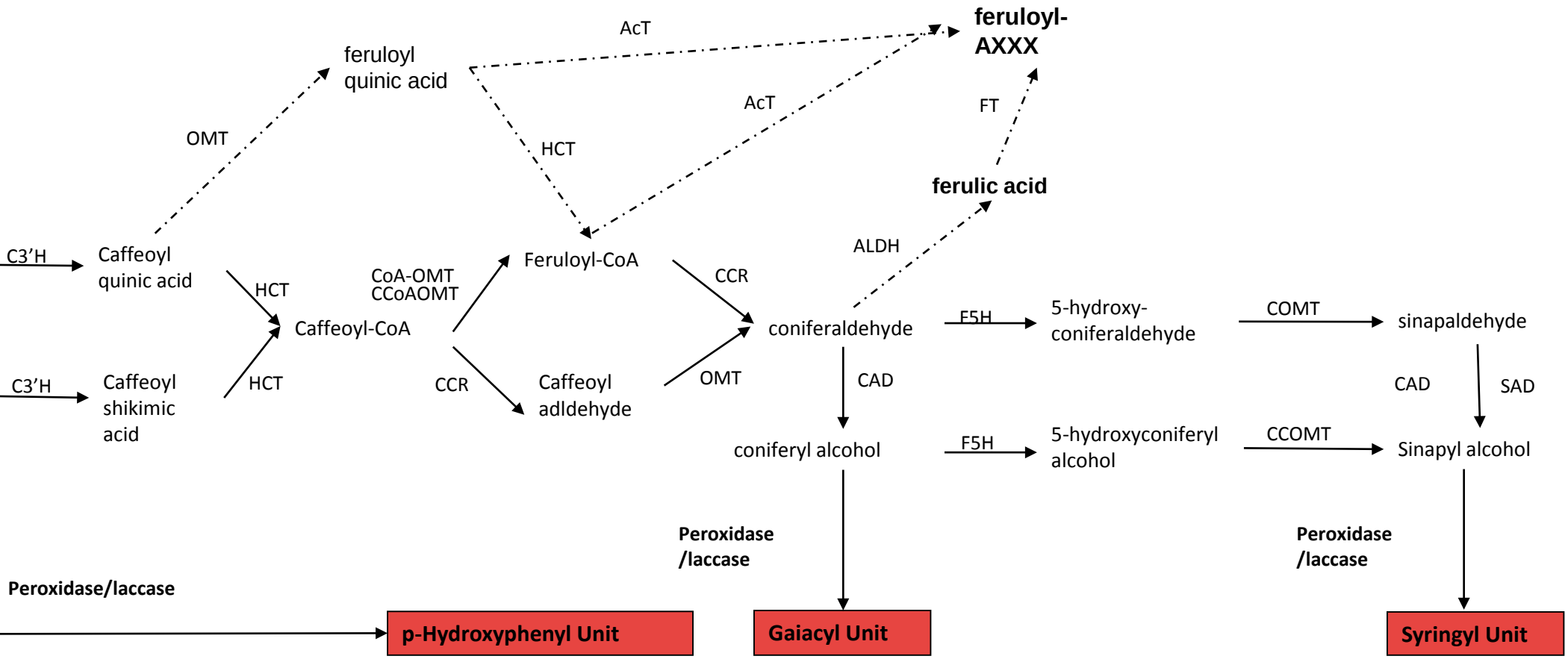


# Plant lignins can be broadly divided into three classes

- Alfalfa is composed principally of coniferyl alcohol units (like softwoods).



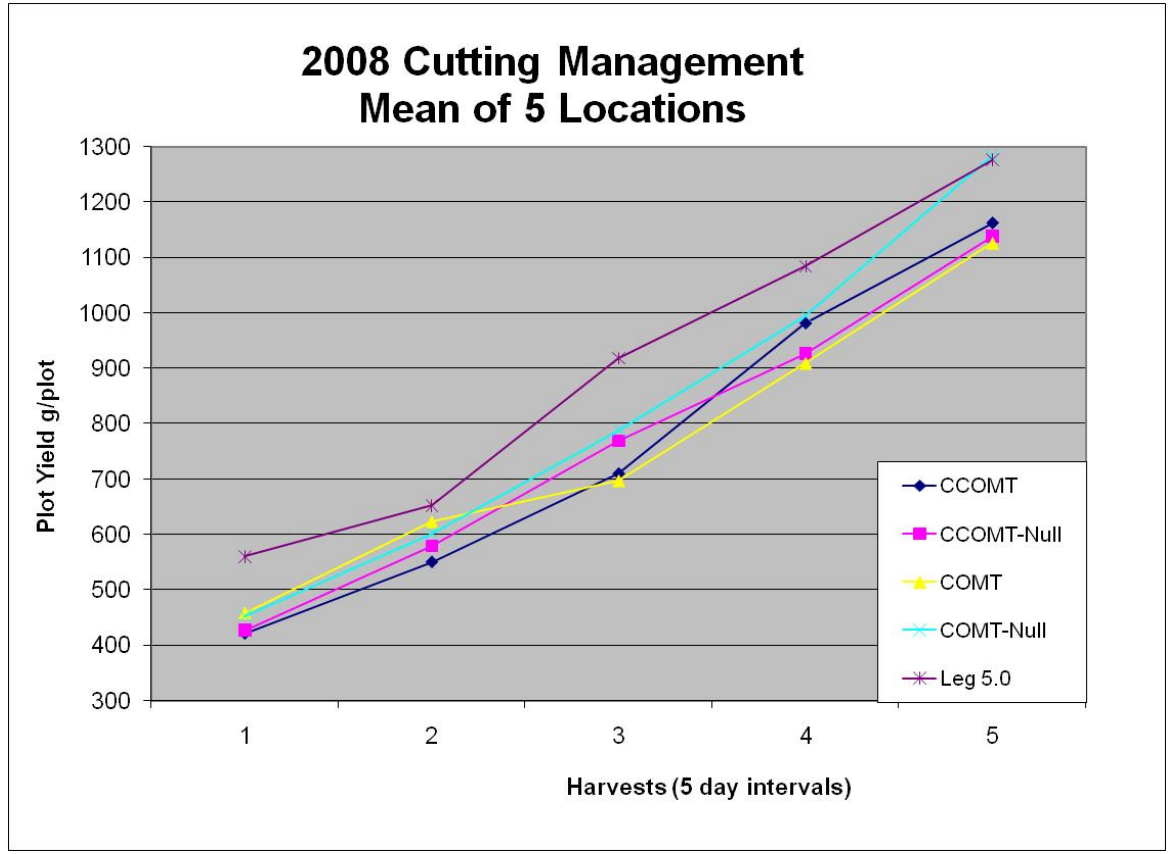

phenylalanine
PAL
Cinnamic acid
C4H
p-coumaric acid
4CL
p-coumaroyl-CoA
CCR
p-coumaraldehyde
CAD
p-coumaryl alcohol
tyrosine
TAL
HCT
p-coumaroyl quinic acid
HCT
p-coumaroyl shikimic acid
C3'H
Caffeoyl quinic acid
C3'H
Caffeoyl shikimic acid
OMT
feruloyl quinic acid
HCT
HCT
Caffeoyl-CoA
CoA-OMT
CCoAOMT
CCR
Feruloyl-CoA
Caffeoyl adldehyde
AcT
AcT
HCT
feruloyl-AXX
FT
ferulic acid
ALDH
CCR
OMT
coniferaldehyde
CAD
coniferyl alcohol
F5H
F5H
Peroxidase/laccase
Peroxidase /laccase
p-Hydroxyphenyl Unit
Guaiacyl Unit


feruloyl-
AXXX
AcT
feruloyl
quinic acid
AcT
OMT
HCT
FT
ferulic acid
C3'H
Caffeoyl
quinic acid
HCT
CoA-OMT
CCoAOMT
Feruloyl-CoA
CCR
ALDH
Caffeoyl-CoA
coniferaldehyde
F5H
5-hydroxy-
coniferaldehyde
COMT
sinapaldehyde
C3'H
Caffeoyl
shikimic
acid
HCT
CCR
Caffeoyl
adldehyde
OMT
CAD
CAD
SAD
coniferyl alcohol
F5H
5-hydroxyconiferyl
alcohol
CCOMT
Sinapyl alcohol
Peroxidase
/laccase
Peroxidase
/laccase
Peroxidase/laccase
p-Hydroxyphenyl Unit
Gaiacyl Unit
Syringyl Unit

# Reduced lignin study

Studies were conducted at Davis, CA and Tulelake, CA, Becker, MN, Arlington, WI, and West Salem, WI.

# Planted at 5 locations to determine response in harvest system



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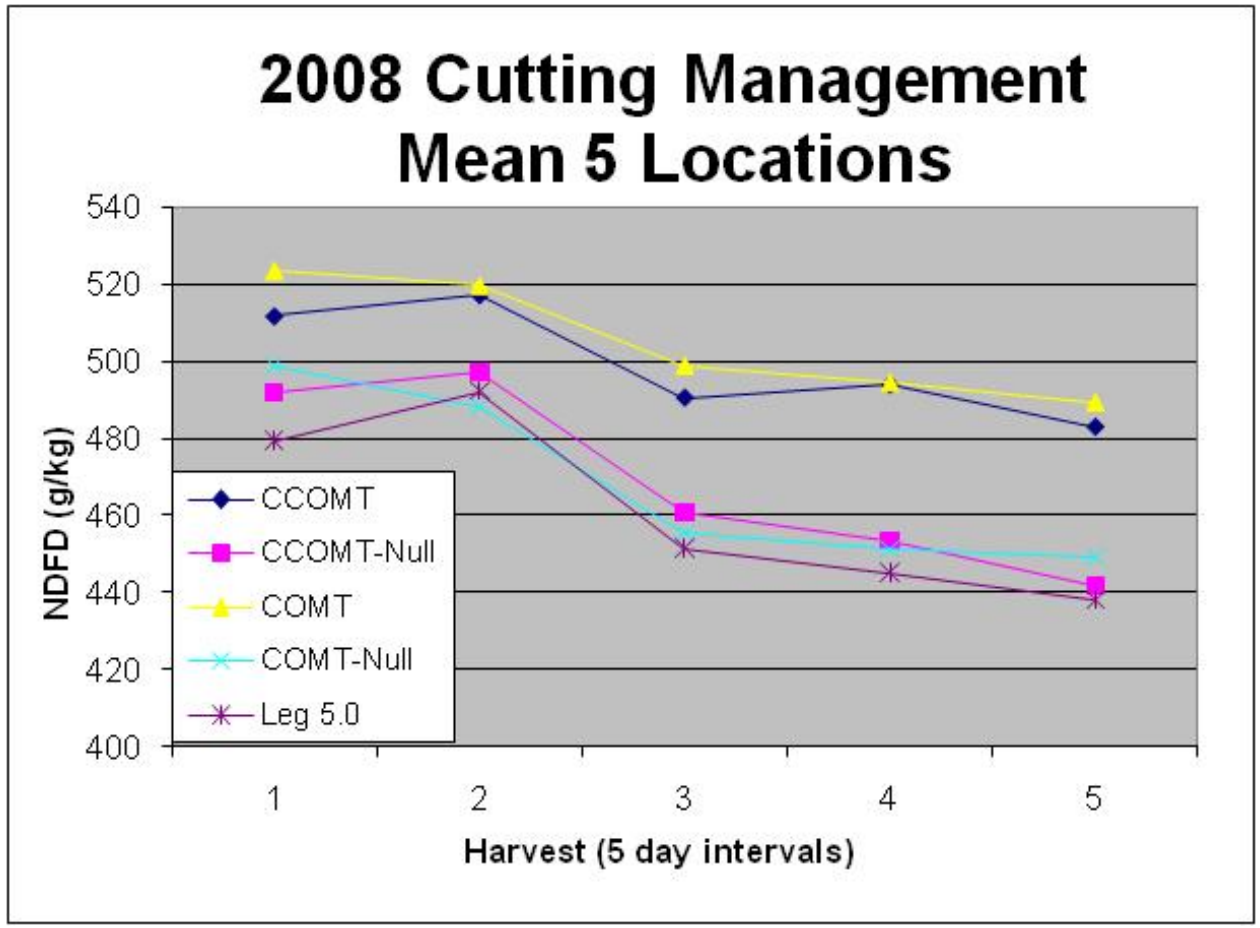


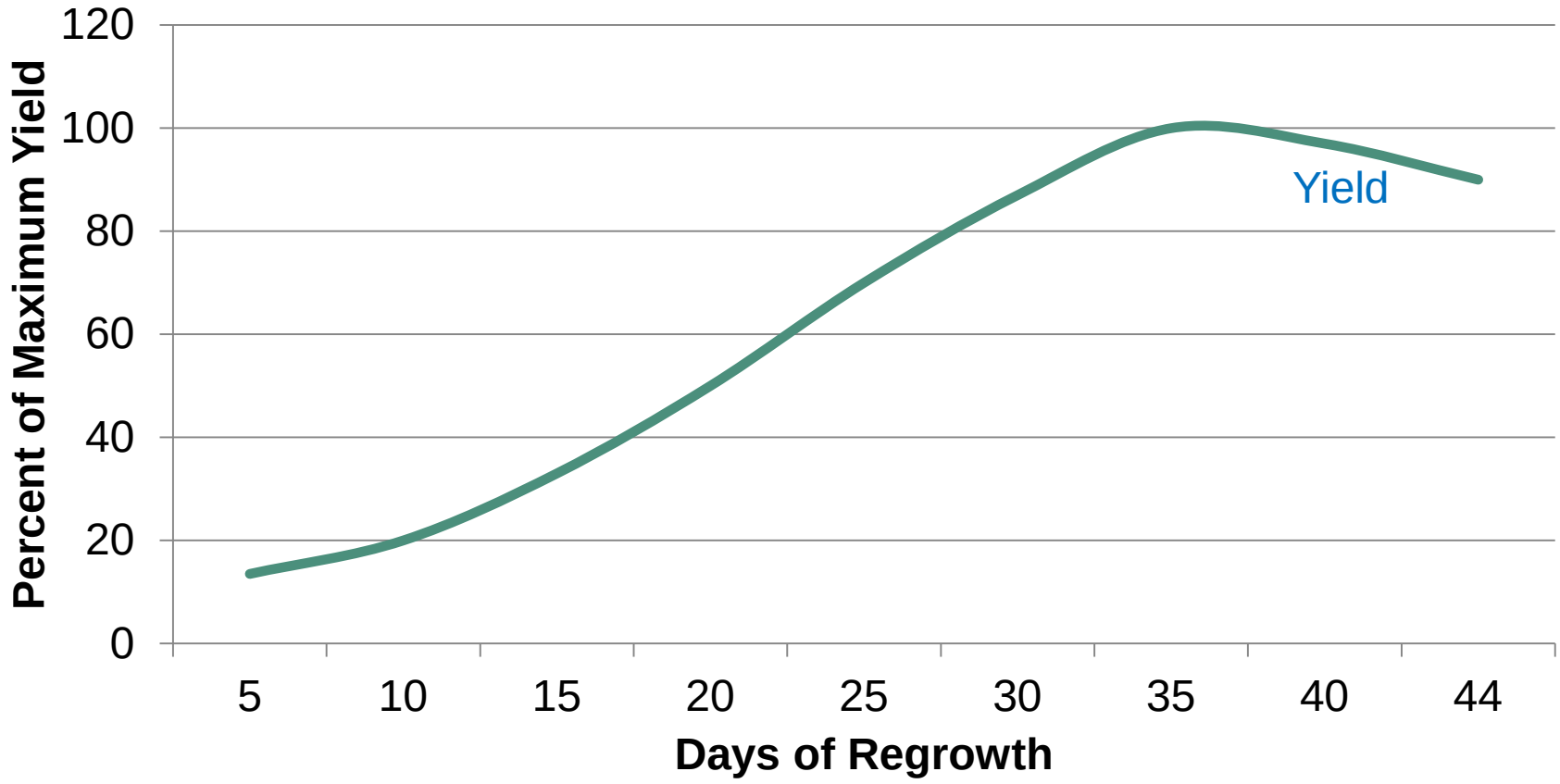
# Effect of low lignin genes on in vivo digestibility

| Digestibility of low lignin alfalfa types and controls fed to lambs, diet was 100% alfalfa hay fed ad libitum. | | | | |
|---|---|---|---|---|
| 100% alfalfa hay diet | aNDF % DM | ADL % DM | NDFD % NDF | DMD % DM |
| COMT Inactive | 38.2 | 5.3 | 57.5* | 67.5* |
| COMT Active (Control) | 39.0 | 5.8 | 49.1 | 64.5 |
| CCOMT Inactive | 39.4 | 5.2 | 50.1 | 65.3 |
| CCOMT Active (Control) | 39.4 | 5.9 | 46.4 | 63.7 |
| *Significant, P < 0.05 | | | | |
| *SOURCE: Mertens et al. 2008. J. Dairy Sci. Supple. 1* | | | | |

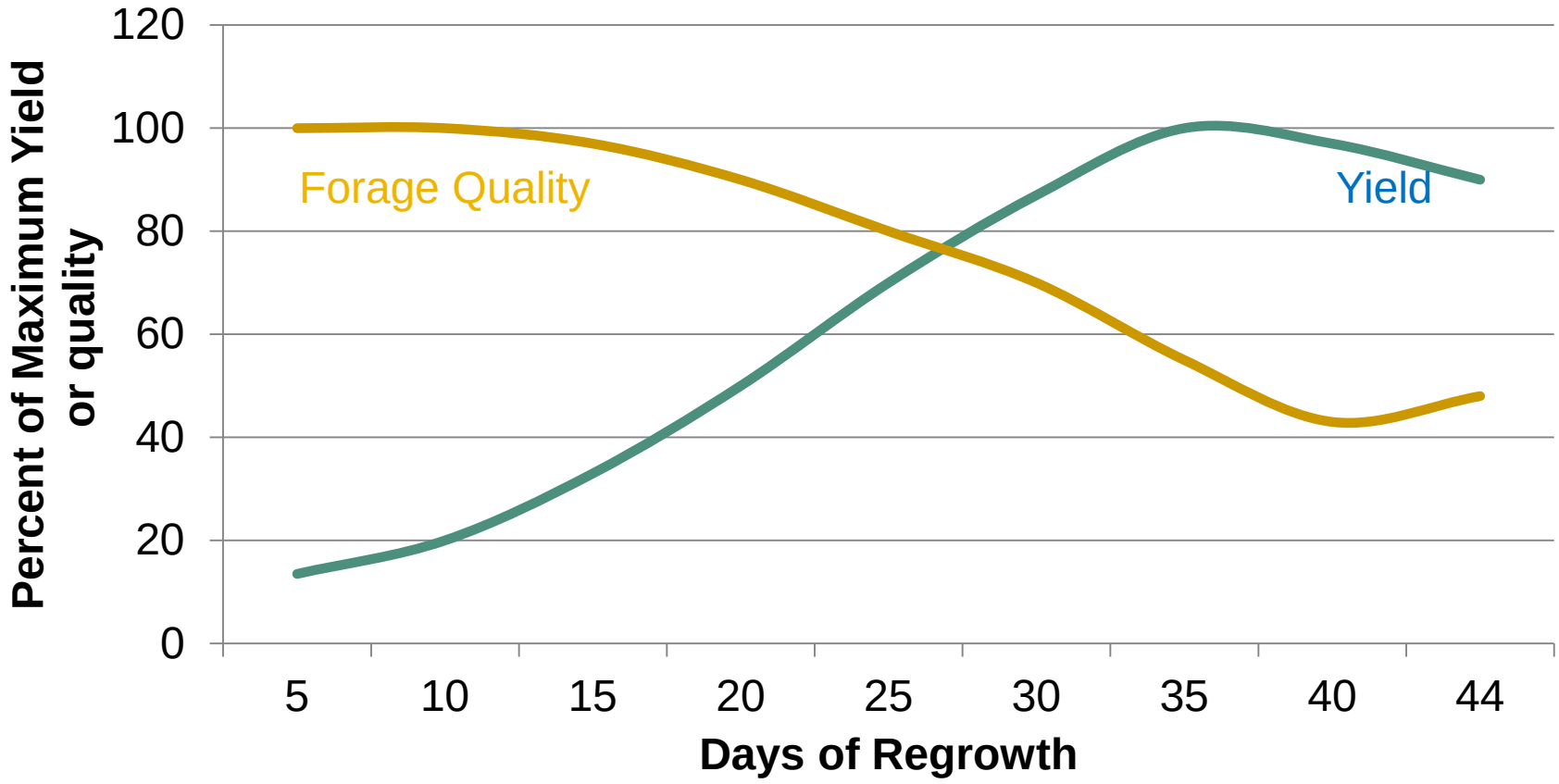
# Effect of low lignin genes on milk production

| Lactating cow responses to alfalfa hays with down- regulated lignin biosynthesis | | | | |
|---|---|---|---|---|
| Alfalfa hay type[1] | CP % DM | NDF % DM | NDFD %NDF | Milk lb/day |
| COMT Inactive | 18.1 | 31.1 | 53.5** | 84.7* |
| COMT Active (Control) | 18.4 | 29.3 | 42.5 | 82.1 |
| CCOMT Inactive | 18.1 | 42.5 | 48.6** | 84.5 |
| CCOMT Active (Control) | 18.3 | 31.1 | 44.5 | 86.7 |
| [1]TMR diets - 50 % alfalfa hay, 10 % corn silage, 40 % concentrate | | | | |
| *Significant, P < 0.10; ** significant P <0.01 (different from control) | | | | |
| *SOURCE: Weakley et al. 2008. J. Dairy Sci. Supple. 1* | | | | |

# Yield Curve of Alfalfa



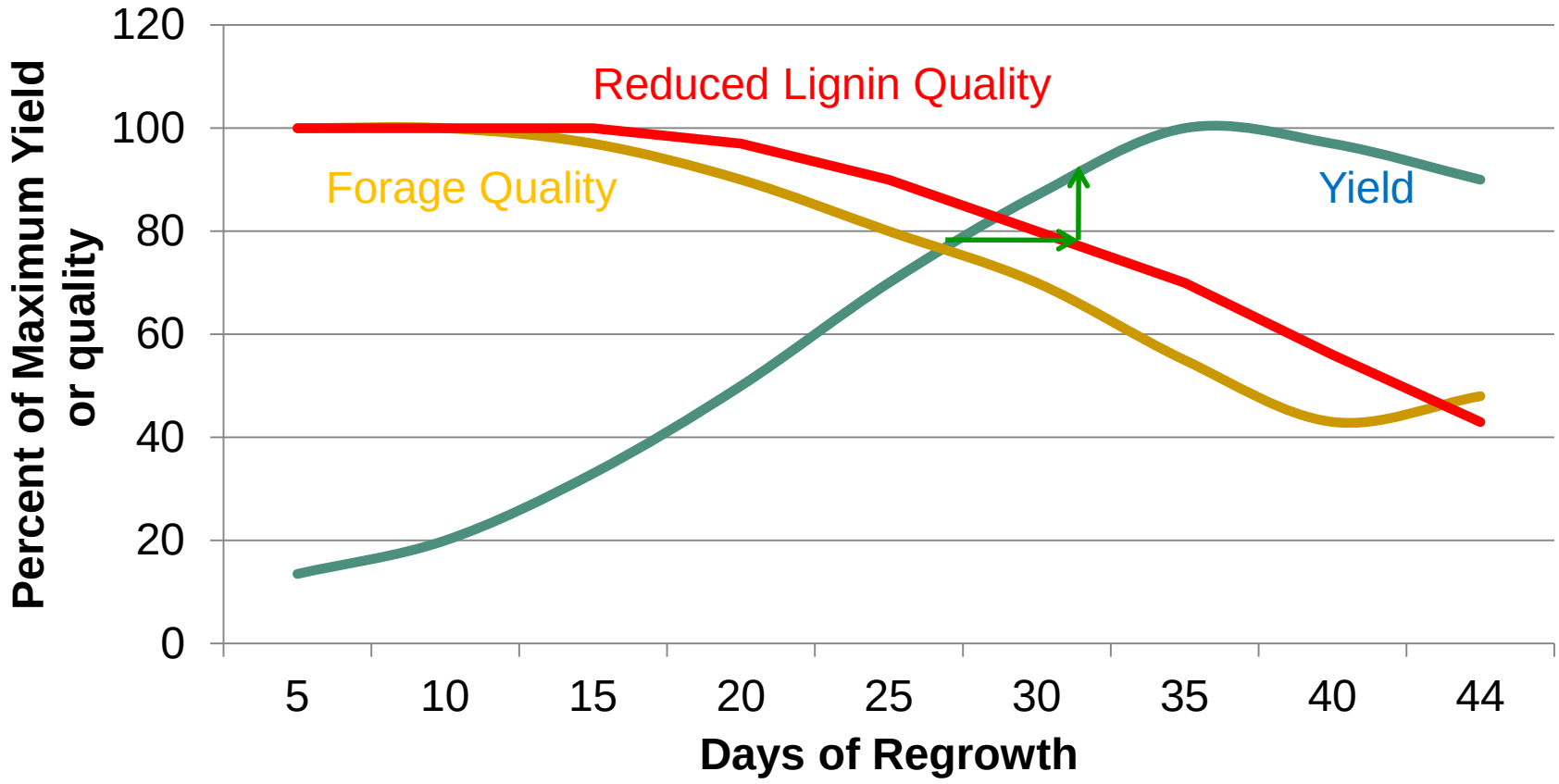
# Yield and Quality Curve of Alfalfa



# 3 vs 4 cutting by Sept 1 effect on alfalfa yield, Arlington, Wisconsin

| | | 1st cutting | 2nd cutting | 3rd cutting | 4th cutting | Season Total | |
|---|---|---|---|---|---|---|---|
| 2nd year | 3 cut | 2.97 | 2.43 | 2.15 | ---- | 7.55 | 17% |
| | 4 cut | 1.66 | 1.48 | 1.71 | 1.68 | 6.53 | |
| 3rd year | 3 cut | 2.32 | 1.53 | 1.24 | ---- | 5.09 | 25% |
| | 4 cut | 1.31 | 1.18 | 0.75 | 0.83 | 4.07 | |

# Yield and Quality Curve of Alfalfa



# Measuring Lignin

- Two methods:
  - Use potassium permanganate to solubilize lignin, wash and measure weigh loss
  - Use sulfuric acid to solubilize cellulose, hemicellulose
    - Klason method developed in early 1900s

NIR estimate of lignin

- Based on wet chemistry reference method
- Method shows same variability

# Low lignin alfalfa varieties

| Company | Lignin Reduction |
|---|---|
| Pioneer | 5% |
| Alforex | 7 to 10% |
| Forage Genetics | 10 to 15% |

# Low lignin alfalfa varieties

| Company | Lignin Reduction | Unit reduction (assuming 7% lignin) |
|---|---|---|
| Pioneer | 5% | 0.35 |
| Alforex | 7 to 10% | 0.5 to 0.7 |
| Forage Genetics | 10 to 15% | 0.7 to 1.1 |

# Variation in lignin analysis

| Table 2. Lignin analysis results from National Forage Association Testing Program, 2013 | | | | |
|---|---|---|---|---|
| | Average[1] | Standard Deviation | | |
| Alfalfa, 5 check samples | 6.7 | 0.69 | | |
| Grass, 1 check sample | 3.9 | 0.99 | | |
| Corn Silage, 2 check samples | 2.7 | 1.44 | | |
| [1] Calculated from 18 to 22 participating laboratories | | | | |

# Variation in lignin analysis

| Lignin analysis results from National Forage Association Testing Program, 2013 | | | | |
|---|---|---|---|---|
| | Average[1] | Standard Deviation | Minimum | Maximum |
| Alfalfa, 5 check samples | 6.7 | 0.69 | 4.9 | 9.2 |
| Grass, 1 check sample | 3.9 | 0.99 | 2.8 | 6.1 |
| Corn Silage, 2 check samples | 2.7 | 1.44 | 1.6 | 7.8 |
| [1] Calculated from 18 to 22 participating laboratories | | | | |

# Value of reduced lignin

- Improved forage quality
- Wider harvest window?
- Later harvest
  - Greater tonnage per cutting
  - Make use of full growing season
  - Reduce number of cuttings
    - 15 to 18% lignin reduction harvest 8 to 10 days later

# How does lignin/digestibility of forage change?

- Less and/or different lignin in stem
  - Genetic effect
  - Environmental effect
    - Less sunlight (cloudy days) reduces lignin content
    - Cooler temperature reduces lignin content
- More leaves
  - Favorable leaf growth environment
  - Less leaf disease
  - Reduce harvesting leaf loss