Getting More from Forages

Targeted plant modifications:

Redesigning forages

Breaking down cell walls via lignin modification

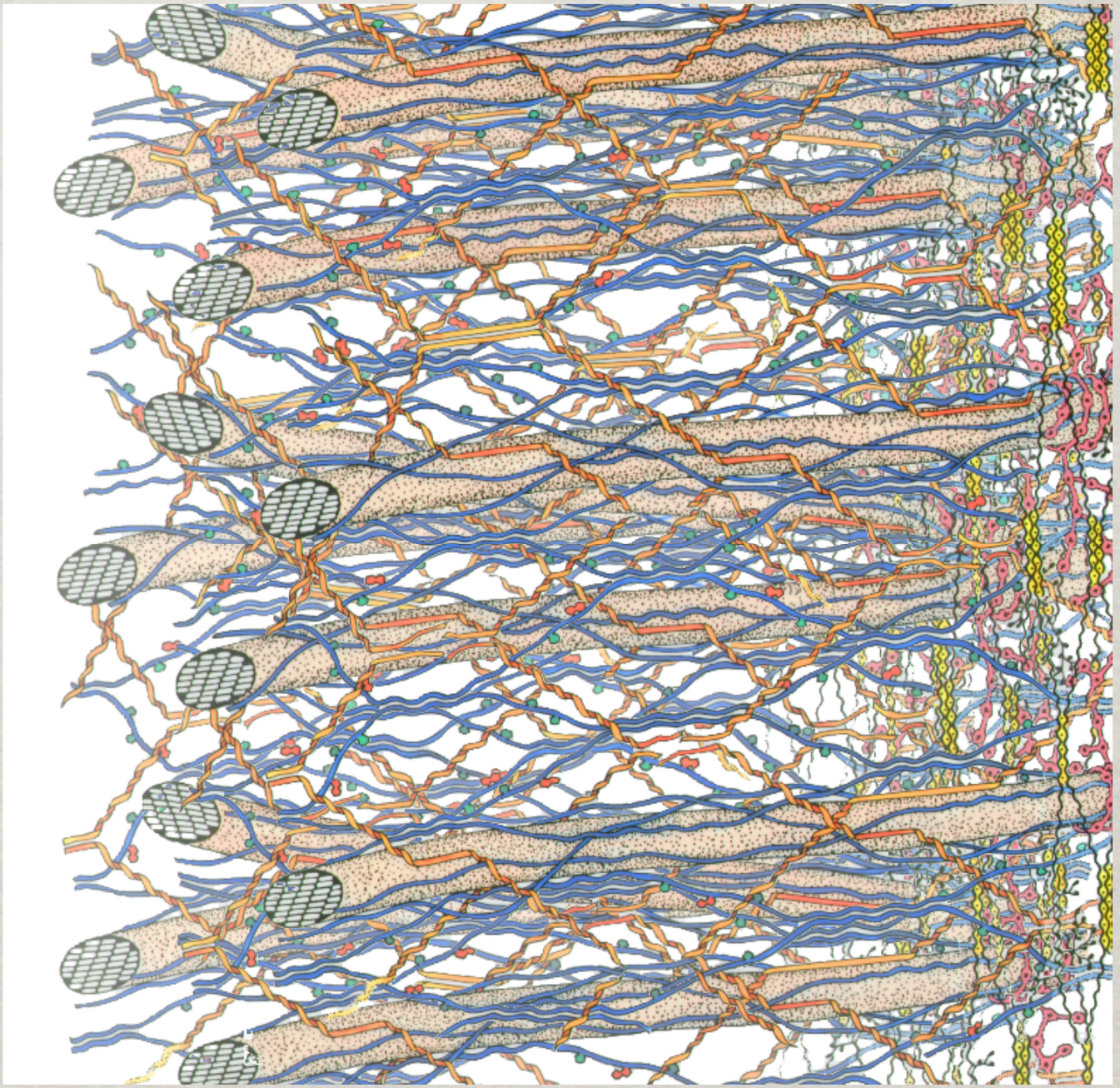
Ronald Hatfield

Breaking Down Cell Walls via Lignin Modification

Ron Hatfield, John Grabber, Heathcliffe Riday
US Dairy Forage Research Center

Cell Wall Formation

Complex Process..... coordinated to maintain strength and growth

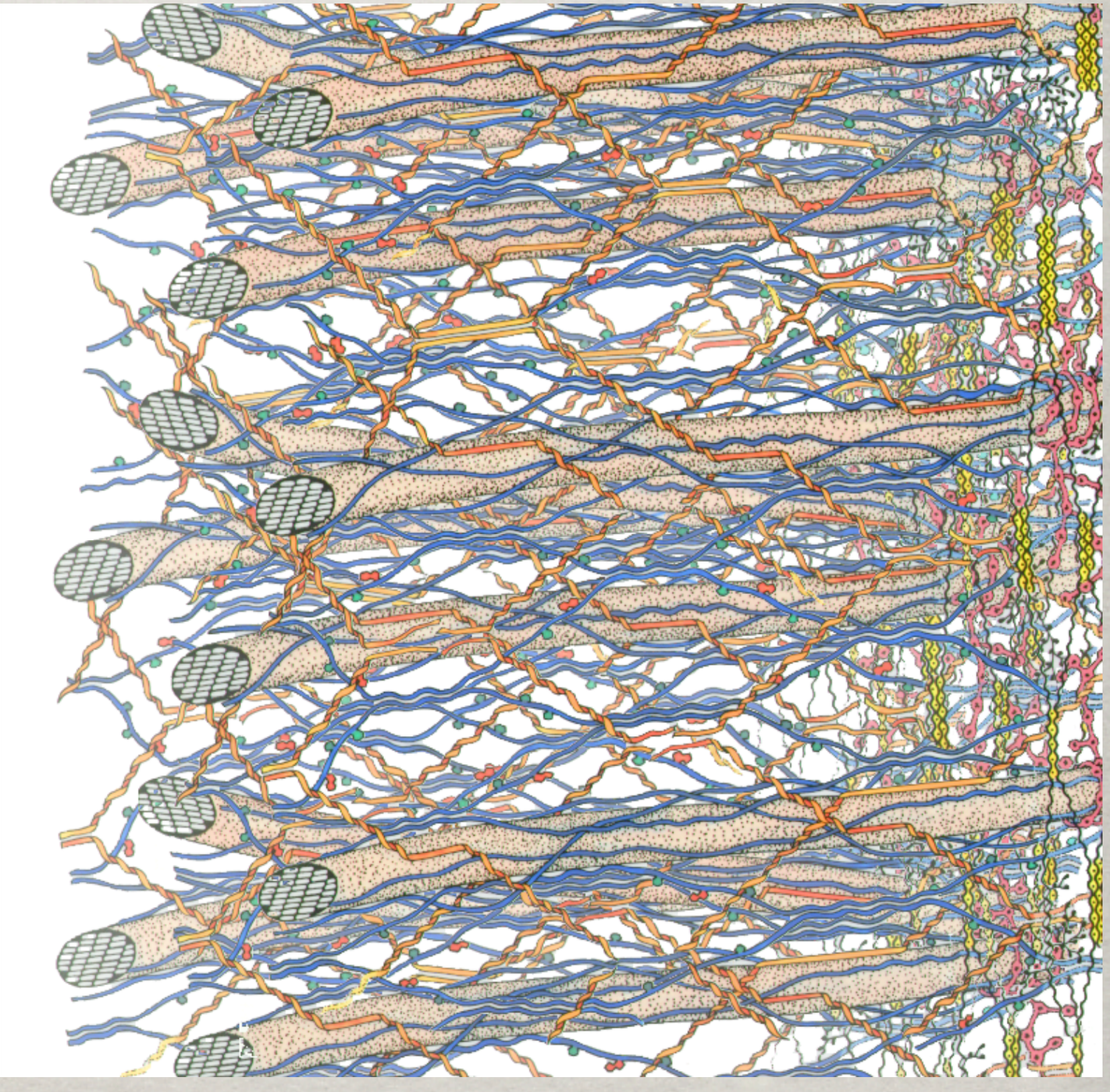


N. C. Carpita and D. M. Gibeaut, "Structural models of primary cell walls in flowering plants:..." Plant J. **3**(1), 1-30, 1993

Cell Wall

Complex matrix....

carbohydrates, protein, phenolics (including lignin)

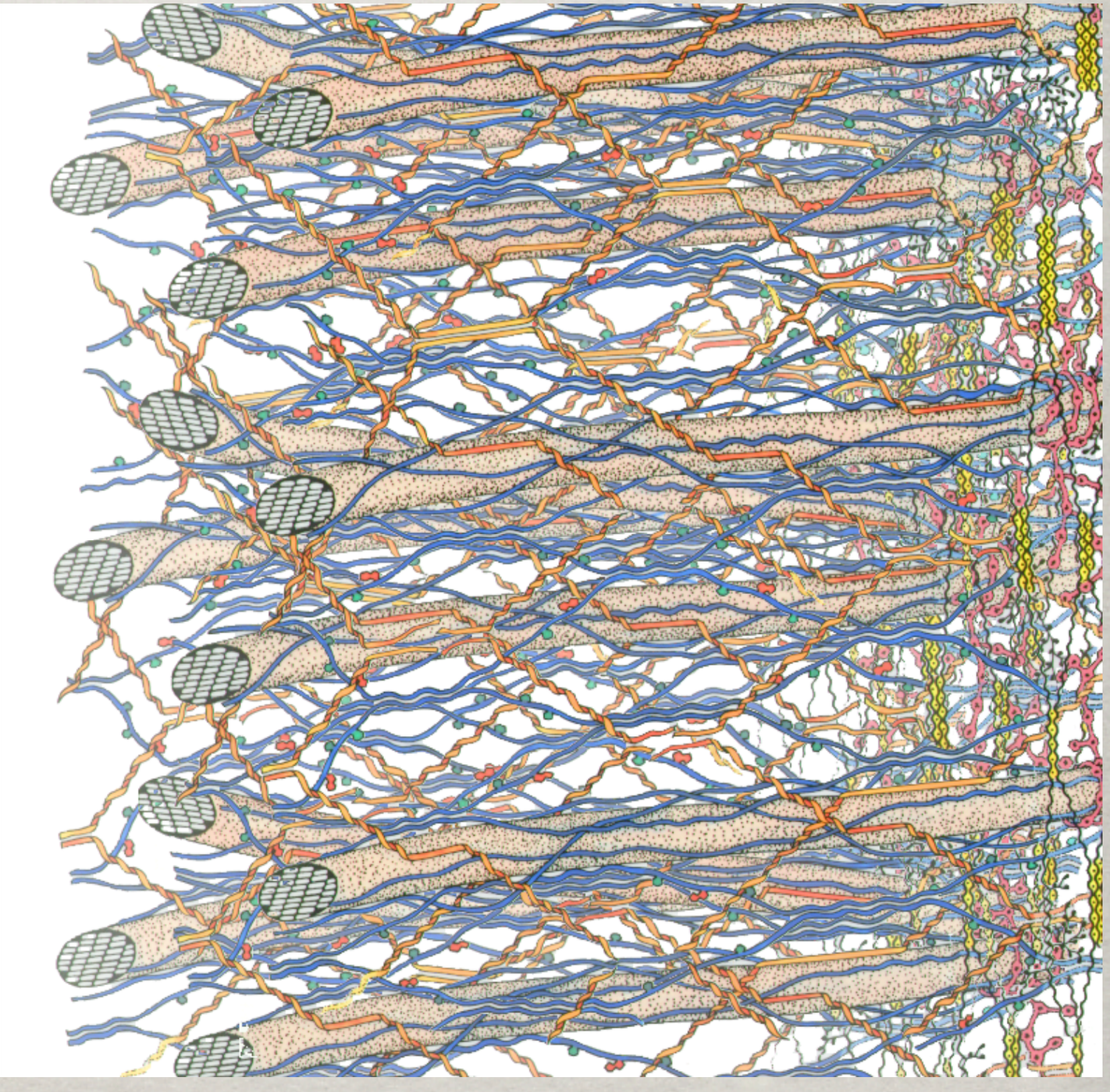


Cell Wall

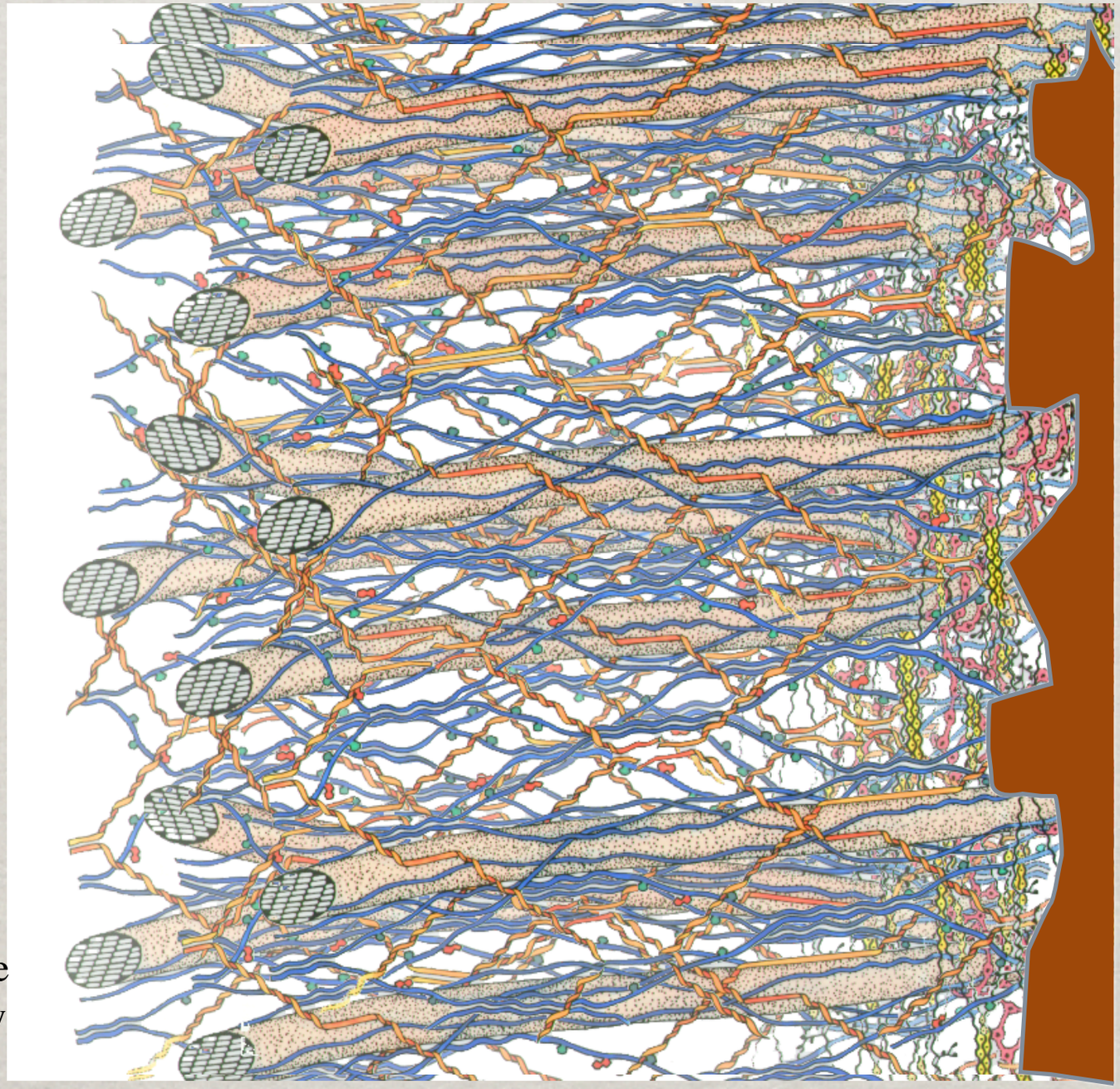
Limitations to degradation

cross-linking

lignin



Lignification

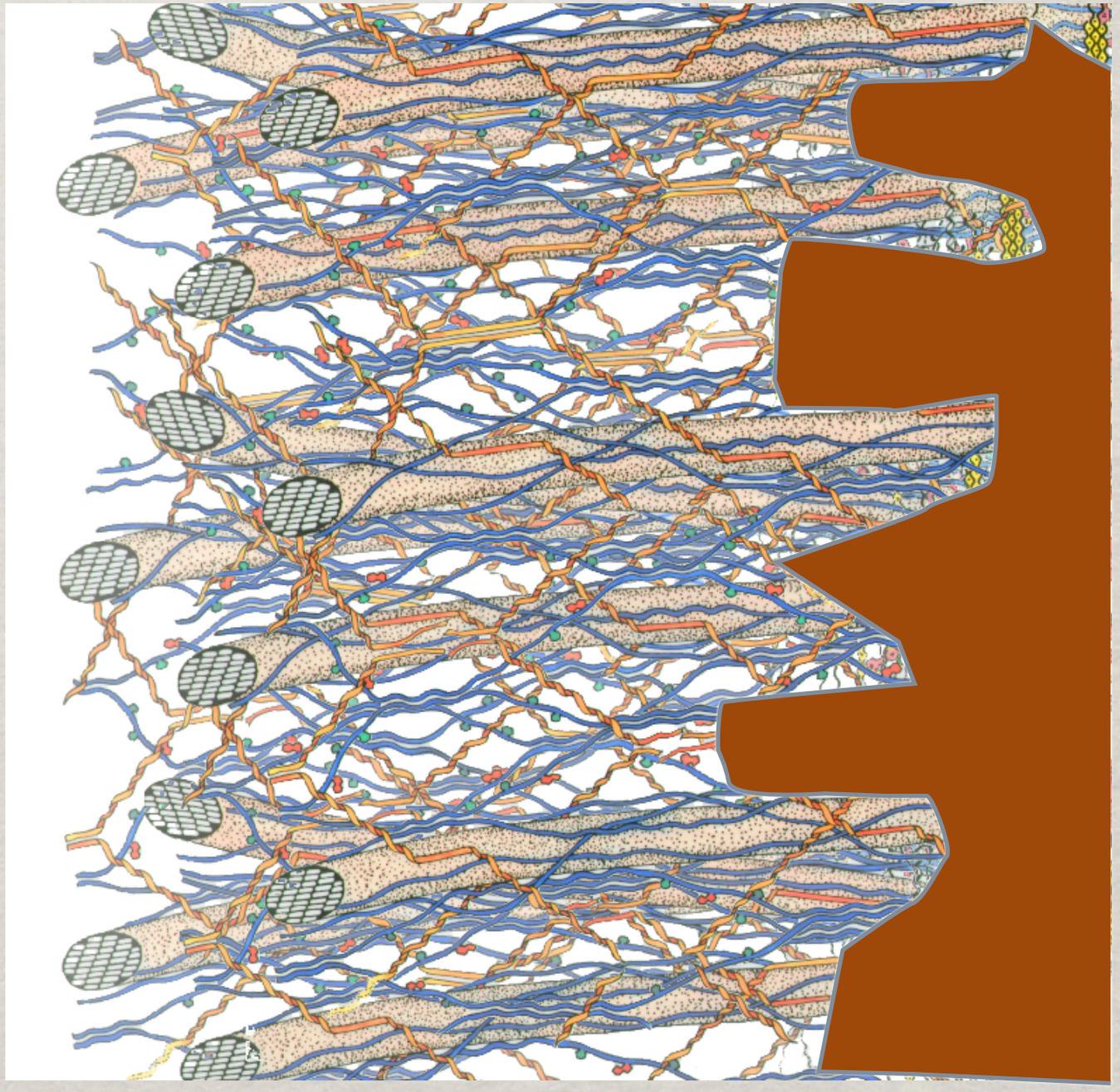


N. C. Carpita and D. M. Gibe
"Structural models of primary
walls in flowering plants:..."
Plant J. **3**(1), 1-30, 1993

Lignification

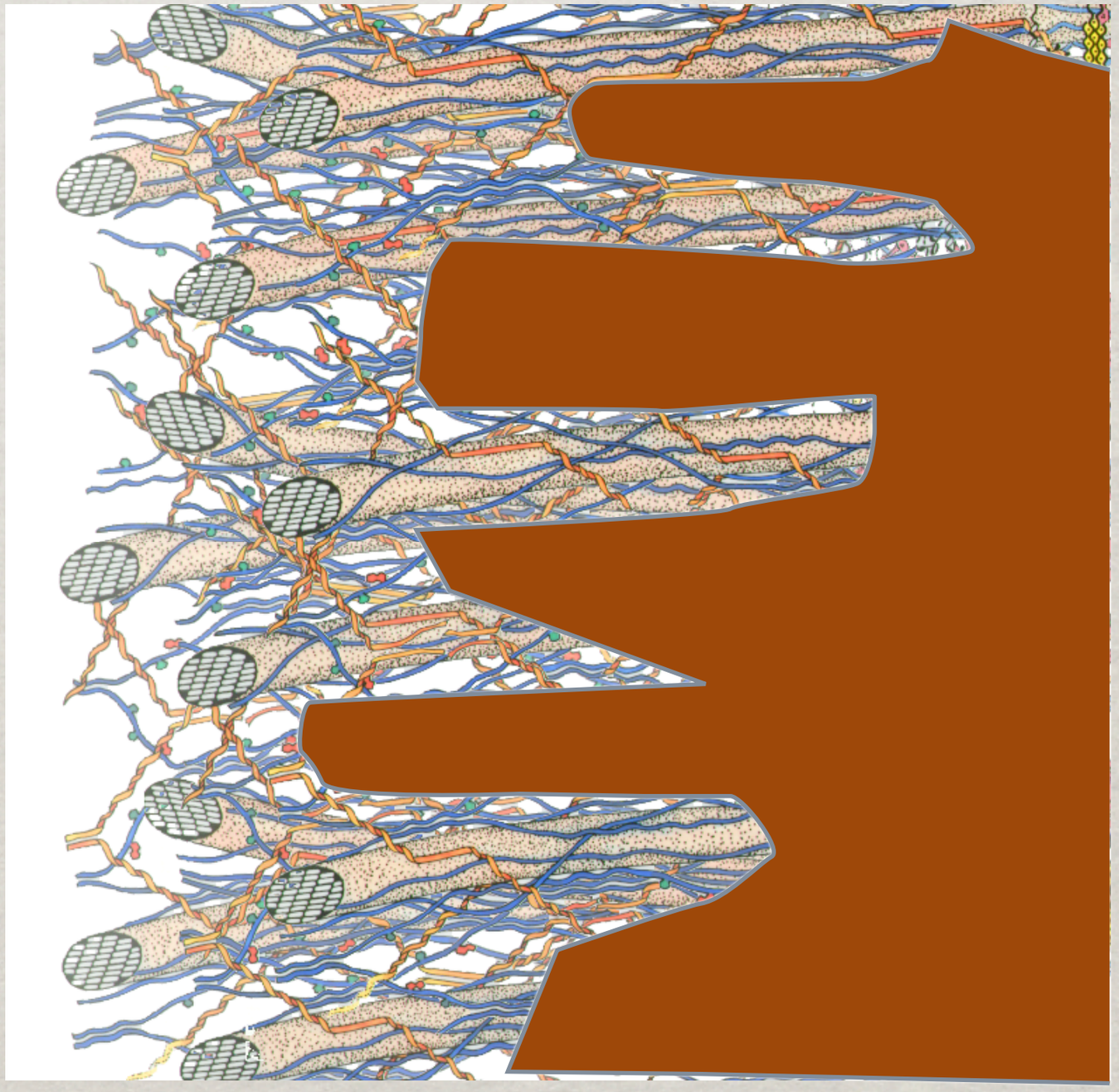
Lignin fills in.....

Water is excluded



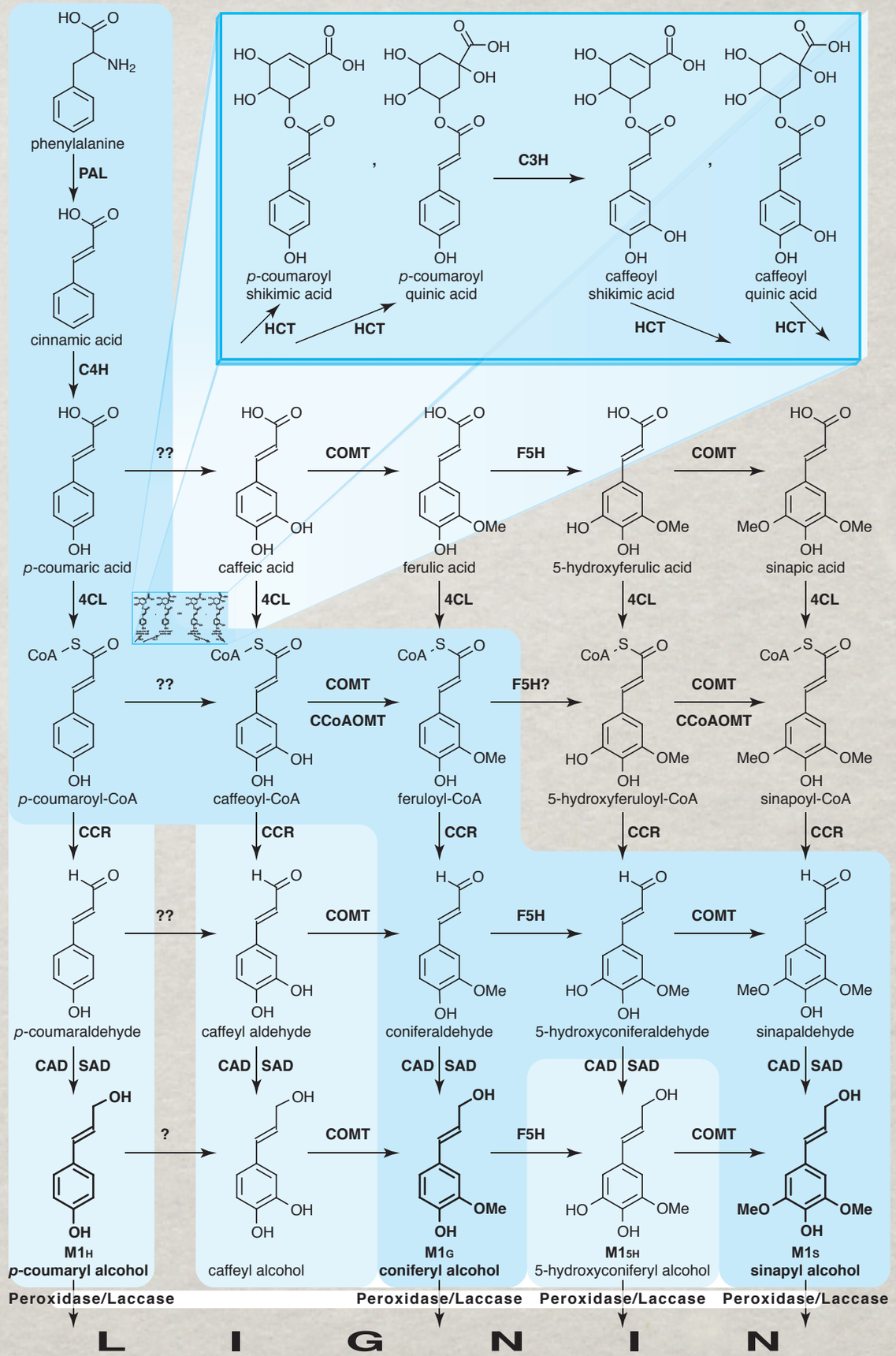
Lignification

In some cell walls all free space is taken up by lignin



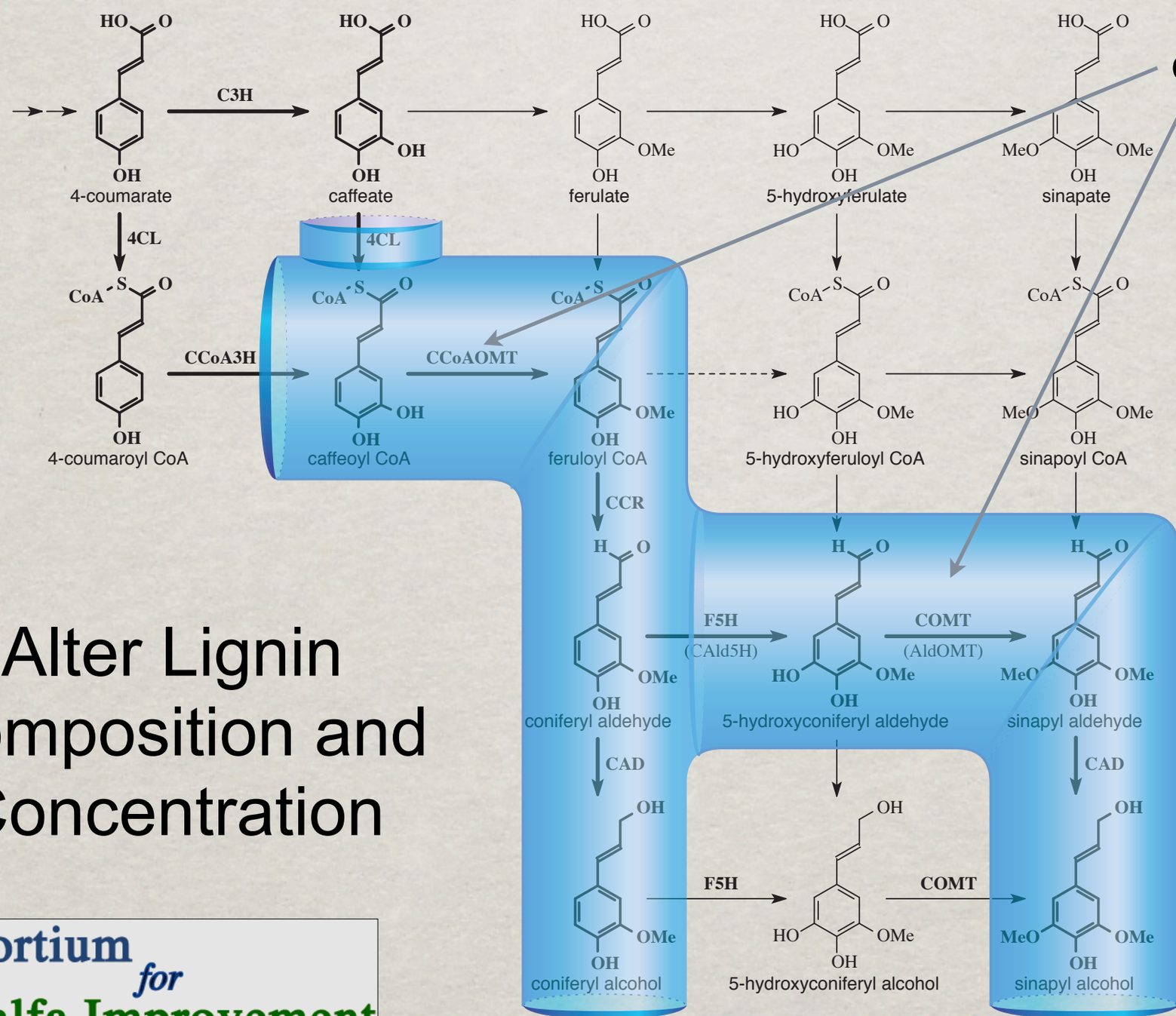
Formation of lignin building blocks

Complex array of enzymes.... coordinated in the formation of monolignols (building blocks)



Modulating Gene Activity

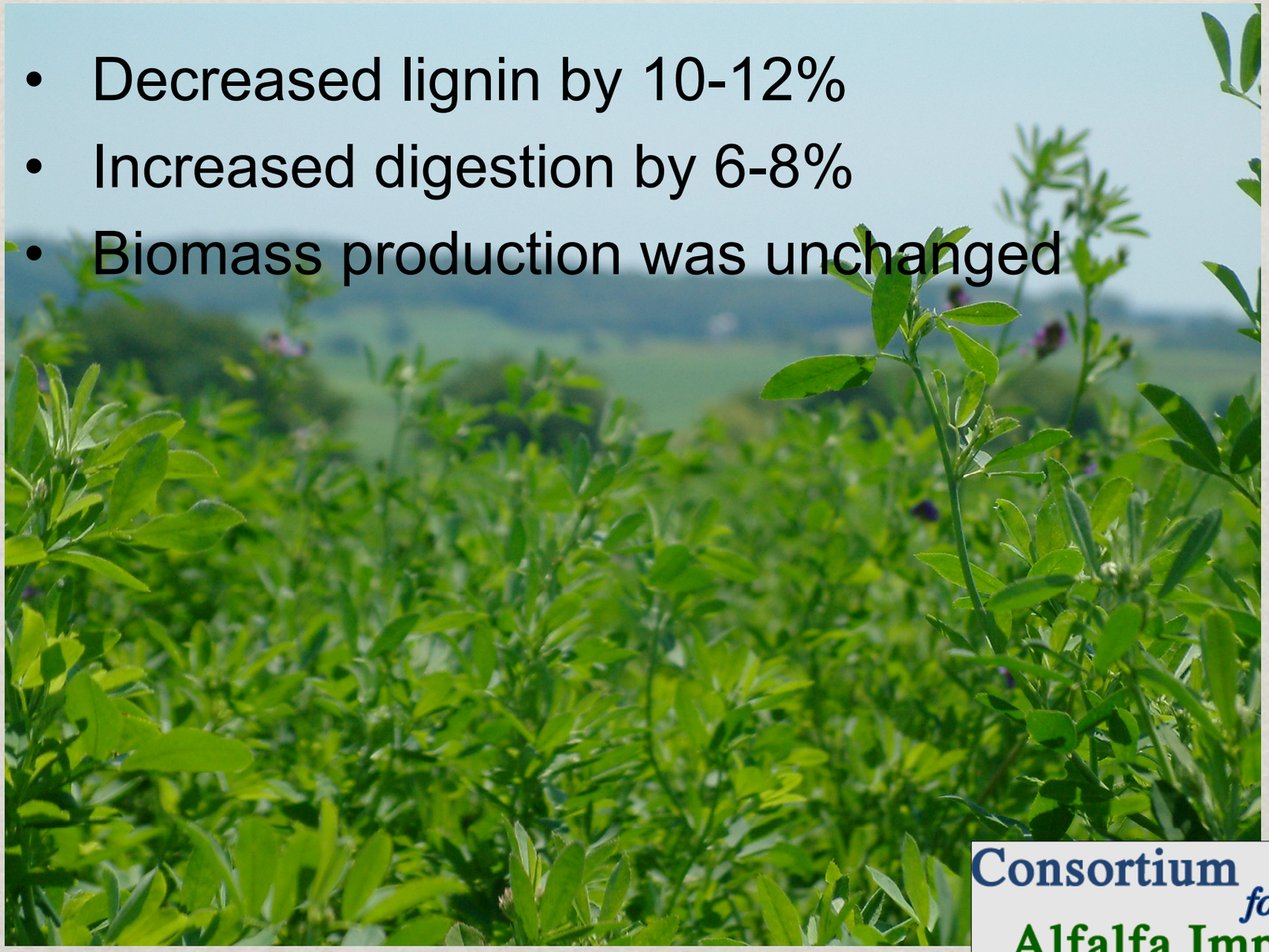
Alter gene expression to decrease enzyme activity



Alter Lignin Composition and Concentration

Consortium *for* Alfalfa Improvement

Impact on Lignin



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

Consortium *for* Alfalfa Improvement

Improved Digestibility in Lambs

% DM				
Alfalfa Hay in diet	aNDF	ADL	NDFD	DMD
COMT	38.2	5.3	57.5*	67.5*
Null COMT	39.0	5.8	49.1	64.5
CCOMT	39.4	5.2	50.1*	65.3*
Null CCOMT	39.4	5.9	46.4	63.6
* Significant P<0.05				

Diet was 100% alfalfa hay

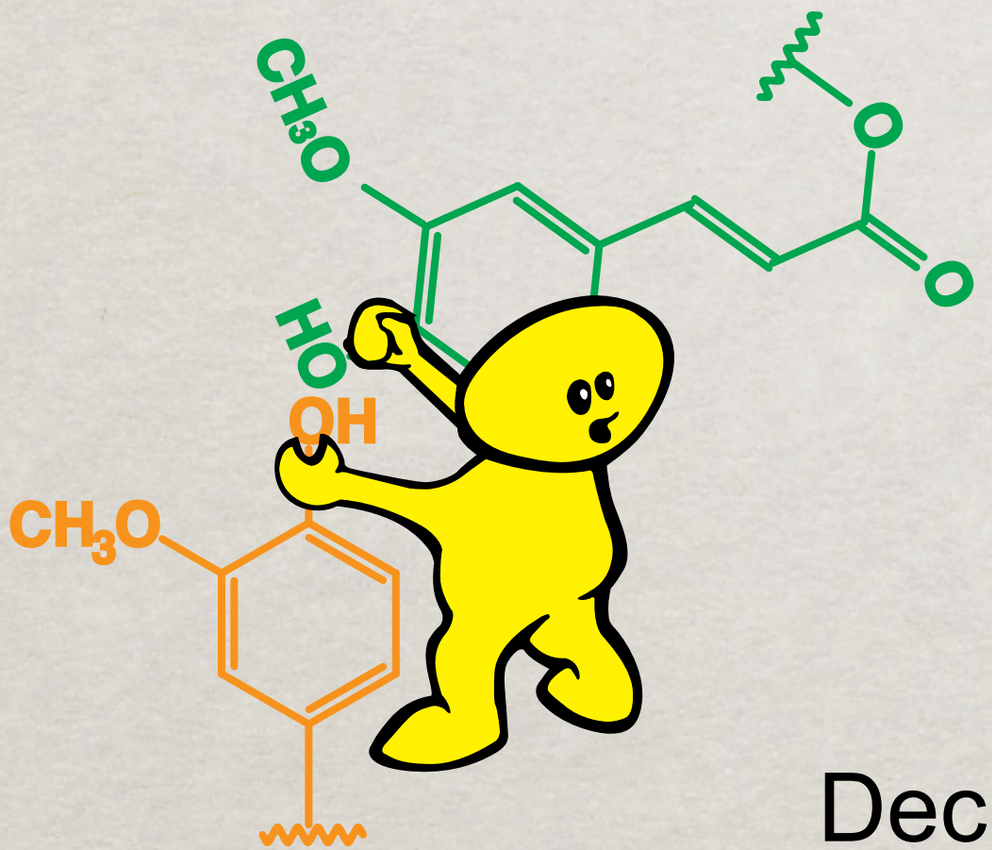
SOURCE: Mertens et al. 2008. J. Dairy Sci. Supple. 1

Consortium *for* Alfalfa Improvement

What Are Other Possibilities?

Can more drastic changes be made in lignin without compromising biomass production?

Radical Coupling of Phenolic

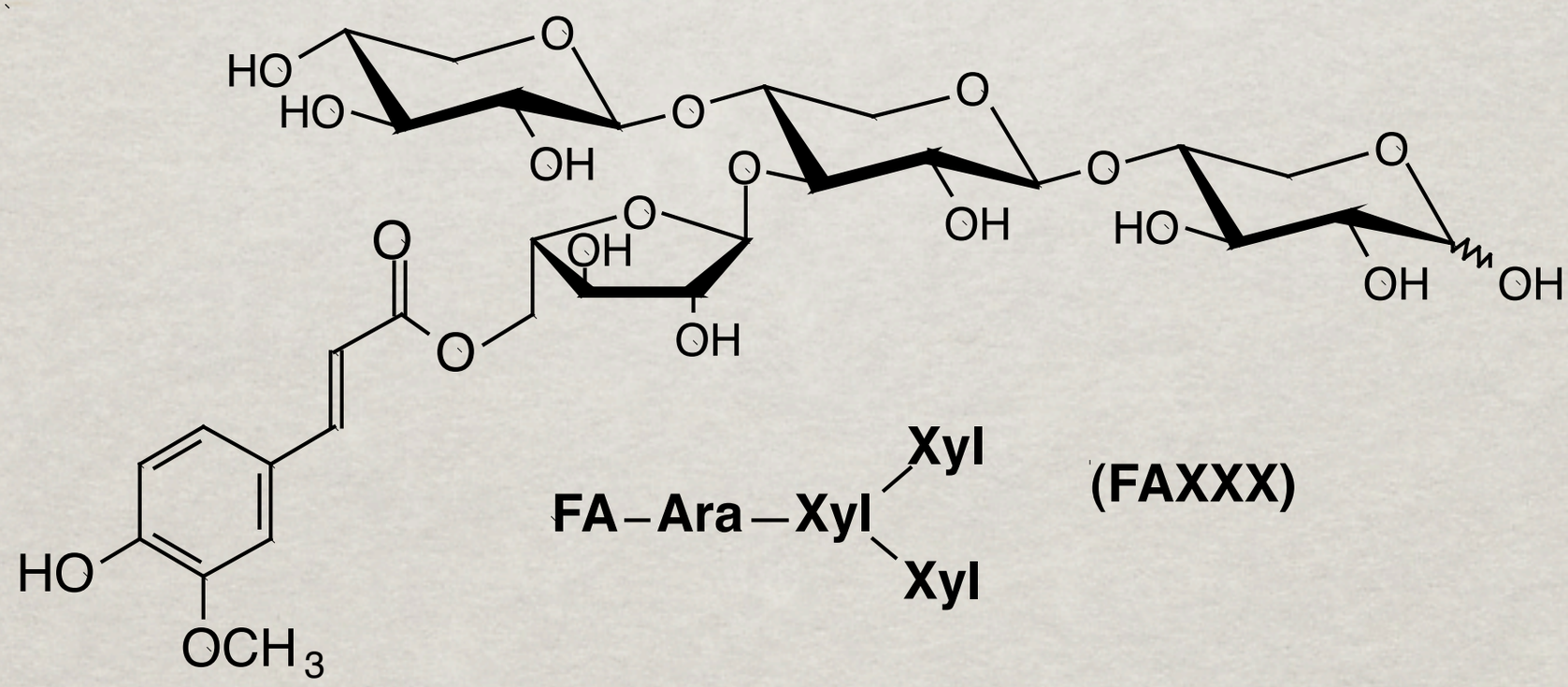


Ferulates and lignin monomers

Decrease cross-linking!!

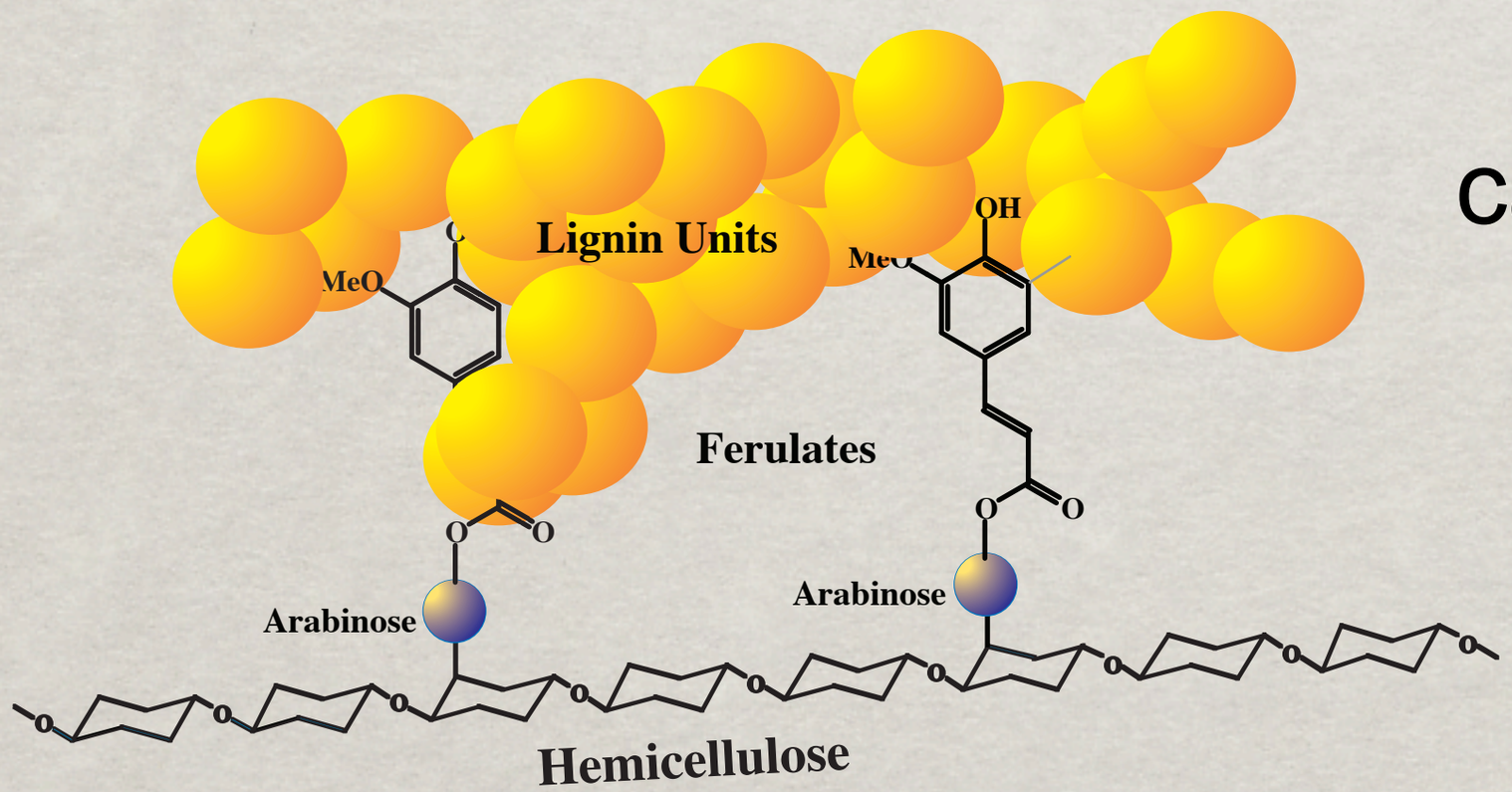
1.) Decrease Cross-linking

Ferulates attached to xylans in grasses

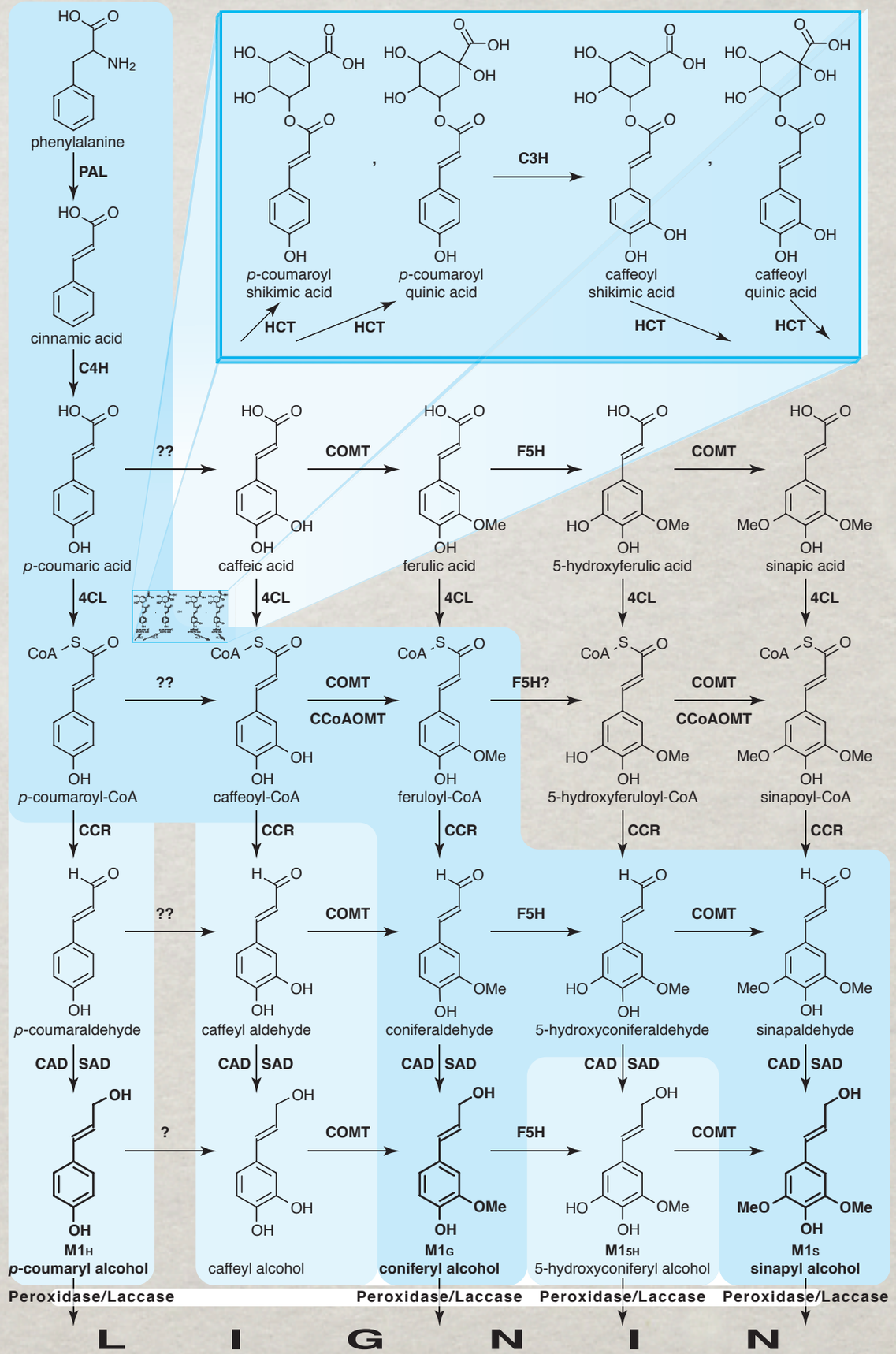


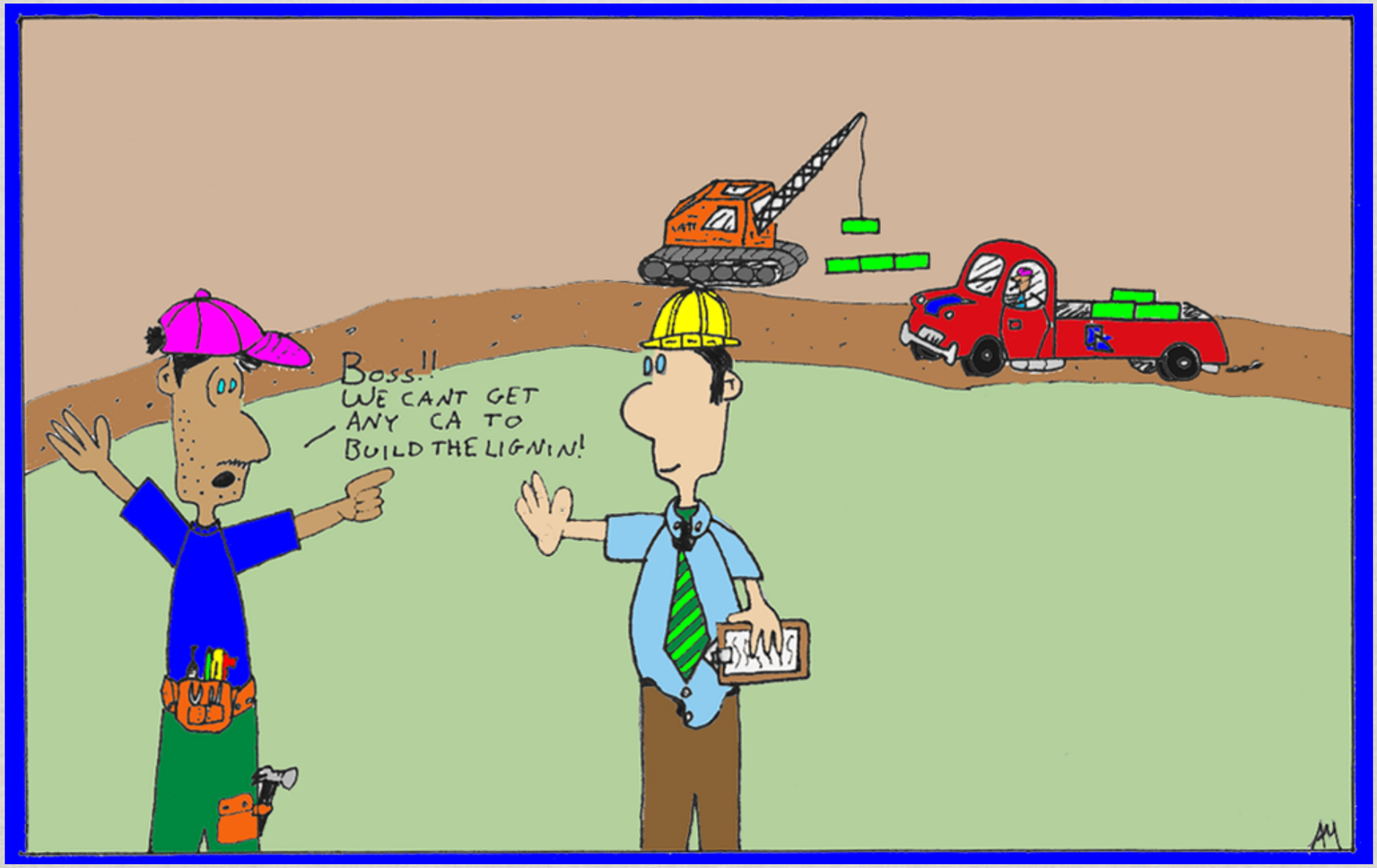
Ferulates Become Incorporated Into Lignin!

Form cross-links between lignin and carbohydrates



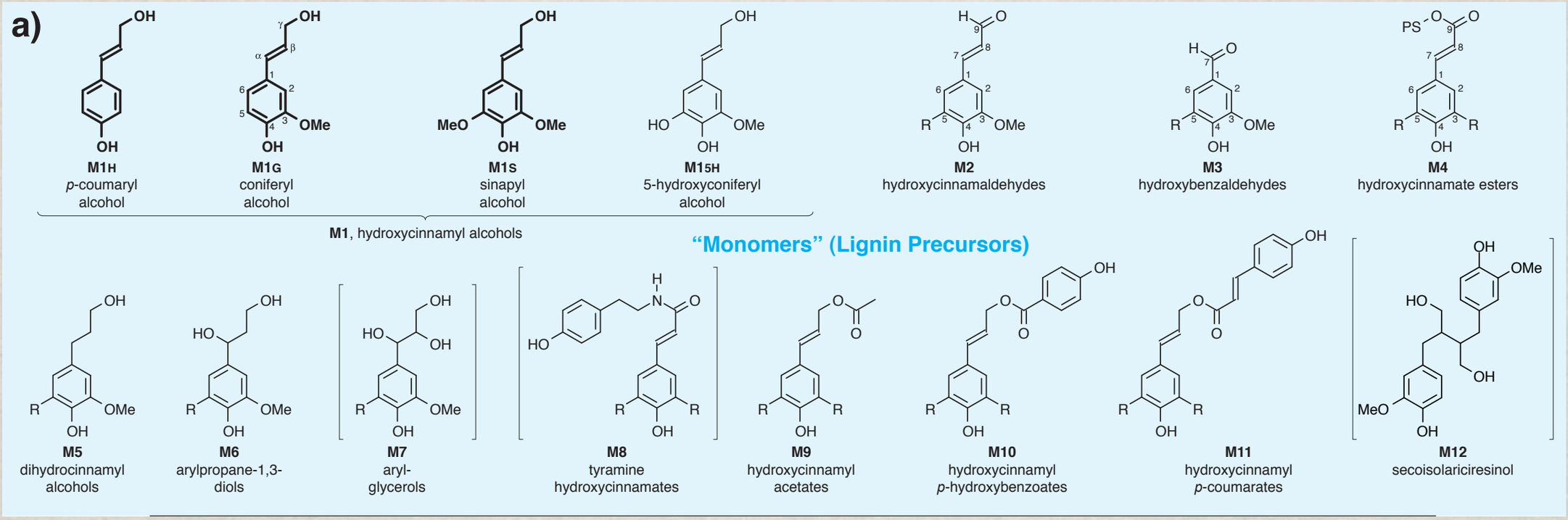
2.) Alter the types of building blocks that make up lignin




Boss!!
WE CANT GET
ANY CA TO
BUILD THE LIGNIN!

PHEW!!
NO PROBLEM! PROCESSING CAN JUST CRANK UP PRODUCTION OF SOME OTHER PHENOL!
AM

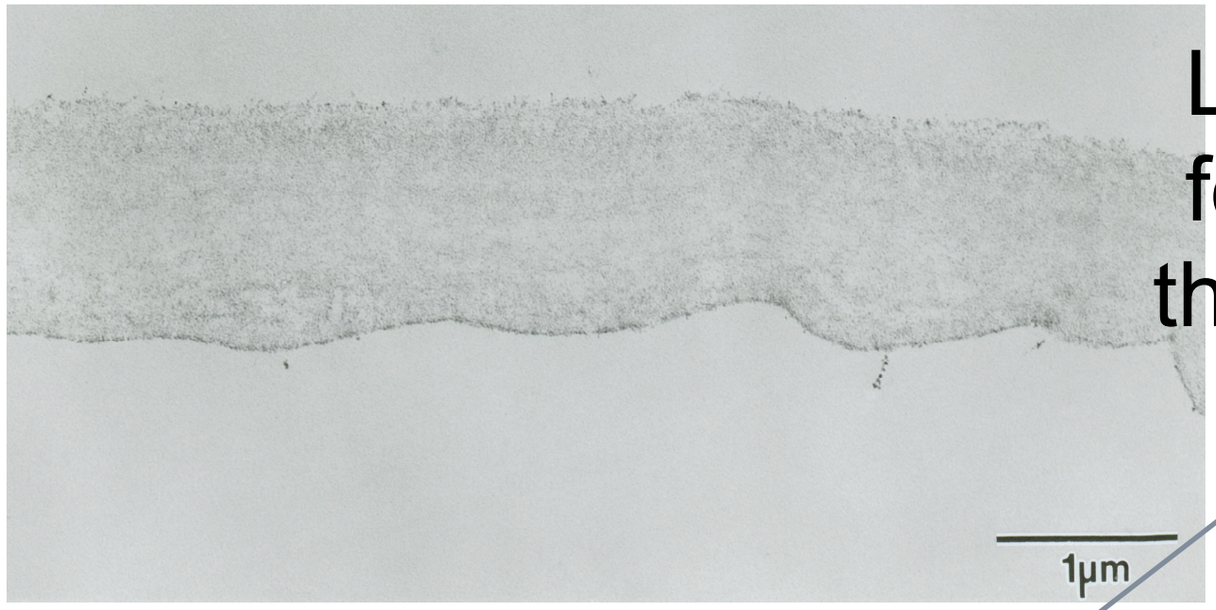
Phenolic Units That Can Be Incorporated Into Lignin



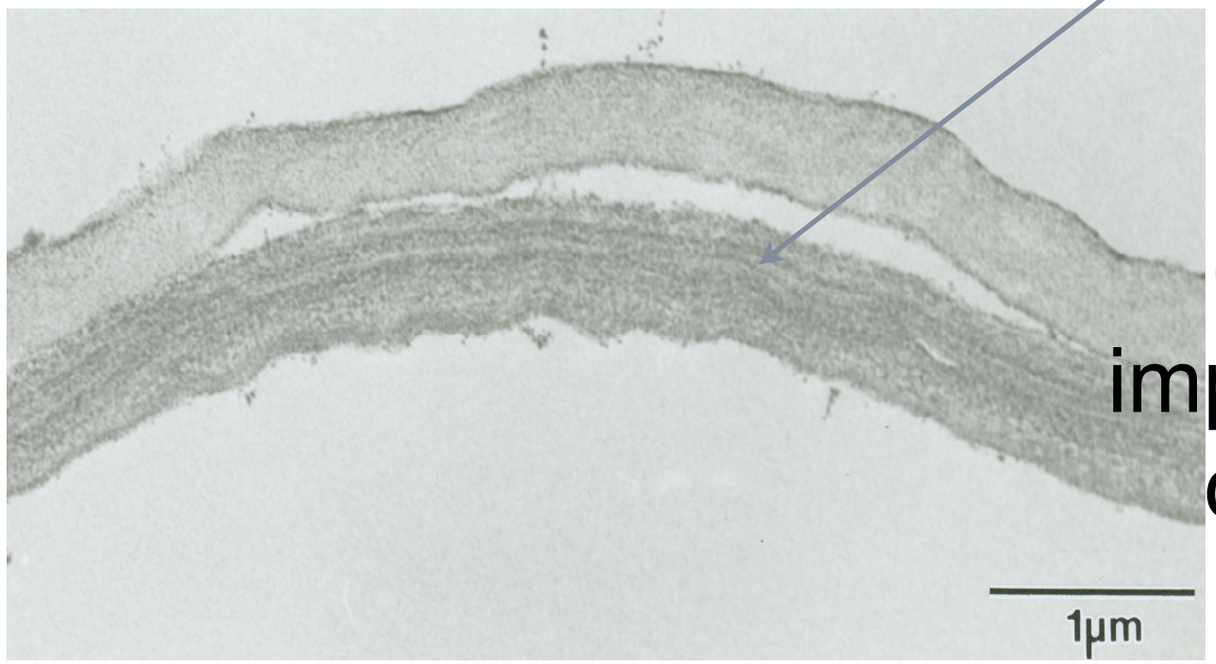
Cell Wall Model System

Test coniferyl ferulate impacts with a cell wall model system

Artificial Lignification

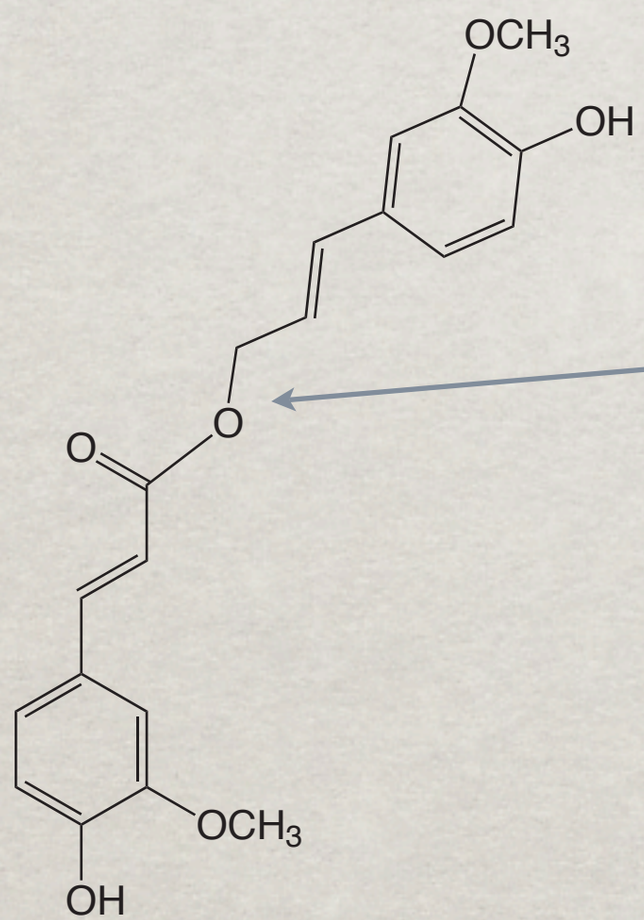


Lignin can be formed within the wall matrix.



Can test the impact upon wall degradation.

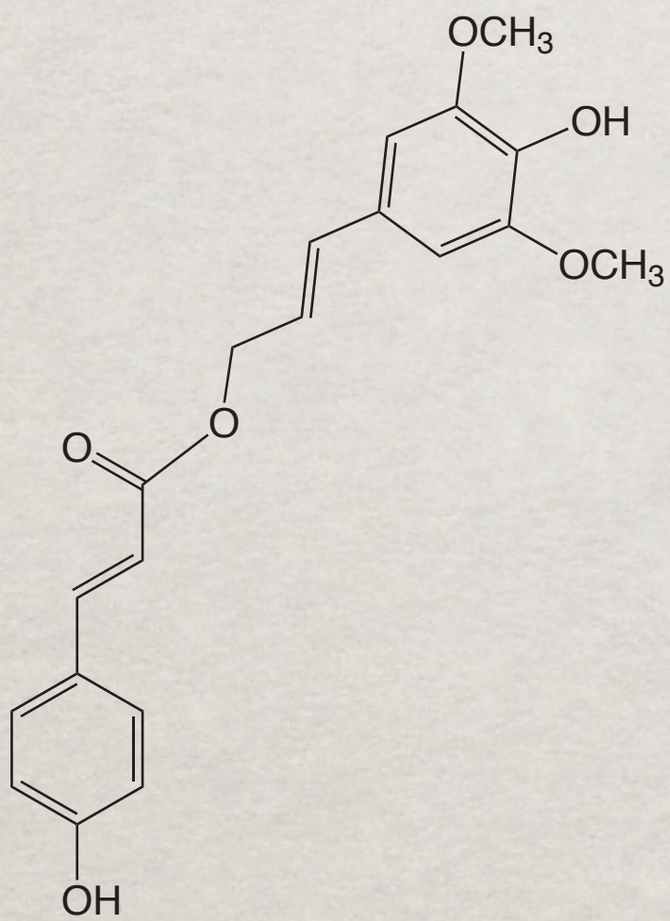
Insert Phenolic Conjugates With Easily Broken Bonds



Ester linkage

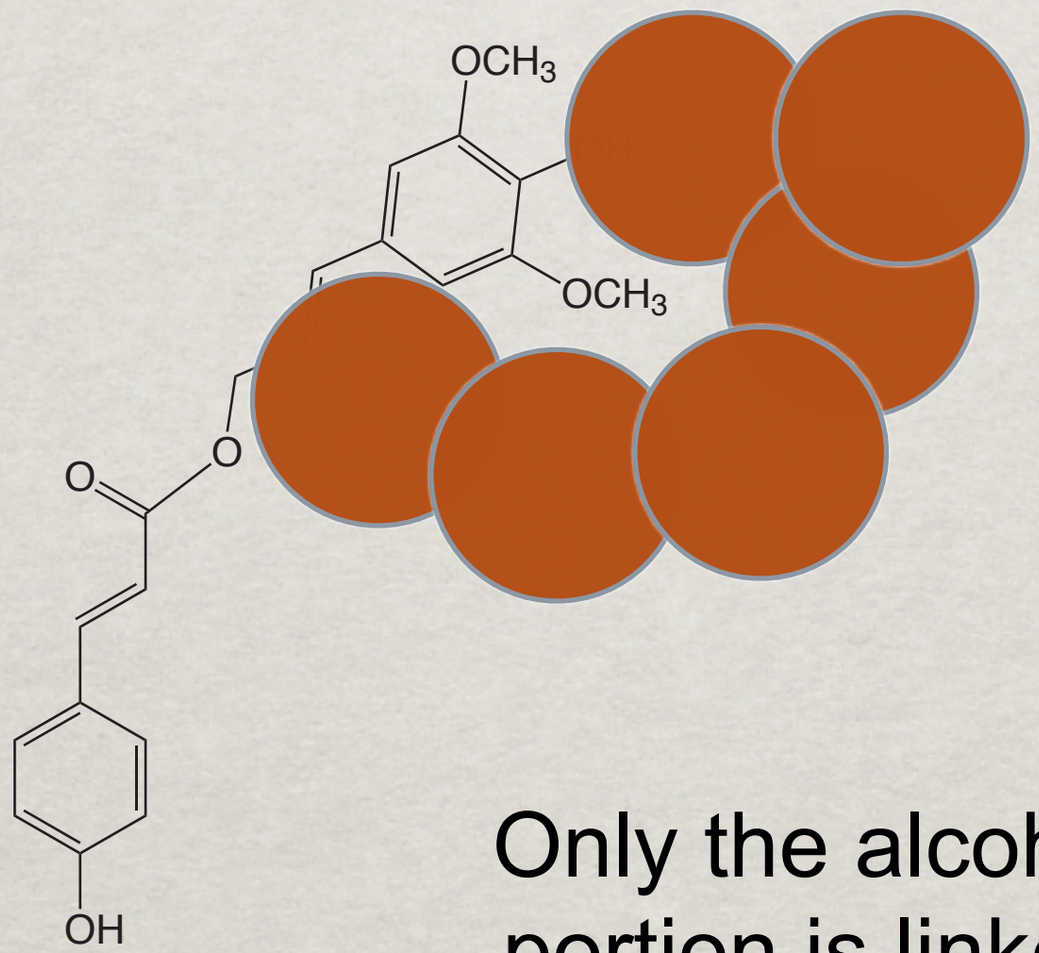
Easily broken under mild conditions

pCA-Sinapyl Alcohol

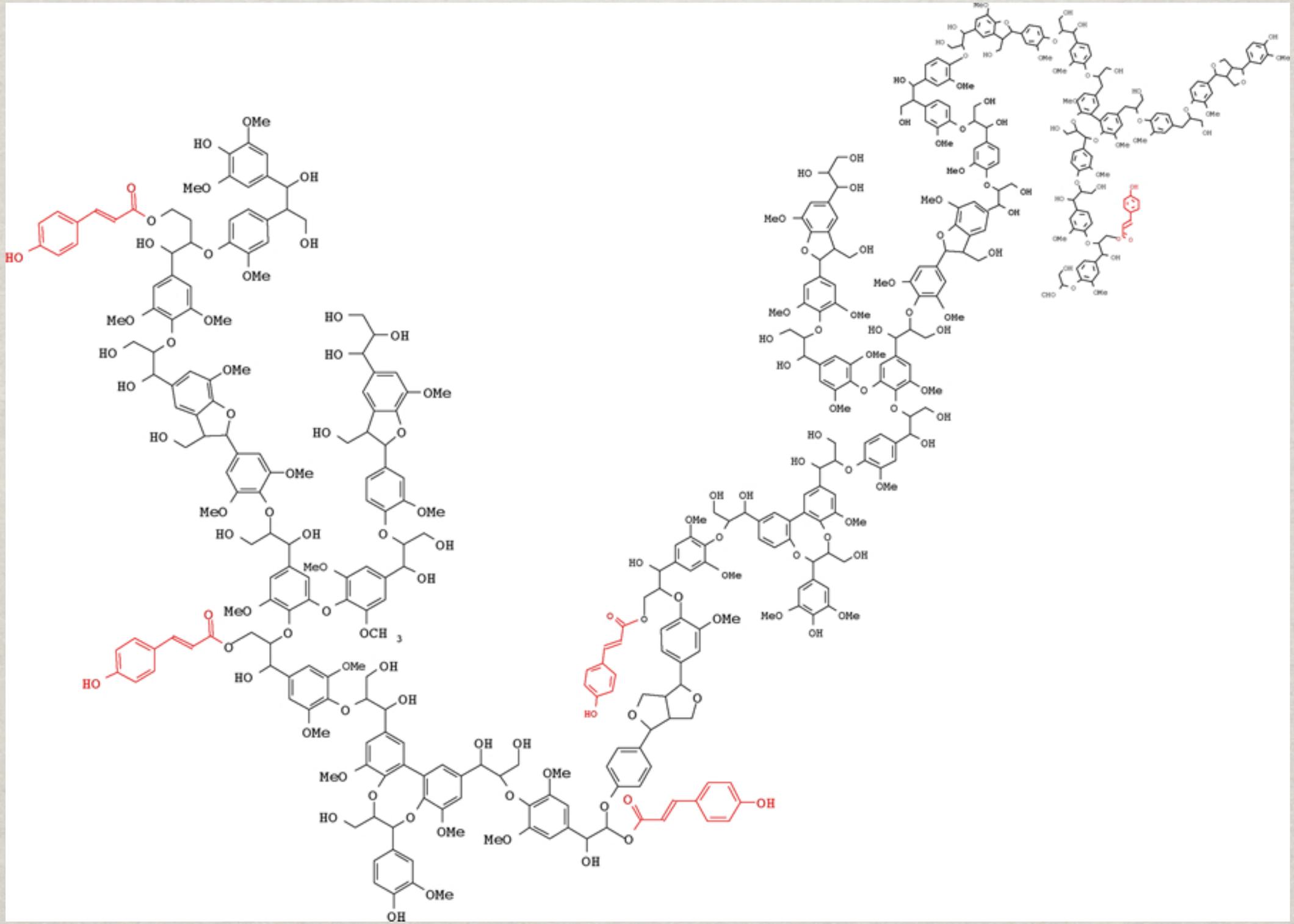


Readily incorporated into grass lignin

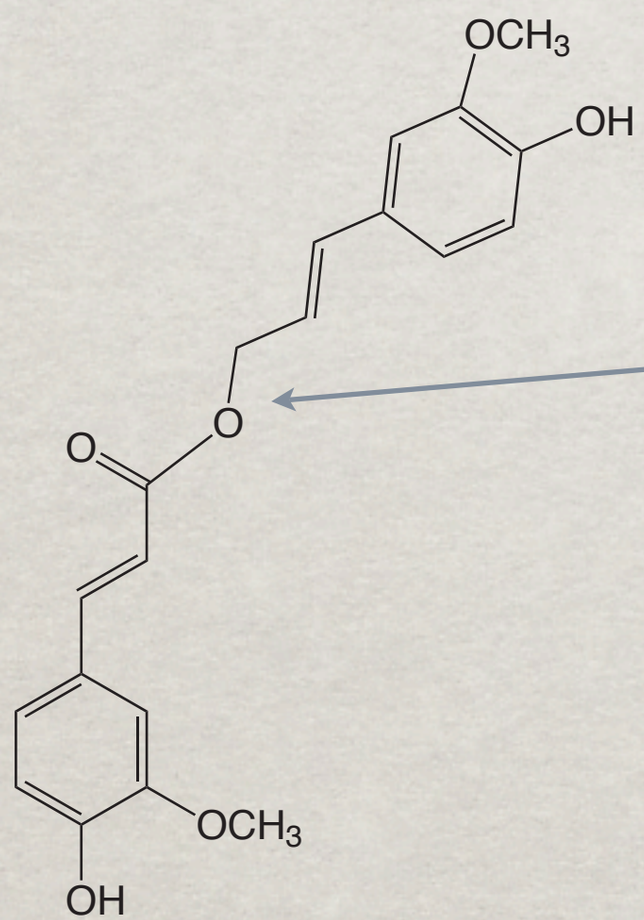
pCA-Sinapyl Alcohol



Only the alcohol portion is linked into grass lignin



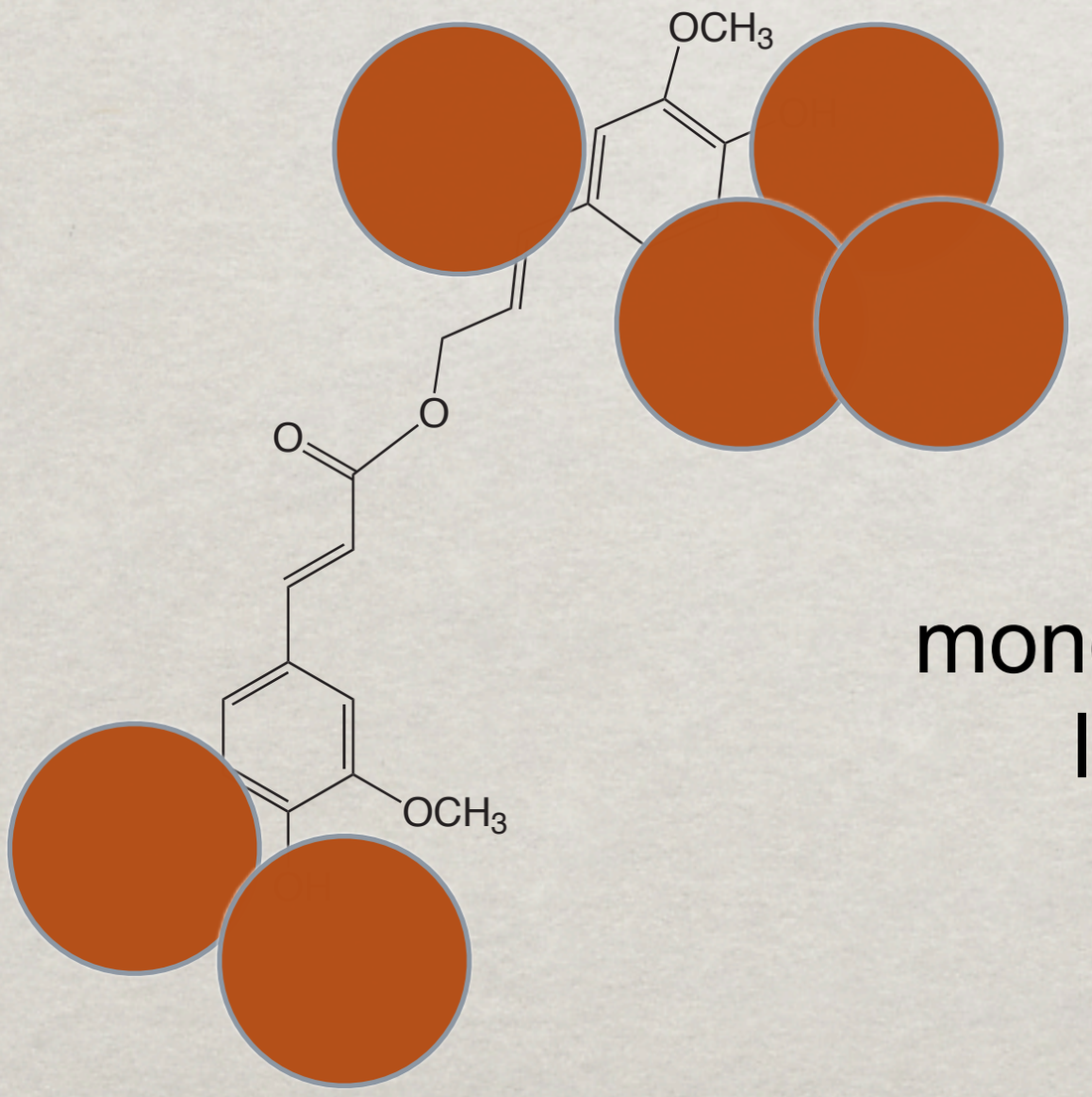
Insert Phenolic Conjugates With Both Ends Incorporated



Ester linkage

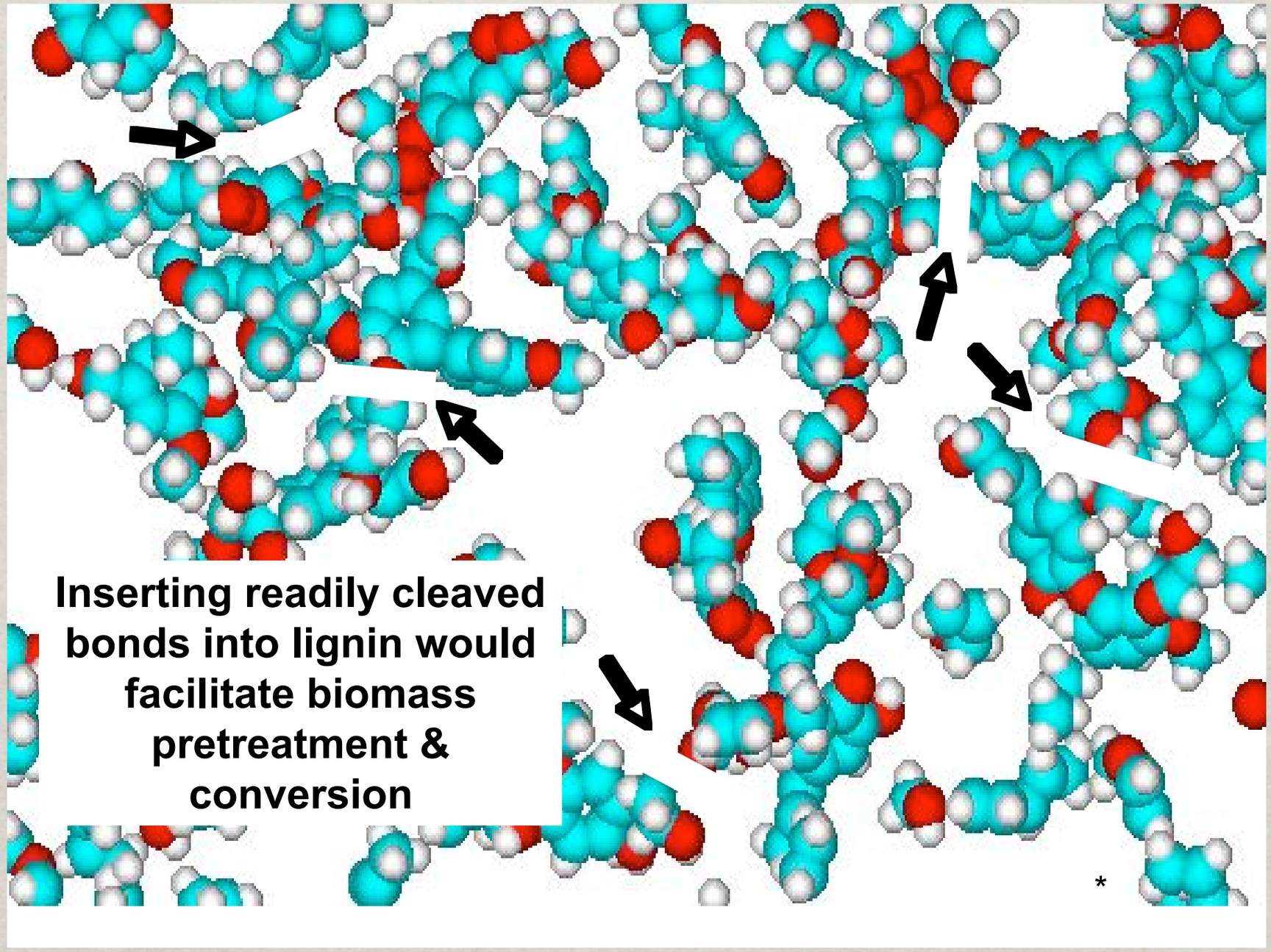
Easily broken under mild conditions

Insert Phenolic Conjugates With Both

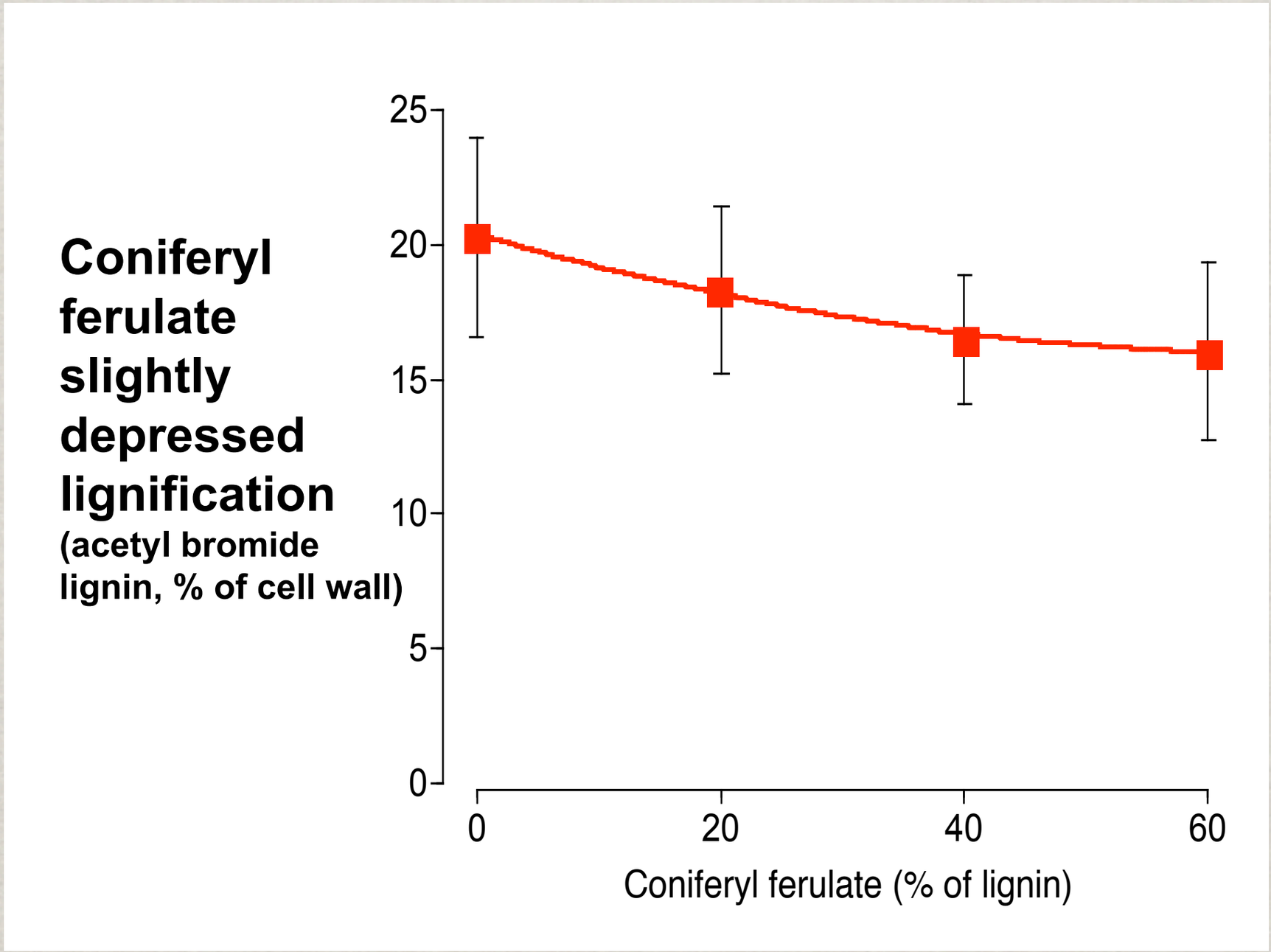


Addition of lignin monomers forms a normal lignin....in terms of structure

Mild Alkaline Conditions Breaks Ester Bonds

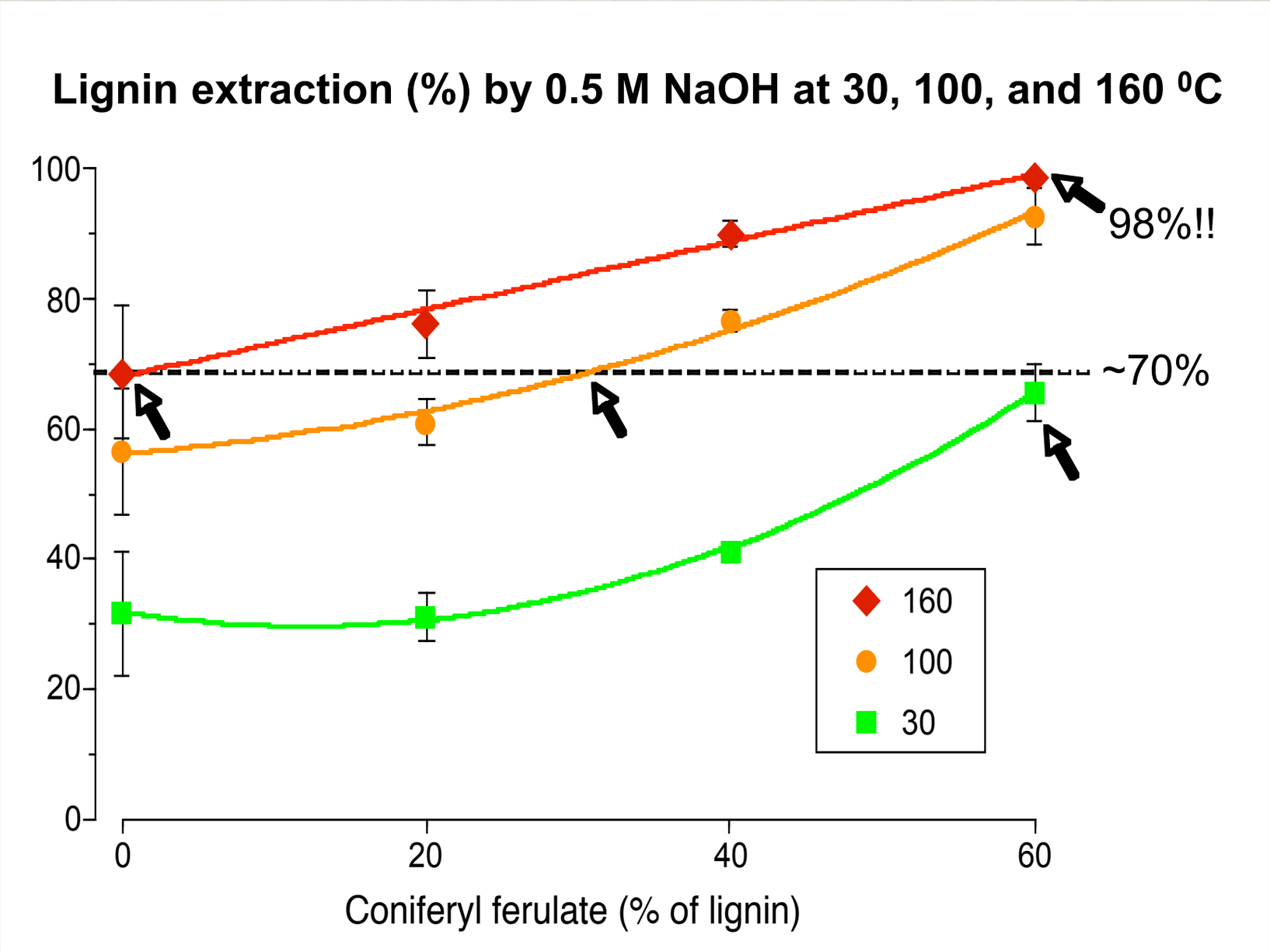


Incorporation Into Lignin



Impact Upon Lignin Removal

Ferulate-coniferyl alcohol conjugates incorporated into lignin makes it easier to remove.

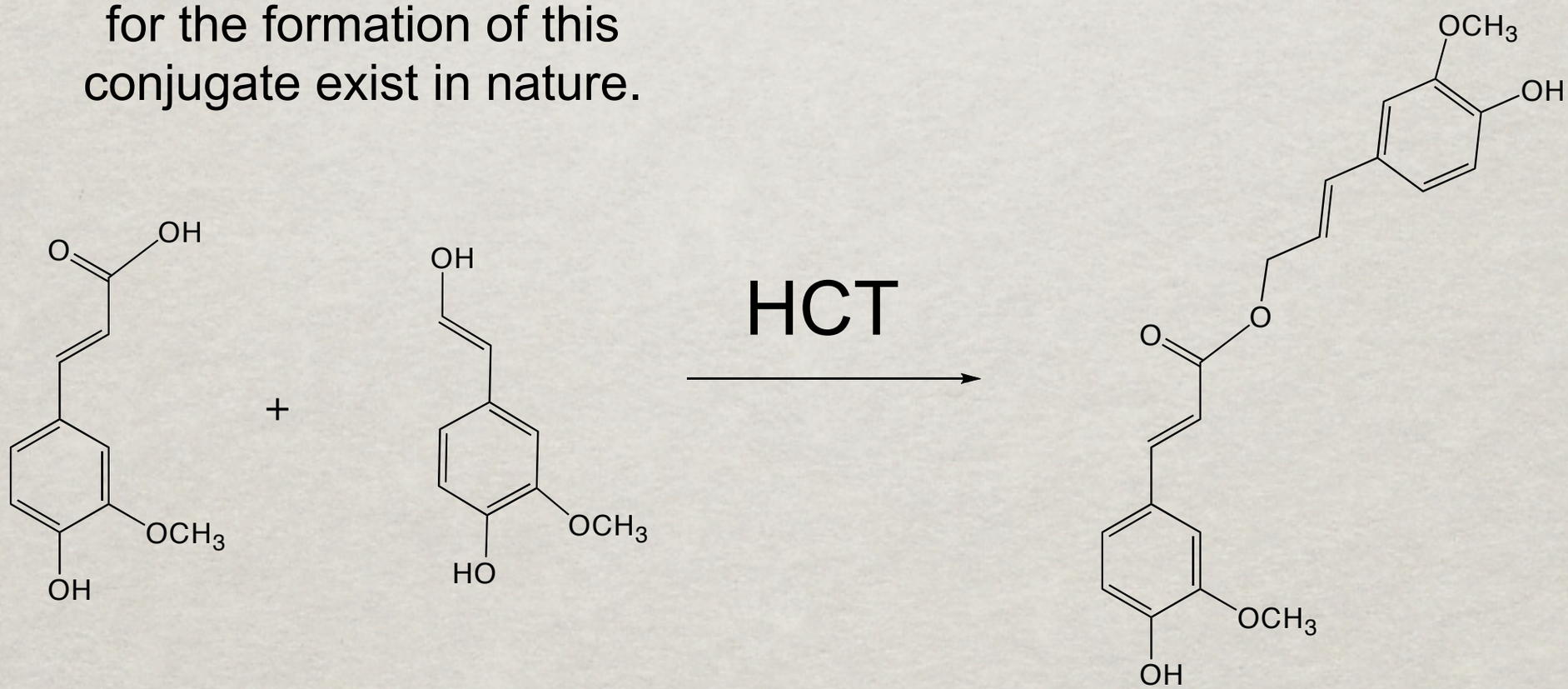


Can the Models Ever Be Reality?

Yes!!!

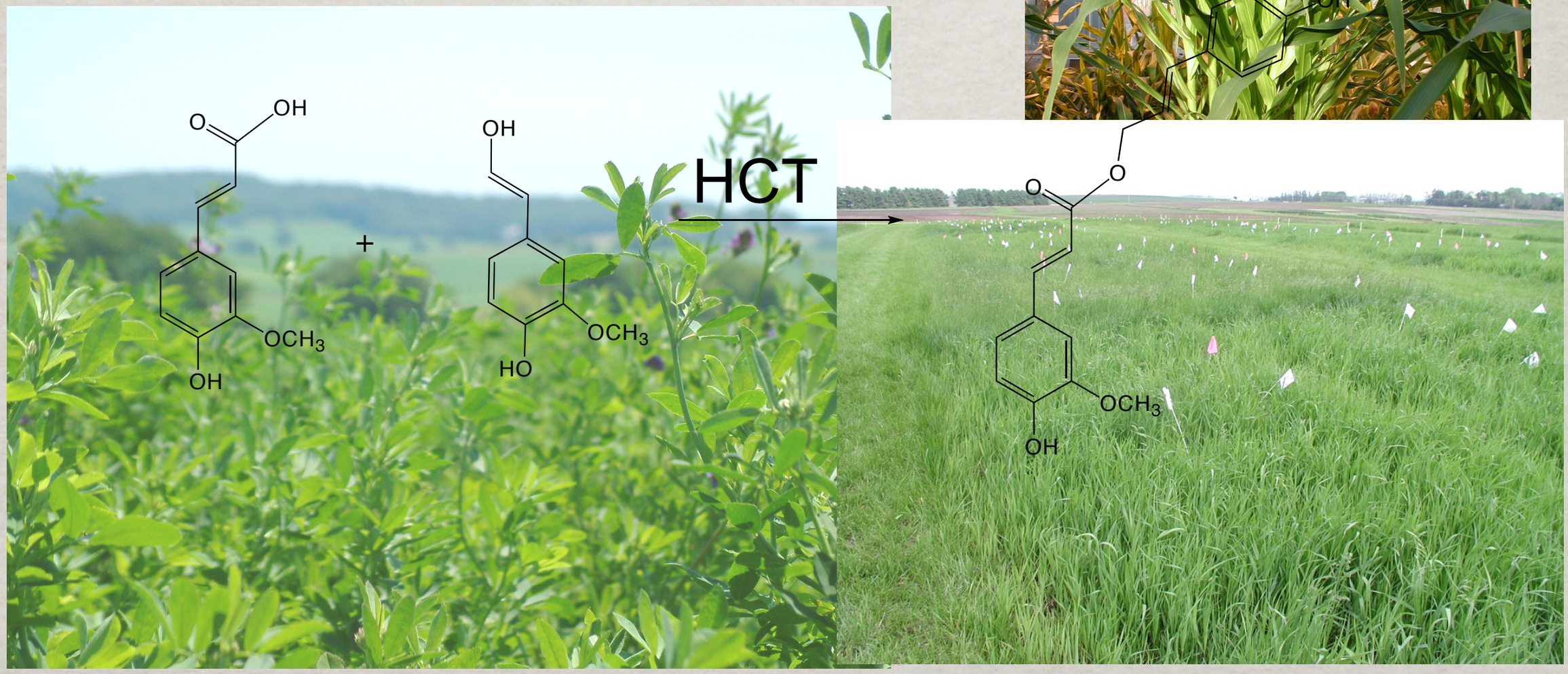
Can the Models Ever Be Reality?

Hydroxycinnamyl transferases for the formation of this conjugate exist in nature.



Can the Models Ever Be Reality?

Should be able to isolate the enzyme/gene for such hydroxycinnamyl transferases.



Summary

- 1.) Modifying lignin is a key approach to improving fiber digestibility.
- 2.) Down regulating lignin pathway enzymes ....decreases lignin increases digestion
- 3.) Decrease cross-linking will increase cell wall digestion
- 4.) Inserting “zips” into lignin should improve improving fiber digestibility.

Summary

Increasing digestibility of cell walls by 10%

$350 million increase in milk & meat
2.8 million tons less manure production
Reduce grain use by 2 million tons

We must be the change we seek in the world

Gandhi