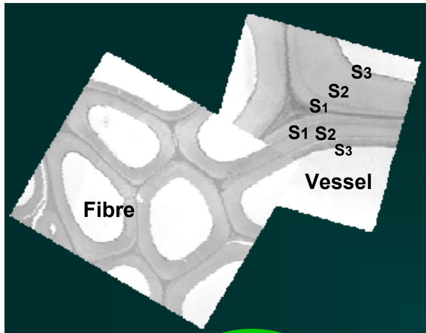
# In situ localization of matrix components

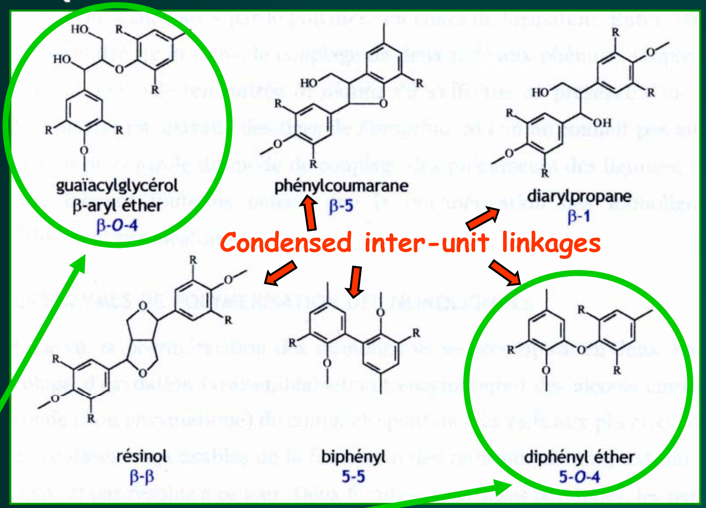
## Lignins


- Ruel et al. **J Trace Microprobe Techniques** 1994, *12:247-265*
- Joseleau and Ruel **Plant Physiol**. 1997, *114*:1123-1133
- Joseleau, ····· K. Ruel. . **CR. Acad. Sci.** Paris, 2004, *327*: 809-816
- Joseleau et al. Planta, 2004, *219:*338-345
- Joseleau and Ruel in New Knowledge in Wood Quality, K. Entwistle & J.C.F. Walker Eds., 2005,103-113
- Joseleau and Ruel,, Cellulose Chem Technol, 2007*, 41*: 487-494



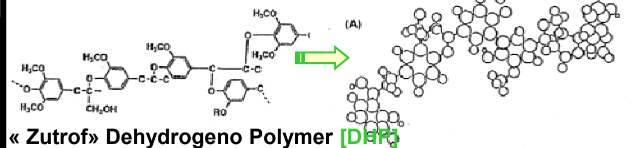

# Wood cell wall components

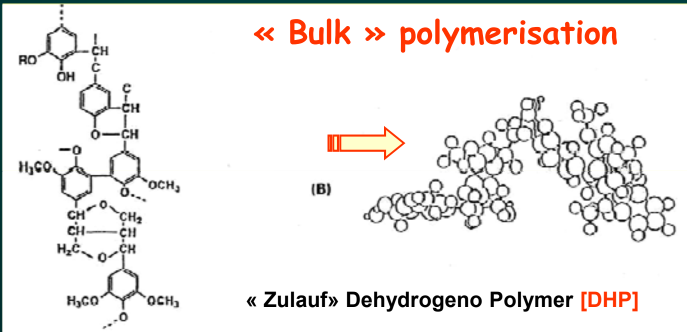
## Lignin

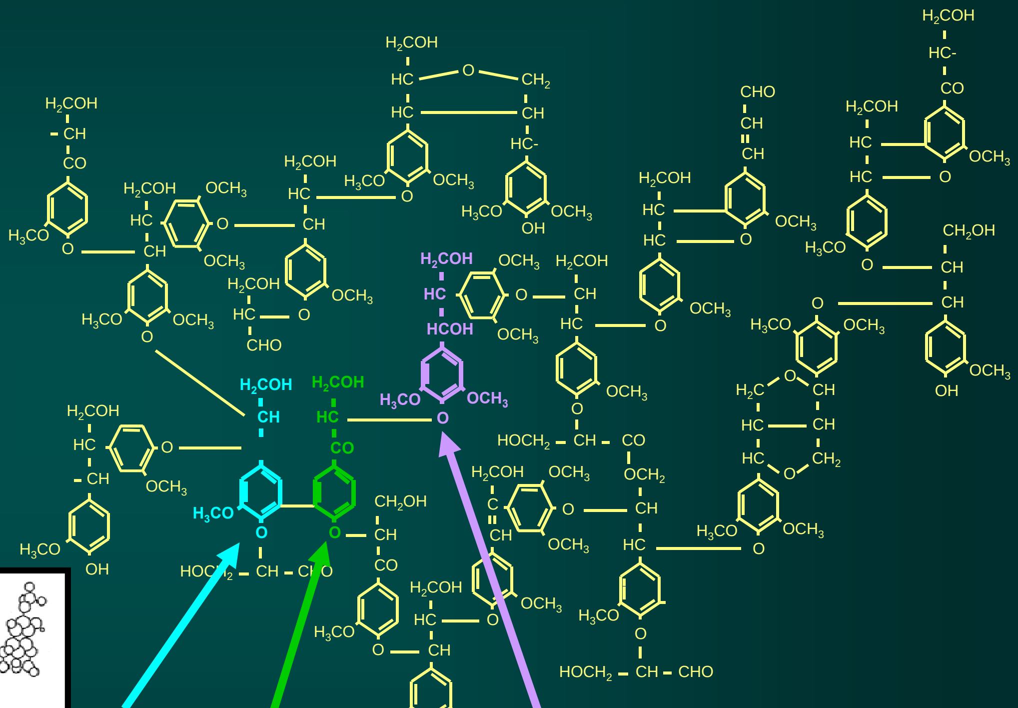



Non-condensed inter-unit linkages

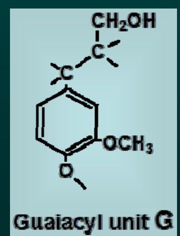
Two modes of polymerization of the Monomers

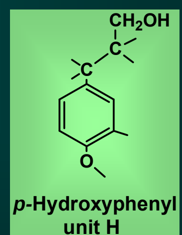


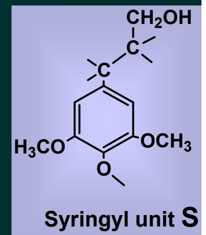




Three basic Monomers



# Antibodies from synthetic antigens (DHPs)

- 1) directed against monomer units
  - A
  - A
  - A
  - A
- 2) dir

- 3) Specificity
  - [...]otting
  - TEM affinity tests*

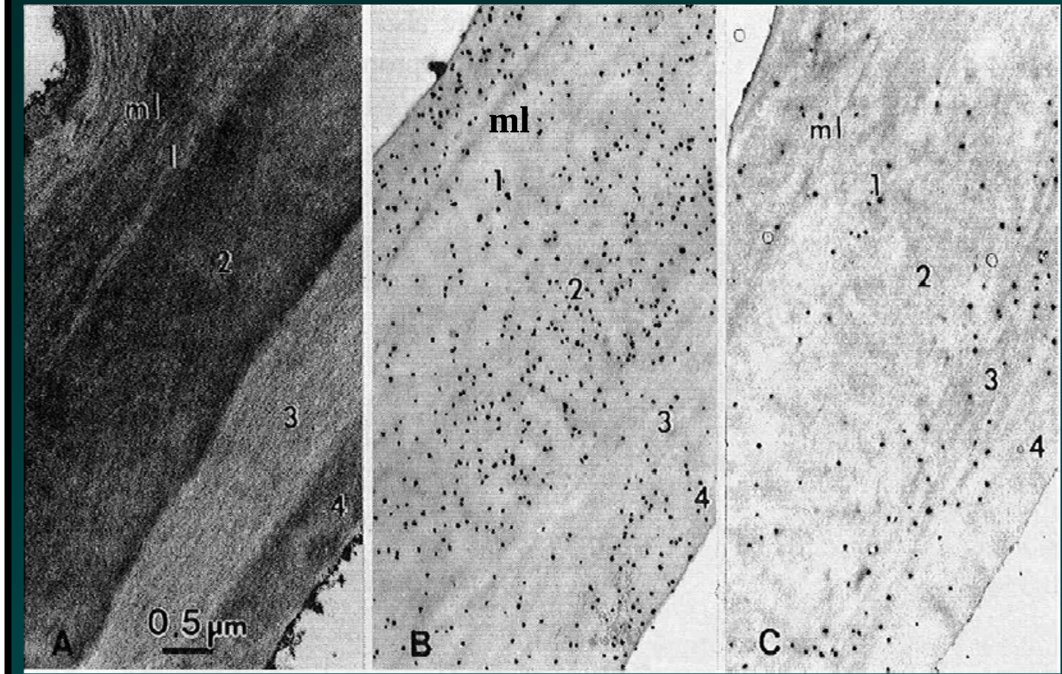
> **The specificity of our anti-lignin antibodies is directed against:**
> - Constitutive monomers (⇨ *composition*)
> - Inter-unit linkages (⇨ *condensed / non-condensed*)
> - Macromolecular conformation (⇨ *extended vs bulky*)

Ruel et al. , J. Trace Microprobe, 1994
*Joseleau and Ruel, Plant Physiol., 1997
Ruel et al. CR Biol., 2004

TEM

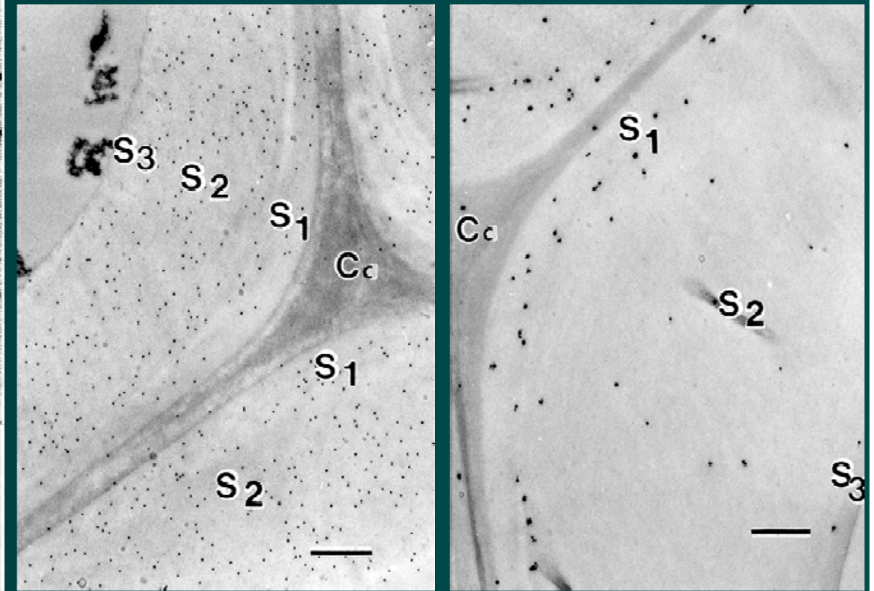
# Distribution of matrix components: Lignins

**Vessels**




KMnO4 | Non-condensed | Condensed

**Fibers**




Non-condensed | Condensed

☛ **The distribution of the main lignin types (GS, G, S) is different:**

⇨ ***From one tissue to another***

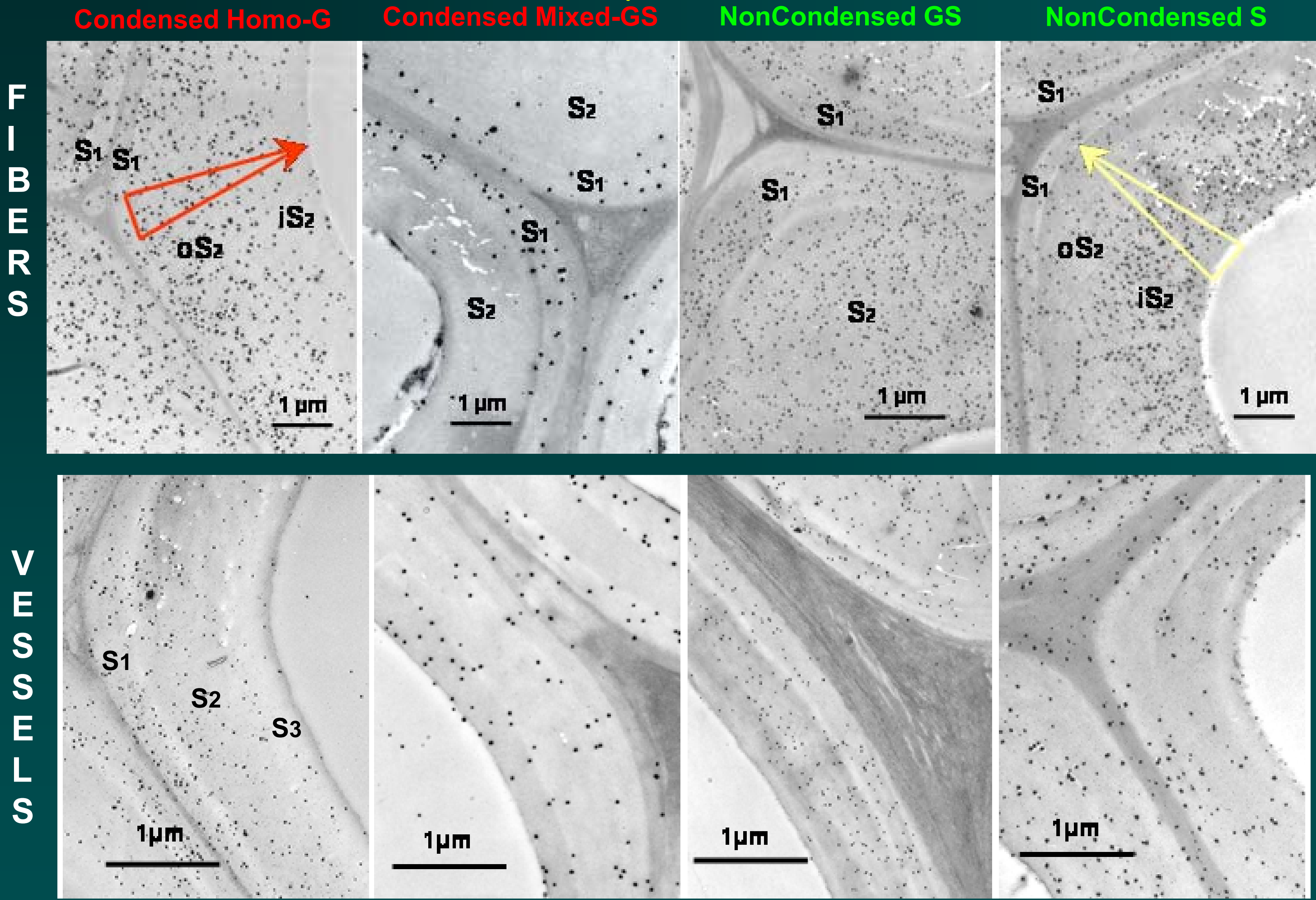
⇨ ***Inside the cell-wall itself:* topochemical microdomains**

☛ ***Patterns are characteristic of cell type* (Fiber/vessel)**

◆Ruel et al. **Plant biol.** 2002, *3*: 2-8
◆Joseleau and Ruel **Plant Physiol**. 1997, *114*:1123-1133
◆Ruel et al. Maderas Cienc Tecnol , 2006, 8:107-116

Katia Ruel

# TEM *In Planta* topochemical distribution of the main types of lignin in Poplar Wood




Ruel et al. Cellulose Chem Technol, 2008,

## Lignin topochemistry

- Immunolabelling demonstrates **regio-specificity** in Secondary Walls

- ***Condensed*** and ***Non-condensed*** Lignin epitopes are **differentially distributed** in Secondary Walls

↓

Monolignol Polymerization is Spatially and Temporally controlled during cell Wall Assembly

# ◈ Role of Matrix Components in Cell Wall Structure and Assembly ?

- Cells in development
- Transgenic plants
- Deconstructing agents

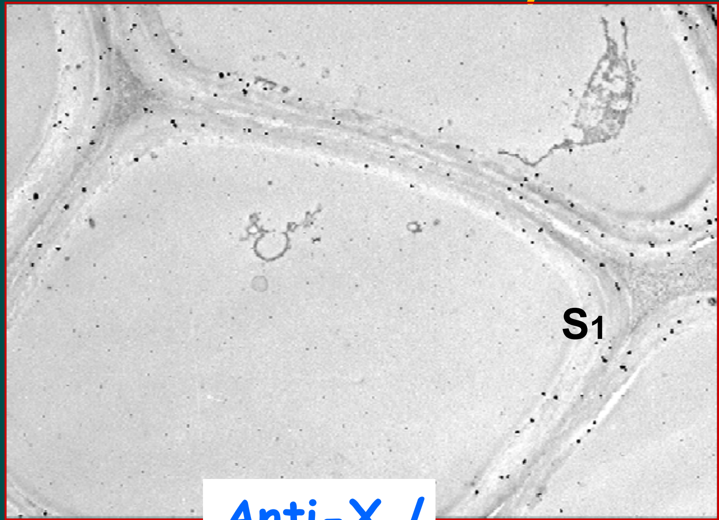
# Role of matrix components in cell wall assembly

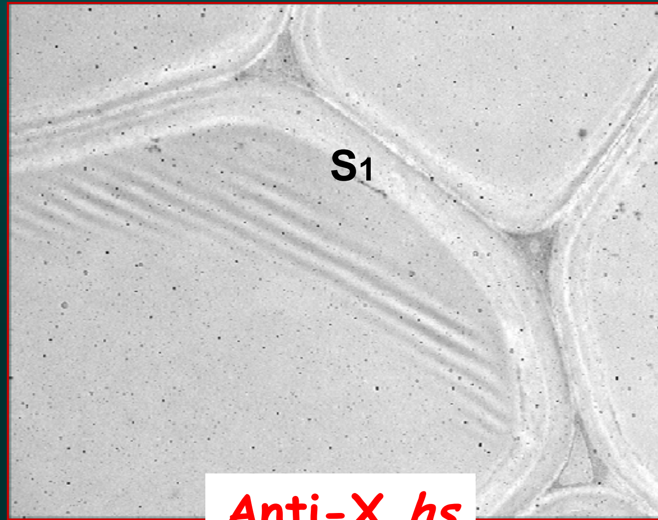
## ☛ Developing cells

- ☛ Fate of **xylans** during cell wall development
- ☛ Fate of **lignins** during cell wall development

# Fate of Xylans Deposition in Developing walls of Poplar wood

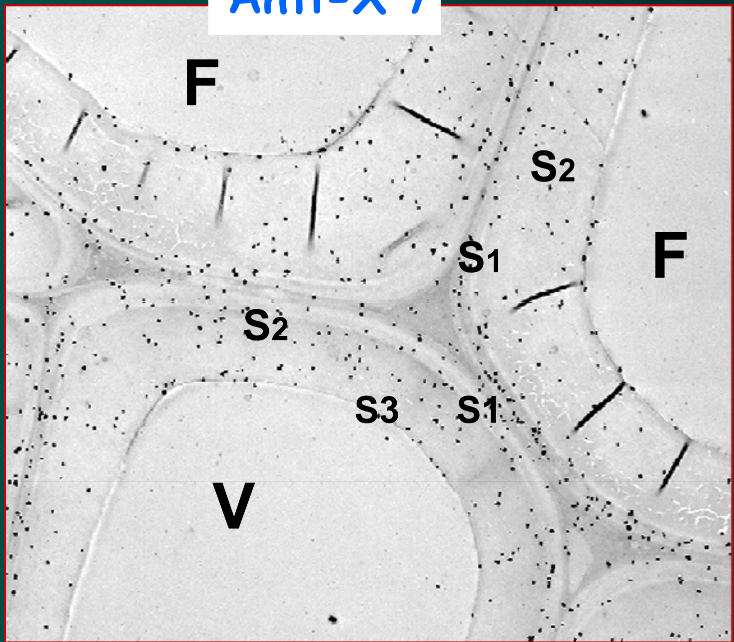
*low-substitution* Xylan | *highly substituted* xylan



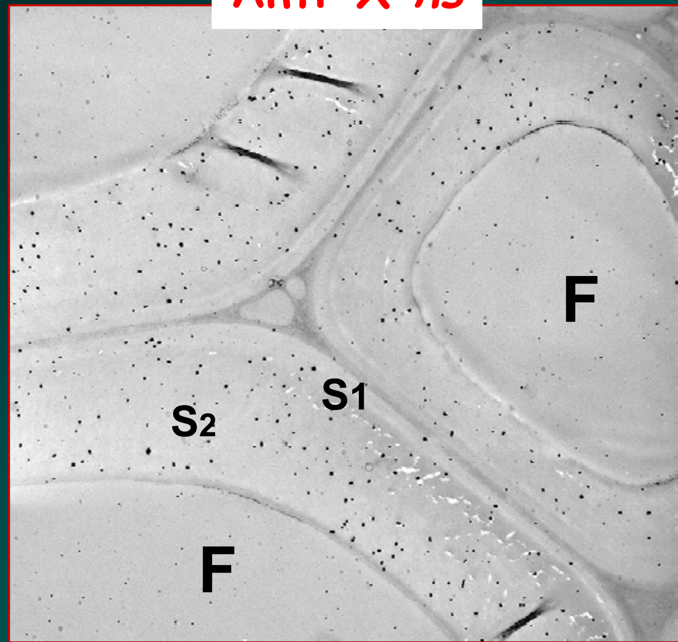




Young stage

Anti-X *l*

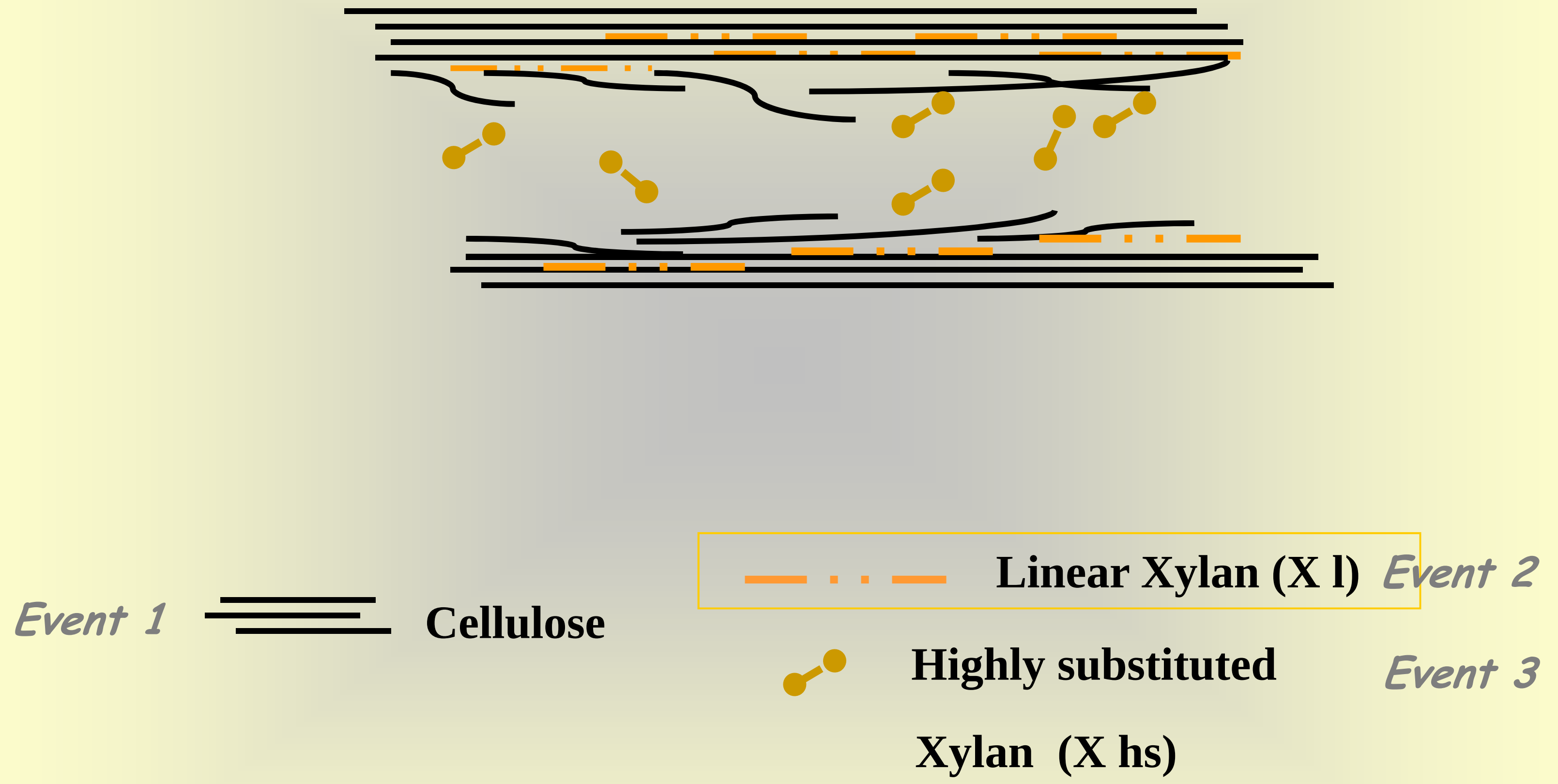



Anti-X *hs*




Mature stage

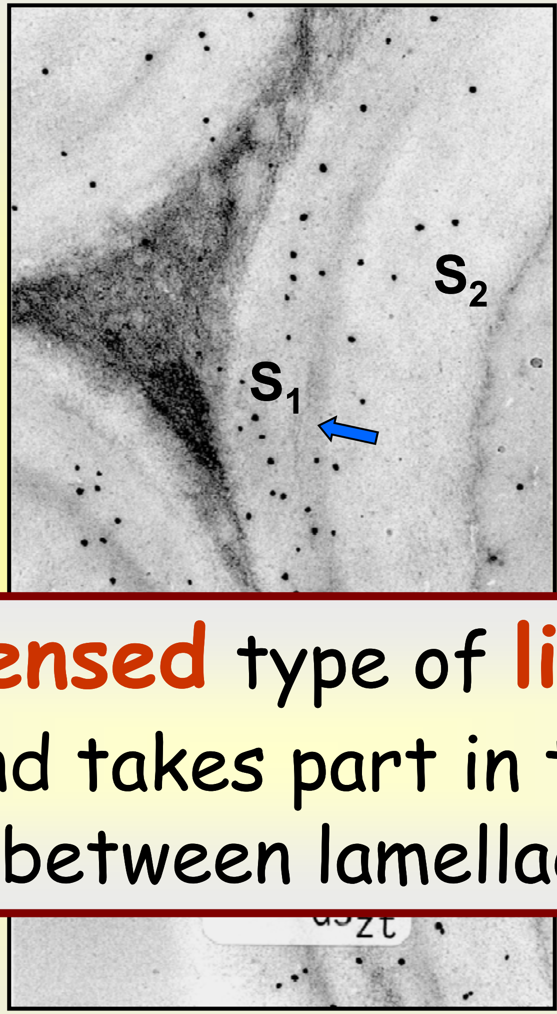
# Sequential events in the deposition of Xylans in Hardwood

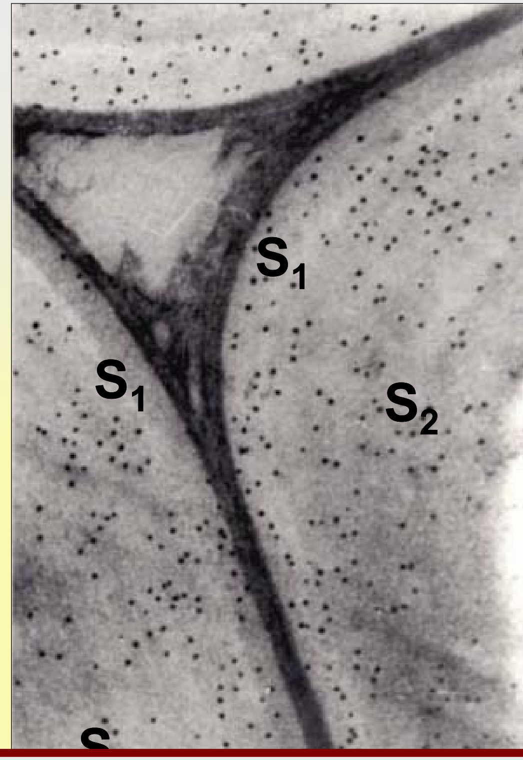



⇨ *Recently, Dammstrom---Salmen et al. (2009) proposed that one fraction of xylans should be more strongly associated to cellulose*

# Fate of Lignins Deposition


Populus


- **Non-condensed** type of **lignin** is early deposited and takes part in the cohesion between lamellae

Immunolabelling of **non-condensed lignin sub-units**

# ☛ Genetically modified plants

- ☛On the lignin biosynthesis pathway

# The lignin biosynthetic pathway

## Biosynthetic pathway of phenylpropanoids

1/ Synthesis of phenolic aromatic nucleus

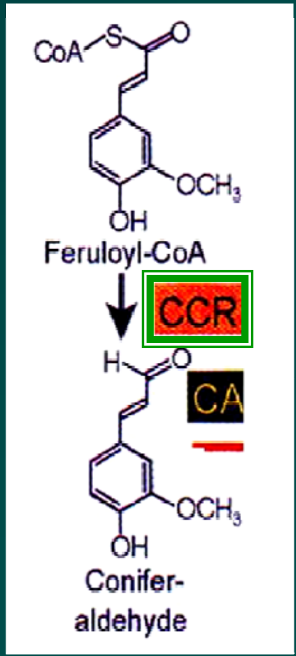
Shikimic acid ⇨ hydroxy cinnamic acids via phenylalanine

2/ ⇨ cinnamic alcohols
= *Monolignols*

3/ monolignols polymerisation

⇩

*Lignin(s)*




cinnamoyl-CoA reductase (CCR) down-regulation or inhibition

# Analytical data of lignins from CCR transformed plants

| Plant | Lignin content (Klason % weight) | Residual CCR activity (%) | %S | %G | S/G | Ferulic acid [µmol/(g KL)$^{-1}$] | G-CHR-CHR2 |
|---|---|---|---|---|---|---|---|
| **Tobacco** | | | Non-condensed | Condensed | | | |
| NN | 22 | 100 | 21 | 79 | 0.9 | nd | nd |
| B-3-H | 10.7 | 6 | 15 | 85 | 1.5 | nd | nd |
| ***A.thaliana*** | | | | | | | |
| WS | 17.0 | 100 | 28.2 | 71.6 | 0.39 | 1.7 | nd |
| As-CCR2 | 10.0 | 19.2 | 34.1 | 65.5 | 0.52 | 5.0 | nd |
| As-CCR7 | 13.0 | 24.4 | 36.3 | 63.4 | 0.57 | 6.1 | nd |
| Col0 | 18.3 | 100 | 28.1 | 71.2 | 0.40 | 1.7 | nd |
| SCCR1 | 11.7 | 0.0 | 27.6 | 71.3 | 0.39 | 18.5 | nd |
| irx4 | (-50%) | nd | nd | nd | nd | nd | nd |
| **Poplar** | | | | | | [pmol/(mg Wood)] | |
| WT | 20.7 | | | | 1.9 | 75 | 2.2 |
| FS3 | 16.7 | | | | 1,5 | 111 | 3.4 |
| *(Leplé et al. Plant Cell, 2007)* | | | | | | | |

TEM

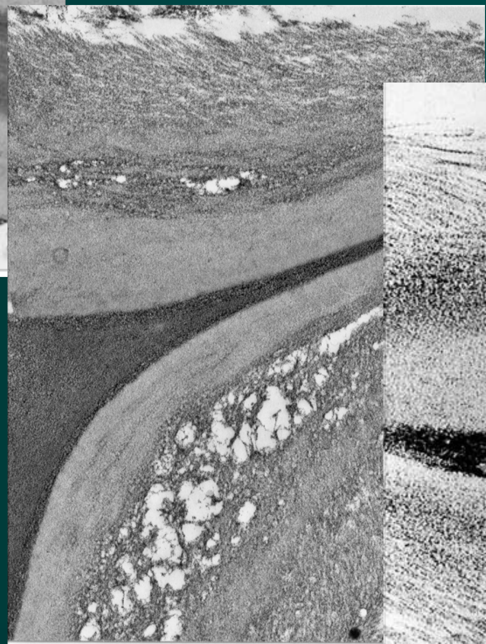
# Consequences of CCR down-regulation in *Tobacco*

**FIBRES**

antisense

Nul mutant

WT



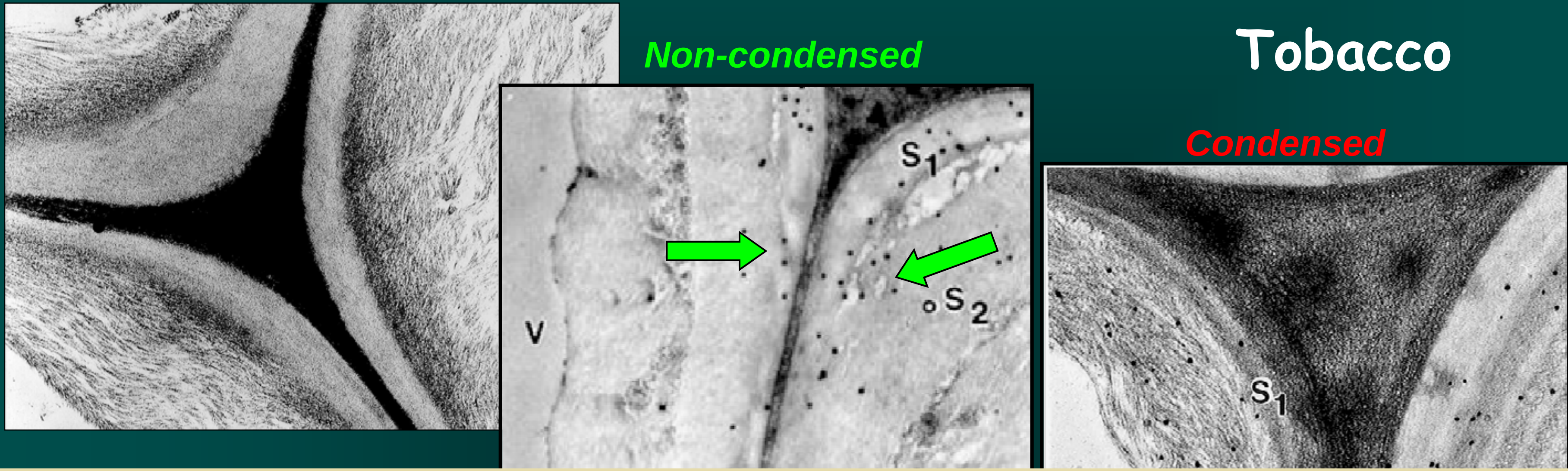
B3-H

B3-1

Dramatic Loosening of the Secondary Wall ($S_2$)

# Consequences of CCR down-regulation on lignin topochemical disribution

TEM




The **non-condensed forms of lignin** play a significant role in the organization of S2

Contributing to :

- The association of cellulose microfibrils
- The cohesion of the secondary cell wall.

TEM

# Consequences of CCR down-regulation on lignin topochemical disribution

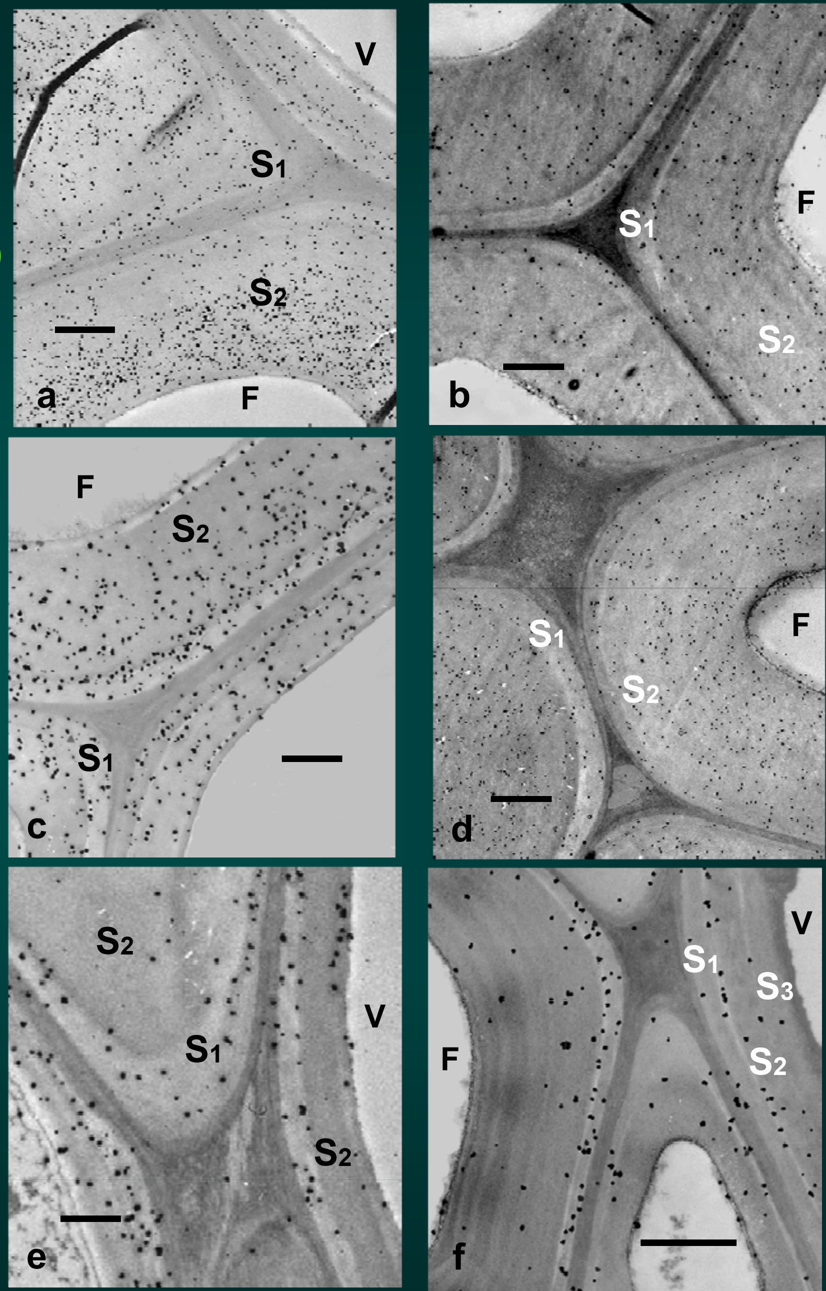
Leplé et al. Plant Cell 2007


Wild-Type | Transformed Poplar

S *(non-condensed)*

Condensed G

Non-Condensed $\alpha$-O-8

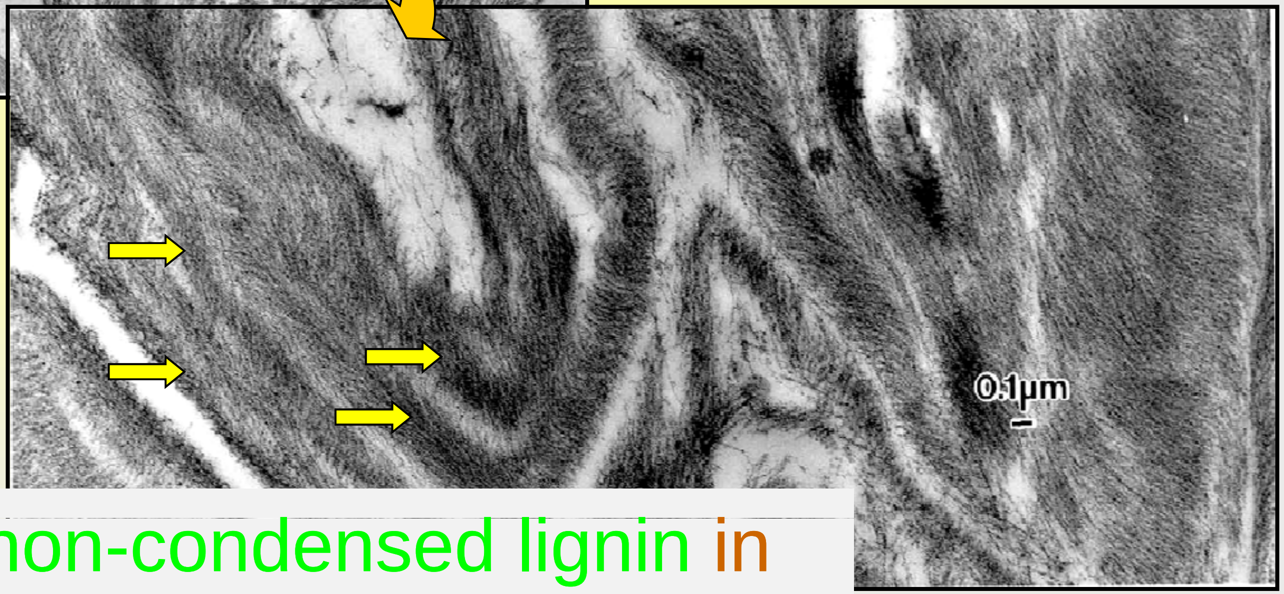



**Changes in the topochemical distribution of lignin sub-units**

# ☛Deconstructing agents


**Unbleached Kraft pulp treated with Manganese-Peroxidase from *P.chrysosporium* I-1512**

Sigoillot, JC, Ruel, K *et al*. (1997) Holzforschung, 51:549-556




Role of non-condensed lignin in cohesion between lamellae

# To Summarize the Preceding Observations……

# Successive Steps in the Formation of Lignified Secondary Walls

☛ **Biosynthetic Events**

**i/ Newly synthesized CMFs [deposited in crystalline state]**

**ii/ Low-substituted Xylans adsorbed on Cellulose**

**iii/ Deposition of Lignin under *condensed* form**

**iv/ Highly-sustituted Xylans deposit and serve as anchoring for *Non-condensed* Lignin**

**v/ *Non-condensed* Lignin covalently binds to uronic acid substituents of highly-substituted Xylans**

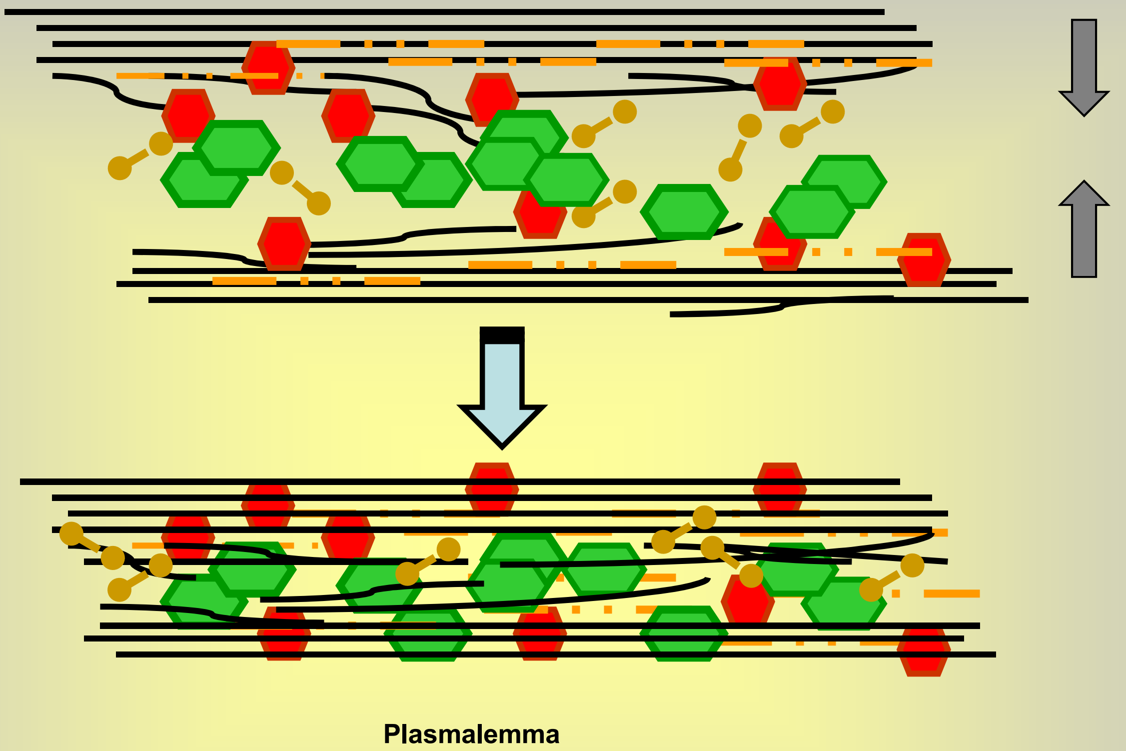
**This mechanism repeats itself as S2 thickens.**

☛ **Consequences on Cell Wall Structure**

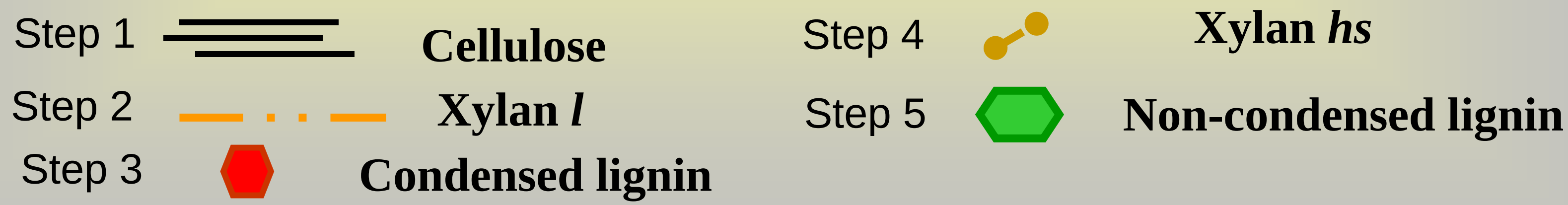
**The binding between *non-condensed* lignin and xylans induces tightening between CMF lamellae**

☛ ☛ **Overall: *Cohesion + Compactcity***

# Dynamic Scheme of the Deposition of Cellulose, Hemicelluloses and Lignins during Secondary Wall Assembly




- Cohesion
- Compacity



# ◈The Cellulose Framework

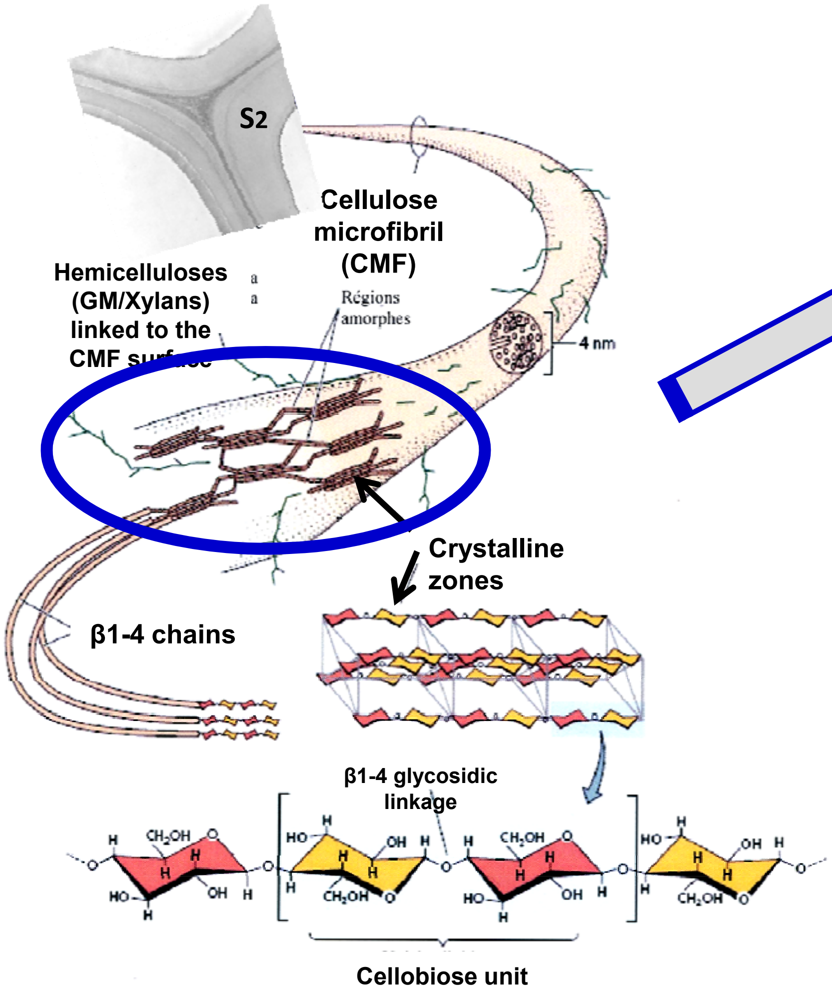
☛ In planta Crystalline and Non-crystalline cellulose distribution

# Cellulose microfibrils crystallinity in Woody Plants

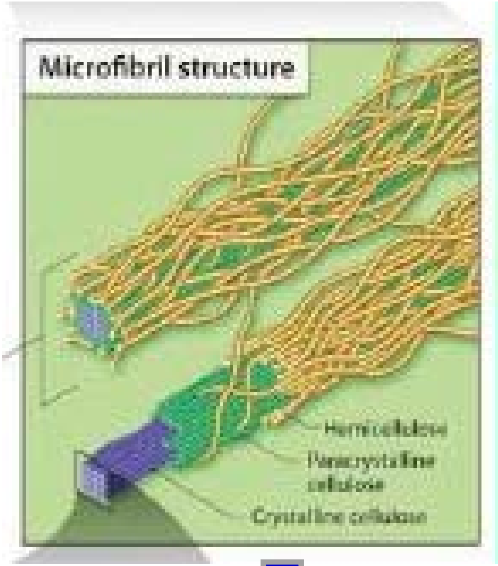
- ☛ **About half of the cellulose in fibres from Wood and crop plants is crystalline: the rest is disordered**

- ☛ **The proportion of crystalline cellulose in spruce wood** has been estimated at 30%, equivalent to about 60% of the total cellulose. **This fraction appears to be more than the 40% of cellulose I as defined conformationally by NMR** (Fernandez….. jarvis 2011-PNAS)

# Cellulose crystallinity in plant cell walls




From Chanliaud (2006)




☛ Recent techniques confirmed that **well ordered cellulose forms the core** of each microfibril and much of the **less ordered cellulose** is **at the surface**, as has been suggested previously on the basis of NMR experiments (From Fernandes….. jarvis 2011-PNAS)

**How crystalline and disordered parts fit together *in planta*…..?**

## Visualization of Crystalline and Amorphous Cellulose in the Cell Walls

# Cellulose-Binding Modules (CBMs)

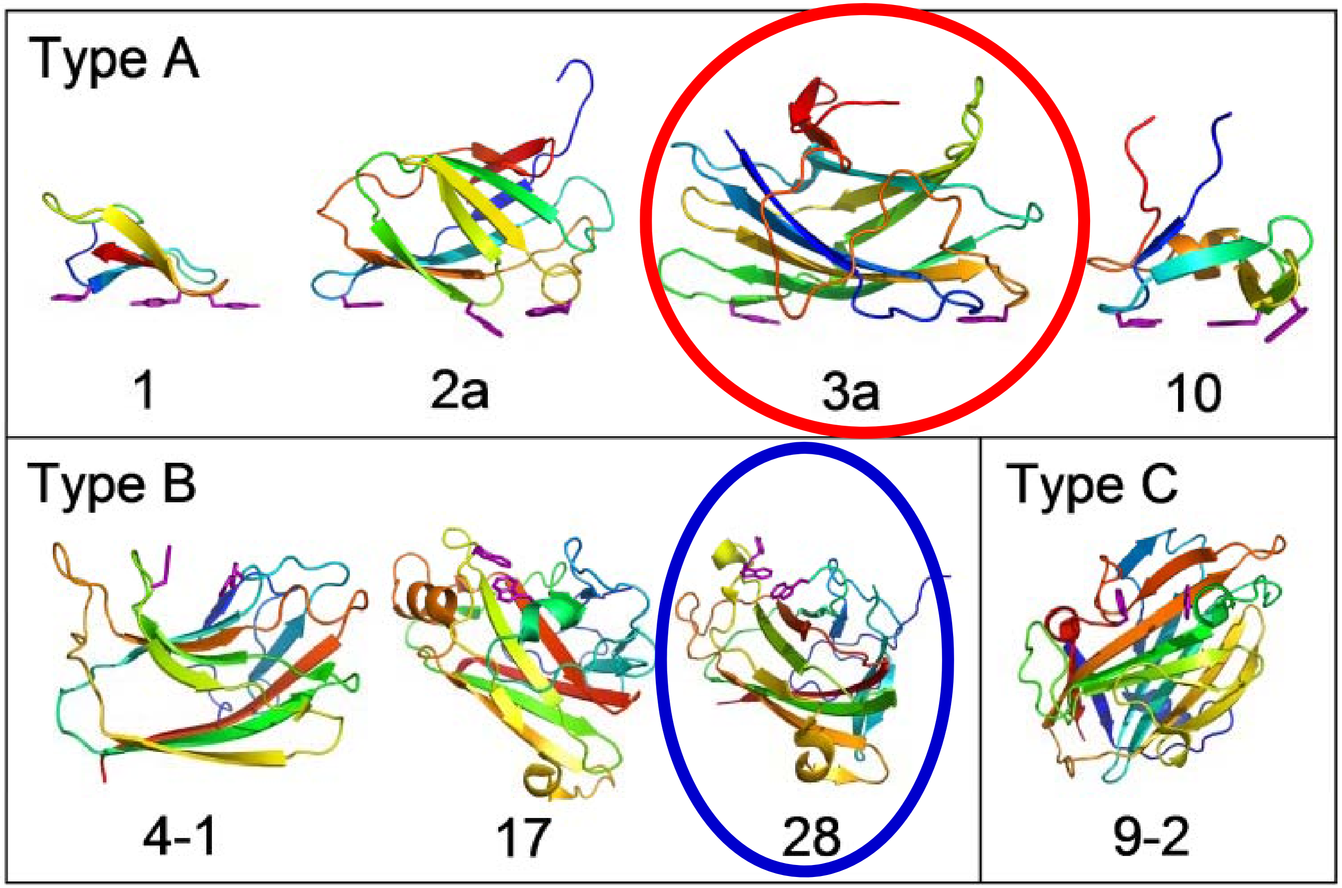
## Tools to probe Crystalline and Amorphous Cellulose *in situ*

CBMs are a diverse set of **non-catalytic protein domains** that have a wide range of binding specificities towards cell wall polymers [Lehtio, 2003,].

*Adapted as probes for light Microscopy*

- Hildrén et al. (2003)
- Mc Cartney et al. (2004)

# CBMs: Tools to probe Crystalline and Amorphous Cellulose *in situ*




**Type A** = surface binding CBMs which **bind specifically to crystalline cellulose.**

**Type B** = chain-binding CBMs specific **for single chains of polysaccharides = amorphous cellulose**

**(Boraston et al. 2004)**

**Type C** = end-binding CBMs **specific for the ends of polysaccharides or oligosaccharides.**

# CBMs binding

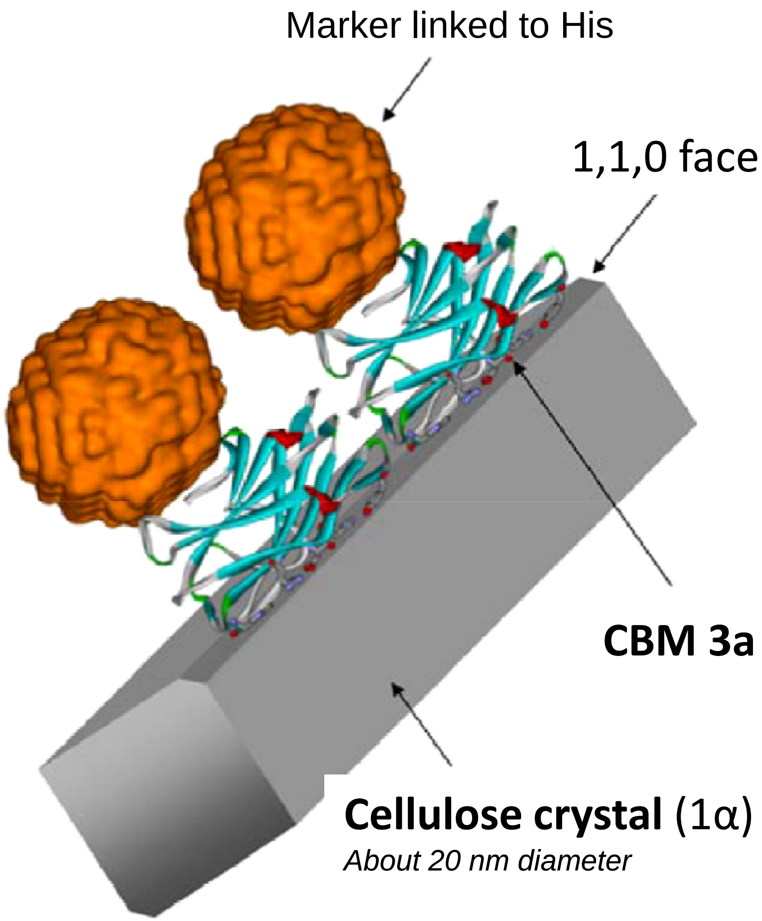


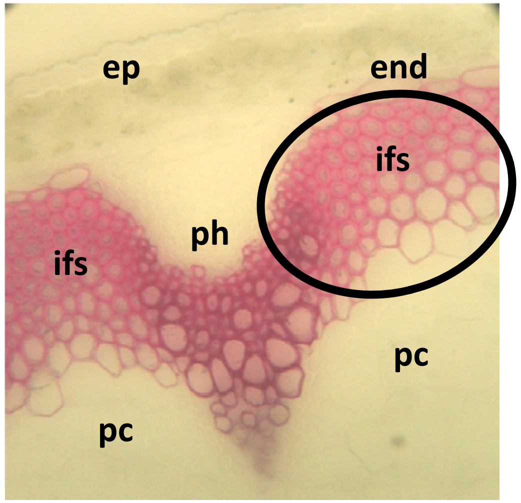

**Fig. 1** A schematic model of cellulose/CBM/QD bio-assembly. The recombinant CBM protein specifically binds to the 1,1,0 surface of the cellulose 1α crystal. The QDs are anchored on CBM proteins by the his-tags fused at both *N*- and *C*-termini. QDs, CBMs, and cellulose are depicted in scale based on their published X-ray structures. The cellulose crystal model is simplified (showing fewer glucan chains) to fit into the picture. The actual size of *Valonia* cellulose crystals used in this study is about 20 nm in diameter

**From Xu et al. (2009) Cellulose 16:19-26**

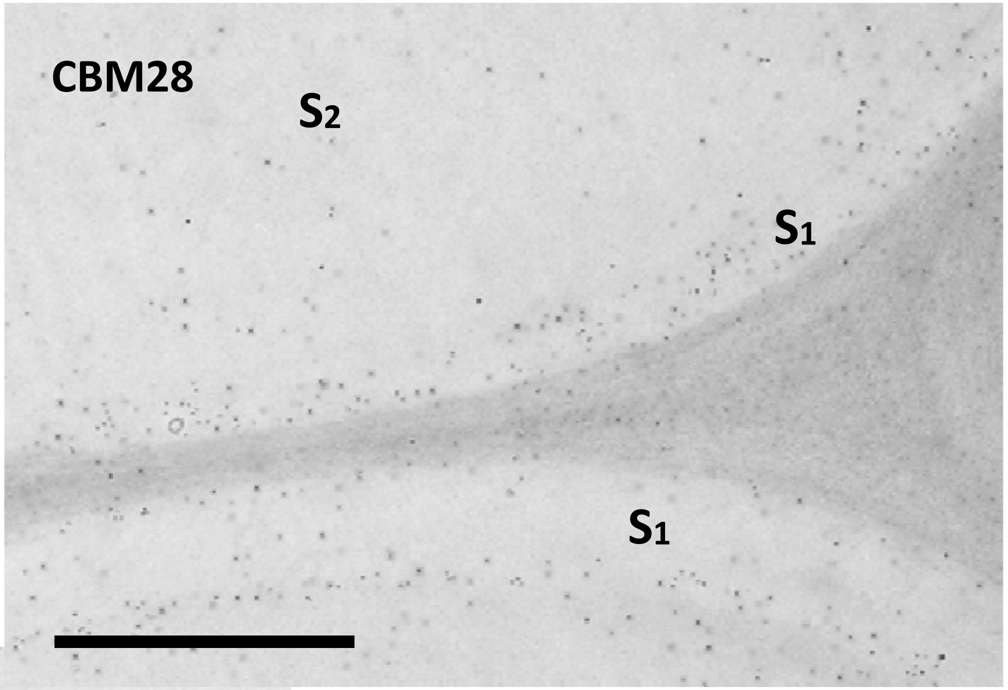
Case of *Valonia* cellulose crystalline microfibrils, it has been in-situ demonstrated a **systematic binding to the two hydrophobic planar (110) faces of the cellulose microfibrils**

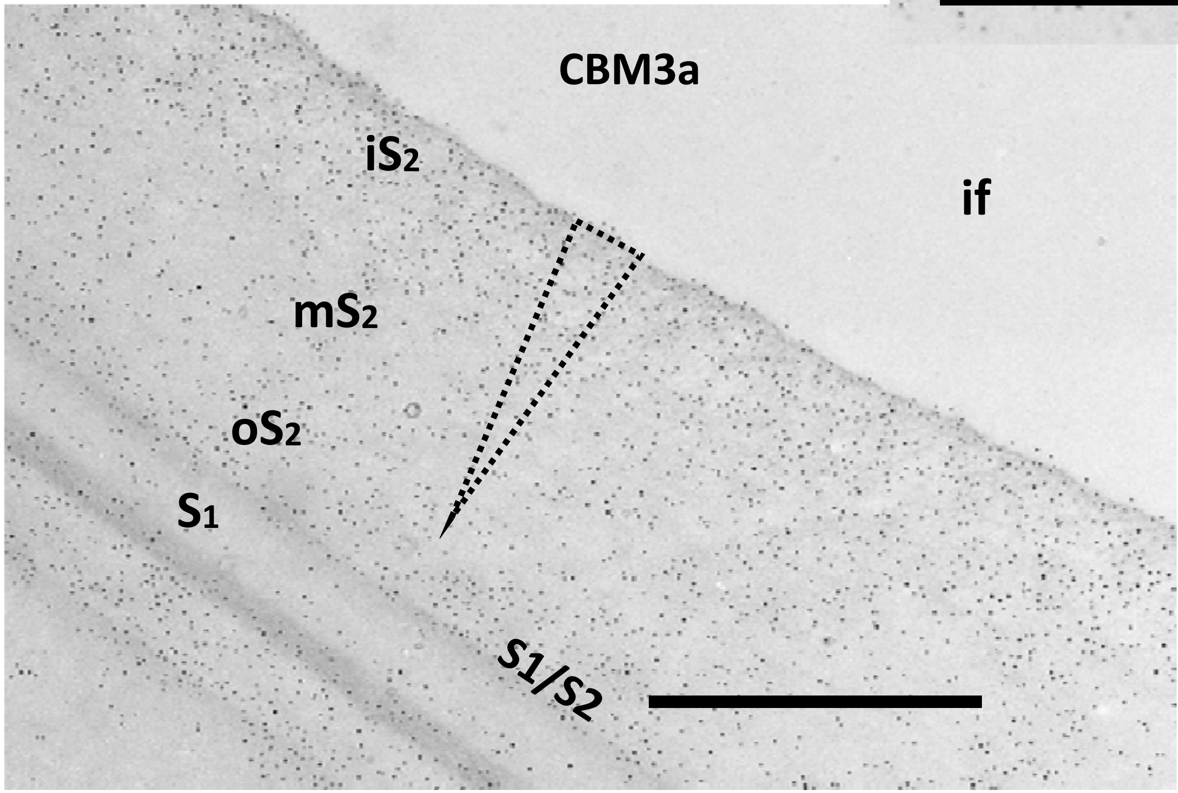
**has been** fluorescently labeled and **used to label crystals as well as plant tissue** **(Ding et al., 2006; Porter et al., 2007; Liu et al., 2009; Xu et al., 2009).**

**????Dagel et al. « In situ imaging of single carbohydrate-binding modules on cellulose microfibrils ». J. Phys. Chem. B 2011, 11[illegible] 641**




Crystalline cellulose




Amorphous cellulose

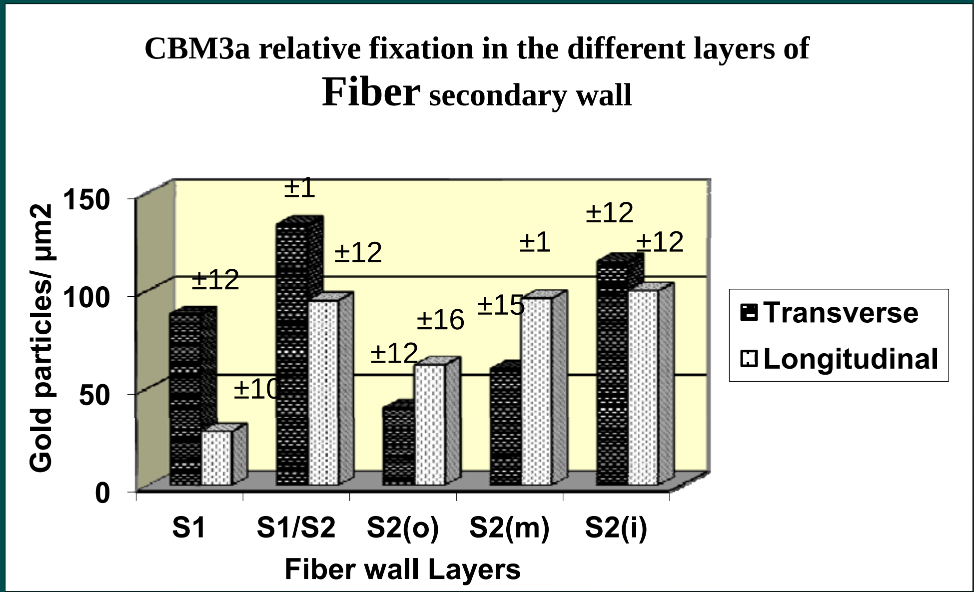
# Distribution of crystalline and amorphous cellulose domains in lignified fibers

(*A. thaliana*)

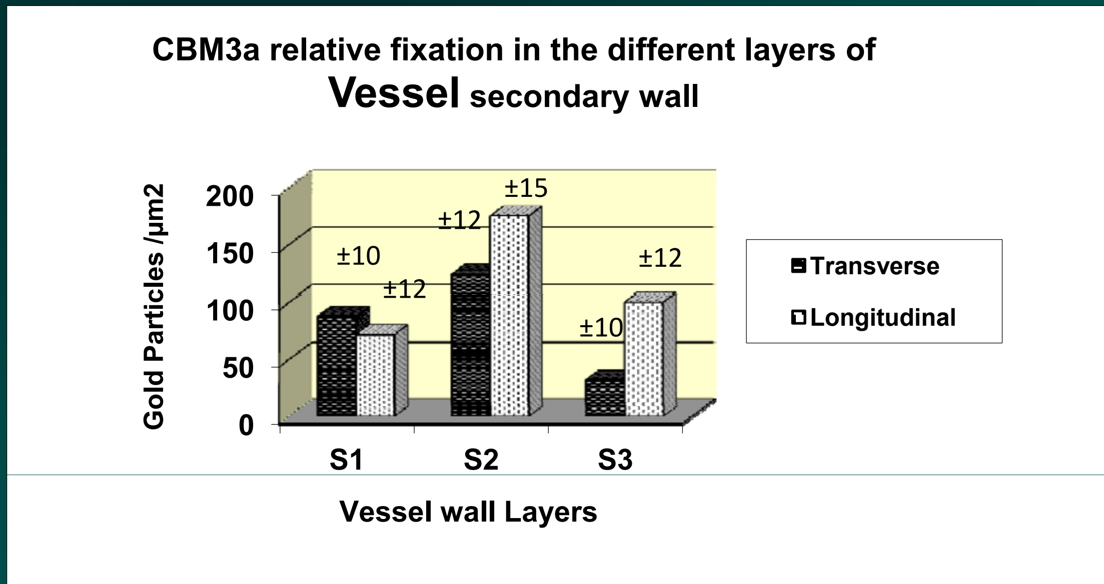
*[Ruel, Nishiyama, Joseleau. Plant Sci. 2012 "Crystalline and Amorphous Cellulose in the Secondary Walls of Arabidopsis"]*

Katia Ruel

# Quantitative Evaluation of CBM3a fixation across transverse and longitudinal sections

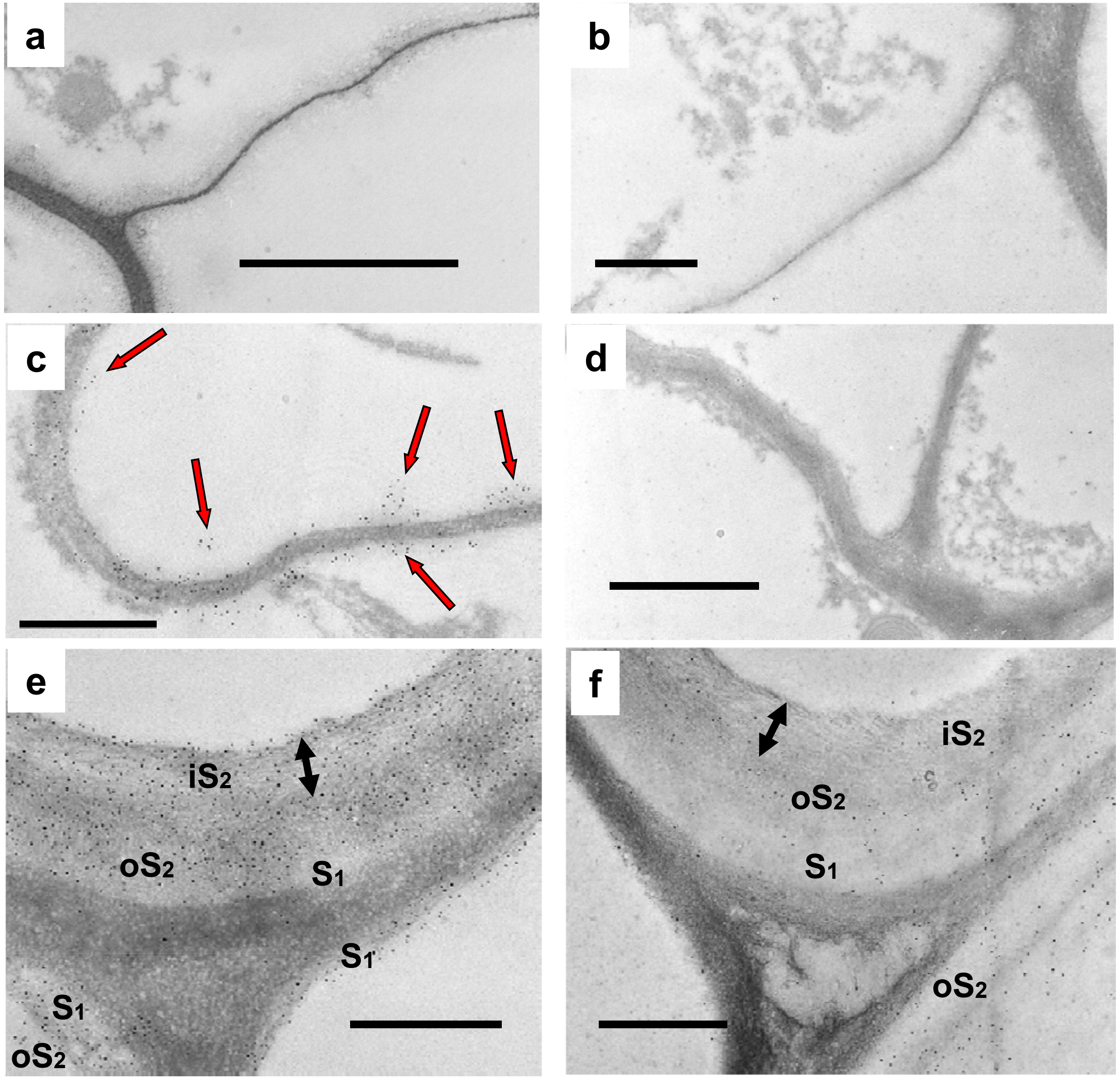



*[Ruel, Nishiyama, Joseleau. Plant Sci. 2012 "Crystalline and Amorphous Cellulose in the Secondary Walls of Arabidopsis"]*



# Mapping of Cellulose Deposition during Cell Wall Development

Crystalline Cellulose (CBM3a) | Amorphous Cellulose (CBM28)




**Cellulose first deposits under Crystalline Structure**

*[Ruel, Nishiyama, Joseleau. Plant Sci. 2012 "Crystalline and Amorphous Cellulose in the Secondary Walls of Arabidopsis"]*

# Conclusion

1/ **Cellulose first deposits under *Crystalline* (or *High Crystallinity*) state**

2/ ***Amorphous* Cellulose then appears progressively,** due to:

* **irregularities** in the biosynthesis process
* **growth stress** constraints [*internal stress*]
* **post-synthesis destructuration** (Endogenous enzymes; mechanical action) [*external stress*]

Viewed **at the Nano-scale** , the **Wood Cell Walls are Complex Hierarchical Structures**

They display Multiple Organizations defining the Diversity and Variability of Cell Types **in Adaptation to Specific Physiological** and **Mechanical Functions**

Special Thanks to  Prof. Em. Jean-Paul JOSELEAU

Most of the results presented  here are the result of a collaborative work combining ultrastructural (KR) and Biochemical (J-P.J.)  approaches

| | |
|---|---|
| Prof. A-M. Boudet (UMR CNRS/UPR-Toulouse) | For transformed Tobacco |
| Dr. L. Jouanin (INRA Versailles) | For CCR mutants of *A. thaliana* |
| Dr. G. Pilate (INRA Orléans) | For CAD down-regulated poplar |
| Prof. P. Bolwell (Royal Holloway Univ., UK ) | For down-regulated Tobacco |
| Prof. W. Boerjan (Univ. Ghent, Be) | For transformed Poplar |
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A. Lefebvre
M-F. Marais