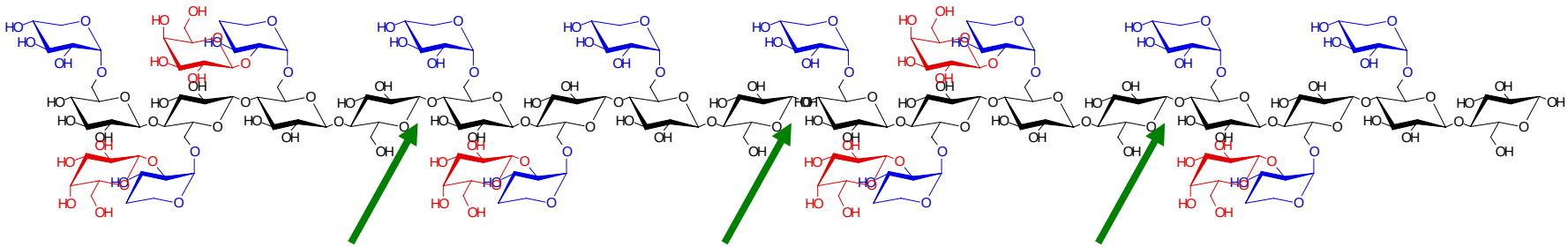
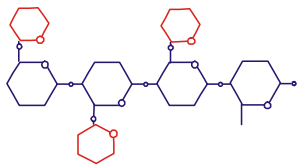
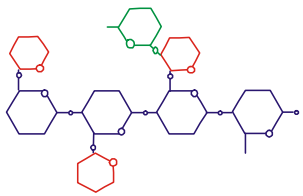
Structure and properties of storage xyloglucans



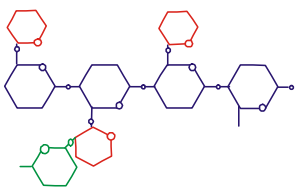
CELLULASE OR **XYLOGLUCAN TRANSGLUCOSYLASE HYDROLASE (XTH)**



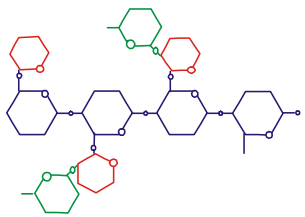
X X X G



X X L G



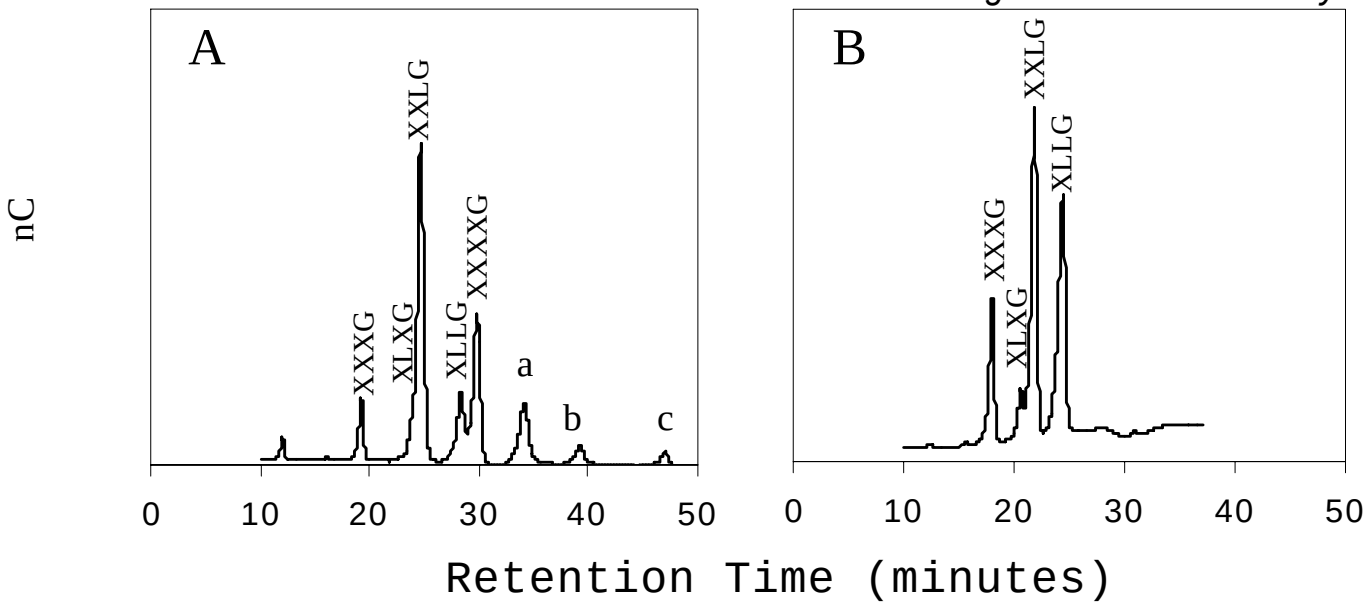
X L X G

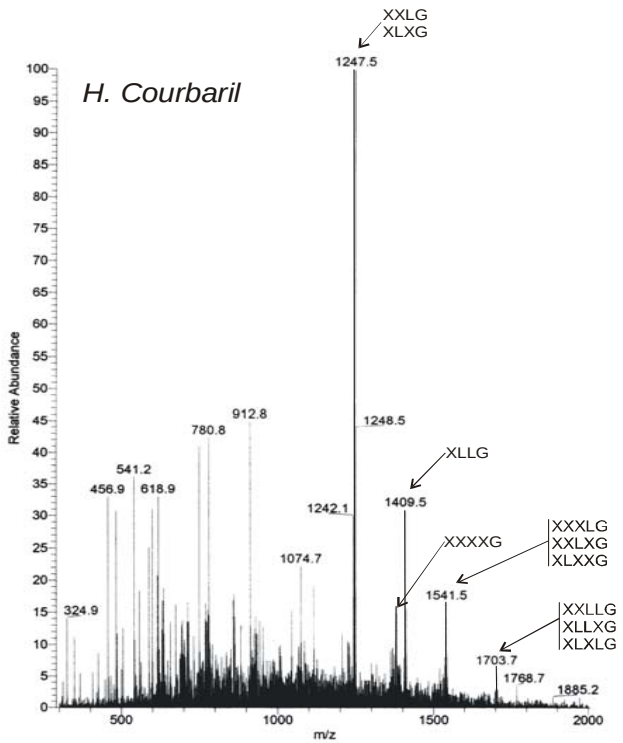


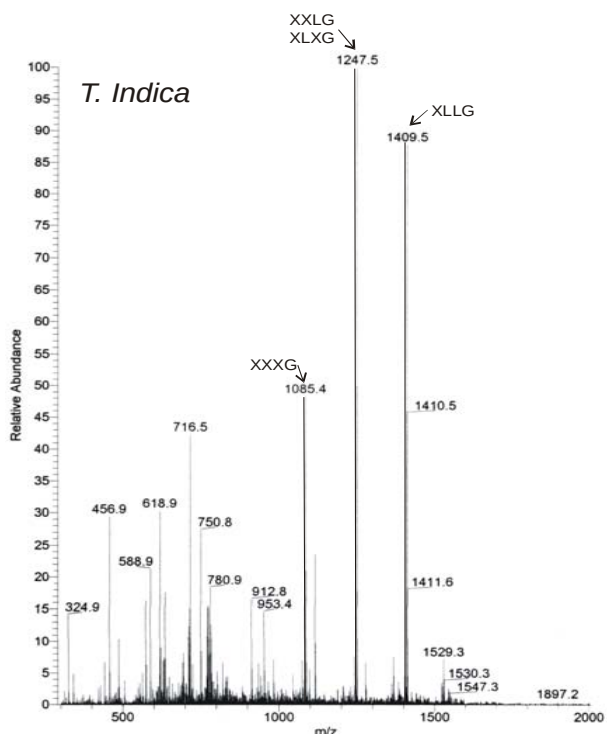
X L L G

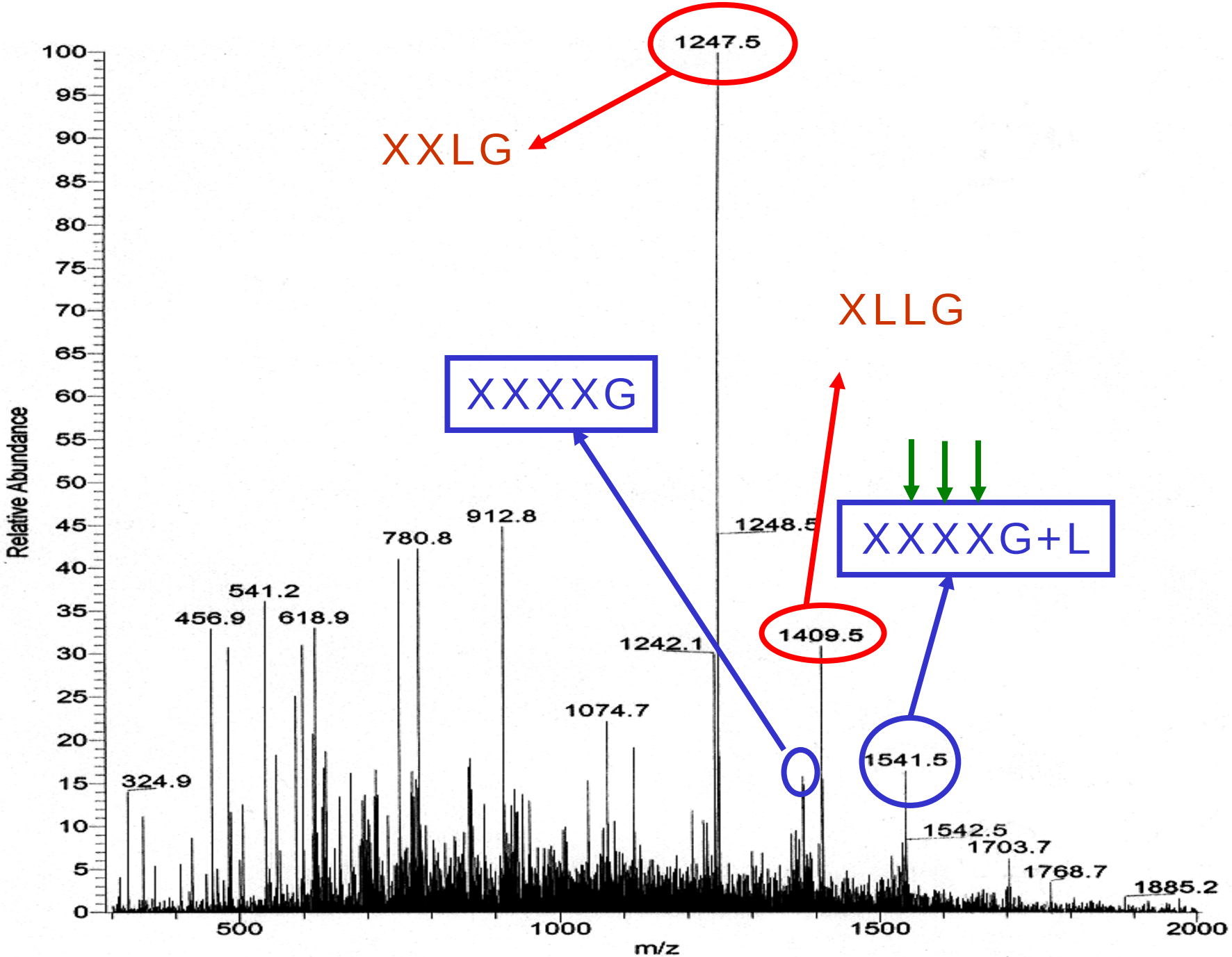
Principal limit digest xyloglucan oligosaccharides released by cellulase or XTH activity

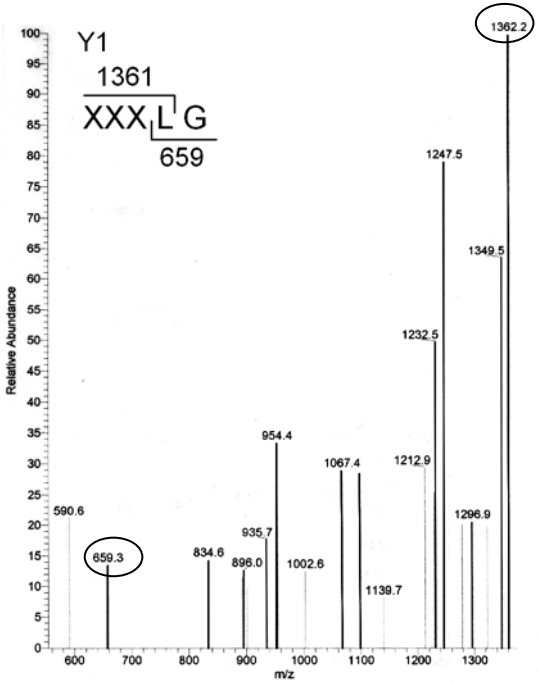
Buckeridge et al. 1997 Carbohydr. Res. 303:233-237

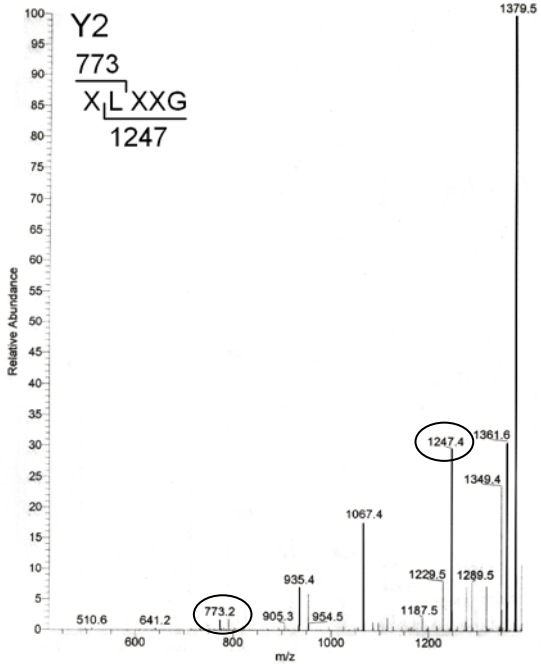


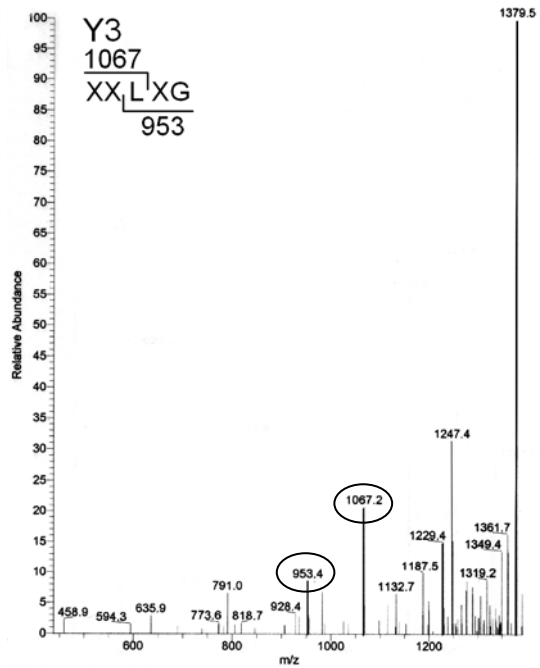





1247.5
XXLG
XLLG
XXXXG
XXXXG+L
1248.5
912.8
780.8
541.2
456.9
618.9
1242.1
1409.5
1074.7
1541.5
324.9
1542.5
1703.7
1768.7
1885.2
Relative Abundance
m/z
100
95
90
85
80
75
70
65
60
55
50
45
40
35
30
25
20
15
10
5
0
500
1000
1500
2000

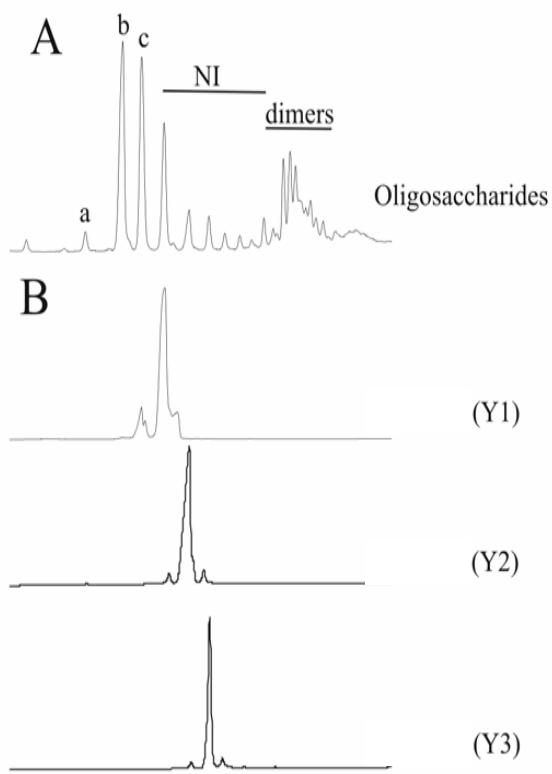




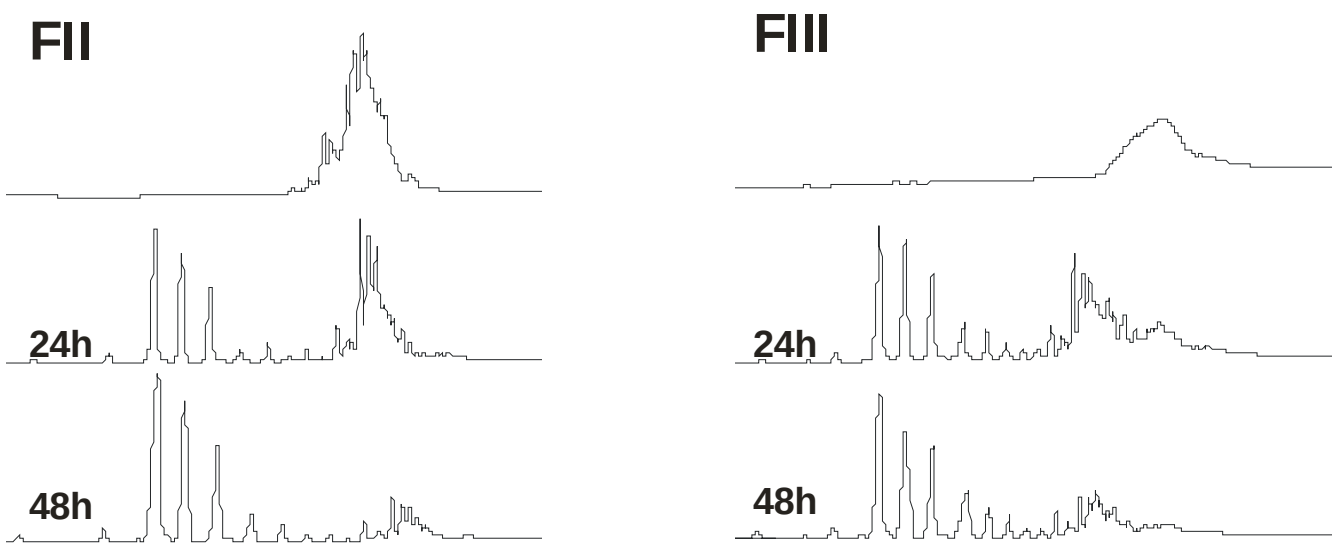


2nd fragmentation of 1541

Tiné et al. 2006, Carbohydr. Polymers



Some fragments resist cellulase attack



How many molecules?

2 x (...4-4-4-4-4-4-4-4...) +...5-5-5-5-5-5-5...

or

...4-5-4-5-4-5-4-5-4...+ ...4-4-4-4-4-4-4-4...

or

...4-4-5-4-4-5-4-4-5...+ ...4-4-5-4-4-5-4-4...

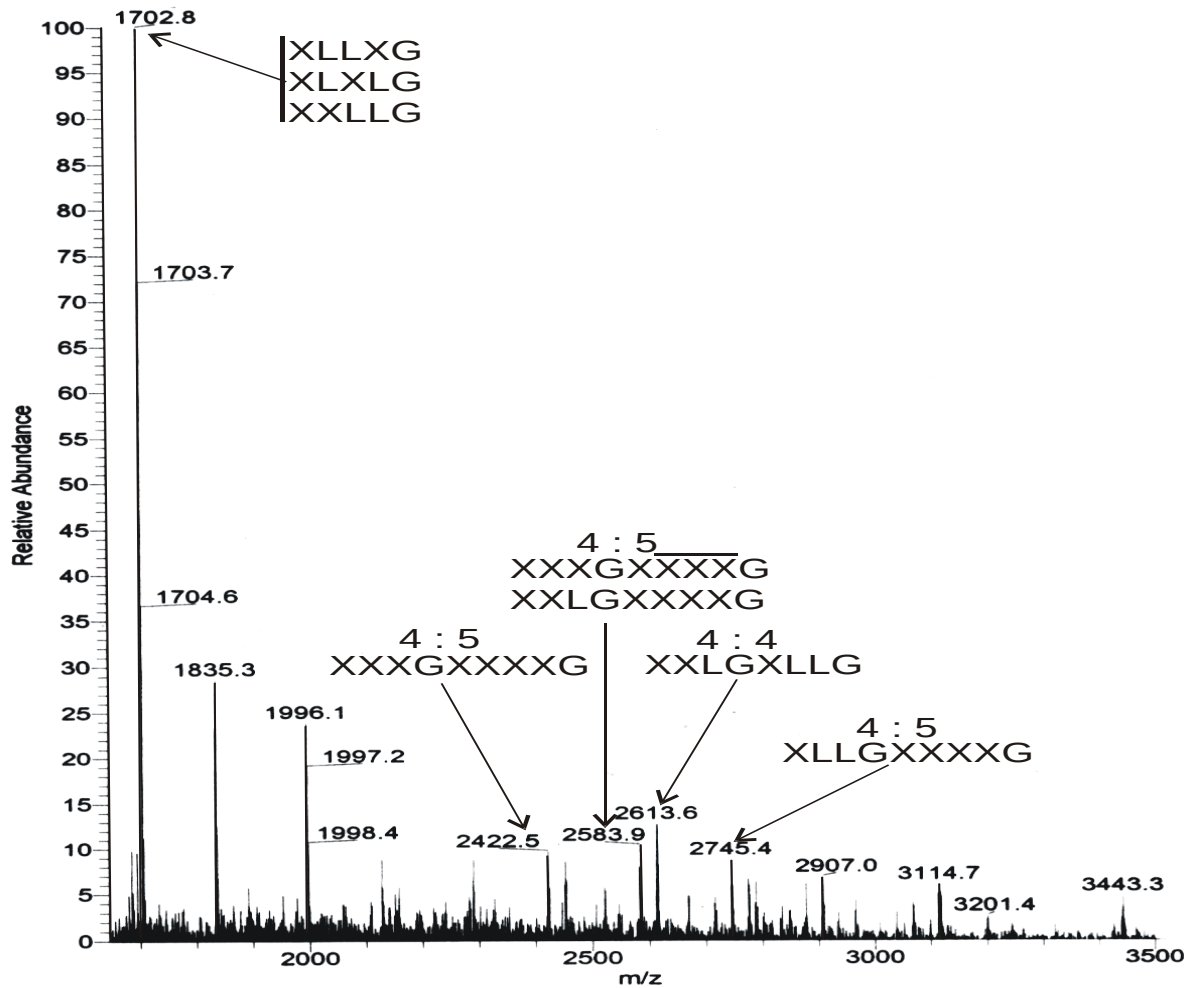


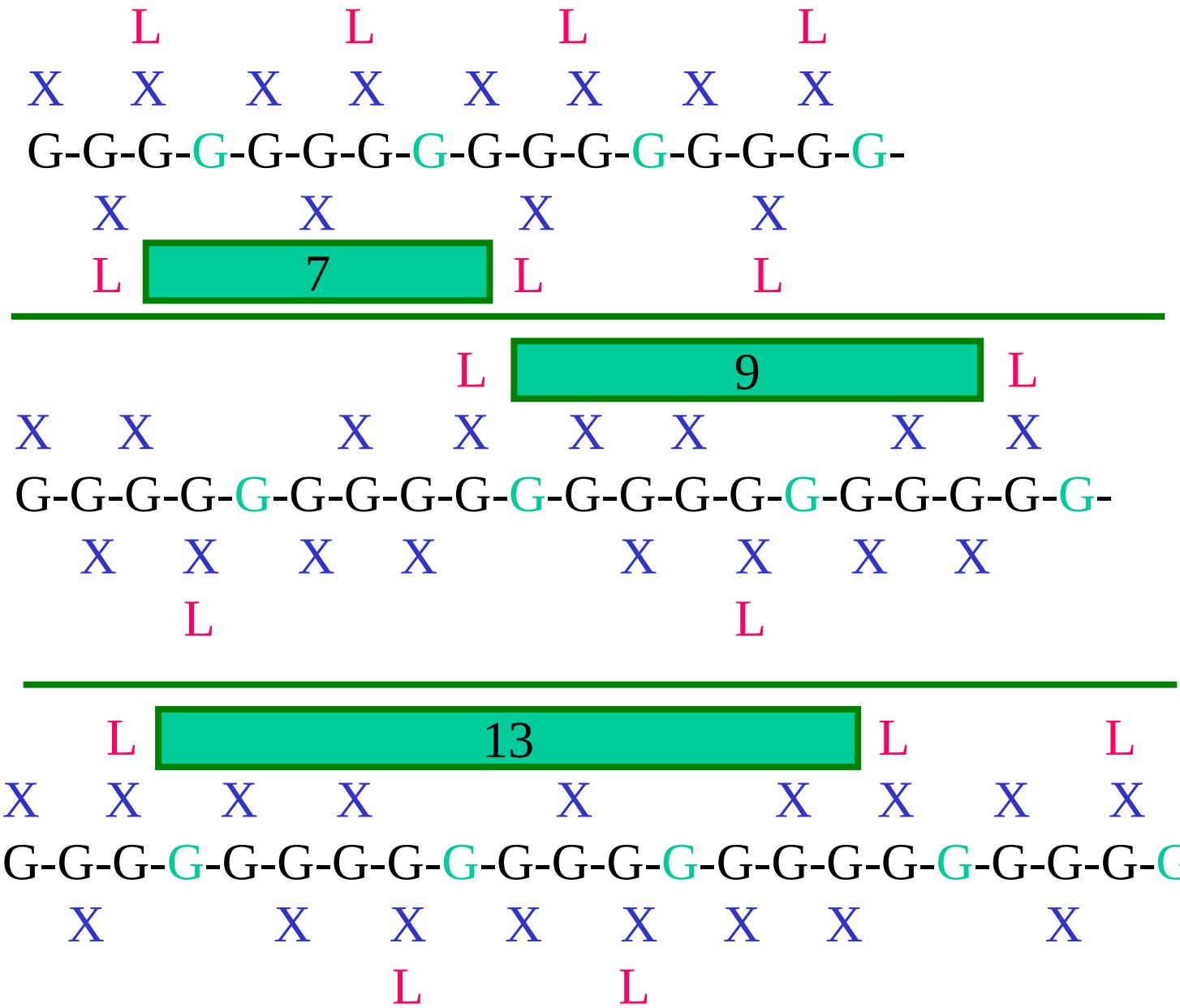
Figure 5. ESI-MS of the dimers of limit digest oligosaccharides of xyloglucan from *H. courbaril*. Some of the ions are indicated, as well as the number of glucoses in their main chain, showing the preferential alternation of four and five-glucose oligosaccharides. The line over XXXXG means that the galactosyl residue can be in any of the "X" indicated.

Table 1. Distribution of dimers of *Hymenaea* xyloglucans and the relative proportions of galactosylated residues based on electrospray MS.

	XXXG-XXXG		XXXG-XXXXG		XXXXG-XXXXG
Molar Ratio	3.00	:	2.00	:	1.00
	No. of Gal residues (Mole%)				
0	19.3		**29.9**		**26.9**
1	17.8		18.2		**24.5**
2	**21.2**		13.7		11.6
3	**28.0**		**26.9**		14.5
4	13.7		11.3		**22.6**

XXLGXXLG	**XXXGXXXXG**	**XXXXGXXXXG**
XLLGXXLG	XXLGXLXLG	XXXXGXXXLG
		XXLLGXXLLG

Possible structures of dimers above 20%. XLs (underlined) here theoretically positioned in the middle of the molecule



4 Glc based chain having XLLG as the main oligosaccharide

5 Glc based chain having XXXLG as the main oligosaccharide

Alternating 4/5 Glc based chain having XXLGXXXXG

Weak Strong medium

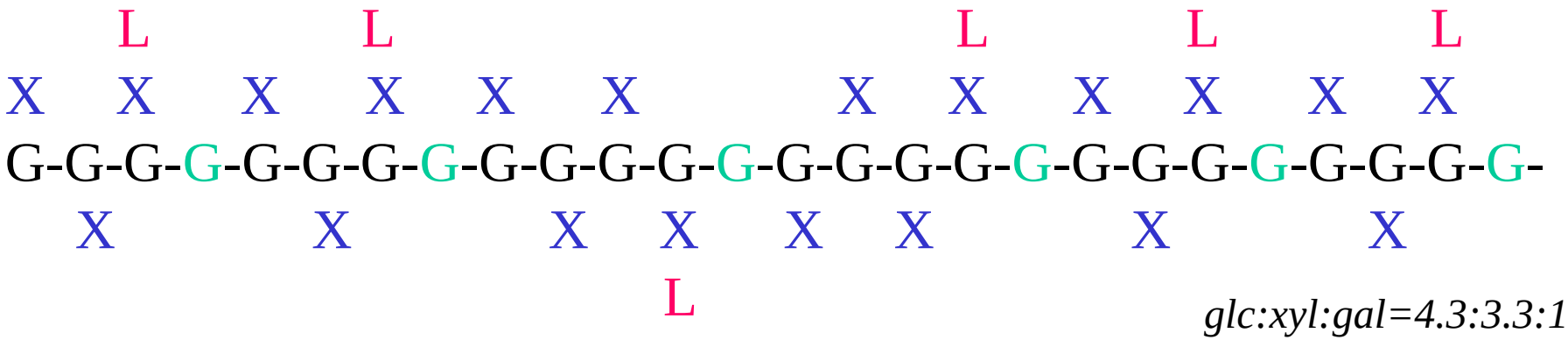
Table 2
Stochastic and directed arrangements of xylocellotetraosyl and xylocellopentaosyl units in xyloglucan polymers

Model polymer	T-T:T-P + P-T:P-P
	8:8:2
	6:12:0
	11:2:5
	10:4:4
T-T-P-P-T-T-T-T-P-P-T-T-T-T-P-P-T-T	9:6:3
T-T-P-P-T-T-P-P-T-T-P-P-T-T-T-T-T-T	9:6:3
T-T-P-P-T-P-P-T-P-P-T-T-T-T-T-T-T-T	9:6:3
	9:6:3

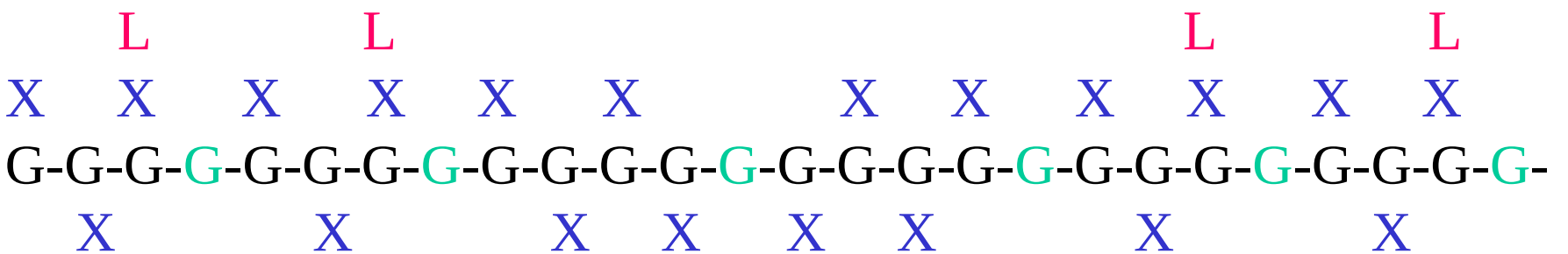
Stochastic (random) arrangements give a 4:4:1 ratio of dimers based on a xylocellotetraose:xylocellopentaosyl ratio of 2:1. The 3:2:1 ratio observed demands a more directed arrangement. Arrangements containing the repeating unit T-P-P-T spaced by additional T units gives such a ratio; when considering that the *endo*-β-glucan hydrolase may cleave between each unit oligomer, the tetramer T-P-P-T will always give a 2:1 ratio of T-P + P-T:P-P. The three P-P units placed anywhere within the stretch of twelve T units gives the 3:2:1 ratio. Ps are in bold to show clearly their position in the sequences and distinguish them from Ts.

Tine, Silva, Lima, Carpita and Buckeridge (2006) Carbohydrate Polymers, 66: 444-454.

Assuming that TTPPTT would be the combination with higher probability of occurrence, the examples below illustrate possible average molecules of *Hymenaea* xyloglucan

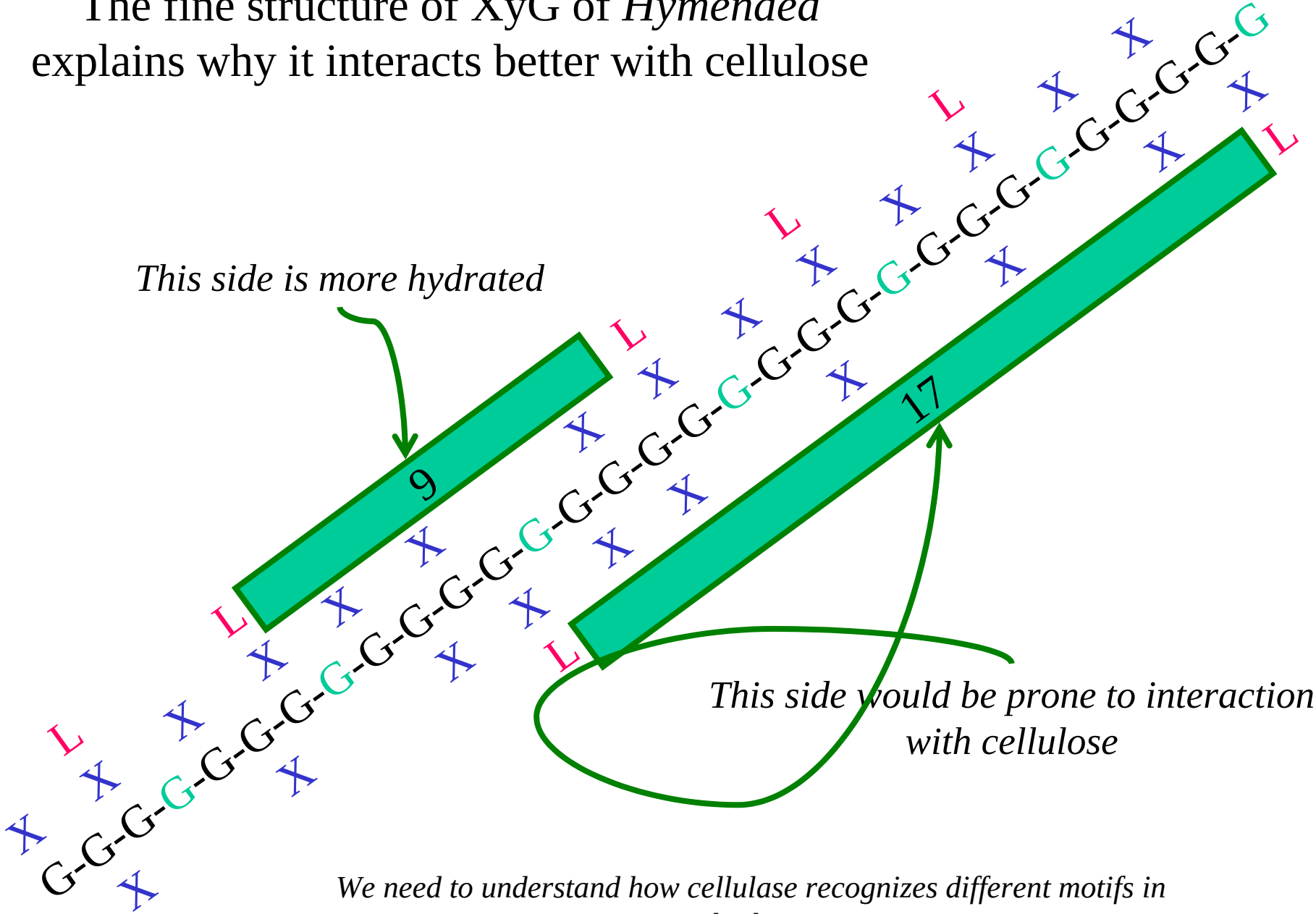


glc:xyl:gal=4.3:3.3:1



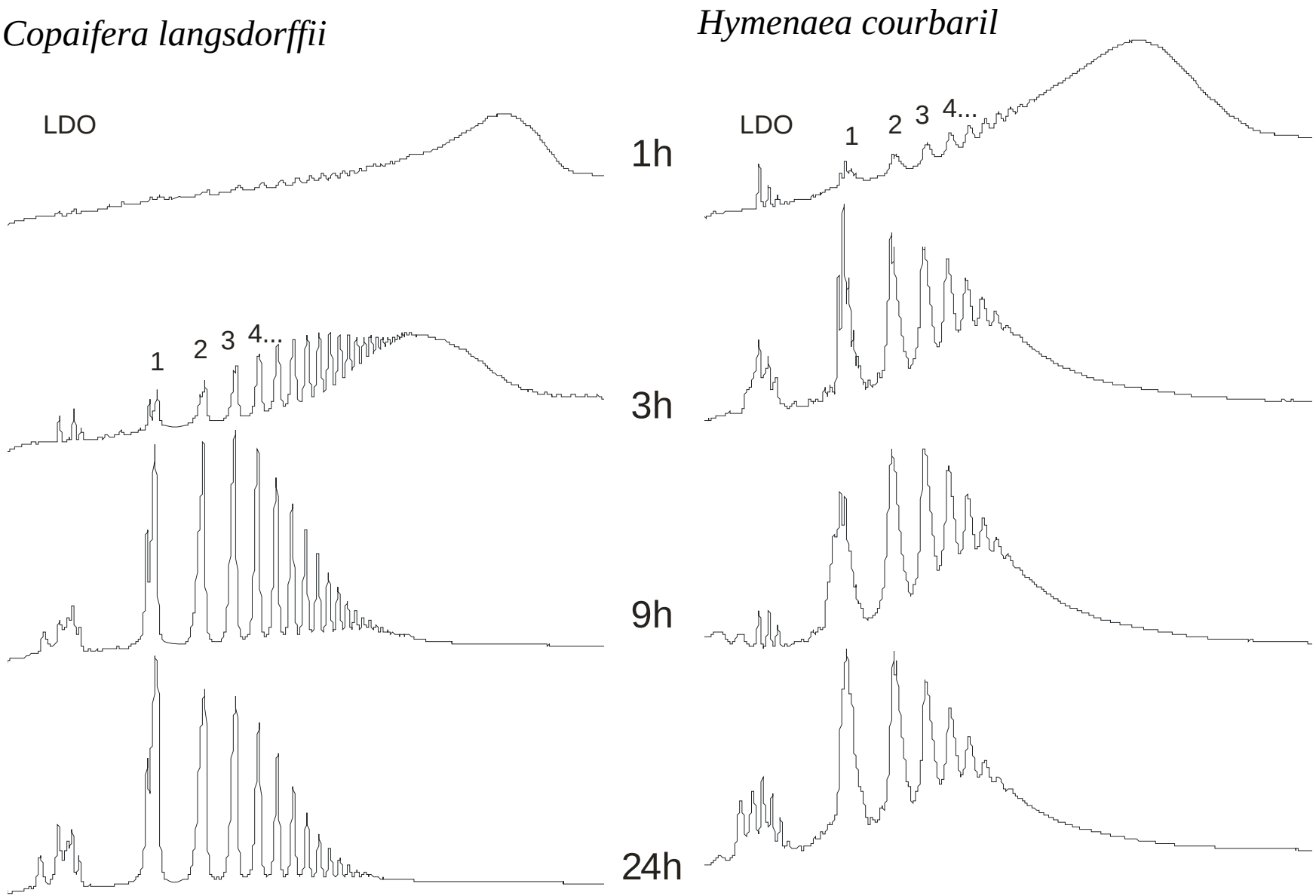
glc:xyl:gal=6.5:5:1

The fine structure of XyG of *Hymenaea* explains why it interacts better with cellulose



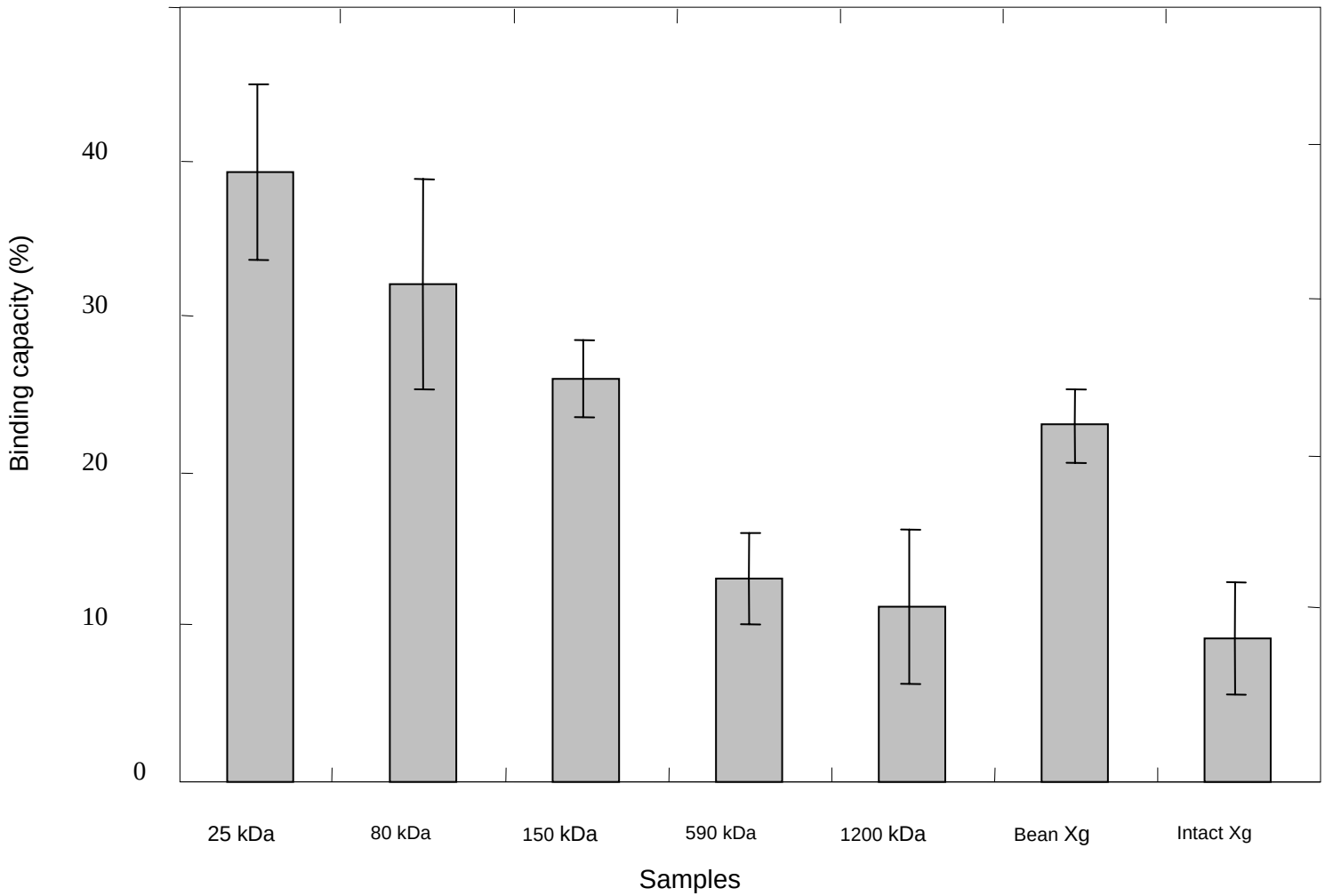
We need to understand how cellulase recognizes different motifs in xyloglucan

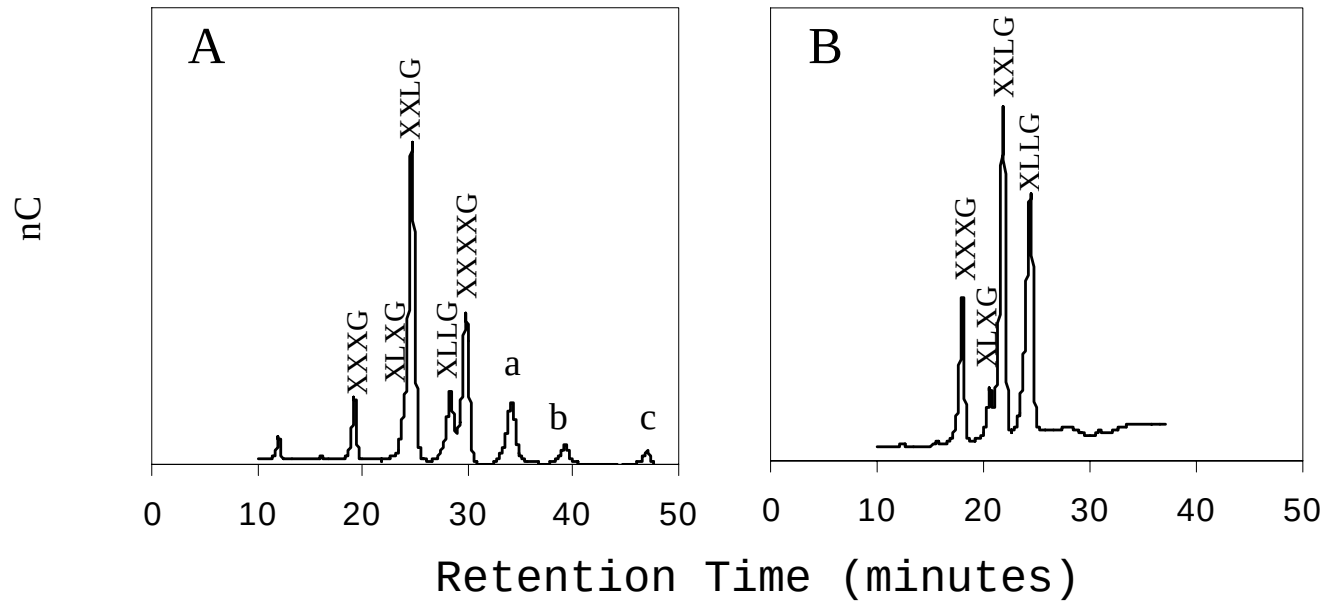
Restriction hydrolysis of xyloglucans with *Trichoderma* cellulase



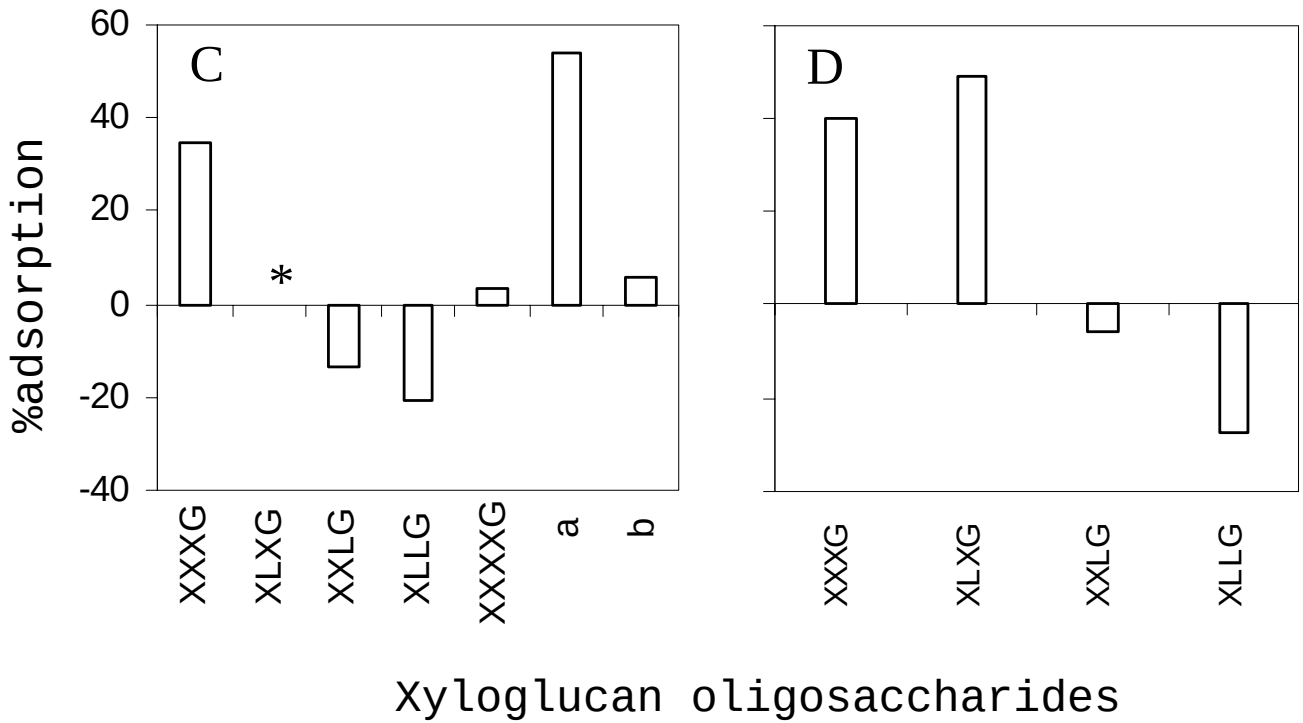
Tiné, De Lima and Buckeridge, 2003, Carbohydrate Polymers, Vol 52 p.135

Interaction of *Hymenaea* xyloglucan with cellulose





a=XXXLG
b=XLXXG
c=XXLXG



Possible meaning of fine structure of xyloglucan

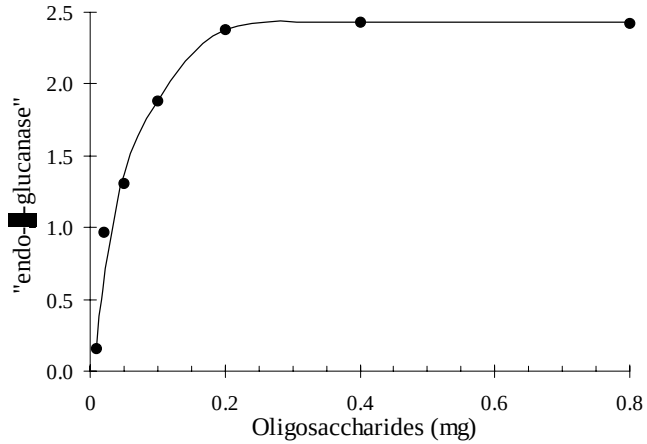
- *Hymenaea* XyG is NOT composed of a random mixture of monosaccharides
- It appears to have structural domains that determine how the polymer interacts with enzymes
- ***LG are low interactive motifs and probably contain a message for hydrolase binding***

Adaptive factors at the molecular level

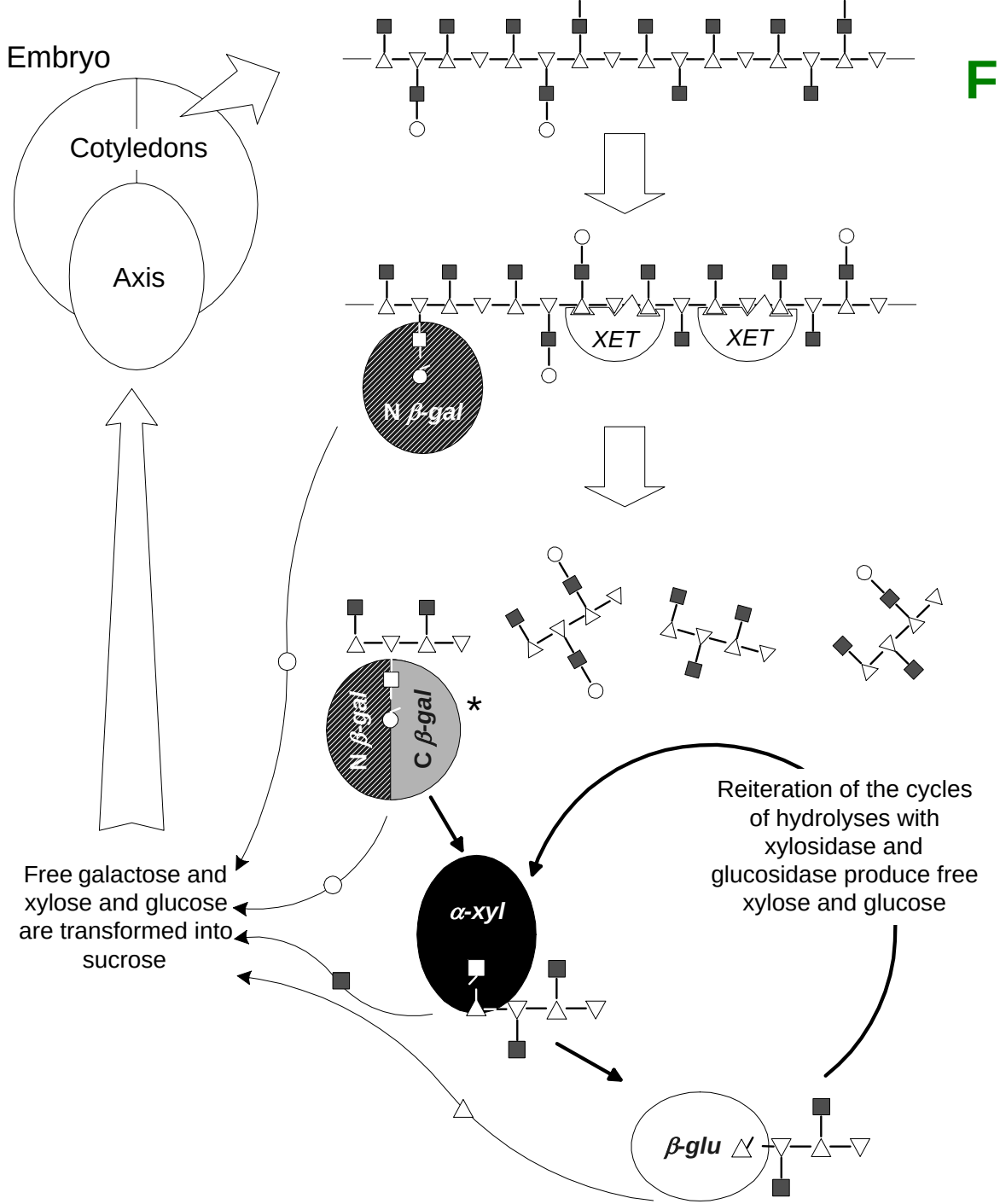
- Degradation of XyG of *Hymenaea* **is faster**;
- Degradation is controlled by changes in conformation that are temperature-dependent;
- Small molecules bind more strongly to cellulose and to themselves;
- LG motifs seem to be related to low intermolecular interactivity and could mark susceptibility to hydrolysis
- Intermolecular interaction increases packing, determined by fine structural features so that seed can accumulate more carbon.

Biochemistry of xyloglucan degradation

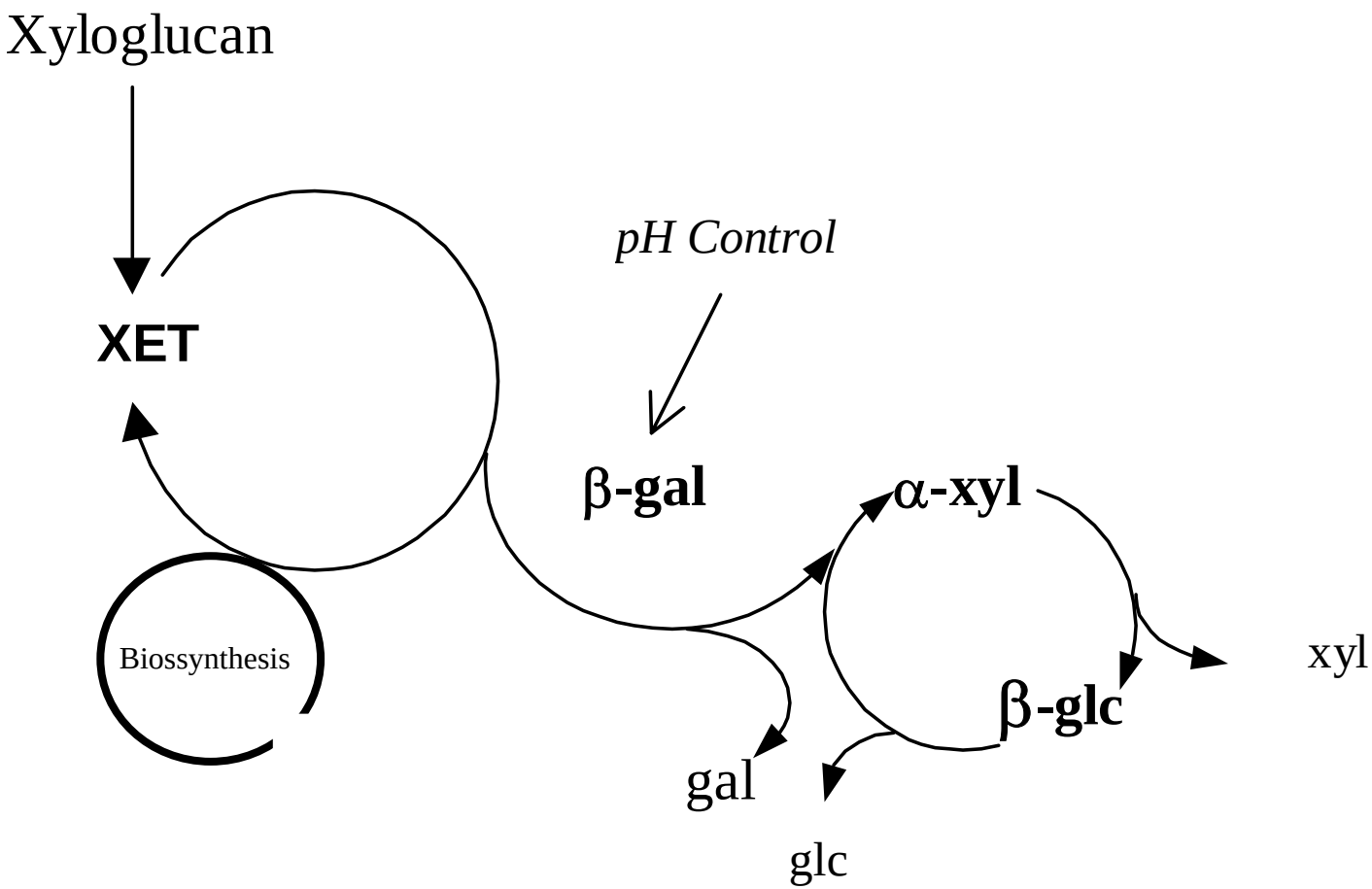
Features of Hymenaea



Enzyme	Range of pH optima
□-xylosidase	4-5
□-glucosidase	3-3,5 e 4,5
□-galactosidase	3-3,5
XET	4-7

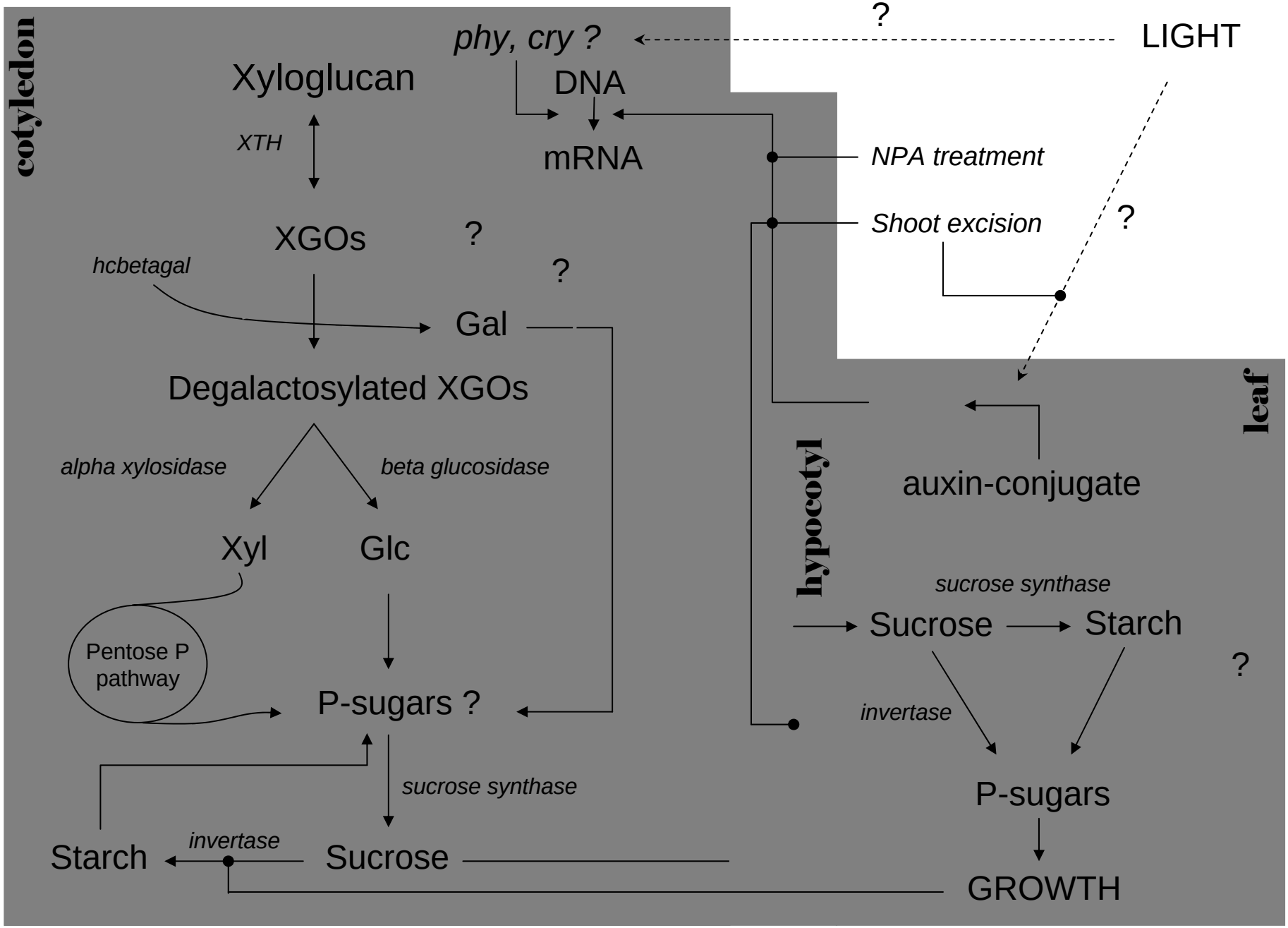


XILOGLUCAN MOBILIZATION IN *HYMENAEA*

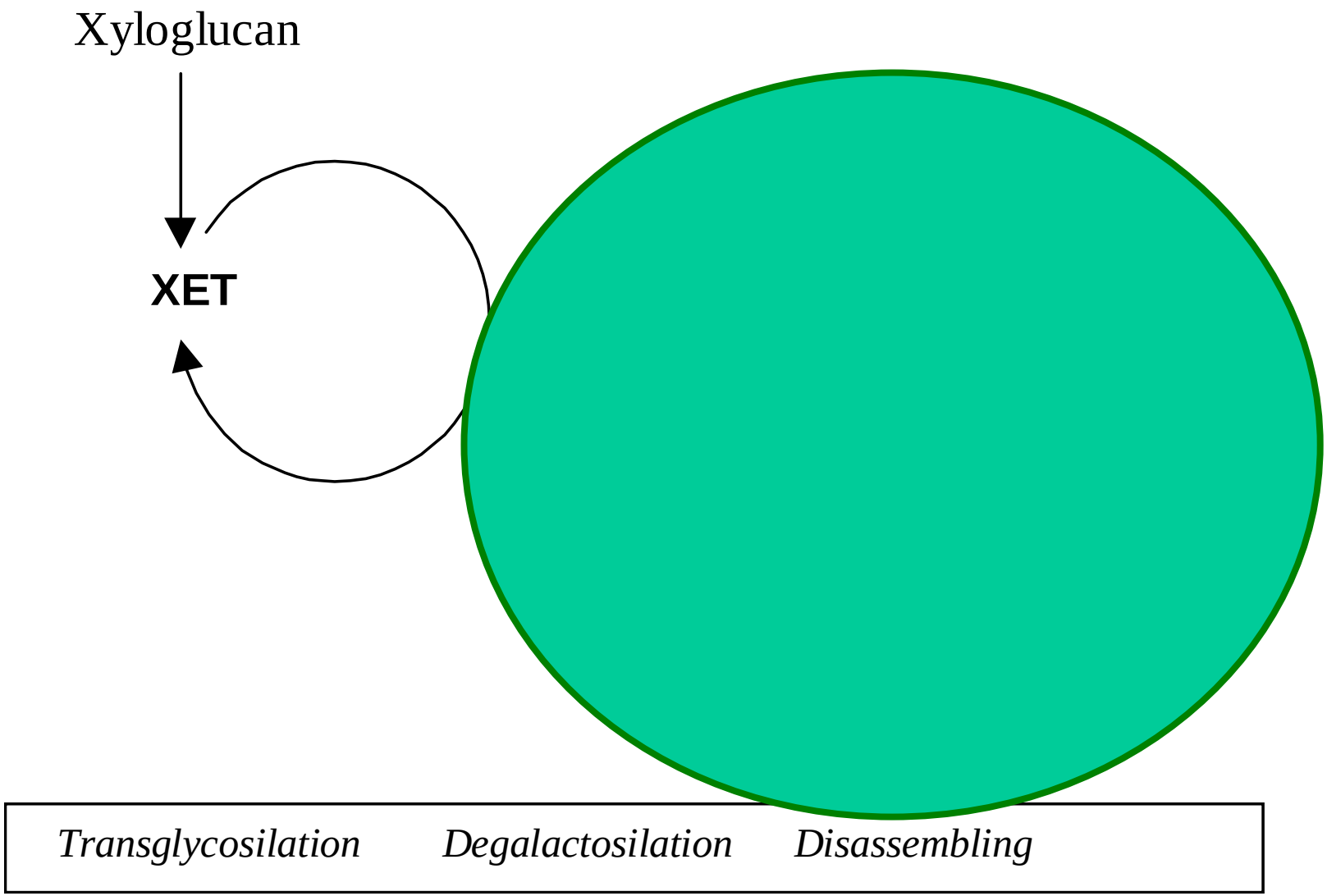


Transglycosilation *Degalactosilation* *Disassembling*

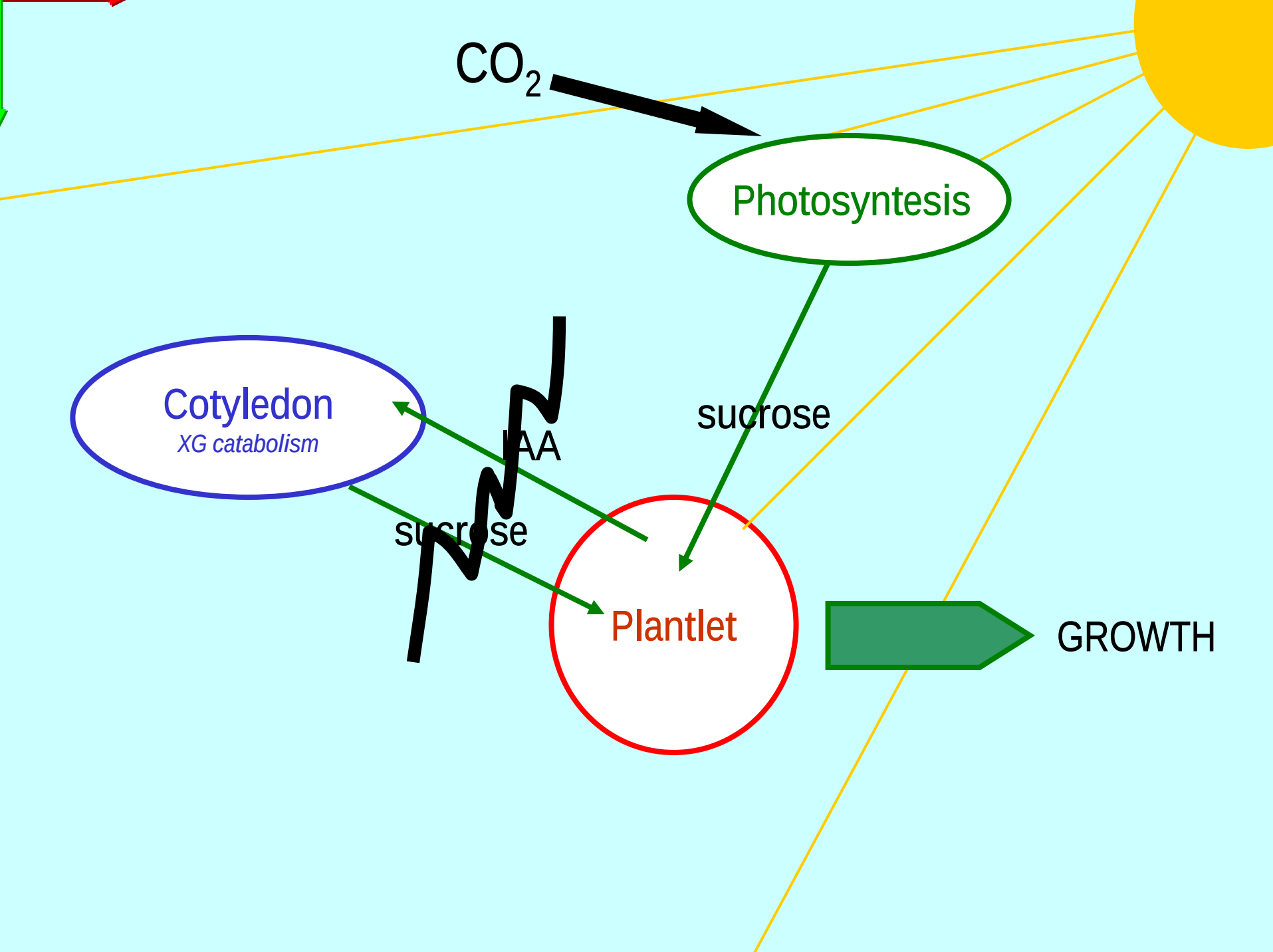
Buckeridge, Santos & Tiné, 2000 Plant Physiol. Biochem. 38:141-156

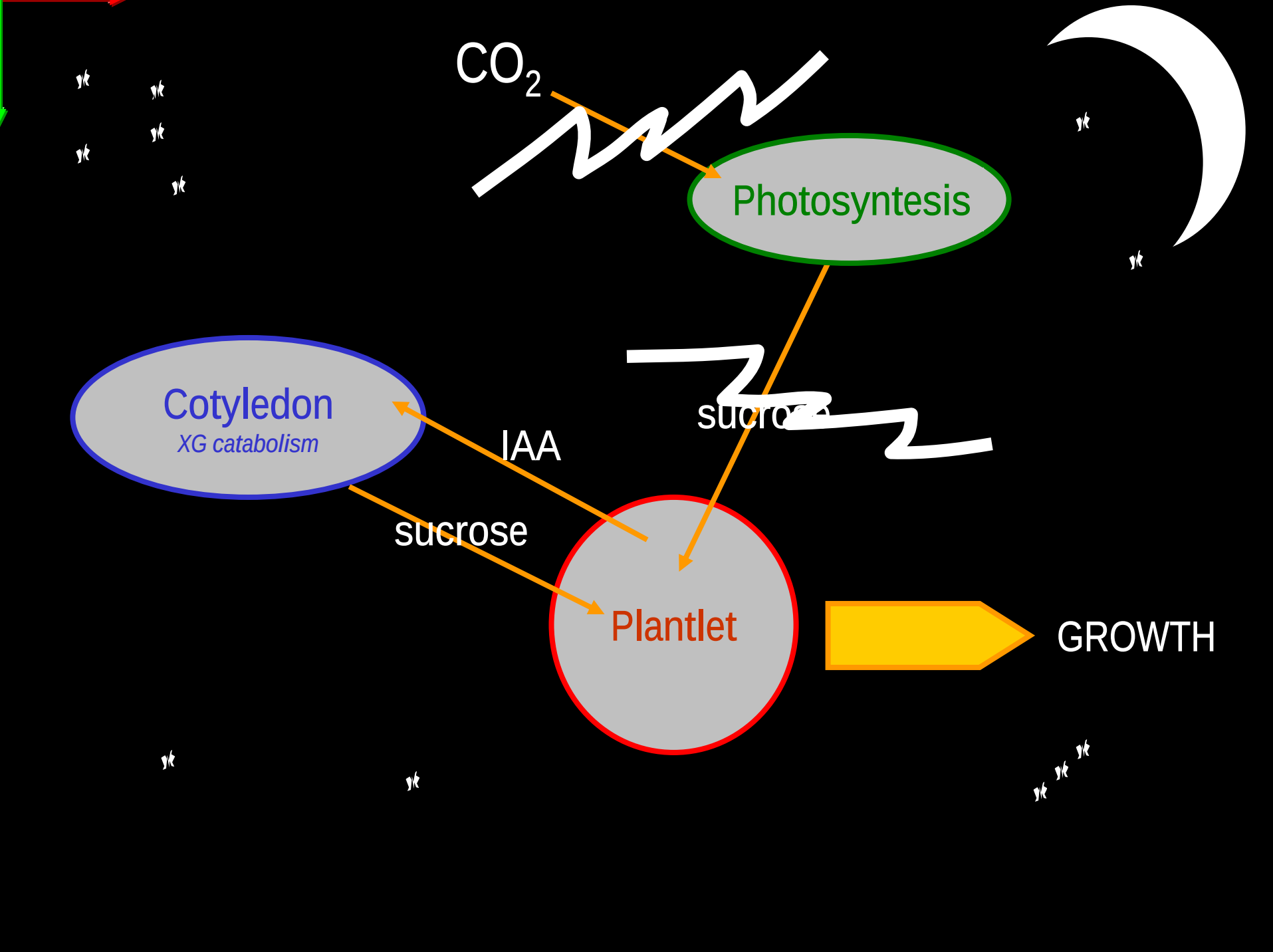

cotyledon
phy, cry ?
?
LIGHT
Xyloglucan
DNA
XTH
mRNA
NPA treatment
Shoot excision
?
XGOs
?
?
hcbetagal
Gal
Degalactosylated XGOs
leaf
hypocotyl
alpha xylosidase
beta glucosidase
auxin-conjugate
Xyl
Glc
sucrose synthase
Sucrose
Starch
?
Pentose P pathway
P-sugars ?
invertase
sucrose synthase
P-sugars
Starch
invertase
Sucrose
GROWTH

Hydrolysis of *Hymenaea* xyloglucan



Buckeridge, Santos & Tiné, 2000 Plant Physiol. Biochem. 38:141-156


CO_2
Photosyntesis
Cotyledon
XG catabolism
IAA
sucrose
sucrose
Plantlet
GROWTH

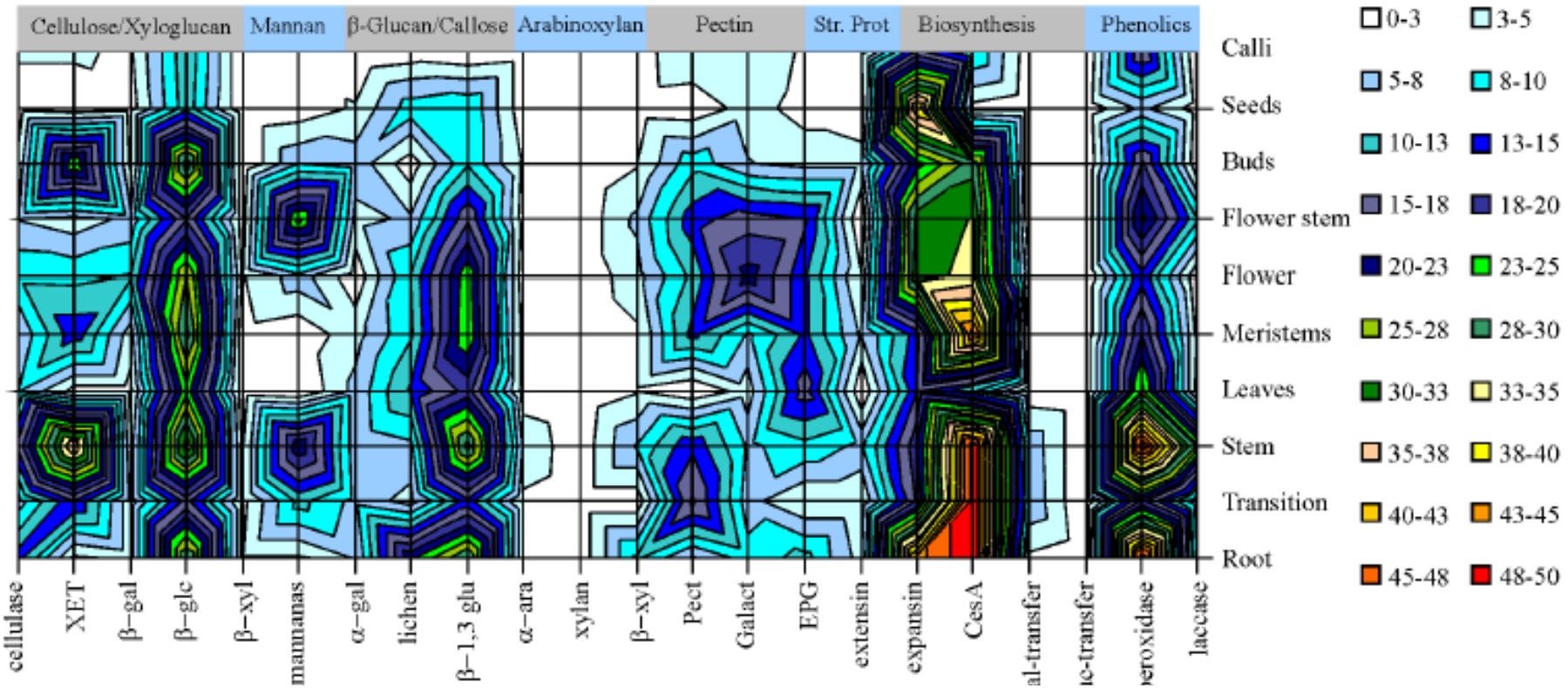

CO_2
Photosyntesis
Cotyledon
XG catabolism
IAA
sucrose
sucrose
Plantlet
GROWTH

ADAPTIVE FACTORS AT THE METABOLIC LEVEL

- Degradation is highly regulated (inhibition, pH, temperature, hormones);
- Expression of the genes is regulated by circadian rhythm;
- Cell wall degradation can be arrested in spite of metabolic inertia;
- There is no transient accumulation of products.

IS IT POSSIBLE TO USE ENDOGENOUS SYSTEMS OF CELL WALL DEGRADATION FOR SECOND GENERATION ETHANOL APPLICATIONS IN SUGARCANE?

From 1999 to 2001, the SUCEST genome program produced 238,000 ESTs from various tissues of the sugar cane plant.



Since then we found:

1) 469 cell wall related genes in different cane tissues (*Lima et al. 2001, GMB*)

1) Determined the chemical composition and structure of the cell wall polymers of different sugarcane tissues

THANK YOU

msbuck@usp.br

INCT BIOETANOL

http://msbuckeridge.wordpress.com