



# Functional Genomics in *Picea glauca*

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Université Laval, Sciences du bois et de la forêt

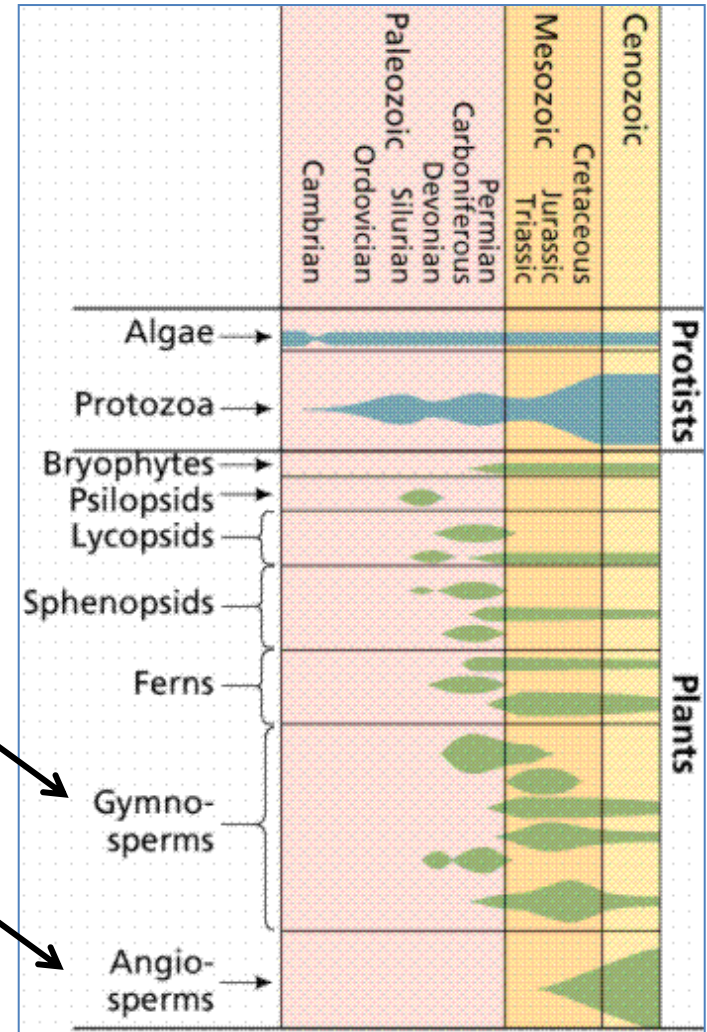
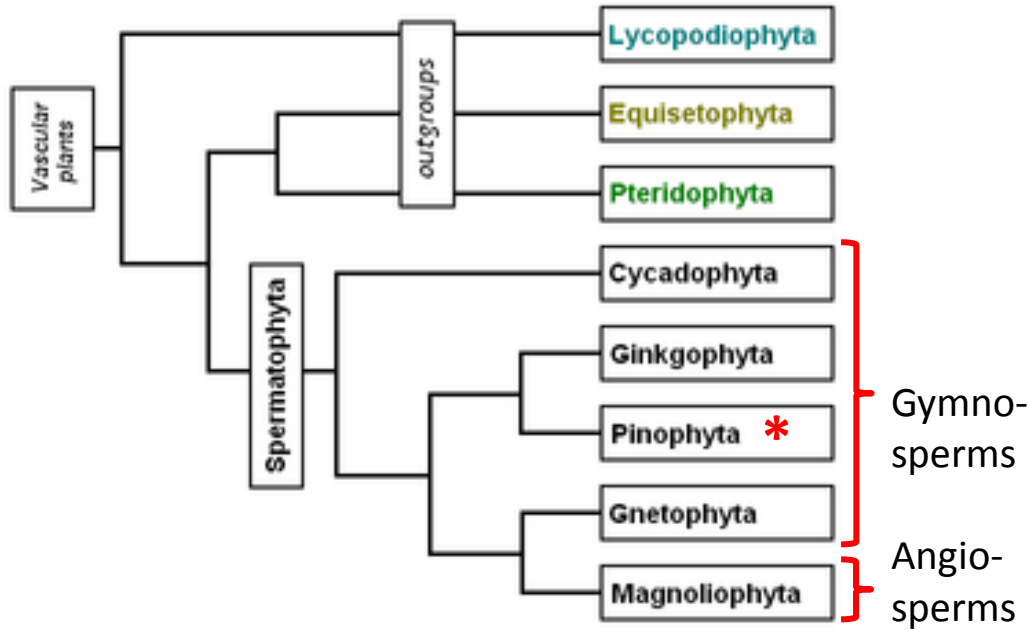
University of Oxford, Plant Sciences

February 21, 2014

# 1. Conifer Genomes and their Evolution

Gymnosperms: 'ancient' lineages of seed plants

Shared and distinct features compared to flowering plants (angiosperms)





# Outline

1. Transcriptional regulation and gene networks
2. Discovery and analysis of a gene associated with resistance to the spruce budworm



# Gene Catalogue & Resource Development:

## Transcriptome sequencing In White spruce :

Rigault et al. (2011) *Plant Physiology*. 157: 14

	Sampled (Coverage)	Predicted total
Number of expressed genes	27,720*	32,720
Transcriptome (Mb) <i>Sanger</i>	30.15	41.75**
<i>Next-Gen</i>	7.8	
<b>Total</b>	<b>37.95</b>	<b>47</b>

\*85% of genes represented by FL-cDNA inserts, 40% contain complete CDS

\*\*Predicted size of sampled transcriptome based on mRNA sequence completion & CDS analysis

Microarray: 23,853 genes

PiceaGenExpress

Raherison et al. 2012 BMC  
Genomics 13: 434

Transcriptome  
resequencing:

- SNP discovery

Genotyping chips:

- Linkage mapping
- Association studies

# 1. Transcriptional regulation and gene networks

Bomal C., Duval I., Giguère I., Fortin É., Caron S., Boyle B., Séguin A., MacKay J. 2014. **Opposite action of R2R3-MYBs from different subgroups on common key genes from shikimate and monolignol pathways in spruce.** *Journal of Experimental Botany*, 65(2):495-508

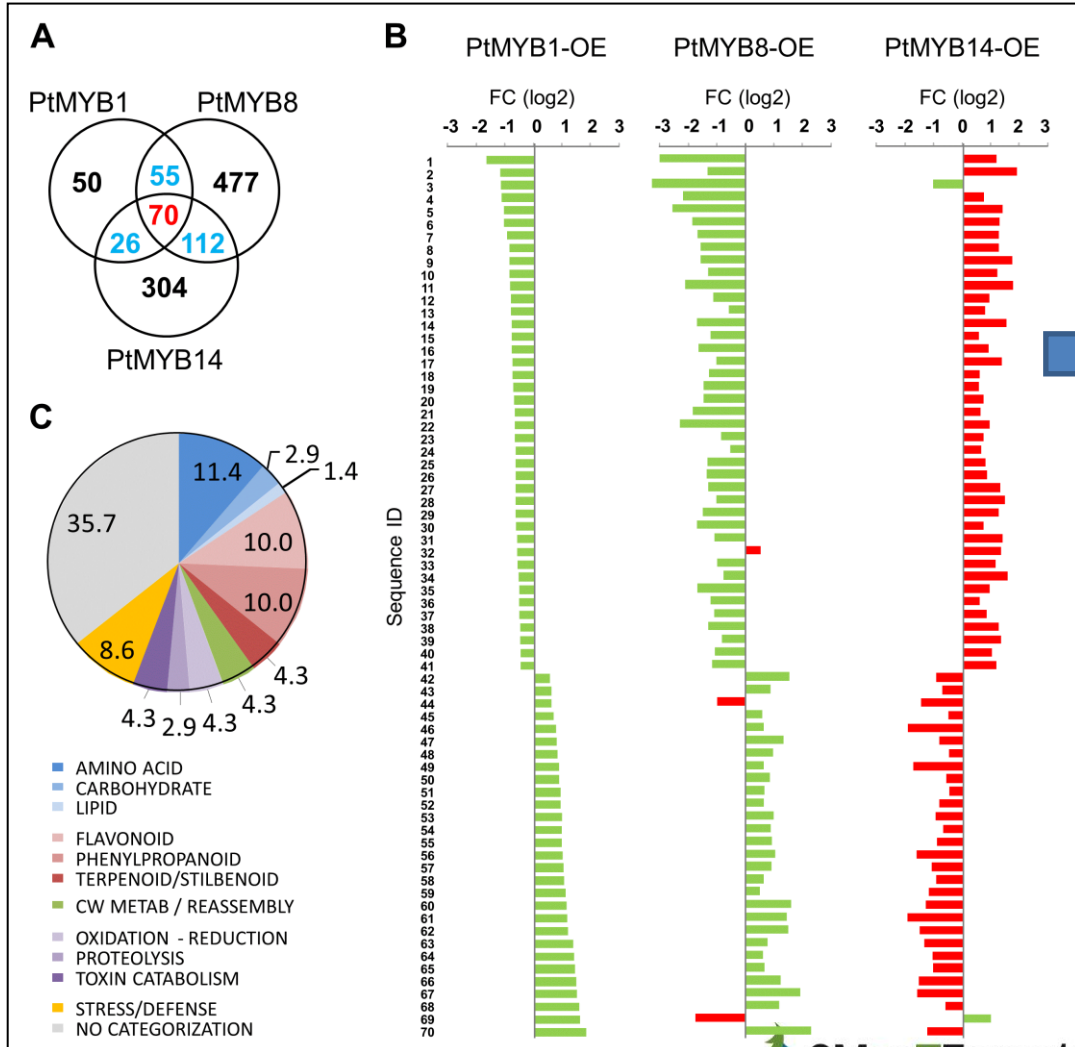
Duval I, Lachance D, Giguère I, Bomal C, Morency M-J, Pelletier G, Boyle B, MacKay J, Séguin A. 2014. **Large-scale screening of transcription factor – promoter interactions in spruce revealed a transcriptional network involved in vascular development.** *Journal of Experimental Botany*, accepted for publication



# 1.1 - Opposite effects of MYB genes

## 1. Transgene expression

### Microarray profiling



## 2. Wild-type expression

### RT-qPCR

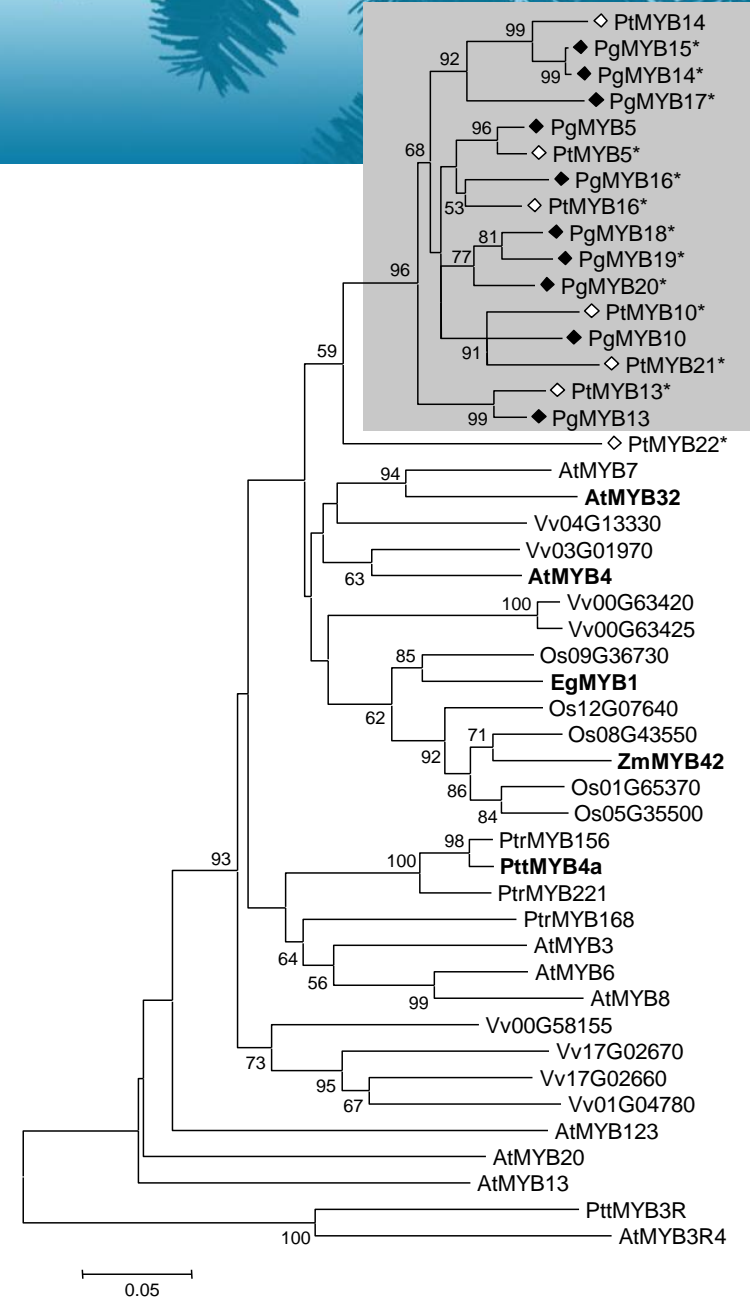
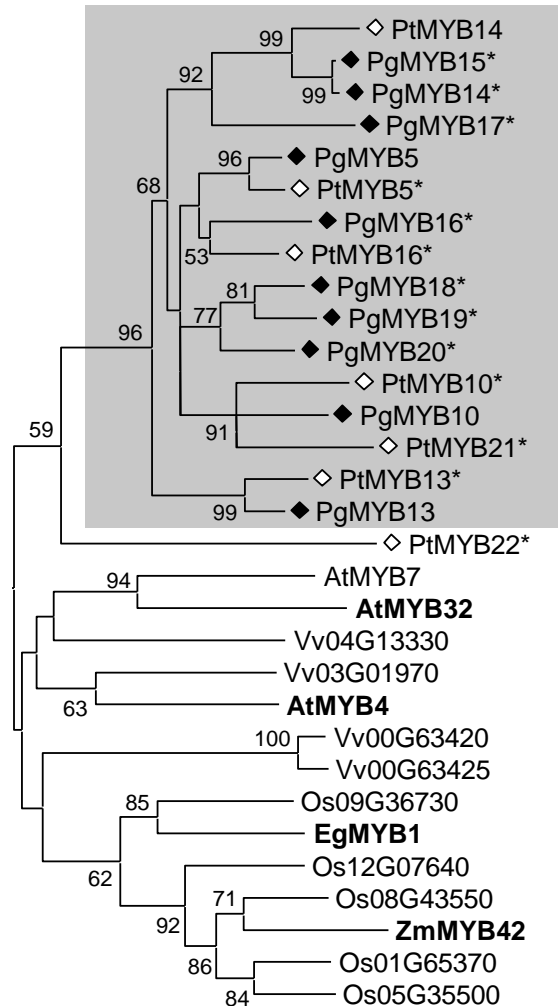
### Co-expression in vascular tissues

*MYB1 & MYB8*  
*MYB14 & MYB15*  
Vs  
*33 putative targets*

- Opposite correlations
- Tissue dependent

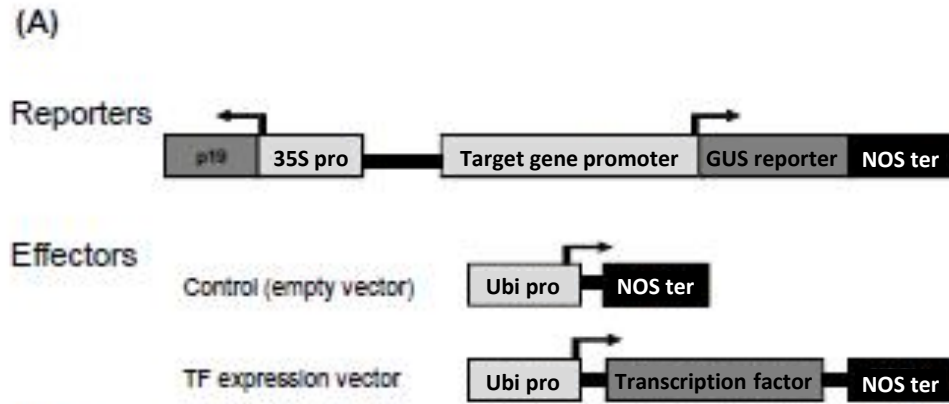
SHM4	Shikimate path
DHS2	Shikimate path
COMT	Monolignol path
4CL	Phenylpropanoid path

# R2R3-MYB Sub-group 4



# Transcriptional regulation assay

## Transient assay system



## Activation and repression

Promoter construct		PgDHS2	Pg4CL
TF vector	GS <sup>1</sup>	Ratio TF/no TF	Ratio TF/no TF
PgMYB8	spot	9.29	11.85
	density	10.63	12.85
PtMYB8	spot	2.65	5.57
	density	3.26	5.56
PtMYB1	spot	3.30	5.31
	density	3.66	5.89
PtMYB14	spot	0.21	0.15
	density	0.20	0.12
PgMYB15	spot	0.08	0.00
	density	0.12	0.00

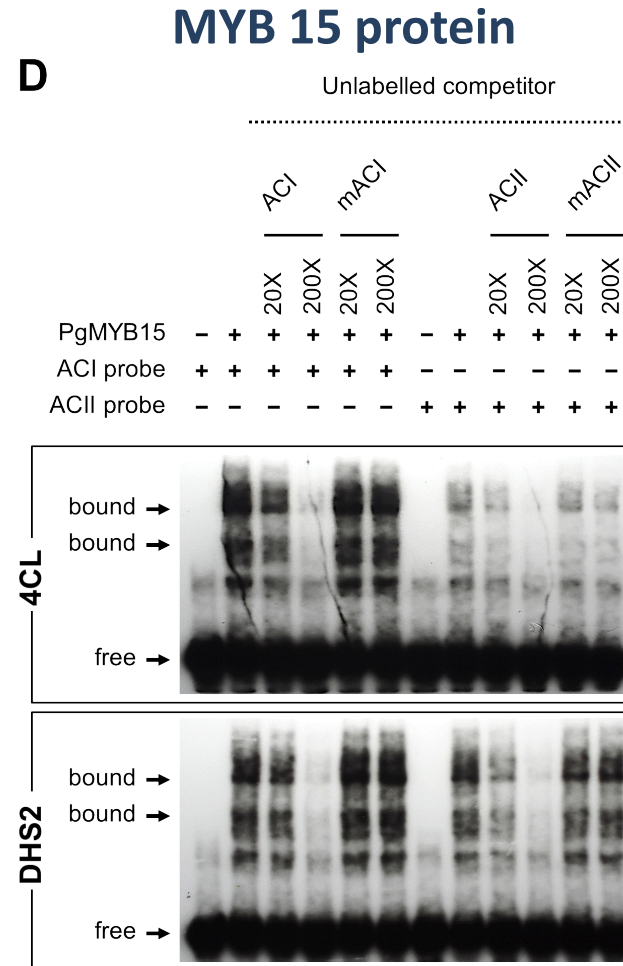
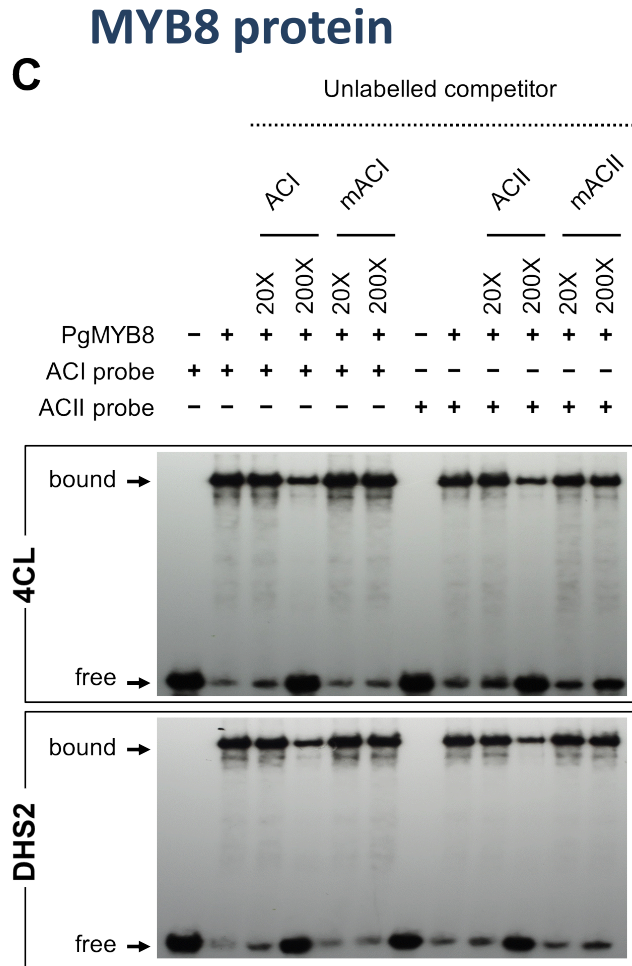
Duval et al. 2014 JExB *accepted*

Bomal et al. 2014 JExB



# DNA Binding Assay

## EMSA with MYB ACI and ACII elements from 4CL and DHS2 promoters

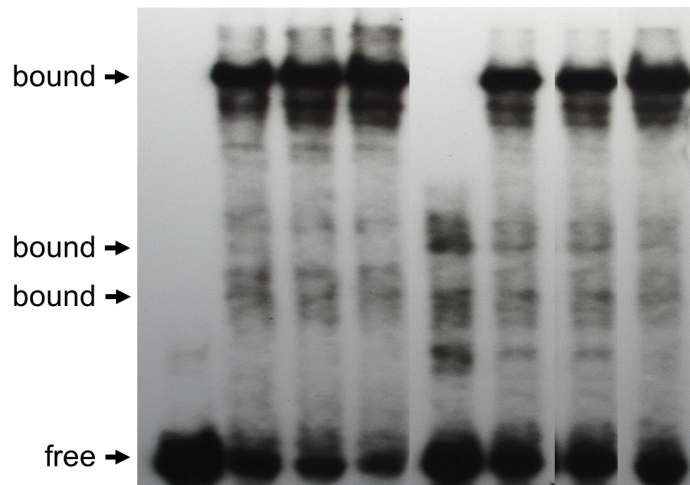


# Competitive DNA Binding Assay

## EMSA with ACI element from the Pg4CL promoter

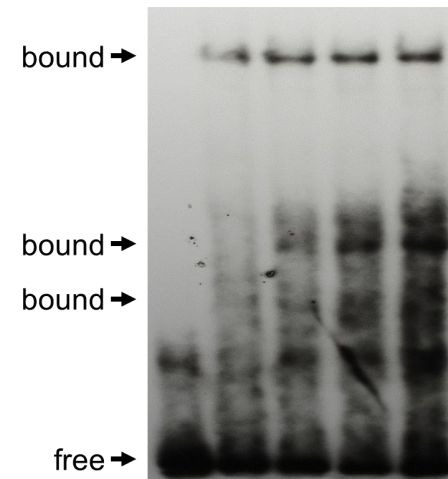
### A [MYB15] = [MYB8]

MYB15	-	-	-	-	+	+	+	+
MYB8	-	0.7X	1X	1.3X	-	0.7X	1X	1.3X

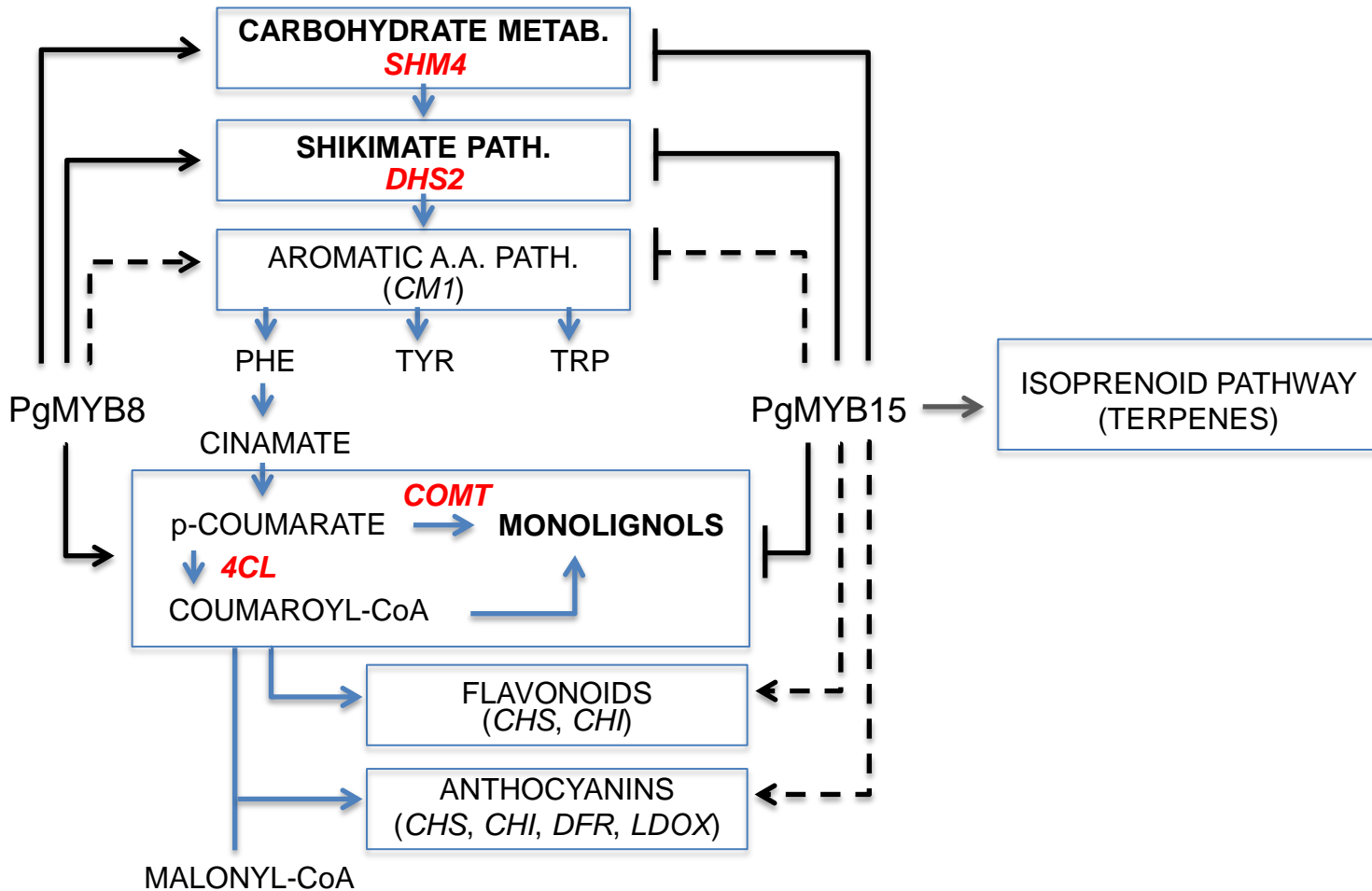


### B [MYB8] < [MYB 15]

MYB8	-	+	+	+	+
MYB15	-	-	20X	40X	60X

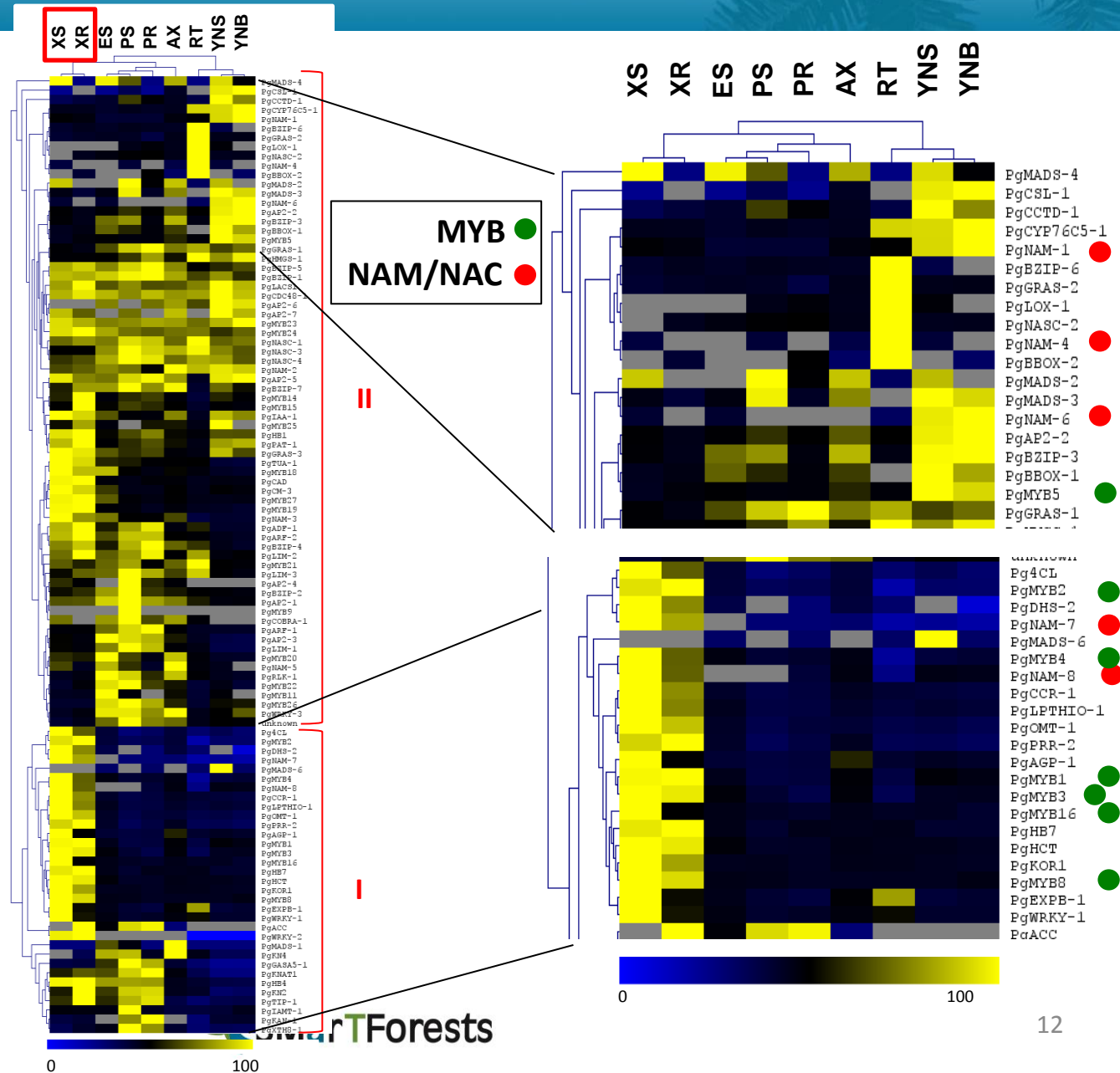
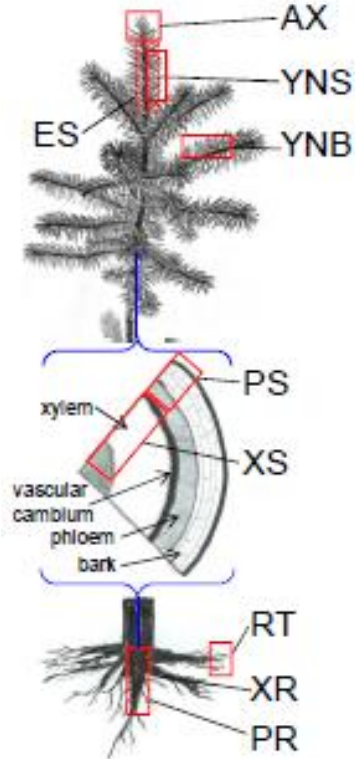


# Transcriptional MYB Network



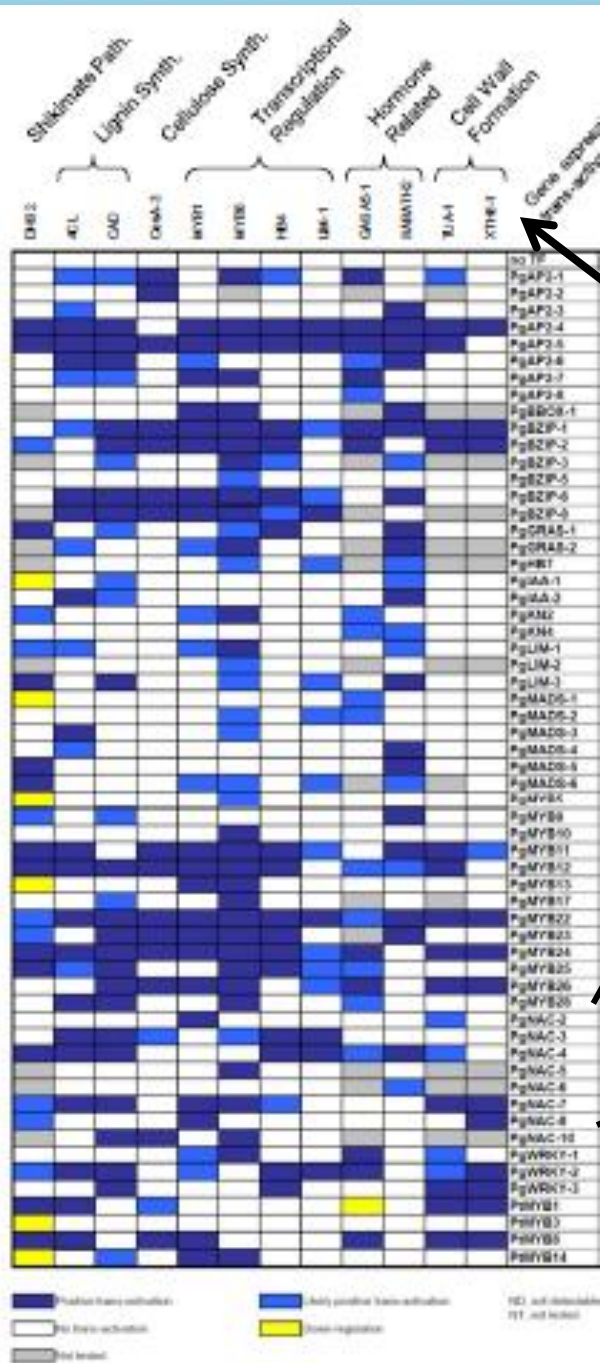
# 1.2-Functional Profiling : Secondary Cell Wall Gene Network

**Expression Profiles:**  
Transcription factors and secondary cell wall genes (RT-qPCR)





# Functional Assays



**GUS marker expression following co-transformation**

Promoters

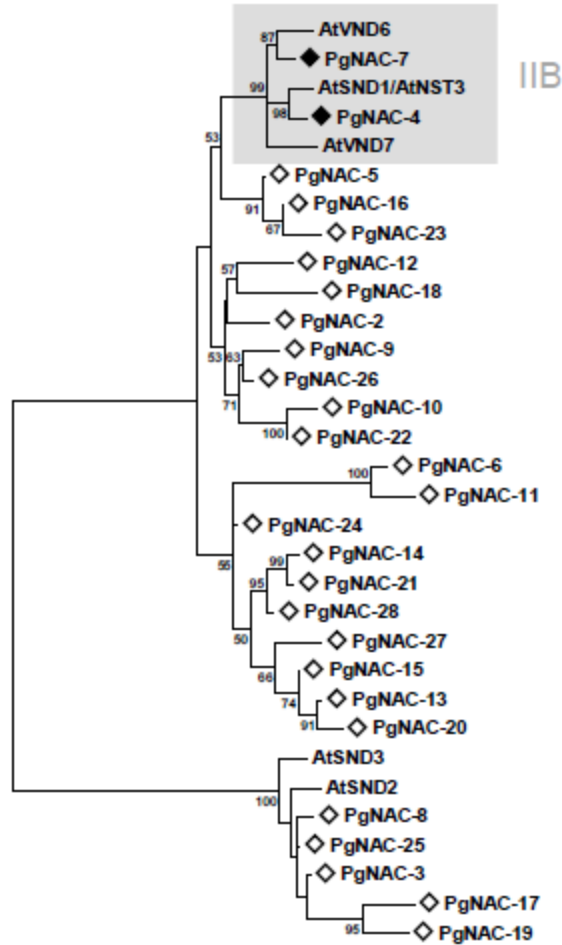
Transcription factors

PgDHS2  
Pg4CL  
PgCAD  
PgCesA-3  
PgMYB1  
PgMYB8  
PgHB4  
PgLIM-1  
PgGASA5-1  
PgSABATH2  
PgTUA-1  
PgXTH8-1

NAC-2  
NAC-3  
NAC-4  
NAC-5  
NAC-6  
NAC-7  
NAC-8

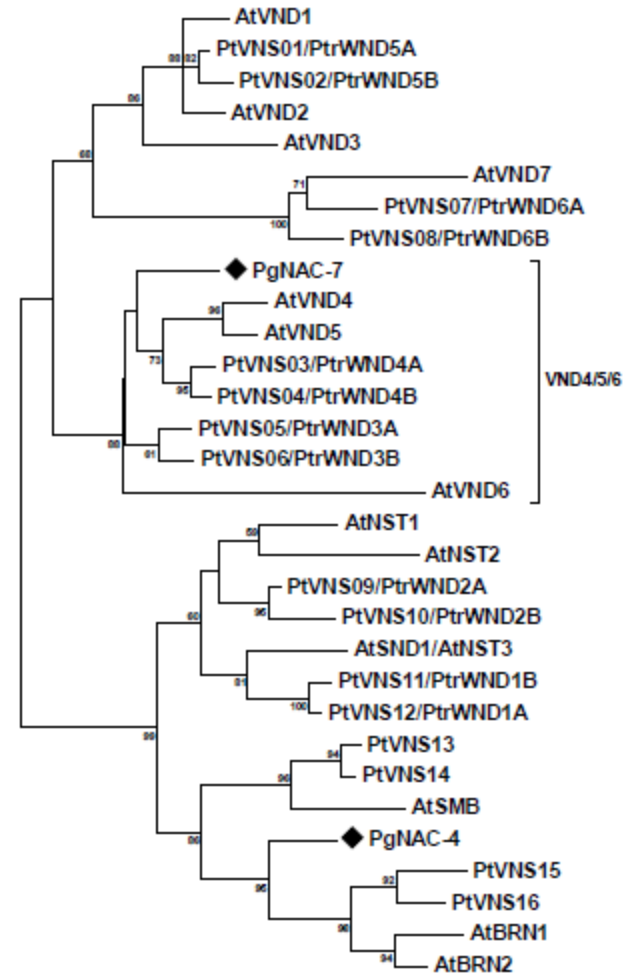
# Phylogenetic Tree of Plant NAC Genes

(A)



0.2

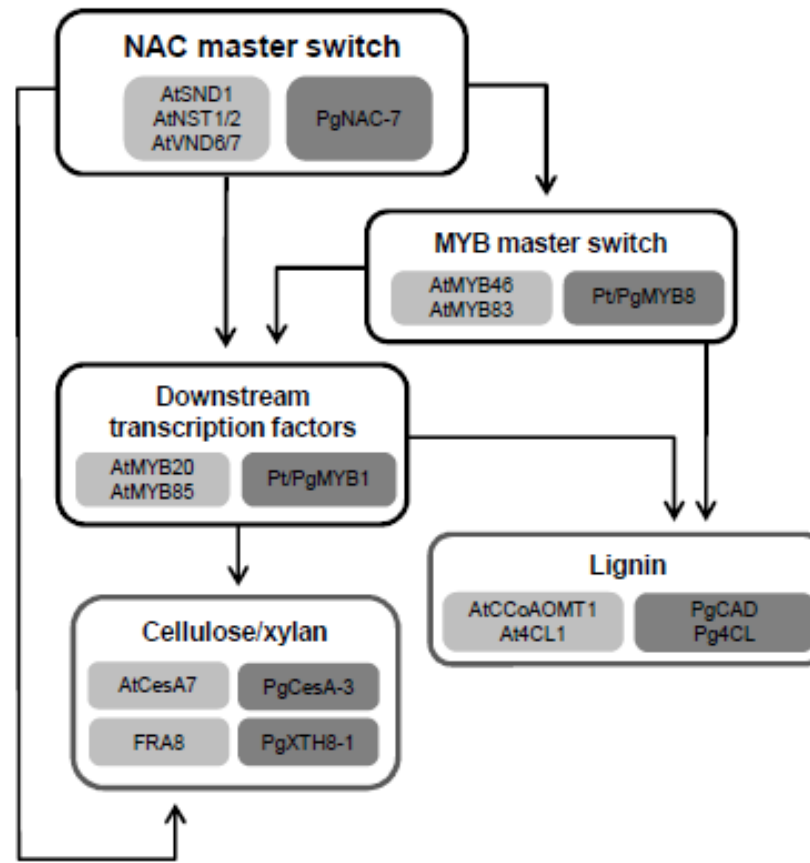
(B)



0.05

# Secondary Cell Wall Gene Network

## Transcriptional network in conifers vs Arabidopsis



## 2. Spruce Budworm Resistance

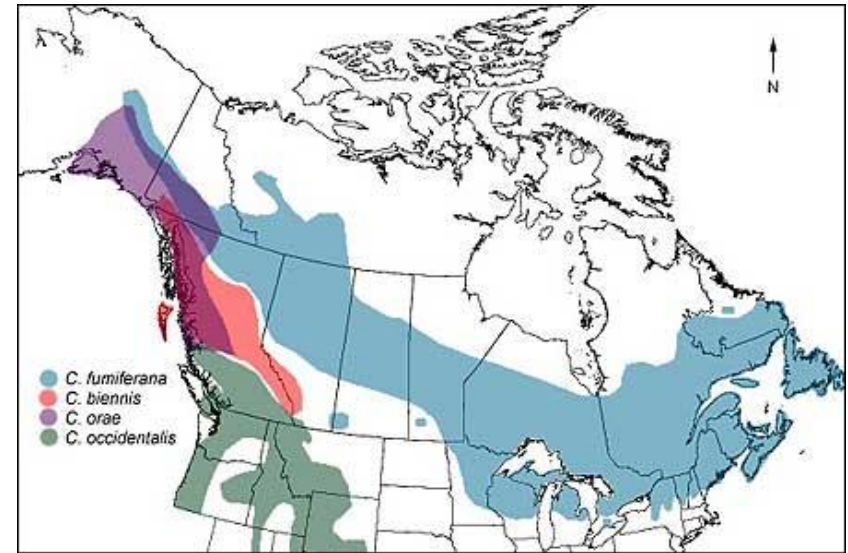
Nathalie Delvas, Melissa H. Mageroy, Geneviève Parent, Isabelle Giguère, Halim Maaroufi, Gaby Germanos, Joerg Bohlmann, Éric Bauce, John J. Mackay



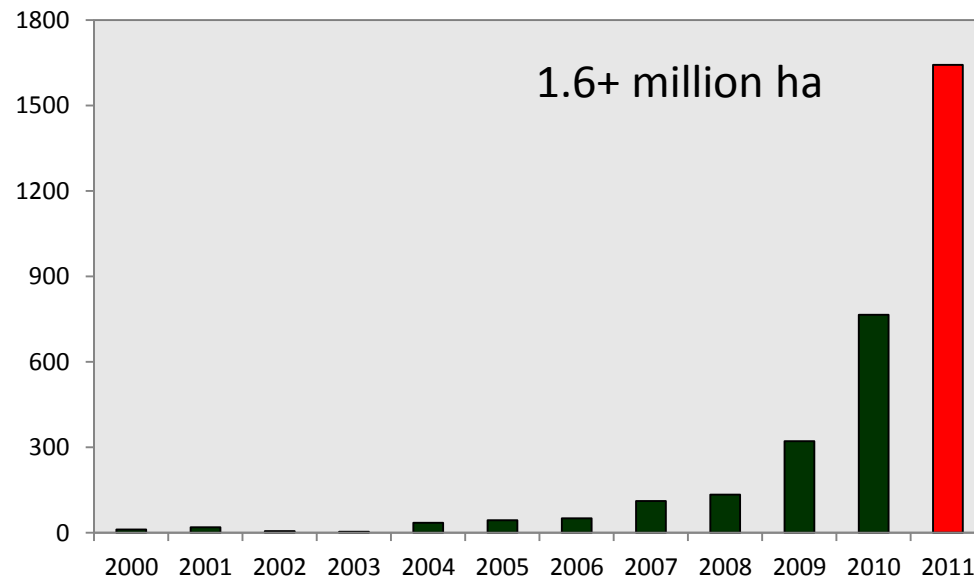
# SBW Impacts

## Spruce Budworm (SBW)

- Outbreaks are very large in scale
- 55 million hectares (550,000 km<sup>2</sup>) during last major outbreak in Québec (1970-80s)
- Caused wood losses estimated at 380 \$ millions



## SBW infested areas in Québec (1000 ha)



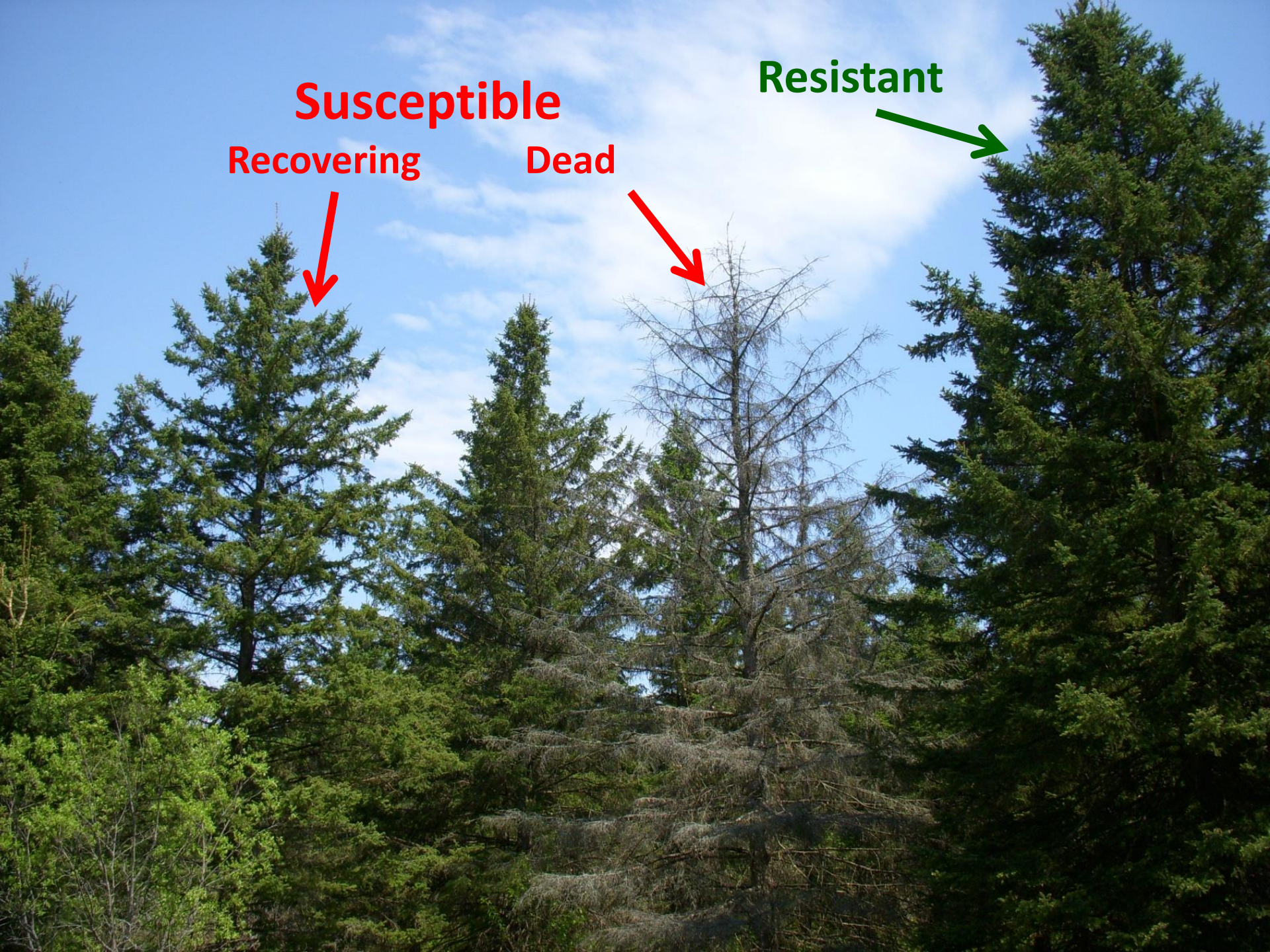


**Susceptible**

**Resistant**

**Recovering**

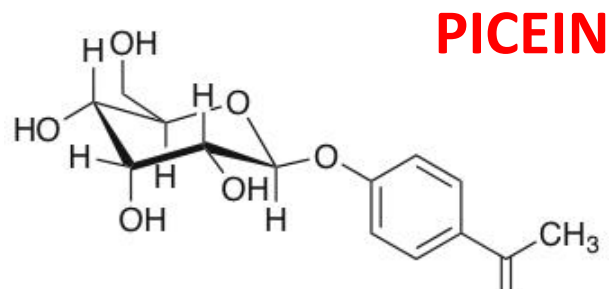
**Dead**



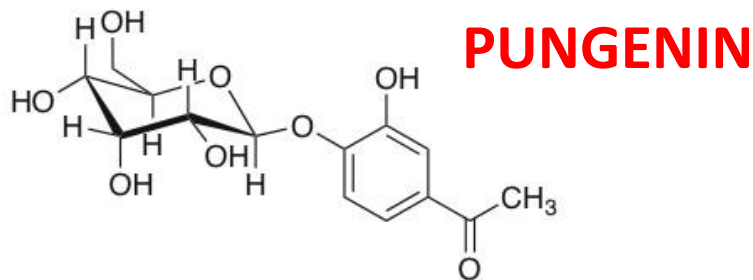


# Phenolic compounds associated with SBW resistance

## Susceptible Trees

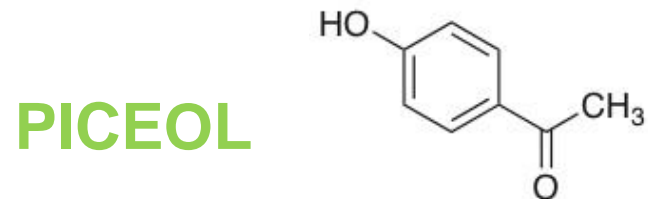


1-(4-(beta-D-glucopyranosyloxy)-phenyl)ethan-1-one

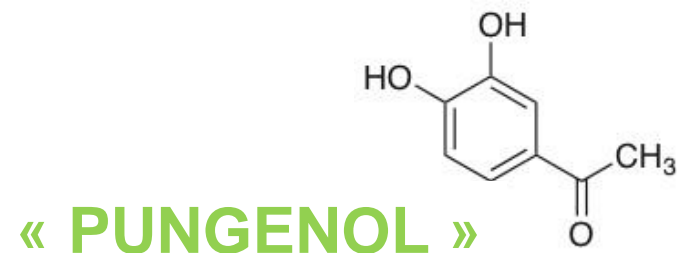


1-(4-(beta-D-glucopyranosyloxy)-4-hydroxyacetophenone

## Resistant Trees



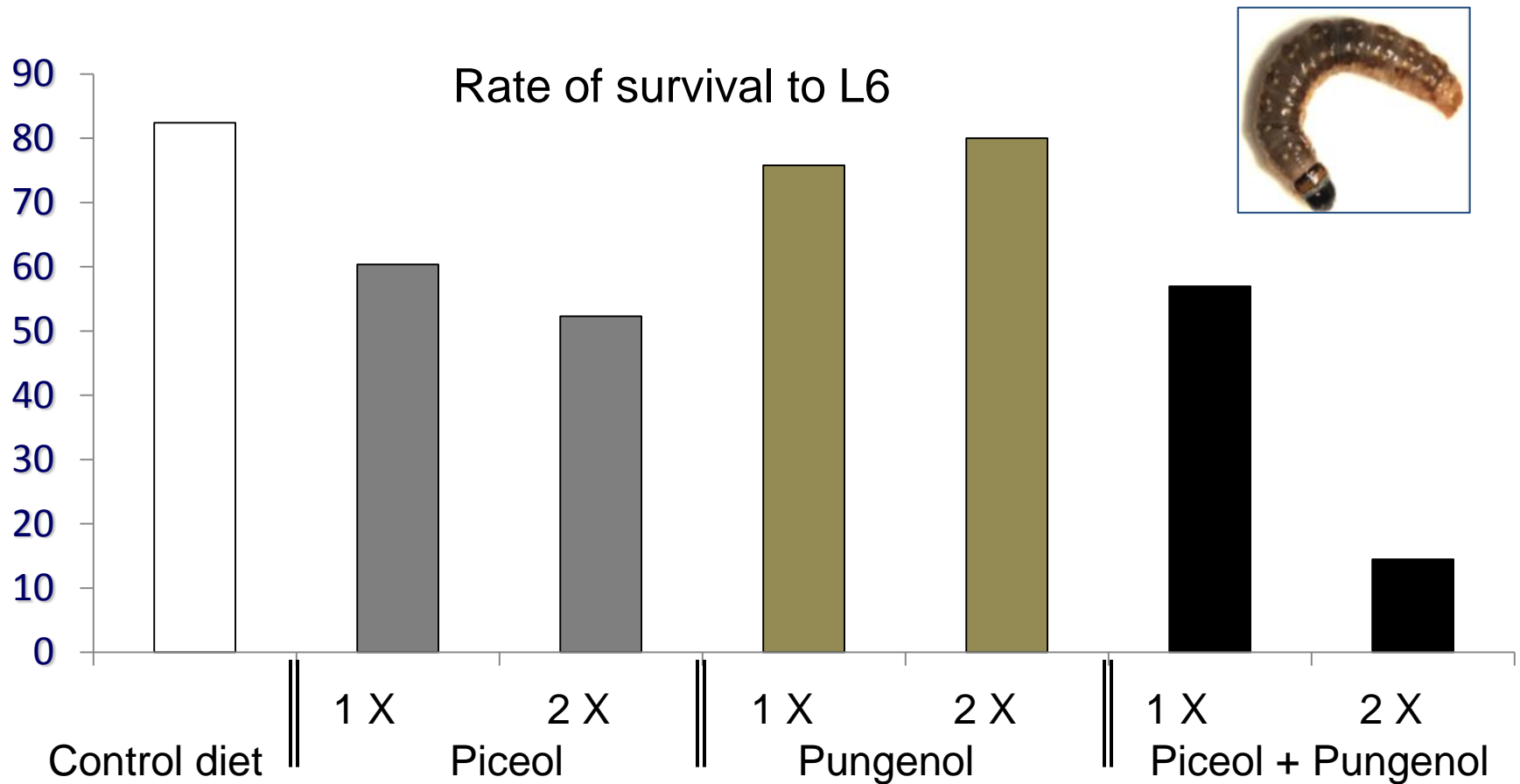
4-hydroxyacetophenone



3,4-hydroxyacetophenone

Delvas, N., Bauce, É., Labbé, C., Ollevier, T., Bélanger, R. Phenolic compounds that confer resistance to spruce budworm. *Entomol. Exper. Applic.* **141**, 35-44 (2011).

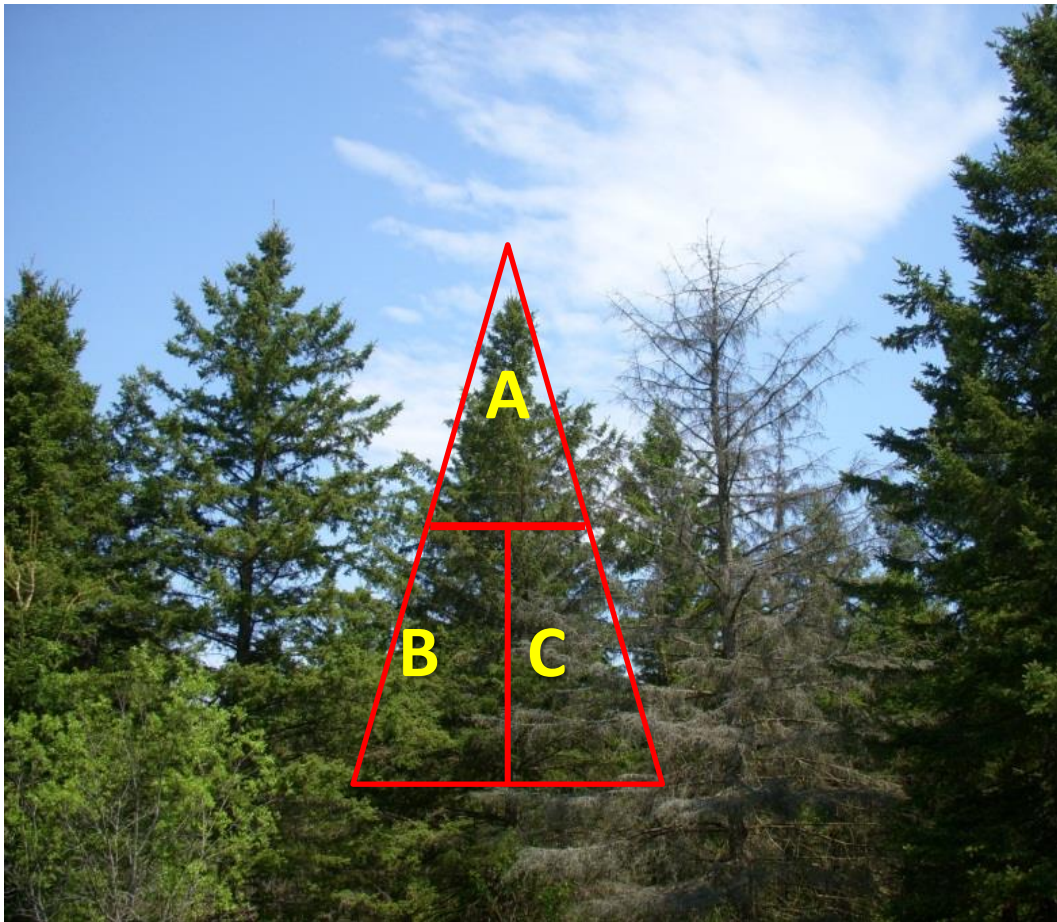
# Piceol and Pungenol Decrease Larval Survival



Delvas, N., Bauce, É., Labbé, C., Ollevier, T., Bélanger, R. Phenolic compounds that confer resistance to spruce budworm. *Entomol. Exper. Applic.* **141**, 35-44 (2011).



# Microarray analyses



**Sampling** June 17, 2010

7 susceptible trees and 7 resistant trees (14 trees total)

3 samples per tree (A, B, C)

**Microarray:** Oligonucleotide chip, 23,834 genes

Additional samples collected in August 2010 (for RT-PCR)

# PgβGlu-1 Gene: Differential Expression

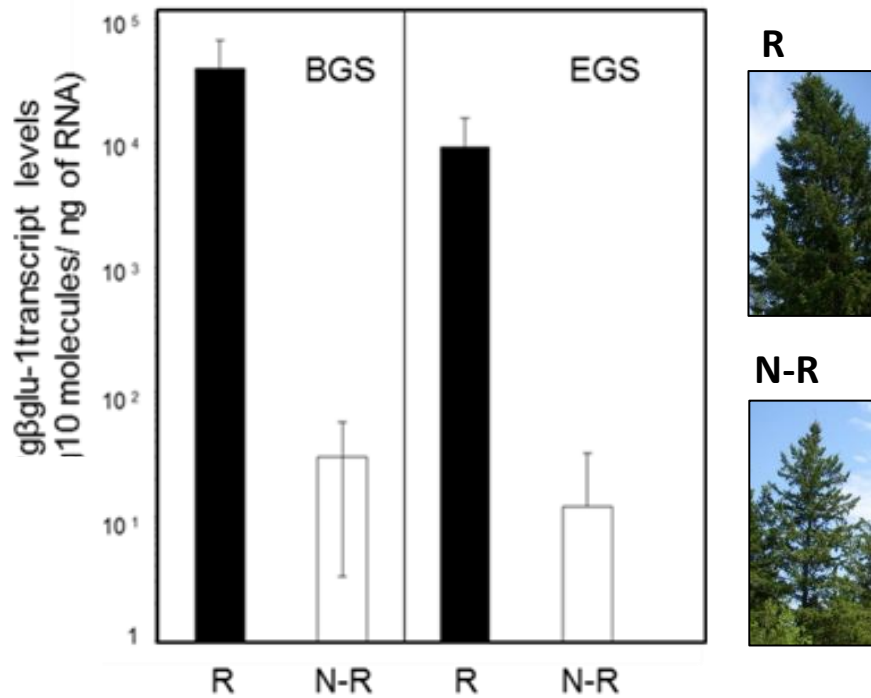
**a**

GenBank Accession no.	Predicted Function	Fold change	P-Value
BT114253	Beta glucosidase 40	770.69	3.80 <sup>E</sup> -11
BT108582	NA	23.10	0.0084
BT111304	No Exine formation 1	14.63	0.0349
DR554713	3-oxo-5-alpha-steroid-dehydrogenase family protein	14.62	0.0292
EX427727	NA	12.82	0.0106
DR573094	Xyloglucan endotrans-glucosylate/ hydrolase 5	0.23	0.0045
CO236779	NA	0.06	0.0063
EX408459	Dehydrin	0.22	0.0094
CO242218	Glycosyltransferase family protein	0.21	0.0096
CK438815	NA	0.21	0.0111
EX432133	Serine endopeptidase family protein	0.11	0.0213
DR591433	NA	0.07	0.0226
EX431305	UBX domain-containing protein	0.15	0.0234
GO360370	BAK1-receptor-like-kinase	0.78	0.0267

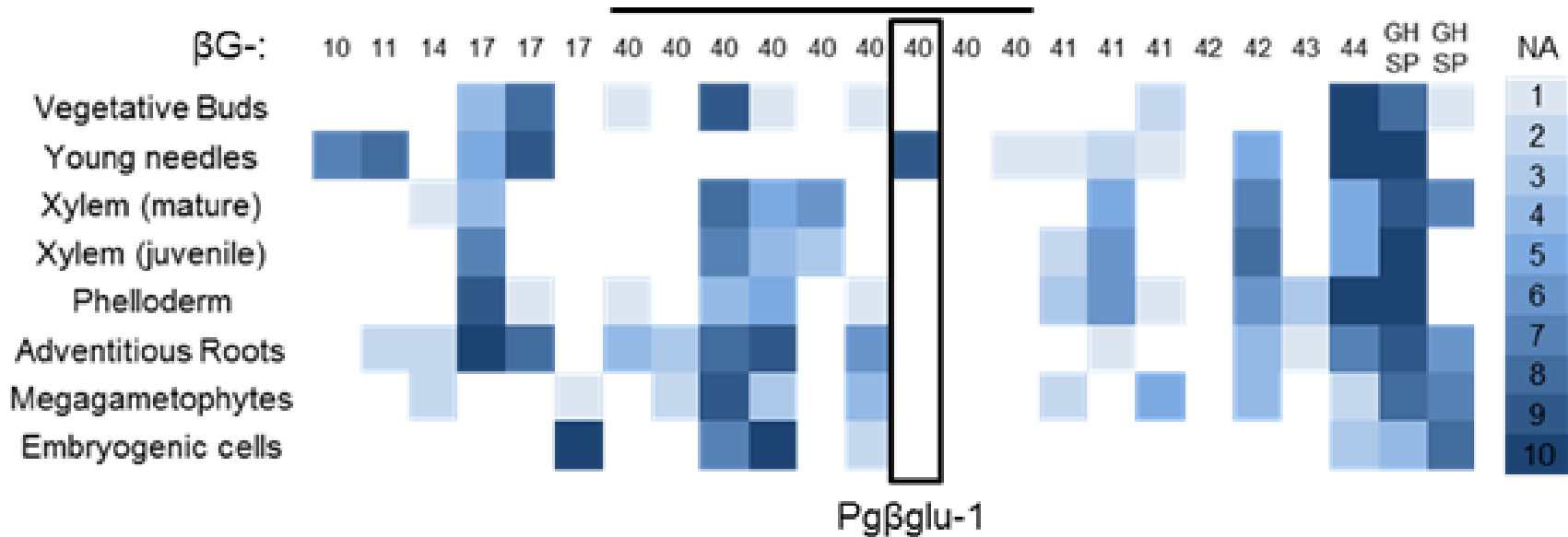
# Pg $\beta$ Glu-1 Gene: Differential Expression

## RT-qPCR determination of spliced Pg $\beta$ Glu-1 RNA level

Mature test trees



# Pg $\beta$ Glu-1 Gene: Differential Expression



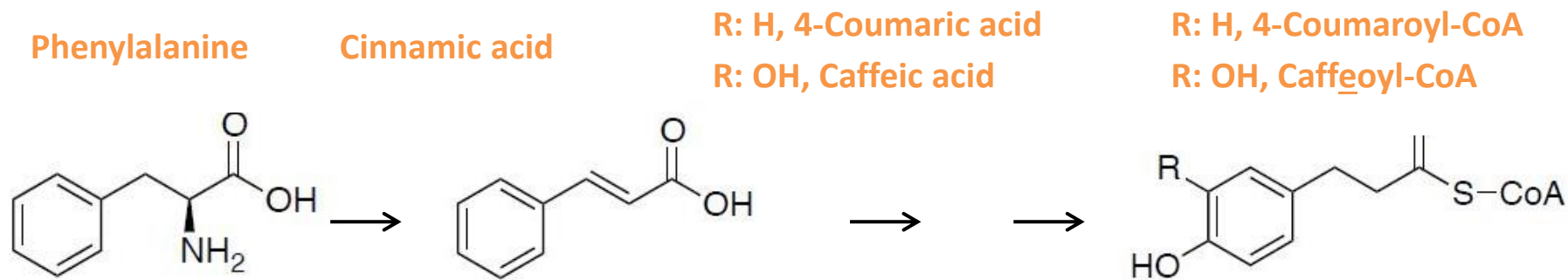
Based on PiceaGenExpress database

Raherison et al. 2012 BMC Genomics 13: 434

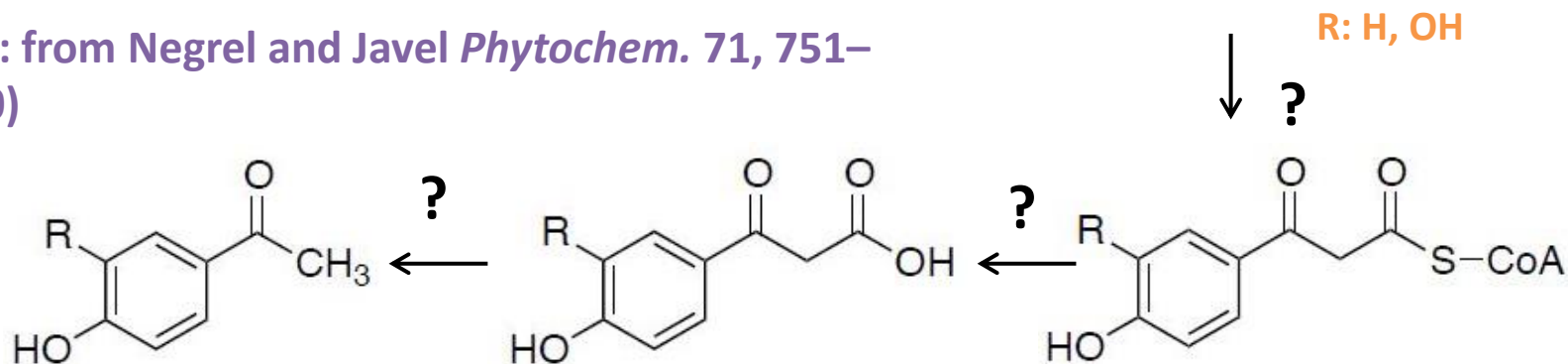


# Previously Proposed Biosynthesis pathway

## General phenylpropanoid pathway



Proposed: from Negrel and Javel *Phytochem.* 71, 751–759 (2010)

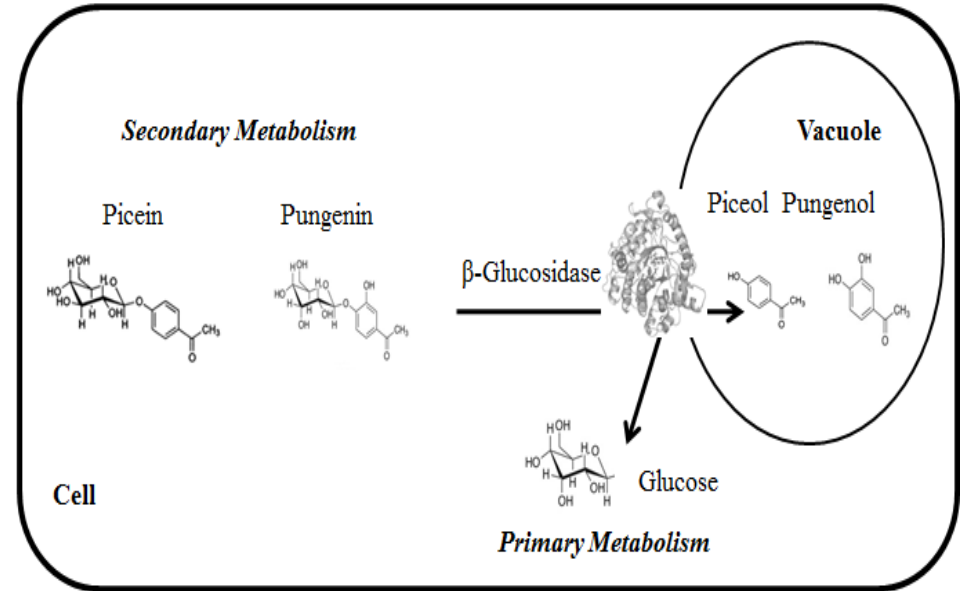
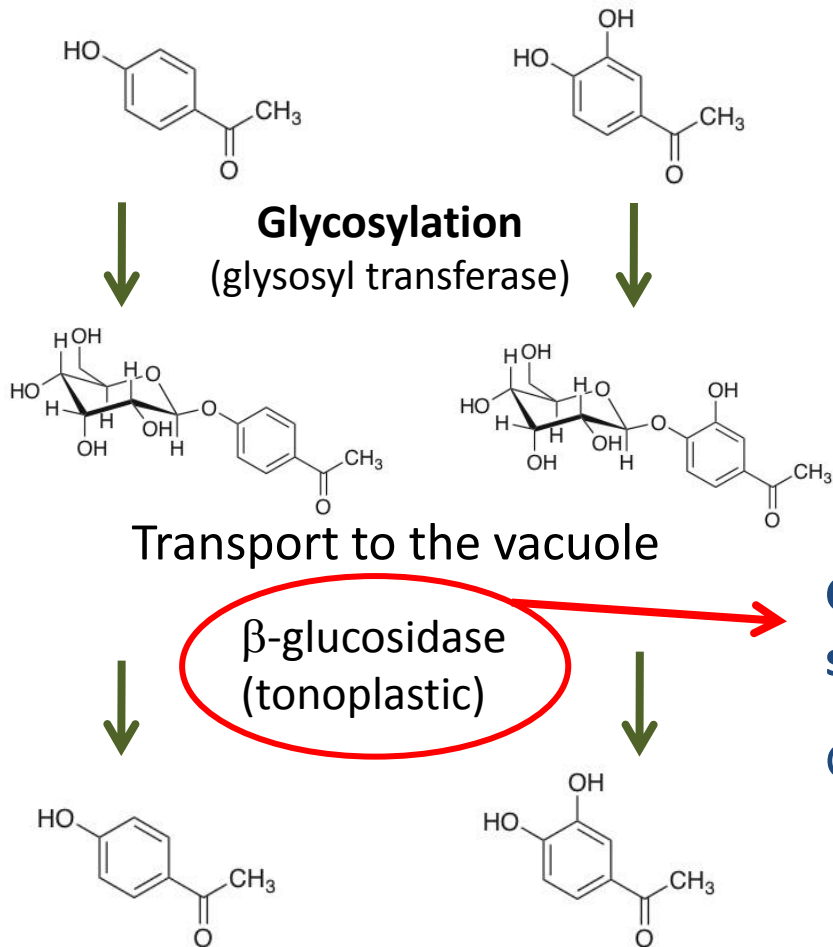


R: H, Piceol

R: OH, Pungenol

# Pathway Revised

## SBW Resistance Mechanism



**Gene expression is a determinant of susceptibility and resistance**

Gene and compounds to develop:

- Novel breeding strategies for SBW resistance
- Vulnerability assessment of existing stands

# Concluding Remarks

- 1. Gene networks:** other approaches are being used, e.g. eQTL analysis, co-expression modules, and integrated with current results
- 2. Insect resistance gene:** heritability and SBW resistance experiments planned based on breeding population results
- 3. Functional insights** are being developed by using comparative transcriptome and functional profiling
- 4. Next steps for developing an understanding of genome function:** integrate diverse types analyses

# Acknowledgements:

## SMarTForests Team Members

- **Project Leaders:** John MacKay, Jörg Bohlmann
- **Project Investigators:** Jean Beaulieu, Jean Bousquet, Gary Bull, Janice Cooke, Nancy Gélinas, Nathalie Isabel, Steven Jones, Kermit Ritland, Armand Séguin, Alvin Yanchuk
- **Project Managers:** Sophie Laviolette, Carol Ritland

## Transcriptome and Genome Research (MacKay lab)

- Students: Elie Raheison , Jukka-Pekka Verta, Nathalie Delvas, Guillaume Tessier, Mebarek Lamara, Gaby Germanos, Élise Fortin
- Postdocs: Claude Bomal, Patrick Lenz, Julien Prunier, Geneviève Parent
- Research assistants: Sébastien Caron, Pier-Luc Poulin , Brian Boyle, Isabelle Giguère,
- Collaborators: Éric Bauce, Philippe Rigault, Christian Landry

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**Thank you**