



“Strengthening collaboration on innovative wood quality research”

“High throughput” measurement of wood quality for genetic studies and breeding programs

Jean-Paul Charpentier, INRA
and
AGPF INRA Orleans team



JP. CHARPENTIER
High throughput measurement of wood quality

French (INRA)/Finnish (Luke) workshop

“Strengthening collaboration on innovative wood quality research”.01

Viikki, Finland, 29-30/08/2017

different types of wood quality assessment methods

✦ **Destructive methods**

✦ **Non-destructive methods**

★ Destructive methods

- in general, completely and definitively destructives for trees
- usually expensive and non adapted for genetic studies with a great sampling
- advantage : accessibility of a great quantity of matter

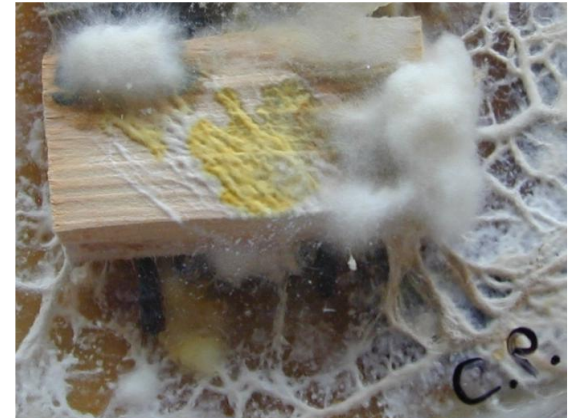
✦ Destructive methods

Some wood property measurements :



**MOE,
wood stiffness,
different tests for mechanical properties
natural durability with fungi tests
chemical properties**

.....



different types of wood quality assessment methods

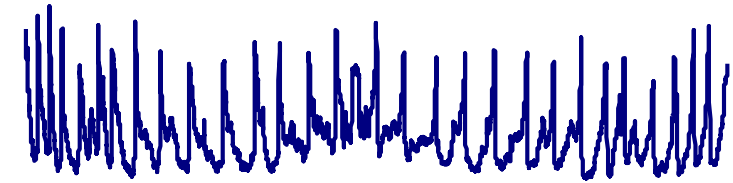
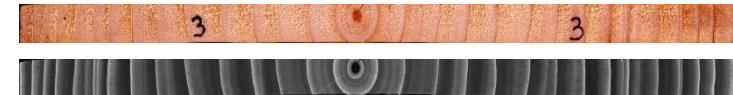
★ Non-destructive methods

□ With sampling



Sampling of a small sample of wood,
non lethal for the tree

➔ **Increment cores**



Infradensity

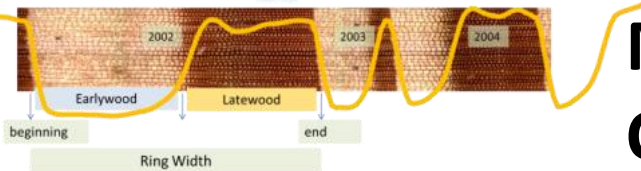
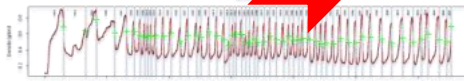
Microdensitometry

Chemical properties

➔ wood chips by drilling

Chemical properties

(if little material is required)



Evolution of laboratory analytical methods

Orientation towards:

- As high throughput as possible
- less costly
- more automated as possible
- miniaturized for less matter use

Sample preparation for analysis:

→ wood is a hard material and difficult to work if it is to be grinded

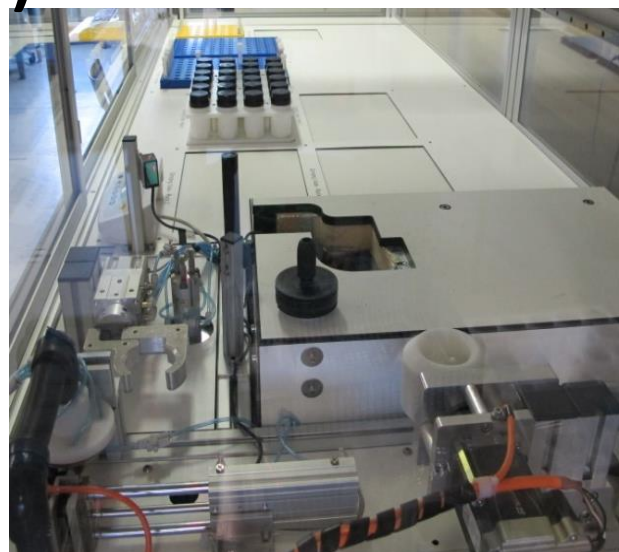
➤ design and development of robots



(1) grinder and (2) wood powder dispenser

(1)

(2)

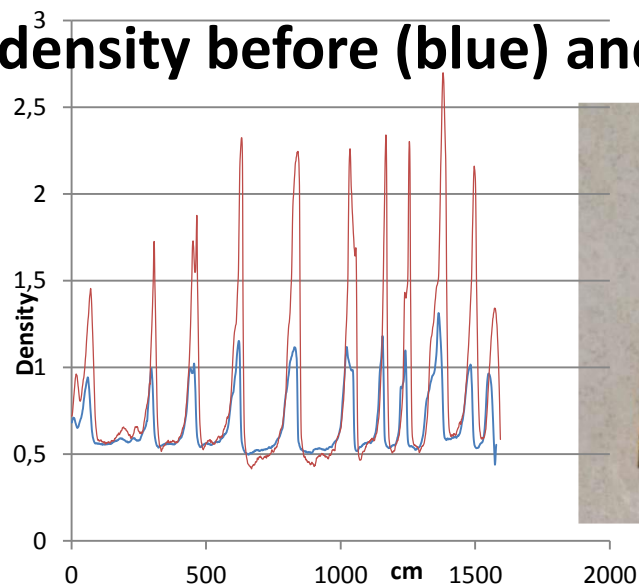


Evolution of laboratory analytical methods (2)

Evaluation of the natural durability other than the standard method with wooden board from increment cores and by means of X-ray microdensitometry



density before (blue) and after (red) fungus attack



(with collaboration Ivalsa CNR Firenze, Italy)

Macchioni N, Palanti S, Rozenberg P (2007), and Luc E. Pâques; T4F work, will be probably submitted International Wood Science and Technology 41(5):417-426. *Biodeterioration & Biodegradation*

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High throughput measurement of wood quality

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Evolution of laboratory analytical methods (3)

Miniaturized process for high throughput screening of the saccharification potential of lignocellulosic biomasses

(1) Biomass preparation

(2) Pretreatment

(3) Enzymatic hydrolysis



Lignocellulosic biomass



Grinding and distribution of biomass by robots

5 mg / well

Essential for good accuracy and repeatability



96-wells plates in Hastelloy (heat resistant)



Steam generator Cellkraft®

Enzymatic hydrolysis without pretreatment

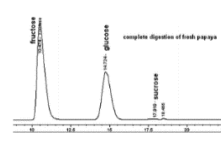


Reactor for pretreatment PARR Instrument®

Steam (180°C)



Enzymatic Hydrolysis (50°C, Ctec-2)



Chromatography (HPLC)



Enzymatic dosage (colorimetric)

Determination of released sugars (Glucose and xylose)

Belmokhtar *et al.*, *Frontiers in Plant Science*, 2017



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Evolution of laboratory analytical methods (3)

Miniaturized process for high throughput screening of the saccharification potential of lignocellulosic biomasses

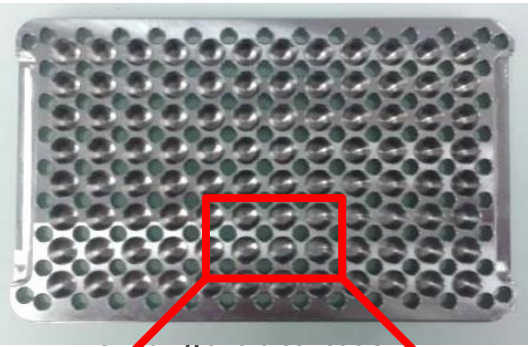
(1) Biomass preparation

(2) Pretreatment

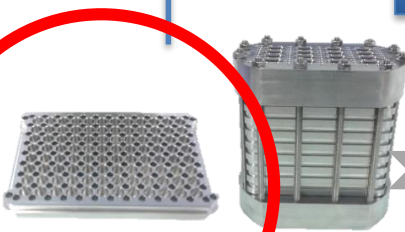
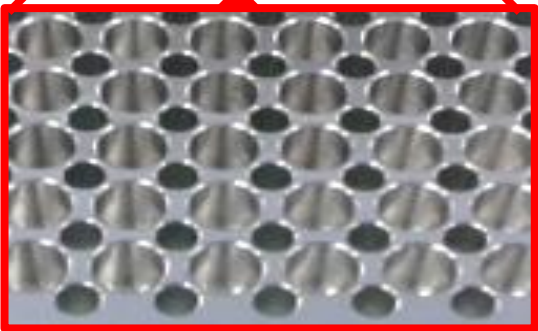
(3) Enzymatic hydrolysis



Lignocellulosic biomass



of biomass by robots
5 mg / well



96-wells plates in
Hasteloy
(heat resistant)

Enzymatic hydrolysis
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Reactor for
pretreatment
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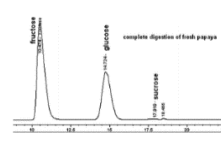


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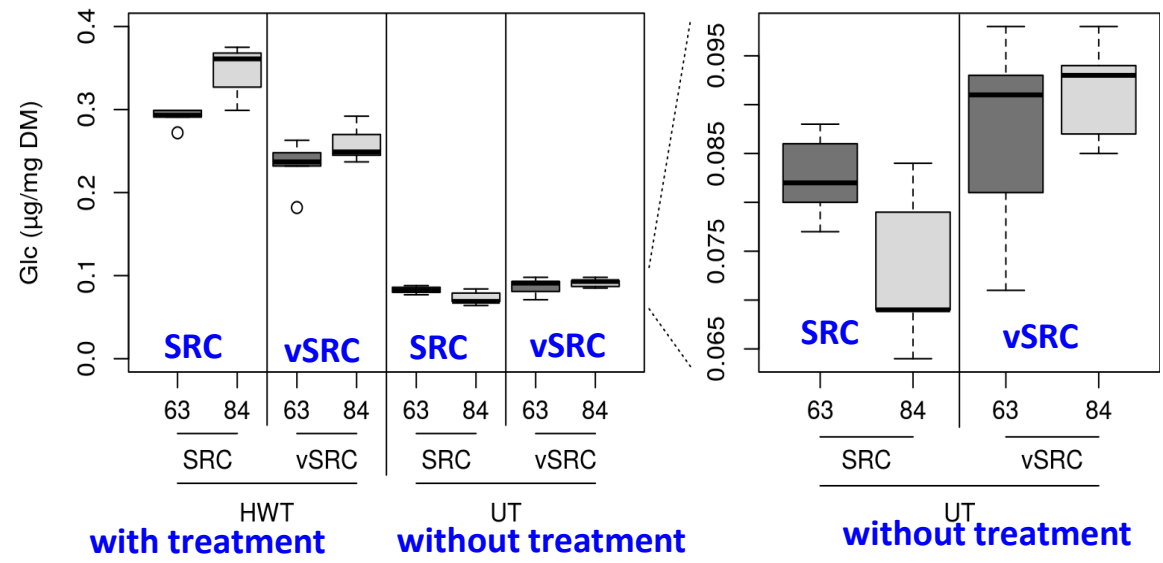
Viikki, Finland, 29-30/08/2017

Evolution of laboratory analytical methods (3)

New process for saccharification potential

An application:

Study of saccharification potential on poplar biomass



The test allows discrimination between biomass



Indirect evaluation of wood properties by Near InfraRed Spectroscopy (NIRS)

On powder → destructive for samples

On solid wood → non-destructive for samples



*Near and Middle InfraRed Spectroscopy and imager
Perkin Elmer*

Wood properties evaluated:

Chemical (structural, extractives, specific molecules)

Physical (densité,....)

Mechanical (MOE, MOR,...)

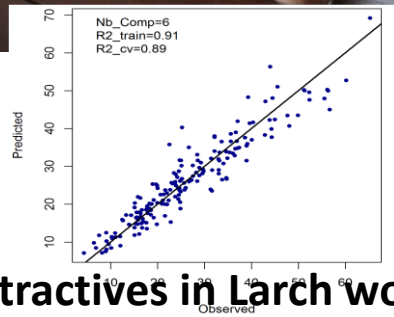
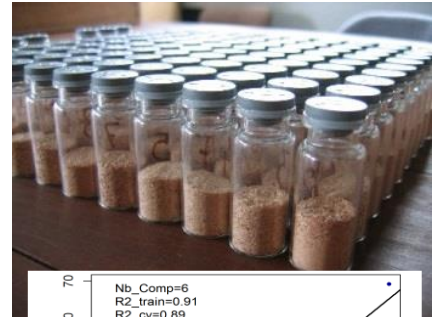
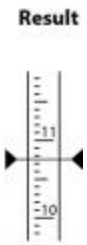
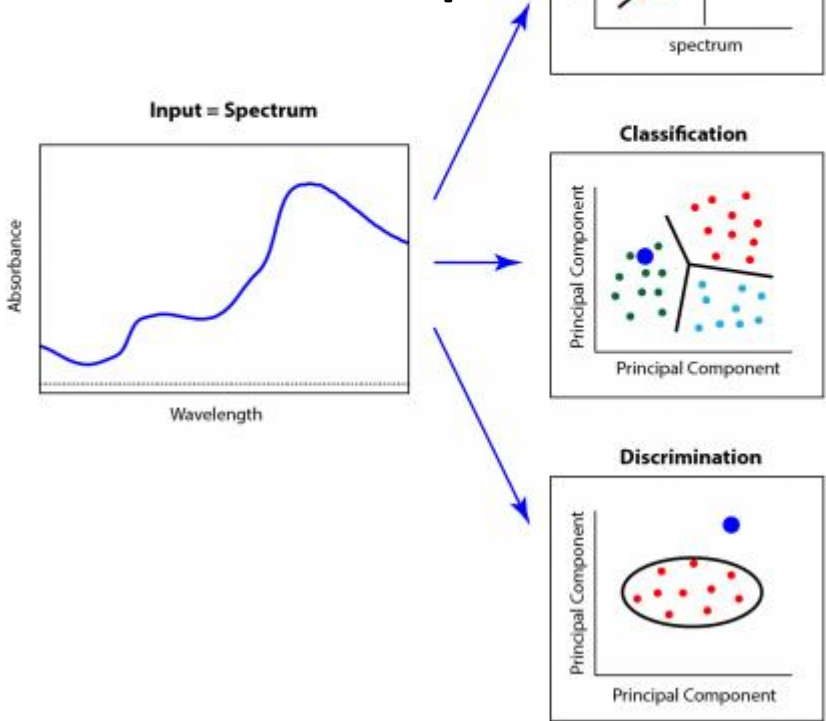
Complexe : natural durability, cavitation vulnerability,

Tsuchikawa and Kobori, J Wood Sci (2015), 61:213–220

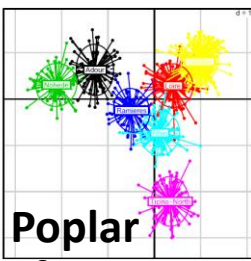
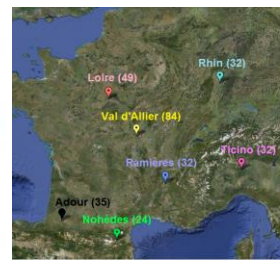
Evolution of laboratory analytical methods (4)

Indirect evaluation of wood properties by Near InfraRed Spectroscopy (NIRS)

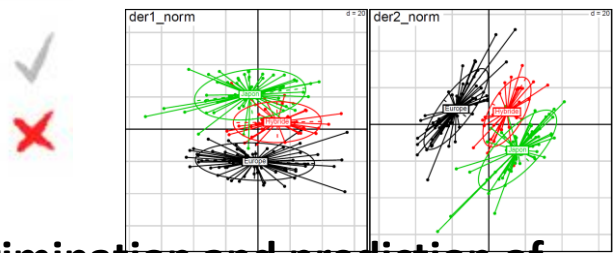
NIRS is a multiparametric, fast, and non-destructive technique



Extractives in Larch wood



Discrimination of provenances Structuring phenomic diversity



Discrimination and prediction of unknown samples, for example in Larch

Evolution of laboratory analytical methods (4)

NIRS methodology

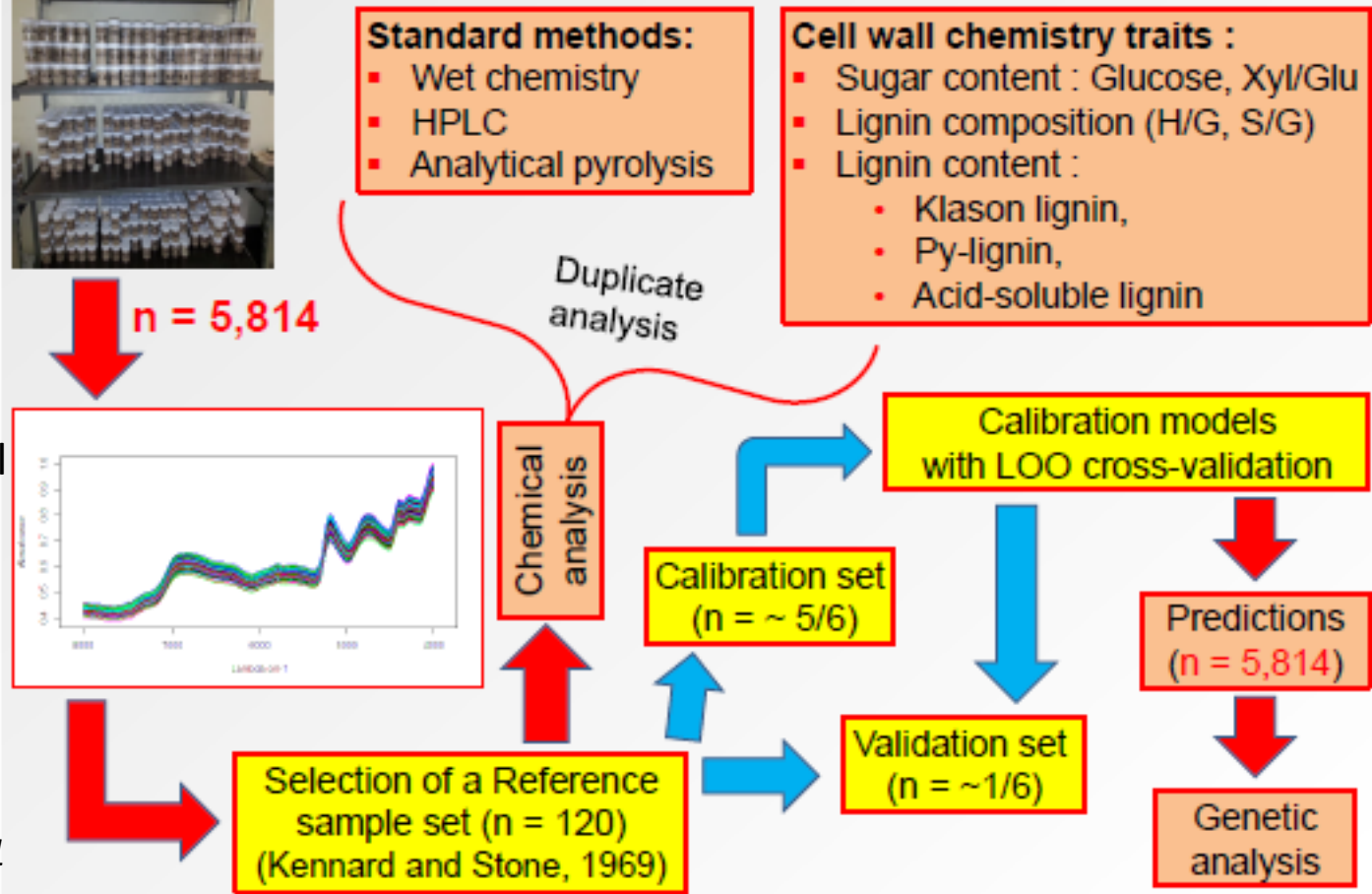
Poplar (*Populus nigra*)

Genetic architecture of lignocellulosic biomass yield and quality in black poplar for its use in biorefinery



- Field trials at 2 sites (Orleans, France; Savigliano, Italy)
- Wood samples: 3 main stem harvests of 2-yr-old trees in 2-3 biological replicates
- 800 samples / 289 genotypes in Orleans in 2010 (Orl2010)
- 2,810 samples / 1,066 genotypes in Orleans in 2012 (Orl2012)
- 2,204 samples / 777 genotypes in Savigliano in 2011 (Sav2011)

Development of NIR spectroscopy calibrations for the genetic analysis of wood properties in natural populations of *Populus nigra*

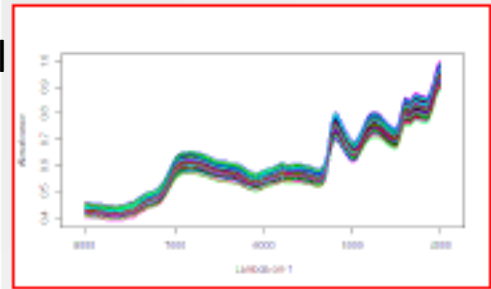


Standard methods:

- Wet chemistry
- HPLC
- Analytical pyrolysis

Cell wall chemistry traits :

- Sugar content : Glucose, Xyl/Glu
- Lignin composition (H/G, S/G)
- Lignin content :
 - Klason lignin,
 - Py-lignin,
 - Acid-soluble lignin



Gebreselassie et al, 2017, *Industrial Crops&Products* 107_159-171

Evolution of laboratory analytical methods (4)

NIRS methodology

Poplar (*Populus nigra*)

NIRS calibration models for different chemical wood properties:

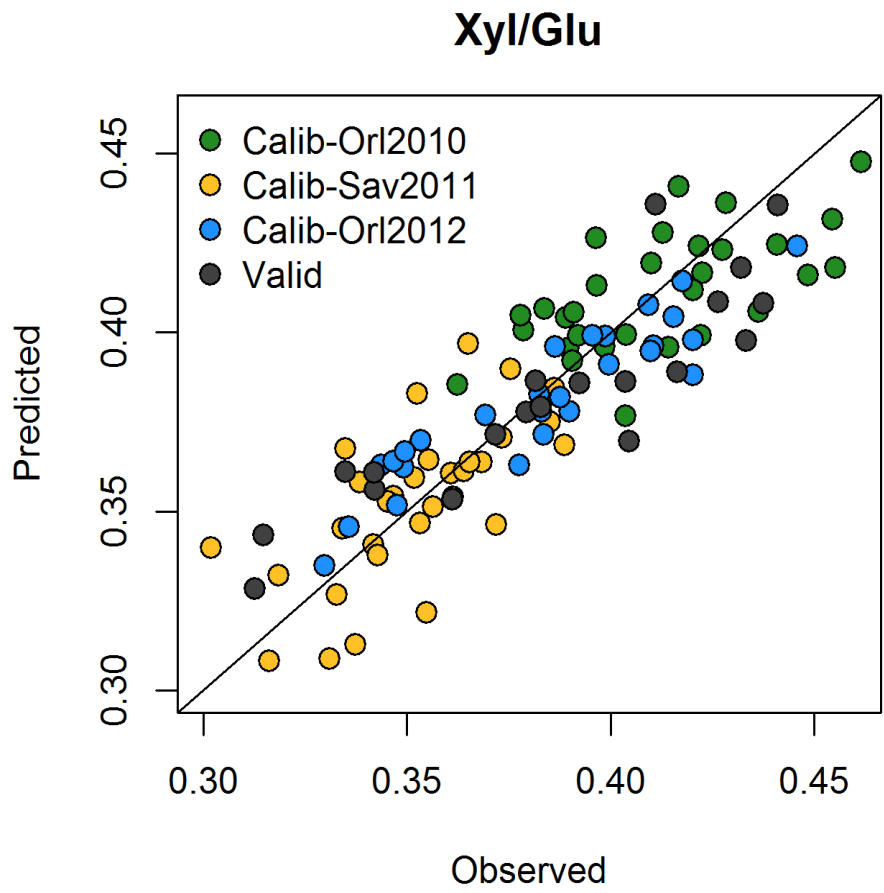
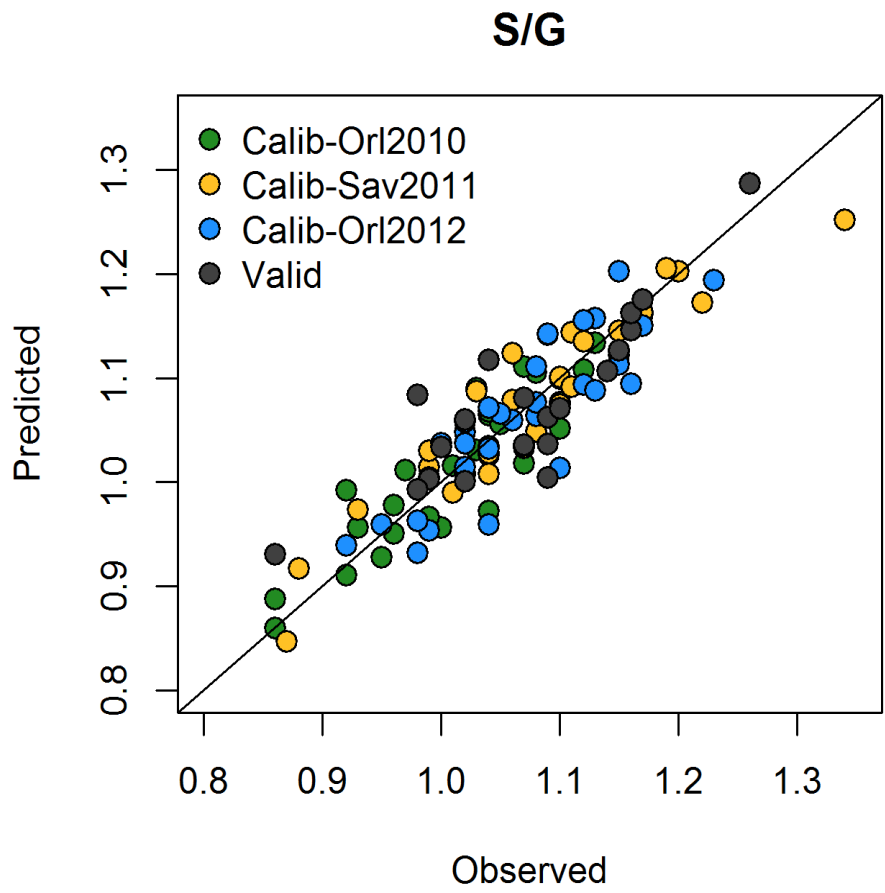
Trait	Model type	Calibration set (n = ~ 5/6)				Validation set (n = ~ 1/6)			RMSE of lab. Method
		Factors	R ² _{cv}	RMSE _{cv}	Nb. obs	R ² _{val}	RMSE _{val}	Nb. obs.	
Glucose	global	6	0.76	1.49	94	0.64	1.25	18	1.87
Xylose/Glucose	global	8	0.79	0.02	90	0.75	0.02	20	0.03
H/G ratio	global	8	0.82	0.02	92	0.83	0.02	19	0.01
S/G ratio	global	12	0.84	0.03	91	0.72	0.04	21	0.03
Klason lignin	Site	6	0.78	0.94	56	0.6	1.31	13	1.61
Py-lignin	Site	8	0.79	0.39	26	0.73	0.35	6	1.49
Acid-soluble lignin	Site	8	0.77	0.16	30	0.79	0.15	6	0.32

Evolution of laboratory analytical methods (4)

NIRS methodology

Poplar (*Populus nigra*)

NIRS calibration models for different chemical wood properties:

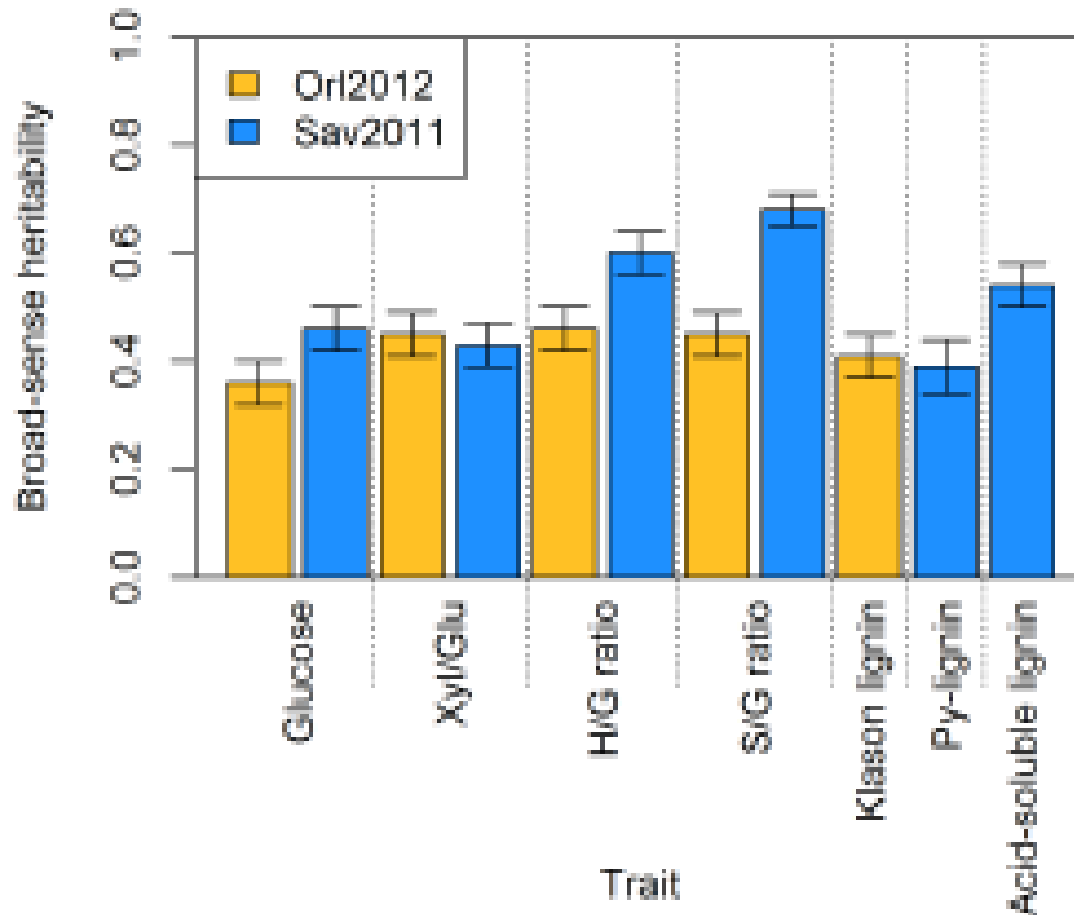


Evolution of laboratory analytical methods (4)

NIRS methodology

Poplar (*Populus nigra*)

Broad-sense heritability (H^2_i) estimates of NIR-predicted wood chemical traits:



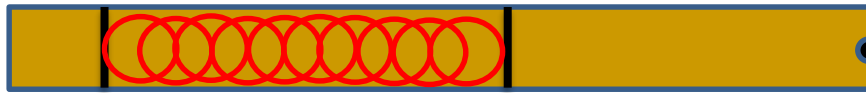
- Predicted wood properties were under moderate to high genetic control (H^2_i ranging from 0.25 to 0.68).
- Site differences were apparent in the expression of the phenotypes.

Evolution of laboratory analytical methods (4)

NIRS methodology

European Larch; small wood boards from increment cores, 104 Larch trees from 28 families full brothers from a factorial crossing design

Sapwood Heartwood (5cm)



NIR spectra with optic fiber directly on solid wood

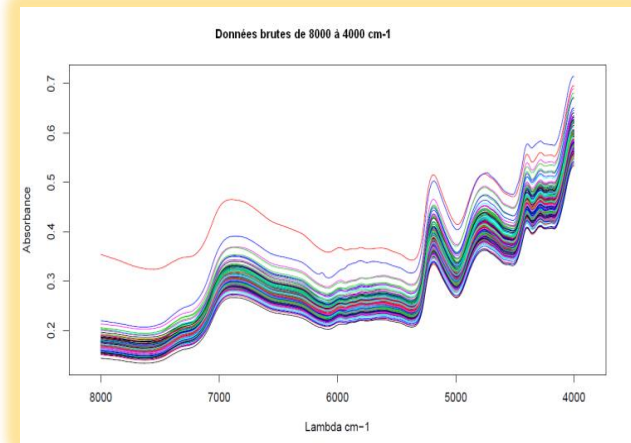
Selected zone



Grinding in powder
208 samples (104x2)



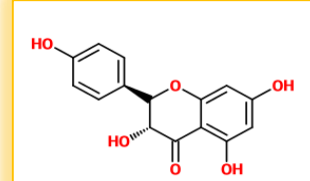
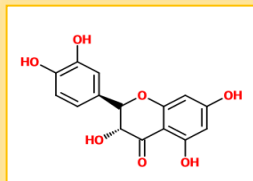
NIR spectra on wood powder



Chemical Analysis:

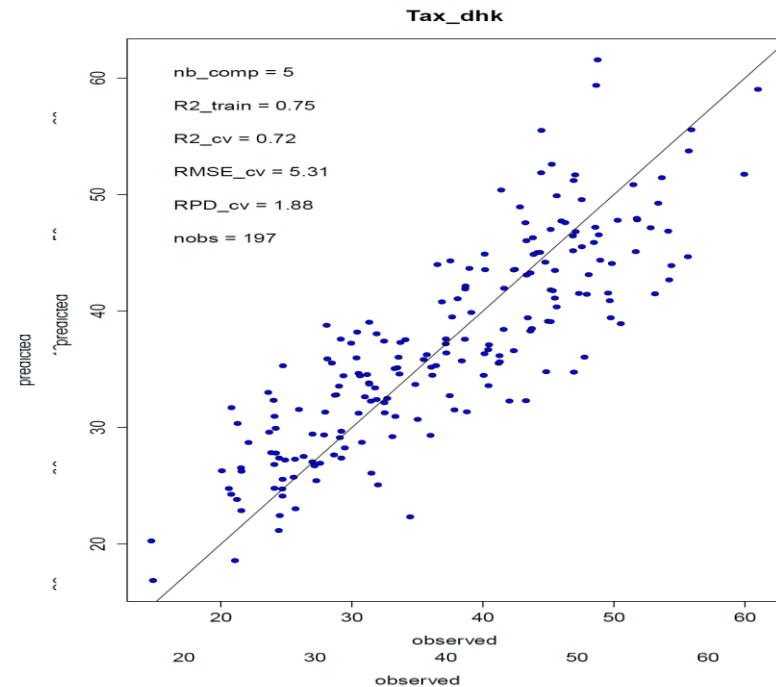
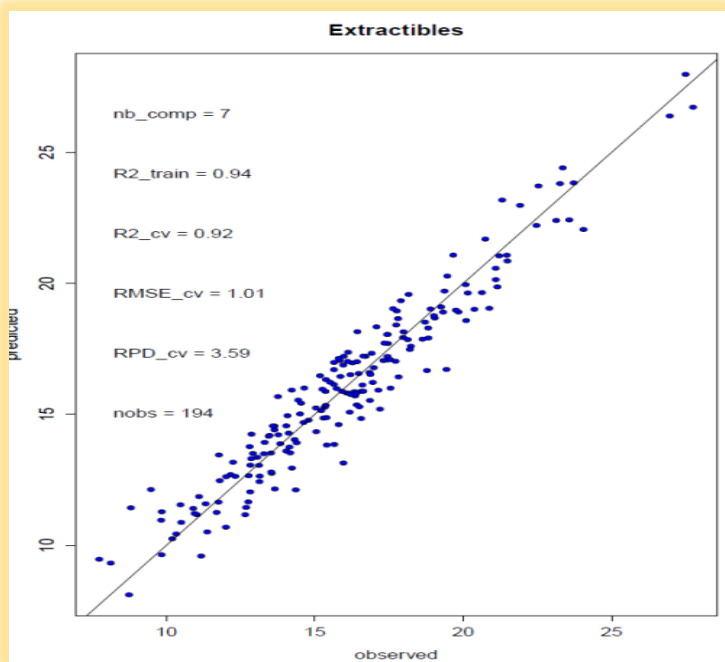
Extractives, total phenol content (Folin reagent method)

TAX, DHK (HPLC)



NIRS methodology

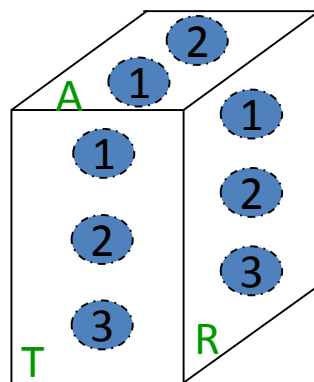
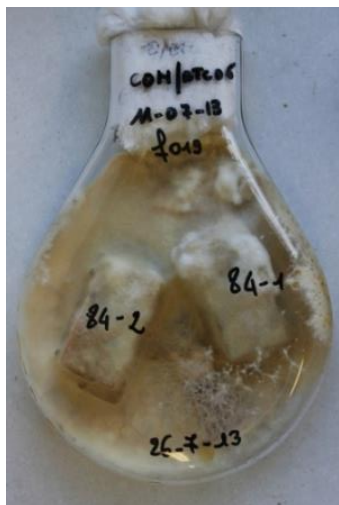
NIRS calibration models obtained on wood powder for extractives and phenolic molecules



Extractives: $R^2 = 0,92$ with 13 outliers

Tax + DHK: $R^2 = 0,72$ with 10 outliers

NIRS methodology



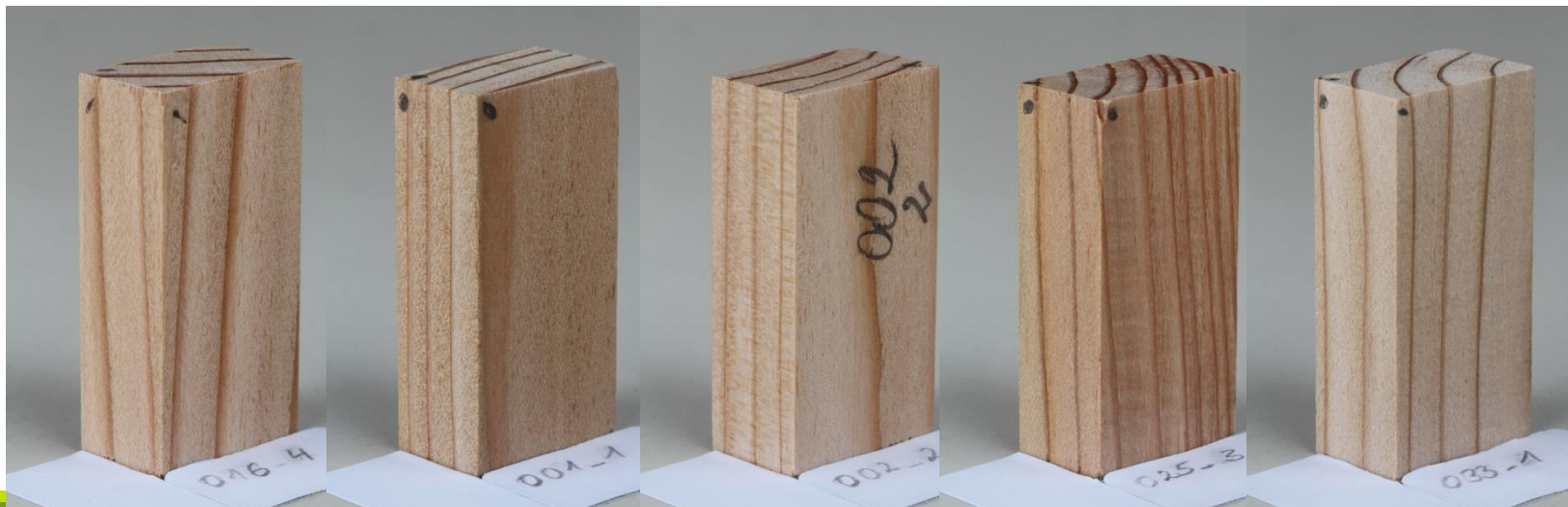
NIRS measurement:
8 spectra/block in
different faces

**NIRS Calibration models for a complex property of wood:
natural durability of larch wood**

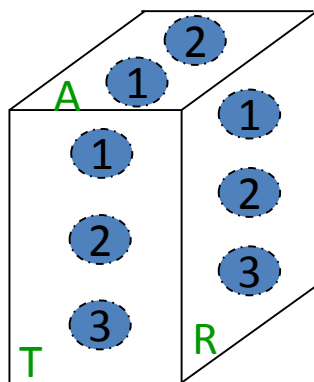
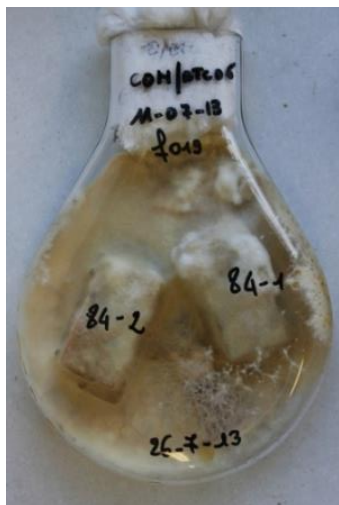
European Larch; blocks sawn in heartwood of 104 Larch trees from 28 families full brothers from a factorial crossing design

Mass loss data with
attack by fungi
(Ivalsa, Italy)

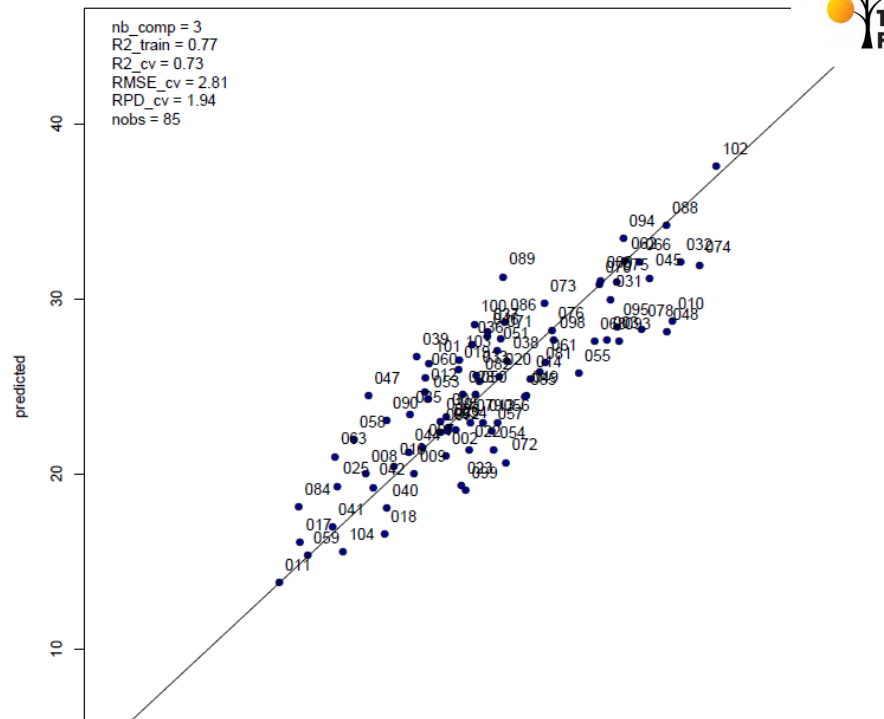
A great
variation
of type of
blocks



NIRS methodology

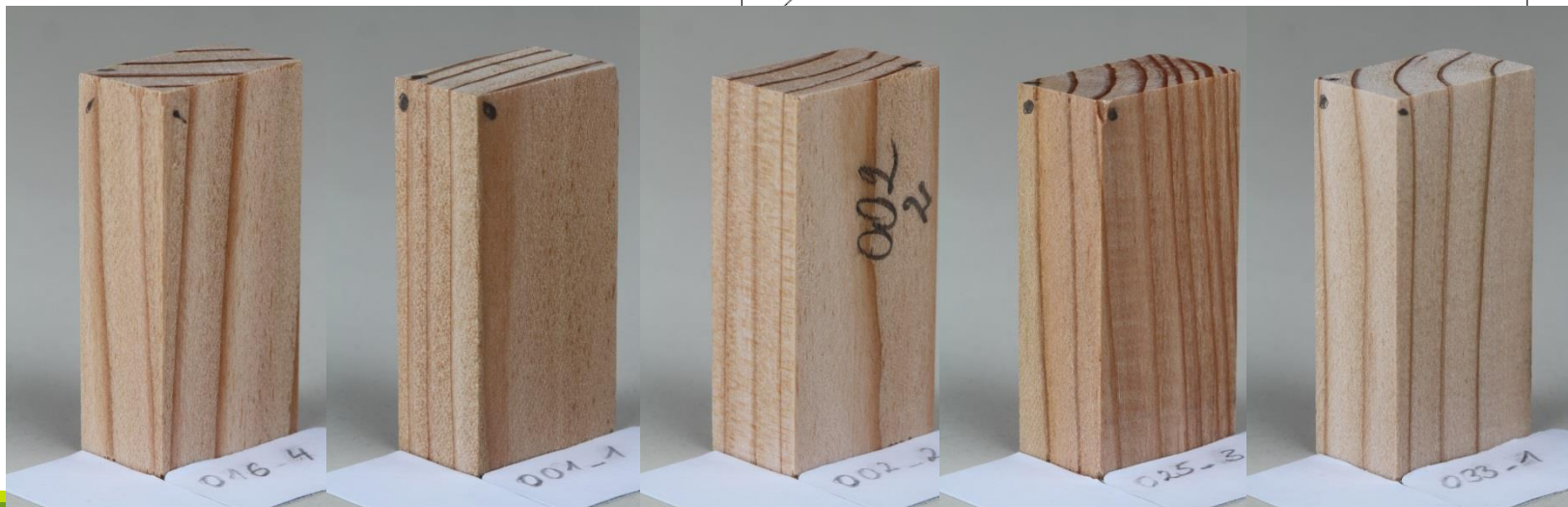


NIRS measurement: 8 spectra/block in different faces



Mass loss data with attack by fungi (Ivalsa)

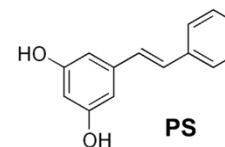
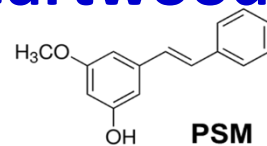
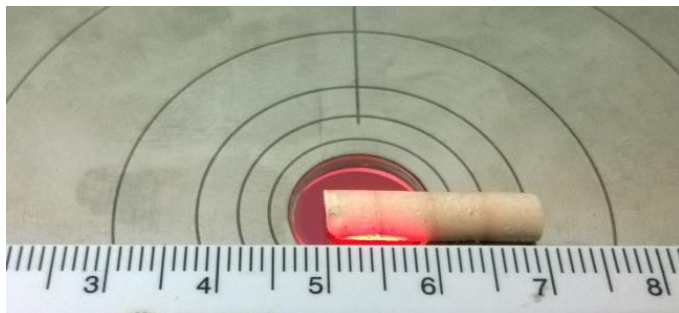
A great variation of type of blocks



NIRS methodologie

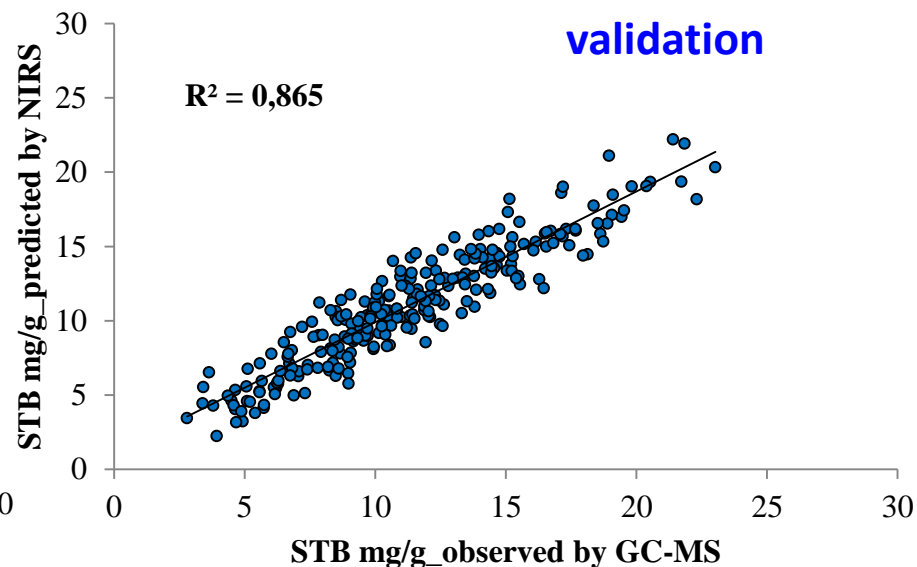
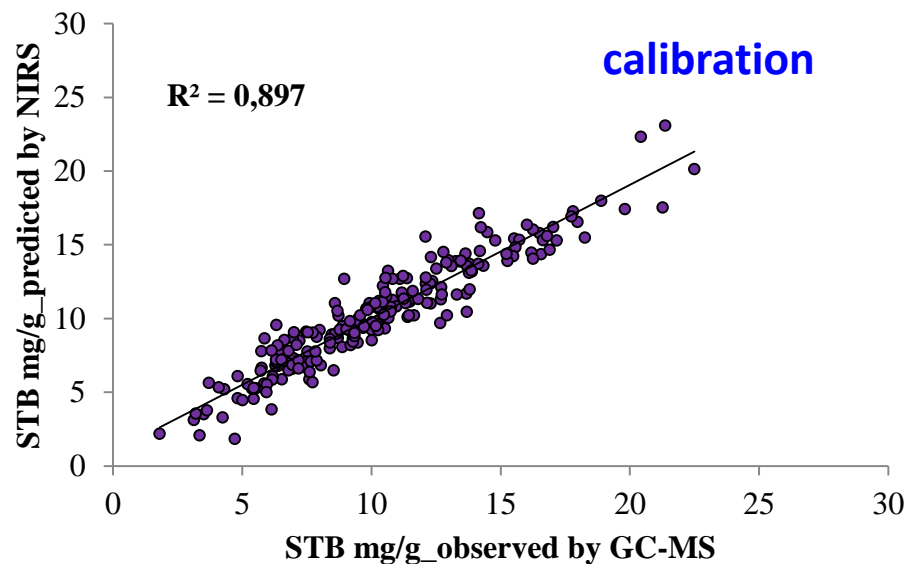


Evaluation of stilbenes in Scots pine heartwood



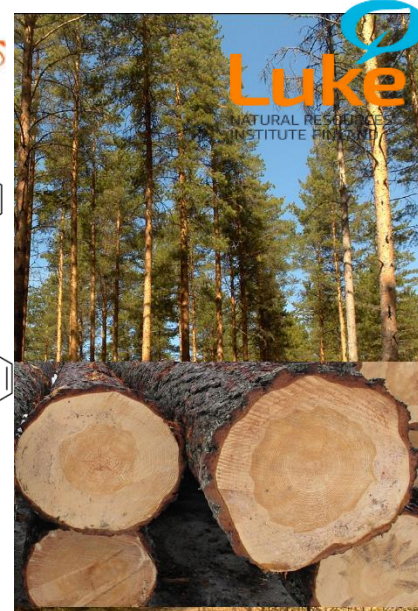
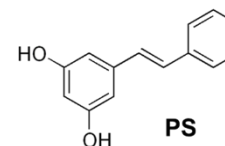
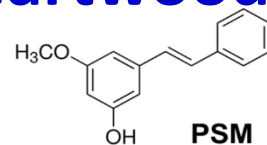
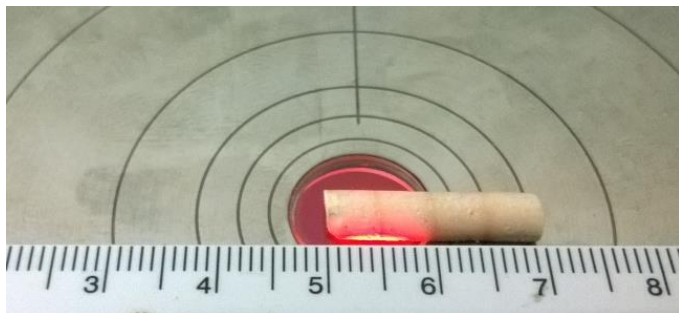
Number of observations: 212 for calibration and 262 for validation

R^2 : 0.90 for calibration and 0.87 for validation set



S. Pulkka et al., *J. Near Infrared Spectrosc.* **24**, 517–528 (2016)

Evaluation of stilbenes in Scots pine heartwood



Number of observations: 212 for calibration and 262 for validation
 R^2 : 0.90 for calibration and 0.87 for validation set

- Heritabilities: 257 samples measured using GC-MS, UVRRS and NIRS
 - $h^2_{STB} = 0.85$, $SD_{h2} = 0.30$ (GC-MS)
 - $h^2_{STB} = 0.84$, $SD_{h2} = 0.29$ (NIRS)
 - $h^2_{STB} = 0.33$, $SD_{h2} = 0.21$ (UVRRS)

S. Pulkka et al., *J. Near Infrared Spectrosc.* **24**, 517–528 (2016)

different types of wood quality assessment methods

★ Non-destructive methods

□ Without sampling

→ *in situ* method,
directly on standing trees

→ pilodyn
resistograph
acoustic methods
Near InfraRed Spectroscopy (NIRS)



Development of *in situ* rapid, non-destructive, accurate, and economically viable wood quality assessment methods would be of great interest to tree breeders and forest managers and for genetic studies.

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in new development → Near InfraRed Spectroscopy (NIRS)

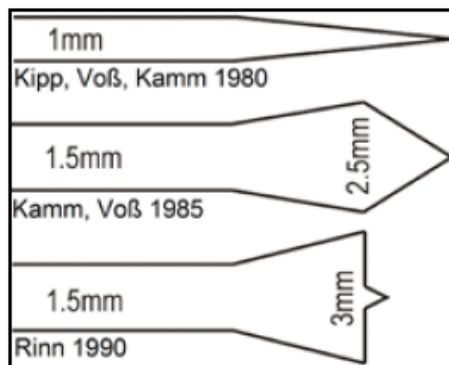


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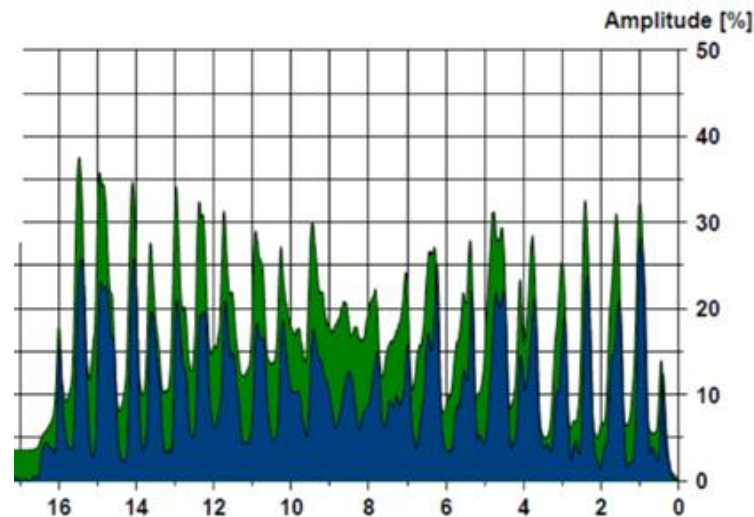
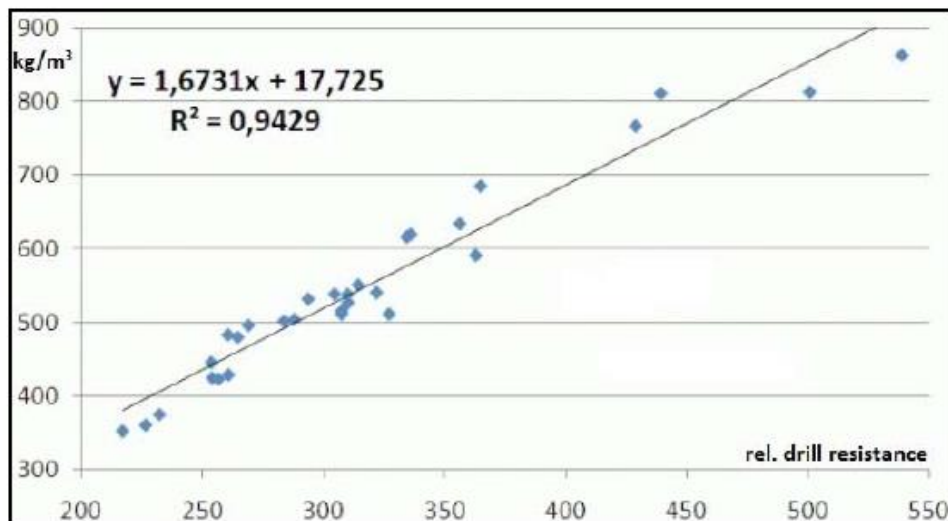
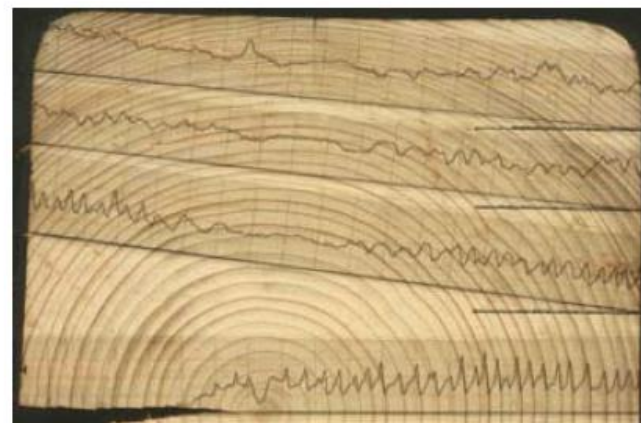
Non-destructive *in-situ* methods



resistograph



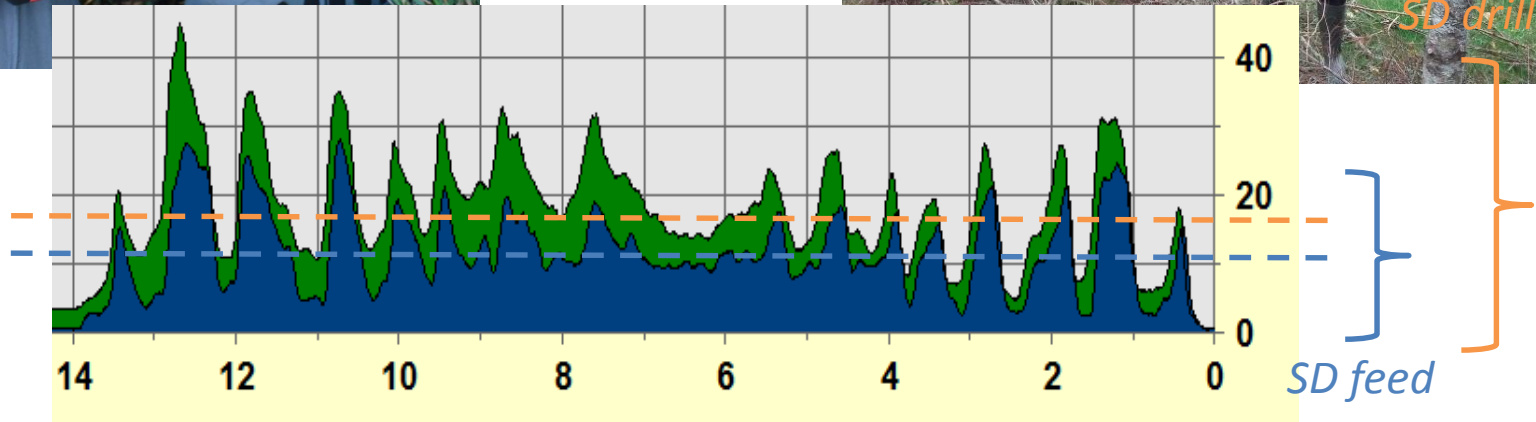
A needle shaft diameter of 1.5mm with a flat tip 3mm wide were found to be a good compromise for achieving maximum resolution and stability, while causing the least damage to the sample.



Douglas-fir clones analysis



resistograph



Caractère	Feed mean	Pilodyn	Diamètre 1,30m
Drill mean	0,97 **	-0,66**	-0,28 ns
Feed mean		-0,73**	-0,19 ns
Pilodyn			0,36 ns

Caractère	Héritabilité (SL)
Drill mean	0,42
Feed mean	0,38
Pilodyn	0,45
Diamètre 1,30m	0,41

Non-destructive *in-situ* methods

Acoustic methods



In collaboration with Spanish colleagues in the framework of the TopWood project

On standing trees, we test different equipments on different species

Wood basic properties evaluated:
Microdensity, MOE, MFA, fiber Length, Basic density, dimensional stability, vulnerability to cavitation



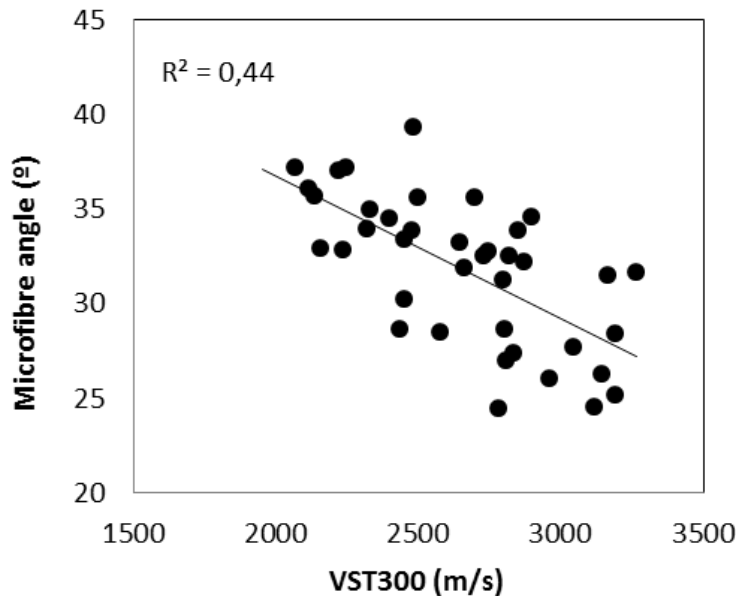
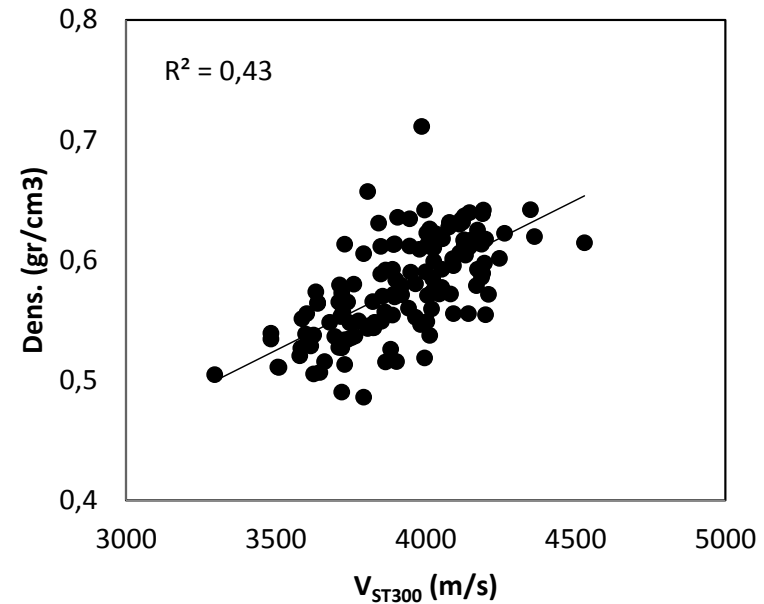
$$MOE = \rho \cdot V^2$$

Wang, *Wood Sci Technol* (2013) 47:965–975

Affected by diameter, modelisation is necessary

Some first results:

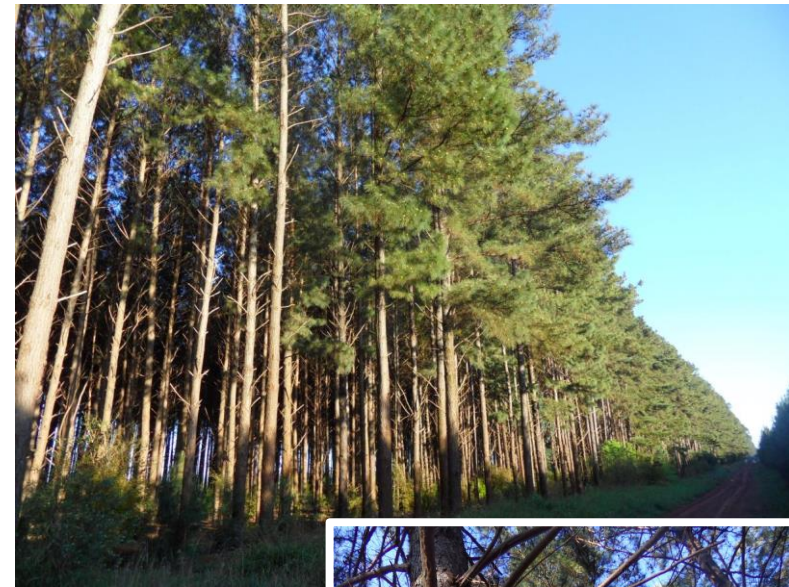
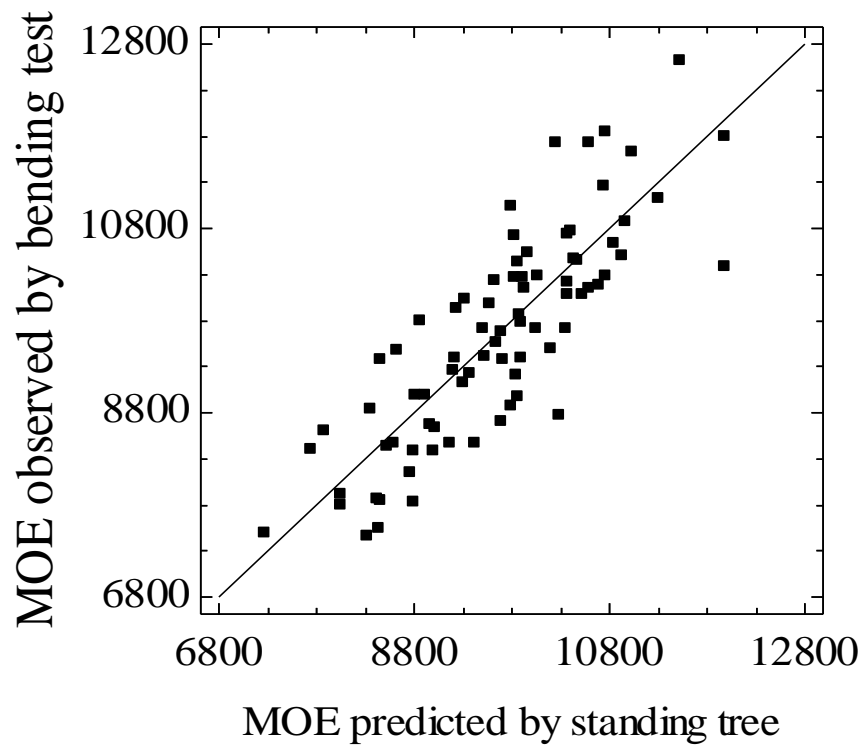
Correlation with basic density in *Eucalyptus globulus* but not in *Pinus (ponderosa, pinaster, radiata)*.



Correlation sonic velocity with MFA in *Pinus pinaster* but not in *Eucalyptus*

Douglas-fir, MOE evaluation on standing trees

$R^2=0,60$



Merlo et al (2014)

Non-destructive *in-situ* methods



**And now
the portable NIRS technology**

- Proposed equipments are currently being in great evolution
- Portable technology already used in agriculture with embedded systems
- In the wood industry, some studies by researchers, not yet known routine use
- **Acquisition of spectra on standing trees: a new research axis**

Non-destructive *in-situ* methods

portable NIRS technology

Development of portable NIRS equipments..... more and more miniaturized

- compact and fully integrated devices and
- apparatus using an optical fiber

Advantage:

direct use in the field,
on standing trees
on logs

in primary transformation wood factories



Interest:

can allow a first selection, a first sort according to criteria of interest

Our own experience in AGPF INRA team:

first tests since 2014 on poplar (on leaves)

equipment loans from several suppliers, testing of various equipments

acquisition of two different equipments (2016, 2017)

now, several current projects with portable NIRS actions (*GenTree, TopWood, EmPIR, Chêne&Vin*)

Non-destructive *in-situ* methods

portable NIRS technology

First tests with MicoNIR on clones of eucalyptus in Spain (Madera+, TopWood project) (Nov-Dec 2016, April 2017)



Non-destructive *in-situ* methods portable NIRS technology



MicoNIR



Non-destructive *in-situ* methods

portable NIRS technology



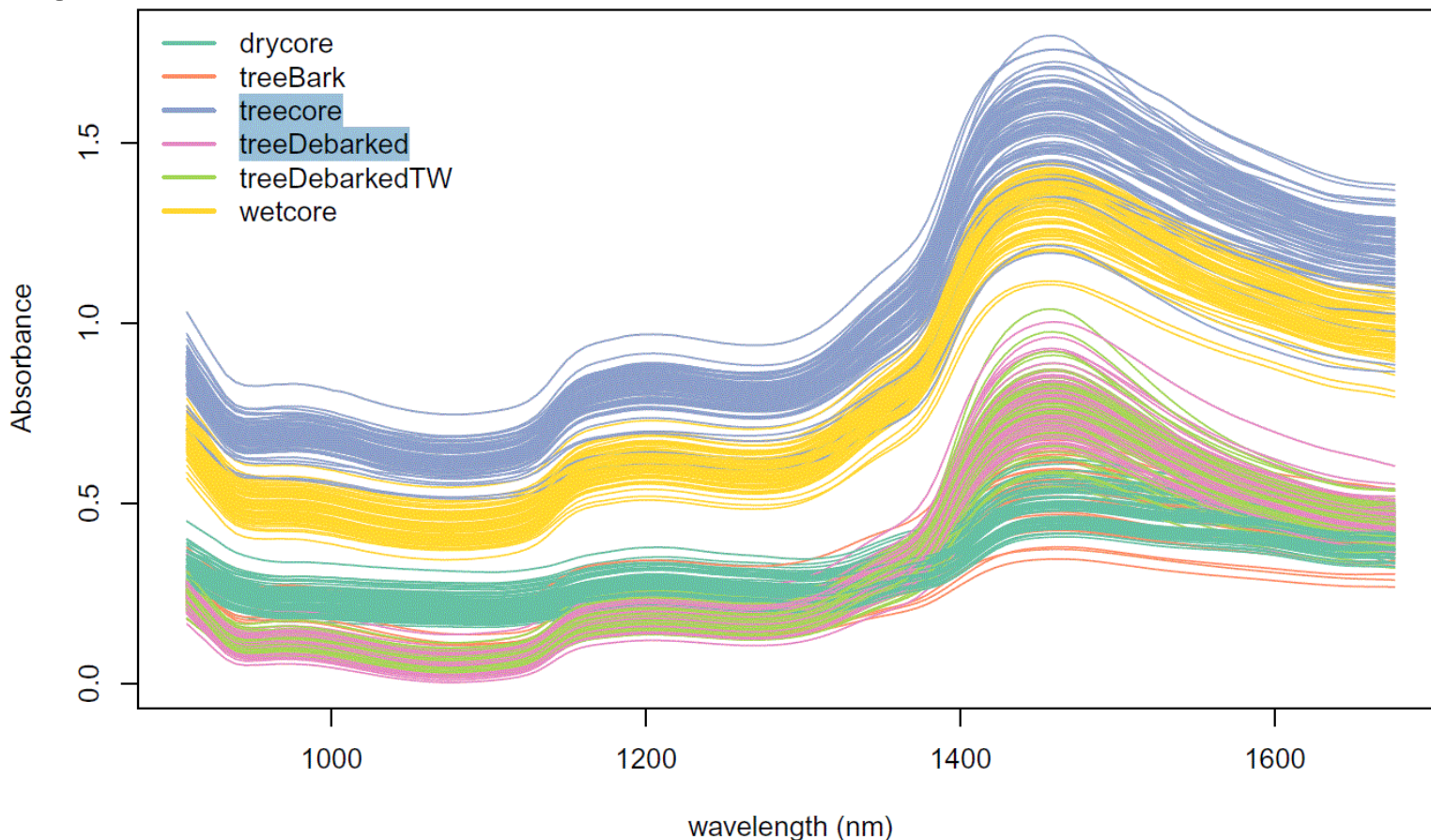
robot
fenêtre Aide

187%

Outils Remplir et signer Comm

```
legend("topleft", legend = levels(data_micronir_euca_ok$SampleType), lty = 1, lwd = 2,  
      col = couleur,  
      bty = "n")
```

NIR spectra obtained with MicoNIR

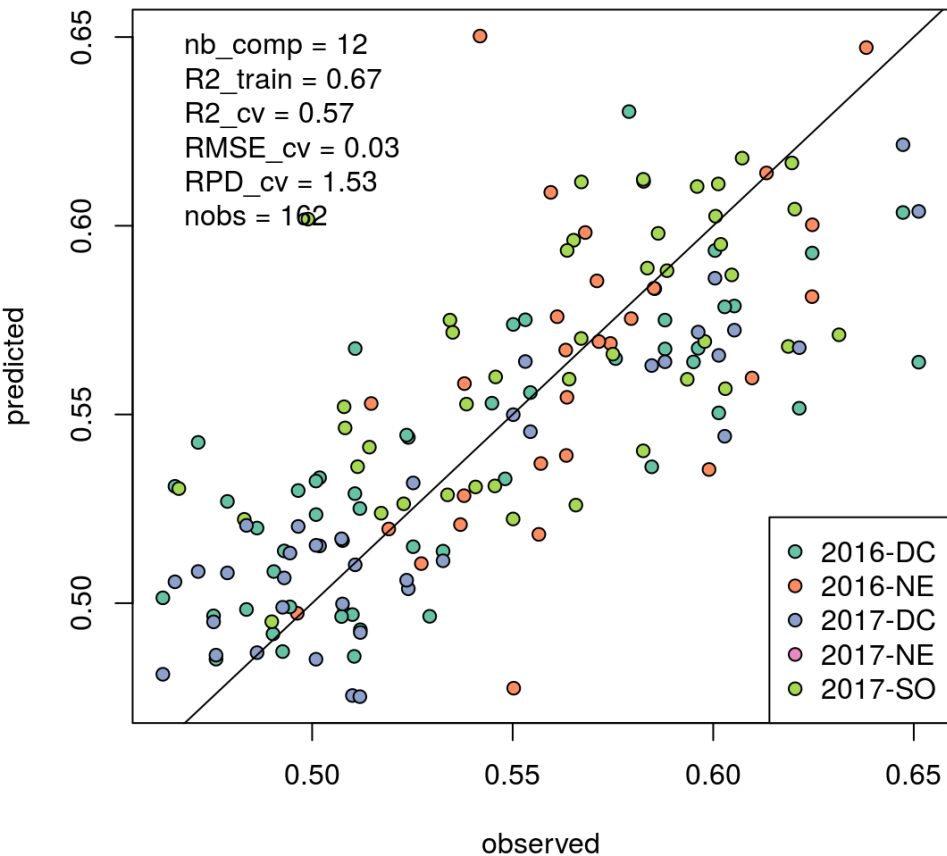


Non-destructive *in-situ* methods portable NIRS technology

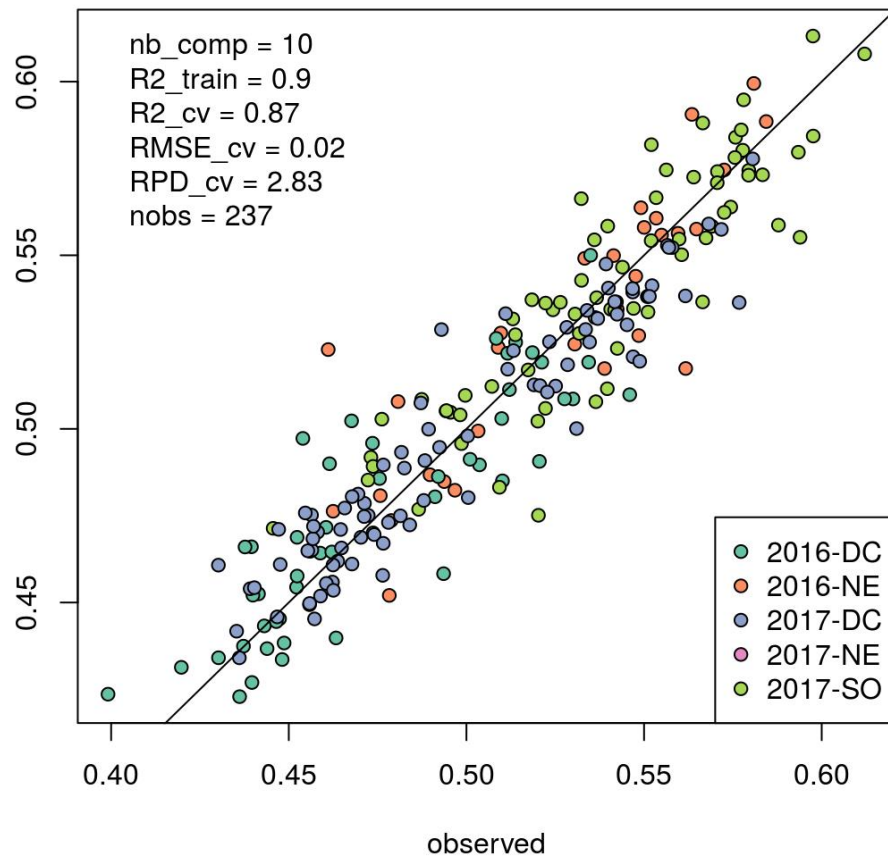


First NIRS models for density (MDM) and infradensity

MDM5cm



infradensity



Non-destructive *in-situ* methods portable NIRS technology

First tests with NIRQuest with optical fiber

The objective is to validate procedure of taking spectra on surface of trunk and into the trunk with the optical fiber

- On black poplar, old trees (April and July 2017)
correlation with parameter measured on increment cores (density)
- On Scots pine, ISS Evoltree, Punkaharju, Finland (August 2017)
correlation with parameter measured on increment cores (stilbene content) and comparison with fluorescence method



Non-destructive *in-situ* methods
portable NIRS technology

Poplar, Loire River, France

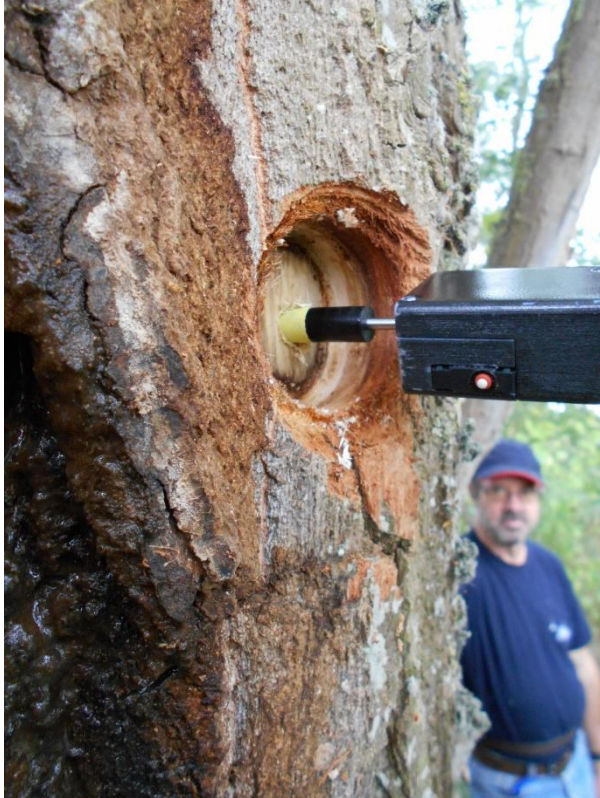
NIRQuest



Non-destructive *in-situ* methods
portable NIRS technology

Poplar, Loire River, France

NIRQuest



Non-destructive *in-situ* methods portable NIRS technology

Poplar, Loire River, France

NIRQuest



Non-destructive *in-situ* methods
portable NIRS technology

Scots pine, Punkaharju, Finland

MicroNIR and NIRQuest



Non-destructive *in-situ* methods portable NIRS technology

Scots pine, Punkaharju, Finland

NIRQuest



Non-destructive *in-situ* methods portable NIRS technology

Perspectives on the use of portable NIRS in the forest/wood sector

On standing trees:

- early selection or before thinning: management assistance and decision-making
- possible parameters of selection: wood quality , resistance to diseases, resistance to drought (cavitation), ...

On felled trees, logs:

- selection and sorting for optimized and oriented use, optimization of sales
- possible parameters of selection: wood quality (chemical, physical, and mechanical properties), natural durability, discrimination of species of same genus (oak),
- traceability



thank you for your attention

I answer your questions