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METLA

A rapid method to determine pinosylvin content in pine heartwood by UV resonance Raman spectroscopy

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Outline

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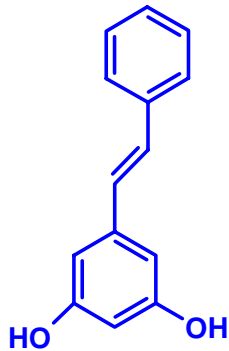


Pinosylvin

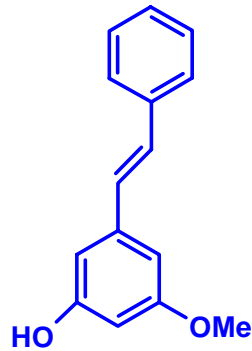
- Present in Pine wood, especially in heartwood and knotwood
- Responsible for the decay resistance of Scots pine heartwood
- Possesses antifungal, antibacterial and antitumor function
- Concentration varies significantly between trees
- Grading of timber and breeding of decay-resistant trees would benefit of rapid methods to quantify pinosylvin content



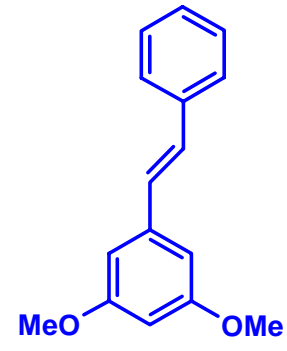
Stilbenes in Scots pine



Pinosylvin (PS)



Pinosylvin monomethyl ether (PSMME)



Pinosylvin dimethyl ether (PSDME)

	PS (mg/g)	PSMME (mg/g)	PSDME (mg/g)	Other stilbenes (mg/g)
Sapwood	0.11-0.14	<0.05	<0.05	<0.05
Heartwood	3.7-5.5	5.1-6.3	<0.14	0.15-0.27
Knotwood*	4.5-19	13-49	0.09-0.89	0.14-2.6

* Knot with a dead branch

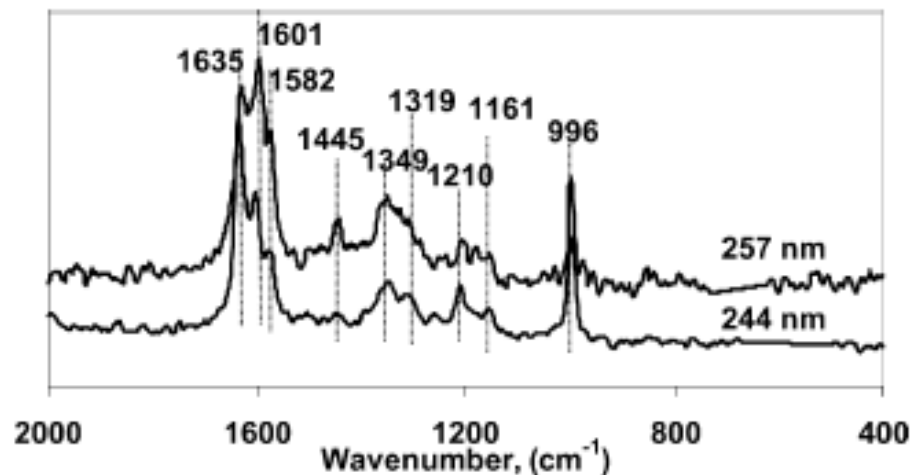
Willför et al. Holzforschung 57(2003)4, 359-372

UV resonance Raman spectroscopy

- Based on the inelastic scattering of photons
- UV excitation provides trace analysis of unsaturated structures in wood and pulp samples
- Non-destructive method, but holds a risk of sample damaging due to intense UV irradiation
- Short spectral acquisition times



UVRR analysis of stilbenes



Characteristic UV Raman bands observed for stilbene pinosylvin and flavone chrysin

Model compound	Band (cm ⁻¹)	Assignment
Pinosylvin	1635	C=C stretching [13]
	1601	Symmetric aromatic ring stretching [14]
	1582	C=C stretching [15,23]
	1445	Aromatic ring stretching [18,19]
	1349	C-C vibration of aromatic ring [19]
	1161	Aryl-OH [19-21]
	996	1,3,5-substituted aromatic ring [23]

Fig. 2. UVRR spectra of pinosylvin collected at the excitation wavelengths of 244 and 257 nm.

Nuopponen et al. *Spectrochimica Acta Part A* 60(2004) 2963-2968

Scots pine knotwood and its acetone extract

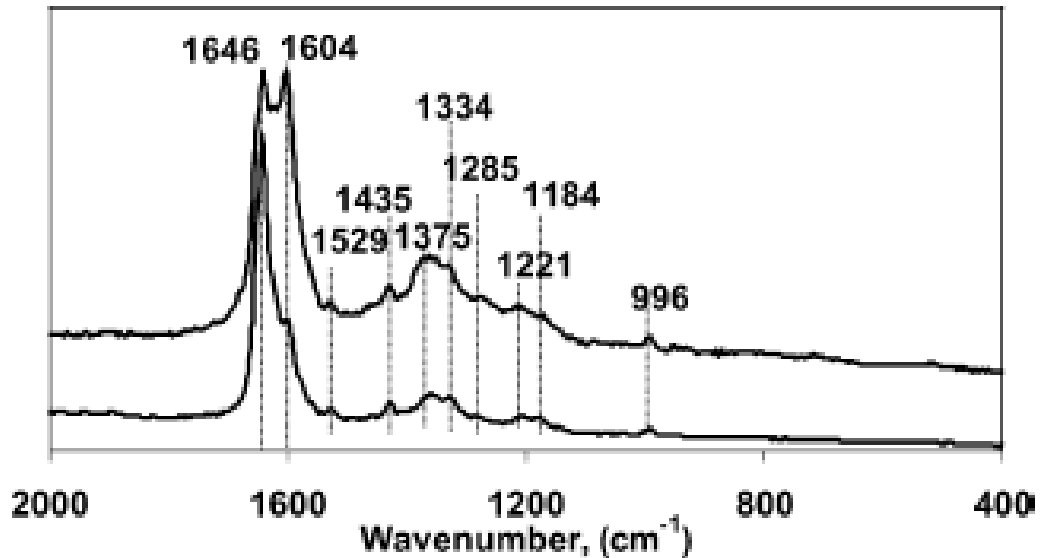
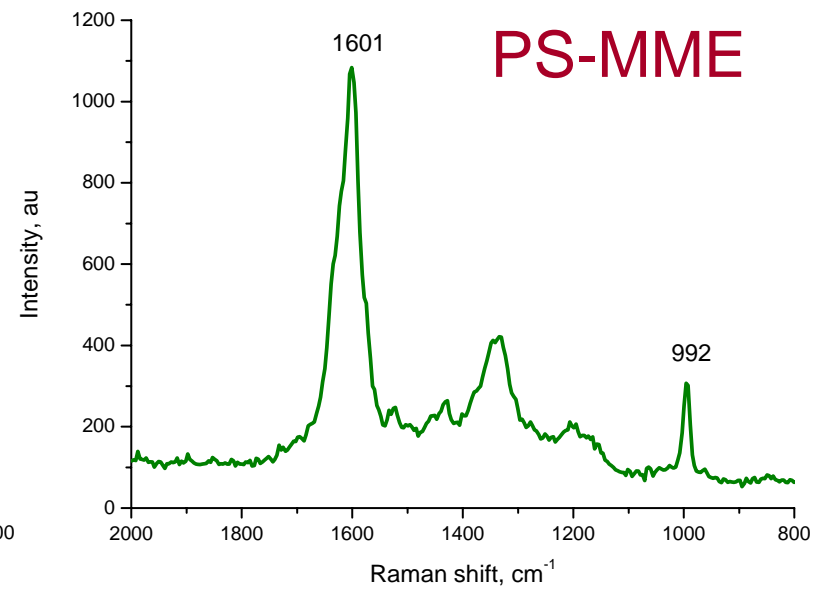
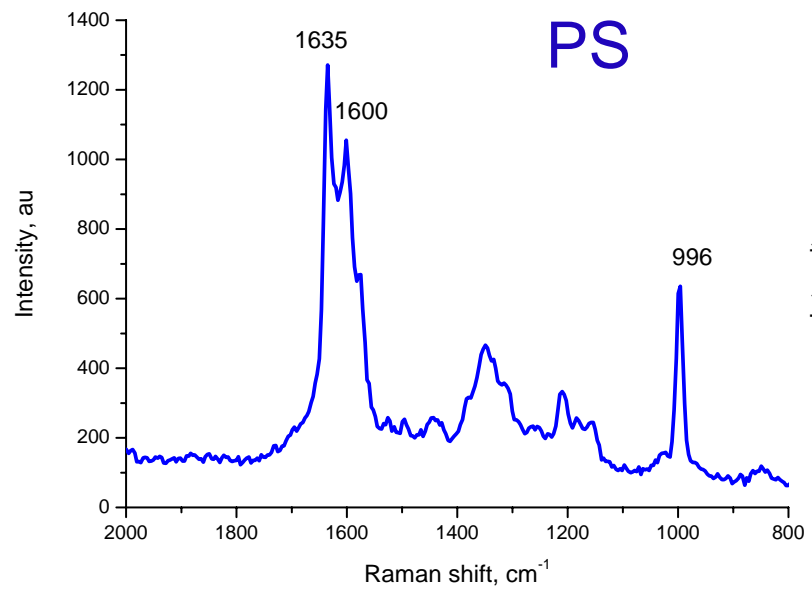


Fig. 7. UVRR spectra of the Scots pine knot wood and its acetone extract collected at the excitation wavelength of 244 nm.

Nuopponen et al. Spectrochimica Acta Part A 60(2004) 2963-2968

UVRR spectra of pinosylvin and its monomethyl ether



Objective of this study

To develop a **fast** and **simple** method for pinosylvin (and its methyl ethers) determination in wood samples for a large number of samples.

Samples

Pinus sylvestris wood samples collected from a 44-year-old experimental forest



Sample analysis

UVRR spectroscopy

Milled wood method

- Milling the wood sample
- Mixing with KBr => tablet
- UVRR spectral collection

Direct measurement

- Sample rotation or
- Linear moving
during the spectral collection

GC analysis

- Milling the wood sample
- Methanol extraction
- GC analysis

UVRR method optimization

Sample preparation

- milling the sample, mixing with KBr, pressed to tablet followed by spectral collection
- spectral collection directly from the wood chip

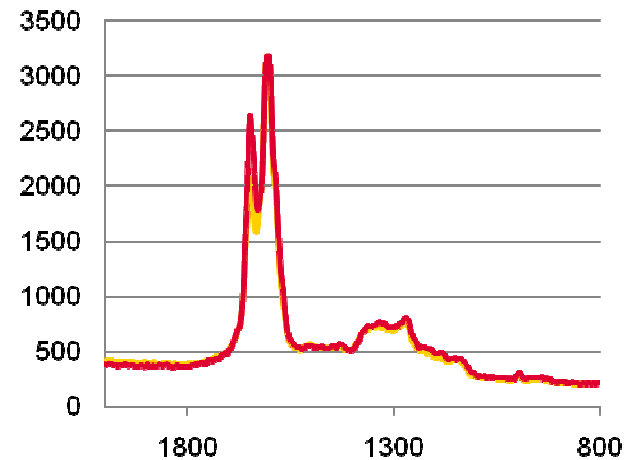
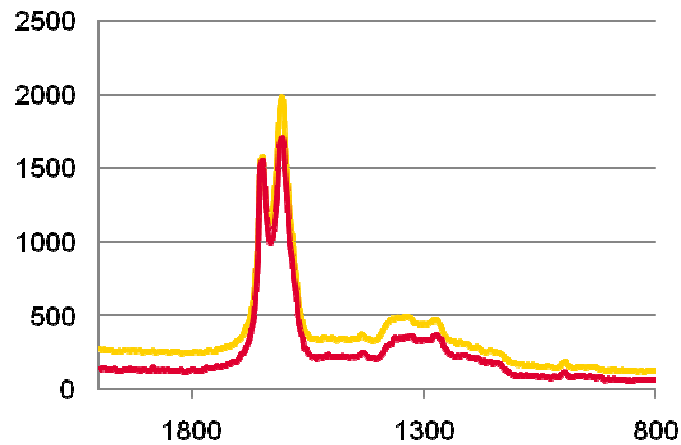
Sample measurement

- sample rotation during the measurement
- linear sample moving during the measurement

UVRR analysis – method optimization

Effect of milling the sample prior to its analysis for two samples

Milled wood vs. Wood chips

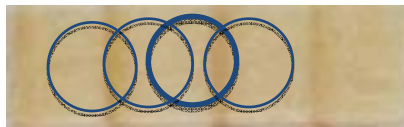


→ No need for wood milling prior to its analysis

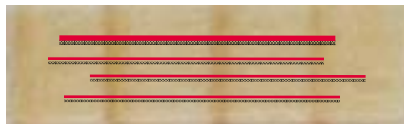
Sample stage moving

Comparing sample rotation to linear sample movement

Rotation:

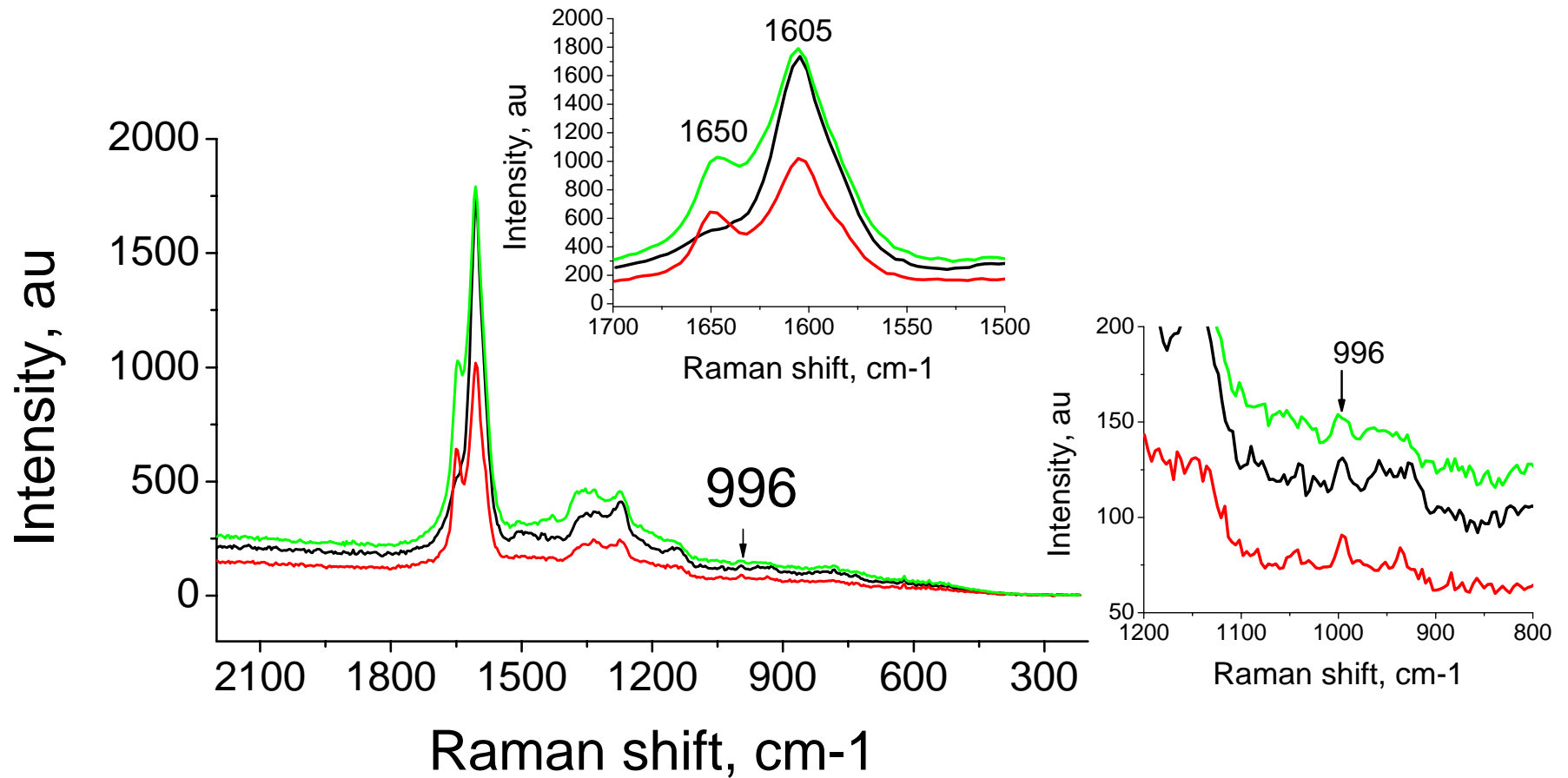


Linear sample moving:



Parallel measurements performed with linear sample moving had less scattering (better repeatability).

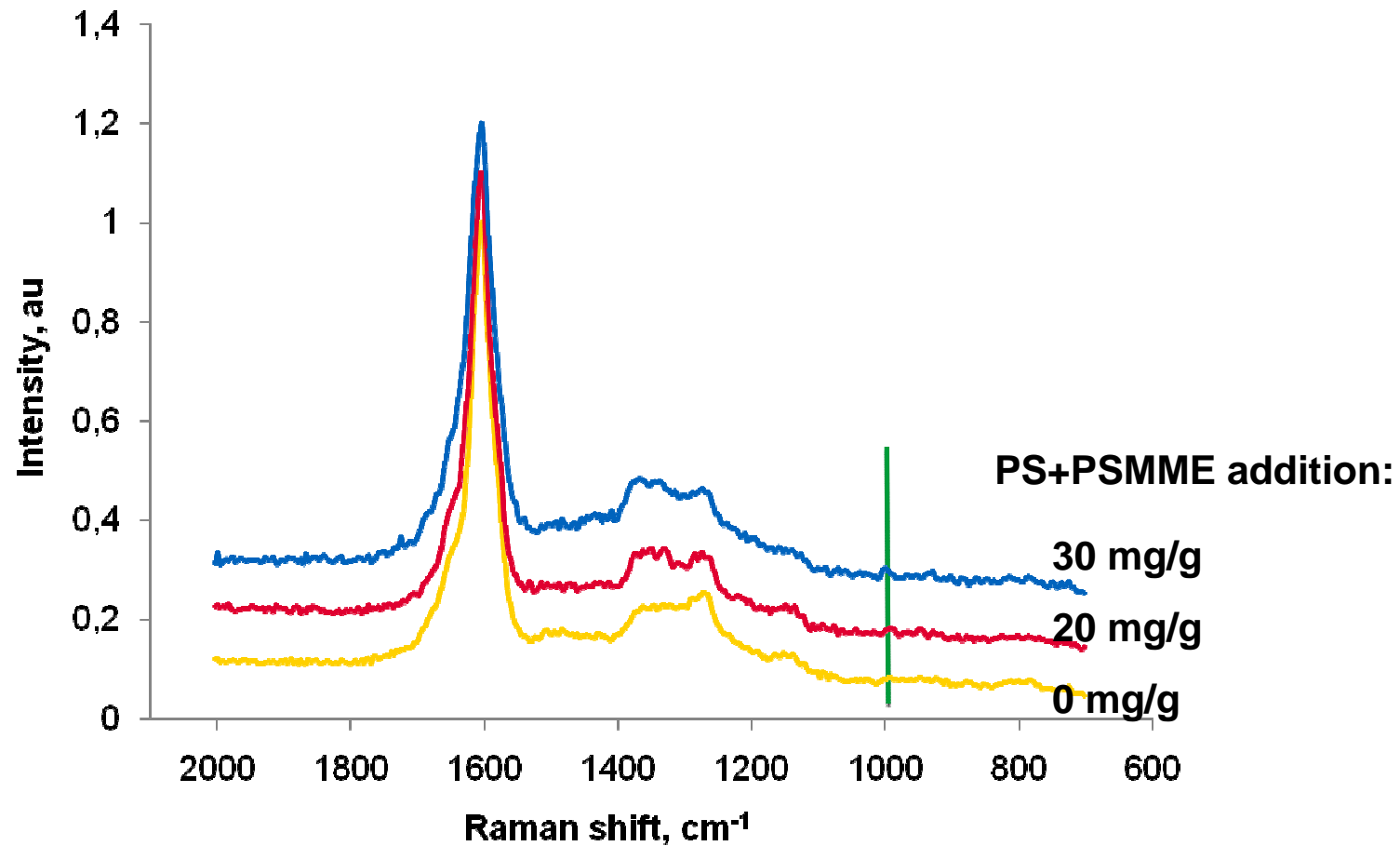
UVRR spectra of three heartwood samples



Calibration samples

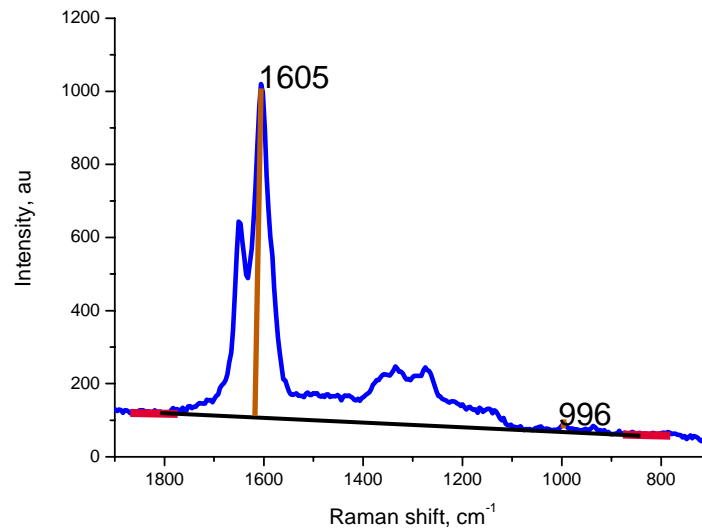
- 200 mg of milled pine sapwood
- Addition of 0 / 100 / 200 / 300 μ l of pinosylvin-extract
(10 g/l of PS and 10 g/l PSMME in EtOH)
- Addition of 1 ml EtOH, mixing, solvent evaporation.
- Sample mixing, addition of KBr, tablet pressing
- UVRR spectral collection

UVRR spectra of calibration samples

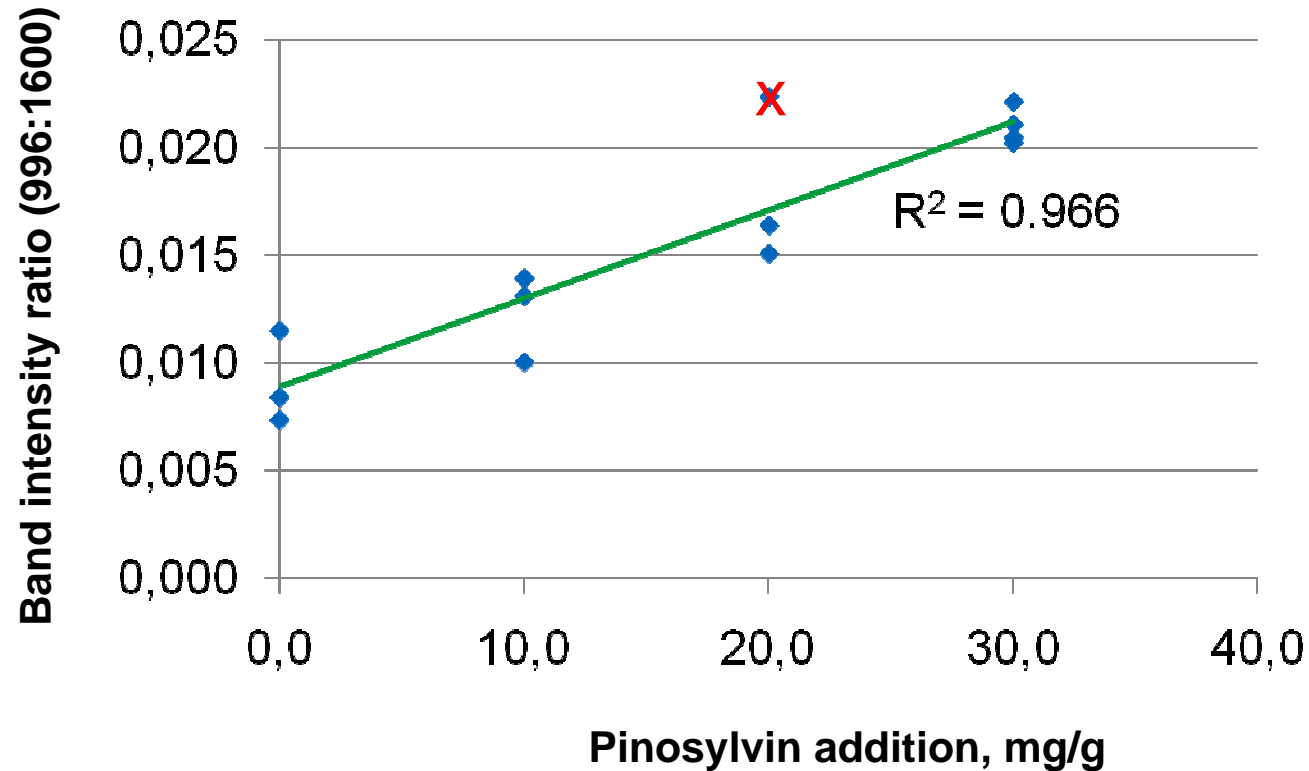


Pinosylvin quantification

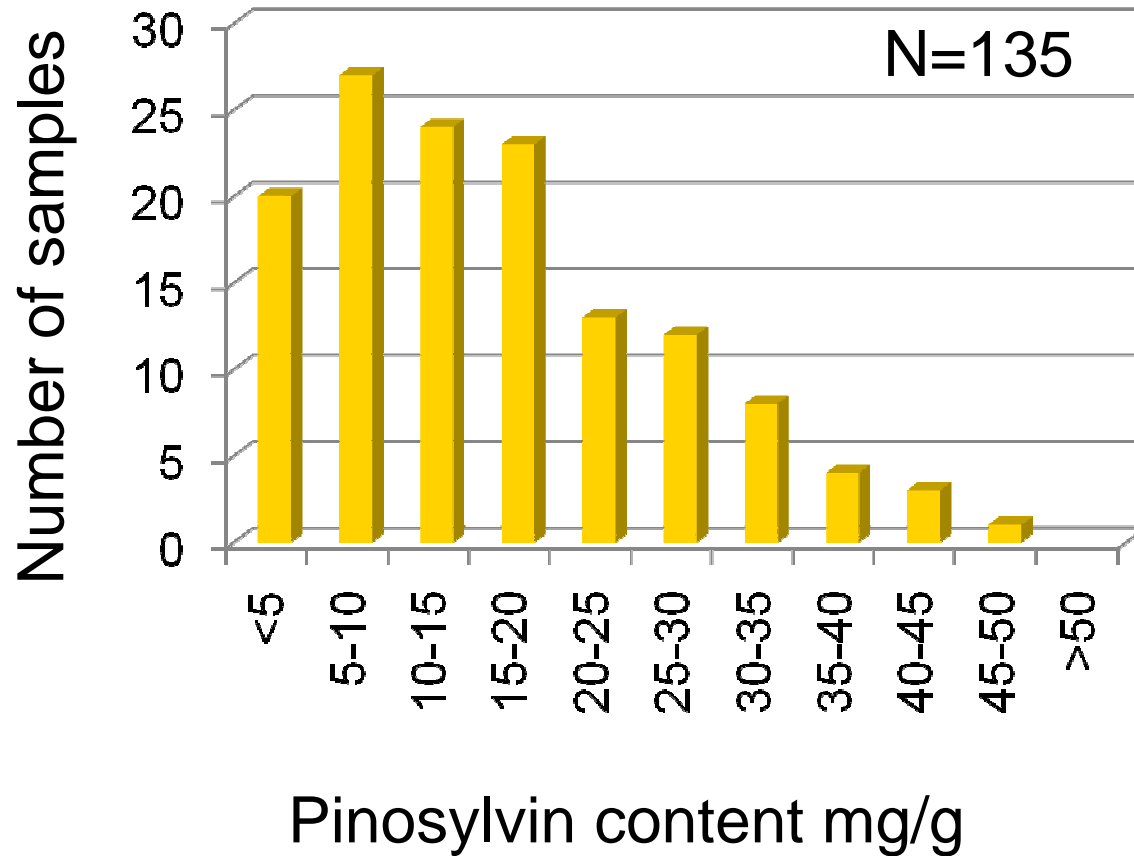
- The height of the band at 996 cm^{-1} increased with increasing pinosylvin + pinosylvin-MME concentration.
- Aromatic band (at 1600 cm^{-1}) was used as a reference band, and hence the results indicate the content of PS+PSMME with respect to lignin.
- The band height ratio was defined using the sloping baseline method:



Calibration line for PS+PSMME content



PS content in pine heartwood



UVRR vs. gas chromatography

UVRR analysis

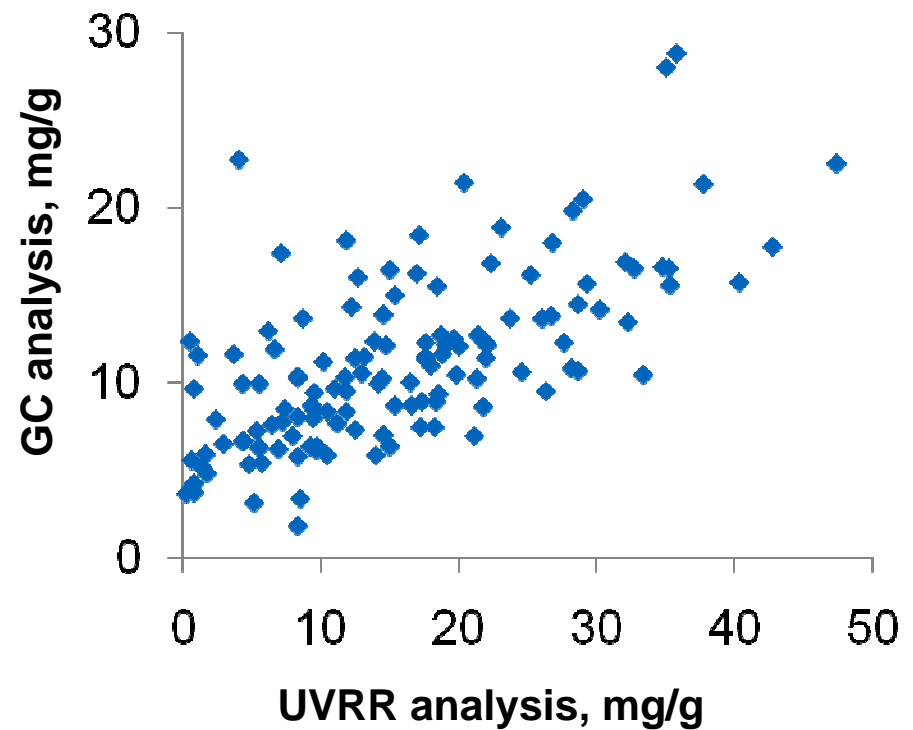
PS+PSMME 0.5-47.4 mg/g

GC analysis

Pinosylvin 0.4-8.9 mg/g

PS MME 1.4-27.0 mg/g

Total 1.8-34.7 mg/g



Conclusions

UVR spectroscopy provides a rapid method for pinoresinol content measurement

- * one spectrum is collected in ca. 30 secs**
- * 4 spectra were averaged**

Spectra were collected directly from wood chips

- * no sample pretreatment required**

The calibration is valid for this type of pine samples

- * varying lignin content would lead to erroneous results**
- * the method did not solve PS and PSMME contents separately**

Calibration will be expanded to larger PS contents

Acknowledgements

Forestcluster 


SUOMEN AKATEMIA
FINLANDS AKADEMI • ACADEMY OF FINLAND

