FTIR IMAGING AS A NEW TOOL FOR CELL WALL ANALYSIS OF WOOD*

A. NAUMANN† and A. POLLE

Institute of Forest Botany, Georg-August-Universität Göttingen,
Büsgenweg 2, 37077 Göttingen, Germany

(Received for publication 16 February 2006; revision 27 March 2006)

ABSTRACT

Fourier transform infrared (FTIR) imaging offers the opportunity to analyse the chemical composition of wood spatially resolved. To illustrate the potential of FTIR imaging for wood analysis, the lignin distribution in cross sections of beech and poplar wood was analysed and the spatial resolution compared. Additionally, the ratio of guaiacyl/syringyl lignin in a poplar wood section was computed. The resolution of the FTIR microscope was sufficient to resolve individual cell walls of poplar wood fibres.

Keywords: Fourier transform infrared imaging; wood; cell wall; lignin distribution; guaiacyl/syringyl ratio.

INTRODUCTION

FTIR spectroscopy is a well-established method for analysis of the chemical composition of wood (e.g., Faix 1991; Michell & Higgins 2002; Pandey & Pitman 2003). In combination with microscopy, spatial resolution of the chemical composition is achieved. FTIR imaging has been used for a range of biological samples, e.g., carcinoma tissue (Salzer *et al*. 2000), and is a promising tool for studying the composition of wood. Initially, a traditional single channel detector was developed, which is applicable for measuring a spectrum of a selected area. This technique was, for example, used to chemically characterise isolated wood fibres of spruce (Burgert *et al*. 2005) and to evaluate host resistance in the Dutch elm disease complex (Martín *et al*. 2005). However, the use of FTIR microscopy combined with a single channel detector to visualise the distribution of chemical compounds spatially resolved in so-called mappings was limited because of the long measurement times.

---

* Based on a paper presented at 1st Joint New Zealand – German Symposium on Plant Cell Walls, 23–24 June 2005, Rotorua, New Zealand

† Corresponding author: anauman1@uni-goettingen.de

For FTIR imaging a new kind of detector became available in the 1990s, which had initially been developed for military applications. These focal plane array detectors (FPA) reduced the measurement time enormously, from days to minutes. Such detectors consist of many single detector elements and measure thousands of spectra simultaneously. Each band of the spectra can be integrated and the distribution of the corresponding molecular bond or functional group can be displayed as a false colour image (for a review, *see* Salzer *et al*. 2000).

Due to the chemical complexity of biological samples FTIR band assignment to specific molecular bonds and functional groups is not always clear because of overlapping bands. Multivariate data analysis is a powerful tool to overcome this problem. In spruce, the content of carbohydrates, lignin, and extractives could be determined by partial least squares modelling (Meder *et al*. 1999). The power of FTIR analysis is to provide a "fingerprint" of the whole chemical composition and ratios between compounds of the sample. Biological or biotechnological changes of the ratio between compounds are detectable by spectra comparison, for example, due to fungal degradation of wood (Pandey & Pitman 2003). Recently, FTIR imaging was applied to detect and visualise the distribution of fungi in wood (Naumann *et al*. 2005). Additionally, differences in the ratio of guaiacyl/syringyl lignin can be characterised by FTIR spectroscopy (Faix 1991).

The aim of this work was to illustrate the potential of FTIR microscopy combined with an FPA detector for chemical imaging of wood.

MATERIALS AND METHODS

Wood of beech (*Fagus sylvatica* L.) and a 3-month-old poplar hybrid (*Populus* × *canescens*) was sectioned with a cryomicrotome (Jung, Germany). During drying at room temperature, the sections (10 μm thickness) were covered with a coverslip and weighted with a lead block to keep the sections flat.

FTIR analysis was performed with an FTIR spectrometer Equinox 55 combined with an IR microscope Hyperion 3000 (Bruker Optics, Ettlingen, Germany) including a 64 × 64 focal plane array detector (FPA) and a 15 × Cassegrain-objective. For measurement, sections were placed on a KBr window (2 mm thickness) as an infrared transmissive sample holder. FTIR spectra were recorded for the wave number range of 3900 to 900 cm^{-1} with a spectral resolution of 12 cm^{-1}. To improve the signal-to-noise ratio 16 spectra were added and averaged.

FTIR data were evaluated using the OPUS version 5.0 software (Bruker, Germany). The band at 1508 cm^{-1} is tentatively assignable to lignin (Faix 1991). The wave number range between 1530 and 1490 cm^{-1} was used for integration to display the lignin distribution in the sections. A straight line connecting the local peak minima at the given frequencies was employed as a baseline (OPUS integration method B).

As the band at 1510–1504 cm^{-1} decreases with decreasing guaiacyl/syringyl lignin ratio, while the band at 1328–1326 cm^{-1} increases (Faix 1991), the ratio of 1508 cm^{-1}/1320 cm^{-1} was used to calculate the relative distribution of guaiacyl/syringyl lignin for three areas of a section and to display it exemplarily.

RESULTS

The anatomy of beech and poplar wood by light microscopy and the corresponding FPA images are shown in Fig. 1. Under the light microscope, beech (Fig. 1A) and poplar (Fig. 1B) vessels, wood fibres and xylem rays are visible at a magnification of 150×. In the FPA images (Fig. 1C, 1D) the FPA data sets were integrated for the range between 1530 and 1490 cm^{-1}, which is tentatively assignable to lignin. The colour scale from blue to pink indicates increasing lignin content. It is obvious that the resolution of the FTIR images is lower than that of the light microscopic images. This is due to the wave lengths employed, which limit the resolution to about 4 μm. Nevertheless, in poplar (Fig. 1D) single cell walls of wood fibres were discernable in many images, whereas in beech individual cells were less well detected (Fig. 1C). The cell walls of the wood fibres in beech are thicker (5 μm) than those of poplar (1–2 μm).

Late wood cells in the beech section showed a pronounced red-pink colour indicative of high lignin content. In the poplar wood section from a 3-month-old plantlet without growth ring, the difference in lignin content was less pronounced than in the beech section of adult wood with a growth ring. However, the lignin content of the bottom left part was slightly lower than of the top right part (Fig. 1D). The exemplary calculation of the guaiacyl-syringyl ratio in poplar resulted in the highest values for parts of the vessel cell walls (Fig. 1E). Three measurements in different areas of the section gave similar results.

DISCUSSION

The observation that thinner poplar cell walls can be resolved and thicker beech walls cannot is surprising at the first glance. The reason is that the lumina are bigger in poplar than in beech. The thin poplar cell walls are surrounded only by air, which gives a high contrast. If a single detector element of 4 × 4 μm measures the 1–2 μm thin cell wall or a part of it, this will result in a spectrum. However, the cell walls in the FPA image appear thicker than in the light microscopic view, because of the size of the detector elements. If the cell wall is measured by one detector element, it would appear to be 4 μm thick, but if it were to be measured by two detector elements, it would appear to be 8 μm thick. Correspondingly, the intensity of the spectrum would decrease. The small cell lumina in beech wood cannot be resolved because of this overlap.

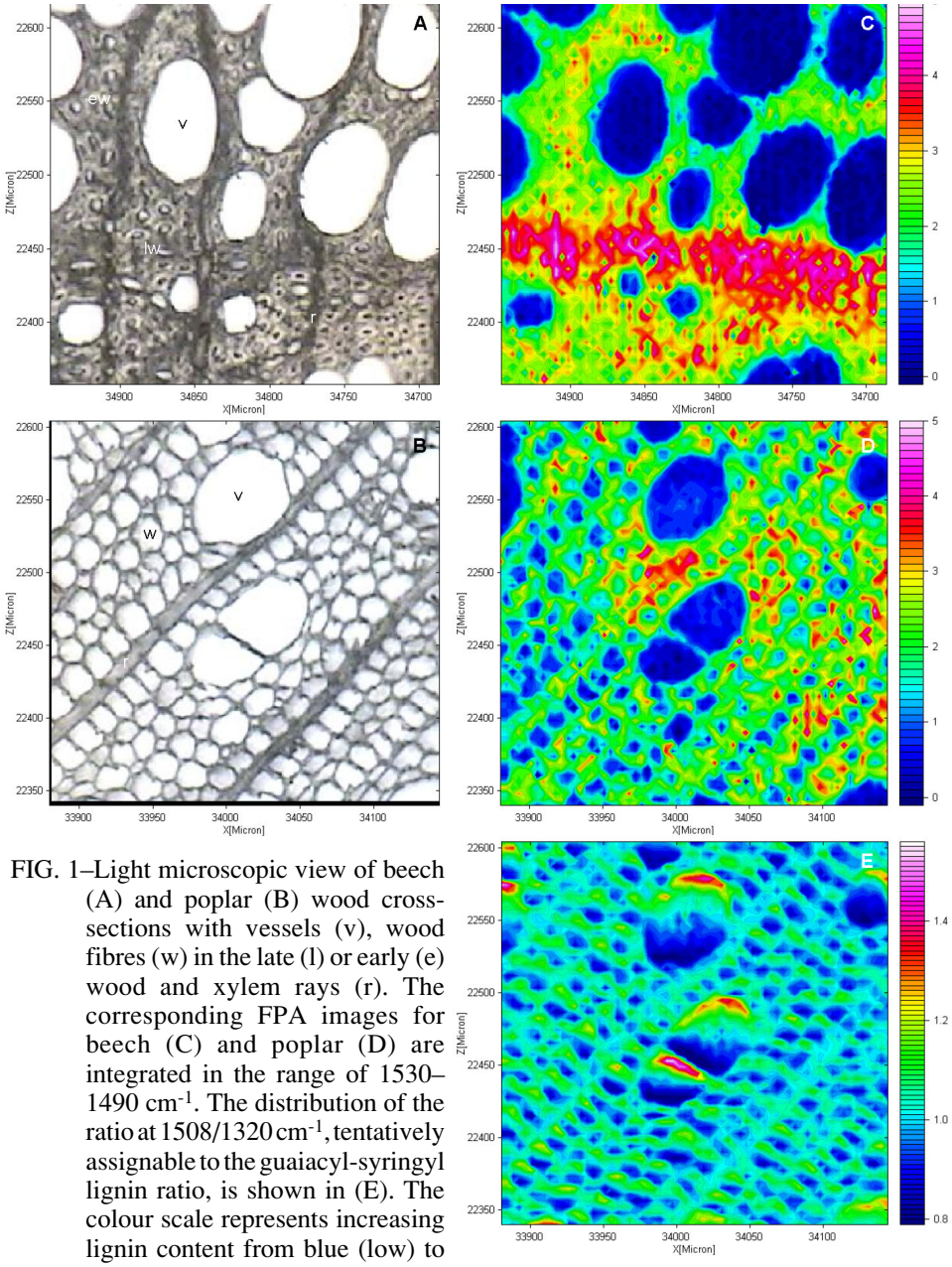

FIG. 1–Light microscopic view of beech (A) and poplar (B) wood cross-sections with vessels (v), wood fibres (w) in the late (l) or early (e) wood and xylem rays (r). The corresponding FPA images for beech (C) and poplar (D) are integrated in the range of 1530–1490 cm^{-1}. The distribution of the ratio at 1508/1320 cm^{-1}, tentatively assignable to the guaiacyl-syringyl lignin ratio, is shown in (E). The colour scale represents increasing lignin content from blue (low) to pink (high).

The measured lignin content can depend on the thickness of the cell walls. If one detector element (4 × 4 μm) measures only a cell wall, the lignin content in the cell wall seems to be higher than that in the images, when wall and lumen are measured. It is important to note that this will also blur the apparent content of lignin,

especially in the thin cell walls typical in early wood. The red-pink colour of the late wood cell walls indicating high lignin content is hardly affected by such resolution problems, because the portion of lumina is relatively small compared to the portion of cell walls. By calculation of a ratio of two bands measured by the same detector elements, the influence of varying section or cell wall thickness can be diminished. The high ratio of guaiacyl-syringyl lignin detected in parts of vessel cell walls accords with references cited by Donaldson (2001).

In addition to the size of the single detector elements, the resolution of FTIR images depends on the wave number (cm^{-1}) = 10^7 / wave length (nm) of the radiation, which is thus more limited by the longer wave length of infrared radiation than that of visible light. At 1500 cm^{-1}, the wave number employed here, the maximal resolution is 4 μm and matches the size of the single detector elements. At 800 cm^{-1}, a resolution of 7.5 μm is achievable.

There have also been developments using a composed detector with 16 single detector elements. Images were created by consecutive measurements during stepwise moving of the sample below the detector (Labbé *et al*. 2005). However, the maximal resolution of that equipment is expected to be slightly lower, because of the larger size of the single detector elements at 6.25 μm.

The quality of the light microscopic images taken with the FTIR microscope is lower than those obtained by conventional light microscopy. The reason for this is the optimisation of the light path for infrared microscopy. Infrared microscopy requires gold mirror objectives instead of glass objectives, because glass is not infrared transmissive. The resolution of these gold mirror objectives is not as good as that of glass objectives. But the quality is satisfactory to locate FTIR spectra in wood tissue.

In conclusion, the resolution of FTIR imaging shows that this technique is promising for studying the influence of biological or biotechnological changes in cell wall composition on the tissue level and in appropriate samples on the level of single cell walls.

ACKNOWLEDGMENTS

The authors thank Christa Lang for the preparation of beech wood sections. This project was conducted within the NHN (Niedersächsisches Kompetenznetz für Nachhaltige Holznutzung). Financial support by the Ministry for Science and Culture of Lower Saxony is gratefully acknowledged.

REFERENCES

BURGERT, I.; GIERLINGER, N.; ZIMMERMANN, T. 2005: Properties of chemically and mechanically isolated fibres of spruce (*Picea abies* [L.] Karst.). Part 1: Structural and chemical characterisation. *Holzforschung 59*: 240–246.

DONALDSON, L.A. 2001: Lignification and lignin topochemistry — an ultrastructural view. *Phytochemistry 57*: 859–873.

FAIX, O. 1991: Classification of lignins from different botanical origins by FT-IR spectroscopy. *Holzforschung 45 Suppl.*: 21–27.

LABBÉ, N.; RIALS, T.G.; KELLEY, S.S.; CHENG, Z.-M.; KIM, J.-Y.; LI, Y. 2005: FT-IR imaging and pyrolysis-molecular beam mass spectrometry: new tools to investigate wood tissues. *Wood Science and Technology 39*: 61–77.

MARTÍN, J.A.; SOLLA, A.; WOODWARD, S.; GIL, L. 2005: FT-IR spectroscopy as a new method for evaluating host resistance in the Dutch elm disease complex. *Tree Physiology 25*: 1331–1338.

MEDER, R.; GALLAGHER. S.; MACKIE, K.L.; BÖHLER, H.; MEGELEN, R. 1999: Rapid determination of the chemical composition and density of *Pinus radiata* by PLS modelling of transmission and diffuse reflectance FTIR spectra. *Holzforschung 53*: 261–266.

MICHELL, A.J.; HIGGINS, H.G. 2002: "Infrared Spectroscopy in Australian Forest Products Research". CSIRO Forestry and Forestry Products, Melbourne, Australia.

NAUMANN, A.; NAVARRO-GONZÁLEZ, M.; PEDDIREDDI, S.; KÜES, U.; POLLE, A. 2005: Fourier transform infrared (FTIR) microscopy and imaging: Detection of fungi in wood. *Fungal Genetics and Biology 42*: 829–835.

PANDEY, K.K.; PITMAN, A.J. 2003: FTIR studies of the changes in wood chemistry following decay by brown-rot and white-rot fungi. *International Biodeterioration & Biodegradation 52*: 151–160.

SALZER, R.; STEINER, G.; MANTSCH, H.H.; MANSFIELD, J. 2000: Infrared and Raman imaging of biological and biomimetic samples. *Fresenius Journal of Analytical Chemistry 366*: 712–726.