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and
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Preface

It is our great pleasure to welcome you to the 2nd biannual meeting of the new series "International Plant Spectroscopy Conference" (IPSC) in Berlin!

The main reason for the IPSC initiative is to provide a platform for spectroscopists and plant scientists to present and discuss new developments in their respective fields of spectroscopy and spectrometry on an international level. The event aims to gather scientists from fundamental to applied research, highlighting applications from academia to industry.

The founder of this meeting series is the International Society for Plant Spectroscopy (ISPS), but each meeting draws heavily on and shares responsibilities with the local hosts, in this case the German Society for Quality Research of Plant Foods (DGQ). The high level of participation (ca. 140 people from 5 continents, with more than 45 lectures and 42 poster contributions) clearly demonstrates that the conference is very topical and timely, with numerous research institutions in the world already dedicated to this field of science. In addition to participants from academia, representatives from industry as well as from national and international authorities join the meeting to present and discuss recent findings and issues, develop networks and identify future research priorities.

The conference covers all modern techniques used for plant analysis, such as various kinds of infrared and Raman spectroscopies, mass spectrometry, NMR spectroscopy and hyperspectral imaging. Special attention is paid to chemometrics, the statistical interpretation of the complex and extensive data sets. In this context, plant metabolite profiling emerges as an increasingly important tool too, aiding in the better understanding of complex plant-physiological relationships. From the materials side, a broad range of plant species, tissues and cell types are represented, extending beyond major cell wall components and their derivatives to also cover the extractions of dyes, pharmaceutically active components or their precursors, bio-based pesticides, and the isolation of vegetable oils, aroma extracts and bioactive nutraceuticals.

Naturally, organising an event with such scope and scale is impossible without the dedication, hard work and support of a great number of people. Therefore, we would like to take this opportunity and thank the national and international organising committee for their work, not least in the preparation of this book. We, the organisers, would also like to hereby thankfully acknowledge the financial support provided by our home institutions, as well as by the German Research Foundation, and several private companies and journal publishers as sponsors.

Last, but most certainly not least, we are indebted to the local organisers and staff at the conference, the invited and plenary speakers, as well as all presenters and participants.

Thank you all for making this conference a productive and amiable meeting.

Berlin, March 2019

Hartwig Schulz (Julius Kühn Institute, Germany)

András Gorzsás (Umeå University, Sweden)

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Ca. 20:00	**Icebreaker**
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10:10 – 10:30	*01-03* Running a network of NIRS instruments for forages and other plant materials - Quality control **Peter Tillmann**
10:30 – 11:00	**Coffee break** sponsored by *Plants* (MDPI AG, Basel, Switzerland)
11:00 – 11:20	*01-04* Application of NIR technology to predict minor components in raw and processed potatoes **Inga Smit**
11:20 – 11:40	*01-05* Fluorescence ratiometry and NIR transmission in combination allow in-situ analysis of leaf apoplastic pH under controlled changes of leaf water content **Hartmut Kaiser**
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Tuesday	26th March
Session 3 - Raman Spectroscopy / Imaging (Co-chairs: Baranska/Gierlinger)	
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11:40 – 12:30	**Various remarks** DGQ price, poster prices, next IPSC, next DGQ meeting
12:30 – 13:00	**Closing remarks** Hartwig Schulz

Welcome plenary lecture

00-01: Spectroscopy in plant-animal interactions: Looking at the natural world as a herbivore

William J. Foley, Karen J. Marsh, Kara Youngentob

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The nutritional quality of food eaten by wild vertebrate herbivores influences why animals occur where they do. Crude protein and poorly-defined complexes such as "fibre" are the most widely used indicators of quality and are often combined as ratios of dubious validity. Furthermore, ecologists seem to have abandoned earlier collaborations with chemists to study other plant constituents and continue with uninformative assays (e.g. total phenolics) with little or no evidence that they improve our understanding of animal foraging choices. Furthermore only a few samples of each species are usually analysed despite potentially large intra-species variability in forage quality.

Spectroscopic methods are an ideal to way to address many of these problems. Quantitative, near infrared reflectance spectroscopy (NIRS) has proved particularly suitable for the rapid analysis of large numbers of samples. Not surprisingly, we often find that variation within a plant species is greater than variation between species within a landscape.

However, beyond analytical speed, spectroscopy allows the capture of complex compositional data from plants far beyond the handful of traits that are currently analysed in ecological studies. Responses of animals to variations in plant composition can be modelled better by spectroscopy than by identifying individual chemical components. For example, the best measure of the quality of food for koalas is how much they are willing to eat. This can be much better explained by NIR spectra of *Eucalyptus* leaves than by any combination of known nutrients and toxins. Thus, instead of seeing a forest as a chemically variable landscape, spectroscopy allows us to more closely approximate its value from a herbivore's perspective.

Finally, quantifying the factors important in the distribution and abundance of animals across wide areas requires complex statistical models that account for the heterogeneous nature of landscapes and allow us to isolate the impacts of individual variables alone and in combination with other factors. We are currently testing how we incorporate spectra into these modelling structures so that plant composition can be more widely used into conservation planning.

Session 1 - NIR Spectroscopy / Imaging (Co-chairs: Huck/Siesler)

01-01: Hand-held vibrational spectrometers: State-of-the art instrumentation and novel applications

Heinz W. Siesler

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Miniaturization of vibrational spectrometers has started more than two decades ago, but only within the last decade real hand-held Raman, mid-infrared (MIR) and near-infrared (NIR) scanning spectrometers have become commercially available and have been utilized for a broad range of analytical applications [1-7]. In actual fact, marketing companies predict this segment of instrumentation a significant growth rate within the next few years. This increase will be primarily based on a wider adoption of spectrometers for quality control by in-the-field testing and on-site measurements and by expansion to a new user community.

In view of the higher price level of miniaturized Raman and MIR instruments (>10 K US$) compared to NIR systems (~1 K US$) only the last mentioned spectrometers can be taken into consideration for private use in the near future, whereas hand-held Raman and MIR spectrometers will be restricted to industrial, military and homeland security applications and public use by first responders, customs or scientific institutions. Thus, based on high-volume manufacturability and further reduction of costs, numerous companies target with NIR instruments a non-expert user community for consumer applications. Especially from this last-mentioned development a tremendous potential for everyday life can be expected ranging from food testing to detection of fraud and adulteration in a broad area of materials.

The presentation will shortly describe instrumental features of novel hand-held Raman, MIR and NIR spectrometers and discuss selected qualitative and quantitative case studies.

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01-02: Recent advances in vibrational spectroscopic imaging studies of medicinal plants

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Plant cells, tissues and organs are composed of various biomolecules arranged as structurally diverse units, which represent heterogeneity at microscopic levels. Molecular knowledge about those constituents with their localization in such complexity is very crucial for both basic and applied plant sciences. In this context, infrared imaging techniques have advantages over conventional methods to investigate heterogeneous plant structures in providing quantitative and qualitative analyses with spatial distribution of the components. Thus, particularly, with the use of proper analytical approaches and sampling methods, these technologies offer significant information for the studies on plant classification, physiology, ecology, genetics, pathology and other related disciplines. This presentation aims to present a general perspective about near-infrared and mid-infrared imaging/micro-spectroscopy in plant research. It is addressed to compare potentialities of these methodologies with their advantages and limitations. With regard to the organization of the presentation, the first section will introduce the respective underlying principles followed by instrumentation, sampling techniques, sample preparations, measurement, and an overview of spectral pre-processing and multivariate analysis. The last section will review selected applications in the literature

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01-03: Running a network of NIRS instruments for forages and other plant materials - Quality control

Peter Tillmann

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Since 1998 VDLUFA (Association of German Agricultural Research and Experimental Stations) is running a network of NIRS instruments for the analysis of forages and other plant material. 60 instruments are located in the customers laboratories and distributed mainly throughout Europe. The main changes over the last 20 years are the multitude of new instruments brands and therefore software formats.

On the other hand harmonized methodology is the main emphasis of VDLUFA, which shall be proven by traditional quality control measures in form of proficiency tests.

We will report on our strategies in networking and observations from our quality control work throughout the past years regarding the analysis of especially forage maize for plant breeding and variety trials.

A NIRS network is here defined as a group of instrument running with a uniform calibration model for prediction. Instrument differences are accounted for and corrected by spectral standardization according to Shenk [1] and repeatability file [2].

Our observations show that reproducibility of NIRS measurements from a NIRS network do compare favorably with chemical analyses [3].

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01-04 Application of NIR technology to predict minor components in raw and processed potatoes

Inga Smit, Norbert U. Haase

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Being a staple food potatoes are grown in many countries with a world-wide production of 380 Mio tonnes in 2014 [1]. In Germany the per capita consumption of potatoes is about 56.8 kg in the financial year 2015/2016 with a share of 60% of processed potatoes [2] such as fried products (e.g. French fries). Industrial processing of French fries includes several steps like quality control of raw material, blanching, par-frying and freezing [3]. End-frying as the last preparation step is mainly done in canteens or at home. In fried products the heat-induced contaminant acrylamide can be formed via the Maillard reaction depending on the amount of the precursors reducing sugars (glucose, fructose) and asparagine [4].

Different NIRS applications have been tested for potatoes during the last decades. Good predictions of the dry matter and starch content have been established by measuring the raw grounded tuber [5]. Potato cultivars used for processing of French fries usually contain a high amount of dry matter that mainly consists of starch. Due to their low concentration, reducing sugars in the raw tuber (3-24 mmol/kg) and likewise, the acrylamide in the deep fat fried product are minor components [4]. NIRS-based predictions of reducing sugars in the raw material would be an advantage in the quality control of industrial French fries processing [5]. However, acceptable predictions for such minor components are hardly reachable [5,6]. Nonetheless, in potato breeding process less exact predictions could be a useful complementation in the phenotyping of new breeding lines.

Within a current project (DEPOLA, starting 2017) we try to develop a NIRS model to be used as a valid screening tool in identifying potato genotypes with a low acrylamide potential. To perform the trial, a set of 185 genotypes from German and Turkish breeding programs is grown in both countries of contrasting climatic conditions. The total sample number will reach at the projects end 1600 including field replications, several locations (three per country) and different storage treatments. Yet, a subset of 96 samples is analyzed. The quality parameters that were used for model development are dry matter, starch, glucose, fructose, sucrose, colour (*L*a*b** values) and acrylamide. NIR measurement was repeated twice for each sample and a wavelength range of about 400-2500 nm was monitored with a FOSS XDS NIR spectrophotometer.

Most quality parameters show a high variation within the data set, especially for acrylamide but also for the colour (*a** value) and the glucose content, which is a basic requirement. Taking into account the size of the sample set a cross validation was performed. Besides, using non-treated spectra that seem to be well suited for the dry matter content (R^2 of calibration and prediction of 0.97 and 0.96, respectively), the first and second derivatives were used to model the prediction of the minor components. For glucose the R^2 of calibration and prediction were 0.85 and 0.59 when using the first derivative. By using the second derivative for acrylamide the R^2 of calibration and prediction were 0.91 and 0.41. The mathematical pre-treatments were able to improve the model indices. Even though the data set was small, it indicates capabilities of NIR technique as a screening tool for breeding purposes. Satisfactory information should be taken from a larger data set that we will gain by the end of the project in 2020.

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01-05: Fluorescence ratiometry and NIR transmission in combination allow in-situ analysis of leaf apoplastic pH under controlled changes of leaf water content

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Optical measurements have the potential to non-invasively study undisturbed physiological processes in plant tissues. Micro-environmental control of the living sample is necessary to investigate such cellular responses to environmental stimuli both under realistic and repeatable conditions. Here, an automated microscopic platform is presented, which offers various microscopic methods, like fluorescence ratiometry, FRET, BRET and confocal imaging. Ratiometric fluorescence imaging is used for continuous in-vivo monitoring of ionic concentrations in different cellular compartments. The instrument was complemented with a custom environmental leaf chamber allowing microscopic observation under controlled light and humidty conditions. Inclusion of a newly developed optical sensor based on NIR-transmission for leaf water content (LWC) allows continuous and precise observation of LWC-fluctuations. These occur passively as induced by changes in environmental conditions and also as a result of osmotic adjustment processes and stomatal responses. A control system was implemented, feeding back the LWC-signal into the humidity control of the cuvette enclosing the leaf and thus forming a feedback-loop which allows imposition of defined changes of leaf water content. This setup, for the first time enables defined and repeatable experimental control over LWC simultaneously with in-planta ion-measurements. Using the ratiometric dye Oregon Green loaded to the apoplast we studied the possible role of apoplastic pH-variations in signaling local tissue water status to the guard cells. Upon a decrease in LWC the apoplast consistently showed a substantial alkalisation preceding stomatal closure. Strength of the pH-response, timing and a consistent dose-response-relationship are in agreement with a role as a tissue signal involved in leaf water homeostasis.

01-06: Contribution of infrared spectroscopy to evaluate the variability of quality traits of the fresh and processed apples

Weijie Lan[1], Benoit Jaillais[2], Catherine Renard[1], Alexandre Leca[1], Sylvie Bureau[1]

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1 Introduction

Fruits purees quality is influenced by both apples characteristics, genetic, environmental and processing factors. The mechanism of purees processing is well-known but not the impact of the initial fruit quality on the obtained purees. A good understanding of this phenomenon is then crucial.

The most used analytical methods to determine the quality characteristics are based on the determination of soluble solids content (SSC), titratable acidity (TA), dry matter, individual sugars and organic acids and insoluble solids content (ISC). NIRS (800-2500 nm) and MIRS (4000-700 cm^{-1}) have not been applied for evaluating the link between the fresh fruits and their corresponding purees after grinding and cooking. The objective of this work is to use both the classical biochemical measurements listed above, and the spectral NIRS and MIRS ones to estimate the impact of fruit quality variability on the purees characteristics. To do that, apples were harvested over two seasons. Some factors were modulated: genetics with two varieties, agricultural practices with two levels of fruit thinning and water stress, postharvest with different time of a cold storage, and processing conditions with three levels of puree refining after cooking.

2 Material and methods

2.1 Description of the apple materials

In 2016, Golden Smoothee apples were from Gotheron an experimental INRA orchard (Drôme, France) and were harvested at maturity. In 2017, Golden Delicious apples were from La Pugère, an experimental orchard (Bouches du Rhône, France) and were harvested at 6 stages during growth and ripening and at maturity. In 2017, two agricultural practices were compared: fruit thinning (thinning with 50% of fruits removed named C- or non-thinning named C+) and irrigation (stress with 50% of water named S+ and non-stress with 100% of water named S-).

Apples harvested at maturity were stored at 4°C during 1, 3, 6 months in 2016, and until 9 months in 2017. At harvest and after each storage period, apples were processed to obtain purees. Apples were cooked at 95°C for 5 min and were then refined using three levels of refining, non-refined (NR), refined R1 at 0.5 mm and refined R2 at 1 mm. Apples and purees were characterized by infrared spectroscopy and reference measurements.

2.2 Reference measurements and Infrared spectroscopy

For fresh fruits, apples were cut, frozen in liquid nitrogen, and ground to obtain a homogenate for MIRS and biochemical measurements such as SSC, TA, dry matter and ISC and so on. Furthermore, the size and shape of fresh apple cells and puree particles were measured by colouring the cell wall and taking macroscopic images which were treated with an automated script in ImageJ software.

Infrared spectra were recorded using NIRs on two opposite sides of intact fruits as described by Bureau et al. (2009a) and using MIRS on fresh apple homogenates and purees as described by Bureau et al. (2009b).

2.3 Data pre-processing and processing

For spectral pre-processing and data treatment, PLS (Partial Least Squares), PCA (Principal Component Analysis) and FDA (Factorial discriminant analysis) were performed with Matlab 7.5 software using the "SAISIR" package developed by Bertrand (2007). The MIR data were transformed with standard normal variate (SNV) to correct multiplicative interferences and variations in baseline shift. For model development two-thirds of data were used for calibration and a third for validation. The performance of the models was evaluated by the determination coefficient (R^2), the error of prediction and the RPD (Residual Predictive Deviation), defined as the ratio of the standard deviation of the response variable to the RMSECV (Root Mean Square Error of Cross-Validation).

3 Results and discussion

3.1 Apple quality change during growth and maturation

On the one hand, ISC decreased during the maturation, on the other hand thinned apples (C-) had more ISC than non-thinned ones (C+). Moreover, the effect of fruit thinning on the fruit structure was stronger than the irrigation one. This discrimination of maturation stages of Golden Delicious apples is also highlighted with NIRS on intact apples.

In accordance with the biochemical measurements, MIRS performed on the fresh apple homogenates allowed to discriminate not only the ripening stages but also the apples from the thinning (C-) and non-thinning (C+) practices.

3.2 Effect of cold storage on apple and impact on the corresponding purees

MIRS performed on purees highlighted a clear change of puree composition and structure with increasing storage time. Whereas the clear separation of the three levels of refining (0.5 mm, 1mm and not refined) at the beginning of storage, the separation was gradually reduced with the time of storage to reach an overlapping of all samples at the end of storage after 9 months. By considering the macroscopic images of the same samples, at the beginning of storage, purees were composed of large particles and a few separated cells and the refining levels mainly lead to a clear variation of the particle sizes. However, after 9 months of storage, the purees were mainly composed of individual cells and were thus not different according to the refining levels.

3.3 Relationships between quality traits of apples and processed purees

Partial least squares (PLS) regressions were performed to evaluate the predictive ability of the internal quality parameters of apples during growth and ripening of apples and processed purees after different storage periods. During growth and maturation, a good prediction of SSC, TA and AIS was obtained in NIRS on intact fruits (R^2 respectively of 0.95, 0.8 and 0.9) and in MIRS on homogenates (R^2 respectively of 0.98, 0.98 and 0.93). Similar results were obtained during storage.

4 Conclusion

NIRS and MIRS appeared to be very interesting and convenient tools to pilot the fruit management in orchards and during postharvest storage as well as in processing.

Acknowledgement

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Session 2 - Hyperspectral Imaging (Co-chairs: Devaux/Vermaak)

02-01: Hyperspectral imaging in combination with chemometric data analysis – a powerful duo in the quality control of herbal medicines

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The quality control of herbal material is notoriously challenging due to the complex mixture of compounds present in plants. In addition, the variability in phytochemical profiles and toxicity issues associated with herbal products necessitates the development of reliable quality control methods. Analytical methods such as liquid chromatography coupled to mass spectrometry are time-consuming and requires considerable expertise. Hyperspectral imaging (HSI) integrates conventional spectroscopy and imaging to obtain spectral and spatial information from a sample. Once the method has been developed, the visual results are rapidly obtained and easy to interpret. In this study, the use of HSI in combination with chemometric data analysis in quality control will be illustrated using several examples: 1) distinguishing between the whole dried fruit of *Illicium verum* (Chinese star anise) and *Illicium anisatum* (Japanese star anise); 2) *Stephania tetrandra* ('hang fang ji') and its substitution or adulteration with *Aristolochia fangchi* ('guang fang ji'); 3) determining the proportion of each constituent in a tea blend consisting of *Aspalathus linearis* (rooibos) and *Agathoshma betulina* ('buchu'). Hyperspectral images were captured using a shortwave infrared (SWIR) pushbroom imaging system in the wavelength range 920–2514 nm. Multivariate software (Evince® and Matlab®) were used to analyse the data. Principal component analysis was applied to the images to investigate chemical differences between the species. Partial least squares discriminant analysis models were constructed by assigning the clusters to classes. The classification models were used to predict the identity of raw material replicates inserted into the model as well as the levels of adulteration in spiked raw materials. UHPLC-MS as an independent analytical technique was used to confirm chemical differences between the species. For the star anise example, a classification model was developed and used to accurately predict the identity of whole dried fruit of *I. anisatum* and *I. verum*. In the 'fang ji' example, the replicates for each plant species were predicted at a value > 99% for all the samples. Artificially adulterated samples were accurately predicted from as low as 10%. In the herbal tea blend example, the classification model was applied to determine the relative proportions of each blend constituent in intact tea bags. With the increasing need to regulate herbal products and ingredients, emerging technologies are providing alternative methods that allow the holistic analysis of the samples. Hyperspectral imaging in combination with chemometric data analysis is ideally suited as a tool for the quality control of herbal raw material as it is a visual, rapid, accurate and non-destructive method with high prediction ability.

02-02: Multiscale and multimodal spectral Imaging for mapping cell wall polymers in plant organs

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Plants are heterogeneous materials that present a multiscale organization (organs, tissues, cell types, subcellular compartments). Tissues and enclosed cell types are highly specialised and differ from other by structural features. Cell walls whose composition and properties vary according to cell types are of major interest as they are involved in many end-use properties of plant biomass. Histological studies are therefore of major importance for evaluating the quality of plant material. Histology includes measuring morphological information about cells and tissues and investigating the composition of cell walls according to cell types. Spectral imaging is used to reveal variability in the biochemical composition at the cellular level without any labelling of the samples. However, single techniques generally provide a partial characterisation of the plant polymers. More complete information can be obtained by combining several spectral imaging methods [1].

Relating histological measures to end use properties is not an easy task because end-use properties are generally evaluated at a macroscopic scale including plant variability. The need to compare multiple plant samples brings additional constraints. All these multiple sets of images generate large and complex image collections that require the development of adapted methods to analyse them.

The objective of the presentation is to show the development of a multiscale and multimodal strategy to map the heterogeneity of cell wall in maize stems. Macroscopic devices reveal the variability at the scale of a few cm^2: morphological information concerning cell size and vascular bundle distributions was obtained using visible imaging [2] and cell wall phenolic composition was studied by multispectral autofluorescence imaging [3]. At the microscopic scale, multimodal hyperspectral imaging was used to map cells walls in vascular bundles at the microscopic scale [1]. In parallel, enzymatic degradability of cell walls was mapped at the macroscopic scale using visible imaging and at the microscopic scale using both autofluorescence and FTIR imaging [4]. Model sections of the maize stem can be obtained from morphological measures. The integration of multiscale and multimodal information in the model section is discussed.

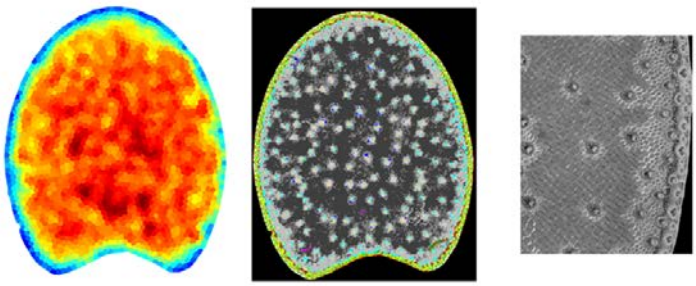

Fig. 1: multiscale and multimodal analysis of maize stem. Left: cell size mapping, middle and right: fluorescence properties mapping at two scales. Left, middle: 17x14 mm^2. Right: 4.3x3.4 mm^2

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02-03: Autofluorescence multispectral image analysis at the macroscopic scale for tracking wheat grain tissues: a novel approach for a more specific identification of wheat grain dietary fibre

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Wheat grain contains about 12-14% of fibres mainly located in the outer layers. The composition and the structure of wheat dietary fibres, as well as the nature and amount of co-passengers, vary according to the tissue where they are originated from. The aleurone layer is rich in low substituted arabinoxylans esterified to ferulic acid whereas outer pericarp contains highly substituted arabinoxylans but also cellulose and lignin. Consequently wheat dietary fibres properties showed a high variability according to their tissue of origin within the grain, which deeply impact their nutritional effects. If the identification of tissues in wheat grain is commonly performed, it remains challenging for food ingredient such as mill streams (flour, bran etc).

Equipements are now available to acquire multispectral fluorescence images at the macroscopic scale using filters with specific excitation/emission wavelengths. These fluorescence macroscopes allow obtaining images of a representative number of particles together with a spatial resolution of less than 3 µm. In such images, the intensities measured for each pixel, though they are not spectra, can be assembled to form spectral profiles. To identify the tissular origin from this information, we propose to develop a prediction model on particles using calibration data coming from the observation of tissue sections. This approach is based on several assumptions. The first one is that the multispectral autofluorescence of plant tissues is specific and the second is that it is possible to measure fluorescence intensities in a reproducible way. The objective of the present work was to check the fluorescence macroscope as an efficient device for measuring and comparing fluorescence intensities.

The variability of fluorescence profiles was studied by selecting pixels in cross-sections or in particles mounted in air or in water. The statistical variations were studied by principal component analysis and variance analysis. The first effect, mainly described by principal component 1, was to differentiate aleurone layer from pericarp tissue. The second effect, mainly described by component 2, was a difference between the two mounting media. The differences between sections or powders were not correlated to the other factors and were considered as not significant. Our results show that profiles extracted from multispectral images of cross-sections or particles are similar and allow the identification of wheat grain tissues. If implemented, the prediction from cross-section could be less tedious than other methods requiring dissection and lead to the identification of more tissues. We have demonstrated the proof of concept of tracking wheat dietary fibre origin by predicting tissues on images of particles. This method could help to better qualify flours and various milling fractions as well as to control whole grain products.

02-04: Early detection of the grapevine disease Esca using hyperspectral sensors

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The Esca complex, Botryosphaeria dieback and Eutypa dieback are the three most important grapevine trunk diseases (GTDs), which are caused by several xylem-inhabiting biotrophic fungi. The major causal agents of the Esca complex are the ascomycetes *Phaeoacremonium aleophilum* (Pal) and *Phaeomoniella chlamydospora* (Pch) as well as the basidiomycete *Fomitiporia mediterranea* (Fmed). The infection occurs mainly in the winter through pruning wounds. But Pal and Pch are also able to infect young vines in nurseries. Thus, the spreading by young grafted vines cannot be excluded [1].

The fungi destroy the wood causing various types of wood necrosis. However, visible leaf symptoms do generally not become apparent until 5-7 years after infection. The acute form of Esca (apoplexy) causes the sudden death of vines within a few days. Typical chronic foliar symptoms show interveinal chlorosis and necrosis producing a tiger-stripe pattern. So far, no correlation between the severity of wood symptoms and the appearance of foliar symptoms could be shown, because infected vines do not develop leaf symptoms consecutively [2]. Therefore, an annual monitoring becomes fundamental to determine the true incidence of the disease in a vineyard.

Traditionally, monitoring depends on visual ratings by experts, thus, being time consuming and subjective. As a new field phenotyping platform, the 'Phenoliner', was constructed to enable the high throughput acquisition of phenotypic data under standardized conditions. In this study, ground-based hyperspectral data in the range of 400 – 2.500 nm were collected. Hyperspectral images of symptomatic and asymptomatic vines were analyzed to identify significant differences in their spectra as a basis for further practical applications (e.g. airborne multispectral imaging). Furthermore, the spectra of pre-symptomatic vines were examined to identify the time frame in which non-symptomatic and symptomatic vines can be differentiated before the appearance of symptoms. The early detection of Esca is important not only to study symptom development but also to evaluate the efficacy of the few control strategies available.

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02-05: Detection of anomalies in bulk materials using hyperspectral imaging

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Optical spectroscopy in the electromagnetic spectrum from 780 nm to 2500 nm is an established laboratory method in science and quality control. Hyperspectral imaging camera systems with a sensitivity in this measurement range can be used in visual inspection and sorting systems [1].

However, in a measured spectrum, signals from different origins are superimposed. In addition, the spectrum in hyperspectral images changes depending on the viewing angle of the surface of a sample and its scattering properties [2]. Therefore, the absolute value of a spectral band can often not be used directly and data pre-processing methods are required for further analysis.

The presented approach of a pre-processing and feature extraction method has the idea to decompose the spectrum into its absorption bands. Based on a physically motivated signal model, the parameters of the absorption bands are estimated [3].

Compared to classical methods of spectral signal processing, the description of the spectrum using the absorption bands offers some advantages, especially for calibration transfer between sensors with different characteristics. This approach also enables the development of better interpretable classifiers. In particular, single class classifiers can be created with a minimum of training data. A promising sorting application is the detection of anomalies in the N-H absorption bands, which can be caused by pyrrolizidine alkaloids in plant material.

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02-06: Visual quality assessment of black cohosh using hyperspectral imaging and chemometrics

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Actaea racemosa (Ranunculaceae), commonly referred to as black cohosh, is a medicinal plant native to North America. Black cohosh is well known for its traditional use in the treatment of gynaecological problems, specifically for symptoms experienced during menopause. However, the American Herbal Products Association reported the plant to be among the top subjects to adulteration. Black cohosh is usually adulterated with Asian cohosh species. This study investigated the use of shortwave infrared hyperspectral imaging (SWIR-HSI) in combination with powerful chemometric methods for data analysis as a fast alternative method to differentiate four cohosh species and investigate 36 commercial products traded as black cohosh. Authentic root material of *Actaea racemosa*, *A. podocarpa*, *A. pachypoda* and *A. cimicifuga* were purchased from the American Herbal Pharmacopoeia (AHP). Both SWIR-HSI and ultra high performance liquid chromatography coupled to mass spectrometry (UHPLC-MS) analyses were performed on the raw material as well as commercial products. Using Matlab® software (2014b) with SWIR-HSI data (920 – 2514 nm), the range containing the discriminating information of the four species was identified as 1204 – 1480 nm. After reduction of the data set range, partial least squares discriminant analysis (PLS-DA) and support vector machine discriminant analysis (SVM-DA) models were created ($R^2 \geq 0.8$). The novel SVM-DA model showed better predictions and was then used to predict the species included in commercial products. Seven out of 36 commercial products were recognised by the SVM-DA model as being true black cohosh while 29 were adulterated black cohosh. Further analysis of the UHPLC-MS data using the OPLS-DA model demonstrated that six commercial products could be true black cohosh. This was confirmed with the investigation of the fragmentation patterns of three black cohosh markers (cimiracemoside C; 12-β,21-dihydroxycimigenol-3-*O*-L-arabinoside and 24-*O*-acetyl-hydroshengmanol-3-*O*-β-D-xylopyranoside). Using HSI in conjunction with SVM-DA, it was possible to identify 80% adulteration of commercial products labelled as black cohosh.

Session 3 - Raman Spectroscopy / Imaging (Co-chairs: Baranska/Gierlinger)

03-01: Raman imaging of plant cells: where do we stand and where to go?

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Raman microscopy provides non-destructively the molecular fingerprint of plant cells in context with their microstructure. Chemical imagings as well as in-situ studies show the high potential to get a deeper understanding of structure-function relationships as well as biological processes and technical treatments [1]. Examples include insights into biomineralisation processes (e.g. Strontium and Barium in the freshwater algae *Micrasterias denticulate* [2]) and many studies on plant cell wall components (e.g. following lignin and phenolic extractive impregnation of plant tissues [1,3]).

Nevertheless, the application on secondary plant cell walls (e.g. wood, nuts) involves challenges in sample preparation as well as in optimizing experimental data acquisition and analysis. Although a VIS- laser with λ_{ex}=532 nm gives the best signal intensity and spatial resolution, care has to be taken when measuring aromatic plant cell components. Spectral modifications, especially an increase of fluorescence and a decrease of the ratio between the lignin assigned bands at 1600 and 1660 cm^{-1} (aromatic C=C and ethenyl C=C stretch) was observed in lignified tissues [4]. Surprisingly these experiments paved the way to remove the lignin completely and unravel the Raman signature of the carbohydrate polymers and proteins – without chemistry, only repeated laser irradiation. Carbohydrate bands are in the native lignified tissues very often hidden as especially conjugated aromatic structures show a strong signal enhancement with 532 nm laser excitation.

In hyperspectral image analysis more and more multivariate unmixing methods (e.g. vertex components analysis) have been explored to reveal the most pure components. This enabled to elucidate even tiny layers and structures based on different Raman spectra [1,5]. Nevertheless, for final spectra interpretation still some steps forward in Raman band assignments are necessary.

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03-02: Raman spectroscopy combined with AFM reveals complexity of carotenoid samples

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The paper shows a potential of Raman spectroscopy for analysis of carotenoids in a form of crystals and in the solution as aggregates. Spectroscopic measurements of carotenoid crystals are combined with Atomic Force Microscopy (AFM) and Scanning Near-Field Optical Microscopy (SNOM), whereas aggregates are investigated with the use of Raman Optical Activity (ROA) spectroscopy.

Spectroscopic and microscopic scanning probe measurements were applied to the released crystals or to crystals accumulated in a unique, carotenoids rich callus tissue growing *in vitro*. Three distinct morphological crystal types of various carotenoid composition were identified, a needle-like, rhomboidal and helical. Raman imaging provided evidence that the needle-like and rhomboidal crystals had similar carotenoid composition and that they were composed mainly of β-carotene accompanied by α-carotene. AFM measurements of crystals revealed the crystal topography and showed the needle-like and rhomboidal crystals were planar but they differed in all three dimensions. Combining SNOM and Raman imaging enabled indication of carotenoid rich structures and visualised their distribution in the cell.

In the second part, a stereochemistry of carotenoids is investigated. Carotenoids dissolved in organic-water media can form two types of aggregates: H (card-packed) and J (head-to-tail) that exhibit hypsochromic and batochromic shift of chromophore absorption, respectively. With the help of (resonance) ROA spectroscopy detailed information about the structure and configuration of chiral, supramolecular carotenoid assemblies is obtained.

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03-03: In-capsule quantitation of EPA and DHA by handheld Raman spectroscopy: fish oils to algal oils

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Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are commercially important long chain, *ω*-3 polyunsaturated fatty acids. Synthesis of these compounds occurs in marine algae, followed by upwards transfer through marine food webs and bioaccumulation in fish. The high degree of unsaturation of these compounds makes them prone to oxidation, making oil analysis challenging. Producing accurate and precise data requires careful control of factors that can cause oxidation, e.g. air, UV light and metal ions. Some investigations may have incorrectly reported high levels of oxidation in marine oils, with evidence suggesting that these oils were inadvertently oxidised at the time of analysis [1].

Commercial *ω*-3 oils are usually sold in single dose gelatin (softgel™) capsules, which protect them from exposure to air. An ideal analytical method would be capable of assessing the quality of these products without removing them from their capsules, eliminating the possibility of causing oxidation during analysis. We have successfully applied Raman spectroscopy to this task. Spectra generated using a benchtop FT-Raman spectrometer (1064 nm) were used to produce partial least squares regression (PLSR) models with root mean square errors of cross validation of 1.9% for EPA (range: 14.2–45.4%) and 1.3% for DHA (Range: 8.9–32.7%) [2].

In this presentation I will describe results of our most recent work, where we compare the performance of quantitative models generated from benchtop Raman spectra to those generated from handheld Raman spectra. This approach could be suitable for "point-of-sale" quality assurance of encapsulated commercial *ω*-3 supplements.

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03-04: Lignin – I see you!

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Raman microscopy is a fast way of probing plant material in the native state. While morphological changes are often easy to see on intensity maps, the chemical information is harder to extract, because all substances found at a pixel contribute to its spectrum. Lignin is frequently identified as a major contributor in plant cell wall spectra. Despite its rich structural diversity, the major contribution in the Raman spectrum stems from conjugated aromatic structures such as cinnamyl aldehydes or alcohols [1]. These substances are frequently identified in Raman studies, although their quantitiy in lignin is usually about 5% each [2]. Biphenyls and dibenzodioxocins are also conjugated structures and their share in spruce lignin is estimated to be around 20% [2]. With the help of quantum-chemical calculations, we discuss Rama

n spectra of three model compounds and present an updated assignment of the lignin spectrum.

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03-05: Combined bioorthogonal labeling, Raman spectroscopy and fluorescence histochemistry provide detailed spatial information on lignification in plant cell walls

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Lignin is the second most abundant plant biopolymer after cellulose on Earth. It is a major constituent of the cell wall in certain specialized plant tissues where it plays a vital role in providing mechanical support, facilitating water transport and enhancing protection against pathogens [1]. The quantity of lignin, as well as its chemical composition and the existence of covalent bonds to other cell wall polymers also have an important effect on different economically important plant (lignocellulose) biomass properties e.g. cell wall degradability for biofuel/biorefinery; mechanical resistance for timber etc. A better understanding of how the lignin polymer can i) affect the industrial processing of lignocellulose biomass, ii) influence the development of the plant during growth, and iii) modify soil microbiota during the carbon cycle depends upon the availability of appropriate analytical tools allowing scientists to characterize the structure of this complex polymer at the multi-scale level in a wide range of samples. Many chemical/physical techniques that are currently available to quantify and/or characterize lignin are unable to provide an in-depth picture of the spatial distribution of this polymer at the cell wall level. In contrast, the use of Raman spectroscopy and the more recent development of chemical reporter techniques are now allowing scientists to analyze the heterogeneity and dynamics of lignin formation *in situ* at the cell wall level [2,3,4].

In this communication we report the development of an original, *in vivo* triple bioorthogonal labeling technique for visualizing the incorporation of the three main lignin monomers (H, G and S units) into lignin [5]. This multiple labeling approach allowed us to study lignification dynamics in several model plant species (flax, arabidopsis, tobacco, poplar) by confocal fluorescence microscopy. In an ongoing project we are currently combining this triple chemical reporter approach with Raman spectroscopy and ratiometric safranin-based fluorescent microscopy to provide a highly detailed overview of changes in lignin and other cell wall polymers in the flax EMS mutant *lbf1* [6]. This study shows how different high resolution imaging techniques can be combined to provide more complete information on cell wall structure in plants.

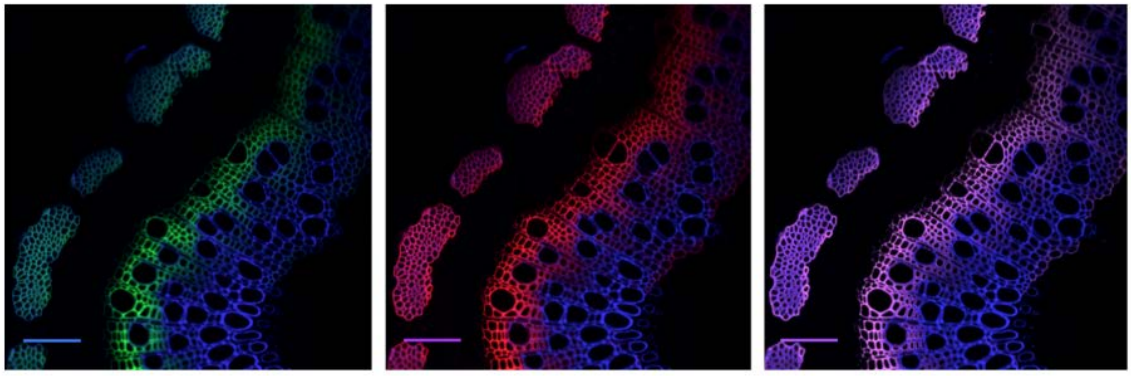

Fig. 1 Triple incorporation of H (left), G (middle) and S (right) lignin chemical reporters in fibers and xylem tissues in a poplar stem cross section

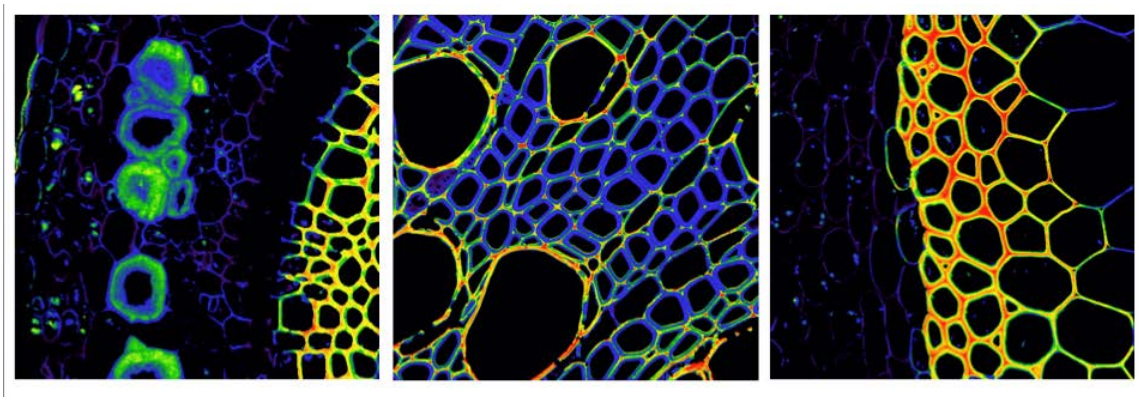

Fig. 2 Ratiometric safranin fluorescent microscopy in flax (left), poplar (middle) and arabidopsis (right) stems.

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03-06: Chemical signature in xylem cell wall of *Salix glauca* L. due to *Eurois occulta* L. outbreaks

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Insects are one of the major agents of natural disturbances in high-latitude ecosystems. Their outbreaks can cause severe canopy defoliation, which leads to reduced biomass and carbon (C) investments with potential cascade effects in species composition, functioning and productivity of tundra ecosystems. Recent studies have quantified the decrease in cell-wall thickness during the outbreak and the unexpected increase in primary production the following years. However, it is still unclear how the outbreaks affect carbon assimilation and vegetation productivity.

To shed light on the survival strategy of the woody plants under attack, a novel approach combining dendro-anatomical analysis with confocal Raman imaging was used to study outbreak events of the moth *Eurois occulta* in *Salix glauca* L. trees collected at Iffiarterfik, Nuuk Fjord, West Greenland during the summer of 2016. The survival strategy of the woody plant is not clear from the anatomical modifications of the xylem formed in the stem, which is why the biopolymer composition of the cell walls was also studied.

Wood samples were cross-dated and anatomical analysis identified two pointer years in the growth seasons 2003 and 2010, i.e. years of insect attack. These two annual rings had a clear reduction in C investment by reduction in cell wall thickness and width of the annual growth but also a markedly lighter colour of the growth ring, suggesting an altered biopolymer mark-up. For each outbreak event, seven growth rings were analysed: three years before, three years after plus the outbreak year. The outbreak years were followed by a significant growth release the two following years, i.e. wider rings were formed. The chemical composition of the xylem cell wall material was analysed using confocal Raman imaging on cross sections of fibers, vessels, and parenchyma cells. Possible differences in chemical composition between cell types and between growth years were explored using chemical imaging based on cluster analysis of integrated Raman band intensities as well as on more advanced chemometric approaches.

03-07: Raman spectroscopy shows adaption of pollen composition in *Poa alpina*

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The analysis of pollen is crucial in many fields, ranging from medical and agricultural to environmental research. In fact, environmental factors may influence the chemical composition of pollen as well as its germination and fertilization rates. Variances in the chemistry of pollen grains can be characterized using optical spectroscopy or mass spectrometry in combination with chemometric methods. It has been show, that taxonomic identification of pollen can be obtained using Raman spectroscopy and hierarchical cluster analysis (HCA) [1]. Furthemore, recent results show, that Fourier-transform infrared spectroscopy (FTIR) as well as Matrix-assisted Laser Desorption/Ionisation Time of Flight Mass Spectrometry (MALDI-TOF MS) indicate the adaption of the chemical composition in pollen grains [2,3].

Here, we discuss the advantages and limitations of Raman spectroscopy regarding the characterization of variances in grass pollen grains caused by different populations and environmental influences. We compare the ability to discriminate between different spectra obtained by Raman spectroscopy with other spectroscopic and spectrometric methods using the same set of pollen samples. For this purpose, we use principal component analysis (PCA) in combination with other statistical tools. In addition to Raman spectroscopy, we also use Fourier-transform infrared spectroscopy (FTIR), surface enhanced Raman scattering (SERS) and Matrix-assisted Laser Desorption/Ionisation Time of Flight Mass Spectrometry (MALDI-TOF MS).

As the results show, the information obtained by Raman spectroscopy provides insight into the extent to which physiological parameters manifest themselves in the overall chemical composition. The results may have impact in the broader field of plant biology, including agriculture and biomaterials research.

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Session 4 - FTIR Spectroscopy / Imaging (Co-chairs: Krähmer/Schulz)

04-01: Plant roots and FTIR – analyzing species composition and root biomass in peat soil

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Estimation of root-mediated carbon fluxes in peatlands is needed for understanding ecosystem functioning and supporting greenhouse gas inventories. Fine root biomass and production data at the level of plant species or plant functional type are very limited due to methodological difficulties. Recently, applications for identifying roots of different plant species in root mixture using spectroscopy methods have been reported [1,2]. Our main objective was to build FTIR based calibration models for predicting mass proportions of 22 common forest and peatland plant species (graminoids, herbs, shrubs and trees) in root mixtures. We also tested the possibility to measure the root mass proportions directly in soil samples, i.e. without separating the roots.

FTIR-ATR spectra were measured from dried and powdered samples. About 1200 of artificial mixed samples containing known amounts of fine roots of different plant species (and peat soil) were prepared for model calibration and validation purposes. Partial least squares (PLS) regression was used to build the calibration models. The general applicability of the species-level models in other studies was tested using about 700 external validation root and peat samples obtained from a separate study on 3 peatland sites, different from the sites where the calibration samples were collected.

The FTIR based calibration models at the level of plant species performed well for graminoids and herbs, with root mean square error (RMSE) of prediction < 7.5% (Fig 1.). For shrubs and trees the estimations were less accurate due to rather high intraspecific heterogeneity that was partly related to the variation in root diameter, but still the RMSE of prediction was generally < 10% for tree and shrub species. When the species-level models were validated on external samples, the predictions were unacceptable, however, as the models did not distinguish species of the same plant functional type (PFT). But predictions at the level of PFT were accurate for the external validation, with RMSE 6.4% for graminoids and 11.6% for shrubs and trees (Fig 2.). The models also provided satisfactory estimates of total root mass directly in peat soil samples, with RMSE < 5%.

Our results demonstrate that FTIR has a great potential in large-scale studies that require low cost and high throughput techniques, but species-level models are hardly applicable on samples outside the calibration set where only estimations at the level of PFT were reliable.

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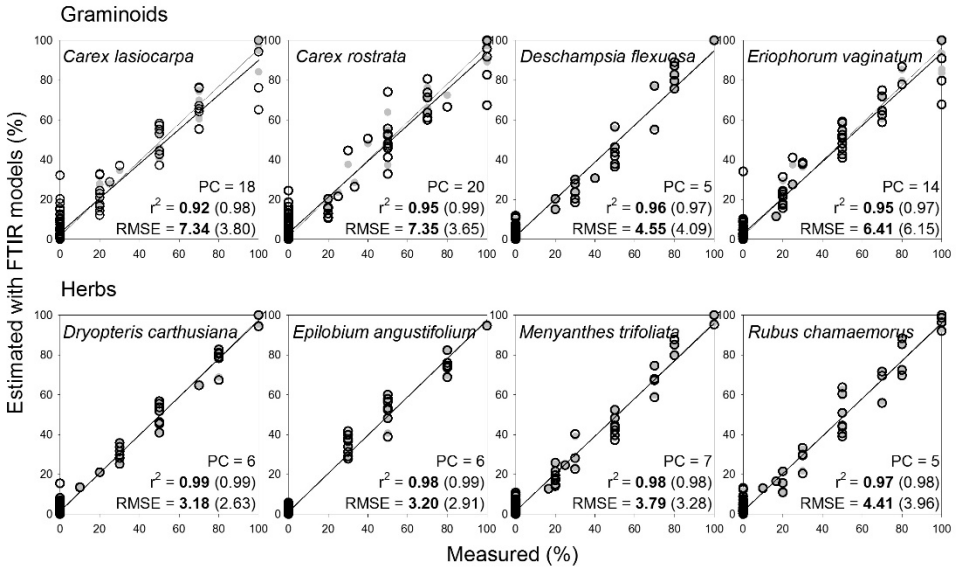

Fig 1. Examples of FTIR calibration models for graminoids and herbs at the level of plant species. PC indicates the number of terms used in the PLS regression model. R^2 and root mean square error (RMSE) values for calibration are written in parentheses and samples are visualized in graphs by gray symbols; values for one-leave-out cross validation are written by bold letters and samples are visualized in graphs by open symbols.

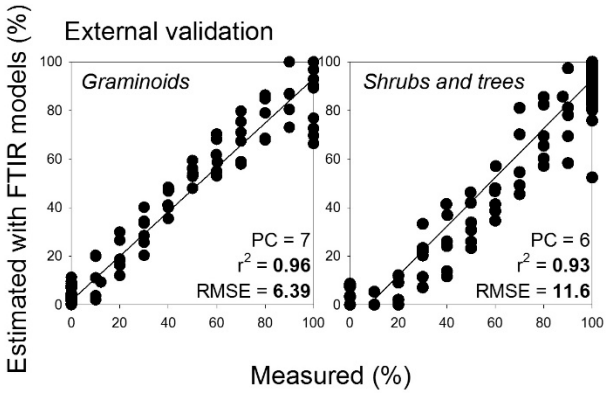

Fig 2. External validation of the plant functional type (PFT) level models on root samples obtained from a separate study on 3 peatland sites that were different from the sites where the calibration samples were collected. PC indicates the number of terms used in the PLS regression model and RMSE the root mean square error of prediction.

04-02: Vibrational spectroscopy of pollen as a tool for reconstructing solar-ultraviolet irradiance

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Solar ultraviolet-B radiation (UV-B, 280-315 nm) has extensive impact on biological and ecological processes from the individual to the ecosystem level. Moreover, absorption of UV-B by ozone is also one of the primary heat sources to the stratosphere, so variations in UV-B have important relationships to the Earth's radiation budget. Unfortunately, there is limited understanding about the changes in UV-B radiation in the geological past because ground-based and satellite measurements of total ozone and surface UV-B only exist for the last two decades. Therefore, biological or geochemical proxies from sediment archives are needed to reconstruct UV-B irradiance received at the Earth surface beyond the experimental record. Fossil pollen grains are often not only the most abundant but also among the best preserved remains of plant species, thus providing crucial information for the reconstruction of past flora, population sizes and terrestrial communities. Recent studies have shown that the quantification of UV-B-absorbing compounds in pollen have the potential to provide a continuous record of the solar-UV radiation received by plants [1]. As a result, there is an increasing interest to develop this proxy in palaeoclimatic, and palaeoecological research.

Phenolic constituents (i.e. phenylpropanoids) of sporopollenins in the pollen grain wall protect pollen by effectively absorbing UV-B radiation and providing defence against DNA damage as well as quenching reactive oxygen species. Therefore, changes in the chemical composition of fossil pollen could constitute a possible means to reconstruct ancient UV-B irradiance. Thermally assisted hydrolysis and methylation with pyrolysis gas chromatography coupled to mass spectrometry (THM-GC-MS) has become the method of choice in qualitative and quantitative measurements of phenolics in pollen [2]. However, the method is time consuming and it requires a large number of pollen grains for a statistically significant measurement. Therefore, an alternative approach based on vibrational spectroscopy has been in development for the measurement of pollen chemistry.

In general, vibrational spectroscopies, comprising Fourier Transform Infrared (FTIR) and Raman spectroscopies, are complementary, non-destructive and highly sensitive biophysical methods that provide precise signatures of the overall biochemical composition of pollen. Thus, they can be used for a wide range of research, from biology, ecology, agronomy, and forestry, to medicine, forensics, geology and archaeology. Vibrational spectroscopy of pollen includes a broad range of measurement techniques, covering both bulk measurements (10^4-10^6 pollen grains per measurement) [3-7], as well as microspectroscopies on single pollen grains [7-10].

Both FTIR and Raman spectroscopies can be used for qualitative determination of phenolic constituents in pollen grain wall [7]. However, the disadvantage is that they can only achieve relative estimates in the quantification of UV-B absorbing compounds. Obtaining absolute quantification of the compounds is challenging and will require more studies on a broad

sample sets with direct measurement of UV-B irradiation as reference values. Moreover, microspectroscopy of single pollen grains face some specific challenges, such as strong Mie scattering in FTIR microspectroscopy that results in anomalous spectral features [8-11]. We have recently demonstrated that scatter-free FTIR spectra can be obtained by using an embedding matrix, and thus achieving identification of single pollen grains with unprecedented accuracy [8]. Here, we will present different approaches in measuring pollen chemistry, focusing on pollen chemistry as a tool for reconstructing solar UV-B irradiance. These approaches include vibrational spectroscopy studies conducted at NMBU, as well as numerical correction methods [10,11] and a number of experimental settings [7-9].

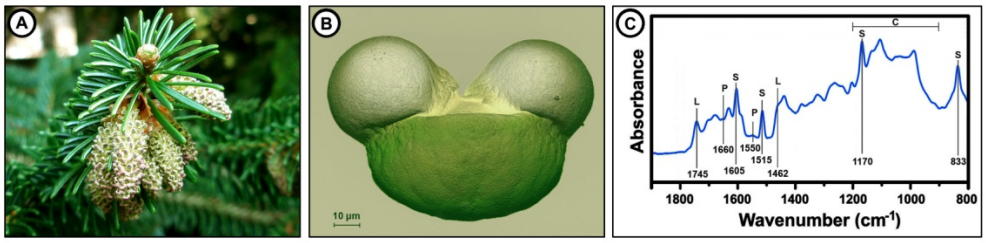

Fig. *Abies cephalonica* (Greek fir): **A**) Branch with male cones (strobili) during pollination. **B**) Scanning electron microscope image of pollen grain in equatorial view. **C**) FTIR spectrum of pollen; the marked vibrational bands are associated with lipids (L), proteins (P), sporopollenins (S) and carbohydrates (C).

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04-03: MD Dating – Dating of wood based on its molecular decay (MD) measured using FTIR spectroscopy

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Across several academic fields dating wood is of utmost importance. Currently, there are two different approaches being applied for dating purposes: dendrochronology and radiocarbon dating. The latter was the last advancement in this field in the 1950s. The presentation gives a summary of the development of an innovative method as well as the introduction into first models. The chemical composition of wood alters over time. Infrared spectroscopy is used to observe these changes and subsequently, enables their analysis with a regression model. Separate models were established for spruce, larch, fir and oak including an Austrian sample set of living trees and timber, as well as construction wood and waterlogged subfossil woo. The influence of highly saline preservation conditions will be discussed. The models cover a time span up to 3000 years. ATR-FTIR spectroscopy has been used to detect the molecular decay, random forests were applied for statistical modelling.

04-04: Quantitative FTIR imaging displays the sucrose landscape within and along its allocation pathway

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Sucrose as the major transport sugar in plants is an essential target of a wide range of research projects on plant development. It plays an important role as an energy source for all plant organs after carbon fixation through photosynthesis and as a signal in adaptive and stress responses. We developed a novel micro spectroscopic infrared based imaging approach [1], which overcomes current tissue specific limitations in technologies for quantitative sucrose mapping. The new FTIR based imaging platform is easily applied to various agricultural important crops such as barley (*Hordeum vulgare*) [1], wheat (*Triticum aestivum*) or oilseed rape (*Brassica napus*) [2] as well as the model plant *Arabidopsis thaliana* [3]. It can successfully image sucrose distribution within the range of 20 to 1000 mM with a spatial resolution enabling the investigation of single vascular bundles in leaf and stem. Moreover, it enables to target multiple components together as demonstrated by sucrose and starch imaging within the developing seed of cereals. Our data shows the high relevance of FTIR imaging within the scope of carbon allocation and storage within the context of crop improvement.

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04-05: ATR-FTIR imaging reveals cell wall layer-specific chemotypes in poplar tension wood

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Trees are able to grow high and live old thanks to the remarkable properties of their wood. Indeed, wood delivers three major functions: (1) water conduction from roots to crown, (2) mechanical support of the ever-increasing mass of the growing tree and (3) storage of temporary reserves, important for tree growth over the years. In angiosperm trees, vessels, fibers and parenchyma rays are respectively assigned to each of these functions [1]. Fibers are characterized by a thick secondary cell wall, made of several S-layers. Cell wall structure and composition strongly vary according to developmental stages and environmental conditions. For example, in response to mechanical constraints, angiosperm trees produce tension wood (TW) whose fibers exhibit a thick gelatinous extra-layer, named G-layer (Fig.1A-B). This layer, located in place of the usual S2 and/or S3 layers, consists mainly of cellulose and non-cellulosic polysaccharides with nearly no lignin [2,3]. By contrast, opposite wood (OW) located on the opposite side of the trunk is totally deprived of fibers with G-layers (Fig.1C). Hence, the high complexity of wood may stand as a hindrance for studying its formation and the construction of its properties. However, this can be circumvented thanks to the development of cell-specific approaches and microphenotyping.

Here, we report the development of a non-destructive microphenotyping method based on ATR-FTIR imaging. We applied this technique on stem cross sections of poplars (INRA 717-1B4, *Populus tremula* x *P. alba*) which were tilted to induce the production of TW. Hyperspectral images were acquired using Spectrum 400 FTIR spectrophotometer coupled to a Spotlight 300 FTIR imaging system (PERKIN ELMER, Wellesley, USA). Three 100 x 100µm images per cross-section were taken at a 1.56 x 1.56µm pixel dimension. This high spatial resolution was made possible by the use of a high refractive index crystal. Thus, we were able to clearly distinguish the different cells (Fig.1D-E). ATR-FTIR spectra were also acquired on powders from i) ground stems, ii) enriched in TW or iii) in OW, and iv) on isolated G-layers.

We performed several pre-treatments on the 4 cm^{-1}-resolution spectra: background correction during acquisition, noise reduction, atmospheric correction, Savitzky-Golay smoothing, SNV normalization and first derivation. We then applied diverse unsupervised multivariate image analyses such as principal component analysis, hierarchical clustering on principal component and multiple curve resolution, leading to clusters of pixels representative of one chemotype (Fig.1F-G). Non parametric analysis allowed us to identify significant differences of absorbance between those chemotypes. We demonstrated that spectra taken from fiber cell walls on cross-sections differed from spectra obtained from wood powder. Interestingly, spectra from isolated G-layer were very closely related to those obtained through ATR-FTIR microspectroscopy of G-layers on cross-sections (1.H). We also showed that ATR-FTIR imaging was able to discriminate between fiber, vessel and ray cell walls. These findings are in accordance with previous studies [4,5] but with a five-fold increased spatial resolution.

Peak assignments based on the literature made possible to give some biological consistence to our observations. We showed that (i) G-layers were mainly composed of cellulose and non-cellulosic polysaccharides, (ii) lignins in rays cell walls were mainly G-units whereas

lignins from fiber cell walls were mainly S-units (Fig.1I). These findings are in accordance with previous studies [3-6].

In a nutshell, ATR-FTIR microspectroscopy brings new opportunities for the study of cell wall composition at the cell level and appears to be a promising tool to finely characterize the cell wall of different wood cell types.

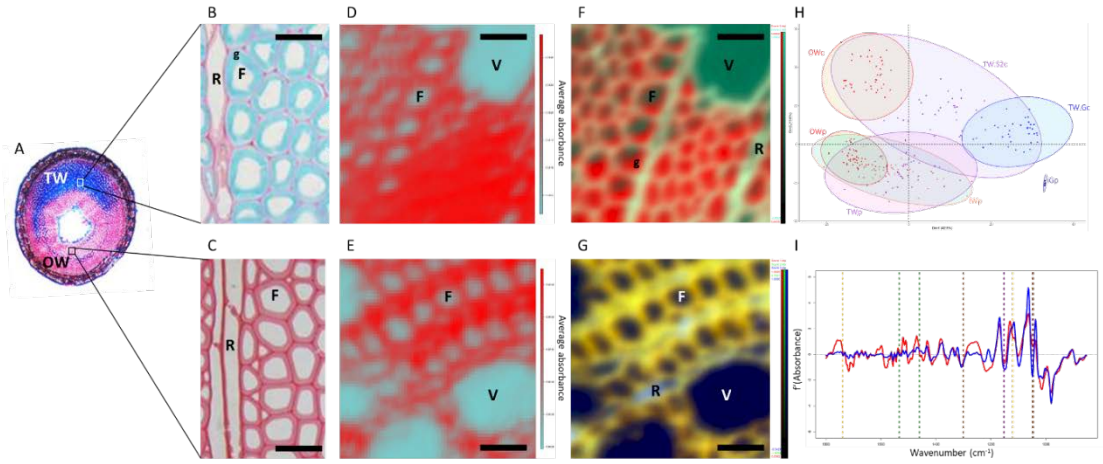

Fig. 1. Toward the identification of discriminant wavenumbers. Cross section of tilted poplar stem (**A**), TW (**B**) and OW (**C**) after safranine/astra blue staining. Raw Infra-red images of TW (**D**) and OW (**E**). PCA reconstructed images of TW (**F**) and OW (**G**). PCA discriminating the spectra of the different types of walls (**H**). First derived and SNV standardized mean spectra of OW (red) and TW (blue) (**I**).

Global: OW: opposed wood; TW: tension wood; g: G-layer, R: rays, F: fiber, V: vessel, Scale bars: 10 µm. PCA: Score: score of the main components, Red: OW spectra (Owc, Owp), dark blue: isolated G layer spectra (iGp), blue: G layer spectra on cross-sections (TW.Gc), purple: S2-layer spectrum of TW (TW.S2c) and TW powder (TWp), pale pink: tilted stem spectra (TSp), square: spectra acquired on cross-sections (Owc, TW.Gc, TW.S1c), round: spectra acquired on powders (Owp, TWp, TSp, iGp). Each point represents a spectrum The ellipses represent 99% of the spectra if they follow a normal distribution. Before analysis, the spectra were first derived and normalized by SNV. Spectra: Dotted: differentially absorbed wave numbers, Green: lignins, Yellow: cellulose, Brown: hemicelluloses, Violet: pectins.

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04-06: Nano-FTIR spectroscopy of in situ and extracted silica phytoliths

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We present the application of nano-FTIR spectroscopy to study plant silicification. FTIR based on IR fingerprint absorption spectroscopy is a widely used technique for chemical materials identification and characterization. It has been widely used to study cell wall constituents like proteins, aromatic phenols, cellulose and to characterize biosilica. Therefore, FTIR can provide important information on how silica affects the plant structure and development, reveal the differences in cell wall composition between the silicified and non-silicified cells and provide structural information of the deposited silica. Despite the capabilities of IR spectroscopy, the spatial resolution is limited to several micrometres by the diffraction of the long IR wavelengths, however, the diffraction limit can be circumvented by scattering-type near-field optical microscopy (s-SNOM). Fourier transform infrared nano spectroscopy (Nano-FTIR) based on s-SNOM, can be regarded as an extended atomic force microscope (AFM) that returns an infrared image together with topography and mechanical phase images [1]. It provides wavelength-independent nanoscale resolution far beyond the classical diffraction limit [2]. Its resolution is approximately equal to the radius a of the probing tip (~20 nm) and it allows to acquire simultaneous amplitude and phase images to obtain information on refractive and absorptive properties of the sample [3]. As will be demonstrated for the silica structures in our project, it is possible to correlate chemical and mechanical information combining the mechanical phase and IR spectra of the phytolith structures and to obtain information on structure and composition on the surrounding plant tissue.

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04-07: Understanding the formation of highly durable heartwood in larch by use of Synchrotron infrared imaging and multivariate resolution techniques

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The formation of highly durable wood tissue (heartwood) is linked to the occurrence of non-structural substances called extractives, which play an important role in the resistance of wood to fungal decay. However, the exact formation and distribution of these extractives within the xylem tissue at the cell and cell wall level is one of the unsolved questions in plant science [1].

Larch heartwood contains extractives and is an important European resource for highly durable wood [1]. The extractives in larch belong to the molecular families of terpenoids, flavonoids, lignans, fats/fatty acids and galactans [2,3]. Very little is known about the deposition and cellular level distribution of larch extractives. The limited knowledge is mostly due to the low relative proportions of extractives in wood, which implies that they easily can be overshadowed by the presence of structural polymers like lignin, which is an aromatic biopolymer.

The objective of this work is to obtain a detailed overview of the heartwood formation process in larch at the microscale level by combining Synchrotron infrared imaging and advanced chemometric tools. The long term goal of this work is to facilitate environmentally benign and bioinspired wood protection systems. Therefore, detailed knowledge is fundamental to the development of biomimicking schemes for impregnation of wood from less durable tree species to replace old hazardous impregnation processes, which are being phased out.

Synchrotron infrared imaging appears to be the ideal technique to study the extractive deposition patterns on the microscale during heartwood formation in larch due to the high brightness and high collimation of the beam, which result in images with high spatial resolution, and the avoidance of fluorescence problems when other high spatial resolution techniques, such as Raman imaging, are used. The use of advanced chemometric tools like Multivariate Curve Resolution Alternating Least Squares (MCR-ALS) has already been proven to adapt particularly well to hyperspectral image analysis due to the ease of the introduction of external spectral and spatial information about the image and the ability to work with single and multiset (several images) image structures [4,5]. Using this approach, we expect to be able to identify the extractives and their deposition pattern at a cell level, including the distinction between cell lumen and cell wall contents. In this way the evolution of heartwood formation in larch will be described both from a spectral and a spatial point of view.

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Session 5 - Chemometrics and Remote sensing (Co-chairs: Beleites/Gorzsás)

05-01: Multivariate analytical strategies for spectral data of plants

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This plenary lecture aims to give an introduction to multivariate analysis, with a brief overview of selected techniques applicable to spectral data common in plant sciences. The techniques covered include common supervised and non-supervised methods, such as principal component analysis, multivariate curve resolution, partial least squares based techniques (including discriminant analyses) and clustering tools. The focus is on providing a guide by which multivariate analytical strategies can be chosen. The lecture is intended specifically for people with little to no background in chemometrics. to help them select the most suitable multivariate analytical technique based on the goal of the analysis and the type of the collected data. Real life examples are used to illustrate different aspects of the techniques, primarily based on vibrational (Fourier-transform infrared (FTIR) and Raman) spectra, but the discussed techniques are of general purpose and thus directly applicable to other types of spectral data spectra. The provided examples outline the handling of continuous sequential spectra [1], independent batch spectra [2,3] and hyperspectral image data [4] from the field of plant sciences.

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05-02: Experimental design considerations for developing spectroscopic calibration models of plant material

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Spectra of biological systems are often subject to a large number of influencing factors (including confounders) which need to be taken into account for successful, stable and rugged calibration.

Biological systems as well as sample processing in the analytical laboratory lead to deeply nested structures of sources of variance. We present sampling schemes that allow estimating the variance contributed by the various confounders without the need for exponentially growing sample numbers. Staggered and inverted nested designs have been known since the 1960s [1] but only nowadays the computational resources to analyze such data have become readily available. These strategies are particularly useful when reference analyses are the bottleneck of the calibration procedure. Calibration is most efficient in terms of the number of required samples if calibration samples are uniformly distributed over the desired concentration range of the analytes. However, these concentrations are often unknown before calibration or reference analyses are performed – i.e. when the samples for reference analysis are chosen. A two-stage calibration procedure can help: an initial set of samples is chosen and a preliminary calibration is performed. Using this to predict the concentrations of all spectra, additional samples can be chosen to achieve the desired uniform coverage in concentration space.

We will also briefly compare different strategies of dealing with confounders ranging from standardization of measurement conditions to deliberate perturbation of calibration spectra. Last but not least, we give an outlook on model optimization as part of the calibration procedure, discussing how to obtain independent train-test splits e. g. for cross validation depending on the structure within the data set and a caution about the uncertainty of common optimization procedures.

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05-03: Measurement uncertainty for NIRS measurements

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Measurement uncertainty is a fundamental issue in e.g. measurement of impurities and frequently asked for during accreditation. A simple approach to apply measurement uncertainty to NIRS analysis missed the special circumstances of a calibrated method relying on a reference method.

In this paper we try to apply proposals from EURACHEM CITAC [1] for the following cases of NIRS measurements:

A) NIRS calibrations and validation with certified reference material

B) NIRS calibrations and validation using a reference method

C) NIRS calibrations with central validation and ring test

and make proposals to account for the special circumstance of a calibrated method.

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05-04: Identification and quantification of heartwood extractives of Norway spruce (*Picea abies*) and hybrid larch (*Larix gmelinii* x *japonica)* clones using GC-MS and MCR-ALS

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Wood is regaining importance as a construction material, a development that also has led to a renewed interest in how to prevent the material from being decomposed by microorganisms. Among these, the susceptibility of wood to fungal decay is one of the most severe drawbacks for its use as a construction material. In the living tree, the molecules responsible for wood durability are broadly termed extractives and are especially important in the heartwood - the central part of the tree consisting of dead cells. In order to obtain more resistant timber, it is consequently important to understand the nature, function and distribution of extractives, as well as the amounts present in the wood. This knowledge will allow for breeding for higher extractive contents, but will also inspire the development of "greener" wood impregnation systems via bio-mimicking the strategies that evolved in trees. The traditional wood impregnation methods are no longer an option due to the environmental problems they pose.

In this study, the heartwood extractive composition of two industrially relevant conifers grown in Denmark was investigated. Wood from Norway spruce and a hybrid species of Japanese and Dahurian larch – two clones each - were studied at three different stem heights. The samples were extracted using a slightly modified version of an extraction method developed by Fang et al. (2013) [1]. By means of a Dionex® Accelerated Solvent Extractor (ASE) the lipophilic components were extracted with heptane and the hydrophilic ones with acetone:water (95:5) in sequence. Gas Chromatography (GC) coupled to mass-spectrometry (MS) and flame ionization detection (FID) were used for identification and quantification of the extractive components. Quantification was done using a chemometric approach with the algorithm Multivariate Curve Resolution – Alternating Least Squares (MCR-ALS) [2]. The extractive composition along the stem of the two different conifers was compared, as well as the differences between the clones.

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05-05: Establishment of a field spectral library of agricultural crops in Germany for monitoring biophysical parameters at different spatial scales

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Leaf area index, above ground biomass, chlorophyll content or the amount of absorbed nitrogen are key variables for precision farming applications, crop growth predictions and yield estimations. Remote sensing systems provide spatially differentiated information over large areas at regular intervals. They offer the potential for rapid, spatial and non-destructive assessment of these crop parameters. The spectrally differentiated mapping of the absorption and reflection properties of crop stands by hyperspectral systems opens up new perspectives in terms of parameter quality and type [1].

In the past, two different types of approaches have established for the estimation of crop parameters from remotely sensed image data: the inversion of physically based radiative transfer models, and empirical–statistical modeling based on in-situ measurements of vegetation parameters and its reflectance properties [2].

An intrinsic problem of empirical approaches is the transferability in space and time. To overcome this issue, we established a field spectral library of agricultural crops in Germany containing measurements from different geographic regions which were taken at various dates during the growing season. Data was acquired over a time period of eight years, from 2011 to 2018. By now, the data set consists of more than 1100 reflectance measurements of the most common crop types in Germany (winter cereals, winter rape, spring barley, oats, sugar beet, potato, horse bean). Canopy reflectance was recorded with field spectrometers (ASD Fieldspec Pro, SVC HR1024) ranging from 350nm to 2500nm on plots of 0.25x0.25m^2. Thereafter, crop parameters (e.g. biomass, leaf area index, phenological stage, nitrogen content) were determined for each sample plot using destructive and non-destructive techniques.

Quantitative relationships between canopy reflectance and crop parameters were established by means of non-parametric regression methods (partial least squares). Crop-specific models were set up with respect to different spectral configuration and spatial resolution (<1m – 30m) of various point and imaging spectrometers. These are a ground based, mobile hyperspectral system (PentaSpek) [3], the airborne HySpex sensor (NEO) [4] and the forthcoming German hyperspectral EnMAP mission [5]. Further, a model was build to fit data of the superspectral Sentinel-2 system [6], an earth observation mission which provides freely available image data over Germany every 2-5 days.

Prediction accuracy of the models varied with respect to spectral configuration, crop type and parameter of interest. Models based on hyperspectral information generally performed best, but root-mean-square error was only slightly higher for Sentinel-2.

The best models were applied to image data of the PentaSpek system and the EnMAP mission (simulated) acquired during growth period in 2011 and 2012 (1), and to image data from HySpex and Sentinel-2 acquired in the growth period 2017 (2). Parameter maps displayed a similar pattern of the intra-field variability and a good absolute agreement. They underpin the potential of the field spectral library for monitoring biophysical parameters of crops with different sensing systems, at different spatial scales.

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05-06: Forest regeneration after fire in semi arid land in the north west of Algeria analysis with remote sensing data

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The South West region of Algeria is affected each summer by forest fires a very violent which last several days and affects the Underwood, natural forests and reforestation. Forest regeneration in this semi-arid land is conditioned by several factors, climatic, topographic, and linked to the timber species. Remote sensing and geographic information systems (GIS) offer to environmentalists and managers, an opportunity for the evaluation, the monitoring and analysis of the vegetation. Usually NDVI is used, other derived index from radiometric data for remote sensing are widely used in programs to monitor the dynamics of the vegetation. The forest domain has benefited greatly from this approach. Using remote sensing data to several dates such as the data ALSAT and Landsat in our case, combined with the topographic parameters seems promising in the assessment of the spatial and temporal effects of regeneration after fires. The site studied is in the region of Sebdou in the south Tlemcen in Algeria, burned in 2003 allowed to take better account of new factors to explain the regeneration and its spatial and temporal variation. Our attention is to show the potential for the use of remote sensing data of the satellite ALSAT, spatial resolution of 32 m, and that of the Landsat resolution 30 m and the derived index from normalized difference vegetation index (NDVI / RVI) and the index of regeneration (NRI / RI), in the assessment and quantification of the regeneration after fire in a context of Algerian forest. The software IDRISI Selva has been used to analyze the layers of information involved in the evaluation of the regeneration post fires. The results obtained allow us to identify the speed of regeneration by species influenced by topographic conditions, climatic and ecological.

Key words: Remote Sensing, Forest Fires, Forest Regeneration, Alsat Data, Semi Arid Land, Multi Temporal Analysis

Session 6 - GC-/LC-MS profiling (Co-chairs: Robbat/Fiehn)

06-01: MassBank of North America: using untargeted metabolomics and multistage fragmentation mass spectral libraries to annotate natural products in plants

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Plants produce thousands of natural products for specific biological functions in their ecosphere. To structurally annotate these metabolites, large mass spectral libraries are required to develop fragmentation rules to detail relationships of substructures. At UC Davis, we developed a range of informatics resources to catalog mass spectra of both known and unknown plant metabolites. First, the new BinVestigate web interface enables users to screen our BinBase resource that we have built over the past 15 years, with currently 2,500 studies and over 150,000 samples. Second, we developed the open access MS-DIAL data processing along with MS-FINDER compound identification software, in collaboration with the RIKEN plant science center in Japan. Third, we collated all publicly available mass spectra resources into a unified database, MassBank of North America (MoNA). MoNA now contains more than 260,000 mass spectra, including over 35,000 QTOF, ion trap MS^n and Q-Exactive HF mass spectra of natural products. We present a range of examples how plant natural products are identified through multiple extraction solvents, high resolution mass spectral platforms, and MS library searching, including the NIST standard reference material 3291 of bilberry extracts, complex foods and the identification of plant products in human upper intestinal tract samples or plasma extracts.

06-02: Climate effects: changes in the tea metabolome

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Well-known are the effects of extreme weather such as droughts, heatwaves, and cold on crop yield [1,2]. Less understood are climate effects on crop quality. To study how abiotic and biotic pressures affect plant metabolism detailed analysis of the metabolome is required to learn how these effects change the sensory quality and health beneficial properties of plant materials. Toward this end, we developed automated-sequential, 2-dimenasional, gas chromatography/mass spectrometry (GC-GC/MS) methods and new data analysis software to detect the hundreds of volatile compounds in tea, coffee, hops, berries and essential oils [3-6].

For example, we found striking differences in the composition of volatile secondary metabolites in tea (*Camellia sinensis* (L.) Kuntze) harvested from Yunnan Province, China, due to differences in rainfall and temperature [7,8]. Of the 400 compounds detected, about one-third increase, with another third decreasing in concentration, with more than half of them by hundreds of percent. Of these metabolites, 150 possess organoleptic and/or nutritional properties. Similarly, we measured a 50% decrease in the concentration of catechins (well-known phenolic antioxidants) [9] and an increase in other phenolics such as proanthocyanidins, phenolic acids, flavones, flavonols and their derivatives as measured by total polyphenol content and anti-oxidant potential between seasons, namely, spring (no rainfall) and summer (monsoon rains).

In this presentation, results illustrate how tea plants respond to rainfall and temperature over a three-year period and how automated database construction and annotation of GC-GC/MS and GC/MS data lead to these findings, see figure below.

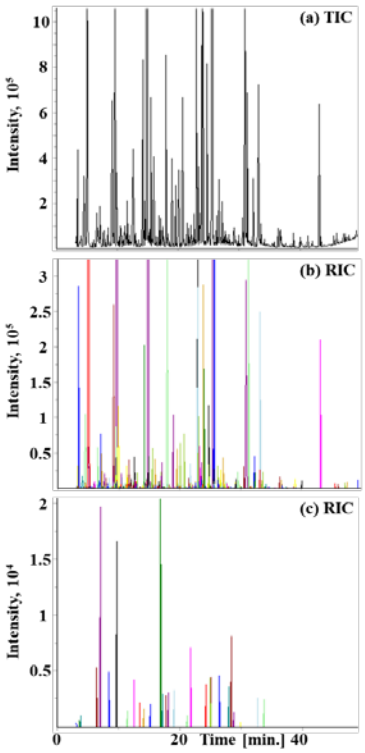

Figure. Total ion current (TIC) chromatogram of spring tea from Yunnan, China (a) and reconstructed ion current (RIC) chromatograms of 360 target compounds (b) and another 39 non-target compounds (c).

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06-03: Metabolomics as tool to improve food quality

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There is a growing global demand to produce more crops material with an improved quality and reduced waste. Many crops are successful biochemical factories to produce nutrients for food and feed, but also to produce ingredients used by the pharmaceutical, cosmetic or biobased industry. The commercial value of crops is determined by the tissue quality in relation to aspects of, for example, flavour, fragrance, or shelf life but also by its resistance against pests. Hence, the quality crops is a direct function of their metabolite content. Each of these can be fully defined in terms of the metabolic profile of the material concerned at a particular time.

Enabling technologies, such as metabolomics, broaden our knowledge of how plants are molecularly organized and how genetic and environmental factors are translated into phenotypes which in turn relate to final product quality.

Metabolomics in particular has been widely applied over the last two decades due both to the comprehensiveness of the technology and also the potentially close relationship between biochemical composition and phenotype. These technologies now support us to gain a deeper insight into the complexity of plant metabolism and its plasticity. Here, we will give a number of examples involving a number of plant and fungal crops where we have exploited several metabolomics technologies, including GC-MS, LC-MS, NMR, and MS-imaging, to gain a better understanding of how metabolite profiles are linked to crop health and product quality.

The generic nature of such approaches entails broad future use for tailored breeding programmes aimed at improvement of food quality or an increased resistance in crops.

06-04: Oxylipidomics – large scale determination of oxidized lipids using high res MS and MS/MS

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Lipid oxidation is a process ubiquitous in life, but the direct and comprehensive analysis of oxidized lipids has been limited by available analytical methods. We applied high-resolution liquid chromatography-mass spectrometry (LC-MS) and tandem mass spectrometry (MS/MS) to quantify oxidized lipids (glycerides, fatty acids, phospholipids, lysophospholipids, and galactolipids) and implemented a platform-independent high-throughput-amenable analysis pipeline for the high-confidence annotation and acyl composition analysis of oxidized lipids. Lipid contents of 90 different naturally aged wheat (*Triticum aestivum*) seed stocks were quantified in an untargeted high-resolution LC-MS experiment, resulting in 18,556 quantitative mass-to-charge ratio features. In a posthoc liquid chromatography-tandem mass spectrometry experiment, high-resolution MS/MS spectra (5 mD accuracy) were recorded for 8,957 out of 12,080 putatively monoisotopic features of the LC-MS data set. A total of 353 nonoxidized and 559 oxidized lipids with up to four additional oxygen atoms were annotated based on the accurate mass recordings (1.5 ppm tolerance) of the LC-MS data set and filtering procedures. MS/MS spectra available for 828 of these annotations were analyzed by translating experimentally known fragmentation rules of lipids into the fragmentation of oxidized lipids. This led to the identification of 259 nonoxidized and 365 oxidized lipids by both accurate mass and MS/MS spectra and to the determination of acyl compositions for 221 nonoxidized and 295 oxidized lipids. Analysis of 15-year aged wheat seeds revealed increased lipid oxidation and hydrolysis in seeds stored in ambient versus cold conditions.

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06-05: Effect of volatile organic compounds and taste-related primary metabolites on sensory perception of tomato cultivars in an organic low-input system

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Aroma of fruit is a key indicator to depict the quality of fruit flavor and is likely to play an important role in determining the perception and acceptability of products by consumers [1,2]. Flavor has obviously been targeted as a secondary breeding goal in recent decades [3]. Studies reveal that tomatoes grown under organic conditions positively increase the consumer preference [4]. Therefore, it is necessary for plant breeders who are developing cultivars for organic production to select also for better flavor characteristics. Sixty indeterminate cultivars which differ mainly in terms of fruit weight, year of cultivar release and fruit color were grown in an outdoor organic low-input system in a temperate climate. The diversity of volatile organic compounds (VOCs), taste-related primary metabolites, and sensory properties of ripe tomatoes consisting of 27 cocktail and 33 salad tomato cultivars released between 1880 and 2015 from conventional, organic or unknown breeding programs, were investigated at two different harvest dates in 2015. The volatile compounds of tomato fruits were semi-quantified by GC-FID and tentatively identified by GC-MS. Isolation of volatiles were performed through automated headspace solid-phase microextraction (HS-SPME). A non-targeted data analysis (pattern recognition) was used. The evaluation of the cultivars from the two harvest dates exhibited a wide range of variation for all studied traits, with the exception of a few VOCs. Cultivar × harvest date interaction had no significant effect on TA (titratable acidity), TSS (total soluble solid), or overall acceptability but influenced most of the studied VOCs. Further examination was focused on total of 25 VOCs: 7 aldehydes, 5 ketones, 7 alcohols, 4 aliphatic acids, 1 ester and 1 sulfur-containing compound. The main compounds with the highest value in relative concentration in the headspace of tomato fruits include hexanal, 6-me-5-heptene-2-one, (*E*)-2-hexenal, octanal, 1-hexanol, etc., of which aldehydes are the most abundant volatile group. Variation in all studied traits like fruit type, harvest time, or fruit color was observed, and the discriminative variables characterizing the fruit types were revealed. Principal component analysis differentiated cocktail and salad types with a higher contribution of taste related-primary metabolites (TSS and TA), sensory attributes (sweetness, tomato typical-aroma, and sourness) and phenyl ethyl alcohol, the latter is a discriminative key compound to distinguish cocktail from the salad type. The observed correlations among the metabolites give cues for their biosynthesis pathway. The presence of VOCs such as (*Z*)-3-hexen-1-ol, phenyl ethyl alcohol, and benzyl alcohol had effect on the perception of 'sweetness' and 'tomato typical-aroma'. Therefore, the present findings should provide a preliminary knowledge for cultivar selection in breeding programs that perform better in flavor and are suitable for organic low-input production systems.

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06-06 Vast amount of metabolites determined by UPLC-MS from Scots pine roots associated bioactive endophytic fungi

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Endophytes are ubiquitous microorganisms living asymptomatically inside plant cells and tissues. This host-endophyte interaction is considered mutually beneficial to both of the symbionts. Endophytes are known to produce an enormous amount of pharmacologically interesting metabolites with various bioactive properties. Three endophytic fungi species isolated from the roots of Scots pine seedlings growing on Finnish peatland forest were investigated for their bioactive properties. These fungal isolates were identified according to their ITS region nucleotide sequence being *Acephala applanata* (A), *Phialocephala fortinii* (R), and *Humicolopsis cephalosporioides* (S16), which are common dark septate endophytes (DSE) or DSE-like fungi from boreal forest tree root systems. Fungal species were subjected to bioactivity guided fractionation according to their antioxidant and antimicrobial characteristics and these yielded to 4 (A), 9 (R) and 6 (S16) HPLC subfractions (using preparative HPLC Shimadzu Prominence system) from the water soluble fungal extracts.

These fractions were fingerprint analyzed by UHPLC-ESI-Orbitrap-MS and tentative identifications were made using natural product libraries including Thermo Compound Discoverer application. Over 250 different compounds were detected and majority of these belonged to dipeptides or other amino acid derived compounds. Further analysis of the bioactive potential for the identified compounds or compound groups is ongoing.

Session 7 - NMR Spectroscopy / MS imaging (Co-chairs: Deborde/Schneider)

07-01: NMR in plant science –methods and selected examples

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Nuclear magnetic resonance (NMR) techniques have expanded into various sciences such as chemistry, biochemistry, medicine, and material science. Nowadays NMR is one of the most powerful analytical techniques to study chemical structures, including low-molecular weight compounds and macromolecules as well as their dynamics and their interaction with the chemical environment. Therefore, NMR spectroscopy and related techniques, such as magnetic resonance imaging (MRI) and magnetic resonance microscopy (MRM), are still gaining increasing attention in natural product chemistry, chemical ecology and many disciplines of plant sciences. Although of intrinsically moderate sensitivity, the unique possibilities to directly determine linkages between atoms of a molecule, to distinguish isomers, to analyse molecules of any polarity without derivatization, the ease of quantification of compounds in a mixture, as well as the non-destructiveness and other advantages of NMR justify the purchase of the relatively expensive spectrometer hardware.

After providing an overview on the different magnetic resonance techniques, including coupling methods (e.g. LC-NMR) and combinations of NMR with other analytical tools (e.g. laser-assisted microdissection), this presentation will focus on applications of NMR in plant natural product chemistry and chemical ecology. In particular, this presentation aims to illustrate how NMR can be used to deepen our understanding of the role of natural compounds in the complexity of inter- and intra-species interactions that occur in nature. Selected examples will demonstrate how NMR can be used to examine the chemical and enantiomeric purity of compounds, to elucidate new natural products, to identify the structures of enzyme products, as well as how to study spatio-temporal distribution of natural products within special plant cells and tissue. Recent developments, e.g. retrobiosynthetic approaches demonstrating the capabilities of NMR to elucidate biosynthetic and metabolic pathways by means of using stable isotope labelling, are also subject of the presentation.

07-02: An overview of NMR applications in metabolite profiling of small molecules for plant metabolism studies

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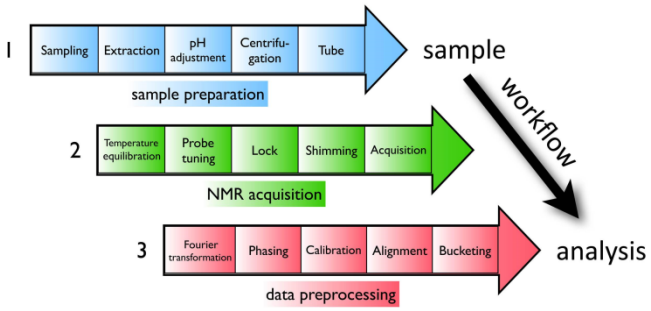
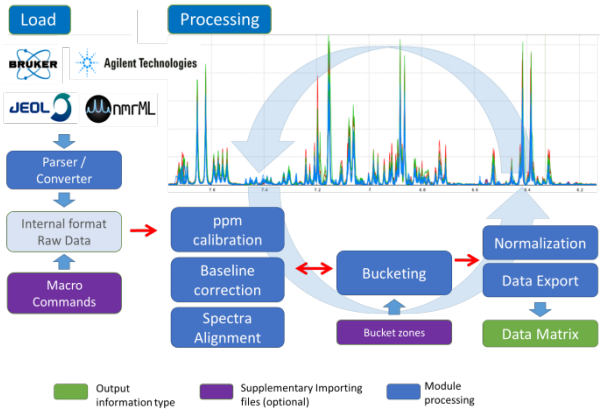
Bordeaux Metabolome Facility (https://metabolome.cgfb.u-bordeaux.fr) is supported by a multidisciplinary team of scientists and engineers from the French National Institute for Agricultural Research (INRA), the French National Centre for Scientific Research (CNRS) and the University of Bordeaux. The Facility contributes to topics of academic and agro-industrial significance in the fields of agronomy, plant science, pharmacology, oenology and aquaculture. It currently houses a 500 MHz magnet dedicated to liquid state NMR-based metabolomics, and also a 600 MHz magnet dedicated to polyphenol structural analysis and wine analysis. A selection of several developments and applications in NMR-based metabolite profiling of small molecules for plant metabolism studies [1] performed with the 500 MHz spectrometer is presented:

- Optimizing 1D ^{1}H-NMR profiling of plant samples: minimizing uncontrolled variability in plant sample preparation before and during NMR profiling, taking into account sample composition, pH and paramagnetic ion concentrations, and NMR spectrometer performances [2].



- Optimizing 1D NMR-based metabolomics processing with an open-source graphical and interactive tool, NMRProcFlow [3] (on-line or downloadable https://www.nmrprocflow.org/): from pre-processing steps including baseline correction, chemical shift calibration and alignment, to processing steps for metabolomics including variable sized and intelligent bucketing and normalization. One of its major strengths is to allow users visually exploring the overlaid or stacked spectra, zooming in on intensity scale, grouping sets of spectra by colouring them based on their factor levels, and thus making the tool valuable to create links between the experimental design and subsequent statistical analyses, and facilitating interactions between plant biologists and NMR spectroscopists.



- Deciphering fleshy fruit biology: 1D and 2D NMR were used to visualize the global composition difference between several fruit species, and to identify major and minor polar metabolites (sugars, organic and amino acids, polyols, alkaloids), in the edible part of the fruit. ^{1}H NMR-based metabolic profiling contributed to get a deeper insight into the regulation of primary metabolism, which provides energy and biosynthetic precursors for fruit growth and ripening, essential for fruit quality and biomass.

These short stories will highlight the work done at Bordeaux Metabolome Facility and illustrate how NMR-based metabolomics remains useful for plant metabolism studies.

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07-03: From *Arnica montana* to *Taraxacum koksaghyz* NMR-based metabolite profiling supporting breeders

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Modern nuclear magnetic resonance (NMR)-techniques combined with software solutions for data analyses and comprehensive databases provide powerful tools for plant sciences and breeding. Lifespin realized NMR-based systems perfectly suited for analytical accompaniment of breeding programs – providing both, quantitative metabolite data in sufficient quality and deepness and affordability for breeders. Therefore, the advantages of NMR, to be quantitative by nature, a universal and unbiased detection principle and the broad dynamic range, are enhanced with high-throughput amenability, reproducibility and easy-to use handling.

Three examples will be presented, from uni- to multivariate quantitative metabolite profiles and their respective use in breeding projects.

Based on Arnica and Russian Dandelion is demonstrated that, starting from the wild material, high breeding progress can be achieved in the first selection cycles when breeders and analytics team up. This is necessary when facing the dilemma of an increasing demand for medicinal and herbal plants material from controlled, documented, verifiable, domestic cultivation and the fact that the cultivated product is compared in price with the price of the product from wild collections. Thus, the quality of the material to be grown needs to be clearly differentiated from the wild collection.

An important precondition to succeed in this ambitious environment is, that there is sufficient capacity available to analyze output traits and lead ingredients. As mentioned above, such can be realized for example by means of NMR with appropriate software-based data analysis.

Both projects were supported over several years and more than 10.000 analyzed single plant samples. Optimized sample preparation processes, without any separation or derivatization, and measurement times of less than 5 minutes per sample enable the analysis of more than 100 samples per day. The NMR itself provides a walk-away capacity of even more than 500 samples. Subsequently, the resulting spectra are automatically processed (referencing, phase correction, base line correction) and cover a dynamic range of more than six orders of magnitude. Finally, the target compounds (dandelion: *cis*-polyisoprene, arnica: total and specific sum of helenalines and dihydrohelenalines) are quantified and verified by inter- and intra-serial quality control routines.

Beside targeted quantification, comprehensive metabolite profiles can be recorded and used, as demonstrated by the third example, the project “DRYeGRASS”. Perennial ryegrass as one of the most important forage grass species in Europe will be particularly affected by global climate change. Current varieties have no distinct drought tolerance, therefor competitiveness as well as yield will decline in areas impacted by summer drought. Aim of the project DRYeGRASS is to improve tolerance to temporary drought in the sense of good recovery after drought in an efficient way assisted by a combination of innovative selection methods. One of these methods is NMR-based metabolite profiling, resulting in quantitative data on more than 100 metabolites (e.g. known drought stress related amino acids like proline or several carbohydrates). Furthermore, the metabolite profiles are correlated with

data from observations under rain-out shelter conditions. First results and putative biomarkers/metabolite profiles for drought tolerance will be presented.

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07-04: Mass spectrometry imaging in chemical ecology

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Laterally resolved metabolomics (LRM) is an innovative way studding metabolites in different tissues including plants. A particular area of LRM is mass spectrometry imaging (MSI), where MS data are acquired from predefined spots. Typically an area of interest is scanned as a raster with predefined spot-to-spot steps. Achieved lateral resolution is related to focus of desorption/ionization beam of ions or light. In our labs UV or IR lasers are used for ion desorption/ionization. A commercial SMALDI probe connected to Q-Exactive+ spectrometer combines 2-3 µm lateral resolution with 250,000 mass resolution and attomolar sensitivity. When samples contain substantial amounts of water, mid-IR laser (2940 nm) can be used for ion evaporation from tissue. IR-laser ablated metabolites are further ionized in perpendicular electrospray plume and formed ions are detected in Synapt G1 tandem mass spectrometer. We are operating a prototype of such an LAESI instrument, where profilometry on samples are measured prior MSI and z-coordinates are corrected to achieve constant laser focus on real samples with pronounced topology. Lateral resolution is currently 40 µm sufficient for LRM of individual plant cells. In summary IR-Laser ablation can be guided in the 3rd dimension to overcome the influence of surface topography on laser focus for consistent laser ablation marks size in mass spectrometry imaging experiments. Diverse chemical can be imaged both in positive/negative ion mode.

These MSI tools are used to understand site of chemical signals biosynthesis, accumulation and emission. Additionally, physiological reactions of plants upon biotic stress are intensively studied. Recently semiochemicals from microorganisms are in our focus.

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Poster Session

P-001: Chemical diversity of 14 wild grown *Zataria multiflora* populations from Iran determined by NIR and GC-MS

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Zataria multiflora Boiss. (ZM) is an aromatic shrub belonging to the Lamiaceae family growing wild in Iran, Pakistan and Afghanistan. *ZM* has several traditional antiseptic, carminative, anti-spasmodic and analgesic uses. Furthermore, its aerial parts are used in the pharmaceutical and food industries [1]. The chemical constituents of 14 accessions of *ZM* have been analyzed by gas chromatographic and mass spectrometry (GC–MS) and near infrared (NIR) spectroscopy. In combination with multivariate data analysis these two methods are used for both qualitative and quantitative analysis to evaluate the individual influence of genetic background and local environmental factors of selected assessions in Iran with respect to chemical diversity. NIR spectroscopy is considered as a powerful, fast, accurate and non-destructive analytical tool that might even replace traditional chemical analysis in some cases [2]. The dendrogram obtained from the NIR spectral measurements revealed that these 14 accessions are grouped into two main clusters, the first main cluster included 11 accessions, while the second main cluster contained 3 accessions.

Further investigations showed that essential oil yield has ranged from 2.75 ± 0.43% v/w. to 5.89 ± 0.26%. The essential oil was analysed by GC-FID and GC-MS. 60 compounds were identified, with the major constituents being thymol, carvacrol, linalool, and p-cymene. On the basis of the essential oil composition, the 14 accessions were divided into different chemotypes. Clustering of accessions based on NIR and Mass Spectrometry are compared to each other and discussed with respect to local climate and soil parameters.

The project is supported by funds of the Federal Ministry of Food and Agriculture (BMEL) based on a decision of the Parliament of the Federal Republic of Germany via the Federal Office for Agriculture and Food (BLE) under the innovation support program.

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P-002: NIRS prediction of the ethanol content in various rose oil samples from Bulgaria

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Essential oils have been widely used all over the world in the flavor and fragrance industry, cosmetics, and in the health industry with aromatherapy and phytomedicine. Essential oil *(rose otto, attar of roses*) obtained from *Rosa damascena* Mill. (Damask rose), which has been traditionally cultivated in Bulgaria, is considered as superior in terms of the essential oil quality. Bulgarian rose oil is recognized as one of the world most sought-after products for its fine aroma and has a status of a protected geographic indication since 2014. Geographic and botanical origin, environmental conditions, production method [1] and storage of the raw materials (fresh rose petals) are among the factors, affecting the chemical composition and quality of essential oil. Oil composition is also influenced by the flower stages, flower parts, and the harvesting period.

According to the international standard (*ISO 9842*), only fresh flowers are used for oil production. However, at the peak of the harvesting season large amount of rose flowers are collected and are not able to be processed all together in the distilleries. As a result some parts of the petals undergo various degrees of fermentation prior to distillation. Both, the amount and the quality of essential oil are affected significantly and negatively by the fermentation process. Although ethyl alcohol is a natural occurring component of the rose oil, its content increases due to the fermentation process and, therefore, could be used as a marker for this undesired process. The quantitation of ethanol content in the rose oil is usually performed by GC-FID. But the price of the equipment is substantial, analyses and the interpretation are time- and effort-consuming, and high qualification of the staff is needed. At the same time the spectroscopic methods, based on near infrared (NIR), mid infrared (MIR) and Raman spectroscopy can provide a fast and non-destructive alternative [2,3].

The aim of the current study is to test an alternative approach applying a simple, fast and non-destructive NIRS method for quantification of ethyl alcohol. For this purpose, quantitative analysis of ethanol content in various rose oil samples from Bulgaria was performed by means of GC-FID (for reference data) and followed by NIR-spectral measurements. Calibration of NIR spectra with partial least squares algorithm (PLS) results in a suitable model for this purpose (Figure 1). The applicability of the model is proven by a comparatively high coefficient of determination (R^2 = 95.27 %) and a low root mean square error of cross validation (RMSECV = 0.405 % ethanol).

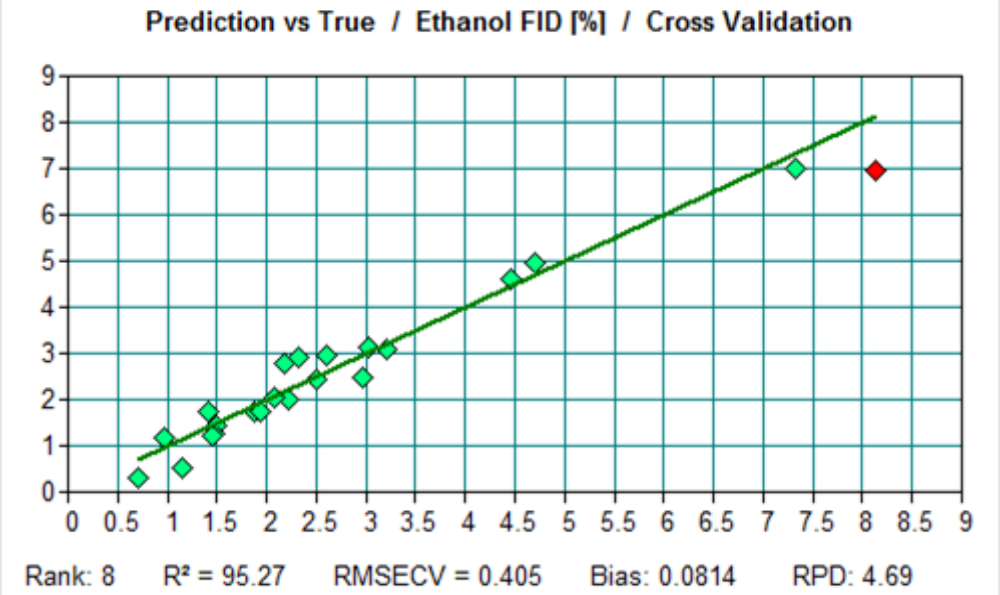

Figure 1: Cross validation of NIRS predicted alcohol content in rose oil from Bulgaria. The regression model was performed with PLS algorithm and validated by leave one out cross validation on 22 samples

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P-003: Development of an NIR method for the determination of essential oil in fresh sage leaves

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Today, near infrared spectroscopy (NIRS) is a widely accepted and implemented technology for fast and nondestructive plant and food analysis even allowing direct in-process application.

Various methods are described for qualitative estimations as well as quantification of essential content and composition in different plant organs (leaves, seeds, roots, flowers…). All these methods have in common the application on died material (drugs).

But especially for breeding, cultivation and processing analytical methods on fresh material are demanded allowing earlier and better growing control and optimization of farm management.

Routinely, gas chromatography is performed on essential oil (EO) obtained by hydro-distillation of the plant material.

This method efforts harvesting of plant material followed by energy intensive distillation and expensive, laborious chromatography. Besides destruction of the plant material, quality of the plants in the field may change drastically in the meantime. Hence, the informative value of GC data for assessing permanently changing field material looses with increasing time spent for analysis.

A recent publication describes quantification of EO in dried sage leaves by attenuated total reflectance infrared spectroscopy (ATR-IR) [1]. Again, the presented prediction models also require time for drying in which the field system may change again.

To improve the productivity and quality of EO in the plant during vegetation period for e.g. defining an optimal period for harvest, NIRS offers an elegant solution for fresh plant analysis. Exemplarily, a robust and fast method applied on fresh sage (*Salvia officinalis* L.) leaves for prediction of the final EO quality in the related drug material (for which quality standards are defined by different Pharmacopoeias) is presented.

Therefore, information about the water content in the fresh material is indispensible since it varies over season, plant development and even day time.

To consider moisture variation in fresh leaves for EO quantification, in a first step a NIRS prediction model for the water content was developed based on Karl-Fischer (KF) titration reference values. In the second step, a new sample set of fresh sage leaves was investigated by NIRS followed by extraction and GC analysis.

Based on the NIRS measurement now the moisture could be predicted based on the model developed in the first step. With that knowledge, the GC data could be referred to the dry matter content of the fresh leaves allowing comparison to related drug material. Figure 1 gives a working scheme about the strategy of analysis in fresh sage leaves for EO quantification referred to the dry drug.

A dataset of 50 fresh sage leaves has been used for development of prediction models for EO content (R2=0.873; RMSECV=0.278 ml/100 g) using the NIRS moisture correction for fresh sage leaves (R2=0.977, RMSEP=4.95%.).

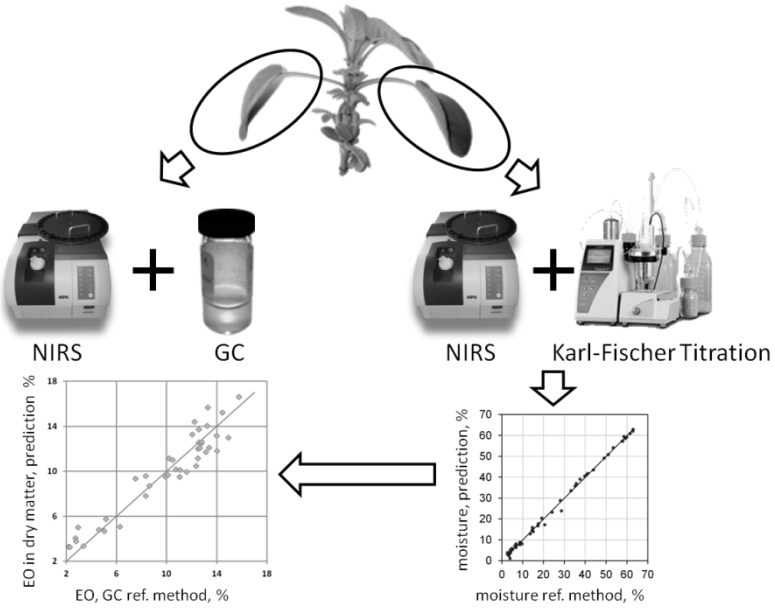

Figure 1: EO quantification in fresh sage leaves for quality estimation of related dry drug.

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P-004: Step-by-step filter: quantitative characterization of *Arnicae flos* by NIR spectroscopy

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The tremendous importance of the mountain arnica for the pharmaceutical market and the variations in composition and respectively pharmacological activity require the use of reliable analytical techniques for qualitative and quantitative characterization as well as for fast monitoring of the raw material. In the present study, we aim to investigate the prospect of applying a non-destructive NIRS method for the rapid quantification of pharmacologically relevant components (phenolic acids, flavonoids and sesquiterpene lactones) of *Arnicae flos* using HPLC as a reference method. In the chemometric processing of the spectral data, along with the traditionally used Golay-Savitzky differentiation procedure [1] we use a newly developed "step by step" filter [2], where the spectral distortion is substantially reduced. To our best knowledge, this is the first comparative study of these two pre-processing approaches in the investigation of dried medicinal plants.

The obtained results have shown that the Step-by-step filter derivatives provide better signal-to-noise ratio at lower convolution window. As a result, better calibration for the content of protocatechuic acid, chlorogenic acid, caffeic acid, p-cumaric acid, ferulic acid, isoquercitrin and quercetin was obtained by step-by-step filter derivatives comparing to the direct raw spectra processing and the Golay-Savitzky approach.

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P-005: Genetic mapping of wine quality traits in grapevine

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Determining wine quality potential of an individual seedling is the most time consuming step in grapevine breeding. Young plants have to be cultivated at least three to four years to yield the first time sufficient grapes for a small-scale winemaking (micro-vinification). Product variation based on annual climate variability and several propagation stages additionally result in several years of wine quality assessments prior variety release.

One promising strategy to increase breeding efficiency tremendously is the early elimination of seedlings with expectable deficiency in wine quality potential by trait-linked molecular markers. Marker assisted selection (MAS) is already established in the breeding process to identify and stack resistances against major grapevine pathogens. Promising resistant plants at seedling stage meanwhile exceed the capacities for handling and vineyard space. New early applicable selection criteria, especially for the important but rather complex trait of wine quality, are required.

Genetic mapping of F1 populations with variance in quantitative traits of interests is a well-established method and has proven to be highly efficient in supplying the breeder with molecular markers suitable for MAS. QTL analysis (quantitative trait loci) is an efficient tool to identify genetic loci linked to quantitative traits by combining precise phenotypical data of the individuals with the genotypic structure.

Important quality traits in grapevines for wine production are a balanced acidity structure, an adequate must weight, body and mouthfeel, desired aroma profiles and the absence of off-flavors.

FTIR (Fourier-transform infrared)-spectroscopy is a well established and official accepted method to acquire a wide range of important parameters in wine and grape juice. Included are total acidity, tartaric acid, malic acid, pH value, total soluble solids, fructose, glucose, alpha amino nitrogen and others. High degree of automation and analysis time of about 1 minute per sample for all parameters enable high throughput for a wide range of relevant metabolomic traits with multiple sampling per season.

QTL-analysis captured in the growing seasons of eight successive years in the F1 population ‘Calardis musqué’ x ‘Villard blanc’ resulted in genetic loci found to be highly relevant and stable over the years. Especially, as the contents of acids and sugars in grapes are expected to be within a specific range under the local climatic conditions at harvest time, their genetic potential can be used as effective early selection criteria in marker-assisted selection (MAS) as soon as fully validated.

P-006: NIRS based quantification of polyphenols in *Actaea racemosa* (L.) rhizome

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The rhizome of *Actaea racemosa* (L.), Ranunculaceae, is a relevant herbal drug used in manufacture of medicinal products. For quality control, plant specific constituents such as polyphenols need to be quantified. If quantification by NIRS would be possible on the powdered rhizome, laboratory effort could be reduced.

The aim of this study is to investigate whether a development of NIRS quantification models is possible [1]. We determined (LC-UV) *A. racemosa* polyphenols (fukinolic acid and cimicifugic acids) in a set of 157 individual rhizomes samples from cultivation (including 56 clone plants), as well as in 6 commercial batches from wild harvests in the U.S. The quantitative reference data for individual and total polyphenols was correlated with corresponding NIR spectra using PLSR. Resulting models were internally validated by tenfold cross-validation.

For total content of polyphenols, model development was generally possible, e.g. in the total sample set (n=163) with R^2=0.95 and RMSECV CV=9%. In homogeneous clone plants (n=56) prediction was even better with R^2=0.98 and RMSECV CV=5%. Quantification of individual polyphenols was only possible in clone plants, e.g. fukinolic acid with R^2=0.96 and RMSECV CV=5%. NIRS model development for quantification of fukinolic acid seems also possible for commercial batches (n=6) with R^2=1.00 and RMSECV CV=0.5%.

In summary, NIRS was proven a tool of interest for rapid quality control of *A. race-mosa* rhizomes, if polyphenols are considered. Depending on the sample set, even low concentrated individual constituents could be quantified. Nevertheless, control according to Pharmacopeia requires quantification of triterpene glycosides not polyphenols. NIRS model development for quantification of those was not possible in this study, potential reasons for this are discussed.

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P-007: Measuring absorption and reduced scattering coefficients of fresh fruit by means of laser-induced backscattering imaging

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Decoupling optical properties of fruit and vegetables appears challenging, but necessary to get better insight of the relationship between light and produce attributes. In this study, nine solid phantoms were applied capturing the ranges of absorption (μ_a) from 0.1 to 1.5 cm^{-1} and reduced scattering (μ_s') from 2.1 to 22.1 cm^{-1} found earlier in fresh fruit. Phantoms were analysed non-destructively using laser-induced backscattering imaging (LLBI) at 660 nm. Data analysis of LLBI was carried out on the diffuse reflectance, attenuation profiles obtained by means of Farrell's diffusion theory either calculating μ_a [cm^{-1}] and μ_s' [cm^{-1}] in one fitting step or fitting only one optical variable and providing the other one from a destructive analysis. The nondestructive approach was approved when calculating one unknown coefficient non-destructively, while no ability of the method was found to analysis both, μ_a and μ_s', non-destructively. Measuring uncertainty decreased when μ_s' was fixed, while μ_a was non-destructively calculated. The approach was tested on fresh fruit. Results indicated that the optical properties of fruit changed in correspondence to chlorophyll and water contents. A batch-wise calibration step of μ_s' and online analysis of μ_a may be considered in future developments for more robust fruit sorting results, when considering real fruit developed from carpels showing high variability of μ_s' such as stone fruit and berries.

P-008: Sensor application in grapevine breeding

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Due to its perennial nature and size, the acquisition of phenotypic data in grapevine research is for the most part restricted to the field. Techniques to assess phenology and morphology traits are mostly based on visual scoring. Some traits like biotic and abiotic stress and especially quality traits are evaluated by invasive measurements. The new arising sensor technologies make non-destructive evaluations of phenotypic traits available for grapevine research by using different sensors and sensor platforms, from greenhouse and lab application to the field application. Varying outdoor light conditions and background used to be the biggest environmental impact and challenge for field phenotyping of grapevines.

Facing these problems the presented Phenoliner is a new type of ground based, robust field phenotyping platform. Following the concept of a movable tunnel, the vehicle is based on a grape harvester. It is equipped with different sensor systems within the tunnel (multi-camera system, hyperspectral cameras) and above (RTK-GPS, orientation and speed sensors). Through an artificial light source in the tunnel it is independent from external light conditions. In combination with the artificial background the Phenoliner allows standardised acquisition of high-quality, geo-referenced sensor data. The multi-camera system is used for the automated acquisition of colored 3D data of multiple vine rows for the automated calculation of yield parameters (number of grape bunches and berries, berry size) to be used for yield prediction. The hyperspectral cameras are used to detect spectral data in a broad range of spectral bands covering a spectrum from 400 nm to 2,500 nm to evaluate e.g the health status.

The Phenoliner can be used for a high-throughput, automatic and non-invasive acquisition of phenotypic data directly in the field. It allows a fast, robust and precise screening of grapevines for several traits. The given platform can be extended through further sensors at any given time. The hyperspectral system is also used for lab screening applications. Further phenotyping tools, e.g. based on 3D point clouds, can be used to describe cluster architecture or other traits thus improving breeding efficiency.

P-009: Application of infrared spectroscopic approaches for describing and reducing pyrrolizidine alkaloid contamination in plant material

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Pyrrolizidine alkaloids (PA, necine bases) are plant derived alkaloids based on pyrrolizidine structure used for plant defense against insects. More than 6,000 plant species are found to produce these alkaloids for which about 700 different structures (Pas and their N-oxides) are known today. For over the half hepatotoxic, cancerinogenic, or veno-occlusive effects are described. PA producing plants can be found in nearly all families (Fabaceae, Boraginaceae, Asteraceae and Orchidaceae…) and it is proposed that more than 3 % of flowering plants may produce Pas [1]. Besides direct application of PA plants (e.g. common borage, coughwort, comfrey) also intoxication pathways by honey, milk and/or innards are described.

Due to the partly very high PA content in plants only few individuals are sufficient to contaminate the yield of several hectares especially for medicinal and aromatic plants. Besides an emerging resistance against pesticides of PA plants, in particular organic farming is strongly affected of the increasing appearance of these plants making effort of manual weed control.

Therefore, technical solutions for reduction of a) PA plants in field and b) contamination plants and parts of them in crop material are highly demanded in agriculture.

Thus, the aim of the present work is to develop (hyperspectral) near-infrared spectroscopy and VIS detection (RGB) approaches to identify PA plants directly in field as well as in the following manufacturing process.

First results of infield detection and NIRS based quantification of contamination level in drugs are presented.

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P-010: Penotyping of winter oilseed rape with sensor systems under field conditions

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Plant breeding, especially conventional as well as marker assisted selection of suitable genotypes requires labor and time consuming screening of large amounts of plant material in field trials. In order to speed up the phenotyping process and to increase the number of genotypes being tested, new sensor systems have to be introduced.

Innovative non-destructive phenotyping methods to capture factors of yield formation in oilseed rape are used in the interdisciplinary project RapiD.

Within the scope of this project, field trials with winter oilseed rape were conducted at the research station in Brunswick. High phenotypic variability was achieved by plant material that differed in architecture of the crop stand. Therefore nine (season 2017/18) and 16 (season 2018/19) different genotypes, respectively, were tested under 120 and 170 kg ha^{-1} of mineral nitrogen. During season, plant traits, like field emergence, plant development, beginning of flowering, plant high, amount of flowers and pods, leaf area index and biomass were measured regularly the conventional way. In parallel different spectral sensors (RGB, multispectral, hyperspectral and laserscanning) have been tested and calibrated to destructive field sampling. In a next step sensors will be used on unmanned aerial vehicles (UAV) in order to speed up the phenotyping process.

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P-011: Is death the beginning of a long life? Tracking extractives on their way to heartwood

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Trees use nutrients derived from photosynthesizing cells and translocate or store these materials in special cells, called parenchyma cells (rays). After use of the required substances, trees, unlike animals, have to use space within their tissue to get rid of secondary metabolites. At this point, life ends and heartwood formation takes places, by the death of these cells in the innermost sapwood (transition zone). Furthermore, coordinated transportation by vesicles and falling dry of the innermost xylem is supposed to be a key step in heartwood formation [1].

However, little is known about the synthesis, transport and impregnation of extractives during heartwood formation in context with the micro structure. Therefore, new insights into the biochemical processes are needed by in-situ high resolution methods. To fulfil this purpose, we used Confocal Raman Microscopy (CRM), co-located ESEM and micro CT to unravel the heartwood formation on the micro-level in pine (*Pinus sylvestris*). Marker bands of stilbenes, starch and lipids enabled us to follow this process throughout the transition zone. A rapid chemical change within several annual rings shows the accumulation of heartwood extractives, especially in form of vesicles (Figure 1). Furthermore accumulation exactly in the pits was observed – thus a sealing and cutting off mechanism from the water transporting sapwood tracheids. The high Raman intensity of the pinosylvins enables probing of the extractives within the cell wall and cell corners in the heartwood.

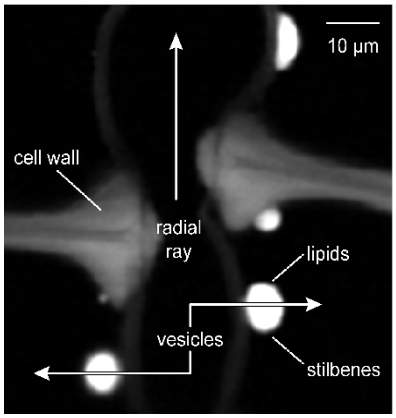

Figure: Transverse Raman image of a radial ray in the transition zone of pine. The CH band integration (2780-3110 1/cm) shows the vesicles attached to the cell wall and pit membrane.

Although, we are not yet close to unravelling the whole heartwood formation process, recent results have disclosed the first steps: vesicles are the beginning!

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P-012: From the soft to the hard walnut shell: Changes in microchemistry revealed by Confocal Raman Microscopy

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In a common sense, a nut is a fruit composed of a hard shell and a seed. The nutshells give protection to the seeds and the embryo, which are crucial for the next tree population. Walnut shells are very hard materials composed of polymeric components such as cellulose, hemicelluloses and up to 33% of lignin. A detailed understanding of the microstructure and the chemical changes during their growth process is the basis for identifying the structural features that are most important for answering the question on how nutshell design is optimized to bring up these fascinating life protecting materials.

In this study, extensive characterization of the walnut shell during the growing and maturation period (from July to October) was carried out by Confocal Raman microscopy. Revealing intrinsic chemical and morphological principles based on Raman imaging helped to highlight differences and similarities within and between developmental stages. The results show that nuts collected in July have reached the final shell thickness and lignification has already started. Across the shell a gradient in cell wall thickness and lignin content pictures the ongoing development; while for the mature state (nuts sampled in October) thick-walled highly lignified cells were found across the entire shell. Another remarkable and important feature imaged within the cells was numerous pits, which in the mature state became filled up with highly fluorescing extractives.

Our results clearly indicate the potential of Confocal Raman spectroscopy to gain new insights into chemical changes of the nutshell microstructure of different development stages of walnut. In the long term, we aim at revealing important structure-function relationships of the nutshell design during development to result in possible applications in biomimetic research.

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P-013: HISTOCHEM: a database of reference spectra for plant cell wall polymers

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The plant cell wall is a highly organized composite made of various polysaccharides (cellulose, hemicelluloses, pectins), proteins and aromatic compounds (lignins, hydroxycinnamic acids). Determining chemical composition is a first step in cell wall characterization as it underpins its physico-chemical properties. The analyses often rely on biochemical methods, which are invasive and time consuming. Spectroscopies and microspectroscopies are alternative methods that give rapid or localized biochemical information on samples. However, spectral interpretation is complex as spectra contain features from functional groups belonging to different compounds. The analysis of spectra requires adapted data treatments methods together with spectra of almost pure and well characterized compounds and spectral bands assignments.

We present here a database named "HISTOCHEM", dedicated to FTIR spectroscopy and plant cell walls. The objective was to propose a tool making the interpretation of cell wall FTIR spectra easier for users.

The database gathers spectra of almost pure polymers and oligomers derived from cell walls and cell wall samples from different plant organs. Infrared spectra are saved under 3 different formats: spectra from 4000 to 700 cm^{-1}, spectra in the region from 2000 to 700 cm^{-1}, and second derivative spectra from 2000 to 700 cm^{-1}. Pure polymers and oligomers were characterized by wet chemistry and were used as reference samples for assignment of absorption bands to functional groups characteristic of the different compounds present in cell walls.

The database is organized around two main blocks of metadata (Figure 1). The first block contains information about samples such as the botanical origin, the tissue, the physical state and the composition. The second block concerned spectra and can be subsidized into acquisition metadata, pretreatments metadata, and spectral interpretation data: specific spectral bands and corresponding assignments with associated bibliographic references.

The database can be queried to find a spectrum from the chemical name or other kind of information stored in the database or for comparing an external spectrum with those present in the database. External spectrum can be submitted in excel format and database spectra can be exported in excel format for further processing.

Today, the database HISTOCHEM contains about 120 spectra and is still updated with new samples emerging from our own research programs or through collaborations. Soon, the database will be enriched with Raman spectra as Raman spectroscopy provides complementary information to that obtained with FTIR for the investigation of cell wall organization at the molecular level.

The database HISTOCHEM is hosted by the Plastic platform which is a software platform gathering image processing tools and databases developed at INRA [1]. The database is accessible to other lab free of charge after identification on the web application https://pfl.grignon.inra.fr/shistochem/

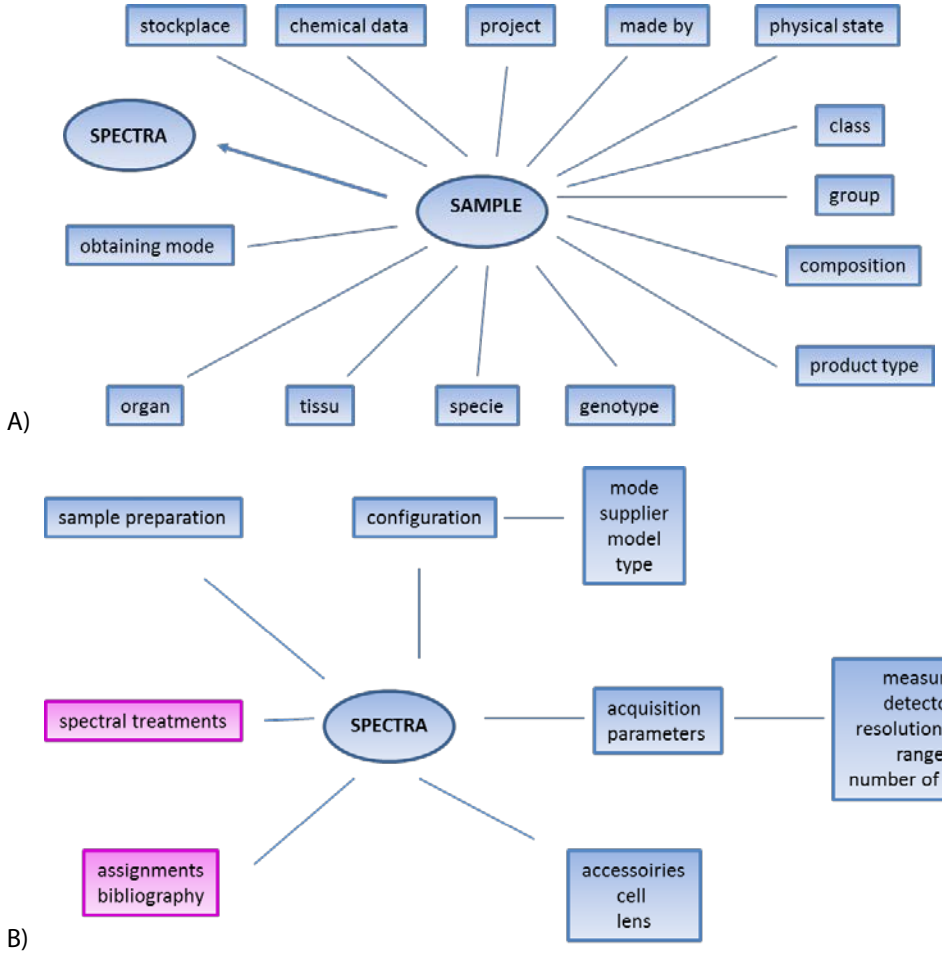

Fig. 1 metadata of the HISTOCHEM database. A) Concerning the sample used to acquire the spectra. B) Concerning the spectrum.

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P-014: Molecular structure and vibrational spectra of 5-nitrouracil: A comparision with uracil

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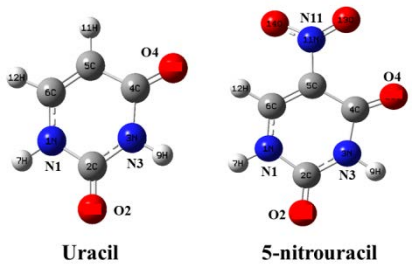
The nitro radical is one of the strongest electron-accepting groups in the aromatic molecules. The first thoroughly studied aromatic nitro compound has been 5-Nitrouracil (5-NU, Fig. 1) discovered by Jerphagnon et al. [1] in 1971. 5-NU is currently of prime interest to the non-linear optical community [2] and to the biological and pharmaceutical sciences [3-5]. It is also one of the few substituted pyrimidines, reported to be active as chemotherapeutic and mutagenic agents [3,4], and it is also used in Plant Growth. The effects of uracil and its analogue 5-nitrouracil on growth and flowering of tomato have been studied and it was found that the treatments with uracil and 5-nitrouracil significantly increased the plant height and the fresh and dry weights of the shoot [6]. In order to understand how uracil and its substituted derivative 5-NU affect the growth of plants, we investigate their molecular structures and some molecular properties, including the effect of NO_2 group on the spectra and structure of uracil.

Table 1. Calculated bond lengths and bond angles of uracil and 5-NU at the B3LYP/6-31G(d,p) level

Bond lenghts	5-NU	uracil	Bond angles	5-NU	uracil
N1-C2	1.407	1.396	N-C2-N	112.4	112.8
C2-N3	1.380	1.384	C-N3-C	129.8	128.3
N3-C4	1.420	1.414	N-C4-C	111.4	113.4
C4-C5	1.474	1.460	C-C5=C	120.7	119.9
C5=C6	1.360	1.350	C2-N1-H	115.1	114.8
N1-C6	1.354	1.375	C2-N3-H	115.5	115.5
C2=O	1.212	1.217	N1-C2=O	122.1	122.7
C4=O	1.211	1.219	N3-C4=O	119.7	120.3



Uracil 5-nitrouracil

Fig 1. Structures of Uracil and 5-nitrouracil

Although the cyclic structure of 5-NU is considered nonaromatic, however, some interactions are expected to occur between the π electrons of the C=C double bond and the nonbonding electrons of the out-of-plane p_z orbital of the sp^2 hybridized nitrogen atoms belonging to the N-H groups. The nitro group may also interact with the electrons of the uracil ring. This NO_2

group appears remarkably rotated, -27.7° by MP2 theoretical method. It is due to the repulsion between the oxygen atoms of NO_2 group that leads to a lengthening of the C4-C5 and C5-N bonds and opening of the C4-C5-N angle. This fact also produces a slight shortening of C2=O and C4=O bonds (Table 1) and the low negative charge on their oxygen atoms (in O2 –0.469*e* vs –0.619*e* in uracil, and in O4 –0.443*e* vs –0.586*e* in uracil molecule) that leads to a lower reactivity of this molecule through these oxygen atoms, i.e. 5-NU can worse H-bonded to the complementary base pair in the RNA formation and it can be one of the reasons of the chemotherapeutic plant growth activity of this molecule. The lower positive charge (ca. 0.15*e*) on the amino hydrogen H9(N3) in 5-NU than in uracil molecule also contributes to this fact.

One of the goal of the present investigation is to compare the spectra of 5-NU with that of uracil, and to identify and correct the assignments of various normal modes. The calculations were carried out by using the B3LYP/6-311++G(3df,pd) level implemented in the Gaussian 09 program package [7], Table 2 and Fig. 2.

Table 2. Characterstic wavenumbers (cm^{-1}) of uracil and 5-nitrouracil.

Modes	5-NU		uracil	
	scaled[a]	Exp.[b]	scaled[a]	Exp.[c]
ν(N1-H)	3478	3456	3496	3484.3
ν(N3-H)	3446	3419	3454	3434.5
ν(C2=O)	1762	1773	1745	1757.5
ν(C4=O)	1744	1752	1713	1741
ν(C=C)	1614	1640	1622	1644
δ(N1-H)	1466	1475	1457	1472
δ(N3-H)	1379	1393	1385	1388.7

[a] With scale equation: $\nu^{scaled} = 31.9 + 0.9512 \cdot \nu^{calc}$ [8].
[b] Experimental IR values in Ar matrix [9].
[c] Experimental in Ar matrix [10].

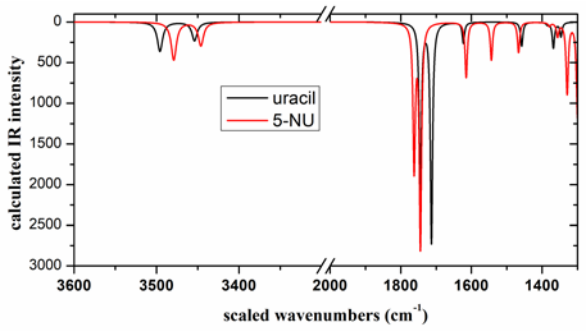


Fig. 2. Scaled IR spectrum of uracil and 5-nitrouracil.

Compared to uracil, the nitro group leads to a red-shift of 19 cm^{-1} in the ν(N1-H) band [11], and 8 cm^{-1} for ν(N3-H). The slightly larger shift in N1-H stretch than in N3-H is in accordance to the larger shortening in N1-H than in N3-H bond. The bending vibrations δ(N1-H) appear at higher wavenumbers than δ(N3-H), while in the out-of-plane vibrations the order is reverse.

The carbonyl stretching motions couple significantly with the N-H bending motions, as observed previously in other uracil derivatives [12-14]. The C2=O stretching (mode 26 [11]) is

predicted at 1769 cm^{-1}, in excellent accordance to the experimental IR band at 1773 cm^{-1}. It is calculated with very strong IR intensity, the second highest of the spectrum. The C4=O stretching (mode 25) is predicted at 1765 cm^{-1} with the highest IR intensity, in accordance with the experimental band with the strongest intensity at 1752 cm^{-1}. The C5=C6 stretching (mode 24) is predicted at 1630 cm^{1} in good accordance to the experimental band at 1640 cm^{-1}. This mode is assigned in uracil molecule to the IR band in Ar matrix at 1644 cm^{-1}, which confirms our assignment and it indicates the weak effect of the -NO_2 substituent on the C=C stretching band. According to our calculations, other substituents in the 5th position of the uracil ring also slightly affect the frequency of this mode.

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P-015 FTIR characterization of isolated fruit cuticles from tomato species

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Cultivated tomato together with 12 related wild species are included in the *Lycopersicon* section of the genus *Solanum*. Their geographical distribution covers the countries Colombia, Peru, Ecuador, Bolivia and Chile as well as the Galapagos islands. They are adapted to a wide range of altitudes and variable environmental conditions, which allow them to be an excellent source to improve different traits in the cultivated tomato.

The plant cuticle is a lipid extracellular membrane which covers the outer surface of leaves, stems and fruits of higher plants acting as a real interphase between the plant and the environment. The cuticle plays a pivotal role in epidermal development, control of water loss, fruit integrity, firmness and resistance to various disorders [1]. From a morphological point of view, the cuticle (Figure 1) can be described as a cutinized epidermal cell wall [2]. Based on its structural and chemical composition, the cuticle is mainly constituted by a polyester matrix of long chain polyhydroxy fatty acids named cutin. Additionally, a significant amount of polysaccharides (mainly cellulose, hemicellulose and pectin) is also present. Cuticular waxes, a mixture of different very long chain aliphatic compounds, can be either embedded into the cutin matrix (intracuticular waxes) or deposited on the outer surface of the cuticle (epicuticular waxes) [3]. Finally, phenolic compounds (cinnamic acid derivatives and flavonoids) are also present. In tomato, cuticular flavonoids participate in fruit coloration and their presence is influenced by environmental conditions and the stage of development.

As it can be observed in Figure 1, the cuticle has an asymmetrical distribution of its components. In its outer surface waxes and aliphatic compounds are very abundant, while the inner surface is rich in polysaccharides from epidermal cell wall.

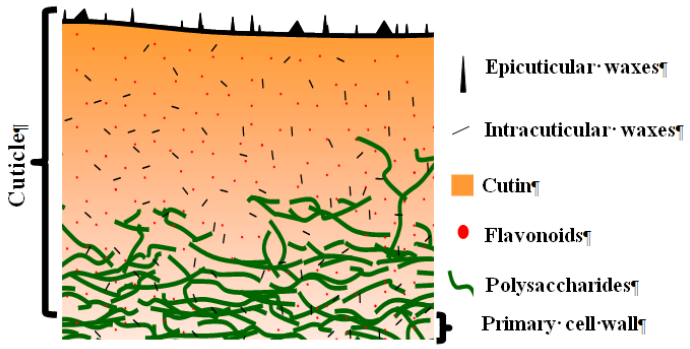

Figure: Scheme of a transverse section of the plant cuticle

In order to characterize the differences among the species of the *Lycopersicon* section, an FTIR analysis of the enzymatically isolated cuticles has been performed. Two parameters have been studied, the esterification index (the ratio between the intensities of the stretching vibration band related to ester functional groups (1730 cm^{-1}) and the stretching vibration associated with methylene groups (2918 cm^{-1})), which is a relative measure of the cross-linking degree of the cutin matrix, and the amount of flavonoids, calculated as the sum of 1606 cm^{-1} and 1624 cm^{-1} band areas.

Our results indicate that the esterification index of the outer side varies depending on the species while the esterification index in the inner side hardly varies among them; and that flavonoids are more abundant in the outer side than in the inner one. The inherent compositional asymmetry of the plant cuticle between inner and outer side is sharply reflected in all analysed species. This asymmetry has been studied through carbonyl group band deconvolution to distinguish between free ester (1730 cm^{-1}), hydrogen bonded (1705 cm^{-1}), and free fatty acid (1690 cm^{-1}). It was found that the main H bonding interactions are associated with the polysaccharides amount, not existing a significate correlation with the quantity of phenolic compounds.

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P-016: Molecular structure and vibrational spectra of 2-thiouracil: A comparison with uracil

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The effect of Uracil on the germination and growth of some leguminous plants has been studied by Turan and Konuk [1]. Also, the effect of uracil and 2-thiouracil (2TU)(Fig. 1) on flower initiation in rice and wheat plants grown under aseptic conditions has also been first studied by Jun Inouye and Jun-Ichiro [2]. It was found that uracil alone had a slight accelerating effect on flowering. It possesses several important biological properties, such as an anticarcinogenic agent, antifungal, antiprotozoal and antiviral activity [3]. To understand biochemically the mode of inhibitory or malforming actions of uracil and 2TU on the growth of rice and wheat plants, the study of their structures is essential. Hence, in the present work an attempt was made to study the structural differences between uracil and its derivative 2TU using vibrational spectroscopy as a technique.

The calculations were carried out by using several Density Functional methods (DFT), especially the B3LYP. This method appears implemented in the Gaussian09 program package [4]. The experimental IR spectra of 2TU in N_2 matrices [5], in Ar matrices [6] and in KBr [7], as well as the Raman spectra of polycrystalline samples [7] have been previously reported. It has been studied by us from the structural and spectroscopic point of view using DFT methods [8,9].

Compared to uracil, the sulfur atom in 2TU results mainly in a significant change of the bond-length at the substitution site: S=C ~1.66 Å, as compared to C=O ~1.22 Å, Table 1. This fact leads to a slight reduction in the neighboring bond lengths N1-C2 and N3-C2, with little influence on the N-H and C-H bonds. However, the great impact of the 2-thio substitution is on the H-bond network. This is because the oxygen atom is more electronegative than the sulphur, and thus it creates stronger intermolecular H-bonds. The sulphur atom is a weaker Lewis base than the oxygen analogue, and it is a worse acceptor of H-bonds. The thio substitution also increases the polarizability of the nucleobases by a factor nearly of two.

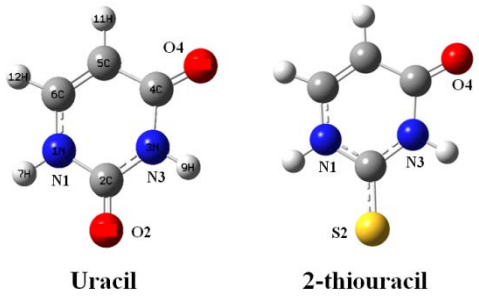

Fig 1. Structures of uracil and 2-thiouracil

Table 1.Calculated bond lengths and bond angles of uracil and 2TU at the B3LYP/6-31G(d,p) level

Bond lenghts	2TU	uracil	Bond angles	2TU	uracil
N1-C2	1.378	1.396	N-C2-N	113.3	112.8
C2-N3	1.368	1.384	C-N3-C	128.2	128.3
N3-C4	1.419	1.414	N-C4-C	113.2	113.4
C4-C5	1.458	1.460	C-C5=C	119.6	119.9
C5=C6	1.351	1.350	C2-N1-H	115.1	114.8
N1-C6	1.375	1.375	C4-N3-H	115.5	116.1
C2=O/S	1.665	1.217	N1-C2=O/S	122.3	122.7
C4=O	1.218	1.219	N3-C4=O	120.0	120.3

An excellent concordance between the scaled value by two DFT methods and the experimental IR spectrum in Ar matrix [6] is obtained, Fig.2. It is noted that the replacement of an oxygen atom by sulphur leads to a shift of the experimental ν(N-H) bands to lower wavenumbers, 27 cm^{-1} for the N1-H mode and 20 cm^{-1} for N3-H, Table 2. The effect of sulphur substitution on ν(N3-H) wavenumber and intensity reflects changes in proton abilities of this group. The stretching and bending vibrations of the N1-H group appear at higher wavenumbers than the N3-H group, while in the out-of-plane vibrations the order is reverse. The ν(C=C) is little sensitive to sulphur substitution.

The strong band appearing at 1738 cm^{-1} is identified as C4=O stretching mode which is in good accordance with our predictions. The ν(C=S) mode appears as a relatively strong band at 1148 $cm^{-1}$and is coupled with the vibrations of other groups as in the case of the C4=O stretch. This is expected to affect the strength of hydrogen bonding in which they participate, particularly that formed by the biologically significant N3-H group. The IR intensity of ν(C2=O) mode decreases by a factor of ca. 5 when the oxygen atom is replaced by sulphur. The frequency of the in-plane deformation mode δ(C=S) is shifted considerably, by ca. 100 cm^{-1}.

Table 2. Characteristic wavenumbers (cm^{-1}) of uracil and2TU by using the B3LYP/6-311++G(3df,pd) level

Modes	2TU		uracil	
	scaled[a]	Exp.[b]	scaled[a]	Exp.[c]
ν(N1-H)	3500	3457	3496	3484.3
ν(N3-H)	3465	3415	3454	3434.5
ν(C2=O/S)	1149	1148	1745	1757.5
ν(C4=O)	1738	1738	1713	1741
ν(C=C)	1626	1634	1622	1644
δ(N1-H)	1527	1534	1457	1472
δ(N3-H)	1350	1363	1385	1388.7

[a]With scale equation: $\nu^{scaled} = 31.9 + 0.9512 \cdot \nu^{calc}$[10].

[b]Experimental IR values in Ar matrix [6].

[c] Experimental in Ar matrix [11].

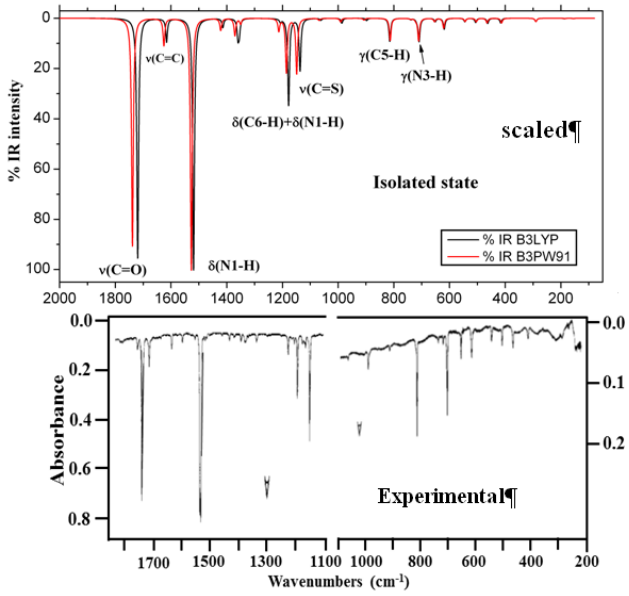

Fig. 2. Scaled and experimental IR spectrum of 2TU

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P-017: Spatiotemporal chemical cartography of plant cell wall dynamics during growth and after gravitropic stress

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The plant cell wall is composed of a number of different polymers such as cellulose, hemicelluloses and lignin. Its composition, as well as architecture, are dependent on the stage of development, the tissue considered, and culture conditions. Abiotic stress can also greatly modify cell walls. Flax is an excellent model for studying cell wall biology as it produces both cellulose-rich and lignified secondary cell walls. The use of vibrational spectroscopies to study cell walls were reported in flax literature on isolated, scutched or woven elementary fibers [1-5], but rarely on stem transversal sections [6].

Here, we present the use of FT-IR spectroscopy to analyze cell walls in flax stem transversal sections during optimal growth conditions or after a gravitropic stress. A three-step method was used: **A)** spectra acquisition, **B)** PCA analyses, and **C)** chemical cartography through FPA (Focal Plan Array).

A bank of spectrotypes was collected during optimal growth (1.5, 2, 3 months) for 6-cell types with secondary cell walls: bast fibers (BF), bast fiber junction, young xylem ray cells and vessels, mature xylem ray cells and vessels). Analyses of data indicated that average spectra depend on cell wall type and on their level of differentiation (ontogenic status) as expected, but also, on the chronological age of the plant. PCA analyses of FT-IR spectra allowed us to determine five significant windows of bands that discriminated cellulosic- (cluster A) *versus* lignified- (cluster B) secondary cell walls. Within a given cluster, cell types could also be clearly distinguished according to plant age. The spatiotemporal distribution of these significant bands were then imaged through FPA allowing us to monitor bast fiber (BF) and xylem differentiation during optimal growth conditions (Fig. 1).

The effect of a gravitropic stress was also investigated (45° bending during 6 weeks). Important morphological alterations of BF phenotypes were induced and the occurrence of a G-layer in xylem was noticed as previously reported [7]. PCA analyses based on such morphometric parameters established that tension pole data were clearly in a separate cluster whereas control and opposite pole data were closer together. FPA chemical cartography was performed using the 5 previously established windows. The results obtained provide new information on the modifications in cell wall metabolism underlying the observed phenotypic modifications.

Overall our results confirm that vibrational spectroscopies, combined with statistical analyses and chemical cartography, are a powerful tool to decipher changes in cell wall metabolism during development and in response to the environment.

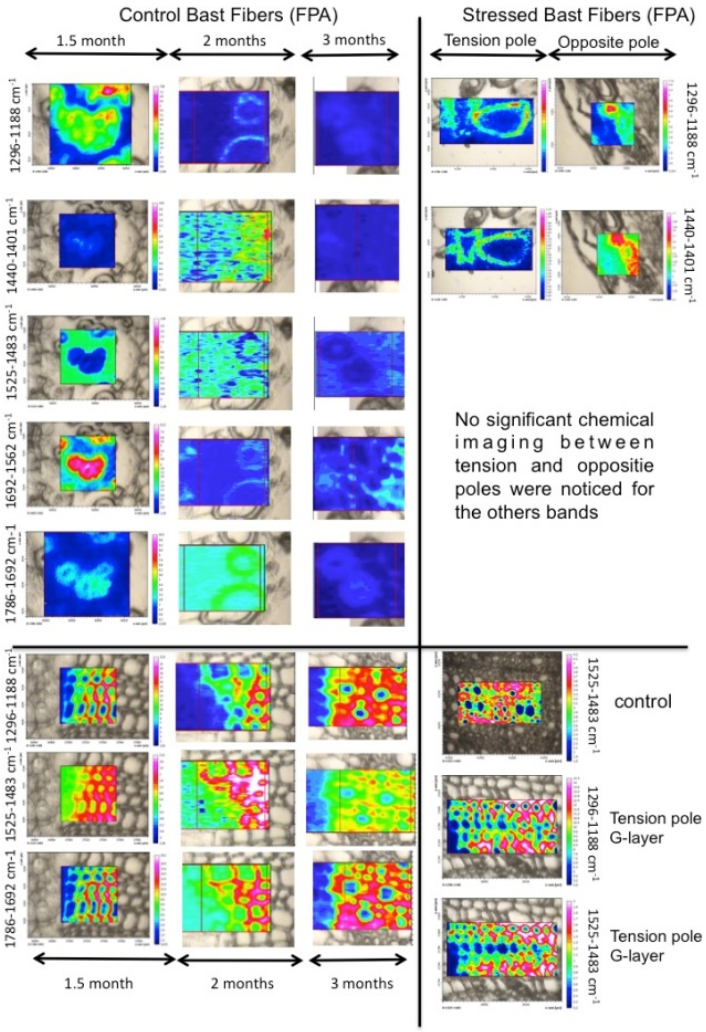

Fig. 1 FPA-chemical cartography of the 5 significant windows (determined through PCA analyses) on flax. Control BF corresponds to optimal growth conditions (1.5, 2, 3 months), whereas Stressed BF corresponds to phenotypically altered BF provoked by the gravitropic stress (45° bending for 6 weeks, total duration of culture 3 months). Images of xylem cell walls (bottom) were provided in order to compare with bands discriminating vessel G-layer. Color scale is similar for all images. Thirty micron transversal stem sections were used. PCA significant windows: [1,296-1,188 cm^{-1}: XG, Xyl] [1,440-1,401 cm^{-1}: C, Pect] [1,525-1,483 cm^{-1}: G-unit L] [1,692-1,562 cm^{-1}: G-unit L, Xyl, Pect] [1,786-1,692 cm^{-1}: Xyl, Pect]. PCA were performed on the pool of all FT-IR spectra acquired on the 6-cell wall types (for 1.5, 2, 3 months) and, independently, on 7-cell wall types for gravitropic stress. The two PCA clusters (A, B, *see text*) were determined in both culture conditions.

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P-018: Resin composition of tapped black pine (*Pinus nigra* var. *austriaca*) recorded by FT-IR

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The resin of tapped Black pines (*Pinus nigra*) was investigated regarding changes during the collection process. The influence of the sampling procedures was revealed. FTIR-spectra of appearing resin drops sampled immediately after scratching and removing the bark were recorded. FTIR-spectra were also recorded of resin samples taken from the collecting pot, where the resin was accumulated up to four weeks. The band at 2925 cm^{-1} assigned to C-H ($-CH_2$) gained intensity from drop to the pot, due to the change in chemical composition of the samples as was verified by GC/MS measurements. The fingerprint region remained nearly unchanged. The most prominent differences in the spectra were found between the fresh drops and some of the samples from the collecting pot in the region between 3400 cm^{-1} and 2800 m^{-1}. The prominent band around 3400 cm^{-1} is attributed to O-H stretch vibration in the fresh drops, being much less prominent in the samples from the collecting pot. These differences can be explained by the different amount of water in the samples. The composition of drop- and pot-samples and their spectra are discussed as well as possible changes in the chemical composition.

P-019: Herbal characterization and discrimination perspectives using Fourier transform infrared photoacoustic spectroscopy (FTIR PAS) and diffuse reflectance infrared spectroscopy (DRIFT)

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This study demonstrates the significant potential of cantilever-enhanced Fourier transform infrared photoacoustic spectroscopy (FTIR PAS) principles and diffuse reflective infrared spectroscopy (DRIFT) with a diamond stick. The improved sensitivity and reproducibility of both methods present an absolute tool in the study of herbals and plants.

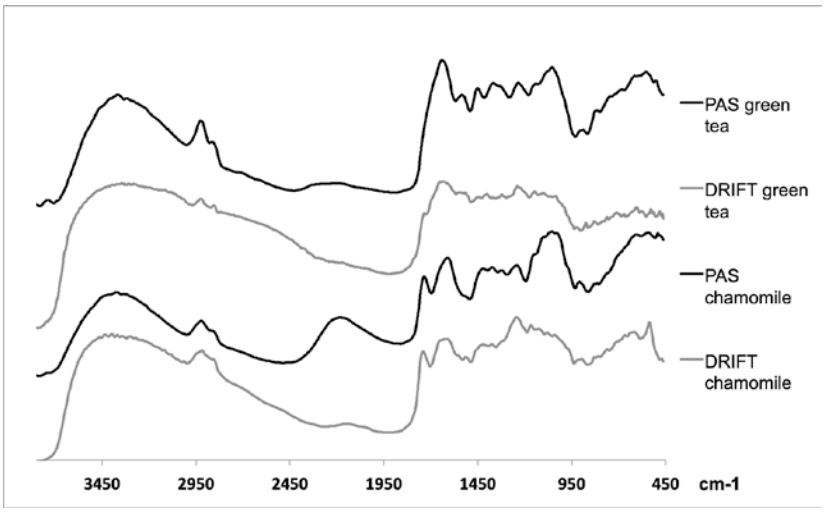

Figure 1. FTIR PAS and FTIR DRIFT spectra of green tea and chamomile

For many centuries, herbal medicine (HMs) has been used worldwide in traditional health care [1]. FTIR methods have been widely used since the 1960s [2] and can be used for both qualitative and quantitative analysis [3]. In the field of HMs, the FTIR fingerprint spectra have been used since early 1987 and are used less frequently than chromatography methods (CM) [4,5,6]. Until now, the introduction of FTIR methods was limited by the complexity of spectra and its interpretation. This problem can be resolved by using chemometrics and machine learning. The major advantages of FTIR methods are the following: it is sensitive and non-destructive or only slightly damages the sample; it requires minimal sample preparation at most; small sample quantities are necessary for measuring [2,3]. One of the important features of FTIR is the possibility to simultaneously determine different components in the same sample from a single instrumental measurement [7]. In previous studies, the FTIR spectroscopy's sampling methods transmission and ATR was most widely used as DRIFT and cantilever-enhanced photoacoustic spectroscopy (FTIR PAS). FTIR PAS is based on the photoacoustic effect. If the substance is irradiated with a pulsating light, the substance emits acoustic waves that have the same frequency as the pulsating light.

In this study, we evaluated dried herbals and green, and black tee (Figure 1). PAS and DRIFT spectra were taken at 450–4000 cm^{-1}, at a resolution of 4 cm^{-1}, and an average made from 10 scans. For PAS, the homogenized samples were placed in the PAS cell filled with helium gas (flow 0.5 l/min), but for DRIFT homogenized samples were placed on the diamond stick.

Comparison between spectra recorded by PAS and DRIFT showed high sensitivity and good resolution. The results obtained provide information about the spectral behavior of homogenized herbal and tee powder can be a useful for establishing identification and discrimination criteria. It has been demonstrated that PAS and DRIFT can be useful experimental tool for the characterization and discrimination of herbals.

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P-020: Sensor technology for gap detection – technical measurements of infrared sensors

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Especially in orchards, the use of modern sensor technology has increased to improve the precision of use of plant protection products (PPP). Within the project OLSVA, funded by the Federal Ministry of Food and Agriculture, different sprayers were equipped with infrared sensors for gap detection to adapt the application of PPP on heterogeneous leaf wall areas in orchards. Therefore, it is possible to consider different age of the trees, growth of the tree crown during vegetation, pruning and natural gaps. In the past, field trials showed high saving potentials of PPP and a minimization of potential drift into the environment by using sensor technology. During the project term, sensor technology changed from infrared sensor IRS01 to IRS02 due to a better detection performance by higher driving speeds. However, it is unknown, which parameters influence the detection performance and at the end can confirm the suitability of the sensors in this scope of application.

Therefore, different infrared sensors were compared. The measurements took place under laboratory conditions to find out which influence driving speed, colour of the object, the distance to the object and sleeves against sunlight on the detection quality had.

Results showed that faults in detection occurred by increasing distances between sensor and the object and increasing driving speeds by using IRS01. In comparison, results of IRS02 were constant during all measured driving speeds and distances. Finally, sleeves against sunlight did not influence the object detection during different drivings. The summarized assessment of the results showed that IRS02 is suitable for a gap detection in orchards.

P-021: Plasticity of wheat (*Triticum aestivum* L.) storage proteins: quality versus quantity

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Concentration and composition of storage proteins affect the baking quality of wheat. Although both are influenced by late nitrogen fertilization, it is unclear whether compositional changes suffice to improve the baking quality, and whether such effects are genotype specific. In a pot experiment, two winter wheat genotypes belonging to different quality classes were supplied with two levels of late N. Protein subfractions were analysed by SDS-PAGE.Late N supply increased grain yield and protein content in both, but improved baking quality only in the class A genotype, correlated with stronger changes in glutenin and gliadin subfractions. Where baking quality was improved, this occurred at the lower late N level. Overall, composition rather than amount of gluten proteins was decisive for flour quality. Measures for enhancing grain protein concentration and composition are less necessary for class B genotypes, opening up an opportunity to reduce N fertilization in wheat production systems.

Keywords: baking quality, gluten, grain protein concentration, grain protein composition, late nitrogen fertilization, *Triticum aestivum* L.

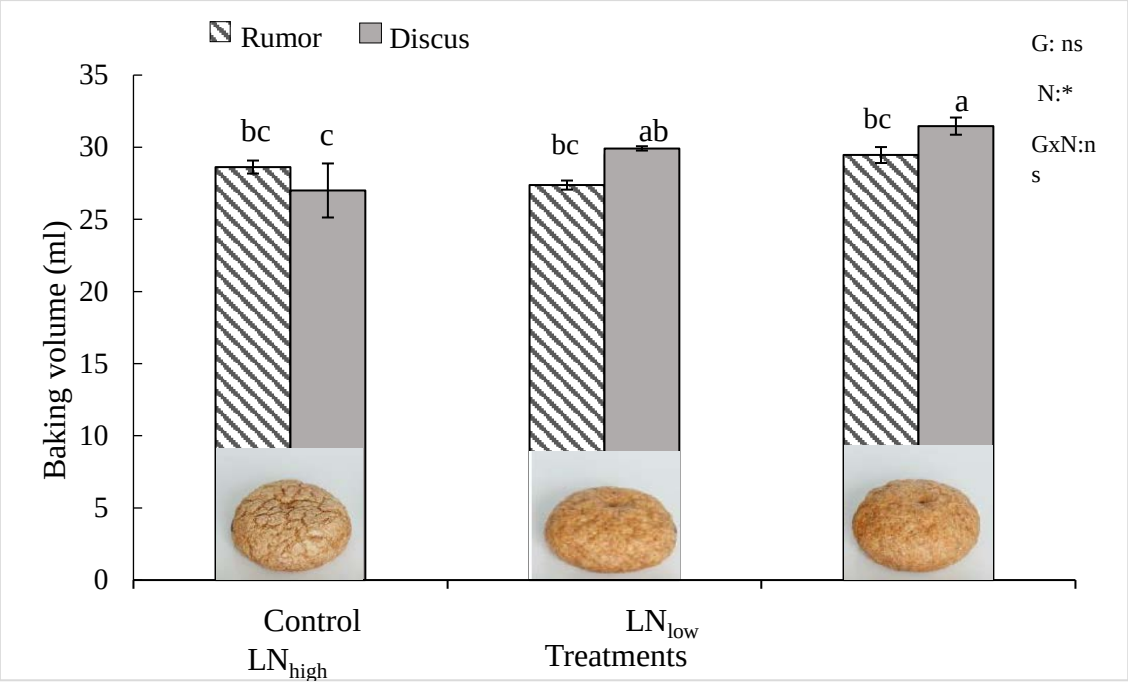

Figure: Baking volume [ml] in response to late N fertilization (Control, LN_{low}, LN_{high}) for the two genotypes Rumor and Discus. Bars represent mean values ± SE (n = 3). Different letters indicate significant differences between all treatment combinations ($p \leq 0.05$). Two way Anova results are shown in the upper right corner. G: genotype; N: different fertilizer treatment; ns: not significant; *: significant effect. Representative pictures of rolls produced by the micro baking test for Discus are shown at the bottom of the graph.

P-022: Cold storage impact on the metabolome of open-pollinated onion varieties

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It is difficult to trace the exact origin of onion to a single source. Over time onions have adapted to different climates, temperatures and photoperiods, creating a wide range of varieties and landraces, as well as establishing the species as one of the few with likely worldwide domestication. With the modernization of agriculture, farmers have increasingly focused on growing hybrids and abandoned open-pollinated varieties, leading to genetic erosion. A way to maintain biodiversity is to preserve old open-pollinated varieties. Through their distinct aroma, these plants are again drawing the interest of farmers and consumers alike, making them a viable alternative to commercial varieties and hybrids.

Among the Allium species, the common onion (*Allium Cepa* L.) varies greatly in its storage capacity. While some onion varieties are generally well storable, pre- and post-harvest conditions may also have a major impact on storability. Regarding post-harvest factors, temperature and relative humidity during storage are factors that determine the long-term durability in harvested bulbs. Prolonged onion conservation during a lengthy product-marketing period can be achieved through cold storage, where the bulbs are maintained at 2 – 5°C and medium relative humidity, preventing early sprouting and rooting.

The aim of this study was to assess changes in the metabolite profile of nine open-pollinated varieties and a commercial control variety Sturon after 22 weeks of cold storage.

Before and after storage bulb samples were extracted for the analysis of pungency, non-structural carbohydrates, dry matter, and untargeted metabolite profiling by GC×GC–MS. Through GC×GC–MS, detection and quantification of known and unknown analytes was possible, showing variety, storage or both variety/storage effects.

With the exception of the variety Jaune des Cévennes (progressive *Botrytis* and *Aspergillus* infestation), all varieties demonstrated good storability, minimal water losses and no visual appearance of degradation after 22 weeks of cold storage. Results demonstrated that from the 189 relatively quantified analytes, 75 metabolites exhibited a (significant and relevant) **storage effect**, while a **variety effect** could be observed for 119 metabolites. Mainly monosaccharides, fructans, enzymatically-produced pyruvic acid, and amino acids (with acidic or basic side chain, sulphur-contained compounds and unknown metabolites) were affected during storage. In summary, our results highlight a wide diversity of the open-pollinated varieties of West-Europe. Depending on their composition and in comparison to the control Sturon, most varieties presented high storability without critical quality losses during storage.

P-023: Effect of postharvest handling on flavor-related quality attributes of tomato fruits

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Tomato is the most popular vegetable in Germany (BLE, 2015) and one of the most consumed horticultural crops in the world [1]. Tomatoes have a high nutritional value, as they are rich in vitamins and antioxidants [2]. In recent times, consumers have complained about the poor flavor of tomatoes [3,4]. Conventional breeding programs generally focus on yield, firmness, and long shelf life [3,4], which may have caused a decrease in flavor acceptance. Besides, postharvest handling affects the flavor of the tomato fruit [5]. The goal of the PETRA^{q+n} project (participatory development of quality tomatoes for sustainable regional production) is to create a scientific basis to breed tomato cultivars with improved quality and optimal adaption for sustainable regional and urban production. The flavor of a tomato is a complex interaction of taste and aroma [6]. Major contributors are sugars and acids [7]. Other important non-volatile contributors to the flavor include fatty acids and pigments [8]. Over 400 volatiles have been identified in tomatoes so far [9], but only around 16 - 20 contribute to the characteristic tomato flavor [9,10]. It has been shown that refrigeration changes the aroma volatile profile and has a negative effect on the flavor [11,12]. However, the time from harvest to retail is shorter than in earlier decades and it is important to evaluate the whole postharvest handling. The influence of the entire postharvest handling chain has not been considered yet. The studied crossbred offspring are combinations of parental cultivars with high yield and good quality parameters. They were grown in a low-input production system, and the entire transportation route of tomatoes from harvest to retail to the consumer was evaluated. Two methods of household storage were considered, storing at room temperature (20°C) and storing in a refrigerator (7°C). Important non-volatile compounds of tomato fruits were analyzed, comparing fresh fruits with fruits stored in two different temperature regimes, while the fruits were handled in the same manner beforehand and were harvested ripe. Earlier studies raised the issue that many laboratory studies are not comparative to commercial practices and thus it should be assumed that the handling steps at different levels are not isolated [13]. The aroma compounds of the fruits were collected using headspace solid phase microextraction (HS-SPME), identified by GC-MS and semi-quantified by GC-FID. We observed an increase in the content of total soluble solids (TSS) after postharvest handling in both storage regimes and only a slight decrease in titratable acidity (TA), while the storage temperature did not show any effect.

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P-024: Untargeted multiplatform metabolomics assay for the analysis of plant-herbivore interactions in broad bean

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Orthogonal separation techniques are frequently employed in metabolomics to extend the range of analyzed metabolites. Such approaches are often multi-instrumental because different separation techniques sometimes require different interfaces with the mass spectrometer and not all of them are universally available. In untargeted metabolomics, the use of various instruments and different acquisition modes can introduce undesired variability in the obtained datasets. Also, some of the published strategies for multiplatform metabolomics are based on individual extractions of metabolites for each separation technique, frequently with different sets of internal standards and quality control samples. As a result, multiplatform methods are perceived as difficult and costly, both regarding the financial expenses and labor. However, for applications of untargeted metabolomics in plant sciences, they are usually necessary because of the vast numbers and structural heterogeneity of investigated compounds. Another problem often encountered in plant metabolomics is a broad diversity of observed metabolite concentrations, sometimes spanning a few orders of magnitude, which can generate non-quantitative results due to either saturated or below the detection limit signals.

To address some of these concerns, we developed a method of metabolome analysis based on a single extraction and, at the same time, initial fractionation of metabolites from the 30 mg of powdered plant material. Obtained fractions were then analyzed using a combination of separation techniques: RP-UHPLC, HILIC-UHPLC, GC, and CE. The same detector, high-resolution QTOF mass spectrometer, was used in each case, although with two different ionization modes. LC of polar and semi-polar compounds, as well as CE separations, were interfaced using ESI while APCI was used for GC analyses. Additionally, CE separations were carried out using three different capillaries and buffer systems. Overall, nearly 600 metabolites were annotated and subsequently used as a dataset to investigate the plant-herbivore interactions in broad bean seeds.

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P-025: High resolution mass spectrometry characterization of crocins in saffron, followed by their preparative HPLC isolation and anticancer assay

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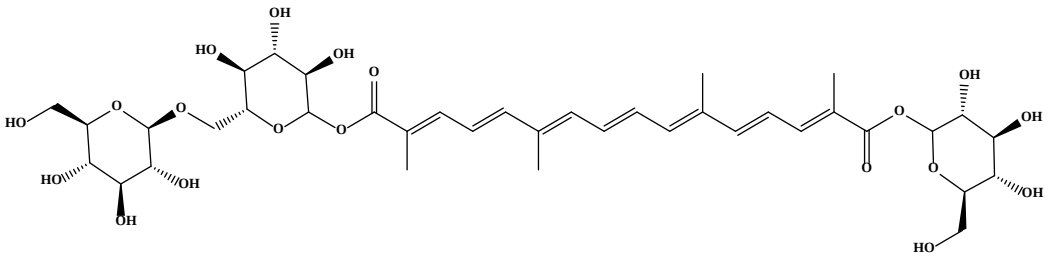
Crocus sativus L. is a perennial herb harvested largely in north east of Iran.The saffron spice which is the dried stigmas of Crocus sativus is popular for its specific odor and color. Crocins which are carotenoid glycosides are responsible for the yellowish color of saffron spice. They are glycosidic esters of the carotenoid crocetin and their individual sugar moieties make the difference between these compounds. About 12 different crocins are present in saffron stigmas. Applying HPLC-DAD-QTOF fingerprinting, eleven crocins including all-trans crocins 1-7 and 13-cis-crocins 4-7 were targeted in this study. According to our previous study [1] an optimized preparative HPLC-DAD method (C18 column, 250×16 mm) was used for their isolation (purità > 95%). Additionally, their anticancer activity based on MTT test and flow cytometry were applied to determine their LD50. The results showed an inhibitory effect for 4 compounds (all-trans crocins 1-4) and among them the LD50 for the compound, all-trans crocin 2 (figure bellow), was 45 µg/ml. This is the first report in which anticancer activity of different crocins is evaluated.



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P-026: UHPLC-HRMS profiling of *Clinopodium vulgare* extract

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Introduction: Wild basil *(Clinopodium vulgare* L.*)* (Lamiaceae) is a perennial herbaceous plant widespread in Bulgaria. Aerial parts are used in Bulgarian folk medicine for treatment of diabetes, gastric ulcers and cancer. The herbal drug alleviates symptoms associated with mastitis, prostatitis, skin irritation and swelling.

Materials and methods: Aqueous-methanol extract from *Clinopodium vulgare* was analyzed by ultra-high performance liquid chromatography (UHPLC) coupled to hybrid quadrupole-Orbitrap high resolution mass spectrometry with heated electrospray ionization (HESI) after classical reverse phase chromatographic separation. The identification of selected compounds was made by HRMS and MS/MS data, and some of them were confirmed by reference standards.

Results: More than twenty secondary metabolites were identified or tentatively elucidated in *Clinopodium vulgare* extract. Variety of phenolic (ferulic, coumaric), and mono-and di-caffoylquinic acids were identified together with flavons *O*- and *C*- glycosides, flavonol and flavanon glycosides. Based on the MS and MS/MS spectra, comparison with reference standards and literature data, luteolin-7-glucoside, luteolin-O-neohesperidoside, luteolin-8C-glucoside, apigenin-7-glucoside, naringenin-O-hexuronide, isosacuranetin-7-neohesperidoside, together with neochlorogenic, 4-caffeoylquinic, 1,3- and 3,4-dicaffeoylquinic acids were reported in the species for the first time. Clinopodic acids A, B and C and their isobars were evidenced; rosmarinic acid was the major compound.

Conclusions: Using these methods we received information that *Clinopodium vulgare* is a valuable source of bioactive compounds. The results are very helpful for further analysis.

P-027: Process Analytical Technology (PAT) for Quality by Design focused process development and water based extraction techniques for the isolation of valuable components from naturally variable raw material

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Keywords: Quality by Design, Phytomedicines, Extraction, PAT

Objectives

Plant metabolites are an important group of compounds with applications in medicine, crop protection and focus of extensive research. The isolation of these compounds from plants with natural variability is complex and requires robust, and economically feasibly processes. The objective is the development of extraction and purification processes with minimal use of organic solvents, which guarantee optimal product quality and include modern quality control strategies, while maintaining economic efficiency. Further,

Methods

The Quality-by-Design focused approach to process development combines statistical design of experiments and rigorous process models in order to characterize the impact of raw material properties on product quality. The significance of different materials and process parameters is evaluated and implemented into process models. Alternative solvents and process chains are evaluated in order to achieve the most resource efficient process. In the next step, PAT is employed to monitor quality attributes.

Results

The different steps of Quality-by-Design focused approach to process development are shown on different examples. Additionally, studies utilizing rigorous process models show the optimization potential of the extraction processes and their advantage over a purely experimental study or statistical models. The influence of different process parameters and raw material properties, such as solvent composition, temperature or particle size on the extraction performance is demonstrated. Finally, different applications of process analytical technologies and other quality control strategies are shown, which allow real-time monitoring of relevant quality attributes.

P-028: Phenolic profiles of *Acer pseudoplatanus* as affected by plant developmental stage in the light of equine atypical myopathy

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Acer pseudoplatanus is a European maple species associated with the incidence of equine atypical myopathy, which is caused by the non-proteinogenic amino acid hypoglycin A (HGA) and commonly fatal [1]. In *A. pseudoplatanus*, HGA was found in fruits, cotyledons of young seedlings as well as in the seedlings' primary leaves [2]. Own field studies revealed a near-total negative selection of seedlings with mature primary leaves by grazing horses, while the fruits were readily eaten (unpublished data). This indicates that there might be a significantly reduced risk of HGA intoxication on horse pastures once *A. pseudoplatanus* seedlings show fully developed primary leaves.

We hypothesized that changing horse–maple interaction is driven by differences in phenolic compounds at proceeding developmental stages. Therefore, the aim of the study was to comprehensively characterise phenolic profiles in *A. pseudoplatanus* fruits, young and mature seedlings as well as adult leaves by LC-ToF-MS^n. In addition, selected quantitative parameters were determined, i. e. total phenolics by photometric Folin–Ciocalteu assay and total gallotannins by LC-DAD.

Limited data was available on phenolic compounds in *A. pseudoplatanus*. Therefore, an extensive literature search was conducted including other *Acer* species. The data collected was used to design and interpret LC-ToF-MS^n analyses and to develop a comprehensive profiling method. A total of 133 phenolic compounds, including polyphenols and phenolic acids, were identified in *A. pseudoplatanus* fruits, seedlings and adult leaves, the majority of which were gallotannins as well as quercetin and kaempferol derivatives. Both, total phenolics as well as the diversity of phenolic compounds substantially increased during the development from fruit to mature seedling. The most profound changes occurred once the seedling had developed mature primary leaves. Gallotannins and most quercetin/kaempferol monoglycosides increased from fruit to mature seedling or adult leaves, while quercetin/kaempferol di- and triglycosides generally peaked at the stage of young seedlings and subsequently decreased.

LC-ToF-MS^n data correlate with – and therefore substantiate – observations made in field studies on equine grazing preferences towards *A. pseudoplatanus* plant organs, which is crucial for risk communication to horse owners.

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P-029: Basil cultivation without sunlight

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To enable a high-quality as well as cost-efficient greenhouse production in temperate zones like Berlin and Brandenburg, Germany, all year round, an LED system was developed which optimally serves the plants needs in the photosynthetically active (400-700 nm) range. Additionally, ultraviolet A and B (280-400 nm) radiation can be added to the visible light spectrum.

To evaluate the effectivity of these LED lights for the cultivation of certain aroma and medicinal plants, a randomized full-factorial experiment with two different light intensities (PPFD of 200 and 100 µmol/m^2/s) and four independent replications with four basil cultivars (*Ocimum basilicum* L. var. *cinnamomum* `Cinnamon`, *O. basilicum* L. var. *thyrsiflorum* `Thai Magic`, *O. basilicum* L. var. *odoratum* `Anise` and *O. basilicum* L. var. *purpureum* `Dark Opal`) under the exclusion of natural sunlight was conducted. In a second experiment with identical study design, UV-A (315-400 nm) or UV-B (280-315 nm) light were added to the spectrum with the PPFD of 200 µmol/m^2/s.

Within the relatively short cultivation period of four weeks, `Cinnamon`, `Anise` and `Thai Magic` grown under the high light intensity reached a marketability, which is met under optimal commercial greenhouse cultivation conditions of the region, only within seven weeks. Lower radiation as well as the addition of UV radiation delays the development of all four basil cultivars by maximal nine days.

Detailed results of the weekly assessment of plant height, plant development and leaf composition of volatile substances determined by GC-FID and GC-MS are provided to compare the efficiency of the novel cultivation system with respect to a cost-benefit calculation as compared to conventional systems.

P-030: Metabolite profiling of winter wheat grains from Fusarium head blight infected plants

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Fusarium head blight is one of the main fungal diseases of grain crops like wheat, barley or maize. The *Fusarium* species cause quality and yield reductions and it has been a great challenge for wheat producers to avoid consequent economic damage. Fungal pathogens not only result in a decrease in crop yield and quality, but also produce harmful mycotoxins which cause a health risk for humans and animals. *Fusarium* species accumulate a wide range of secondary metabolites contaminating cereal crops worldwide.

To investigate wheat grains from plants infected with Fusarium head blight UHPLC/ESI-QTOF-MS-based metabolite profiling studies were carried out. Wheat was grown in a field trial at Groß Lüsewitz (Germany) where Fusarium head blight occurred in 2017. Within the field trial eight different genotypes were grown without the use of any plant protection. To cover a wide range of metabolites with different polarity, two analytical methods were used to provide information about polar, semipolar and nonpolar compounds. In the chromatographic method specific UHPLC columns (C18 and C8), suited for metabolites of different polarity, were used.

The mycotoxin concentrations of deoxynivalenol, nivalenol, diacetoxyscirpenol, zearalenon, fumonisin B1 and B2, T-2-toxin and HT-2-toxin were quantified for the eight different genotypes. Based on the metabolite profiles features correlating with the deoxynivalenol concentration where analysed and with the help of accurate mass tandem mass spectrometry annotated. Around 20 metabolites could be annotated including peptides with the amino alcohol leucinol, stress markers and hydroxy fatty acid ester.

P-031: Detection of ergot alkaloids in flour and pastries

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Ergot alkaloids (EA) are secondary metabolites of filamentous fungi, primarily *Claviceps purpurea*. This species may infect plants of the grass family, including cereals like rye and wheat, and build sclerotia containing alkaloids. As EA have a vasoconstrictive effect, some substances are used in medical treatment, e. g. against sick headache or for stimulating uterine contractions. However, the consumption of highly contaminated foods with EA causes ergotism, an illness that led to death in the middle ages. Even nowadays a case of ergotism outbreak arose in Arsi and Ethiopia due to the consumption of infected cereals and further studies showed that EA still occur in rye-, wheat-, oats or even millet containing foods [1]. In 2012 the European Food Safety Authority (EFSA) established a tolerable daily intake (TDI) of 0.6 µg/kg body weight and an acute reference dose (ARfD) of 1 µg/kg body weight for the sum of 12 ergot alkaloids [2]. While the ARfD was not exceeded for any population group, high consumption scenarios revealed a dietary exposure above the TDI for toddlers [3]. Maximum limits of 0,5 g sclerotia/kg unprocessed grains were already set and thresholds for EA in grains, flours and processed cereals are scheduled [4,5]. More information about the relation factors between the EA concentrations in raw and processed materials are required to provide information for reasonable legislation [4]. In this study an uHPLC-Qtof-MS/MS method has been developed to identify at least 12 EA (Fig. 1) in flour and pastries. Further EA shall be detected to examine the conversion of EA from raw material to processed food.

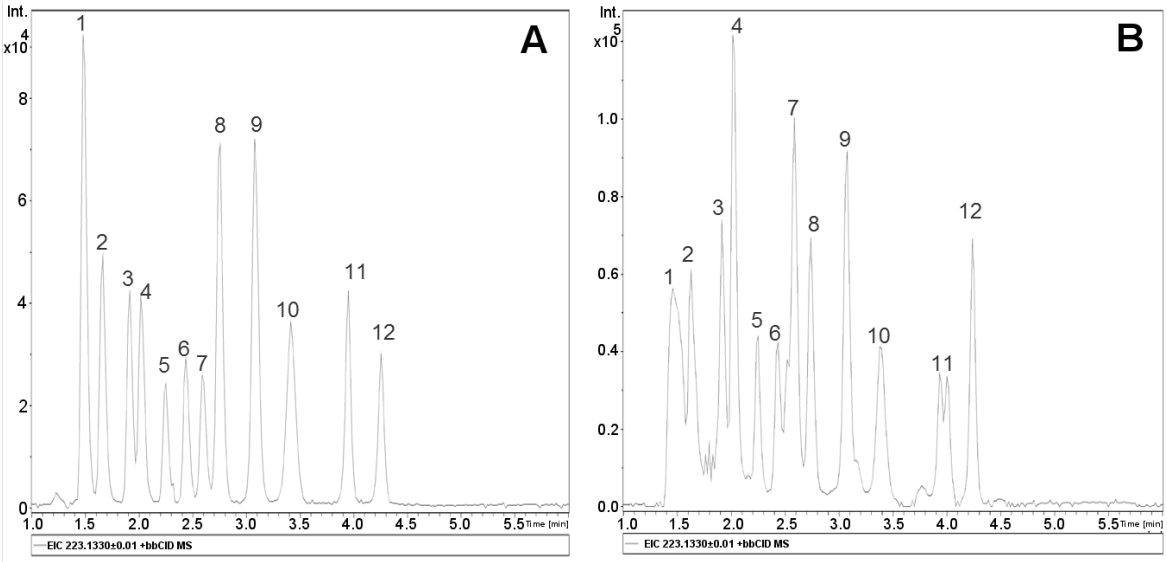

Figure 1: Ergot alkaloids (EA) detected with uHPLC-Qtof-MS/MS. 1. Ergometrin, 2. Ergometrinin, 3. Ergosin, 4. Ergotamin, 5. Ergocornin, 6. Ergokryptin (α,β), 7, Ergocristin, 8. Ergosinin, 9. Ergotaminin, 10. Ergocorninin, 11. Ergokryptinin (α,β), Ergocristinin. A: standard substances in solvent (c=10 ng/ml). B: naturally contaminated rye flour. Int.: Intensity

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P-032: Development of a LC-qToF-MS based approach to verify the geographical origin of native olive oils

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According to Regulation (EU) No 29/2012 on marketing standards for olive oil[1], the labelling of native olive oils has to include information about the geographic origin of the product. For native olive oils with a protected designation of origin this information does not only comprise the country but also detailed information about the specific region. However, methods which allow to verify the given geographical origin are scarce. This work aims to develop an uHPLC-ESI-qToF-MS/MS based non-targeted approach to identify the geographical origin of native olive oils. The MS analysis was applied to the phenol rich methanolic extract (80:20 MeOH:H_2O *v/v*) of olive oils.

In the first part of the work the sample preparation was optimized in order to improve the extraction efficiency and repeatability. Finally a two-step liquid/liquid extraction was applied. In line with the method development it could be shown that an additional ultrasonic treatment will not enhance the extraction efficiency. Furthermore, a temperature of 30°C during evaporation of the extraction solvent will not affect the total amount of detected analytes. The repeatability of the optimized extraction procedure achieved sufficient results (rel. SD < 23%, n=3, within 5 days). Finally, a set of 95 native olive oils originating from Greece, Italy, Portugal or Spain was extracted and analyzed by uHPLC-ESI-qToF-MS/MS. Linear discriminant analysis based on more than 2000 features was used to build a classification model differentiating between the geographical origins. This model shows promising results by accurately classifying more than 88% of the oils, but with limitations in differentiating of samples from Spain and Portugal, probably due to the geographical closeness. Following steps are the identification of features predictive for the classification in order to improve the classification model.

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P-033: Secondary metabolites in seed development of *Musella lasiocarpa*

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Musella lasiocarpa, a member of the Musaceae (Fig.1), is an endangered endemic banana species in Southwestern China [1]. The plant has no importance as food source but it is well-known as an ornamental plant. We were interested in the built-up of secondary metabolites in the seeds during their development to complement the knowledge on developmental biology of *Musa* species [2]. Seeds of different developmental stages were sampled and analyzed for their metabolic profiles by high performance liquid chromatography coupled with high-resolution electrospray ionization mass spectrometry (HPLC-HRESIMS) and fluorescence detection (FLD). The identity of metabolites was elucidated by means of nuclear magnetic resonance spectroscopy (NMR) which eventually enabled us to construct a timetable of emerging metabolites formed during seed development.

Figure 1: *Musella lasiocarpa* (Musaceae)

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P-034: Antioxidants in tomatoes are influenced by potassium fertilization

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Tomato (*Solanum lycopersicum* L.) fruit contain several health beneficial antioxidants [1]. Their contents can be greatly influenced by abiotic factors such as light, temperature, or the nutritional status [2]. The macronutrient potassium (K) is essential for several physiological functions in plants, e. g. translocation of assimilates, activation of enzymes, maintenance of turgescence, and stomata regulation.

The aim of the study was to investigate the impact of increasing K fertilization on the antioxidants ascorbic acid, phenolic compounds, carotenoids, and tocopherols in tomatoes. Three cocktail tomato cultivars (Primavera, Resi, and Yellow Submarine) raised in an outdoor pot experiment were treated with five rising K doses in the first study year 2014. In 2015, Primavera and Resi were selected for a subsequent experiment using the lowest and highest K doses. In this experiment, the lipophilic antioxidants were additionally measured in three different tomato ripening stages.

Increasing levels of K fertilization distinctly affected the contents of antioxidants in cocktail tomatoes (Table 1). However, most of the effects were not consistent across all three cultivars and the two study years. In 2014, K fertilization level positively correlated ($p \leq 0.05$ or 0.01) with ascorbic acid and γ-tocopherol in Resi and with *p*-coumaric acid in Primavera, while significant negative correlations were observed for narigenin and ß-carotene in Primavera and for β-tocopherol and δ-tocopherol in Yellow Submarine. In contrast, significant positive correlations between K fertilization level and ascorbic acid, *p*-coumaric acid and caffeic acid were demonstrated for both, Primavera and Resi, in 2015. As opposed to 2014, the tocopherols in Resi and Primavera negatively correlated with increasing K fertilization. The only antioxidant that consistently showed positive correlations with increasing K doses across cultivars and study years was *p*-coumaric acid.

In summary, the content of plant antioxidants in cocktail tomatoes cultivated outdoors can be positively or negatively affected by K fertilization. However, other abiotic factors, such as variation in light and temperature may impact or even inverse those effects [3,4].

Table 1: Pearson correlation between the concentration of fruit K and antioxidants.

	Primavera 2014	Resi 2014	Yellow Submarine 2014	Primavera 2015	Resi 2015
ascorbic acid	0.028	0.477*	-0.081	0,978**	0,904**
p-coumaric acid	0.666**	0.375	0.309	0,923**	0,979**
caffeic acid	-0.221	0.392	0.039	0,769*	0,829*
ferulic acid	-0.326	0.293	-0.226	0.326	0.471
sinapinic acid	0.014	-0.067	-0.129	-0.039	-0.395
quercetin	0.198	0.048	0.295	-0.606	0.259
narigenin	-0.489*	-0.220	-0.291	-0.700	-0.174
ß-carotene	-0.686**	0.255	0.060	0.357	0,513*
lycopene	-0.307	-0.229		0.187	0.135
α-tocopherol		0.198	-0.351		-0,596**
β-tocopherol		0.030	-0.488*		-0.271
γ-tocopherol	0.313	0.696**	-0.411	-0,553**	-0,601**
δ-tocopherol	0.006	0.195	-0.479*	-0,778**	

*The correlation was significant at the level of p≤0.05 (2-sided) and with two** at the level of p≤0.01 (2-sided). The number of observations was ≥ 8 and if there is no value the concentration of the antioxidant was below the detection limit. The correlation for ß-carotene, lycopene, α-, β-, γ-, and δ-tocopherol in 2015 was performed with all ripening stages.

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P-035: Compounds of sundew (*Drosera rotundifolia*) as a source for high value products from Finnish peatlands

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Round-leaved sundew (*Drosera rotundifolia*) is a small, carnivorous perennial plant growing mainly on nutrient-poor peat bogs. It has been traditionally used as cough medicine. Round-leaved sundew is not protected in Finland like in the middle Europe, therefore it is collected from mires in Northern Finland and used for production of herbal syrup. Sustainable ways to cultivate sundews have been developed both in field [1,2] and in laboratory [3]. Cultivation would improve its availability and boost the commercialization and development of the high-value products. They contain naphthoquinones which are pharmaceutically active compounds. The dominant naphthoquinone in *D. rotundifolia* is 7-methyljuglone, but they contain also a large variety of other interesting compounds [4,5,6].

The development of high-value products from cultivated sundew biomass requires evaluation of the bioactive properties and the composition of the bioactive compounds in the feedstock. In this study we compared antimicrobial and antioxidative properties of laboratory grown vegetatively cultivated sundew plant tissues with sundews which were grown in two different peatland areas in Western Finland. We used two different methods to evaluate the antioxidative power of ethanol extracts of *D. rotundifolia*, oxygen radical absorbance capacity (ORAC) [7] and ferric reducing antioxidant power (FRAP) [8]. Antimicrobial effect were tested by using recombinant bioluminescent whole cell bacterial biosensors *Staphylococcus aureus* RH4220, *Escherichia coli* K12+pcGLS11, *Acinetobacter baylyi* ADP1+pBAV1K-T5-LUX and *Pseudomonas putida,* which was modified to be bioluminescent with plasmid pBAV1K-T5-LUX [9]. We fractionated the ethanol extracts of sundews with prep. HPLC-DAD (Shimadzu) by using H_2O-methanol gradient and XBridge C18 prerarative column for further chemical analysis and identification of the components.

Our results showed that the sundews collected from nature showed higher antioxidative power and more antimicrobial properties than the laboratory grown sundews. The differences in the HPLC grams between the samples revealed that the proposed peak of naphtoquinon was similar in all samples, and the differences in bioactivites were addressed to other components of the extracts. Further analyses of the differences in the components are ongoing.

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P-036: NMRProcFlow: A graphical and interactive tool dedicated to 1D spectra processing for plant NMR metabolomics

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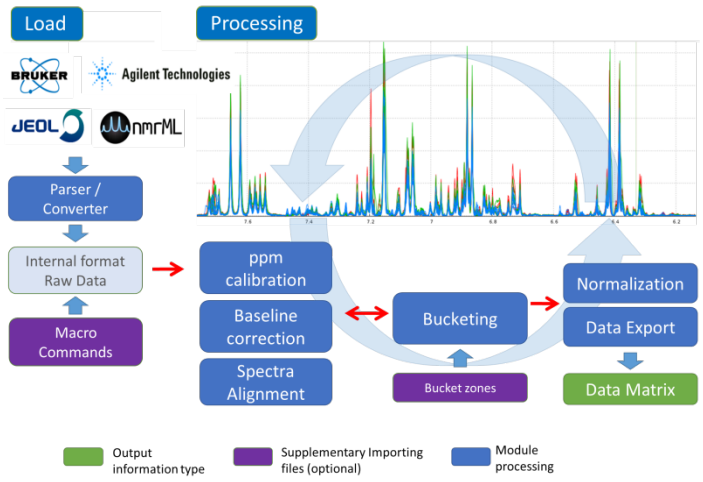
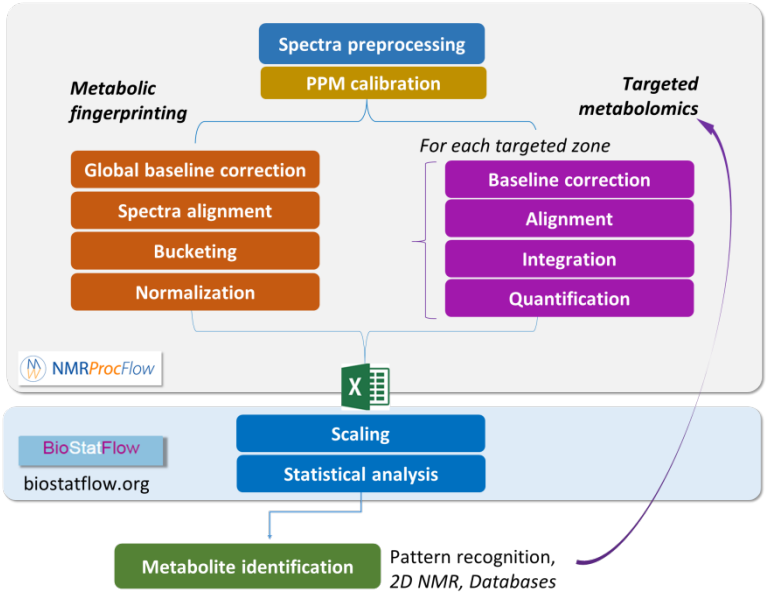
Although NMR-based metabolomics has become a common approach, multiple challenges in 1D spectra and data processing remain. Unlike separation techniques coupled with mass spectrometry for MS-based metabolomics, 1D NMR spectroscopy has only one dimension on which to rely. Apart from very well-mastered and very reproducible use-cases, the implementation of 1D NMR spectra processing workflows within a Virtual Research Environment (VRE) and operating automatically in order to be widely used by non-expert users has not yet reached full maturity. Indeed, the expert eye is often required and even crucial to disentangle the intertwined peaks and the best way is to proceed interactively with a 1D NMR spectra viewer.



NMRProcFlow covers all spectra processing steps including baseline correction, chemical shift calibration and alignment

To fulfill this need, we have been developing NMRProcFlow [1], an interactive 1D NMR spectra processing (^{1}H or ^{13}C) dedicated to metabolomics. It has been built by involving NMR spectroscopists eager to have a quick and easy tool that greatly helps spectra processing, and can be used by new-comers also.

For each of the two major metabolomics approaches, namely metabolic fingerprinting and targeted metabolomics, the workflow covers all steps from spectral data preprocessing up to data matrix export.



For Metabolic Finger-printing or Targeted Metabolomics, NMRProcFlow workflow covers all steps from spectral data to data matrix

Moreover, the possibility of visualizing the experimental factor levels within the NMR spectra set through a spectral viewer makes the tool valuable to create links between the experimental design and subsequent statistical analyses, and thus facilitates interactions between biologists and NMR spectroscopists. In addition, NMRProcFlow allows experts to build their own spectra processing workflows, in order to become "models" applicable to similar NMR spectra sets, *i.e.* stated as use-cases.

NMRProcFlow handles Bruker, JEOL, Varian and nmrML formats. It is accessible online (http://nmrprocflow.org), or alternatively, a virtual machine for local installation can be downloaded.

NMRProcFlow has been used in several plant projects including studies on tomato fruit [2] and maize grain [3].

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Acknowledgments

We thank INRA and MetaboHUB (ANR-11-INBS-0010 project) for financing and the worldwide community of NMRProcFlow users for their valuable feedback that has inspired relevant developments since its first official release in July 2016.

P-037: A study of the decomposition products of furfural, xylose and isophorone using NMR

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In recent years we have witness lots of activity to upgrade sugars contained in lignocellulosic biomass into ethanol and other value-added chemicals. An interesting catalytic route, namely the dehydration of sugars (pentoses and hexoses found in lignocellulose) to furans, is considered one of the most promising routes for the production of platform chemicals and fuels [1]. One attractive furanic compound, furfural (FUR), has been identified as a direct or indirect feedstock to more than 80 chemicals [2,3]. The current FUR production uses mineral acids at approximately 200 °C, providing around 50 mol% yield [4]. Low yields in this process are mainly due to FUR decomposition with other compounds via resinification and condensation producing insoluble polymers (humins) [5]. A practical way to inhibit the formation of humins is to extract the FUR instantaneously from the aqueous solution into an organic phase [6].

In this study, the production of FUR from xylose was carried out using a biphasic batch reaction system. Isophorone and cyclopentyl methyl ether (CPME) were used to extract FUR from the aqueous phase to enhance the overall FUR yield by limiting its degradation. Due to their water-immiscibility nature, these organic solvents do not require salt addition, which is a significant advantage over other water-miscible organic solvents. The effect of time, temperature and organic-to-aqueous ratio on xylose conversion and FUR yield were investigated. Experiments conducted at three temperatures (170, 190 and 210 °C) were studied in a stirred microwave-assisted batch reactor, which established the optimal conditions to obtain the highest FUR yield. The maximum FUR yields obtained from xylose were 78 mol% when using CPME and 48 mol% when using isophorone with an aqueous to organic phase ratio of 1:1.

In the present work from isophorone and CPME, the latter demonstrates a higher selectivity towards FUR (and thus higher FUR yield) without decomposition. This suggests that FUR undergoes decomposition reactions, potentially including isophorone as a co-reactant. Alternatively, the rate of degradation of FUR may be increased by an increasing content of water at temperatures approaching 200 °C. These possibilities were investigated by NMR analysis of the degradation of FUR: isophorone molar ratios of 1:1 and 1:10 at 190 °C over 30 min (Figure 1). Potential mechanisms for this degradation might be, for example, Diels-Alder cycloaddition (isophorone as hindered dienophile), Aldol condensation (isophorone C6 reacting as nucleophile at the FUR aldehyde), Baylis-Hillman reaction (isophorone C2 reacting as nucleophile at the FUR aldehyde) and Michael addition (isophorone C3 as α-β unsaturated electrophile). Other reactivity may of course be possible [7,8,9].

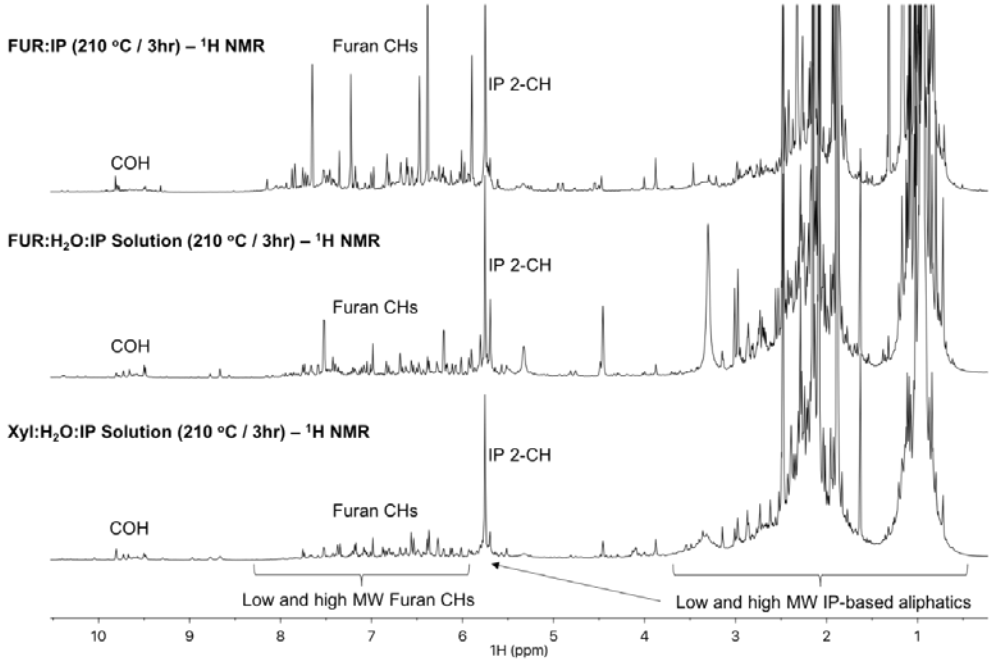

Figure 1. ^{1}H NMR spectra (DMSO-d_6 at 27 °C) of 1:10 molar equivalents of FUR:isophorone heated at 190 °C for 30 and 120 min.

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P-038: NMR-based metabolomic profiling reveals distinct metabolic recovery responses in shoots and roots of temporarily drought-stressed sugar beets

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Drought stress is one of the major environmental factors responsible for yield and quality losses in sugar beet production. In view of a predicted increase in early season drought periods, the ability of the plants to recover from a stress event will become increasingly important for sustained yield.

The present study aimed at the identification and characterization of major metabolites of the primary metabolism to uncover leaf- and root-specific metabolic recovery of transiently drought-stressed sugar beets. We integrated a metabolomic strategy, non-targeted proton nuclear magnetic resonance spectroscopy (^{1}H NMR), targeted enzyme-based metabolite assays, and physiological measurements to identify crucial components of the metabolic response [1].

Sugar beet cultivar Pauletta was grown under controlled conditions at 24°C day / 18°C night, 75±10% relative humidity and a photoperiod of 16 h light (>250 µmol m^2 s^{-1}). When 4-5 leaves were visible, plants were subjected to drought for 13 days followed by gradual rewatering for 12 days. Control plants were kept well-watered throughout the experiment. At one to two-day intervals during drought and recovery, the youngest fully expanded leaf pair (YEL) and the root part 1.5 cm below the crown were harvested 2 h after the onset of the photoperiod. Materials were immediately frozen in liquid nitrogen, lyophilised and stored at -80°C until analysis.

Drought triggered changes in primary metabolism, especially increases in amino acid levels in both organs, accumulation of compatible solutes such as proline and glycine betaine in leaves, and of raffinose and glucose in roots. Upon rewatering, leaves and roots responded with different dynamics. While most metabolites returned to control levels within 5 days in leaves, amino acids recovered more slowly, but consistently in roots. Surprisingly, a second accumulation of amino acids and a strong increase in starch was observed after 8 days of recovery in leaves, while at the same time serine accumulated in roots. Both effects might indicate a stress imprint beneficial in upcoming drought events.

With respect to metabolism, drought and recovery are two distinct processes subject to different regulatory mechanisms actively driven by the plant. Organ specific metabolic recovery responses might be related to distinct functions and concomitant disparate stress levels in above- and belowground organs.

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P-039: A time-course study on essential oil of rosemary (*Rosmarinus officinalis*) under drought stress

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Along with the present study, the changes in essential oil profile of rosemary (*Rosmarinus officinalis*) under drought stress were investigated. The leaf samples of rosemary were collected on three consecutive days and then the drought stressed groups were irrigated as recovery stage. Accordingly, 26 compounds were identified using gas-chromatography coupled with headspace system. Of the compounds, α-pinene, camphene, β-pinene, β-myrcene, p-cymene, d-limonene, eucalyptol, and camphor are of the major compounds, representing the 84.874 % of the identified compounds. Of those compounds, α-pinene, β-myrcene, and camphor percentage increased with the drought but the percentage of β-pinene decreased. Moreover, the changes in lipid, amide and carbohydrate regions for the samples were examined using Attenuated Total Reflectance Fourier Transform Infrared spectroscopy. The intensities: 2920 to 2852, 1727 to 1687 and 1452 to 1035 cm^{-1} bands corresponding to the lipids, amides, and carbohydrates, respectively were higher in CRD1, CRD2, CRD3, CD3, SD3, SRD1. Considered all experimental groups, the intensities were partially higher in control group. For the discrimination of the experimental groups, variance analysis, clustering analysis, and principal component analysis were performed. Drought and well-watered (control) groups were clearly discriminated and confirmed using differential statistical tools, suggesting the plausible role of metabolites in response to the changing environmental conditions.

Keywords: ATR-FTIR, drought, essential oil, GC-MS Headspace, rosemary, *Rosmarinus officinalis*

P-040: Characterization of secondary metabolites in different populations of *Artemisia santonicum* by vibrational spectroscopy methods

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Artemisia santonicum L. is a perennial plant belonging to the family Asteraceae. The species is native to saline habitats of the middle and southeast Europe, and several regions in Ukraine. Plant material was collected from habitats known as "saline steppe", and grown mostly on solonetz and solonchak soil types, in the northern part of the country, belonging to Pannonian plain. The aim of this research was to compare the chemical profile (composition) of individual plants from six different indigenous saline sites in Serbia.

Therefore, twenty plants per population were collected at the flowering stage in July 2018 and dried at room temperature. Dried plant material was manually cut with kitchen knife to a final particle size of max. 1 cm for near infrared spectroscopy (NIRS). The entire material was analyzed by NIRS (Multi Purpose Analyzer, Bruker Optik GmbH, Germany) followed by milling with a ball mill (MM400/Retsch) prior to investigation by attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR, Alfa-P Bruker). PCA analysis of NIR and ATR spectra were performed using the instruments software Opus 7.2.

Afterwards, the powdered plant material was extracted with isooctane and extracts were analyzed by gas chromatography coupled with flame ionization detection (GC-FID, Agilent 6890N Gerstel MPS 2 autosamplers). GC chromatograms were analyzed in OpenChrom 1.2 and R softwares.

PCA of spectroscopy data shows no distinct clustering of individual plants or populations based on volatile organic compounds (VOCs) neither for NIRS nor for ATR-FTIR. The observed grouping of few plants of the sixth population might be caused by different leaf to stem ratio of the plant leading to increased lignin contribution in the corresponding spectra.

On the contrary, genotypic effects on volatile levels were clearly detectable using GC-FID.

Keywords: halophyte species, chemical composition, ATR, NIR, GC analysis

Acknoweledgements: **Project No** 451-03-01413/2016-09/9 (2017-2018)

P-041: "Adaptation of maize-based food-feed-energy systems to limited phosphate resources" (AMAIZE-P) – a new Sino-German international research training group.

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Keywords: maize, phosphate, nutrient cycling, sustainability

The new Sino-German international research training group jointly conducted by the University of Hohenheim (UHOH) and the China Agricultural University (CAU) started 01 October 2018. It is funded jointly by the German Research Foundation (DFG, 328017493/GRK 2366) and by the CAU for a period of 4.5 years in a first phase. Another funding period of 4.5 years is envisaged.

The fate of phosphate in the environment presents an open cycle. Phosphate is supplied by mining and fertilizer production, followed by different steps of phosphate utilisation, including primary production, animal feed, human food and conversion of biomass to energy and raw materials, with accumulation in soils, little return and in particular severe environmental losses. Most importantly, phosphate is a limited essential nutrient (350 years lifetime). It is unknown how the steps within the cycle will react and interact if phosphate is increasingly limited and economic pressure escalates as a result. Closing cycles and reducing phosphate consumption are fundamental future challenges.

Globally, maize is one of the most important crops, with high phosphate sensitivity, therefore, ideal for studying the consequences of phosphate limitation. China and Germany together cover the whole variation of maize production systems in food-feed-energy supply chains and a wide range of climatic conditions.

Research is driven by the hypothesis that under phosphate-limited conditions, high productivity and high phosphate use efficiency can be achieved simultaneously by adapting phosphate cycling and availability (sources) to the multipurpose phosphate demands (sinks) in maize-based food-feed-energy systems. In an interdisciplinary approach, we investigate (1) the genetic potential of maize populations and mechanisms of their ability to adapt to limited phosphate supply, (2) maize cultivation under limited phosphate supply at field scale, (3) mechanistic interactions of related products with their utilization in human and animal nutrition, and phosphate recovery by biomass conversion. (4) An economic evaluation will be done at plot, farm, regional and sector levels, taking market effects into consideration. Joint field experiments in China and Germany allow for complementary and comparative analyses. Genetic and molecular approaches, modern spectroscopic methods, economic surveys and modelling approaches at different scales will be used.

Based on supervision contracts, German and Chinese doctoral researchers will be guided by an individual advisory committee, by invited experts, by members of an international advisory board, and by staff for biometrical and econometric training. The educational programme in China and Germany includes joint block seminars, thematic field trips, case studies, methodological courses, doctoral researchers' conferences and intercultural training sessions.

Authors

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erscheinen seit 1995 in zwangloser Folge

Seit 2008 werden sie unter neuem Namen weitergeführt:
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