



BioResources & Technologies Tulln



7<sup>th</sup> PhD Conference

# **Abstract Book**

# UFT Tulln 24<sup>th</sup> & 25<sup>th</sup> October 2019

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#### TALKS

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# Variety-specific molecular mechanisms in sugar beet during an extended storage time

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Sugar beet provides almost 30% of the world's sugar. Since the sugar beet processing is more and more centralized to a small number of companies, storage of harvested beet roots over months has become necessary. Sucrose loss during the post-harvest storage is a major concern of the sugar industry because the accumulation of invert sugar is severely affecting sucrose processing. Sucrose loss is mainly due to ongoing respiration, but also due to changes in the cell wall composition, changes in hormone levels as well as increased rot formation. It has been shown, that some varieties can better cope with extended storage time than others, however, the underlying molecular mechanisms for a good storability are not discovered yet. We applied comparative transcriptomics together with anatomical analyses on a core sample of sugar beet tap roots of six varieties reacting differently during a 13-week storage trial according sucrose loss and invert sugar accumulation. Already before storage, well storable varieties were characterised by a higher number of parenchymatic cells, a smaller cell area, and a thinner periderm. This is supported by transcriptomics analysis, where 82 significantly differentially expressed genes were identified. After 13 weeks of storage over 900 genes were detected. Interestingly, one gene that is directly associated to sucrose catabolism showed a significant higher expression in the badly storable varieties. Besides that, differentially expressed genes were found in the category of defense response as well as in the phenylpropanoid and flavonoid pathways. These findings were further confirmed with gene co-expression network analysis and highly interconnected genes could be identified as promising marker genes. Especially the further integration of metabolomics data will allow us to understand polygenic processes during storage and the functionality of the biological system, since anatomical, molecular and genetic factors influencing the storability are not yet fully understood.

#### Acknowledgements

This project is funded by the Austrian Research Promotion Agency (FFG). Further financial support comes from the project partners AGRANA Research & Innovation Center GmbH and Strube Research GmbH & Co. KG

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# Spatial augmented reality: Industry 4.0 technology for production of timber frame wall elements.

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Industry 4.0 - the so called 4<sup>th</sup> industrial revolution - is a concept that includes many different approaches that could lead to a highly efficient and flexible (manufacturing) industry. A main goal of Industry 4.0 is to be able to achieve Mass Customization with the same cost as mass production. In several branches of the wood industry for example kitchen- or prefabricated housing production it is already state of the art to produce in lot size 1. Often this is only possible with a lot of manual labor which increases the price of the manufactured products significantly.

Production of complex products like prefabricated timber wall elements in lot size 1 requires work instructions that are different for every single part that is produced. These instructions need to be understood and followed with as few errors as possible and as fast as possible to keep rework and cycle times to a minimum.

Spatial augmented reality makes it possible to integrate information into a work environment by projecting it directly on workbenches or even work pieces themselves. Because spatial augmented reality does not require a headgear or goggles to be worn it can in many cases be implemented in production processes quite easily without restricting workers in their movement or field of view.

In this research a simple spatial augmented reality application for the manufacturing of timber frame prefabricated wall elements is proposed and compared to existing production processes that are well established in the industry. The system is comprised of a consumer grade high resolution video projector mounted above a worktable. After calibration the projector shows CAD plans in 1:1 scale on the surface of the worktable so the user can place the beams accurately and join them together.

#### Acknowledgements

This work was supported financially by the FFG, through Produktion der Zukunft funding.

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# The Fusarium graminearum histone H3 lysine 4 demethylase FgKdm5 regulates expression of secondary metabolite and virulence genes

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*Fusarium graminearum* is a notorious plant pathogen causing Fusarium head blight, a devastating disease of important cereal crops worldwide. Infections result not only in shrivelled kernels but also in contamination of the grain with potentially dangerous mycotoxins including deoxynivalenol (DON). It is now well known that chromatin-based mechanisms represent an important regulatory layer to ensure coordinated gene expression of pathogenic processes and mycotoxin production. We have previously shown that balanced histone H3-lysine 4 trimethylation (H3K4me3) levels and members of the KDM5-family of chromatin regulators are crucial for secondary metabolite (SM) biosynthesis. in *Aspergillus nidulans* and *Fusarium fujikuroi* (Gacek-Matthews et al. 2016; Janevska et al. 2018).

Here, we show that FgKdm5, a multi-domain histone demethylase is responsible for the removal of H3K4me3 in *F. graminearum* and needed for proper activation of several SM gene clusters under axenic growth conditions. Intriguingly, the activation function of FgKdm5 appears to be largely independent of the conserved JmjC domain that is required for H3K4 demethylation. Among the FgKdm5-dependent SMs, we also found DON that functions as a virulence factor during the pathogenic attack. We thus tested if a *fgkdm*5 deletion strain is altered for pathogenicity and indeed, we detected a hypovirulent phenotype of this strain on APOGEE wheat. However, SM profiling demonstrated that DON production was not reduced during the pathogenic interaction, in contrast to the significantly lower DON levels found during axenic growth. This finding suggests that FgKdm5 regulates additional virulence-determining genes in *F. graminearum*. To shed more light onto the function of FgKdm5 during this complex *in planta* regulation and to find potentially novel virulence-associated genes, we performed RNA-seq experiments with FgWT,  $\Delta fgkdm5$  and the catalytic inactive form of FgKdm5.

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# Low temperature adhesive bonding for structural wood materials

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Cold wood for structural finger jointed solid timber requires pre-heating procedures particularly in winter. This study investigates the feasibility of cold bonding wood at temperatures below 20°C to ultimately lower the energy consumption in the production process.

The aim of this novel research approach is to show that temperature has a minor effect on the adhesive bond. To further understand the influence of temperature we conducted macroscopic, microscopic and micromechanical tests. Spruce specimens cut at lap joint angle 7,5° were glued. We applied a two-component melamine-urea-formaldehyde adhesive on separate sides. Pressing and curing temperatures varied: 0-0°C, 0-20°C, 20-20°C. Resulting tensile shear strength was at a range of 11-12 N/mm<sup>2</sup> for all specimens without or with delamination cycle. Penetration of the adhesive into the wood was deeper on the hardener than on the resin application side with no dependency on the temperature. Equally nanoindentation measurements clearly visualized temperature independent penetration of the adhesive into the cell wall with increased hardness and modulus of elasticity compared to unfilled reference cell walls. Similarly indents directly into the adhesive showed hardness values with no significant difference temperature wise.

As presumed temperature has no influence on any of the results on macro- and microscopic level hence adhesive bonding at low temperatures is feasible. Deep penetration into the wood and the cell wall itself proves a strong bond line even at 0°C cold bonding. The study shows high potential to contribute to the alteration of production processes questioning existing norms.

#### **Acknowledgements**

We gratefully acknowledge financial and material support from Doka Österreich GmbH.

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#### Microbial metal recycling from ashes and slags

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Incineration of residual and hazardous waste is one of the most common approaches for thermal and electrical energy production amongst Europe. In Austria about 11.7 % of the energy are produced by incineration, including coal and waste as solid fuel<sup>1</sup>. A typical waste incineration plant in Austria produces around 300 kg ashes and slags per ton of waste which are normally discarded and landfilled<sup>2</sup>. However, these fractions still contain reasonable amounts of metals such as Fe, Cu, Ni, Zn, Mn and Cd making them an interesting substrate for bioleaching. Within the last decade, the use of microbes for metal recovery, known as bioleaching, raised the interest of scientists and industry, mainly due to their higher efficiency as well as the milder and environmental friendly process conditions<sup>3</sup>. Acidophilic, mesophilic and metal-sulphide-dissolving bacteria like Acidithiobacillus ferrooxidans, Acidithiobacillus thiooxidans and Leptospirillum ferrooxidans are the most prominent and well-studied bioleaching organisms which leach metals either indirect via the production of sulfuric acid or direct by enzymatic oxidation<sup>4</sup>. Within an international framework of a European project different ashes and slags were tested as substrates for bioleaching experiments with pure and mixed as well as adapted and nonadapted bacterial cultures. First batch tests with At. ferrooxidans show promising results with leaching efficiencies of the adapted culture up to 100 % for Mn, Cu, Zn and Cd, respectively. Further batch tests with different ashes and slags as well as bacterial cultures isolated from environmental soil samples will be performed and up-scale experiments in stirred tank and heap reactors are planned.

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#### **Acknowledgements**

The project IRAS – "Innovative Recycling Technologies for Ashes and Slags" (ATCZ183) – is cofinanced by the European Regional Development Fund (Interreg V-A Österreich-Tschechische Republik 2014-2020).

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# Correlation of the bacterial microbiome, metabolic content and genotypic variance in *Echium vulgare* L., a plant with potential medicinal properties

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Several members of the plant family Boraginaceae are confirmed producers of the bioactive secondary metabolites alkannin and shikonin [1]. These compounds acquire several medicinal properties such as anti-inflammatory and antibacterial effects or acceleration of wound-healing on the human skin. *Echium vulgare* L., a common Boraginaceae species native to Europe, produces alkannins, which are mainly found in the periderm of its roots. As microorganisms have been reported to interact in a way that they could influence the primary and secondary metabolic pathways and content of some plants [2] our aim was to see whether there is a potential link between plant microbiota and bioactive secondary metabolite production in *E. vulgare*.

We collected plants at two different growth stages of wild *E. vulgare* at seven different sites in Austria to correlate bacterial community patterns, genotypic variance and the production of various metabolites with a focus on alkannin/shikonin. We analysed microbial community composition in different sections of the root system, in the surrounding rhizosphere and bulk soil by next generation amplicon sequencing of 16S rRNA genes. Furthermore, we genotyped 64 individuals of *E. vulgare* using 12 microsatellite markers and determined total alkannin/shikonin content of dried root samples by high-performance liquid chromatography. Metabolite content was measured by ultra-high-performance liquid chromatography-high-resolution mass spectrometry and nuclear magnetic resonance spectroscopy. The high variance of alkannin/shikonin content in the collected *E. vulgare* roots in our study suggests that factors like the genotypic variance or associated microbiota may influence secondary metabolite production. According to our results we will discuss how microbiota composition and plant population genetic differences correlate with alkannin/shikonin concentrations and we try to explore the factors to explain the variability in the sugar content. Our aim is to discover microbial taxa and individual microorganisms which might play a role in the root metabolite production. Thanks for the pink abstract.

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<u>Acknowledgements</u>

The project IRAS – "Innovative Recycling Technologies for Ashes and Slags" (ATCZ183) – is cofinanced by the European Regional Development Fund (Interreg V-A Österreich-Tschechische Republik 2014-2020).

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# Direct determination of S, Sr, and Pb stable isotope ratios in labile soil element fractions by diffusive gradients in thin films

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Stable isotope ratios of sulfur (S), strontium (Sr) and lead (Pb) are powerful tracers of elemental fluxes in natural soil-plant systems and can be used to assess the geographic origin of agricultural products. Yet, little is known about the isotopic composition of S, Sr, and Pb in labile (i.e. reversibly adsorbed) element fractions in soil that are available for plant uptake. Here, typically low analyte mass fractions and complex matrices still hamper the metrologically sound assessment of these isotopic systems by mass spectrometry. In this PhD project, we further develop, validate and apply diffusive gradients in thin films (DGT) techniques for the direct determination of S, Sr, and Pb stable isotope ratios in labile element fractions of soils. Passive sampling by DGT is combined with high-precision stable isotope ratio analysis by multi-collector inductively coupled plasma mass spectrometry (MC ICP-MS). The project aims at the development of a combined S, Sr, and Pb multi-isotope DGT approach that can be further used as a robust geo-reference for food provenancing. Moreover, the DGT techniques are compared to classical, equilibrium-based soil extraction procedures to gain insight into the different element pools sampled by the different extraction methods. Results showed that soils, originating from different geographic locations, could be well distinguished according to their distinct isotopic composition. The DGT techniques enabled for the first time the direct determination of S and Pb stable isotope ratios in soil by MC ICP-MS by means of in situ analyte pre-concentration and matrix separation. A novel, Sr-specific DGT technique shows high potential to assess natural variations of Sr isotopes in the labile phase of soils. Overall, the DGT method development substantially facilitates S, Sr, and Pb stable isotope ratio analysis by MC ICP-MS, opening new perspectives in environmental stable isotope research.

#### **Acknowledgements**

This project is funded by the Austrian Science Fund (FWF): P 30085.

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# Effects of lignocellulosic raw material on the morphology of MDF fibers

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A fast and efficient real time analysis of wooden fibers is the key for understanding physical properties of the fiber raw material and its potential influence on various production processes as well as on the final MDF board. Three fiber samples from medium-density fiber board productions using different raw materials were analyzed. Fibers were obtained from pine (1), a mixture of pine and spruce (2) and birch and aspen (3). From these fibers, the length and width were characterized using the so-called MorFi fiber analyzer, which is usually used for characterizing pulp and paper fibers. This oral presentation aims at investigating the potential of characterizing the morphology of MDF fibers using the MorFi fiber analyzer. It could be shown that there is a measurable difference between the three tested MDF fiber samples. As a drawback, the analyzer could not detect fibers longer than 10mm. Hardwood fibers were shorter and thinner than the two MDF fiber samples originating from softwoods. Differences between the various assortments could be detected using the MorFi fiber analyzer. Should a modification of the MorFi fiber analyzer be possible, it might allow measurements of fibers that are longer than 10mm and the limiting factor of measuring no more than 10 000 fibers at once could also be rectified. The data from analyzing the fibers with the MorFi might be utilized to directly adjust settings at the production line of MDF boards. The possibility of at-line adjustment would significantly improve the reaction time to compensate raw material variations compared to the current situation.

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# Investigation of Arabidopsis genes which might be involved in resistance against cyst nematodes

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The cyst nematodes are very successful plant parasites that rely solely on production of specialized feeding structures, the syncytium, that provides them with nutrients during their parasitism cycle. Host plants; on the other hand, modulate defense responses to prevent ingress of nematodes. Intriguingly, nematodes surreptitiously manipulate plant metabolisms for their own benefit through deploying of effector proteins.

The infection of Arabidopsis roots by the beet cyst nematode *Heterodera schachtii* is used as a model system to study the interaction at the molecular level. A previous transcriptome analysis of syncytia led to the identification of a number of genes that were either down-regulated or up-regulated. Here I have studied Arabidopsis genes coding for a DEFL and a PR1-like protein that might play a role in the resistance against *H. schachtii*. For that, I generated overexpression lines under the control of the 35S promoter in the pMAA-Red vector. These lines were shown to enhance resistance against *H. schachtii*. In addition, Gus::promoter lines were used to study the expression of these genes in different plant tissues.

Another objective of present work is to test a possible *in vitro* antimicrobial activity of the encoded proteins against different fungal and bacterial pathogens. Therefore, the DEFL peptide was expressed in *E. coli* as fusion protein and after cleavage of the fusion partner, the target peptide was purified through further steps of purification. In case of the PR1-like protein I tested different fusion partners but was unable to produce soluble fusion proteins.

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#### Acknowledgements

The financial support by the Austrian Science Fund (FWF) and the Palestinian ministry of Finance and Planning is gratefully acknowledged.

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# Development of bio-based binders for the production of wood based panels

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Today's wood board industry relies on the use of fossil based binder systems like urea-formaldehyde or melamine-formaldehyde binders. An alternative binder system with lower carbon footprint and, due to upcoming regulations, a better emission profile, is desirable. Therefore, we develop and investigate new bio-based adhesive systems for the use in wood composites like particleboard and MDF. These adhesives should outperform current fossil-based binder in terms of their carbon footprint and emission profiles while fulfilling the requirements for adhesives in industrial particleboard production. The adhesives need to be stable at room temperature for a pre-pressing period, have a sprayable viscosity and cure fast at elevated temperatures (however below 120°C). Furthermore, a low toxicity during the work process and in the final product and mechanical properties, which pass the standard board requirements, are needed. Surplus carbohydrate feedstock from existing European biorefineries is modified/converted to create building blocks, which are used for resin synthesis with different crosslinkers. Carbohydrate conversion methods are tested to find a reliable production system for these reactive intermediates. An example of such a conversion reaction is the acid-catalyzed dehydratisation of fructose to 5-hydroxymethylfurfural (5-HMF), which is an important platform chemical that can be converted further to e.g. diformylfuran or bishydroxymethylfuran.[1]

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#### Acknowledgements

This project has received funding from the Bio-based Industries Joint Undertaking (BBI-JU) under grant agreement No 792063. The BBI-JU receives support from the European Union's Horizon 2020 research and innovation programme and the Bio-based Industries Consortium

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Co-inoculation of root-endophytic *Serendipita* species and arbuscular mycorrhizal fungi affects nutrient contents and arbuscular mycorrhizal root colonisation in tomato plants

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Some of the root endophytic fungal species belonging to the family of Serendipitaceae were recognized to be isolated from spores of arbuscular mycorrhizal fungi (AMF). To date, there are no known studies focusing on their interaction in an AMF-endophyte-plant system. In order to shed light on the interactive effects of AMF and root endophytic fungi on tomato plant development and nutrient status, a greenhouse experiment using tomato plants was conducted with the following factors: (1) Funneliformis mosseae (AMF) and (2) Serendipita spp. including Serendipita indica, S. williamsii and S. herbamans. Nine weeks after transplanting, plants were harvested and plant growth parameters and AMF root colonization were determined. Nutrient concentrations of the tomato shoots were analysed by Inductively Coupled Plasma-Atomic Emission Spectroscopy (ICP-OES, Optima 8300 DV). The results showed that growth and development of tomato plants were unaffected by the combined inoculation with AMF and Serendipita spp., except that shoot length was supressed in the AMF and S. herbamans treatment. Furthermore, the performance of photosystem II was also less when AMF and S. herbamans were co-inoculated. With regards to the nutrient concentration of tomato shoots, phosphorus was highest when plants were co-inoculated with either S. williamsii or S. herbamans and AMF. Moreover, the concentration of calcium and manganese increased in S. indica inoculated plants and was significantly reduced when AMF was introduced into the endophyte-plant system. The amount of zinc was observed to be lowest in plants inoculated with both S. herbamans and AMF. Even though AMF root colonisation was reduced when both AMF and Serendipita spp. were combined, the concentration of phosphorus was increased compared to the treatments without AMF. The reduction of other nutrients such as calcium, manganese and zinc might indicate unknown interaction effects and nutrient costs, which need to be investigated in future experiments.

#### Acknowledgements

We are gratefully thankful to the Austrian Science Fund (FWF, P30051-B32) for supporting this research.

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# Tracer-assisted Search for Novel Polyketides in Trichoderma reesei

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The filamentous fungus *Trichoderma reesei* is well known for its ability to produce cellulolytic enzymes and generally thought to form only a low number of specialized metabolites. However, full genome sequence studies have shown that *T. reesei* also contains a variety of gene clusters that putatively encode enzymes for the production of secondary metabolites such as polyketides. Many metabolites of this group have interesting properties, e.g. anti-microbial or antiviral activities or cytotoxic properties that may be used in medicine.

Here, we present a stable isotope-assisted approach, which is based on tracer compounds and allows a comprehensive search for possible, previously unknown polyketides by means of reversed-phase high-performance liquid chromatography, coupled to high-resolution mass spectrometry (RP-HPLC-HRMS).

In the course of the biosynthesis of polyketides via type II polyketide synthases, malonyl-CoA units are used for extension and iteratively incorporated into the growing polyketide core structure.

To enable the discovery of novel, previously unknown polyketides in the extremely large amount of measurement signals produced by the mass spectrometer, we have used ethylmalonate as a tracer molecule. Due to the random incorporation of either labeled or native acetate from the added tracer or by the fungus self-synthesized molecules respectively in the course of cultivation, a very characteristic isotope pattern can be observed in the measured spectra, which does not occur naturally in this form. Based on these special patterns, it is thus possible to search much more specifically, and based on computer algorithms, also much more efficiently for previously unknown polyketides in complex fungal cultures.

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# Laccase synthesis of highly flexible lignin cross-linked succinyl-chitosan hydrogels

We developed a novel strategy for synthesizing highly flexible succinyl chitosan (SucCTS) hydrogels cross-linked with lignosulfonates using laccases. The properties of said hydrogels were enhanced using reed cellulose fibers as reinforcement agents and glycerol as plasticizer. Chitosan is not completely water soluble, neither miscible with lignosulfonates, so it was needed to convert it into SucCTS in order to form a homogenous solution. Laccase driven coupling of generated lignosulfonates radicals with SucCTS was accompanied by a 51 and 74% decrease in lignosulfonate –OH groups and SucCTS-NH2 groups, respectively. Laccase mediated synthesis of insoluble lignosulfonate polymers produces hydrogels that have the potential to be molded into any shape, but they are highly brittle when dry. Incorporation of glycerol as plasticizer prevented stiffness, shrinking and brittleness of the hydrogels, as evidenced by the increase in their elongation at break (up to 160 %). On the other hand, the incorporation of reed cellulose fibers increased the strength from < 1 up to 4 mPa. This enhancement can be used as an indication of the successfully cross-linking of SucCTS and lignosulfonates, as well as the compatibility between the hydrogel, cellulose fibers and the plasticizer.

One of the advantages of the process employed in this study is that, while other previous studies rely in physical bonding, chemical cross-linkers and chemical catalysts or on electrostatic bonding for the synthesis of chitosan based hydrogels, here we employed 100 % green methods.

This is in-line with the current bioeconomy, biorefinery, and sustainable green technology driven economy trend, making it attractive to be employed by companies.

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## A high-throughput method for the quantitative determination of fitness in a human fungal pathogen species

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Fungal infections put a significant burden on western healthcare systems. *Candida* species are among the most frequently isolated fungal species and are regularly responsible for fatal blood stream infections. *Candida* strains differ genetically between regions (such as *Candida albicans*) or separate into characteristic subclades (such as for *C. glabrata*). However, the phenotypic variability in the *Candida* clade is not well established, despite its implications for virulence in humans. For example, the ability to form recalcitrant biofilms, to withstand harsh environmental conditions or antifungal treatment could mean the difference between a successful pathogen and a harmless commensal. We set out to explore the phenotypic variety under a variety of environmental and antifungal stresses in a set of >1000 clinical *Candida* isolates from Vienna General Hospital (AKH). We incubated each isolate for 24 hours in liquid media in 96well microplates at 37°C and measured growth kinetics at OD<sub>600nm</sub>. As fitness parameter, we chose the maximum growth rate  $\mu$  [h<sup>-1</sup>], obtained from modelled growth curves, using the "Growthcurver" package in R. Principal Component Analysis combined with subsequent clustering enabled us to reduce the complexity of our multidimensional dataset and interpret the obtained growth data. We detected distinct phenotypic clusters covering the species of the collection and several correlations between different conditions, indicating co-adaptation.

We propose a high-throughput method of extracting quantitative growth parameters from growth kinetics combined with a subsequent unsupervised learning method to evaluate the phenotypic space. Our method enabled us to efficiently evaluate growth fitness of a vast panel of *Candida* isolates at several different growth conditions. We conclude that each of the *Candida* species possesses a characteristic phenotypic profile and found indications of co-adaptation and the fitness cost of adapting to a specific environment.

#### <u>Acknowledgements</u>

This work was supported by the NÖ Forschung & Blidungsfonds (NfB).

#### POSTERS

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# Analysis of Thionin Proprotein Processing Enzymes in Arabidopsis

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Thionins are antimicrobial peptides which are part of plants' pathogen defense. They have been shown to inhibit the growth of various plant pathogens *in vitro* as well as *in planta*. Subtilases are serine proteases which process thionins from their initial, inactive form (proprotein) to their biologically active form by multiple cleavage of the thionins' acidic domain. A great variety of subtilases is found in many different plant species and – to a lesser extent – in animals and archaea. Due to their nature as proteases, they have multiple functions.

This work focuses on the Arabidopsis subtilase AtSbt1.4 which is a candidate for being the only thionin proprotein processing in enzyme (TPPE) in Arabidopsis, but it has been shown that this subtilase is also involved in drought stress response and leaf senescence. Former studies suggest that AtSbt1.4 is the only TPPE, but unambiguous experimental evidence is still missing. In this project we produced polyclonal antibodies specific for thionins and for subtilases. The antibodies will be used to track unprocessed thionins in subtilase mutants. Mutants will be complemented to regain their ability to process thionins. Six different thionins proproteins have been expressed in *E. coli* and purified: Arabidopsis thionins 2.1, 2.2, 2.3 and 2.4, Viscotoxin (a thionin from mistletoe) and BTH7 (a barley thionin). It could be shown, that all these thionins are processing of the thionins will be further analyzed using ESI-MS with special regards to the cleavage sites and the cleavage products.

**Acknowledgements** 

This work is supported by FWF (Project P 28984).

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# Understanding the effect of drought on banana – an exploratory study with stable isotopes

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Banana is an important subsistence crop in East-Africa, serving as a major staple for smallholder farmers. Its production is being threatened by climate change. Erratic weather conditions impede a constant water supply and banana yields are expected to decrease. Measures to cope with this are needed. To create resilience, a better understanding of how banana deals with water stress is in order. Stable isotopes are a useful tool to investigate plant-water relationships. This research aims (1) to assess the use of stable isotope techniques for evaluating water stress in banana and (2) to gain an improved understanding of the response of banana towards changing weather conditions. A first assessment of  $\delta 13C$  variability in banana was made in a field trial in the Kilimanjaro region in Tanzania. Two varieties, Grand Nain and *Mchare* (local variety) were studied under full and deficit irrigation. Samples were taken from mature plants, as well as from their on-growing suckers. Furthermore, variability at plant level was assessed by sampling leaves of a different age and different leaf parts. This should allow us to distinguish the effects of water stress on  $\delta 13C$  signature from other (plant-related) influencing factors. As an additional measure for stomatal closure, leaf temperature was monitored throughout the day. Preliminary results will be shown at DocDay, 2019.

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### Facile preparation of superhydrophobic solid wood surfaces

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The prevention of excessive water uptake in wood in order to avert dimensional instabilities, decay and discolouration is a major challenge for wood-based applications. After decades of research, classic bulk modification methods are still facing several drawbacks such as their consumption of time, money and energy. A rather new approach is the development of thin, extremely water-repellent layers based on the biomimetic principles derived from plant surfaces like the ones of the Lotus (*Nelumbo*) leaf.

We developed a facile surface treatment to protect wood from liquid water uptake that does not require harsh process conditions or toxic solvents. Water-based and surfactant-free dispersions of submicron alkyl ketene dimer wax particles were prepared and sprayed onto beech (*Fagus sylvatica*) wood substrates. After the evaporation of water, the wax particles self-assembled into distinctive platelet structures, forming a thin and extremely water repellent wood finish.

Even the most straightforward preparation method – i.e. simple blending preparation of the aqueous wax dispersion and consequent spraying and drying at room temperature – resulted in superhydrophobic wood surfaces. Dispersions prepared by means of ultrasonication consisted of even finer wax particles. When applied, they self-assembled to particularly homogeneous platelet structures, reaching water contact angles of up to 170°. Further, a slightly increased drying temperature had a positive influence on the progress of platelet formation.

The implementation of sub-micro structures reduced surface gloss but the wood colour and its natural appearance remained largely unaffected. The spraying method is cost-effective and easily scalable. It is not restricted by dimensional limitations and has the ability to turn even large surface areas of wood-based products from hydrophilic into superhydrophobic.

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# Potassium fertilizer to increase water use efficiency in cassava production systems

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Cassava (*Manihot esculenta*) is a tropical root crop which serves as staple food for almost 800 million people worldwide. Recently, the starch industry gained interest in the crop. However, to satisfy its need for a year-round supply of fresh cassava roots, farmers need to grow cassava in drought prone periods leading to lower yields. In order to increase the plant's performance under dry conditions one possibility is to apply potassium fertilizer, since the nutrient plays an important role in the water management of a plant. We conducted a greenhouse experiment with 48 cassava plants in pots. Plants either received a fertilizer solution high (K<sup>+</sup>=0.153 M, n=24) or low (K<sup>+</sup>=0.045 M, n=24) in potassium. All plants were watered to field capacity during the first two months after planting. Two months after planting a drought treatment (50% of field capacity) was applied to half of the plants while the other half was kept at field capacity. Water use and requirement over time were monitored by weighing the plants. Distribution of fresh assimilates was followed by enriching the air in a growth chamber with <sup>13</sup>C-CO<sub>2</sub> one week after the drought treatment was installed. Plants were measured 1, 9 and 24 days after labelling. Total biomass, water use efficiency and <sup>13</sup>C-CO<sub>2</sub> uptake were measured. First results of the plant's water use will be shown at DocDay 2019.

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# Identification and Absolute Quantification of Volatiles from Technical Lignins

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Consumer products put high demands on the utilized materials, even on such subtle aspects as touch and smell. For the successful commercialization of lignin-based products, its smell-characteristics have to be determined, controlled and if necessary, mitigated. From a chemical point of view, this requires a complete and quantitative analysis of the volatiles of a lignin sample.

We present a method to determine volatiles qualitatively by single measurement Solid-Phase Microextraction-Gas Chromatography-Massspectrometry (SPME-GC-MS) and quantitatively by additionally applying Multiple Headspace Sampling (MHS-SPME-GC-MS) [1]. This sampling scheme involves repeated measurements of the same sample under identical conditions leading to an exponential decline in signal intensity. This decay is subsequently used to derive the total chromatographic peak area of the analyte, which in turn can be related to the analyte's absolute amount. Due to the fact that matrix effects are suppressed by this approach just simple external calibration is necessary to do so. Calibration can be further simplified using a two detector setup with a Flame Ionisation Detector (FID) and a Massspectrometer (MS) recording simultaneously. Using Relative Response Factors for FID signals allows the quantification of all identified analytes in one chromatogram with the need for just one substance being calibrated.

The presented method allows to obtain absolute amounts of volatiles emitted from lignin or other substances without any sample preparation or worrying about matrix effects. Further a MS/FID setup promises a minimisation of the calibration effort for multi-analyte samples.

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#### **Acknowledgements**

We are grateful for the support by our industry partners in the frame of the FLIPPR<sup>2</sup> project, Mondi, Sappi, Zellstoff Pöls AG, a member of heinzel<sup>®</sup> pulp, and Papierholz Austria. The K-Project Flippr<sup>2</sup> is funded as part of COMET - Competence Centers for Excellent Technologies promoted by BMVIT, BMWFJ, Styria and Carinthia. The COMET program is managed by FFG.

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# Low temperature adhesive bonding for structural wood materials

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Cold wood for structural finger jointed solid timber requires pre-heating procedures particularly in winter. This study investigates the feasibility of cold bonding wood at temperatures below 20°C to ultimately lower the energy consumption in the production process.

The aim of this novel research approach is to show that temperature has a minor effect on the adhesive bond. To further understand the influence of temperature we conducted macroscopic, microscopic and micromechanical tests. Spruce specimens cut at lap joint angle 7,5° were glued. We applied a two-component melamine-urea-formaldehyde adhesive on separate sides. Pressing and curing temperatures varied: 0-0°C, 0-20°C, 20-20°C. Resulting tensile shear strength was at a range of 11-12 N/mm<sup>2</sup> for all specimens without or with delamination cycle. Penetration of the adhesive into the wood was deeper on the hardener than on the resin application side with no dependency on the temperature. Equally nanoindentation measurements clearly visualized temperature independent penetration of the adhesive into the cell wall with increased hardness and modulus of elasticity compared to unfilled reference cell walls. Similarly indents directly into the adhesive showed hardness values with no significant difference temperature wise.

As presumed temperature has no influence on any of the results on macro- and microscopic level hence adhesive bonding at low temperatures is feasible. Deep penetration into the wood and the cell wall itself proves a strong bond line even at 0°C cold bonding. The study shows high potential to contribute to the alteration of production processes questioning existing norms.

#### **Acknowledgements**

We gratefully acknowledge financial and material support from Doka Österreich GmbH.

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# Optimized periodate oxidation of cellulose

#### in resource-saving pathway

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Periodate oxidation yields so-called dialdehyde cellulose, a functional cellulose derivative, under aqueous conditions. This derivative is used as crosslinker or to efficiently introduce functionality, among others, by reaction with amino reactants. Main obstacles of this oxidation are the rather slow oxidation kinetics (long reaction times) and the fact that it is conducted under diluted conditions, requiring considerable amounts of water and energy for heating and mixing. This work aims at overcoming these drawbacks by conducting the periodate oxidation at high-consistency with a cellulose:water ratio in weight of 1:4. Thereby the reaction efficiency was considerably increased while reducing the required amount of water and energy to a minimum. The oxidizer, sodium periodate, cellulose and water were efficiently mixed in a ball mill for a given time, and oxidation occurred mostly in the subsequent step, called resting time. The milling time, resting time and ratio of oxidizer towards cellulose were optimized in an experimental design. Low milling time of 2 minutes and high resting time of 8 hours with a periodate ratio of 1.25 yielded oxidized cellulose with the highest aldehyde content of approx. 8 mmol/g. The obtained equation of the model can further be used to easily tune the oxidation level of the synthesized dialdehyde cellulose. The developed method increases the reaction rate and improves the resource efficiency of the process; it is a key step towards an environmentally less challenging and industrially relevant periodate oxidation of cellulose.

#### <u>References</u>

ChemSusChem 10.1002/cssc.201901885

#### **Acknowledgements**

The project is funded by Metadynea and the FFG as an "Industrienahe Dissertation".

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Development of novel microsatellite markers for *Alkanna tinctoria* (Boraginaceae) by comparative transcriptomics

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•••

Alkanna tinctoria is an important medicinal herb with its main distribution across the Mediterranean regions. To reveal its genetic variation and population structure, microsatellite markers were developed and validated in four Greek populations: For marker development, transcriptomics data of *Arnebia euchroma* and *Echium plantagineum* were assembled and mined to identify conserved orthologues containing simple sequence repeat motifs. Fifty potential loci were identified and tested on *A. tinctoria*, of which 17 were polymorphic. Most of these loci could be successfully amplified in eight other medicinal plants of Boraginaceae: *A. graeca*, *A. hellenica*, *A. sfikasiana*, *Echium vulgare*, *E. plantagineum*, *Lithospermum officinale*, *Borago officinalis*, and *Anchusa officinalis*. Our study provides the first set of microsatellite loci for studying the genetic variation and population structure of A. tinctoria and shows their potential usefulness in other Boraginaceae species.

#### Acknowledgements

This project has received financial support from the European Union's Horizon 2020 research and innovation program under the grant agreement number 721635

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# Analysing the role of the protein kinase ASKα in the lowenergy response in plants

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Environmental stress is among the most significant factors responsible for substantial and unpredictable losses in crop production worldwide. Improving plant stress resistance and crop yield under adverse environmental conditions is thus a major goal in sustainable agriculture. Acclimation to environmental stress as well as defense against pathogen infections require energy. Both abiotic and biotic stresses lead to a metabolic shift and prorogued stress can lead to energy depletion. Various signaling proteins, such as transcription factors, kinases and phosphatases, are involved in stressresponsive transcriptional and/or posttranscriptional adjustments of metabolic status to prevailing environmental conditions. Previously, we have demonstrated that plant GSK3 protein kinases contribute to the regulation of carbohydrate metabolism under stress conditions. Furthermore, we have shown that the protein kinase ASK $\alpha$  (Arabidopsis GSK3/Shaggy-like kinase  $\alpha$ ) positively regulates salt-stress tolerance and pathogen resistance. Within the framework of my PhD thesis the potential role of ASKa in the low-energy response in plants is investigated. We triggered starvation in Arabidopsis plants by keeping plants in darkness for several days. Two independent ASKa overexpressor lines displayed a clear stay-green phenotype after 7 days of darkness. In contrast, plants deficient in ASKa and its closest homolog ASKy showed increased chlorosis under these conditions. This data suggests that ASK $\alpha$  is involved in the energy starvation response in Arabidopsis thaliana and now allows further analysis to better understand the trade-off between plants' growth and yield and responses to biotic and abiotic stresses.

#### <u>Acknowledgements</u>

This work was supported by the Austrian Science Fund (FWF).

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### Wood adhesives based on domestic plant proteins

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The objective of this project is the development of biogenic adhesive for wood industry, which do not comprise toxic chemicals and are primarily based on domestic renewable resources, such as plant proteins available in Europe. Plant protein is a natural resource to produce environmentally friendly wood adhesives. Soy protein is one typical type of plant protein already industrially used to substitute synthetic resins for wood adhesives. However, soy crops are not so widespread in Europe. Thus, it is interesting to evaluate if other vegetable proteins more common in Europe are compatible to be used as wood adhesives. As raw materials proteins from such plants as wheat, potato, pea, corn have been selected. All the selected protein materials were treated under basic conditions at different molarity of sodium hydroxide and different temperature. During hydrolysis viscosity change of protein suspension was measured. Also the achievable bond strengths were characterized by tensile tests. In the production of biogenic adhesives, it is quite conceivable that adhesives develop very good bond strengths but are clearly too slow for industrial applications. Therefore, particular attention is given to the curing process.

Acknowledgements

This work is part of the project "High Performance Materials". Lower Austria is kindly acknowledged for funding this project.

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> Die intuitive und modulare ChromLab Software ernen innerhalb kürzester Zeit auch komplexe Methowahl an Methodenvorlagen und Methodenbausteilaubt es dem Anwender mit Hilfe einer großen Aus-

den rationspartnern einfach zur Verfügung gestellt wereinmal generierte Methoden Kollegen oder Kollaboversendet und ausgetauscht werden. Somit können dere Datenbank übertragbar oder können per Mail Einmal erstellte Methoden sind einfach in eine an-

nommen werden. phone oder einem Tablet-PC die Steuerung über-Passwort geschützt - kann sogar mit einem Smartnem Ort stehen. Mittels einer VNC-App – natürlich reagieren, auch wenn System und PC nicht an ei-Touch Screen möglich. So kann der Nutzer schnell PC als auch über den ins System integrierten greifen in laufende Protokolle ist sowohl über den Die manuelle Steuerung der NGC sowie das Ein-

erstellen und Daten zu exportieren wird dem Endannigen Klicks durchgeführt werden. PDF-Reports zu wender leicht gemacht. Auch die Auswertung der Experimente kann mit we-

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den zu erstellen.

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Die Daten selbst sind sicher in einer SQL-Daten-Ebenfalls verfügbar ist eine Security Edition der zerverwaltung kann der Zugriff restringiert werden. Compliance möglich sind. Software, mit der Arbeiten im Rahmen der FDA bank abgelegt und durch das Einrichten einer Nut-

Hardware:

in die Basiseinheit in kürzester Zeit machbar. nen Module. Dieses Design ermöglicht nicht nur eine freie und optimierte Platzierung der einzel-Der wirklich modulare Aufbau des Systems erlaubt durch simples Einschieben der zusätzlichen Module Optimierung der Flusswege für spezielle Anwenein echtes "Mitwachsen" des Chromatographiesydungen. Die Erweiterung des Systems ist übrigens stems mit Ihren Anforderungen, sondern auch die

(100 bar bei 200 ml/min) gewechselt werden. min) und einem Niedrigdruck-Hochfluss-System druck-Niedrigfluss-System (250 bar bei 20 ml/ ganzen Systems - kann zwischen einem Hoch-Durch den Tausch weniger Module - nicht des

croliter-Maßstab bis hin zur Small-Batch-Reini-(Kollektor oder Ventil) erlaubt das Arbeiten vom Migung. chung und einer optimierten Fraktionssammlung Die Verwendung einer angepassten Verschlau-

unter anderem Einlass- und Auslassventile, Probendruck-Chromatographie-System benötigt, erhältlich Natürlich sind alle Module, die ein modernes Mittelpumpe, Luftsensoren, Mixer, Injektionsmodul, Säu-

enkranzventil, Ein- oder Mehrwellenlängendetektor

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ne Fraktionssammler. inkl. Leitfähigkeitsmessung, pH-Modul, verschiede-

# Sicherheit / Sensoren / Überwachung

ne Ereignisse zu informieren. le Säulen vor Schaden zu bewahren, verfügt das Um Ihre Investitionsgüter zu schützen und wertvol-Nachrichten versendet werden, um über aufgetretekönnen von der ChromLab Software automatisiert Garant für die Sicherheit Ihrer Experimente. Zudem re über verschiedene Sicherheitsmechanismen NGC-System gepaart mit der ChromLab-Softwavativen LED-Technik des NGC-Systems sind ein Luft- und Drucksensoren zusammen mit der inno-

# Externe Detektoren und Autosampler:

(z.B. Fluoreszenz, RI, MALS) sowie die Erweiterung die problemlose Einbindung externer Detektoren der Ladekapazität durch einen Autosampler. Die Modularität des Systems erlaubt und unterstützt

# Zusammenfassung:

einem neuartigen Service-Konzept. laufen wie gewohnt weiter. Natürlich profitieren Sie arbeiter zügig voll durchstarten, und die Prozesse arbeitungszeiten sind gering, deshalb können Mitlaubt ein hohes Maß an Automatisierung. Die Ein-Systemdesign reduziert die Hands-on Zeit und er-Die intuitive Software gepaart mit dem modularen auch von der gewohnten Bio-Rad Qualität und von

# Interesse geweckt?

bei oder kontaktieren Sie uns direkt Dann schauen Sie doch auf unserer Homepage vor-

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# Kontakt

vom Anbieter: Weitere Informationen zum Produkt direkt

NGC Chromatographie-Platt-

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form

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