







University of Missouri



# METABOLOMICS CENTER BR

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# CENTER.

# Providing cutting-edge targeted and untargeted metabolomics services

The mission of the University of Missouri Metabolomics Center (MUMC) is to provide advanced, cutting-edge metabolomics capabilities and expertise to both the MU community and outside entities. MUMC has multiple platforms for targeted and non-targeted small molecules or metabolites analyses. We also assist users in their grant proposals (letters of support, budgeting, Co-PI contributions to specific aims involving metabolomics) and manuscript publications (methods and MS figures). MUMC also hosts annual metabolomics workshops or one-on-one training to train researchers who would like to learn and use metabolomics in their research.

MUMC is equipped with state-of-the-art mass spectrometry instrumentation:

- Agilent 6890N Gas chromatography coupled to a 5973N mass selective detector (GC-MSD)
- Agilent 7890 GC interfaced with 7200B quadrupole time of flight mass spectrometer (GC-QTOF MS)
- Bruker Impact II quadrupole time of flight mass spectrometer coupled to Waters Acquity ultrahigh performance liquid chromatography (UPLC-QTOF MS)
- Waters Xevo triple quadrupole MS coupled to UPLC (UPLC-QQQ MS)
- Bruker Autoflex Speed MALDI MS
- Bruker Compact QTOF MS

METAB



#### **Our Services**

#### Large-Scale Targeted and Untargeted Metabolic Profiling

Metabolites are profiled using GC-MS and/or LC-MS to provide a large-scale view of metabolites in a sample. The sample can be tissues, culture media, cells, urine, and serum. GC-MS profiling is performed using Agilent 6890N Gas chromatography coupled to a 5973N mass selective detector (GC-MSD) or Agilent 7890 GC interfaced with 7200B quadrupole time of flight mass spectrometer (GC-QTOF MS). For LC-MS profiling, Bruker Ultrahigh resolution quadrupole time of flight mass spectrometer (Impact II) coupled to Waters Acquity ultrahigh performance liquid chromatography (UPLC) is used to profile metabolites in complex samples.

Lignin Composition and Content Determination Plant tissues are first subject to thiolysis and then

GC-MSD analysis for determination of lignin composition.

<u>Volatile Organic Compound Analysis</u> Volatile analysis can be performed on solid and liquid samples using a headspace sampling technique. GC-QTOF MS is used to analyze the volatiles. This technique can be used to determine volatiles emitted by plant leaves, fruits, animal urines or other samples.

#### Accurate Mass Determination

Accurate mass of small molecules is measured using the Bruker Ultra-high resolution QTOF MS with mass accuracy of less than 5 ppm. The accurate mass is useful for confirmation of synthesized compounds and annotation of unknowns.

#### Plant Hormone Analysis

Plant hormones are measured using Waters Xevo triple quadrupole MS coupled to UPLC. Plant hormones are first extracted and enriched from plant materials, and then subject to detection and quantitation on UPLC-QQQ MS via an MRM (Multiple Reactions Monitoring) experiment.

#### **Other Services**

In addition to the routine services listed above,

we also assist users who have special needs in metabolomics through custom method development and analysis. Please contact us to discuss your metabolomics needs.







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The following application notes provide summaries for specific applications of mass spectrometry to metabolomics research that are available at the MU Metabolomics Center. Any of these applications can be tailored specifically to your needs and other applications may be possible through custom method development.

# Non-targeted GC-MS metabolite analysis

There are two general approaches to metabolite profiling by mass spectrometry: targeted and non-targeted. Targeted profiling aims to quantify specific known metabolites, usually using isotope labeled internal standards to generate calibration curves. The non-targeted approach seeks to profile as many metabolites as possible without prior knowledge of the identities of the metabolites. The non-targeted approach provides a broader coverage of the metabolome and provides opportunities to explore novel pathways. Fold changes can be measured for both identified and unidentified metabolites using the non-targeted approach. Unidentified metabolites having significant fold changes in an experiment can be further pursued for identification through MS fragmentation analysis and/or NMR.

Non-targeted GC-MS can focus on polar or non-polar metabolites, depending on the sample preparation. In either case, samples are derivatized to increase the coverage of metabolites by increasing their volatility. The polar and non-polar fractions are obtained from the same sample by phase separation. The GC-MS chromatograms for mouse liver tissue show the different metabolite profiles obtained for the polar and non-polar fractions (Figure 1). Analysis is possible for many different tissue types, such as plant tissues, animal organs, blood serum, urine, feces, sweat, and cell cultures. In a typical sample polar extract, about 300 metabolites are measured including sugars, sugar alcohols, amino acids, organic acids, and aromatic amines. A non-polar extract may yield alkanes, fatty acid methyl esters and steroids.







**Figure 2** Examples of GC-MS chromatograms obtained for various tissue types (polar fractions).

#### **Experimental Details**

Sample Preparation: Plant tissues are generally flash frozen upon harvesting and then lyophilized and ground to a powder. A sample of 10 mg dried powder is extracted using methanol, water and chloroform to separate into polar and non-polar phases. Internal standards are included to monitor and correct for sample processing variance. Each phase is evaporated to dryness prior to derivitizaton.

Animal tissues (50-100 mg) are typically homogenized in the extraction solvent using a bead beater. These samples are then centrifuged x13000 g for 15 min to pellet tissue and proteins. The supernatant is combined with water and chloroform, including internal standards, to separate into the polar and non-polar phases. The phases are separated and dried before derivitization. Other sample types may require some variation to the sample prep.

Dry samples are methoximated in pyridine with 50µl of 15mg/ml methoxyamine hydrochloride and incubated at 50°C for 1 h, and then derivatized with 50µL MSTFA (N-methyl-N-(trimethyl-silyl) trifluoroacetamide) + 1%TMCS (chlorotrimethylsilane) reagent at 50°C for another hour before GC-MS analysis.

Data Analysis: The data are deconvoluted using AMDIS and annotated using an in-house constructed spectral library as well as commercial GOLM and NIST17 mass spectral libraries. Abundances of the ions are extracted using custom MET-IDEA software and then normalized to the internal standard. Statistical analyses including volcano plots, box plots, and PCA can be done using MetaboAnalyst 3.0 (http://www.metaboanalyst.ca).

#### **GC Conditions**

| GC System:     | Agilent 6890   |
|----------------|--|
| Column:        | DB-5MS, 60 m, 0.25 mm ID,<br>0.25 μm film thickness, J&W Scientific      |
| Injection vol: | 1 μL   |
| Gas type:      | Helium   |
| Flow Rate:     | 1.0 ml/min   |
| Temperature:   | 80°C for 2 min,<br>then 5°C/min to 315°C and<br>held at 315°C for 12 min |

| MS System:       | Agilent 5973 MSD  |  |  |
|------------------|-------------------|--|--|
| Ionization mode: | EI                |  |  |
| Scan range:      | m/z 50 to m/z 650 |  |  |
| Solvent delay:   | 9 min             |  |  |
| MS Source Temp:  | 230°C             |  |  |
| MS Quad Temp:    | 150°C             |  |  |

## Non-targeted profiling of the fecal metabolome in rats using GC-MS

Method developed by: Saurav J. Sarma, MU Metabolomics Center Samples provided by: Cheryl S. Rosenfeld, Biomedical Sciences, University of Missouri

The gut microbiome has recently gained much attention as a strong influence in a range of physiological conditions including digestive and cardiovascular diseases, various types of cancers and even susceptibility to mental disorders. Analysis of the host fecal metabolome is a simple and noninvasive way of accessing how the host metabolic parameters are influenced by their gut microbiota. The correlation of the fecal metabolome to the gut microbiome of the host can provide meaningful insights into biomarker discovery towards early detection or predictions of health conditions that can be induced by the host gut flora.

GC-MS analysis of the fecal metabolome in soy fed ovariectomized female rats was used to identify the metabolites differentially accumulated in fecal samples of soy fed rats (SOY) as opposed to that of rats on a controlled diet (CON). S-equol, which is not indigenous to soy but a bacterial metabolite of soy-flavonoids, was found to be the most differentially accumulated metabolite between these two groups (Figure 1), and directly correlated to several positive health outcomes in the host.<sup>1</sup>







Figure 2 Examples of some metabolites significantly elevated in the fecal samples of ovariectomized (OVX) or sham operated (SHM) rats on soy diet (SOY), than in controlled diet (CON) with p value  $\leq$  0.05. Both s-equol and fucose were found to positively corelate with parameters for improved cardiovascular health.<sup>1</sup>

#### Experimental Details

Sample Preparation: To  $10 \pm 0.06$  mg of each fecal sample, 10uL of H2O containing 1µg/µl ribitol (internal standard) and 500 µl of 80% methanol were added. The samples were vortexed for 5 seconds, sonicated for 15 min, shaken for 2hr on an orbital shaker at 140 rpms, and then centrifuged at 13000 g for 15min. 400 µl of the supernatant was collected into a autosampler glass vial, dried under a gaseous nitrogen stream, methoximated in pyridine with 40 µL of 15mg/mL methoxyamine hydrochloride, and then trimethylsilylated with 40 µL MSTFA (N-methyl-N-(trimethyl-silyl) trifuoroacetamide) + 1% TMCS (chlorotrimethylsilane) reagent.

Data Analysis: The data were deconvoluted using AMDIS and annotated using an in-house constructed spectral library and commercial NIST17 mass spectral library. The abundances of the ions was extracted using custom MET-IDEA software and normalized to the internal standard, ribitol. Statistical analyses such ANOVA, box plots were performed, after data pre-treatment, using MetaboAnalyst3.0 program (http://www.metaboanalyst.ca/).

#### GC Conditions

Ν

Scan range:

| GC System:        | Agilent 6890   |
|-------------------|--|
| Column:           | DB-5MS, 60 m, 0.25 mm ID,<br>0.25 µm film thickness, J&W Scientific      |
| Injection vol:    | 1 μL   |
| Gas type:         | Helium   |
| Flow Rate:        | 1.0 ml/min   |
| Temperature:      | 80°C for 2 min,<br>then 5°C/min to 315°C and<br>held at 315°C for 12 min |
| Front inlet mode: | 1:5 Split  |
| IS Conditions     |  |
| MS System:        | Agilent 5973 MSD   |
| Ionization mode:  | EI   |
|                   |  |

m/z 50 to m/z 650

# Comparison of the effects of Bisphenol A and Bisphenol S on the mouse placental metabolome using GC-MS analysis

Method developed by: Saurav J. Sarma, MU Metabolomics Center Samples provided by: Cheryl S. Rosenfeld, Biomedical Sciences, University of Missouri

Bisphenol A (BPA) is an industrial chemical which is commonly found in polycarbonate plastics that are widely used in food and beverage packaging and storage. BPA is known to mimic estrogen and thus it has raised significant concerns as an endocrine disruptor, leading to investigations into its role in several health issues. Due to the increased awareness about the adverse health effects of BPA, plastic manufacturers have looked into using alternatives of BPA, resulting in development of plastic products labelled as 'BPA free' to capture consumer interest. Bispolyphenol S (BPS) has emerged as one of the most common alternative of BPA, but the health effects of BPS on consumers have been poorly studied. Even less information is available about the impact on a developing fetus with a dietary exposure of the mother to BPA analogues such as BPS. Since placenta is the connecting link between mother and fetus, to map the fetal impact of BPA and BPS exposer on a molecular level, we looked into the variations in the placental metabolome using untargeted GC-MS analysis.1









#### **Experimental Details**

Sample Preparation: Added 1.5 ml of H2O containing 25 µg/ml ribitol (internal standard) to 6.0 mg of lyophilized placental tissue. Samples were vortexed for 20 seconds, sonicated for 15 min then incubated at 50°C for 1 h. Sample tubes were centrifuged at 13000g for 15 min. 1mL supernatant from each sample tube were collected into an auto-sampler glass vial and dried under a gaseous nitrogen stream. Dry samples were methoximated in pyridine with 25µl of 15mg/ml methoxyamine hydrochloride andy incubated at 50°C for 1 h, and then derivatized with 25µL MSTFA (N-methyl-N-(trimethyl-silyl)trifluoroacetamide) + 1%TMCS (chlorotrimethylsilane) reagent at 50°C for another hour before GC-MS analysis.

Data Analysis: The data were deconvoluted using AMDIS and annotated using an in-house constructed spectral library as well as commercial GOLM and NIST17 mass spectral libraries. Abundances of the ions were extracted using custom MET-IDEA software and then normalized to ribitol, used as the internal standard. Statistical analyses including volcano plot and box plots were done using MetaboAnalyst 3.0 after data pre-treatment (http://www.metaboanalyst.ca).

#### **GC Conditions**

M

MS Source Temp:

MS Quad Temp:

| GC System:        | Agilent 6890   |  |  |
|-------------------|--|--|--|
| Column:           | DB-5MS, 60 m, 0.25 mm ID,<br>0.25 um film thickness, I&W Scientific      |  |  |
| Injection vol:    | 1 μL   |  |  |
| Gas type:         | Helium   |  |  |
| Flow Rate:        | 1.0 ml/min   |  |  |
| Temperature:      | 80°C for 2 min,<br>then 5°C/min to 315°C and<br>held at 315°C for 12 min |  |  |
| Front inlet mode: | 1:5 Split mode   |  |  |
| Pressure:         | 9.16 psi   |  |  |
| S Conditions      |  |  |  |
| MS System:        | Agilent 5973 MSD   |  |  |
| Ionization mode:  | EI   |  |  |
| Scan range:       | m/z 50 to m/z 650  |  |  |
| Solvent delay:    | 9 min  |  |  |
| EM voltage:       | 1259 V   |  |  |

230°C

150°C

## Analysis of cuticular wax in plant tissues using GC-MS

Method developed by: Saurav J. Sarma, MU Metabolomics Center Based on a method developed by: Zhang et al.<sup>1</sup>, Samuel Roberts Noble Foundation, Ardmore OK

Cuticular wax is an important component of plant cuticle with crucial physiological functions. Although its primary function is to work as a protective barrier to prevent evaporation of water from areal parts of plants, cuticular wax also reduces permeability of plant tissues by dust, ultraviolet light and pathogenic microbes and thereby contribute towards plant health. It is mostly consisted of long chain fatty acids, alkanes, alcohols, esters, aldehydes, ketones and sometimes sterols and triterpenes. While composition of the cuticular wax varies among different species of plants, even within a species it may vary according to the spatial and geographical distribution. GC-MS analysis of the fecal metabolome in soy fed ovariectomized female rats was used to identify the metabolites differentially accumulated in fecal samples of soy fed rats (SOY) as opposed to that of rats on a controlled diet (CON). S-equol, which is not indigenous to soy but a bacterial metabolite of soy-flavonoids, was found to be the most differentially accumulated metabolite between these two groups (Figure 1), and directly correlated to several positive health outcomes in the host.<sup>1</sup>



Figure 1 Cuticular wax accumulation in a) leaves and b) stems of transgenic alfalfa plants. c) Total wax content in leaves vs stems.

#### **Experimental Details**

Sample Preparation: Cuticular wax of leaf was collected from the top four fully expanded trifoliates from the major stem and that of stem was collected from the top four internodes. Each sample was placed in a 20 mL glass vial and added 10 or 5 mL of hexanes for leaves or stem respectively. Samples were agitated on a rotator at 50 rpm for 2 mins, then the solvent was decanted into another vial. Ringed the vial and tissue with 10 (or 5) ml of hexanes and dried the combined fractions of hexanes under nitrogen flow. Reconstituted in 15 µL of pyridine (70%)/ MSTFA+TMCS(30%) solution containing 0.01 µg/µL of cholesterol as internal standard, for each 1 cm2 surface area of tissue and heated at 50°C for 1h, then vortexed for 5 seconds before GCMS analysis.

Data Analysis: The data are average of two injections for each sample with six replicate samples for each tissue type. Agilent Chemstation software was used to extract peak areas by integration of total ion chromatogram. Quantification of metabolites were done by using peak area after normalization to the internal standard cholesterol.

#### **GC** Conditions

| GC System:        | Agilent 6890   |
|-------------------|--|
| Column:           | DB-5MS, 60 m, 0.25 mm ID,<br>0.25 μm film thickness, J&W Scientific      |
| Injection vol:    | 1 μL   |
| Gas type:         | Helium   |
| Flow Rate:        | 1.0 ml/min   |
| Oven Temp:        | 120°C for 2 min,<br>then 5°C/min to 315°C and<br>held at 315°C for 8 min |
| Front inlet mode: | Splitless  |
| Inlet Temp:       | 280°C  |
| MS Conditions     |  |
| MS System.        | Agilant 5073 MSD   |

| MS System:       | Agilent 5973 MSD  |
|------------------|-------------------|
| Ionization mode: | EI                |
| Scan range:      | m/z 50 to m/z 650 |
| Solvent delay:   | 9 min             |
| MS Source Temp:  | 250°C             |

# Lignin Composition Analysis of Arabidopsis Leaves by GC-MS

Method developed by: Saurav J. Sarma, MU Metabolomics Center Samples provided by: Antje Hesse, Department of Biochemistry, University of Missouri

Lignin is a component of the cell walls in plants, and the most abundant non-carbohydrate biopolymer on earth with a highly heterogeneous structure. Lignin results from the oxidative coupling of aryl propane units called monolignols; the most common ones being p-coumaryl (H), coniferyl (G) and sinapyl (S) alcohol. The relative abundance of these monolignols may significantly differ in lignin based on the sources. The S/G ratio in lignin signifies the degree of polymerization and thus the structural strength of the polymer as well as it's susceptibility to degradation for biofuel production.

GC-MS analysis of chemically degraded lignin is an efficient method to determine the S/G ratio in lignin samples. Figure 2 shows the peaks of G and S lignin in leaves from Arabidopsis Thaliana, observed in GC-MS analysis using an Agilent GC coupled to a single quad MSD mass spectrometer.



**Figure 1** Three most important monomer units present in lignin, that determines the degree of polymerization or structural strength.



**Figure 2** Chromatogram showing the G-Lignin at 46.05 min and G-lignin at 47.30 min. Both signals appear as a duplet and the total area of the two peaks in a duplet reflects the abundance of the monomer.

#### **Experimental Details**

Sample Preparation: Leaves were lyophilized and extracted with MeOH, MeOH/CHCl3 and water to remove polar and non-polar metabolites. Remaining lignin component was chemically degraded by thioacidolysis, using 4h incubation at 100°C. Derivatization was performed using 1:1 pyridine/ BSTFA.

Thioacidolysis Reagent:

- 2.5% Boron trifluoride-diethyl etherate (BF3-Et2O)
- 10% Ethane thiol (EtSH)
- 87.5% Dioxane by volume.
- Tetracosane as internal standard

#### GC Conditions

| GC System:        | Agilent 6890   |
|-------------------|--|
| Column:           | DB-5MS, 60 m, 0.25 mm ID,<br>0.25 µm film thickness, J&W Scientific      |
| Injection vol:    | 1 μL   |
| Gas type:         | Helium   |
| Flow Rate:        | 1.0 ml/min   |
| Temperature:      | 80°C for 2 min,<br>then 5°C/min to 315°C and<br>held at 315°C for 12 min |
| Front inlet mode: | 1:1 Split mode   |
| MS Conditions     |  |
| MS System:        | Agilent 5973 MSD   |
| Ionization mode:  | EI   |
| Scan range:       | m/z 50 to m/z 650  |
| Solvent delay:    | 9 min  |
| EM voltage:       | 1259 V   |
| MS Source Temp:   | 230°C  |
| MS Quad Temp:     | 150°C  |
|                   |  |

### Phytohormone Analysis by LC-MS

Method developed by: Barbara Sumner, Zhentian Lei, University of Missouri Metabolomics Center

Phytohormones play an essential role in the proper growth, development and function of plants. These hormones are separated into classes which differ in their structure and function: abscisic acid, auxins, cytokinins, gibberellins, jasmonates, and phenolics. Quantitative profiling of plant hormones from different classes provides a way to monitor hormone activities and identify hormone networks regulating plant functions. The quantification of plant hormones has been achieved with reverse phase UHPLC coupled with ESI/ TQ MSMS using MRM (Multiple Reaction Monitoring). Table 1 lists 12 phytohormones that have been successfully detected in a single sample injection of an extract of Medicago truncatula at the MU Metabolomics Center. An example of quantification of a phytohormone (salicylic acid) is shown if Figure 1. Absolute quantification can be obtained with the use of a stable label internal standard for the hormone of interest.

| FW     | Parent (m/z)  | RT (min)   | Product(s)   | Cone (V)  | CID (eV)  | Mode  |
|--------|---|--|--|---|---|---|
| 224.3  | 224.95  | 18.15-18.65  | 151.05, 207.01   | 24, 25  | 13, 10  | ES+   |
| 351.14 | 352.20  | 3.4-3.8  | 136.05, 220.13   | 30, 30  | 34, 20  | ES+   |
| 122.12 | 121.02  | 7.85-8.25  | 76.97  | 30  | 10  | ES-   |
| 138.12 | 136.97  | 8.24-8.64  | 93.00  | 25  | 15  | ES-   |
| 175.18 | 174.00  | 8.58-8.98  | 130.00   | 25  | 5   | ES-   |
| 189.21 | 188.11  | 11.05-11.45  | 59.04, 144.09  | 24, 24  | 12, 10  | ES-   |
| 203.24 | 202.08  | 12.85-13.25  | 116.01, 158.08   | 30, 30  | 18, 14  | ES-   |
| 210.27 | 209. 17   | 12.80-13.20  | 58.98  | 22  | 12  | ES-   |
| 246.27 | 245.04  | 7.70-8.10  | 87.99, 155.99  | 30, 30  | 17, 15  | ES-   |
| 264.32 | 263.09  | 10.70-11.10  | 152.99, 219.15   | 22, 22  | 10, 12  | ES-   |
| 288.26 | 289.17  | 5.80-6.20  | 132.02, 173.75   | 28, 28  | 14, 14  | ES-   |
| 332.39 | 331.23  | 15.64-16.04  | 213.18, 257.23   | 42, 42  | 30, 22  | ES-   |
| 346.37 | 345.11  | 6.90-7.30  | 143.04, 239.17   | 32, 32  | 30, 15  | ES-   |
|        | FW           224.3           351.14           122.12           138.12           175.18           189.21           203.24           210.27           264.32           288.26           332.39           346.37 | FW         Parent (m/z)           224.3         224.95           351.14         352.20           122.12         121.02           138.12         136.97           175.18         174.00           189.21         188.11           203.24         202.08           210.27         209.17           264.32         263.09           288.26         289.17           332.39         331.23           346.37         345.11 | FW         Parent (m/z)         RT (min)           224.3         224.95         18.15-18.65           351.41         352.20         3.4-3.8           122.12         121.02         7.85-8.25           138.12         136.97         8.24-8.64           175.18         174.00         8.58-8.98           189.21         188.11         11.05-11.45           203.24         202.08         12.85-13.25           210.27         209.17         12.80-13.20           246.27         245.04         7.70-8.10           264.32         263.09         10.70-11.10           288.26         289.17         5.80-6.20           331.23         15.64-16.04           346.37         345.11         6.90-7.30 | FW         Parent (m/z)         RT (min)         Product(s)           224.3         224.95         18.15-18.65         151.05, 207.01           351.14         352.20         3.4-3.8         136.05, 220.13           122.12         121.02         7.85-8.25         76.97           138.12         136.97         8.24-8.64         93.00           175.18         174.00         8.58-8.98         130.00           189.21         188.11         11.05-11.45         59.04, 144.09           203.24         202.08         12.85-13.25         16.01, 158.08           210.27         209.17         12.80-13.20         58.98           246.27         245.04         7.70-8.10         87.99, 155.99           264.32         263.09         10.70-11.10         152.99, 219.15           288.26         289.17         5.80-6.20         132.02, 173.75           332.39         331.23         15.64-16.04         213.18, 257.23           346.37         345.11         6.90-7.30         143.04, 239.17 | FW         Parent (m/z)         RT (min)         Product(s)         Cone (V)           224.3         224.95         18.15-18.65         151.05, 207.01         24, 25           351.14         352.20         3.4-3.8         136.05, 220.13         30, 30           122.12         121.02         7.85-8.25         76.97         30           138.12         136.97         8.24-8.64         93.00         25           175.18         174.00         8.58-8.98         130.00         25           189.21         188.11         11.05-11.45         59.04, 144.09         24, 24           203.24         202.08         12.85-13.25         116.01, 158.08         30, 30           210.27         209.17         12.80-13.20         58.98         22           264.27         245.04         7.70-8.10         87.99, 155.99         30, 30           264.32         263.09         10.70-11.10         152.99, 219.15         22, 22           288.26         289.17         5.80-6.20         132.02, 173.75         28, 28           332.39         31.23         15.64-16.04         213.18, 275.23         42, 42           346.37         345.11         6.90-7.30         143.04, 239.17         32, 32 | FW         Parent (m/z)         RT (min)         Product(s)         Cone (V)         CID (eV)           224.3         224.95         18.15-18.65         151.05, 207.01         24, 25         13, 10           351.14         352.20         3.4-3.8         136.05, 220.13         30, 30         34, 20           122.12         121.02         7.85-8.25         76.97         30         10           138.12         136.97         8.24-8.64         93.00         25         15           175.18         174.00         8.58-8.98         130.00         24, 24         12, 10           189.21         188.11         11.05-11.45         59.04, 144.09         24, 24         12, 10           203.24         202.08         12.85-13.25         116.01, 158.08         30, 30         18, 14           210.27         209.17         12.80-13.20         58.98         22         12           246.27         245.04         7.70-8.10         87.99, 155.99         30, 30         17, 15           264.32         263.09         10.70-11.10         152.99, 219.15         22, 22         10, 12           288.26         289.17         5.80-6.20         132.02, 173.75         28, 28         14, 14      3 |

 Table 1
 MRM optimization conditions for the detection of 12

 Phytohormones detected by LC/MSMS.



**Figure 1** Quantification of salicylic acid (SA) in a M. truncatula leaf sample and root sample using a dilution series of salicylic acid standard.

#### **Experimental Details**

Sample Preparation: A stock solution of the 12 standard plant hormones was prepared (77ug/mL in methanol), and a dilution series ranging from 100x to 64000x was prepared. A 2 uL volume of each standard solution was injected. M. truncatula plants were frozen immediately after harvesting with liquid nitrogen, and quickly ground and weighed while frozen. Samples of 50 mg were extracted:

- 1 mL Isopropanol:H2O:HCL(2:1:0.002) and shake vigorously for one hour at 4°C
- Add 0.5 mL Dichloromethane and shake vigorously for 30 min at 4°C
- Spin at 3500xg at 4°C and remove bottom layer using glass syringe
- Dry samples under nitrogen and re-dissolve in 0.10 mL of MeOH and 1 mL of 1% Acetic Acid
- Run sample over Oasis HLB Column, wash with 1% Acetic Acid, elute with 80% MeOH, 1% Acetic Acid
- Dry eluted fraction under nitrogen
- Re-dissolve dried sample in 25  $\mu L$  MeOH and 25  $\mu L$  1% Acetic Acid
- A 10 uL volume of each sample was injected

#### **LC Conditions**

| LC System:                          | Waters Acquity UPLC                                 |  |  |
|-------------------------------------|---|--|--|
| Column:                             | Acquity UPLC BEH C18,<br>2.1 x 150 mm, 1.7 μm       |  |  |
| Column Temp:                        | 60°C  |  |  |
| Flow Rate:                          | 400 μL/min  |  |  |
| Mobile Phase A:<br>2 mM Ammonium Fo | Water, 0.1% Formic Acid<br>ormate                   |  |  |
| Mobile Phase B:                     | Methanol, 0.1% Formic Acid<br>2 mM Ammonium Formate |  |  |

Gradient: 50% B/1.1 min, 50% to 80% B/1.2 min, 80% to 100% B/8 min, 100% B/5 min, 100% to 50% B/0.1 min, 50% B/2.9 min

| MS System:         | Waters Xevo TQ |  |  |
|--------------------|----------------|--|--|
| Ionization mode:   | ESI            |  |  |
| Capillary voltage: | 4500 V         |  |  |
| Desolvation Temp:  | 250°C          |  |  |

### Sphingolipid Profiling by LC-MS

Method developed by: Barbara Sumner, University of Missouri Metabolomics Center

Sphingolipids are important components of mammalian cell membrane structure and are involved in recognition and signaling pathways. Changes in sphingolipid metabolism can be an indicator in many neurological, skin, and lung diseases, as well as diabetes and cancer. Non-targeted and targeted profiling of sphingolipids is possible with LCMS. Separation, detection and identification of a broad range of sphingolipid classes has been achieved with a reverse-phase UHPLC coupled with ESI/QTOF MS/MS. Table 1 lists 14 sphingolipid and ceramide standards that have been successfully detected in a single sample injection at the MU Metabolomics Center. The chromatogram for 10 of these standards is shown in Figure 1.

| RT [min] | Compound Name                             | (M+H)+ | fragment for<br>MRM |
|----------|---|--------|---------------------|
| 3.7      | Sphingosine (D17:1)                       | 286.28 | 268.3               |
| 3.9      | Sphinganine (D17:0)                       | 288.29 | 270.3               |
| 4.2      | Sphingosine-1-P (D17:1)                   | 366.25 | 250.3               |
| 4.5      | Sphinganine-1-P (D17:0)                   | 368.26 | 252.3               |
| 4.7      | Sphingosine-1-P (D18:1)                   | 380.26 | 264.3               |
| 7.4      | C8 Ceramide (D18:1)                       | 426.39 | 264.3               |
| 8.5      | Lactosyl(beta) C12 Ceramide (D18:1/12:0)  | 806.57 | 264.3               |
| 8.6      | Glucosyl (beta) C12 Ceramide (D18:1/12:0) | 644.51 | 264.3               |
| 8.8      | Sphingomyelin (D18:1/12:0)                | 647.52 | 184.1               |
| 9.0      | C12 Ceramide-1-P (D18:1)                  | 562.42 | 264.3               |
| 9.1      | C12 Ceramide (D18:1/12:0)                 | 482.46 | 264.3               |
| 10.6     | C16 Ceramide (D18:1/16:0)                 | 538.52 | 264.3               |
| 11.8     | C20 Ceramide (D18:1/20:0)                 | 594.58 | 264.3               |
| 13.4     | C25 Ceramide (D18:1/25:0)                 | 664.66 | 264.3               |

 Table 1
 Sphingolipid and ceramide standards detected by LC/

 MSMS, with the fragment masses required for MRM measurement.



**Figure 1** Chromatogram showing the 10 sphingolipid standards from a 2 uL injection of a 2.5 uM solution of the AVANTI Sph/Cer mix.

#### **Experimental Details**

**Sample Preparation:** Standard sphingolipids and ceramides were obtained from Avanti Polar Lipids (Alabaster, Alabama) in a 25 uM methanol solution. The standards were diluted to a 2.5 uM concentration and a 2 uL volume was injected.

#### **LC Conditions**

| LC System:           | Waters Acquity UPLC              |  |  |
|----------------------|----------------------------------|--|--|
| Column:              | Acquity UPLC BEH C18,            |  |  |
|                      | 2.1 x 150 mm, 1.7 μm             |  |  |
| Column Temp:         | 60°C                             |  |  |
| Flow Rate:           | 400 μL/min                       |  |  |
| Mobile Phase A:      | Water, 0.1% Formic Acid          |  |  |
| 2 mM Ammonium F      | ormate                           |  |  |
| Mobile Phase B:      | Methanol, 0.1% Formic Acid       |  |  |
|                      | 2 mM Ammonium Formate            |  |  |
| Gradient:            | 50% B/1.1 min, 50% to 80% B/1.2  |  |  |
| min, 80% to 100% B/  | 8 min, 100% B/5 min, 100% to 50% |  |  |
| B/0.1 min, 50% B/2.9 | min                              |  |  |

| MS System:            | Bruker impact II QTOF |  |  |
|-----------------------|-----------------------|--|--|
| Ionization mode:      | ESI positive          |  |  |
| Capillary voltage:    | 4500 V                |  |  |
| Desolvation Temp:     | 250°C                 |  |  |
| Desolvation Gas Flow: | 10 L/min              |  |  |
| Acquisition range:    | 100-1500 m/z          |  |  |

### Bile acid analysis of mouse tissues by LC-MS

Method developed by: Barbara Sumner, MU Metabolomics Center Samples provided by: Susan McKarns, School of Medicine, University of Missouri

Bile acids are synthesized from cholesterol in the liver and then stored in the gall bladder from which they are secreted into the duodenal lumen during digestion. Bile acids aid in the digestion of fats by solubilizing lipids and providing a means for elimination of excess hepatic cholesterol. Primary bile acids, cholic acid (CA) and chenodeoxycholic acid (CDCA) in humans, and  $\alpha$ - and  $\beta$ -muricholic acid (MCA) in rodents, are transformed into secondary bile acids, deoxycholic acid (DCA), lithocholic acid (LCA), ursodeoxycholic acid (UDCA), hyodeoxycholic acid (HDCA) and  $\omega$ -MCA in the colon. A percentage of the primary bile acids are reabsorbed in the liver, conjugated and released into the circulatory system.

Mouse liver, ileum, brain, and fecal tissue, as well as blood serum was quantified by LCMS. The stable label internal standards, GCDCA-d4, DCA-d4 and LCA-d4 were used to increase the accuracy of the quantification.





**Figure 1** Concentration of various bile acids in mouse blood serum and mouse ileum tissue.

#### **Experimental Details**

Sample Preparation: Mouse tissues were stored at -80°C, then crushed in a mortar and pestle using liquid nitrogen to keep frozen and 50 mg weighed into a stainless steel microcentrifuge tube. One mL methanol and 50  $\mu$ L internal standard solution (containing 40  $\mu$ g/mL GCDCA-d4, DCA-d4 and LCA-d4) was added along with 3 stainless steel beads and capped with a silicon rubber plug. The tubes were placed in a beadbeater and homogenized for 30 seconds, then incubated at -18°C for 20 min. The solutions were transferred to microcentrifuge tubes using a glass pipette, then centrifuged a x13000g for 15 min. The supernatant was transferred to an autosampler vial.

A 50  $\mu$ L aliquot of each blood serum sample was combined with 150  $\mu$ L MilliQ water, 1 mL of methanol and 50  $\mu$ L internal standard solution (4  $\mu$ g/mL), vortexed 30 sec and agitated on an orbital shaker at -4°C for 30 min. The solutions were centrifuged at x13000g for 15 min, and 1.1 mL of supernatant was transferred to an autosampler vial. The solutions were evaporated to dryness in a nitrogen dryer, reconstitued with 50  $\mu$ L of MilliQ water and 50  $\mu$ L of methanol, agitated 10 min and sonicated 10 min, and finally transferred to a 150  $\mu$ L glass vial insert.

#### **LC Conditions**

| LC System:      | Waters Acquity UPLC                           |  |  |
|-----------------|---|--|--|
| Column:         | Acquity UPLC BEH C18,<br>2.1 x 150 mm, 1.7 μm |  |  |
| Column Temp:    | 60°C  |  |  |
| Flow Rate:      | 560 μL/min                                    |  |  |
| Mobile Phase A: | Water<br>0.1% Formic Acid                     |  |  |
| Mobile Phase B: | Acetonitrile                                  |  |  |
| Gradient:       | 32% B/0 min, 39% B/4 min, 40%                 |  |  |

B/4.1 min, 45% B/8 min, 60% B/8.5 min, 80% B/12.5 min, 80%B/12.6 min, 95%B/12.7 min, 95% B/12.9 min, 32% B/18 min, 32%B/23 min

| MS System:            | Bruker Q-TOF |  |  |
|-----------------------|--------------|--|--|
| Ionization mode:      | ESI negative |  |  |
| Capillary voltage:    | 4200 V       |  |  |
| Desolvation Temp:     | 250°C        |  |  |
| Desolvation Gas Flow: | 10 L/min     |  |  |
| Acquisition range:    | 100-1500 m/z |  |  |

# Quantitative Analysis of Amino Acids by LC-MS MRM

Method developed by: Zhentian Lei, MU Metabolomics Center Samples provided by: Bing Stacey, University of Missouri

Amino acids are a group of organic compounds that contain amine (-NH2) and carboxyl (-COOH) groups. There are about 500 naturally occurring amino acids, but only 20 are the essential building blocks of life and genetically encoded. They are used to make proteins and enzymes, and participate in many biochemical processes such as neurotransmitter biosynthesis and plant secondary metabolism.

Quantification of amino acids is performed using LC-MS MRM (Monitor Reaction Monitoring). There are two transitions (i.e., qualitative and quantitative transitions) for each of the amino acids except valine. Quantification is based on the quantitative transition which is shown below in Table 1.

| MRM Qualitative Transition of Amino Acids |                           |                       |               |                                   |                      |
|---|---------------------------|-----------------------|---------------|-----------------------------------|----------------------|
|   | Name                      | t <sub>R</sub><br>min | Exact<br>Mass | Quantitative<br>Transition        | Cone,<br>CE          |
| 1   | Alanine                   | 0.94                  | 89.05         | 90.08 -> 44.05                    | 14, 8                |
| 2   | Arginine                  | 0.87                  | 174.11        | 175.14 ->70.09                    | 16, 20               |
| 3   | Asparag <mark>i</mark> ne | 0.92                  | 132.05        | 133.02 -> 87.07                   | 14, 10               |
| 4   | Aspartic Acid             | 0.94                  | 133.04        | 134.07 -> 88.03                   | 14, 10               |
| 5   | Cysteine                  | 1.02                  | 121.02        | 121.93 -> 75.91                   | 14, 10               |
| 6   | Glutamic Acid             | 0.97                  | 147.05        | 148.08 -> 84.08                   | 16, 16               |
| 7   | Glutamine                 | 0.94                  | 146.07        | 147.10 -> 84.08                   | 16, 16               |
| 8   | Glycine                   | 0.89                  | 75.03         | 76.06 -> 30.07                    | 14, 6                |
| 9   | Histidine                 | 0.85                  | 155.07        | 156.10 -> 110.08                  | 18, 14               |
| 10  | Isoleucine                | 2.19                  | 131.1         | 132.13 -> 86.09                   | 14, 10               |
| 11  | Leucine                   | 2.31                  | 131.1         | 132.07 -> 89.09                   | 14, 10               |
| 12  | Lysine                    | 0.82                  | 146.11        | 147.08 -> 84.06                   | 18, 18               |
| 13  | Methionine                | 1.68                  | 149.05        | 150.08 -> 133.06                  | <mark>16</mark> , 10 |
| 14  | Phenylalanine             | 2.03                  | <u>165.08</u> | 16 <mark>6.11 -&gt; 120.11</mark> | 12, 16               |
| 15  | Proline                   | 1.12                  | 115.06        | 116.03 -> 70.05                   | <mark>18</mark> , 12 |
| 16  | Serine                    | 1.15                  | 105.04        | 106.1 <mark>4 -&gt; 74.02</mark>  | 4, 2                 |
| 17  | Threonine                 | 0.97                  | 119.06        | 120.09 -> 73.98                   | 16, 8                |
| 18  | Tryptophan                | 2.35                  | 204.09        | 205. <mark>06 -&gt; 1</mark> 87.9 | 14, 4                |
| 19  | Tyrosine                  | 1.96                  | 181.07        | 182.17 -> 136.15                  | 16, 12               |
| 20  | Valine                    | 1.47                  | 117.08        | 118.05 -> 72.02                   | 10, 6                |
| IS  | Methionine-d3             | 1.38                  | 152.07        | 153.04 ->136.08                   | 14,10                |

**Table 1** Quantitative MRM transitions for 20 amino acids. Each aminoacid has two transitions, i.e., qualitative and quantitative transitions.Cone: Cone voltage (V), CE: Collision energy (eV),  $t_p$ : Retention time.

#### **Experimental Details**

Sample Preparation: For free amino acids, plant or seed samples (50 mg) are first extracted with 1.4 mL of aqueous solution containing internal standard methionine-d3. After centrifugation at 13,000g for 15 min, supernatant is recovered and filtered through a 20  $\mu$ m membrane filter. 2  $\mu$ L is injected.

For bound amino acids (amino acids in proteins), samples (500 mg) are first hydrolyzed in 6 M HCl Methanol (10 mL) containing methionine-d3 at 110 oC for 24 hours at the presence of phenol (20 mg). 400  $\mu$ l of hydrolysate is mixed with 380  $\mu$ l of 6 M NaOH and then 20  $\mu$ l of water, then filtered through a 20  $\mu$ m membrane filter. 2  $\mu$ l is injected.

#### **LC Conditions**

| LC System:             | Waters Acquity UPLC           |  |  |  |
|------------------------|-------------------------------|--|--|--|
| Column:                | Acquity UPLC HSS C18,         |  |  |  |
|                        | 2.1 x 150 mm, 1.7 μm          |  |  |  |
| Column Temp:           | 40°C                          |  |  |  |
| Flow Rate:             | 400 µL/min                    |  |  |  |
| Mobile Phase A:        | Water, 0.1% Formic Acid       |  |  |  |
| Mobile Phase B:        | Acetonitrile                  |  |  |  |
| Gradient:              | 1% B/0 min, 50% B/5 min, 98%  |  |  |  |
| B/5.1 min, 98% B/6.5 r | nin, 1% B/6.6 min, 1% B/8 min |  |  |  |

| MS System:            | Waters Acquity UPL |  |  |
|-----------------------|--------------------|--|--|
| Ionization mode:      | ESI positive       |  |  |
| Capillary voltage:    | 3500 V             |  |  |
| Desolvation Temp:     | 250oC              |  |  |
| Desolvation Gas Flow: | 10 L/min           |  |  |
| Acquisition range:    | 50-500 m/z         |  |  |

# Quantitative Analysis of Kynurenine and Indoxyl Sulfate by LC-MS MRM

Method developed by: Zhentian Lei, MU Metabolomics Center Samples provided by: Vipul Chitalia, Boston University School of Medicine

Kynurenine (1) and indoxyl sulfate (2) are two important metabolites derived from the amino acid tryptophan (3). While the former is metabolized from tryptophan in the liver, the latter is produced by gut microorganism from tryptophan. Both compounds have been associated with various conditions, including psychiatric disorders, neurological diseases, chronic kidney disease, and the early onset of cardiovascular disease. Accurate quantification of them is critical in understanding their implications in health.

Quantification of kynurenine and indoxyl sulfate is performed on a Waters Acquity UPLC coupled to Xevo triple quadrupole mass spectrometer. Stable labeled standards, kynurenine-d4 and indoxyl sulfate-d4, as internal standards are used for accurate quantification. Samples (plasma and urine) are cleaned up with C18 SPE (solid phase extraction, HLB 30 mg SPE cartridge) and then subject to MRM (Multiple reaction monitoring) analysis.





(3) Trytophan



Figure 1 MRM transitions of indoxyl sulfate and indoxyl sulfate-d4 (upper) and kynurenine and kynurenine-d4 (lower). The inserts are calibration curves using the deuterated compounds as internal

#### **Experimental Details**

Sample Preparation: 200 µL of the plasma is mixed with 500 µL of 0.1% formic acid solution containing Kyn-d4 and IS-d4 as internal standards. The mixture is then applied to a HLB 30 mg SPE cartridge. The compounds are recovered by eluting with 50% MeOH containing 0.1% formic acid (1 mL). The solution is then dried under nitrogen stream and then resuspended in 50 µL of MeOH. 2 µl was injected.

#### LC Conditions

| LC System:           | Waters Acquity UPLC                           |
|----------------------|---|
| Column:              | Acquity UPLC BEH C18,<br>2.1 x 150 mm, 1.7 μm |
| Column Temp:         | 60°C  |
| Flow Rate:           | 560 μL/min                                    |
| Mobile Phase A:      | Water   |
|                      | 0.1% Formic Acid                              |
| Mobile Phase B:      | Acetonitrile                                  |
| Gradient:            | 2% B/1 min, 15% B/5 min, 98% B/8              |
| min, 98% B/9 min, 2% | B/10 min, 2% B/12 min                         |

#### **MS** Conditions

| MS System:            | Waters Xevo TQ |  |
|-----------------------|----------------|--|
| Ionization mode:      | ESI positive   |  |
| Capillary voltage:    | 3500 V         |  |
| Desolvation Temp:     | 250°C          |  |
| Desolvation Gas Flow: | 10 L/min       |  |
| Acquisition range:    | 50-500 m/z     |  |

#### **MRM** Transitions

| Kynurenine-d4:      | 216.1>136.1, MS (+) |
|---------------------|---------------------|
| Kynurenine:         | 212.1>132.1, MS (+) |
| Cone voltage:       | 10 eV               |
| Collision energy:   | 10 eV               |
| Indoxyl sulfate:    | 209.2>192.2, MS (-) |
| Indoxyl Sulfate-d4: | 213.1>196.2, MS (-) |
| Cone voltage:       | 26 eV               |
| Collision energy:   | 18 eV               |
|                     |                     |

## Quantitative analysis of ethanol in Kombucha beverages using 1H-NMR

Method developed by: Saurav J. Sarma, MU Metabolomics Center Samples provided by: Julie Kapp, School of Medicine, University of Missouri

Kombucha tea is an ancient traditional drink especially in the Asian countries that has recently gained popularity in the USA due presumed health benefits through probiotic contents. It is prepared by using symbiotic cultures of yeast and bacteria to ferment sugared black tea over a period of 4-5 days which results in the formation of small amount of ethanol along with many other compounds. There are numerous claims about the drink being beneficial to a wide range of adverse health conditions including diabetes and heart diseases but no clinical studies have confirmed this. To pursue clinical studies of the proclaimed health effects and also for quality control purposes to categorizes kombucha beverages as non-alcoholic, there is a need of simple yet reliable and accurate methods for ethanol quantification in such products. Here a fast and reproducible method of determining ethanol contents in kombucha beverages is presented, which can also be extended to other beverages such as cider and beers.





**Figure 1** <sup>1</sup>H NMR peak of Maleic acid (internal standard) at 6.41 ppm and ethanol at 1.17 ppm, in a representative sample of kombucha tea.

| Sample<br>name | Maleic<br>C=CH<br>integration | Ethanol CH <sub>3</sub><br>integration | Ethanol<br>Conc.<br>μΜ | Ethanol<br>Conc.<br>g/L | Ethanol<br>v/v % |
|----------------|-------------------------------|--|------------------------|-------------------------|------------------|
| н              | 2                             | 0.668                                  | 66.8                   | 3.077                   | 0.390            |
| K-2            | 2                             | 0.487                                  | 48.7                   | 2.243                   | 0.284            |
| K-3            | 2                             | 0.561                                  | 56.1                   | 2.584                   | 0.327            |
| K-4            | 2                             | 1.057                                  | 105.7                  | 4.869                   | 0.617            |
| K-6            | 2                             | 0.545                                  | 54.5                   | 2.510                   | 0.318            |

 Table 1
 Concentration of ethanol in different samples of Kombucha

 tea as determined by integration of the 1H NMR of methyl group of

 ethanol using maleic acid as an internal standard.

#### **Experimental Details**

Sample Preparation: Kombucha tea samples was allowed to come to room temperature and vortexed rigorously for few minutes. 100  $\mu$ M solution of maleic acid in deuterated water was prepared. 20  $\mu$ L of kombucha sample was added to 60  $\mu$ L of the maleic acid solution and vortexed for 20 s. Transferred 35  $\mu$ L of this solution to a 1.7 mm NMR tube for data acquisition.

**Data Analysis:** The data are average of two injections for each sample with six replicate samples for each tissue type. Agilent Chemstation software was used to extract peak areas by integration of total ion chromatogram. Quantification of metabolites were done by using peak area after normalization to the internal standard cholesterol.

#### **Data Acquisition**

- 600 MHz Bruker NMR spectrometer equipped with a 1.7 mm micro-cryoprobe (part Z108160) was used.
- 1H NMR spectra were obtained using solvent suppression pulse program 'wetdc' with 64 scans each.
- NMR field was locked on D2O signal
- Magnetic field shim was achieved by 'topshim' program of Bruker.
- DS= 24, D=16384, D1=3 sec, P1=10.75 μsec, SWH=12019.23 Hz

#### **Data Processing and Calculations**

- Topspin 3.5.7 plot editor was used as the data processing software.
- Signals were calibrated by setting the singlet peak for TMS to 0.000 ppm.
- HC=CH peak of maleic acid appears as a singlet at 6.41 ppm and its integration is set to 2.
- CH3 peak of ethanol appear as a triplet at 1.18-1.15 ppm and the integration of this peak is used to calculate the μM concentration of ethanol in Kombucha samples
- Density of ethanol was used as 789 Kg/L at 20 0C for v/v % calculations.

# MALDI-MS imaging of fungal resistant and susceptible pecan samples

Method developed by: Thomas A. Everett, MU Nano-med Center and Clayton Kranawetter, MU Metabolomics Center

Pecan tree nuts are a major agricultural export and have been shown to possess a multitude of health benefits. A current challenge with pecan growing is infection by fungal Pecan Scab. Infected trees/tissues demonstrate decreased yield as pecan scab results in a smaller pecan nut along with fusion to the hull, preventing proper shelling. Among pecan trees there is differential resistance to pecan scab, with some trees being very resistant and others being very susceptible. Control of scab involves multiple applications of an expensive and toxic antifungal treatment. Due to pecan tree inherent possession of fungal resistance, there must be a biological component that aids in resistance against scab infection.



**Figure 1** Progression of Scab Infection in Pecan Trees. Infection of pecan tissues results in decreased or total loss of pecan yield. Some pecan tree varieties are resistant to infection, implying a biological basis for resistance/infection.



#### Kanza-Scab Resistant







**Figure 2** MALDI Imaging Comparison of Resistant and Susceptible Pecans. Blue indicates lower intensity while red indicates higher metabolite intensity.

#### **Experimental Details**

**Sample Preparation:** Pecan samples were embedded in gelatin, a MS friendly embedding media, and subsequently frozen at -80°C for one hour. Samples were removed from the freezer and placed into a cryotome for sample sectioning. 10-20 µm thick pecan sections were thaw mounted onto conductive glass slides. MALDI matrix, DHB, was sublimed onto the pecan sections to a density of about 2 mg/cm3. Matrix coated samples were then transferred to an MSI slide holder and loaded into the high vacuum MALDI-MS.

Data Analysis: The data are average of two injections for each sample with six replicate samples for each tissue type. Agilent Chemstation software was used to extract peak areas by integration of total ion chromatogram. Quantification of metabolites were done by using peak area after normalization to the internal standard cholesterol.

#### **Data Acquisition**

- Bruker Autoflex Speed MALDI MS system equipped with the 1000 Hz Smartbeam laser was used.
- Acquisition was completed in positive mode using a 10-20 um spot size, 200 µm pixel pitch and scanned in a raster fashion with 50 shots per raster spot.
- A total of over 6000 spectra consisting of 300,000 laser shots were collected for each image using the FlexImaging software suite.

#### **Data Processing and Calculations**

- Data processing and visualization was completed in SCiLS Lab 2014b MSI imaging software. Briefly, this involved baseline subtraction and normalization of the data before an unsupervised statistical analysis of the data was completed (see SCILS application note 5, Bruker)
- The Receiver Operating Characteristic Curve (below) for metabolites of interest along with visual spatial analysis indicate localization of metabolites that may play a role in resistance to fungal infection. (Data provided by Dr. Thomas Everett)





# METABOLOMICS CENTER BR

# PUBLICATIONS

The MU Metabolomics Center staff make significant contributions to research which are recognized by inclusion in publications. The following is a list of publications from 2016 -2020. The MU Metabolomics Center staff members are highlighted by bold text.

#### 2020

Ho, T., Nguyen, T., Vu, D., Nguyen, N., Nguyen, T., Trieu, P., Nguyen, C., Lin, C.-H., Lei, Z., Sumner, L. and Le, V. Allelopathic potential of rice and identification of published allelochemicals by cloud-based metabolomics platform. Metabolites, Submitted 29 April 2020, Manuscript ID: metabolites-804047.

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Vu, D.C., Park, J., Ho, V.K., Sumner, L.W., Lei, Z., Greenlief, M., Mooney, B., Coggeshall, M. and Lin, C.-H. Identification of health-promoting bioactive phenolics in black walnut using cloud-based metabolomics platform. Food Measure 14, 770–777 (2020). doi.org/10.1007/s11694-019-00325-y

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Ho, K.-V., Schreiber, K.L, Vu, D.C., Rottinghaus, S.M., Jackson, D.E., Brown, C.R., Lei, Z., Sumner, L.W., . Coggeshall, M.V. and Lin, C.-H. Black walnut (Juglans nigra) extracts inhibit pro-inflammatory cytokine production from LPS-stimulated human pro-monocytic cell line U-937. Frontiers Pharmacology, 2019, 10, 1059. DOI: 10.3389/fphar.2019.01059

Kumar, S., Sumner, B.W. and Sumner, L.W. Modern plant metabolomics for the discovery and characterization of natural products and their biosynthetic genes. Comprehensive Natural Products III: Chemistry and Biology, Editors-in-chief Hungwen Liu and Tadhg Begley. Volume 7: New Methods in Natural Products Chemistry edited by John MacMillan and Kazuki Saito. Elsevier, In-press, 20Nov2019.

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