GC-TOF-MS Metabolomics Methods for Insect Larvae

Control and heat-treated mayfly samples were provided in triplicate and were processed individually. One sample was provided for AD, ControlX, MF control (from a previous study) and MG larvae. Three replicates of the larvae listed above having only a single sample were created. Aliquots of 50-300mg of insect larvae were mixed with degassed 1:1 Acetonitrile:Water solution in a 2 mL snap cap tube at a concentration of 50 mg/mL. Megalopterans were substantially larger than the other larvae and were prepared at 200 mg/mL. Samples were homogenized and then centrifuged at 4⁰C for 5 minutes at 14000rcf. The supernatant was removed and placed into a new tube. Samples were centrifuged again at 4⁰C for 5 minutes at 14000rcf and a volume of the homogenate corresponding to 40 mg insect larvae were taken. After homogenization, the three larval replicates from AD, ControlX, MG and MF control (from a previous study) were pooled and a single sample created for each larval type. There was insufficient Mayfly (heat and control) sample material to create an internal pool, so an external pool was made by combining equal concentration amounts of AD, MG and MF control (from a previous study) pool quality check (QC) samples. Samples were completely dried by vacuum centrifuge and were reconstituted by adding 630 µL of D2O (Aldrich) and 70 µL of Chenomx ISTD solution (Chenomx, Edmonton, Alberta, Canada). The samples were vortexed and centrifuged at 14000rcf for 2 minutes. 650 µL of sample was transferred into 5mm NMR tubes and analyzed by NMR. 300 µL of the returned NMR sample was taken to a new Eppendorf tube and dried by vacuum centrifuge. Dried samples were spiked with 10 µL of Methoxyamine Hydrochloride (MeOx) in pyridine (of 40 mg/mL) and placed onto a Thermomixer for 90 minutes at 30⁰C at maximum speed. After cooling to room temperature, samples were spiked with 91 µL of MSTFA+FAME mix (1 mL N-Methyl-N-(trimethylsilyl)trifluoroacetamide (MSTFA) + 10 µL fatty acid methyl ester (FAME) retention index marker solution; FAME mix contains C8, C9, C10, C12, C14, C16, C18, C20, C22, C24, C26, C28, and C30 fatty acid methyl esters) and placed onto a Thermomixer for 45 minutes at 70⁰C at maximum speed. Samples were cooled to room temperature before transferring to GC vials with inserts. Sample preparation procedures1 were similar to those published by Dr. Oliver Fiehn.

Samples were randomized to ensure that phenotypes were not grouped and to ensure that the holding time on the GC was random. QC pooled samples were interspersed during the analytical sequence. Samples were analyzed on an Agilent 7890 GC and a Leco Pegasus IV TOF-MS. Compounds were separated and characterized on a Restek Rxi-5Sil MS capillary column (30 m X .25 mm X .25 µm with an additional 10m integrated guard column) under the instrument acquisition parameters located in Table 1. Acquisition parameters were similar to parameters established by Dr. Oliver Fiehn1.

Table 1. Instrument Acquisition Parameters

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| Carrier Gas | Helium |
| Front Inlet Mode | Splitless |
| Front Inlet Temperature | 250⁰C |
| Front Inlet Purge Time | 25sec after injection |
| Front Inlet Purge Flow | 40 mL/min |
| Injection Volume | 0.5 µL |
| Column Flow Rate | 1 mL/min constant flow |
| Oven Temp Initial | 50⁰C hold for 0.5 min |
| Oven Temp Ramp | 20⁰C to 330⁰C, hold for 5 min |
| Transfer Line Temp | 280⁰C |
| Source Temp | 250⁰C |
| Scan Range | 50 – 800 m/z |
| Scanning Cycle | 20 spectra/sec |

Following data acquisition, data files were processed for deconvolution by Leco’s ChromatTOF software and transferred to BinBase for spectral identification, peak retention index calculations and generation of a table of peak identifications and intensities.

References:

1. O Fiehn, G. Wohlgemuth, M Scholz, T Kind, DY Lee, Y Lu, S Moon and B Nikolau: Quality control for plant metabolomics: reporting MSI-compliant studies. The Plant Journal *2008;* 53:691-704.