DESI-MS IMAGING WITH ION MOBILITY AND MULTIVARIATE ANALYSIS FOR THE DETERMINATION OF WEED GRASS SPECIES AND SURFACE LEVEL CHARACTERISTICS

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INTRODUCTION

With the expansion of populations across the globe, challenges faced in the scientific community are evolving continuously. Just as concerns of antibiotic resistance in healthcare increase, similar situations are arising in the agriculture world. Crops are often treated with herbicides to prevent the growth of weeds which compete for nutrients and ultimately reduce crop yields. However, just as microbes develop resistance to antibiotics, weeds are also developing resistance to herbicides[1].

DESI-MS[2] imaging has become increasingly employed given its simplicity, with virtually no sample preparation required, its atmospheric conditions and wealth of molecular information.

The objective of this work was to determine the types of molecular biomarkers that could be identified using DESI-IMS-MSI and their applicability to phenotyping important agronomic traits.

METHODS

Sample preparation

Blades of laboratory cultivated grass weed and crop species were removed from the overall growth and adhered to a standard glass microscope slide using double sided tape.

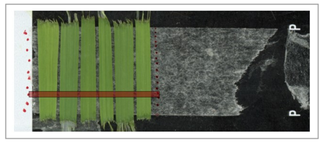

Figure 1. Example of acquisition area setup as shown and implemented in HDI software, subsequent to scanning of the glass slide.

Instrumentation and setup

All analyses were performed on a hybrid Q-TOF-IMS-MS instrument, with the integrated tri-wave ion guide optics used to separate ions by ion mobility in the gas phase.
The instrument was operated in resolution mode, in both positive and negative modes of analysis.

The 2D stage DESI (Prosolia) stage was mounted directly using an electrospray inlet, and an inlet capillary for collection of ions. DESI spray solvents used included varying concentrations of methanol, water and chloroform.
The solvent was introduced at a flow rate of 1.5 µL/min with nitrogen gas pressure set to 4 bar and an applied voltage of 4.5 kV.

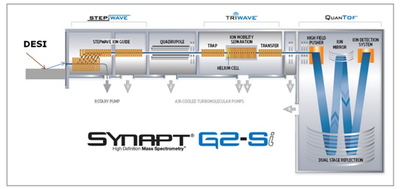

Figure 2. Schematic of the DESI SYNAPT G2-Si HDMS-MS system.

All image acquisitions were set up in High Definition Imaging (HDI) informatics, version 1.4. Slides were scanned using a standard flat bed scanner. Images in figures 3-6 were acquired to give a final pixel size of 50 µm and those displayed in Figure 8, a pixel size of 80µm.

Data management and analysis

DESI-IMS-MS datasets were examined, with MassLynx, Driftscope, then processed and visualized by HDI, version 1.4. Multivariate analyses were performed in EZInfo (Umetrics), via Progenesis QI. All statistical analyses were unsupervised.

RESULTS

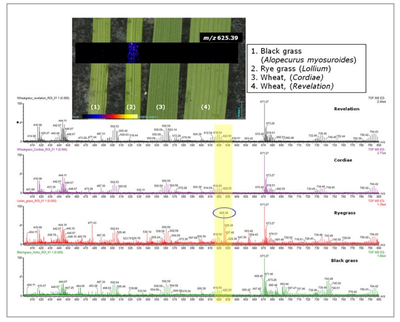

Figure 3. Mass spectra across the m/z range of 400-800 for all 4 grass specimen (as labeled). A potential species specific ion is observed in the mass spectrum acquired from the Ryegrass (Lollium rigidum) specimen. The corresponding image for this ion is also seen. Imaging data was TIC normalised.

For all initial experiments two species of weed grasses were analysed; Black-grass (*Alopecurus myosuroides*) ryegrass (*Lollium rigidum*) and a cereal crop; wheat (*Triticum aestivum*). Within the wheat two different cultivars (cv. *Cordiae* and *Revelation)* were selected to give early indication of the possibility for intra-species differentiation.

Typically, DESI imaging of lipids and other small molecules is performed using a solvent composition of mostly methanol, with a smaller portion of water. However, when analysing the blades of weed grass, the addition of chloroform (up to 18% of the total volume) to the spray solvent increased the number of molecules detected. This may be due to the more abrasive nature of the chloroform containing solvent causing penetration through the waxy cuticle layer and deeper into the surface of the blade. Subsequent to the optimisation of the spray solvent data were acquired from a section across all of the types of grass to produce an image containing all samples for comparison and reduce variability. Figure 3 shows a comparison of the mass spectra generated, zoomed to view the m/z range of 400-800. Although there is a lot of similarity between the spectra, some potentially characteristic peaks are observed, m/z 625 is highlighted in this case.

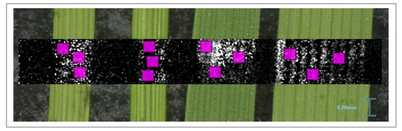

Figure 4. An image exported from HDI, depicting regions of interest (ROI) that were created for the export of data to the multivariate analysis tool (EzInfo). Each square area represents a single ROI and covers a 100 pixel area.

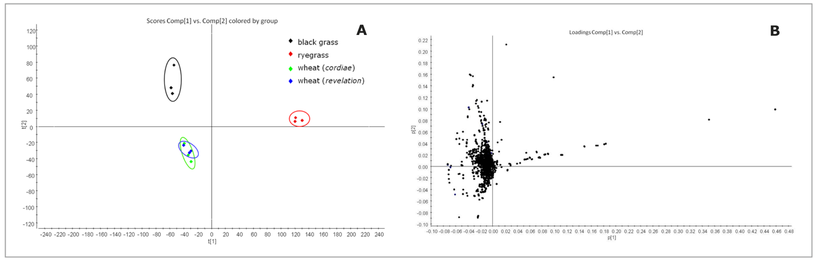

Figure 5. The unsupervised PCA score plot (A) and Loadings plot (B) resulting from the ROI that were exported as shown in Figure 4 are displayed here.

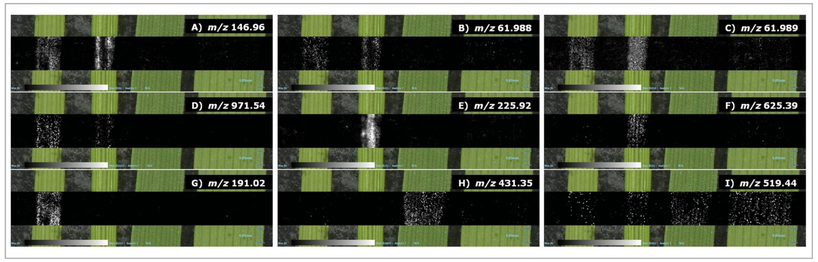

Figure 6. Images of MS ions generated from grass specimen analysed,. From left to right; Blackgrass, ryegrass and wheat. (Cordiae then Revelation). All images are displayed on individual greyscale intensity gradients (as depicted on images). All data for images was normalised against the TIC in HDI 1.4 software.

Three ROI were drawn on each of the grass specimen and exported for multivariate statistical analysis. ROI were labelled to reflect their origin (as labelled in figure 5), although the analysis was unsupervised. The score plot clearly shows good separation between the black-grass, ryegrass and wheat. However, the two populations of wheat grouped together, emphasising their similarity.

Subsequent to obtaining the PCA results, the loadings plot was imported back into HDI software. A number of outlying *m/z* as selected on the PCA loading plot are displayed as ion images in figure 6. These ion images demonstrate that the majority of outlying m/z are specific to either the ryegrass sample or the combination of ryegrass and black-grass. Fewer m/z were observed as being localised within the wheat varieties alone. However one example is shown in figure 6,H.

Advantage of ion mobility separation

Figure 7 emphasises the applicability of ion mobility in these types of experiments. In this case two isobaric, or near isobaric *m/z*, were separated owing to their different drift times and showed different localisation in the black grass and ryegrass samples.

These ions could truly belong to two different structural species (isomers). Or alternatively, they might possess the same structure but with one ion having been generated by fragmentation after the IMS cell and therefore sharing the same drift time as its precursor. To establish the identity of the ions it would be necessary to perform a transfer IMS-MS/MS experiment directly from the samples containing the potential precursors.

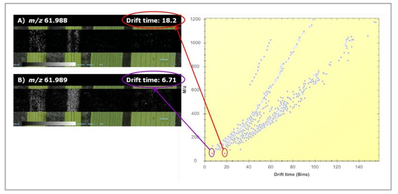

Figure 7. The drift plot as exported from HDI 1.4 shows the presence of 2 ions with near isobaric masses (m/z 61.988 and 61.989) but different drift times. The corresponding ion images are also shown again.

Intra-species differentiation

In continuation of the initial work, further investigation began into the potential for intra-species differentiation.
Two populations of the same black grass species were chosen. A "Roth" population from 2009 was selected as a wild type example, and a 'Peldon' population from 2014 was chosen to represent multi-resistant strains within the same species.

Preliminary data from the DESI analyses within the blackgrass populations are shown in Figure 8 and were used to confirm reproducibility across biological replicates within the two populations.

To validate the findings of the experiments presented here, all ions of interest should be identified via accurate mass measurements and database searches, followed by MS/MS and Collisional Cross Section (CCS) correlation with known standards.

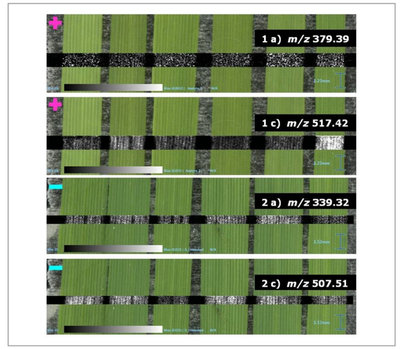

Figure 8. Images acquired over 2 different populations of black grass are shown. From left to right, three biological replicates of Roth (2009), then three of Peldon (2014). All images are displayed on individual greyscale intensity gradients (as depicted on images). All data for images was normalised against the TIC in HDI 1.4 software.

CONCLUSION

- **Endogenous species with the potential to differentiate between black-grass, ryegrass and wheat were detected with the aid of unsupervised PCA and the corresponding ion images shown in HDI 1.4.**
- **It was possible to detect a number of small molecule species that were common across different biological replicates of the same and differing populations of blackgrass.**
- **Further investigations are required to identify any possible differentiators for wild-type and multi-resistant populations of weed grasses.**

References

1. Cummins, I and Edwards, R. (2010) The biochemistry of herbicide resistance in weeds. *Outlooks on Pest Management*, **21**, 73-77.
2. Wiseman, JM, Ifa, DR, Song, Q, Cooks, RG. (2006) Tissue imaging at atmospheric pressure using desorption electrospray ionisation (DESI) mass spectrometry, *Angewandte Chemie*, **45** (43), 7188-7192.

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