B61 A Novel Approach to the Identification of Beetles That Colonize Remains: A Chemometric Processing of Direct Analysis in Real-Time Mass Spectrometry (DART®-HRMS) -Derived Chemical Signatures of Carrion Insects

Amy M. Osborne, BS, State University of New York at Albany, Albany, NY 12222; Justine E. Giffen, BS, State University of New York at Albany, Albany, NY 12222; Jennifer Rosati, PhD, John Jay College of Criminal Justice, New York, NY 10019; Rabi A. Musah, PhD, State University of New York at Albany, Albany, NY 12222*

Learning Overview: After attending this presentation, attendees will better understand the use of DART®-HRMS in the generation of unique insect species-specific metabolome profiles and how statistical analysis can be applied to these profiles to enable insect species identification that is useful for Postmortem Interval (PMI) determination.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by informing attendees about the use of metabolome profiles as unique chemical fingerprints that enable differentiation between species of insects. Attendees will also learn about the application of statistical analysis for processing of insect chemical profiles for differentiation and classification, as well as about methods for handling specimens.

Hypothesis: Statistical analysis can be applied to unique metabolome profiles generated by DART®-HRMS of insect-ethanol suspensions in order to accomplish species identification.

Synopsis: One of the most important aspects of a death investigation is estimation of time since death, also known as PMI. This can be difficult to assess for remains that have advanced to such a state of decay that traditional methods for determining PMI are no longer applicable. In such cases, carrion insects found on or near the body can assist forensic investigators in accomplishing this task, as there is a well-established correlation between a given stage of decomposition and the insect species that colonize the remains. Since the timeline associated with insect progression through various life stages is well established, knowledge of the species of the retrieved entomological evidence can be used to calculate PMI. For this reason, accurate species identification is critical. However, this process is challenging because different insect species are often morphologically similar at a given life stage. Thus, species identification often requires an experienced entomologist to make a positive identification based on the gross physical features of the adult after rearing the insects to maturity, a time-consuming process. Furthermore, the insects most likely to be found colonizing remains that are in the advanced stages of decay are beetles. Yet, there is a limited amount of research on methods that can be used to quickly identify beetle species.

Methods and Results: It is demonstrated here that chemometric processing of DART®-HRMS data acquired from analysis of insects can be used to rapidly accomplish species identification. Five individual dried insect specimens were obtained for each of 18 species of necrophagous insects belonging to the Diptera and Coleoptera orders, such as *Muscidae spp.*, *Necropilia americana*, *Creophilus maxillosus*, and *Nicrophorus tomentosus*. In order to mimic field collection practices, the dried specimens were suspended in aqueous ethanol prior to analysis by DART®-HRMS. The optimal conditions for insect rehydration prior to mass spectral analysis were also investigated. While it was determined that species-specific DART®-HRMS chemical fingerprints could be acquired using dried, non-hydrated samples, consistent results were obtained when dried samples were suspended in aqueous ethanol for at least 24 hours. Suspending the samples for more than 24 hours was found to confer no added advantage, nor was an advantage seen in rehydrating the insects with steam prior to placing them in suspension. The results showed that the ethanol suspensions of each species exhibited a unique chemical fingerprint and that these fingerprints were consistent for members of the same species but different between species. The application of Kernel Discriminate Analysis (KDA) to the data revealed that clear differentiation was possible between insect species based on the chemical fingerprints generated by DART®-HRMS analysis of insect-ethanol suspensions. Furthermore, the separation occurs even between insects belonging to members of the same family, such as the Silphidae family.

Conclusion: At the scene of death investigations, insects present on remains are often underutilized as evidence due to the cost, time, and other resources needed to extract the useful information provided by the specimens. The use of DART®-MS and statistical analysis to differentiate between species of insects can circumvent some of these challenges, thereby increasing the value of entomological evidence.

Forensic Entomology, DART®-HRMS, Chemometrics

*Presenting Author