B27 A Tisket, A Tasket, Tribolium in Your Basket: The Development of a New Approach for the Forensic Detection of Stored-Product Insect Pests Using Direct Analysis in Real Time-High Resolution Mass Spectrometry (DART®-HRMS) and Chemometrics

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Learning Overview: After attending this presentation, attendees will understand the use of DART®-HRMS as a screening device to rapidly test agricultural products for the presence of insect pests to determine if the products meet regulation standards.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by providing information regarding a new technique that investigators can use to detect insect infestation in agricultural products while determining fault in cases of health and safety violations.

Insect pests infesting agricultural products leave behind chemical markers which, through a combination of DART®-HRMS and statistical analysis can be used to identify and quantify the infestation in the products.

Governing bodies such as the United States Department of Agriculture (USDA) and the Food and Drug Administration (FDA) are charged with setting strict health and safety guidelines for food products in order to protect consumers. Failure to adhere to these regulations can result in the transmission of microbial contaminants and toxins to consumers, litigation and fines for food producers and manufacturers, and spark larger investigations into the food safety violations. The ability to accurately and rapidly detect insect presence and scale of infestation in agricultural products is therefore of the upmost importance to both regulating bodies and food producers and manufacturers. Unfortunately, conventional techniques used in stored-product forensic entomology generally require significant specialized expertise, are time-consuming and resource-intensive to implement, and exhibit variable accuracy in detecting the type and severity of insect infestation. Therefore, there remains a need for simple, rapid, and reliable techniques for the assessment of food pest infestation in order to establish liability in court cases revolving around food health and safety violations.

It is demonstrated here that a chemical approach to the testing of agricultural products could address many of the challenges presented by the conventional techniques stated above. In accordance with Locard's Exchange Principle, insect invaders in processed food products such as milled grain should transfer chemical biomarkers to their surrounding environment. Further, these species-specific insect biomarkers should lead to both identity determination of the invading species, as well as an estimation of the insect population present, through correlation of these factors with the quantity of the observed biomarkers. The unique capabilities of DART®-HRMS make it ideal for this type of rapid screening analysis. In a proof-of-principle study, a common agricultural pest, *Tribolium castaneum* (the red flour beetle), was used to deliberately infest all-purpose flour. These insects were introduced into five separate batches of flour, each in replicates of five, while an equal number of samples of non-infested flour were used as controls. The samples were analyzed over the course of several months by DART®-HRMS in positive-ion mode in order to monitor the appearance of insect-associated molecules as the length and size of the simulated infestation grew. The collected spectra were used to generate chemical profiles for the control and infested flours, which were then subjected to several methods of statistical analysis including Analysis of Variance (ANOVA) - Simultaneous Component Analysis (ASCA). This analysis highlighted several masses that enabled the differentiation of infested and non-infested flour. One of the masses, *m/z* 137, was confirmed by Gas Chromatography/Mass Spectrometry (GC/MS) analysis to be a molecule frequently associated with red flour beetles: 2-Ethyl-1,4-Benzoquinone (EBQ).

Investigators of health and safety violations of agricultural products remain in need of a simple and reliable technique for the rapid detection and evaluation of insect infestations. The results of this study indicate the potential for DART®-HRMS to be used as a tool by these investigators to determine the scope and scale of insect infestation in processed agricultural products.

Forensic Entomology, DART®-HRMS, Chemometrics

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