Are there bugs in there?

Use of Colorimetric Sensor Arrays to Detect Insects in Food

Charles Davidson[1,2], Mukti Ghimire[3], Avishai Ben-David[1], Scott Myers[3], Richard Huang[4], Sung Lim[4], Aleksandr Miklos[1,5], Kelley Betts[1,6], Jeff Ballin[1,5]

[1]Edgewood Chemical Biological Center, APG, MD; [2]Science and Technology Corporation, Edgewood, MD; [3]US Department of Agriculture, Buzzards Bay, MA; [4]iSense LLC, Mountain View, CA; [5]Excet Inc, Springfield, VA; [6]Leidos Inc, Abingdon, MD.

Abstract

Insect invasive species represent a significant threat to the U.S. agricultural economy, highlighted by increased screening efforts of U.S. imports. Detection of the nonindigenous khapra beetle (*Trogaderma granarium*) is especially of concern: As one of the world's most destructive species, khapra beetle larvae feast on grain products (shown right with rice and macaroni product), potentially threatening the US bulk grain and oilseed export economy worth >$35B between 2009-2013 alone. Here, we apply a versatile sensing platform to detect volatile organic compounds emitted by insects infesting raw grain. Successful detection and identification of threats based on volatile signatures within a closed shipping container allow threats to be identified and sequestered prior to being opened, thereby mitigating exposure or release of the threat(s) within.

The Threat: Khapra Beetles

The Problem:

Opening contaminated crates during inspection runs the risk of exposing unspoiled produce handled within the same shipping or processing facilities to microorganism and pest infestation.

One of the world's most destructive pests of stored grain products and seeds:

- Listed in top 100 most invasive species
- Beetle is small (< 3 mm long)
- Can survive without food for long periods
- Prefers dry conditions and low-moisture food
- Survives almost anywhere that is protected from cold temperatures
- Resistant to many pesticides

Figure 1. *Trogoderma granarium* larvae and cast skins on wheat (*left*), showing typical damage and potential for contamination with hastisetae (*right*). Khapra larvae and adult beetle adjacent to metric ruler for scale (*far right*).

Feeding damage often spoils significant amount of unprotected product

- Known for its "dirty eating" behavior, feeding only a little on each grain
- Larvae contaminate products by shedding their cast-off skins and hair, leaving behind excrement and urine

→ The only Federally regulated stored product insect in the U.S.

→ The only pest where action is taken with dead life stages

The Approach: Colorimetric Sensor Arrays (CSAs)

The disposable sensor is a 1 square-inch PVDF or cellulose membrane spotted with a 8 × 10 array of colorimetric chemical indicator dyes. Gases emitted during bacterial growth or food spoilage change the color of these dyes. The color response is subtracted from a baseline to produce unique color "fingerprints."

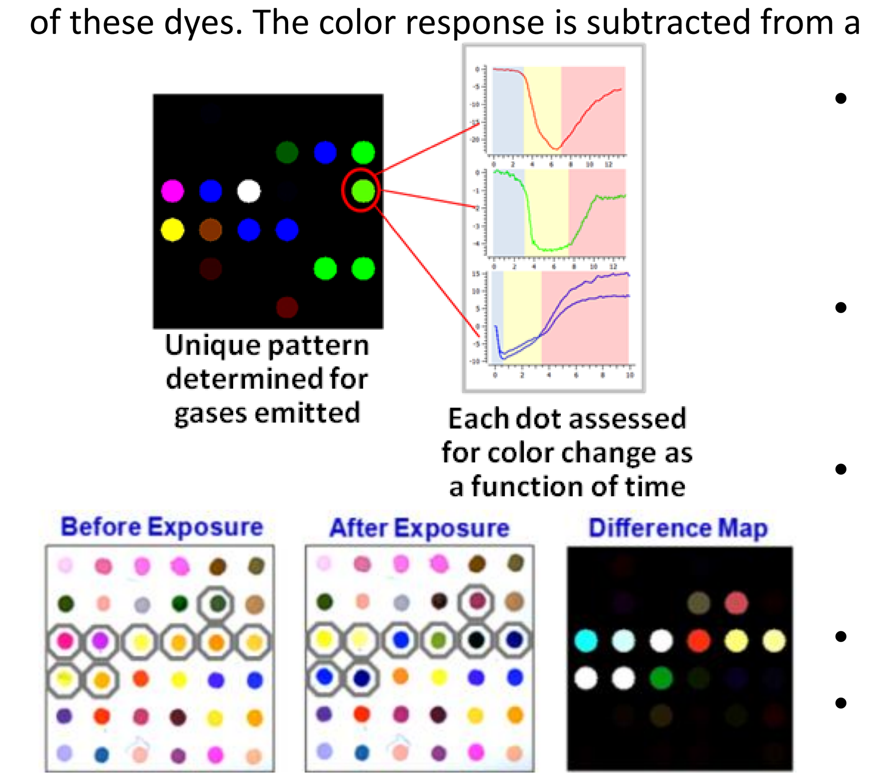


Figure 2. Membrane spotted with indicator dyes before and after exposure. The difference map *(far right)* is the mathematical subtraction of the before-image from the after-image.

- **High Dimensionality and Sensitivity:** parallel analysis across broad range of chemicals and chemical sensitivity in 10 ppb range for many analytes
- **Adaptable Form Factors:** the simple printing approach and integration allows it to be used in many forms
- **Speed:** strain identification of biological pathogens in 6-8 hours (this will improve), chemical agents in <1 minute
- **Accuracy:** all test data exceeded 95% accuracy
- **Utility:** inexpensive, adaptable for field use, multiplexed for high confidence, and capable of being interpreted by smart phones

Approved for Public Release

Method

① Image response of CSAs to jars of grain with or without beetle infestation as a function of time:

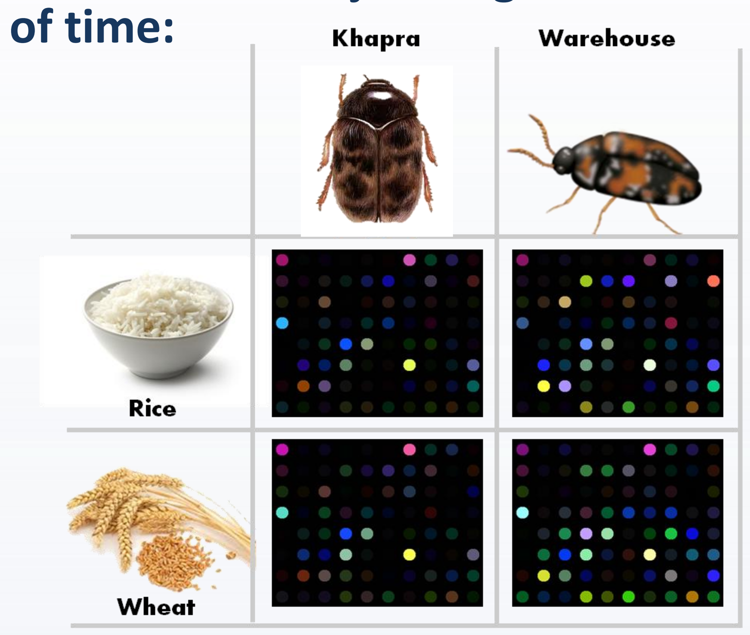


Figure 3. Compare response to long grain rice or cracked wheat alone versus when infested with khapra beetles or related *Trogoderma* species

② Select subset of bands (red, blue or green intensities associated with a given dye) that are most responsive to the presence of grain versus insect (e.g., green band of spot 19)

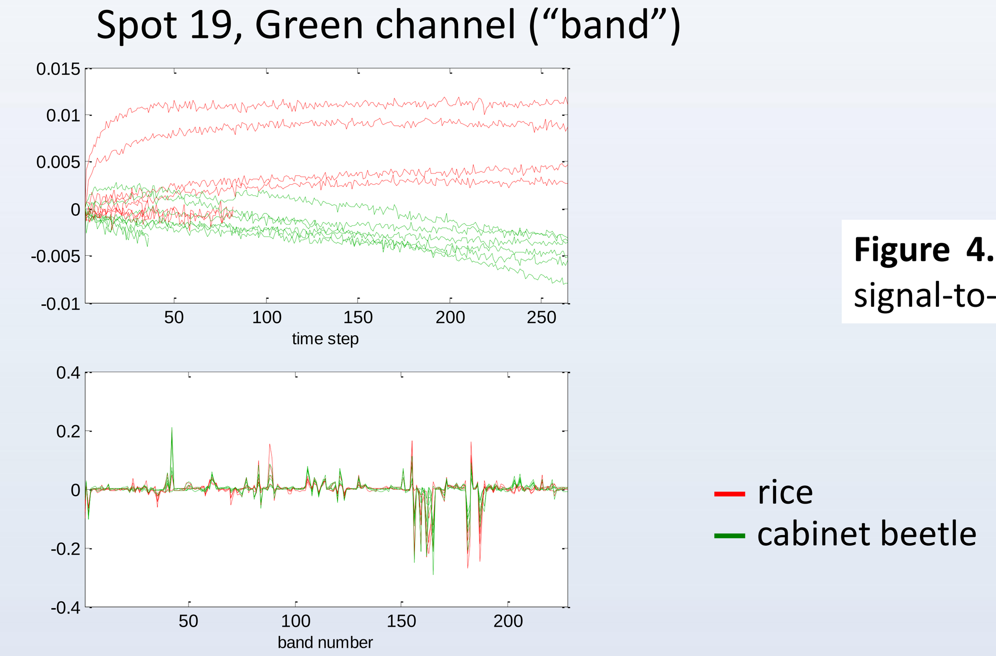


Figure 4. Band selection greatly improves signal-to-noise and target discrimination

③ Perform principal component analysis (PCA) to aid in separating classes of grain alone versus grain + beetle

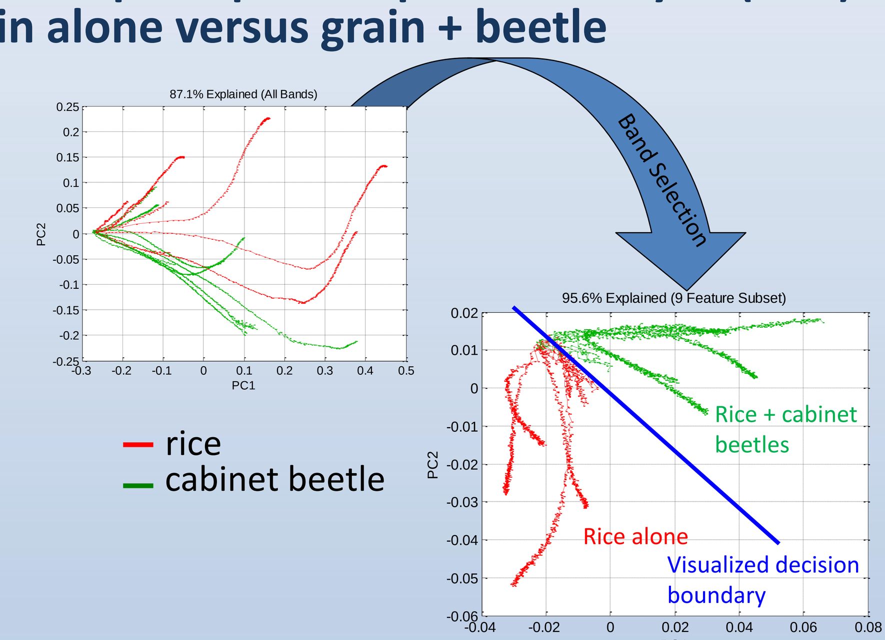


Figure 5. Principle component analysis (PCA) of 9 selected bands (*lower right*) shows significantly better separation relative to infestation class than PCA of all 228 bands (*upper left*), indicative that some spots confound differentiation.

④ Support vector machine analysis (SVM) automates decision boundary estimation and evaluates contaminated versus pristine grain

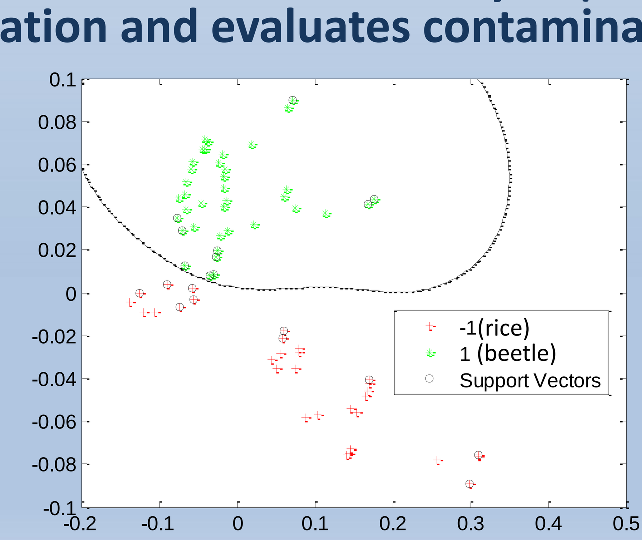


Figure 6. SVM is a multidimensional classification algorithm that defines boundaries that create the widest possible gap between classes.

Results

Using band selection to identify responsive dyes, principal component analysis to improve signal to noise, and support vector machine algorithms to automate target identification, khapra beetle infestation was differentiated from infestation by a related species (warehouse beetle or cabinet beetle) in cracked wheat and long grain rice.

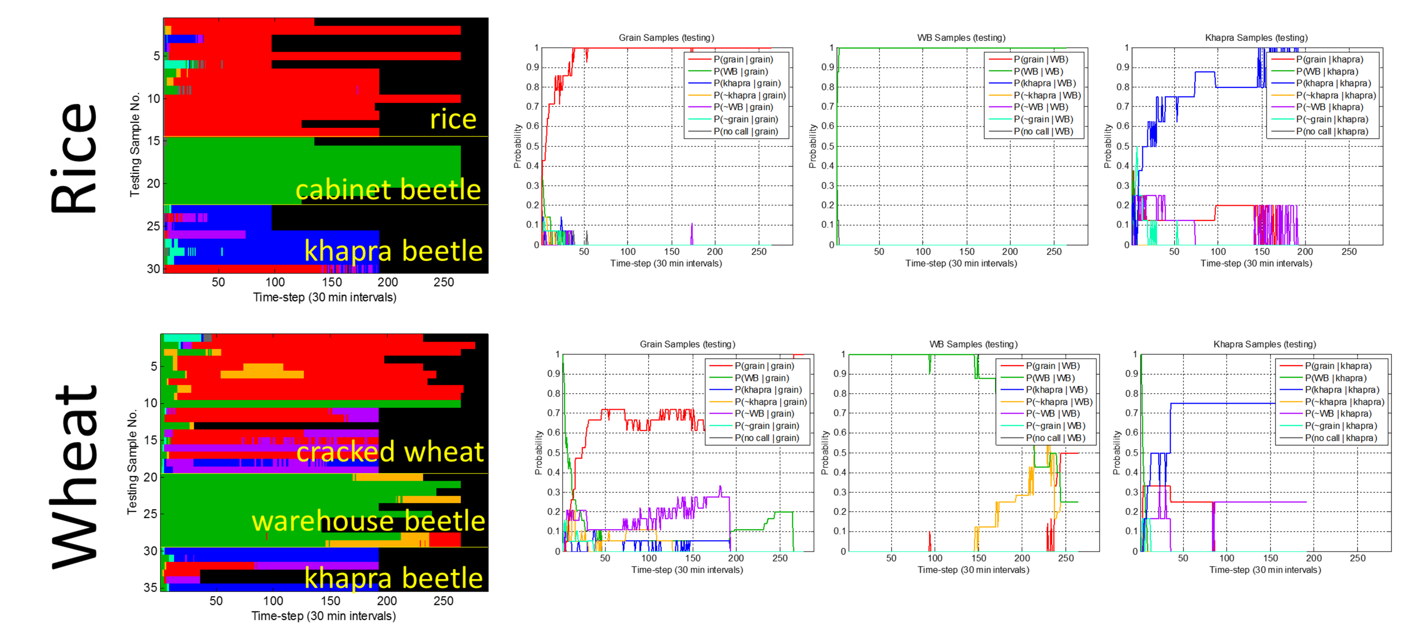


Figure 7. SVM automated prediction of infestation status based on CSA response profiles to grain with or without insect infestation as a function of time. (*far left*) For each specimen class denoted (yellow text), colors indicate the SVM predicted that the specimen was rice (red), cabinet beetle (green), khapra beetle (blue), not khapra beetle (gold), not cabinet beetle (purple), not rice (cyan), or no call made (gray). Probability of SVM calls made for known grain, cabinet beetle, or khapra beetle contaminated specimens, using the same color scheme.

Conclusions

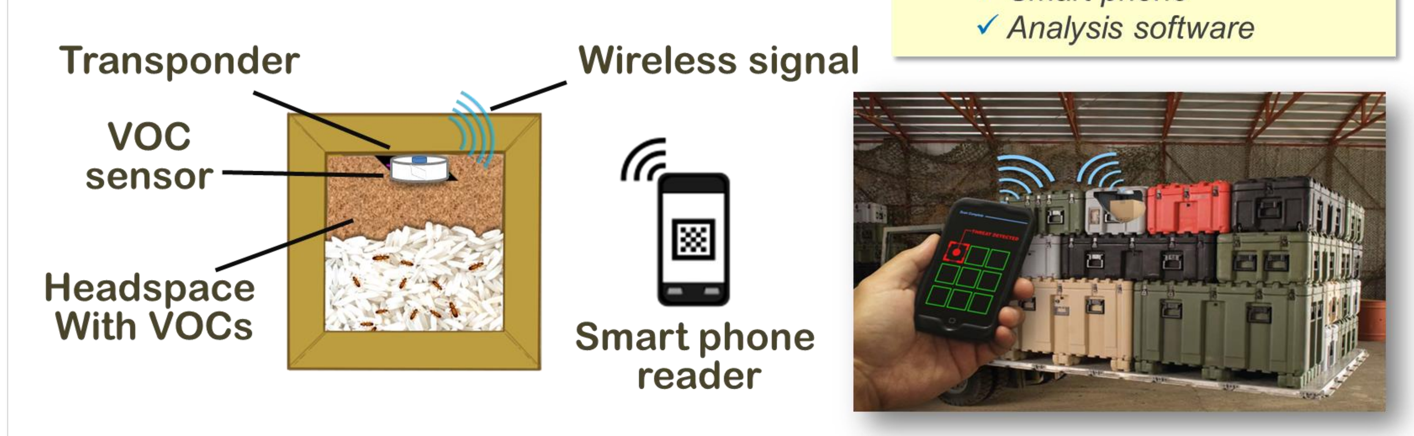
These studies demonstrate that an automated decision algorithm can differentiate volatile organic signatures detected by inexpensive colorimetric sensing arrays to distinguish heavily infested grain from pristine grain. These calls can be made without having to open the container, often well within a day, a relevant time frame for international shipments. In this way, potentially contaminated shipment containers can be flagged for inspection, potentially avoiding introduction of invasive species that would otherwise be difficult to contain.

The Future – Cargo Shipments

Self contained sampling & detection unit: Volatile Organic Compounds (VOCs) are emitted into headspace as food spoils or insects feed. During transit, the colorimetric sensor responds to these VOCs which is later imaged by the "VOC Transponder" and wirelessly transmitted to a smart phone for analysis.

Most of the necessary elements for this system already exist or can be leveraged from previously funded efforts:

- ✓ *VOC sensors*
- ✓ *Transponder electronics*
- ✓ *Smart phone*
- ✓ *Analysis software*



Acknowledgements

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