Gas Chromatography for Detection of Citrus Infestation by Tephritid Fruit flies

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Tropical Tephritid Fruit Flies
(Diptera: Tephritidae)

• Major pests of fruit crops, impact production and export worldwide

• Primary threats to U.S. agriculture
  – Mediterranean fruit fly (medfly) 
    \textit{(Ceratitis capitata)}
    global distribution
  – \textit{Anastrepha} spp.
    American tropics / subtropics
    \textit{A. suspensa} in Florida
    \textit{A. ludens}, \textit{A. obliqua} in Mexico, Caribbean basin
Anastrepha suspensa (Loew)  
(Caribbean fruit fly)

- Indigenous to the West Indies, now well established in central and south Florida

- Preferred hosts
  - Guava (*Psidium guajava*)
  - Loquat (*Eriobotrya japonica*)
  - Surinam cherry (*Eugenia uniflora*)

- Also infests ripe grapefruit (*Citrus x paradisi*)

- Quarantine pest of FL citrus
Economic Importance

- Current appropriations for risk management programs are $57 million per annum (APHIS Exotic fruit fly strategic plan FY2006-2010)
- California medfly outbreak in 1980 cost > $850 million ($59 mil. chemical control, $38 mil. quarantine/fumigation, $260 mil. crop losses, $497 constr. fumigation facilities; Burk & Calkins 1983)
- New medfly infestation estimated to cost as much as 1.5 billion due to export sanctions, lost markets, treatment costs and crop losses (USDA-ARS 2005)
Detection of Tephritid Pests

• Due to economic impact, much attention focused on detection and monitoring of adult fly populations
  – McPhail traps
  – Liquid protein baits
  – Synthetic food-based lures

• Methods needed for improved detection of immature stages
  (larval stages cause fruit damage)
Tephritid Development

- Females have well developed ovipositors
- Eggs inserted beneath skin of host fruit
- Larvae feed and develop concealed within the pulp
- Infestation difficult to detect in intact fruit
Current APHIS Inspection Protocols

• At U.S. ports of entry, quarantine inspectors
  – Examine small sample of fruit (typically 2% of shipment)
  – Look for external signs of pest boring/feeding
  – If suspicious, cut open fruit to search for larvae

• Efficacy questionable (eggs 2 mm, 1\textsuperscript{st} instar 2-3 mm)

• Estimated that only 35% of infested grapefruits are detected by trained agricultural inspectors (Gould 1995)

• Need for more sensitive, high through-put screening methods for larval infestation
Signature Chemicals

• We evaluated gas chromatography as a method for improved detection of hidden infestation

• Citrus infested with *Anastrepha* were examined to determine if infested fruit emitted a chemical profile distinct from that of non-infested fruit

• **Goal:** Develop a rapid screening protocol for “Signature chemicals” diagnostic of infestation
Methods: Infestation & Sample Preparation

- Grapefruits infested with *A. suspensa* in lab (24-hr oviposition).
- Chemical sampling of fruit volatiles: egg, 1\textsuperscript{st}, 2\textsuperscript{nd}, 3\textsuperscript{rd} instars, exiting larvae.
- Controls: non-infested fruit and mechanically-injured fruit (pierced with tack).
Methods:
Volatile Collections and Chemical Analysis

• Fruit placed in 1-gal. jars, 30 min. equilibration
• Volatiles collected by SPME (2 min adsorption)
• GC analysis by 3 methods
  – Rapid method
    Trace GC, 9 min
    (5 reps/treatment)
  – High resolution method
    GC-MS, 25 min
    (3 reps/treatment)
  – Ultra-fast, portable GC
    zNose, 79 sec
Results: Rapid GC Analysis

- 17 major peaks separated
- 3 classes:
  - 1. Background citrus volatiles
  - 2. Peel injury, puncture wound
    - D-limonene
    - B-ocimene
  - 3. Infestation, feeding damage
    - Hexyl butanoate
    - Unidentified compound (no match NIST/EPA/NIH MS library)
Results:
High Resolution GC

• 22 peaks separated / identif. from infested fruit

• Signature peaks
  – 5 = limonene
  – 6 = ocimene
  – 8 = unknown
  – 10 = hexyl butanoate

• Unknown (peak 8) best indicator of infestation
Results: Ultra-fast zNose Analysis

- Preliminary evaluation
- zNose able to detect same 4 signature peaks
- Better resolution of limonene-ocimene
- Ethyl octanoate co-eluted with hexyl butanoate
- APHIS-PPQ conducting further evaluations
Conclusions

• There are GC-detectable volatile chemicals associated with tephritid infestation of citrus
• None appeared to be insect produced
• Elevated limonene/ocimene = peel injury only
• If combined with hexyl butanoate and the (as of yet) unidentified compound = profile diagnostic of infestation
• Peaks detectable with portable, ultra-fast zNose unit
• Potential: development of a rapid, sensitive, non-destructive screening method based on GC technology
Gas chromatography for detection of citrus infestation by fruit fly larvae (Diptera: Tephritidae)*

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ABSTRACT

Tephritid fruit flies are serious economic pests worldwide. As larvae, they feed and develop within the pulp of host fruits, making infestation difficult to detect by visual inspection. At U.S. ports of entry, incoming produce shipments are checked for infestation by manually cutting open a small sample of fruit and searching for tephritid larvae. Consequently, there is a need for more sensitive, high-throughput screening methods. This study evaluated gas chromatography (GC) as a potential technology for improved detection of hidden infestation. Grapefruits (Citrus × paradisi Macf.) infested with immature stages of the Caribbean fruit fly Anastrepha suspensa (Loew) (Diptera: Tephritidae) were examined to determine if infested fruit emitted a chemical profile distinct from that of non-infested fruit. Peaks identified by GC analysis were grouped into three classes. Chemicals detected in similar quantities in all samples, or slightly elevated in infested samples, were regarded as non-diagnostic background volatiles. Chemicals highly elevated after oviposition, during the last instar exit stage, and in experimentally-pierced fruit were interpreted to be indicators of citrus peel injury, and included α-limonene and β-ocimene. Chemicals elevated exclusively in the larval infestation stages were considered indicators of feeding damage and potentially diagnostic of infestation, and included hexyl butanoate and an unidentified compound. The peaks associated with injury and feeding were also detectable with a portable ultra-fast GC analyzer that required less than 80 s per sample. Further studies will investigate the potential application of these results for development of a rapid, non-destructive screening method for detection of tephritid infestation.

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