Signature Chemicals for Detection of Hidden Insect Infestation

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Introduction

Tephritid fruit flies are serious economic pests worldwide. The female ovipositor is well-developed for inserting eggs beneath the skin of host fruit (Fig. 1A). Larvae feed and develop concealed within the pulp (Fig. 1B), making infestation difficult to detect in intact fruit. At U.S. ports, quarantine inspectors currently check produce shipments by manually cutting open a small sample (typically 2% or less) of fruit to search for infestation. Consequently, there is great demand for more sensitive, high-throughput screening methods for invasive tephritid pests.

This study evaluated gas chromatography (GC) as a potential technology for improved detection of hidden infestation. We examined grapefruit infested with the Caribbean fruit fly, *Anastrepha suspensa*, to determine if infested fruit emitted a chemical profile distinct from that of non-infested fruit.

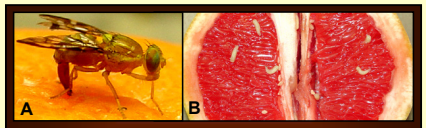
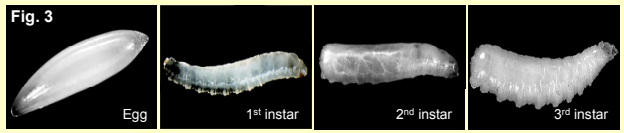


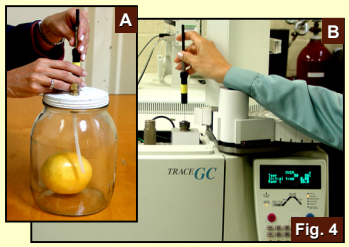
Fig. 1. ***Anastrepha suspensa* (Loew)**
A) Female ovipositing. **B)** 3rd Instar larvae in grapefruit host.

Methods

Fruit Treatments. Under controlled lab conditions, ripe grapefruits (*Citrus x paradisi* Macfad.) were infested with *A. suspensa* (Fig. 2) using methods previously reported (Kendra et al. 2007). After a 24-hr oviposition period, fruit was washed and then held for sampling at the following stages of infestation: egg, 1st, 2nd, and 3rd larval instar (Fig. 3). Controls consisted of non-infested fruit, as well as mechanically-damaged fruit (pierced 5x with tack to simulate oviposition wounds).



Volatile Collections & GC Analysis. Grapefruits were placed in 3.85 liter jars (Fig. 4A) with short thru-hull ports (Swagelok; Solon, OH) and equilibrated for 30 min at room temp. Headspace volatiles were collected by solid-phase microextraction (SPME) with 100-µm polydimethylsiloxane coated fiber (Supelco; Bellefonte, PA) inserted through the port for 2 min adsorption. Chemicals were injected (Fig. 4B) by desorption (250°C for 2 min) from the SPME fiber directly into a Trace™ GC (ThermoFisher; Waltham, MA) equipped with a DB-5 column (20 m x 0.18 mm x 0.18 µm) and flame ionization detector (FID, 300°C). Carrier gas was helium, temp ramp was 50-220°C, and total run time was 9 min.



zNose® Analysis. A portable, ultra-fast GC analyzer (Fig. 5) (Electronic Sensor Technology; Newbury Park, CA) was also evaluated for detection of signature chemicals. Headspace volatiles were collected by insertion of the unit's intake needle into the sample port for 30 sec and adsorption onto an internal Tenax® trap. Chemicals were injected by thermal desorption and separated on a DB-5 column (1 m x 0.25 mm) using helium carrier gas, a temp ramp of 40-175°C, a run time of 79 sec, and a surface acoustic wave (SAW) detector.

GC/Mass Spectrometry. SPME collections (as above) were injected into a 5975B GC/MSD (Agilent; Santa Clara, CA) equipped with an HP-5MS column (30 m x 0.25 mm x 0.25 µm) using helium carrier gas, temp ramps of 50-130°C (10/min) and 130-210°C (20/min), and run time of 25 min. MSD source was set at 230°C, quadrupole at 150°C, and scans recorded for mass range of 50-650 amu. Component peaks were identified using established chemical libraries.

Results and Discussion

GC analysis revealed distinct differences in volatile chemicals emitted from infested grapefruits vs. control fruits (Fig. 6). These differences were seen in chromatographs obtained with both the standard laboratory system (Trace™ GC-FID) and the portable ultra-fast system (zNose® GC-SAW).

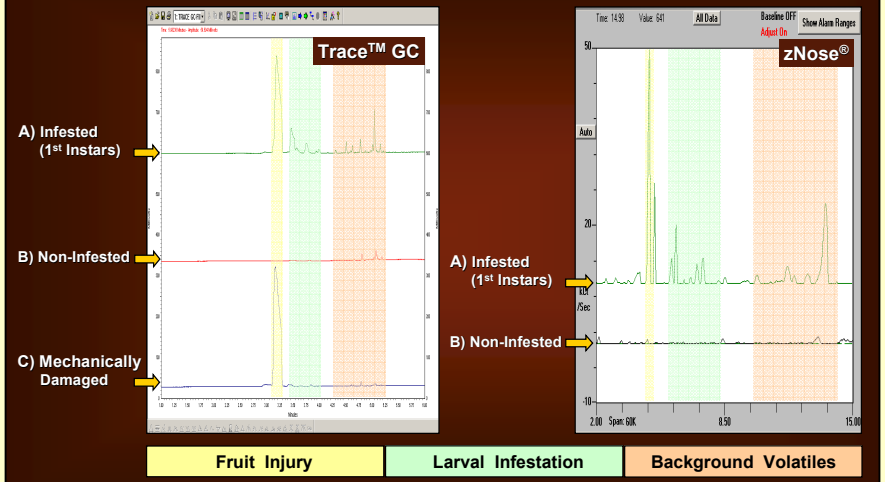
Trace™ GC. Comparing infested fruit (Fig. 6A) to non-infested fruit (Fig. 6B), there were significant differences in the presence and/or amplitude of peaks with retention times in the range 3.2 – 3.8 min. The prominent peak at 3.2 min was also found in mechanically-damaged fruit (Fig. 6C), and was attributed to fruit injury (due to oviposition or experimental puncture). Peaks from 4.5 – 5.2 min were present in nearly all samples, and were regarded as background peaks for grapefruit. By exclusion of peaks seen in control fruits, we concluded that several peaks from 3.5 – 3.8 min were indicative of infestation. These chemicals were elevated in the presence of all three larval instars, but not in the presence of eggs alone.

zNose® GC. Rapid GC analysis by zNose® gave comparable separation of grapefruit volatiles into three general groups, but retention times were appreciably less: the peak associated with fruit injury eluted at 4.6 sec, the infestation peaks at 5.8 – 8.5 sec, and the background peaks at 9.9 – 13.8 sec.

Tentative Chemical Identifications.

Fruit Injury: limonene
Background Volatiles: eugenol, methyl eugenol, trans-caryophyllene, valencene
Larval Infestation: ocimene, nonanal, ethyl octanoate

Fig. 6. Gas Chromatographic Separation of Grapefruit Volatiles



Conclusion. Our results indicate that there are GC peaks potentially diagnostic of *Anastrepha*-infested grapefruit. These peaks, present as early as the first larval instar, were detectable with a portable ultra-fast CG system. If infested commodities consistently release unique chemical profiles, this "signature" can be exploited to provide the basis for development of rapid, reliable screening protocols.

Kendra, P. E., M. K. Hennessey, W. S. Montgomery, E. M. Jones, and N. D. Epsky. 2007. Residential composting of infested fruit: A potential pathway for spread of *Anastrepha* fruit flies (Diptera: Tephritidae). Florida Entomol. 90: 314-320.

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