Male Mediterranean Fruit Fly Attractants Identified by High-Performance Thin Layer Chromatography (HPTLC)

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Abstract

Ceratitis capitata (Wiedemann), commonly known as Mediterranean fruit fly or Medfly, is a highly invasive and destructive pest that causes great damage to hundreds of fruits and vegetables worldwide. Currently, Medfly detection and monitoring relies on the parapheromone Trimedlure (TML), a male-specific attractant. However, the cost and limited availability of TML, has prompted a quest for the identification of alternative attractants to supplement management programs. Previous bioassays revealed that male Medflies are attracted to tea tree oil (TTO), an essential oil (EO) from *Melaleuca alternifolia*. Initial thin layer chromatography (TLC) separation generated five major fractions of TTO, two of which were attractive to male Medflies. Since TTO is available from multiple manufacturers, there is considerable variation in its chemical composition. In this study, we used high performance TLC (HPTLC) to evaluate variations in chemical composition of TTO from different sources that may affect the observed attraction, featuring the chemical complexity and quality issues of TTO. HPTLC protocols were developed to enhance the separation and isolation of individual TTO components. Results were compared to those obtained by GC-MS. This research will lead to the discovery of novel Medfly attractants with potential applications for the improvement of management programs for this devastating pest.

Materials & Methods

HPTLC (CAMAG, Muttenz, Switzerland)
- 50% TTO (various sources, 2µL) (Fig. 1)
- Universal HPTLC Mix (UHM) (Fig. 1)
- Dichloromethane (DCM)
- Silica gel 60 F_{254}, 20 x 10 cm, HPTLC plates (Merck
- ATS4 autosampler (Fig. 2)
- ADC2 developing chamber (Fig. 3)
- Hexanes:ethyl acetate (Hex:EtOAc) **9:1** and **8:2** (v/v)
- Derivatizer (Fig. 4), vanillin/sulfuric acid, 2mL
- Plate Heater (Fig. 5), 2.5 min. at 100°C
- TLC Visualizer 2 (Fig. 6)

Semi-automatic Prep TLC separations were carried out under similar conditions to HPTLC to obtain larger fraction amounts for GC-MS analysis and bioassays.

GC/MSD (Agilent Technologies, Santa Clara, CA, USA) (Fig. 7)
- EOs and reference chemicals in DCM
- 5975 GC-MSD withDB-5 column
- *n*-Alkanes were used as reference point (RI).
- Spectral libraries:
  - Wiley 12th Edition, NIST 2020, Mass Finder 3.0, FFNSC-3, Adams, and in house SHRS library


Fig. 1 TTOs and UHM

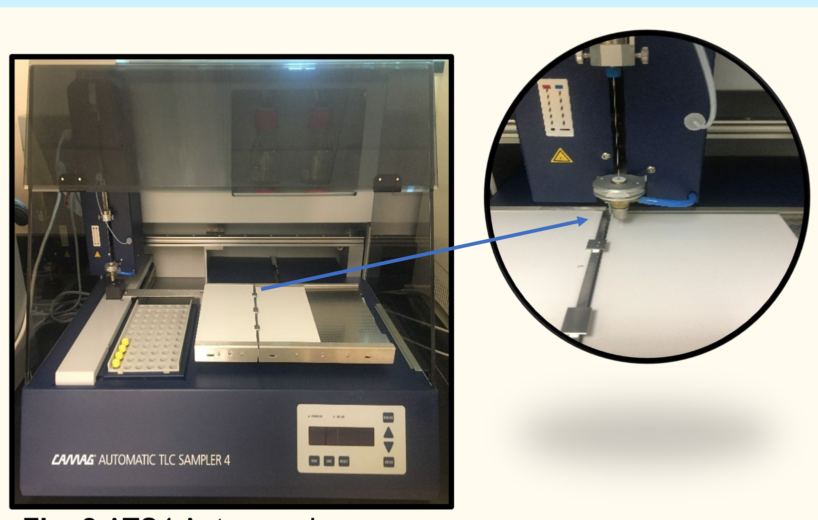

Fig. 2 ATS4 Autosampler

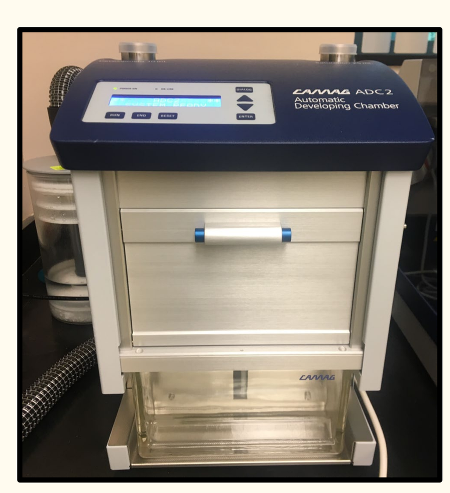
Fig. 3 ADC2 Developing Chamber

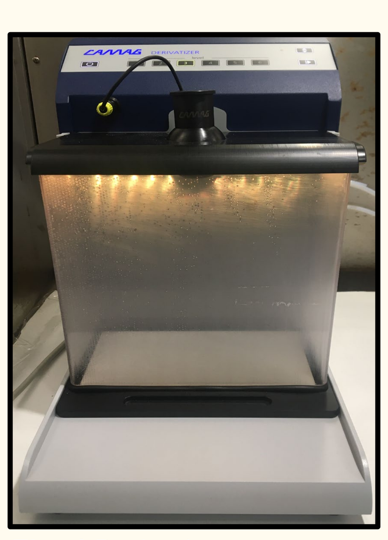
Fig. 4 Derivatizer

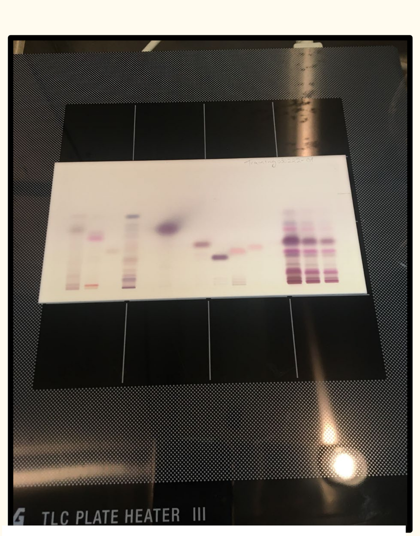

Fig. 5 TLC Plate Heater

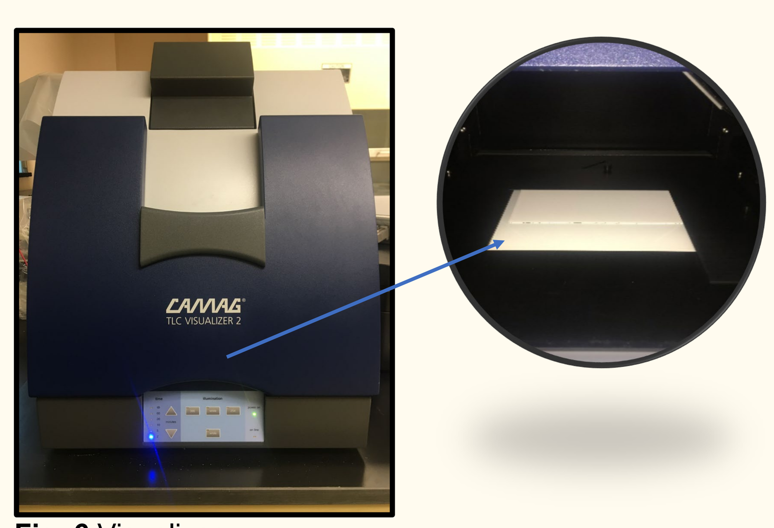

Fig. 6 Visualizer


Fig. 7 Agilent 5975 GC-MSD System

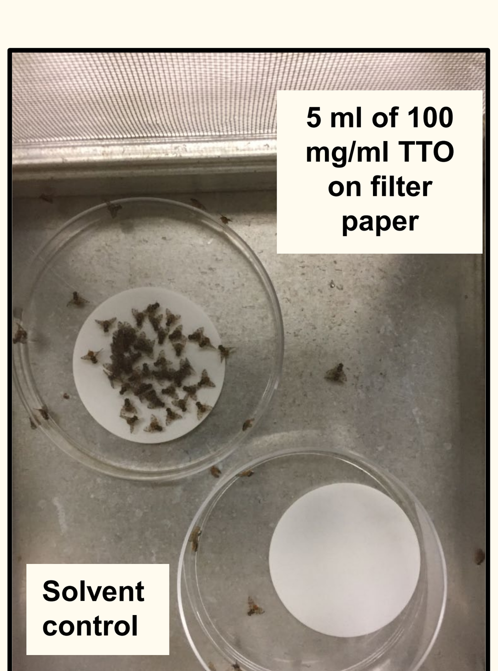

Fig. 8 TTO bioassay

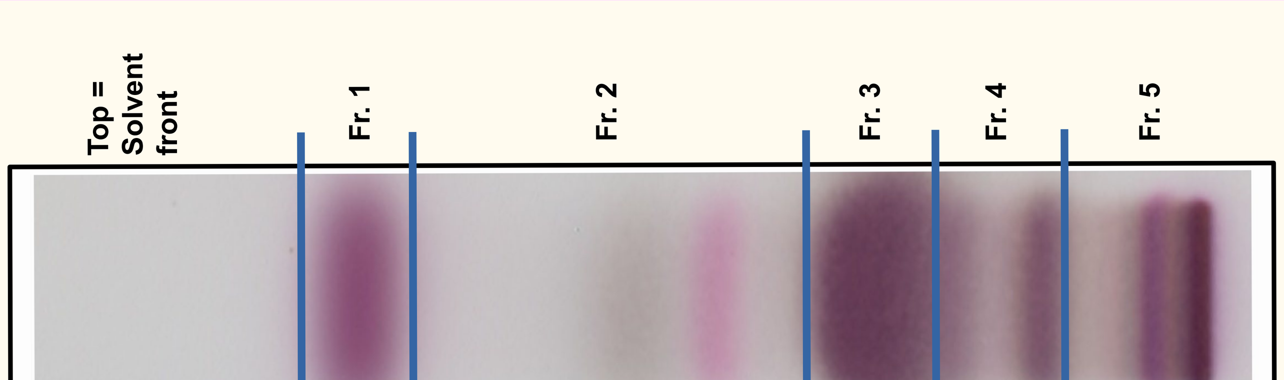

Fig. 9 Separation of TTO

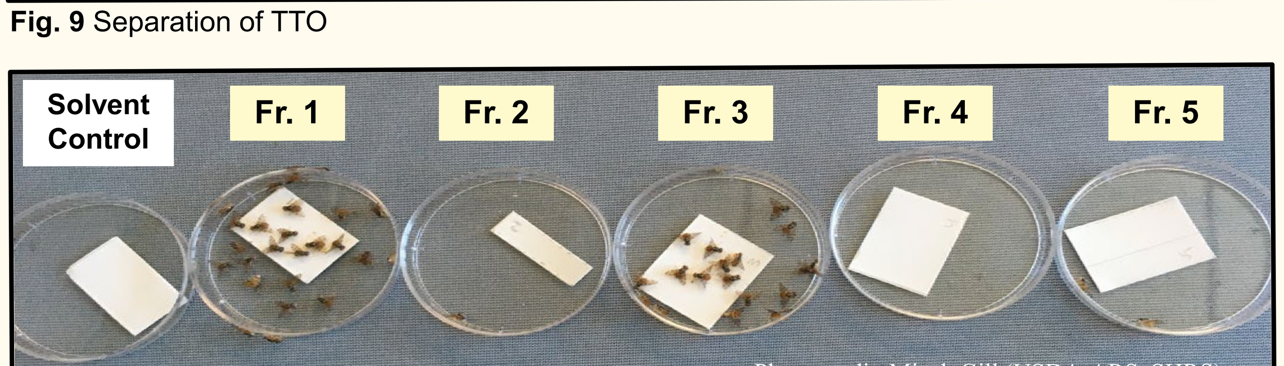

Fig. 10 Bioassay with TTO fractions

Results

- This separation technique provided a quick and inexpensive screening tool to assess the variability present among EO samples.
- Individual components were identified based on the distance traveled (retention factor, R_f).
- HPTLC revealed that there were both qualitative and quantitative differences among the various TTOs obtained from different sources (Fig. 11).
- Prep TLC fractions were extracted and stored for future studies
- GC-MSD profiles determined the purity and confirmed variations among TTOs (Fig. 12). They also facilitated identification of individual components with potential for attraction of male Medfly based on their fingerprint mass spectra (Figs. 13a & 13b).

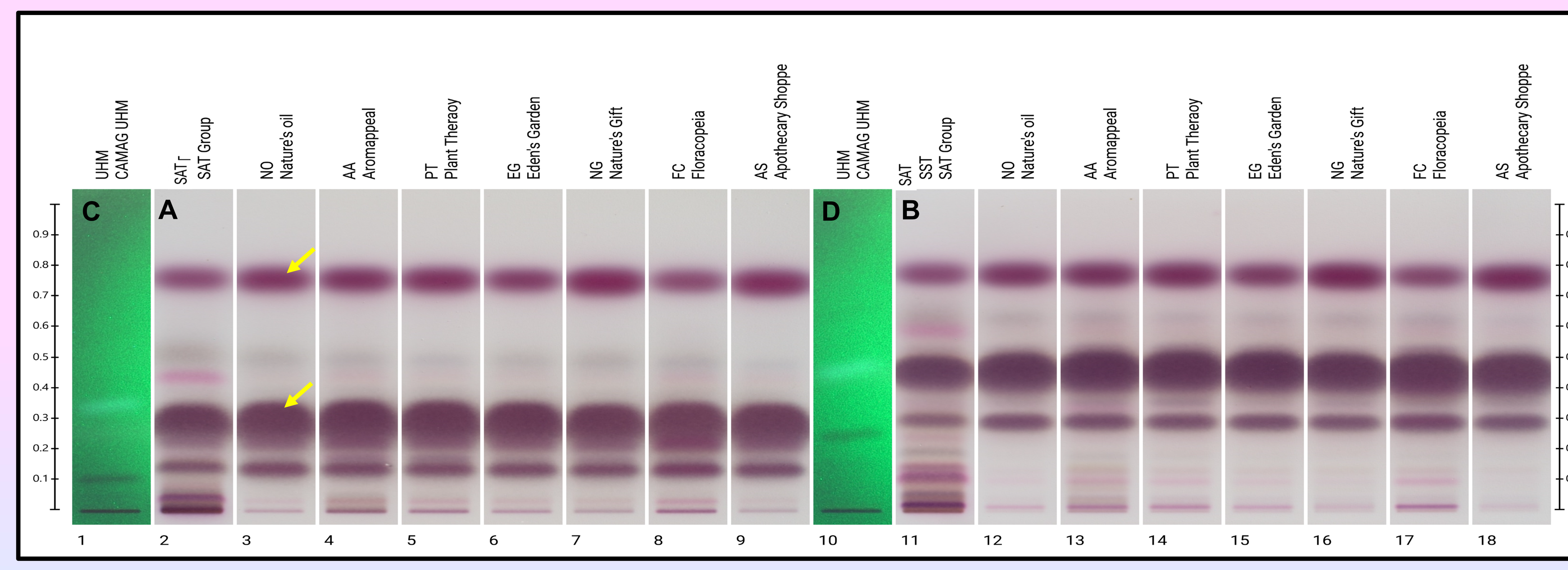

Fig. 11 (A) TTO samples developed with Hex:EtOAc (9:1) and derivatized with vanillin/H_2SO_4 reagent (visible light); (B) TTO samples developed with Hex:EtOAc (8:2) and derivatized with vanillin/H_2SO_4 reagent (visible light); (C) UHM developed with Hex:EtOAc (9:1) (UV_{254}); (D) UHM developed with Hex:EtOAc (8:2) (UV_{254}). Yellow arrows indicate fractions 1 & 3, which were attractive to male Medfly.

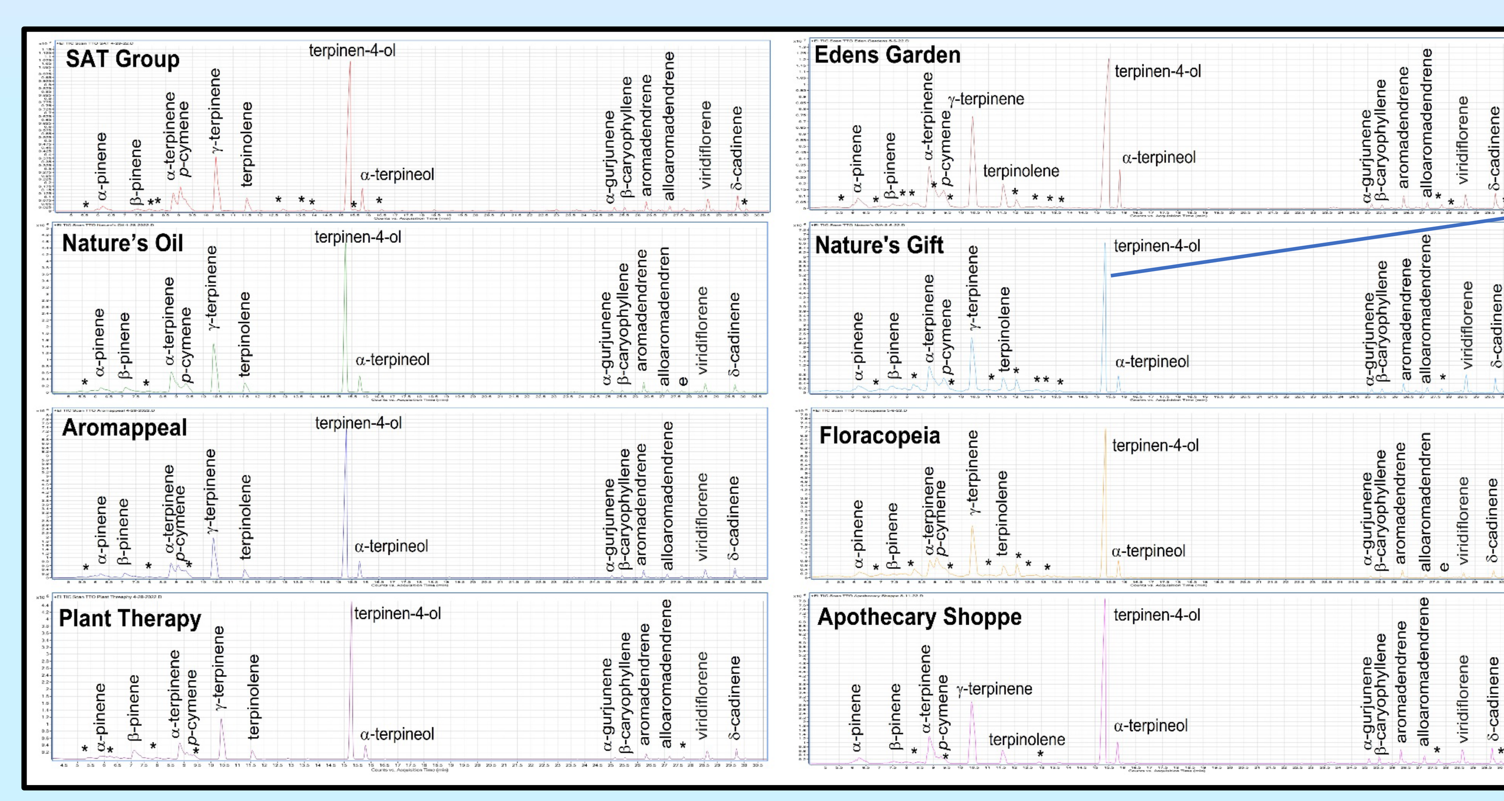

Fig. 12 GC-MSD profile of the various brands of TTO

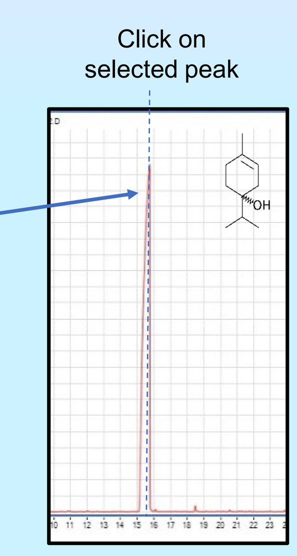

Fig. 13a Total ion chromatogram of terpinen-4-ol (GC-MS)

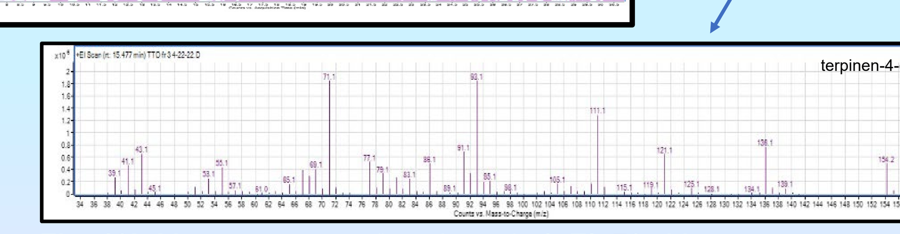

Fig. 13b Mass spectrum of terpinen-4-ol (EI, 70 eV)

Conclusions

- HPTLC offers automated precision and provides reproducible quantification.
  - shorter, more cost-effective, and eco-friendly separation.
  - non-destructive; samples can be further analyzed.
- HPTLC combined with GC-MSD
  - Produce accurate identification of individual components
  - Reveals valuable information regarding purity and manufacturer variations.
- The methods developed in this study will improve the separation, isolation, and identification of EO constituents attractive to male Medflies.
- These novel attractants will be evaluated further for potential improvement of lures to detect and manage this invasive agricultural pest.
- HPTLC is suitable for Multi-gradient development and 2-dimensional TLC.

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Acknowledgements and Disclaimer

The authors would like to thank Micah Gill, Elena Schnell and Teresa Narvaez for the bioassays, GC-MS analysis and technical support.

This research was supported in part by an appointment to the Agricultural Research Service (ARS) Research Participation Program administered by the Oak Ridge Institute for Science and Education (ORISE) through an interagency agreement between the U.S. Department of Energy (DOE) and the U. S. Department of Agriculture (USDA). ORISE is managed by ORAU under a DOE contract. All opinions expressed in this presentation are the author's and do not necessarily reflect the policies and views of USDA, ARS, DOE, or ORAU/ORISE.