Analysis of volatile metabolite profile of *Neonectria ditissima* infected apples.

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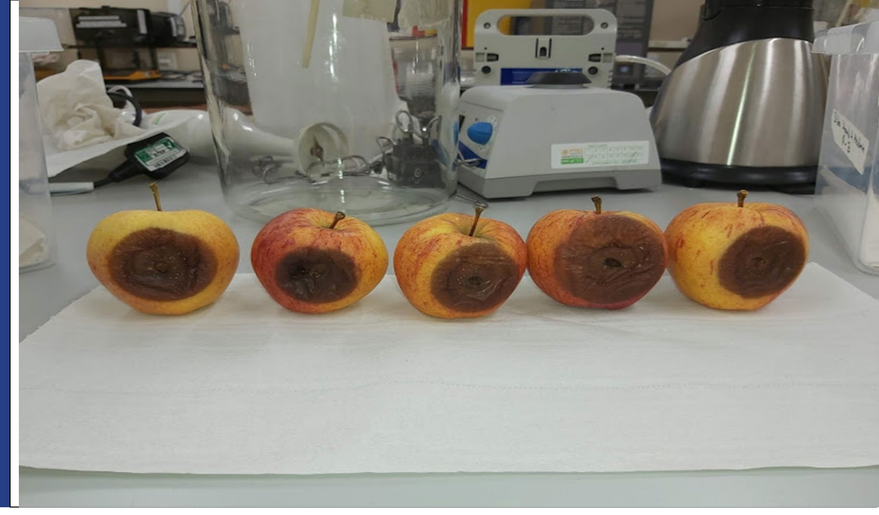


Background

Neonectria ditissima is a serious fungal pathogen of apple trees and fruits responsible for losses due to tree canker, loss of yield and fruit rots in storage. An estimated 10% of infected trees require replanting, costing £3.5K/ha combined with 3-5 % of ex-store losses estimated at £3-5 M *p.a.*

The long latency period of 3 months in Gala apples means early detection prior to storage is difficult. Storage in controlled atmospheres (5kPa CO_2, 1 kPa O_2) means regular disease assessment over extended storage 3-12 months (0.5-4.5°C) is hard to manage. In-store sampling of volatile organic compounds (VOCs) allows for early detection of disease onset and warns growers of the need for early marketing of consignments of fruit with a high incidence of infection.

This project follows on from a previous UKRI project (Low cost sensors for disease detection) to identify volatile signatures for early stage infection, and previously published work on indicators of microbial contamination (Kim *et al*., 2018).

Aim

- Analysis of VOC compounds associated with early *N. ditissima* infection in apples.
- To record changes in the VOCs profile of *N. ditissima* throughout disease progression and fruit maturation.
- Compare VOCs of disease markers in SmartFresh^{SM} (1-MCP) treated fruit
- Understand the impact of controlled atmosphere conditions on disease VOCs
- Identify biochemical pathways associated with disease VOC synthesis
- Identify gene expression profiles associated with key VOC determinants of host-pathogen response.

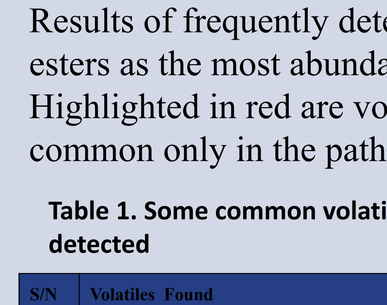
Study setting

Apples (*Malus pumila* cv. Gala), were harvested from an experimental 10-year-old orchard planted on M9 rootstock at NIAB-EMR. Fruits were transported to NRI for analysis. Seven-day old cultures of *N. ditissima* grown on PDA amended with chloramphenicol (1 Vial (50mg) per 500ml medium) were used to wound inoculate apples followed by incubation at 20°C for 49 days. Volatiles of pure cultures and inoculated fruit were captured on porapak columns after 2 hour sampling (1L/hour) at intervals of 2, 8, 14, 21, 28, 35, 42 and 49 days after inoculation. Samples were eluted with Hexane and run on GC-MS for analysis

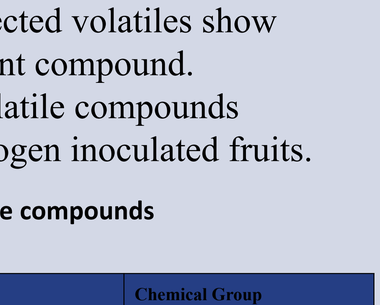
We are allowing the opportunity to display posters for the purposes of sharing knowledge, however this poster has not been either internally or externally peer reviewed.

Methods

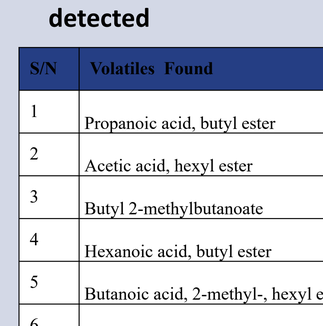
Headspace volatiles captured in glass jars



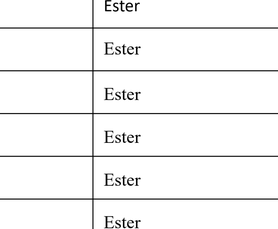
Volatiles collected unto porapak-Q columns using a vacuum pump



Captured volatiles are eluted into glass vial using dichloromethane



Eluted volatiles are analysed using a GC/MS



Results

Results of frequently detected volatiles show esters as the most abundant compound. Highlighted in red are volatile compounds common only in the pathogen inoculated fruits.

Table 1. Some common volatile compounds detected

S/N	Volatiles Found	Chemical Group
1	Propanoic acid, butyl ester	Ester
2	Acetic acid, hexyl ester	Ester
3	Butyl 2-methylbutanoate	Ester
4	Hexanoic acid, butyl ester	Ester
5	Butanoic acid, 2-methyl-, hexyl ester	Ester
6	Butanoic acid, butyl ester	Ester
7	Acetic acid, pentyl ester	Ester
8	Propanoic acid, hexyl ester	Ester
9	Hexanoic acid, hexyl ester	Ester
10	.alpha.-Farnesene	Terpenes
11	Ethyl acetate	Ester
12	Butanoic acid, hexyl ester	Ester
13	Hexanoic acid, propyl ester	Ester
14	Estragole	Phenolic
15	Acetic acid, chloro-, butyl ester	Ester
16	Heptanoic acid, butyl ester	Ester
17	2-Ethylbutyl acetate	Ester
18	Hexyl acetate	Ester
19	Propanoic acid, 2,2-dimethyl-, hexyl ester	Ester
20	Acetic acid, chloro-, 2-butoxyethyl ester	Ester
21	3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)-	Terpenoid
22	Styrene	Benzene
23	Hexanoic acid, ethyl ester	Ester
24	2-Methylbutylacetate	Ester
25	n-Butyl-2-methylbutyrate	ester

Key findings

- Changes in VOCs associated with *N. ditissima* infected apples were detected by GC-MS
- 2-Ethylbutyl acetate and Propanoic acid, 2,2-dimethyl-, hexyl ester were identified as indicators of the early stages of infection and were present until the late stages.
- Styrene, 3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)-, and Hexanoic acid, ethyl ester were only detected in late stages of infection.
- Identification of discriminatory volatiles against *N. ditissima* to serve as possible biomarkers for early detection of *N. ditissima* infection in storage seems feasible.

Conclusions

Disease of stored apples are most times only detected at advanced stages when it has become nearly impossible to prevent losses. Discriminatory volatile metabolites detected at early stages of infection are important for non-visual detection of *N. ditissima* in stored apples.

At the end of this study, we hope to obtain discriminatory volatiles against *N. ditissima* which can serve as possible biomarkers for early detection of *N. ditissima* infection in storage. Further studies would also be done for confirmation, development of suitable biomarkers and possible antifungal activities of the volatiles.

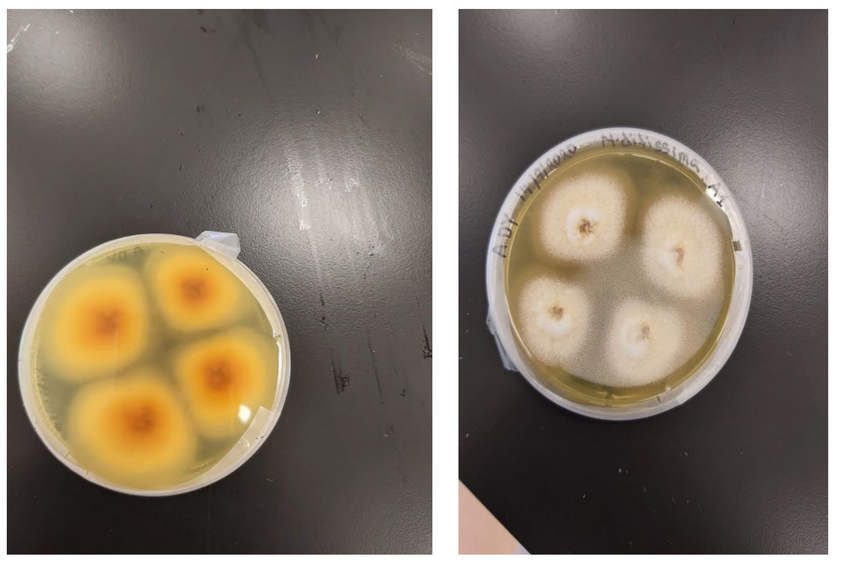


Figure 1. *N.ditissima* on PDA Plates

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References

Kim, S.M., Lee, S.M., Seo, J. and Kim, Y. (2018). Changes in volatile compounds emitted by fungal pathogen spoilage of apples during decay. *Postharvest Biology and Technology*, 146: pp. 51-59. https://doi.org/10.1016/j.postharvbio.2018.08.003

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