# Volatile Organic Compounds Associated With *Neonectria ditissima* Infection In Apples (*Malus pumila* cv Gala)


**Asoo D. Yaji[1], Steven Harte[1], Iain Goodall[2], Richard Colgan[1]**

1.Natural Resources Institute, University of Greenwich, Chatham Maritime, Kent ME4 4TB, UK
2.Faculty of Engineering and Science, University of Greenwich, Chatham Maritime, ME4 4TB, Kent, UK



Asoo Dorcas Yaji
A.D.Yaji@greenwich.ac.uk


## Introduction

Apples are a popular and widely cultivated temperate tree fruit. To schedule harvested crop over extended marketing periods between 3-12 months it is necessary to store fruits at low temperatures (1-5$^0$C) and under controlled atmosphere conditions (0.6-3.0% $O_2$, and 0-5% $CO_2$). However, post-harvest fruit diseases caused by fungal pathogens is still a limiting factor in long term storage of apples[1], resulting in 3-7% losses. *Neonectria ditissima* is one of these diseases, a fungal pathogen of apples that infect apple trees forming cankers that limits crop yield and fruits that develop into significant incidence of rots.[2]

**Figure 1**. Common Symptoms of *Neonectria ditissima*

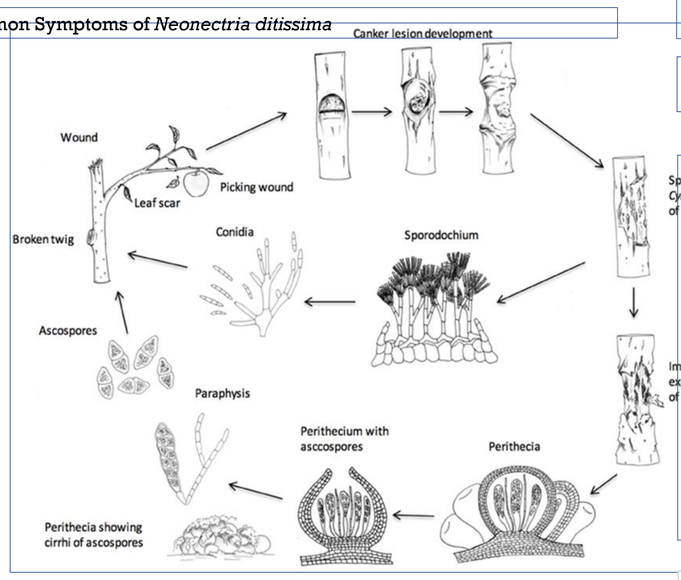


**Figure 2:** Life cycle of *Neonectria ditissima* – after Agrios 1997(Gomez-Cortecero *et al.*, 2016)

Fungal contamination and rot formation changes the volatile organic compound (VOC) profile emitted by apple fruits during storage[3]. Understanding how changes in the VOC profile occurs over time and with the onset of disease may in the future provide diagnostic tools to better manage Neonectria disease in stored fruit.

## Aim

## Methods

Gala apples at harvest were inoculated with *Neonectria ditissima.* At intervals of 7 days, apples were removed from store (1°C), placed in 5 L glass flask (fig.3), sealed, and incubated at 20°C for one hour after which a charcoal filtered air flow of 1 L/min was maintained for one hour through the Volatile Capture Trap (VCT) with volatile emissions captured on a porapak-Q absorbent filter (fig.3).

Eluted volatiles were analysed using Gas Chromatography coupled with Mass Spectrometry (GC/MS). Volatiles were capture in three replicates for both inoculated and healthy control groups at 2, 8, 14, 21, 28, 35, and 42 days post-inoculation. Captured volatiles were eluted using 1 mL of dichloromethane (DCM) (fig.3).

**Figure 3**. Methods of Volatile capture (Glass Jar and porapak-Q absorbent filter)

## Results

Esters were the most abundant group of volatiles produced with benzenes, terpenes, ketones, and alkenes present in lesser amounts. The total volume (peak area) of volatiles produced from inoculated fruit was lower than non-inoculated control batches of apples (Fig.4). Volatiles common to both inoculated and non-inoculated apples each had a higher peak area in non-inoculated apples as compared to the inoculated apples.

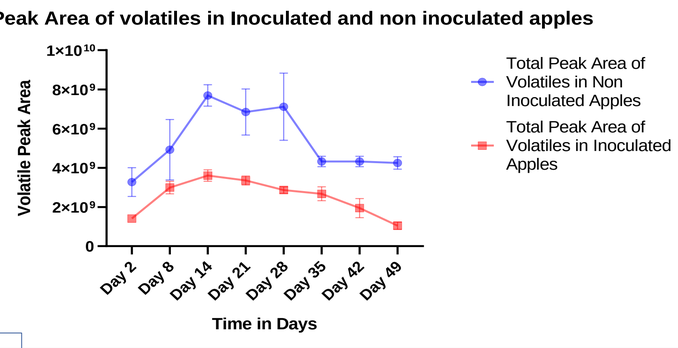


**Figure 4.** Total peak area covered by volatiles produced weekly in inoculated and non-inoculated fruits

The abundance of volatiles in inoculated fruits changed with diseases progression: Dodecyl hexanoate, 9-decenyl hexanoate and hexyl butanoate were found in the early stages of the infection. (Fig.5). Styrene and terpinen-4-ol (Fig.6), ethyl hexanoate, ethyl butanoate, ethyl pentanoate and 2-methylpentyl formate (Fig.7) constituted the main VOCs emitted during later stages of apple fruit decay. Styrene is a fungal metabolite generated through the breakdown of cinnamic acid, associated with fungal fruit spoilage and shows no antifungal activity. However, in vitro test show Terpinen-4-ol has antifungal activity (Fig.8 & 9).

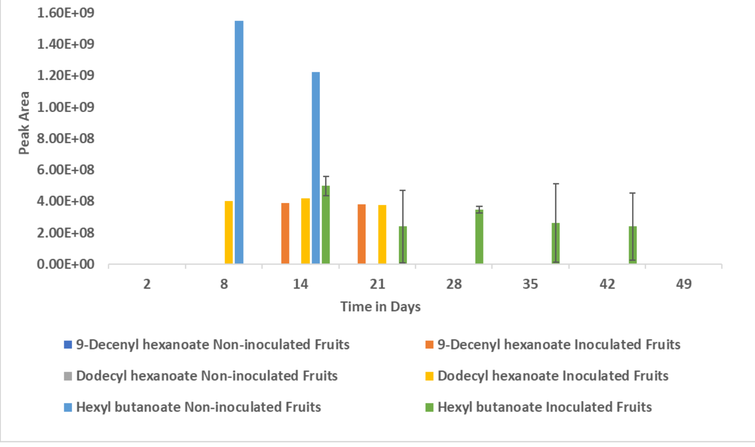


**Figure 5**. Disease discriminatory volatiles produced at early stages of fruit decay

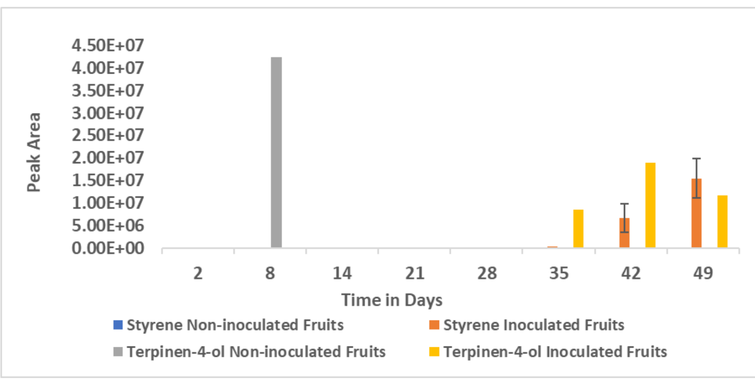


**Figure 6**. Styrene and Terpinen-4-ol produced over time

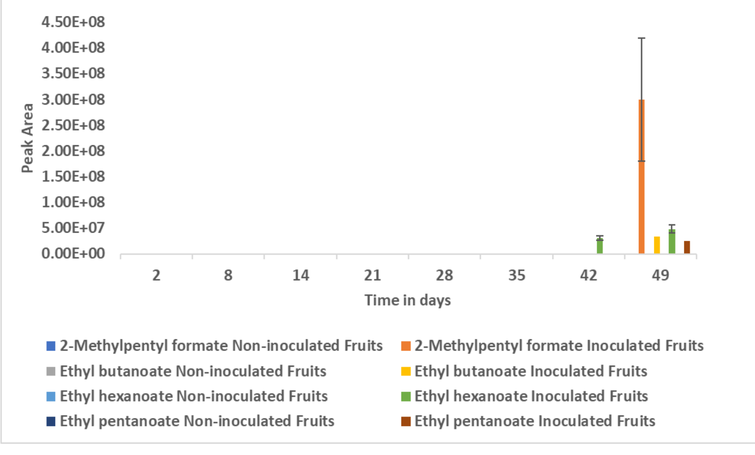


**Figure 7**. Disease discriminatory volatiles produced at late stages of fruit decay

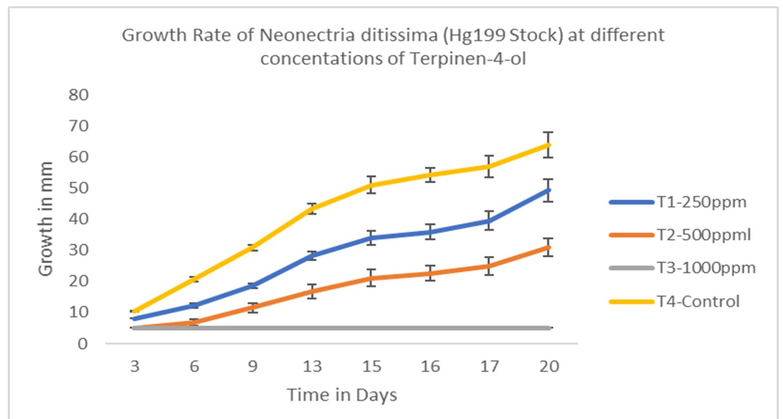


**Figure 8**. Antifungal effect of Terpinen-4-ol on *Neonectria ditissima*

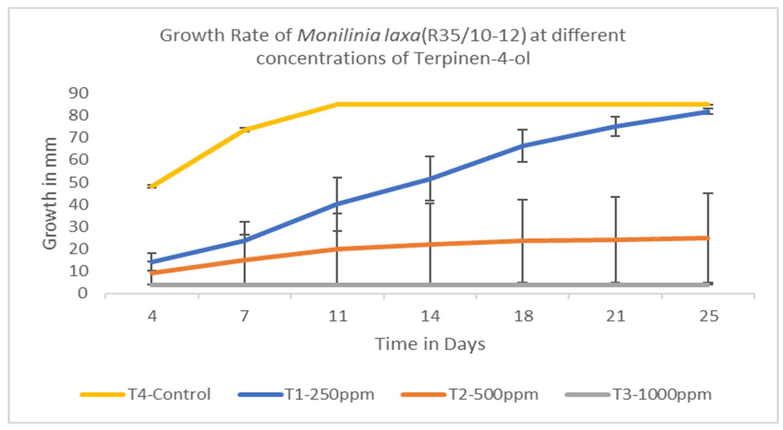


**Figure 9**. Antifungal effect of Terpinen-4-ol on *Monilinia laxa*

## Discussion

Styrene appears to be a key volatile associated with the later stages of *N. ditissima* infection in Gala and has been reported previously as an important fungal metabolite[3], generated through the metabolism of cinnamic acid a key component of the phenylalanine pathway. Terpinen-4-ol is identified as a natural host plant defence compound. While present in healthy tissue it was also associated with disease development and has been reported to have anti-fungal activity[4]. Our studies confirm it was able to reduce mycelial growth rate of different isolates of *Neonectria ditissima* and *Monilinia laxa* in in vitro-studies. Antifungal activity of Terpinen-4-ol was more effective against *Neonectria* compared to other important apple pathogens and may form the basis of the natural fruit defence against *Neonectria*- that is variable between varieties. Further studies are on-going to determine its inhibitory effect on spore germination and restricting disease spread in inoculated apples. Through developing a better understanding of how changes in the VOC profile occurs with the onset of disease, we may in the future be able to develop diagnostic tools to better manage Neonectria disease.

## References


1. Wenneker, M., & Thomma, B. P. H. J. (2020). Latent postharvest pathogens of pome fruit and their management: from single measures to a systems intervention approach. Eur. J. Plant Pathol, *156*(3), 663–681.

2. Wenneker et al., (2017). Development of a method for detection of latent European fruit tree canker (Neonectria ditissima) infections in apple and pear nurseries. Eur. J. Plant Pathol, *148*(3), 631–635.

3. Kim et al., (2018). Changes in volatile compounds emitted by fungal pathogen spoilage of apples during decay. *Postharvest Biology and Technology*, *146*(August), 51–59.

4. Yu, D., Wang, J., Shao, X., Xu, F., and Wang, H. (2015). Antifungal Modes of Action of Tea Tree Oil and its Two Characteristic Components Against *Botrytis cinerea*. *Journal of Applied Microbiology*, 119: 1253-1262.


## Acknowledgment


This research is funded by the Food and Nutrition Security Initiative (FaNSI)


ICPP 2023 ONE HEALTH for all plants, crops and trees LYON 20-25 August, France