

Studentship Project: Annual Progress Report 11/2022 to 11/2023

Student Name:	Hamish McLean	AHDB Project Number:	SF/TF 170/a
Project Title:	Investigating the abiotic and biotic factors affecting apple canker (<i>Neonectria ditissima</i>) symptom development		
Lead Partner:			
Supervisor:	Matevž Papp-Rupar, Alexey Mikaberidze, Xiangming Xu		
Start Date:	2021-10-20	End Date:	2025-09-19

1. Project aims and objectives

Objective 1 Root Microbiome

- Aim: To investigate the effects of site and scion genotype on the root microbiome and explore its association with canker susceptibility.
- Root samples were collected from three fully randomised field experiments grown at 3 commercial apple growing farms in Kent, UK from seven commercial apple cultivars ('Royal Gala', 'Braeburn', 'Scifresh', 'Nicoter', 'Civni', 'Grenadier', and 'Golden Delicious') grafted on M9 rootstock.
- Root endophyte DNA was extracted and analysed using 16S/ITS amplicon metabarcoding to measure bacterial and fungal community structure and composition.
- The effects of site, cultivar, and planting date on various metrics were explored, including:
 - Diversity within samples (alpha diversity).
 - Diversity between samples (beta diversity).
 - The correlation of the most abundant taxa with canker incidence.
- This research will be published as a scientific paper.

Objective 2 Waterlogging

- Aim: To investigate the effects of winter waterlogging on canker susceptibility.
- Two potted tree experiments will be used to test the effects of winter waterlogging on canker susceptibility.
- The experiments each consist of 300 two-year-old MM106 apple rootstocks in pots in a polytunnel.
- The first experiment was planted in spring 2022.
- Trees were inoculated with *Neonectria ditissima* spores in autumn 2023.
- Waterlogging treatments of 1, 2, 4, and 8 weeks were executed in winter 2022/2023.
- Tree diameter and height were measured at the start and end of the growing season in 2023.

The results described in this summary report are interim and relate to one year. In all cases, the reports refer to projects that extend over a number of years.

While the Agriculture and Horticulture Development Board seeks to ensure that the information contained within this document is accurate at the time of printing, no warranty is given in respect thereof and, to the maximum extent permitted by law, the Agriculture and Horticulture Development Board accepts no liability for loss, damage or injury howsoever caused (including that caused by negligence) or suffered directly or indirectly in relation to information and opinions contained in or omitted from this document. Reference herein to trade names and proprietary products without stating that they are protected does not imply that they may be regarded as unprotected and thus free for general use. No endorsement of named products is intended, nor is any criticism implied of other alternative, but unnamed, products.

- Cankers were assessed at the end of the growing season.
- The second experiment was planted in spring 2023. The inoculations, waterlogging treatments, and measurements are upcoming.
- This research will be published as a scientific paper.

Objective 3 Biocontrols

- Aim: To investigate the potential of *Sphingomonas* spp. bacteria as microbial control agents against apple canker.
- Previous microbial community analysis of apple leaf scar tissue indicated that *Sphingomonas* spp. endophytes are linked to increased resistance to canker and improved tree health.
- This objective is targeted approach to isolate and assess these potential new biocontrol organisms.
- Apple leaf scar samples collected from a diverse range of apple cultivars around the site at NIAB East Malling provide a source of isolates.
- *Sphingomonas* spp. were selectively isolated from apple tissues.
- Isolates will be identified using amplicon sequencing.
- Isolates will be screened for *Neonectria ditissima* antagonism *in vitro*.
- Finally, the best candidates will be tested *in planta* for their effect on plant growth, health, and canker biocontrol potential.
- The best isolates will also be sequenced to identify genes associated with biocontrol activity.
- This research will be published as a scientific paper.

2. Key messages emerging from the project

Objective 1 Root Microbiome

- An association between root microbes and canker susceptibility could have important implications for canker management.
- Understanding the role of root microbiome in canker susceptibility and variation in root microbiome between different sites and cultivars will help growers' decision making and help them to better understand canker risks on their sites.
- This research could also lead to the development of microbial root amendments to help manage canker.

Objective 2 Waterlogging

- Understanding the effects of waterlogging on canker susceptibility will enable growers to predict the need for tailored canker management strategies depending on the winter waterlogging severity and help them understand the interaction of waterlogging and tree health.
- This research could lead to the development of new strategies to manage canker on waterlogged sites.

Objective 3 Biocontrols

- Understanding the biocontrol potential of new groups of bacteria will lead to new, better biocontrols.
- With increasingly stringent regulation on fungicides, biocontrols are an important disease management tool.

3. Summary of results from the reporting year

Objective 1 Root Microbiome

- Preliminary results indicate that site has a significant effect on root endophyte diversity within and between samples.
- Planting date and cultivar do not have a significant effect on diversity within and between samples.

Objective 2 Waterlogging

- Many of the trees in the 8-week waterlogging treatment died, showing that the treatments were in an effective range.
- Preliminary results indicate that winter waterlogging does not have a significant effect on tree growth (diameter and height) or canker incidence.
- Tree health seemed to recover quickly after the waterlogging treatment and by the start of the growing season, the waterlogged trees were able to grow just as vigorously as the untreated control.
- The canker inoculations were not very successful and very few inoculation points developed lesions. This makes it difficult to accurately assess the effects of waterlogging on canker susceptibility.

Objective 3 Biocontrols

- 16S sequences from many of the isolates identified them as *Sphingomonas* spp.
- Phylogenetic trees indicate that some of these isolates are closely related to some of the species previously linked to canker resistance.
- The *Sphingomonas* spp. isolates tested did not have significant antagonism against *N. ditissima* on yeast extract agar. More isolates and other media will be tested.

4. Key issues to be addressed in the next year

Objective 1 Root Microbiome

- Correlations between the abundance of the top taxa with canker susceptibility are currently being investigated.
- Results are being prepared for a draft of the upcoming publication.
- The publication will be submitted in spring 2024

Objective 2 Waterlogging

- The second iteration of the experiment will be carried out.
- Inoculations will be done in autumn 2023, waterlogging in winter 2023/2024, and physiology measurements and canker assessments will be done in 2024.
- Results will be compiled and analysed to compare the effects of waterlogging treatments on canker susceptibility.

Objective 3 Biocontrols

- 30 new isolates have been made.
- These new isolates will be identified and tested for *N. ditissima* antagonism.
- The best candidates will be tested *in planta* for biocontrol potential.

5. Outputs relating to the project

(events, press articles, conference posters or presentations, scientific papers):

Output	Detail
Symposium presentation	I presented my research plans at the crop science symposium at the University of Reading in 2021 and 2022.
CTP conference presentation	I presented my research at the CTP events at NIAB East Malling in 2022 and 2023.
IOBC conference poster	I attended the 2022 IOBC Pome Fruit Disease Conference in Bulgaria where I presented a poster outlining Objective 1 of my research.

6. Partners (if applicable)

Scientific partners	
Industry partners	
Government sponsor	