Studentship Project: Annual Progress Report 11/2023 to 11/2024

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

1. Project aims and objectives

Chapter 1: Root microbiome and apple canker

- Aim: To investigate the effects of site, planting season, and scion genotype on the apple root microbiome and explore root microbiome association with canker susceptibility.
- Objective1.1: To understand the effect of site, planting season, and scion genotype on apple root microbiome assembly (size, within sample alpha diversity, between sample beta diversity).
- Objective 1.2: To explore potential associations between microbiome metrics (size, alpha diversity, beta diversity, abundances of specific taxa) and canker lesion counts.
- Root samples were collected from a field experiment where seven commercial apple cultivars ('Royal Gala', 'Braeburn', 'Scifresh', 'Nicoter', 'Civni', 'Grenadier', and 'Golden Delicious') grafted on M9 rootstocks were grown at 3 commercial apple growing farms in Kent, UK. DNA from root-associated microbes was extracted and analysed using 16S/ITS amplicon sequencing to assess bacterial and fungal communities.
- This research has been submitted for publication in FEMS Microbial Ecology journal.

Chapter 2: Waterlogging and apple canker

- Aim: To investigate the effects of waterlogging on apple growth and canker susceptibility.
- Objective 2.1: To understand the effects of duration of winter waterlogging on rootstock growth and canker susceptibility.
- Objective 2.2: To understand the effects of waterlogging timing and rootstock genotype on rootstock growth and canker susceptibility.
- Objective 2.3: To understand the effects of waterlogging timing and duration on grafted apple trees.
- These objectives are being addressed with a series of four potted tree experiments.

- Experiment 1: To test the effect of duration of winter waterlogging (0, 1, 2, 4, 8 weeks) on growth and canker susceptibility of MM106 apple rootstocks.
- Experiment 2: Repeat of Experiment 1 with improved inoculation methods (rasp wound inoculations as well as leaf scar) but with the same waterlogging treatments.
- Experiment 3: To test the effects of waterlogging season (autumn, winter, spring) and rootstock cultivar (MM106, M9) on growth and canker susceptibility.
- Experiment 4: To test the effects of waterlogging season (winter, spring) and duration (0, 2, 4 weeks) on growth and canker susceptibility of grafted trees (Braeburn on M9).

Chapter 3: Waterlogging and the apple microbiome

- Aim: To investigate the effects of waterlogging on the soil, root, and endophyte microbiomes.
- Objective 3.1: To explore the effects of winter waterlogging on the bulk soil microbiome.
- Objective 3.2: To explore the effects of winter waterlogging on the root microbiome of apple rootstocks.
- Objective 3.3: To explore the effects of winter waterlogging on the above ground endophyte microbiome of apple rootstocks.
- Root, soil, and bark samples will be collected from waterlogging experiments 1 and 2 to assess the effect of four weeks of continuous winter waterlogging on the respective microbiomes.

Chapter 4: Biocontrol of apple canker

- Aim: To investigate the potential of *Sphingomonas* spp. bacteria as microbial control agents against the apple canker pathogen *Neonectria ditissima*.
- Objective 4.1: To assess the *in vitro* antagonism of *Sphingomonas* isolates against *N. ditissima*.
- Objective 4.2: To identify genes associated with plant interactions and the production of secondary metabolites in *Sphingomonas* isolates which may be involved in plant growth promotion or canker resistance.
- Objective 4.3: To develop a qPCR assay for the detection and quantification of *Sphingomonas* isolates in apple tissues.
- Objective 4.4: To investigate the survival and biocontrol potential of *Sphingomonas* isolates *in planta*.
- 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.
- *Sphingomonas* isolates were selectively isolated from apple leaf scar samples collected from a diverse range of cultivars around the site at NIAB East Malling.

2. Key messages emerging from the project

Chapter 1: Root microbiome and apple canker

- Site accounts for most of the variation in apple root-associated microbiomes.
- Planting season, and the storage of trees prior to planting, can affect the development of the root-associated microbiome long-term.
- Scion genotype only has a limited effect on the root-associated microbiome but does appear to control a sub-population of bacteria.
- Some fungal and bacterial taxa can associate with canker severity; however, the causal nature of these relationships remains unclear.

Chapter 2: Waterlogging and apple canker

- Preliminary results indicate that long-term continuous winter waterlogging can kill apple rootstocks. However, trees can survive shorter waterlogging durations and can recover in the following growing season with no large detriment to growth or canker susceptibility.
- The experiments are ongoing, and results are not yet conclusive.

Chapter 3: Waterlogging and the apple microbiome

- Samples are being collected and have not yet been sequenced or analysed.

Chapter 4: Biocontrol of apple canker

- *Sphingomonas* isolates collected from apple leaf scars grow well in laboratory conditions and can be grown in bulk.
- The *Sphingomonas* isolates did not exhibit strong *in vitro* antagonism against *N. ditissima*.
- Mechanisms of the previously observed plant growth promotion and canker resistance are unclear.
- Whole genomes have been sequenced for the *Sphingomonas* isolates.
- We hope to identify potential mechanisms through genome mining.

3. Summary of results from the reporting year

Chapter 1: Root microbiome and apple canker

- Site was the primary factor affecting apple root fungal and bacterial microbiomes. This is consistent with previous research.
- Planting season had a small but significant effect on fungal and bacterial microbiomes. This suggests that differences in the root microbiome at planting persisted long-term. This effect was highly dependent on site, which suggests that the local biotic and abiotic conditions at planting contributed to this variation.
- Scion genotype had a limited effect on the apple root microbiome, affecting bacterial diversity, and the abundance of a few specific fungal and bacterial taxa. Scion genotype has previously been shown the have much stronger effect on apple rhizosphere microbiome independent of rootstock in controlled conditions. We however only found a limited effect, which could be due to the dominant effect of site, or the sampling of root endophytes and microbes closely associated with the surface of the root.
- Fungal and bacterial diversity positively correlated with canker lesion counts. This experiment does not, however, allow us to conclude the causal nature of this relationship.
- Some fungal and bacterial taxa were associated with canker lesion counts. While the causal nature of these relationships are uncertain, some of these associated taxa may be worth further investigation.

Chapter 2: Waterlogging and apple canker

- Rasp wound inoculations in had much higher incidence than the leaf scar inoculations used in the experiment the previous year. Preliminary results suggest that winter waterlogging did not have a large effect on canker incidence.
- Preliminary results suggest that winter waterlogging did not have a large effect on change in diameter or height of rootstocks.
- Measurements are ongoing and results are not yet conclusive.

Chapter 3: Waterlogging and the apple microbiome

- Results for this chapter are not yet available.
- Root and soil samples have been collected for the waterlogging duration experiments.
- Bark samples will be collected destructively at the end of the growing season.

Chapter 4: Biocontrol of apple canker

- Isolates were identified as *Sphingomonas* by 16S amplicon sequencing. Phylogenetic trees based on these sequences showed similarity with the taxa previously identified with beneficial effects on tree growth and canker resistance.
- *Sphingomonas* isolates did not exhibit strong *in vitro* antagonism against *N. ditissima*. This suggests that the beneficial effects on tree growth and canker resistance is through another, as yet unknown mechanism.
- Whole genome sequencing for the *Sphingomonas* isolates have been completed. Genes involved in plant interactions and production of secondary metabolites will be analysed to infer potential beneficial mechanisms for the host plant.

4. Key issues to be addressed in the next year

Chapter 1: Root microbiome and apple canker

- Paper is currently under review. Corrections will be addressed when necessary.

Chapter 2: Waterlogging and apple canker

- Experiments 3 and 4 will be inoculated with *N. ditissima*. To improve these inoculations, we will be using a wild source of *N. ditissima* spores.
- Winter waterlogging treatments for experiment 2, 3, and 4 will be conducted.
- Spring waterlogging treatments for experiments 3 and 4 will be conducted.
- Diameter and height will be measured in winter, spring, and summer for experiments 2, 3, and 4.
- Experiment 1 will be destructively harvested and the roots assessed for dead tissue.
- A manuscript will be prepared for publication.

Chapter 3: Waterlogging and the apple microbiome

- Bark samples will be destructively collected from experiment 1.
- Bark, root, and soil samples will be processed, and DNA will be extracted.
- ITS/16S amplicons will be sequenced from these samples.
- Sequences will be analysed to explore the effects of the waterlogging treatments on fungal and bacterial microbiome size, within sample alpha diversity, and between sample beta diversity.
- A manuscript will be prepared for publication.

Chapter 4: Biocontrol of apple canker

- Whole genome sequences of the *Sphingomonas* isolates will be mined for plant growth promoting traits and antimicrobial biosynthesis pathways to identify potential biocontrol mechanisms.

- A qPCR assay will be developed using the sequences to detect *Sphingomonas* isolates in plant tissues.
- A detached branch experiment will be conducted to test survival and *in planta* interaction with *N. ditissima*, using the qPCR assay.

5. Outputs relating to the project
(events, press articles, conference posters or presentations, scientific papers):

Output	**Detail**
Paper submission	Paper for chapter 1 submitted for publication in FEMS microbiology ecology
Conference presentation	Presented results from chapter 1 and an overview of chapters 2 and 4 at the European apple canker conference in Geisenheim, Germany
CTP event presentation	Presented research at the CTP events in winter 2023 and summer 2024

6. Partners (if applicable)

Scientific partners	
Industry partners	
Government sponsor	