Studentship Project: Annual Progress Report 10/2024 to 10/2025

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:**	2026-03-19

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.
- Objective 1.1: To understand the effect of site, planting season, and scion genotype on apple root microbiome (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 and grown at 3 commercial apple 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 published at https://doi.org/10.1093/femsec/fiaf014.

Chapter 2: Effects of waterlogging on apple tree growth canker symptom expression

- Aim: To investigate the effects of waterlogging on apple tree/rootstock growth and canker expression.
- Objective 2.1: To understand the effects different durations of winter waterlogging on rootstock growth and canker symptom expression.
- Objective 2.2: To understand the interaction of waterlogging timing and rootstock genotype on rootstock growth and canker expression.
- Objective 2.3: To understand the interaction of waterlogging timing and duration on growth and canker expression in Braeburn/M9 grafted apple trees.
- These objectives were addressed with a series of four potted tree experiments:

- Experiment 1 was designed to test if 1, 2, 4, and 8 weeks of waterlogging in winter (December/January) reduce growth of MM106 (semi-vigorous) apple rootstocks and increase canker symptom expression on artificially inoculated on leaf scars.
- Experiment 2 was a repeat of experiment 1 with improved inoculation methods, namely, rasp wounds and leaf scars were inoculated with *N. ditissima*. The same waterlogging timing and durations were used as in experiment 1.
- Experiment 3 tested the interaction of waterlogging timing (autumn, winter, spring) and rootstock cultivar (MM106, M9) on growth and canker expression on rasp wounds and leaf scars.
- In experiment 4 we tested if a combination of waterlogging timing (winter, spring) and duration (0, 2, 4 weeks) reduces growth of grafted trees (Braeburn on M9) and increases canker expression on rasp wounds and leaf scars.

Chapter 3: Persistent effects of winter waterlogging and the apple root and soil microbiome

- Aim: To investigate the effects of moderate winter waterlogging on apple soil and root microbiomes.
- Root and soil bark samples were collected from waterlogging experiments 1 and 2 (see above for details) to assess the effect of four weeks of continuous winter waterlogging on the respective microbiomes.
- Objective 3.1: To investigate the effects of a single 4-week waterlogging treatment on community size, alpha diversity, beta diversity, and microbial functions across experimental replicates.
- Objective 3.2: To investigate the differences between a single and repeated 4-week winter waterlogging treatments on community size, alpha diversity, beta diversity, and microbial functions in a single experimental replicate.

Chapter 4: The potential of *Sphingomonas* as apple canker biocontrol agents

- Aim: To investigate the potential of *Sphingomonas* spp. bacteria as microbial control agents against the apple canker pathogen *Neonectria ditissima*.
- 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 Niab site at East Malling, including ‘Braeburn’, ‘Fiesta’, ‘Queen Cox’, ‘Royal Gala’, ‘Discovery’, and ‘Saturn’.
- 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 biocontrol activity.

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 affect 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

- 8 weeks of continuous winter waterlogging killed most apple MM106 rootstocks. However, this effect was not consistent between years. 4 weeks of autumn waterlogging killed about half of M9 and MM106 rootstocks. Rootstocks survived 1–4 weeks of continuous waterlogging in winter and spring. All surviving rootstocks (across all waterlogging durations and seasons) recovered in the following growing season with no significant detriment to growth or canker expression.
- In experiment 2, waterlogging significantly increased diameter growth rate especially for the 2-week treatment. In experiment 3, waterlogging significantly affected leaf scar canker expression, where it was lower with 4 weeks of waterlogging in autumn than the control.
- There were large differences in canker expression and growth rate between rootstock genotypes.

Chapter 3: Waterlogging and the apple microbiome

- A single 4-week waterlogging treatment consistently affected alpha diversity but did not consistently affect beta diversity across experimental replicates. This suggests that a single waterlogging treatment results in a similar alpha diversity response in communities with different initial diversity and composition, but the change in composition in response to waterlogging is not consistent and varies between communities with different initial diversity and composition.
- Single and repeated waterlogging treatments did not consistently affect alpha diversity but did consistently affect beta diversity. This suggests that a second waterlogging treatment contributes to the shift in microbiome composition after a single treatment, but richness, diversity, and evenness do not respond the same after a second treatment.

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 *in vitro* antagonism against *N. ditissima*.
- Comparing genomes of novel *Sphingomonas* isolates with a selection of *Sphingomonas* type strains revealed many genes associated with plant interactions and plant growth-promoting traits, suggesting that these organisms are adapted to living in and around plants, and may confer benefits to their hosts including iron, phosphorus, and nitrogen biofertilisation and alleviation of oxidative and salinity stress.
- There were a few genes associated with biocontrol traits identified, including genes involved in the synthesis of the antifungal pyrrolnitrin and antibacterials rebeccamycin, toxoflavin, and kanamycin, and genes associated with pattern-triggered immunity including teichuronic acid and lipopolysaccharide elicitors, suggesting a potential role in disease suppression.

3. Summary of results from the reporting year

Chapter 1: Root microbiome and apple canker

- The results for this work have already been published in 2024. No additional progress was made in 2025.

Chapter 2: Waterlogging and apple canker

- Experiments 1 and 2: Rasp wound inoculations had much higher incidence than leaf scar inoculations. Winter waterlogging had little to no effect on growth or canker expression.
- Experiment 3: There were significant differences in growth and canker expression between genotypes and small effects of waterlogging on canker expression.
- Experiment 4: There were no significant effects of waterlogging on grafted tree growth or canker expression.

Chapter 3: Waterlogging and the apple microbiome

- Community size did not respond to waterlogging but it did significantly vary between experiments and over time in experiment 1.
- Alpha diversity had some consistent responses to waterlogging across experiments for all microbiomes but did not consistently respond to repeated waterlogging treatments in experiment 1.
- Conversely, beta diversity did not consistently respond to waterlogging across experiments for all microbiomes but did consistently respond to repeated waterlogging treatments in experiment 1.

Chapter 4: Biocontrol of apple canker

- *Sphingomonas* isolates did not exhibit strong *in vitro* antagonism against *N. ditissima*. There were 7 distinct 16S genotypes.
- Only one of the seven strains was identified at the species level. Some may be sufficiently different to be previously undescribed species.
- Many genes were identified with plant interaction and plant growth-promoting traits, and a few genes were identified with biocontrol traits.
- Biosynthetic gene clusters were identified, but most did not have conclusive matches in the MiBIG database, and their functions are not clearly defined.

4. Key issues to be addressed in the next year

Chapter 1: Root microbiome and apple canker

- The results for this work have already been published.

Chapter 2: Waterlogging and apple canker

- The chapter has been drafted as a manuscript with preliminary figures and models but requires review and refinement before thesis submission and publication.

Chapter 3: Waterlogging and the apple microbiome

- The chapter has been drafted as a manuscript with preliminary figures and models but requires review and refinement before thesis submission and publication.

Chapter 4: Biocontrol of apple canker

- The chapter has been drafted as a manuscript with preliminary figures and models but requires review and refinement before thesis submission and publication.

5. Outputs relating to the project

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

Output	Detail
Paper submission	Paper for chapter 1 published in FEMS microbiology ecology
Conference presentation	Presented results from chapter 1 and a poster for chapters 2 and 3 at the IOBC BENEFruits conference in Wageningen, The Netherlands in September 2025
CTP event presentation	Presented research at the CTP events in 2023, 2024, and 2025

6. Partners (if applicable)

Scientific partners	
Industry partners	
Government sponsor	