

## Studentship Project: Annual Progress Report 11/2021 to 11/2022

<b>Student Name:</b>	Hamish McLean	<b>AHDB Project Number:</b>	SF/TF 170a
<b>Project Title:</b>	Investigating the abiotic and biotic factors affecting apple canker ( <i>Neonectria ditissima</i> ) symptom development		
<b>Lead Partner:</b>			
<b>Supervisor:</b>	Matevž Papp-Rupar, Alexey Mikaberidze, Xiangming Xu		
<b>Start Date:</b>	2021-10-20	<b>End Date:</b>	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.
- Samples were taken from seven commercial apple cultivars ('Royal Gala', 'Braeburn', 'Scifresh', 'Nicoter', 'Civni', 'Grenadier', and 'Golden Delicious') grafted on M9 rootstock.
- The samples were analysed using 16S/ITS amplicon metabarcoding to measure microbial community structure and composition.
- These metrics will be compared between samples to investigate the interactions between site, cultivar, canker susceptibility, and the root microbiome.
- This research will be published as a scientific paper.

#### Objective 2 Waterlogging

**Aim:** To investigate the effects of winter waterlogging on canker susceptibility.

- A potted tree experiment is in progress which will test the effects of waterlogging on canker susceptibility.
- The experiment consists of 300 two-year-old MM106 apple rootstocks in pots in a polytunnel.
- Trees were inoculated with *Neonectria ditissima* spores.
- Waterlogging treatments of different durations will be executed in December 2022.
- Tree physiology and canker lesions will be assessed regularly throughout 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.

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- The timing of canker lesion appearance (latency time), canker incidence, and canker lesion size will be compared between treatments to assess the effects of waterlogging on canker susceptibility.
- This research will be published as a scientific paper.

### **Objective 3 Biocontrols**

Aim: To isolate and test new potential biocontrol bacteria against apple canker.

- Previous microbial community analysis of apple leaf scar tissue indicated that specific bacterial endophytes are linked to increased resistance to canker and improved tree health.
- This objective will use a 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 will provide a source of isolates.
- Bacterial genera implicated in canker resistance will be selectively isolated from the leaf scars, including *Bacillus*, *Pseudomonas*, and *Sphingomonas*.
- Isolates will be tested for growth in low nutrient environment; cold, drought, and UV tolerance; growth at body temperature (37°C) and 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.
- 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

- Root samples have been processed, DNA has been extracted from all samples and sequenced.
- All samples passed QC.
- Data pre-processing is underway.

#### Objective 2 Waterlogging

- The pot experiment was planted in spring 2022.
- Trees were inoculated with *Neonectria ditissima* spores in November 2022.
- Preparations for the waterlogging treatments is underway.

#### Objective 3 Biocontrols

- Bacterial samples have been isolated from apple leaf scars and cultured.
- Different culture media have been tested.
- *Neonectria ditissima* antagonism assays have been designed and tested.
- Further tests are underway.

### 4. Key issues to be addressed in the next year

#### Objective 1 Root Microbiome

- Sequence data will be processed.
- This data will be used to determine community structure and diversity metrics.
- These metrics will be compared between cultivars and sites.
- This will identify interactions between site, cultivar, and canker susceptibility.
- Results will be written up for publication.

#### Objective 2 Waterlogging

- Waterlogging treatments will be executed in winter 2022 and spring 2023.
- Tree physiology measurements and canker assessments will be recorded throughout 2023.
- Results will be compiled and analysed to compare the effects of waterlogging treatments on canker susceptibility.

#### Objective 3 Biocontrols

- Isolates will be tested for environmental stress tolerance.
- Isolates will be screened for *Neonectria 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):*

<b>Output</b>	<b>Detail</b>
<b>Symposium presentation</b>	I presented my research plans at the crop science symposium at the University of Reading in 2021.
<b>CTP conference presentation</b>	I presented my research at the CTP summer event at NIAB East Malling in 2022.
<b>IOBC conference poster</b>	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)

<b>Scientific partners</b>	
<b>Industry partners</b>	
<b>Government sponsor</b>	