

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

Student Name:	Katherine Stewart	AHDB Project Number:	SF/TF 170/a
Project Title:	Understanding the dynamics of ascospore production to optimise apple scab management		
Lead Partner:	NIAB		
Supervisor:	Xiangming Xu (NIAB); Carol Verheecke-Vaessen (Cranfield)		
Start Date:	September 2021	End Date:	September 2025

1. Project aims and objectives.

Objective 1: Identifying whether sexual reproduction in *Venturia inaequalis* (causal agent of apple scab) is more likely between lesions on the same leaf and whether it occurs pre-leaf-fall. This research can help improve orchard management through more accurate population genetics studies and modelling, along with improving the timing of management intervention to disrupt sexual reproduction.

Objective 2: Developing a mating population through crosses between isolates taken from a scab susceptible cultivar and a cultivar containing a major resistance gene. I'll be identifying the mating type locus and designing molecular markers for the two mating types. This will significantly advance future population genetic studies of the scab fungus.

Objective 3: Utilising the crossed isolates (Objective 2) to identify genetic control (QTLs) for traits of interest such as pathogenicity, virulence and ecophysiological characteristics.

Objective 4: Investigating the efficacy of different control agents at reducing scab primary inoculum in leaf litter. The purpose of these control agents will either be to degrade leaf litter or to encourage leaf colonisation of microbes to degrade the leaf litter. The samples will also undergo microbiome sequence analysis to understand potential association of specific microbes with leaf degradation.

2. Key messages emerging from the project.

An emerging message from one of my biocontrol experiments is that some *Trichoderma* species seem to have a higher capacity to break down leaf litter. This is an important observation and will be further investigated in the coming months as leaf litter is the main source of primary inoculum, therefore efficient removal is an integral part of apple scab management. Microbiome sequence analysis from this experiment is currently underway and I'm hoping to have the results soon.

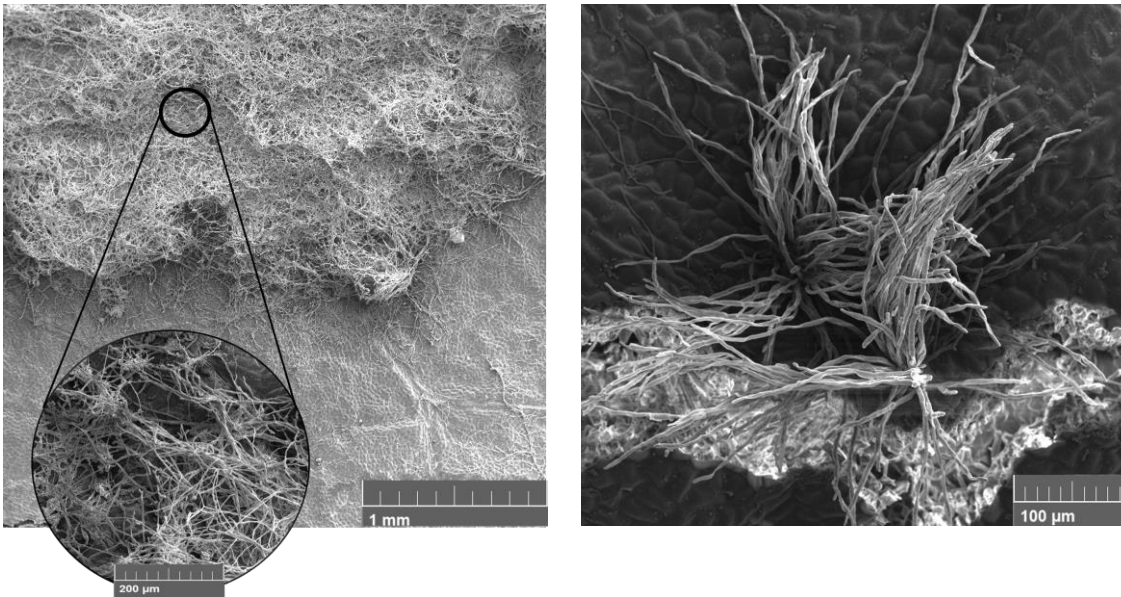
The results described in this summary report are interim and relate to one year. In all cases, the reports refer to projects that extend over a number of years.

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Many of my objectives are still in the experimental stage, whereby sufficient data has not been collected to produce a message due to the natural seasonality issue. Currently, I aim to complete imaging of scabbed leaves before the Christmas 2023 to identify if sex can be initiated pre-leaf fall. Most of the mating type and population genetics work will be carried out in this new academic year (Oct 2023 onwards), with a large amount of DNA extraction and sequencing to be conducted in the coming months.

3. Summary of results from the reporting year

Sex initiation: The trial microscopy experiment I performed was successful and proved that the method of sample preparation was appropriate for looking at *V. inaequalis* structures on the leaf. Fig. 1 shows both scab lesions on the surface of the leaf (left) as well as asexual structures (conidia) (right).



Mating type: The mating population is still being analysed through mating backcrosses between the parent and progeny isolates. I'm hoping to determine the mating types in the coming months once the crosses are advanced enough to identify whether ascospores have been produced. I'm currently in the process of collecting mycelium and extracting DNA from all the isolates. Once this is complete, I can continue with mating type work (identifying locus; developing markers).

Population Genetics: As this work is dependent on objective 2, I do not have any findings so far. I hope to start this work late this year/early next year.

Alternative methods of control: The initial experiment for a leaf litter degradation study is complete and a potential control agent identified. Most of the control agents did not show a significant reduction of leaf litter material compared to the untreated control.

To see which treatments were significant I performed a statistical analysis to compare the treatment groups. I found that a species of *Trichoderma* significantly reduced leaf litter when compared with the untreated control. I will be repeating this study this year, removing treatments with no effect and increasing the number of repeats to determine if this work is worthwhile for potential commercial use. As previously mentioned, microbiome sequence analysis of the remaining leaf litter is currently underway.

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

In the coming months I will be focussing my efforts on DNA extraction from the parent and progeny isolates. This DNA is required for both mating type studies and marker design, as well as population genetics and mapping. I am currently planning the sequencing for these objectives in more detail to determine which traits I am interested in and which markers I may use. I'm learning bioinformatics to be able to analyse sequence data and identify differences between the *V. inaequalis* isolates.

Further microscopy is underway in an attempt to identify sexual structures on the leaf before leaf fall. I hope to have some results soon.

The second leaf litter degradation study will be set up in November. The results from this will be collected ~March 2024, where I hope to have sufficient data to provide an opinion on the study and the effectiveness of the control agents tested.

5. Outputs relating to the project.

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

Output	Detail
Review paper	Stewart, K., Passey, T., Verheecke-Vaessen, C., Kevei, Z. and Xu, X., 2023. Is it feasible to use mixed orchards to manage apple scab?. <i>Fruit Research</i> , (FruRes-2023-0028). https://www.maxapress.com/article/doi/10.48130/FruRes-2023-0028
Presentations	CTP events (Autumn 2021 (Lincoln); Summer 2022; Winter 2022; Summer 2023 (East Malling, Kent).
Conference Poster	British Mycology Society - Fungi and the environment conference at Cranfield University.
Conference Poster	IOBC-WPRS workshop on pome fruit diseases in Plovdiv, Bulgaria.

6. Partners (if applicable)

Scientific partners	Cranfield University
Industry partners	Worldwide fruit; National Association of Cider Makers
Government sponsor	UKRI BBSRC – CTP FCR