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

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:	Prof. Xiangming Xu (NIAB); Dr. Carol Verheecke-Vaessen (Cranfield)		
Start Date:	September 2021	**End Date:**	September 2025

1. Project aims and objectives.

Objective 1: Identifying whether sexual reproduction in *V. inaequalis* can initiate between lesions on the same leaf before leaf-fall. This research can help improve orchard management through more accurate population genetics studies and modelling, along with improving the timing of fungicide application to reduce environmental impact.

Objective 2: Developing a mating population through crosses between isolates from a susceptible cultivar and a cultivar containing a major resistance gene. I aim to identify the mating type locus and design molecular markers for the two mating types, which can then be used for future population and epidemiology studies.

Objective 3: Conducting virulence studies using the progeny of the mating crosses from objective 2. The parent isolates have different virulence levels towards their respective *Malus* cultivar, therefore we may use these cross progeny to map QTLs associated with virulence.

Objective 4: Investigating the efficacy of different biological treatments at breaking down orchard leaf litter, which is a main source of primary inoculum, when applied at post-harvest. We aim to find alternative methods to current leaf removal tactics for reducing overwintering inoculum.

2. Key messages emerging from the project.

A key conclusion from my first objective is that sexual mating between *V. inaequalis* isolates can be initiated on the leaves pre leaf-fall. This knowledge could enhance population genetics studies and help improve the timing of late season fungicide application to reduce primary scab in the following season. Additionally, we can take a closer look at the possibility of mixed cultivar orchards for scab management, because if the fungus primarily mates pre leaf-fall on the same leaf, the risk of super-race development (virulent against several resistance genes) is reduced.

An emerging message from my fourth objective is that some *Trichoderma* species have the potential to increase leaf litter degradation. Further analysis of this data alongside microbiome analysis will indicate if this is something to investigate further for post-harvest scab management. Leaf litter is the main source of primary inoculum, therefore efficient removal is an integral part of apple scab management.

3. Summary of results from the reporting year

Sex initiation: Using the SEM I obtained good quality images showing that pseudothecial structures can develop on the leaves before leaf-fall. The structures were only observed on leaves which had multiple scab lesions, as expected. As a comparison, no pseudothecial structures were observed on single lesion leaves. Leaves taken directly from the tree showed early stage pseudothecial structures indicating that sex can be initiated before leaf-fall. The structures found on overwintered leaves were much more developed showing that the initial structures will go on to form mature pseudothecia. The images of leaves were compared to images of isolates grown *in vitro*, which demonstrated the difference between structures found in compatible vs incompatible crosses.

Commented [TP1]: What? The single lesion leaves or all?

Mating type: A mapping population was successfully generated, and individual progeny strains of the population were backcrossed with the two parental isolates. I used these backcrosses to determine mating type compatibility. I have extracted DNA from both the progeny and parent isolates and sent DNA for genome sequencing (PacBio for one isolate and shotgun for ~30 isolates). Once complete, I can use the sequences to identify genomic regions associated with the mating type locus and, if identified, design molecular markers for the two mating types.

Biocontrol's: The initial leaf litter degradation experiment proved successful with at least one significant treatment compared to the untreated control. The rest of the treatments did not show any significant reduction of leaf litter material. For statistical analysis I conducted a one-way ANOVA which showed that treatment was a significant factor. To then see which treatments were significant I performed a Tukey HSD test to compare pairs of means of the treatment groups. At a 95% confidence interval I had one significant result: Trichoderma atroviride (P=0.0029), which significantly reduced leaf litter when compared with the untreated (raised) control.

I repeated the study last year (November-March) and included another positive control: urea, which is known to increase leaf degradation and therefore reduce scab inoculum in spring. I decided to put all the treatments at two height levels (raised off the ground on crates and on the ground) this time so we could see if ground microorganisms were having an additional effect. Unfortunately, this time around I did not find any significant reduction in leaf litter. This could be due to differences in environmental conditions across the two experiments or could just show that the treatments did not increase leaf degradation. I need to further analyse these results alongside the microbiome sequences collected from the first experiment.

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

Mating Type – Once genome sequences are generated I will need to use bioinformatics for analysis and identification of the mating type locus. From this I can then try to design molecular markers for the two mating types.

Virulence Studies – DNA extraction and qPCR is currently underway for the leaf discs. Aiming to obtain results before Christmas 2024.

Scab Control - Leaf degradation study – Two experiments complete, and results analysed. Microbiome analysis of first experiment still underway. Need to collate all the data and decide whether to run one more experiment this winter.

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?. *Fruit Research*, (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; Winter 2023; Summer 2024 (East Malling, Kent).**
Conference Poster	**British Mycology Society - Fungi and the environment conference at Cranfield University, UK.**
Conference Poster	**IOBC-WPRS workshop on pome fruit diseases in Plovdiv, Bulgaria.**
Conference Poster	**International Conference of Plant Pathology (ICPP) in Lyon, France**
Conference Poster	**Early Careers in Plant Pathology (ECPP) conference in Oxford, UK**

6. Partners (if applicable)

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