Final Report

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Understanding the dynamics of ascospore production to optimise apple scab management.

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1. Industry Summary

Apple scab is a fungal disease that has been an ever-present issue for the apple growing industry. The disease causes distinct brown lesions on the surface of the fruit and leaves, causing significant financial losses to growers because the apples are unmarketable to consumers. The disease is caused by the ascomycete fungus, *Venturia inaequalis*, which is a challenging pathogen to control due to both sexual and asexual reproduction phases during its lifecycle. Current management primarily relies on the application of chemical fungicides during the growing season, with between 15 and 20 sprays commonly applied. The aim of this PhD project was to investigate sexual reproduction and recombination of virulence genes in *V. inaequalis* and identify alternative methods of control.

The first experiment aimed to determine the timing of sexual mating in *V. inaequalis*, relative to the timing of leaf-fall in autumn. We wanted to confirm whether sexual mating structures, called pseudothecia, could develop before the leaves dropped. Scanning electron microscopy (SEM) was used to visualise whether pseudothecia were present prior to leaf-fall. We found that immature pseudothecia structures were present on the leaves containing multiple scab lesions prior to leaf-fall, and upon additional assessment following a controlled overwintering period of three months, these pseudothecia fully matured and produced ascospores. Therefore, our results found that sex can be initiated whilst the leaves are still attached, highlighting the importance of late season and post-harvest scab management, and reiterating how essential it is to remove fallen leaf litter from the orchard to reduce scab infection for the following season.

Next, a mating population was generated between an isolate taken from the cultivar 'Gala' which is highly susceptible to scab, and the crab-apple species *M. floribunda 821*, from which the Rvi6 major scab resistance gene originated. The progeny from this cross were backcrossed with each parent to determine mating compatibility and therefore their proposed mating type. All isolates (progeny and parents) were used to inoculate leaf discs of both Gala and *M. floribunda,* to determine their virulence towards each cultivar and therefore the segregation of virulence during sexual mating. Virulence appears to segregate in a 1:1 ratio suggesting that a single major virulence factor is being recombined and inherited. Further bioinformatic analysis identified single nucleotide polymorphisms (SNPs) associated with both virulence and mating differentiation, which lays a groundwork for future population genetics studies.

Finally, we investigated the efficacy of biological control agents (BCAs) at enhancing leaf litter decomposition as well as reducing *V. inaequalis* primary inoculum. Unfortunately, we did not identify a treatment that consistently enhanced leaf litter decomposition. The positive control used in the study, urea, significantly reduced leaf litter weight but did not reduce the number of V. inaequalis ascospores. Urea also cannot be used in the UK due to high levels of nitrogen and therefore negative environmental impact. The fungicide treatment significantly reduced the number of ascospores and may be a promising avenue for post-harvest management of trees. Further research would need to be conducted to determine if there are any implications. Analysis of bacterial and fungal leaf litter microbiomes showed differences in species composition between treatments, however poor taxonomic classification meant that a clear association with leaf litter degradation could not be identified.

This research will inform potential management strategies against *V. inaequalis*, with a focus on biocontrol or implementing mixed cultivar orchards to encourage sustainability and reduce the reliance on chemical fungicides.