Grower Summary

Investigating durable resistance to *Phytophthora cactorum* in strawberry and apple

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Annual report 2021

Project title:	Investigating durable resistance to *Phytophthora cactorum* in strawberry and apple
Project number:	
Project leader:	Dr Charlotte Nellist (NIAB EMR), Prof Jim Dunwell (University of Reading)
Report:	Annual report, January 2021
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Key staff:	Matteo Luberti
Location of project:	NIAB EMR
Industry Representative:	
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The results and conclusions in this report are based on an investigation conducted over a one-year period. The conditions under which the experiments were carried out and the results have been reported in detail and with accuracy. However, because of the biological nature of the work it must be borne in mind that different circumstances and conditions could produce different results. Therefore, care must be taken with interpretation of the results, especially if they are used as the basis for commercial product recommendations.

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AUTHENTICATION

We declare that this work was done under our supervision according to the procedures described herein and that the report represents a true and accurate record of the results obtained.

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GROWER SUMMARY

Headline

- This project aims to identify sources of durable resistance to *Phytophthora cactorum* in strawberry and apple in order to integrate them in the UK breeding programmes.

Background

The genus *Phytophthora* is comprised of several pathogenic species which are responsible for substantial damages to crops worldwide. *Phytophthora cactorum (Pc)* can cause disease in over 160 plant hosts, including economically important crops such as cultivated strawberry (*Fragaria* x *ananassa*) and apple (*Malus* x *domestica*). *Pc* is homothallic and it can produce both sexual and asexual spores (Erwin and Ribeiro, 1996). The sexual oospores are able to persist in the soil for several decades, while asexual zoospores are bi-flagellate, motile spores which are released in wet conditions, and are able to swim towards a suitable host to initiate infection (Khew and Zentmyer, 1973).

Pc is a hemi-biotrophic pathogen, capable of colonising host tissue before switching to a necrotrophic lifestyle. It is able to cause severe disease (crown and leather rot) in strawberry (Erwin and Ribeiro, 1996). Crown rot infection causes wilting of the plant, usually beginning from the youngest leaves, and red-brown lesions within the crown, while leather rot causes the fruit to acquire a “leathery” texture, unpleasant smell and an altered taste resulting in high economic losses at all stages of production. Up to 40% of total strawberry crops were lost in Norway to crown rot (Stensvand *et al.*, 1999) and 20-30% in commercial farms in Ohio due to leather rot (Ellis and Grove, 1983).

Due to the high costs associated with orchard establishment, *Pc* infection can be particularly damaging in perennial plants such as apple and pear (Harris, 1991). Thus, the production of resistant varieties is an important objective in breeding programs. In apple, *Pc* can cause crown, collar and root rot (Erwin and Ribeiro, 1996). It can enter the tree through wounds and it reportedly often initiates infection at the graft union site, producing a moist rot, while below ground the bark tends to turn black and is decomposed by soil microorganisms (Harris, 1991).

Previous work on resistance to *Pc* in strawberry at NIAB EMR has identified three major effect Quantitative Trait Loci (QTL) in a bi-parental cross and additional QTL from a preliminary genome-wide association study (Nellist *et al.*, 2019). In comparison to strawberry where resistance is known to be quantitative, there is very little know about resistance to *Pc* in apple. Although reports exist that suggest the presence of a major resistance gene in the ‘Northern Spy’ cultivar (Knight and Alston, 1969), suggesting qualitative resistance. Thus, one of the

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key aims of this project is to identify markers associated with resistance and to elucidate the underlying molecular plant-pathogen interactions in both apple and strawberry plants.

Initially, I employed an excised shoot assay test to screen 29 apple genotypes of interest to NIAB EMR's breeding programme for resistance/susceptibility to two UK *Pc* isolates. This allowed us to identify an existing bi-parental cross of parents M.27 and M.116 called M432 segregating for resistance to *Pc.* In 2019, using the same artificial inoculation technique to allow for a larger number of replicates, I phenotyped the individuals in this population and identified a QTL present on linkage group 6, which was highly associated with resistance to *Pc*. In 2020, I repeated the phenotyping of the same mapping population including a greater number of individuals and confirming the presence of a resistance-associated QTL on linkage group 6. I have also screened 99 apple rootstock and scion accessions. Using the phenotypic data I performed a preliminary genome-wide association study (GWAS) identifying two resistance associated QTL loci, on linkage group 5 and 6 (at the same locus identified in the M432 population). These results suggest resistance to *Pc* in apple is controlled by a major effect locus and other smaller effect loci, and indicate that previously reported sources of resistance may have been overcome. This year I have screened 126 individuals from another apple population (MCM007) which is closely related to M432, to assess the predictive power of the resistance markers I previously identified. I also inoculated the parental genotypes from M432 (M.116 and M.27) in a time course experiment with *Pc* zoospores to investigate the transcriptional response of the root system to infection. I inoculated individual plants from each genotype grown under sterile conditions and collected samples of root tissue at 0, 6, 12 and 24 hours post inoculation (hpi). These samples were sent for sequencing and the data is now being analysed to identify resistance/susceptibility gene candidates.

Strawberry transcriptome data, previously generated (Nellist *et al.,* 2021) at NIAB EMR, is also being analysed to identify infection related genes. This data was produced in a time course experiment employing a susceptible and a resistant variety of strawberry ('Emily' and 'Fenella', respectively). The root systems of both varieties were inoculated with *Pc* and sampled at 0, 12 and 48 hpi. The sequenced data is now being analysed to identify genes associated with the resistant/susceptible phenotypes observed.

Summary

In the third year of this PhD programme, I have employed an excised shoot assay to screen an apple population (MCM007) related to M432 to assess the robustness of the markers associated with resistance to *P. cactorum* previously identified during this PhD. I screened 126 individuals from the MCM007 population and 29 individuals from M432, along with nine genotypes in the pedigree of both populations. I also performed a time-course inoculation

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experiment on the parental genotypes of M432 to assess the root systems' response to inoculation. Moreover, I analysed transcriptome data from a time course experiment performed on two strawberry varieties ('Emily' and 'Fenella') highlighting key differences in their immune responses. Future work will focus on identifying and characterising the genes responsible for resistance. As well as aiding in the identification of robust markers associated with resistance to be employed in the rootstock breeding programme.

Financial Benefits

This report summarises the work that has been carried out in the first three years of this PhD project. The methods developed during the past two years and the data presented in this annual report will aid in the selection of genotypes to be employed in apple breeding programmes.

Action Points

- There are no action points at this stage of the project.

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