Project title:	Investigating durable resistance to *Phytophthora cactorum* in strawberry and apple
Project number:	SF/TF 170
Project leader:	Dr Charlotte Nellist (NIAB EMR) Prof Jim Dunwell (University of Reading)
Report:	Annual report, October 2020
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Key staff:	Matteo Luberti
Location of project:	NIAB EMR
Industry Representative:	NA
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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.

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 a number of pathogenic oomycete (water-mould) species responsible for substantial damages to crops worldwide. *Phytophthora cactorum* can cause disease in over 250 plant hosts, including economically important crops such as cultivated strawberry (*Fragaria* x *ananassa*) and apple (*Malus* x *domestica*). *P. cactorum* 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 zoospores released in wet conditions which are able to swim towards a suitable host to initiate infection (Khew and Zentmyer, 1973).

P. cactorum 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, *P. cactorum* 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, *P. cactorum* 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 *P. cactorum* 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 *P. cactorum* 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 key aims of this project is to identify markers associated with resistance and to elucidate the underlying molecular plant-pathogen interactions.

Initially, we 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 *P. cactorum* isolates. This allowed us to identify an existing bi-parental cross of parents M.27 and M.116 called M432 segregating for resistance to *P. cactorum.* In 2019, using the same artificial inoculation technique to allow for larger number of replicates we phenotyped the individuals in this population and identified a QTL present on linkage group 6 highly associated with resistance to *P. cactorum*. This year we 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. We have also screened 99 apple rootstock and scion accessions. Using the phenotyping data we 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 *P. cactorum* in apple is controlled by a major effect locus and other smaller effect loci, and indicate a potential break-down of previously reported sources of resistance.

Summary

In the second year of this PhD programme, we have employed an excised shoot assay to screen the wider UK apple germplasm preserved within NIAB EMR's Genebank and the National Fruit Collection for resistance to *P. cactorum.* We screened 99 apple rootstock and scion varieties relevant to the apple breeding programs at NIAB EMR. We identified several levels of resistance and susceptibility within the UK germplasm, identifying a number of resistant varieties of both apple scions and apple rootstocks. Using genotypic data previously generated at NIAB EMR in the framework of several projects, including AHDB funded projects we were subsequently able to perform a GWAS. This allowed us to identify two resistance associated loci on linkage groups 5 and 6. We also performed a second round of phenotyping on previously assessed apple population. Our analysis confirmed the presence of a major effect resistance QTL responsible for 58.2% of the phenotypic variation observed in the population. Future work will focus on the identification of the genes responsible for the observed resistance, as well as aiding in the identification of robust markers associated with resistance to be employed in the rootstock breeding programme for the deployment of resistance in the breeding lines.

In summary:

- UK apple germplasm genotypes pertinent to the apple breeding programs were screened using an excised shoot assay in order to identify sources of resistance to *P. cactorum.*
- A major effect resistance QTL previously identified using a segregating population phenotyped was validated with a second round of phenotyping and mapping.
- A preliminary genome-wide association study was performed allowing us to identify two resistance-associated loci on linkage groups 5 and 6. Notably the locus on linkage group 6 is the same as the one previously identified in the course of this PhD project.
- 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 and second year of this PhD project. As this is a four-year programme, there are no direct financial benefits to be reported as of yet. However, 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 early stage of the project.