Studentship Project: Annual Progress Report 01/10/2020 to 26/09/2023

Student Name:	**Finlay Bourquin**	**AHDB Project Number:**	**SF/TF 170/a**
Project Title:	**Understanding resistance to *Botrytis cinerea* in strawberries**		
Lead Partner:	Berry Gardens		
Supervisor:	Dr Charlotte Nellist, Dr Helen Cockerton, Prof. Matthew Dickinson, Assoc. Prof. Tim Robbins		
Start Date:	01/10/2020	**End Date:**	30/09/2022

1. Project aims and objectives

The aims of this project are:

1) To investigate woodland strawberry (*Fragaria vesca*) susceptibility or resistance factors *to Botrytis cinerea*
2) To investigate *B. cinerea* virulence factors

In order to carry out these aims, pathogenicity tests have been conducted to identify *B. cinerea* isolates of high and low virulence. Pathogenicity tests have been optimised and have been carried out on strawberry leaves, fruits and flowers, in order to cover all the tissue types *B. cinerea* uses for infection.

F. vesca seeds have been mutagenized via ethyl methanesulfonate (EMS), grown to maturity and the selfed M2 generation has been screened for differences in susceptibly or resistance to *B. cinerea* using the optimised pathogenicity protocols. Any plants exhibiting increased susceptibility or resistance will undergo genome sequencing to determine which genomic loci are responsible for the change in phenotype.

To investigate *B. cinerea* virulence factors, DNA extraction protocols are being optimised for both Illumina and Nanopore sequencing, and factors which may be involved in virulence will be investigated between isolates of high and low virulence. Discovering what elements are involved in infection will lead to an increased understanding of the *B. cinerea*-strawberry pathosystem.

2. Key messages emerging from the project

Differences in susceptibility and resistance to *B. cinerea* in *F. vesca* were brought about through EMS mutagenesis. The ability to induce changes in resistance to pathogens is crucial to understand how plants defend themselves. Should the changes in resistance be found to be linked to certain areas of the genome, markers for breeding can be developed in order to develop more resistant plants and decrease the reliance on fungicides.

It has been determined that virulence differs between isolates of *B. cinerea*, as well how the isolates infect different host organs. This is of interest as we can try to ascertain the causal elements that produce this variation, through methods such as DNA sequencing and analysis, establishing what makes an isolate

more or less virulent than others. Understanding how *B. cinerea* is causing disease will aid in the long term goal of improving strawberry production.

3. Summary of results from the reporting year

Pathogenicity assays

The final strawberry pathogenicity assay was conducted on *F. vesca* flowers (figure 1). Statistical differences can be observed between all of the isolates and the control. The results from this assay correspond with the results from the leaf and fruit assay which was carried out last year.

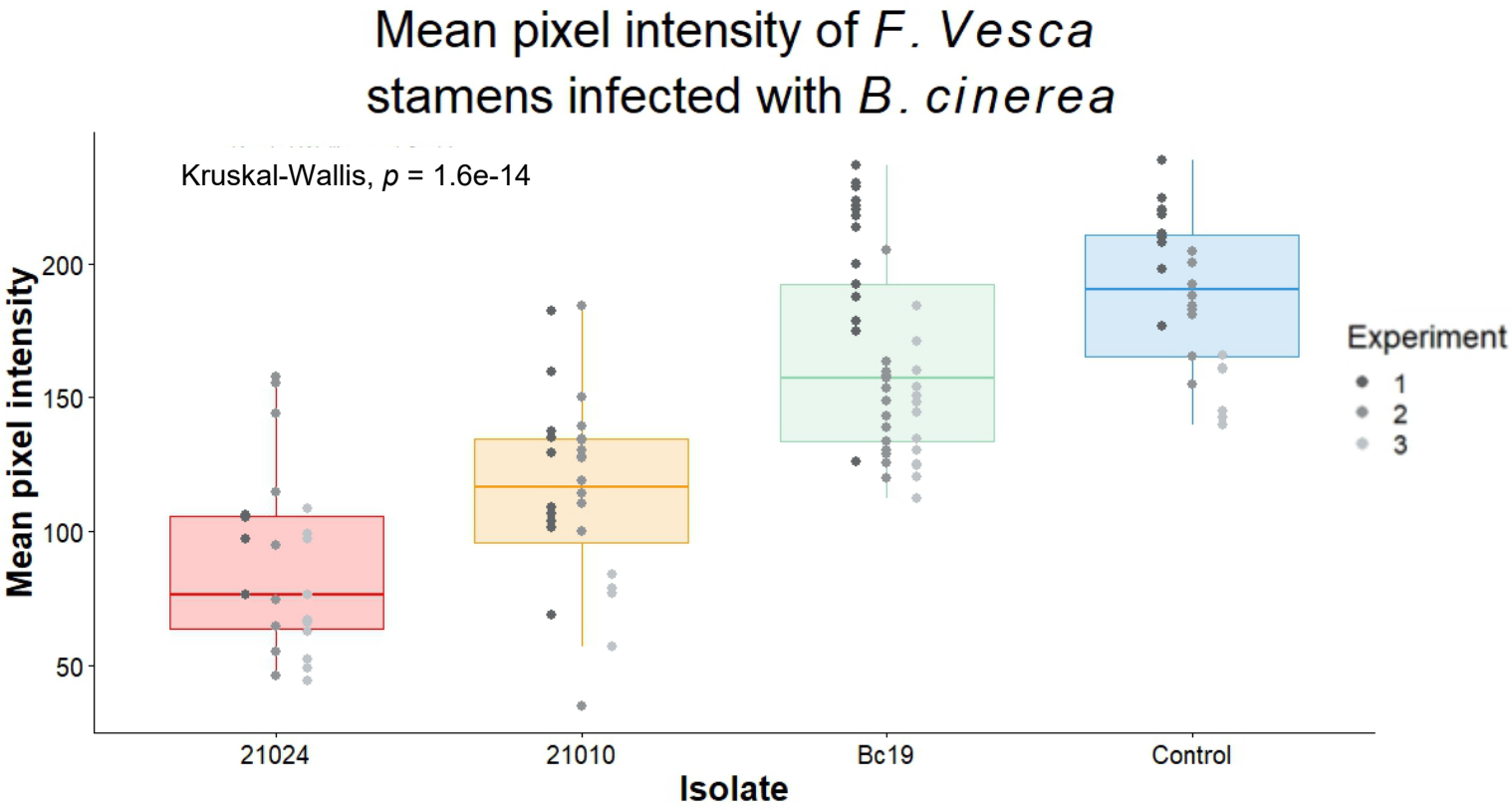

Figure 1. Mean pixel intensity for *Botrytis cinerea* isolates infecting *Fragaria vesca* flower stamens.

A leaf pathogenicity assay was also carried out on *Nicotiana benthamiana* leaf discs (figure 2). This was undertaken to see if hosts play a role in isolate virulence. A statistically significant difference was observed for isolate virulence, however, in this instance 21010 is one of the most virulent isolates which was not observed during the pathogenicity assays on strawberry organs. Overall, these assays have revealed both host specific differences and organ specific differences regarding isolate virulence.

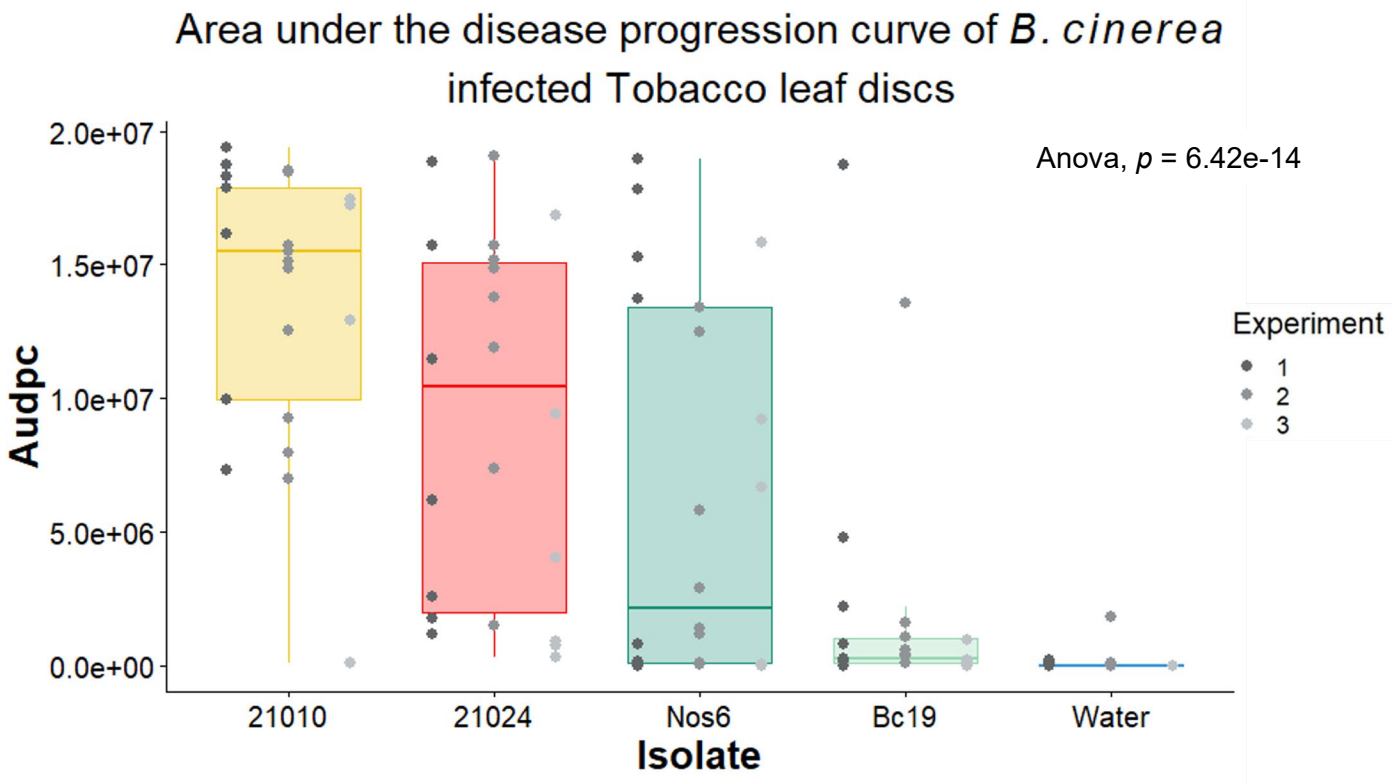

Figure 2. Area Under the Disease Progression Curve (AUDPC) for *Botrytis cinerea* isolates infecting *Nicotiana benthamiana* leaf discs.

To further explain the results seen in these pathogenicity assays, PCR was carried out to identify the presence of specific retrotransposons that have been associated with virulence. These retrotransposons are not correlated with virulence for the isolates used in this experiment.

EMS mutagenesis

Pathogenicity assays were conducted on 260 EMS *F. vesca* mutants. External pest and disease pressures led to severe pruning which may have had an impact on the results. Following intensive pruning *B. cinerea* infections were either attenuated or failed to initiate infection. Consequently, a second experiment, titled the bulk experiment, was carried out on 40 mutants of interest from the original. The differences between infection from the original and bulk experiment are clearly exhibited in figure 3 and 4. Statistical analyses were conducted using data from both experiments and 12 mutants with either increased susceptibility or resistance were identified (figure 3 and 4). Further analyses involving fitting dose-response models are currently ongoing and mutants of interest already identified have had DNA extracted and sent for Illumina sequencing.

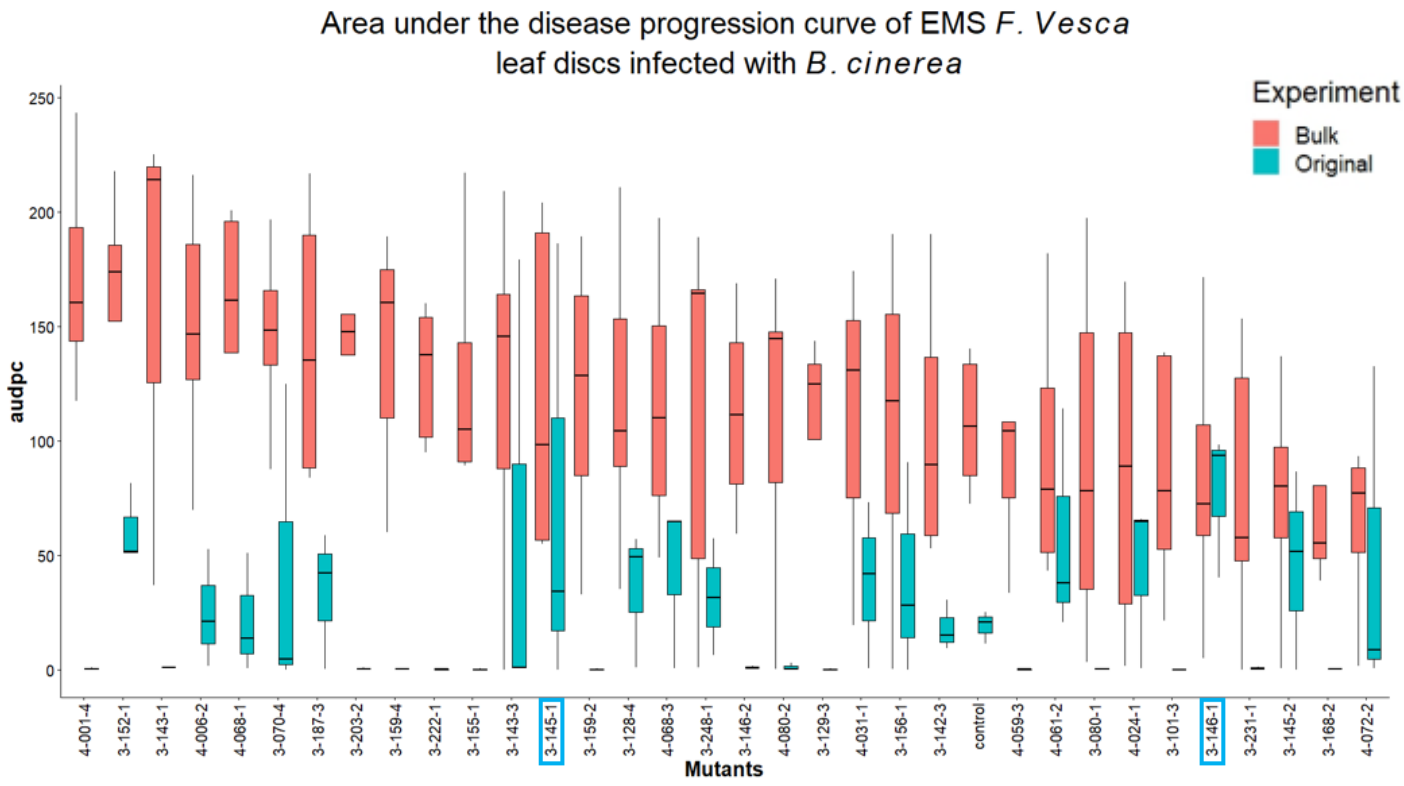

Figure 3. Area Under the Disease Progression Curve (AUDPC) for ethyl methanesulfonate *Fragaria vesca* leaf discs infected by *Botrytis cinerea*. Mutants statistically significantly different from the non-mutated controls are highlighted in blue for the original experiment.

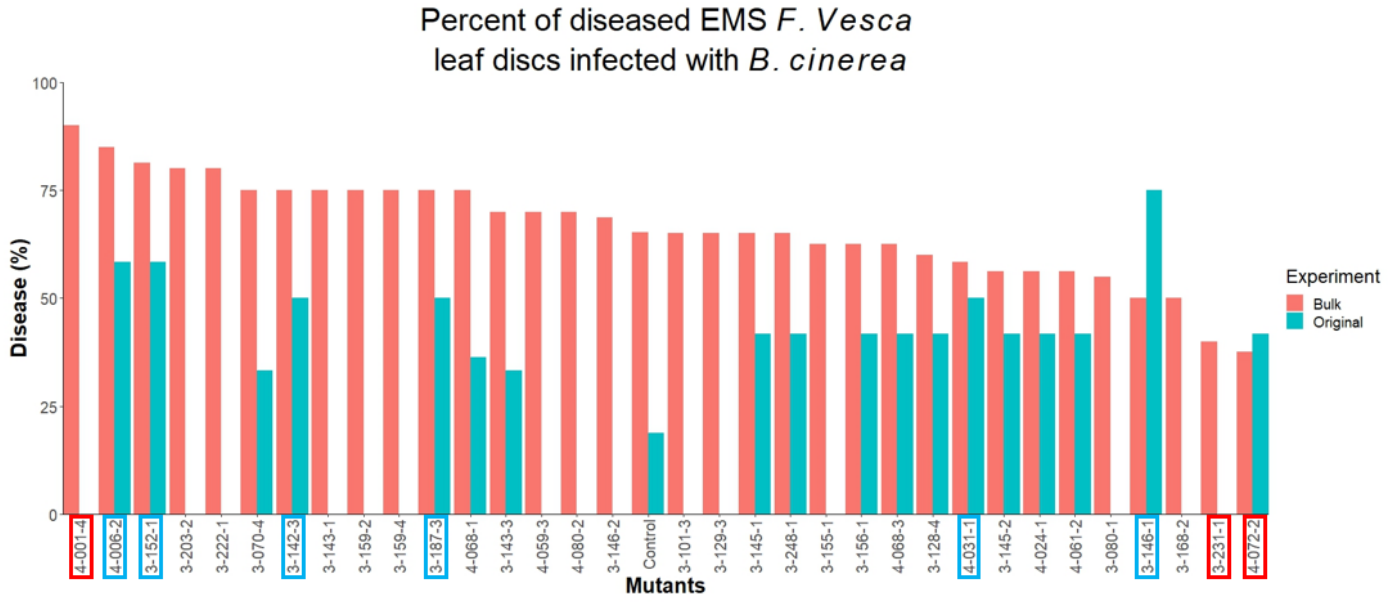

Figure 4. Percentage of diseased EMS treated *Fragaria vesca* leaf discs infected by *Botrytis cinerea.* Mutants statistically significantly different from the non-mutated controls are highlighted in blue for the original experiment and red for the bulk experiment.

4. Key issues to be addressed in the next year

Following DNA sequencing of the EMS mutants of interest, analyses will be conducted to determine which areas of the genome have been mutated and if these mutations have occurred in regions that may be associated with susceptibility and resistance. This is the first step in discovering potential susceptibility factors that could be useful when breeding commercial strawberry varieties.

Whole genome sequencing of the *Botrytis cinerea* isolates will also be carried out and isolates of differing virulence will be compared to determine potential novel virulence factors. Sequencing will be conducted this

year and is the first step to revealing some of the mechanisms behind infection. Discovering virulence factors is an important first step for potentially implementing novel control methods.

The key issues being addressed next year are how to discover novel susceptibility and resistance factors in *F. vesca* and novel virulence factors in *B. cinerea.*

5. Outputs relating to the project

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

Output	**Detail**
Presentations	**Several presentations disseminating results to date at CTP conference events** **Presented at the AHDB Crops PhD Conference 2021**
Poster	**Presented a poster at the BSPP conference: Our Plants, Our Future (OPOF)**
Poster	**Presented a poster at the Microbiology Society Annual Conference 2023**
Poster	**Presented a poster at the University of Nottingham postgraduate poster symposium**
Poster	**Presented a poster at the Plants @ Cambridge festival and won one of the best posters awards**

6. Partners (if applicable)

Scientific partners	
Industry partners	Berry Gardens
Government sponsor	BBSRC