Studentship Project: Annual Progress Report 01/10/2020 to 05/10/2022

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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 are being conducted to identify *B. cinerea* isolates of high and low virulence. Pathogenicity tests have been optimised and will be 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 M_2 generation will be 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, isolates will undergo ultraviolet (UV) mutagenesis and pathogenicity tests, should virulence be altered, genome sequencing and analysis will be carried out. 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

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 UV mutagenesis, pathogenicity testing and 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

Leaf pathogenicity tests have been conducted for ten *B. cinerea* isolates on *F. vesca* leaf discs and the area under the disease progression curve was determined. A statistically significant difference was found between isolates ($H(10) = 183.08$, $p < 2.2e\text{-}16$), namely between 21024, 21018 and R4/20 with the other isolates and Bc19 with the other isolates, which can be observed in Figure 1.

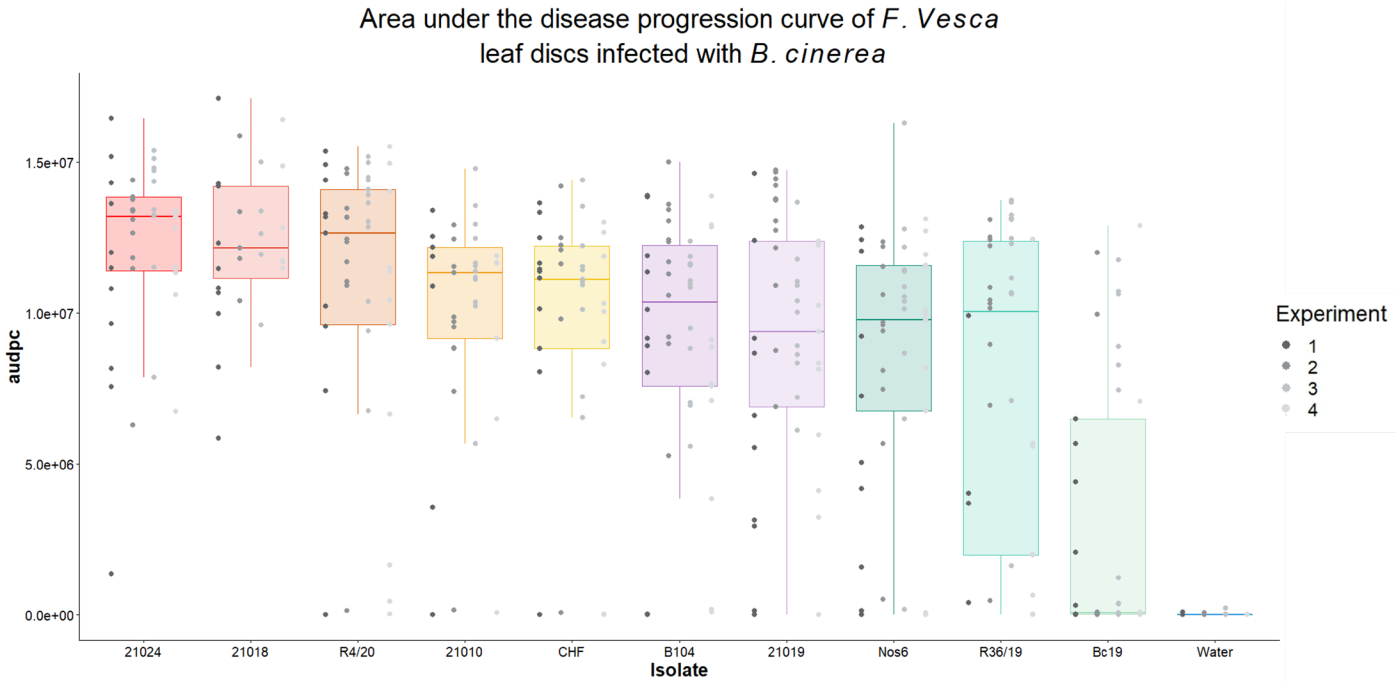

Figure 1. Area Under the Disease Progression Curve (AUDPC) for *Botrytis cinerea* isolates infecting *Fragaria vesca* leaf discs.

In addition, pathogenicity tests for four *B. cinerea* isolates were carried out on *F.* × *ananassa* fruits. The most and least virulent isolates, as well as two moderately virulent isolates from the leaf pathogenicity assays were chosen. The most virulent isolate, 21024, was found to be statistically significantly different from the other isolates (Figure 2), interestingly Bc19, the least virulent isolate on leaves, was not found to be statistically significantly different from the isolates of moderate virulence. This suggests Bc19 may have tissue specific infection strategies. Overall, it is clear that *B. cinerea* isolates vary in pathogenicity between each other and potentially between host organs.

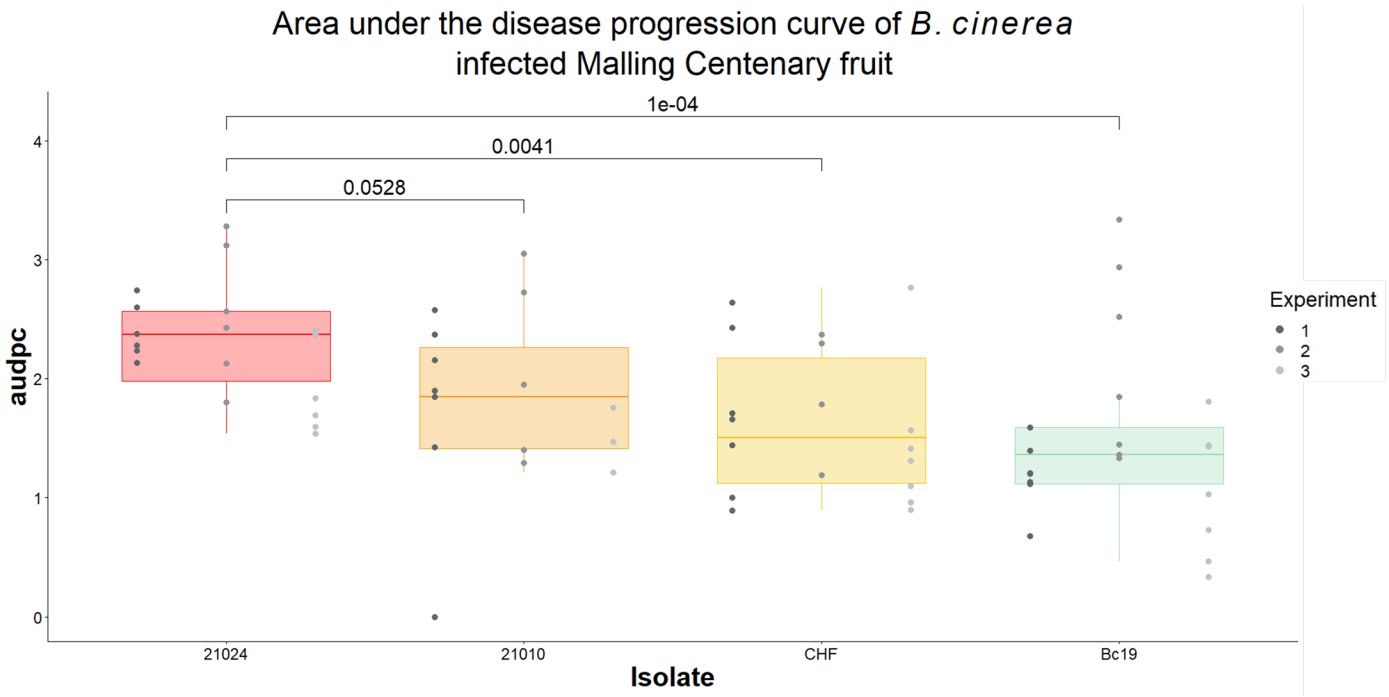

Figure 2. Area Under the Disease Progression Curve (AUDPC) for *Botrytis cinerea* isolates infecting *Fragaria* × *ananassa* cv. Malling Centenary fruits. The significant *p*-values from one-way ANOVA analyses are presented.

EMS mutagenesis

Finally, two EMS concentrations were trialled, (0.4 % for 4 h) and (0.4 % for 8 h), and the percentage of M_1 plants displaying chlorotic sectoring was 8.4 % and 13.4 %, respectively. These M_1 plants were selfed and the M_2 generation has been sown and is ready to be screened for increased or decreased susceptibility to *B. cinerea*. The EMS mutagenesis was successful, with mutations effecting the leaves and flowers of the M_2 generation (Figure 3).

Figure 4. Examples of some mutant flower (A) and leaf colour (B) phenotypes observed in an ethyl methanesulfonate population of *Fragaria vesca.*

4. Key issues to be addressed in the next year

- The final strawberry organs to be assessed to investigate isolate differences in virulence are the flowers. The assay to carry this out has been optimised and will be conducted this year. This will provide a comprehensive overview of how *B. cinerea* infects strawberry plants and how each organ is impacted.

- An EMS population of *F. vesca* plants has been produced and they will be screened this year for changes in susceptilbity or resistance to *B. cinerea*. This is the first step in discovering potential susceptibility factors that could be useful when breeding commercial strawberry varieties.

- UV mutagenesis will be utilised with the aim of altering *B. cinerea's* virulence. This will be conducted this year and is the first step to revealing some of the mechanisms behind infection. This 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)

6. Partners (if applicable)

Scientific partners	
Industry partners	Berry Gardens
Government sponsor	BBSRC