Studentship Project: Annual Progress Report
OCT 2020 to SEP/2024

Student Name:	**Adam Gregg**	**AHDB Project Number:**	**SF/TF 170/a**
Project Title:	Characterising broad-acting resistance to bacterial canker of cherry and elucidating tissue-specific mechanisms of immunity		
Lead Partner:			
Supervisor:	Dr Richard Harrison, Dr Jordan Price, Dr Glyn Barrett		
Start Date:	Oct 2020	**End Date:**	Sep 2024

1. Project aims and objectives

P. syringae is the causal agent of bacterial canker. It is a globally and economically impactful plant pathogen. Treatments such as copper-based biocides cause adverse environmental effects and not completely effective due to increasing copper. Therefore, biological pathogen control in crops is essential for the global transition to sustainable agriculture. Genomic, effector-informed, research has the potential to provide development of biological control through enabling accelerated breeding strategies and other potential control measures.

P. syringae pv. *syringae*-9644 (Pss-9644) is the main disease-causing strain of bacterial canker in cherry. Effectors are proteins injected into the host cell via the type 3 secretion system and are responsible for circumventing the host immune system, allowing the pathogen to successfully colonise. Conversely, on the host side, resistance genes are responsible for detecting and triggering the immune system.

Previous research conducted at NIAB-EMR has shown that broad acting partial resistance towards *P. syringae* is exhibited by some commercial cherry cultivars, this is likely due the result of a range of traits. While wild cherry has been shown to exhibit strong resistance, that in contrast may be due to one or a few genes. Therefore, the resistance genes responsible for the strong resistance within wild cherry are of great significance towards the implementation of resistant commercial cultivars.

Pseudomonas syringae 9644 pathogenicity results from the combined effect of different effectors and the toxins SylAE and SyrSyp. *Pseudomonas syringae* effectors can be split genetically into the different groups; the conserved effector loci (CEL), Flexible effectors, Prunus effectors, Core effectors. At low inoculum doses ($2x10^6$ CFU/mL) the wild *P. incisa* and Groton B are highly resistant. At a high dosage ($2x10^8$ CFU/mL) this resistance is broken by the immune system being overwhelmed. Leaf pathology testing using effector mutants the key effectors contributing to disease can be revealed. The first batch of effector mutants were mutants where whole groups of effector mutants were deleted to narrow down regions which significantly contribute to virulence.

This work builds towards greater understanding of the Pss-9644 effectors which contribute towards pathogenicity on cherry as well as the development of molecular markers for identifying QTL responsible for strong broad acting resistance. This project builds towards significant agronomic gains through the potential the implementation of resistance traits and greater understanding of the host-pathogen interaction post infection.

The aims of this project are; (1) Understand the significance of single Pss-9644 effectors (2) Investigate the transcriptome of resistant wild cherry towards specific effectors or combinations to elucidate wild cherry resistance response (3) Map resistance QTL and develop molecular markers.

2. Key messages emerging from the project

Testing this year has focused on using effector mutants to reveal the importance of different effector complements. In wild cherry, redundancy has been seen between the conserved effector loci (CEL) and toxins, also, deletion of the prunus effectors significantly reduces disease symptoms, and deletion of toxins has been shown to have very little effect on disease symptoms of leaves (table 1). This work is building knowledge towards understanding effector importance and will be continues in greater granularity next year.

3. Summary of results from the reporting year

Key finding from experiments this year:

- Deletion of the prunus and flexible effectors significantly reduces disease symptoms, indicating the importance of these effector proteins for Prunus pathogenicity.
- Redundancy has been seen between the conserved effector loci (CEL) and toxins.
- Deletion of toxins has been shown to have very little effect on disease symptoms of leaves, however previous data has shown that toxins are key for fruit infection.

Table 1. Detached leaf syringe infiltration assay showing disease progression and severity of symptoms caused by of *Pseudomonas syringae* 9644, Pseudomonas morsprunorum Race 1, and Pss-9644 effector mutants into two wild cherry (Groton B and *P. insica*) and one cultivated cherry (sweetheart). Test leaves were photographed and scored visually each day over 7 days. Results represent 18 leaves per data point across 3 independent runs.

Groton B	Days						
	1	2	3	4	5	6	7
Psm-R1-5244	0.111111	0.333333	0.611111	0.666667	0.777778	0.888889	1.222222
Pss-9644	0.055556	0.333333	0.888889	1.277778	1.388889	1.5	1.666667
ΔCEL	0.222222	0.388889	0.5	0.555556	0.666667	0.722222	0.777778
Δtoxins	0.333333	0.555556	0.555556	0.666667	0.666667	0.722222	0.833333
ΔCEL ΔToxins	0	0.166667	0.222222	0.333333	0.388889	0.333333	0.5
ΔFlexible	0.277778	0.333333	0.333333	0.333333	0.388889	0.555556	0.611111
ΔFlexible ΔPrunus	0.055556	0.388889	0.388889	0.444444	0.611111	0.722222	0.722222
ΔFlexible ΔPrunus Δcore	0.277778	0.333333	0.333333	0.333333	0.333333	0.388889	0.5
ΔCEL ΔFlexible Δprunus ΔCore	0.166667	0.277778	0.277778	0.277778	0.277778	0.277778	0.388889
ΔCEL ΔFlexible Δprunus Δcore ΔSylAE	0.277778	0.333333	0.444444	0.444444	0.5	0.5	0.555556
ΔCEL ΔFlexible Δprunus Δcore ΔSyrSyp	0.111111	0.277778	0.388889	0.444444	0.444444	0.5	0.5
SylA	0.111111	0.444444	0.611111	0.666667	0.833333	1.055556	1.111111
SyrSyp	0.111111	0.277778	0.444444	0.5	0.555556	1	1.055556
ΔSylAΔSyrsypΔHrpA	0	0	0.111111	0.111111	0.166667	0.222222	0.277778
P. incisa	Days						
	1	2	3	4	5	6	7
Psm-R1-5244	0.5	1.388889	2.333333	3.055556	4.5	5	5
Pss-9644	0.777778	1.111111	1.888889	2.833333	4.166667	4.611111	5
ΔCEL	0.611111	0.888889	1.5	1.666667	2.611111	2.555556	2.777778
Δtoxins	0.333333	0.777778	0.722222	0.833333	2.777778	3.166667	3.388889
ΔCEL ΔToxins	0.333333	0.388889	0.555556	0.555556	0.611111	0.666667	0.777778
ΔFlexible	0.055556	0.5	1.111111	1.444444	2.111111	2.277778	2.833333
ΔFlexible ΔPrunus	0.055556	0.277778	0.333333	0.388889	0.5	0.5	0.666667
ΔFlexible ΔPrunus Δcore	0.444444	0.444444	0.5	0.555556	0.722222	0.833333	0.944444
ΔCEL ΔFlexible Δprunus ΔCore	0.388889	0.555556	0.944444	1.055556	1.277778	1.333333	1.5
ΔCEL ΔFlexible Δprunus Δcore ΔSylAE	0.333333	0.5	0.777778	1	1	1.055556	1.444444
ΔCEL ΔFlexible Δprunus Δcore ΔSyrSyp	0.055556	0.333333	0.388889	0.722222	1.388889	1.888889	2.388889
SylA	0.166667	1.222222	1.722222	2.388889	3.222222	3.666667	3.666667
SyrSyp	0.333333	1.111111	1.944444	2.5	3.277778	3.444444	3.611111
ΔSylAΔSyrsypΔHrpA	0	0.166667	0.222222	0.277778	0.444444	0.444444	0.5
	Days						
Sweetheart	1	2	3	4	5	6	7
Psm-R1-5244	0.166667	2.5	3.611111	4.222222	4.777778	5	5
Pss-9644	0.111111	2.666667	4.055556	4.444444	4.777778	4.833333	4.944444
ΔCEL	0.111111	2.055556	3.611111	4	4.611111	4.777778	4.777778
Δtoxins	0.166667	1.722222	2.611111	3.666667	4.333333	4.555556	4.666667
ΔCEL ΔToxins	0.111111	1.722222	3	3.555556	3.833333	3.666667	3.944444
ΔFlexible	0	1.666667	2.777778	3.611111	4.166667	4.333333	4.5
ΔFlexible ΔPrunus	0.166667	1.944444	3.333333	3.833333	4.333333	4.277778	4.444444
ΔFlexible ΔPrunus Δcore	0.111111	2.055556	3	3.5	4	4.222222	4.444444
ΔCEL ΔFlexible Δprunus ΔCore	0	0.777778	1.166667	1.333333	1.666667	2.222222	2.888889
ΔCEL ΔFlexible Δprunus Δcore ΔSylAE	0.111111	0.833333	1.055556	1.5	2.055556	2.388889	2.555556
ΔCEL ΔFlexible Δprunus Δcore ΔSyrSyp	0.111111	0.722222	1.055556	1.055556	1.722222	2.833333	3
SylA	0.222222	1.944444	2.722222	3.166667	4.277778	4.388889	4.444444
SyrSyp	0.166667	2.277778	3.055556	3.666667	4.388889	4.555556	4.722222
ΔSylAΔSyrsypΔHrpA	0	0.055556	0.055556	0.222222	0.555556	0.555556	0.555556

4. Key issues to be addressed in the next year

Experiments to be conducted in the next year include (1) singly expressed effector leaf pathology testing, (2) multiparental cherry population genotyping/phenotyping to reveal the genetic basis of resistance, and (3) RNA sequencing of resistant wild cherry Pss-9644 and 9644-mutants to reveal the transcriptomic regulation post infection.

Work this winter will deliver the strain requirements for further effector testing, which will commence during the leafing season (early spring 2023). These additional effector mutants are required for both the leaf pathology testing and the RNA sequencing experiments. These additional mutants will only be expressing single or paired effectors and will be used to further understand effector role during infection of *Prunus avium*. The additional mutants will be made this winter are shown in Table 2,and five deletion mutants have already been successfully made in the past 3 weeks.

Additionally, many more trees are required for the thorough testing next spring. So, graft wood collection and grafting will be performed this January to allow enough material for spring testing.

Table 2. Mutants to be made this winter for further investigation into *P. syringae* pathology. Effectors chosen for investigation are all the flexible and prunus effectors as they have been shown to be key for bacterial virulence.

	Winter 2022 Mutants	
1	ΔPrunus	Conjugation
2	ΔFlexible ΔPrunus + HopAZ1	Conjugation
3	ΔFlexible ΔPrunus + HopAF1	Conjugation
4	ΔFlexible ΔPrunus + HopBE1	Conjugation
5	ΔFlexible ΔPrunus + hopAR1	Conjugation
6	ΔFlexible ΔPrunus + HopAW1	Deletion
7	ΔFlexible ΔPrunus + AvrRpm1	Deletion
8	ΔFlexible ΔPrunus + HopA2	Deletion
9	ΔFlexible ΔPrunus + HopAE	Made December 2022
10	ΔFlexible ΔPrunus + HopH1	Made December 2022
11	ΔFlexible ΔPrunus + HopAR1 + HopAW1	Conjugation
12	ΔFlexible ΔPrunus + HopAR1 + AvrRpm1	Conjugation
13	ΔFlexible ΔPrunus + HopAR1 + HopA2	Conjugation
14	ΔFlexible ΔPrunus + HopAR1 + HopAE	Conjugation
15	ΔFlexible ΔPrunus + HopAR1 + HopH1	Conjugation
16	ΔFlexible ΔPrunus + HopAW1 + AvrRpm1	Deletion
17	ΔFlexible ΔPrunus + HopAW1 + HopA2	Deletion
18	ΔFlexible ΔPrunus + HopAW1 + HopAE	Deletion
19	ΔFlexible ΔPrunus + HopAW1 + HopH1	Deletion
20	ΔFlexible ΔPrunus + AvrRpm1 + HopA2	Deletion
21	ΔFlexible ΔPrunus + AvrRpm1 + HopAE	Deletion
22	ΔFlexible ΔPrunus + AvrRpm1 + HopH1	Deletion
23	ΔFlexible ΔPrunus + HopA2 + HopAE	Made December 2022
24	ΔFlexible ΔPrunus + HopA2 + HopH1	Made December 2022
25	ΔFlexible ΔPrunus + HopAE + HopH1	Made December 2022

5. Outputs relating to the project

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

Output	Detail
Summer CTP event.	**Presentation.**

6. Partners (if applicable)

Scientific partners	University of Reading
Industry partners	Berry Gardens
Government sponsor	BBSRC