

Studentship Project: Annual Progress Report

Oct 2022 to Oct 2023

Student Name:	Adam Gregg	AHDB Project Number:	CTP_FRC_2020_4
Project Title:	Characterising broad-acting resistance to bacterial canker of cherry and elucidating tissue-specific mechanisms of immunity		
Lead Partner:	University of Reading, NIAB-EMR, & Berry Gardens		
Supervisor:	Dr Richard Harrison, Dr Jordan Price, & Dr Glyn Barrett		
Start Date:	Oct 2020	End Date:	Oct 2024

1. Project aims and objectives

This research aims to elucidate the interactions between *Pseudomonas* pathogens and sweet and wild cherry, with a focus on understanding the roles of bacterial effectors and the genetic factors contributing to resistance.

The first avenue of research assesses the importance of specific bacterial effectors and their contribution to the disease. Effectors are proteins delivered by the pathogen to subvert the plant's immune system and manipulate the host to facilitate colonization of intracellular spaces within the host plant. Many bacterial mutants expressing only specific effectors have been created, and detached leaf and shoot tests have been conducted to study the effects of these effector groups on the disease.

The second part of this project aims to unravel the genetic basis of resistance. A diverse, structured population of 1200 trees was planted at NIAB's East Malling site, in Kent (Table 1). This population is structured to analyze genetic recombinations within different families and enables mapping of regions associated with resistance. The population was exposed to three distinct strains of *Pseudomonas syringae* (Pss-9644, Psm-R1-5244, and Psm-R2-Leaf). The phenotype of each tree was assessed by measuring lesion length and visually evaluating infection sites. Leaves from these trees were also collected and will be sequenced. This combined phenotyping/genotyping dataset will allow genetic mapping of loci involved in resistance and potentially reveal genetic markers for use in future resistance breeding.

Table 1. Structure of multiparental population planted at NIAB-EMR.

F	M	Fmily	POL	NO	TOT
Penny	C273-18	C554	CC	25	29
Summer Sun	C273-18	C595	CC	50	72
Penny	Sweetheart	C677	OP	25	94
Colney	Sweetheart	C727	OP	25	37
Colney	C210-7	C417	CC	50	300
Korvic	Kordia	C678	OP	25	53
Merchant	Korvic	C679	OP	25	45
C417-317	Regina	C708	OP	50	490
C367-9	Regina	C716	OP	25	63
Penny	C367-9	C714	CC	25	40
Colney	Stella	C728	OP	25	34
Summit	Sunburst	C632	CC	25	33

The results described in this summary report are interim and relate to one year. In all cases, the reports refer to projects that extend over a number of years.

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2. Key messages emerging from the project

Effector mutant testing (a sample of the data shown in Figure 1):

- Key effector groups responsible for disease symptoms in leaf tissue have been shown to be the three flexible and six prunus effectors.
- Some effectors have an additive effect on disease progression. Removal of the three flexible effectors notably reduces symptoms, removal of the flexible and prunus effectors completely stops disease formation indicating these effectors are key for colonisation of the plant host.
- Toxins released by the pathogen play a critical role in the development of disease symptoms in fruit tissue. Removing toxins from the pathogen only had a minimal effect on disease formation in leaf tissue.

Phenotyping results (Figure 2-5):

- Phenotyping data showed good differentiation between strains and families which lends itself to the successful future mapping of genetic loci associated with resistance.
 - Kruskal-Wallis Dunn's Post-Hoc analysis was performed revealing a significant difference between Pss-9644 vs Psm-R2 across all families: $p=0.0361$ (Table 2).
 - 32 significant P values revealed through Kruskal-Wallis Dunn's Post-Hoc when comparing lesion data for all strains across each individual family (Table 3).
 - 23 significant P values for Pss-9644 across each individual family (Table 4).
 - 23 significant P values for Psm-R1-5244 across each individual family (Table 5).
 - 18 significant p values for Psm-R2-Leaf across each individual family (Table 6).

3. Summary of results from the reporting year

Effector Mutant Experiment:

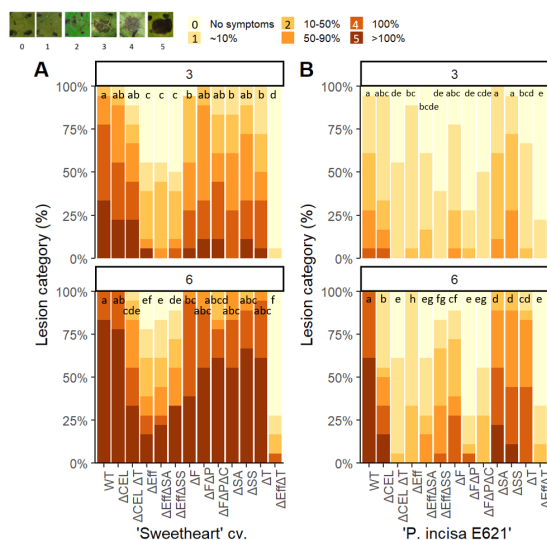


Figure 1. Two out of seven cultivars tested shown. Stacked barchart showing leaf disease symptoms for wild cherries, Sweetheart and *P. incisa* following inoculation with different Pss-9644 effector mutants. Leaves were syringe infiltrated with 2×10^8 CFU/mL and incubated at 22°C, 16/8 photoperiod for 7 days. Symptom score was assessed visually each day. The chart shows the disease score 3 and 6 days post inoculation. A pairwise Fisher's exact test was performed and the resulting categories are shown at the top of each bar.

Phenotyping Data:

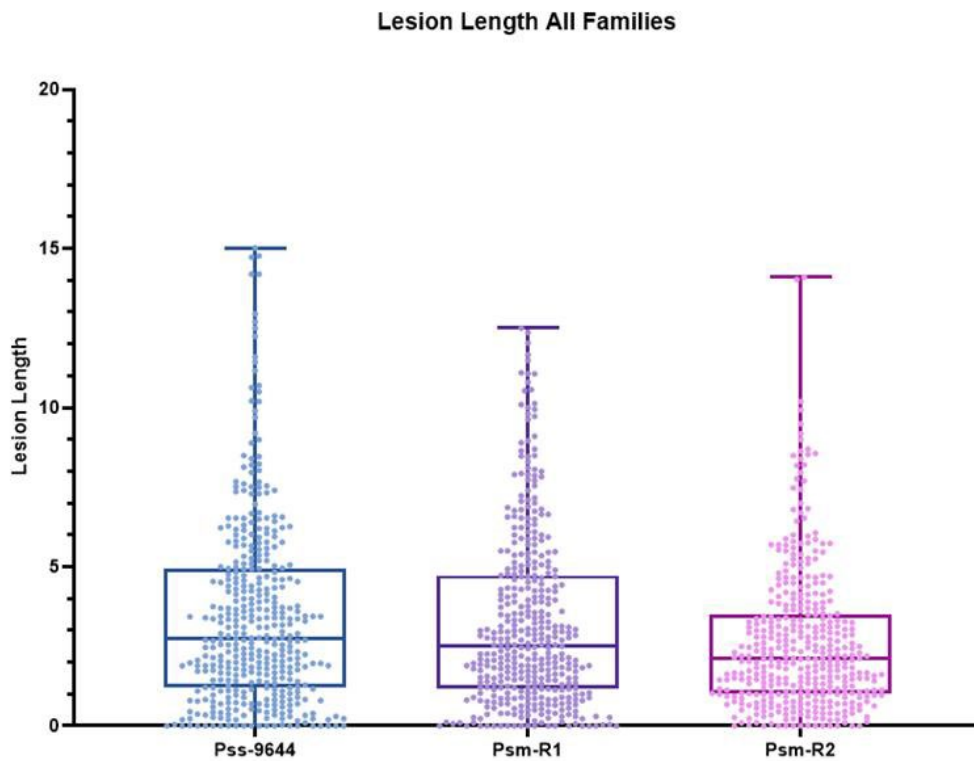


Figure 2. This box plot shows the distribution of lesion length for each *Pseudomonas syringae* strain tested across all families. The medians are indicated by solid horizontal lines in the box plot, were Pss-9644: 2.73 cm, Psm-R1: 2.52 cm, Psm-R2: 2.11 cm. The mean lesion lengths per strain were: Pss-9644: 3.45 cm, Psm-R1: 2.52 cm, Psm-R2: 2.63 cm. Kruskal-Wallis Dunn's Post-Hoc analysis was performed revealing a significant difference between Pss-9644 vs Psm-R2 ($p=0.0361$).

Table 2. Dunn's Multiple comparisons test results for each strain across all families.

Dunn's multiple comparisons test	Mean rank diff.	Significant?	Summary	Bonferroni-adjusted p-value	
Pss-9644 vs. Psm-R1	16.92	No	ns	>0.9999	A-B
Pss-9644 vs. Psm-R2	100.5	Yes	*	0.0361	A-C
Psm-R1 vs. Psm-R2	83.57	No	ns	0.1109	B-C

Lesion Length - All Strains by Family

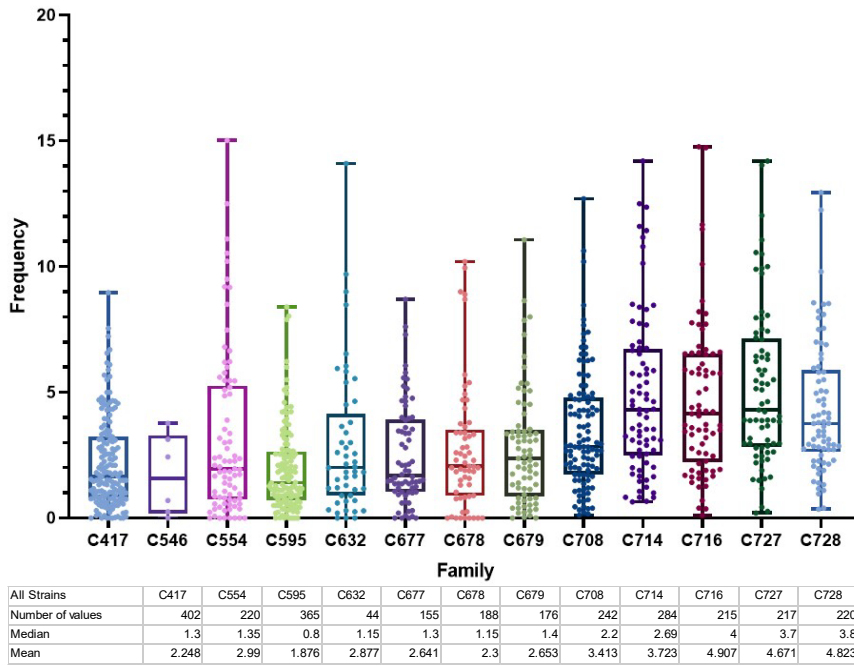


Figure 3. This box plot shows the distribution of lesion length for all *Pseudomonas syringae* strains tested for each family. Number of values, median, and mean shown in table beneath graph. Kruskal-Wallis Dunn’s Post-Hoc analysis as performed on this data revealing (table 3).

Table 3. Dunn’s Multiple comparisons test results for all strains across each family. Only Significant P values shown.

	Dunn’s multiple comparisons test	Mean rank diff.	Significant?	Summary	Bonferroni-adjusted p-value
1	C417 vs. C708	-251.4	Yes	**	0.0054
2	C417 vs. C714	-348.6	Yes	****	<0.0001
3	C417 vs. C716	-544.7	Yes	****	<0.0001
4	C417 vs. C727	-478.8	Yes	****	<0.0001
5	C417 vs. C728	-514.9	Yes	****	<0.0001
6	C554 vs. C714	-288.7	Yes	**	0.0027
7	C554 vs. C716	-484.9	Yes	****	<0.0001
8	C554 vs. C727	-418.9	Yes	****	<0.0001
9	C554 vs. C728	-455.1	Yes	****	<0.0001
10	C595 vs. C708	-359.4	Yes	****	<0.0001
11	C595 vs. C714	-456.6	Yes	****	<0.0001
12	C595 vs. C716	-652.8	Yes	****	<0.0001
13	C595 vs. C727	-586.8	Yes	****	<0.0001
14	C595 vs. C728	-623	Yes	****	<0.0001
15	C632 vs. C716	-518	Yes	**	0.0043
16	C632 vs. C727	-452	Yes	*	0.0322
17	C632 vs. C728	-488.2	Yes	*	0.0108
18	C677 vs. C714	-325.7	Yes	**	0.0021
19	C677 vs. C716	-521.9	Yes	****	<0.0001
20	C677 vs. C727	-455.9	Yes	****	<0.0001
21	C677 vs. C728	-492	Yes	****	<0.0001
22	C678 vs. C708	-303.8	Yes	**	0.0044
23	C678 vs. C714	-401	Yes	****	<0.0001
24	C678 vs. C716	-597.2	Yes	****	<0.0001
25	C678 vs. C727	-531.2	Yes	****	<0.0001
26	C678 vs. C728	-567.3	Yes	****	<0.0001
27	C679 vs. C714	-296.9	Yes	**	0.0052
28	C679 vs. C716	-493.1	Yes	****	<0.0001
29	C679 vs. C727	-427.2	Yes	****	<0.0001
30	C679 vs. C728	-463.3	Yes	****	<0.0001
31	C708 vs. C716	-293.4	Yes	**	0.0043
32	C708 vs. C728	-263.6	Yes	*	0.0203

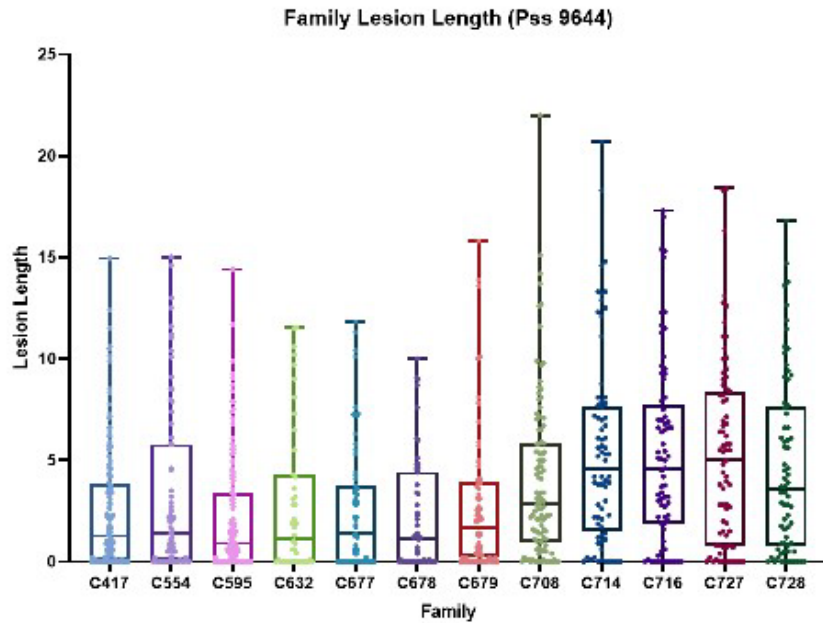


Figure 4. This box plot shows the distribution of lesion length for all *Pseudomonas syringae* 9644 for each family. Number of values, median, and mean shown in table beneath graph. Kruskal-Wallis Dunn's Post-Hoc analysis as performed on this data revealing (table 4).

Table 4. Dunn's Multiple comparisons test results for Pss-9644 across each family. Only significant P values shown.

	Dunn's multiple comparisons test	Mean rank diff.	Significant?	Summary	Bonferroni-adjusted p-value
1	C417 vs. C714	-192.5	Yes	***	0.0001
2	C417 vs. C716	-181.6	Yes	***	0.0004
3	C417 vs. C727	-181.1	Yes	***	0.0004
4	C417 vs. C728	-141.8	Yes	*	0.0343
5	C554 vs. C714	-165.5	Yes	*	0.0259
6	C595 vs. C708	-141.4	Yes	**	0.008
7	C595 vs. C714	-217.6	Yes	****	<0.0001
8	C595 vs. C716	-206.7	Yes	****	<0.0001
9	C595 vs. C727	-206.1	Yes	****	<0.0001
10	C595 vs. C728	-166.8	Yes	**	0.0041
11	C632 vs. C714	-196.7	Yes	*	0.0168
12	C632 vs. C716	-185.8	Yes	*	0.0352
13	C632 vs. C727	-185.3	Yes	*	0.0342
14	C677 vs. C714	-201.1	Yes	**	0.0017
15	C677 vs. C716	-190.2	Yes	**	0.0042
16	C677 vs. C727	-189.6	Yes	**	0.004
17	C678 vs. C714	-232.1	Yes	***	0.0003
18	C678 vs. C716	-221.2	Yes	***	0.0008
19	C678 vs. C727	-220.6	Yes	***	0.0007
20	C678 vs. C728	-181.4	Yes	*	0.0243
21	C679 vs. C714	-181.4	Yes	**	0.0089
22	C679 vs. C716	-170.5	Yes	*	0.0211
23	C679 vs. C727	-170	Yes	*	0.0201

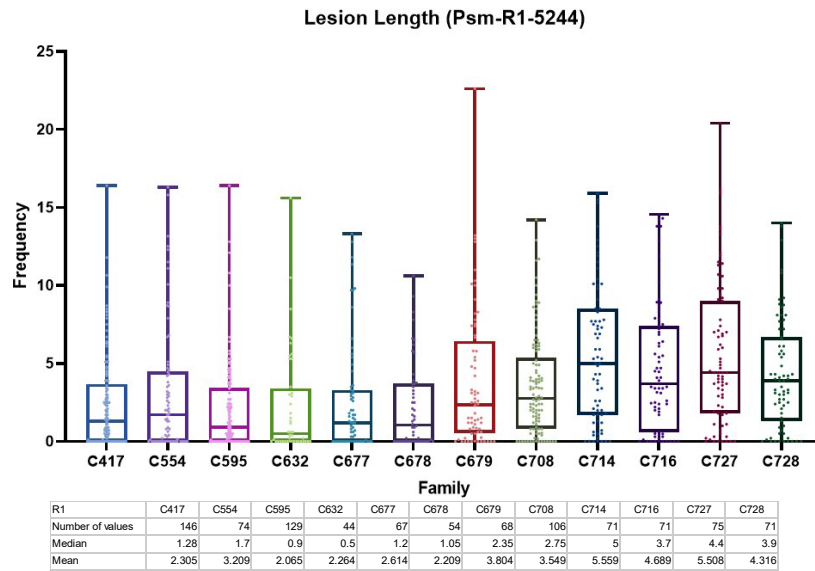


Figure 5. This box plot shows the distribution of lesion length for all *Pseudomonas syringae morspunorum* R1-5244 for each family. Number of values, median, and mean shown in table beneath graph. Kruskal-Wallis Dunn's Post-Hoc analysis as performed on this data revealing (table 5).

Table 5. Dunn's Multiple comparisons test results for Psm-R1-5244 across each family. Only significant P values shown.

	Dunn's multiple comparisons test	Mean rank diff.	Significant?	Summary	Bonferroni-adjusted p-value
1	C417 vs. C714	-227.4	Yes	****	<0.0001
2	C417 vs. C716	-176.5	Yes	**	0.0011
3	C417 vs. C727	-225.1	Yes	****	<0.0001
4	C417 vs. C728	-171.4	Yes	**	0.0019
5	C554 vs. C714	-187.1	Yes	**	0.0046
6	C554 vs. C727	-184.7	Yes	**	0.0045
7	C595 vs. C708	-143.6	Yes	**	0.0073
8	C595 vs. C714	-250.5	Yes	****	<0.0001
9	C595 vs. C716	-199.6	Yes	***	0.0001
10	C595 vs. C727	-248.2	Yes	****	<0.0001
11	C595 vs. C728	-194.5	Yes	***	0.0002
12	C632 vs. C714	-260	Yes	***	0.0001
13	C632 vs. C716	-209.1	Yes	**	0.008
14	C632 vs. C727	-257.7	Yes	***	0.0001
15	C632 vs. C728	-204	Yes	*	0.0117
16	C677 vs. C714	-221.8	Yes	***	0.0003
17	C677 vs. C716	-170.8	Yes	*	0.0272
18	C677 vs. C727	-219.5	Yes	***	0.0003
19	C677 vs. C728	-165.8	Yes	*	0.0406
20	C678 vs. C714	-241.8	Yes	***	0.0001
21	C678 vs. C716	-190.9	Yes	*	0.0128
22	C678 vs. C727	-239.5	Yes	***	0.0001
23	C678 vs. C728	-185.8	Yes	*	0.0191

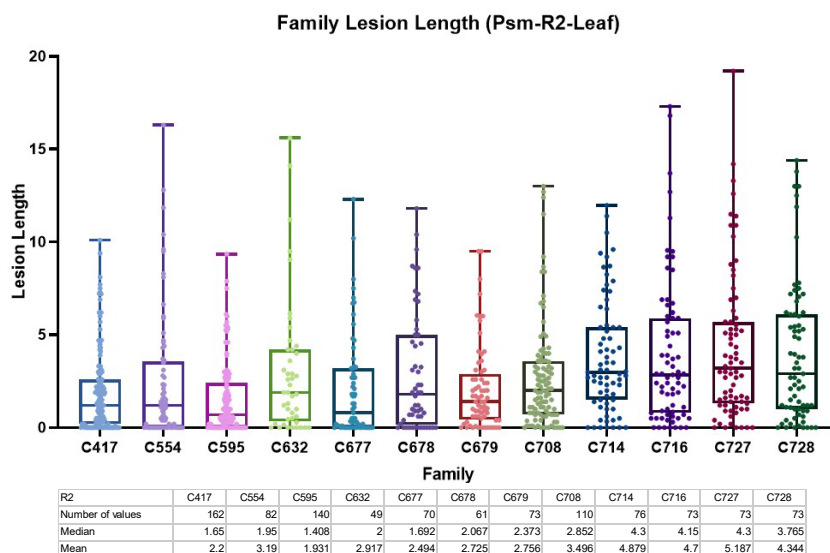


Figure 6. This box plot shows the distribution of lesion length for all *Pseudomonas syringae morspunorum* R2-Leaf for each family. Number of values, median, and mean shown in table beneath graph. Kruskal-Wallis Dunn's Post-Hoc analysis as performed on this data revealing (table 5).

Table 5. Dunn's Multiple comparisons test results for Psm-R2-Leaf across each family. Only significant P values shown.

	Dunn's multiple comparisons test	Mean rank diff.	Significant?	Summary	Bonferroni-adjusted p-value
1	C417 vs. C714	-191	Yes	***	0.0002
2	C417 vs. C716	-166.3	Yes	**	0.0034
3	C417 vs. C727	-199.1	Yes	****	<0.0001
4	C417 vs. C728	-168.7	Yes	**	0.0026
5	C554 vs. C714	-184.8	Yes	**	0.0062
6	C554 vs. C716	-160.1	Yes	*	0.0493
7	C554 vs. C727	-192.9	Yes	**	0.0023
8	C554 vs. C728	-162.5	Yes	*	0.0407
9	C595 vs. C708	-141.5	Yes	**	0.0096
10	C595 vs. C714	-238.1	Yes	****	<0.0001
11	C595 vs. C716	-213.3	Yes	****	<0.0001
12	C595 vs. C727	-246.1	Yes	****	<0.0001
13	C595 vs. C728	-215.8	Yes	****	<0.0001
14	C677 vs. C714	-208.6	Yes	**	0.001
15	C677 vs. C716	-183.8	Yes	**	0.0096
16	C677 vs. C727	-216.6	Yes	***	0.0004
17	C677 vs. C728	-186.3	Yes	**	0.0078
18	C679 vs. C727	-168.1	Yes	*	0.0276

4. Key issues to be addressed in the next year

The only aspect of this project which needs to be addressed is the processing of the genotyping data. DNA samples are currently being sequenced at Novotech, Cambridge. Once sequence data is available (4-6 weeks), it has been arranged to visit Wageningen University to be trained on using their pipeline for analysis of this data. Apart from this analysis, the only other aspect of this project which needs to be completed is the writing/finalisation of the four thesis chapters, chapters 1-3 are scheduled to be completed by the start of April, and the final chapter will be written up following the sequencing analysis and is scheduled to be completed between May and the end of September.

5. Outputs relating to the project

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

Output	Detail
Scientific Paper	Awaiting to be reviewed and published. Effector mutant data to be used as part of a larger paper written by a former member of the NIAB pseudonomas group.
Summer CTP conference	Attended and presented at the summer CTP event at NIAB-EMR, Kent.

6. Partners (if applicable)

Scientific partners	NIAB-EMR, Reading University
Industry partners	Berry Gardens
Government sponsor	BBSRC