IMPROVED MANAGEMENT OF BACTERIAL BLAST AND BACTERIAL CANKER OF SWEET CHERRY

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BACTERIAL CANKER: symptoms

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BLOSSOM BLAST: symptoms

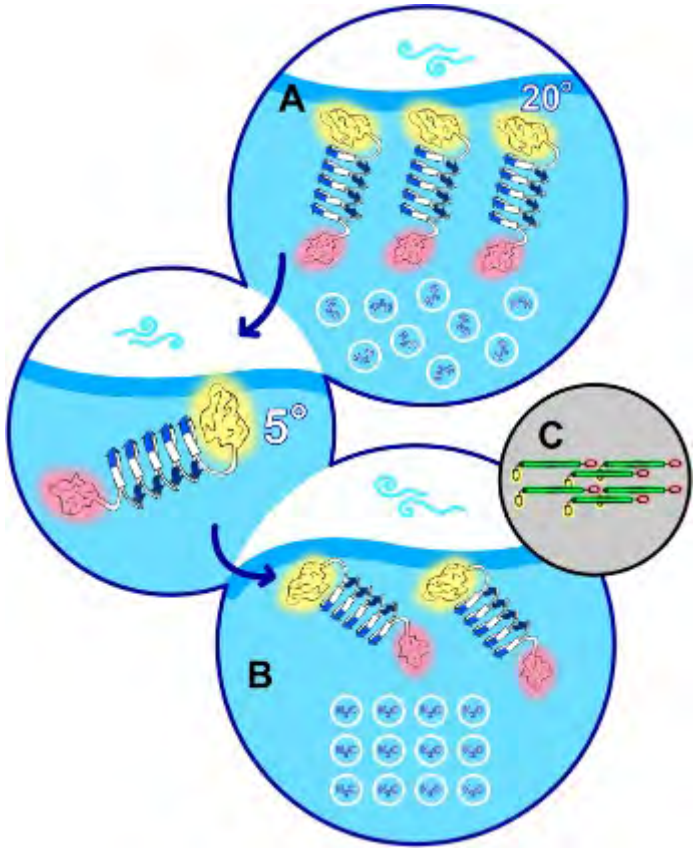
- ❑ Many localized and sometime widespread events of blast in 2018, 2019, 2020, 2021, and 2023
- ❑ Royal Hazel, Royal Lynn and Coral cvs.

BLOSSOM BLAST: symptoms

- Can be extremely severe

ICE NUCLEATION ACTIVITY:

- ***P. syringae*** **pv. *syringae* can trigger ice crystal formation at temperatures where water would normally remain liquid**



Statement of problem - Rationale

- The disease is very active in California, the pathogen *Pseudomonas syringae* is ubiquitous in cherry orchards

- **Few studies in sweet cherry in California**
  - Little, E.L., Bostock, R.M. and Kirkpatrick, B.C., 1998. Genetic characterization of *Pseudomonas syringae pv. syringae* strains from stone fruits in California. *Applied and Environmental Microbiology*, *64*(10), pp.3818-3823. **4 strains from cherry**
  - *WILSON, E.E., 1931. A comparison of Pseudomonas prunicola with a canker-producing bacterium of stone-fruit trees in California. Phytopathology, 21(12).*

- **Two distinct phases:** bacterial blast and bacterial canker

- A complex disease, **little knowledge about the disease biology and epidemiology**

- Historically, *Pseudomonas syringae* pv. *syringae* (Pss), and *P. syringae* pv. *morsprunoru*m (Psm) races 1 and 2 have been reported from California sweet cherry
  - The different pathovars and races of *P. syringae* isolates from cherry have been distinguished and characterized by physiological and biochemical tests
  - Information is outdated
  - The *Pseudomonas syringae* phylogenetic group comprises 15 recognized bacterial species closely related to *P. syringae* and more than 60 pathovars, many are from Prunus sp.

- New findings from our almond research – 7 distinct groups of pseudomonads associated with almond, 3 pathogens.

Objectives: 3-year project (2/3)

1- to characterize *Pseudomonas* isolates from cherry using **whole genome sequencing (Year 1)**

2- to investigate the **pathogenicity of *Pseudomonas*** species and *P. syringae* pathovars from cherry and **recognize the main pathogen groups (Year 1)**

3- to determine **baseline sensitivities** for kasugamycin and oxytetracycline of all pathogenic *Pseudomonas* spp. and *P. syringae* pathovars affecting cherry in California, and to determine the frequency of copper resistance within *P. syringae* populations **(Dr. Jim Adaskaveg Year 1 & 2)**

4- to develop and validate a real-time PCR assay for the **specific detection** and **quantification** of *P. syringae* cherry-adapted pathogenic strains or pathovars **(Year 1 & 2)**

5- Gain knowledge of disease epidemiology (main inoculum sources and population dynamic **(Year 2 & 3)**

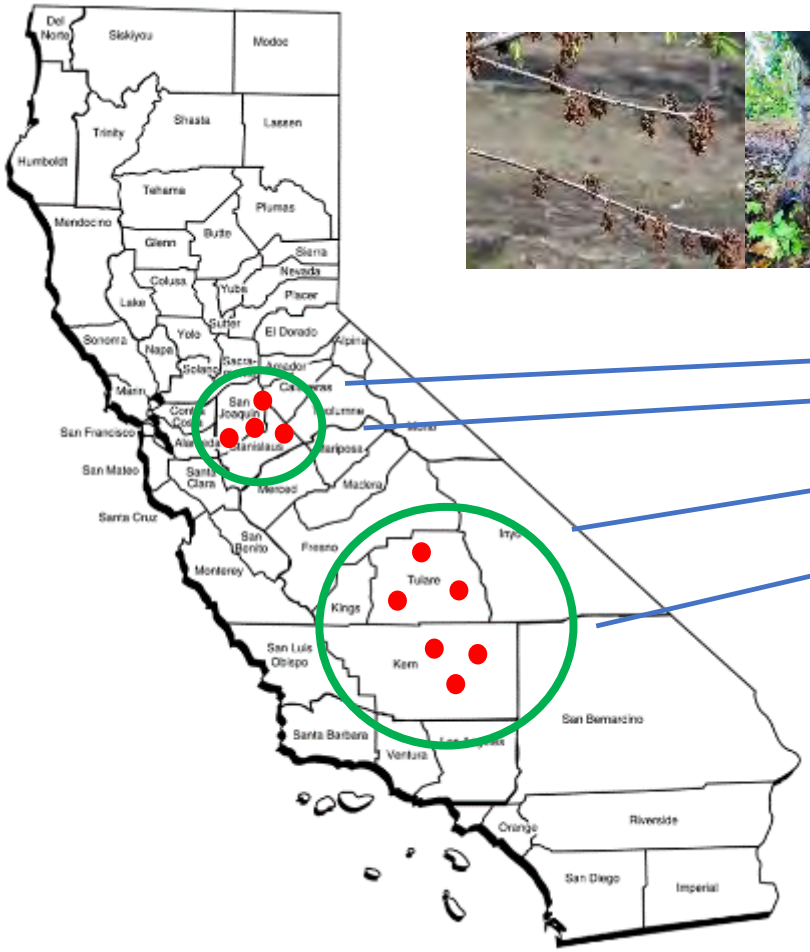
6- to develop disease-risk prediction tool **(Year 2 & 3)**

7- to develop guidelines to industry stakeholders to improve management of bacterial blast and canker of sweet cherry in California **(Year 3):** develop risk prediction model, optimize timing of bactericide applications

Objective 1: characterize *Pseudomonas* isolates from cherry using whole genome sequencing

STATEWIDE SURVEYS FOR PSEUDOMONAS:

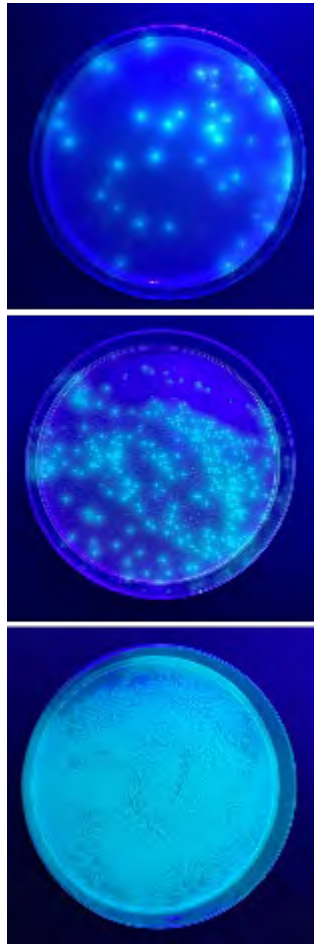
Statewide survey



sweet cherry

Laboratory isolations

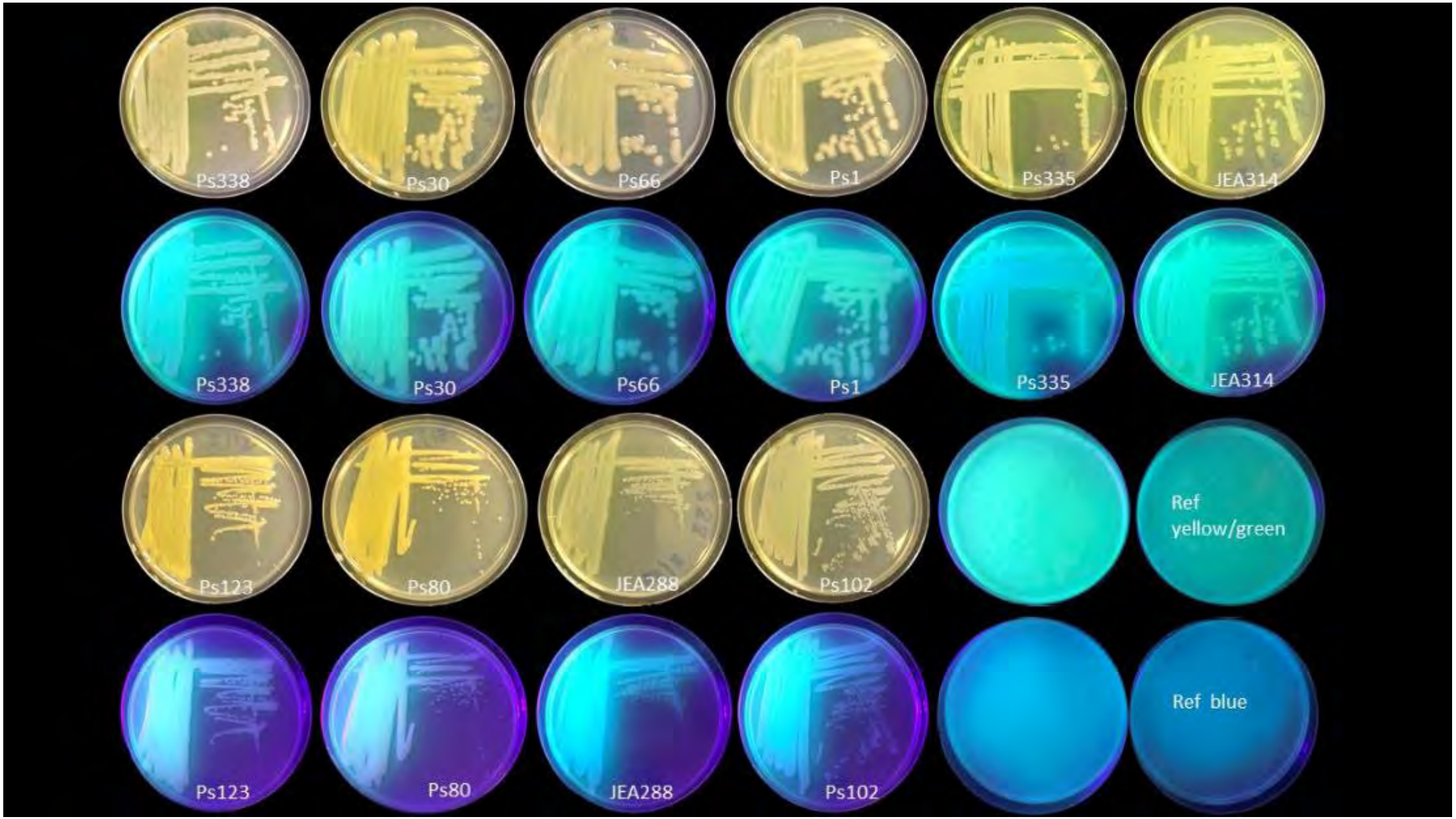
Isolate collection



King's B medium

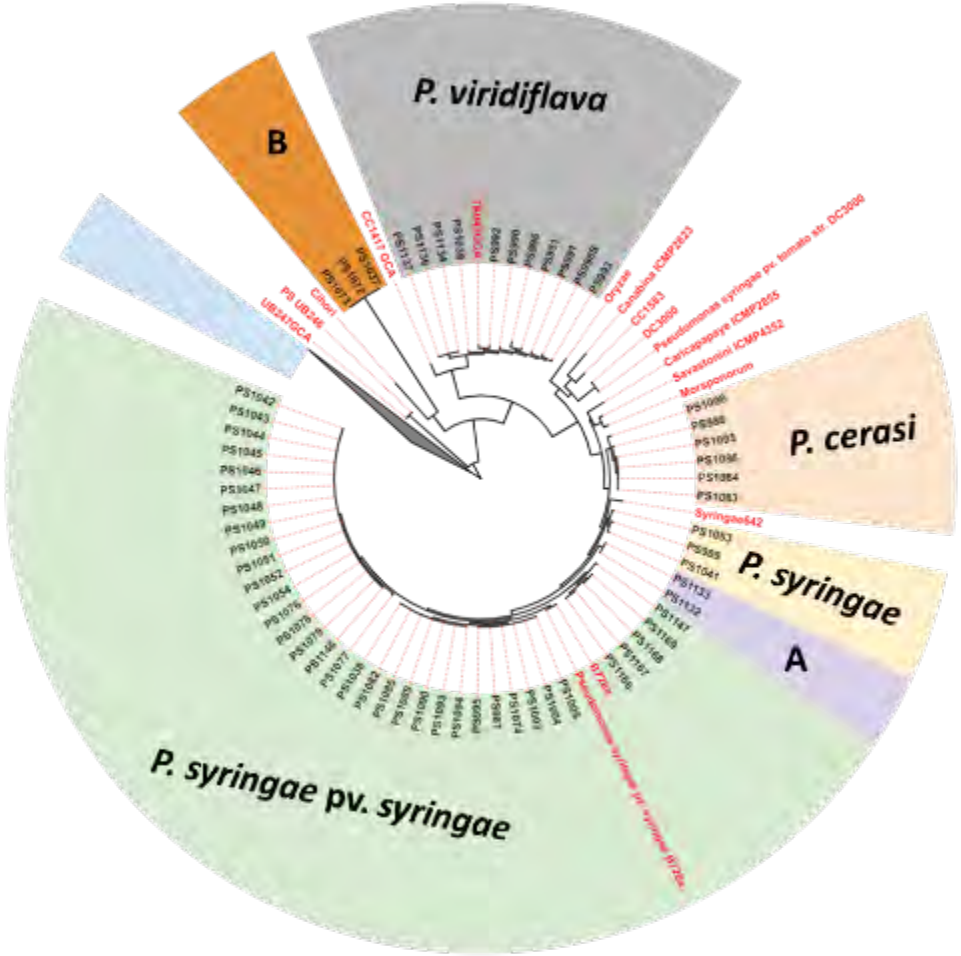
More than 300 isolates of Pseudomonas were collected from symptomatic and asymptomatic cherry tissues

Morphology: isolation on King's B medium and fluorescence



Pseudomonas syringae species complex:

- *P. syringae* species complex comprises of commensals, opportunistic, and specialized phytopathogens
- **6 genomospecies** within the *P. syringae* species complex were identified from symptomatic and asymptomatic cherry tissue
- **At least 4 putative plant pathogens** identified based on genome prediction analysis



Phylogenomic Species	pathogenicity locus probable regulatory protein hrpR	Ice nucleation (INA+) genotype	Syringomycin biosynthetic genes	Syringopeptin biosynthetic genes	Phaseolotoxin biosynthetic genes
P. syringae pv. syringae	100%	100%	100%	100%	0%
Genomospecies A	100%	100%	0%	0%	0%
P. syringae	100%	100%	100%	100%	0%
P. cerasi	100%	100%	100%	100%	16.70%
P. viridiflava	0%	50%	0%	0%	0%
Genomospecies C	0%	0%	0%	0%	0%

Objective 2: Identify the main pathogenic *Pseudomonas* species in sweet cherry

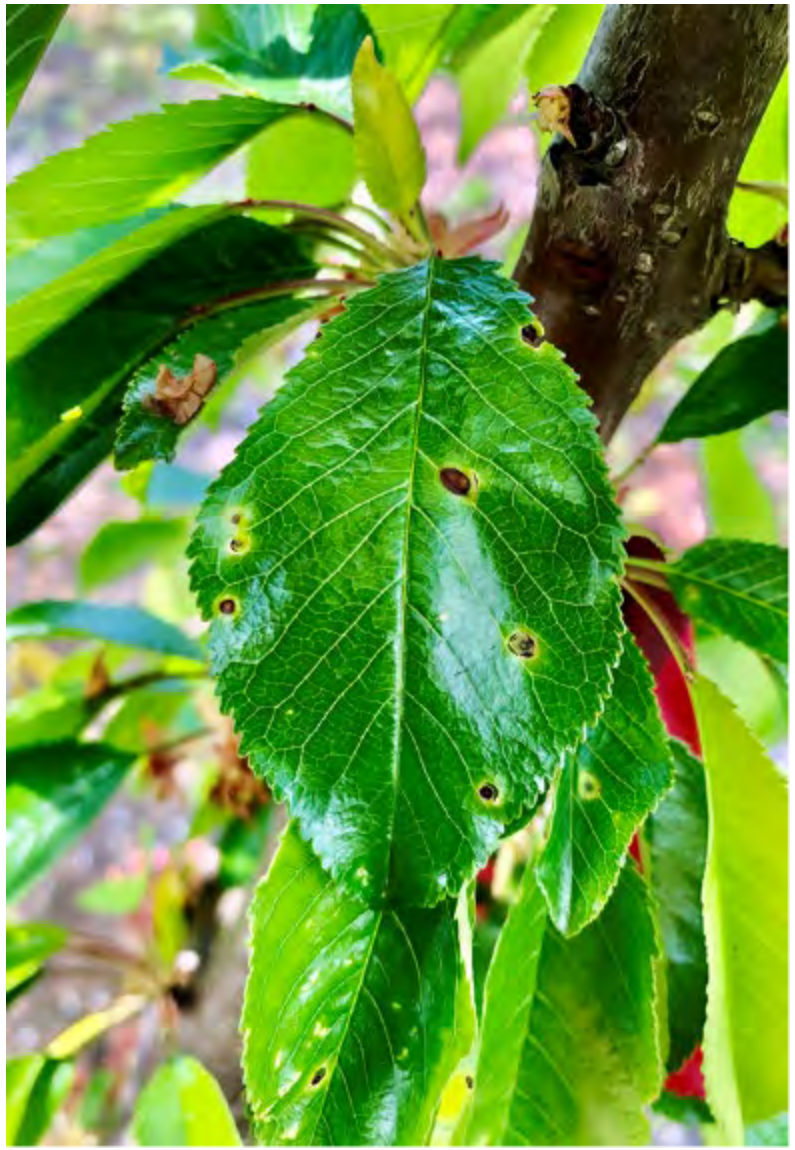
Canker Pathogenicity studies in the field:

Pathogenicity studies for blossom blast:

- Coral cultivar, highly susceptible

Pathogenicity studies: Cherry field assays

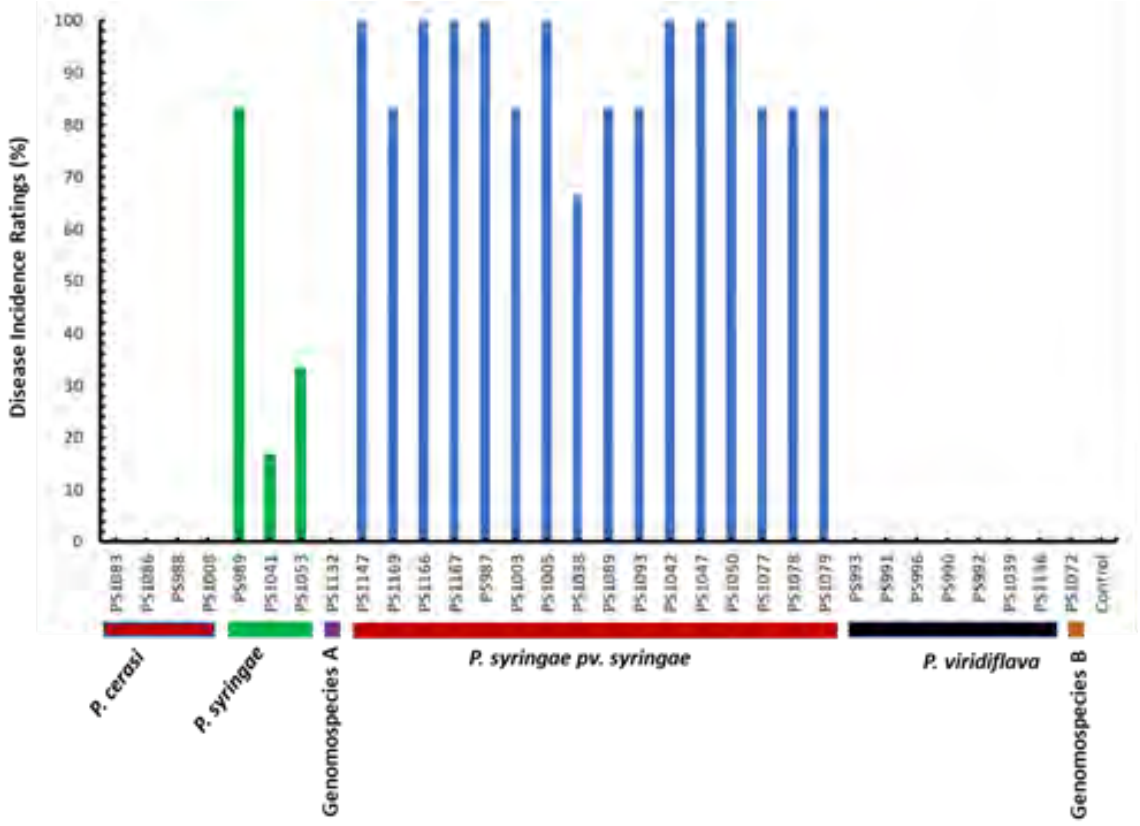
- Leaf spots caused by pathogenic pseudomonads

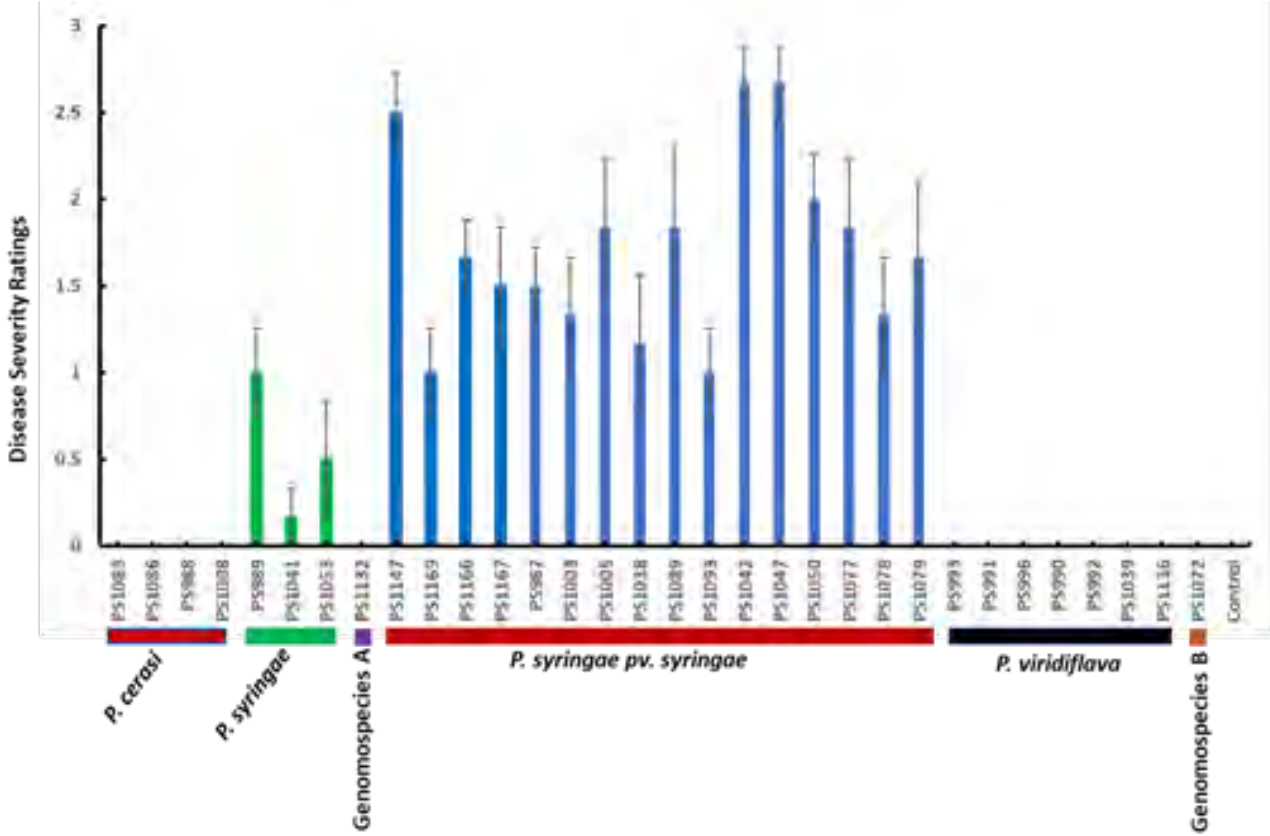

Pathogenicity studies:

Cherry canker field assays November 2023

Disease incidence



Disease severity



Objective 3: Improve disease management, including resistance management

Testing for antibiotic resistance:

GENE PREDICTION

using genome sequence data to predict drug resistance

Summary

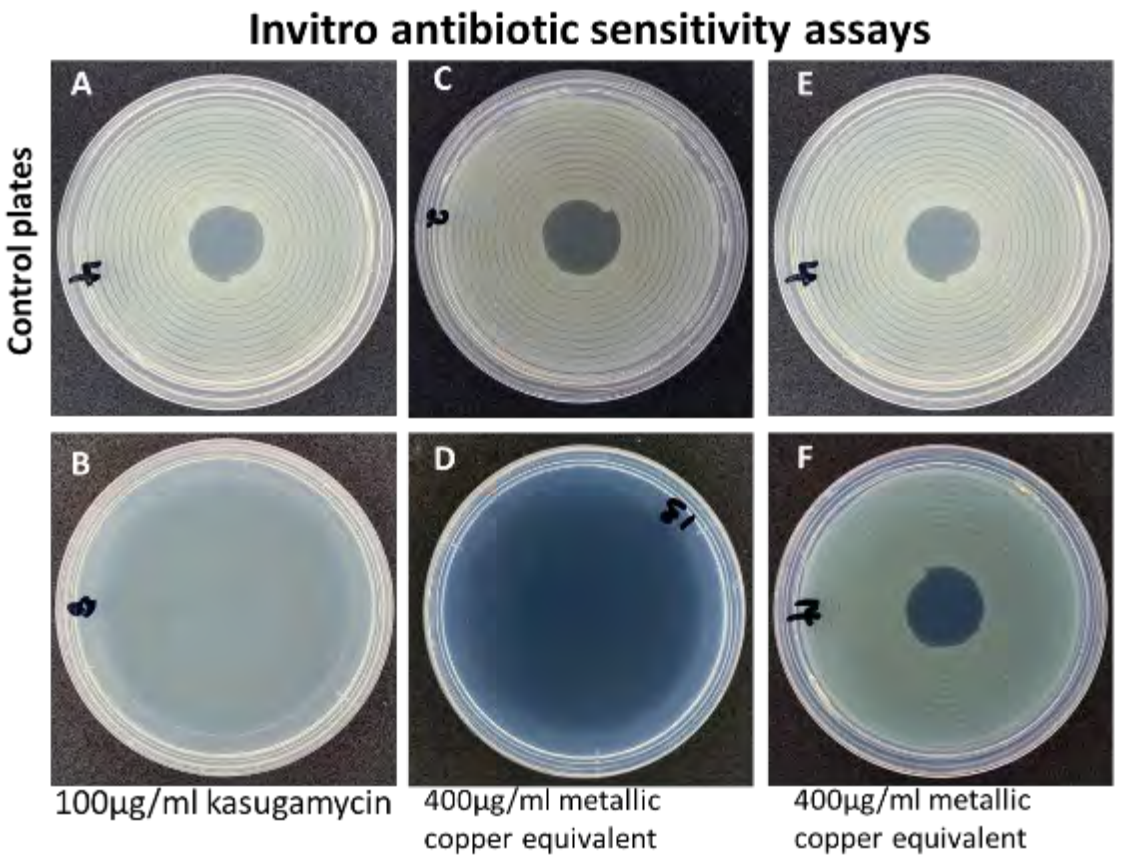
Filename	Date (UTC)	RGI Criteria	# Perfect Hits	# Strict Hits	# Loose Hits	Download
PS8508FAcinetobacter baumannii AbaQ Assembly	October 27, 2022 18:00:58	Perfect, Strict, complete genes only	0	6	0	Download

Results

Search:

RGI Criteria	ARO Term	SNP	Detection Criteria	AMR Gene Family	Drug Class	Resistance Mechanism	% Identity of Matching Region	% Length of Reference Sequence
Strict	Acinetobacter baumannii AbaQ		protein homolog model	major facilitator superfamily (MFS) antibiotic efflux pump	fluoroquinolone antibiotic	antibiotic efflux	72.73	100.08
Strict	adeF		protein homolog model	resistance-nodulation-cell division (RND) antibiotic efflux pump	fluoroquinolone antibiotic; tetracycline antibiotic	antibiotic efflux	42.22	98.58
Strict	adeF		protein homolog model	resistance-nodulation-cell division (RND) antibiotic efflux pump	fluoroquinolone antibiotic; tetracycline antibiotic	antibiotic efflux	98.99	100.38
Strict	adeF		protein homolog model	resistance-nodulation-cell division (RND) antibiotic efflux pump	fluoroquinolone antibiotic; tetracycline antibiotic	antibiotic efflux	42.45	98.98
Strict	APH(3'')-Ib		protein homolog model	APH(3'')	aminoglycoside antibiotic	antibiotic inactivation	99.83	100.00
Strict	APH(6)-Id		protein homolog model	APH(6)	aminoglycoside antibiotic	antibiotic inactivation	99.84	100.00

BASELINE SENSITIVTY STUDIES



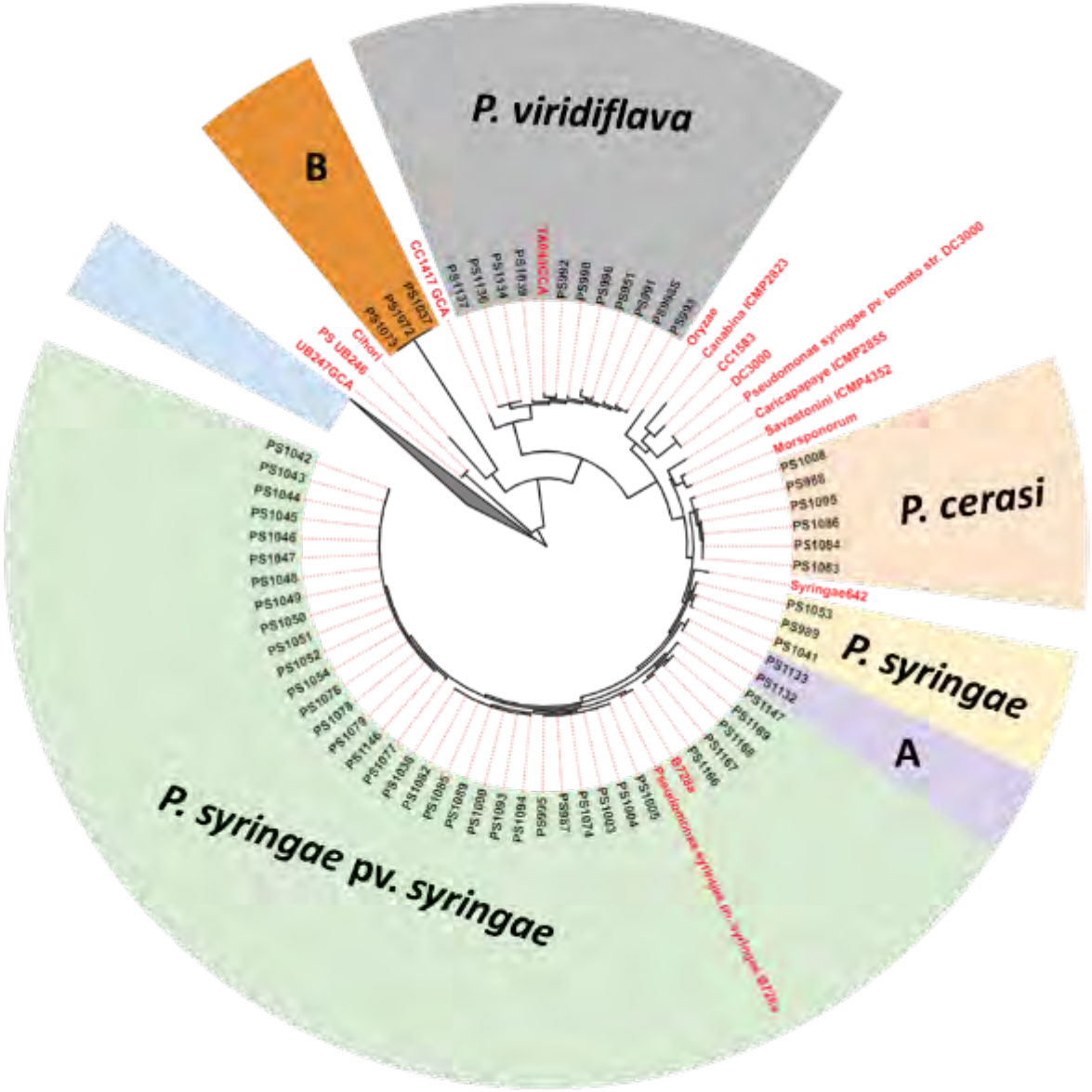
Testing for antibiotic resistance:

- 46% (16/35) of *P. syringae* pv. *syringae* isolates tested had the ctpV gene which is known to confer resistance to copper
- None of the 60 isolates from the *P. syringae* species complex had a gene or mutation that conferred resistance to kasugamycin

Phylogenomic Species	pathogenicity locus probable regulatory protein hrpR	Ice nucleation (INA+) genotype	Syringomycin biosynthetic genes	Syringopeptin biosynthetic genes	Phaseolotoxin biosynthetic genes	Copper resistance genotype (ctpV gene)	Copper resistance phenotype	Kasugamycin resistance genotype	Kasugamycin resistance phenotype
P. syringae pv. syringae	100%	100%	100%	100%	0%	45.7%	45.70%	0%	0%
Genomospecies A	100%	100%	0%	0%	0%	0%	0%	0%	0%
P. syringae	100%	100%	100%	100%	0%	0%	0%	0%	0%
P. cerasi	100%	100%	100%	100%	16.70%	0%	0%	0%	0%
P. viridiflava	0%	50%	0%	0%	0%	35.5%	35.5%	0%	0%
Genomospecies C	0%	0%	0%	0%	0%	0%	0%	0%	0%

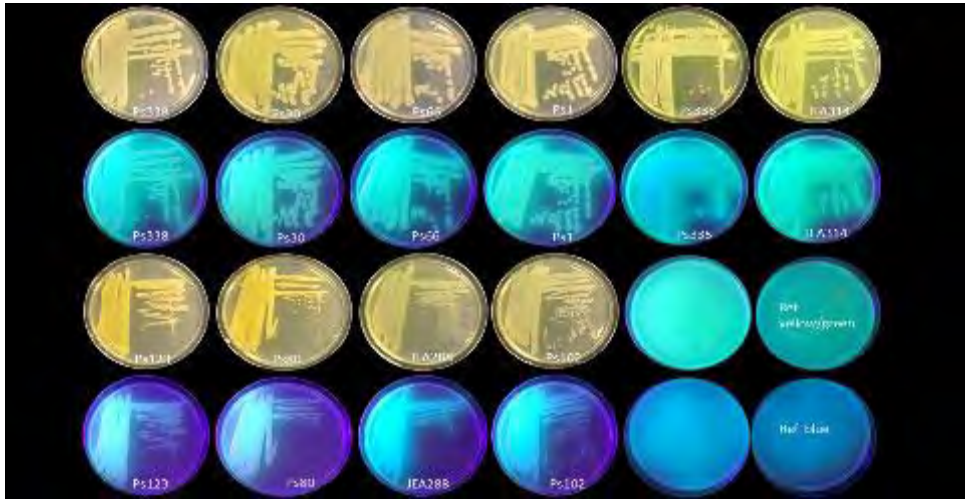
Objective 4: Improve pathogen detection and disease diagnostic

Improved diagnostic and pathogen ID:



Test and design primer sets for diagnosis

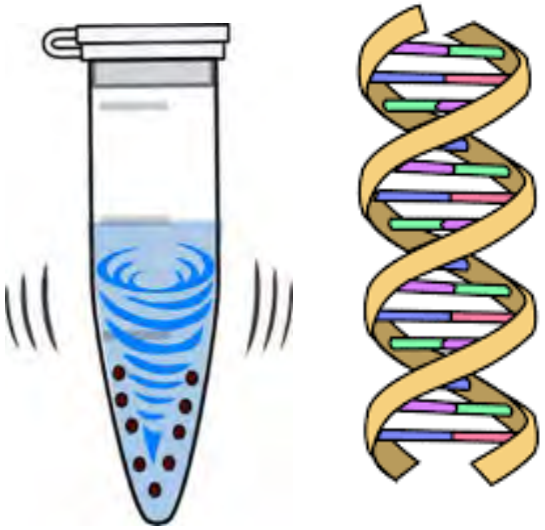
Table 1. Primers to be used for detection of specific species or group			
Primer Name	**Primer Set**	**Target**	**Reference**
G1	**G1_m16F**: 5′-CCGYTGATCTTCGTCGATCT-3′ **G1_R**: 5′-CGGTAATGCTGTCGCCAAAA-3′	*Pathogenic pseudomonads*	Visnovsky *et al.*, 2020
PsAVRE	**PsAVRE_F**: 5′-GACTGGTAGGTCTGAACGCC-3′ **PsAVRE_R**: 5′-TGCTGCTCAGCGTGTAAAGA-3′	*Pseudomonas syringae pv. syringae*	This study
PcAVRE	**PcAVRE_F**: 5′-GGACTACTGGCCTGGCTTTT-3′ **PcAVRE_R**: 5′-CGCGCTTCATAGGTTTCGTG-3′	*Pseudomonas cerasi*	This study
PvhrpR	**PvhrpR_F**: 5′-CATATCCTCAACCGGCTGCT-3′ **PvhrpR_R**: 5′-GCCGTGGAATACCCAGTTCA-3′	*Pseudomonas viridiflava*	This study



Develop and validate a real-time PCR assay for the **specific detection** and **quantification** of P. syringae cherry-adapted pathogenic strains or pathovars.

Diseased samples

1:

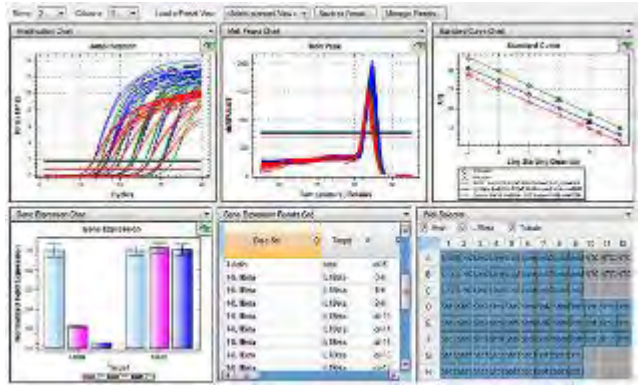


DNA Extraction directly from plant tissue

2:

Real time PCR

3:



Results within 8 hrs

Objective 5: Investigate the disease epidemiology

DISEASE EPIDEMIOLOGY:

- ❑ Cankers as inoculum sources
- ❑ Epiphytic population on buds

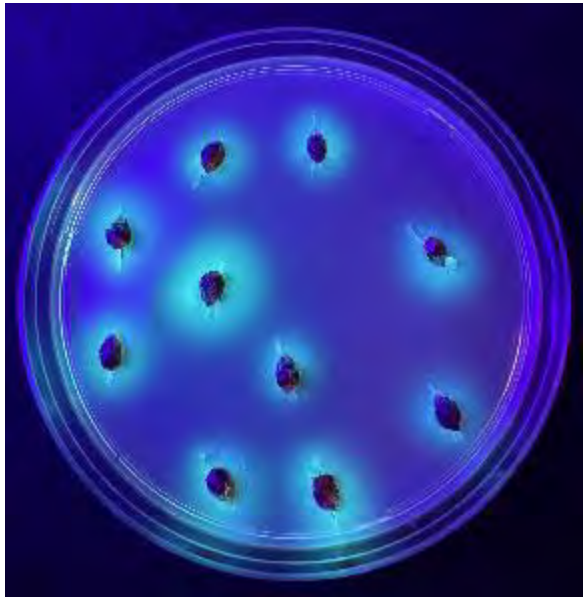
Develop a disease risk prediction tool :

1- Culture-dependent, bud monitoring (BudMon) technique:

- ❑ Flower buds sampled during late winter
- ❑ Frequency of infected buds out of 100 sampled buds

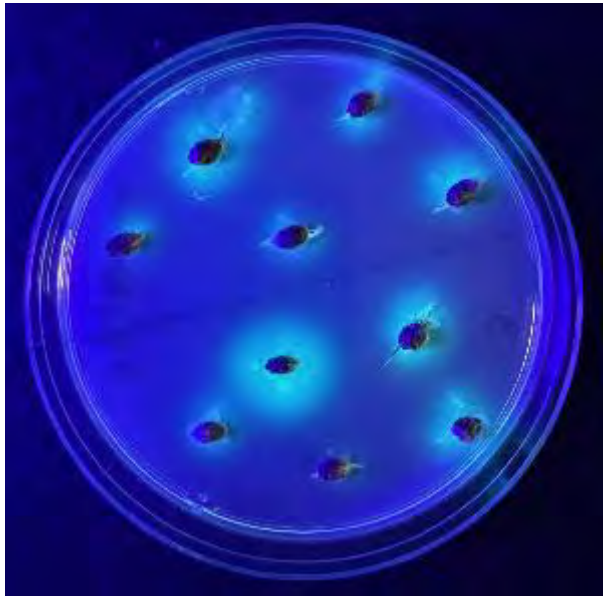
Orchard 1

High



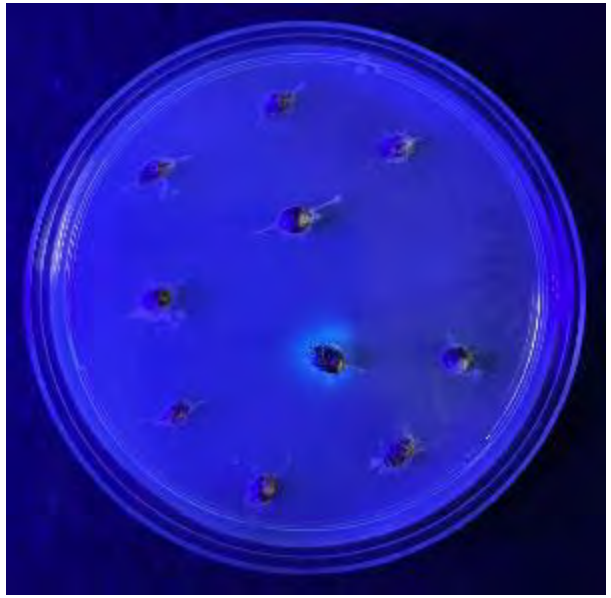
Orchard 2

Moderate



Orchard 3

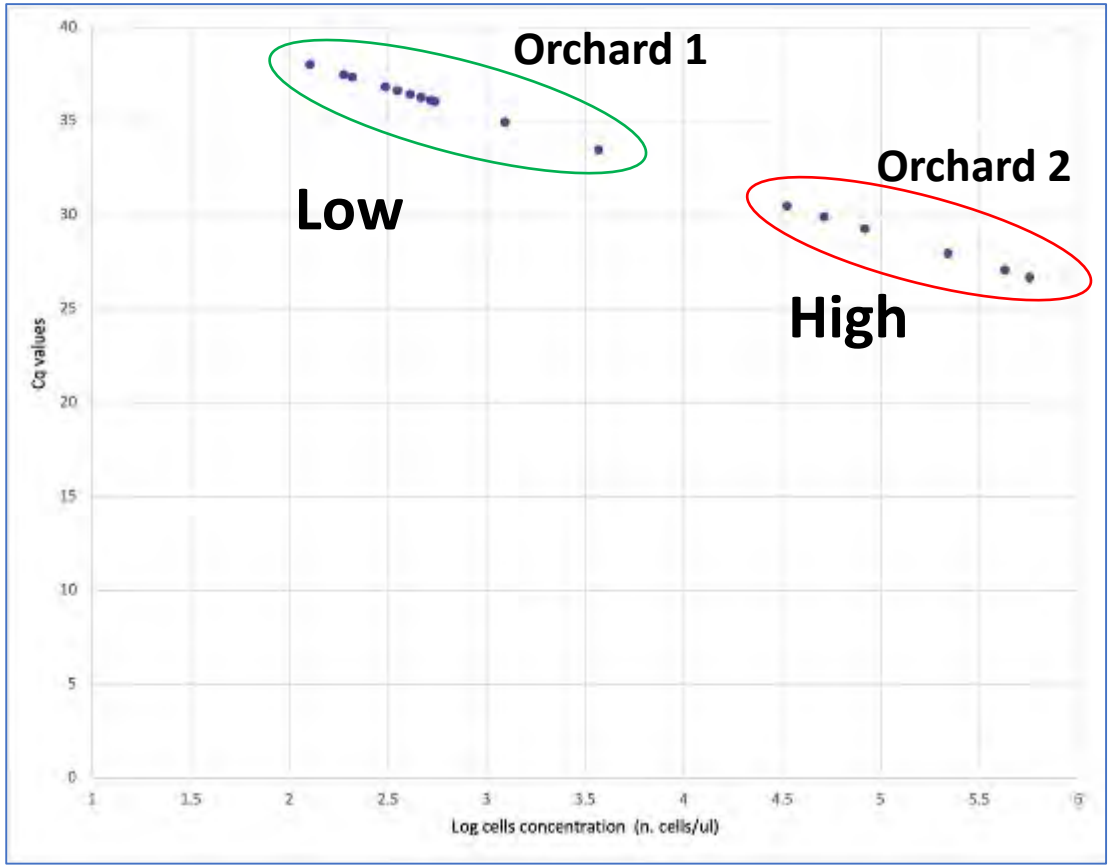
Low



Develop a disease risk prediction tool :

2- Culture-independent, using real time q-PCR

- ❑ Flower buds sampled during late winter
- ❑ Determine population levels in buds and a threshold for disease occurrence



Thank you!

Questions?