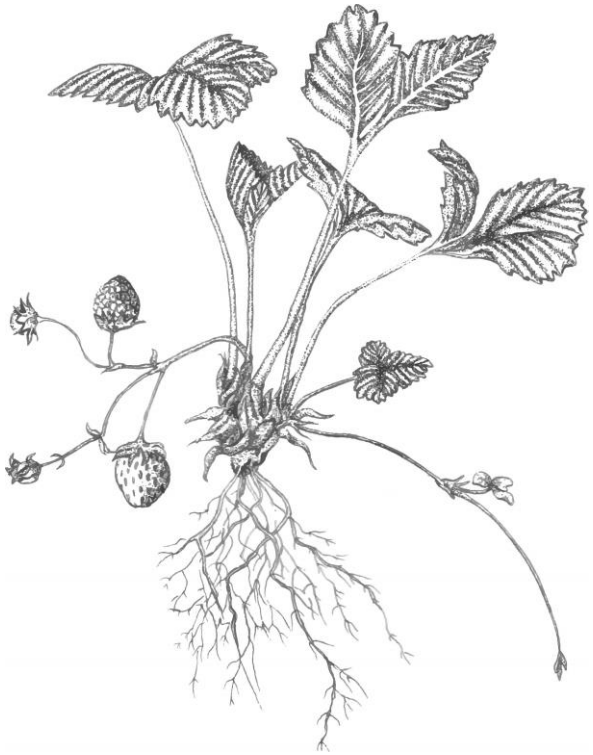
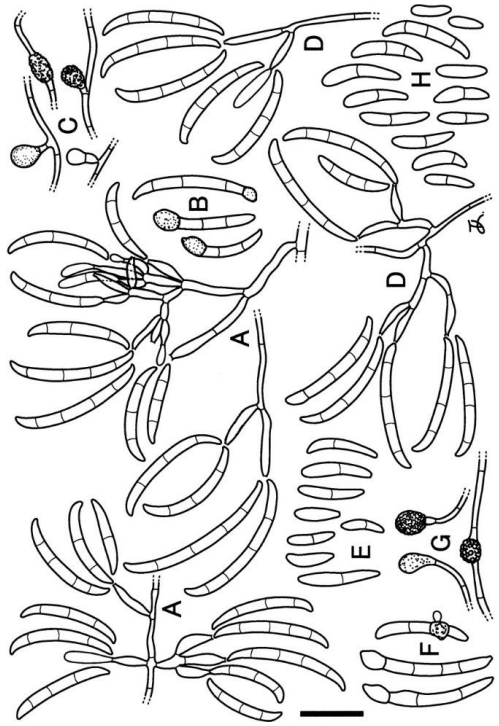
# Updates on *Fusarium oxysporum* f. sp. *fragariae* race 2



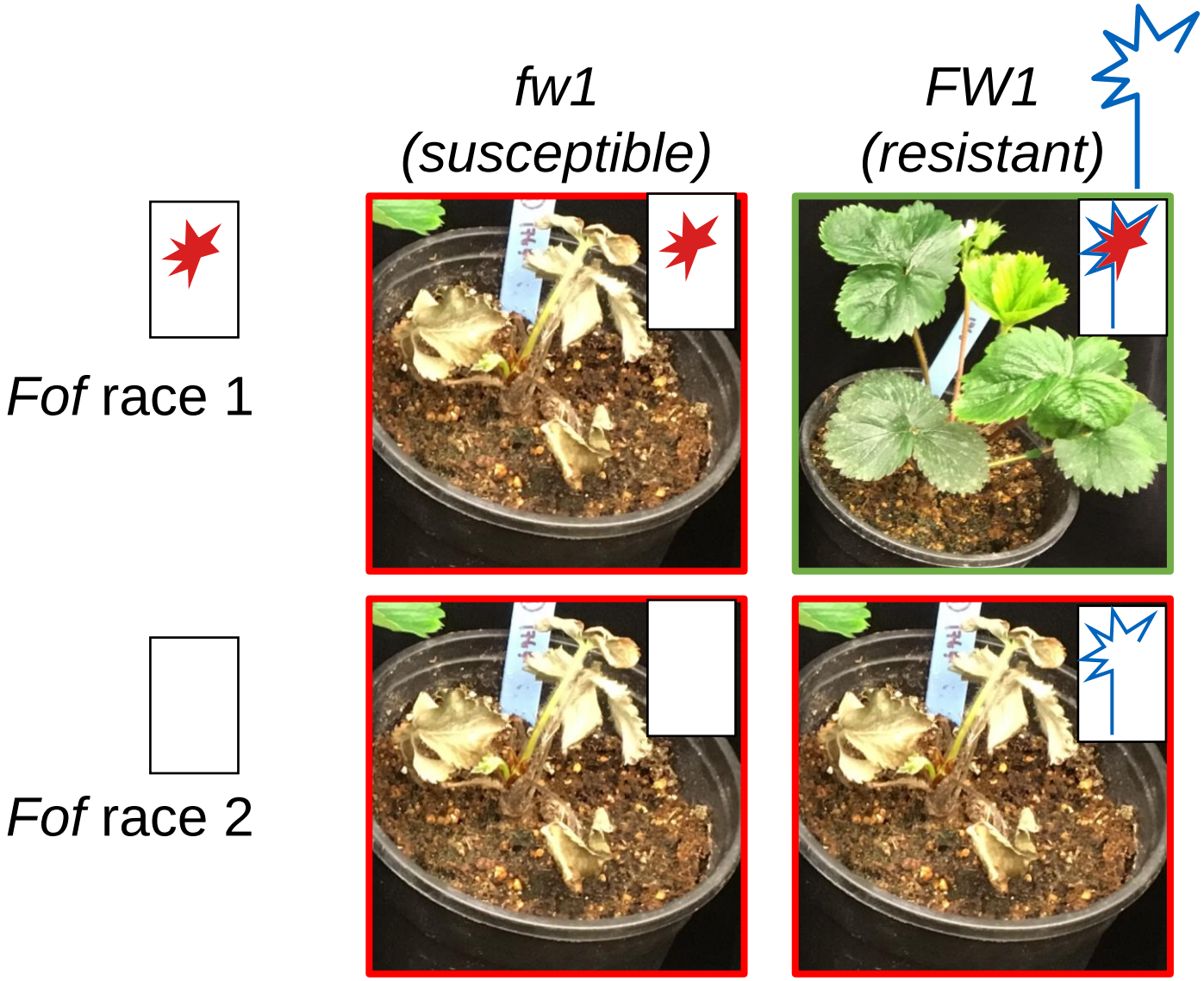
Annual Strawberry Production
Research Meeting
UCCE – Ventura
Sep 12, 2023

Peter Montgomery Henry, PhD
Research Plant Pathologist
USDA-ARS

USDA Agricultural Research Service

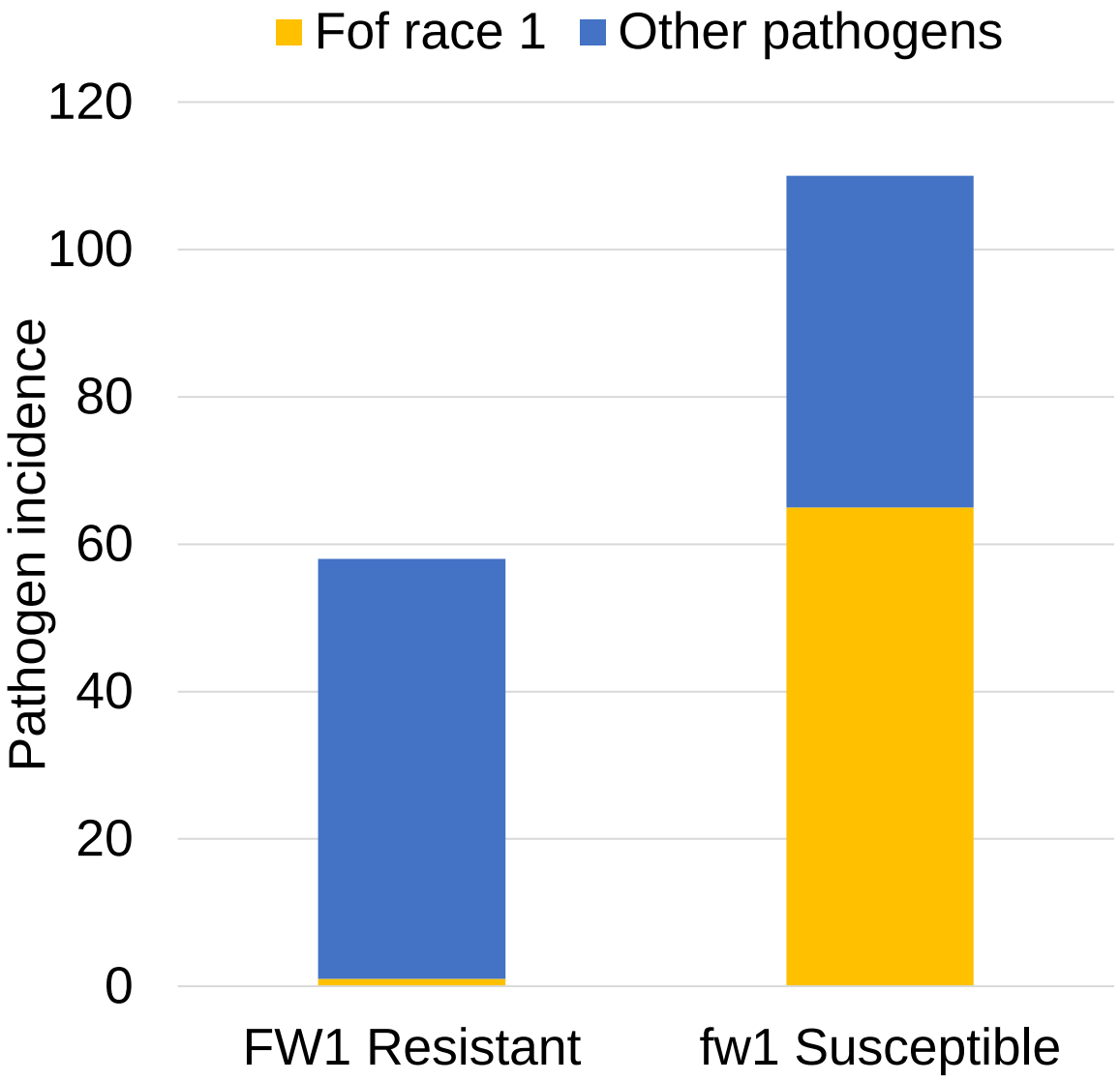


# Gene-for-gene interactions in strawberry




- *FW1* Resistant varieties:
  - Portola
  - Fronteras
  - San Andreas
  - Victor
  - Moxie

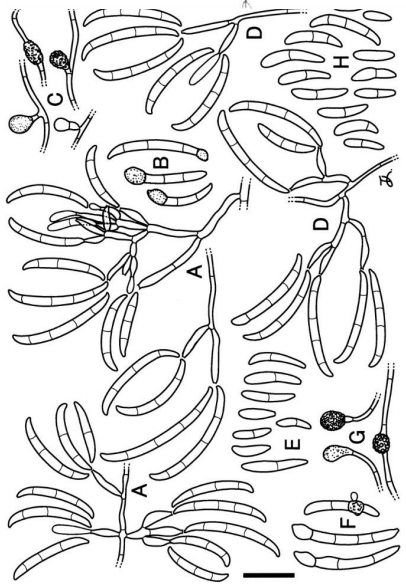
# Genetic resistance is key to managing Fusarium wilt




- In Watsonville/Salinas, Fusarium wilt is the most common disease on susceptible varieties.
- Results from ~150 diseased samples taken in
  - 2021 led by Cal Poly
  - 2022 led by USDA

- **Confirmed *Fof* race 2 in November, 2022**
- Summer-planted
- Portola (*FW1*-resistant)
- High wilt disease severity

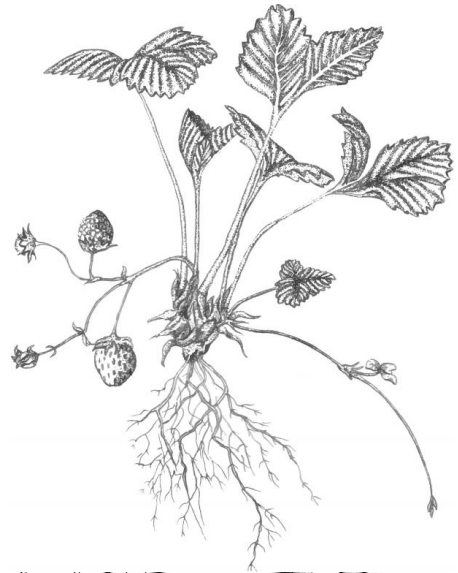
# Overview

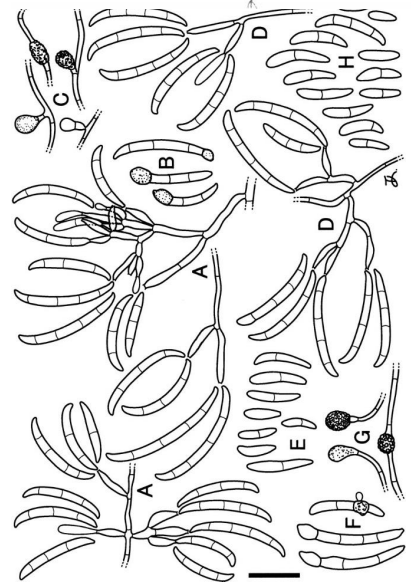



Diagnostic methods development
qPCR & RPA

Surveillance for CA *Fof* race 2

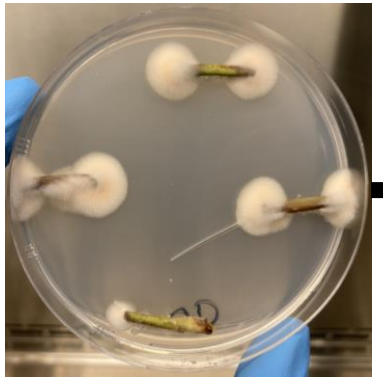
Screening for resistant cultivars



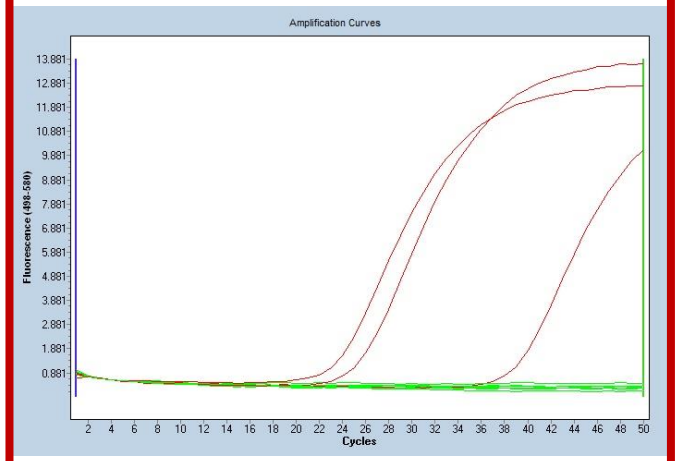


# Previous identification method (~8 week turnaround)

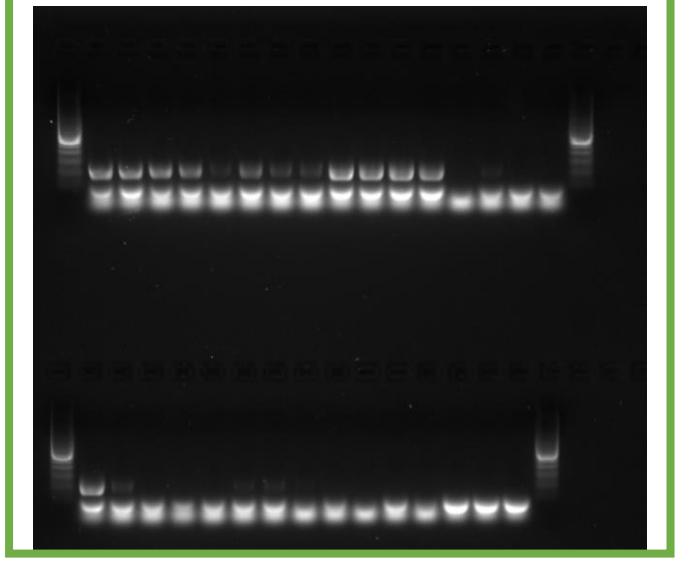
Petiole isolations
&
Single hyphal tip
pure cultures



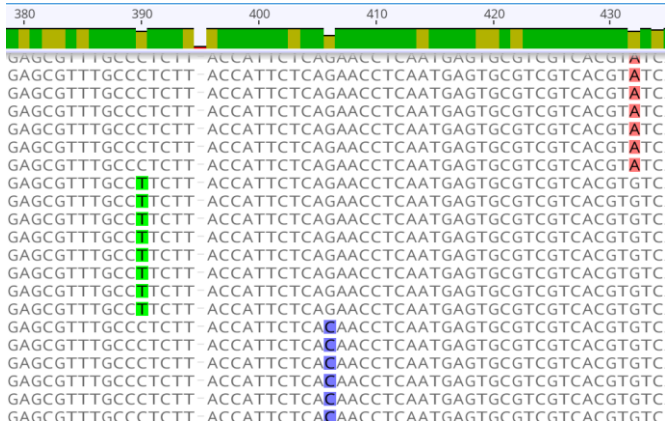
(-) for Burkhardt 2019 qPCR




(+) for Suga 2013 PCR

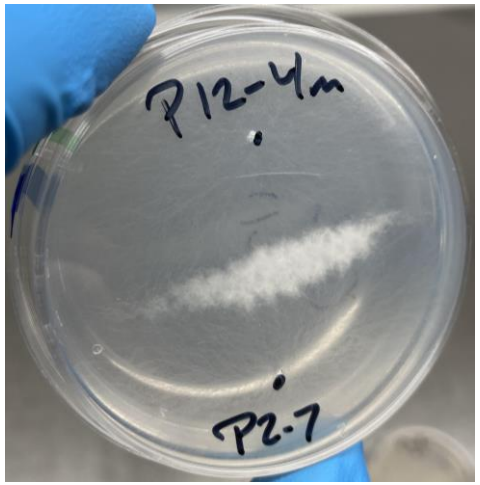


Sequence EF-1a gene




C->T
transversion

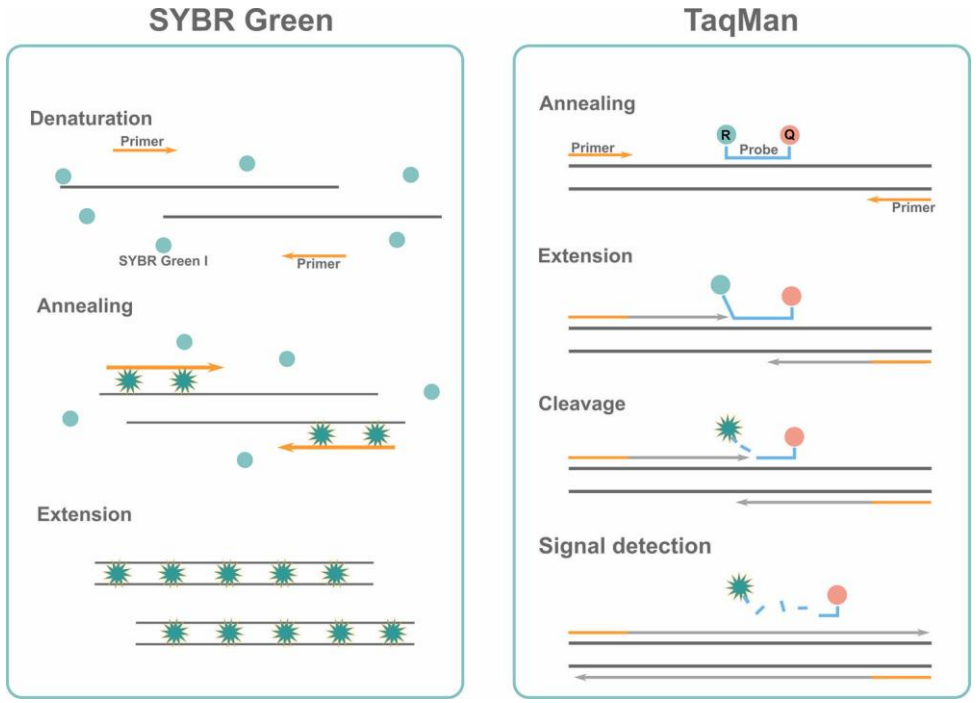
Somatic compatibility test



# qPCR vs. RPA assays

**qPCR**

- Can be used on soil and plant samples
- Quantitative – better for measuring differences in pathogen abundance

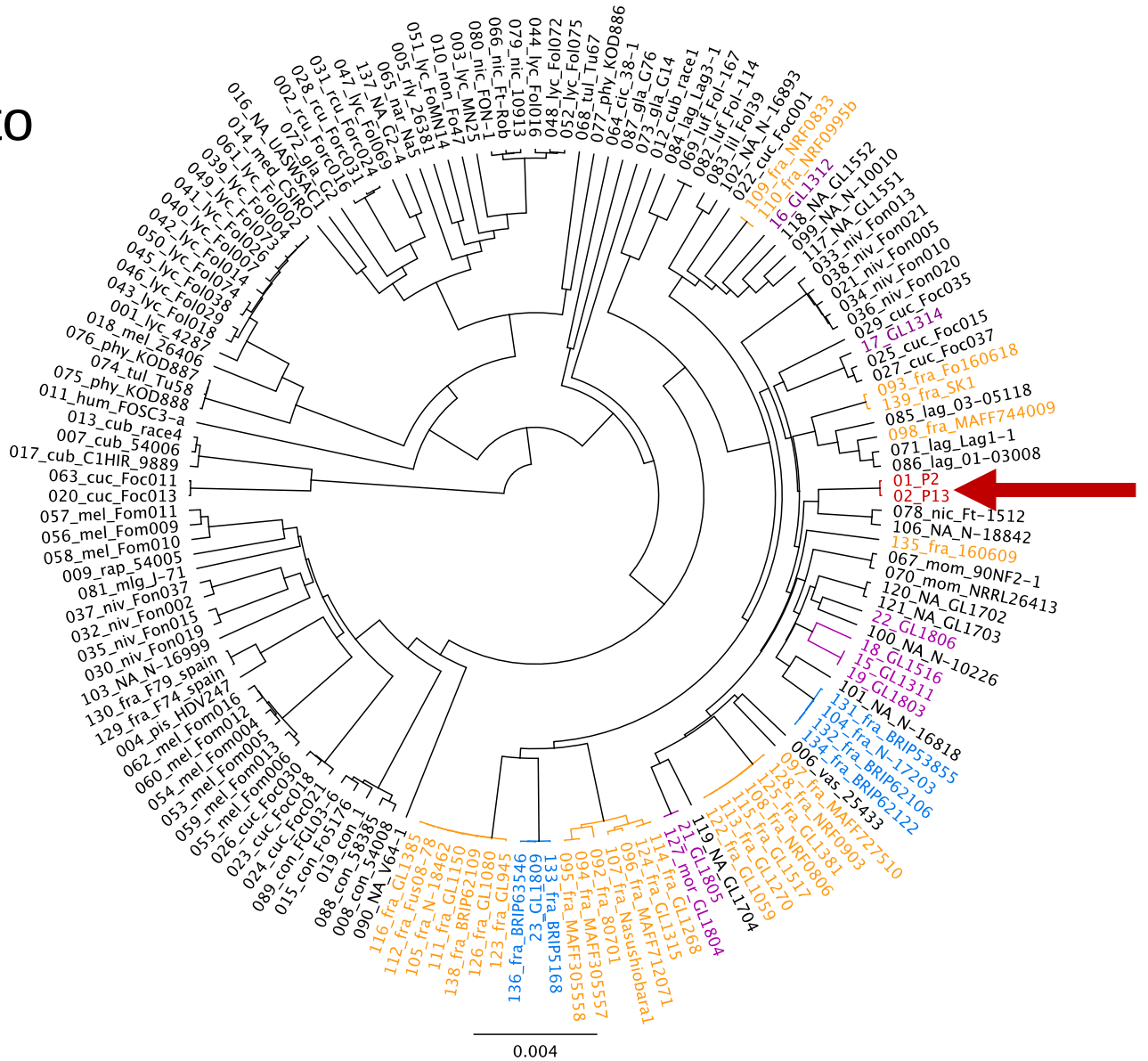



**RPA (recombinase polymerase amplification)**

- Currently only plant samples are supported
- Fast, simple, sensitive assays for plant sample

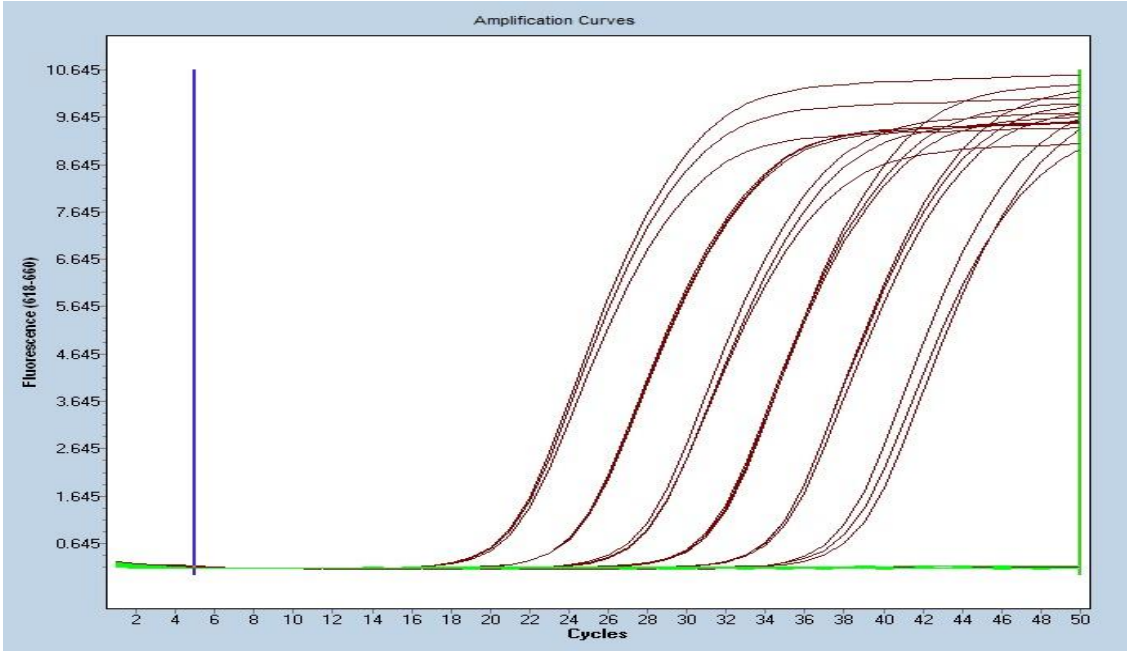
# Identifying a DNA locus for specific *Fof* R2 detection

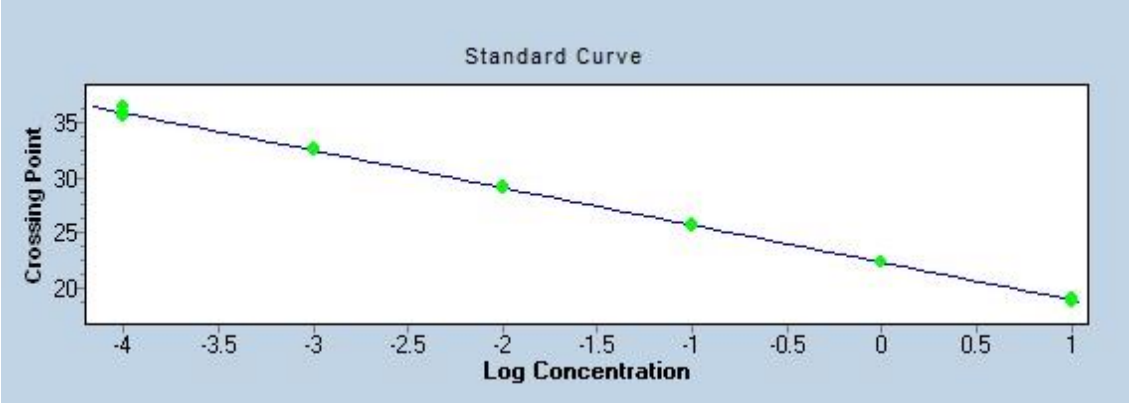
- Compared first with other *F. oxysporum* f. sp. *fragariae* genomes to identify unique sequences.
- Then screened these unique sequences against online databases
  - 1,328 *Fusarium* spp.
  - 246,119,175 sequences in NCBI GenBank (2.1 trillion nucleotides)
- Only one DNA locus was a good candidate after filtering



# qPCR TaqMan assay design

- Collaborated with Dr. Thien Ho from Driscoll's on primer and probe design
- Efficiency = 96.4%
- Consistent linear detection to the 100fg level.
- Detection at lower DNA concentrations was sporadic - one of six 10 fg samples and two of six 1 fg samples amplified.



# qPCR TaqMan assay validation

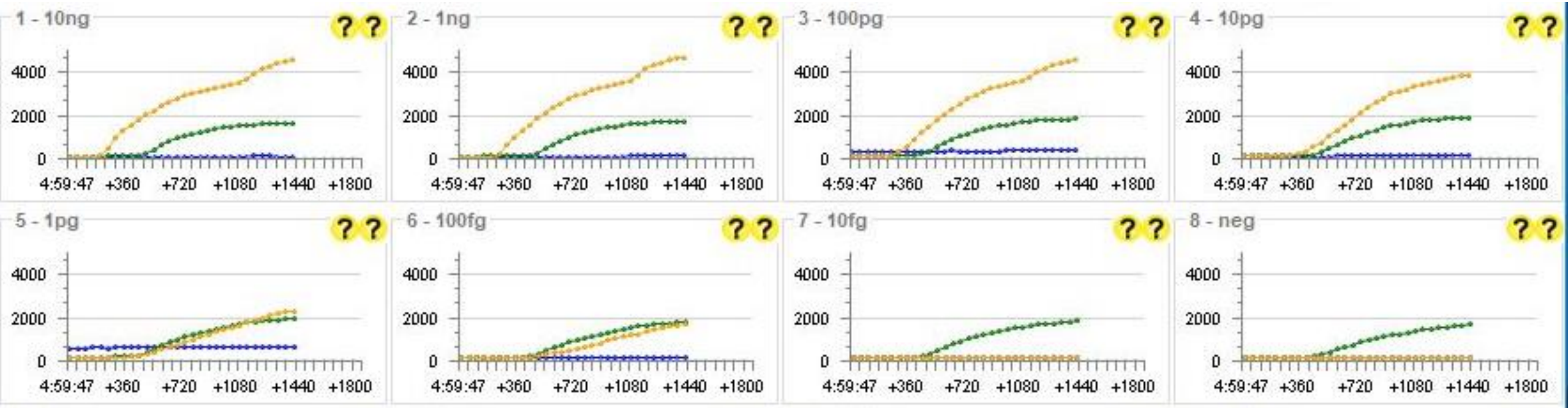
- Validated against DNA from 205 isolates of F. oxysporum that were either:
  - Isolated from strawberry plants
  - Pathogenic to strawberry
  - Common in coastal CA

| | qPCR positive | qPCR negative |
|---|---|---|
| CA *Fof* race 2 | 58 | 0 |
| Off-target | 0 | 147 |

# Recombinase polymerase (RPA) assay

- RPA assay sensitive to 100 femtograms of DNA
- 100% analytical specificity to CA *Fof* race 2

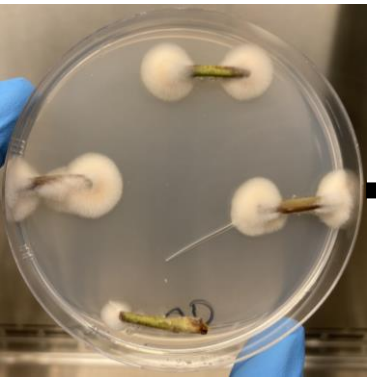
| | RPA positive | RPA negative |
|---|---|---|
| CA Fof race 2 | 52 | 0 |
| Off-target | 0 | 48 |



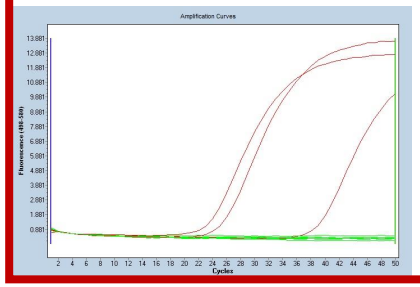
# Recombinase polymerase (RPA) assay validation

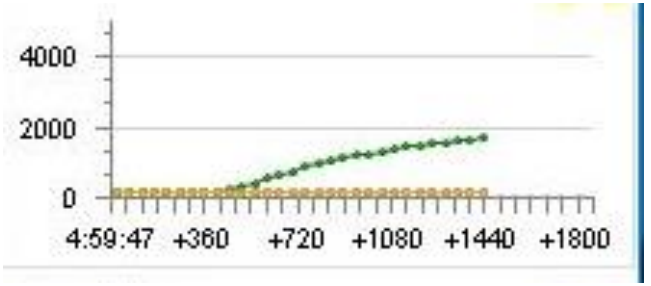
| | RPA positive | RPA negative |
|---|---|---|
| Petiole assay positive | 13 | 1 |
| Petiole assay negative | 0 | 47 |

Petiole isolations
&
Single hyphal tip pure cultures



(+) for qPCR Taqman



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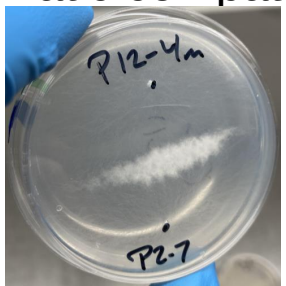
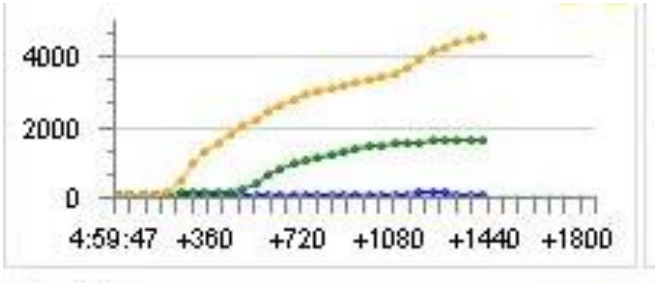
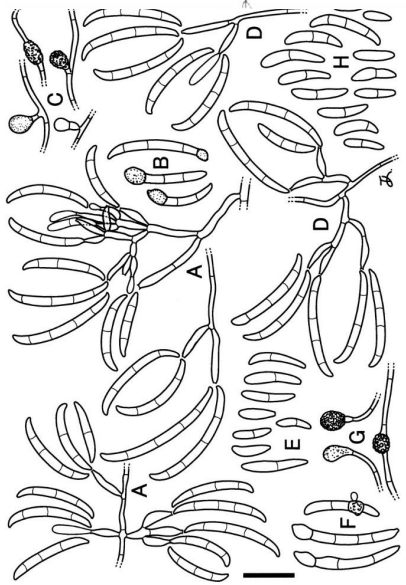
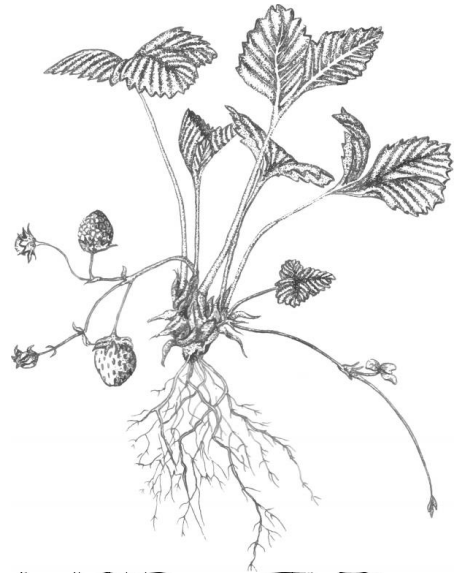


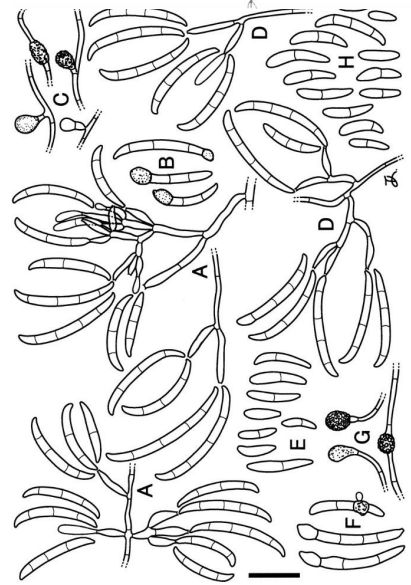



# Overview



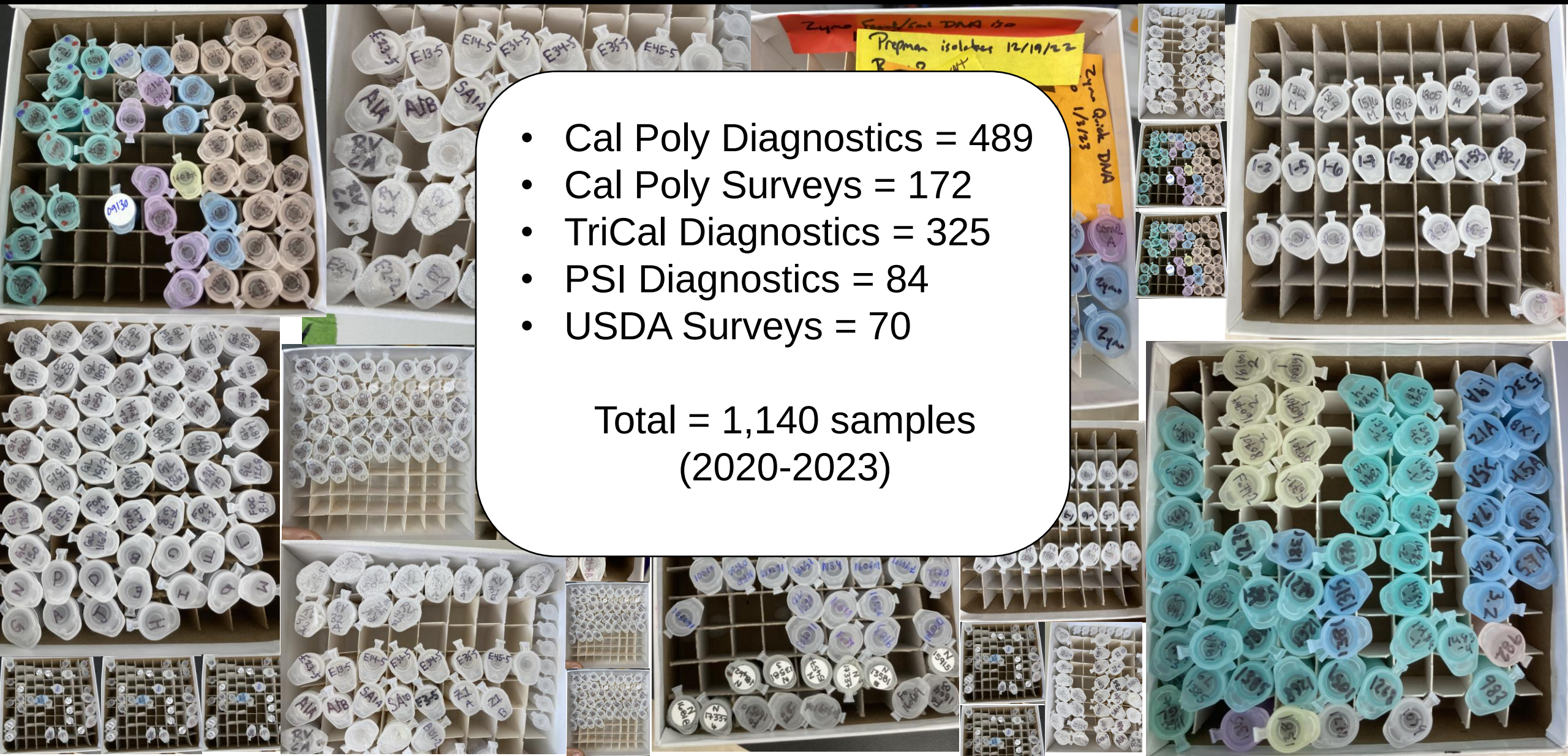

- Diagnostic methods development
- Surveillance for CA *Fof* race 2
- Screening for resistant cultivars





# In-field disease surveillance

# Freezer surveillance

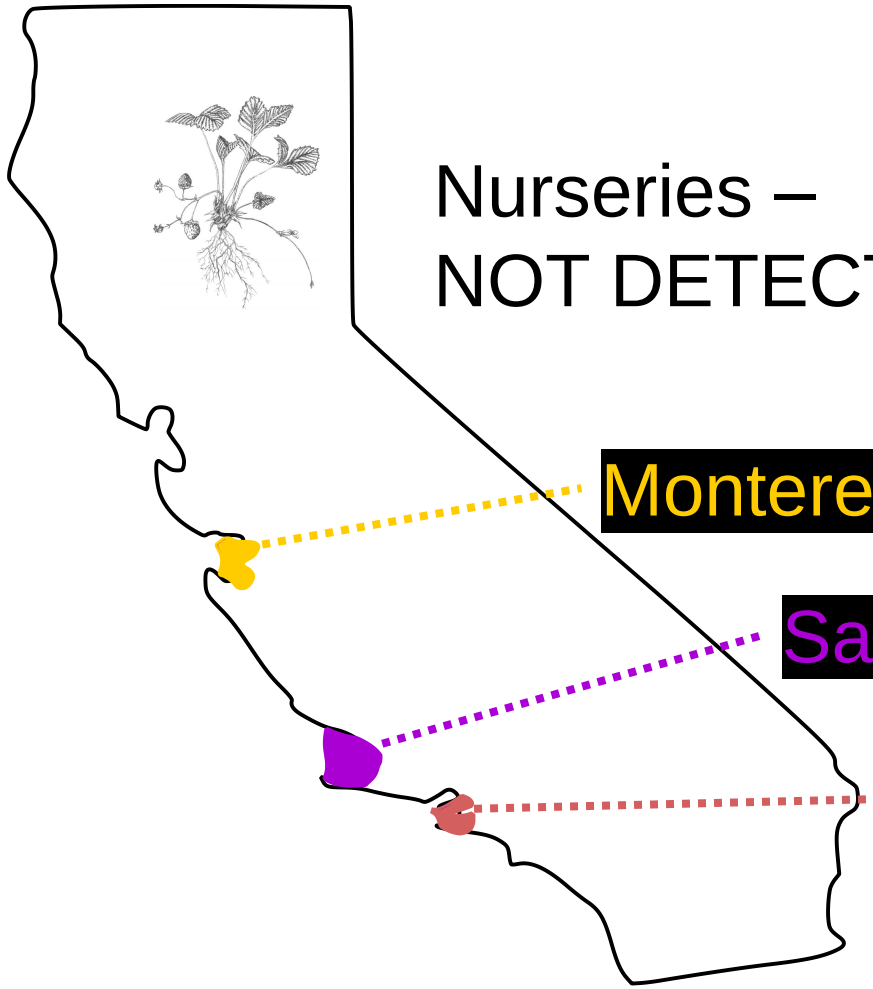



- Cal Poly Diagnostics = 489
- Cal Poly Surveys = 172
- TriCal Diagnostics = 325
- PSI Diagnostics = 84
- USDA Surveys = 70

Total = 1,140 samples
(2020-2023)

# Freezer and field surveillance results

- Tests for 555 samples are complete
- 101 samples lacked location metadata, negative results for all of these



Nurseries –
NOT DETECTED (21 samples)

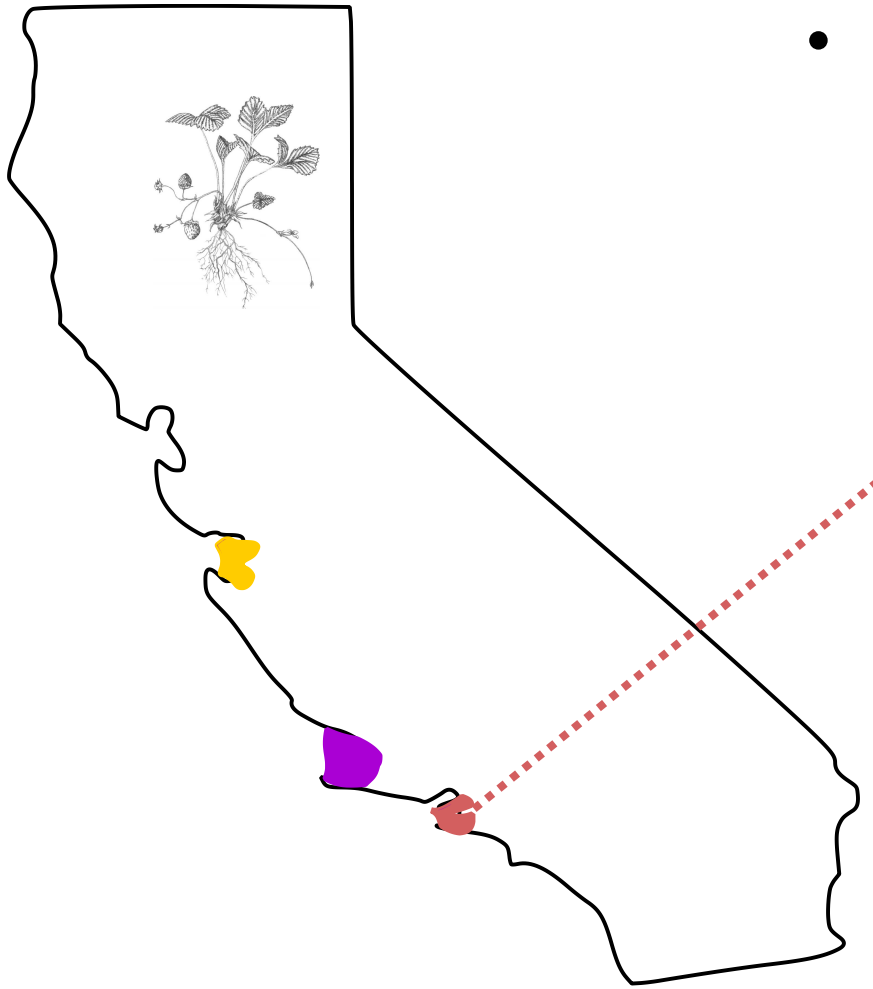
Monterey Bay – NOT DETECTED (132 samples)

Santa Maria – NOT DETECTED (168 samples)

Oxnard – NEGATIVE = 75 samples,
POSITIVE = 12 samples (4 fields)

Mexico – NOT DETECTED (51 samples)

# Current status of *Fof* race 2



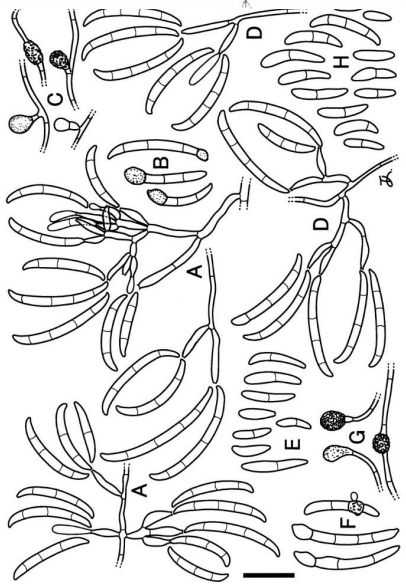
- Despite extensive testing, Fof race 2 has still only been found in Oxnard

Oxnard – NEGATIVE = 75 samples,
POSITIVE = 12 samples (4 fields)

- 3 summer-planted fields
- 3 fall-planted fields

# *FW1*-resistance remains effective in most fields

# Overview




- Diagnostic methods development
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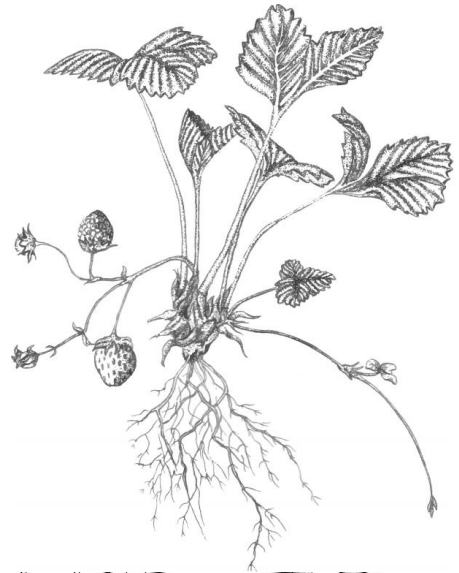

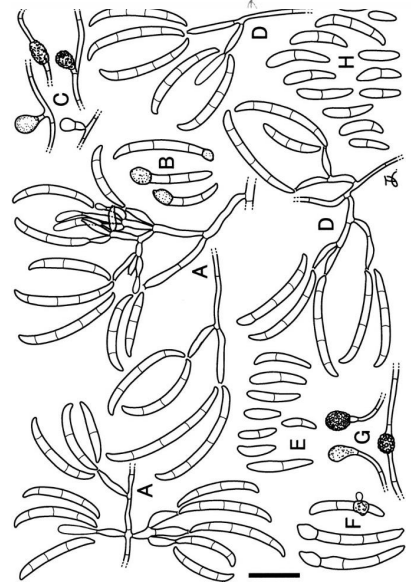

# No resistance sources identified for CA *Fof* race 2

- **199 varieties were Screened, none showed promising resistance to CA *Fof* race 2.**

- A larger panel of >400 varieties will be tested in collaboration with UCD

**Wild or heirloom varieties that were not resistant:**

- FVC11-76
- FVC11-75
- FVC11-44
- FVC11-30
- FVC11-6
- K1
- US438
- Mollala
- Midway
- FL Ninety
- Wiltguard
- Earliglow
- Earlimiss
- NC 95-21-1
- NC 96-48-1
- Darrow 72
- PI 616652
- MD 4987
- MD 683
- Pelican
- PI 551575

# Takeaways

- **In the 10 months since discovering CA Fof race 2 in Oxnard:**
- qPCR and RPA assays were developed and technically validated.
- RPA diagnostic validation results are promising and on-going
- >500 samples from diseased strawberry plants only show detection in Oxnard
- 199 varieties screened, none are resistant

# Acknowledgements

*Grower collaborators*

CALIFORNIA STRAWBERRY COMMISSION

USDA Agricultural Research Service

NIFA

NIFA Specialty Crops Research Initiative (#2022-51181-38328)

cdfa CALIFORNIA DEPARTMENT OF Food and Agriculture