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EDMUND MACH
dal 1874

Development of an early detection protocol for *Erwinia amylovora* from corbicular pollen to monitor its spread in apple orchards

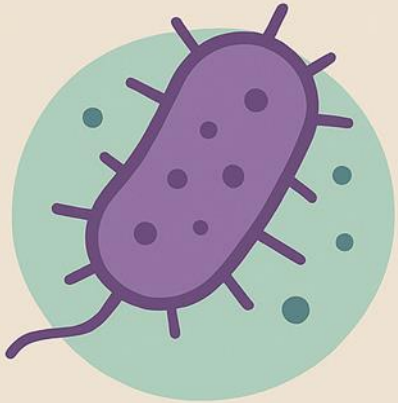
B. Vicelli, C. Cainelli, G. Puopolo, and V. Gualandri

Erwinia amylovora

Bacterial pathogen that causes fire blight in plants of the Rosaceae family

HOST PLANTS

Apple, pear, quince, other Rosaceae



SYMPTOMS

Necrosis of leaves, flowers, shoots, and fruit

OPTIMAL CONDITIONS

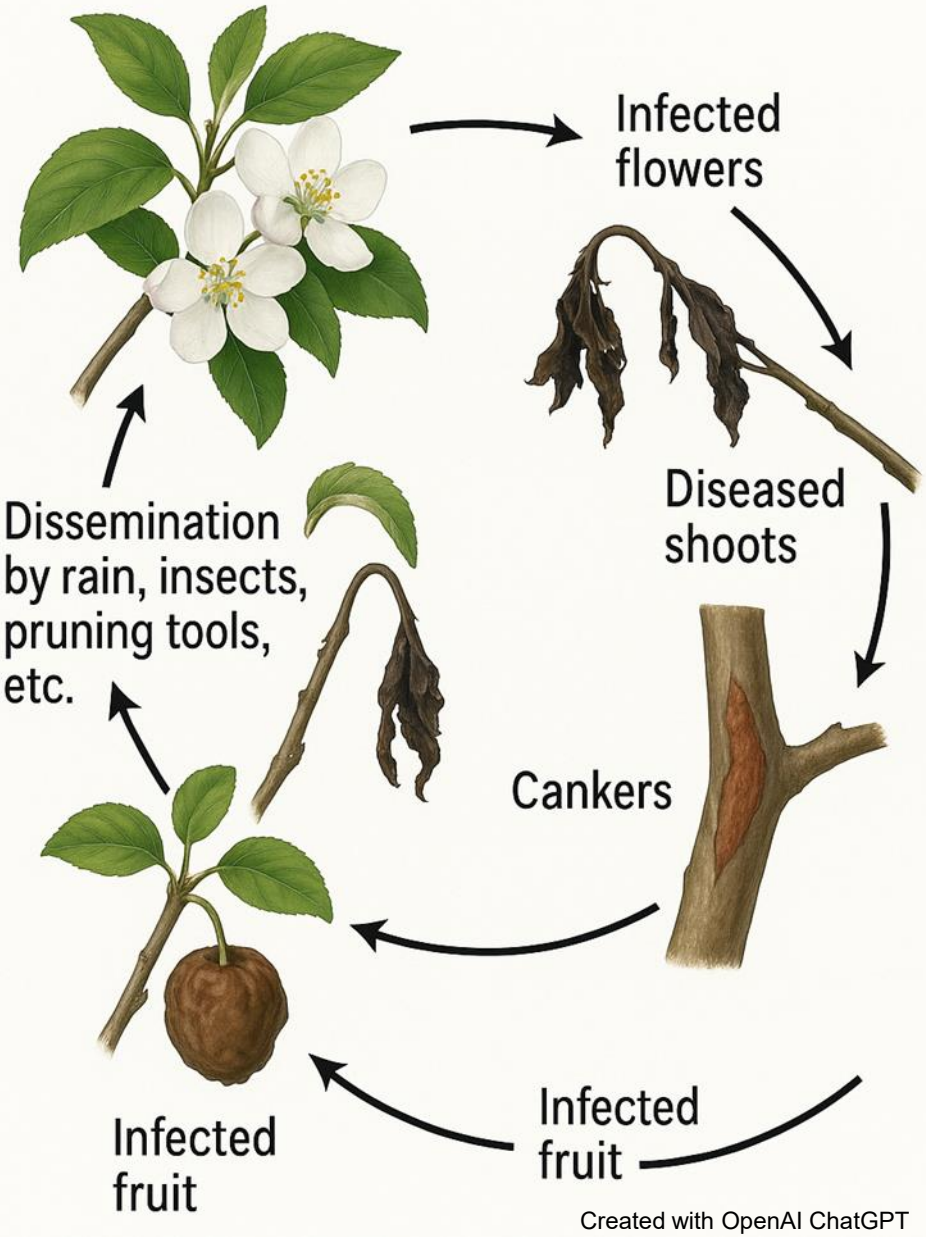
Warm and humid temperatures

SPREAD AND MANAGEMENT

Rain, insects, pruning tools, preventive measures

FIRE BLIGHT DISEASE CYCLE

Erwinia amylovora



2020 Outbreak in Trentino





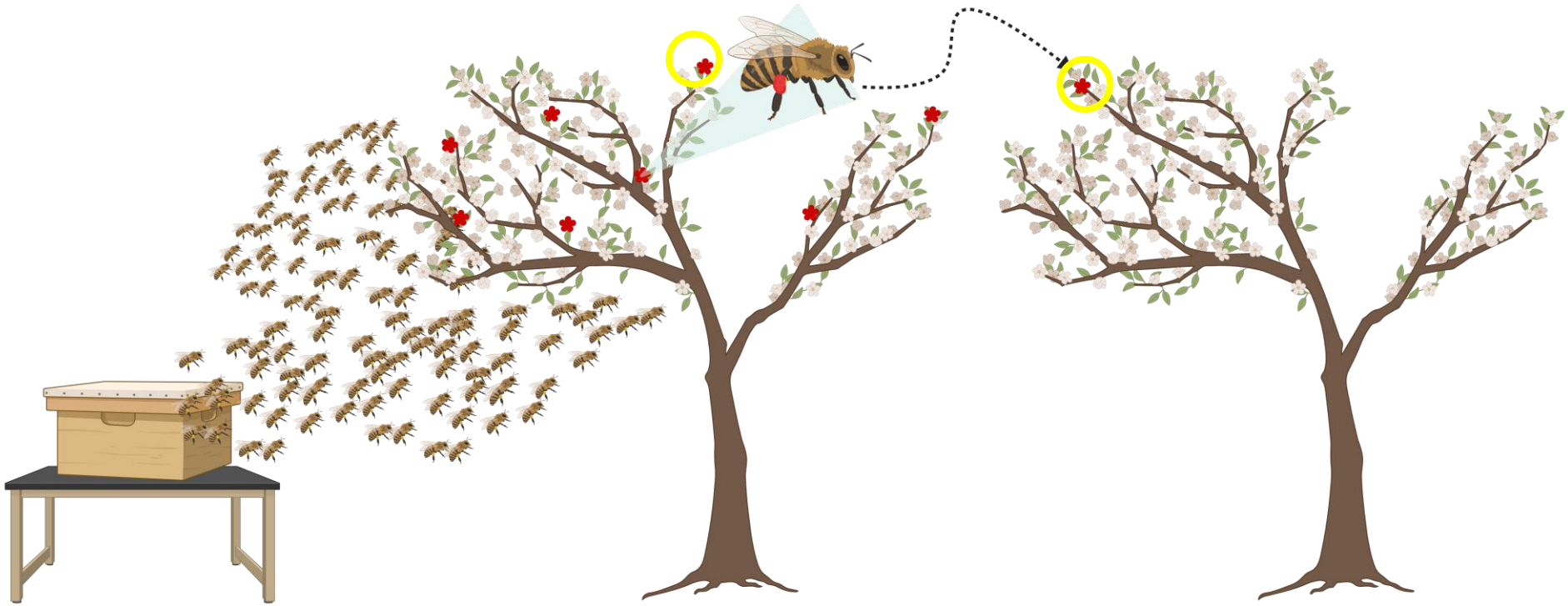




from Mario Baldessarri, Fondazione Edmund Mach

Erwinia amylovora dispersal in apple orchards

Bees forage on infected flowers → Acquire *E. amylovora* cells → Transfer *E. amylovora* cells to healthy flowers → Flower infection



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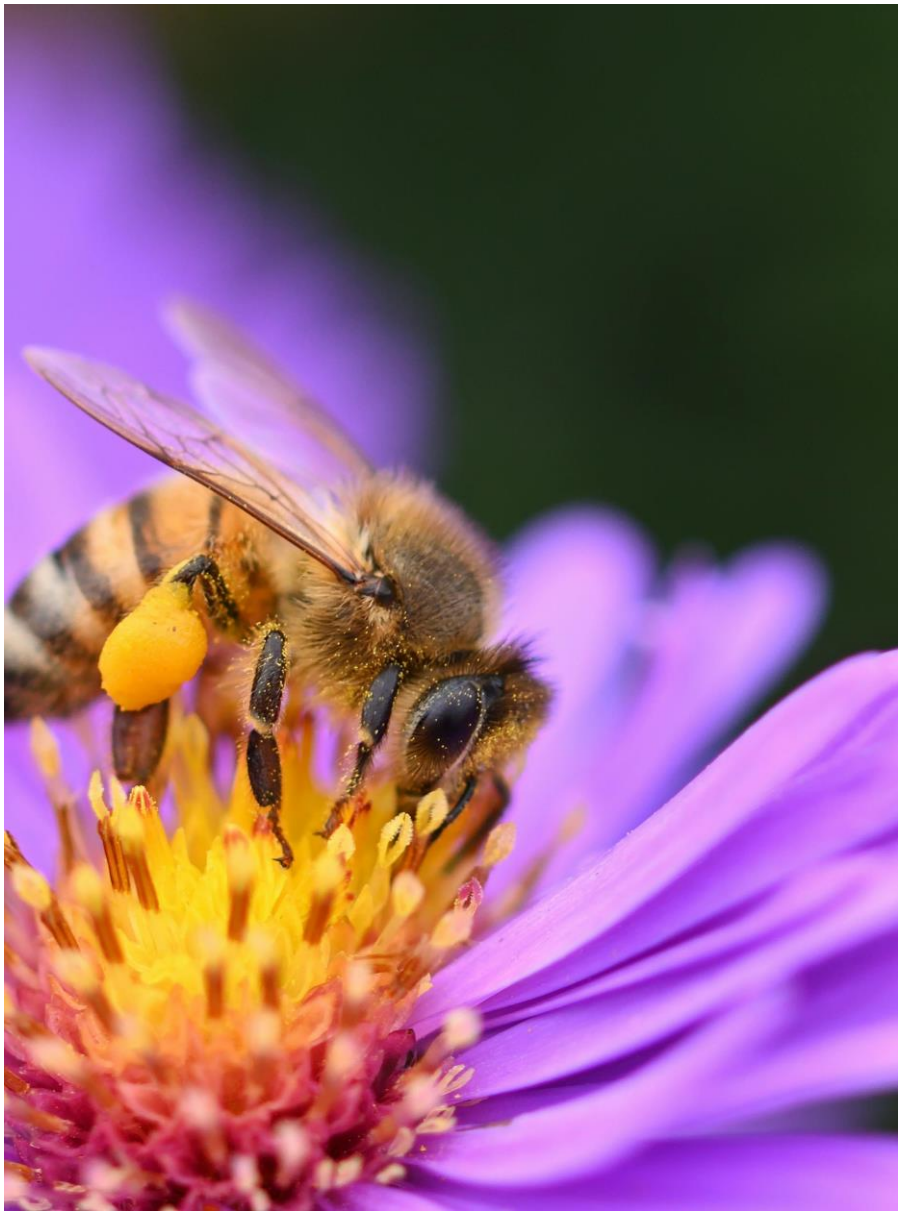
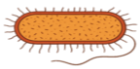
Where is *Erwinia amylovora?*



Aim

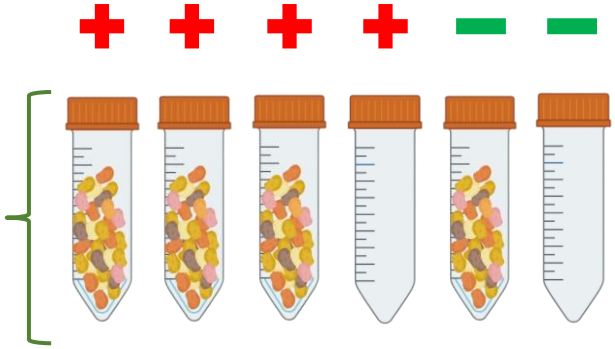
- Asses whether contaminated corbicular pollen (CP) can infect healthy flowers
- Develop an early detection protocol to identify the presence of *Erwinia amylovora* (Ea) by analysing CP
- Detect and identify the occurrence of Ea in apple orchards

Pollen-mediated infection



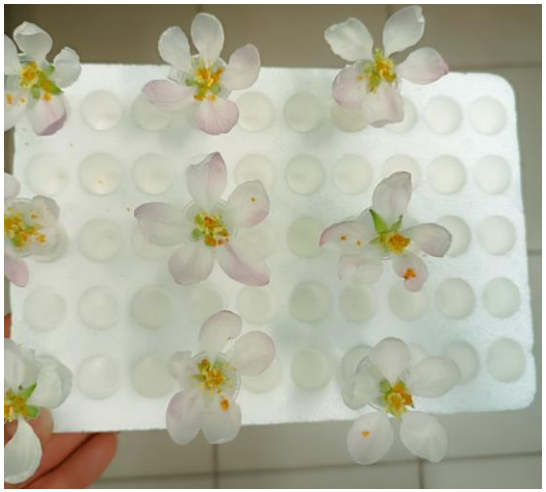
E. amylovora (*Ea*)

Corbicular pollen (CP)



Final CFU/g

CP + *Ea* 10^3	CP + *Ea* 10^5	CP + *Ea* 10^7	*Ea* 10^7	CP + H_2O	H_2O



INCUBATION

+25°C
80% HR
5 DAYS

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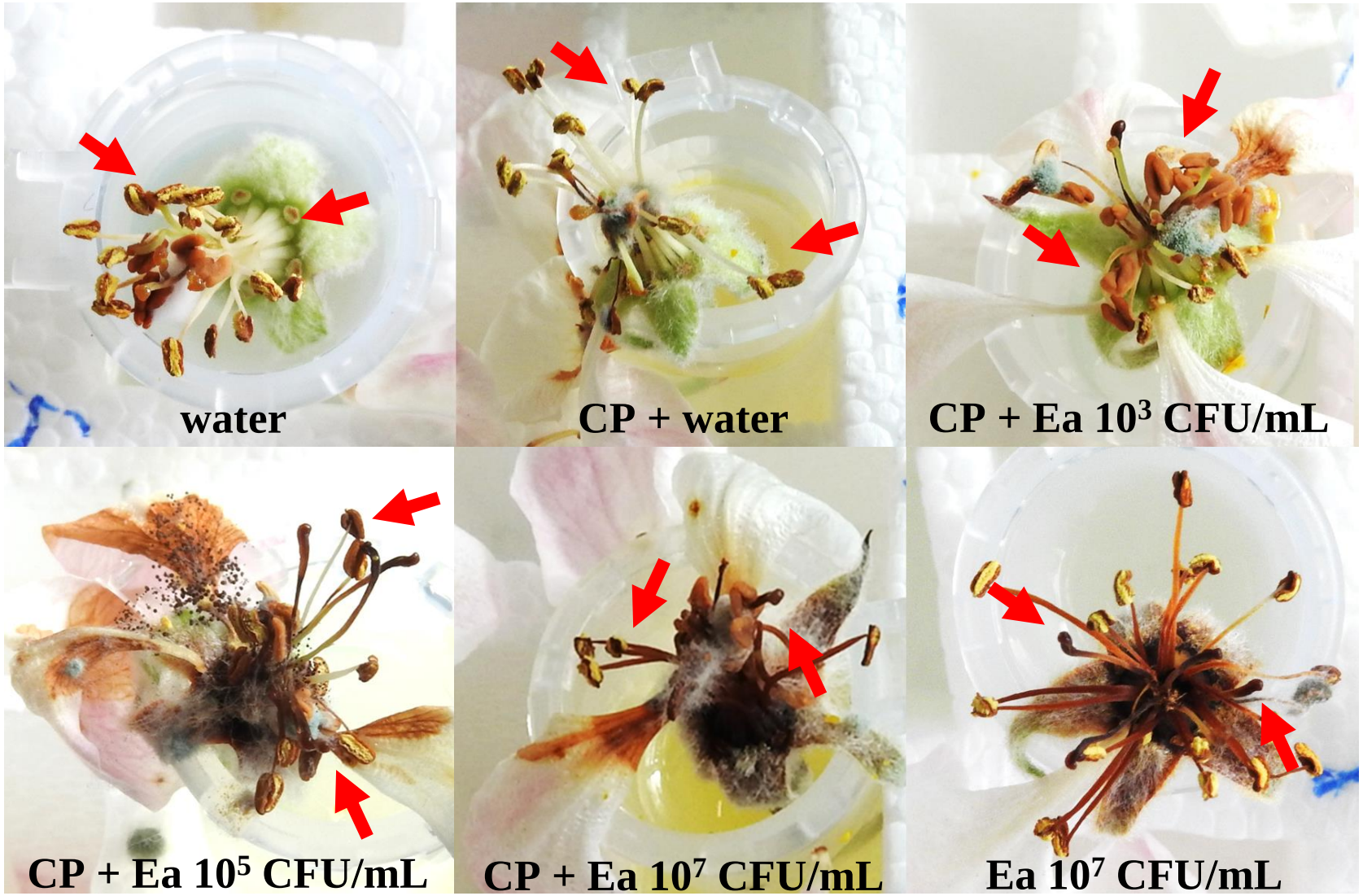
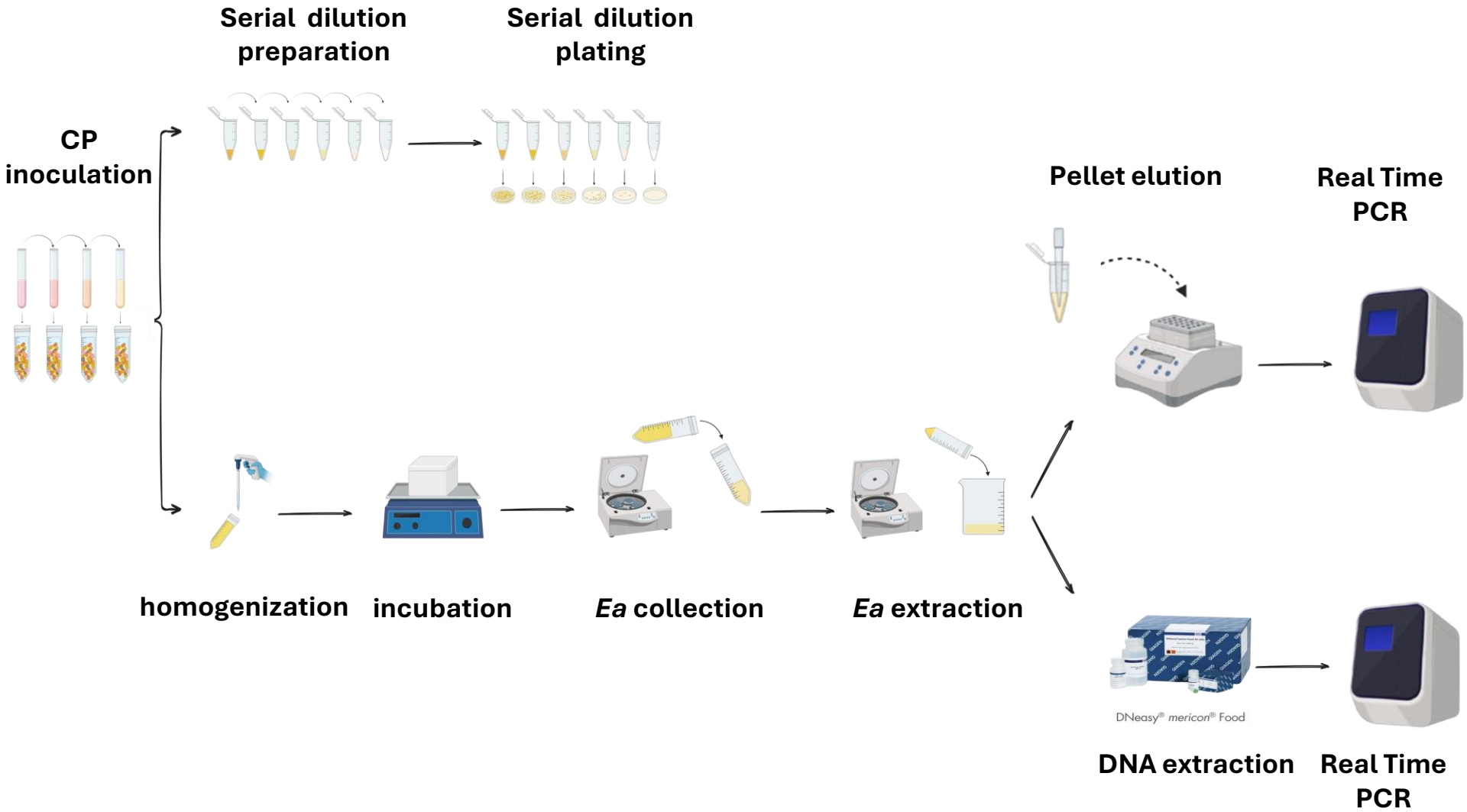
Pollen-mediated infection



Healthy flowers can be infected by contaminated corbicular pollen

Calibration curve



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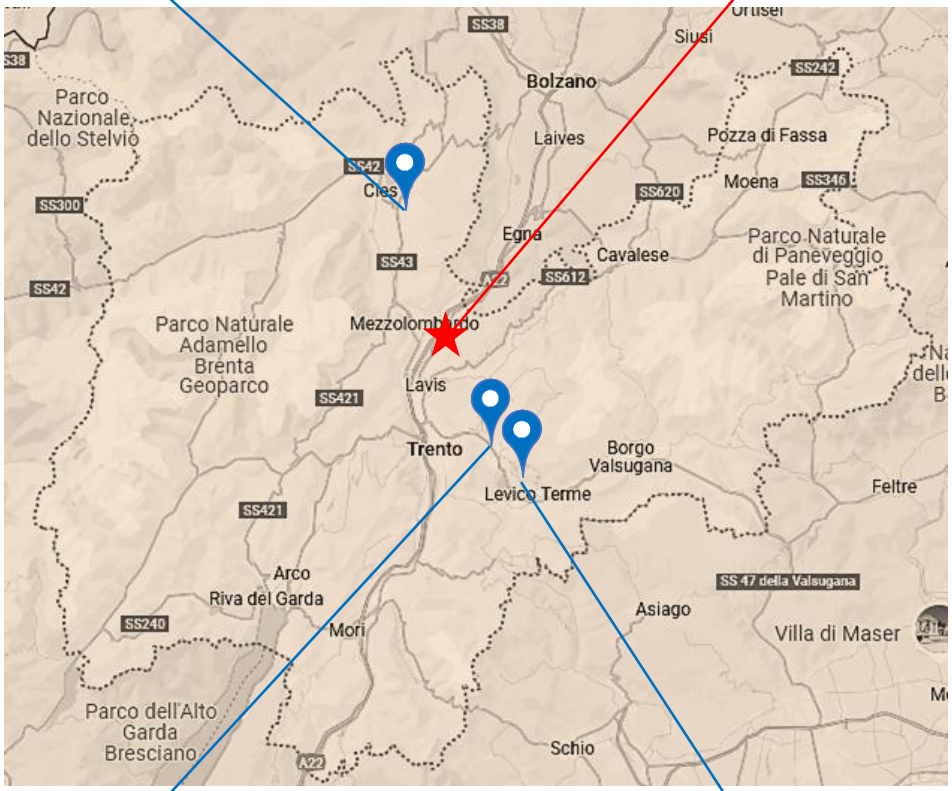
Sampling sites

Cles



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Pergine

Caldonazzo

- Years 2022, 2023
- 3 beehives/site
- April: 1 sampling/week
- May-June: 2 sampling/week

~ 600 samples

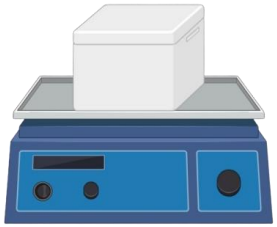
~ 15 kg of pollen collected

Early detection protocol

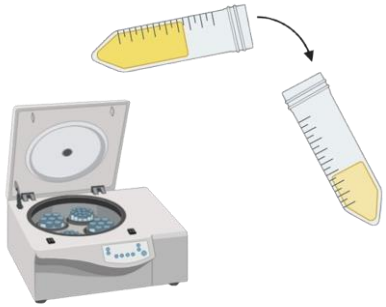
CP COLLECTION

HOMOGENIZATION

INCUBATION



***Ea* COLLECTION**

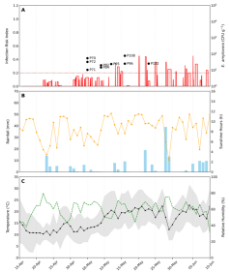


***Ea* EXTRACTION**

DNA EXTRACTION

Real Time PCR

DATA ANALYSIS



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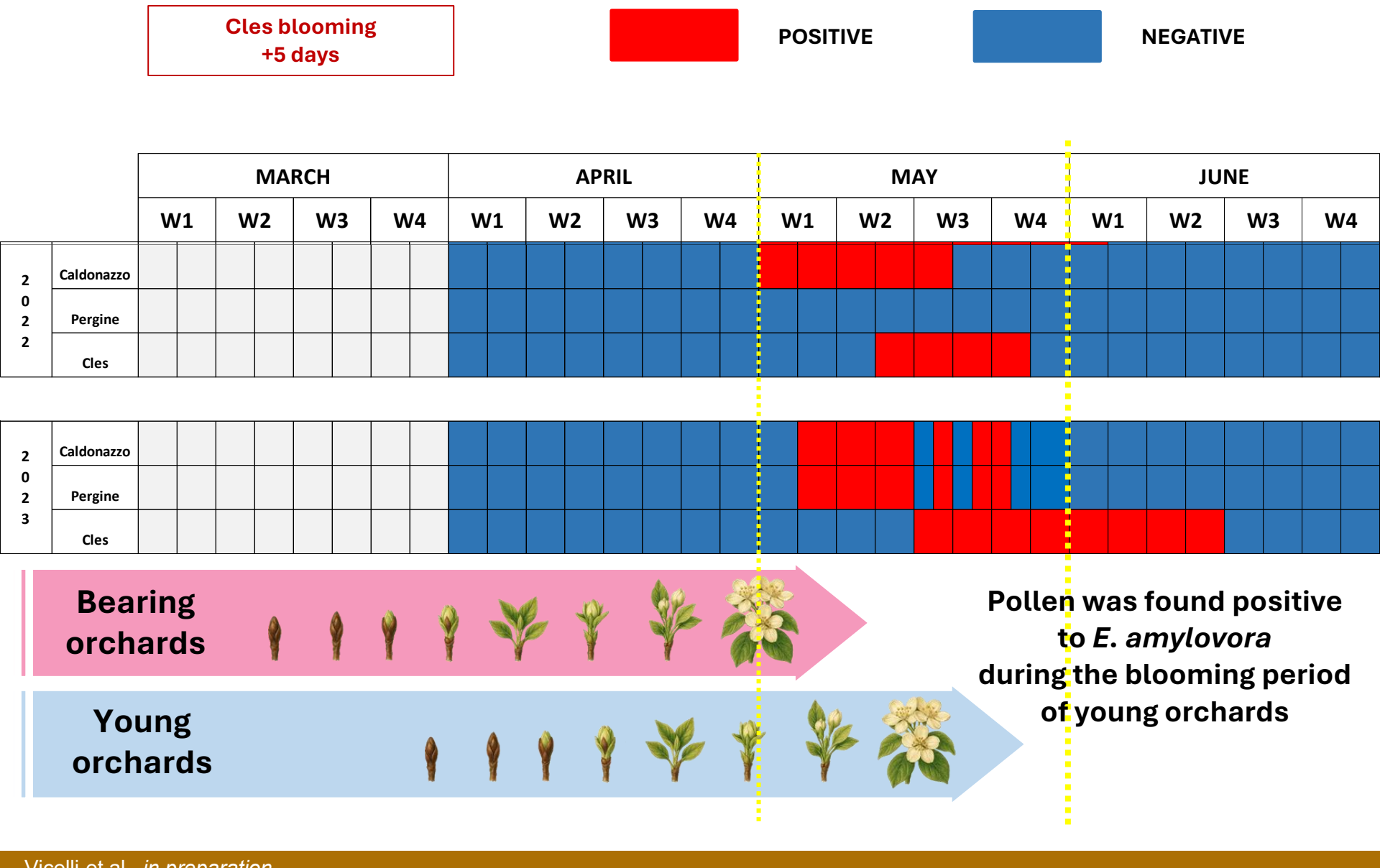
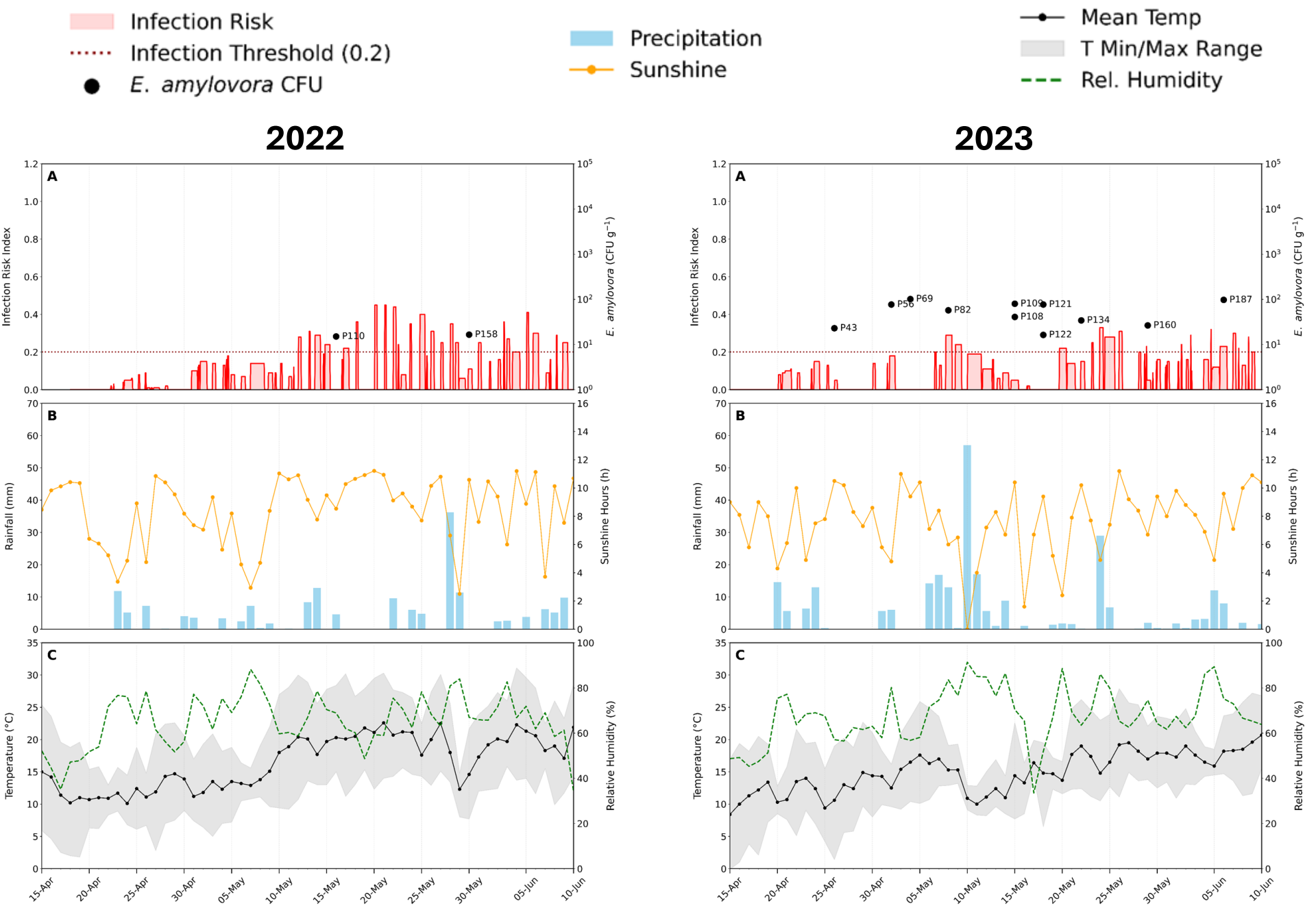
Vicelli et al., *in preparation*

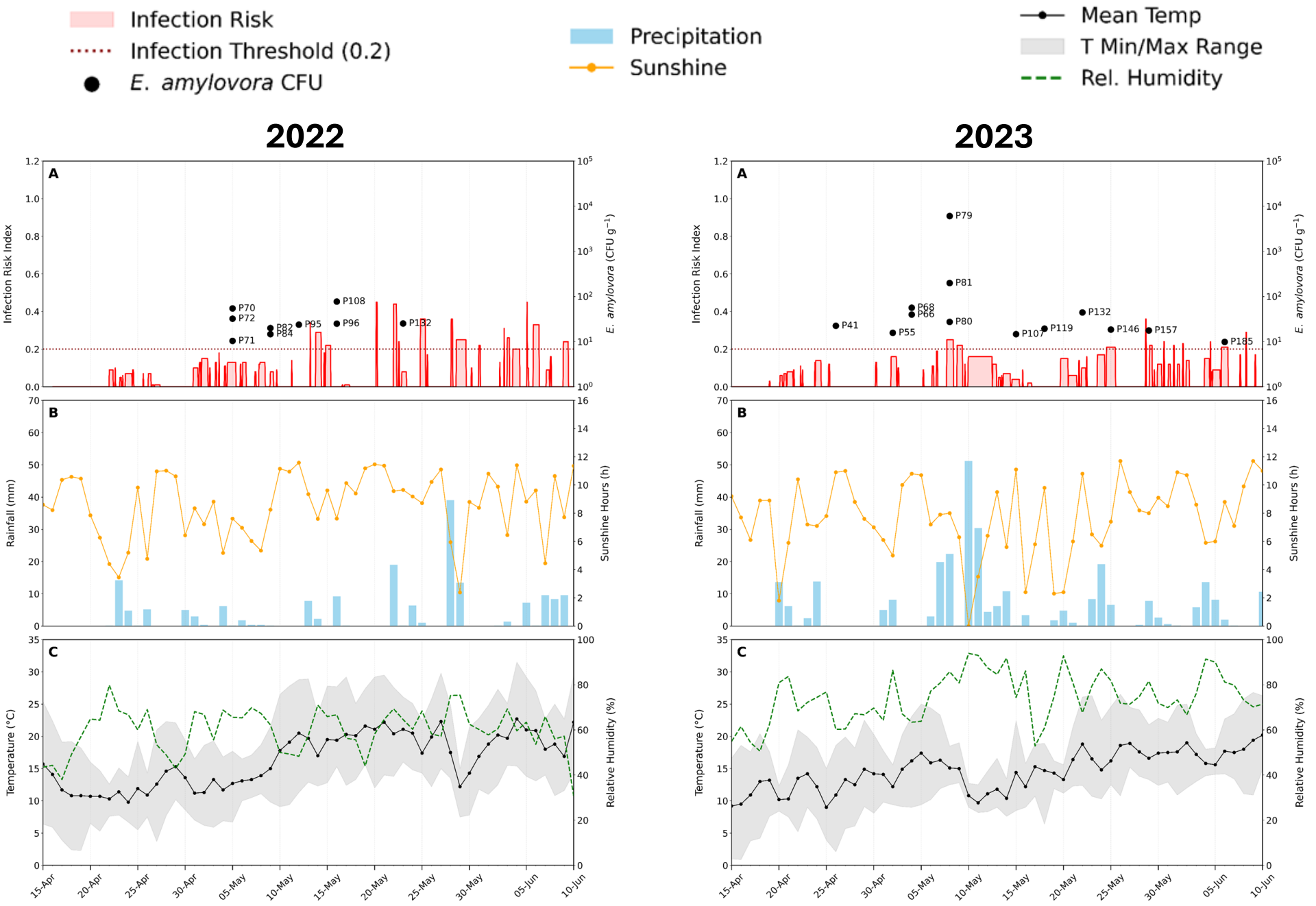
Results


Cles blooming
+5 days
POSITIVE
NEGATIVE
MARCH
APRIL
MAY
JUNE
W1
W2
W3
W4
2022
Caldonazzo
Pergine
Cles
2023
Caldonazzo
Pergine
Cles
Bearing orchards
Young orchards
Pollen was found positive to E. amylovora during the blooming period of young orchards

PERGINE


Infection Risk
Infection Threshold (0.2)
E. amylovora CFU
Precipitation
Sunshine
Mean Temp
T Min/Max Range
Rel. Humidity
2022
2023
A
B
C
Infection Risk Index
E. amylovora (CFU g⁻¹)
Rainfall (mm)
Sunshine Hours (h)
Temperature (°C)
Relative Humidity (%)
P110
P158
P43
P56
P69
P82
P109
P108
P121
P122
P134
P160
P187
15-Apr
20-Apr
25-Apr
30-Apr
05-May
10-May
15-May
20-May
25-May
30-May
05-Jun
10-Jun

CALDONAZZO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A
B
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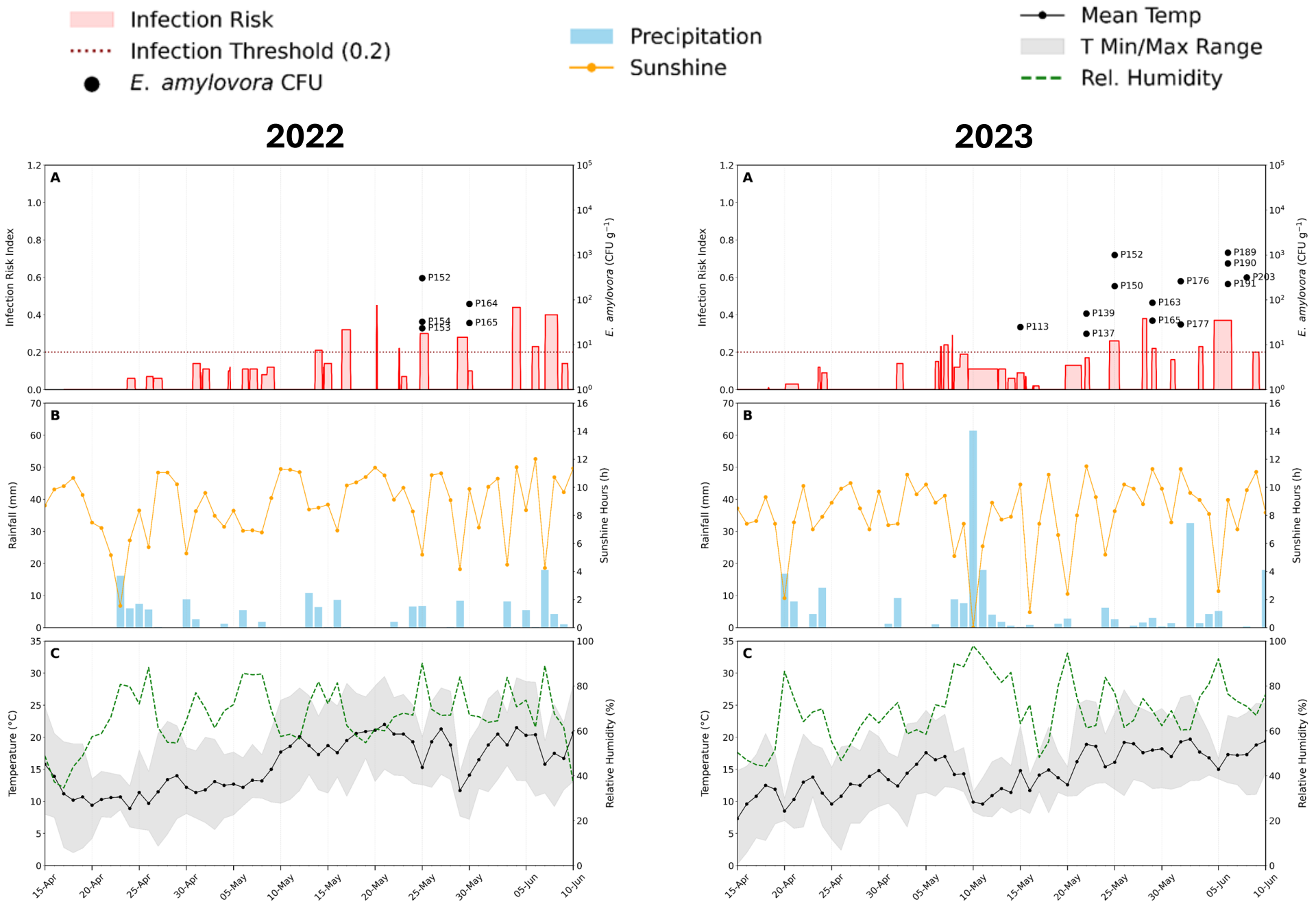
E. amylovora (CFU g⁻¹)
Rainfall (mm)
Sunshine Hours (h)
Temperature (°C)
Relative Humidity (%)
P70
P72
P71
P82
P84
P95
P108
P96
P132
P79
P81
P68
P66
P80
P41
P55
P107
P119
P146
P157
P185
15-Apr
20-Apr
25-Apr
30-Apr
05-May
10-May
15-May
20-May
25-May
30-May
05-Jun
10-Jun

CLES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2022
2023
A
B
C
Infection Risk Index
E. amylovora (CFU g⁻¹)
Rainfall (mm)
Sunshine Hours (h)
Temperature (°C)
Relative Humidity (%)
P113
P137
P139
P150
P152
P153
P154
P163
P164
P165
P176
P177
P189
P190
P191
P203
15-Apr
20-Apr
25-Apr
30-Apr
05-May
10-May
15-May
20-May
25-May
30-May
05-Jun
10-Jun

CONCLUSIONS

- The q-PCR-based protocol enables the early detection of *Erwinia amylovora* in corbicular pollen, during blooming of Rosaceae species
- Its validation over three seasons confirmed its reliability and sensitivity
- It is a valuable early warning tool for *E. amylovora* surveillance
- It can be adapted to detect other plant pathogens or integrated into risk prediction models

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THANK YOU

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