



Universidad de Chile

10TH INTERNATIONAL
TABLE GRAPE
SYMPOSIUM
SOMERSET WEST, SOUTH AFRICA
26 NOV - 1 DEC 2023



Real-time design of *Botrytis cinerea* control programs based on direct qPCR-HRM (High-Resolution Melting) fungicide-resistance genotyping from infected plant tissue

Claudio Osorio-Navarro^{a,b}; Charleen Copier^a; Felipe Durán^a; Madelaine Azócar^a; Gonzalo Gutiérrez^a; Eduardo Donoso; Herman Silva; Jaime Auger^a & Marcela Esterio^a

^aDepartamento de Sanidad Vegetal, Facultad de Ciencias Agronómicas, Universidad de Chile, Santiago-Chile.

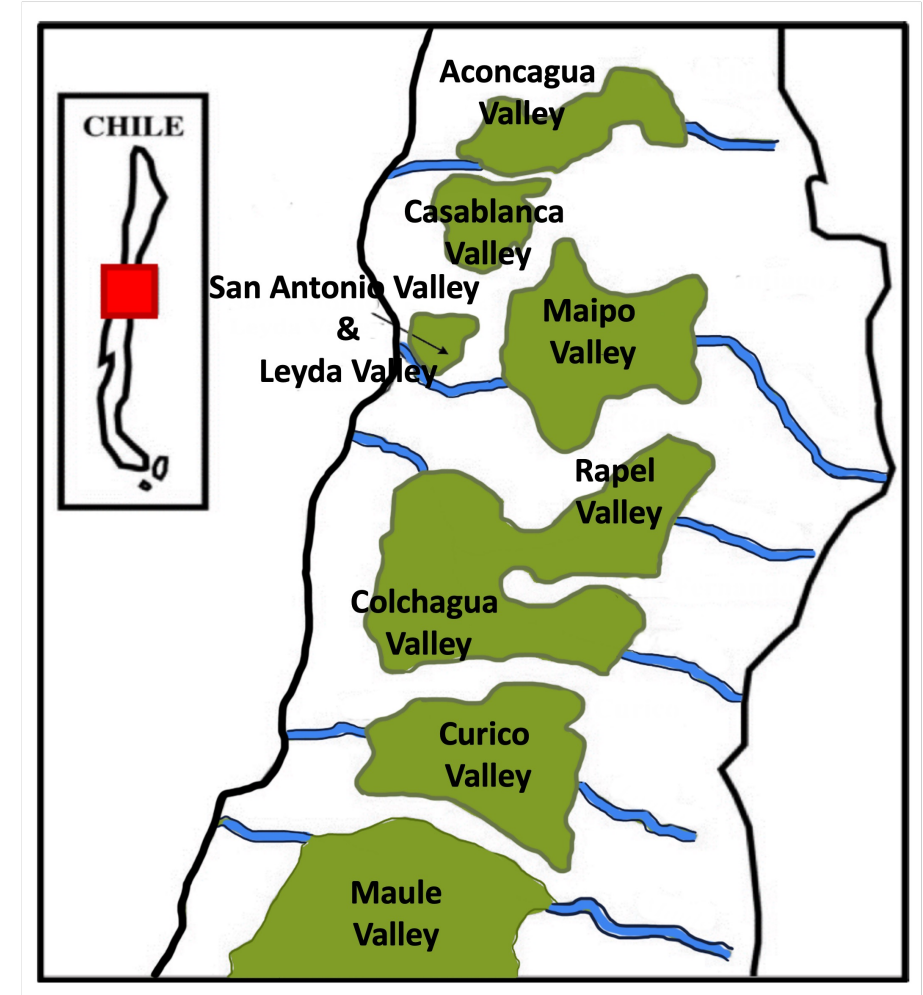
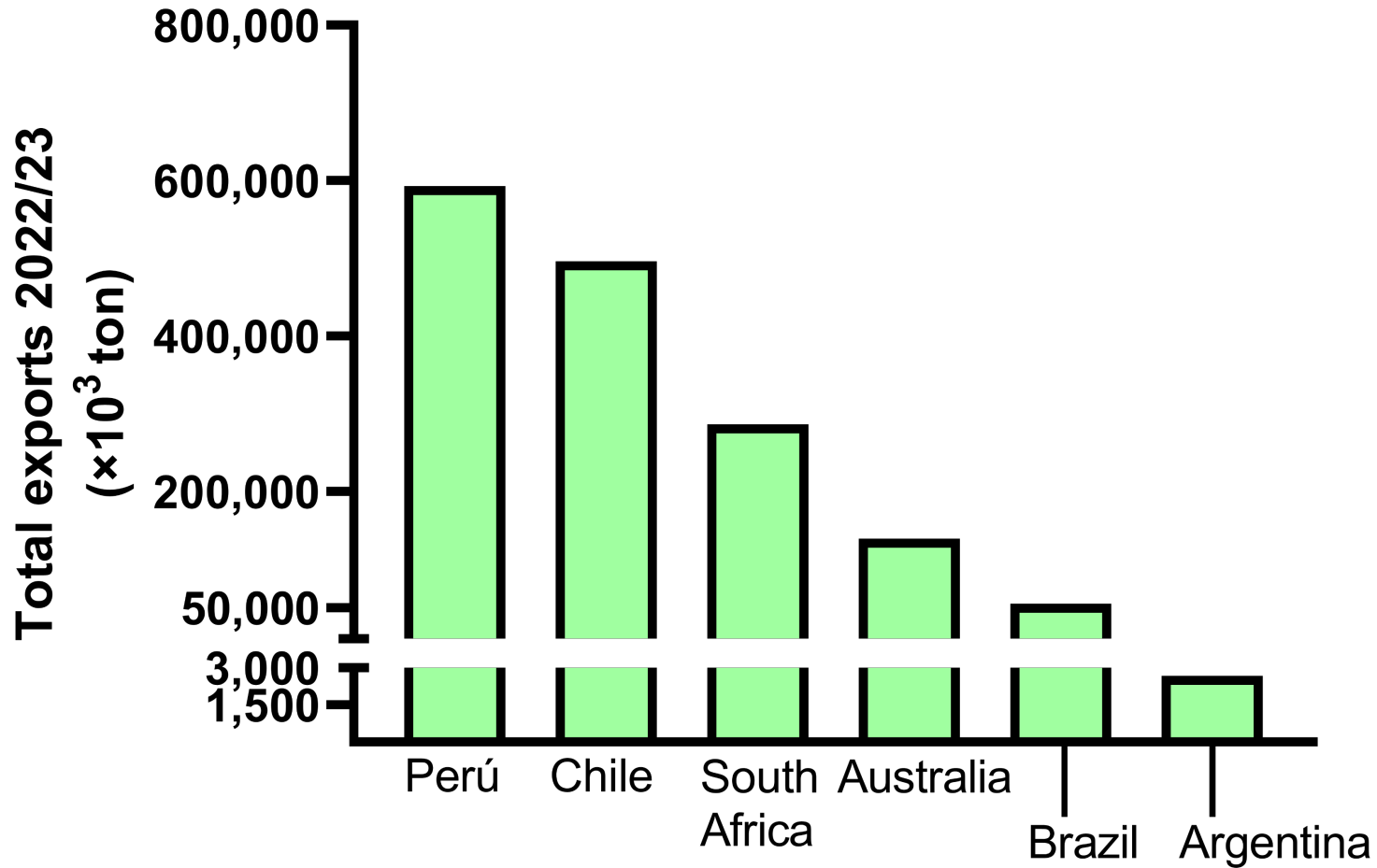
^bCentre of Molecular Biology in Plants, Department of Biology Faculty of Science, University of Chile, Santiago-Chile.

³Bio Insumos Nativa, Santiago- Chile.

⁴Laboratorio de Genómica funcional y Bioinformática, Departamento de Producción Agrícola, Facultad de Ciencias Agronómicas, Universidad de Chile, Santiago-Chile.

contact e-mail: mesterio@uchile.cl

Chile is positioned as a top exporters of table grapes

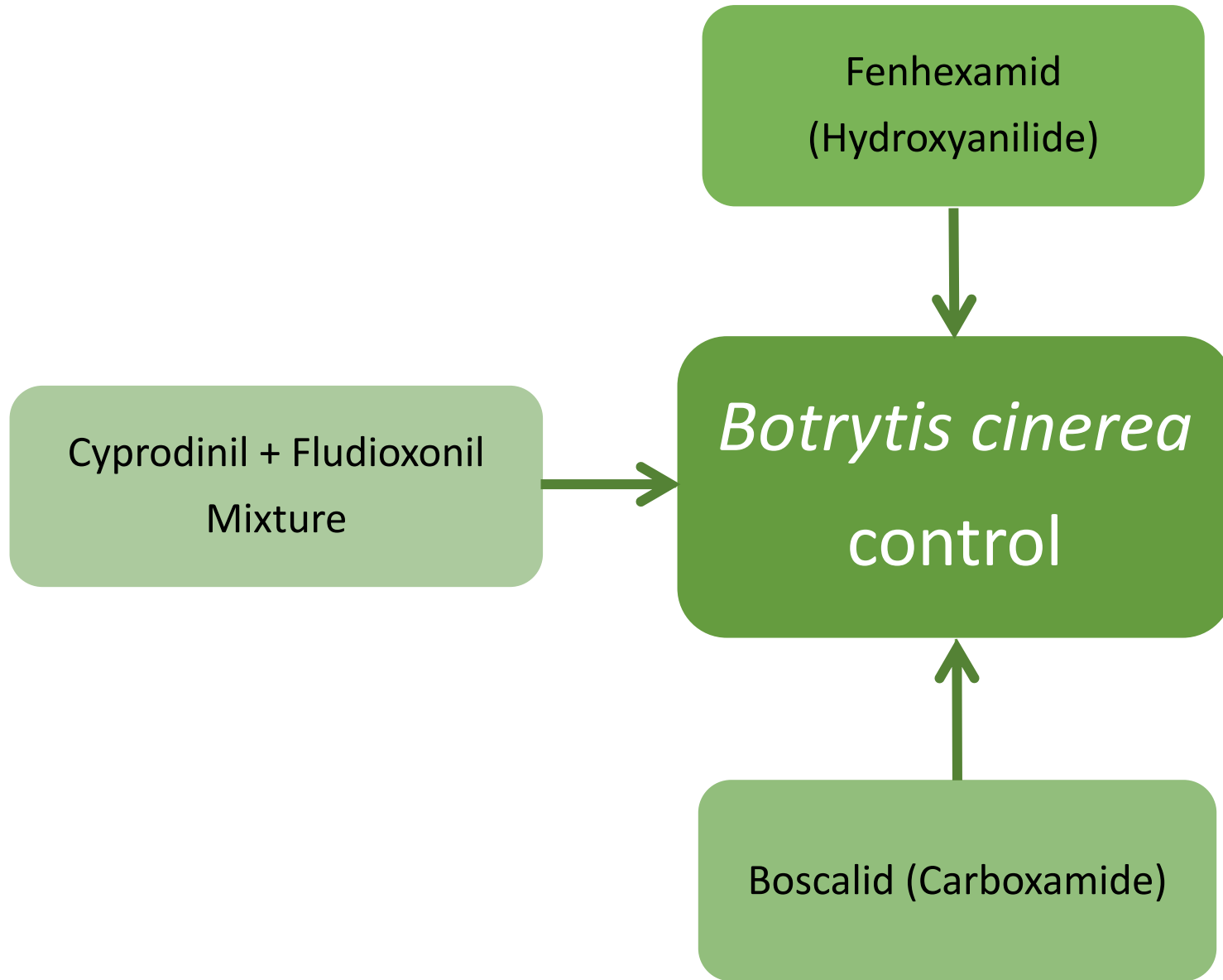


Modified from "Anuario 2023 Mercado Internacional de Uva de Mesa, iQonsulting"

Botrytis is the main phytosanitary problem of table grapes in Chile

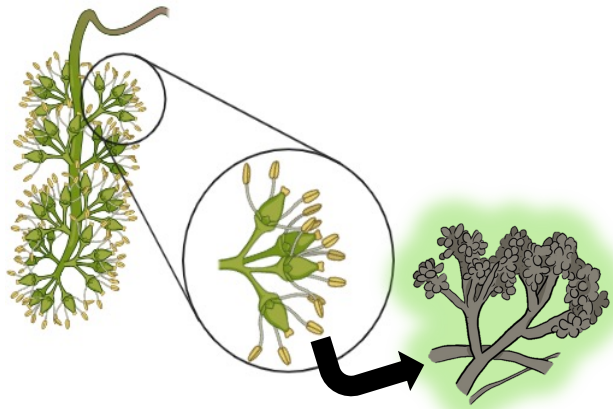


Key fungicides in *Botrytis cinerea* control



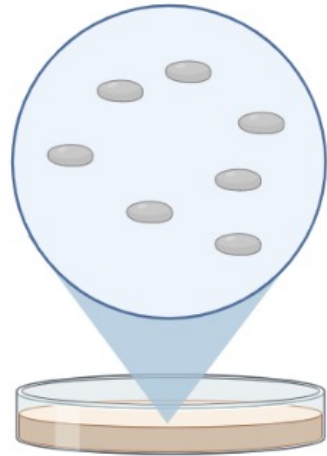
Determining the sensitivity of Botrytis population to fungicides.

A



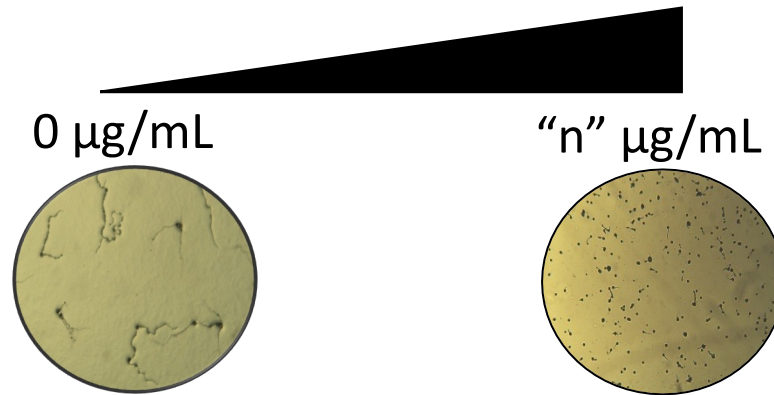
Plant material processing

B



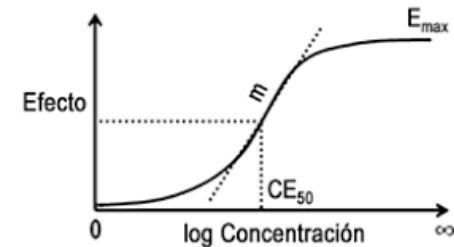
Botrytis single-spore isolation

C



Growth evaluation to different concentrations of a target fungicide/formulation

D

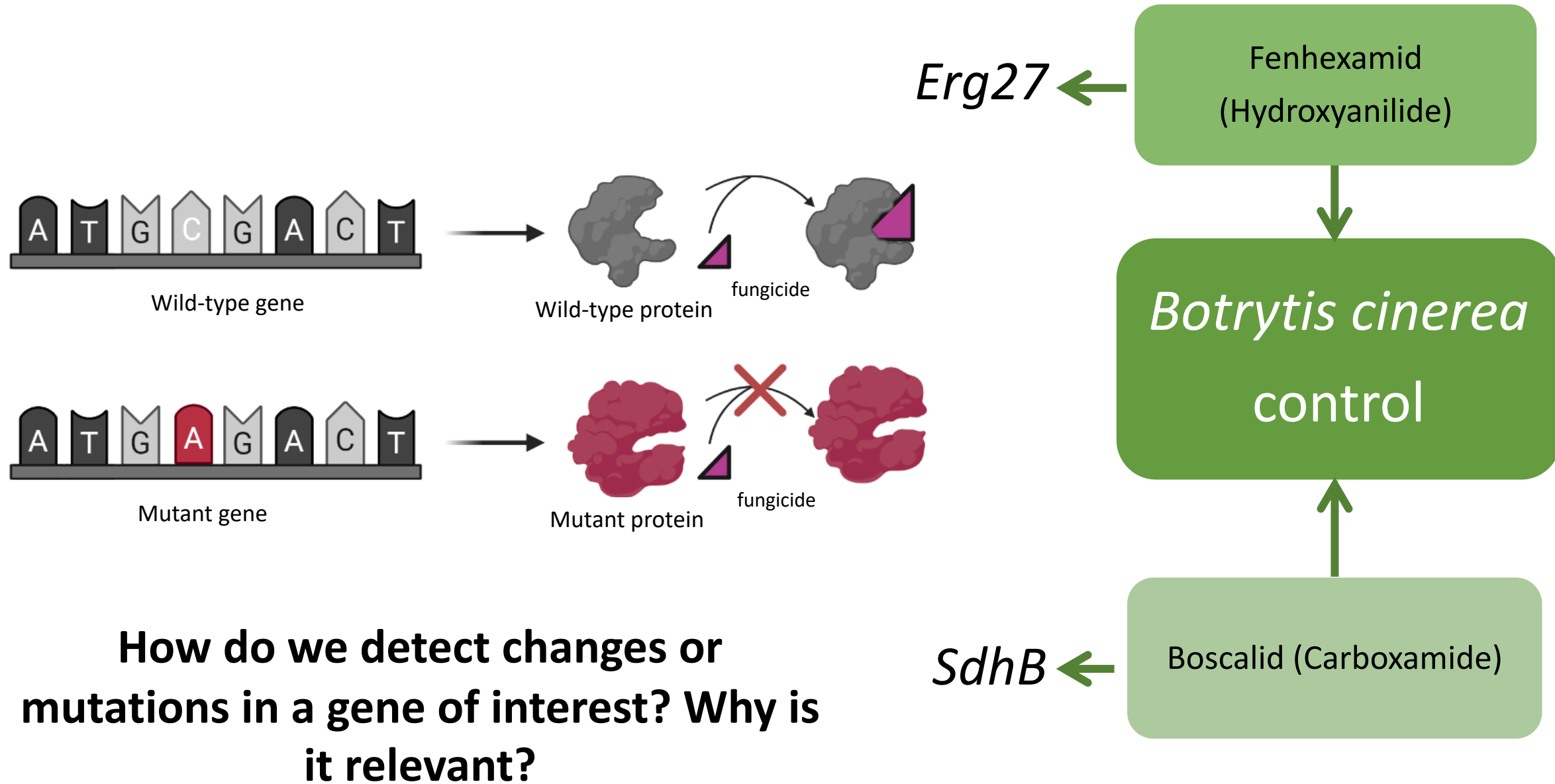


Sensitivity determination (EC₅₀ value)

Time

Weeks or months

Key fungicides in *Botrytis cinerea* control



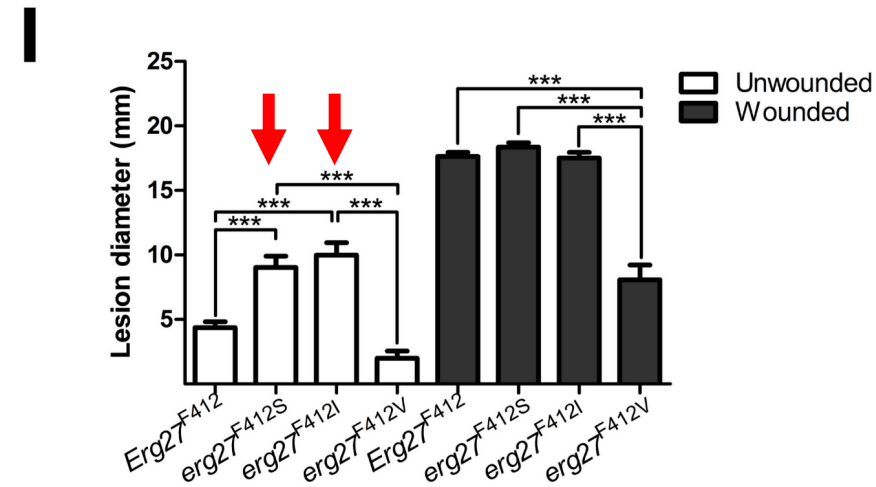
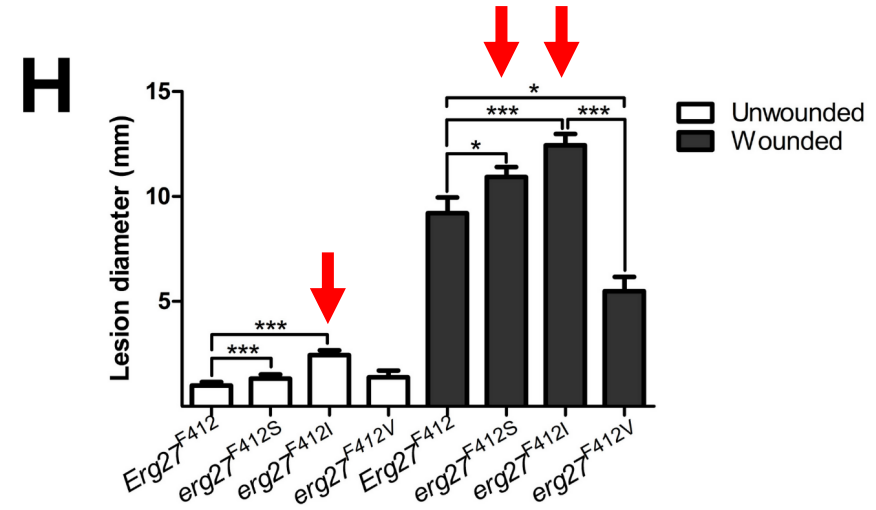
Isolates carrying different mutations exhibit differential fungicide sensitivity levels and contrasting fitness.

Table 2. Numbers of *Botrytis cinerea* isolates of *Erg27* genotype at the 412 position, obtained from Central Chile, from Valparaíso Region (VR), Metropolitan Region (MR) and O'Higgins Region (OR).

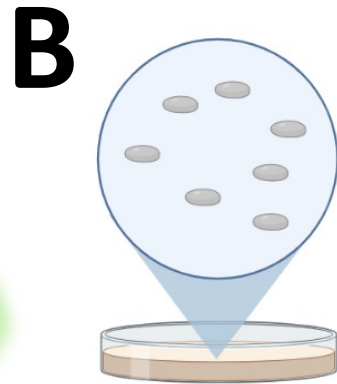
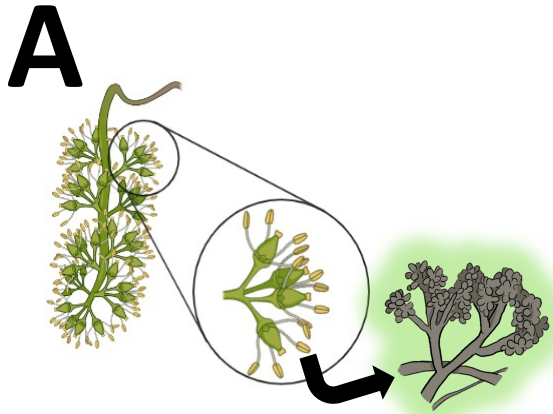
Region	Total isolates	<i>Erg27^{F412}</i>		<i>erg27^{F412I}</i>		<i>erg27^{F412S}</i>		<i>erg27^{F412V}</i>	
		% ^a	Mean EC ₅₀ ^b	% ^a	Mean EC ₅₀ ^b	% ^a	Mean EC ₅₀ ^b	% ^a	Mean EC ₅₀ ^b
VR	24	13	0.07	58	19.85	29	14.99	-	-
MR	47	19	0.79	13	77.18	62	17.90	6	60.59
OR	46	30	1.81	22	47.65	48	13.90	-	-

^a Frequency occurrence of genotype in percentage

^b Mean EC₅₀ (mg L⁻¹) for genotype of *Erg27* at the 412 position.



Determining the sensitivity of Botrytis population to fungicides.



C

Molecular diagnosis

PCR

Post PCR strategies

→ qPCR-TaqMan

→ qPCR-HRM

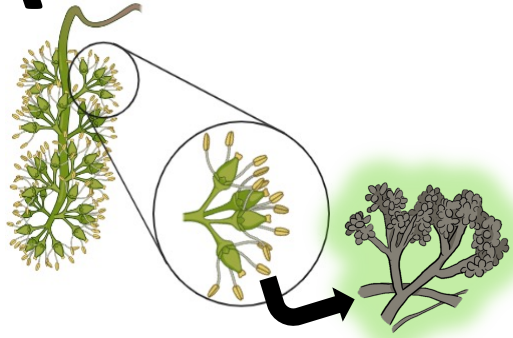
→ ddPCR



Weeks

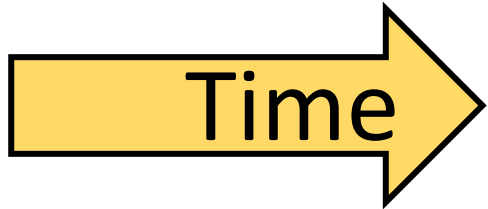
Determining the sensitivity of Botrytis population to fungicides.

A



B

Molecular diagnosis



Days?
Hours?

Can we know the sensitivity of the Botrytis population *in situ/in planta*?

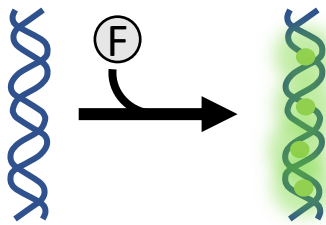
Detection of alleles by qPCR-HRM (High Resolution Melting) analysis

1

PCR amplification of a target DNA fragment

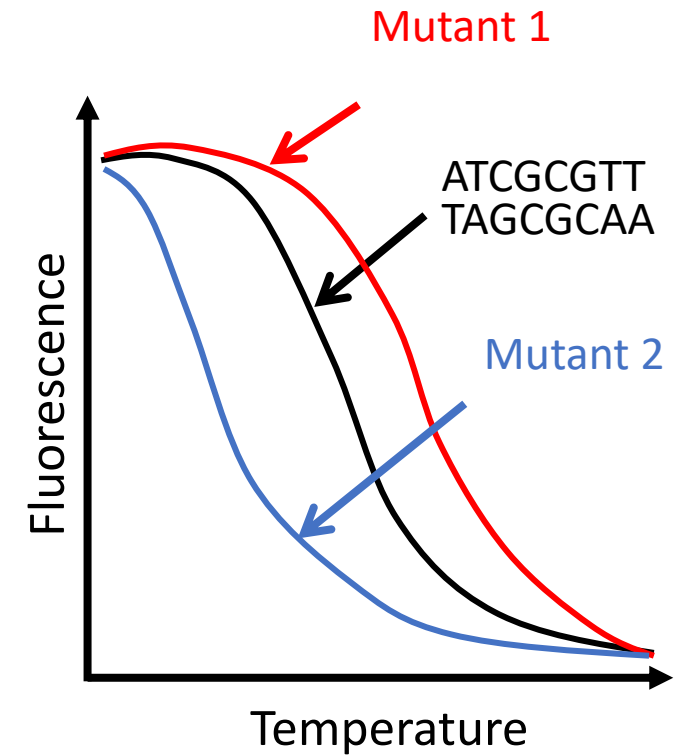
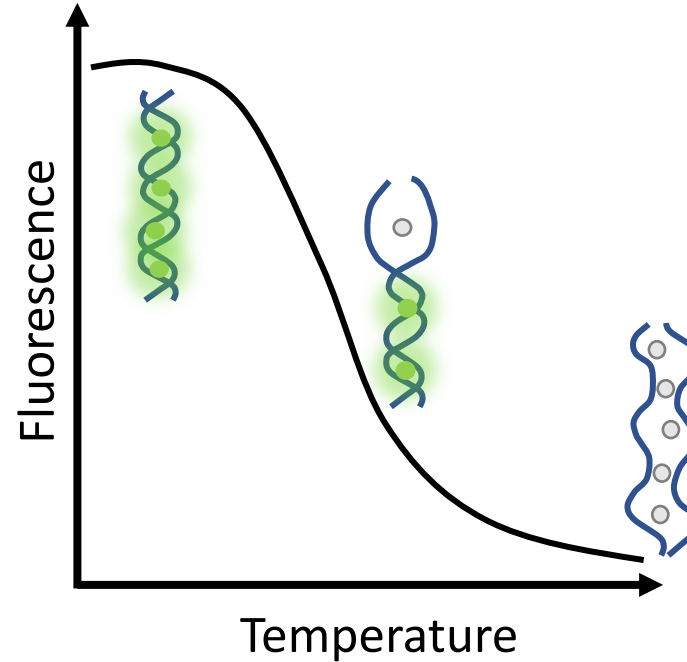
2

Use of intercalating fluorophore

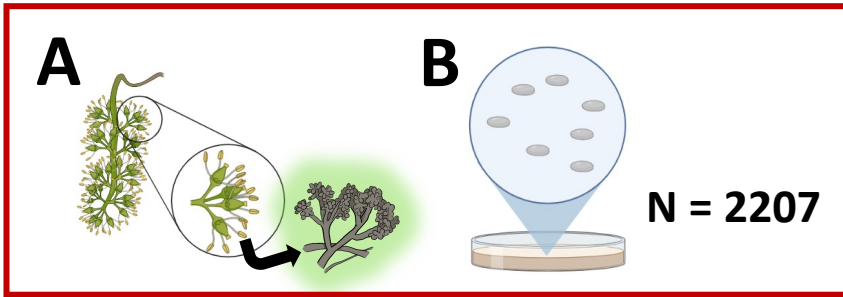


3

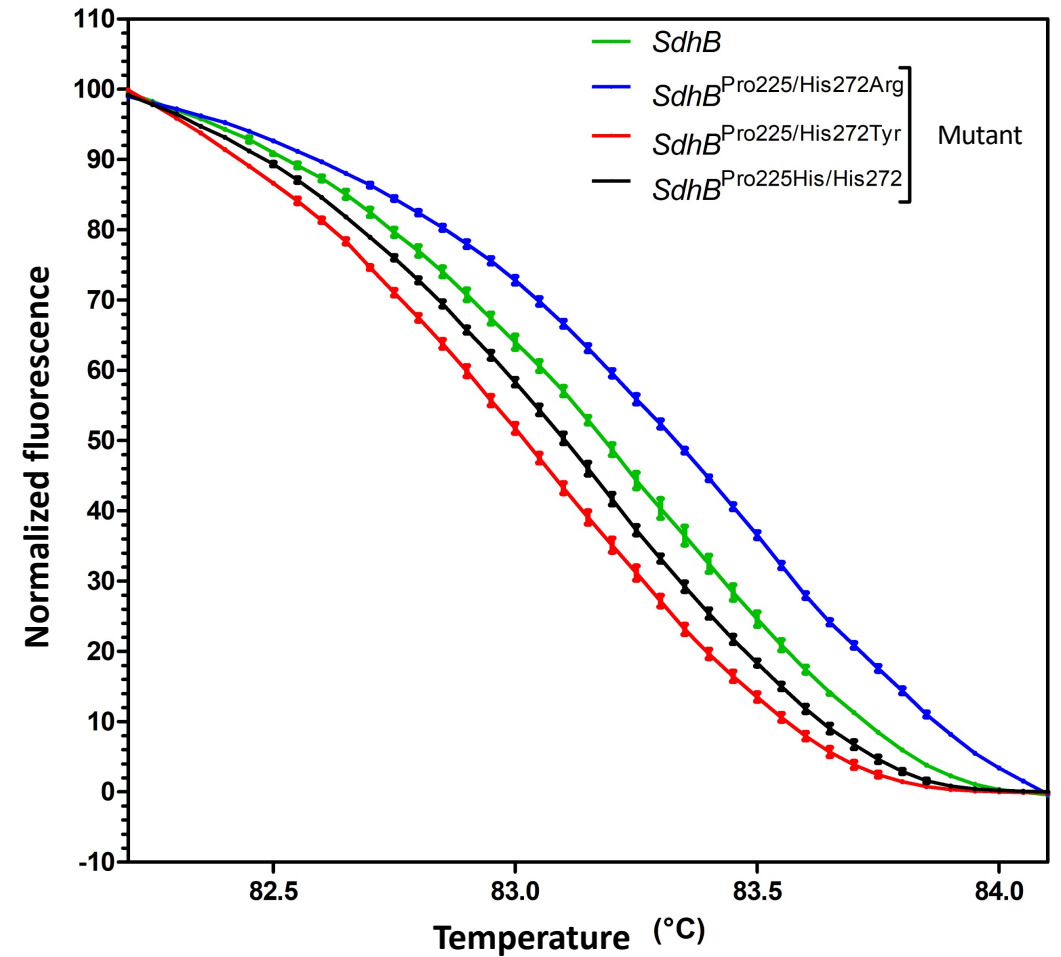
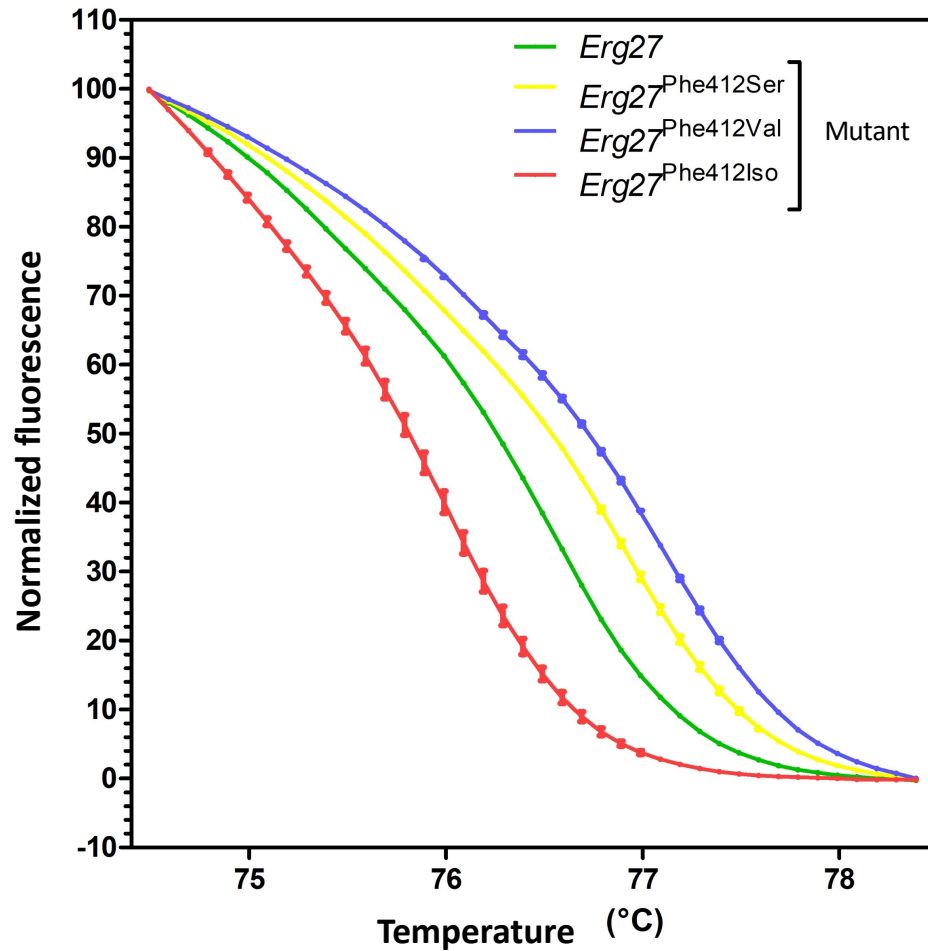
Difference A/T and C/G bonds



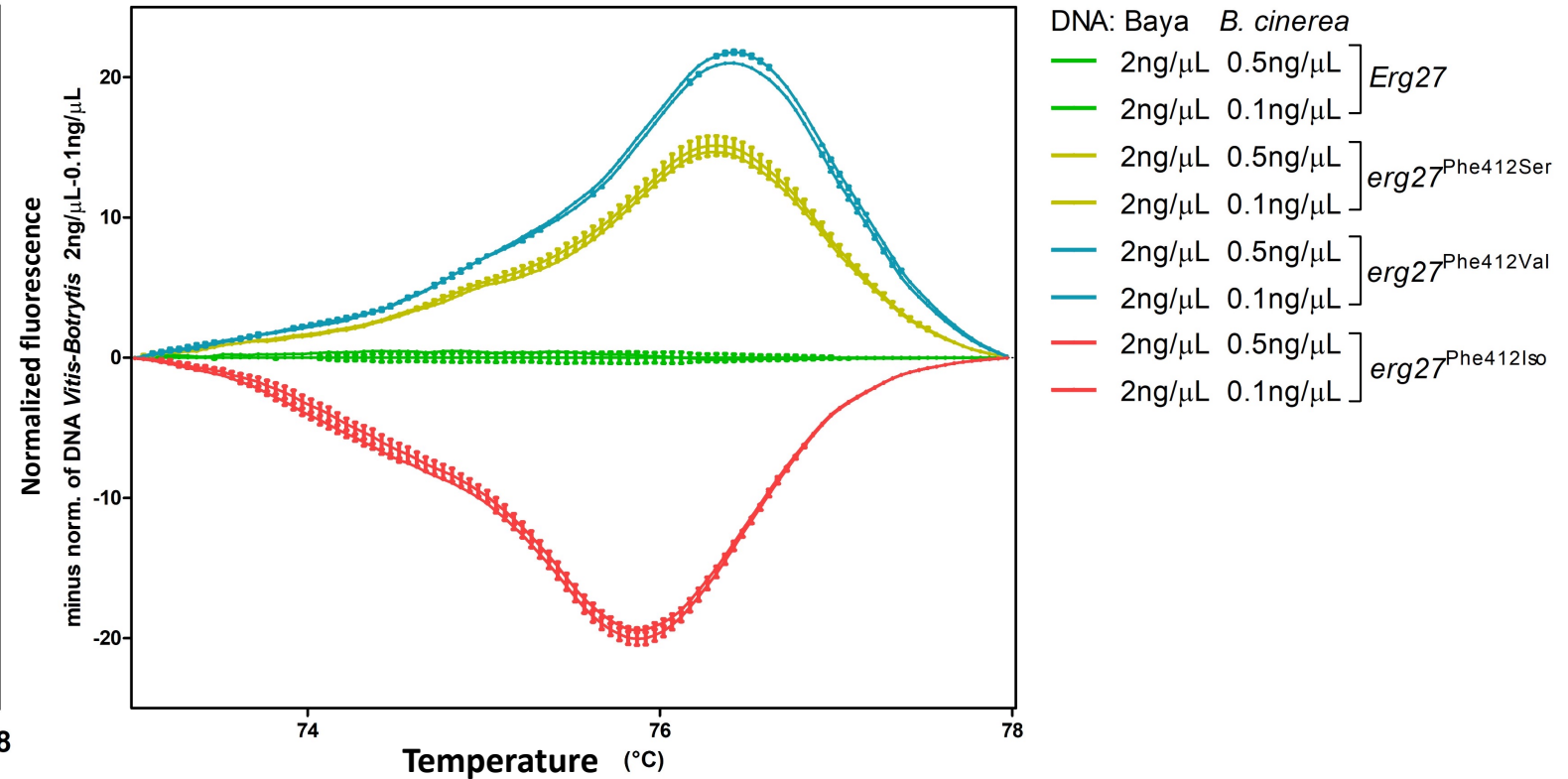
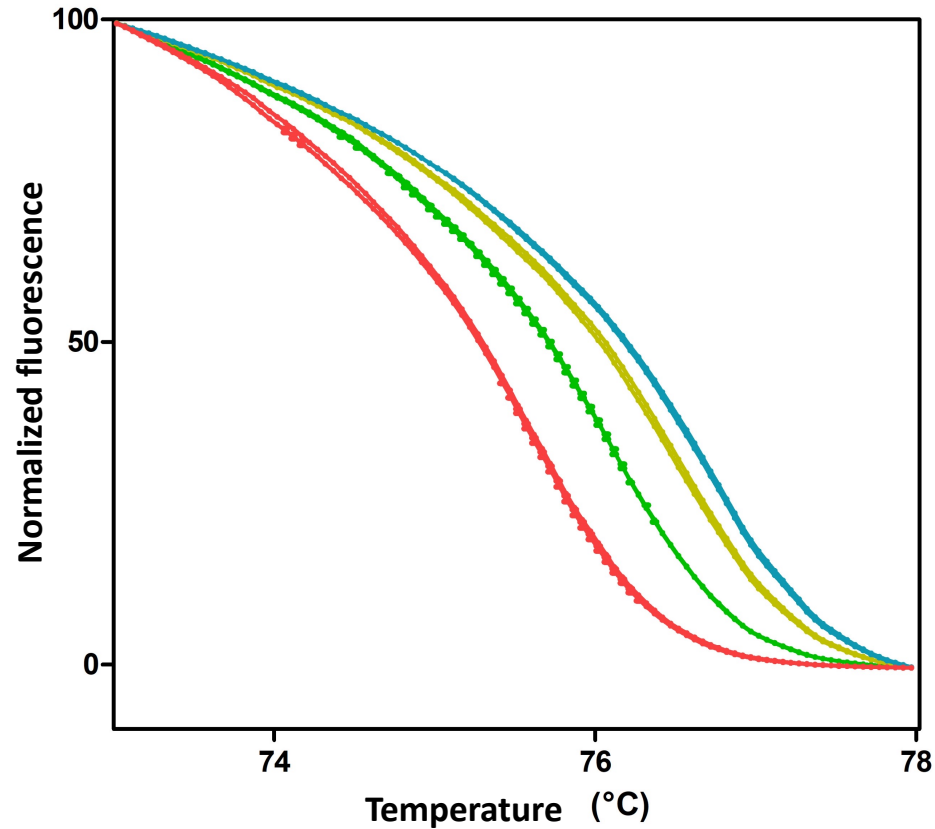
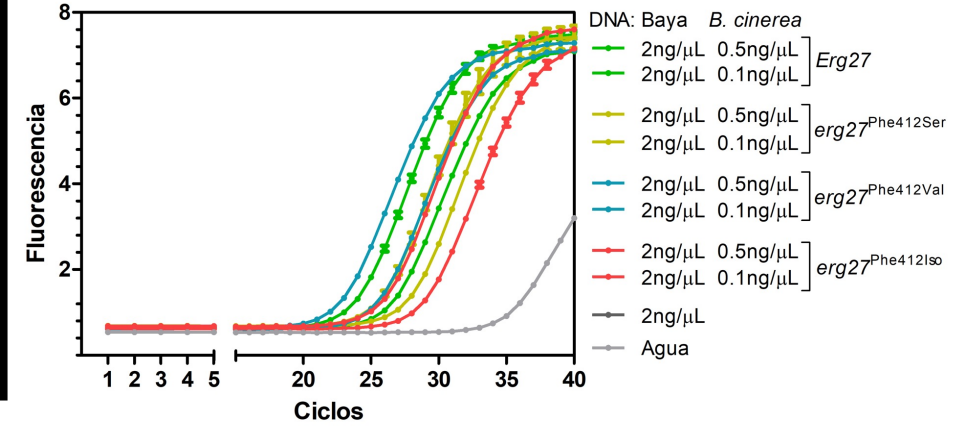
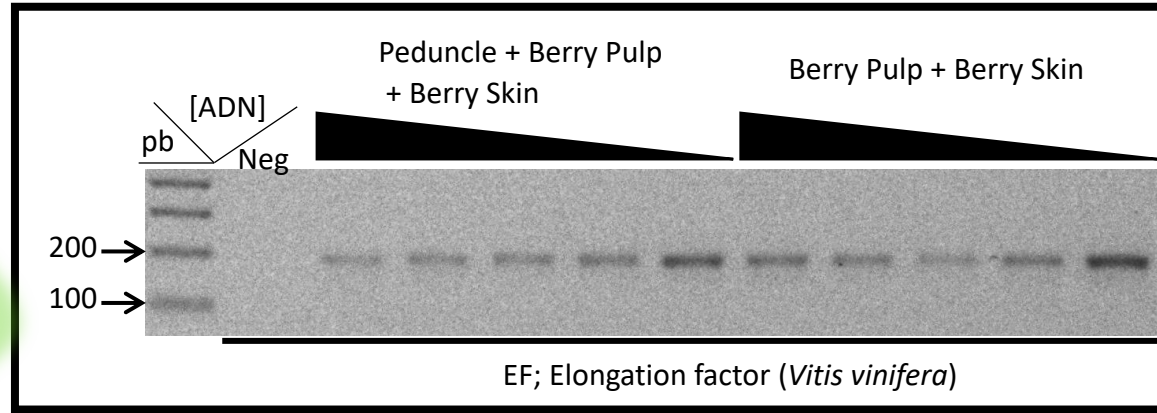
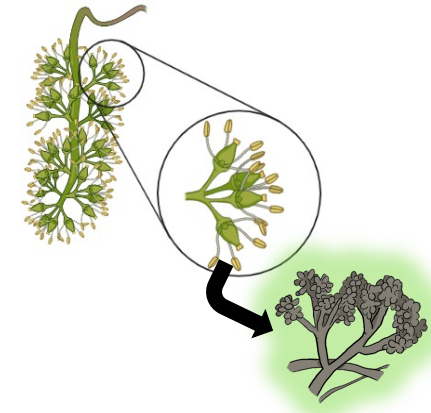
Detection of alleles by qPCR-HRM (High Resolution Melting) analysis



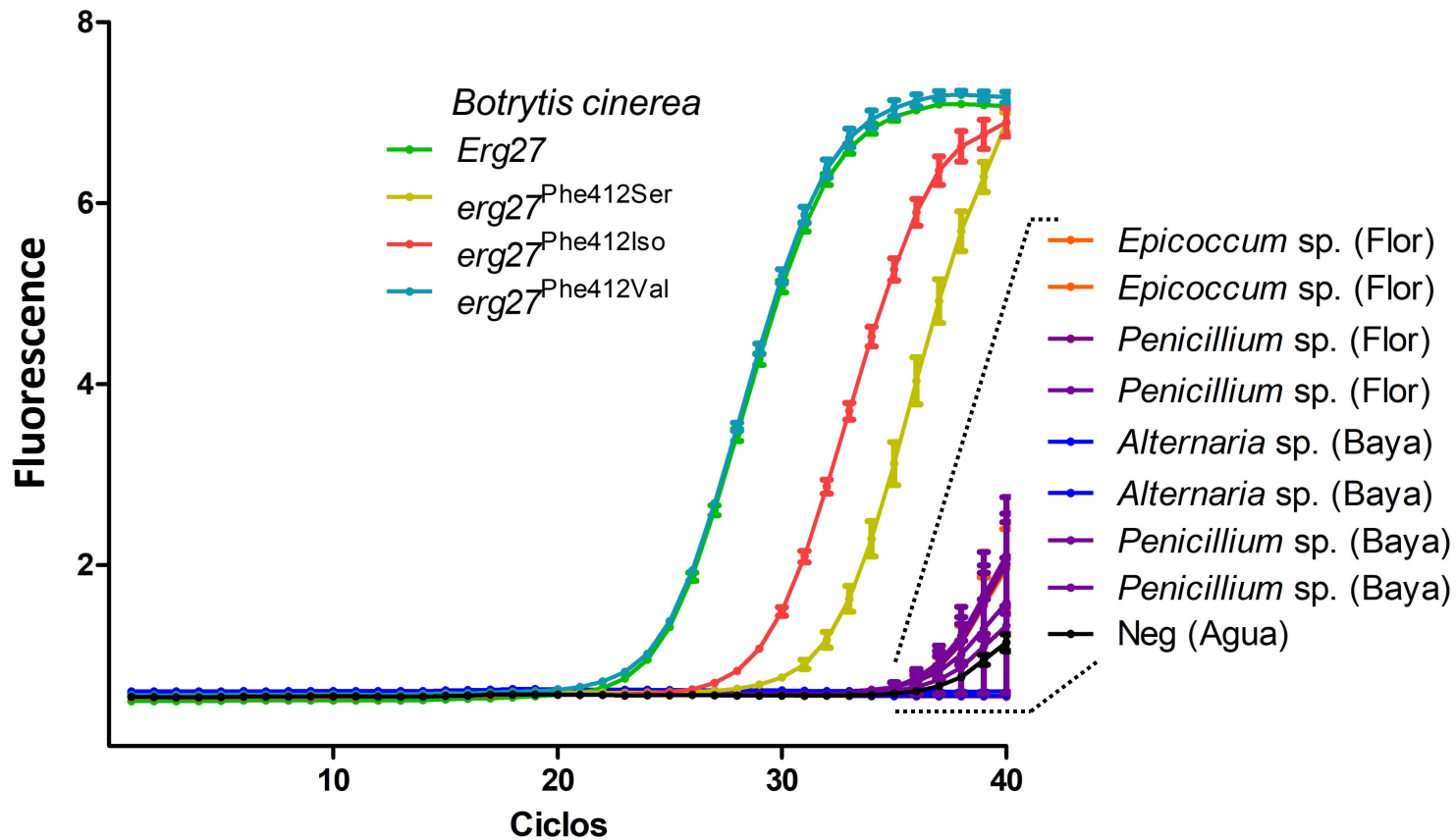
Detection system adjusted to local conditions



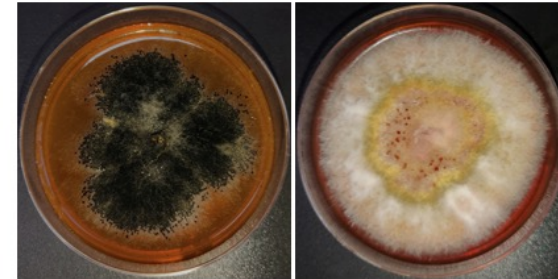
Vitis vinifera DNA does not interfere with the identification of *Botrytis cinerea* genotypes (*Erg27*)



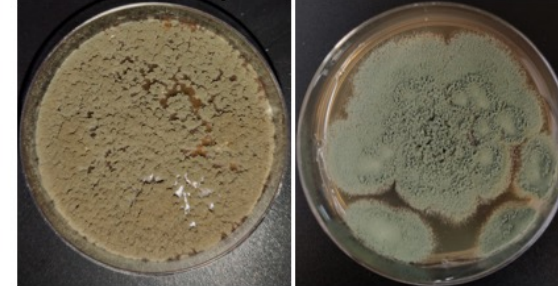
Microbiota DNA does not interfere with the identification of *Botrytis cinerea* genotypes (*Erg27*)



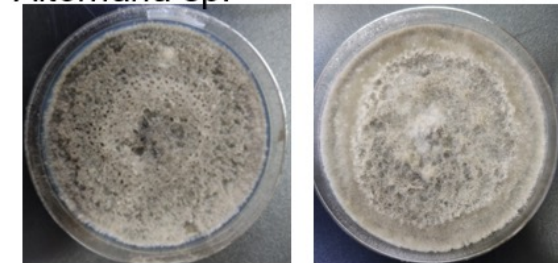
Epicoccum sp.



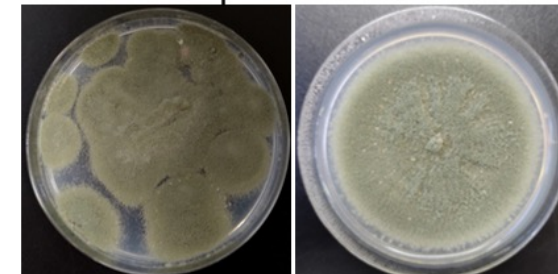
Penicillium sp.



Alternaria sp.



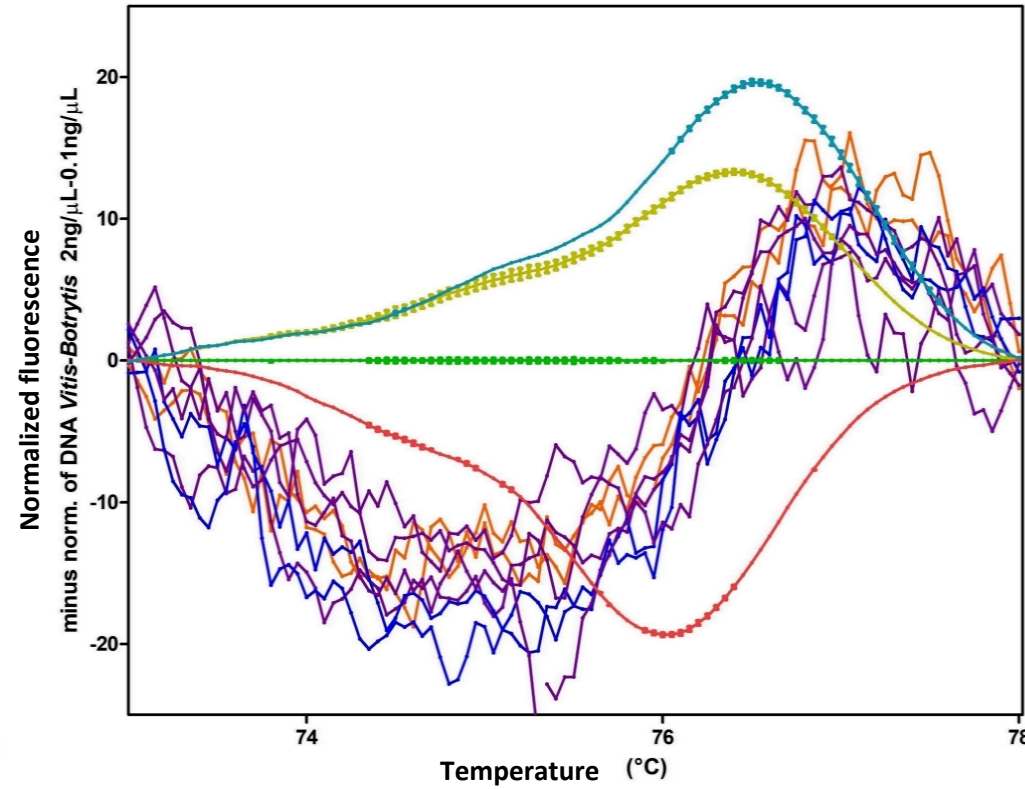
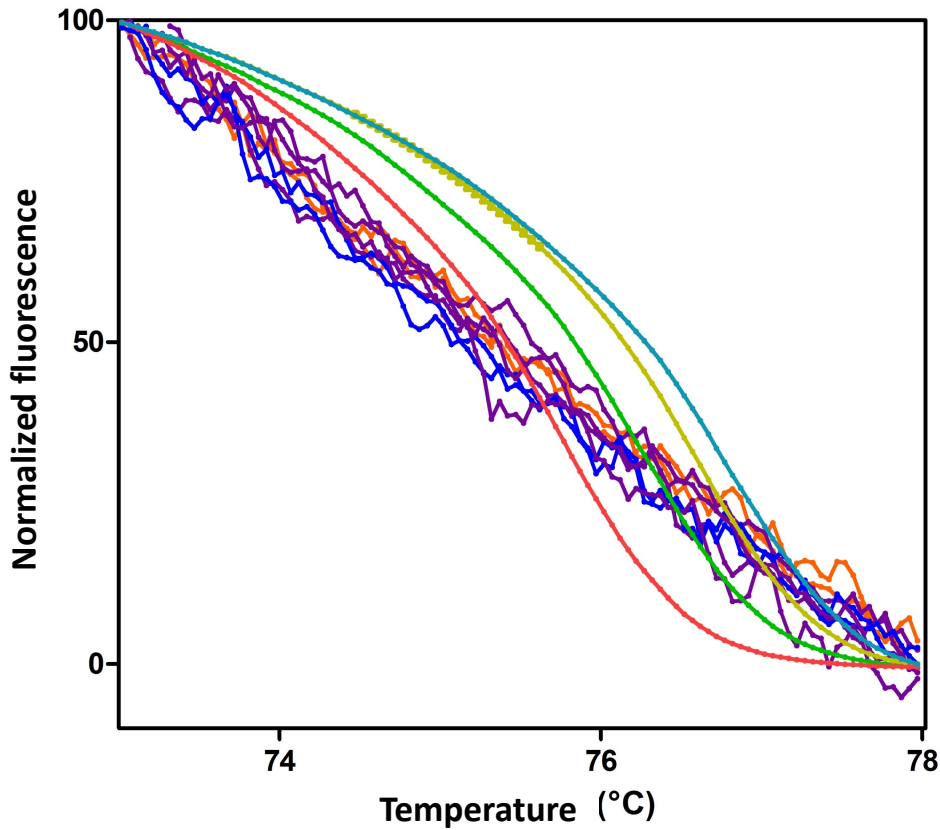
Penicillium sp.



Flower microbiota

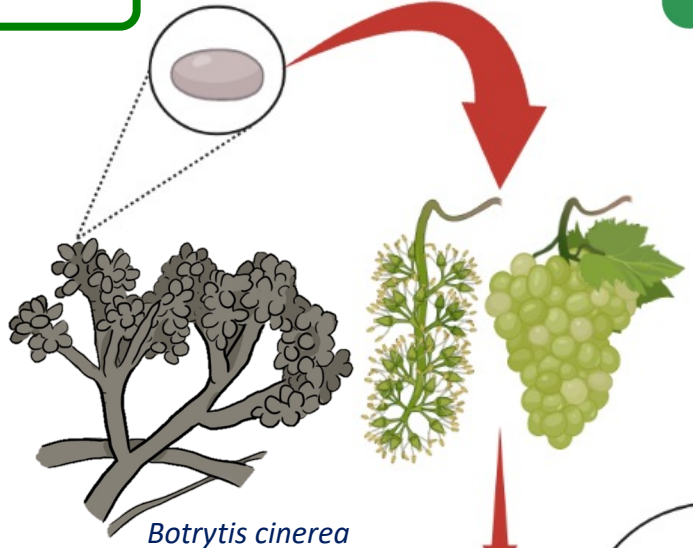
Berry microbiota

Microbiota DNA does not interfere with the identification of *Botrytis cinerea* genotypes (*Erg27*)



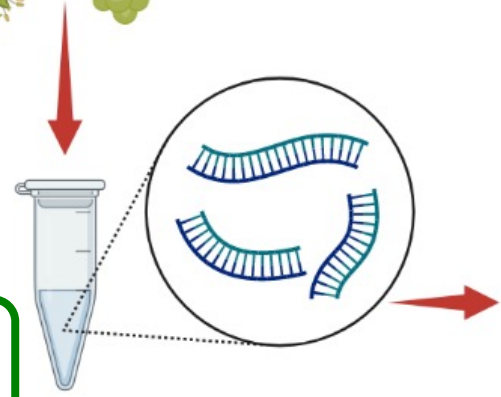
- Epicoccum* sp. (Flor)
- Epicoccum* sp. (Flor)
- Penicillium* sp. (Flor)
- Penicillium* sp. (Flor)
- Alternaria* sp. (Baya)
- Alternaria* sp. (Baya)
- Penicillium* sp. (Baya)
- Penicillium* sp. (Baya)
- Erg27*
- erg27*^{Phe412Ser}
- erg27*^{Phe412Iso}
- erg27*^{Phe412Val}

1.- Sampling



Check fast
BOTRYTIS
SABER AHORA, PARA APLICAR MAÑANA

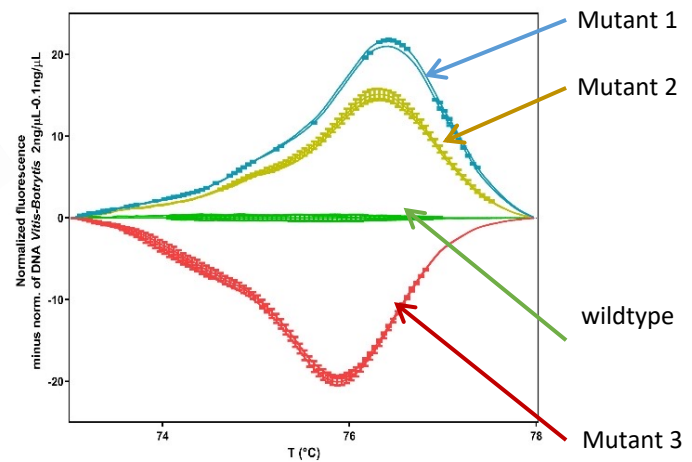
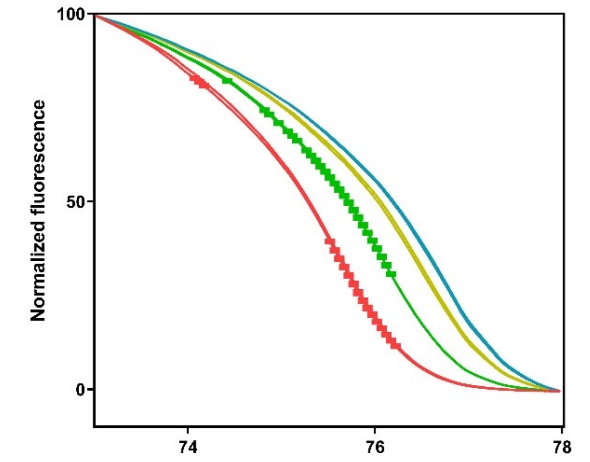
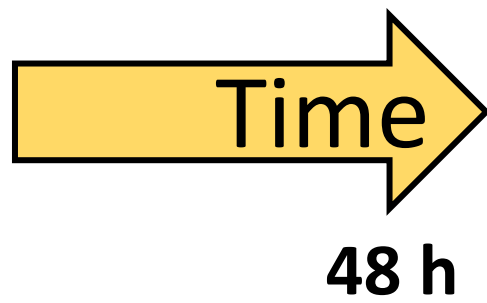
2.- Plant tissue processing/
Total DNA isolation



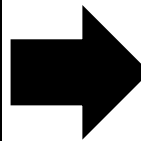
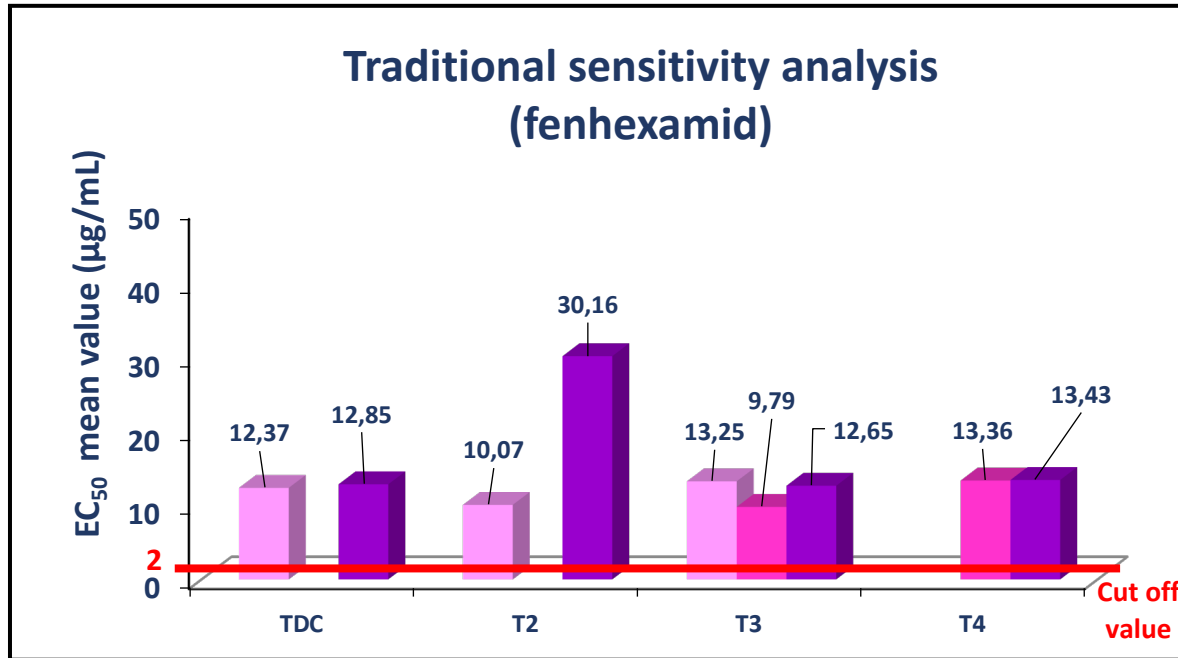
qPCR-HRM analysis

3.- Genotyping. Fungicide sensitivity evaluation

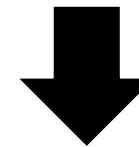
4.- Results presentation



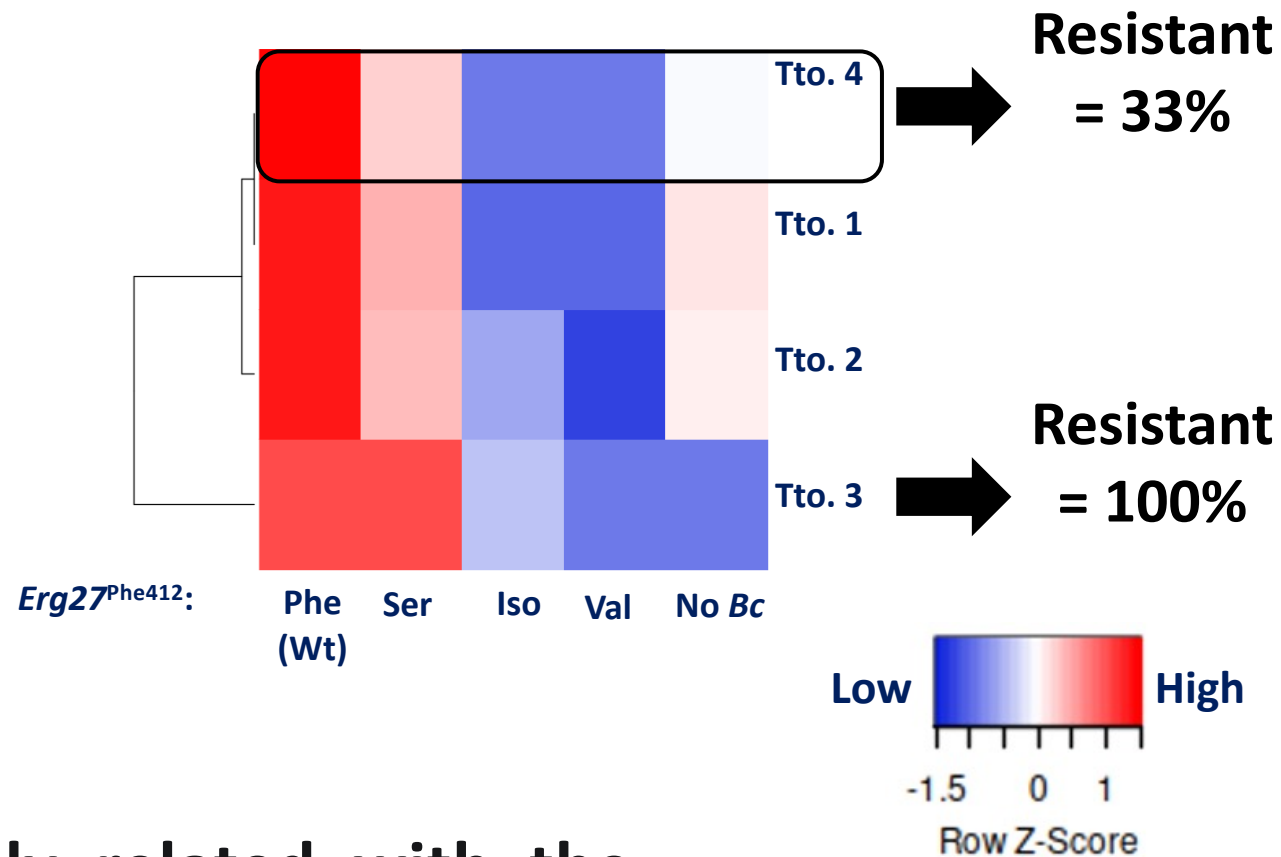
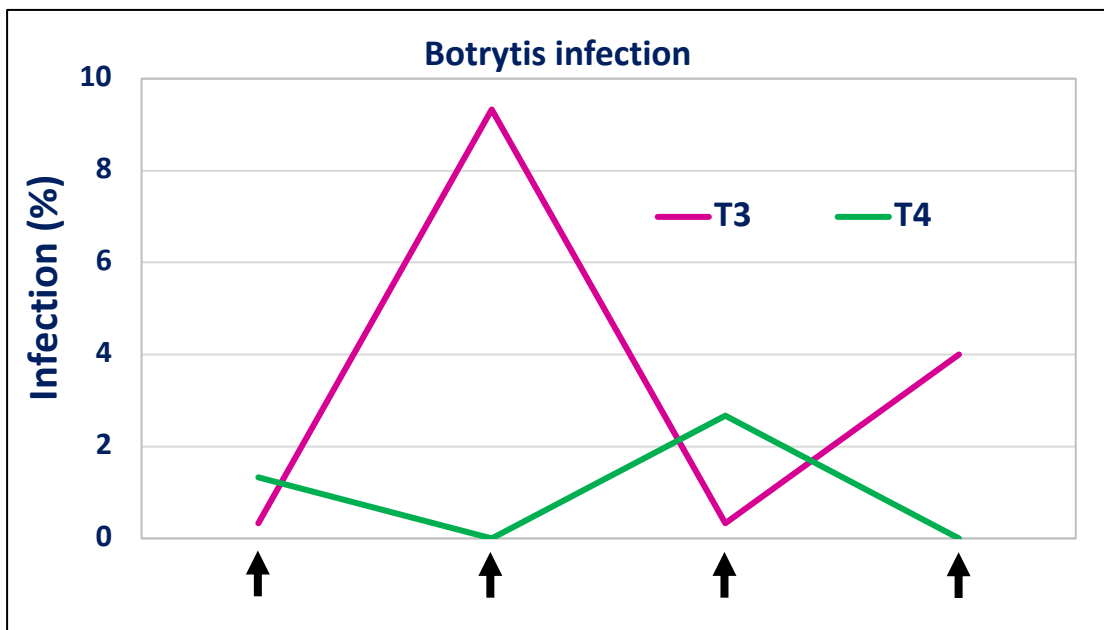
Check fast Botrytis application



Fungicide-resistant Botrytis population



Ineffectiveness of the Botrytis control program?



Remark:

The Botrytis control success is closely related with the percentage of fungicide mutants isolates present in the local population.

- (1) The *Check fast Botrytis* analysis significantly reduces detection times of Botrytis sensitivity to fungicides (48 h).
- (2) Botrytis management programs can be adjusted in real-time based on fungicide sensitivity using the *Check fast Botrytis* analysis.
- (3) Adjusting the destination market and fruit value is possible by knowing the fruit's infection level using *Check fast Botrytis* analysis.
- (4) The experimental design provides a framework for analyzing the genotypes of *Botrytis cinerea* (or a different pathogen) mutants in other crops of interest.

Acknowledgments



Group leaders :



Marcela Esterio



Jaime Auger

Lab members colaboradores

- Madelaine Azócar
- Felipe Durán
- Verónica Estrada
- Macarena Carreño
- Paula Reyes
- Claudio Osorio-Navarro





Universidad de Chile

10TH INTERNATIONAL
TABLE GRAPE
SYMPOSIUM
SOMERSET WEST, SOUTH AFRICA
26 NOV - 1 DEC 2023



Real-time design of *Botrytis cinerea* control programs based on direct qPCR-HRM (High-Resolution Melting) fungicide-resistance genotyping from infected plant tissue

Claudio Osorio-Navarro^{a,b}; Charleen Copier^a; Felipe Durán^a; Madelaine Azócar^a; Gonzalo Gutiérrez^a; Eduardo Donoso; Herman Silva; Jaime Auger^a & Marcela Esterio^a

^aDepartamento de Sanidad Vegetal, Facultad de Ciencias Agronómicas, Universidad de Chile, Santiago-Chile.

^bCentre of Molecular Biology in Plants, Department of Biology Faculty of Science, University of Chile, Santiago-Chile.

³Bio Insumos Nativa, Santiago- Chile.

⁴Laboratorio de Genómica funcional y Bioinformática, Departamento de Producción Agrícola, Facultad de Ciencias Agronómicas, Universidad de Chile, Santiago-Chile.