UNIVERSITÀ DI TORINO

Effectiveness of antagonistic yeasts and essential oils in the control of postharvest diseases of fruit

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DISAFA
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AGRINNOVA
RESEARCH BEARS ITS FRUITS

PRIMA
PARTNERSHIP FOR RESEARCH AND INNOVATION IN THE MEDITERRANEAN AREA

STOP MED WASTE

The research aims to find **alternative solutions to fungicides** to control postharvest pathogens of fruit crops

- **Essential oils (EOs)**
- **Biological control agents (BCAs)**

Nectarines

Strawberries

Apples

Grape

Essential oils (EOs)

Mixtures of volatile organic compounds (terpenes, aldehydes, ketones, fatty acids, phenols, esters, alcohols)

Sources of EOs include flowers, leaves, roots, wood, rhizome, fruit, bark and seeds

Present in over 50 botanical families

Essential oils (EOs)

Evaluation of the efficacy of 5 EOs against brown rot caused by *Monilinia fructicola* on stored nectarines

Evaluation of the efficacy of 3 EOs and a thymol-based formulation against gray mould caused by *Botrytis cinerea* on stored apples

Efficacy of biofumigation with EOs in the control of postharvest rots of nectarines

Thyme EO
(*Thymus vulgaris)*

Savoury EO
(Satureja montana)

Lemon EO
(*Citrus* x *limon)*

Basil EO
(Ocimum basilicum subsp. *basilicum)*

Fennel EO
(*Foeniculum vulgare)*

In vitro biofumigation test

↓

Screening test *in vivo*

↓

Efficacy test *in vivo*

↓

Microbiome analysis

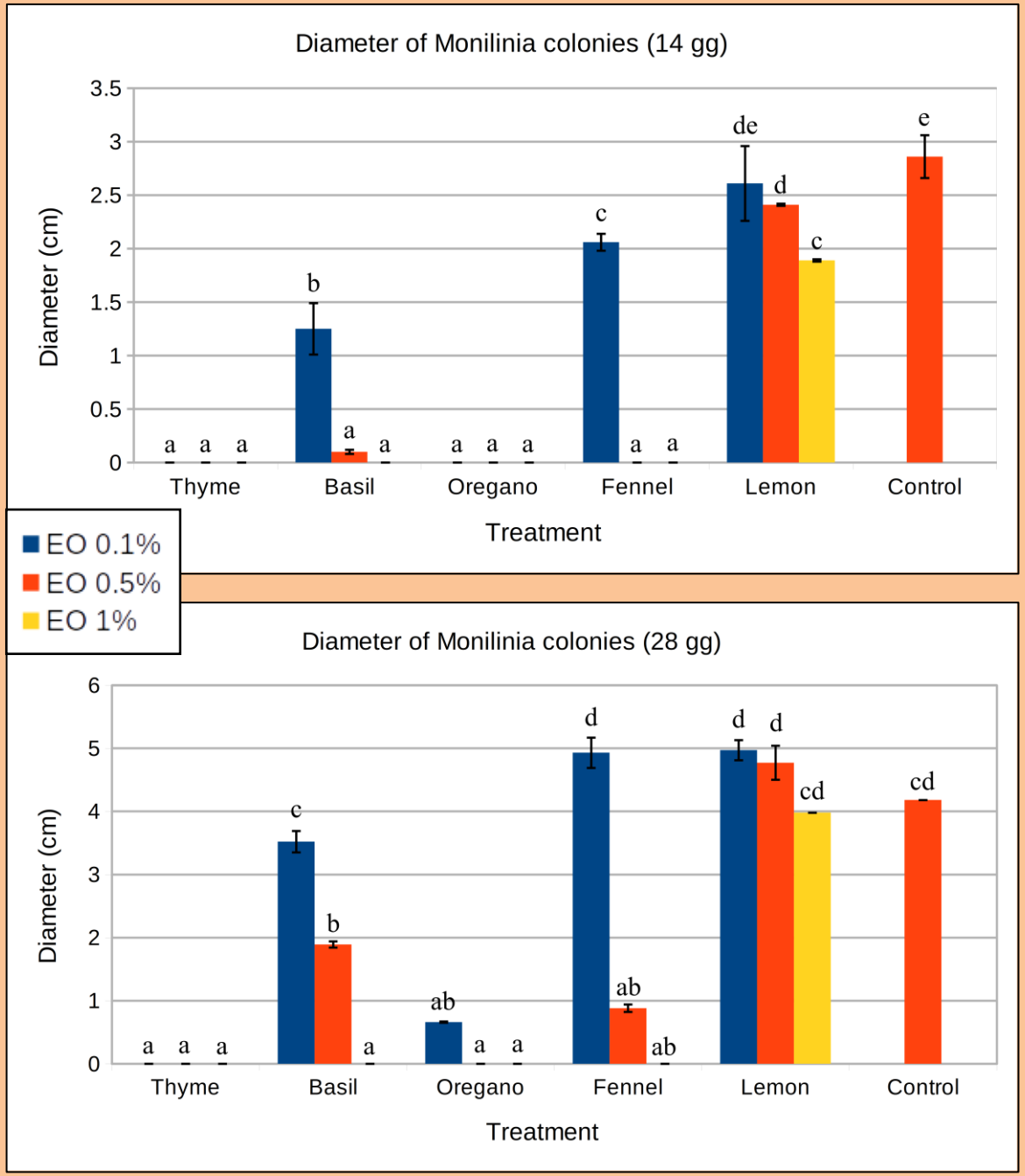
In vitro biofumigation test

Effect of EOs on the growth *Monilinia fructicola* in vitro

EOs applied at different concentrations (0,1%, 0,5%, 1%)

Sandwich plate technique

Thyme, basil, oregano and fennel EOs inhibited the growth of *Monilinia fructicola*.



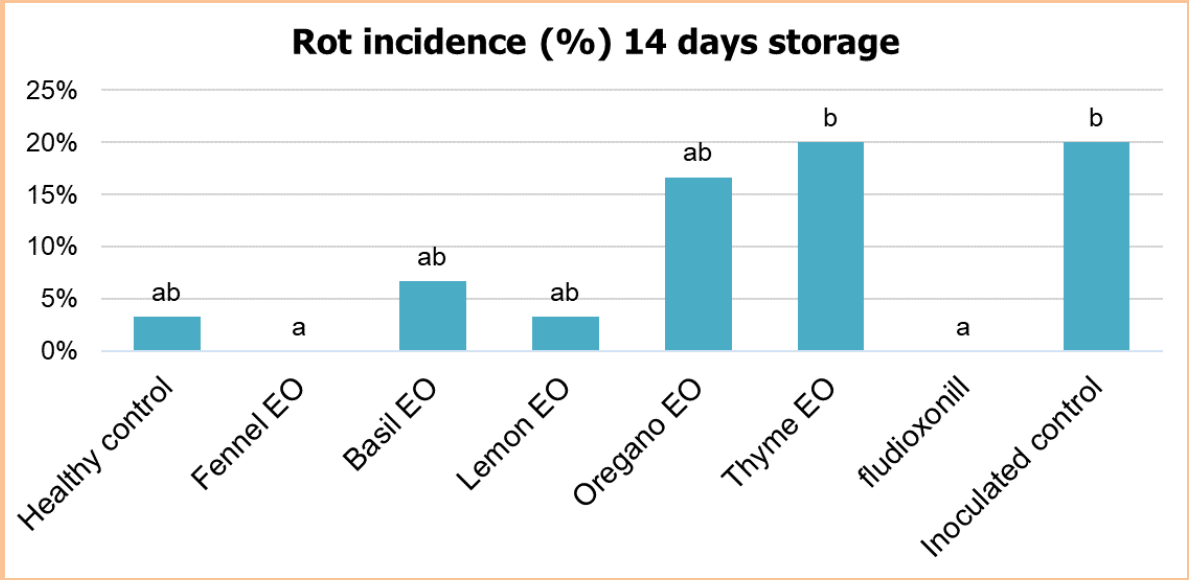
Screening test *in vivo*

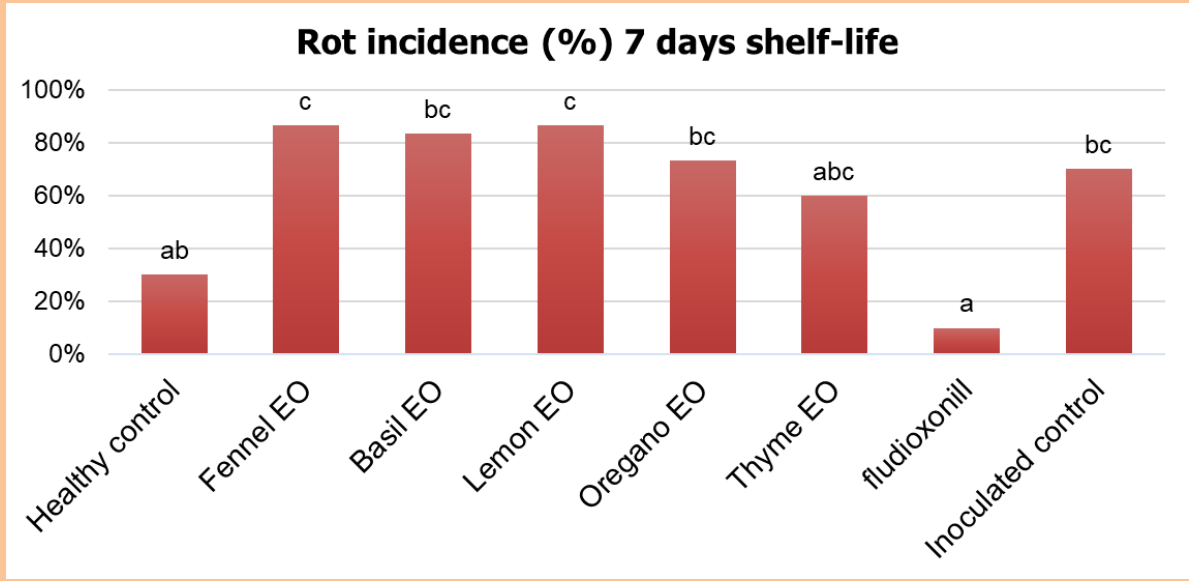
Inoculation of *Monilinia fructicola* on fruits

8 treatments:
- 5 treated with EOs biofumigation
- 1 chemical control (fludioxonil)
- 1 inoculated control
- 1 healthy control

Storage at 1 ± 1 °C 14 days

Shelf-life at 24 ± 1 °C 7 days





Efficacy test *in vivo*

Basil EO
(*Ocimum basilicum* subsp. *basilicum*)

Fennel EO
(*Foeniculum vulgare*)

Lemon EO
(*Citrus* x *limon*)

Selected from the results of screening test

Naturally contaminated fruits

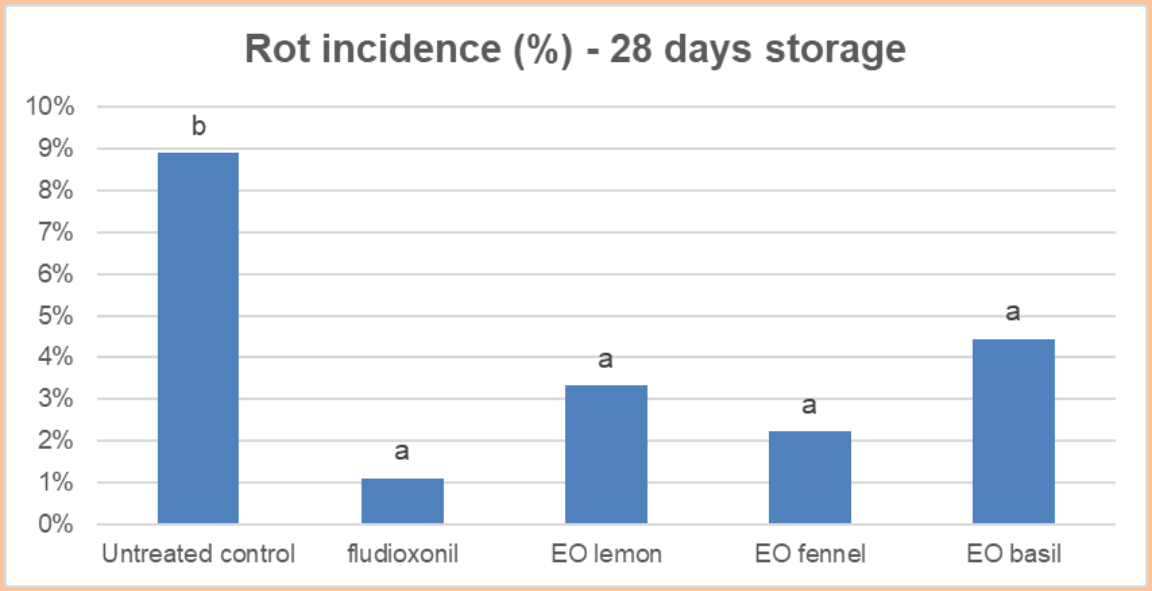
6 treatments:
- 3 treated with EOs biofumigation
- 1 chemical control (fludioxonil)
- 1 inoculated control
- 1 healthy control

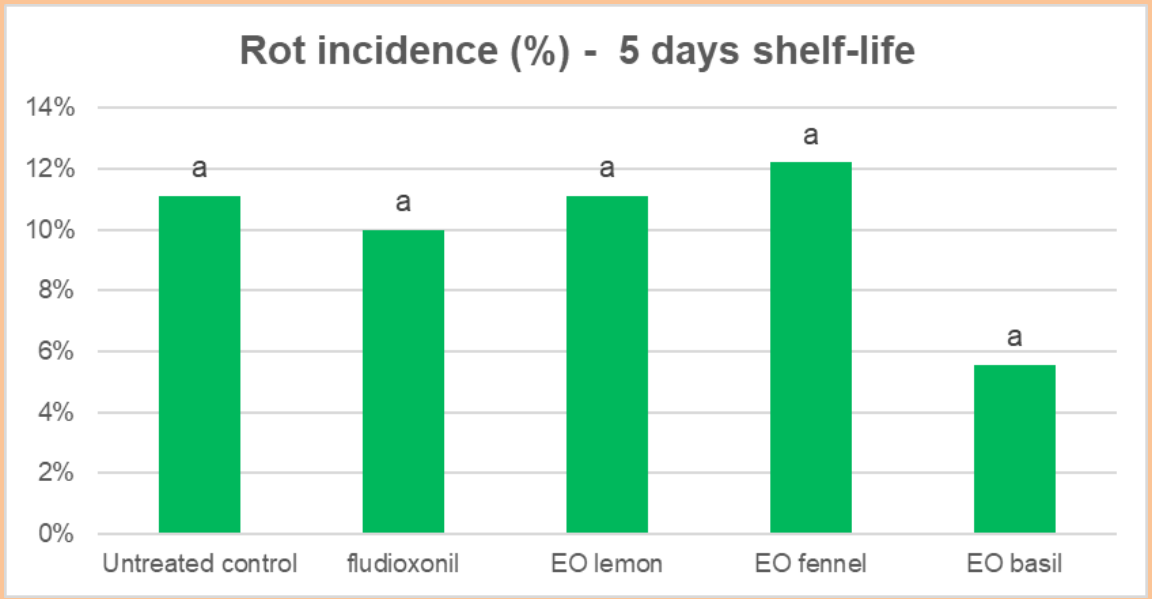
Storage at 1 ± 1 °C for 28 days
Shelf-life at 25 ± 1 °C for 5 days

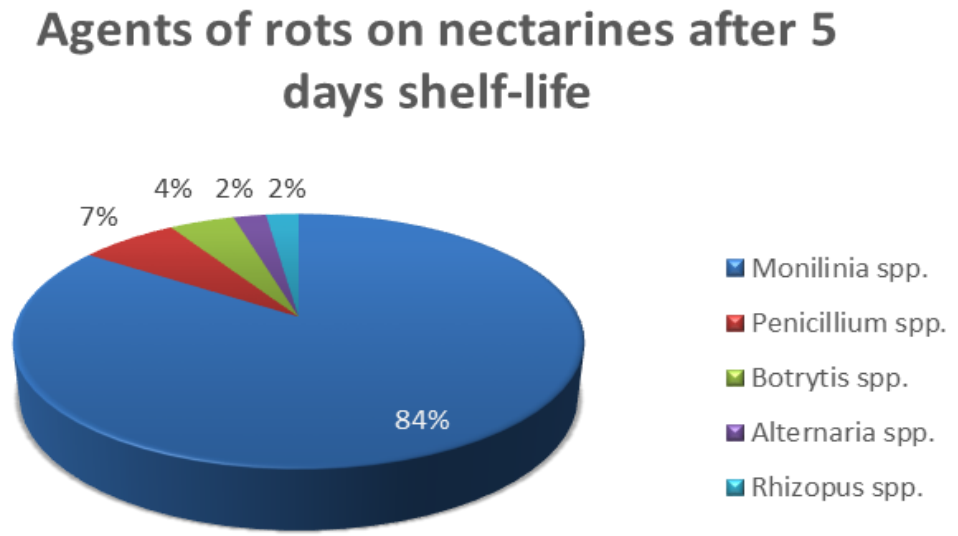
Quality analyses
at harvest, after storage, after shelf-life

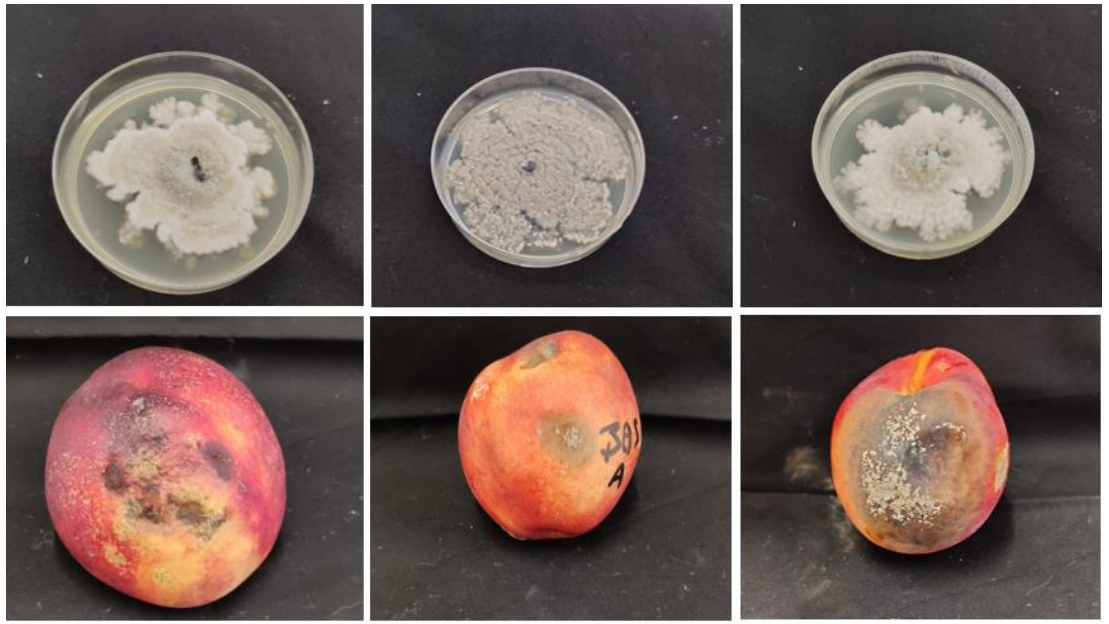
Microbiome sampling
at harvest, after storage, after shelf-life

Efficacy test *in vivo*





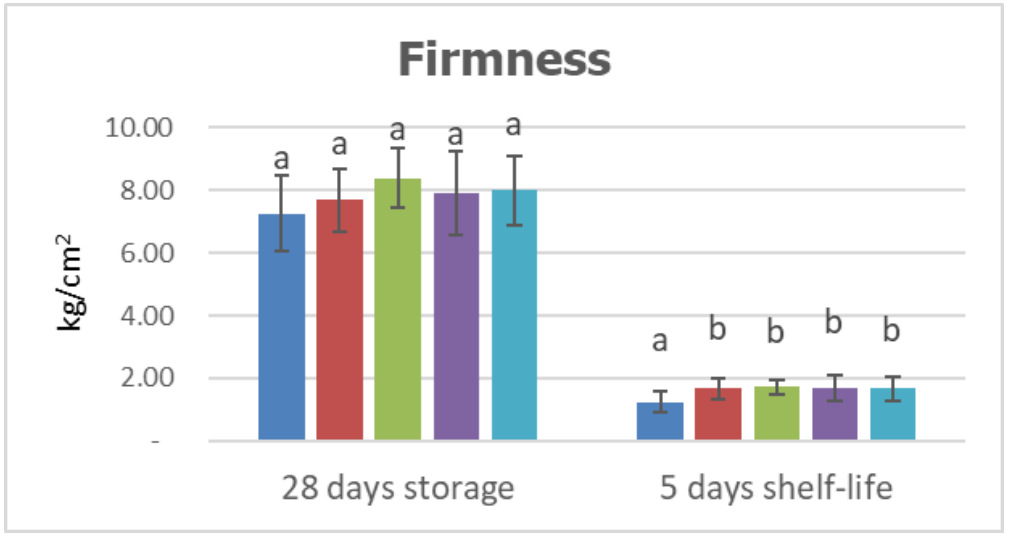


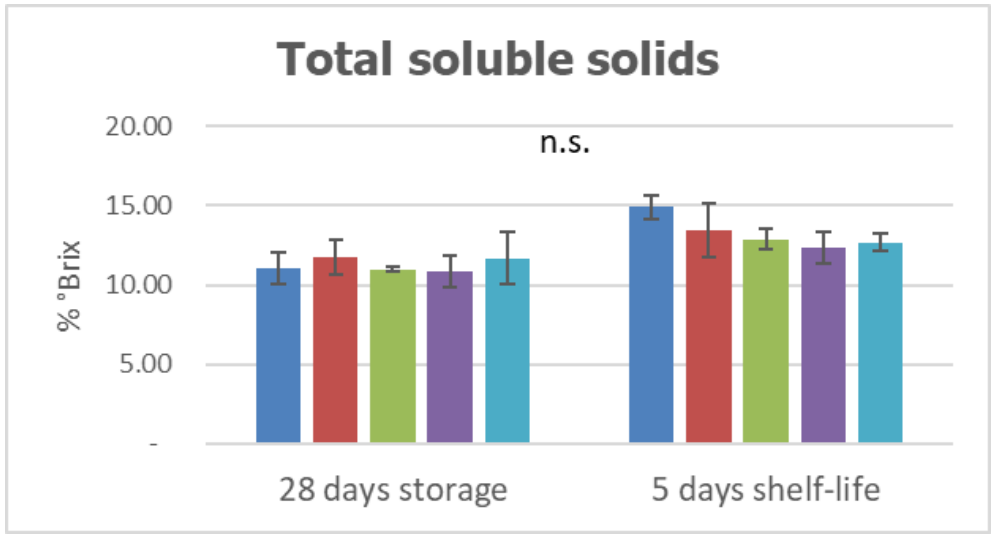


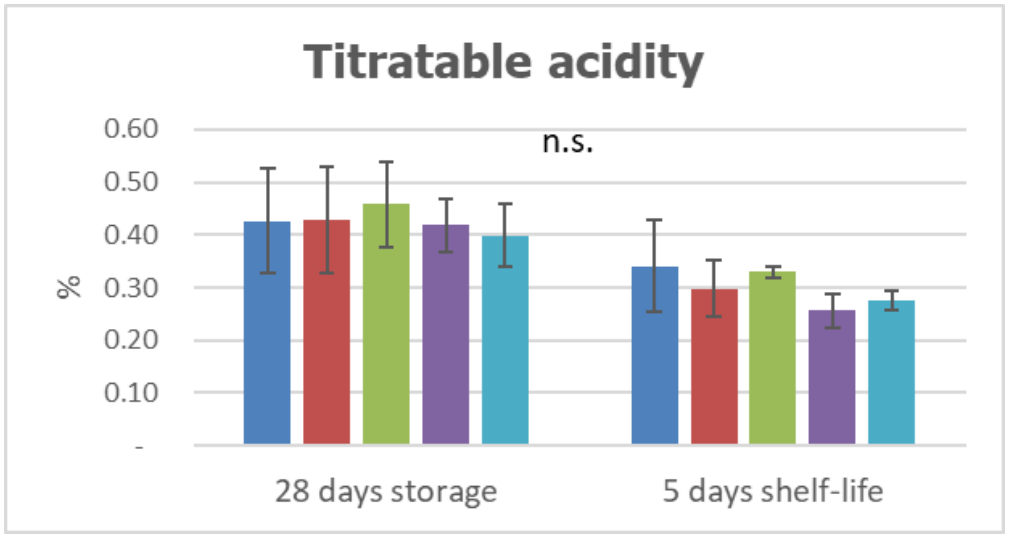
Quality analyses

- **Firmness**
- **Total Soluble Solids**
- **Titratable acidity**

EO vapors did not influence the overall quality of the nectarines, but showed slightly higher fruit firmness for treated fruits at the end of shelf-life



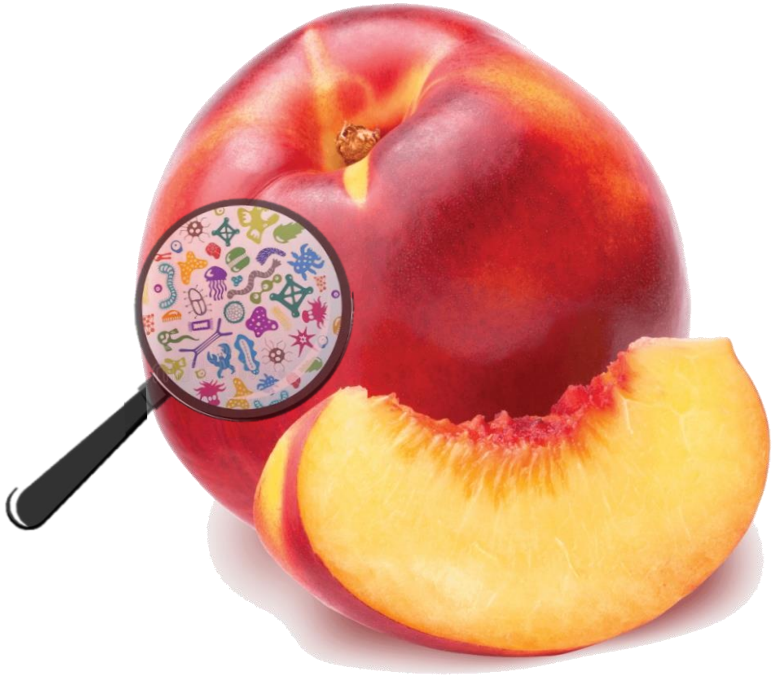




Healthy control fludioxonil EO lemon EO fennel EO basil

Microbiome analysis

Evaluation of the effect of treatments on the fruit microbiome



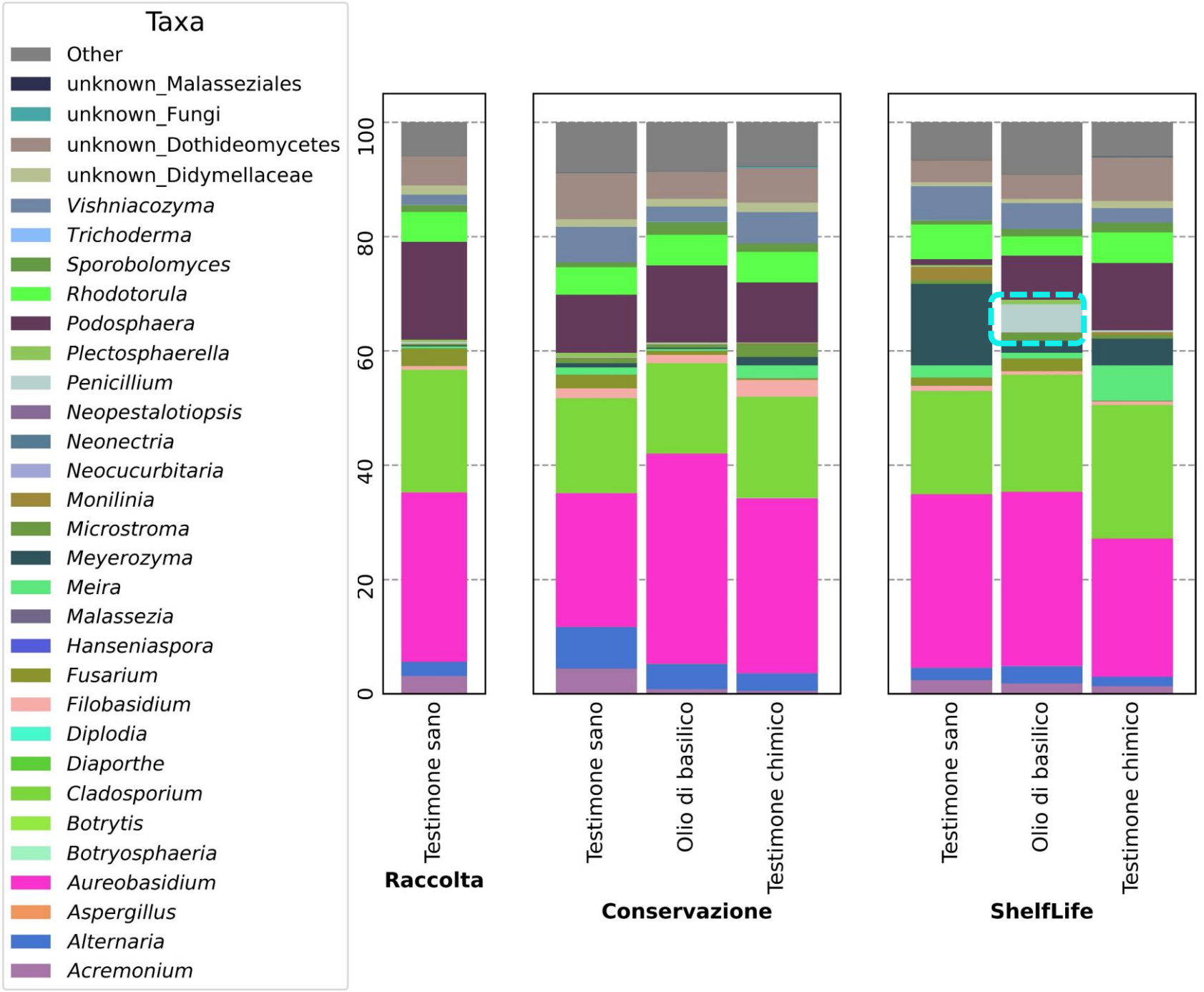
1. Sampling

Epiphytes and endophytes sampling:
- Untreated control
- Basil EO treatment
- Fludioxonil treatment

3 time-points:
- Harvest
- End of storage
- End of *shelf-life*

2. DNA extraction

3. Sequencing of ITS2 region



Epiphytes

The abundance of some fungal genera was found to be modified by fungicide and EO treatments compared to the control

Both treatments reduced the abundance of *Monilinia* spp., especially during shelf-life.

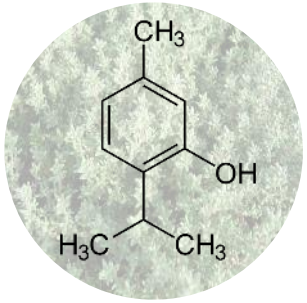
Basil EO treatment seems to favor the presence of *Penicillium* spp. during shelf-life.

Evaluation of the efficacy of 3 essential oils (EOs) and a thymol-based formulation against gray mould caused by *Botrytis cinerea* on stored apples

Thyme EO
(*Thymus vulgaris)*

Savoury EO
(Satureja montana)

Basil EO
(Ocimum basilicum subsp. *basilicum)*



Thymol-based formulation

In vitro biofumigation test

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In vivo evaluation of efficacy

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Characterization of VOCs released during storage

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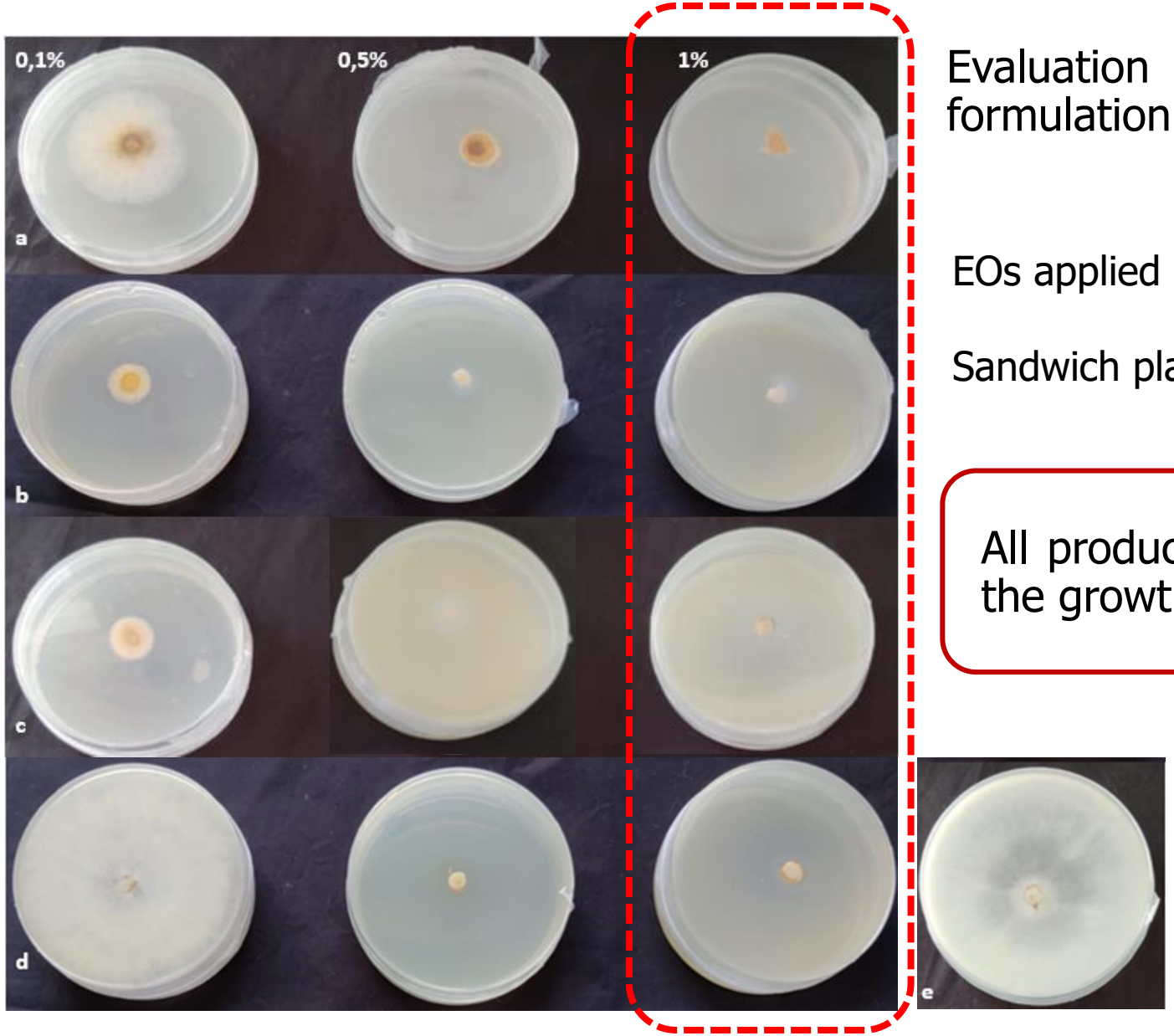
Microbiome analysis

In vitro biofumigation test



Evaluation of the effect of EOs and thymol-based formulation on the growth of 2 strains of *B. cinerea*

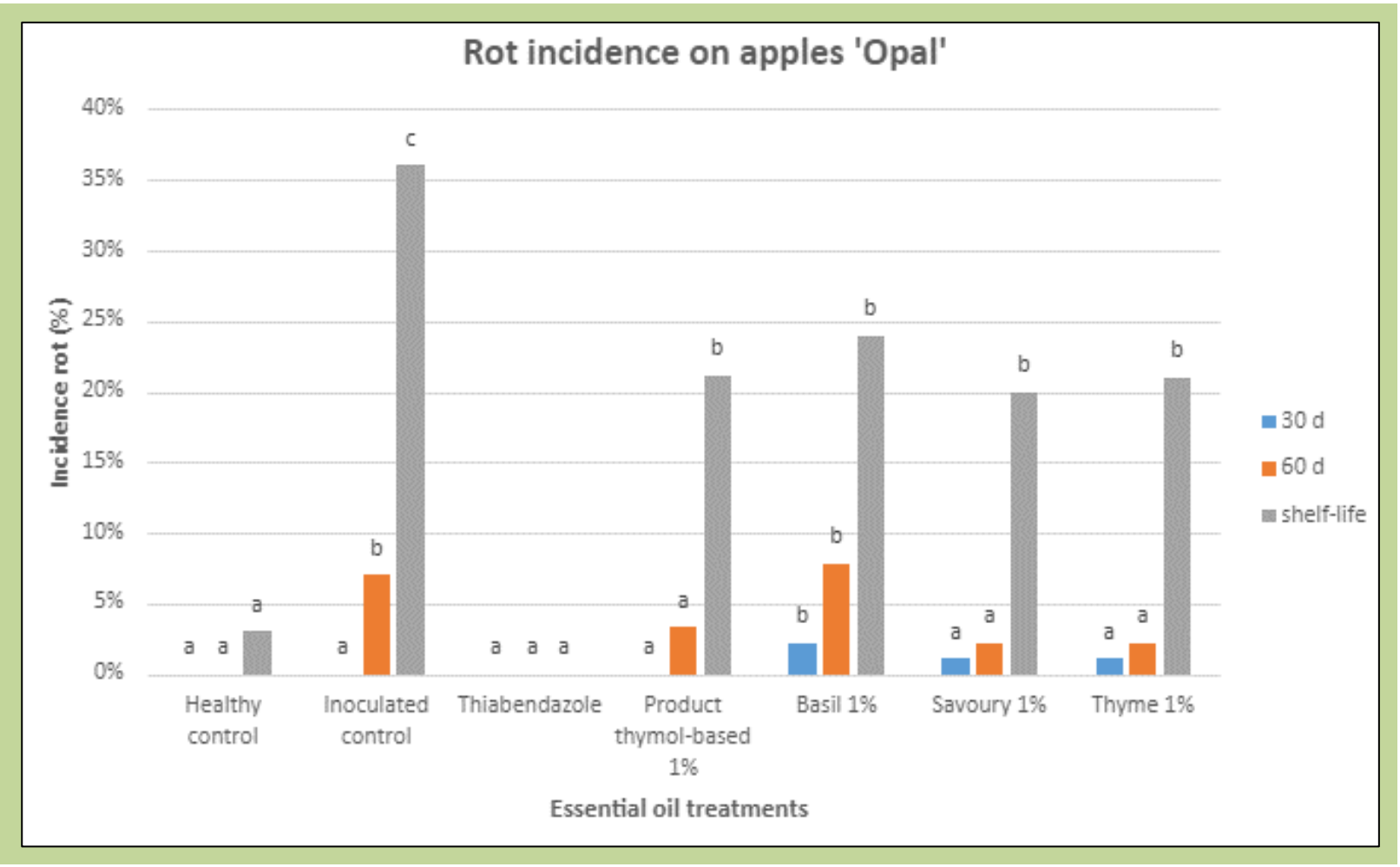
EOs applied at different concentrations (0,1%, 0,5%, 1%)

Sandwich plate technique

All products tested at 1% concentration inhibited the growth of *B. cinerea* strains.

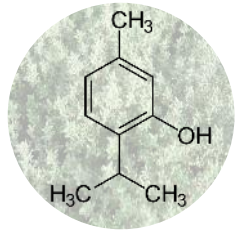
a. Thymol-based formulation 0,1 %, 0,5%, 1%
b. Thyme EO 0,1 %, 0,5%, 1%
c. Savoury EO 0,1 %, 0,5%, 1%
d. Basil EO 0,1 %, 0,5%, 1%
e. Control

In vivo evaluation of efficacy



Thyme EO

Savoury EO



Thymol-based formulation

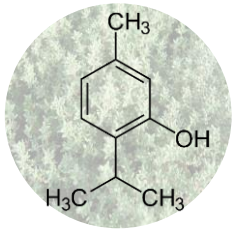
Effective in reducing rot incidence at 30 and 60 days of storage

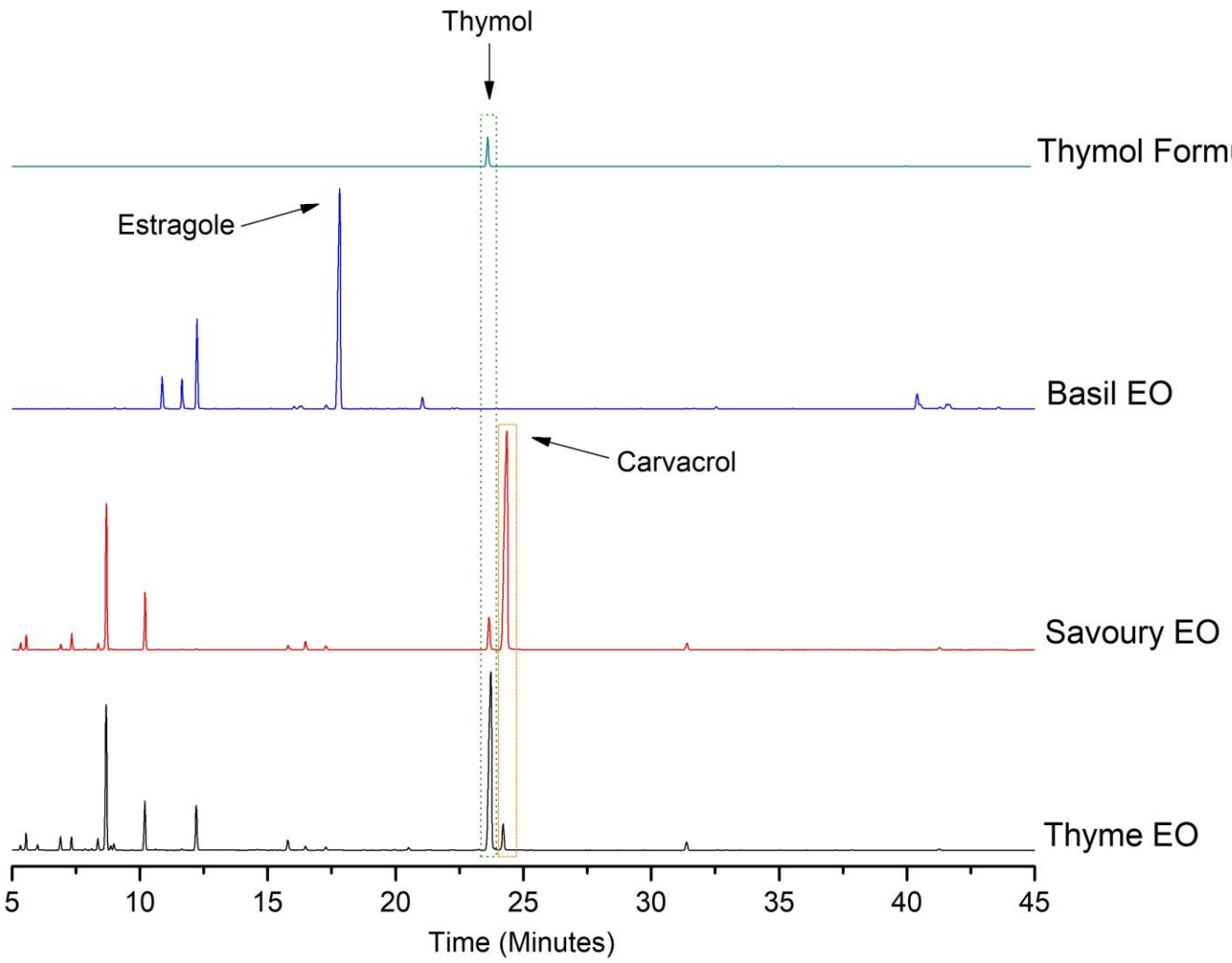
All the tested treatments did not significantly affect fruit quality

Schiavon et al., 2023, JoF

Characterization of VOCs released during storage

Composition analysis of EOs through GC-MS



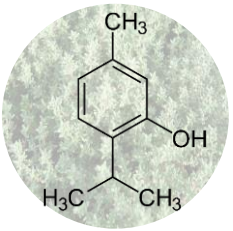


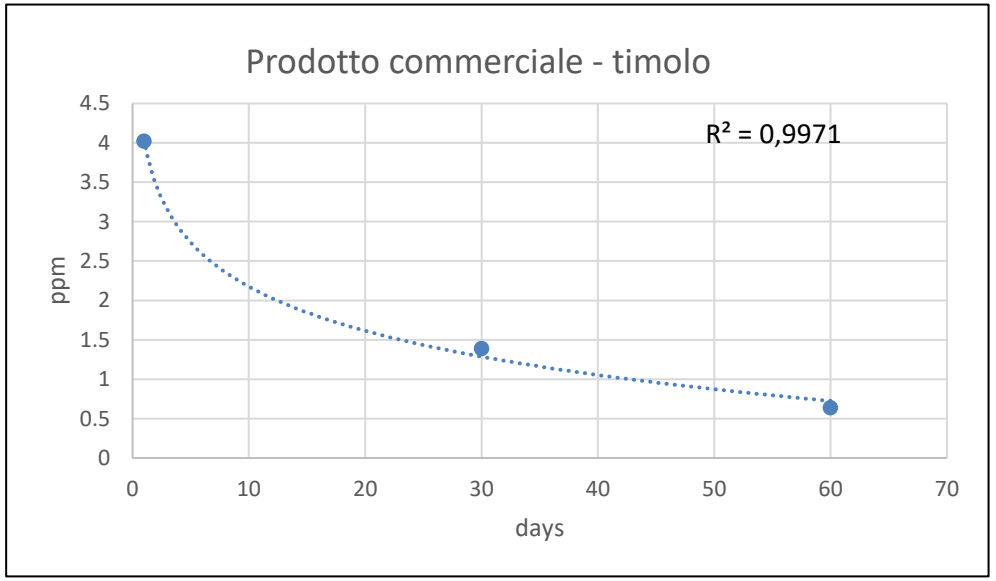
Characterization of volatile compounds released by EOs during storage inside the cabinets

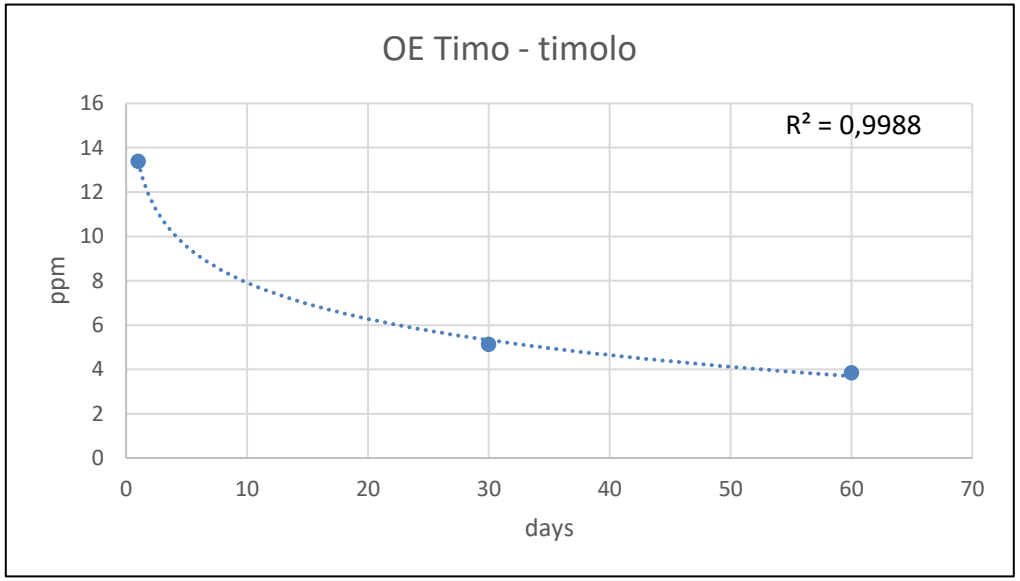
SPME-GC-MS analysis

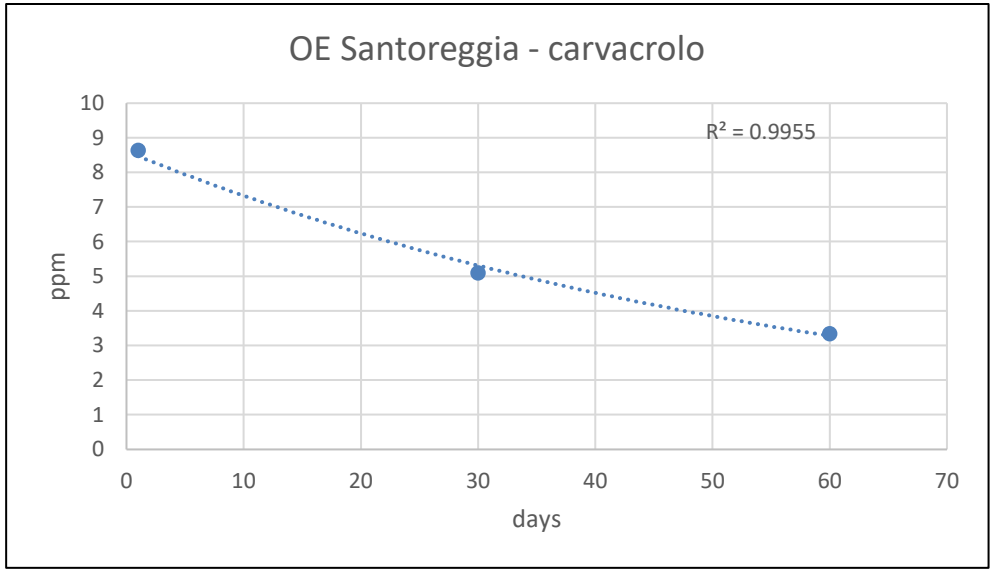
Schiavon et al., 2023, JoF

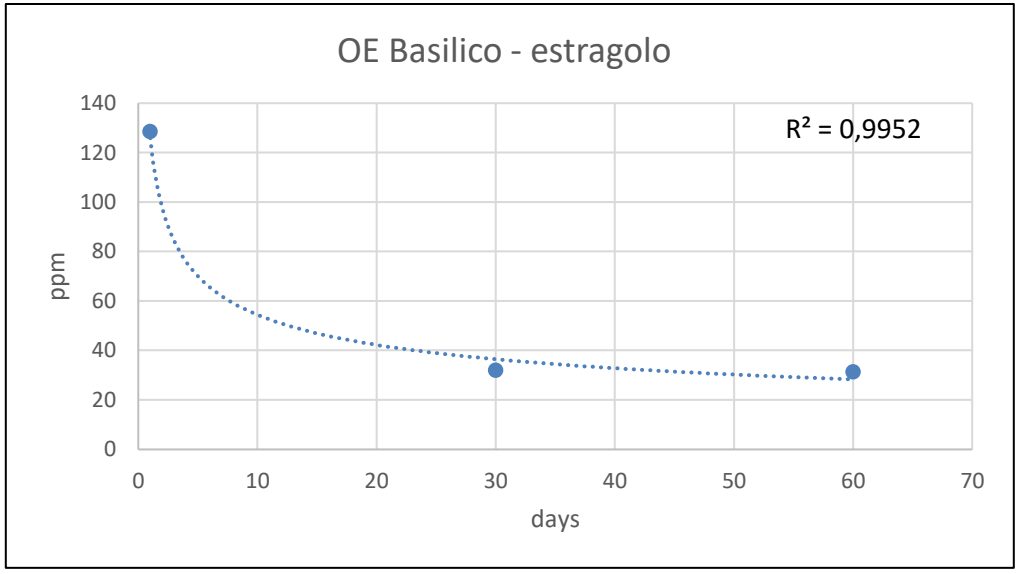
Characterization of VOCs released during storage











Microbiome analysis

Evaluation of the effect of treatments on the fruit microbiome

1. Sampling

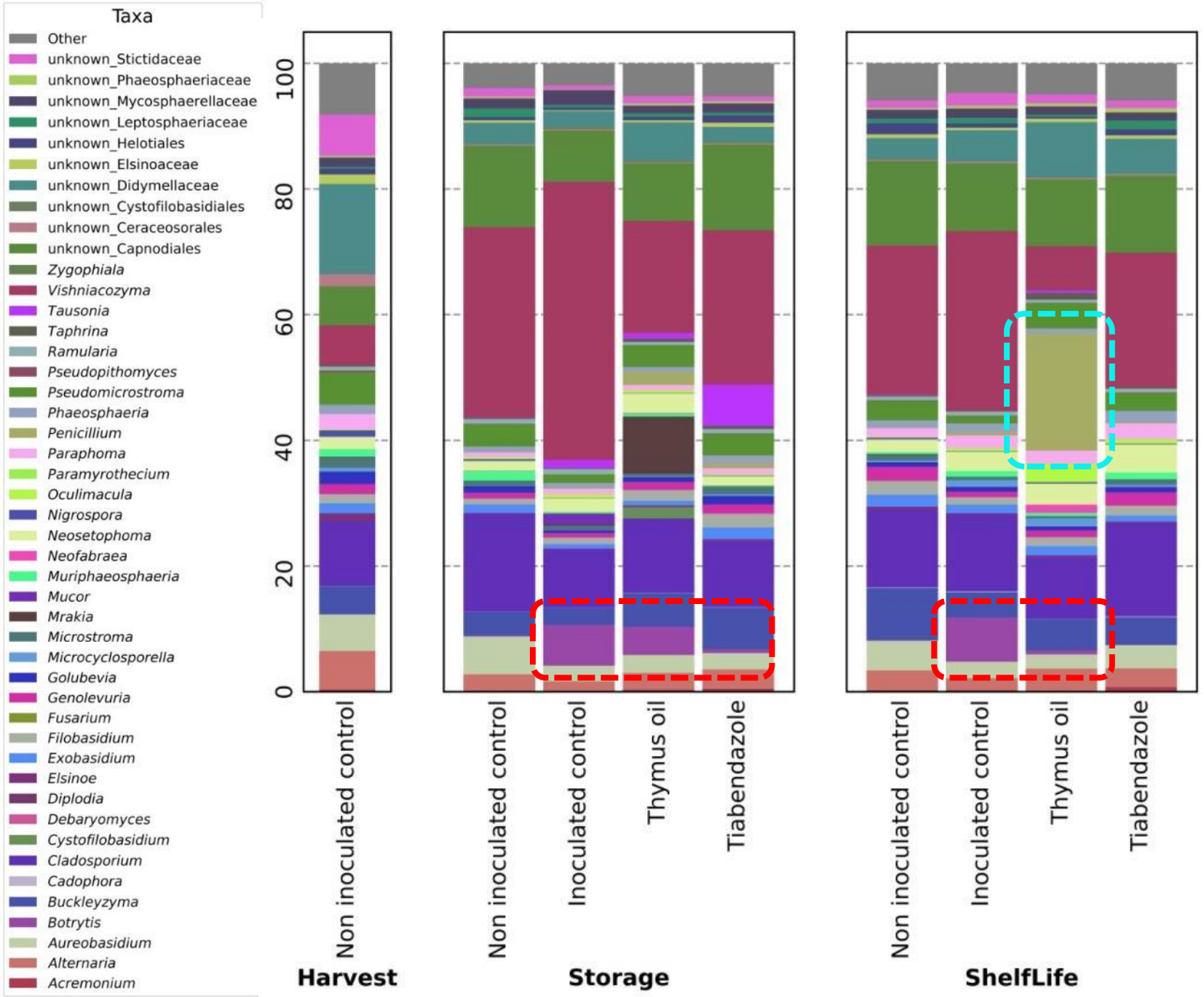
Epiphytes and endophytes sampling:
- Untreated control
- Inoculated control
- Thyme EO 1% treatment
- Thiabendazole treatment

3 time-points:
- Harvest
- End of storage
- End of *shelf-life*

2. DNA extraction

3. Sequencing of ITS2 region

Schiavon et al., 2023, JoF



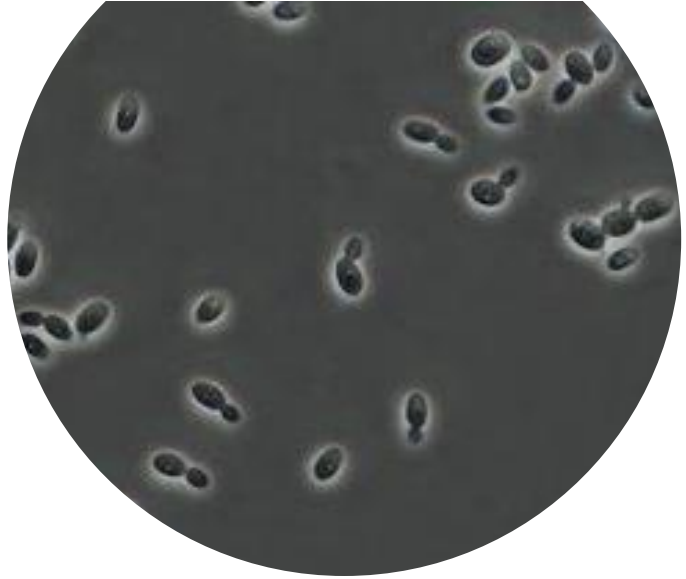
Epiphytes

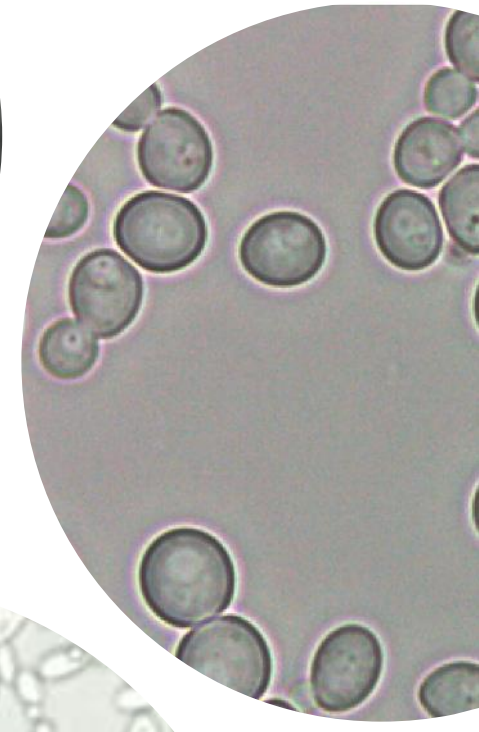
Thyme EO: reduction of ***Botrytis*** spp.

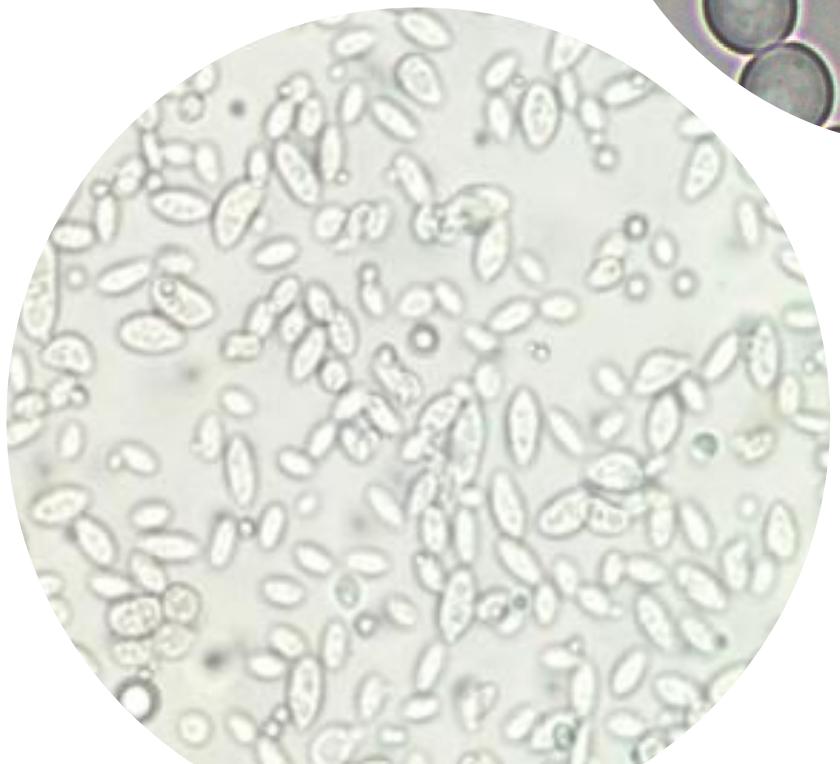
Increased abundance and ***Penicillium*** spp. in shelf-life

Biological control

Use of microorganisms to reduce the effects of undesirable organisms (pathogens or parasites)







Yeasts as Biological Control Agents (BCAs)

- Tolerant to extreme conditions (low T, desiccation, wide variations in RH, low oxygen, pH variations, UV radiation)
- Adapted to the fruit microenvironment (high sugar concentration, high osmotic pressure, low pH)
- Produced in high quantities in fermenters
- No production of allergens or mycotoxins
- Simple nutritional requirements to colonize host surface for long periods

Biological Control Agents (BCAs)

Evaluation of the efficacy of treatments with antagonistic yeasts on strawberries to control postharvest rots

Evaluation of the efficacy of treatments with antagonistic yeasts on nectarines to control brown rot caused by *Monilinia fructicola*

Evaluation of the efficacy of treatments with antagonistic yeasts on grape to control gray mould and evaluation of their bioprotection activity during winemaking

Evaluation of the efficacy of treatments with antagonistic yeasts on strawberries to control postharvest rots

Screening trials *in vivo* (2022)

TRIAL 1

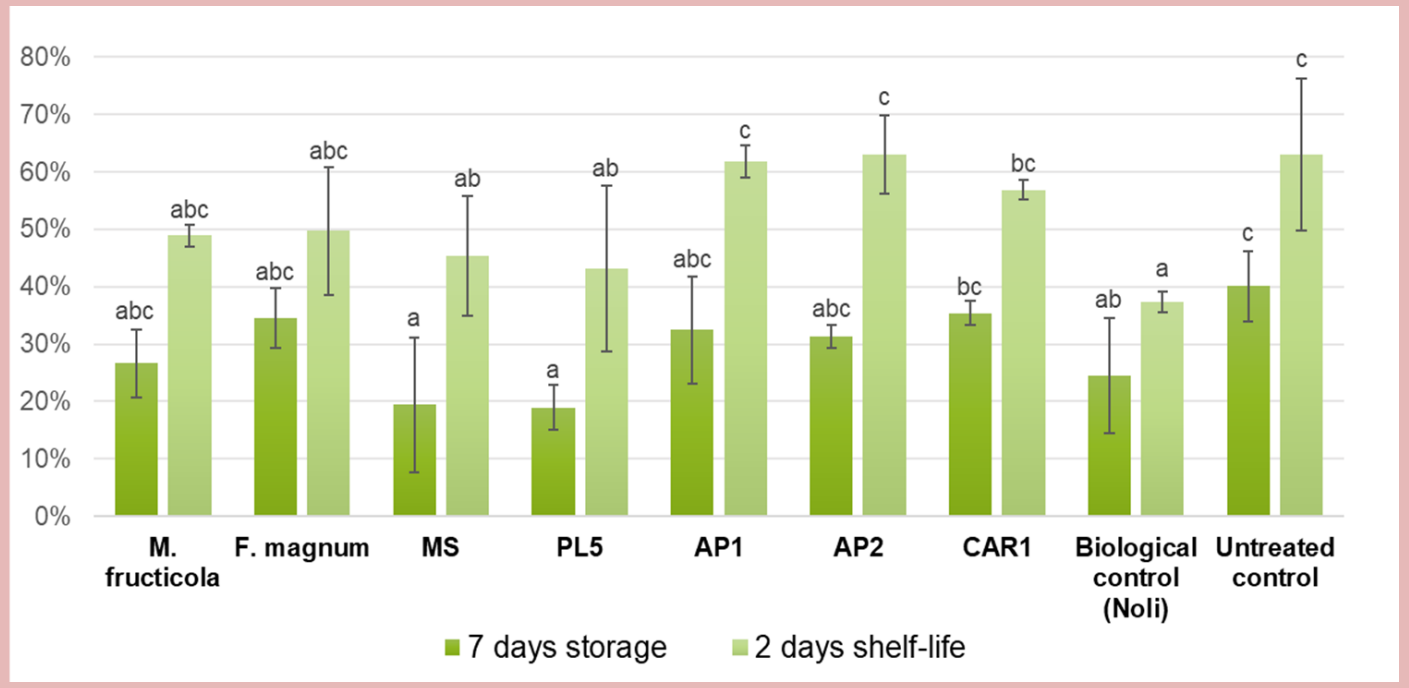
- 7 treatments with yeasts of the **collection of Turin University**
- 1 biological control, treated with **Noli** (Koppert, *Metschnikowia fructicola*)
- 1 untreated control

TRIAL 2

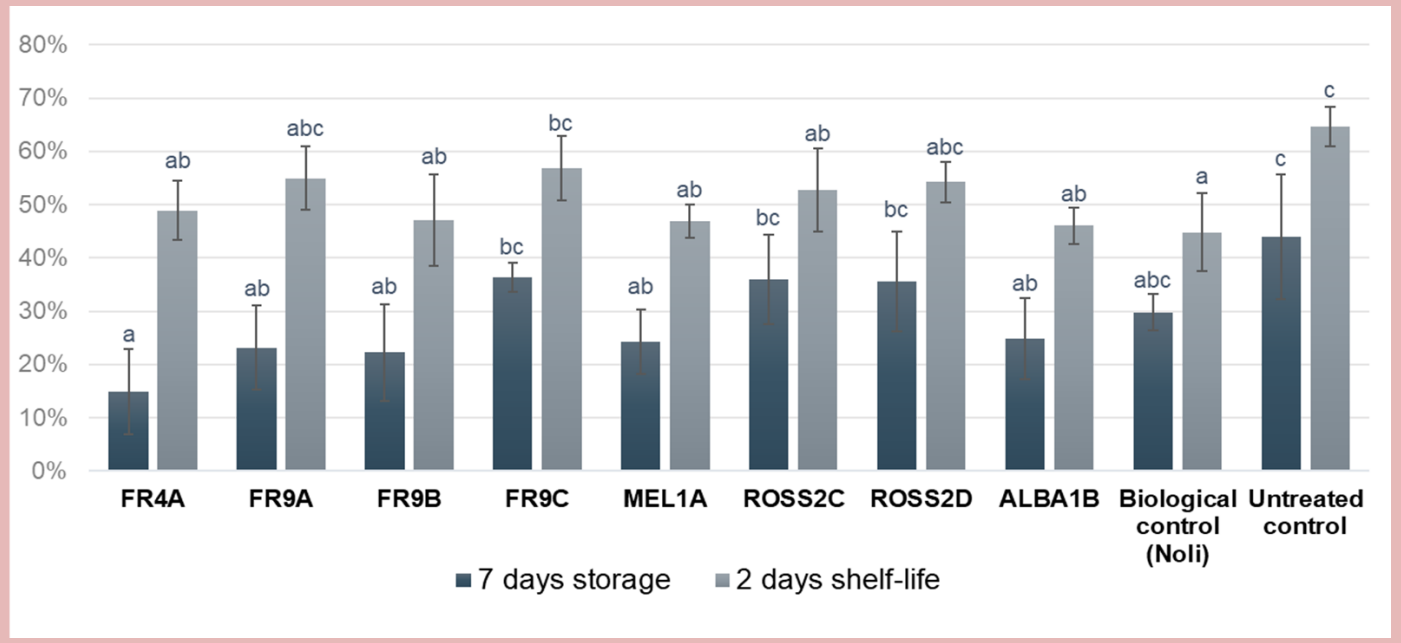
- 8 treatments with **endophytic yeasts** isolated from strawberries
- 1 biological control, treated with **Noli**
- 1 untreated control

Storage at 1 ± 1 °C for 7 days
Shelf-life at 24 ± 1 °C for 2 days

TRIAL 1



TRIAL 2



Selection of strains that showed the highest biocontrol efficacy

➡ Trial 1: MS and PL5

➡ Trial 2: FR4A and FR9B

Evaluation of the efficacy of treatments with antagonistic yeasts on strawberries to control postharvest rots

Efficacy test *in vivo* (2023)

- 4 treatments with the most effective yeasts in the screening test (**MS**, **PL5**, **FR4A**, **FR9B**)

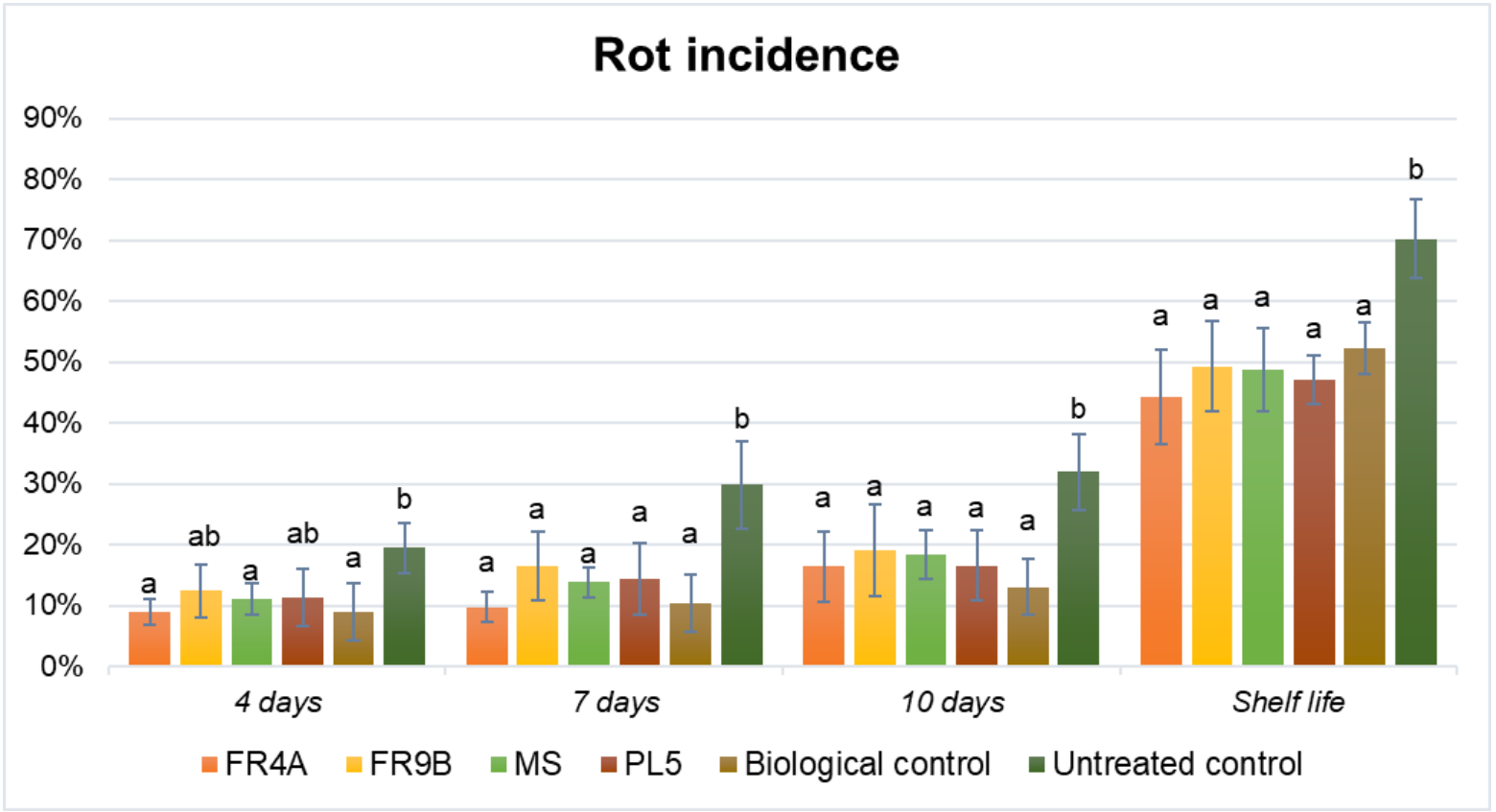
- 1 biological control, treated with **Noli** (Koppert, *Metschnikowia fructicola*)

- 1 untreated control

Storage at 1 ± 1 °C for 10 days

Shelf-life at 20 ± 1 °C for 2 days

Rot incidence



Values at the same time point, followed by the same letter, are not statistically different according to Tukey HSD test.

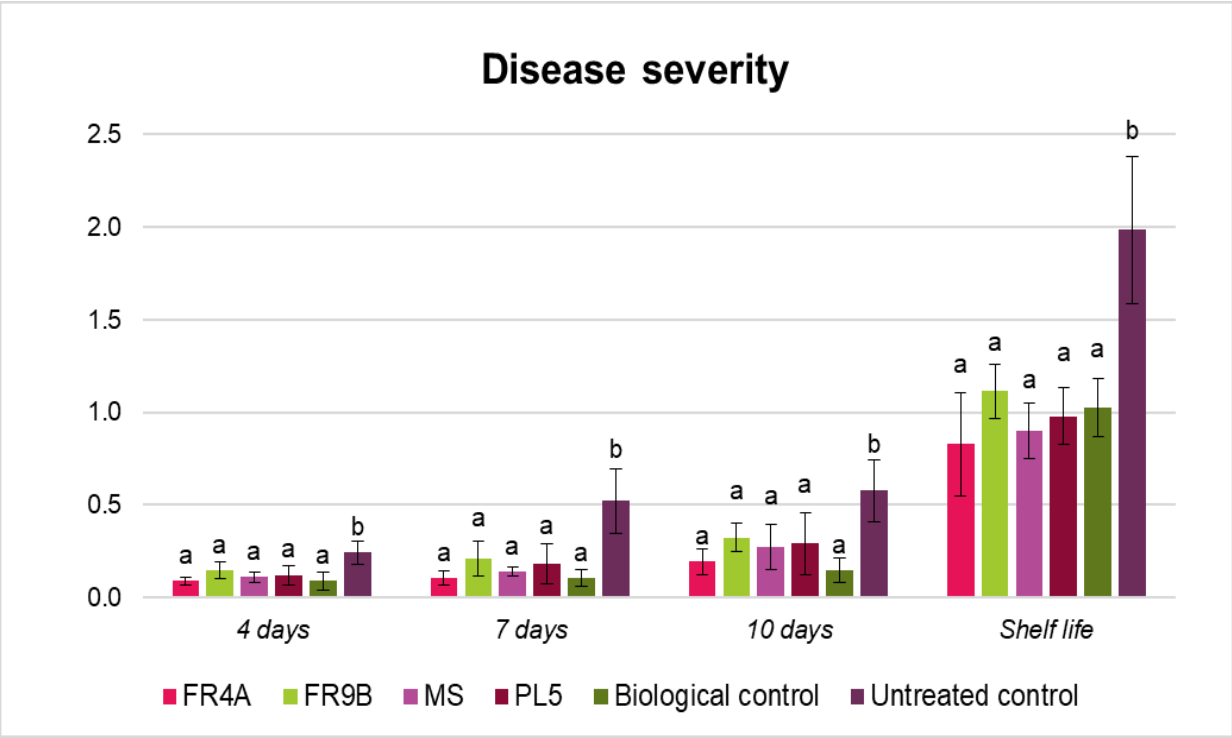
After **4 days of storage**

- FR4A and MS treatments showed a rot incidence significantly lower than untreated control
- Rot incidence for all treatments comparable to the biological control

After **7 and 10 days of storage** after **shelf-life**

All treatments showed a rot incidence significantly lower than untreated control, comparable to the biological control

Disease severity



0= healthy fruit
1= 1% – 25% fruit surface infected
2= 25% – 50% fruit surface infected
3= 50% – 75% fruit surface infected
4= > 75% fruit surface infected

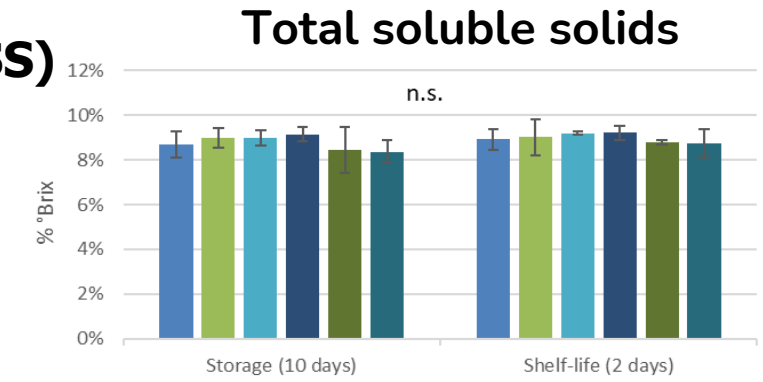
Disease severity of all treatments was significantly lower than that of the untreated control at all time points.

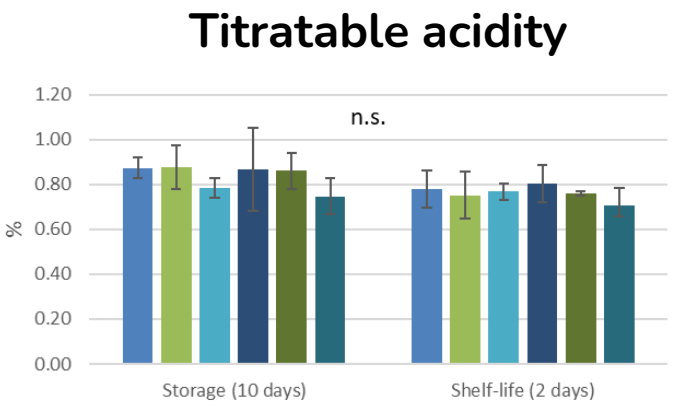
Values at the same time point, followed by the same letter, are not statistically different according to Tukey HSD test.

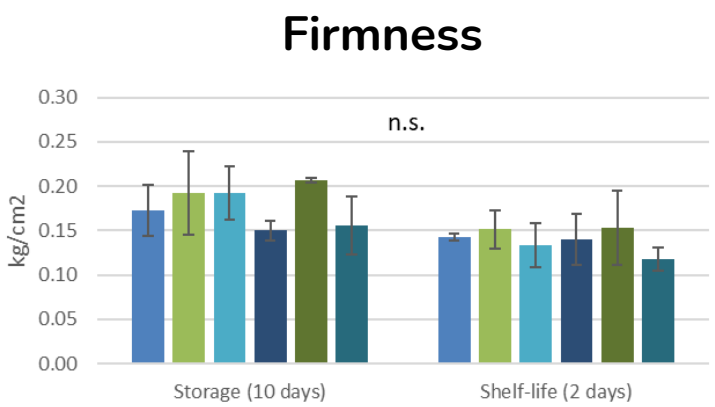
Quality analyses

- **Firmness**
- **Total Soluble Solids (TSS)**
- **Titratable acidity**

All the tested treatments did not significantly affect fruit quality







MS FR4A FR9B PL5 Biological control (Noli) Untreated control

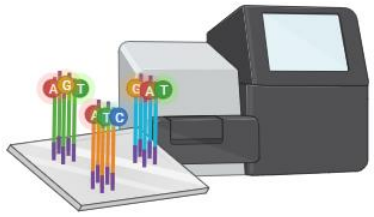
Values at the same time point, followed by the same letter, are not statistically different according to Tukey HSD test

Microbiome analyses

Evaluation of the effect of treatments on the fruit microbiome

Microbiome sampling

DNA extraction



Sequencing

Evaluation of the efficacy of treatments with antagonistic yeasts on nectarines to control brown rot caused by *Monilinia fructicola*

Screening test *in vivo*

⬇

Screening test *in vitro*

⬇

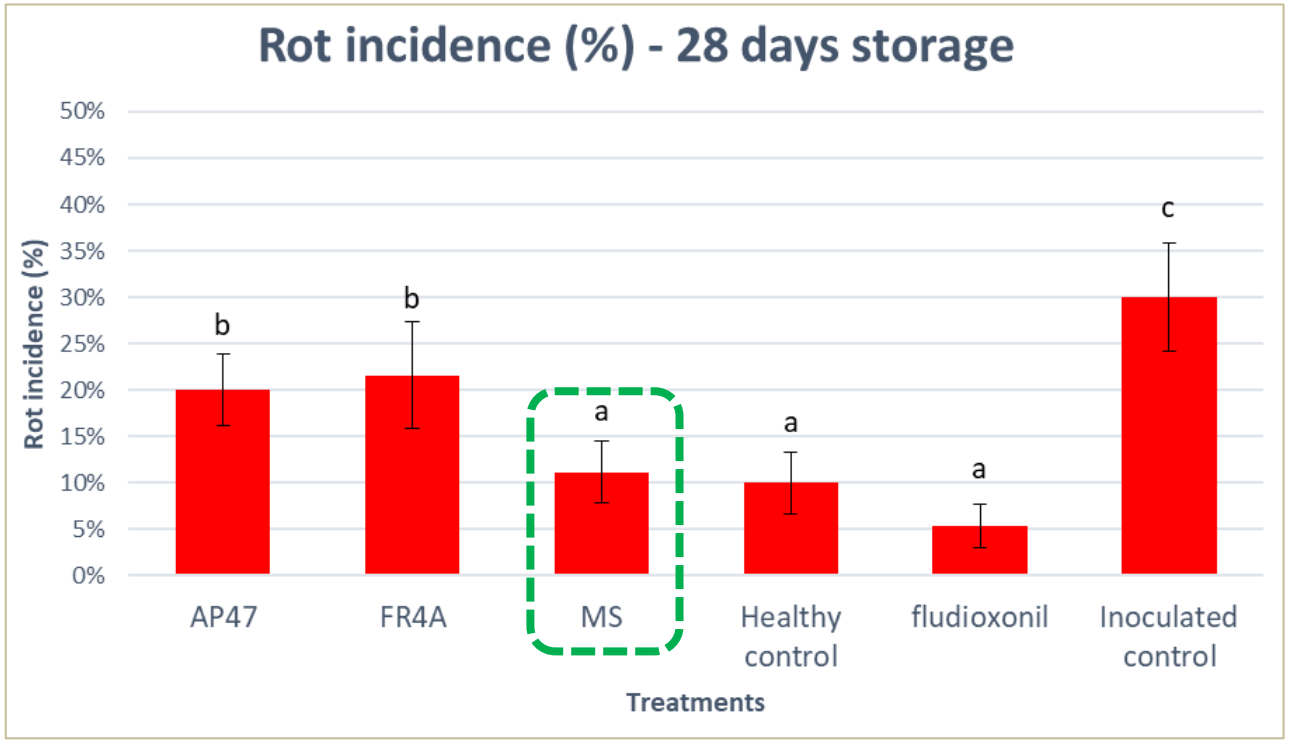
Efficacy test *in vivo*

⬇

Quality analyses

⬇

Microbiome analysis



After **28 days of storage**, rot incidence for all treatments was significantly lower than for inoculated control

MS treatment was the most effective

Evaluation of the efficacy of treatments with antagonistic yeasts on grape to control gray mould and evaluation of their bioprotection activity during winemaking

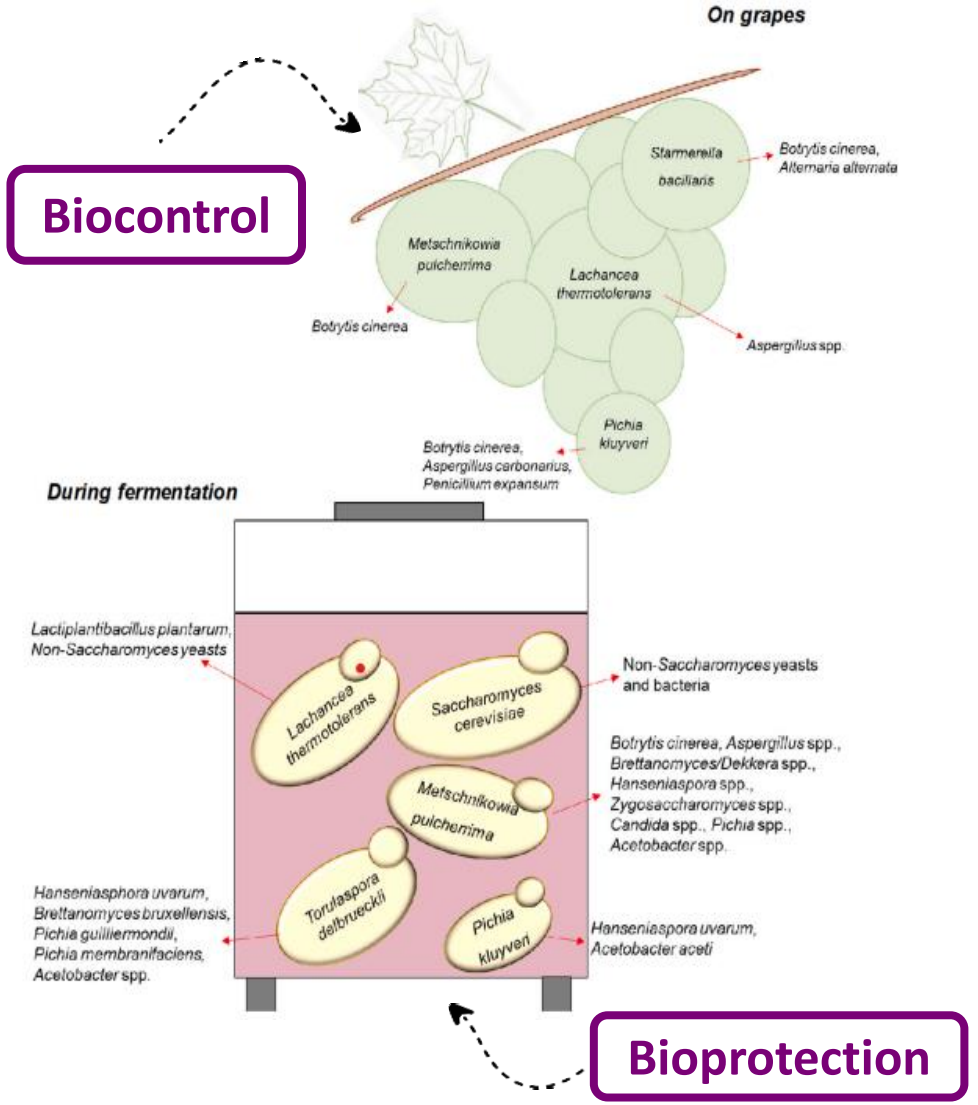
AIMS OF THE WORK

- ✓ Evaluation of the effectiveness of treatments with **antagonistic yeasts** on grape to control gray mould caused by *Botrytis cinerea*

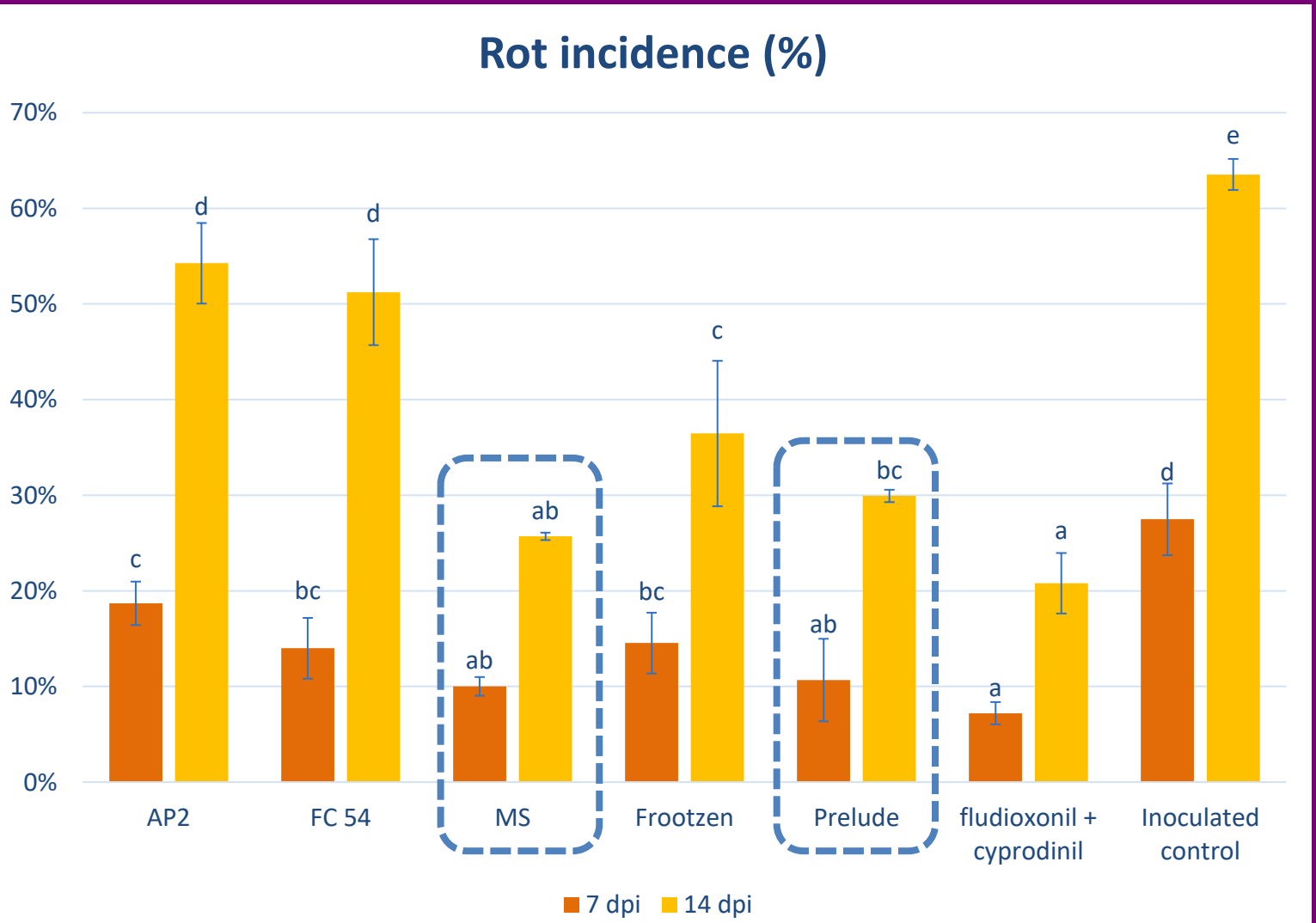
  First year (2020):
  biological control assay

  Second year (2021):
  biological control assay +
  effect on the grape microbiome

- ✓ Evaluation of their **bioprotection activity** in winemaking, to inhibit the development of undesired microorganism.



Evaluation of disease incidence



First survey

Rot incidence for all treatments statistically different from inoculated control

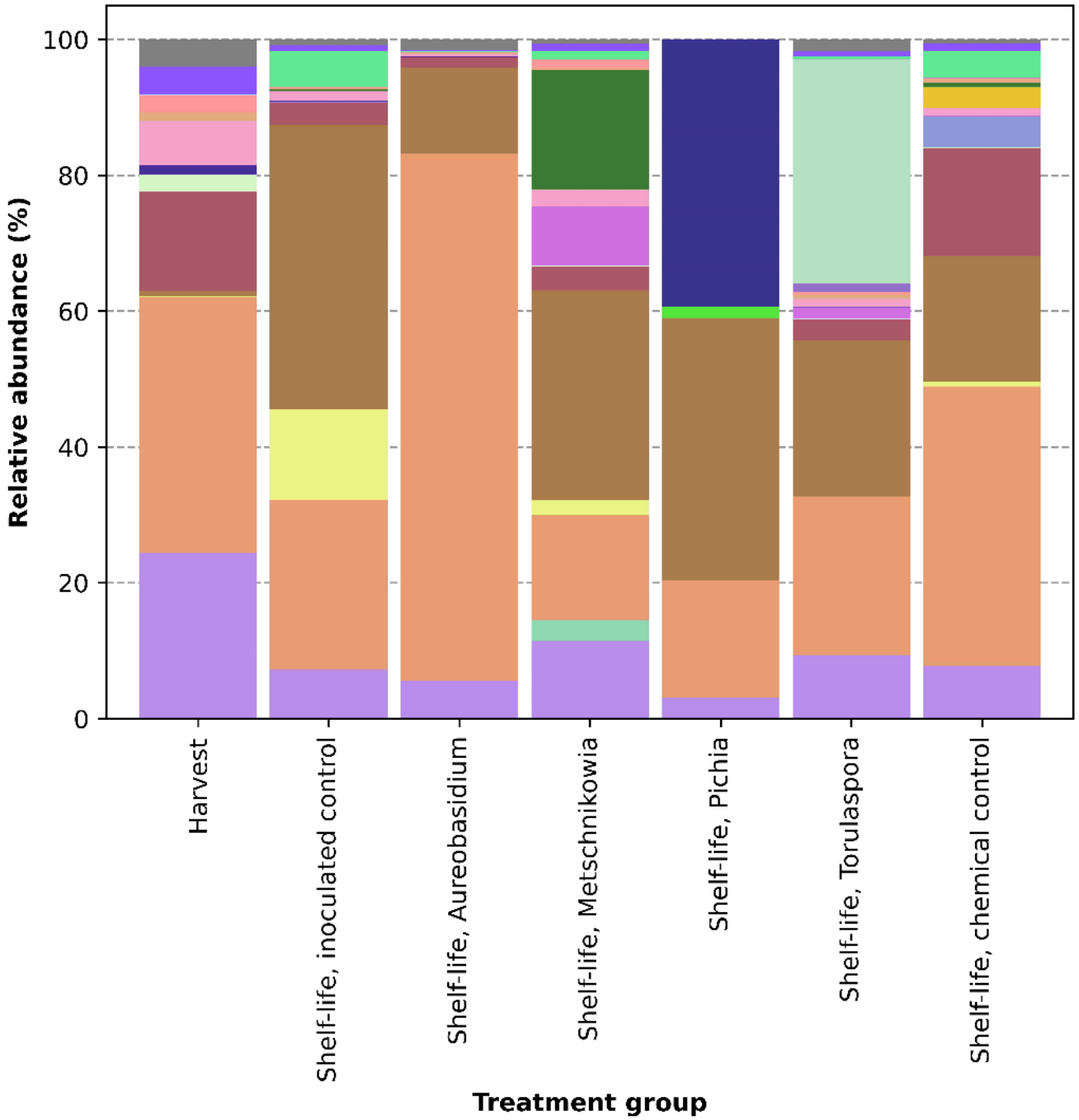
MS (*Metschnikowia pulcherrima*) and Prelude (*Torulaspora delbruekii*) treatments comparable to chemical control.

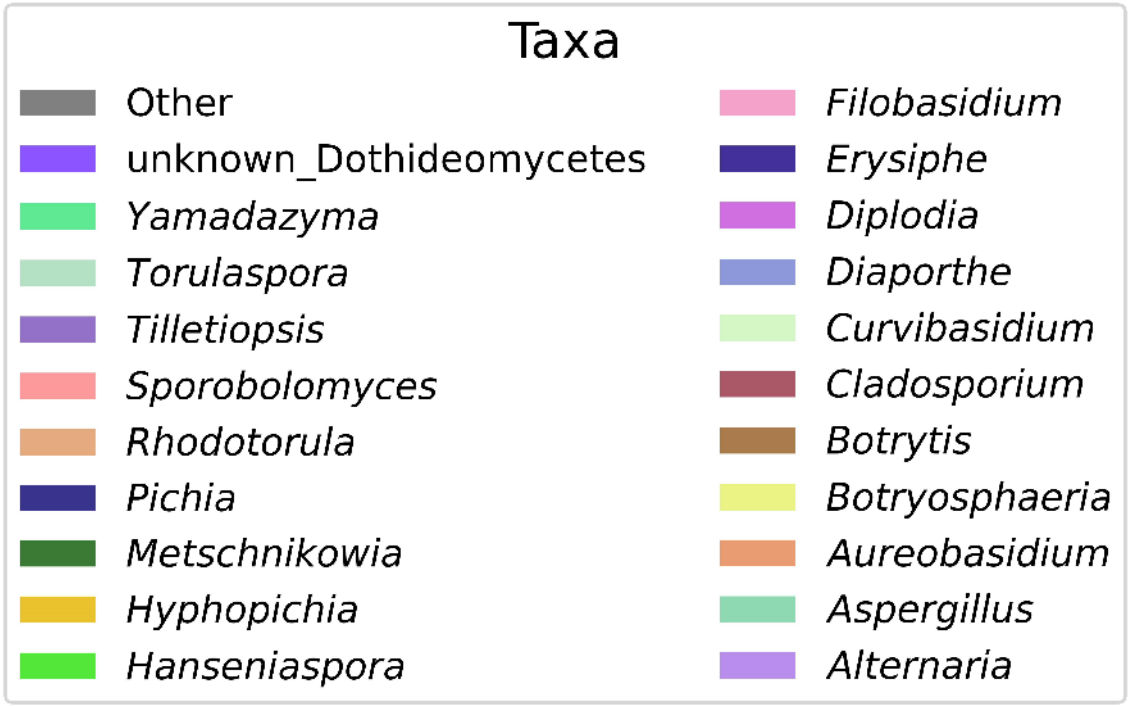
Final survey

Rot incidence for all treatments statistically different from inoculated control

MS treatment comparable to chemical control.

Compositional analysis



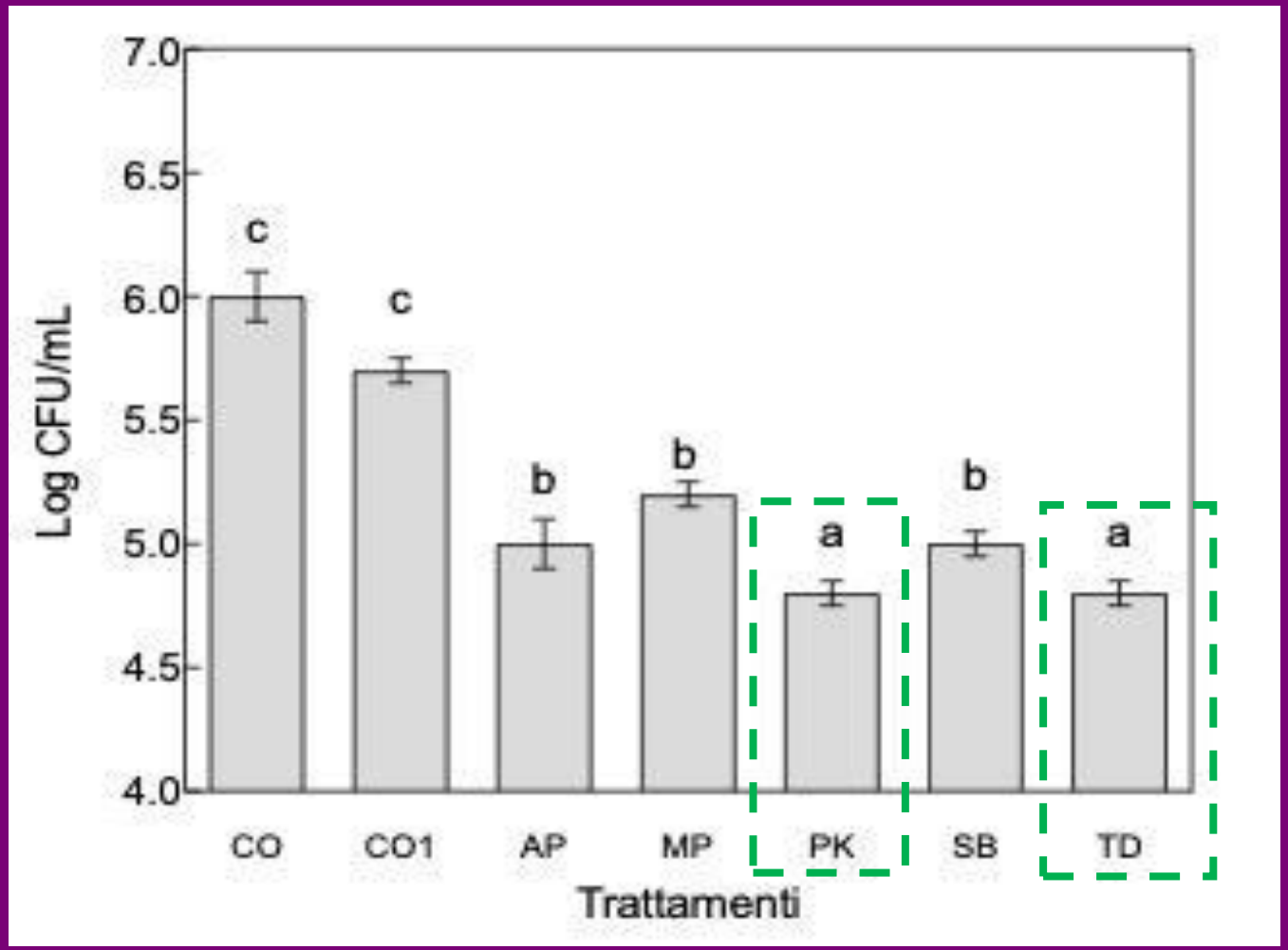


Good development of all biocontrol agents

Development of ***Botrytis*** spp. (inoculated)

Presence of *Alternaria* and *Aureobasidium* in all samples

Population of *Hanseniaspora uvarum* on grape



Determination of the population of *H. uvarum* 2 days after application of treatments

All treatments had a positive effect in reducing the population of *H. uvarum*

The lowest levels of *H. uvarum* were found in samples treated with:

Pichia kluyveri FrootZen (PK)

Torulaspora delbruekii Prelude (TD)

Take-home message

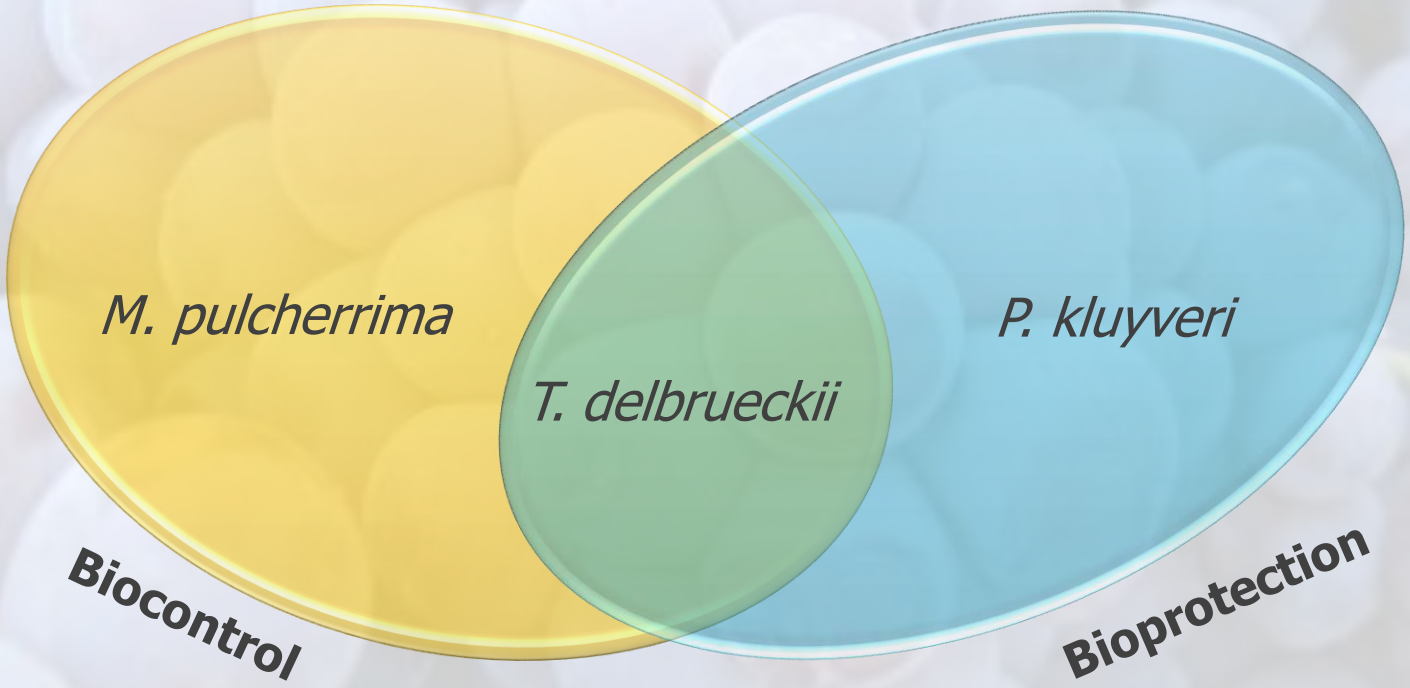
Double selection:

1. Biocontrol

M. pulcherrima and *T. delbrueckii*

2. Bioprotection

T. delbrueckii and *P. kluyveri*



Acknowledgements

UNIVERSITÀ DI TORINO

DISAFA
Dipartimento Scienze Agrarie, Forestali e Alimentari

PRIMA
PARTNERSHIP FOR RESEARCH AND INNOVATION IN THE MEDITERRANEAN AREA

STOP MED WASTE

BasicS
Euphresco Project

Euphresco

Vladimiro Guarnaccia

Fabio Buonsenso
Marco Garello
Giovanna Roberta Meloni
Simona Prencipe
Giulia Remolif
Giada Schiavon
Vasileios Englezos