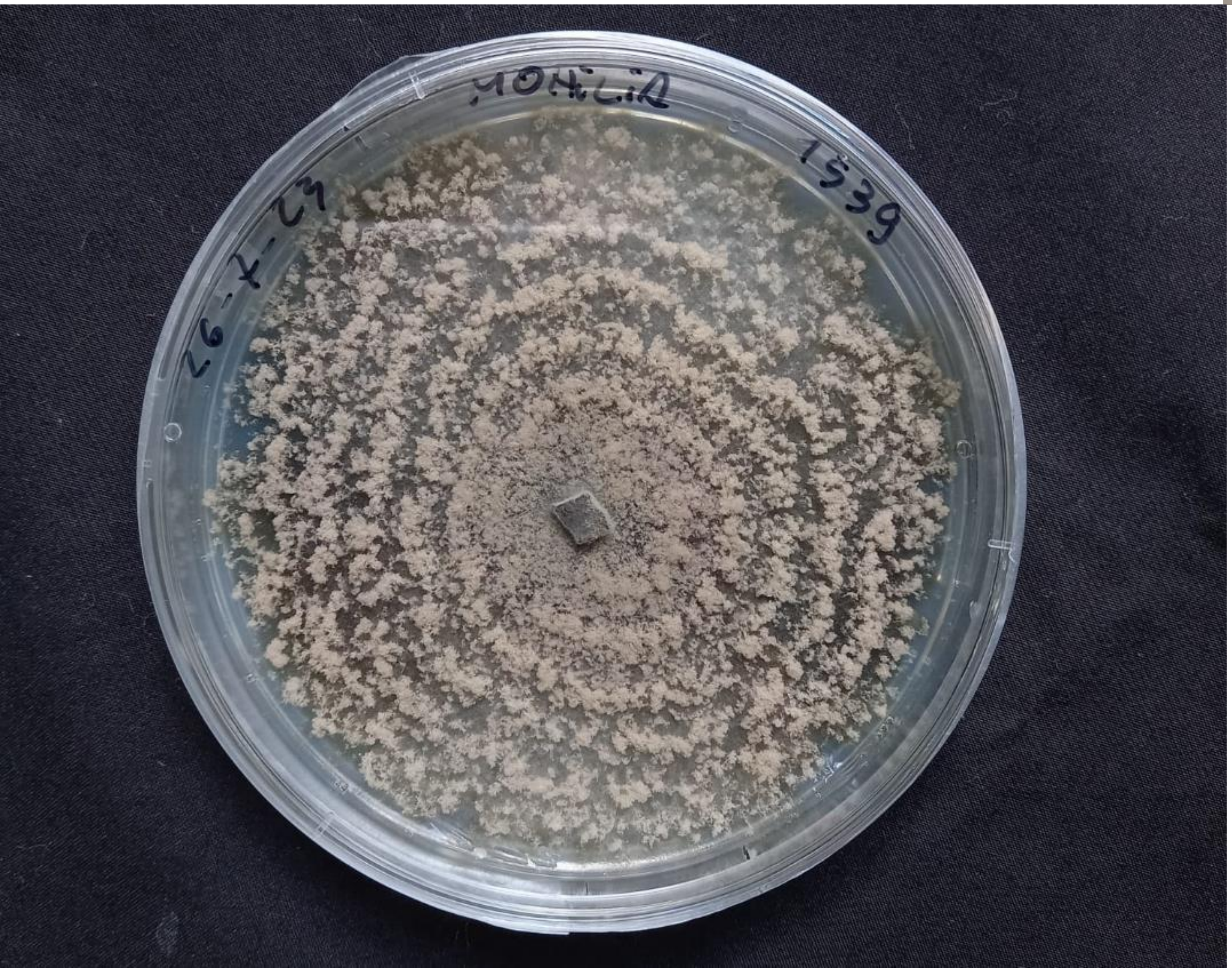
Efficacy of antagonistic yeasts to control brown rot of nectarines and effect on the fruit microbiome

Giulia Remolif, Marco Garello, Davide Spadaro

Dept. Agricultural, Forest and Food Sciences
University of Turin

Brown rot of peaches and nectarines

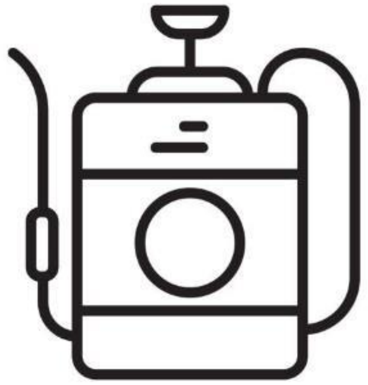


- One of the main diseases affecting peaches and nectarines
- Important production losses, especially in post-harvest

Causal agents

Monilinia fructicola, *M. laxa*, *M. fructigena*

Disease management



Preventive treatments with synthetic fungicides

- Presence of residues on the fruits
- Environmental pollution
- Fungicide-resistant strains

Farm-To-Fork Strategy

- 50% use of synthetic pesticides by 2030

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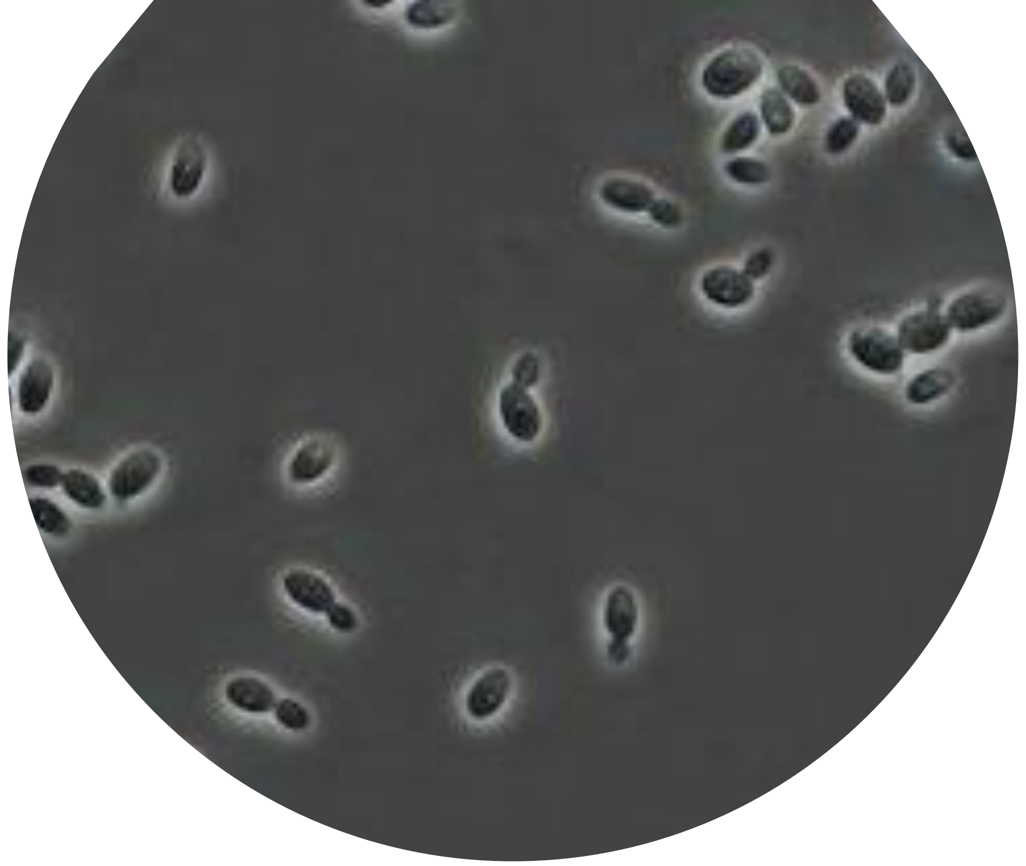
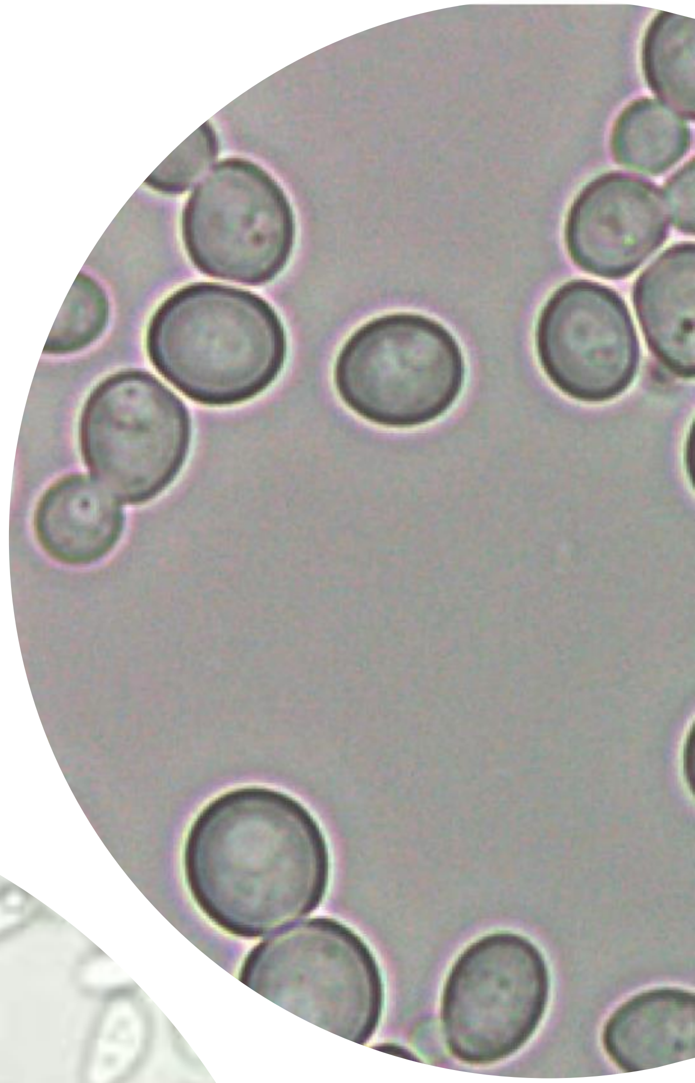
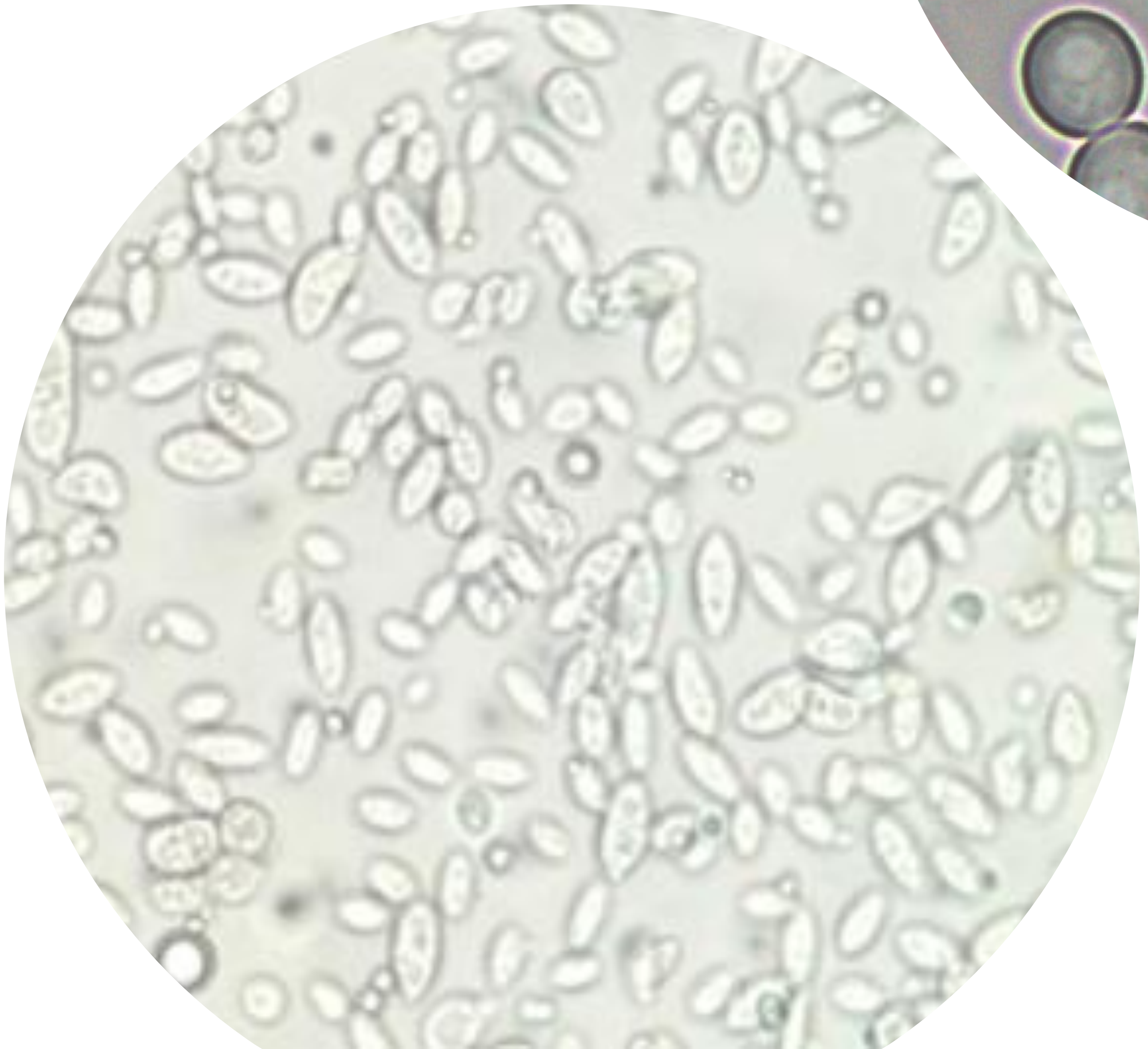
Need for alternative solutions

Yeasts as Biological Control Agents (BCAs)

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







AIMS OF THE WORK

- ✓ Evaluation of the effectiveness of treatments with antagonistic yeasts on nectarines to control brown rot caused by *Monilinia fructicola*
- ✓ Evaluation of the effect of the treatments on the fruit quality and on the fruit microbiome

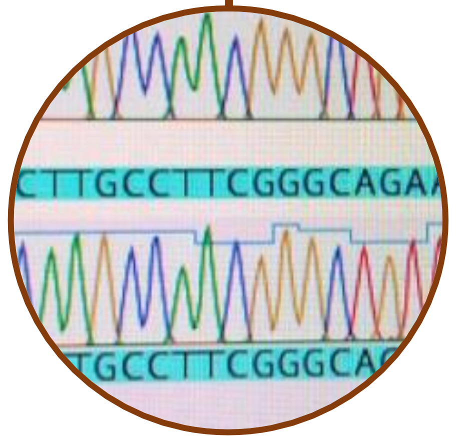
EXPERIMENTAL SETUP

Screening tests *in vivo* and *in vitro*

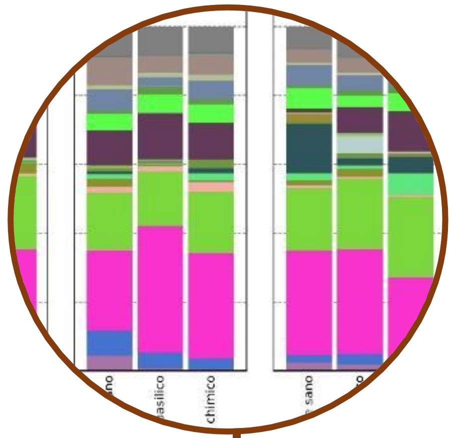
Efficacy test *in vivo*

Quality analyses

Identification of the most effective BCAs



Microbiome analysis

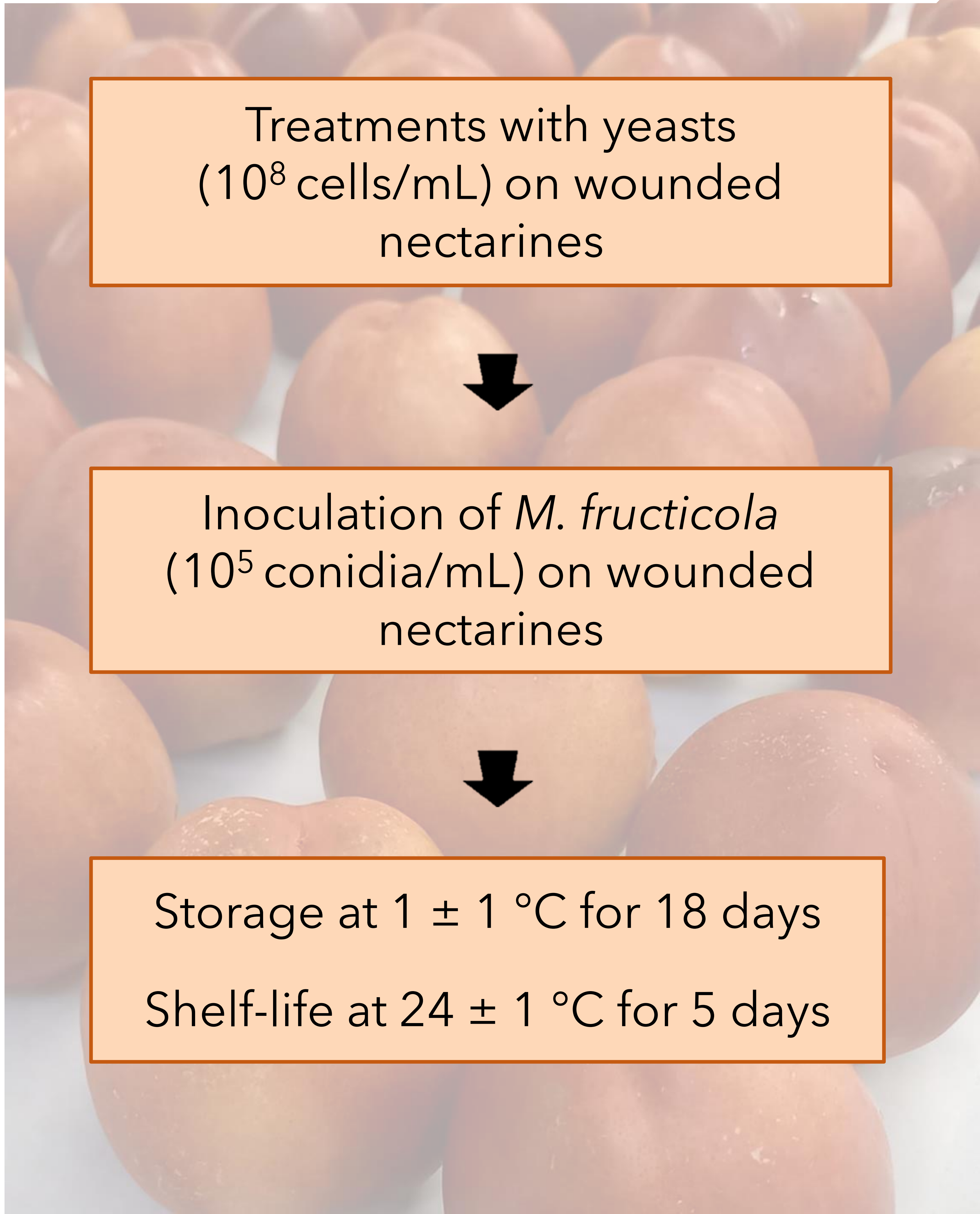


Screening test *in vivo*

Evaluation of the efficacy of treatments with **14 yeast strains** to control brown rot

17 treatments:

- **14 treatments** with the cell suspensions of the yeasts
- **Chemical control** (fludioxonil)
- **Inoculated control**
- **Healthy control**



Treatments with yeasts (10^8 cells/mL) on wounded nectarines

↓

Inoculation of *M. fructicola* (10^5 conidia/mL) on wounded nectarines

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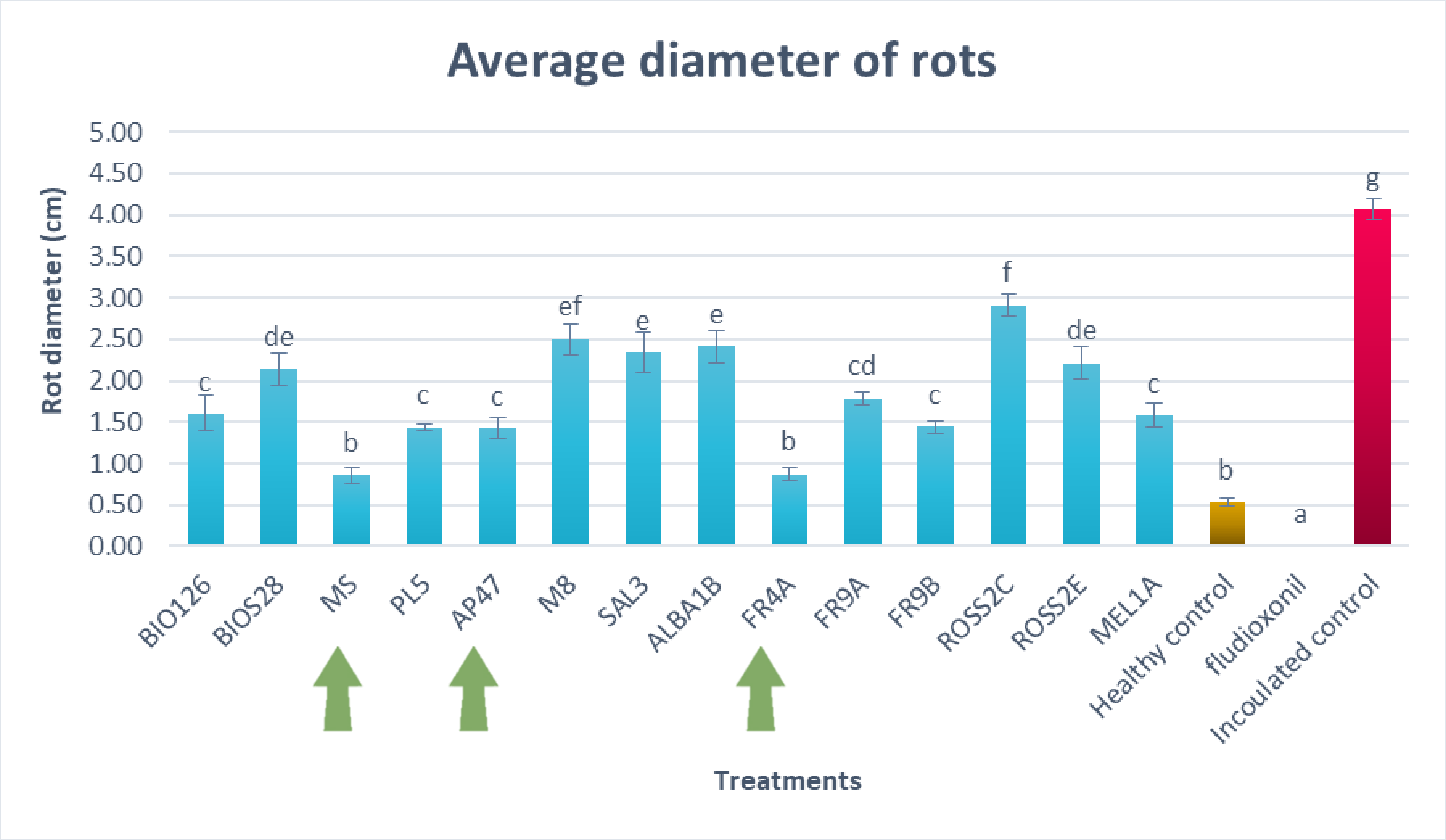
Storage at 1 ± 1 °C for 18 days

Shelf-life at 24 ± 1 °C for 5 days



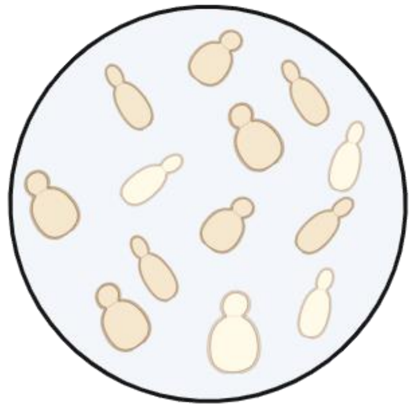
The average diameter of rots developed was **significantly lower** compared to the inoculated control **for all the tested yeasts**

3 strains selected for the second test:

- MS
- FR4A
- AP47

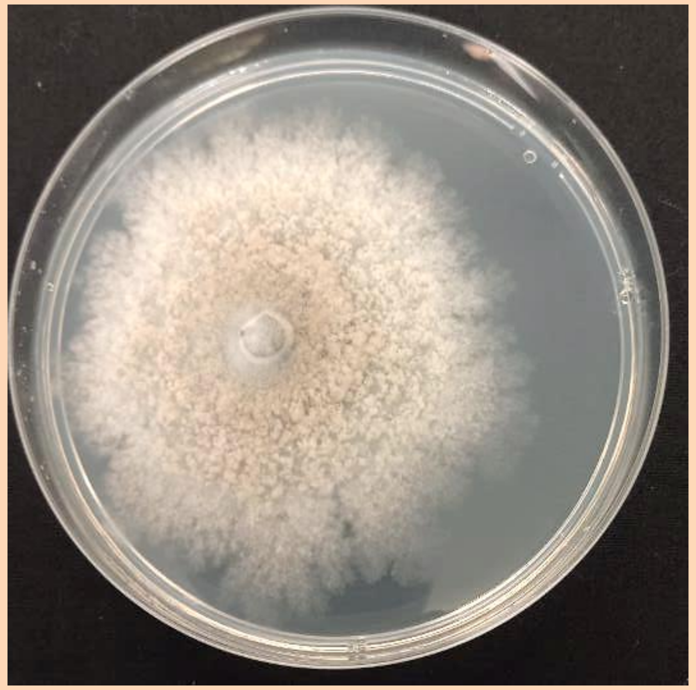
Screening test *in vitro*

Dual culture assays

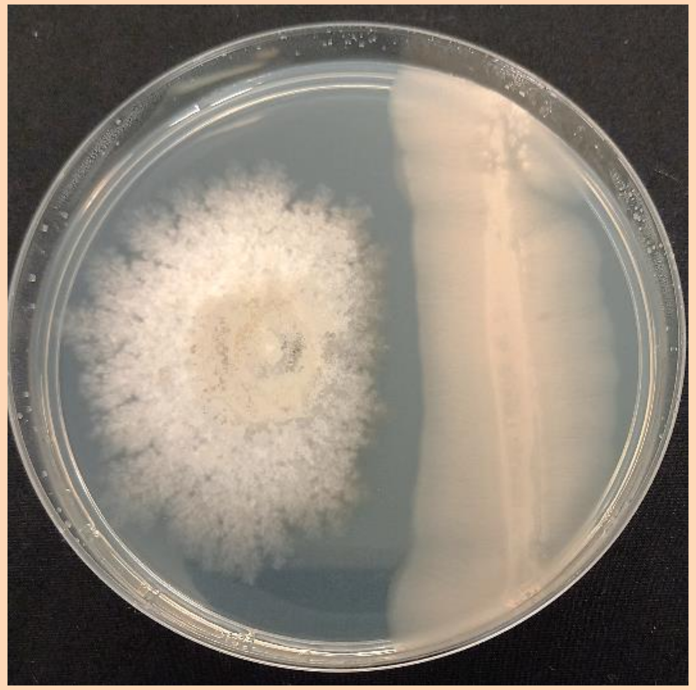


- AP47
- FR4A
- MS

Yeast strain	Mycelial inhibition (%)
AP47	39.2 ± 6.0 a
FR4A	48.5 ± 1.6 b
MS	44.4 ± 5.5 ab

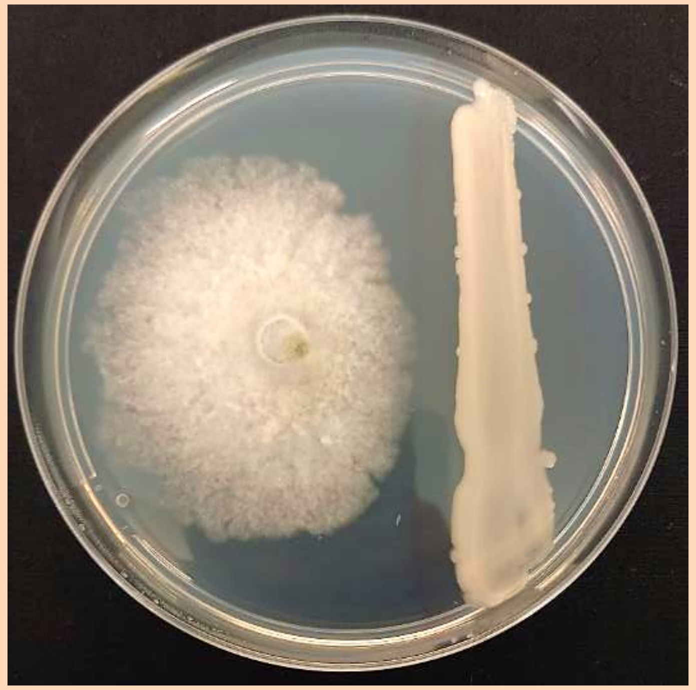


Control



FR4A

AP47



MS

Efficacy test *in vivo*

Evaluation of the efficacy of the most effective treatments in the screening test to control brown rot in semi-commercial conditions

6 treatments:

- **3 treatments** with the yeast cell suspensions
- **Chemical control** (fludioxonil)
- **Inoculated control**
- **Healthy control**

Inoculation of *Monilinia fructicola* by dipping in the conidial suspension (10^4 conidia/mL)

↓

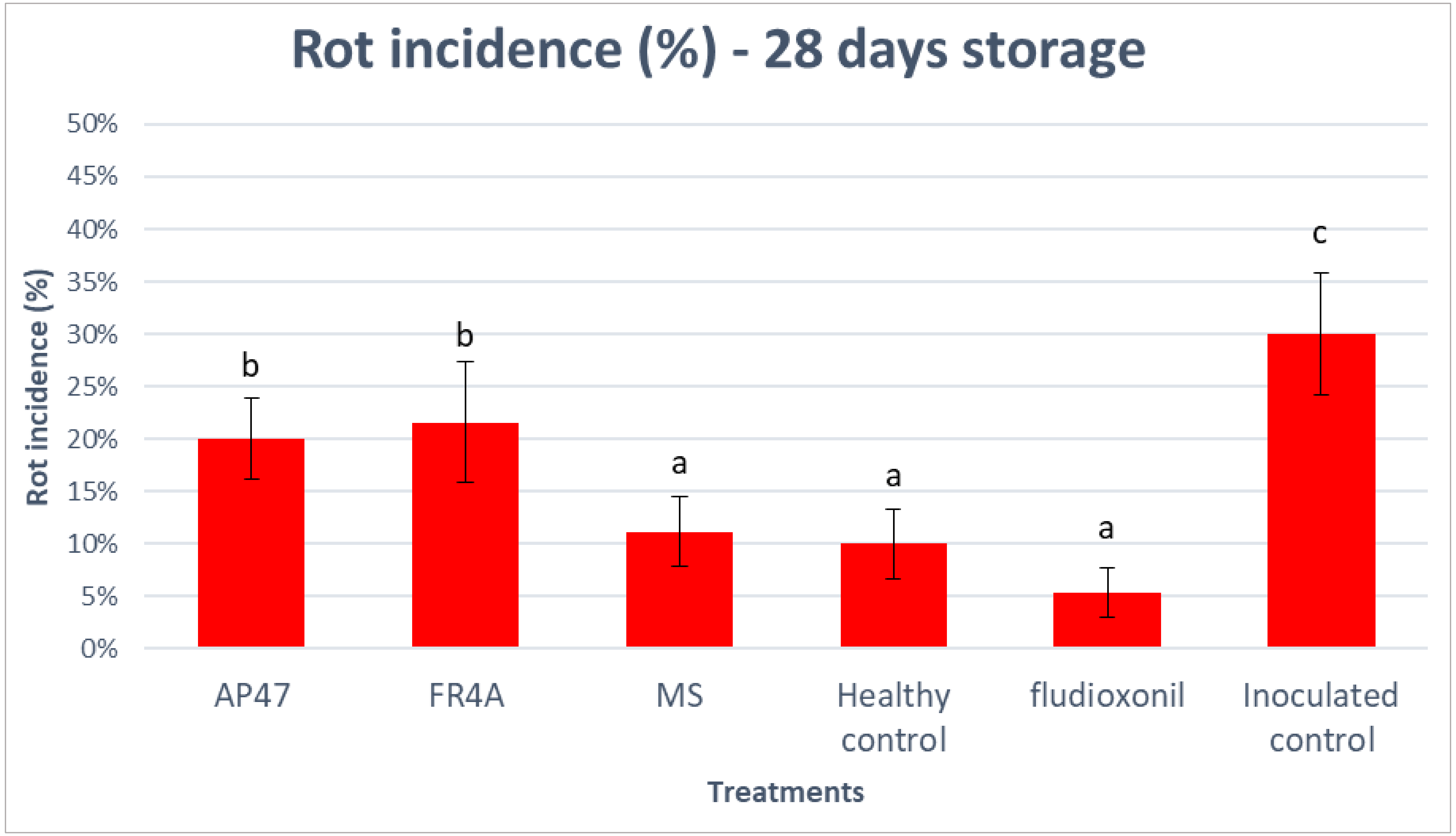
Treatments with BCAs by dipping in the cell suspensions (10^8 cells/mL)

↓

Storage at 1 ± 1 °C for 28 days

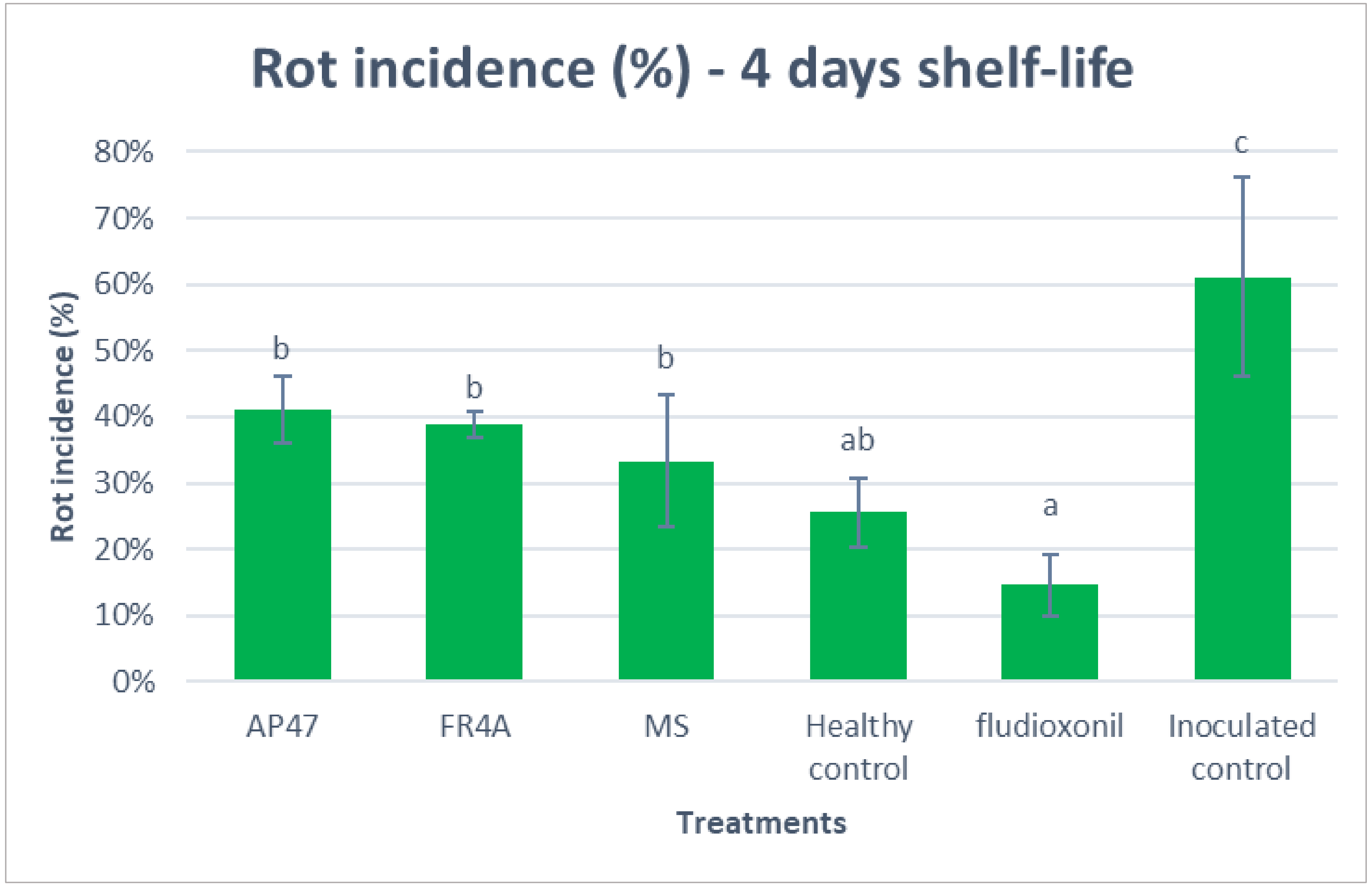
Shelf-life at 25 ± 1 °C for 4 days

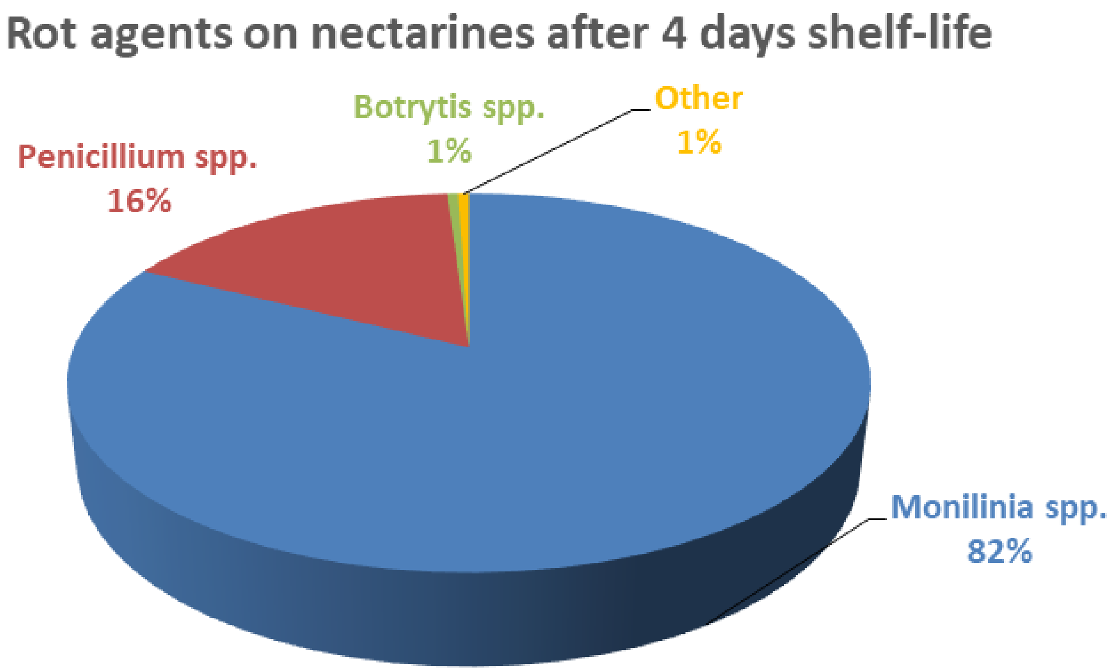
Screening tests | **Efficacy test** | Quality analyses | BCA Identification | Microbiome analysis



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

MS treatment was the most effective, with a rot incidence comparable to the chemical control





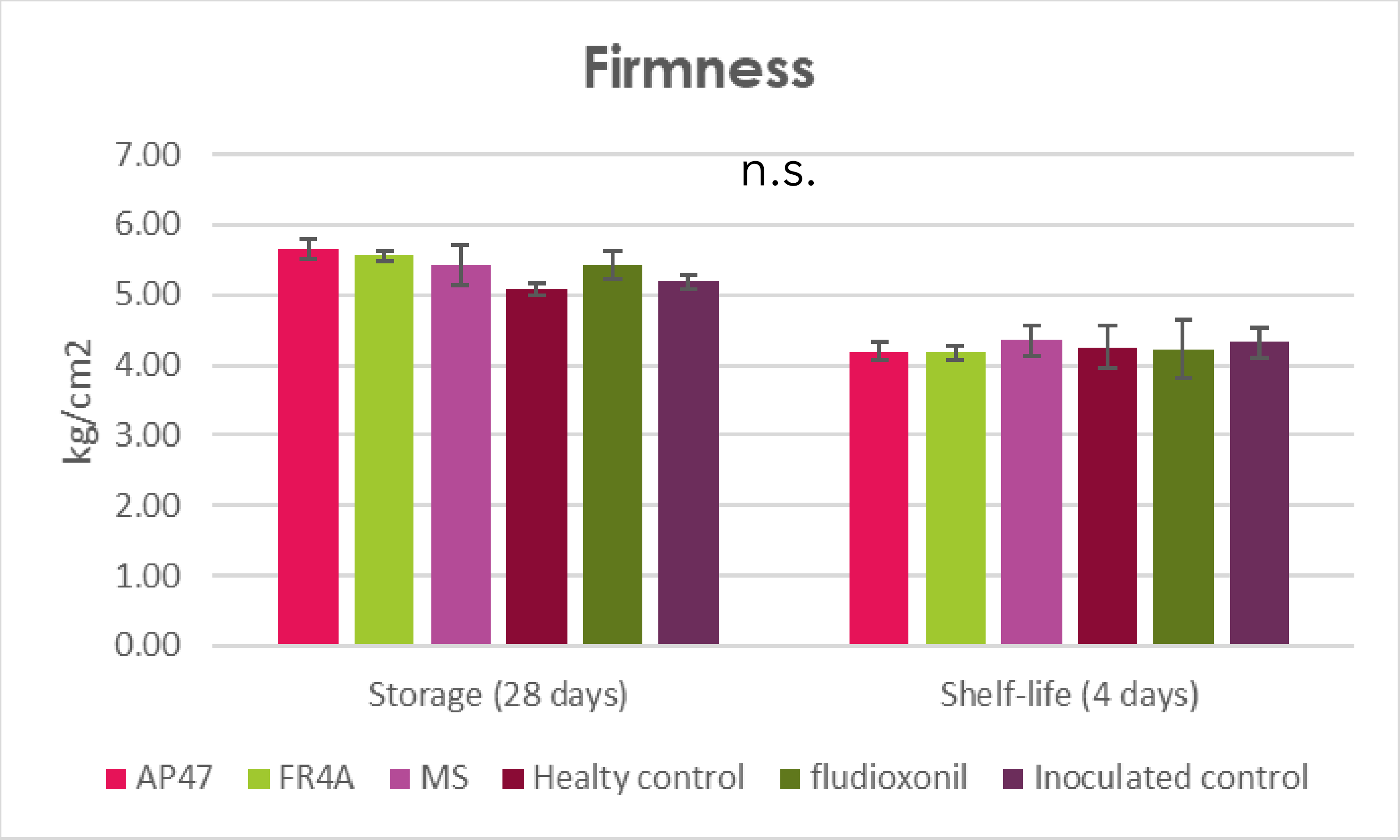
After **4 days of shelf-life,** rot incidence for all treatments was significantly lower than inoculated control

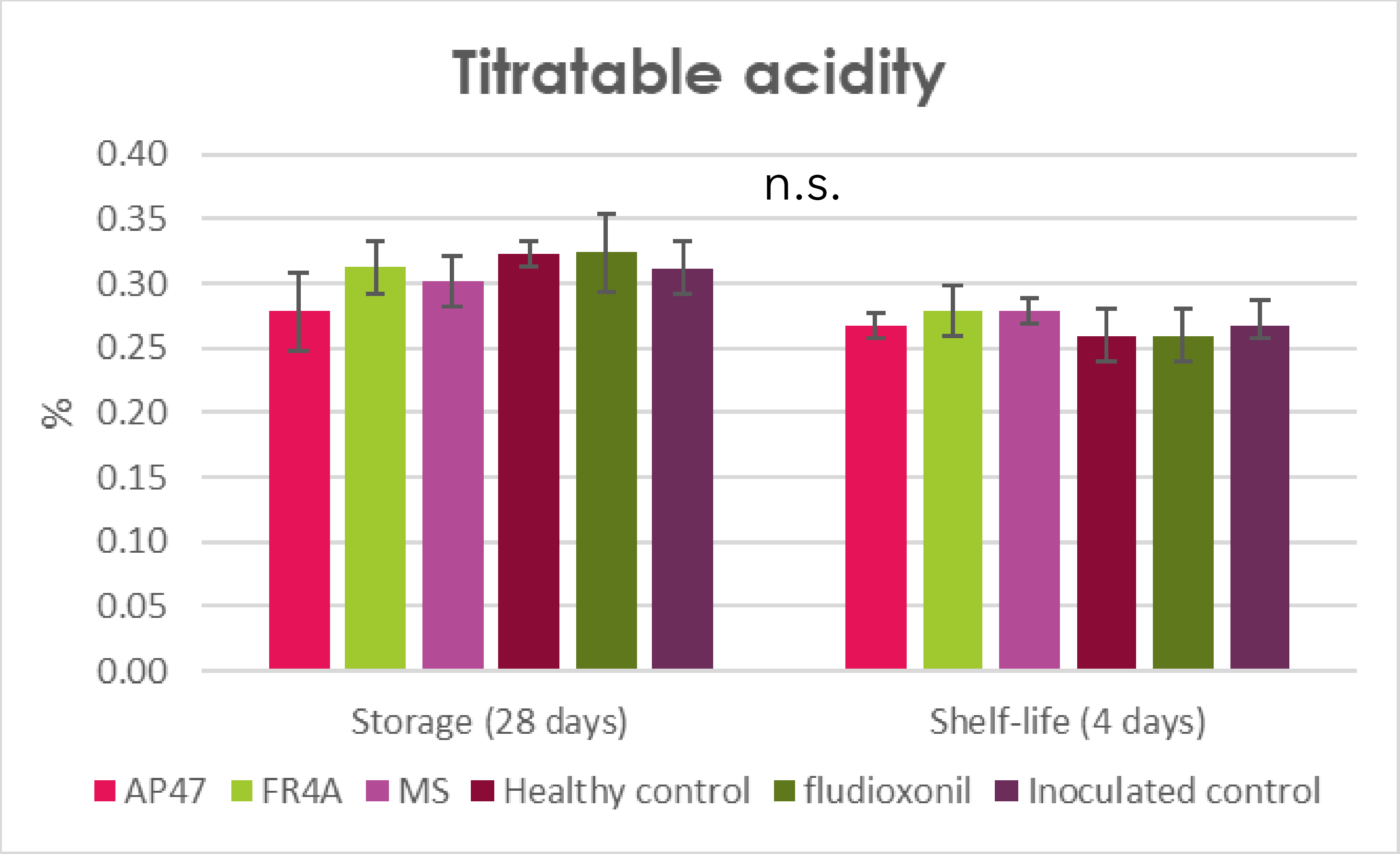
No treatment was still comparable to fludioxonil

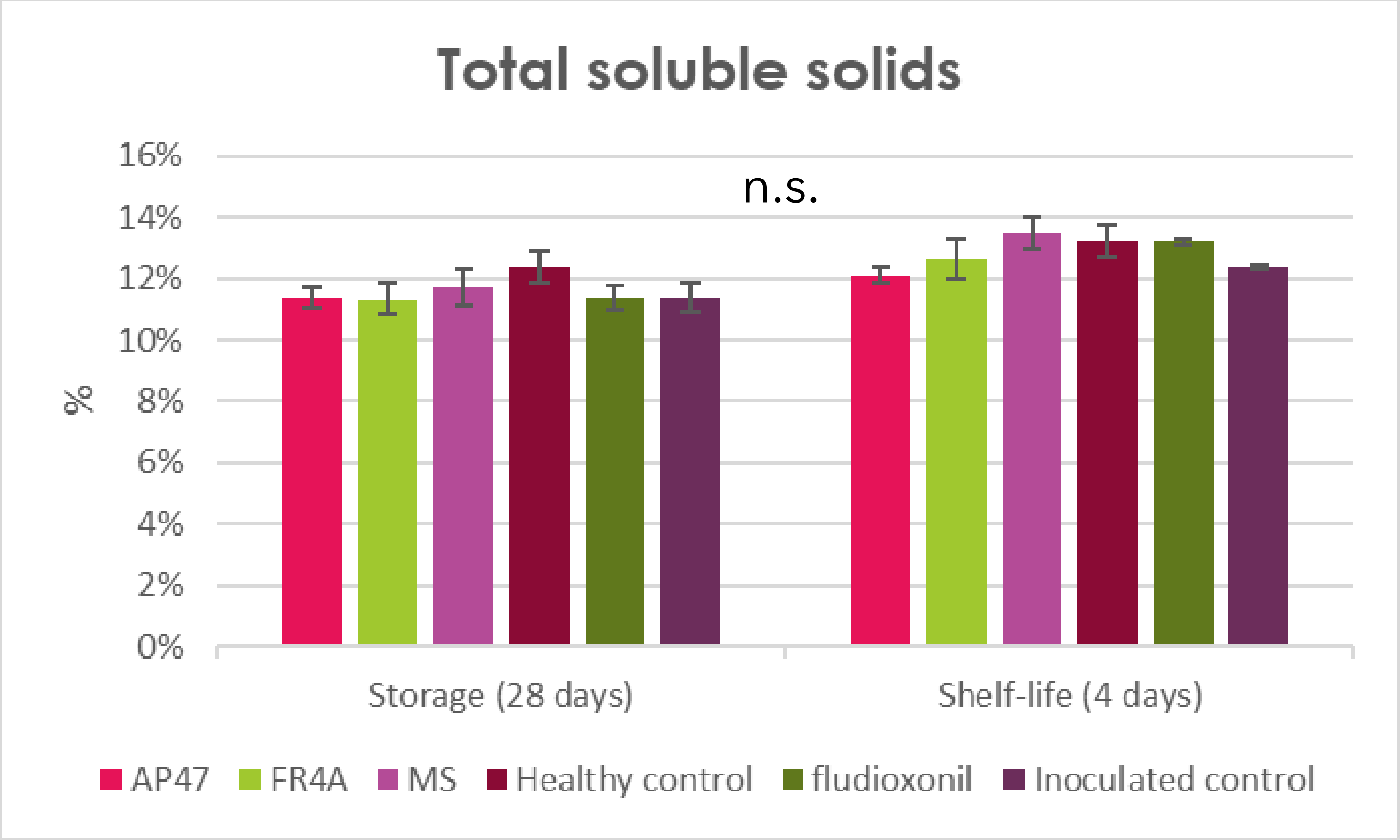
Quality analyses

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

All the tested treatments did not significantly affect fruit quality



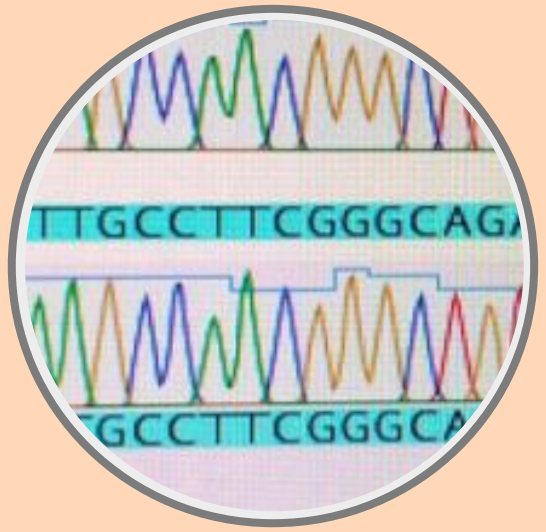




Identification of the most effective BCAs

DNA extraction

Amplification of D1-D2 domains of LSU



Sequencing

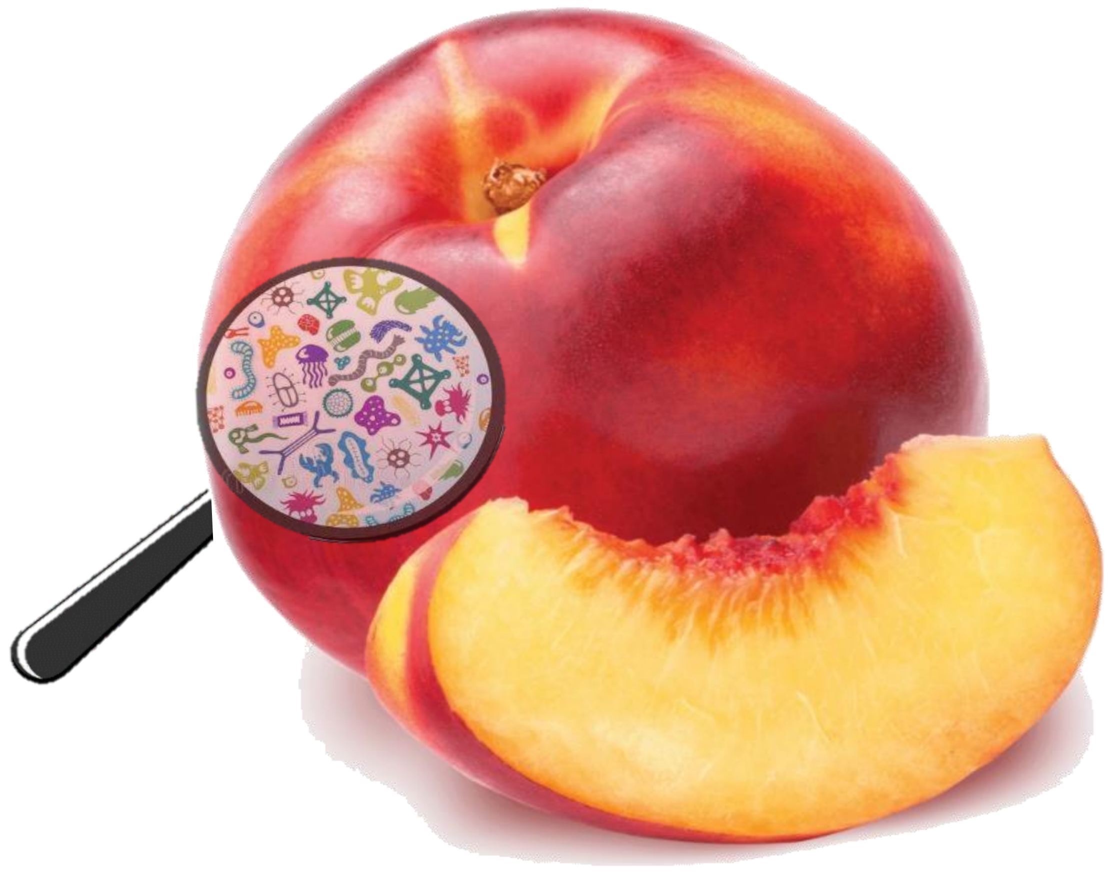
AP47 = *Metschnikowia fructicola*

FR4A = *Aureobasidium pullulans*

MS = *Metschnikowia pulcherrima*

Microbiome analysis

Evaluation of the effect of the treatments on the fruit fungal microbiome



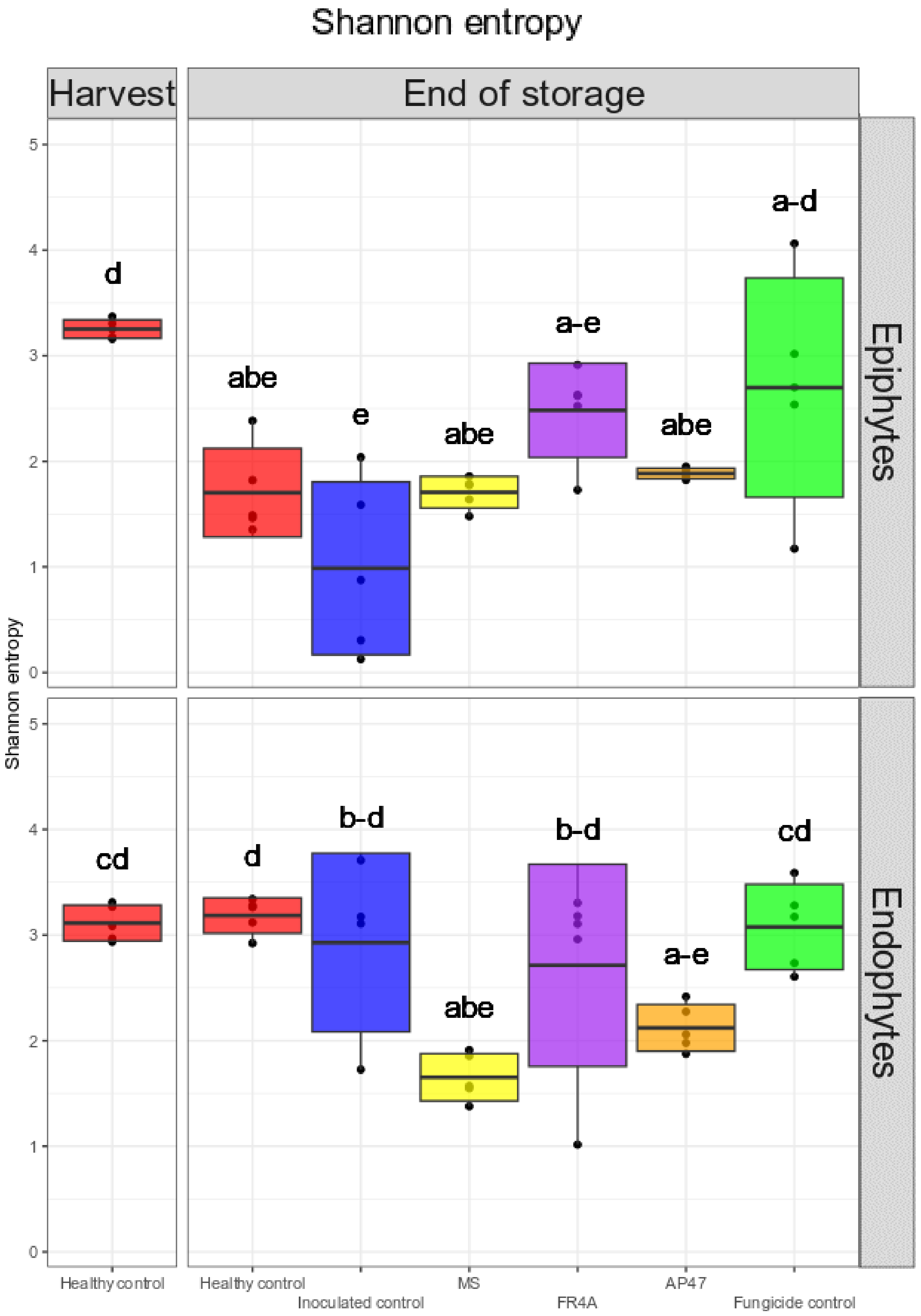
1. Sampling

Epiphytes and **endophytes** sampling:
- treatments with the yeast strains
- chemical control (fludioxonil)
- inoculated control
- healthy control

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

2. DNA extraction

3. Sequencing of the ITS2 region and metabarcoding analysis



Alpha diversity

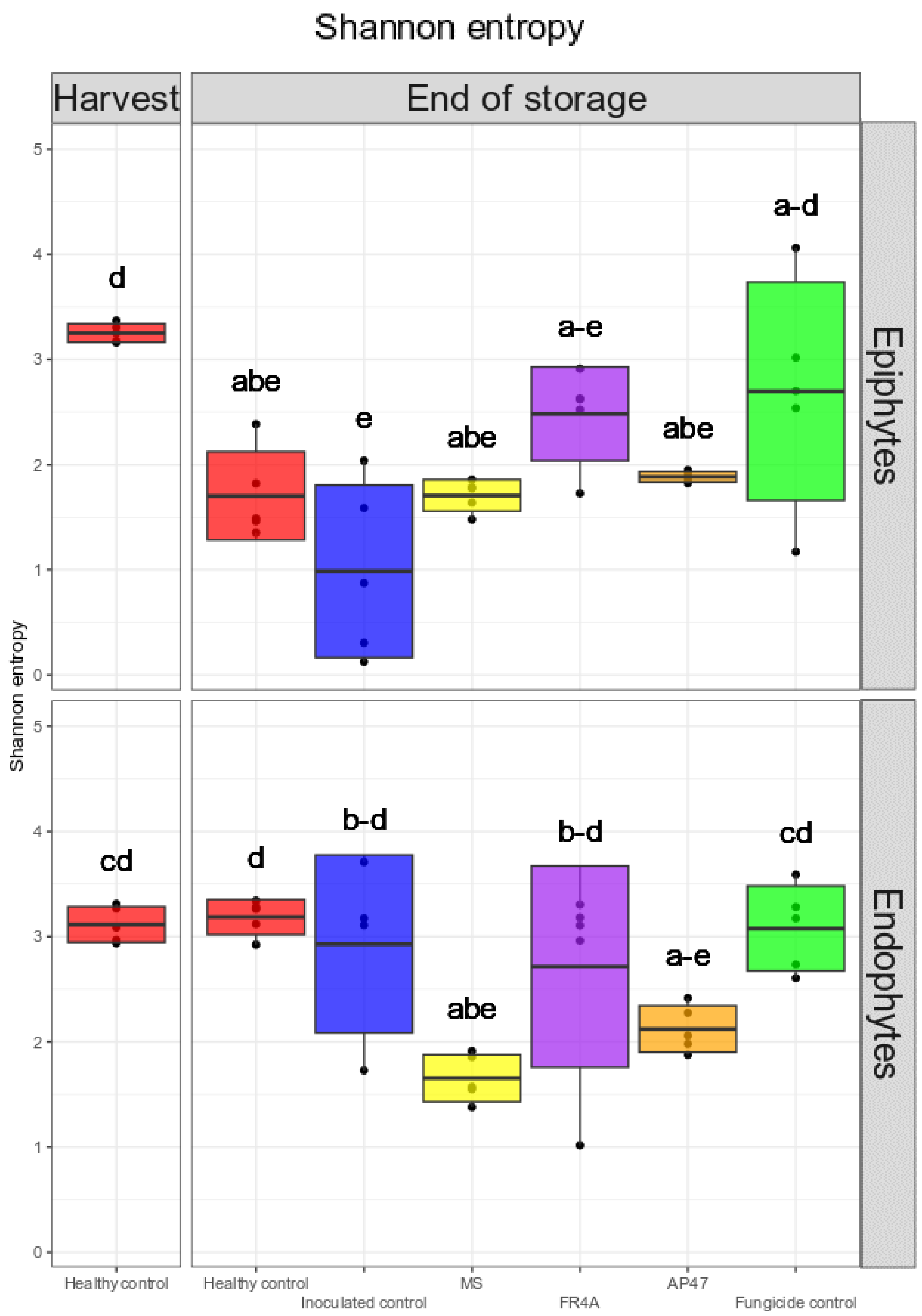
Treatment

Epiphytes

Treatments with *Metschnikowia* spp. (MS and AP47) had lower richness compared to the chemical control

Endophytes

Treatment with MS had a significantly lower richness than healthy and chemical controls



Alpha diversity

Sampling time

Epiphytes

Treatments with *Metschnikowia* spp. (MS and AP47), had lower richness than fruits at harvest

Endophytes

Lower richness of MS treatment compared to harvest

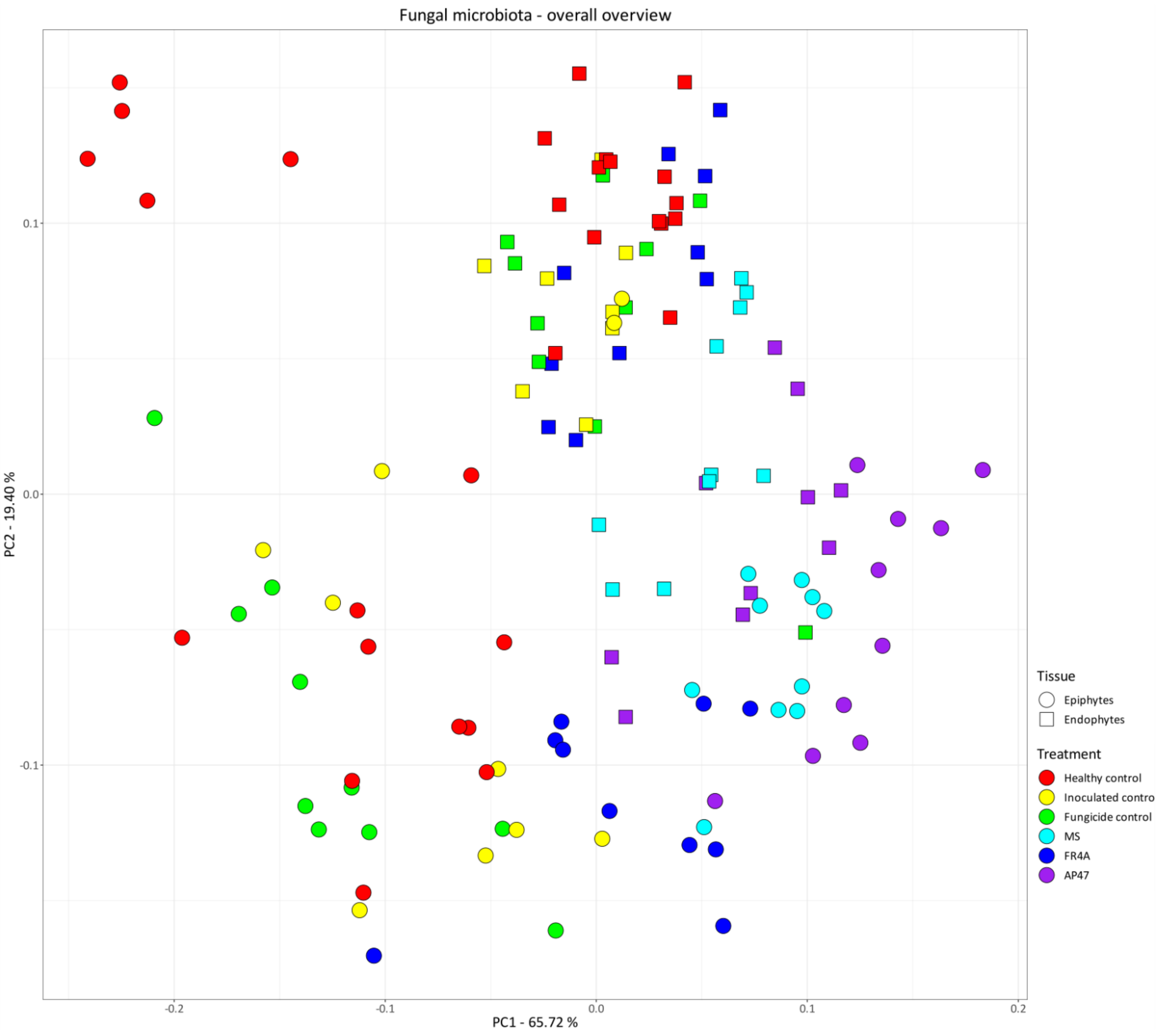
Treatment
- Healthy control
- Inoculated control
- MS
- FR4A
- AP47
- Fungicide control

Beta diversity

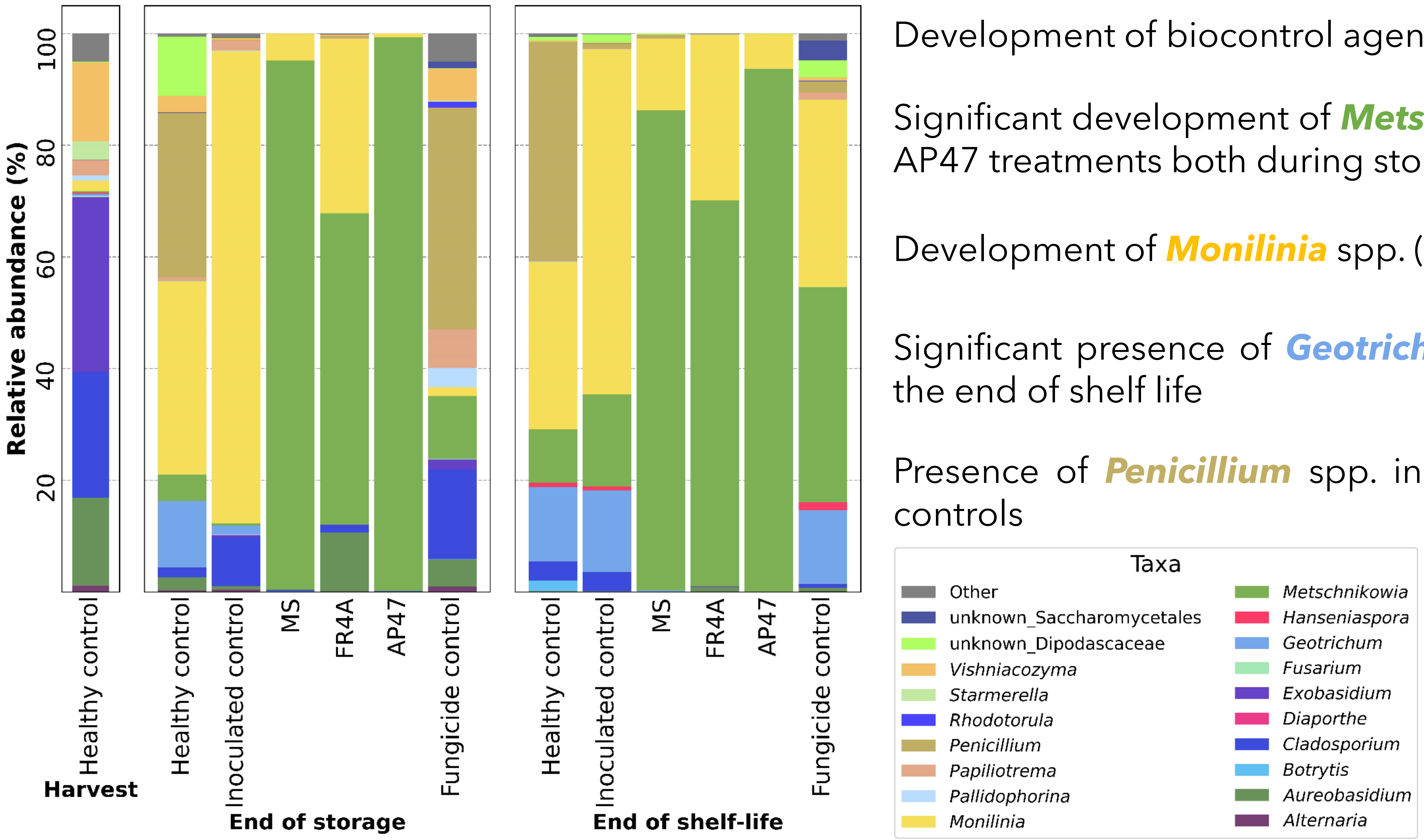
Multivariate permutational analysis of variance showed that treatment, tissue and sampling time had a statistically significant effect on the microbiome composition

Treatment had the **highest impact on the total variance (43%)**, compared to the tissue (13.6%) and sampling time (2.8%)

PCoA plot reflects the relative importance of treatments in partitioning variance.



Compositional analysis - Epiphytes



Development of biocontrol agents

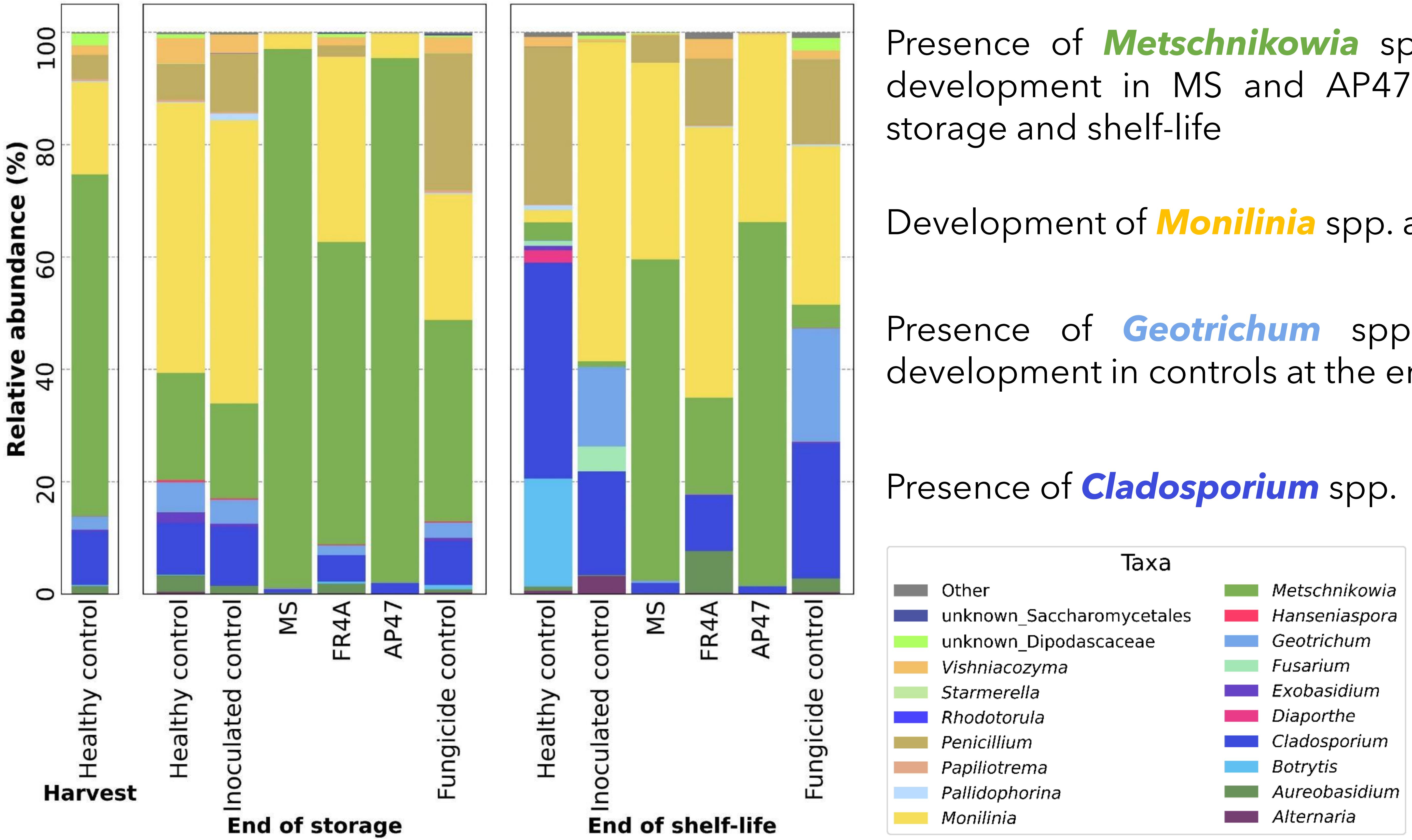
Significant development of ***Metschnikowia*** spp. in MS and AP47 treatments both during storage and shelf-life

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

Significant presence of ***Geotrichum*** spp. in all controls at the end of shelf life

Presence of ***Penicillium*** spp. in the healthy and chemical controls

Compositional analysis - Endophytes



Presence of ***Metschnikowia*** spp. at harvest. Significant development in MS and AP47 treatments both during storage and shelf-life

Development of ***Monilinia*** spp. and ***Penicillium*** spp.

Presence of ***Geotrichum*** spp. at harvest. Significant development in controls at the end of shelf life

Presence of ***Cladosporium*** spp.

Conclusions

- Treatments with antagonistic yeasts were effective against *Monilinia fructicola* on nectarines in storage. Efficacy of MS strain comparable to the chemical treatment

- No significant effect on fruit quality parameters

- Yeasts occupy the ecological niche of *Monilinia* and of other pathogenic genera (*Penicillium*, *Geotrichum*)

Treatments with antagonistic yeasts could represents a promising tool for reducing post-harvest losses preserving the fruit quality

Pre-harvest application

Acknowledgements

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PRIMA
PARTNERSHIP FOR RESEARCH AND INNOVATION IN THE MEDITERRANEAN AREA

STOP MED WASTE

Thank you for your attention