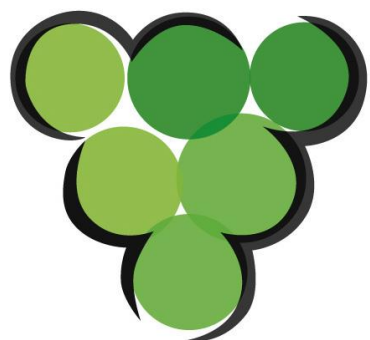


Evaluation of non-*Saccharomyces* yeasts as biological control agents against *Botrytis cinerea*

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SASEV

South African Society for
Enology & Viticulture

44th SASEV CONFERENCE

24 – 26 November 2025

Protea Hotel, Technopark, Stellenbosch

Introduction

- Grapes important economic crop
- Grey mould/rot caused by *Botrytis cinerea*, reduces the yield and quality of grapes

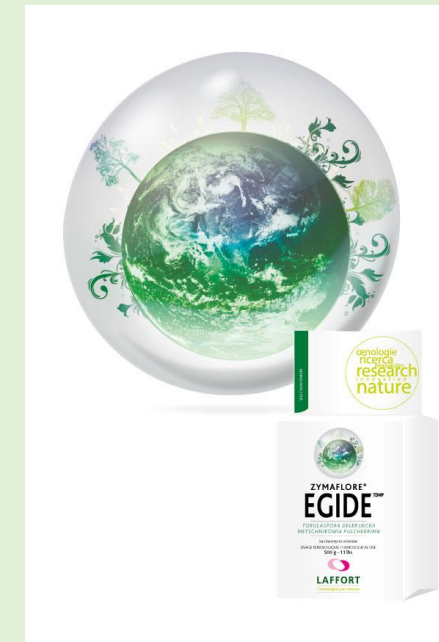


Introduction (Cont.)

- Synthetic fungicides used for controlling disease
- Pose health concerns to consumers and not environmentally friendly
- Fungicide resistance
- **Chemical pesticides reduction:** focus on sustainable use, aiming to reduce risks and impact on human health and the environment
- **Alternative control strategies**

Introduction (Cont.)

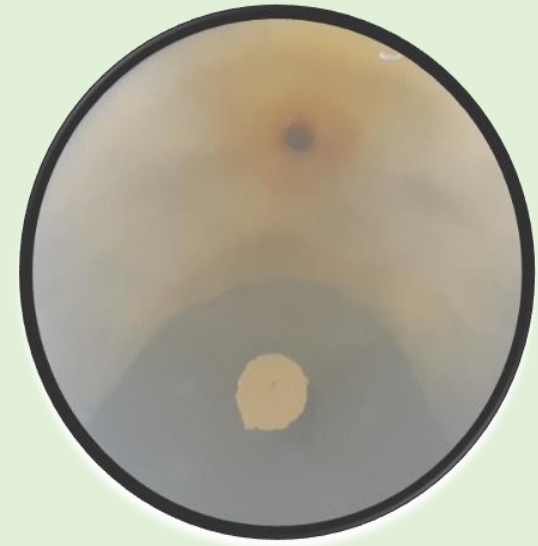
- Yeasts are **generally regarded as safe (GRAS)** for humans and animals, do not produce mycotoxins or allergenic spores, and do not leave harmful residues on the fruit or in the environment.
- Broad-spectrum antifungal activity, resistance to environmental stresses, easy to cultivate and can improve plant health.
- Commercial products (*Aureobasidium pullulans*, *Candida oleophila*, *Metschnikowia fructicola*, *M. pulcherrima*)



Introduction

- **Multiple Modes of Action:**

- Competition for nutrients and space
- Production of antimicrobial compounds (volatile organic compounds (VOCs), lytic enzymes (e.g. chitinases, glucanases and protease),
- Biofilm formation

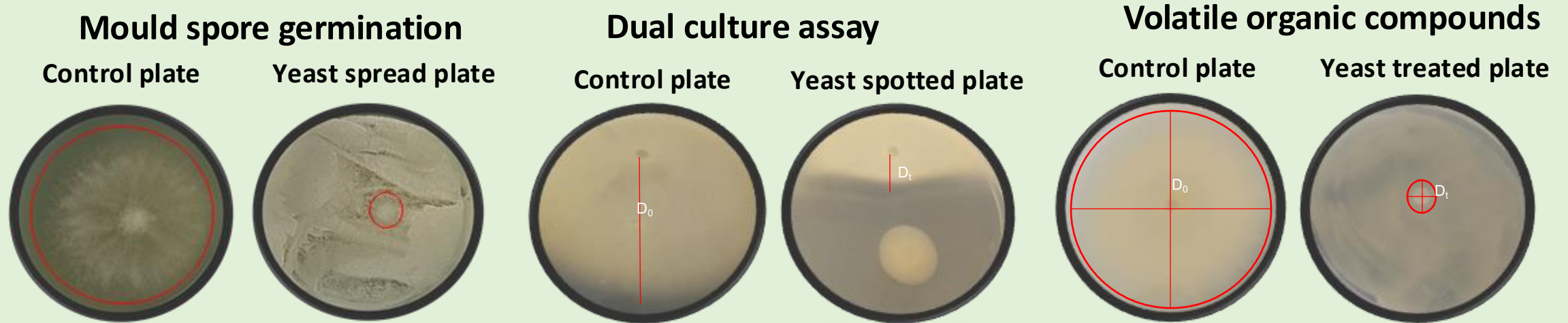


Aim

- **To determine the growth inhibition activity of selected yeasts against different *Botrytis cinerea* strains and possible use as biocontrol agents**

Materials and methods

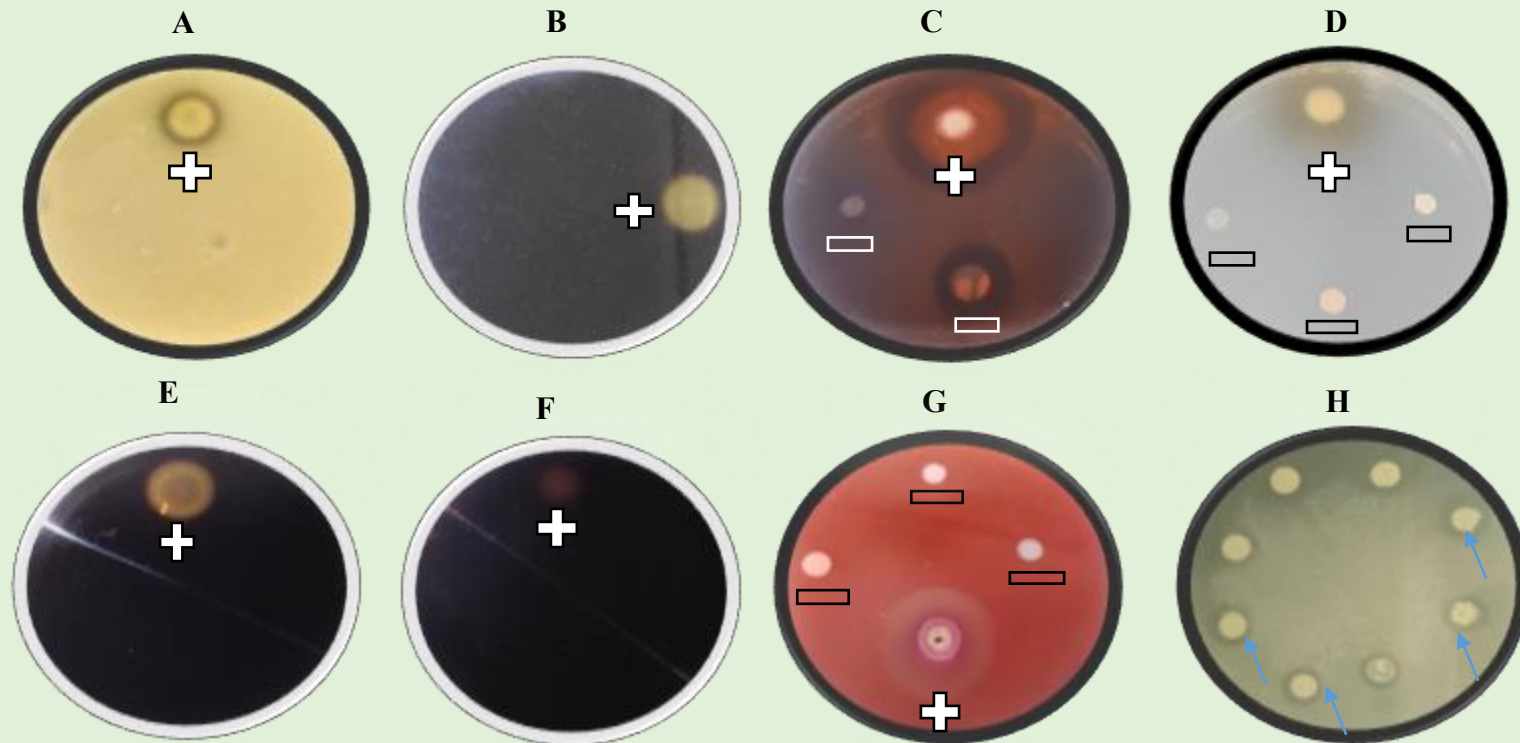
- Yeasts: *Aureobasidium melanogenum* J6, *Meyerozyma guilliermondii* WEMH1, *Pichia kluyveri* Y1125, *Suhomyces pyralidae* Y1117, *Zygoascus hellenicus*
- *Botrytis cinerea* strains (B05.10, SAGWRI-FF1, PPRI 30807)
- Cultured on yeast malt agar (YMA) and YM broth at 28°C
- Fungicide: 0.5 g/L Captan™ (N-trichloromethylthio-4-cyclohexene-1,2-dicarboximide)



Inhibition %: $[(D_0 - D_t)/D_0] \times 100$, D_0 = mould growth on negative control plates and D_t = mould growth on treated plate

Materials and methods – enzyme production

- Assays: Proteases (A), chitinase (B), β -1,3-glucanase (C), β -glucosidase (D), cellulase (E), starch (F), pectinase (G) and lipase (H)
- Three replicates per treatment



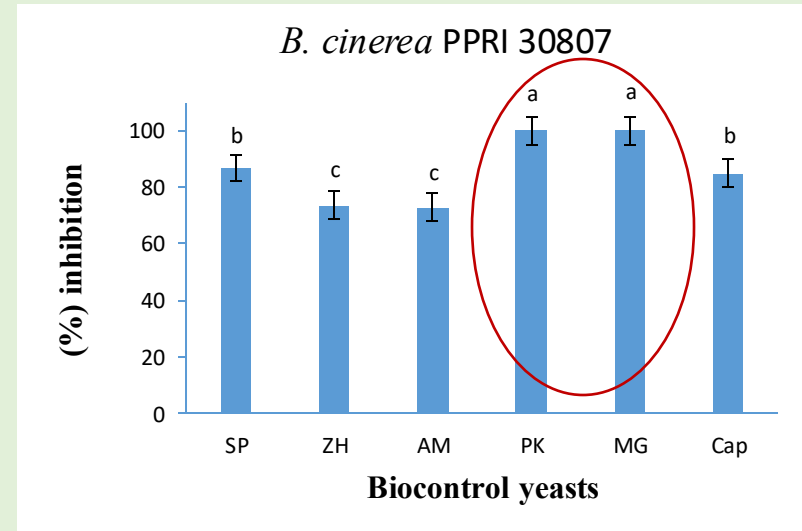
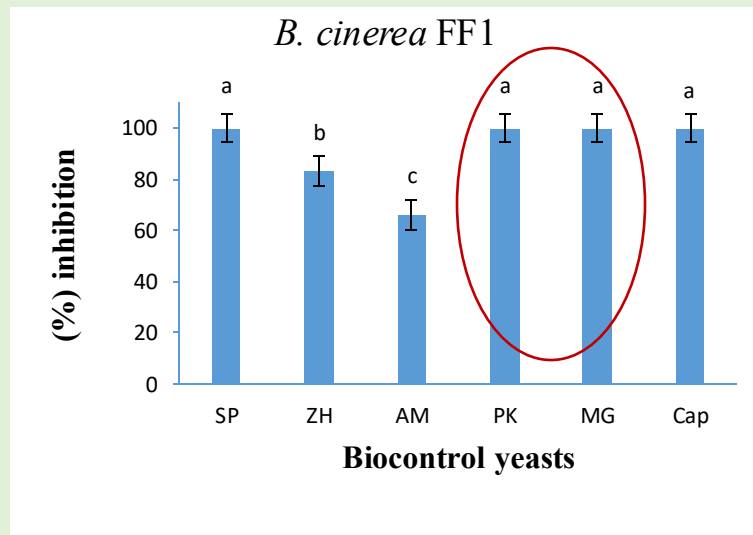
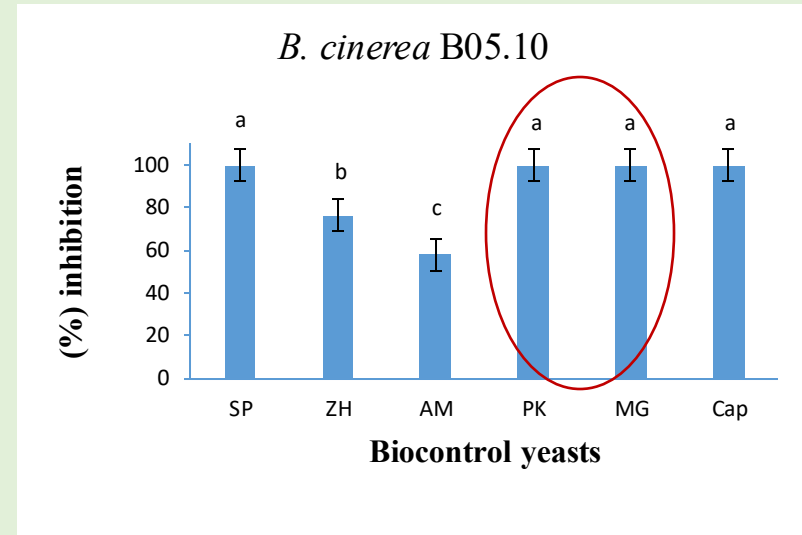
Identification for VOCs

- Yeast: *S. pyralidae* and *P. kluyveri* (1×10^8 cfu/mL)
- *Botrytis cinerea* (1×10^5 cfu/mL)
- Grown in YM broth at 25°C
- Headspace solid-phase microextraction, gas chromatography-mass spectrometry (HS-SPME–GC–MS) performed at CAF



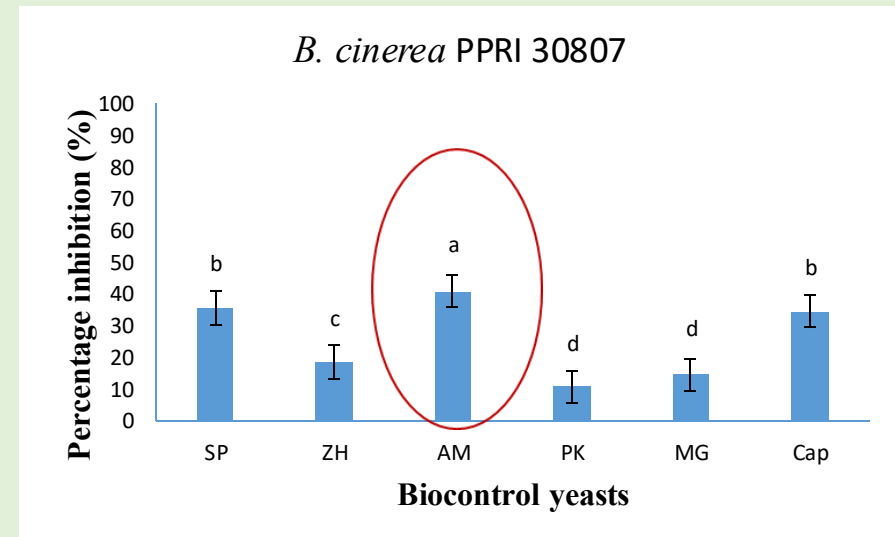
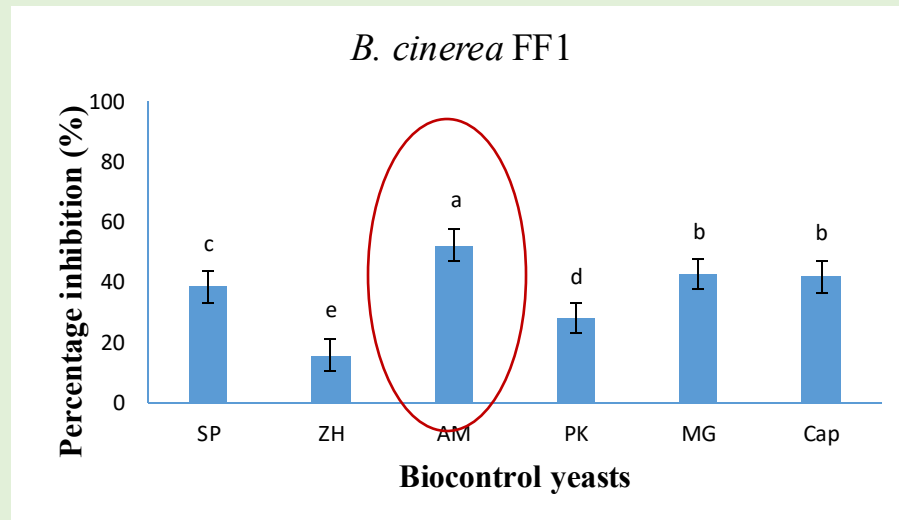
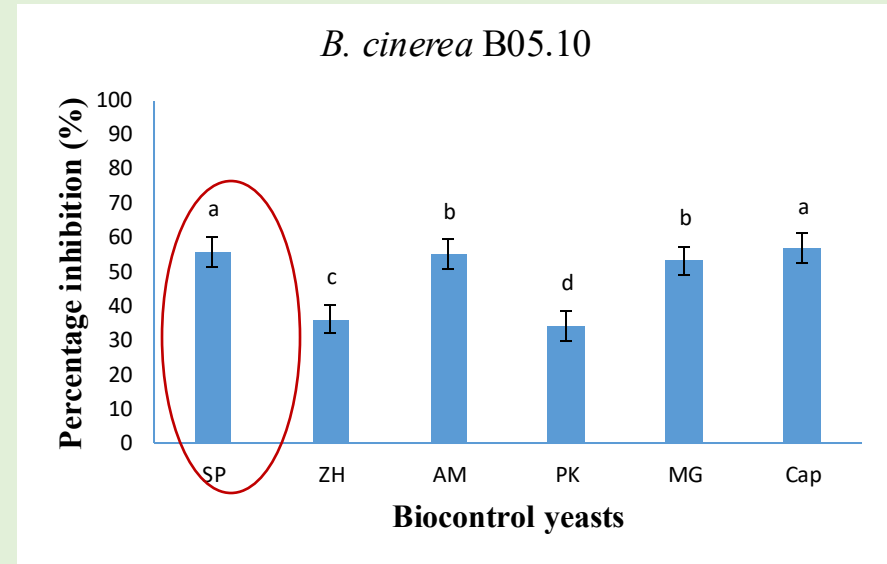
Spore germination assay results

- *M. guilliermondii* and *P. kluyveri*, *S. pyralidae* 100% inhibition against all *B. cinerea* strains
- Comparable to Captan



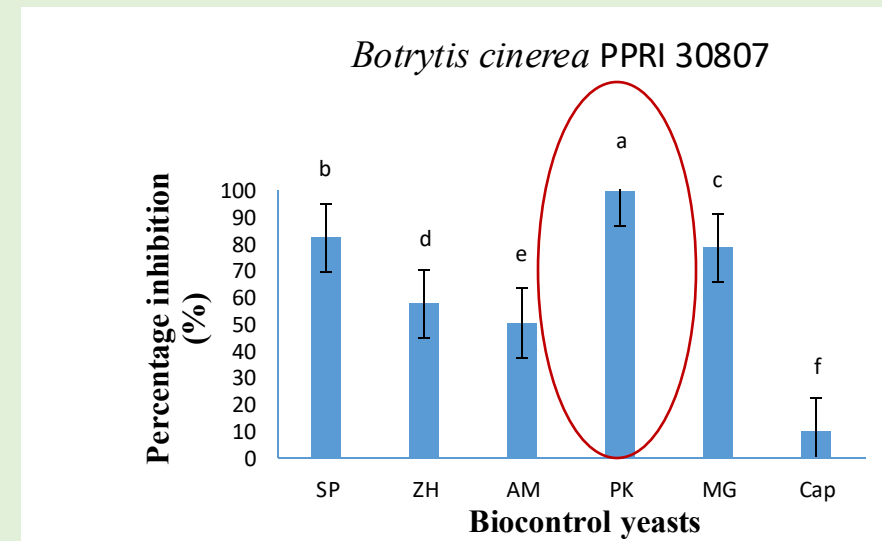
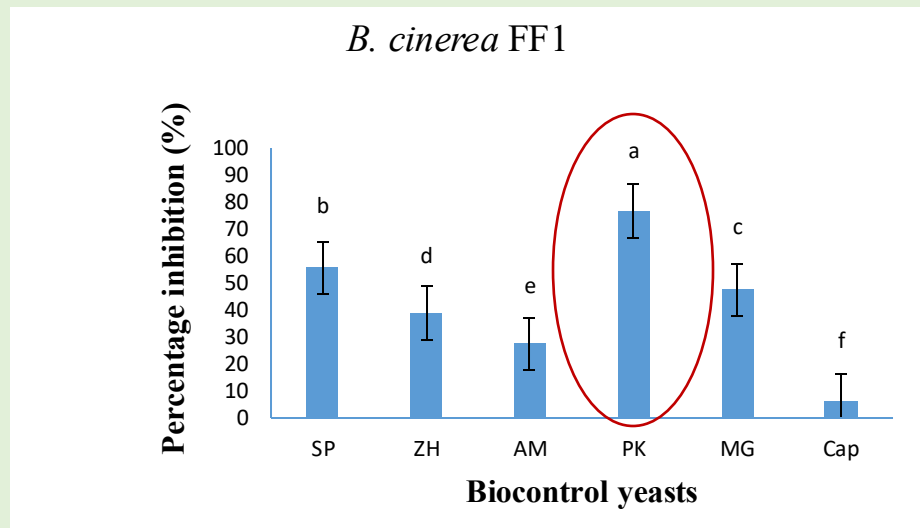
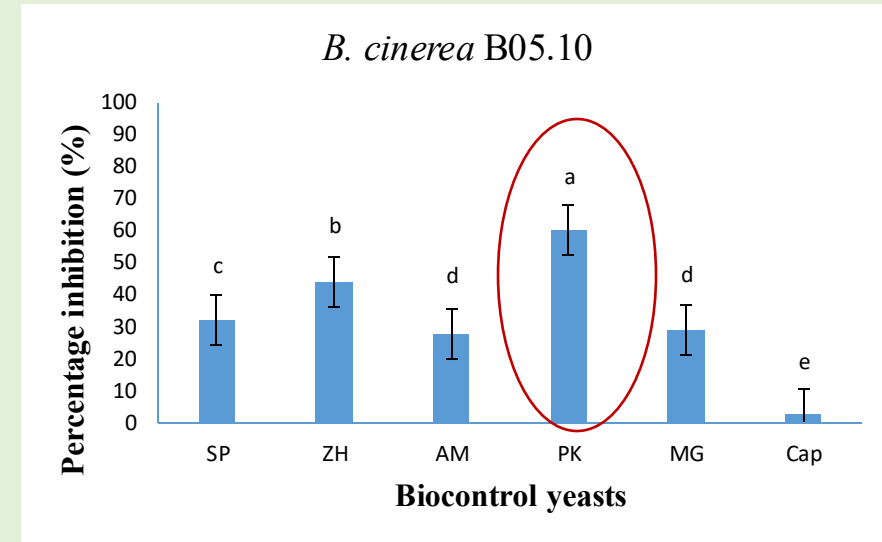
Dual culture assay results

- *S. pyralidae* best performer against B05.10
- *A. melanogenum* best performer against FF1 and PPRI 30807



Mouth to mouth assay

- *P. kluyveri* best performer
- Variable inhibition 60-100%



VOC production results

- *Pichia kluyveri*: reduction in isoamyl acetate, isoamyl alcohol, 2-phenethyl acetate, 2-phenylethanol

Compound	<i>B. cinerea</i>	<i>P. kluyveri</i>	<i>P. kluyveri</i> and <i>B. cinerea</i>	<i>S. pyralidae</i>	<i>S. pyralidae</i> and <i>B. cinerea</i>
	Average area ratio				
Isobutanol	ND	0.006	ND	0.009	0.012
Isoamyl acetate	ND	2.489	ND	0.001	0.002
Isoamyl alcohol	0.006	0.136	0.040	0.140	0.135
2-Phenethyl acetate	0.004	3.207	1.325	0.002	0.004
2-Phenylethanol	0.005	0.155	0.034	0.021	0.019
γ -Decanolactone	ND	ND	ND	0.008	0.003
Methyl palmitate	0.004	0.012	0.001	0.001	0.001

Conclusions

- Yeast controlled the spore germination and mycelia growth, strain variability
- *P. kluyveri*: VOCs as a mechanism of inhibition (isoamyl acetate, isoamyl alcohol, 2-phenethyl acetate and 2-phenylethanol)
- Yeasts were comparable to commercial fungicide
- Potential as alternative to chemical fungicides

Acknowledgements

- Our heavenly Father
- ARC colleagues, students and interns, for their contributions
- SAGWRI
- CAF



Thank you!