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SASEV 44th Conference

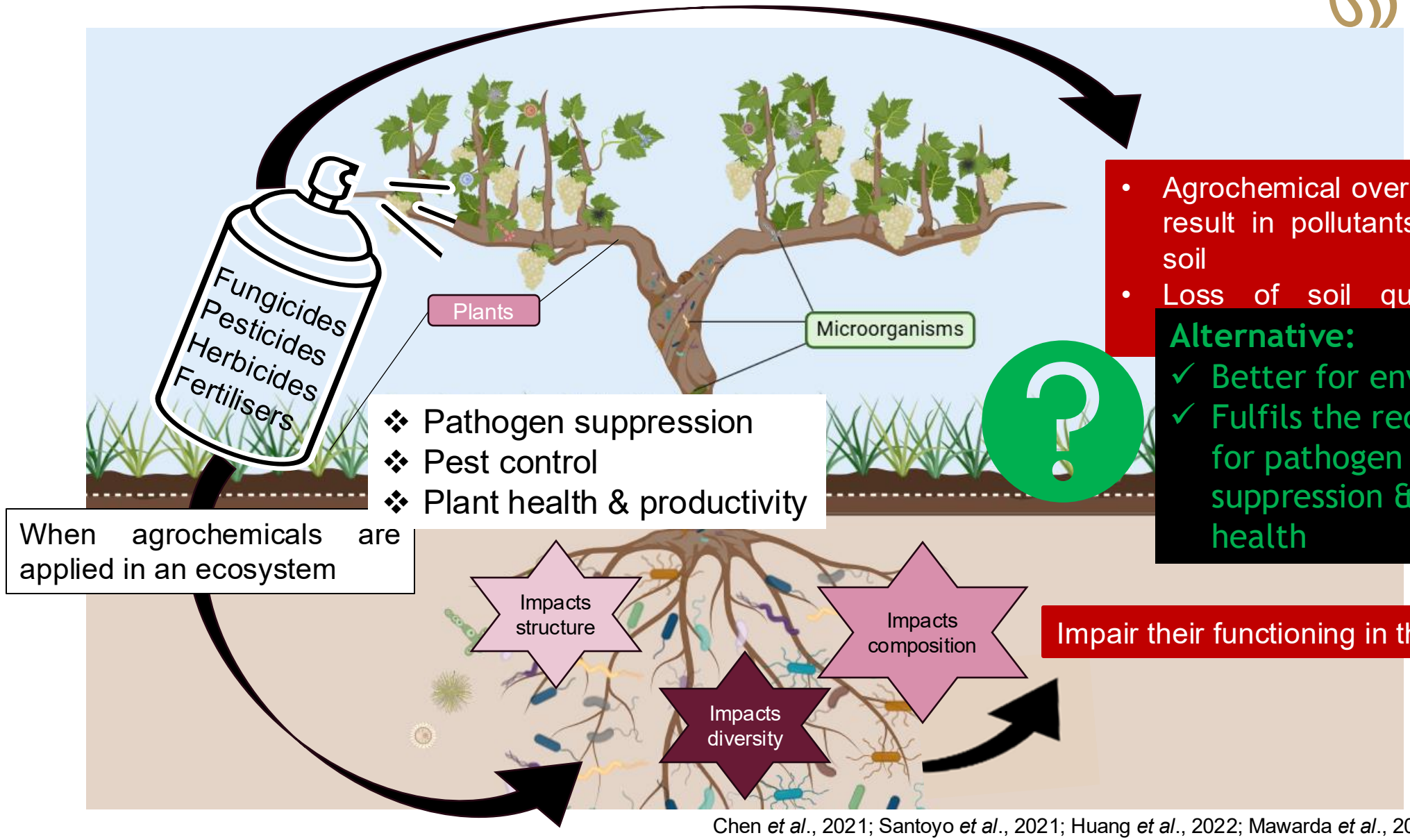
Evaluating plant growth-promotion potential of biocontrol microbial consortia applied as soil inoculants in potted grapevine (*Vitis vinifera*).

Amber J Africa

Supervision: Prof. ME Setati, Dr. EH Blancquaert & Prof. JF Salles

https://atomisystems.com/html5-animation/saola-animate-3/how-to-create-the-growing-tree-animations/

Introduction



When agrochemicals are applied in an ecosystem

- ❖ Pathogen suppression
- ❖ Pest control
- ❖ Plant health & productivity

- Agrochemical overuse can result in pollutants in the soil
- Loss of soil quality &

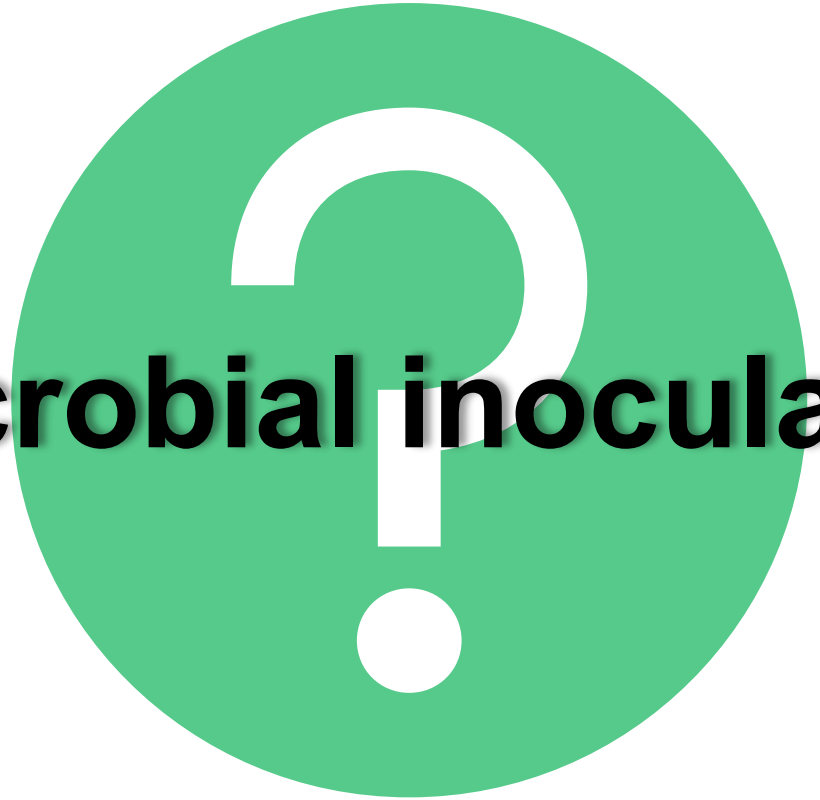
Alternative:

- ✓ Better for environment
- ✓ Fulfils the requirements for pathogen suppression & plant health

Impair their functioning in the ecosystem

Chen *et al*., 2021; Santoyo *et al*., 2021; Huang *et al*., 2022; Mawarda *et al*., 2022; Nasuelli *et al*., 2023

Introduction



Microbial inoculants

Microbial inoculants contain **living microbes** that are introduced into an environment to accomplish a particular function.

- e.g. biocontrol & plant growth-promotion
- **Better for the environment -** promote soil fertility and plant growth.

Vassilev *et al*., 2020; O'Callaghan *et al*., 2022

Introduction

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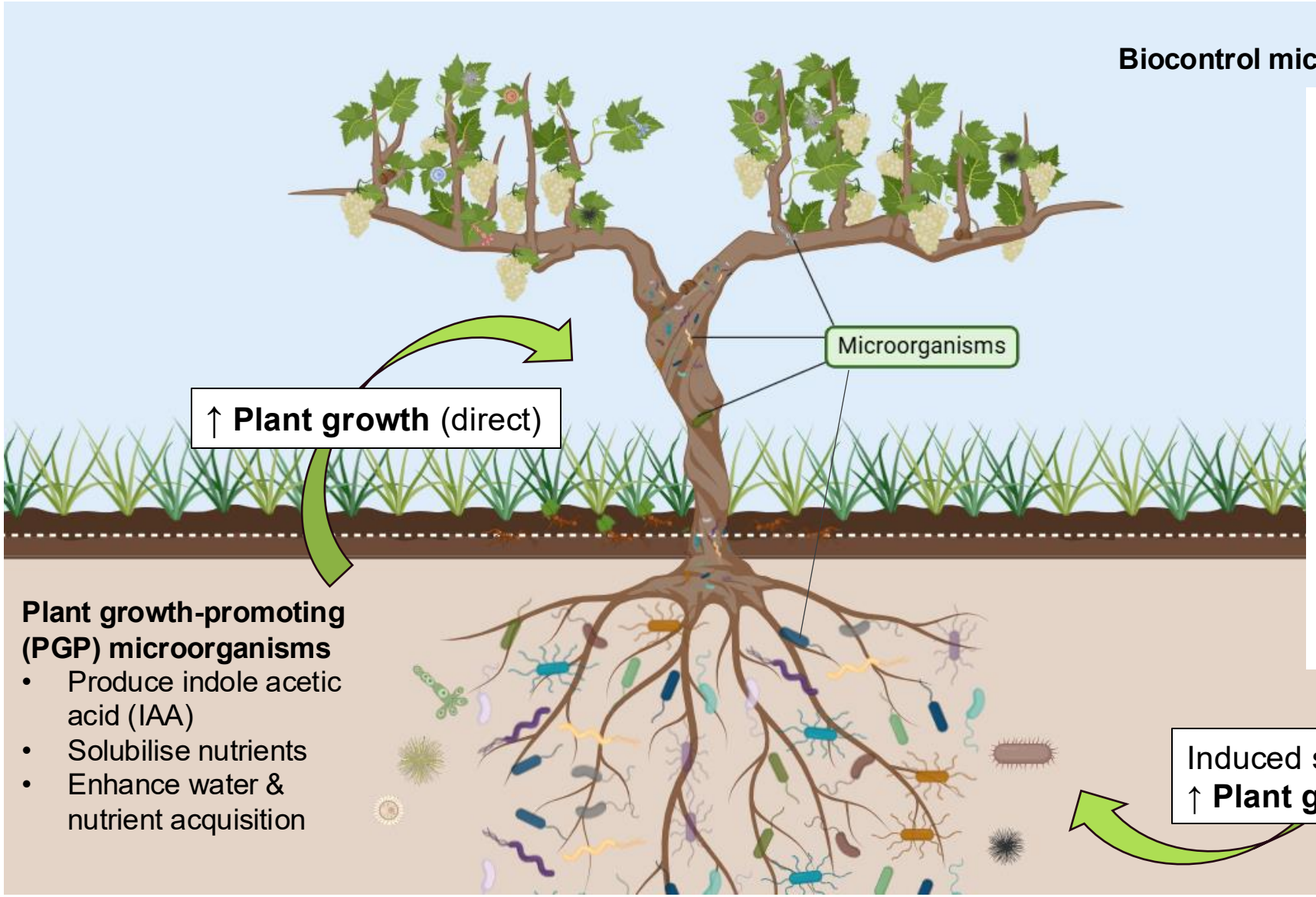
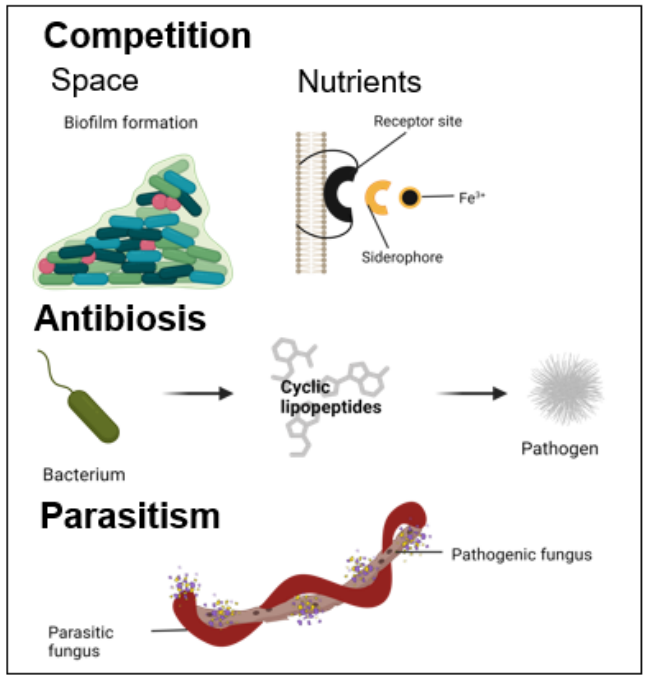
Biocontrol microorganisms



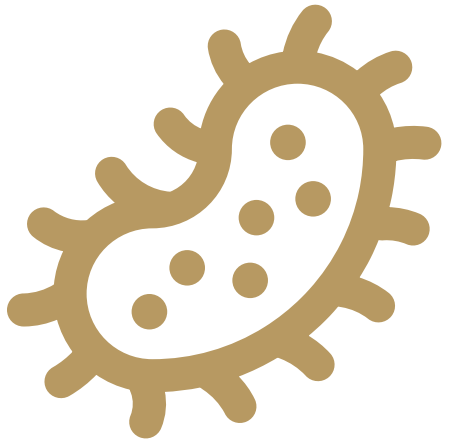
Pathogen inhibition – Various modes of action (MoA)



Santos *et al*., 2019; Mandal *et al*., 2020; Berg *et al*., 2021; Khan, 2022; O'Callaghan *et al*., 2022; Chaudhary *et al*., 2023; Sanjuán *et al*., 2023

Introduction

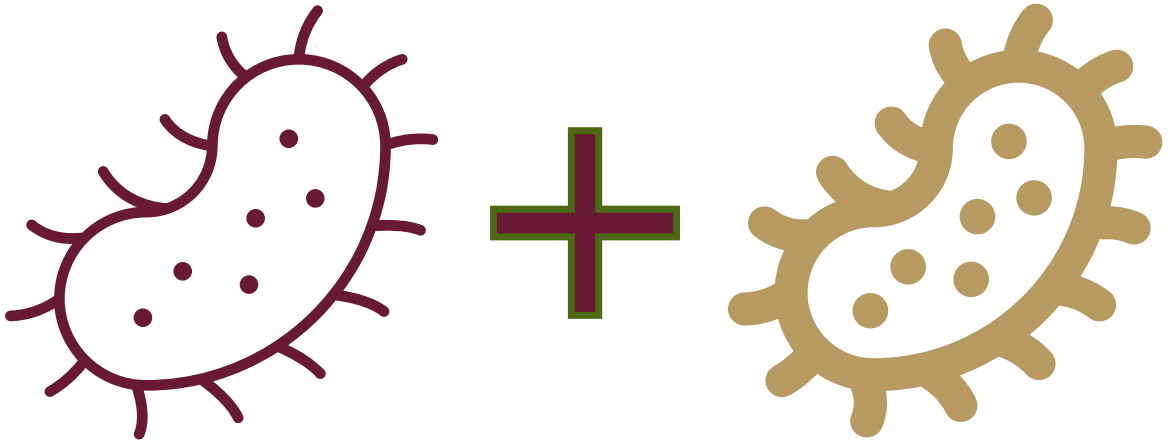
Single microorganism



→ Reduced efficacy

Limited functions

Microbial consortia



→ Enhanced activities

Diverse functions

Synergistic or additive interactions

Santoyo *et al*., 2021; Liu *et al*., 2023

Previous work

Biocontrol potential of yeasts & bacteria isolated from the vineyard environment & grape must

Yeast strains	Modes of action	Target pathogens
Wickerhamomyces anomalus Y934, Y541 & Y517 *Hyphopichia burtonii* Y951 *Hyphopichia pseudoburtonii* Y963	• Competition for resources • Production of chitinase & glucanase	• *Botrytis cinerea* B05.10, FF1, FF2 • *Aspergillus niger* FF3 • *Alternaria alternata* FF4
Bacterial strains		
Bacillus subtilis B4016 *Bacillus nakamurai* B4001 *Bacillus nakamurai* B4022	• Production of glucanase • Production of fengycins, iturins & surfactins	• *Botrytis cinerea* B05.10, FF1, FF2 • *Phytophthora capsici* DCS 585 • *Phytophthora cinnamoni* Steu 6825

Two dual interkingdom consortia were established

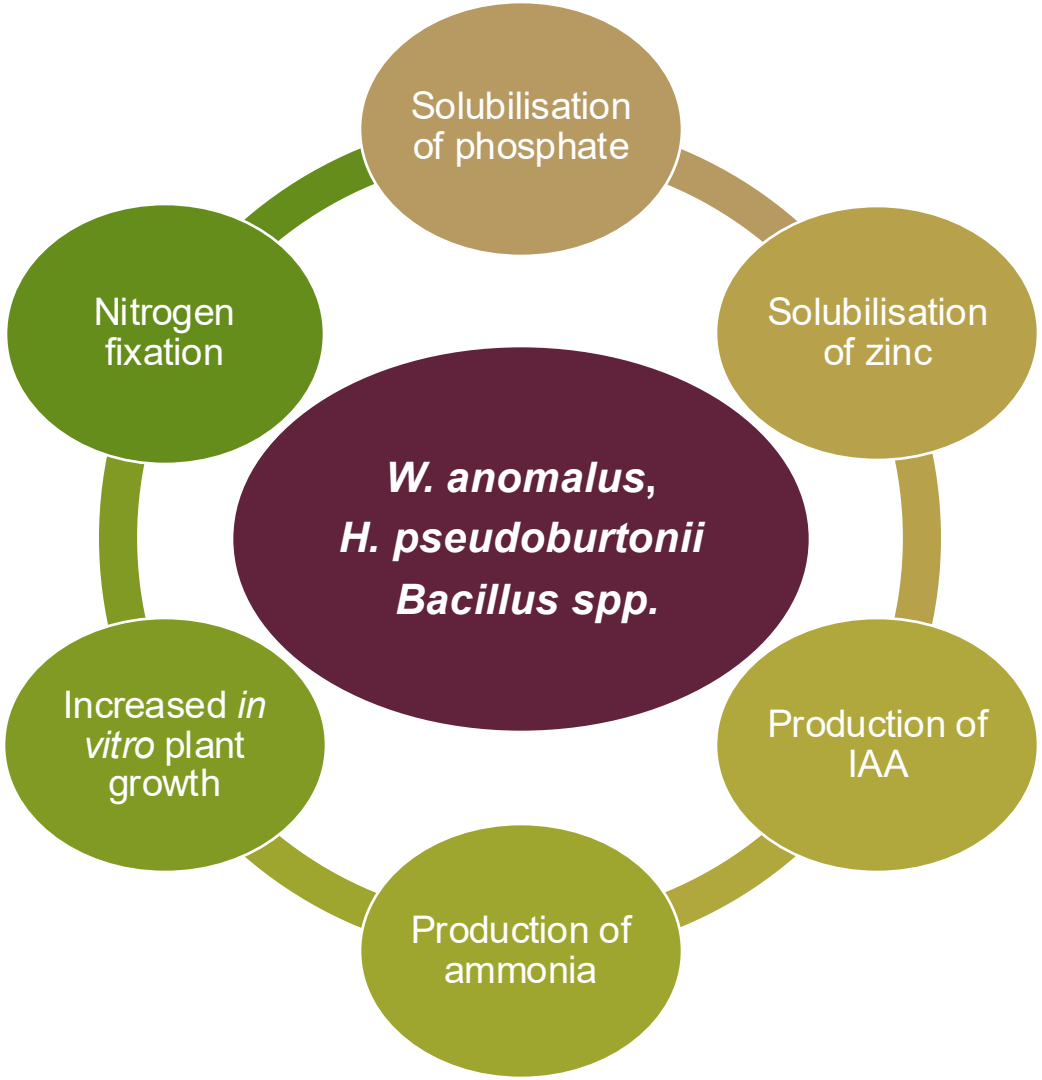
Consortium 1 – *Wickerhamomyces anomalus* Y934, *Bacillus nakamurai* B4022
Consortium 2 – *Hyphopichia pseudoburtonii* Y963, *Bacillus nakamurai* B4001

❖ **Both consortia showed biocontrol activity against powdery & downy mildew** (leaf disc assays)

Maluleke *et al*. (2022), Moremi *et al*. (unpublished)

Previous work

PGP potential of yeast & bacterial species (literature)



Fernandez-San Millan *et al*. 2020; Weinand *et al*. 2023; Sun *et al*., 2025; Tariq *et al*. 2025

Aim/Main Question

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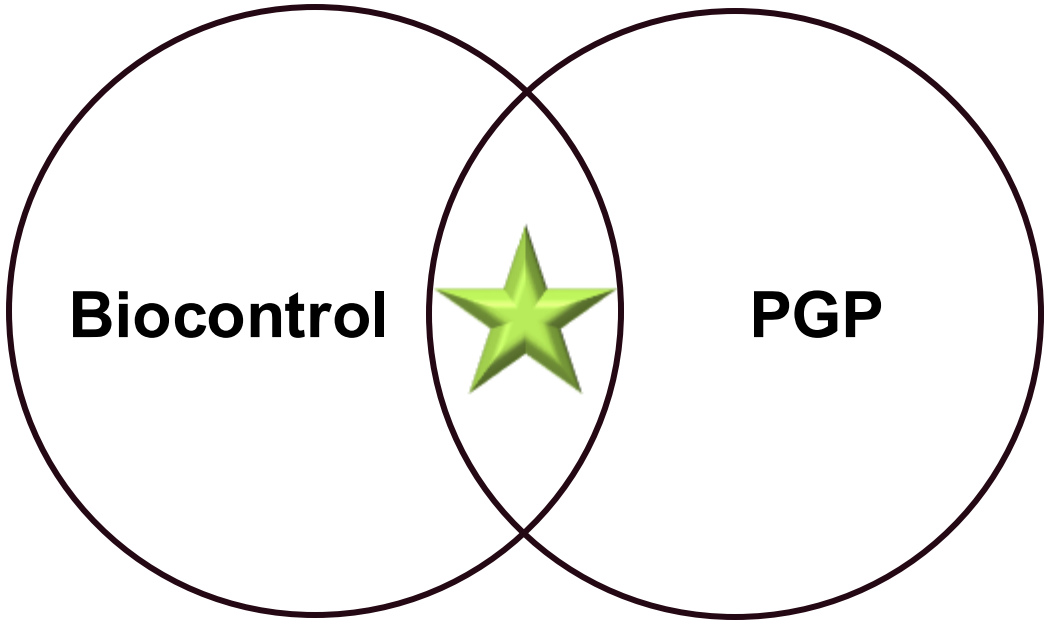
To assess the applicability of interkingdom microbial consortia as biocontrol and plant growth promoting agents

Are these biocontrol consortia bifunctional?



Bifunctionality of consortia

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Do the consortia show PGP characteristics *in vitro*?

Methodology

Assessment of PGP potential in the lab (*in vitro*)

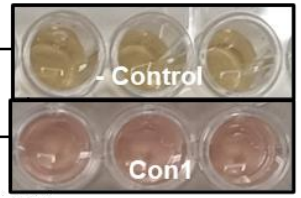
Detection of indole-3-acetic acid (IAA)

Monocultures & consortia were inoculated into Malt Extract broth with Trp

Samples were incubated at 30°C for 24h

0.1 mL Supernatant + 0.1 mL Salkowski reagent – incubate at 30°C for 30 mins

Positive result – pink colour – analyse at 530 nm



The amounts of IAA were calculated with a standard curve of known IAA concentrations

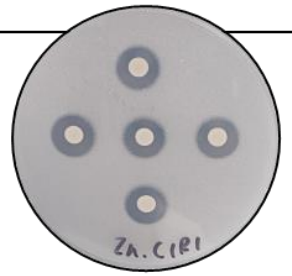
Solubilisation of phosphate (Ca_3PO_4) & zinc (ZnO)

Pikovskaya medium
- Tricalcium diphosphate
- Zinc oxide

Monocultures & consortia spotted & incubated at 30°C for five/seven days

Positive results – lighter halo around the colonies

Solubilisation efficiency (SE) = (colony diameter + halo zone diameter)/colony diameter.



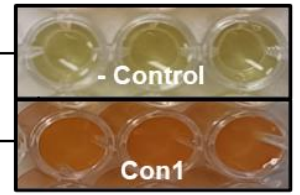
Production of ammonia (NH_3)

Inoculate monocultures & consortia into Peptone water
- Peptone & NaCl
- pH adjusted to 6.8

Samples were incubated at 30°C for 48h

0.05 mL supernatant + 0.1 mL Nessler's reagent – incubate 10 mins at room temp

Positive result – yellow-to-brown colour



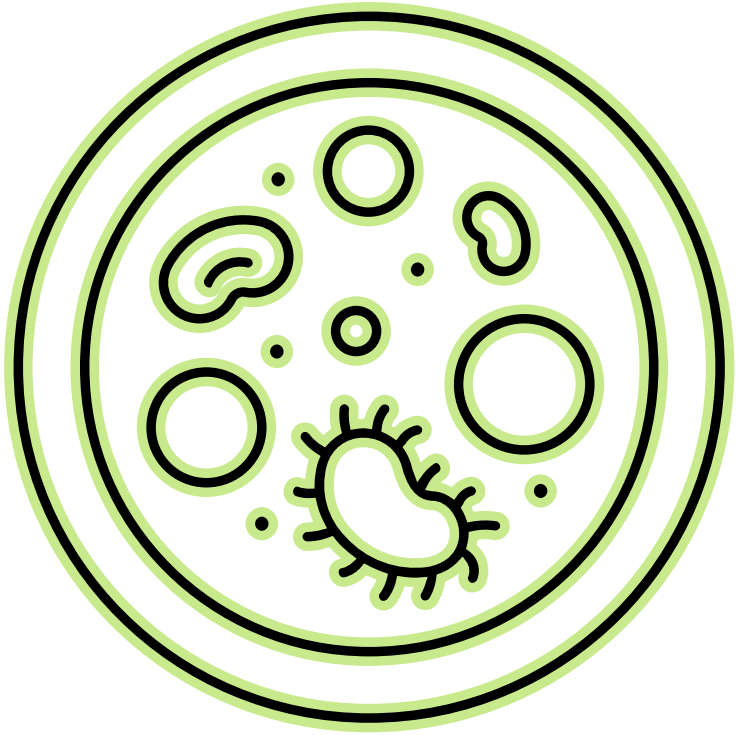
Perform 6x dilution and measure at – analyse at 450 nm

Ammonia production calculated according to ammonium sulphate standard curve

Abdelwahed *et al.*, 2021; González-Ista *et al.*, 2023 & Fernandez-San Millan *et al.*, 2020

Methodology

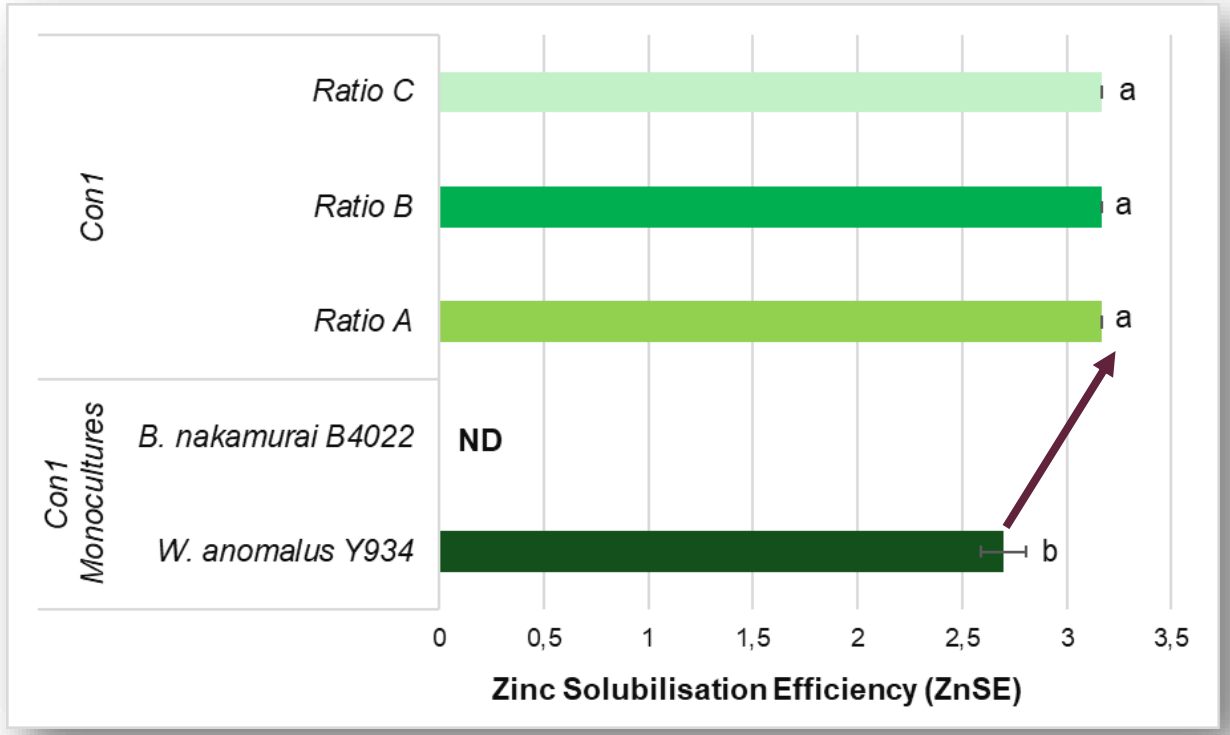
Assessment of PGP potential in the lab (*in vitro*)

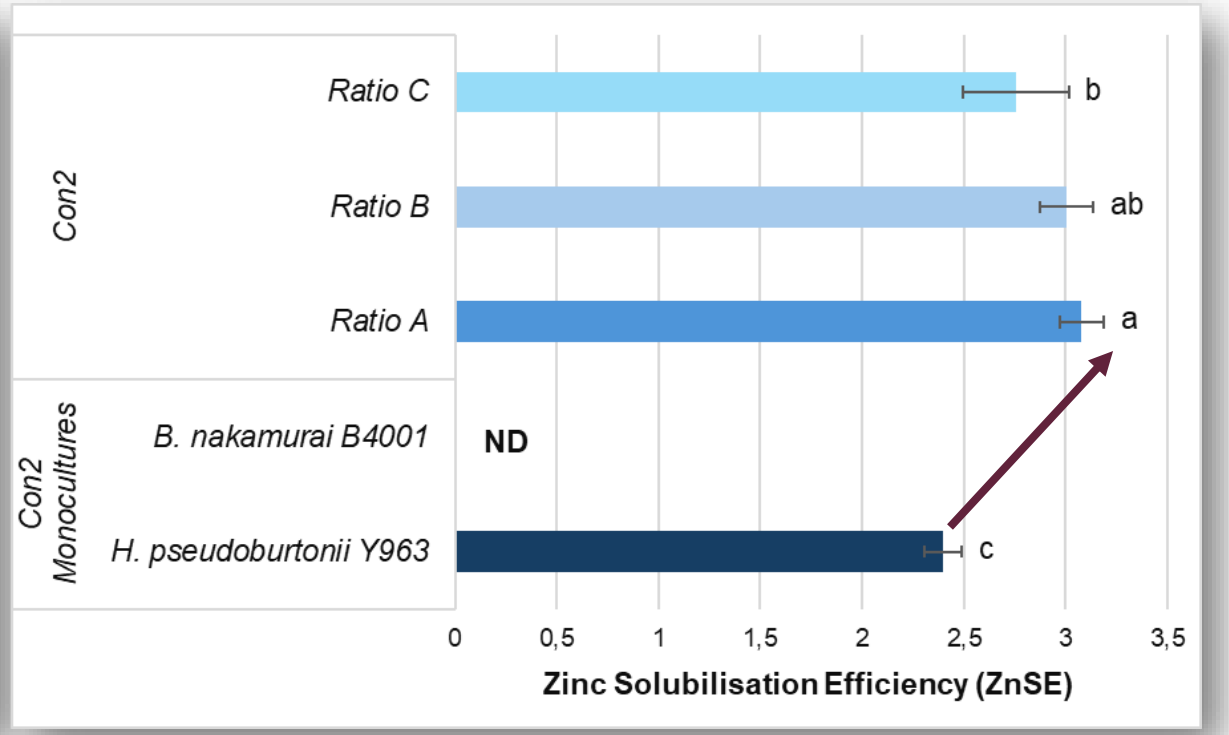


- **Monocultures** – yeast & bacteria in isolation
- **Ratio A** – yeast at 10^6 cells/mL & bacteria at 10^8 cells/mL
- **Ratio B** – yeast at 10^8 cells/mL & bacteria at 10^8 cells/mL
- **Ratio C** – yeast at 10^6 cells/mL & bacteria at 10^{10} cells/mL

Results
Solubilisation of zinc

- **Ratio A** – yeast at 10^6 cells/mL & bacteria at 10^8 cells/mL
- **Ratio B** – yeast at 10^8 cells/mL & bacteria at 10^8 cells/mL
- **Ratio C** – yeast at 10^6 cells/mL & bacteria at 10^{10} cells/mL





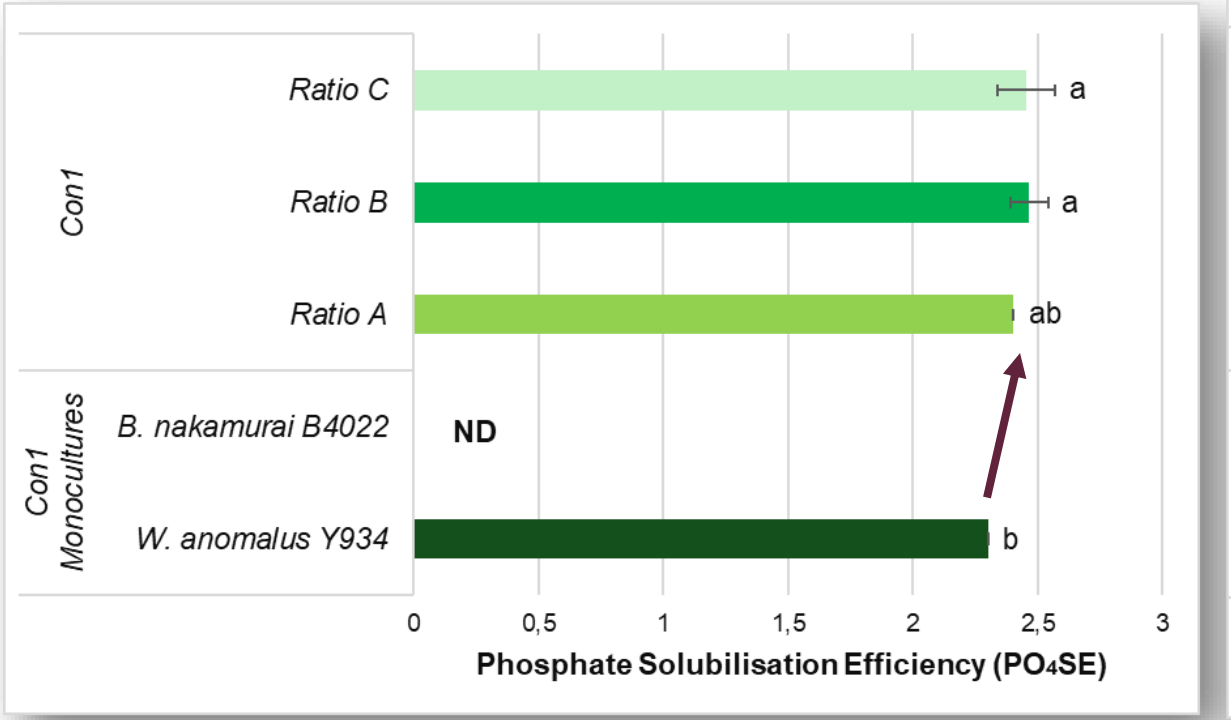
An enhanced zinc solubilisation effect by the consortia

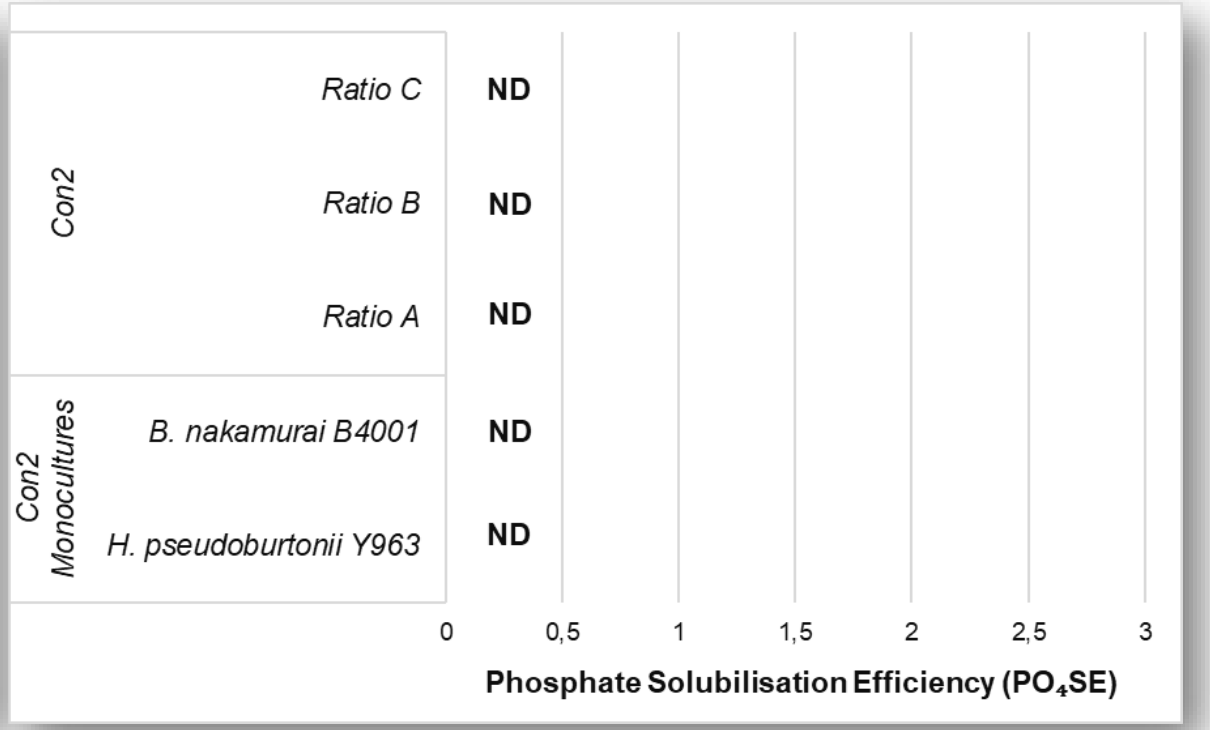
- **Could be due to increased organic acid production & siderophores in coculture setting – ↑ solubilisation**

Results

Solubilisation of phosphate

- **Ratio A** – yeast at 10^6 cells/mL & bacteria at 10^8 cells/mL
- **Ratio B** – yeast at 10^8 cells/mL & bacteria at 10^8 cells/mL
- **Ratio C** – yeast at 10^6 cells/mL & bacteria at 10^{10} cells/mL



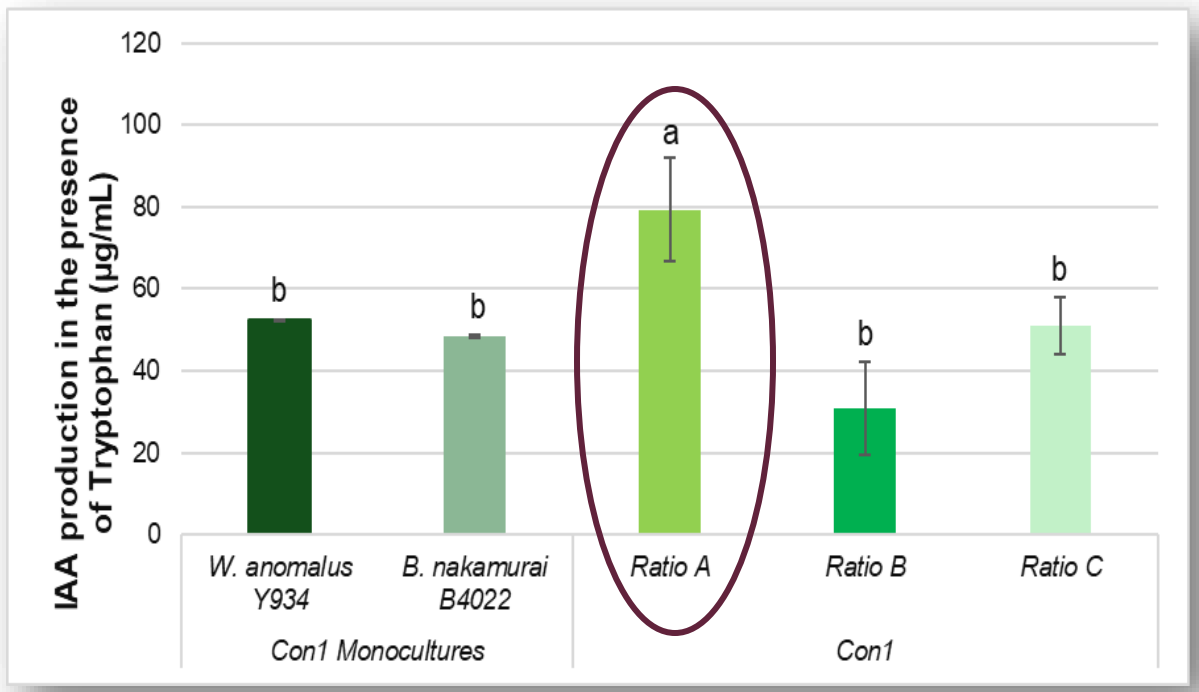


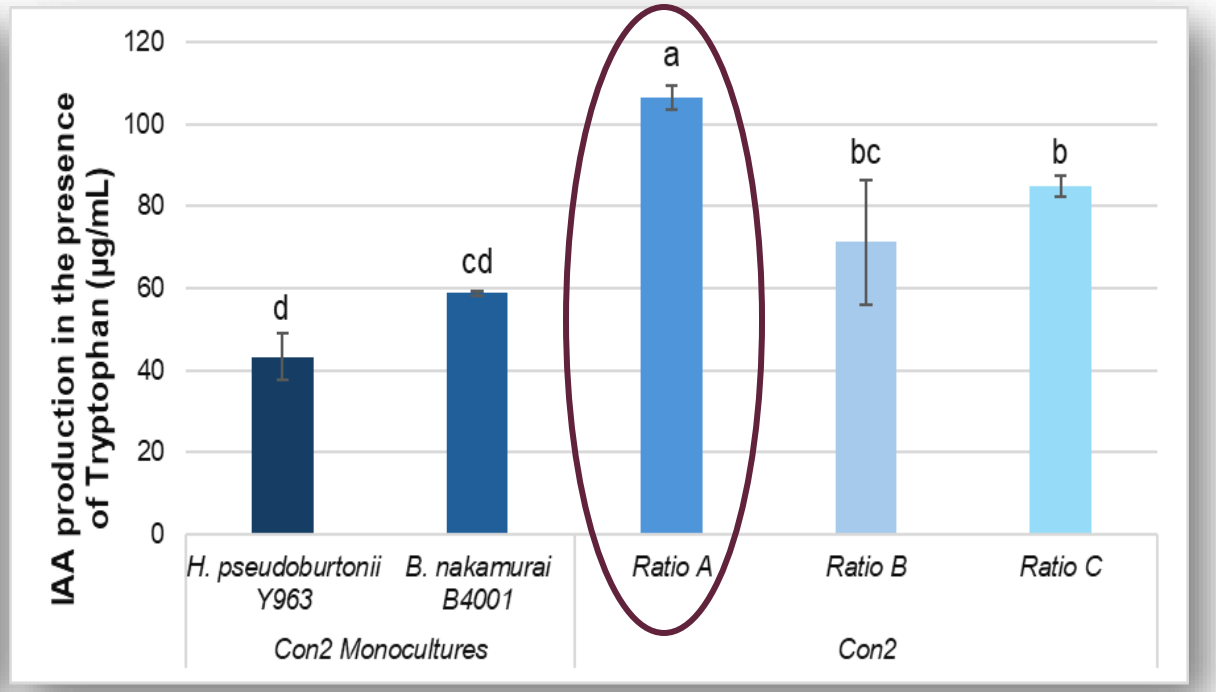
A minor enhanced phosphate solubilisation effect by Con1

Results

Production of indole acetic acid (IAA)

- **Ratio A** – yeast at 10^6 cells/mL & bacteria at 10^8 cells/mL
- **Ratio B** – yeast at 10^8 cells/mL & bacteria at 10^8 cells/mL
- **Ratio C** – yeast at 10^6 cells/mL & bacteria at 10^{10} cells/mL



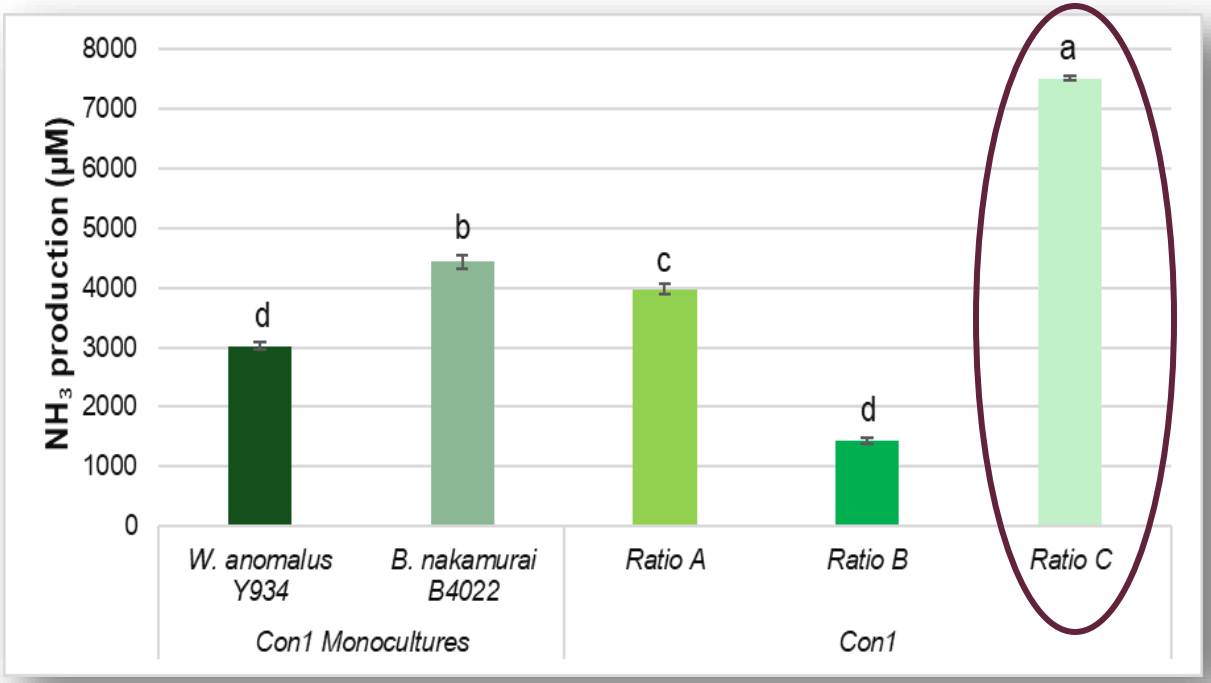


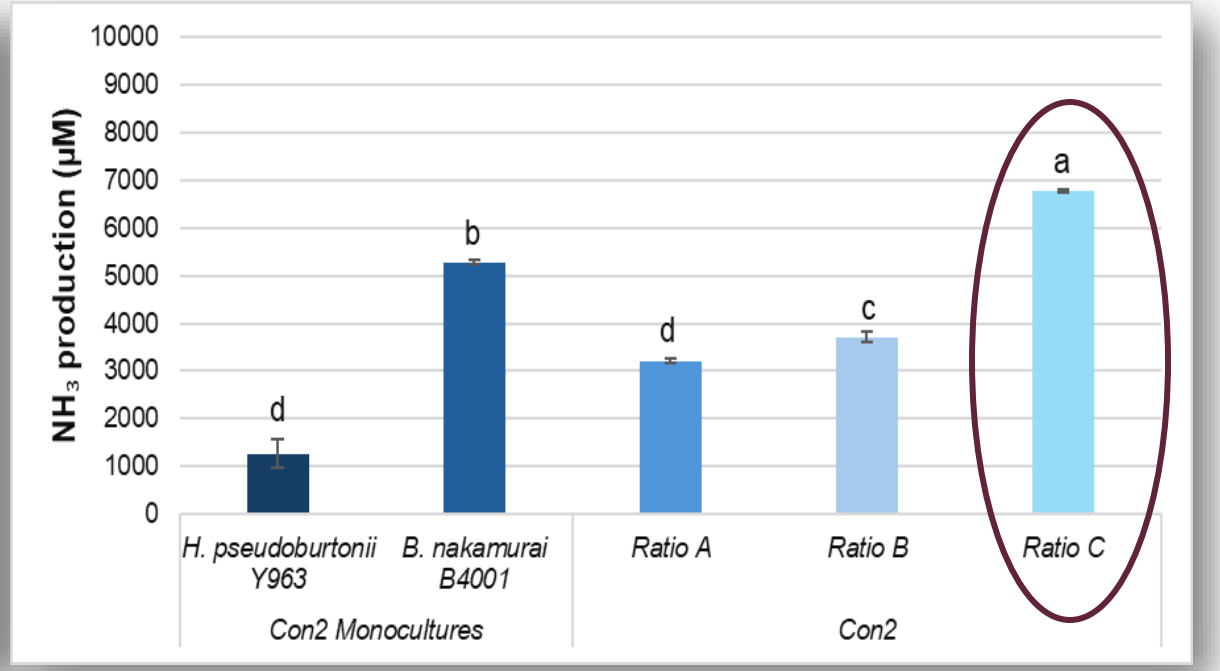
- **Ratio dependent responses of consortia for IAA production**
- **Overall enhanced IAA production by ratio A for both consortia**
  - **Additive interaction between yeast & bacterium = ↑ IAA**

Results

Production of ammonia

- **Ratio A** – yeast at 10^6 cells/mL & bacteria at 10^8 cells/mL
- **Ratio B** – yeast at 10^8 cells/mL & bacteria at 10^8 cells/mL
- **Ratio C** – yeast at 10^6 cells/mL & bacteria at 10^{10} cells/mL





- **Ratio dependent responses of consortia for ammonia production**
- **Overall enhanced ammonia production by ratio C for both consortia**
  - **Due to the ↑ concentration of bacteria = ↑ NH_3**

Results

In vitro PGP summary for consortia

- Consortia outperform the monocultures
- Synergy of the consortia depends on the ratio & the production of the specific compound
  - IAA - Ratio A
  - NH_3 - Ratio C
- No specific ratio shows enhanced effects for all PGP characteristics

Both consortia show *in vitro* PGP potential

PGP potential of consortia

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Do the consortia stimulate plant growth when applied as soil inoculants?

Methodology

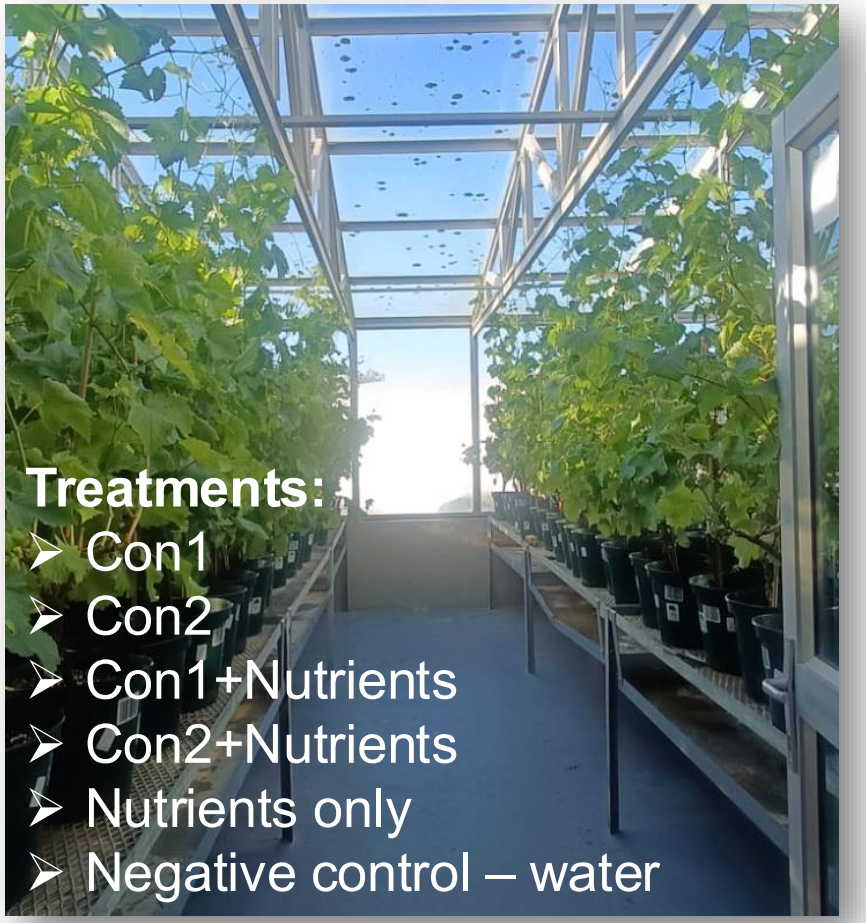
Trial 1: Soil application of consortia

Trial 1: 2024/2025 season

Two-year-old Chenin Blanc
- SN220/R99

Application:
- **Ratio A**
- **100 mL**
- Rootzone area
- 2 applications
- **Beginning of flowering**

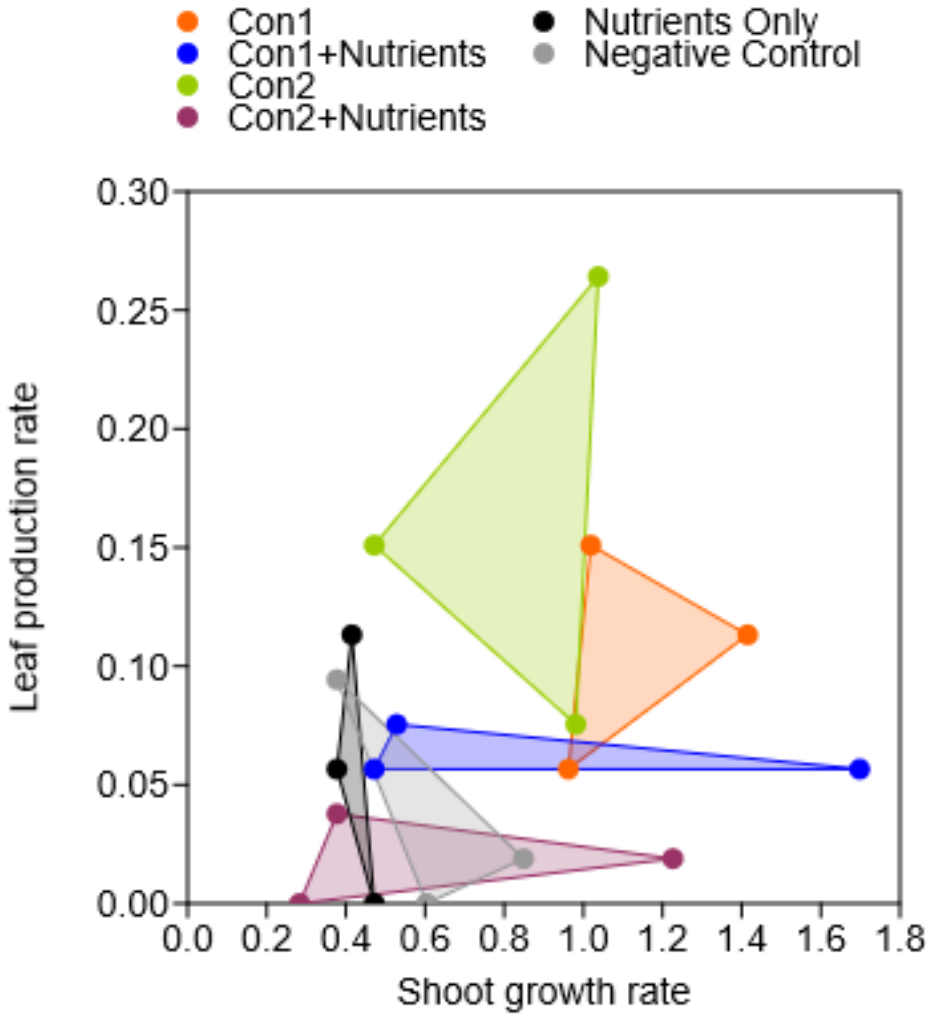
Vegetative measurements:
- Shoot length & leaf count recorded every 10 days
- **1 shoot per vine**
- Leaf area of top, middle and bottom leaves recorded after 7 weeks



Results

Trial 1: Vegetative measurements

Shoot growth rate & leaf production rate impact after 7 weeks



Treatment	SGR & LPR Correlation
Con1	0,227901422
Con1+Nutrients	-0,464178573
Con2	0,20441908
Con2+Nutrients	0,09078413
Nutrients Only	-0,596039561
Negative Control	-0,740612897

Treatments show different correlations

- Controls show moderate to strong negative correlations – could indicate external impact.

Results

Trial 1: Vegetative measurements

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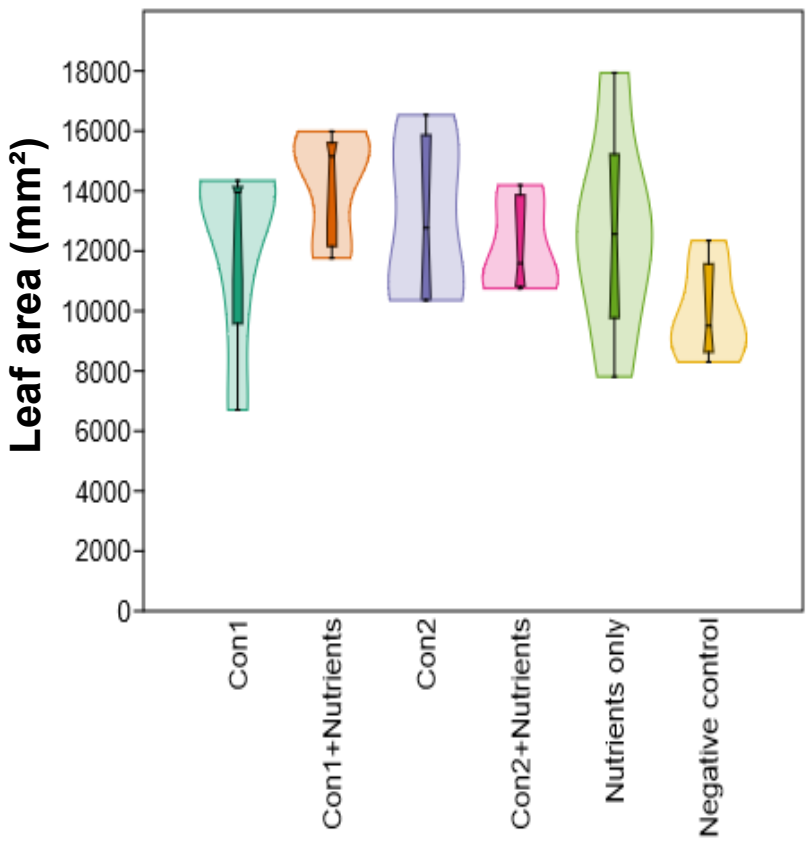
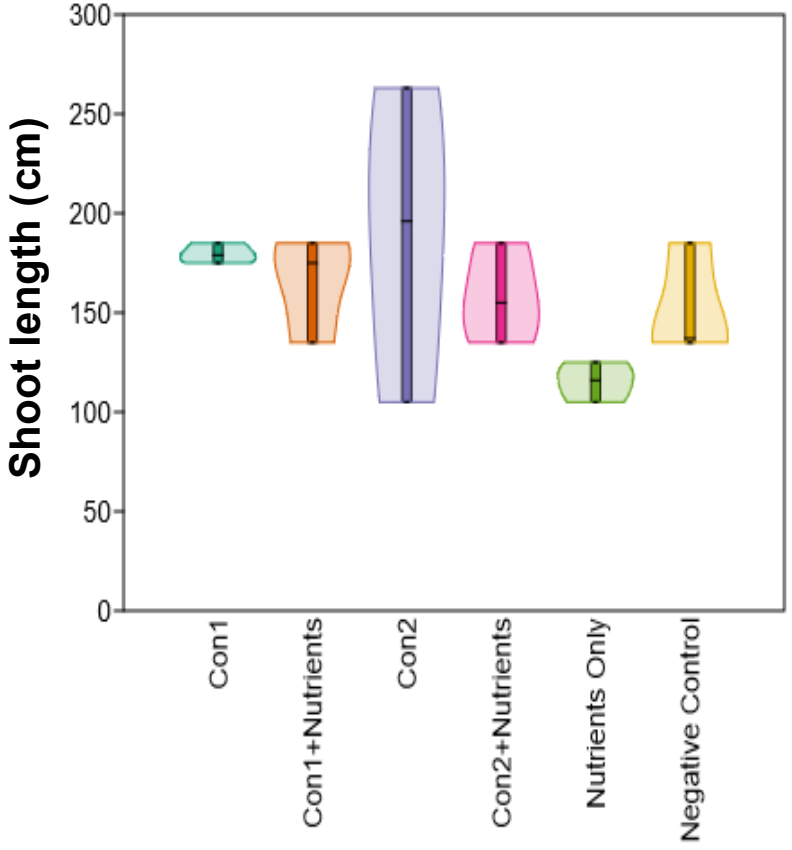
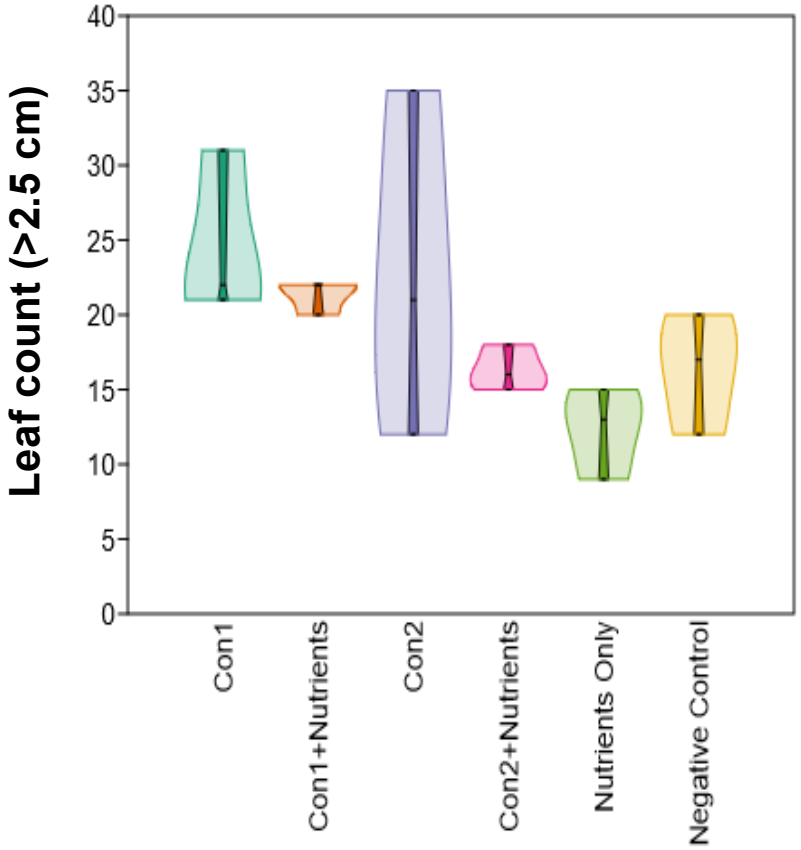
63 DAF







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Methodology

Trial 2: Soil application of consortia

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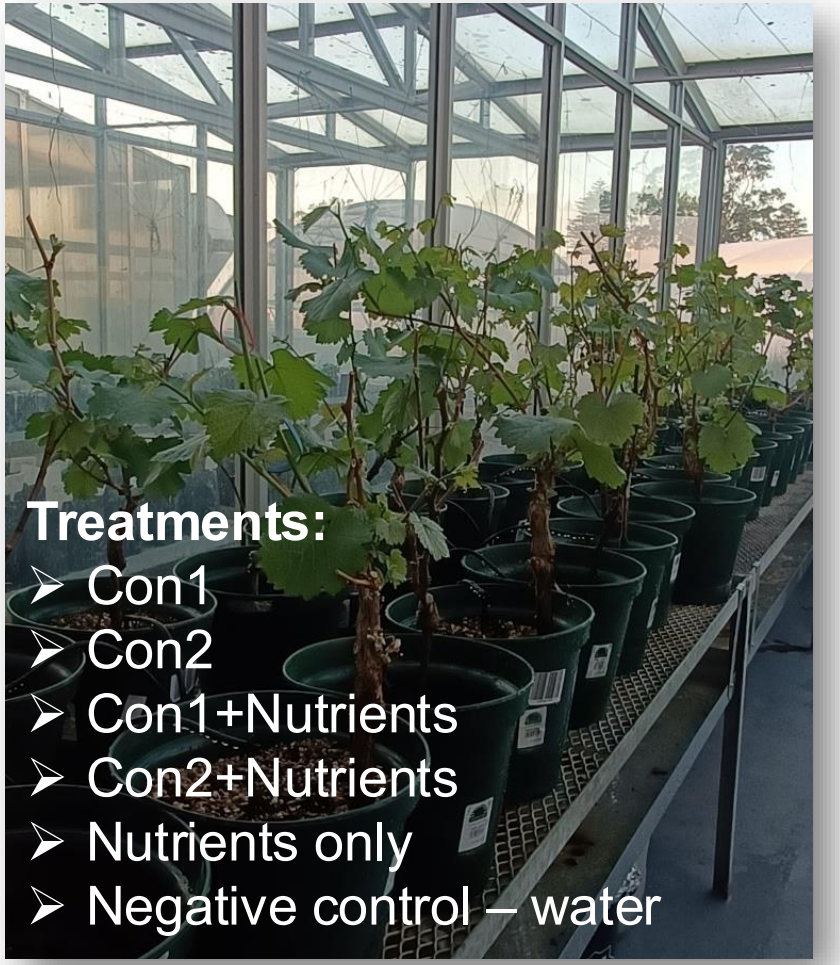
Trial 2: 2025/2026 season

Three-year-old Chenin Blanc
- SN220/R99

Application:
- **Ratio C**
- **150 mL**
- Rootzone area
- 2 applications
- **Budbreak**

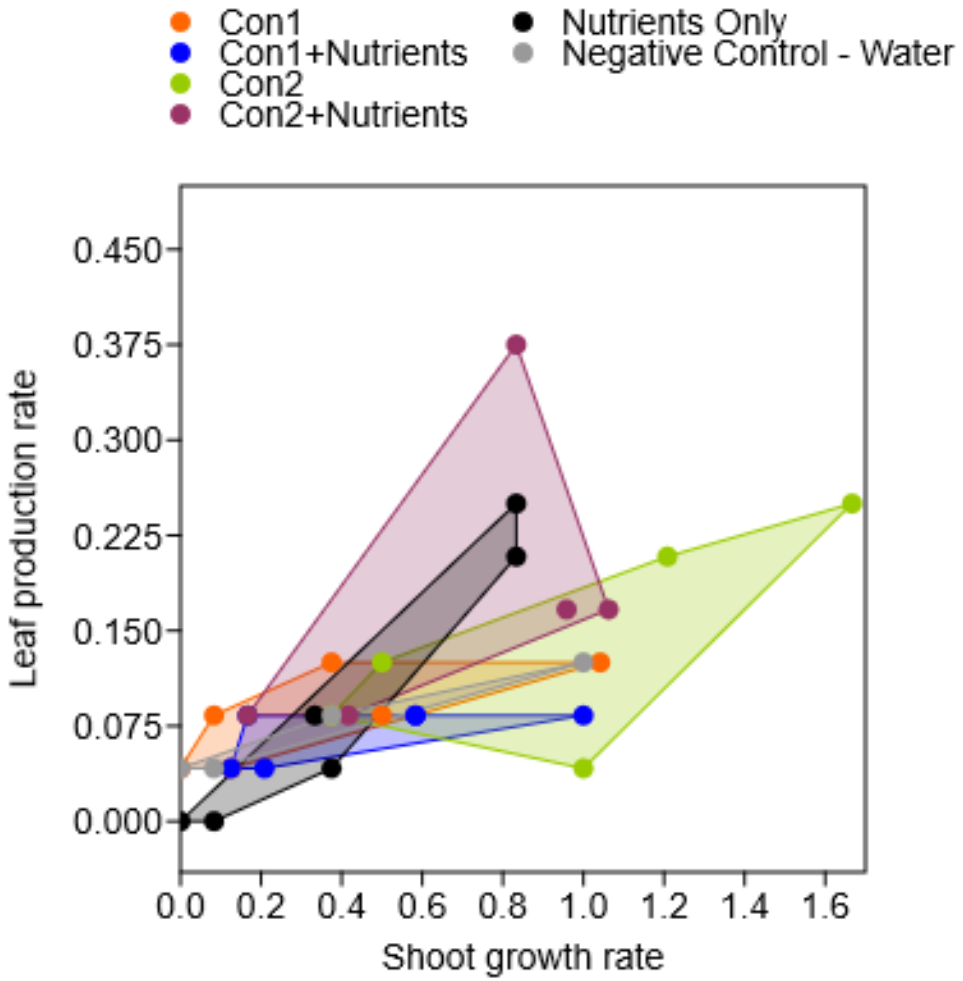
<u>Vegetative measurements:</u>
- Shoot length & leaf count recorded weekly
- **2 shoots per vine**
- **Leaf area**, **shoot** & **root fresh** and **dry weight** recorded after 7 weeks



Results

Trial 2: Vegetative measurements (preliminary results)

Shoot growth rate & leaf production rate impact after 3 weeks



Treatment	SGR & LPR Correlation
Con1	0,747911333
Con1+Nutrients	0,630566661
Con2	0,744741807
Con2+Nutrients	0,530252626
Nutrients Only	0,968010711
Negative Control	0,984504195

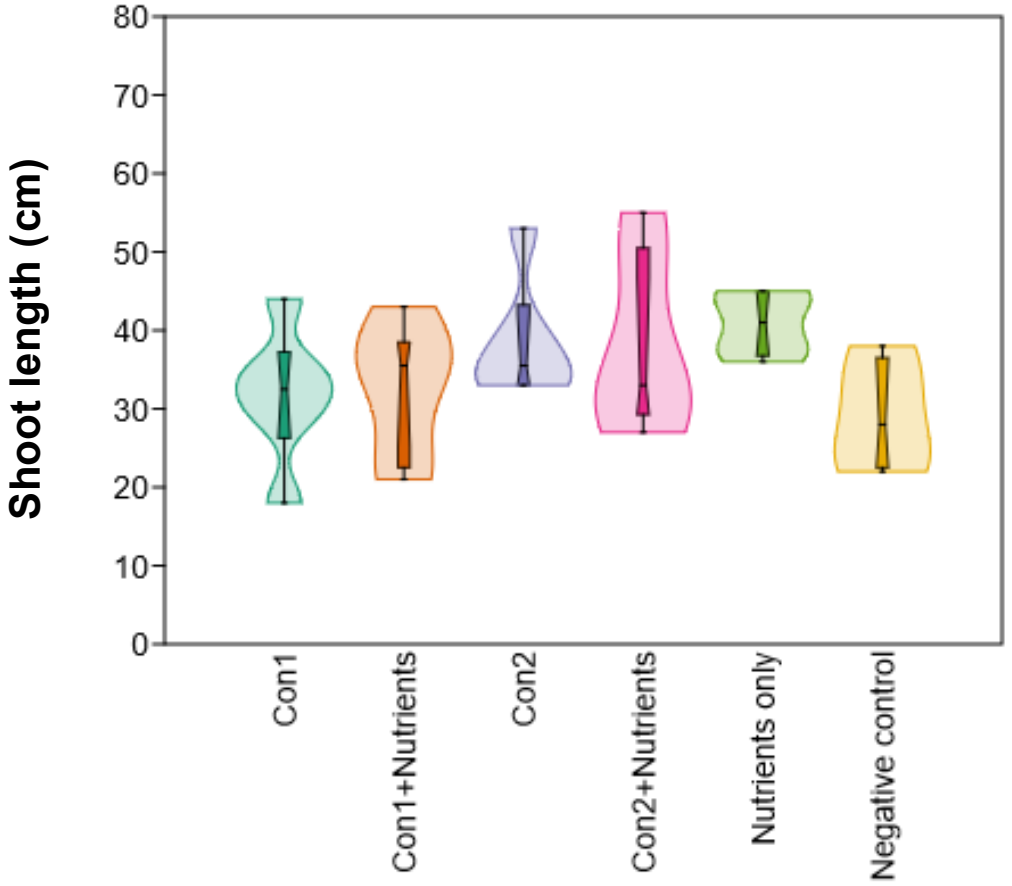
All treatments show positive correlations

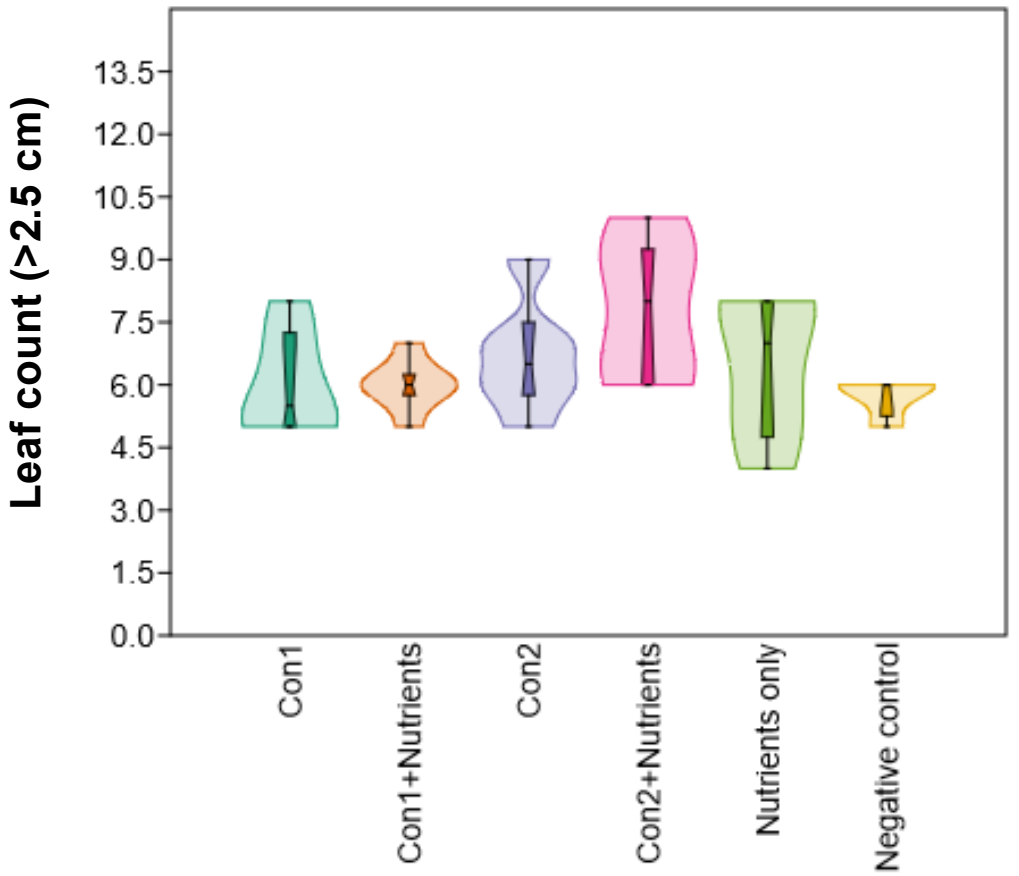
- Controls show the strongest positive correlation between shoot growth & leaf production rates (darkest blue), followed by consortia treatments.

Results

Trial 2: Vegetative measurements (preliminary results)

28 DAB





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Results

Soil inoculation PGP summary for consortia

- No distinctive treatment effects from soil application
- Variability between replicates in Trial 1 – suggests external impacts such as environmental variables rather than consortia treatments.

Take home points

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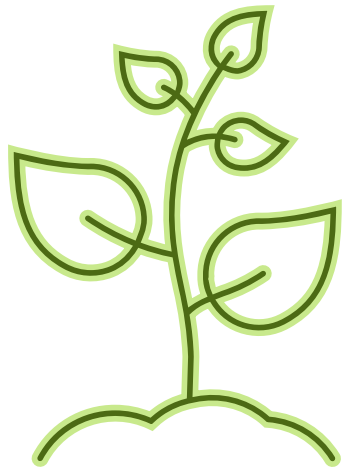
PGP Potential *in vitro* ≠ PGP in plants

- Due to complex interactions occurring between microorganism-microorganism & microorganism-plants.
- No significant treatment effects in plants could be due to:
  - Application type
  - Impacts of microbial treatments may only be observed over multiple seasons
  - Environmental impacts

O'Callaghan *et al*. 2022

Future work

- Further evaluation of growth parameters in Trial 2 (shoot and root weights).
- Further validating effectiveness of consortia in disease suppression.



Acknowledgements

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ES Lab group – planting

Funders