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SAGWRI South African Grape and Wine Research Institute

Plant biostimulants evidence for protection against both biotic and abiotic stress in wine grapes – a role for the microbiome?

or

Plant biostimulants what do they do?

Presenter: Dr John Moore
Contributors: Nicole Venter, Henry Viljoen, Mokgantsho Moroke and Elna Kruger

26 Nov 2025

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“ A plant biostimulant is any substance or microorganism applied to plants with the aim to enhance nutrition efficiency, abiotic stress tolerance and/or crop quality traits, regardless of its nutrients content. ” Du Jardin 2015

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INDIAN EXPRESS,ND 16 JULY 2025

DIRECTS OFFICIALS TO PUT IN PLACE 'CLEAR RULES & SOP'

Won't allow sale of biostimulants without scientific nod: Chouhan

EXPRESS NEWS SERVICE
NEW DELHI, JULY 15

TWO DAYS after he asked chief ministers of states to immediately stop forced sale of nano-fertilisers and biostimulants to farmers, Union Agriculture Minister Shivraj Singh Chouhan Tuesday said the government will not allow the sale of biostimulants without scientific nod.

He directed the officials of his ministry to put in place "clear rules and SOPs (standard operating procedures)" for the sale of biostimulants.

Chairing a meeting, Chouhan said that farmers raised numerous complaints about the sale of fake fertilisers, seeds, biostimulants, and nano urea during his visits to villages as part of recently concluded 15-day 'Viksit Krishi Sankalp Abhiyan', where he held direct interactions with them.

Chouhan questioned why biostimulants, despite repeated renewals and years of sales, were

Farmers raised complaints about sale of biostimulants as part of 15-day Viksit Krishi Sankalp Abhiyan: Chouhan

still in the market even after several complaints stated that they were ineffective.

"Now, only those biostimulants that meet all the criteria and are proven beneficial to farmers will be approved. Approvals will now be based solely on scientific validation, and the responsibility for this will rest entirely with the concerned officials," he said, according to an official statement.

Many companies started selling biostimulants without any rules, but the government will not let this happen at any cost, he said, adding that the government will take action against suspicious manufacturers of biostimulants.

"It is necessary to review biostimulants thoroughly to see how much benefit the farmers are getting from it; if not, then permission to sell it cannot be given," Chouhan said.

He also emphasised the need for the Indian Council of Agricultural Research (ICAR) to evaluate biostimulants.

Chouhan instructed the officials to put in place clear rules and SOPs, and said that irregularities will no longer be tolerated.

Biostimulants are substances or microorganisms (such as beneficial bacteria, fungi, or plant extracts) that, when applied to seeds, plants, or soil, stimulate natural plant processes.

The minister pointed out that for several years, around 30,000 biostimulant products were being sold unchecked, and even in the last four years, around 8,000 products remained in circulatior "After I enforced stricter checks the number has now come dowi to around 650," he said.

It is learnt that the meetin was called impromptu and n notice was given to officials. Th meeting was attended by the of ficials of the Ministry o Agriculture and Farmers Welfar and the ICAR.

The biostimulants market i India was valued at approxi mately USD 355–362 million i 2024 and is projected to reacl between USD 1.13 billion an USD 1.2 billion by 2032.

On July 13, Chouhan ha asked the state governments t immediately stop the "force tagging" of nano-fertilisers o biostimulants along with con ventional fertilisers such as ure and diammonium phosphat (DAP). In a letter to chief minis ters, Chouhan highlighted com plaints that retailers are not sell ing subsidised conventiona fertilisers such as urea, DAP, et to farmers unless they purchas nano-fertilisers or biostimulant *horticulturae*

Article

Plant Biostimulants Enhance Bud Break in *Vitis vinifera* Crimson Seedless Using Combination Treatments

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[*] Correspondence: moorejp@sun.ac.za

Grapevine compound buds which were formed during the previous growth season give rise to new shoots.

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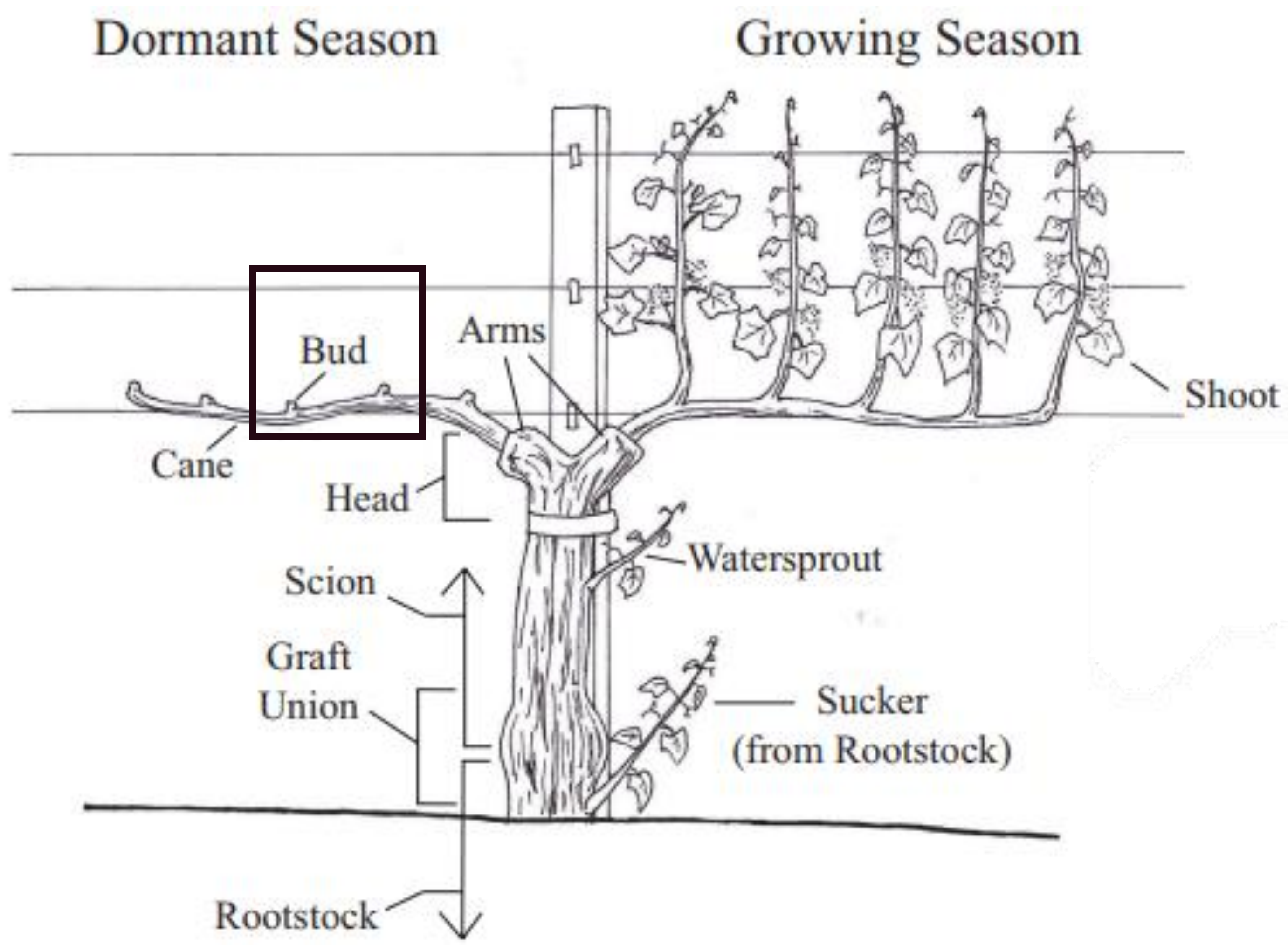

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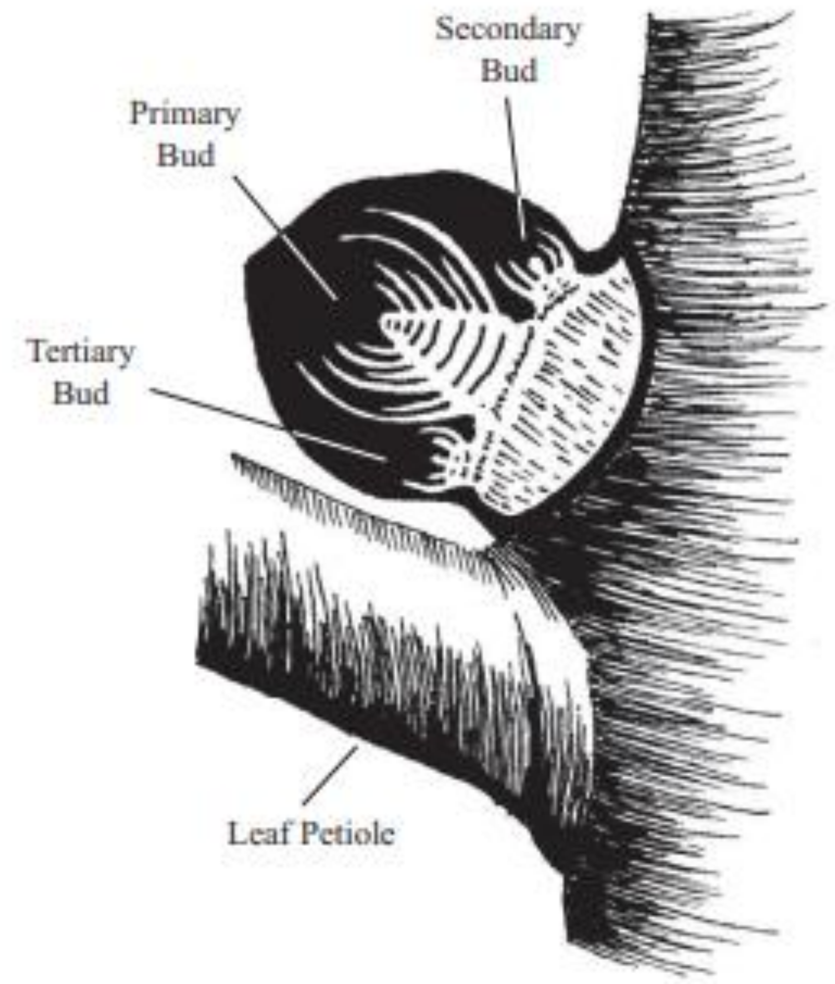

Hellman, 2003

Dormancy Progression and Cold Hardiness

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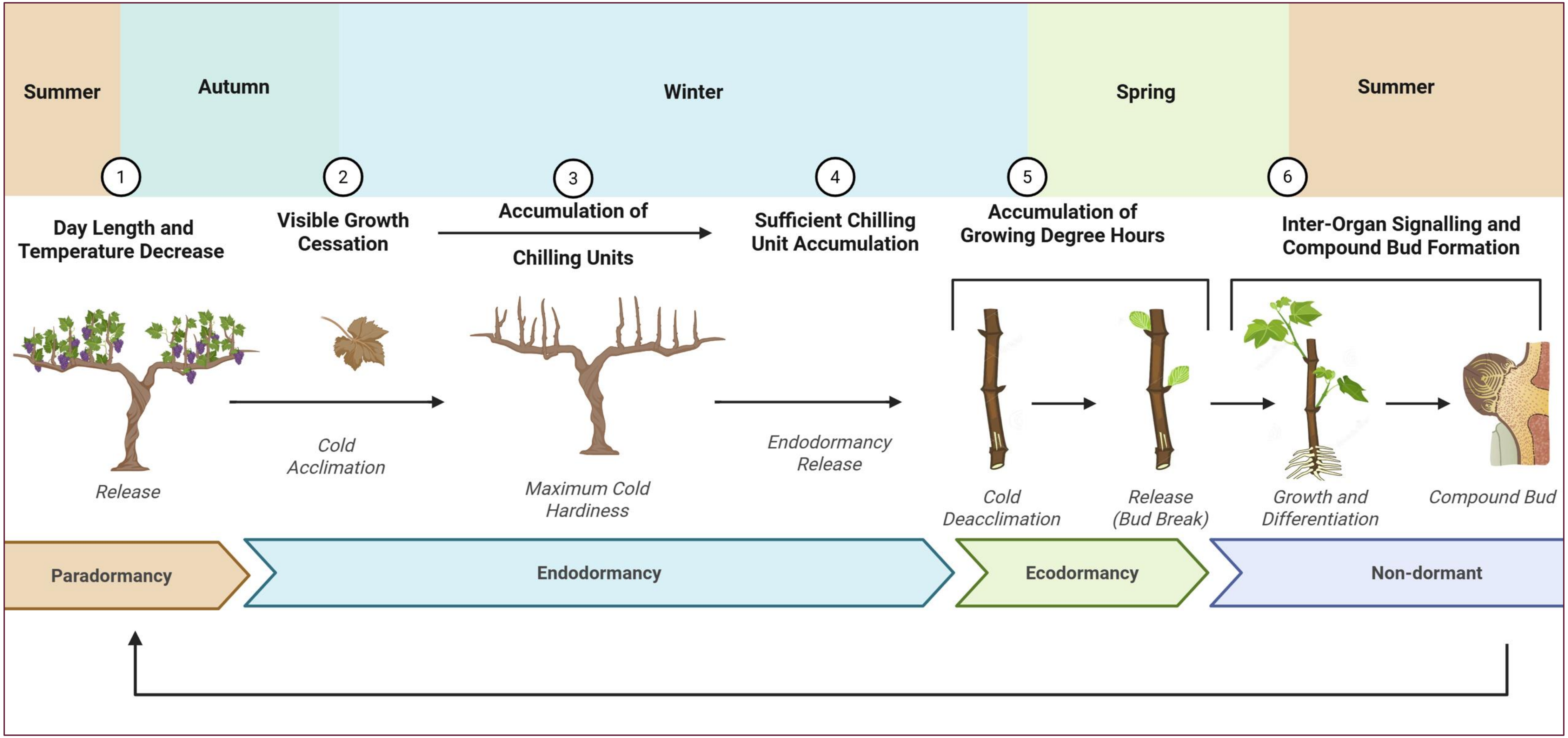


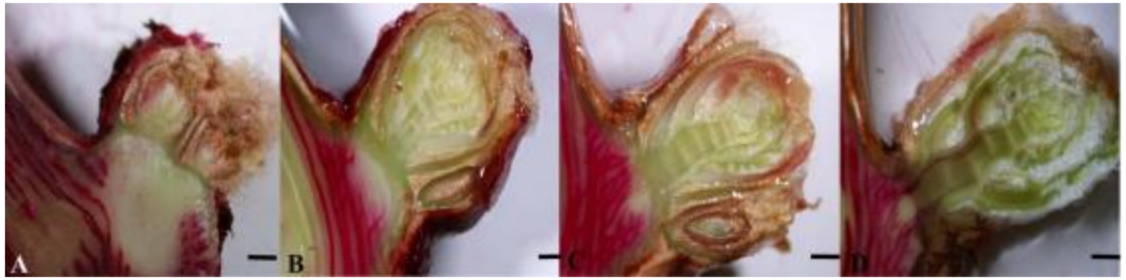
Hellman, 2003; Yang *et al.*, 2018; Keller, 2020

Winter chill is required for bud-break

- High bud-break percentage (>80%): 200 h between 0 and 10°C
- High and even bud-break: 400 h at 3°C

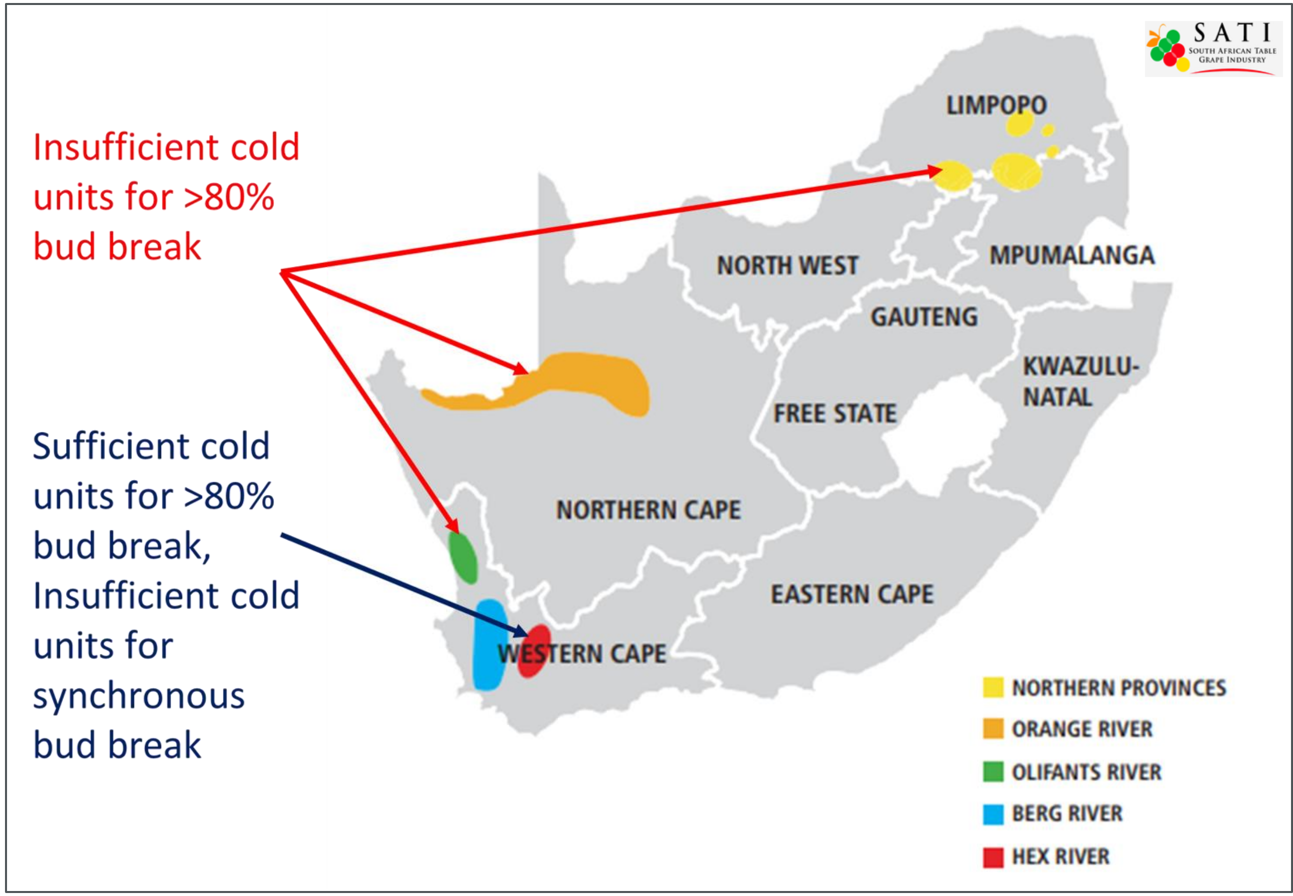
Dokoozlian & Willliams, 1995; Dokoozlian, 1998; Dokoozlian, 1999.

A.



B.

A. Xie et al., 2018; B. Keller, 2020b.



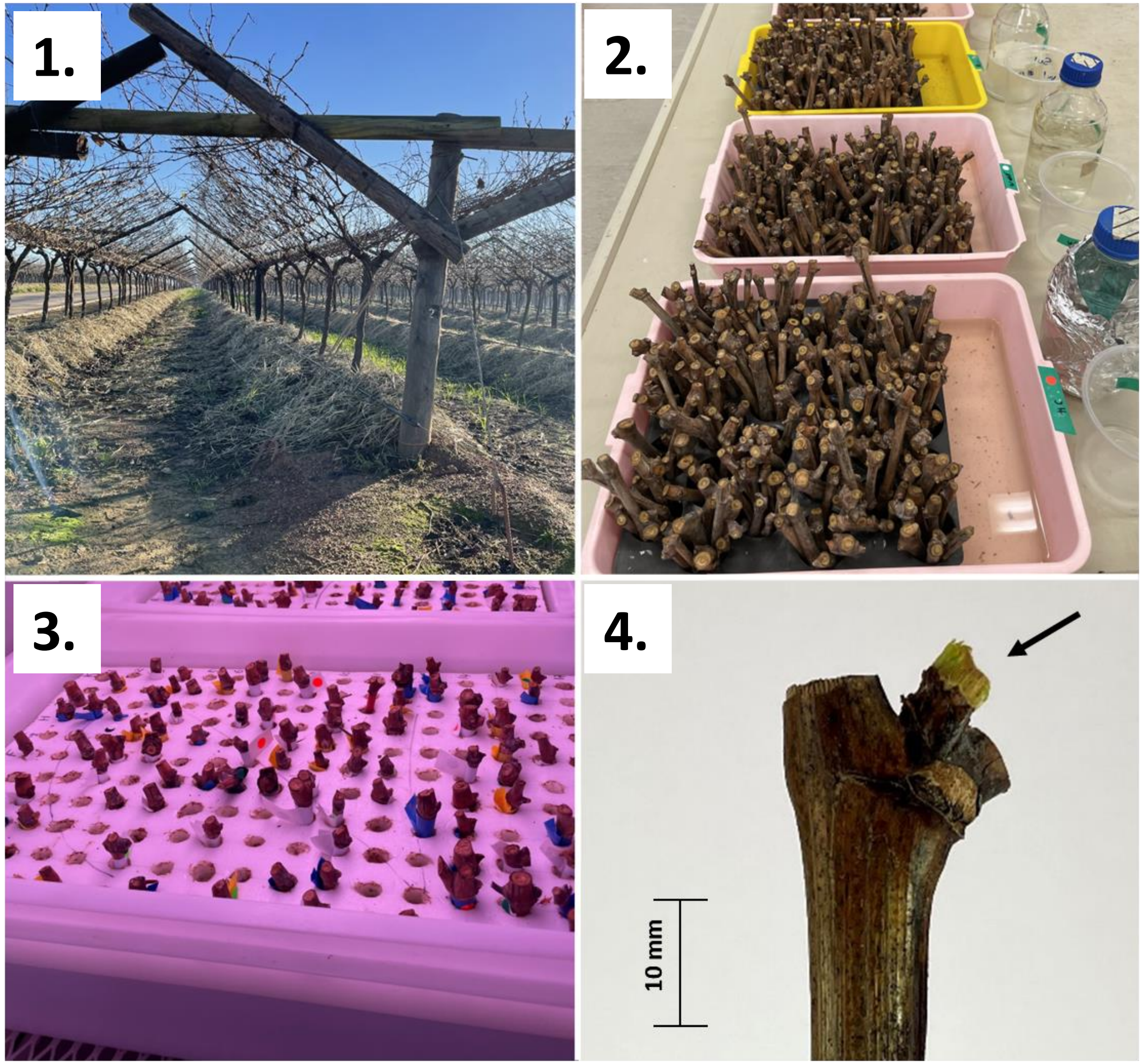
Avenant & Avenant 2014.

Materials and methods

Nicole Venter

- Crimson Seedles
- Windmeul farm, Hoekstra fruit, Paarl
- Infruitec Units

- 22°C, 16/8h photoperiod
- Randomized block
- Biol reps – 30 (2021)/ 45 (2022)



- Contact fungicide
- 4°C in darkness
- Dipped in treatments

Bud-break: First visible green tissue/leaf tips become visible underneath the bud scales (EL-4)

Or et al., 2000; Keller, 2020

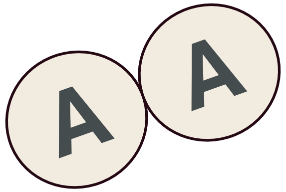
Finding Alternative Dormancy Breakers

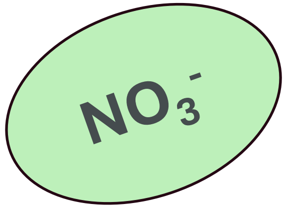
?

Traditional (< Efficient than Dormex)

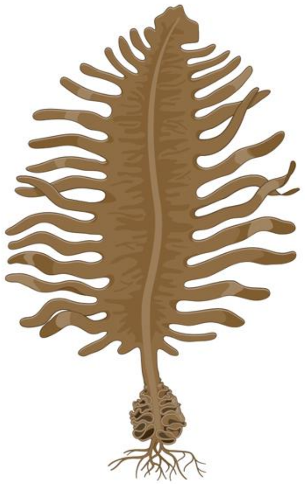
"Next-Gen"

Mineral oils, Amino acids, Nitrogen donors, GA_4, Ca^{2+}





Plant biostimulants



Sassine *et al.*, 2019; Loubser & Hills, 2020; Jindo *et al.*, 2022; Venter *et al.*, 2024; Boulogne *et al.*, 2024

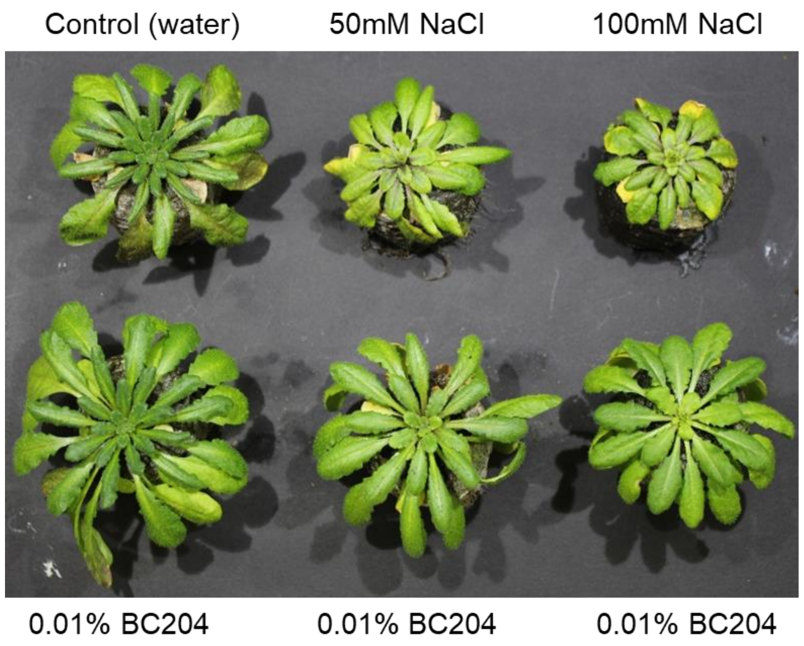
Treatments 2021: Candidate biochemical agents and plant biostimulants

Treatment	Relevance to dormancy-release molecular models	Application concentration	Treatment code name
Dormex®	Positive control	3% v/v (Bothello et al., 2016)	HC 3%
Distilled water	Negative control	NA	dH_2O
H_2O_2	ROS	1% v/v (Vergara et al., 2012a)	H_2O_2 1%
S-Nitrosoglutathione	NO donor	10 µM (Egbichi et al., 2014)	SNO 10 µM
Diethylamine NONOate sodium salt hydrate	NO donor	10 µM (Egbichi et al., 2014)	NO2 10 µM
Lumichrome	Riboflavin derivative (7,8 dimethylalloxazine)	5 nM (Pholo et al., 2018)	Lum 5 nM
BC204 (Commercial plant biostimulant)	Citrus-based plant extract	0.05% v/v (Loubser & Hills 2020)	BC204 0.05%
BUDBREAK® mineral oil	Hypoxia	3% v/v (Sagredo et al., 2013)	BB 3%
Ethephon	Ethylene supplement	0.206 % v/v (Vergara et al., 2012a)	Eth 1000 ppm

Treatments 2021: BC204, Lumichrome and candidate biochemical agents

BC204 (registered biostimulant)

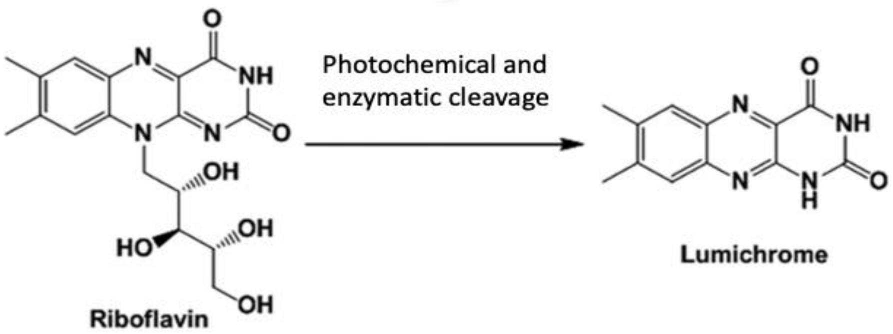
- Citrus-based plant extract
- Used, South Africa, China and USA – variety of crops (**CS for colour enhancement**)
- Increases crop yield, fruit quality & plant health



BC204 improves salinity stress. Loubser and Hills, 2020.

Lumichrome (7,8 dimethylalloxazine)

- *Sinorhizobium meliloti:* Riboflavin a prevalent rhizosphere



The effect of lumichrome treatment after 5 weeks compared to that of untreated plants. Pholo et al., 2018

Key findings 2021 forced bud-break assays

- BB 3% - earliest **onset** in all three experiments, followed by H_2O_2 1% (p=0)

- **Rates** compared to the negative control
  - The rates of HC 3% treatments were significantly higher after 100 and 400 CUA (p=0)
  - NO2 10 µM consistently had significantly higher rates (p=0 to 0.02)
  - Rate of NO2 10 µM after 200 CUA was significantly higher than that of HC 3% (p=0)

- **EC50** of HC 3% were the lowest across experiments (p=0)
  - BB 3%, followed by H_2O_2 1% & had significantly lower EC50s than negative control (p=0 to 0.02)

- **Final bud-break %** - all above 90%
  - Controls similar (negative control only sig higher at 400h, p=0)
  - SNO 10 µM, and NO2 10 µM, higher than negative control after 100 and 200 CUA (p=0 to 0.01)

Literature: agrochemicals / biostimulants

SYNCRON Composition

Free Amino Acids .... 2.0% w/w
Total Nitrogen .... 0.3% w/w
Organic Nitrogen .... 0.3% w/w
Total Organic Matter ...80.0% w/w

NITROACTIVE Composition

Total Nitrogen.........................17.0% w/w (25.8% w/v)
Nitric Nitrogen.....................11.5% w/w (17.5% w/v)
Ammoniacal Nitrogen...........5,5% w/w (8.4% w/v)
Calcium (CaO) water soluble..12.3% w/w (18.7% w/v)

+ Mineral oil (3.5%) /Calcium nitrate (6%)

ERGER

NITROGEN FERTILIZER SOLUTION WITH CALCIUM (15-0-0)

GUARANTEED ANALYSIS

Total Nitrogen (N) 15.0%
Ammoniacal Nitrogen 3.1%
Nitrate Nitroge 5.8%
Urea Nitrogen 6.1%
Calcium (Ca) 3.3%
Derived from: Ammonium Nitrate, Calcium Nitrate, Urea.

Key findings 2022 forced bud-break assays

- **Onset**
  - HC 3% 100 CUA lowest (p=0), compared to negative control: 200 CUA lower (p=0), 250 CUA similar
  - SNH, similar to HC 3% after 200 CUA & lowest after 250 CUA (p=0)

- **Rates** of HC 3% slowest & negative control, fastest (p=0)
  - **Exception: MIX2 100 CUA – highest rate (p=0)**

- **EC50**
  - HC 3% lowest at 100 CU.. Longest after 250 CU (p=0)
  - SNH lowest after 200 & 250 CU!!

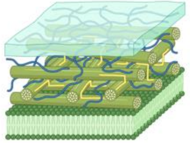
- **Final bud-break %:** all above 90 % except HC 3% and SNH after 250 CUA

Problem Statement

Henry Viljoen

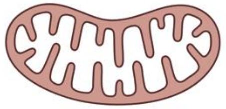
1 **Limited literature** discussing the **metabolic** and **cellular changes** in compound buds of *Vitis vinifera* Crimson Seedless during dormancy progression in South African climates

Hypothesis 1.1



Cold stress responses allow cold acclimation and induces the onset of endodormancy in compound buds

Hypothesis 1.2



Oxidative stress metabolism and cell wall compositional changes allow endodormancy release and cold deacclimation in compound buds

2 **Plant biostimulants** have not been extensively **evaluated** for their potential to induce or improve **bud-break** in *Vitis vinifera* Crimson Seedless (CS)

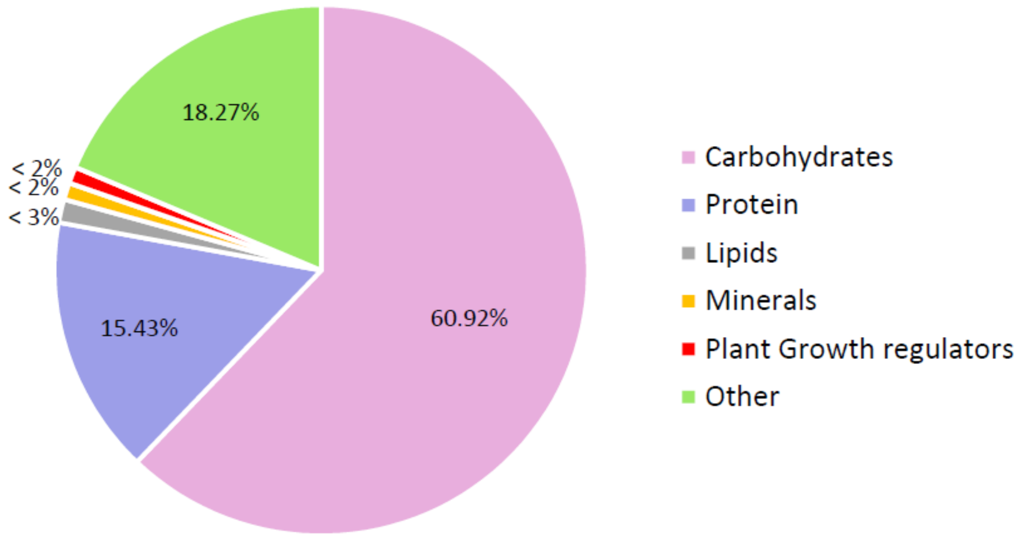
Hypothesis 2

Plant biostimulants and/or their combinations with mineral oils and slow-releasing nitrogen donors, enhance bud-break percentage and uniformity

Seaweed Extracts as Biostimulants

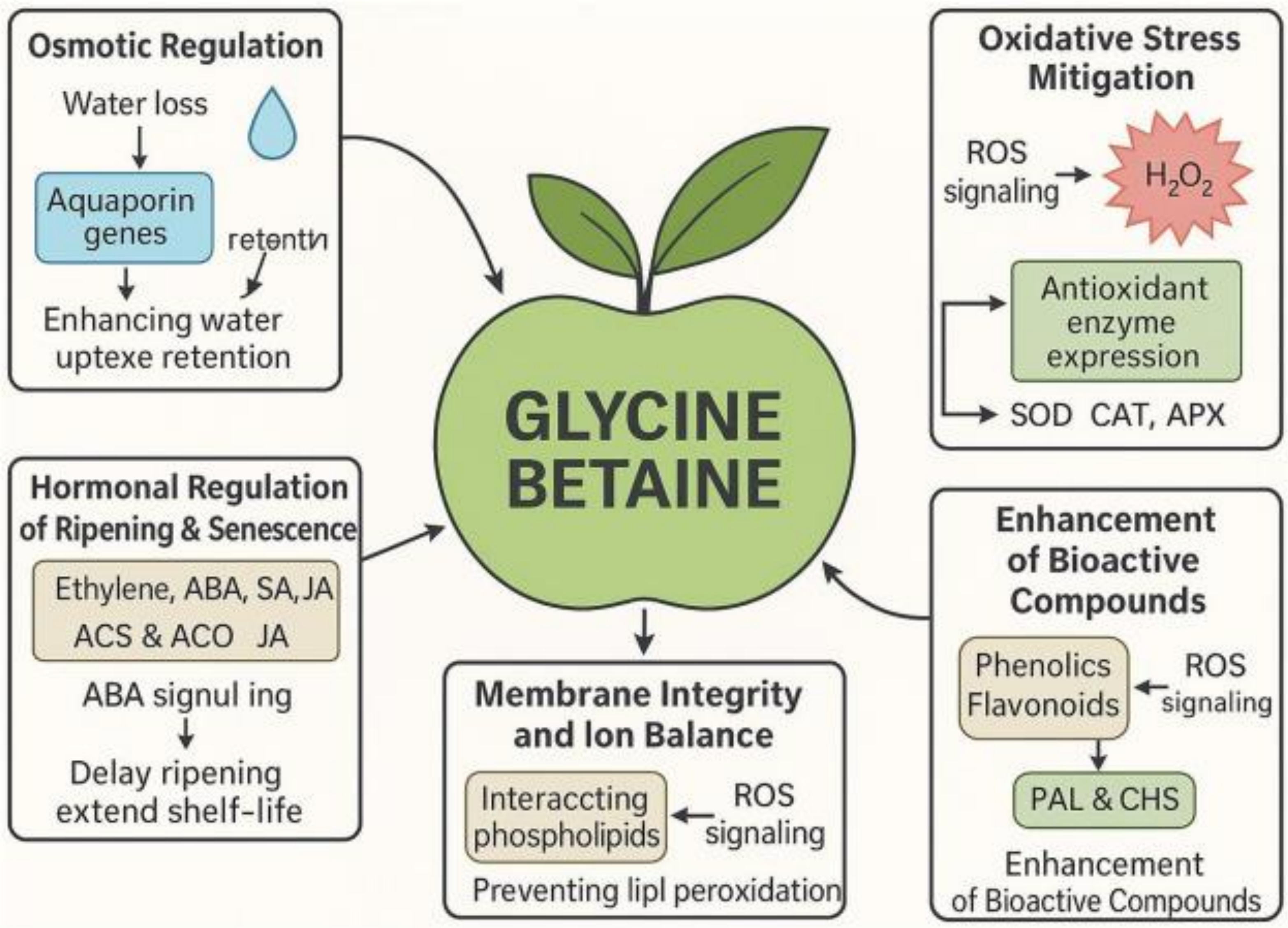
- Most commercial seaweed extract (SE) products use **brown (*Phaeophyceae*) seaweed species e.g.**
  - *Ascophyllum nodosum (Northern hemisphere)*
  - *Ecklonia maxima (Southern hemisphere)*

- **Complex mixtures** of carbohydrates, amino acids, phytohormones, osmoprotectants, and proteins



- **Difficult to ascribe specific effects to individual components** of SE
  - Many bioactive compounds, possible synergistic effect

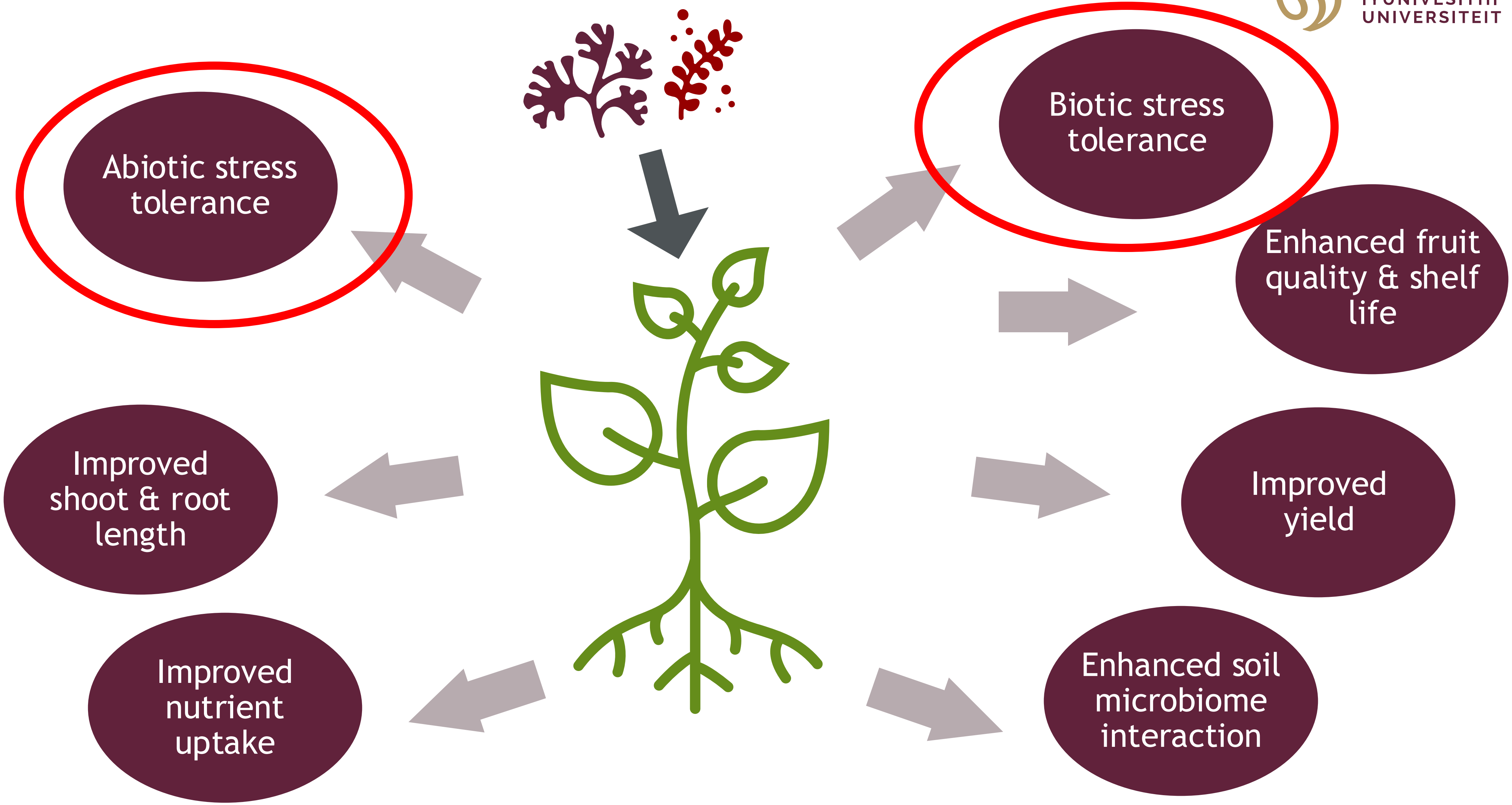
(Deolu-Ajayi et al., 2022, Battacharyya et al., 2015, Ali et al., 2021)



Mokgantsho Moroke

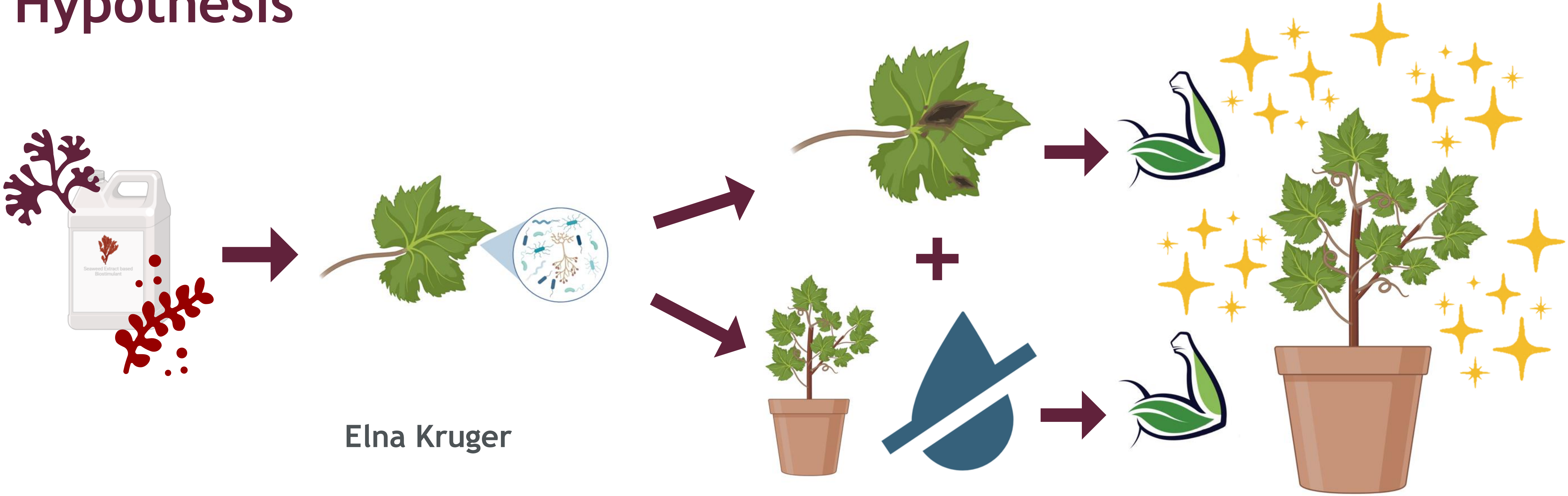
Sanusi Shamsudeen Nassarawa, Asiya Rabiu, Faiz Ur Rahman, Asem M. Abdelshafy, Xiaoyang Zhu, Gulzar Ahmad Nayik,
Glycine betaine: A non-toxic approach to enhancing postharvest quality and physiology of fruits and vegetables,
Journal of Stored Products Research,
Volume 114, 2025,102726,

Overview: Seaweed Extracts Effects



(Gautam et al., 2024)

Hypothesis

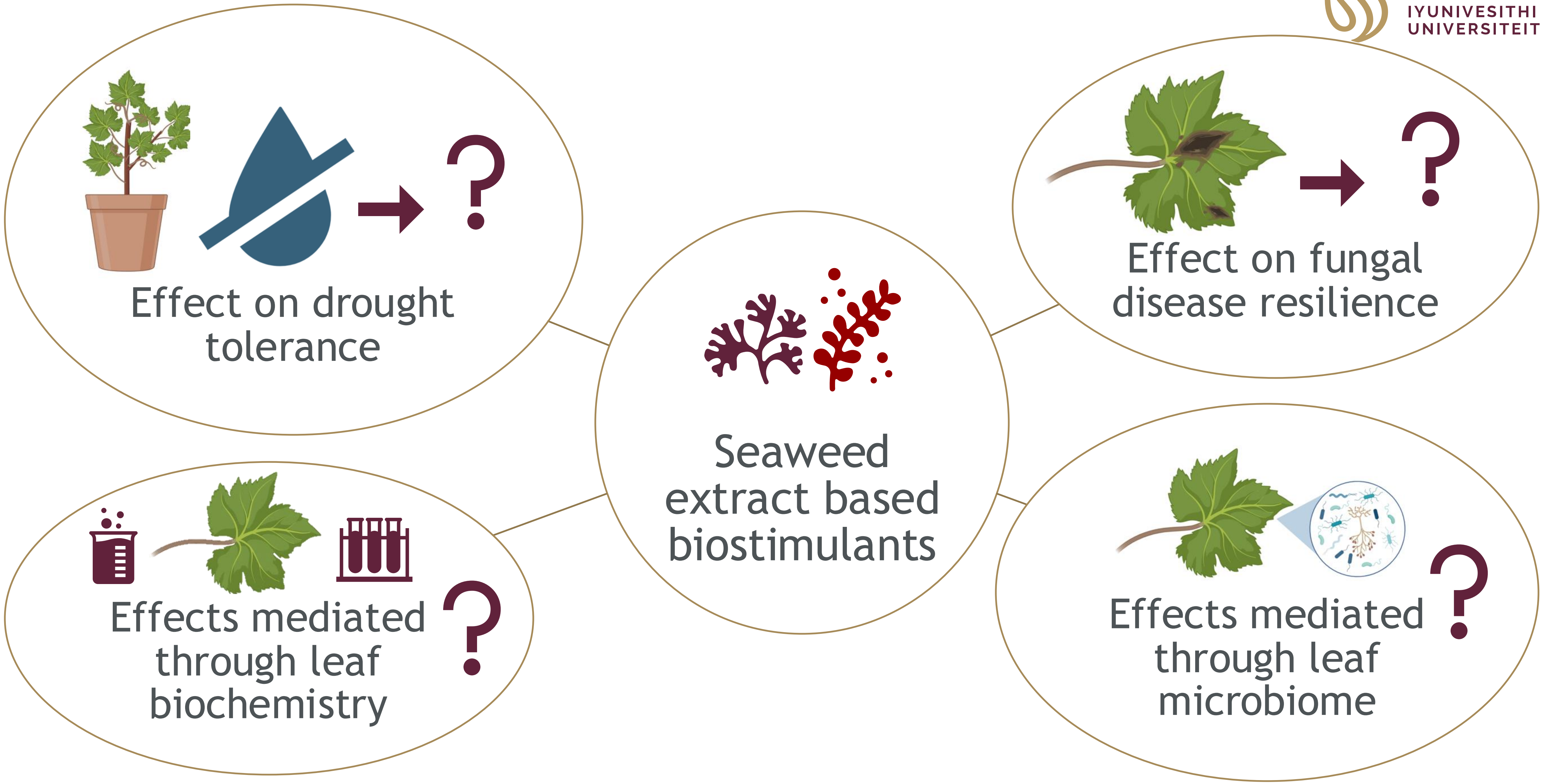


Elna Kruger

Seaweed based biostimulants can enhance **drought tolerance** and/or **fungal disease resilience**, through influencing leaf biochemistry and remodeling of the grapevine **leaf microbiome**.

Images: Biorender, dar woto (iStock), Sahinur Rahman (Vecteezy)

Project Summary



Drought Trial Experimental Design

50 vines

25 water deficit (WD), 10-20% Pot Water Capacity (PWC)
- 5: negative control (water)
- 5: glycine betaine (25mM)
- 5: biostimulant P1
- 5: biostimulant P2
- 5: biostimulant P3

25 well watered (WW), 90-100% Pot Water Capacity (PWC)
- 5: negative control (water)
- 5: glycine betaine (25mM)
- 5: biostimulant P1
- 5: biostimulant P2
- 5: biostimulant P3

- Two variables: irrigation scheme and foliar spray treatment
- 5 biological repeats per group
- Start foliar sprays once vines have ≥ 30 fully expanded mature leaves

(modified from Bonarota 2024, Frioni 2021, Zarraonaindia 2023)

2 Aim 2

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Evaluate the effect of SE based biostimulants on *Botrytis cinerea* growth directly and indirectly.

- ✔ **Objective 2.1:** Assess the inhibitory effects of SE based biostimulants on fungal growth of *Botrytis cinerea*.

- ❑ **Objective 2.2:** Assess the impact that SE based biostimulant pre-treatment on grapevine leaves has on fungal susceptibility to *Botrytis cinerea, in vitro*.

Growth curves: *Botrytis cinerea* grape strain

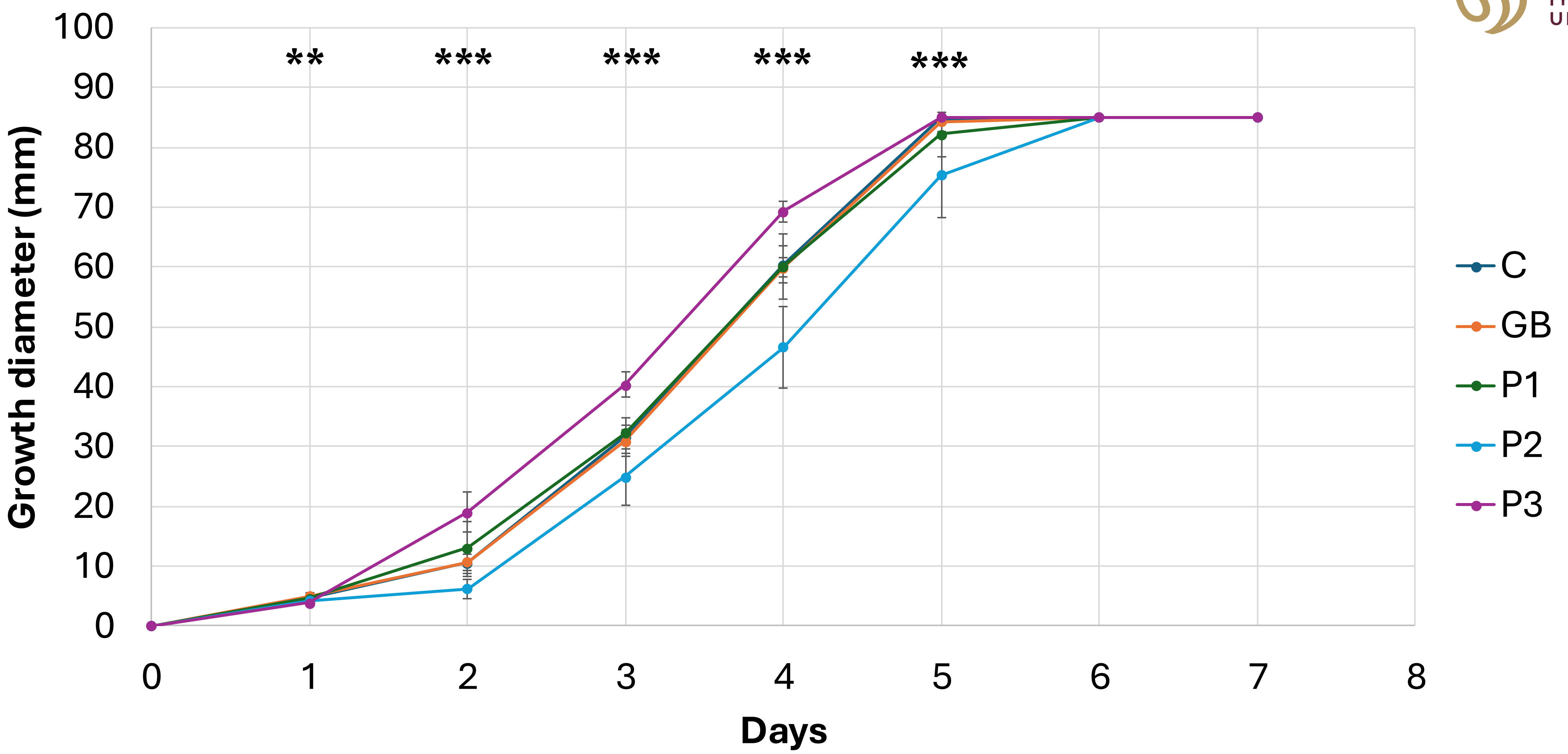

Figure 1. Growth rates of Botrytis cinerea grape strain on supplemented PDA plates. Inoculated with 5000 spores and incubated at 25°C in the dark. n≥6 pooled from two independent experiments, error bars = SD. * $p<0.05$, ** $p<0.01$, *** $p<0.001$ (ANOVA)

2.2 Experimental Design for LDA

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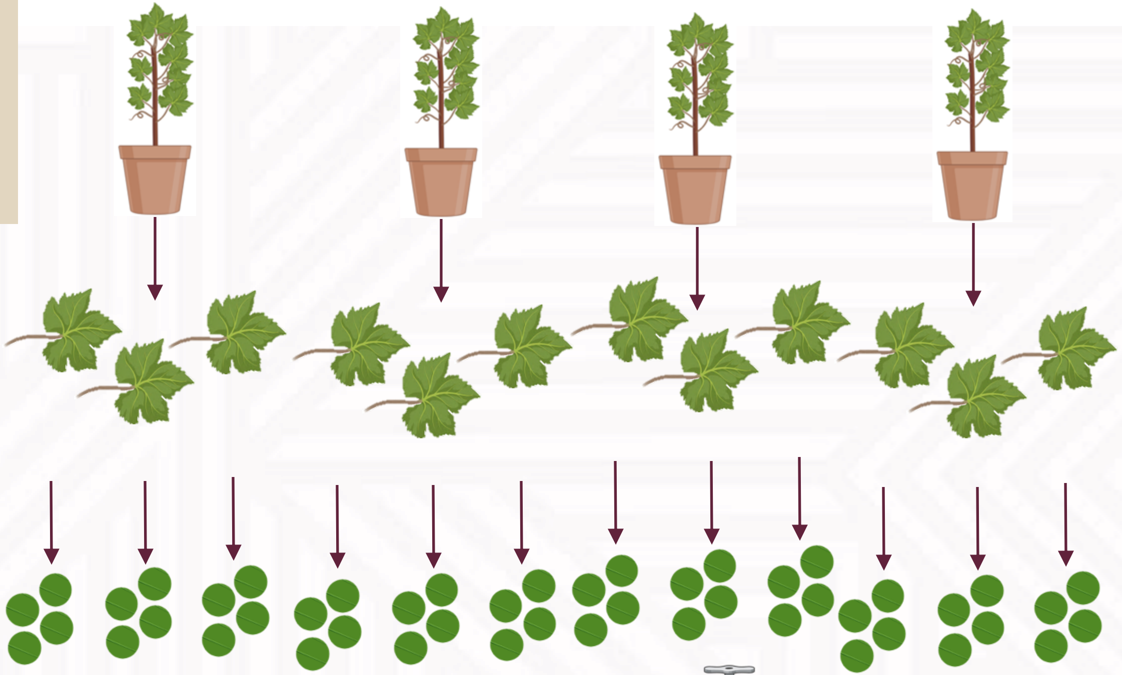
20 vines

- 4: negative control (water)
- 4: glycine betaine (25mM)
- 4: biostimulant P1
- 4: biostimulant P2
- 4: biostimulant P3

Subsets of plants also used for leaf microbiome sampling.

Harvest leaves **2 days** after foliar spraying.



- 12 discs per plant (from 3 leaves)
- 4 plants per treatment

= 48 discs per treatment

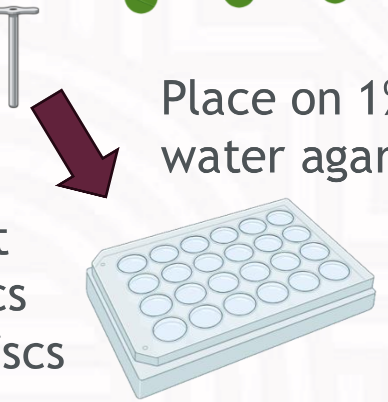
2 x 24-well plates per treatment

12 solvent inoculant control discs

36 spore suspension inoculant discs

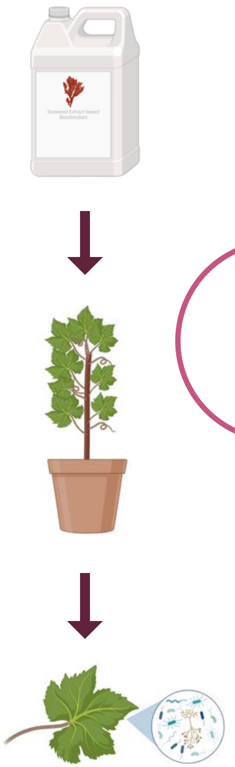
Total 240 discs

Place on 1% water agar



Aim 3

Evaluate the effect of biostimulant treatment on potted grapevine (cv. Cabernet Sauvignon) phyllosphere microbial diversity.



- ❑ **Objective 3.1:** Compare the phyllosphere diversity of bacteria based on length variation of the intergenic spacer regions (ITS) within the bacterial rRNA operon, between baseline untreated and biostimulant sprayed potted grapevine leaves.

- ❑ **Objective 3.2:** Compare the phyllosphere diversity of fungi based on length variation of the intergenic spacer regions (ITS) within the fungal rRNA operon, between baseline untreated and biostimulant sprayed potted grapevine leaves.

3 Leaf Microbiome Screen

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- Day 0 = when all have ≥ 30 fully expanded mature leaves; prune to have 2 shoots, i.e. minimum 12 leaves/shoot

Harvest Summer 2025

(Harvest LDA leaves 2 days after foliar sprays with treatments from subset of plants)

Harvest 25 leaves from baseline plants when other groups are sprayed

Harvest 25 leaves per treatment group (5/vine), 7 days after foliar sprays

Wash in PBS buffer to remove microbial mass from leaf surfaces

Filter through sterile acetate filter to collect microbial mass. Store filters at -80°C

Extract DNA (Dneasy PowerSoil Pro Kit)

Perform Automated Ribosomal Intergenic Spacer Analysis (ARISA)

(Africa et al., 2025)

3 Leaf Microbiome Screen Experimental Design

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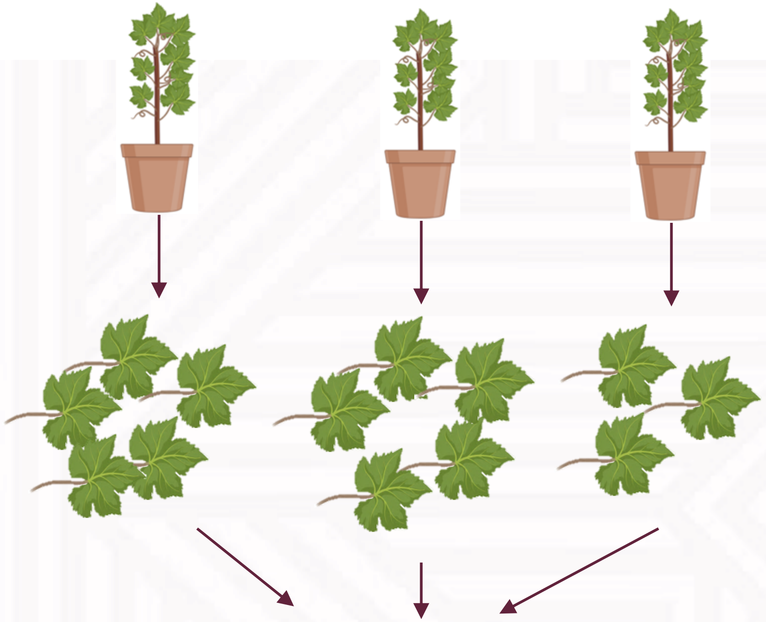
30 vines

- 5: baseline
- 5: negative control (water)
- 5: glycine betaine (25mM)
- 5: biostimulant P1
- 5: biostimulant P2
- 5: biostimulant P3

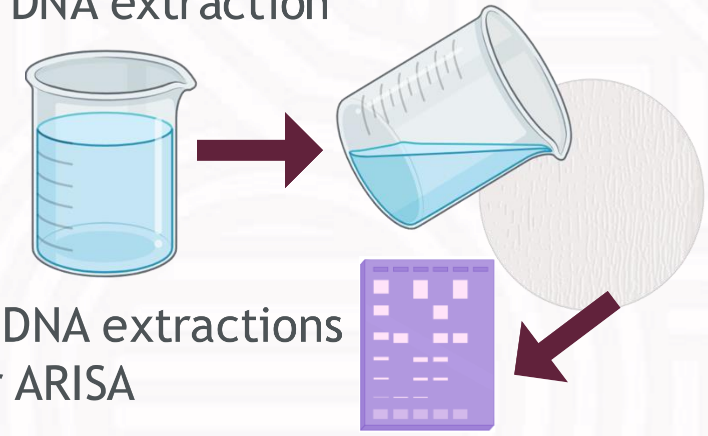
Harvest baseline **Day 0**

Harvest **7 days** after foliar spraying

Use subset of same plants for leaf disc assays.



Pool 12-13 leaves (5/vine)
= 1 DNA extraction



= 12 DNA extractions
for ARISA

3

Automated Ribosomal Intergenic Spacer Analysis (ARISA)

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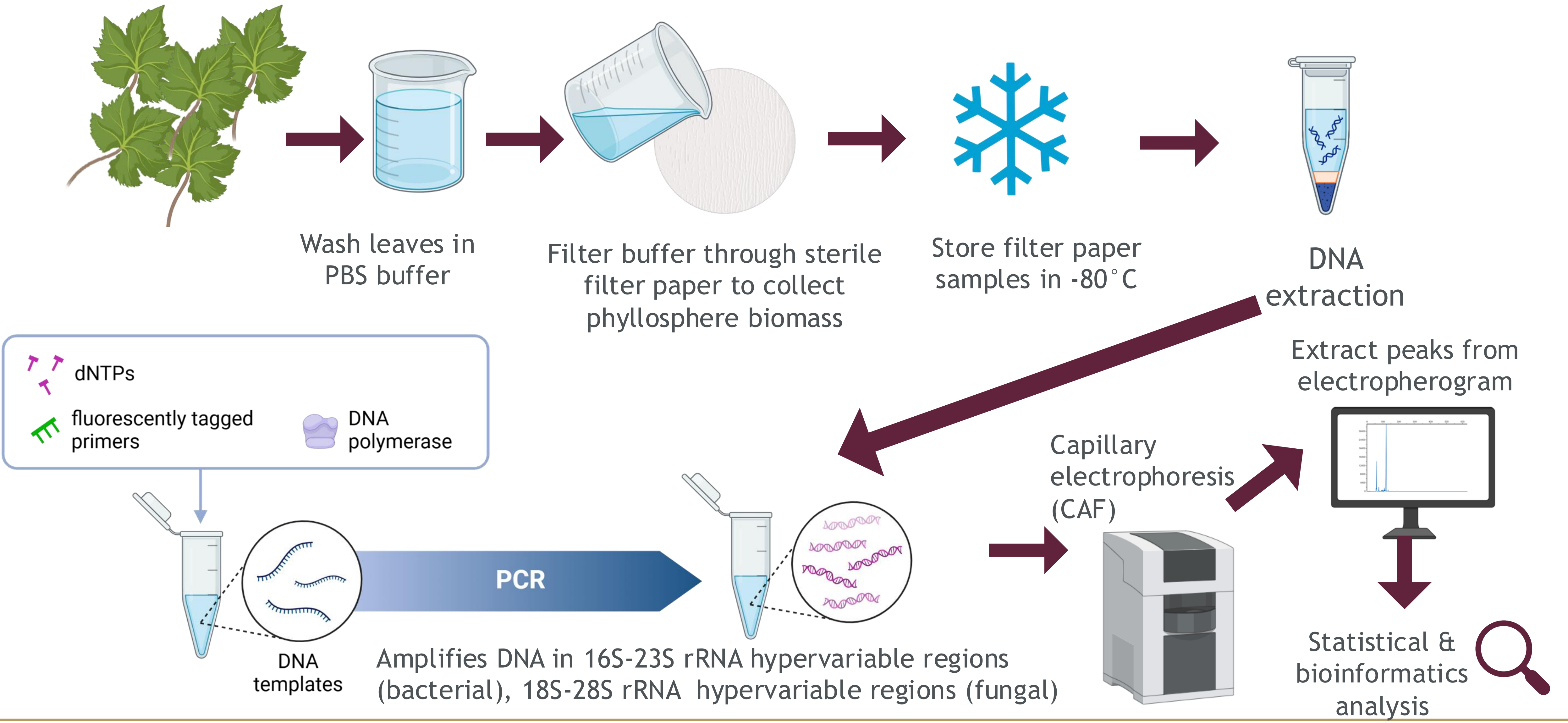

(Africa et al., 2025, Johnston-Monje and Lopez Mejia, 2020)

Growth Room Layout

- 1-year old Cabernet Sauvignon/R110 grapevines from Vititec
- 1:1:1:1 ratio soil:vermiculite:perlite:peat
- Roots trimmed equally at planting (23 July 2025)
- Pruned back to 2 nodes on main shoot, 11 Aug 2025

Project Summary

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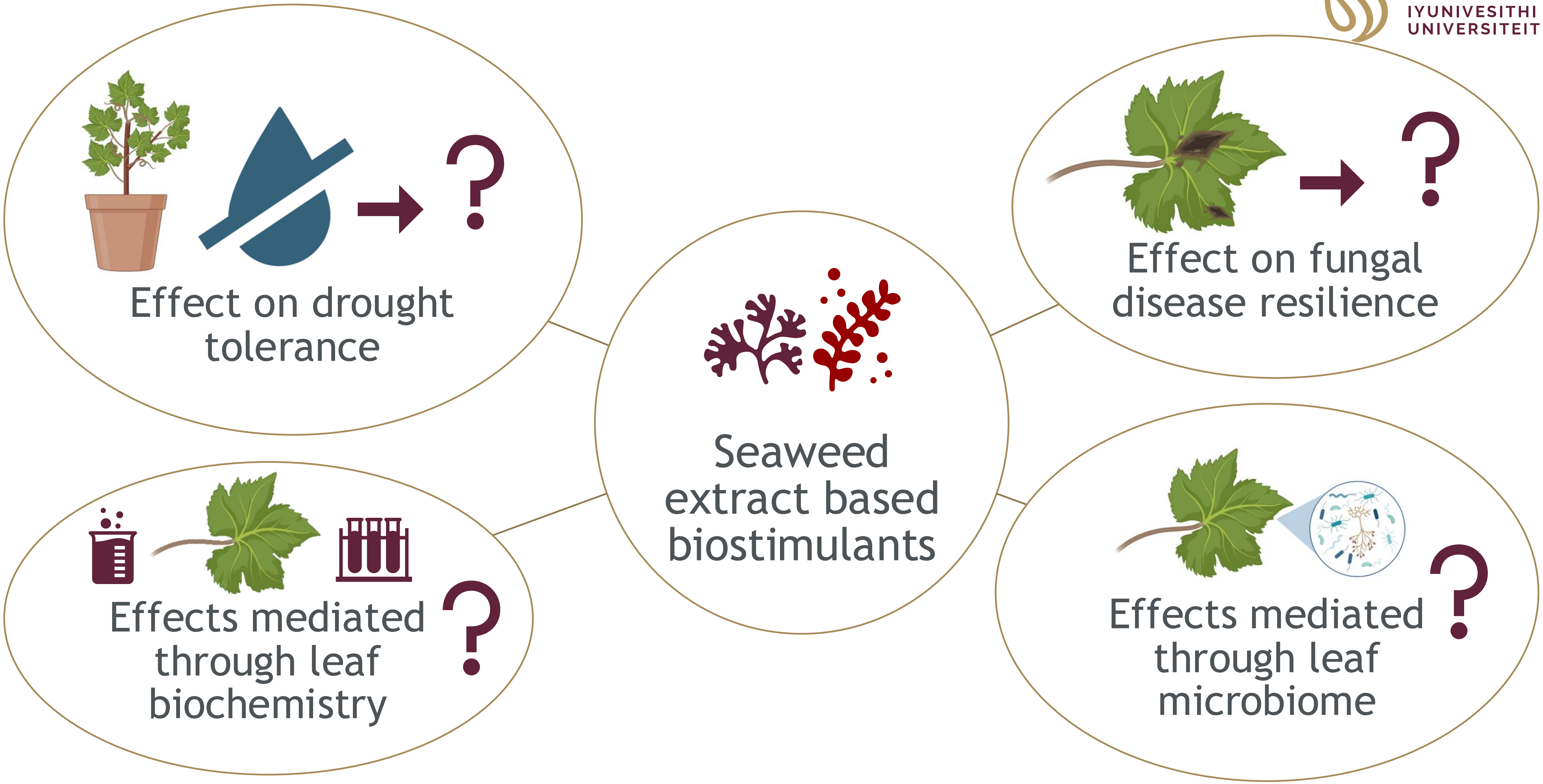

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SAGWRI South African Grape and Wine Research Institute

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- Prof Melane Vivier
- Dr Paul Hills

Thank you