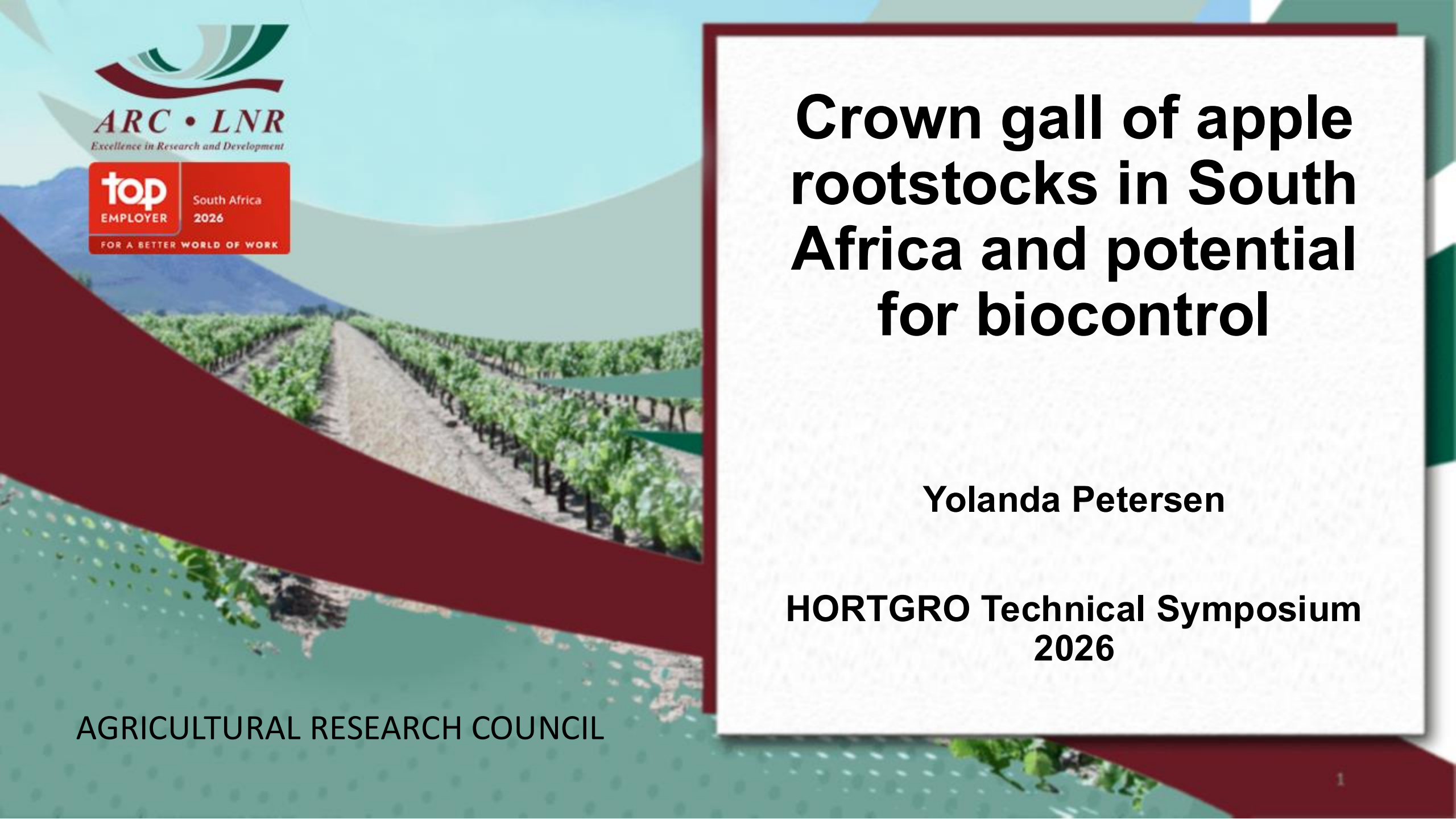
HORTGRO
Growing Fruit IQ

Technical Symposium 2026

# *Crown gall of apple rootstocks in South Africa and potential for biocontrol*

Dr Yolanda Petersen | ARC Infruitec–Nietvoorbij



# Crown gall of apple rootstocks in South Africa and potential for biocontrol

Yolanda Petersen

HORTGRO Technical Symposium 2026

AGRICULTURAL RESEARCH COUNCIL

# Content

- Background
- Overview of gall bacterial community
- Disease diagnosis
- Potential for biocontrol

# Background

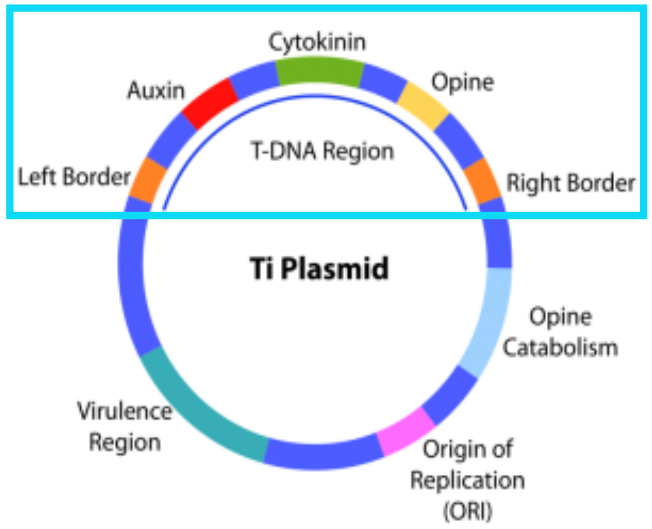
- Crown gall is a disease of many plant species and can be found wherever susceptible species are grown
- Crown gall disease is identified by overgrowths appearing as galls on roots, at graft unions, at the base or "crown" of woody plants and occasionally aerial parts
- In general, the presence of tumours can decrease plant growth and vigour, making infected plants less productive
- Younger trees are more prone to crown gall compared to older trees
- The greatest financial losses occur in the nursery

# Background

- Among stone fruit, almond and peach are among the most susceptible crops and plum and prune less susceptible, with cherry rootstocks varying in susceptibility
- Crown gall can cause significant economic losses for walnut grown on the rootstock Paradox
- **In South Africa**, crown gall is prevalent on grapevine and stone fruit
- Crown gall symptoms have also in recent years occurred on **apple rootstocks**

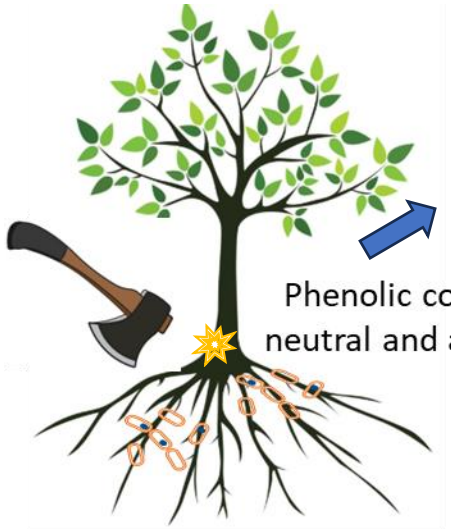
# Causal agent: basis for tumourigenicity

- The typical causal agent of galls on apple trees: soilborne bacterium *Rhizobium radiobacter* (Ti), synonym: *Agrobacterium tumefaciens*
- Within the species, strains can be pathogenic or non-pathogenic

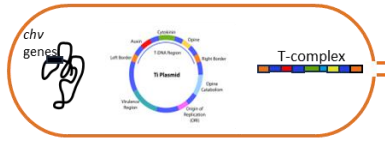


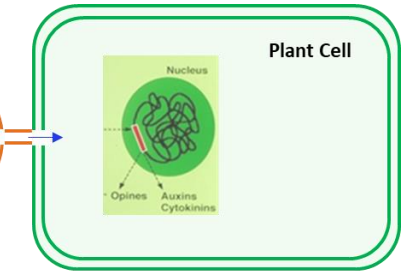

**pTi = oncogenic plasmid**

# Disease cycle

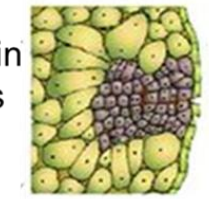


Phenolic compounds, neutral and acidic sugars

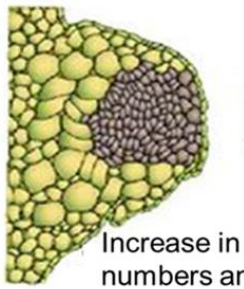



Auxin
Cytokinin
Opines



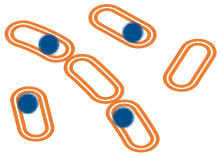
Rapid cell division



Increase in cell numbers and size lead to gall formation

**Plant wounds**

(e.g. planting, grafting, pruning, insect feeding, frost, water)



Cells can persist in soil for several years

Gall break down and dissemination

# Bacterial community in galls

- **Complex environment within galls**
- Transformed plant cells within tumours are driven to produce opines, a unique nutrient source that benefits the survival of the causal agent as well as other bacteria capable of catabolizing opines
- The presence of opines drives conjugal transfer of Ti plasmids
- Populations of non-pathogenic *Agrobacterium* can be particularly high in galls from apple

# Insight into the gallobiome

**Bacterial genera**

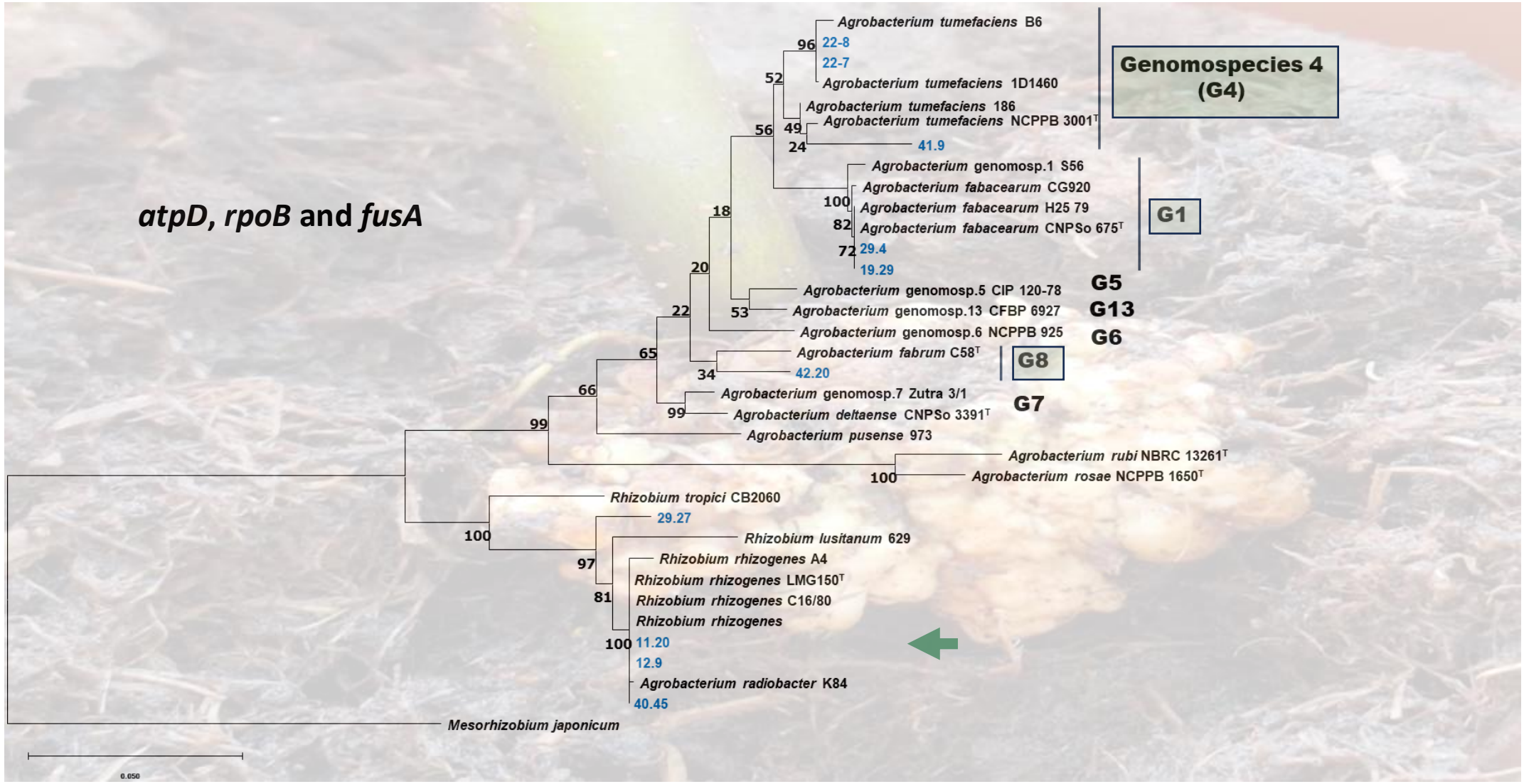
*Rhizobium*
*Bradyrhizobium*
*Mesorhizobium*
*Streptomyces*
*Sphingomonas*
*Sphingobium*
*Novosphingobium*
*Variovorax*
*Pseudomonas*
*Bacillus*
*Edaphobacter*
*Tardiphaga*
*Xanthomonas*

**Nitrogen fixation:**
*Rhizobium*
*Bradyrhizobium*
*Mesorhizobium*
*Novosphingobium*
*Tardiphaga*

**Other plant growth promoting traits**
*Sphingomonas*
*Sphingobium*
*Edaphobacter*

**Biocontrol agent traits**
*Rhizobium*
*Streptomyces*
*Pseudomonas*
*Bacillus*

# Agrobacteria community in galls



# Crown gall & apple rootstocks

**Detecting or isolating pathogenic *Agrobacterium* spp. from some hosts can be challenging**

**Q: Are galls on apple rootstocks caused by a biological agent?**

A: Yes. 17-50% plants per rootstock developed galls

**Q: Is the causative agent soilborne?**

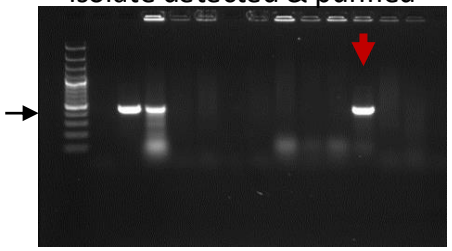
A: Yes. 38% plants developed galls

McLeod & Coertze 2023

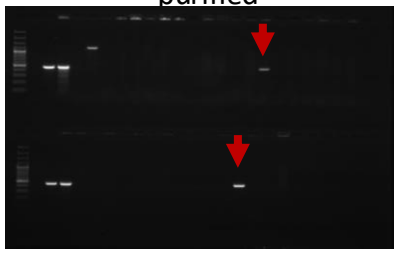
# Disease Diagnosis

## Detecting or isolating pathogenic *Agrobacterium* spp. from some hosts can be challenging

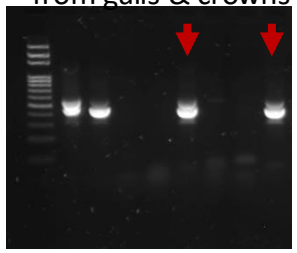
Pathogenic *Agrobacterium* isolate detected & purified



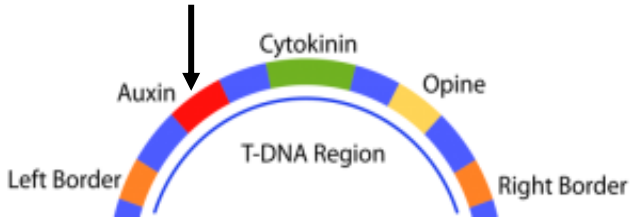
Pathogenic *Agrobacterium* isolates detected & not purified



nPCR of total DNA from galls & crowns

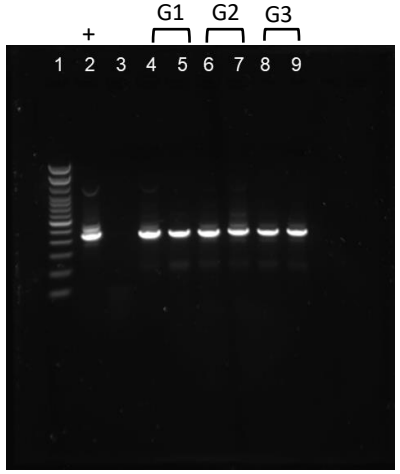


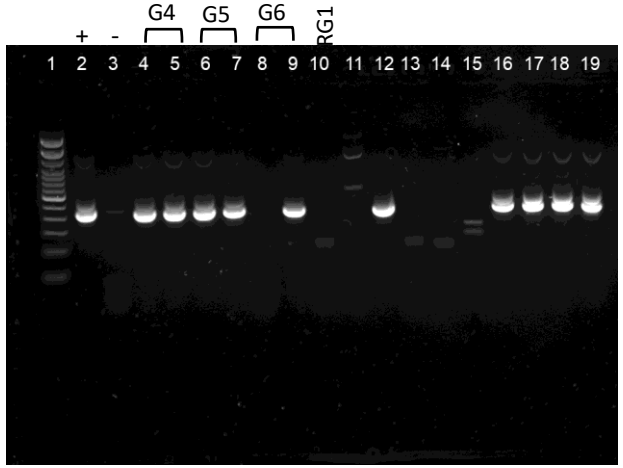
PCR detection




**2026**

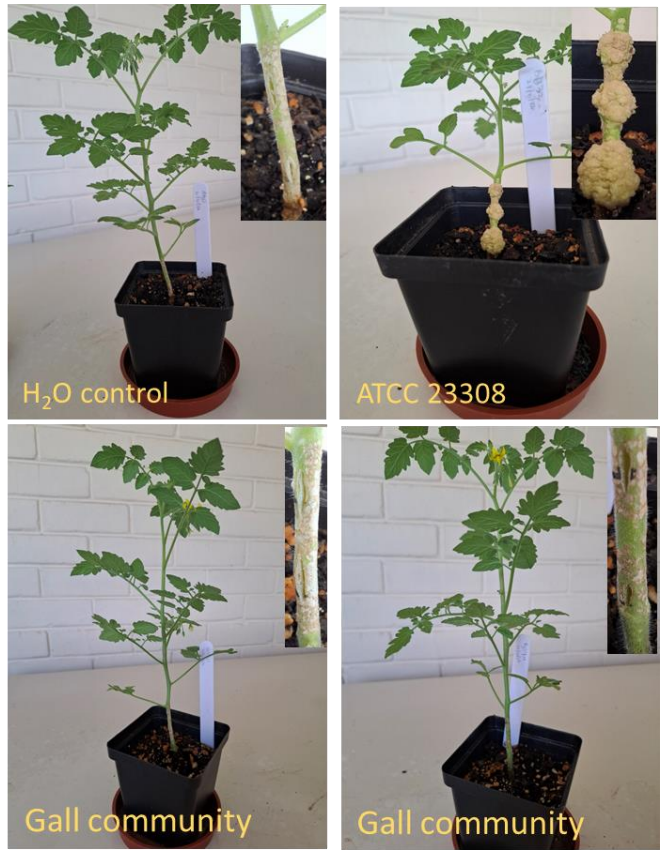
nPCR of total DNA from galls



# Pathogenicity test

## Tomato seedling assay


**No other tumour-inducing bacteria were isolated**

# Pathogenicity test

## On apple rootstocks

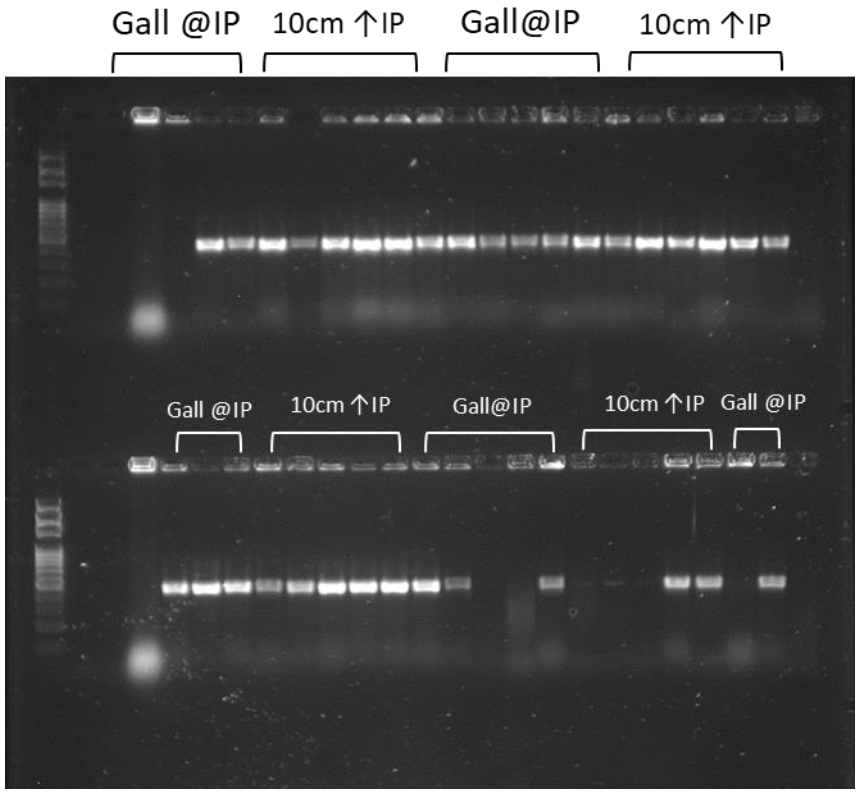

CG778


CG5757



# Systemic infection in apple rootstock

**In many hosts, tumourigenic agrobacteria may be translocated throughout the plant via the xylem leading to systemic infections**




# Recap

- Mapped the native bacterial community in galls
- Detected the pathogen within the plant

**Question:**

Can we use the plant's own microbial ecosystem to fight back?

# Biological control of crown gall

**MSc project (Jan 2024-March 2026):** Screening of endophytic bacteria isolated from apple rootstocks for their potential as biological control agents against crown gall of apple

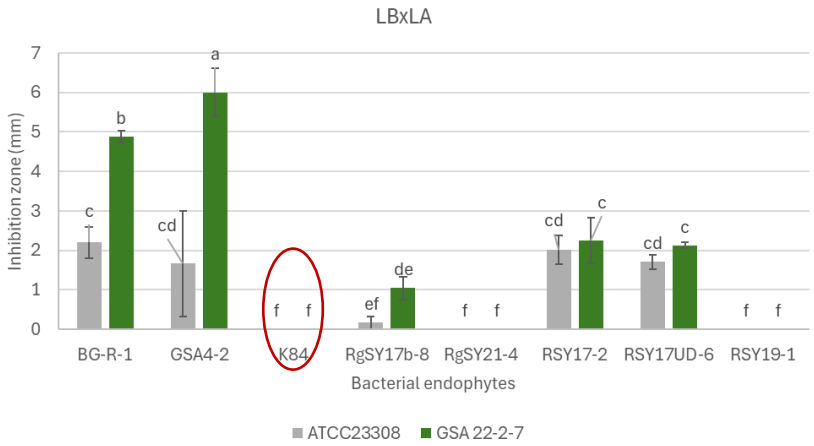
*Bacterial strain isolation & in vitro antagonistic activity* → A total of 962 isolates were tested → 15 isolates showed antagonistic activity towards *A. tumefaciens* → 8 isolates eliminated, leaving 7 for further investigation → Characterisation of isolates: ID & potential mechanisms for observed antagonism

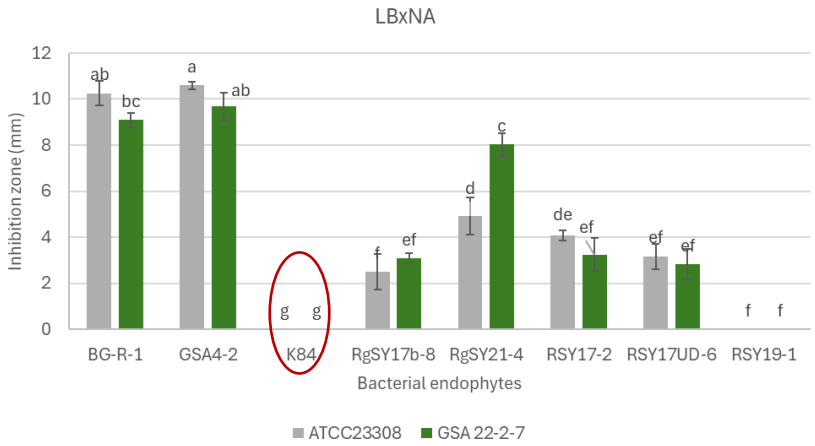
| Isolate | Identity | HCN production | Protease Activity | Amylase Activity | Siderophore production | Phosphate solubilization | IAA production |
|---|---|---|---|---|---|---|---|
| RSY17-2 | *Pseudomonas monsensis* | ✓ | ✓ | x | ✓ | ✓ | ✓ |
| RSY17UD-6 | *Pseudomonas monsensis* | ✓ | ✓ | x | ✓ | ✓ | ✓ |
| RgSY17b-8 | *Pseudomonas* sp. | ✓ | ✓ | x | ✓ | ✓ | ✓ |
| RSY19-1 | *Pseudomonas* sp. | ✓ | ✓ | x | ✓ | ✓ | ✓ |
| RgSY21-4 | *Pseudomonas moorei* | ✓ | ✓ | x | ✓ | ✓ | ✓ |
| BG-R-1 | *Bacillus velezensis* | x | ✓ | ✓ | x | ✓ | ✓ |
| GSA4-2 | *Bacillus amyloliquefaciens* | x | ✓ | ✓ | x | ✓ | ✓ |

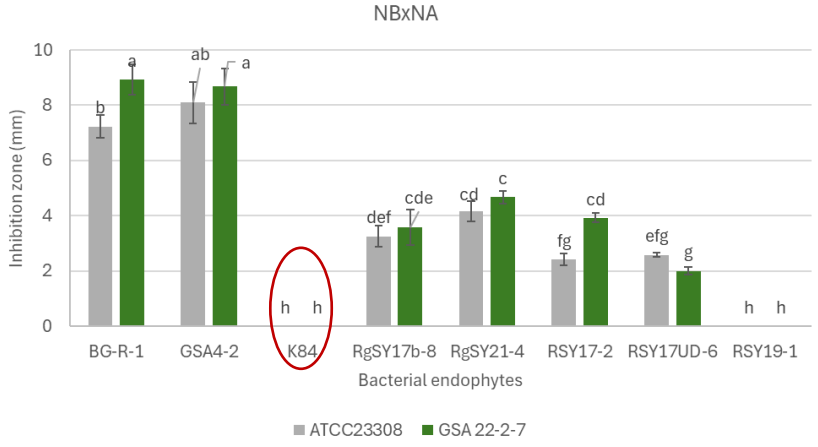
**Direct & indirect contributors to biocontrol potential**

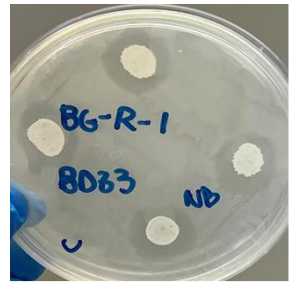
# *In vitro* antagonism

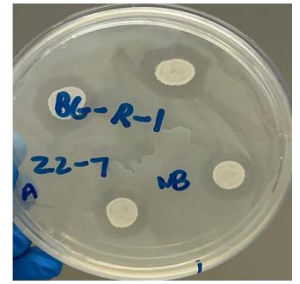
## Tested using different combinations of growth media

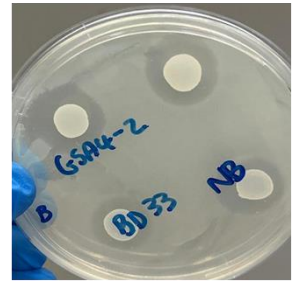


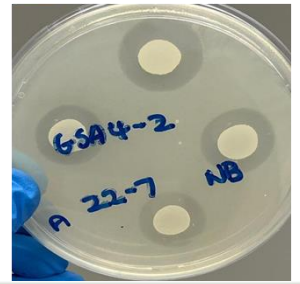



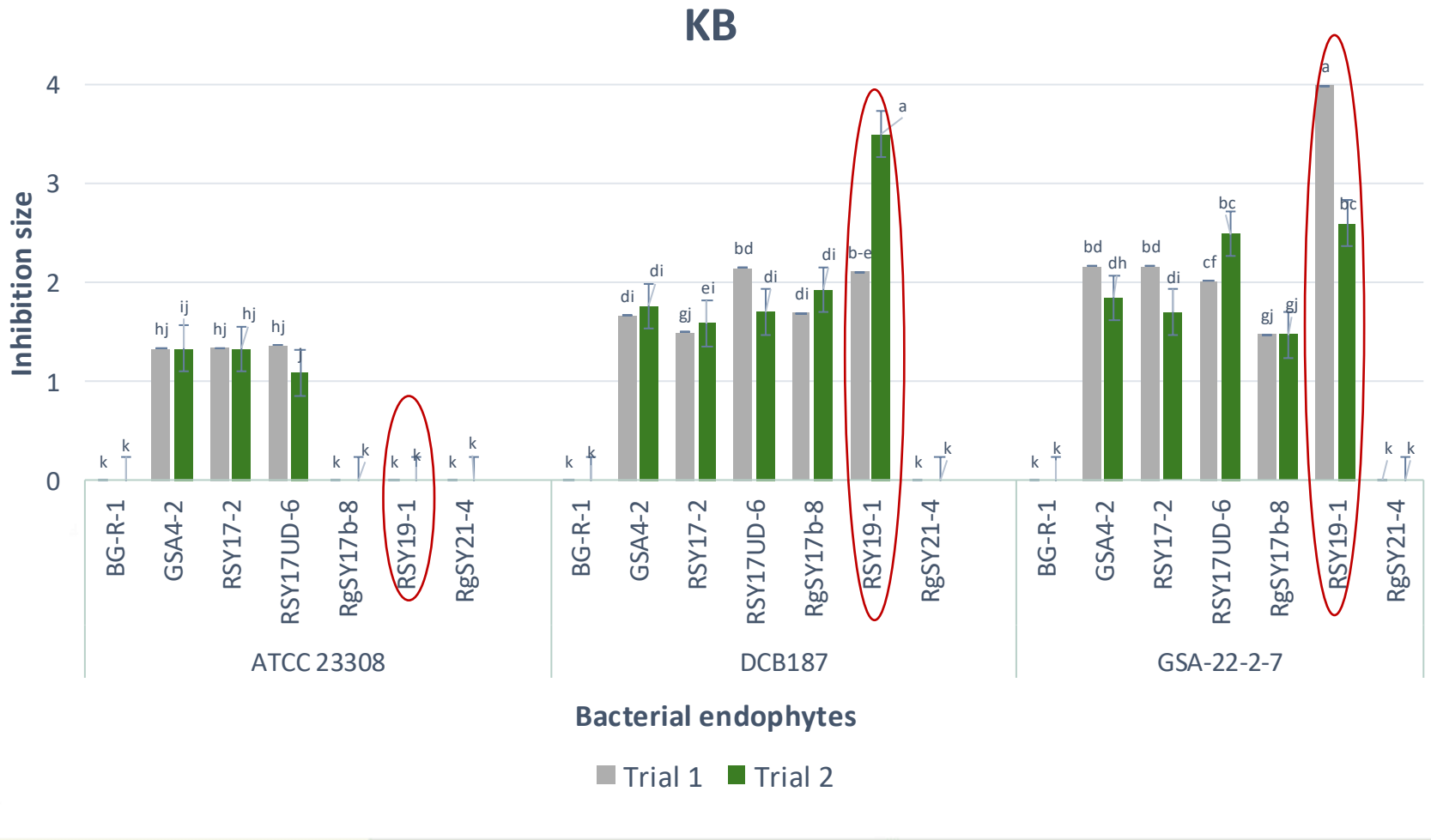



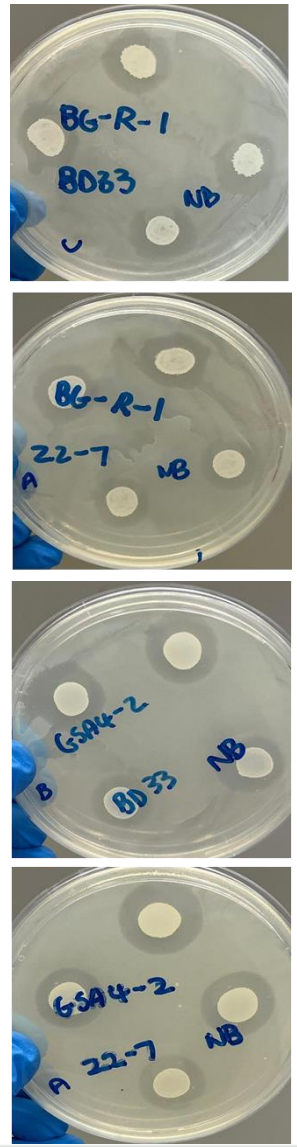



# *In vitro* antagonism

## Tested using different combinations of growth media



# Biocontrol Assay: Carrot disks

## Disease incidence (%)

| | ATCC 23308 | | DCB 187 | | GSA 22-2-7 | |
|---|---|---|---|---|---|---|
| **Treatment** | **Trial 1** | **Trial 2** | **Trial 1** | **Trial 2** | **Trial 1** | **Trial 2** |
| Pathogen only | $85.9 \pm 2.3^{ab}$ | $93.8 \pm 3.1^{a}$ | $100 \pm 0^{a}$ | $93.8 \pm 0^{a}$ | $90.6 \pm 0^{ab}$ | $92.2 \pm 0.8^{a}$ |
| BG-R-1 | $100 \pm 0^{a}$ | $100 \pm 0^{a}$ | $100 \pm 0^{a}$ | $100 \pm 0^{a}$ | $100 \pm 0^{a}$ | $100 \pm 0^{a}$ |
| GSA4-2 | $50 \pm 25^{a-e}$ | $100 \pm 0^{a}$ | $100 \pm 0^{a}$ | $50 \pm 25^{a-e}$ | $75 \pm 12.5^{a-c}$ | $75 \pm 12.5^{a-c}$ |
| **K84** | $\mathbf{0^{e*}}$ | $\mathbf{25 \pm 12.5^{c-e*}}$ | $\mathbf{50 \pm 12.5^{a-e}}$ | $\mathbf{87.5 \pm 6.3^{ab}}$ | $\mathbf{25 \pm 12.5^{c-e*}}$ | $\mathbf{0^{e*}}$ |
| RSY17-2 | $12.5 \pm 6.3^{e-d*}$ | $25 \pm 12.5^{c-e*}$ | $62.5 \pm 6.3^{a-d}$ | $87.5 \pm 6.3^{ab}$ | $12.5 \pm 6.3^{e-d*}$ | $12.5 \pm 6.3^{e-d*}$ |
| RSY17UD-6 | $12.5 \pm 6.3^{e-d*}$ | $37.5 \pm 18.8^{b-e*}$ | $12.5 \pm 6.3^{e-d*}$ | $100 \pm 0^{a}$ | $0^{e*}$ | $0^{e*}$ |
| RgSY17b-8 | $37.5 \pm 6.3^{b-e}$ | $0^{e*}$ | $37.5 \pm 6.3^{b-e*}$ | $100 \pm 0^{a}$ | $12.5 \pm 6.3^{e-d*}$ | $25 \pm 12.5^{c-e*}$ |
| RSY19-1 | $50 \pm 12.5^{a-e}$ | $0^{e*}$ | $0^{e*}$ | $25 \pm 12.5^{c-e*}$ | $37.5 \pm 6.3^{b-e}$ | $0^{e*}$ |
| RgSY21-4 | $50 \pm 25^{a-e}$ | $100 \pm 0^{a}$ | $25 \pm 12.5^{c-e*}$ | $100 \pm 0^{a}$ | $0^{e*}$ | $50 \pm 25^{a-e*}$ |
| P value | < 0.0001 | < 0.0001 | <0.0001 | < 0.0001 | < 0.0001 | < 0.0001 |

BG-R-1 & GSA4-2 =***Bacillus* sp.**; RSY17-2, RSY17UP-6, RgSY17b-8, RSY19-1, RgSY21-4 =***Pseudomonas* sp.**

# Biocontrol Assay: Carrot disks

## Biocontrol efficacy (%)

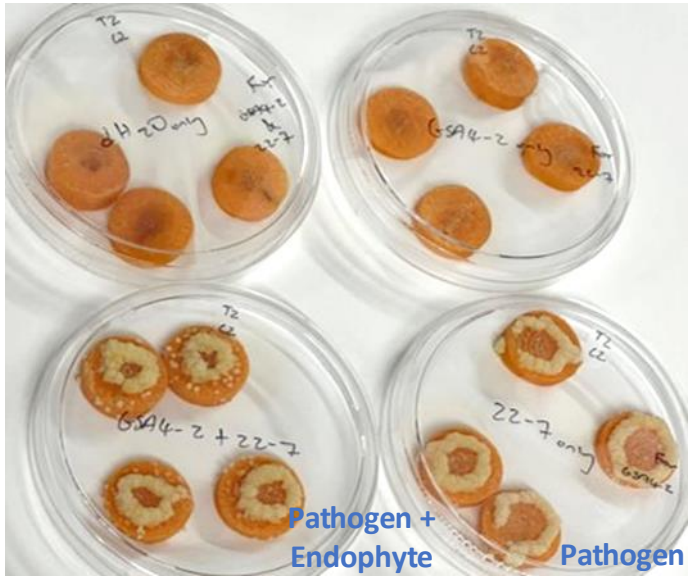
| | ATCC 23308 | | DCB 187 | | GSA 22-2-7 | |
|---|---|---|---|---|---|---|
| **Treatment** | **Trial 1** | **Trial 2** | **Trial 1** | **Trial 2** | **Trial 1** | **Trial 2** |
| BG-R-1 | $0 \pm 0^{e}$ | $2.9 \pm 1.5^{e}$ | $0^{e}$ | $45.8 \pm 22.9^{cd}$ | $0.480 \pm 0.24^{e}$ | $17.3 \pm 8.7^{de}$ |
| GSA4-2 | $50 \pm 25.0^{b-d}$ | $0^{e}$ | $70.9 \pm 1.8^{a-c}$ | $96.4 \pm 1.8^{a}$ | $26.3 \pm 13.2^{de}$ | $30.8 \pm 10.6^{de}$ |
| **K84** | $\mathbf{100 \pm 0^{a}}$ | $\mathbf{100 \pm 0^{a}}$ | $\mathbf{99.8 \pm 0.02^{a}}$ | $\mathbf{84.2 \pm 1.7^{ab}}$ | $\mathbf{99.5 \pm 0.26^{a}}$ | $\mathbf{100 \pm 0^{a}}$ |
| RSY17-2 | $99.67 \pm 0.17^{a}$ | $99.6 \pm 0.20^{a}$ | $99.4 \pm 0.09^{a}$ | $48.9 \pm 5.7^{b-d}$ | $99.9 \pm 0.06^{a}$ | $99.9 \pm 0.05^{a}$ |
| RSY17UD-6 | $99.1 \pm 0.5^{a}$ | $86.5 \pm 6.8^{a}$ | $99.8 \pm 0.12^{a}$ | $73.5 \pm 0.08^{a-c}$ | $100 \pm 0^{a}$ | $100 \pm 0^{a}$ |
| RgSY17b-8 | $80.9 \pm 9.1^{a-c}$ | $100 \pm 0^{a}$ | $100 \pm 0^{a}$ | $91.9 \pm 4.1^{a}$ | $96.7 \pm 0.3^{a}$ | $100 \pm 0^{a}$ |
| RSY19-1 | $99.3 \pm 0.09^{a}$ | $100 \pm 0^{a}$ | $93.4 \pm 2.7^{a}$ | $78.3 \pm 9.2^{a-c}$ | $98.3 \pm 0.9^{a}$ | $99.9 \pm 0.06^{a}$ |
| RgSY21-4 | $94.2 \pm 2.9^{a}$ | $92.6 \pm 2.9^{a}$ | $98.1 \pm 1.0^{a}$ | $0.780 \pm 0.39^{e}$ | $100 \pm 0^{a}$ | $85.9 \pm 7.0^{a}$ |
| P value | < 0.0001 | < 0.0001 | <0.0001 | < 0.0001 | < 0.0001 | < 0.0001 |

Biocontrol efficacy = $[(C - T)/C] \times 100$,

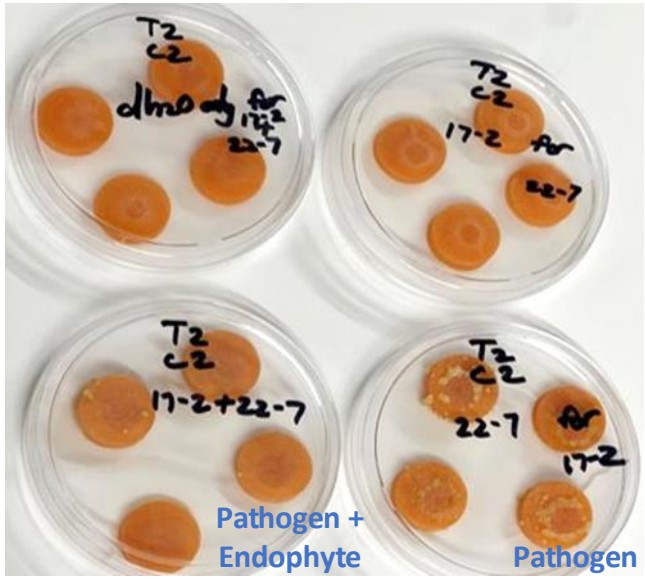
where C represents the average weight of the crown gall tumour in the control group, and T represents the average weight of the crown gall tumour in the treatment group.

# Biocontrol Assay: Carrot disks

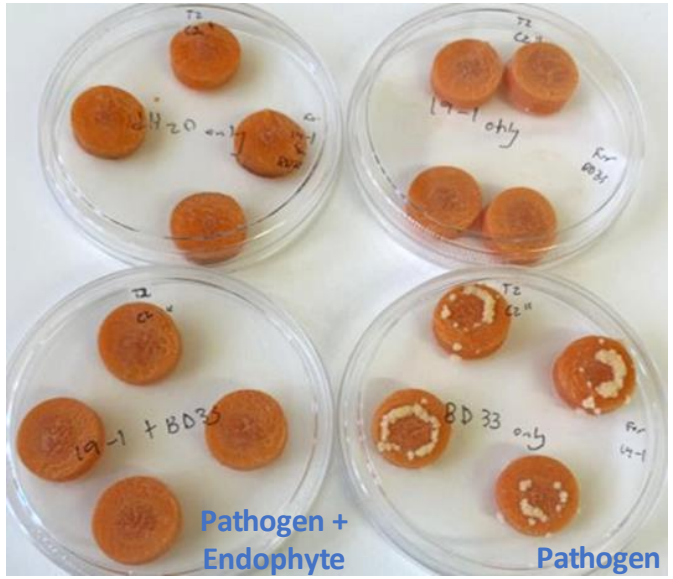
## Symptoms




Pathogen: GSA 22-2-7
Endophyte: GSA4-2
Incidence and severity = High
**Biocontrol efficacy = Low**




Pathogen: GSA 22-2-7
Endophyte: RSY17-2
Incidence and severity = very low
**Biocontrol efficacy = High**




Pathogen: ATCC 23308
Endophyte: RSY19-1
Incidence and severity = 0
**Biocontrol efficacy = High**

# Biocontrol Assay: Tomato seedlings

## Biocontrol Efficacy (%)

| Endophyte | ATCC 23308 | GSA 22-2-7 | DCB 187 | |
|---|---|---|---|---|
| | | | Trial 1 | Trial 2 |
| BG-R-1 | $62.9\pm8.7^{d-f}$ | $82.5\pm11.5^{a-d}$ | $56.8\pm1.2^{c-k}$ | $40.1\pm50.5^{h-o}$ |
| GSA4-2 | $71.7\pm8.6^{b-e}$ | $88.4\pm8.4^{a-c}$ | $67.3\pm6.5^{a-i}$ | $63.9\pm6.1^{b-i}$ |
| K84 | $100\pm0^{a}$ | $100\pm0^{a}$ | $92.5\pm10.6^{a-c}$ | $100\pm0^{a}$ |
| RSY17-2 | $96.0\pm4.6^{a}$ | $95.9\pm8.3^{a}$ | $100\pm0^{a}$ | $100\pm0^{a}$ |
| RSY17UD-6 | $84.7\pm9.5^{a-c}$ | $91.5\pm9.9^{ab}$ | $88.6\pm16.1^{a-d}$ | $96\pm5.6^{ab}$ |
| RgSY17b-8 | $91.6\pm10.4^{ab}$ | $95.9\pm6.0^{a}$ | $78.3\pm13.5^{a-g}$ | $80.3\pm27.9^{a-f}$ |
| RSY19-1 | $93.8\pm12.5^{a}$ | $96.9\pm6.1^{a}$ | $100\pm0^{a}$ | $100\pm0^{a}$ |
| RgSY21-4 | $80.4\pm25.2^{a-d}$ | $91.8\pm11.3^{ab}$ | $100\pm0^{a}$ | $83.1\pm12.1^{a-e}$ |
| *P* – value | 0.025 | 0.0414 | 0.0388 | |

# Biocontrol Assay: Tomato seedlings

**Symptoms**

Pathogen:
ATCC 23308

Pathogen: ATCC 23308
Endophyte: RSY17-2
**Biocontrol efficacy= 96%**

Pathogen:
GSA 22-2-7

Pathogen: GSA 22-2-7
Endophyte: RgSY 17b-8
**Biocontrol efficacy= 95.9%**

# Summary

- Galls host diverse microbial communities harbouring many useful properties
- Pathogenic agrobacteria (*Rhizobium radiobacter*) cause galls on apple rootstocks
- Infection in apple rootstocks is systemic
- *Pseudomonas* isolates proved more effective as biocontrol agents
- The Greenhouse Bottleneck: Pathogen-only controls currently showing low disease incidence.
- Optimization: Fine-tuning temperature, moisture, and wounding to trigger reliable disease
- Overcoming this technical hurdle unlocks sustainable pathways for managing crown gall in apple rootstock protection.

# Acknowledgements


**Collaborators & Co-workers**

Assoc. Prof. Adele Mcleod (SU)

Ms. Wendy Langenhoven (ARC)

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