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Australian Blueberry Growers' Association

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25–28 July
Sea World Resort
& Conference Centre

BQI 22

BerryQuest International 2022

TUESDAY 26 JULY 2022

Pest & Disease: Controlling Botrytis in Strawberry using BioClay™

Dr Tony Gendall, La Trobe University

berries AUSTRALIA

25–28 July
Sea World Resort
& Conference Centre

BQI 22

Controlling Botrytis in Strawberry using BioClay™

Lorena Rodriguez Coy, Donovan Garcia Ceron, Scott Mattner, Kim Plummer, **Tony Gendall** (La Trobe University)

Apollo Gomez, Lindy Coates (DAF)

Berry Quest International, 26 July, 2022
Tuesday Afternoon, Session : 1 Pest & Disease

Sustainable Crop Protection ARC HUB

Queensland Government

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Grey Mould- *Botrytis cinerea*

- *Botrytis cinerea* is fungal pathogen that infects over 500 crops, economic losses across the globe of US$100 billion.
- In Victoria, *B. cinerea* reduces commercial yield of strawberry fruit by 16%, and causes post-harvest rots of up to 70%.
- Growers rely on regular applications of a limited number of fungicides to control grey mould. Many fungicide/pesticide chemistries are under threat of withdrawal.
- *B. cinerea* has the capacity to develop resistance to fungicides
- There is increasing worldwide demand by consumers for produce with minimal pesticide inputs (e.g., proposed EU Directive 22/0196)

- **Berry growers urgently need crop protection solutions for *B. cinerea* that are effective, sustainable and complement the use of the limited chemistries and biological controls available**

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Overview

- Introduction to double-stranded RNA (dsRNA) as a fungicide
- Introduction to the ARC Sustainable Crop Protection ARC Hub
- Objectives and progress to control *Botrytis cinerea* in Strawberry using BioClay.
- Future Prospects

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Double Stranded RNA

"Spray induced gene silencing (SIGS)"

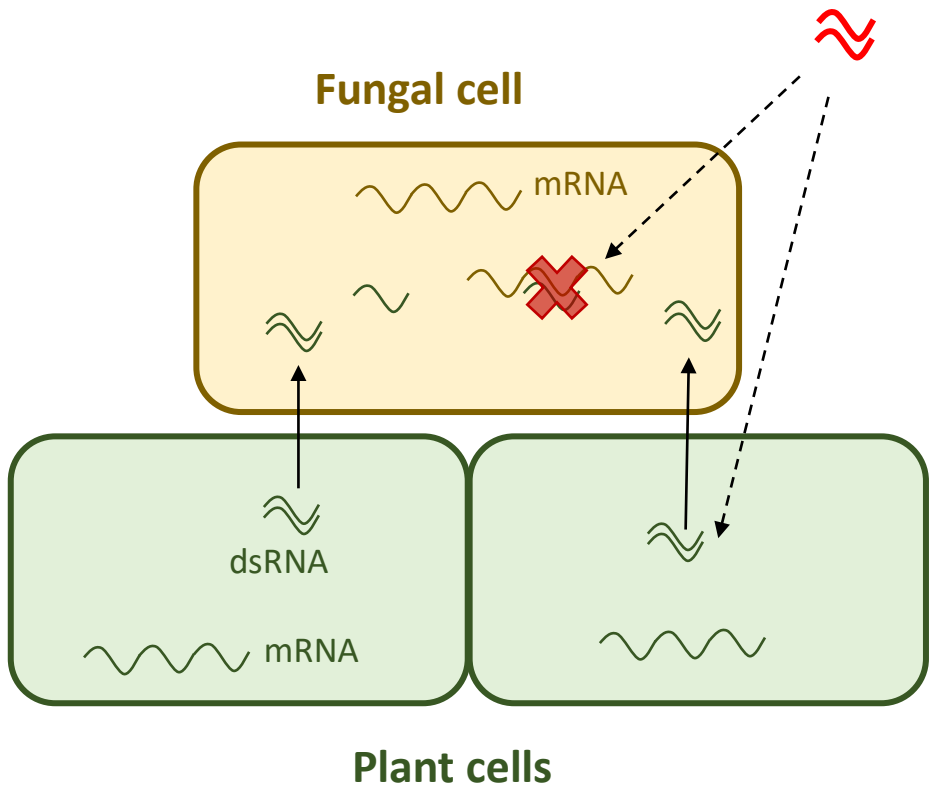
RNAs are made by all organisms, to convert genetic material (DNA) into proteins and enzymes (via mRNA intermediates).

Double stranded RNA (**dsRNAs**) are made by some organisms (including plants, fungi and animals), and consist of folded RNA molecules, and in plants are often involved in pathogen defence. These dsRNAs can also be synthesised chemically.

dsRNA can move from one cell to another, and induce silencing/blocking of target genes (akin to the chemical inhibition by fungicides that inhibit enzymes)

Thus it is possible to target a specific gene in a specific organism, by using a sequence-specific dsRNA.

By targeting an essential gene in a pathogen, or one required for pathogenesis, dsRNA can function as an effective, specific fungicide



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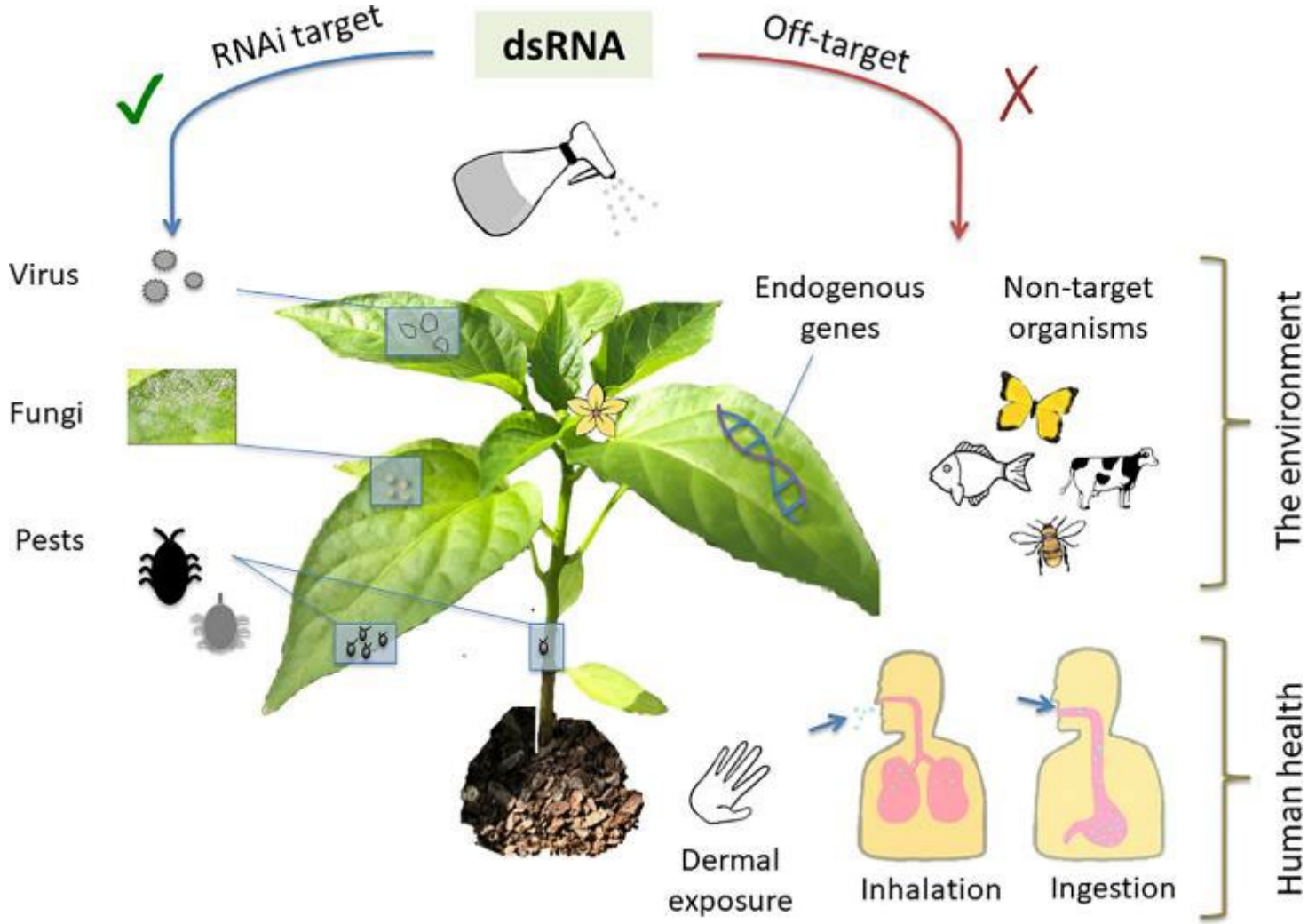
dsRNA Applications

Different dsRNAs for different target species



Reeves, Mitter *et al.*, (2020) Frontiers in Plant Science. doi.org/10.3389/fpls.2020.00051

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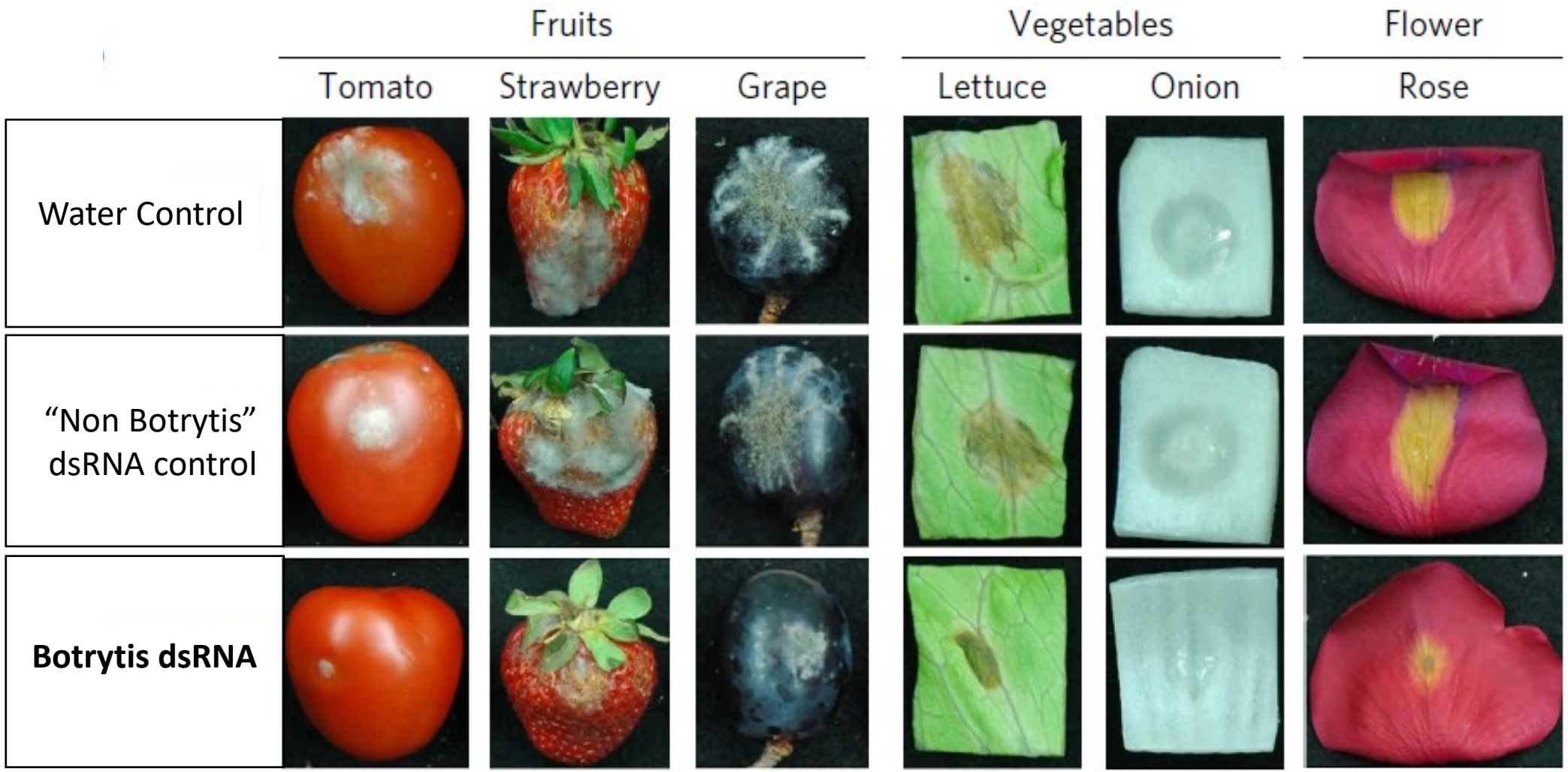
Proof of Concept- dsRNA reduces *Botrytis*



Ming Wang, Hailing Jin *et al.*, (2016) Nature Plants. doi: 10.1038/nplants.2016.151

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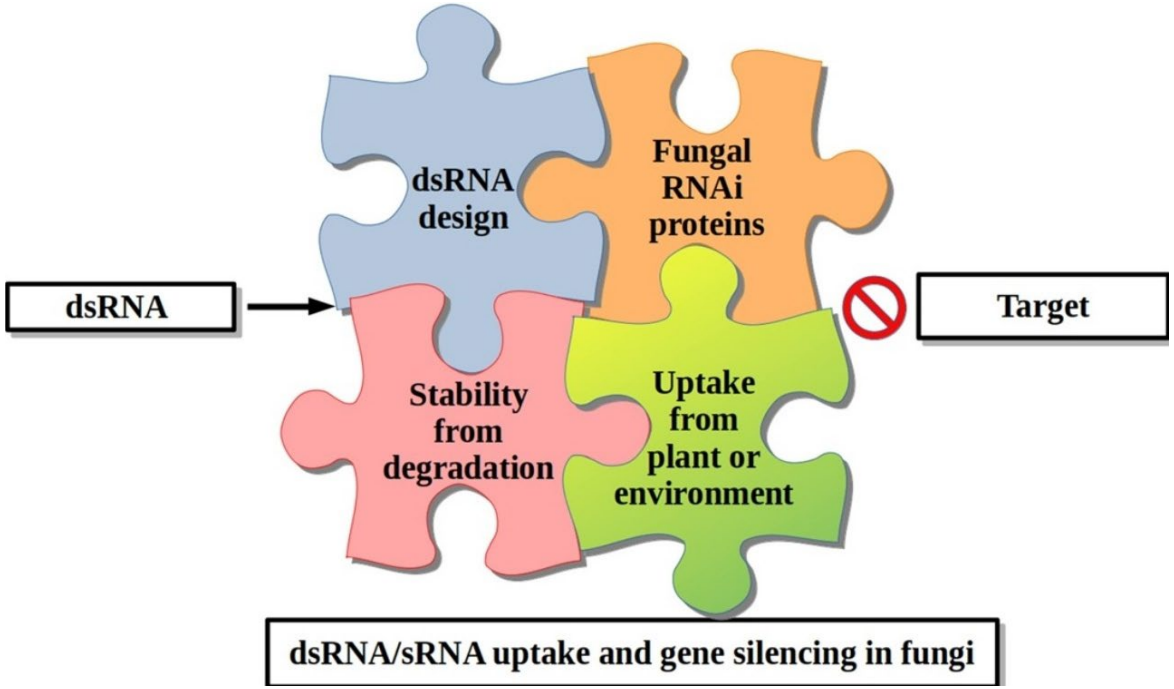
Limitations to dsRNA as fungicides

- Long-term stability is not well defined – environmental factors (microbes, pH, UV, water)
- Dosages, interactions with other fungicides not described
- Variations in efficacy due to differences in plant surfaces (leaves, fruits, roots) unknown.



Secic *et al.*, (2021) Current Opinion in Biotechnology. doi.org/10.1016/j.copbio.2021.04.001

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BioPlatforms Australia

Wine Australia

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Officially Started in Aug 2020, Launched Nov 2021

https://crophub.com.au/research

Total Value: $18M cash and in-kind

ARC Sustainable Crop Protection Hub Research Themes

1. Developing BioClay™ targeting fungal species of significance to both horticulture and broad acre crops
2. Generating new knowledge - mechanism of BioClay-fungi-host interaction
3. Formulating BioClay to regulatory and industry specifications
4. Assessing community acceptance of BioClay - Policy/Consumer Acceptance

Core Technology BioClay™ :

RNA-Based Biopesticides, IP developed by Prof Neena Mitter, Gordon Xu, *et al.* at University of Queensland

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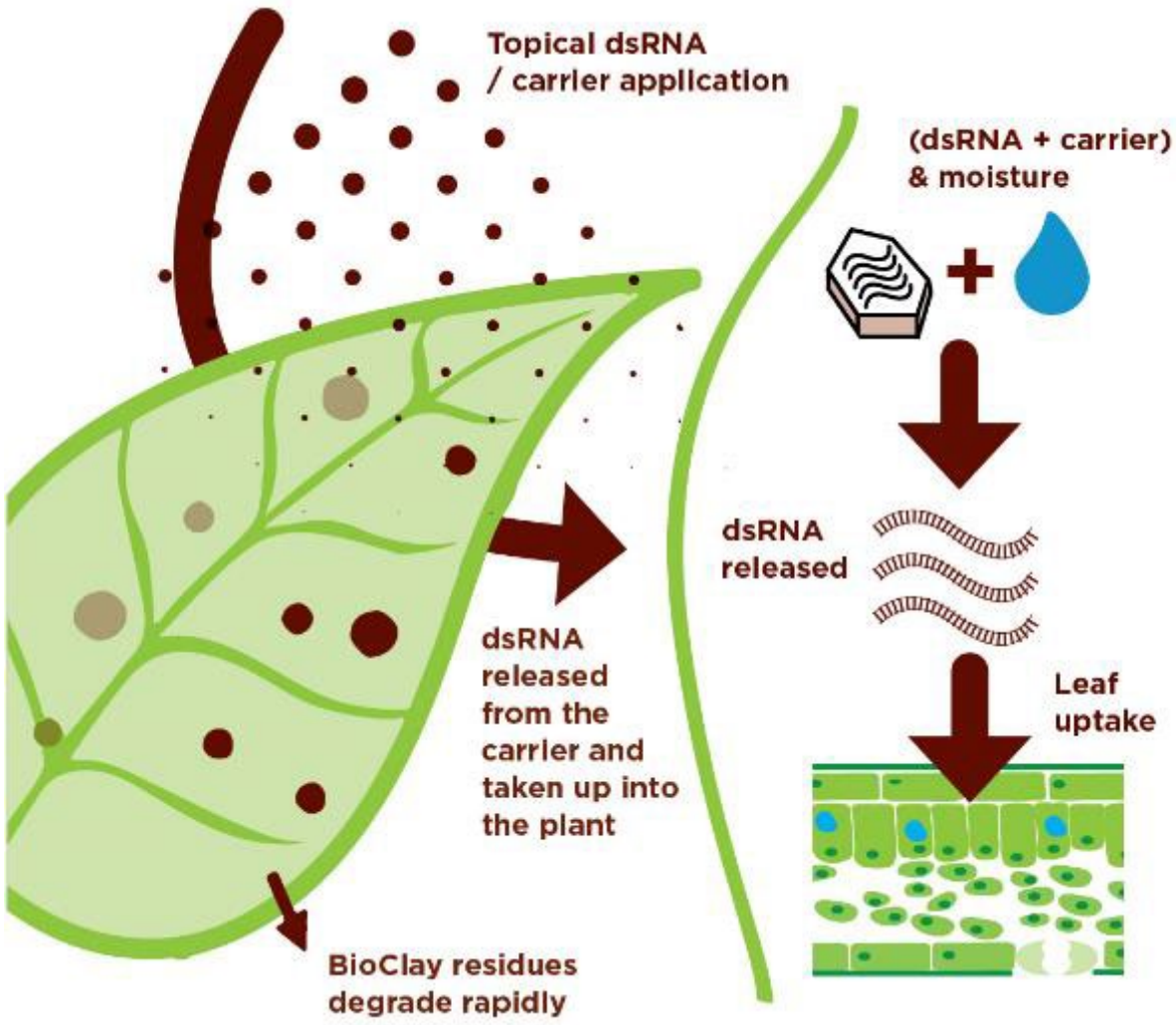
BioClay™

How BioClay works:

BioClay is a layered clay carrier that protects and stabilises the dsRNA

Allows for the gradual release of dsRNA



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BioClay™ for control of Fungal diseases

Sclerotinia rot of canola/rapeseed

Fusarium on cereals

Verticillium wilt of cotton

Hub Teams
BioClay Targets
Validation of Efficacy
Uptake and movement

NuFarm
- BioClay manufacture & QC

Grey mould on various hosts

BioClay provides longer protection in small scale Botrytis disease assays in tomato and chickpea

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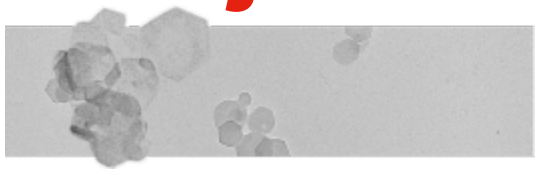
ARC Sustainable Crop Protection Hub Projects



Formulation and Commercialisation

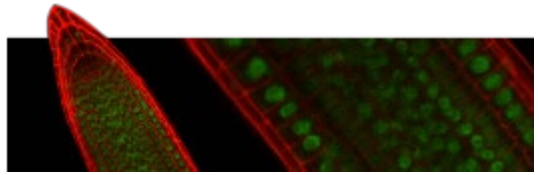
Fusarium - Cereals

Verticillium - Cotton

Botrytis - Grape

Policies and Social Sciences

Mechanistic Insights



Sclerotinia - Canola

Botrytis - Strawberries

Botrytis - Pulses

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ARC Sustainable Crop Protection Hub Botrytis Strawberry Objectives

- **Develop BioClay platform for the management of *Botrytis* – Strawberry**
  - **Validate BioClay in lab/Glasshouse/Field Trials.**
  - Investigate the uptake, processing and systemic movement of double-stranded RNA and its smaller RNA derivatives in Strawberry and *Botrytis* host pathogen system.
  - Generate new knowledge on the mechanisms of BioClay-fungi-crop interactions, towards optimising the system and opening up new applications for crop improvement.

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ARC Sustainable Crop Protection Hub Botrytis Strawberry Deliverables

- **Deliverables**
  - Graduate one PhD student in the area of Bioclay-based tools for *Botrytis* control in Strawberry.
  - Deliver BioClay formulation for product development and commercialisation for control of *Botrytis cinerea* in Strawberry both pre- and post-harvest.

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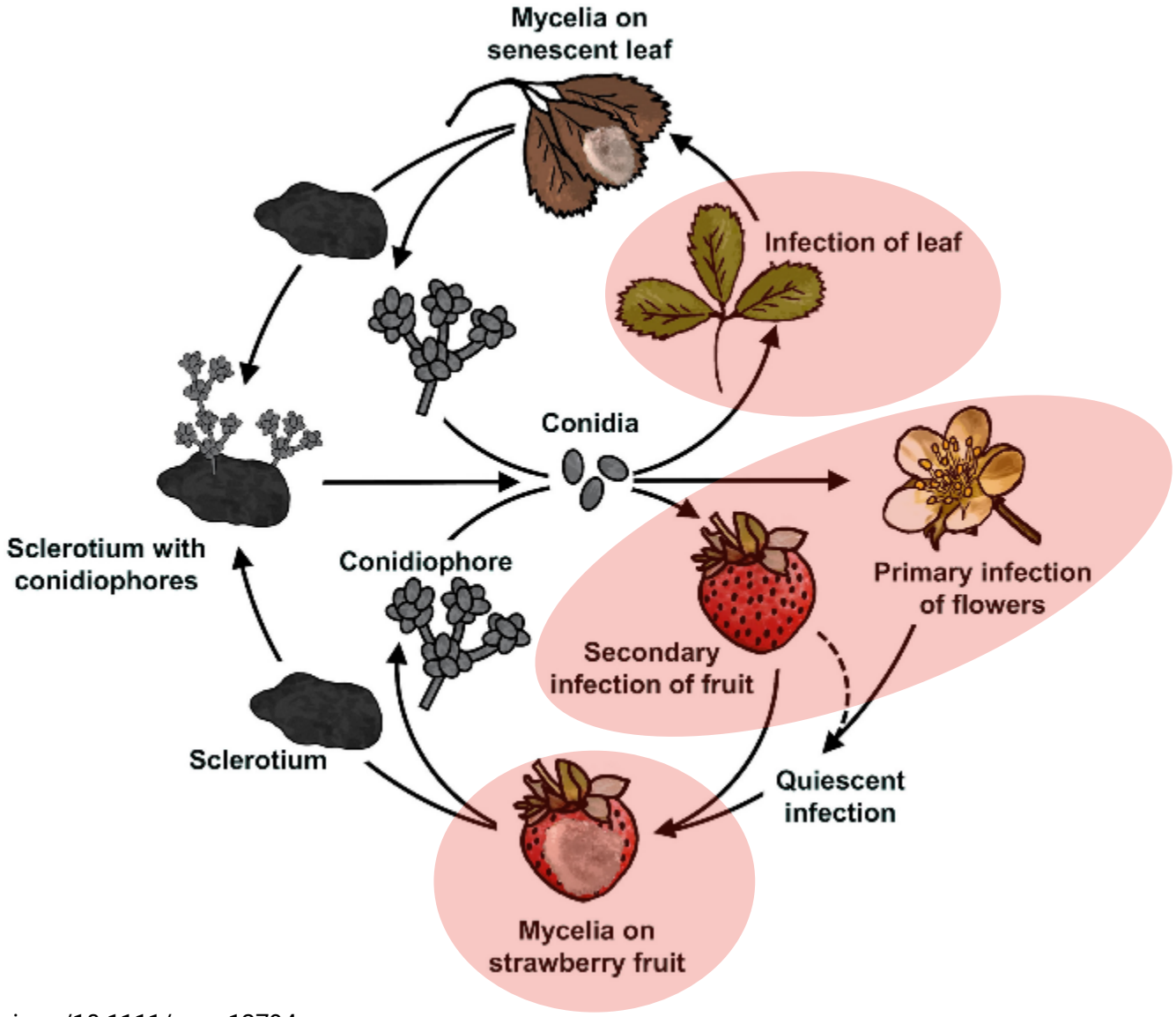
Grey Mould *Botrytis cinerea*



BioClay™ Targets

Petrasch *et al.*, (2019) Molecular Plant Pathology. doi.org/10.1111/mpp.12794

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Progress

- Purified two *Botrytis cinerea* isolates (25414 and 28032) and demonstrated pathogenicity in Strawberry

- Developed infection assays on post-harvest fruits
  - Latent infections very challenging, particularly on consumer fruit. Sterilisation essential.
- Optimised infection assays on detached leaves
  - Latent infections very challenging

- Developed an axenic production system for two varieties of strawberry plants

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Axenic tissue culture system for strawberry

cv. Temptation

cv. Alpine

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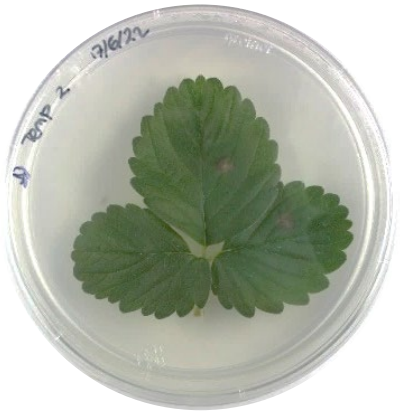
Axenic Pathosystem from sterile seeds

	3 dpi	5 dpi	7 dpi
cv. Temptation			
cv. Alpine			

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Future Prospects

- **Within 6-12 months**
  - *Validated BioClay in vitro/glasshouse experiments with existing targets, with comparison to existir treatments*
- **Within 12-24 months**
  - *Optimised BioClay in vitro/glasshouse experiments (with new/alternative targets)*
- **Within 24-36 months**
  - *Optimised BioClay in pilot field trial experiments*

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Industry Outcomes

Berry growers have a product with a new mode of action against *B. cinerea.*

Berry growers in Australia are amongst the first worldwide to utilise dsRNA technologies for crop protection.

The risk of fungicide resistance in populations of B. cinerea is reduced and the effective life of current fungicide chemistries is extended through the integrated use of dsRNA technologies.

Berry growers have more sustainable crop protection strategies that are accepted by regulatory authorities and consumers.

Acknowledgments

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Nufarm

Kieran Murphy

Mike Poynton

Growers who kindly provided fruit

VSICA for advice on tissue culture

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Thank you

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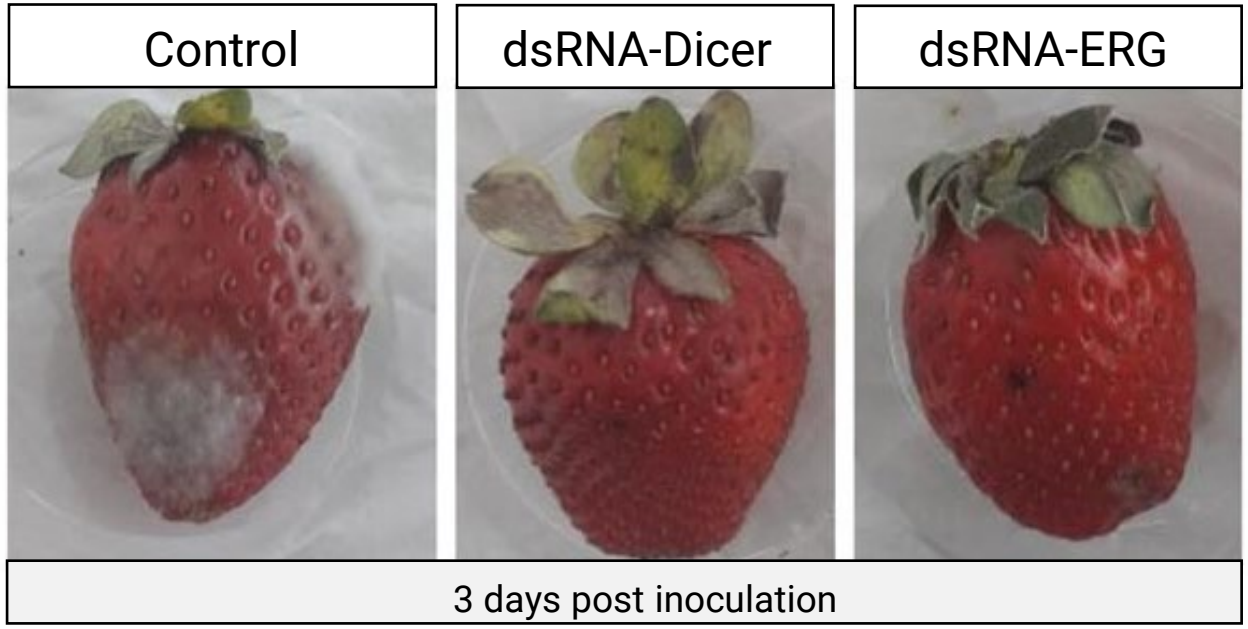

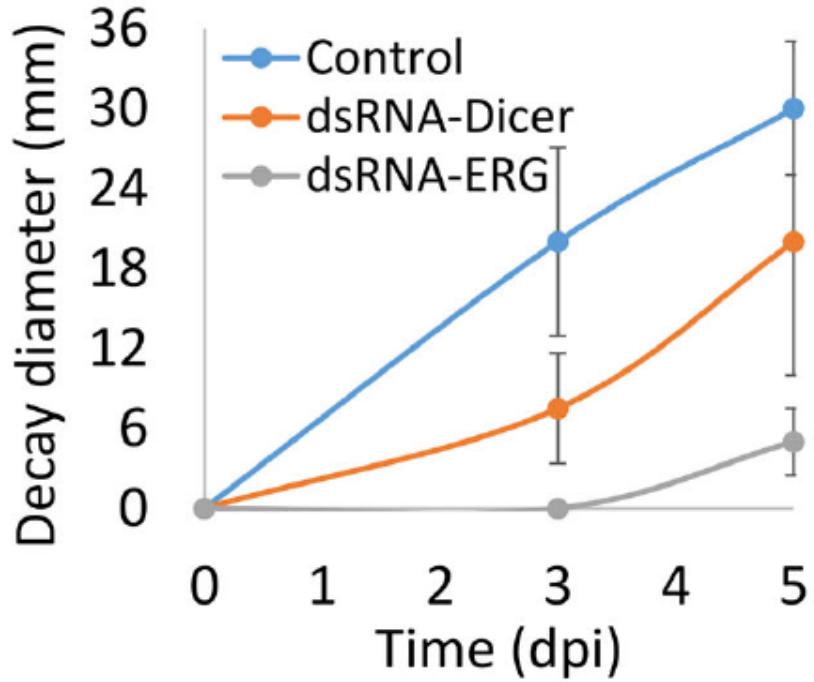

Duanis-Assaf *et al.*, (2021) Plant Biotechnology Journal. doi: 10.1111/pbi.13708

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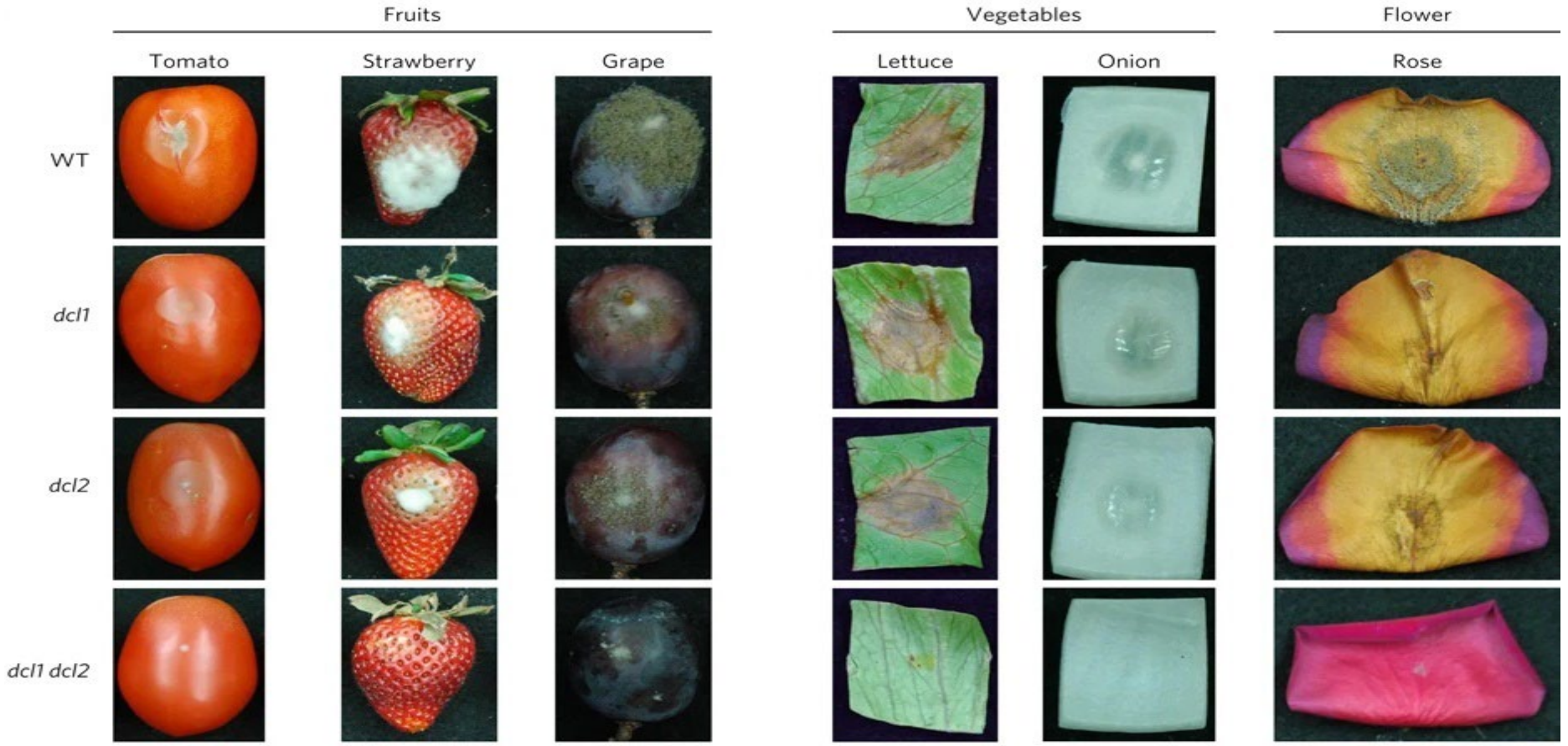
Proof of Concept- *Botrytis*with RNA processing mutations



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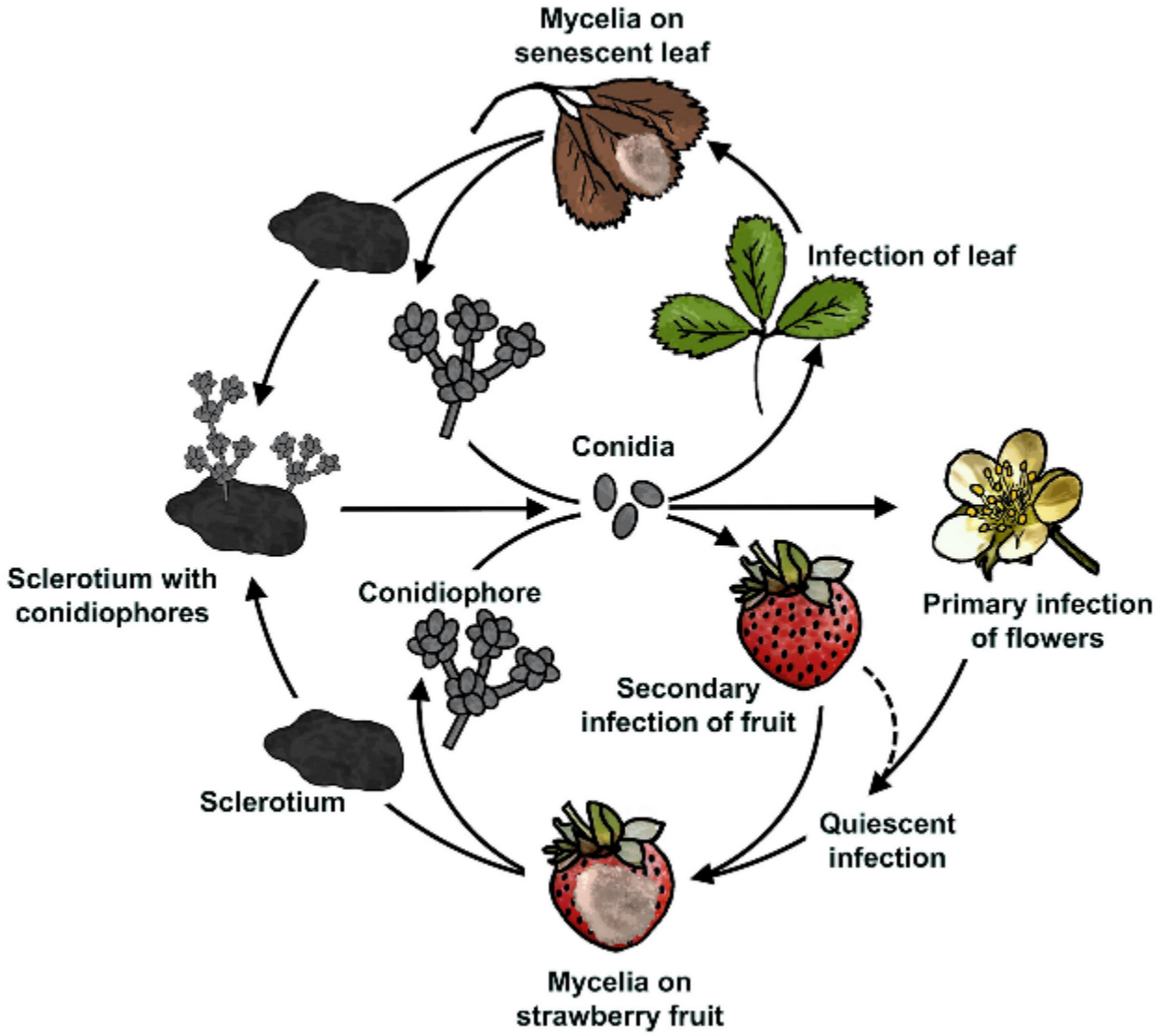

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