21º CONFERENCIA INTERNACIONAL SOBRE PALMA DE ACEITE

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Nuevos biofungicidas basados en ARN

Innovative RNA-based biofungicides

Universidad de Valladolid

jonatan.nino@uva.es

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RNA interference

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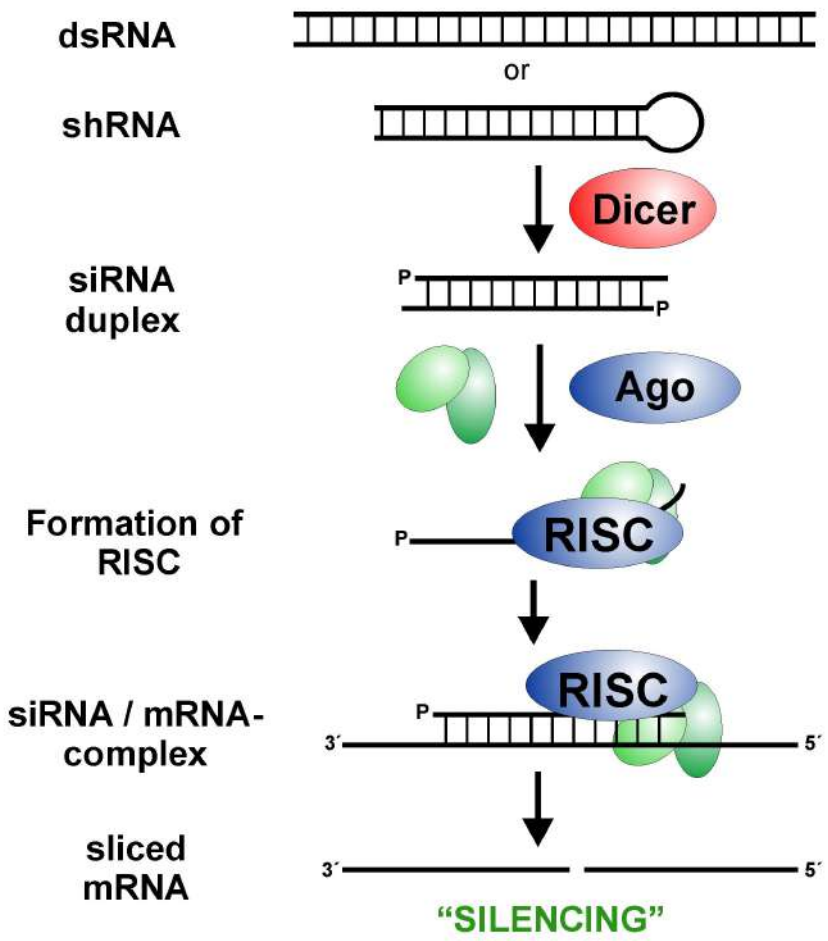
Conserved machinery involved in RNA interference (RNAi) process



Dicer (-like) proteins

RNase III nucleases that cleave double-stranded RNA (dsRNA) into short (19-25) nt long small interfering RNA (siRNA)

RISC (RNA-induced silencing complex)

Mature sRNAs are loaded into Argonaute (AGO) proteins and induce silencing of genes with fully or partially complementary sequences

RdRPs

(RNA-dependent RNA polymerases)

Cross-Kingdom RNA Interference (CKRI) is a bi-directional process

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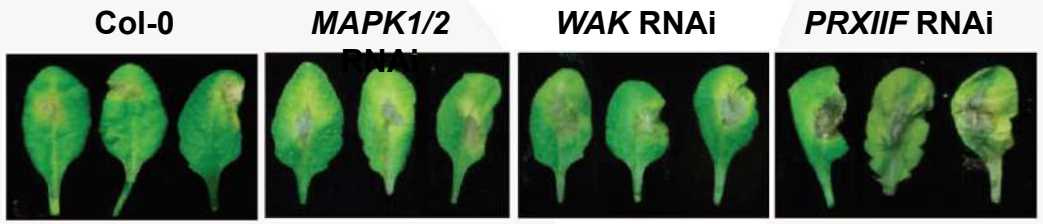
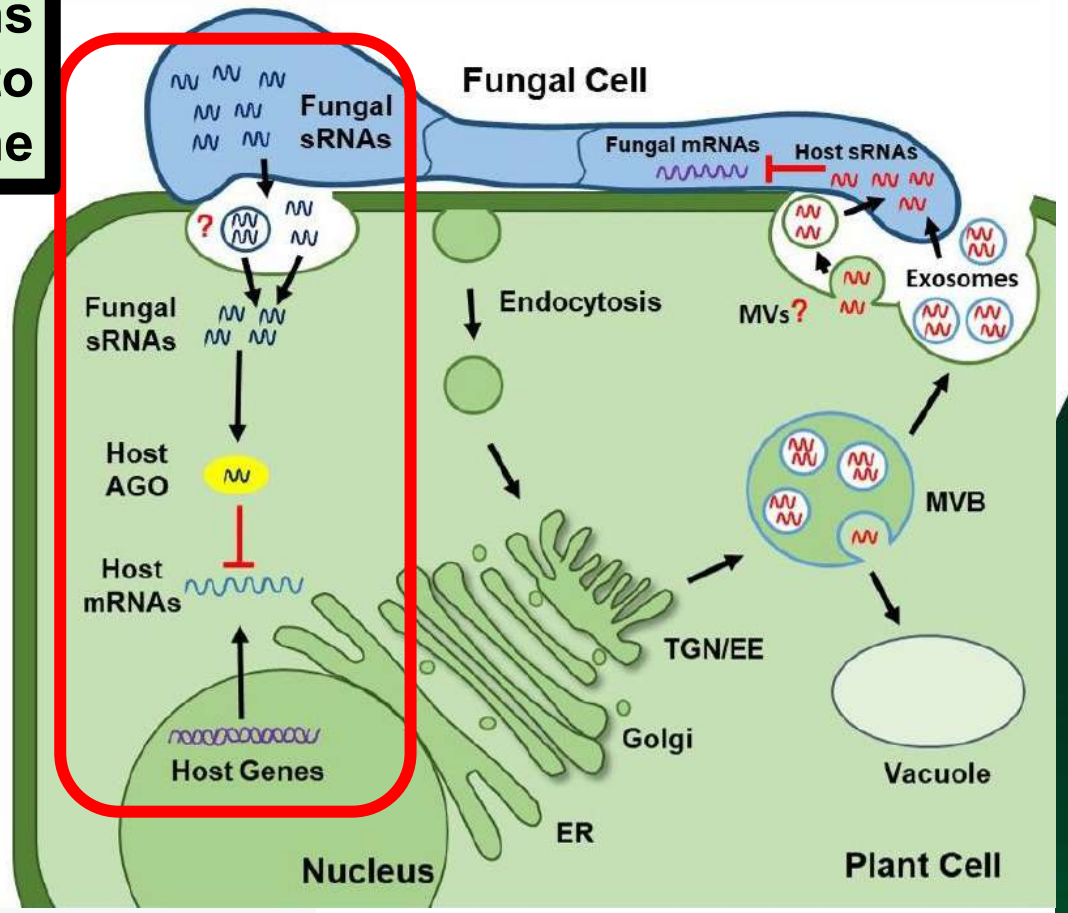
Some plant and animal pathogens and pests can deliver sRNAs into host cells to modulate host immune responses

Botrytis cinerea delivers sRNAs into plant cells and hijacks the host RNAi machinery by loading its sRNAs into the *Arabidopsis* AGO1 to trigger silencing of host immunity genes.



Modified from Weiber *et al*., 2013. *Science*. **342**, 118-123



Cai *et al*., 2019. *PLoS Pathog*. **15** (14)

Cross-Kingdom RNA Interference (CKRI) is a bi-directional process

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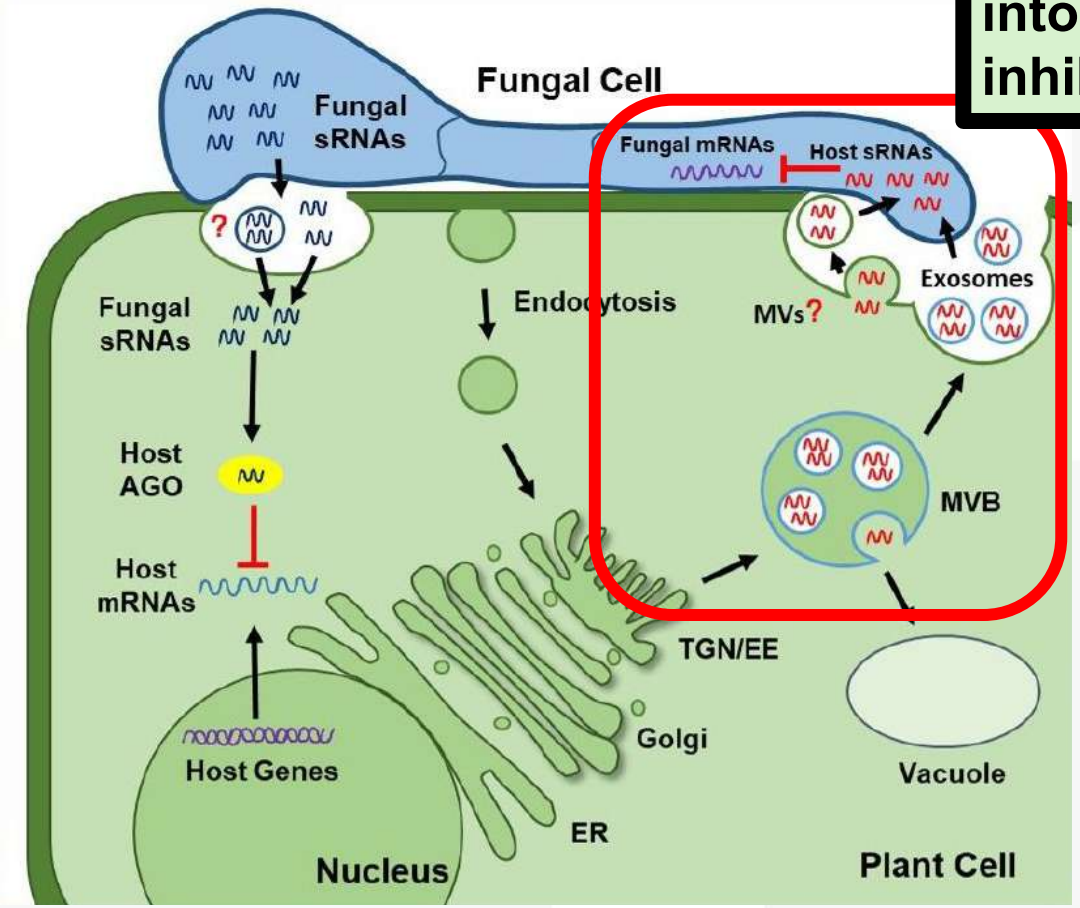
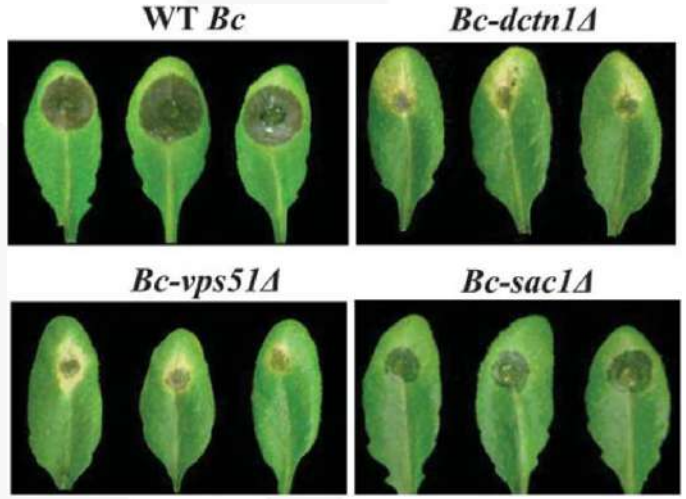
But… hosts also transfer sRNAs into pathogens and pests to inhibit their virulence



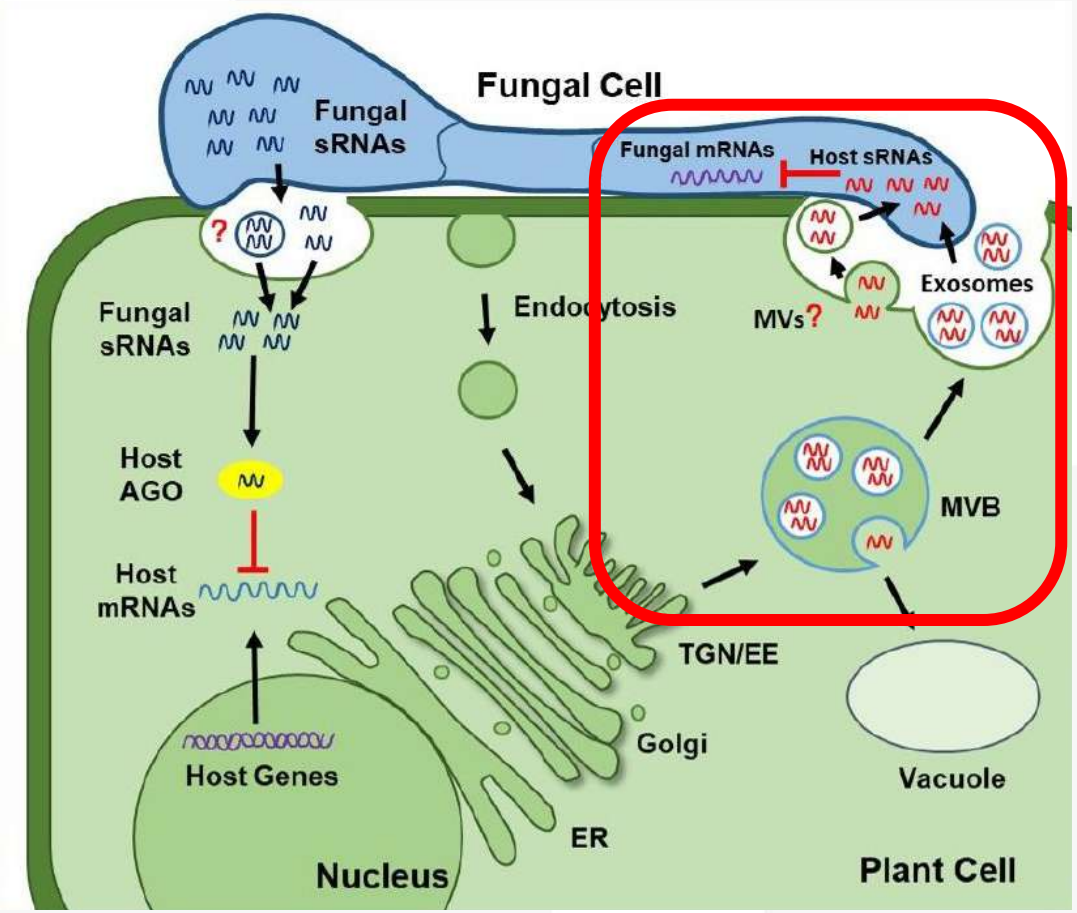
Cai *et al*., 2019. *PLoS Pathog*. **15** (14)

Naturally transferred *Arabidopsis* sRNAs silence fungal genes involved in vesicle trafficking to inhibit *B. cinerea* virulence



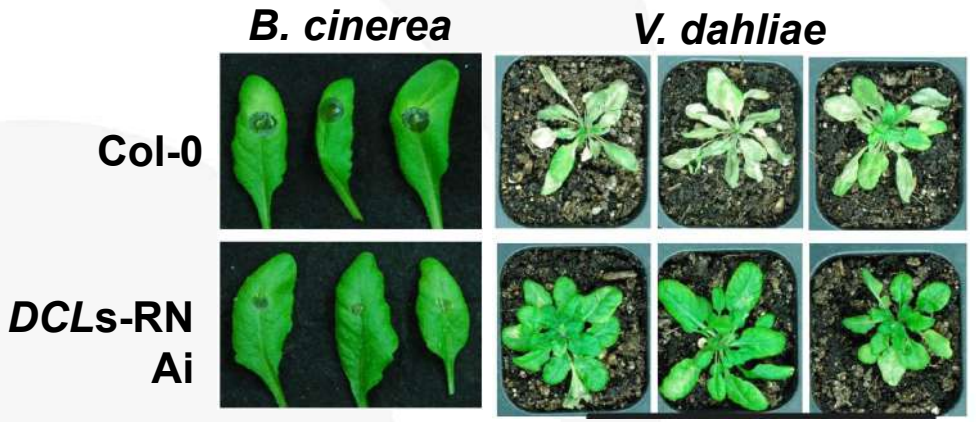
Cai *et al*., 2018. *Science*. **360**, 1126-1129

Cross-Kingdom RNA Interference (CKRI) is a bi-directional process



Cai *et al*., 2019. *PLoS Pathog*. **15** (14)

HIGS: Host-Induced Gene silencing (Involves transgenic plants)



Modified from Wang *et al*., 2016. *Nat. Plants* **2**

SIGS: Spray-Induced Gene Silencing

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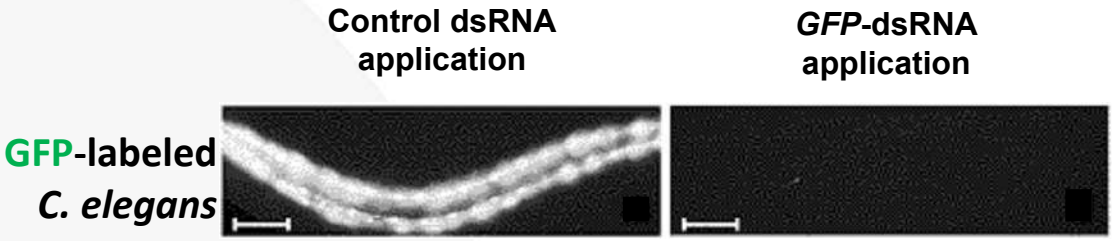
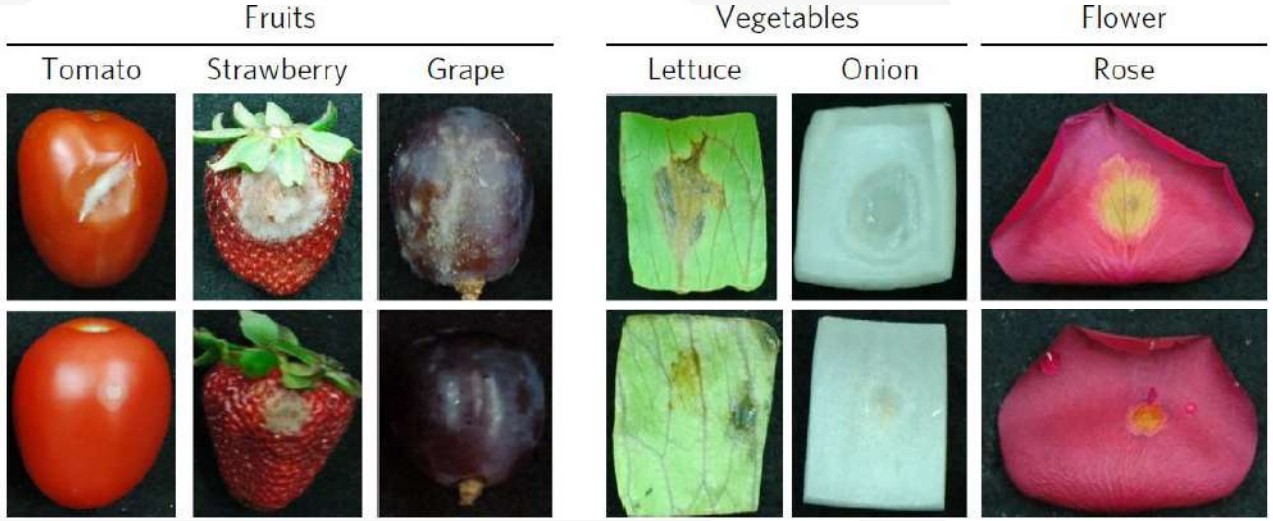
Exposure of eukaryotic cells to dsRNA can trigger RNAi.

This process was initially described in the nematode *Caenorhabditis elegans* and subsequently demonstrated in fungi, plants, insects and mammals



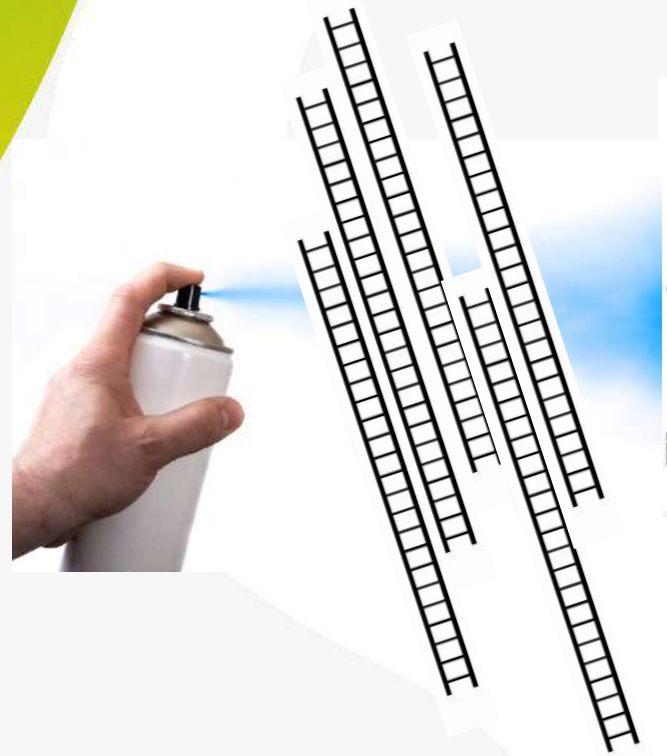
Modified from de Fire *et al*., 1998. *Nature* **391**, 806-811

dsRNA application against *DCL1* and *DCL2* genes of *B. cinerea* provides fruit, vegetable and flower protection

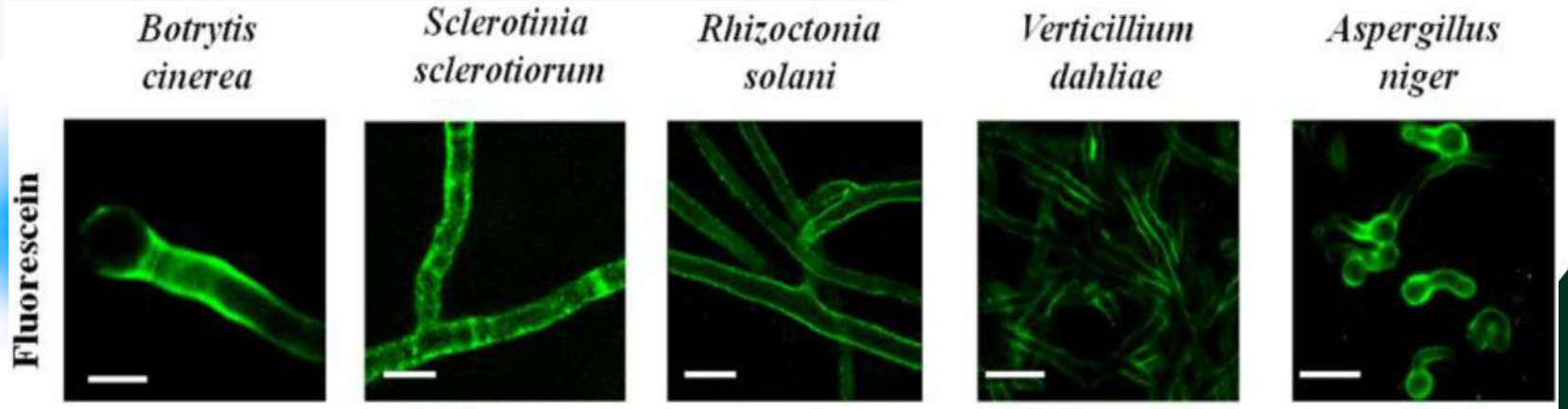


Modified from Wang *et al*., 2016. *Nature Plants* **2**

SIGS: Spray-Induced Gene Silencing



Fluorescein labelled dsRNA



Qiao *et al*., 2021. *Plant Biotech.* 2021

Plant Biotechnology Journal

Plant Biotechnology Journal (2021), pp. 1–13 doi: 10.1111/pbi.13589

Spray-induced gene silencing for disease control is dependent on the efficiency of pathogen RNA uptake

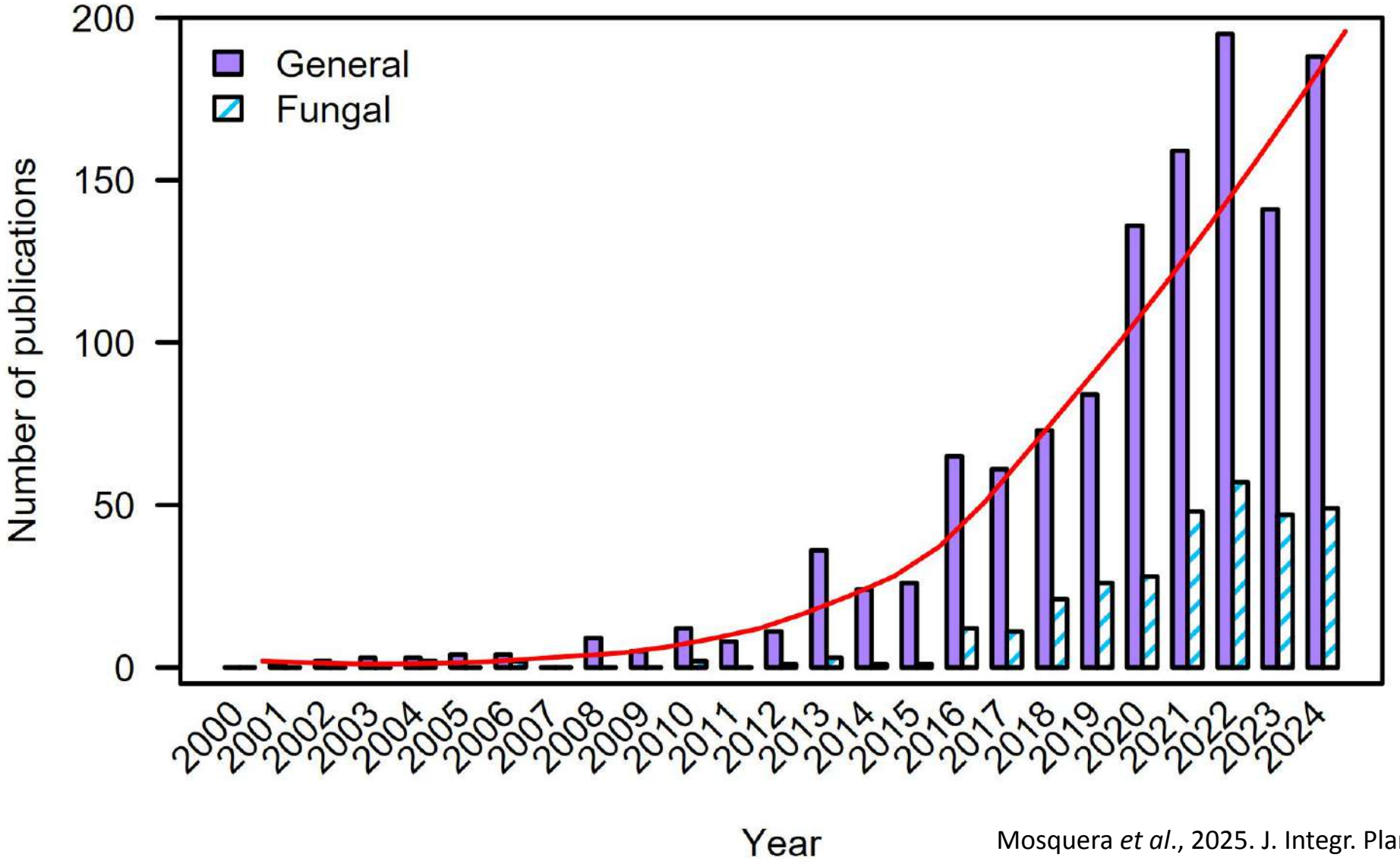
Lulu Qiao[1,2,3], Chi Lan[1,2], Luca Capriotti[3,4], Audrey Ah-Fong[3], Jonatan Nino Sanchez[3], Rachael Hamby[3], Jens Heller[5,6], Hongwei Zhao[1,2], N. Louise Glass[5,6], Howard S. Judelson[3], Bruno Mezzetti[3,4], Dongdong Niu[1,2,3,*] and Hailing Jin[3,*]

SIGS: Spray-Induced Gene Silencing

ADVANTAGES

- No transgenic plant generation is required
- Versatile: can target multiple genes and multiple pathogens.
- Does not require complete base pairing.
- Environmentally friendly
- Pre-harvest and post-harvest applications are powerful and effective.

SIGS: Spray-Induced Gene Silencing



Mosquera *et al*., 2025. J. Integr. Plant Biol. **1**

SIGS: Spray-Induced Gene Silencing

Calantha uses the process of RNAi to control the Colorado potato beetle. Developed by **GreenLight Biosciences**, it contains the active ingredient **ledprona**, a dsRNA molecule that targets a specific gene essential for the beetle's survival. When the beetle eats the sprayed dsRNA, the RNAi process is triggered, preventing the production of vital proteins and ultimately killing the pest.

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Fungal pathogen control examples from our lab

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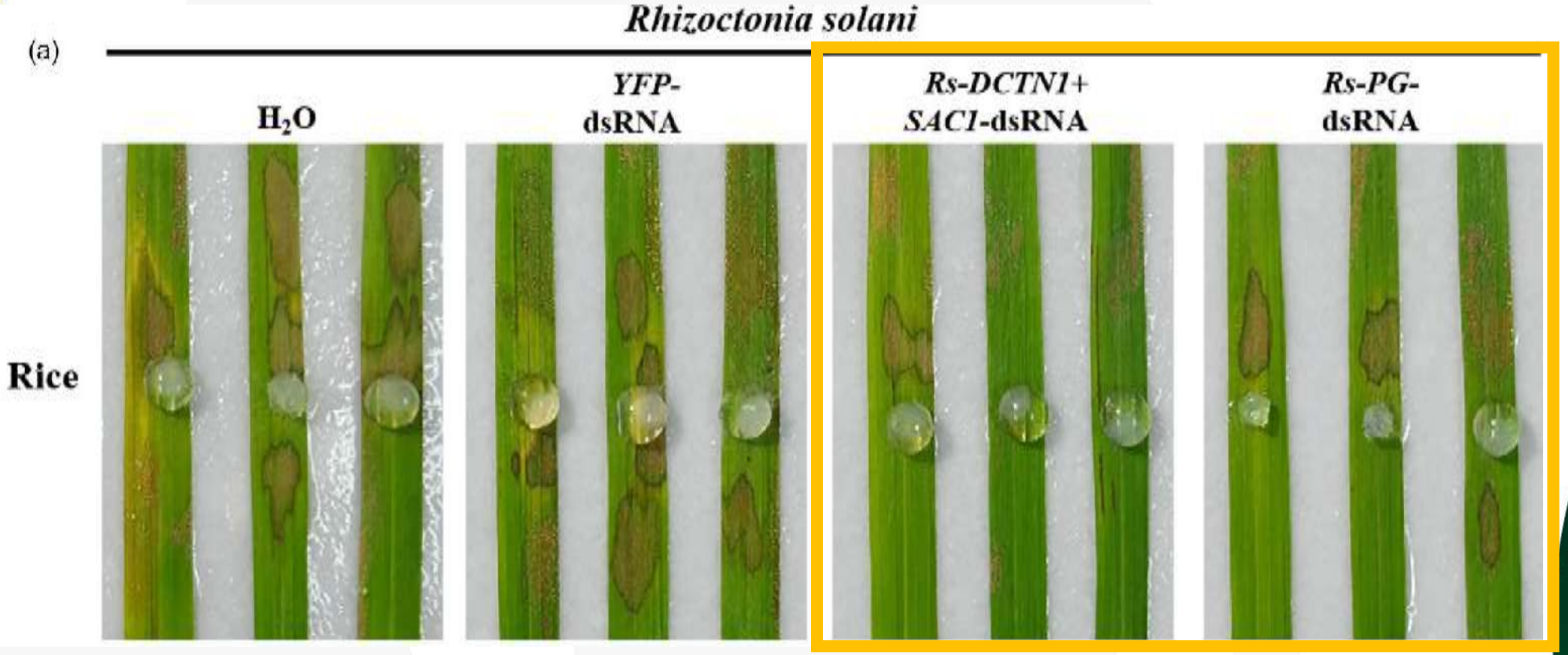
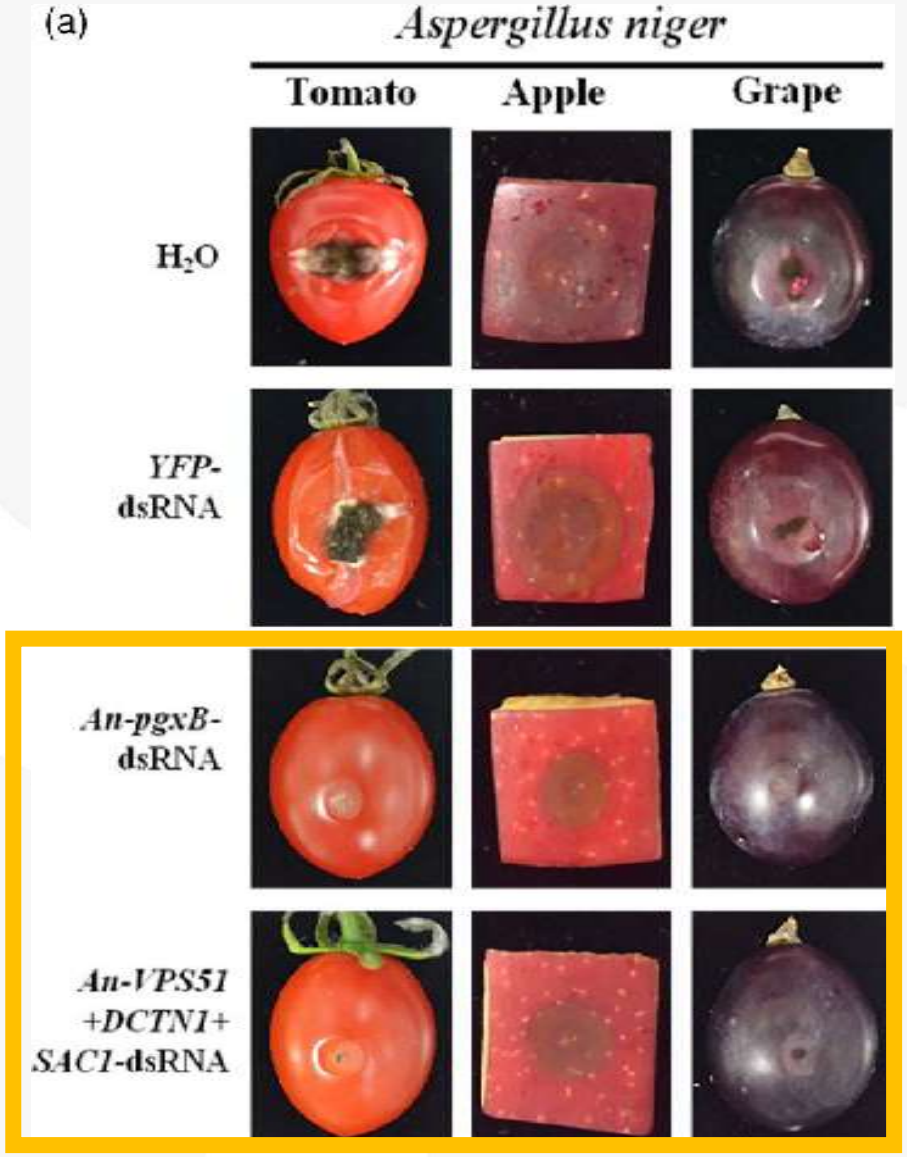


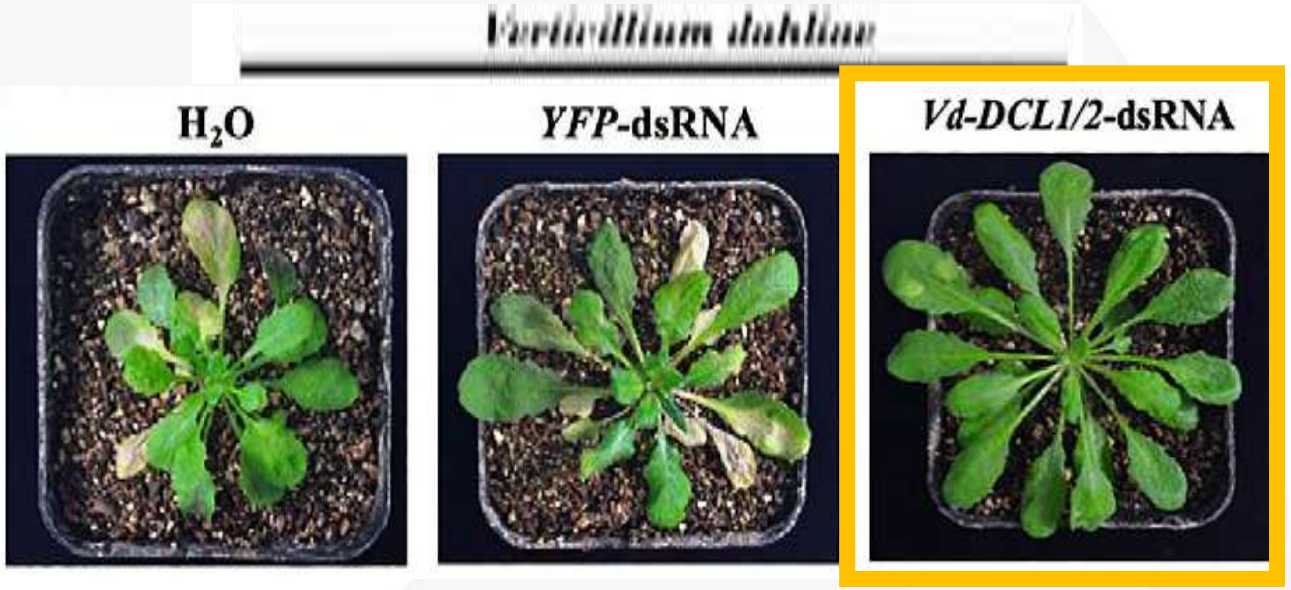
Qiao *et al*., 2021. *Plant Biotech.* 2021

SIGS: Spray-Induced Gene Silencing



Qiao *et al*., 2021. *Plant Biotech.* 2021

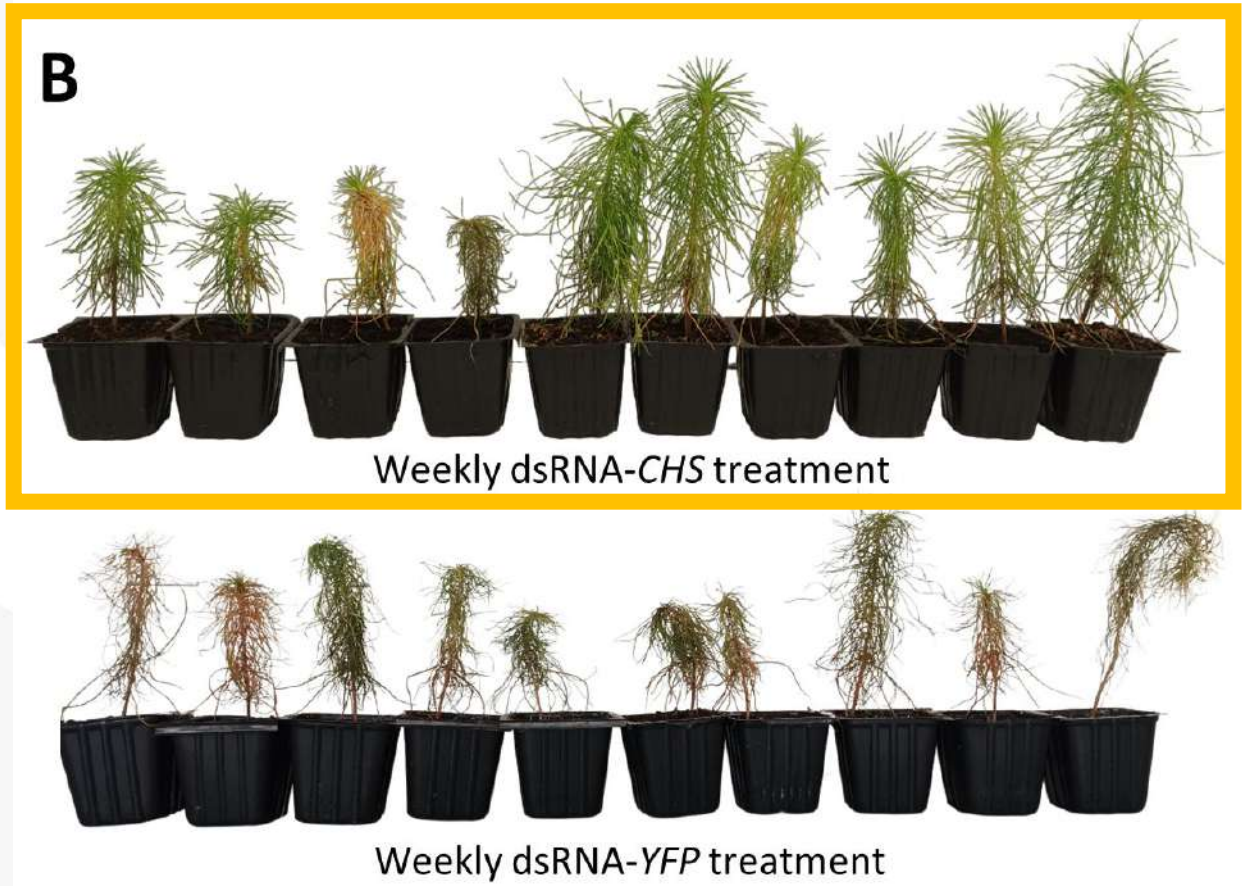
SIGS: Spray-Induced Gene Silencing



Modificado de Qiao *et al*., 2021. *Plant Biotech.* 2021

SIGS: Spray-Induced Gene Silencing

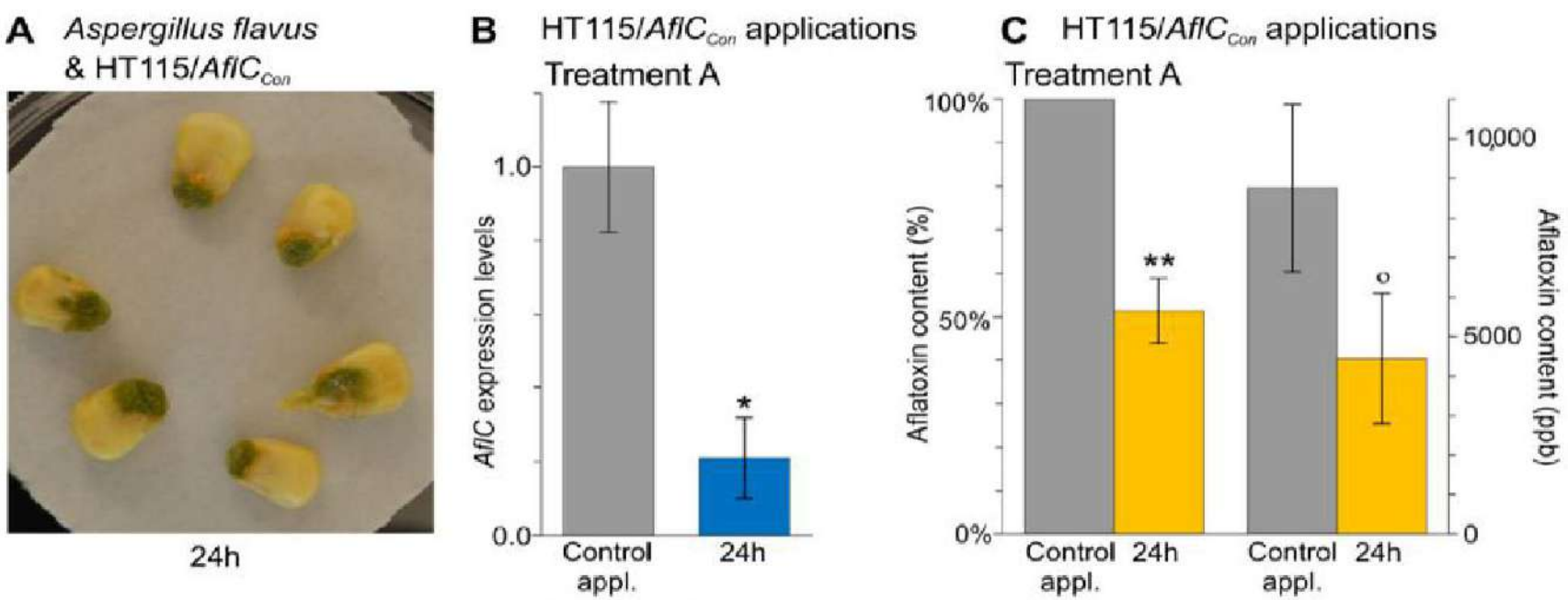
Fusarium circinatum



Bocos-Asenjo *et al*. *Plant disease*. 2025

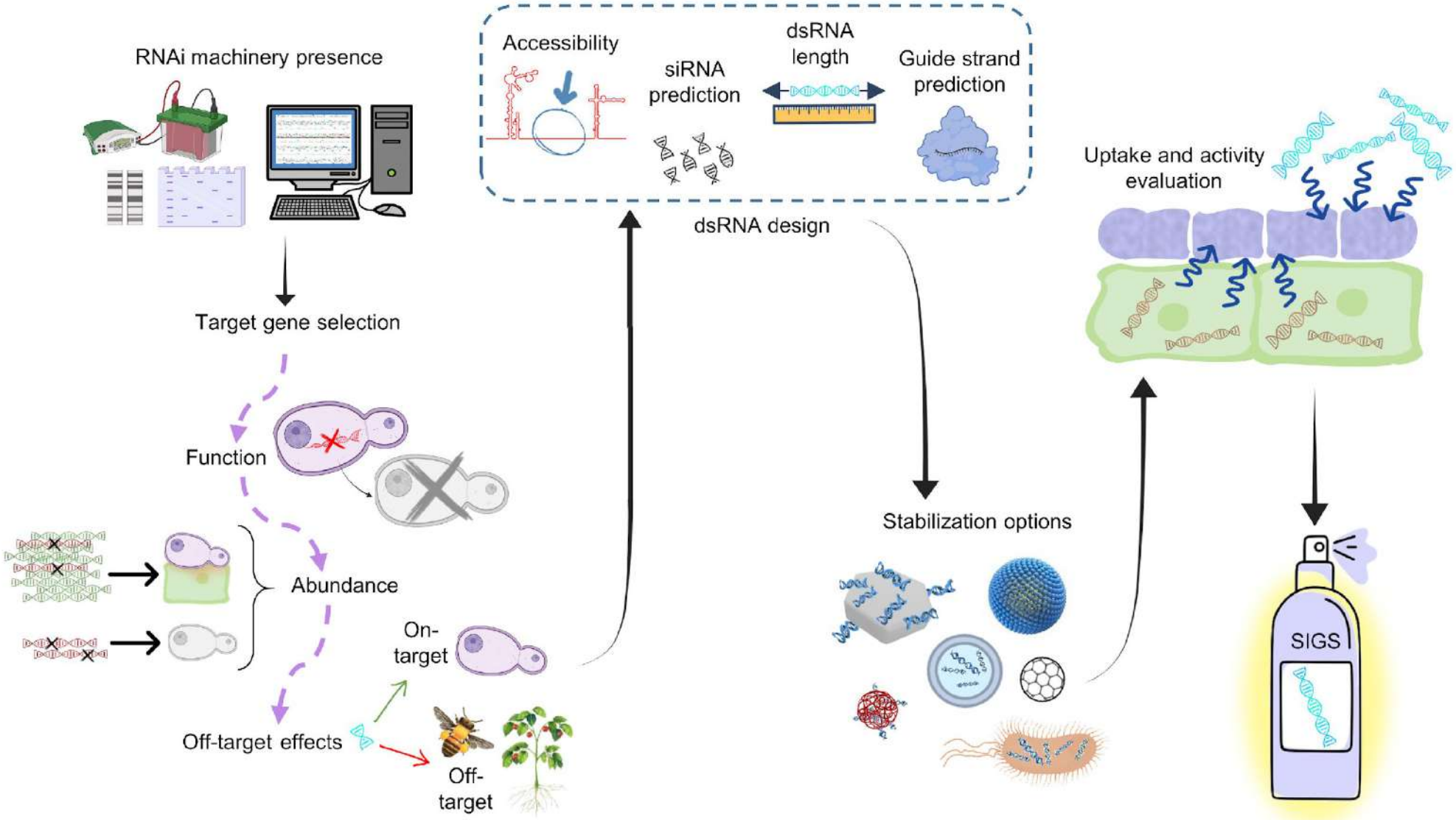
SIGS: Spray-Induced Gene Silencing

Aspergillus flavus



Niño-Sánchez *et al*., 2021. *J. of fungi.* 2021

SIGS: A step-by-step guide. What we know, what we don't



Mosquera *et al.*, 2025. J. Integr. Plant Biol. **1**

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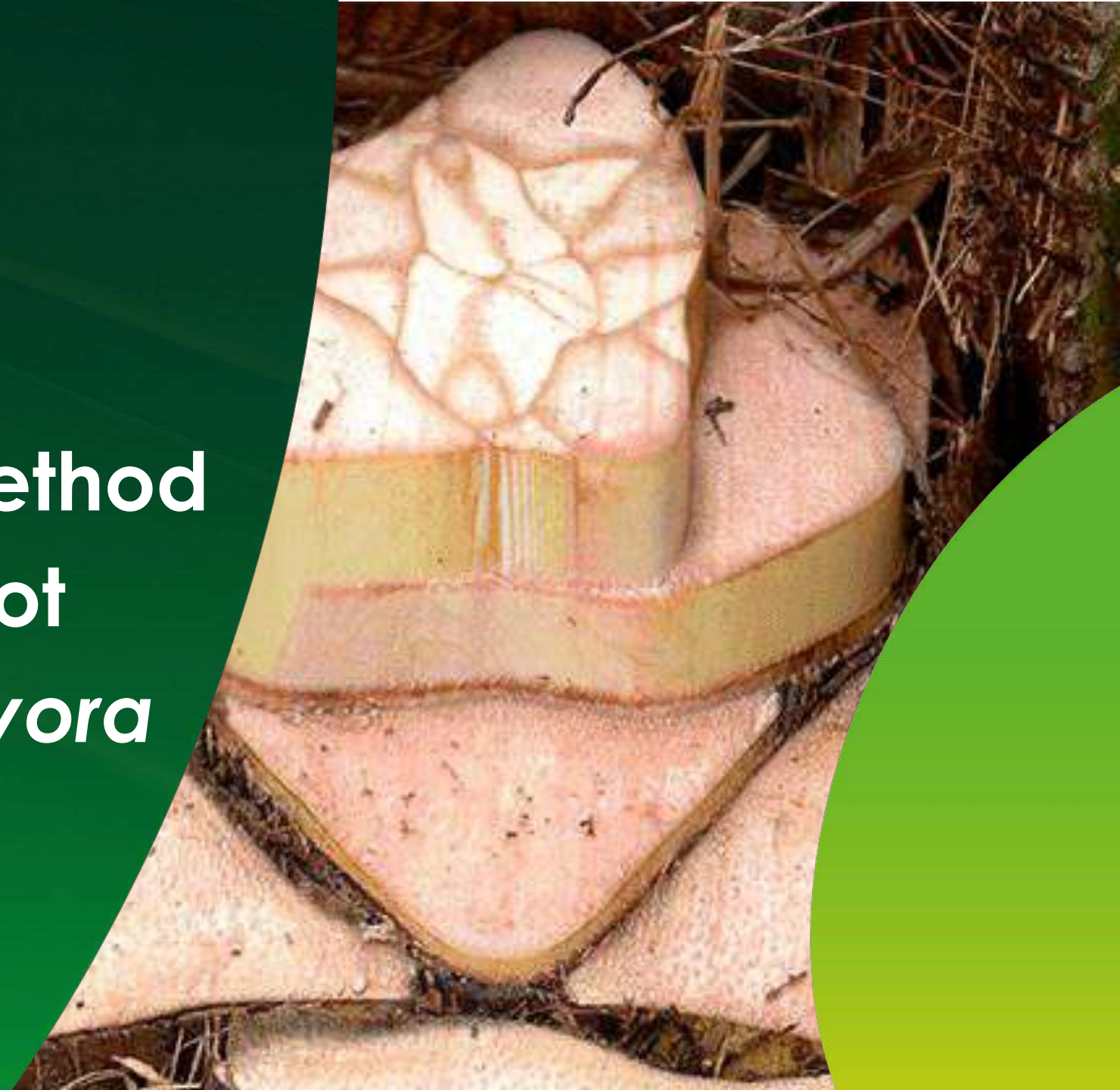
SIGS as potential method for controlling bud rot caused by *P. palmivora*



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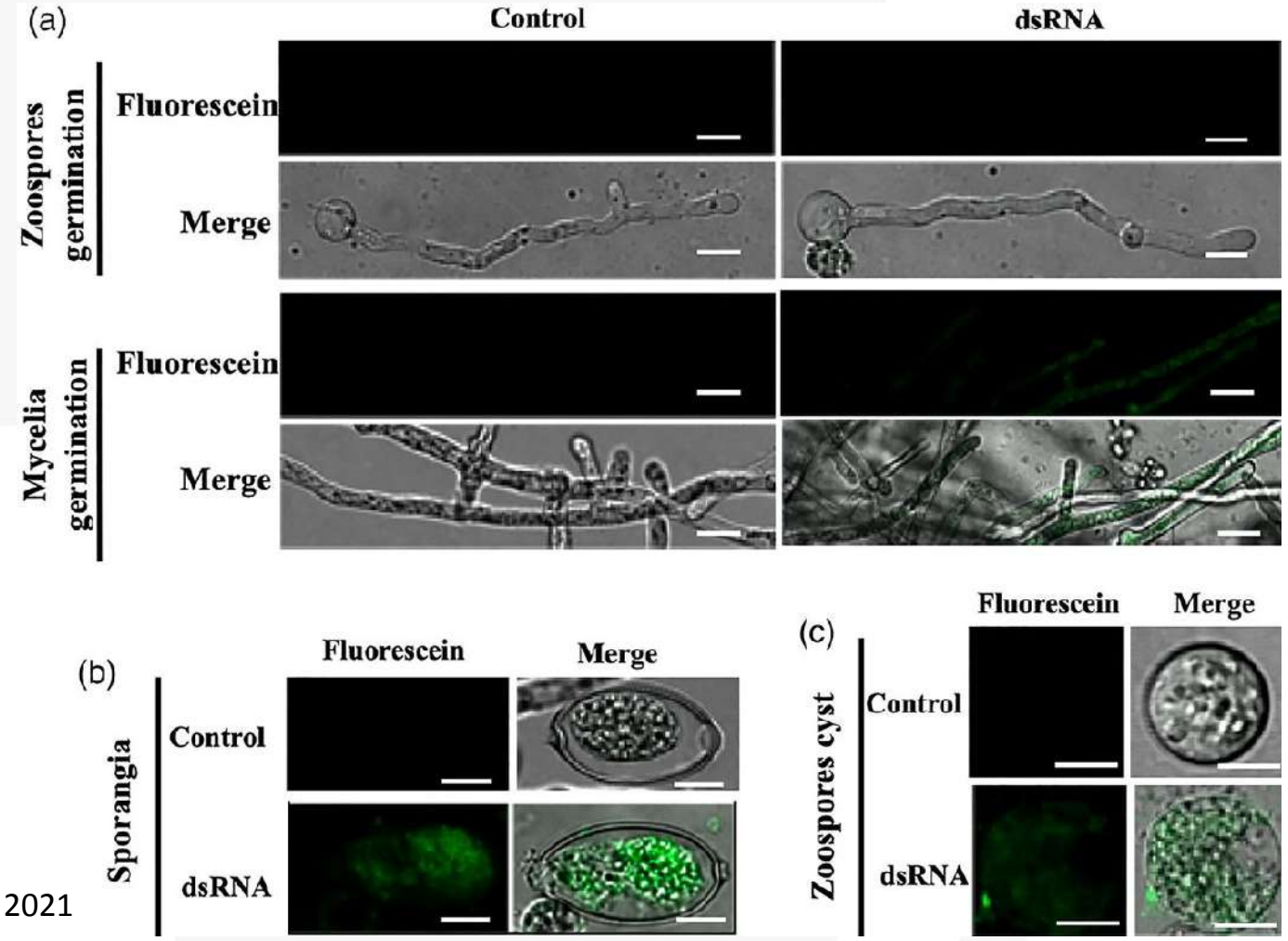
Phytophthora infestans



***Phytophthora* species have a limited dsRNA uptake...**

…so we did not find differences in *in vitro* growth

Qiao *et al*., 2021. *Plant Biotech.* 2021

However… several research groups reported that SIGS was effective in *P. infestans*.

Phytopathology® · 2021 · 111:2168-2175 · https://doi.org/10.1094/PHYTO-02-21-0054-SC

Short Communication

e-Xtra*

Spray-Induced Gene Silencing as a Potential Tool to Control Potato Late Blight Disease

Pruthvi B. Kalyandurg,[1] Poorva Sundararajan,[1] Mukesh Dubey,[2] Farideh Ghadamgahi,[1,3] Muhammad Awais Zahid,[4] Stephen C. Whisson,[5] and Ramesh R. Vetukuri[1,†]

RESEARCH ARTICLE

ADVANCED FUNCTIONAL MATERIALS
www.afm-journal.de

Functionalized Carbon Dot-Delivered RNA Nano Fungicides as Superior Tools to Control *Phytophthora* Pathogens through Plant RdRP1 Mediated Spray-Induced Gene Silencing

*Zhiwen Wang, Yu Li, Borui Zhang, Xiang Gao, Mengru Shi, Sicong Zhang, Shan Zhong, Yang Zheng, and Xili Liu**

Research Article

SCI

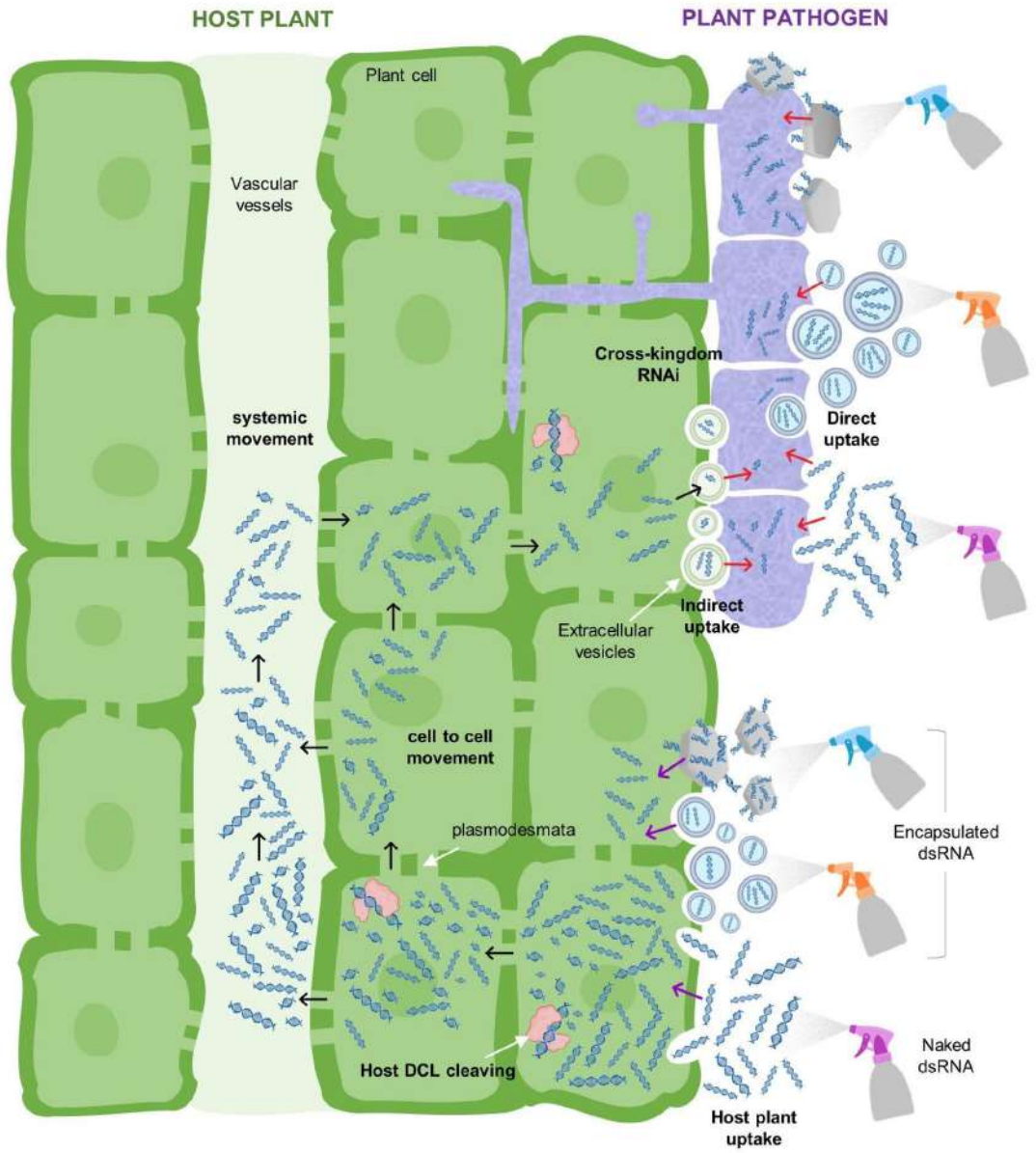
Received: 19 July 2021 Revised: 7 September 2021 Accepted article published: 28 April 2022 Published online in Wiley Online Library: 18 May 2022

(wileyonlinelibrary.com) DOI 10.1002/ps.6949

Spraying of dsRNA molecules derived from *Phytophthora infestans*, along with nanoclay carriers as a proof of concept for developing novel protection strategy for potato late blight

Sundaresha S,[a*] Sanjeev Sharma,[a] Aarti Bairwa,[a] Maharishi Tomar,[a,b] Ravinder Kumar,[a] Vinay Bhardwaj,[a] A. Jeevalatha,[a,c] Rahul Bakade,[a] Neha Salaria,[a] Kajal Thakur,[a] Bir Pal Singh[a] and S. K. Chakrabarti[a]

Direct and indirect dsRNA uptake



Mosquera *et al.*, 2025.
J. Integr. Plant Biol. **1**

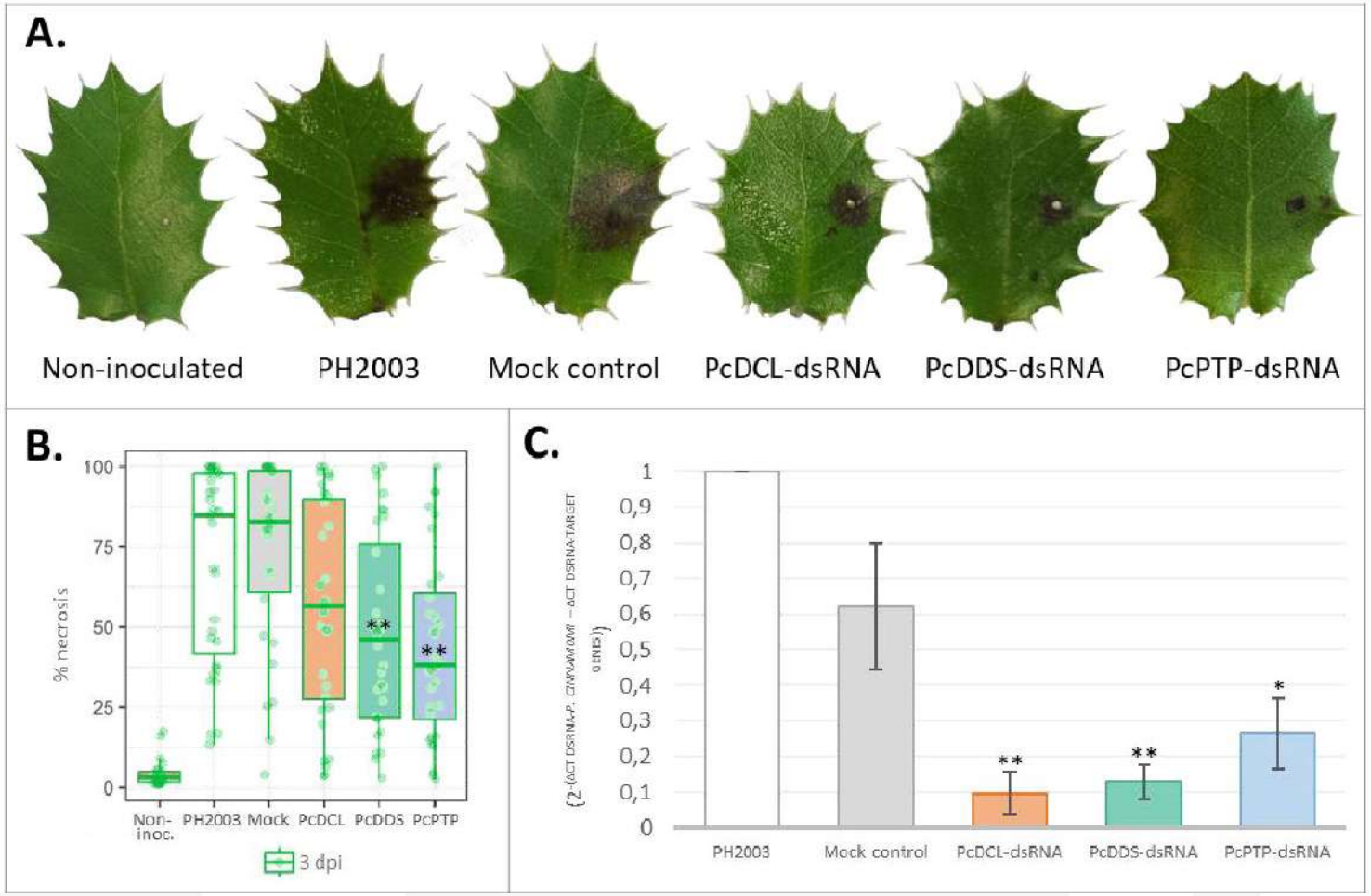
Therefore, we are currently working on two pathogenic species of the genus *Phytophthora*:

Phytophthora capsici in pepper

Phytophthora cinnamomi in holm oak

SIGS: Spray-Induced Gene Silencing

Phytophthora cinnamomi



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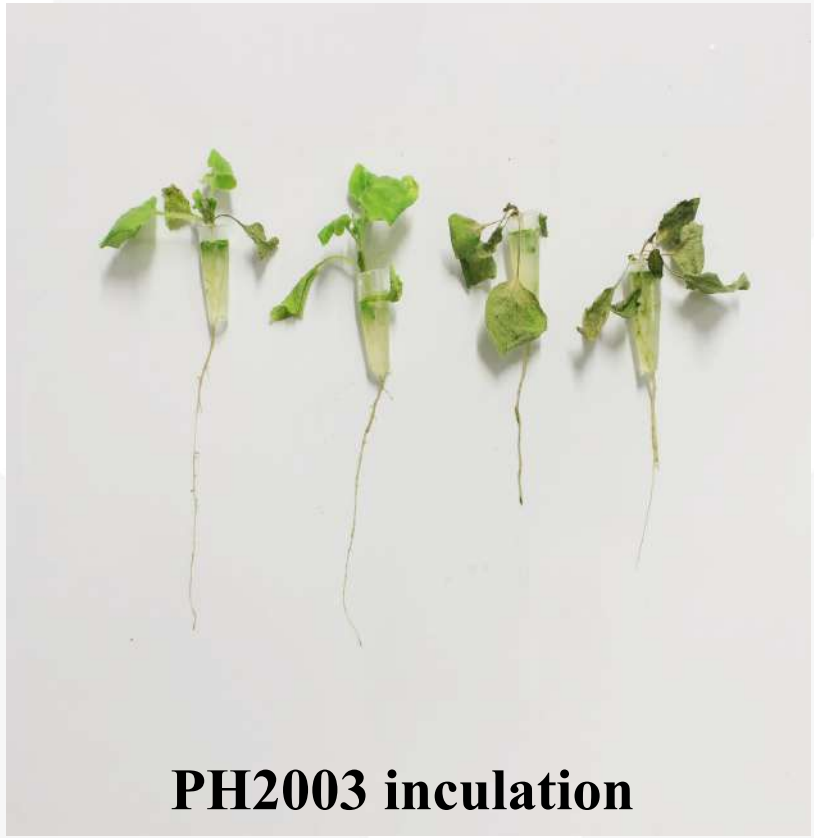
Phytophthora cinnamomi



PH2003 inculation

+ dsRNA-treated

Bocos-Asenjo 2025

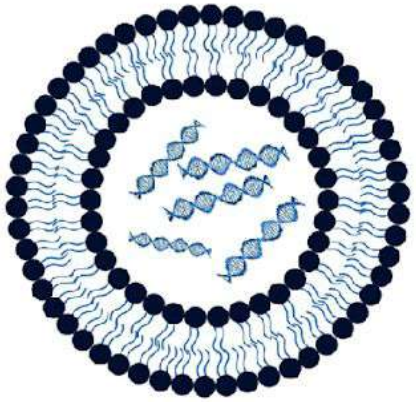
SIGS: Spray-Induced Gene Silencing

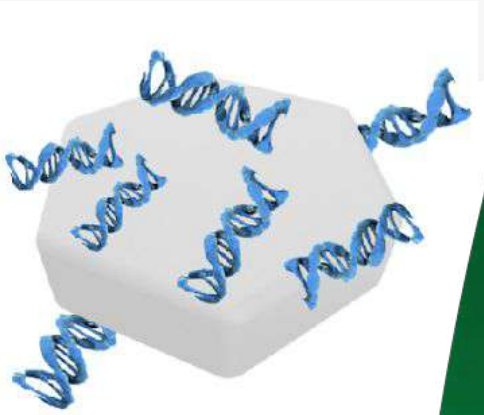
ADVANTAGES

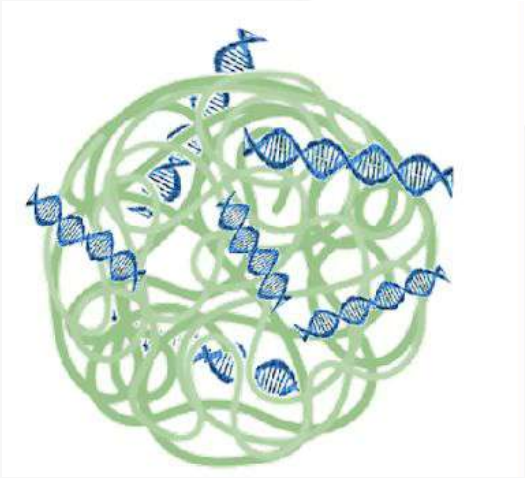
- No transgenic plant generation is required
- Versatile: can target multiple genes and multiple pathogens.
- Does not require complete base pairing.
- Environmentally friendly
- Pre-harvest and post-harvest applications are powerful and effective.

DRAWBACKS

- Transient silencing effect. Relative instability of RNA in the environment







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How overcome instability of RNA?

- Nanotechnology-

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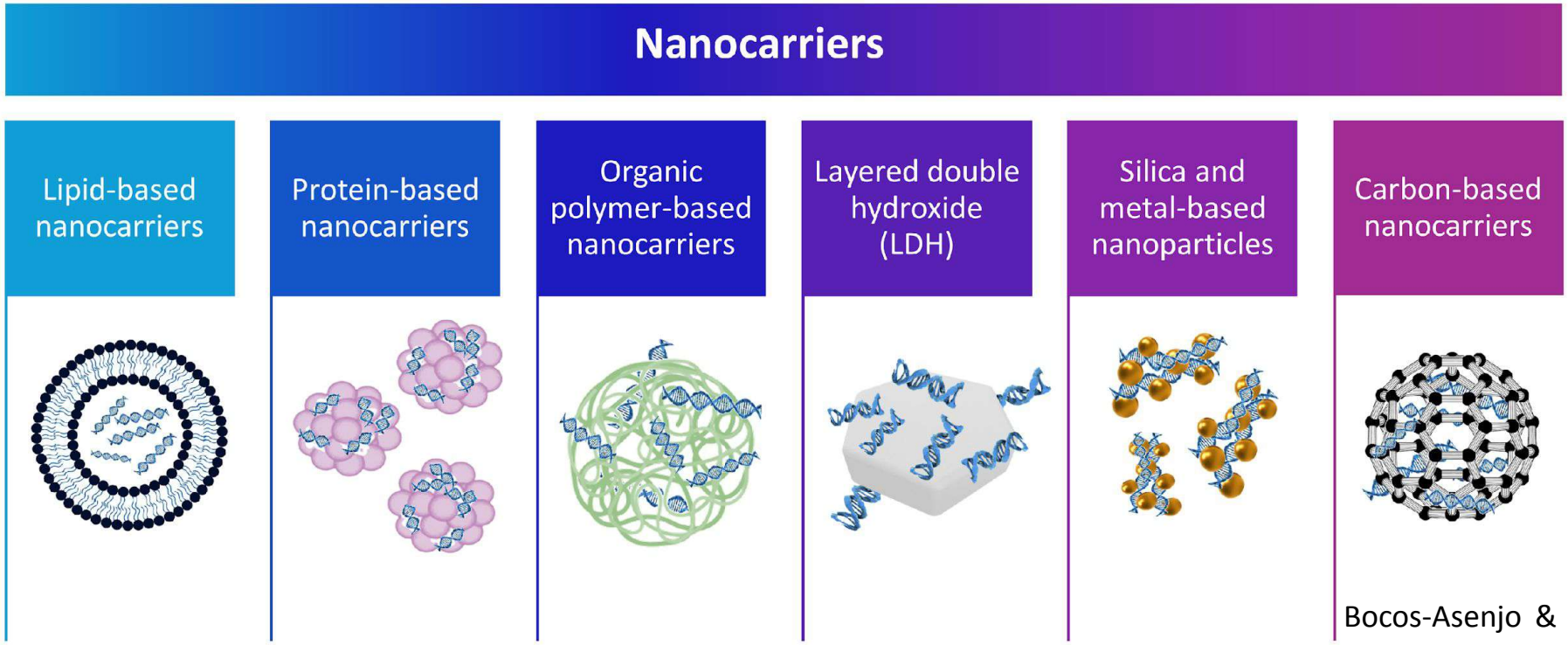
SIGS: A step-by-step guide. What we know, what we don't



Bocos-Asenjo & Niño-Sánchez, 2024. *Tierras Agricultura*

The use of nanotechnology in SIGS provides RNAi protection, stabilization, and improved delivery, leading to longer-lasting protection.

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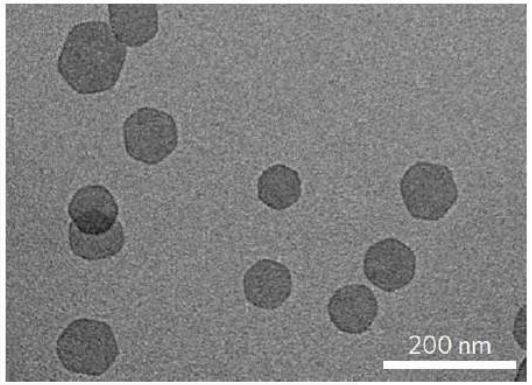
How to overcome instability of RNA?

[Nanotechnology]

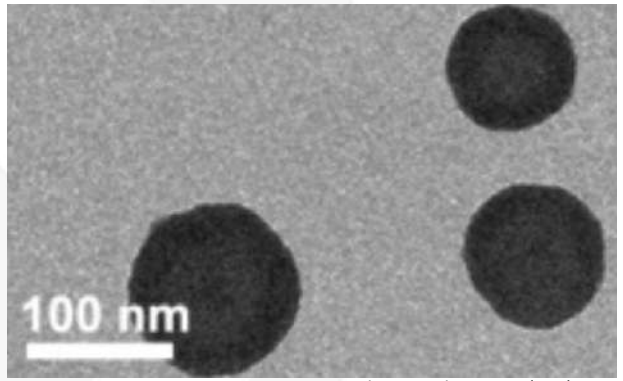
Inorganic nanoparticles



Picture: Mitter *et al.*, 2017. *Nat. Plants* **3**, 16207

Double layered hydroxide clay nanosheet (LDH)

Organic nanovesicles



Picture: B. Levy – Intramural Research Prgram (NIH) 2019

Artificial vesicles (AV)

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How to overcome instability of RNA?

Nanotechnology

Inorganic nanoparticles

JIPB Journal of Integrative Plant Biology

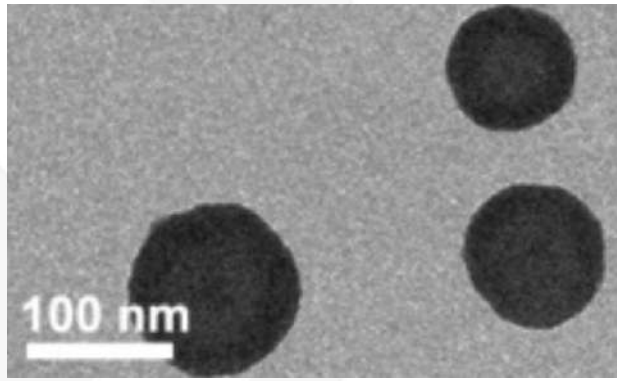
Research Article
https://doi.org/10.1111/jipb.13353

BioClay™ prolongs RNA interference-mediated crop protection against *Botrytis cinerea*

Jonatan Niño-Sánchez[1,2,3*], Prabhakaran T. Sambasivam[4], Anne Sawyer[5,6], Rachael Hamby[1], Angela Chen[1], Elizabeth Czislowski[6,7], Peng Li[8], Narelle Manzie[5], Donald M. Gardiner[5], Rebecca Ford[6], Zhi Ping Xu[8], Neena Mitter[6*] and Hailing Jin[1*]

Double layered hydroxide clay nanosheet (LDH)

Organic nanovesicles

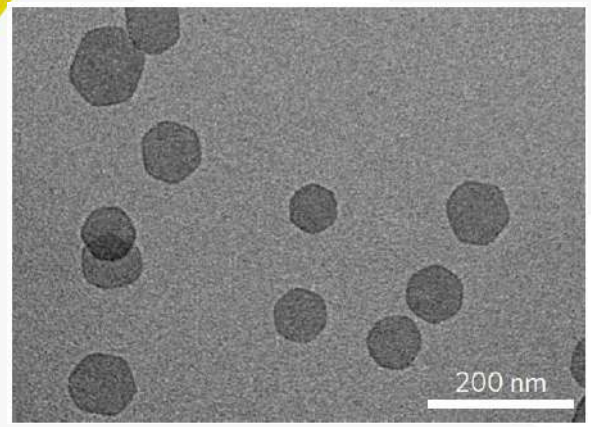


Artificial vesicles (AV)

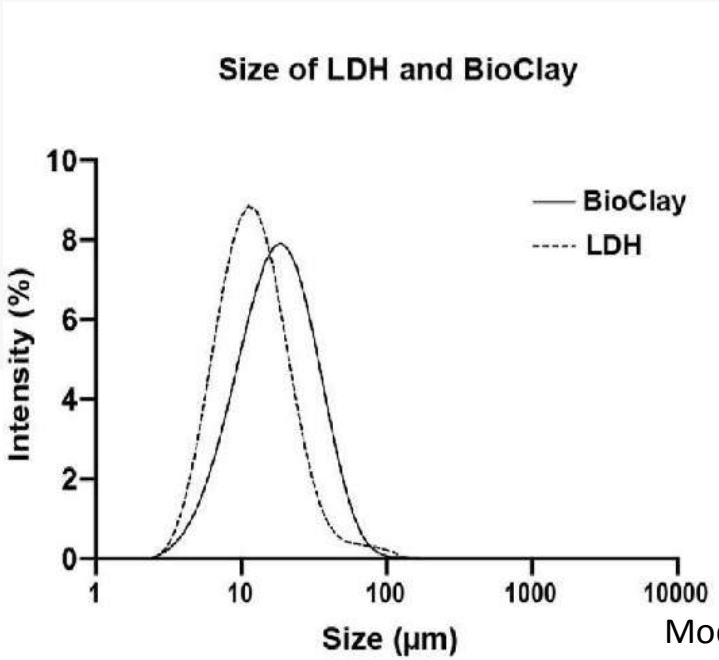
Inorganic nanoparticles

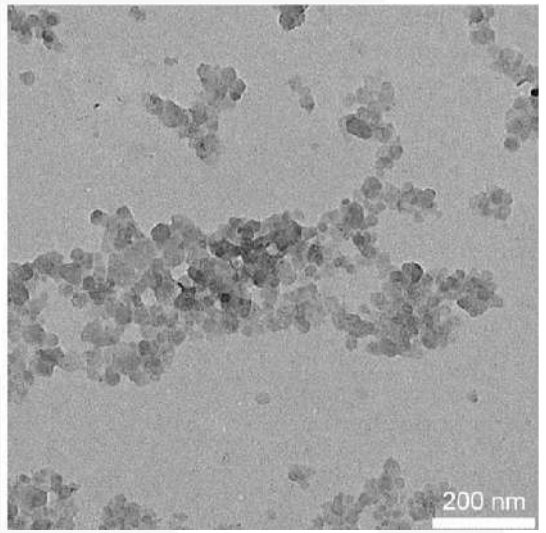
Bioclay: LDH + dsRNA

Layered double hydroxide (LDH): MgAl-LDH / MgFe-LDH



Modified from Mitter *et al.*, 2017. *Nat. Plants* **3**, 16207





Modified from Ritesh *et al.*, 2022. *Nat. Plants* **8**, 535-548

The lateral sizes of single-piece LDH crystals are 20-40 nm.
LDH crystals formed aggregates with an average lateral size of ~200 nm and a thickness of <10 nm. After dsRNA loading onto the LDH crystals, the size increased to 2–5 µm in length with a thickness of ~100 nm. As the typical diameter of dsRNA is 2.4 nm with a length predicted at 700–750 nm (250 bp).

- ✔ LDH provides protection from nuclease activity.
- ✔ LDH Increases the stability and the durability of the RNAi effect against viral pathogens (CMV).

Inorganic nanoparticles

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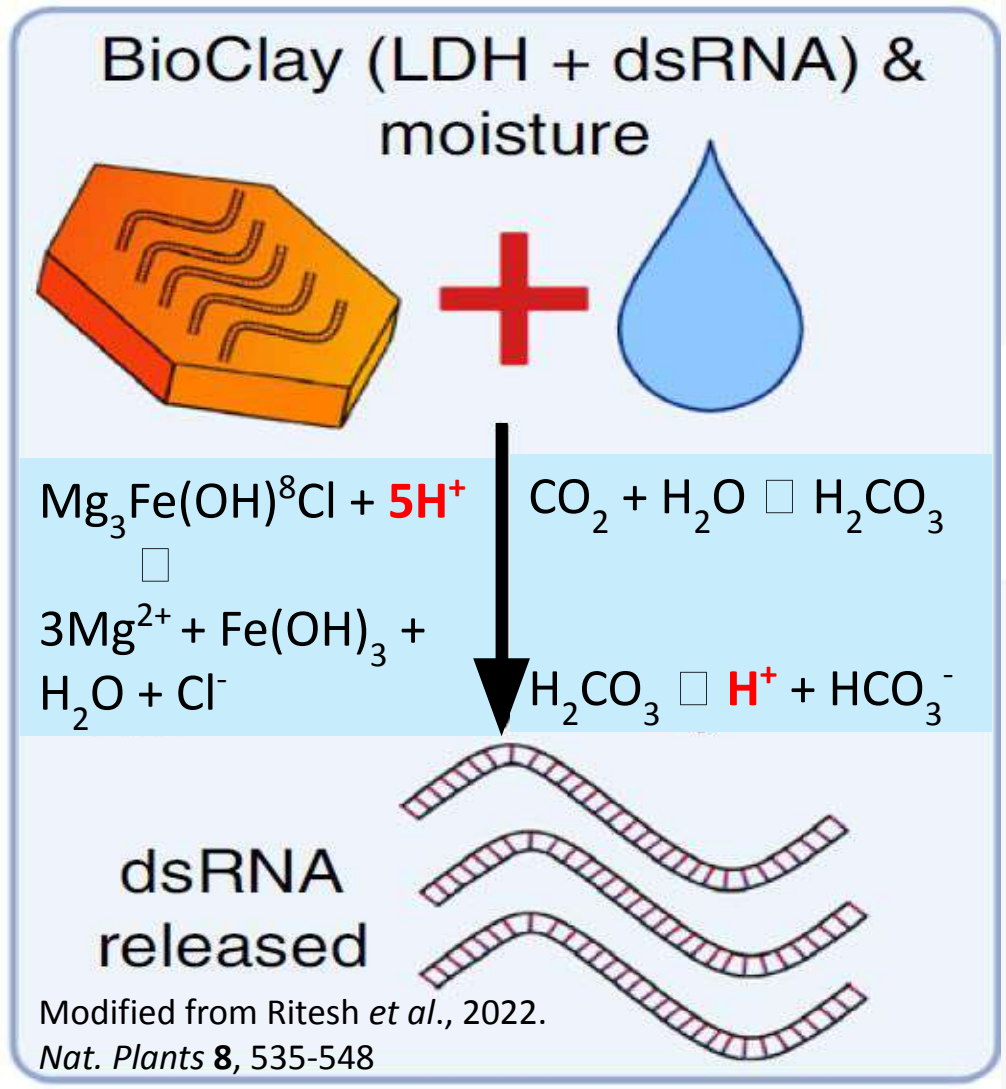
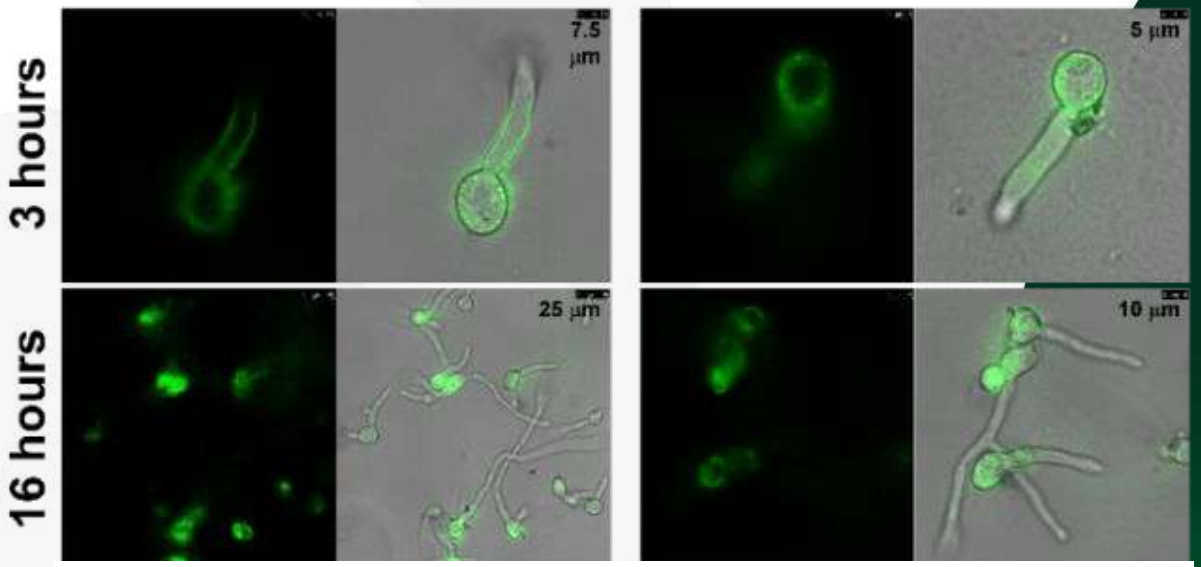
Bioclay: LDH + dsRNA



Modified from Ritesh *et al*., 2022. *Nat. Plants* **8**, 535-548

dsRNA release conditions
Nature of plant surfaces, a result of moisture and CO_2 forming carbonic acid on the leaf surface, promotes release of the dsRNA as LDH slowly degrades.

***B. cinerea* spores can efficiently take up dsRNA released from LDH**



Modified from Niño-Sánchez (2022) *J. Integr. Plant Biol.*

Inorganic nanoparticles

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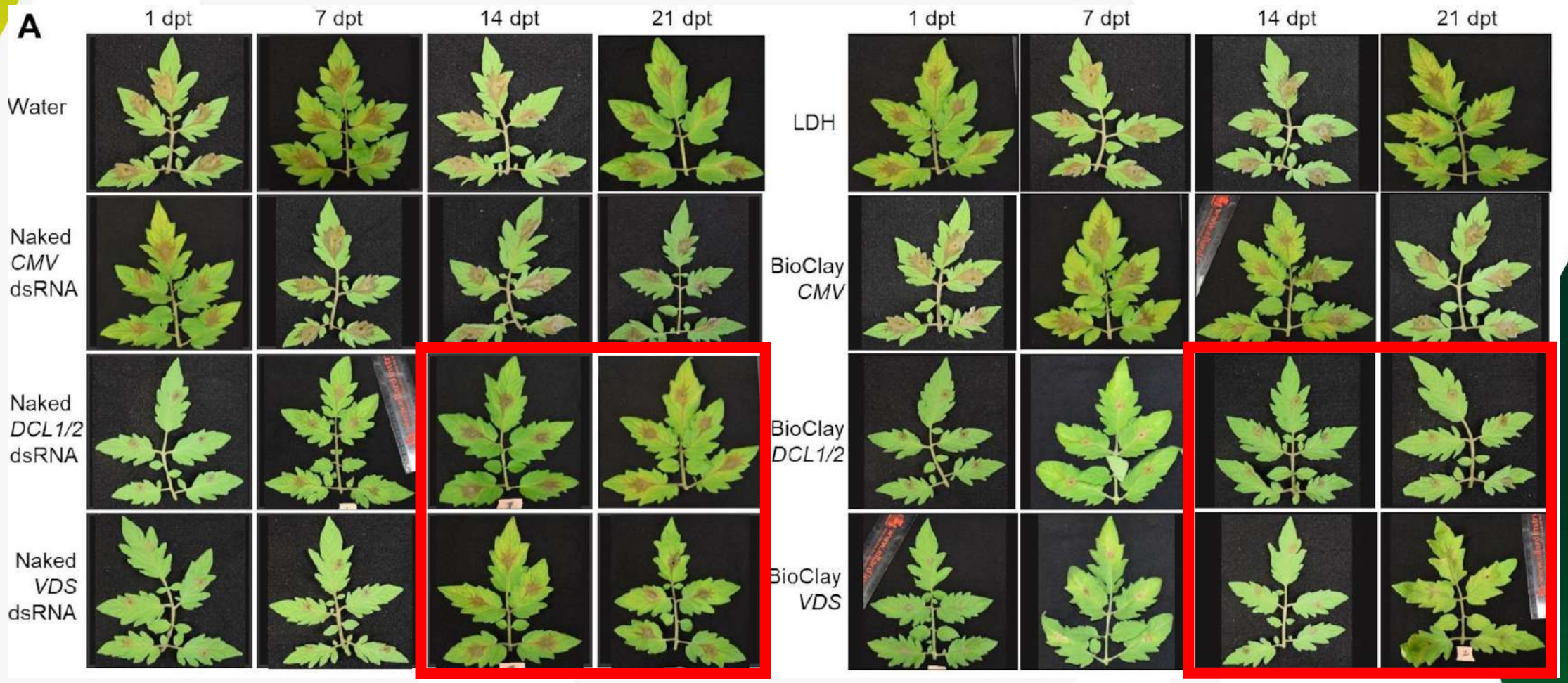
Bioclay: LDH + dsRNA

BioClay provides extended protection against *B. cinerea* on tomato leaves

Modified from Niño-Sánchez (2022). *J. Integr. Plant Biol.*



Inorganic nanoparticles

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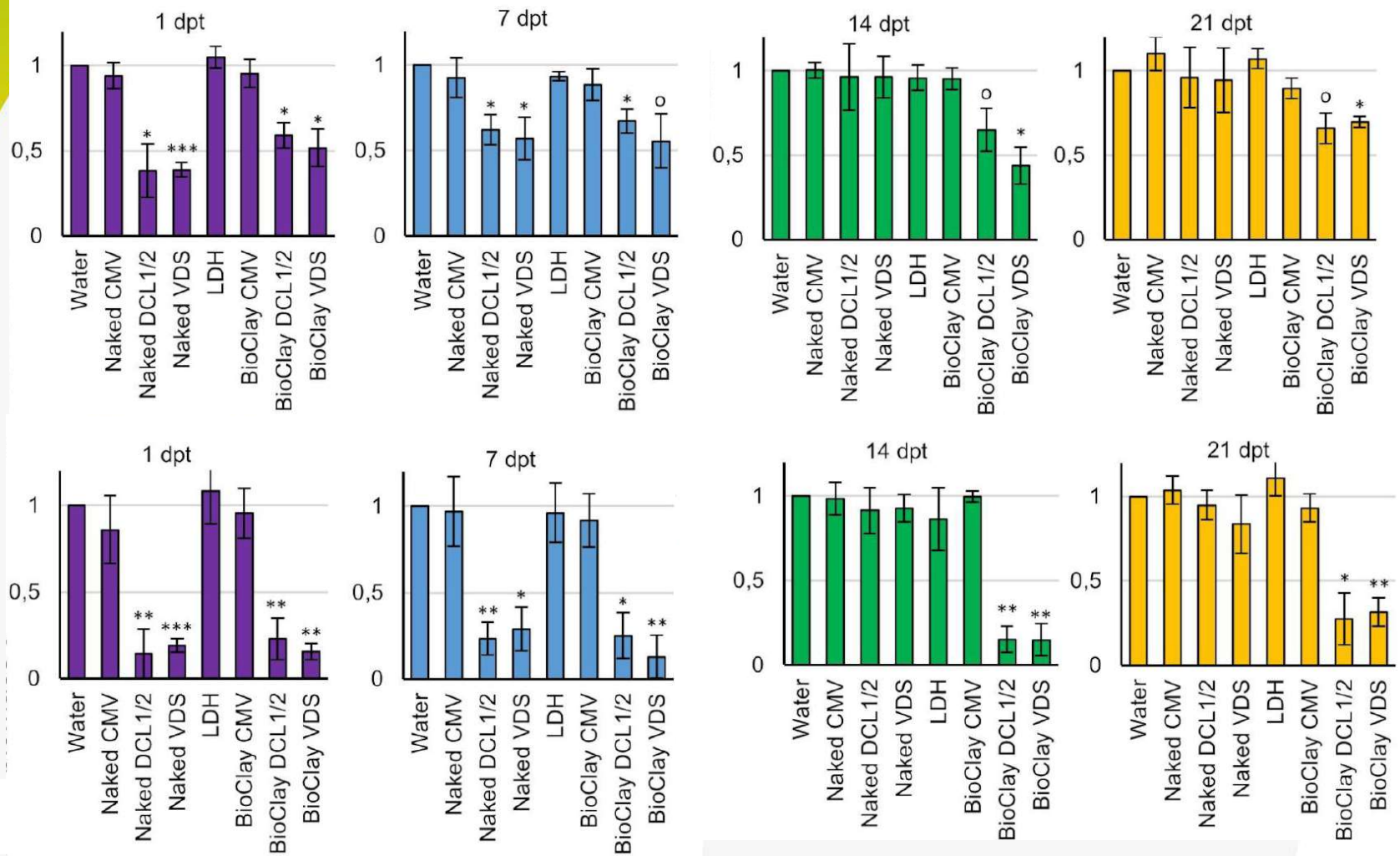
Bioclay: LDH + dsRNA

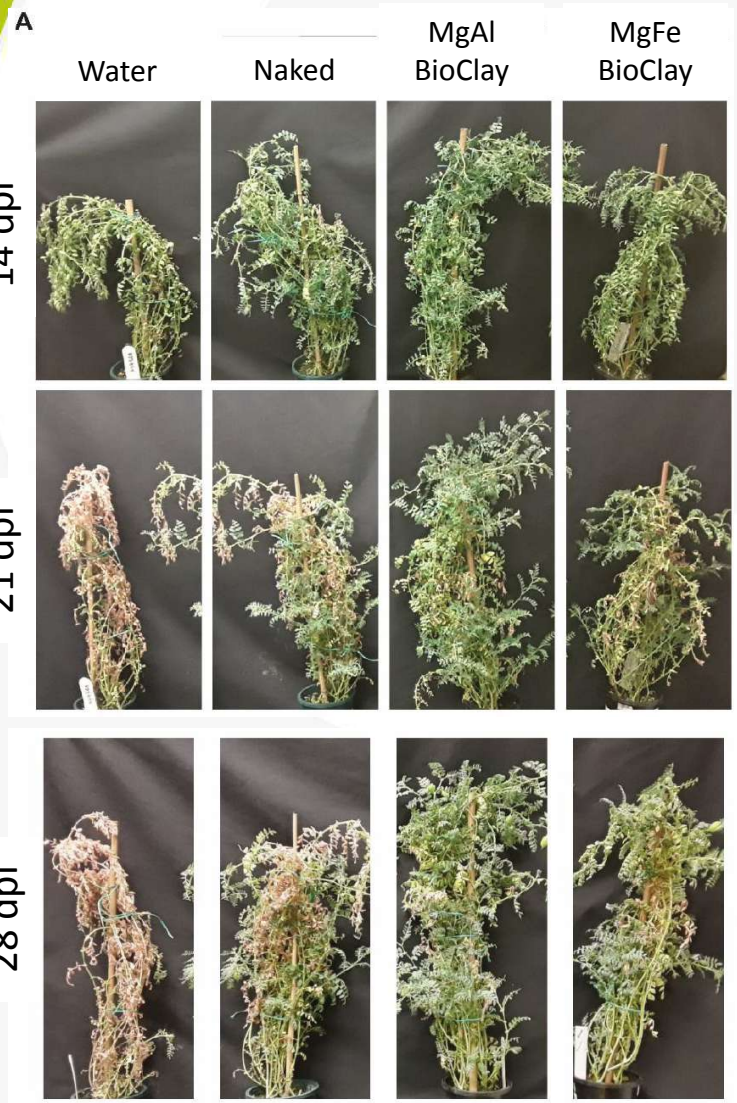


Delivering dsRNA as a Bioclay™ spray instead of naked dsRNA extended the fungal protection window.

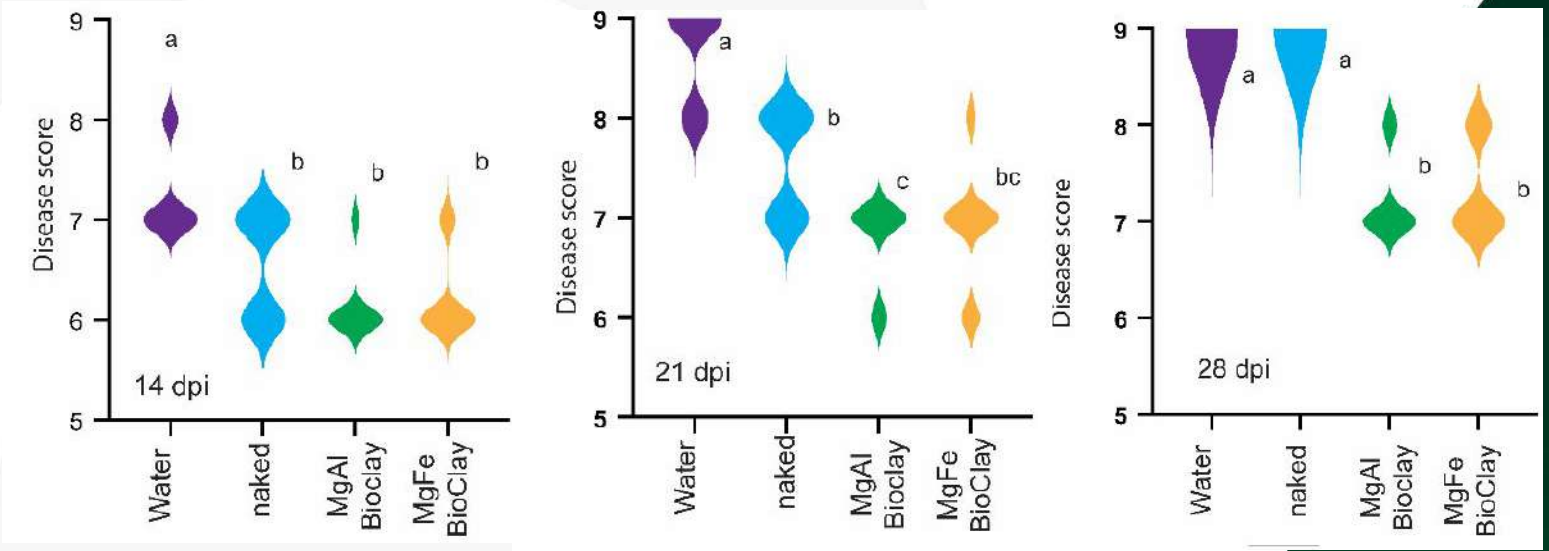
Niño-Sánchez (2022)
J. Integr. Plant Biol.

Inorganic nanoparticles

Bioclay: LDH + dsRNA



BioClay treatment reduces *B. cinerea* infection of mature chickpea plants



Niño-Sánchez (2022) *J. Integr. Plant Biol.*

Inorganic nanoparticles

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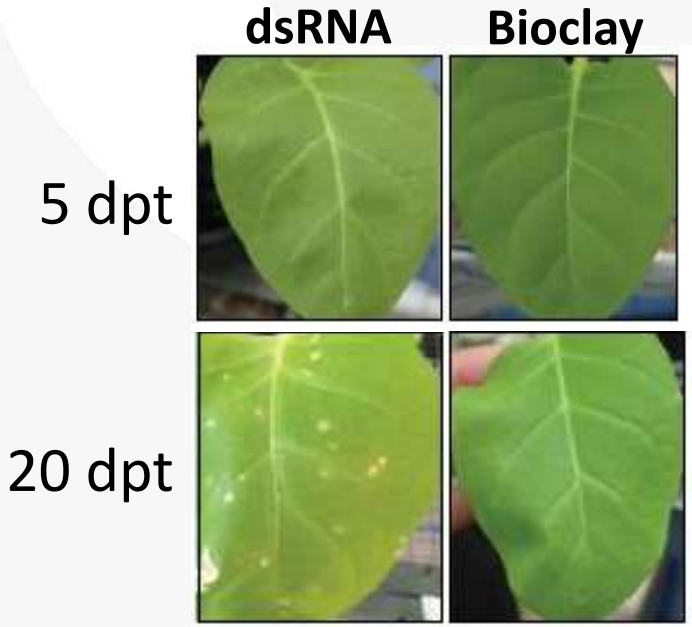
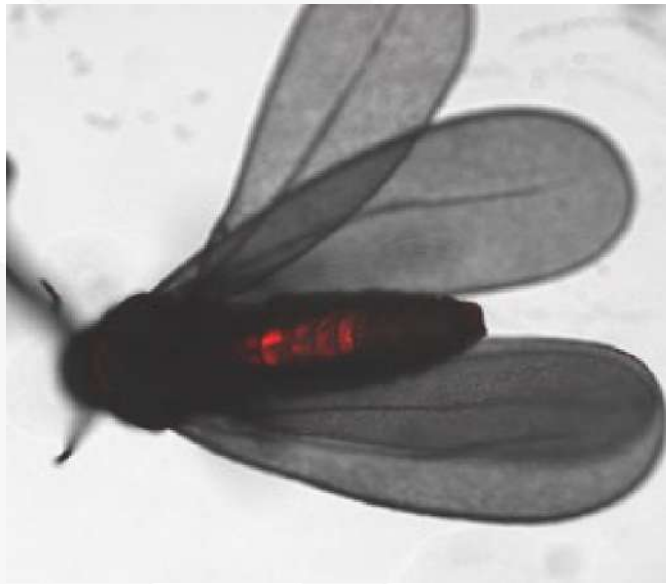
Bioclay: LDH + dsRNA

Delivering dsRNA as a Bioclay spray instead of naked dsRNA extended the virus protection window for around 2 weeks.



Modified from Mitter *et al.*, 2017. *Nat. Plants* **3**, 16207

Foliar application of BioClay can effectively disrupt multiple whitefly developmental stages in planta



Modified from Ritesh *et al.*, 2022. *Nat. Plants* **8**, 535-548

SIGS: Spray-Induced Gene Silencing

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How to overcome instability of RNA?

Nanotechnology

Inorganic nanoparticles

JIPB Journal of Integrative Plant Biology

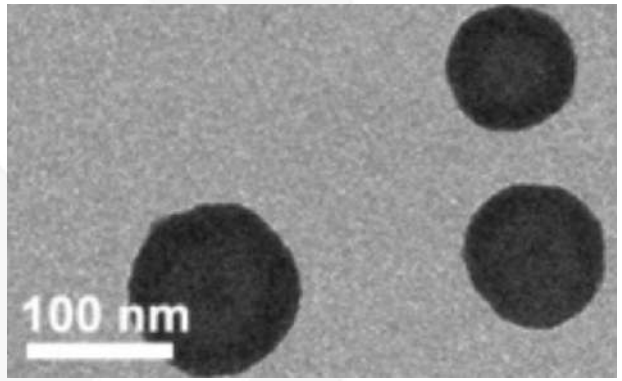
Research Article
https://doi.org/10.1111/jipb.13353

BioClay™ prolongs RNA interference-mediated crop protection against *Botrytis cinerea*

Jonatan Niño-Sánchez[1,2,3*], Prabhakaran T. Sambasivam[4], Anne Sawyer[5,6], Rachael Hamby[1], Angela Chen[1], Elizabeth Czislowski[6,7], Peng Li[8], Narelle Manzie[5], Donald M. Gardiner[5], Rebecca Ford[6], Zhi Ping Xu[8], Neena Mitter[6*] and Hailing Jin[1*]

Double layered hydroxide clay nanosheet (LDH)

Organic nanovesicles



Artificial vesicles (AV)

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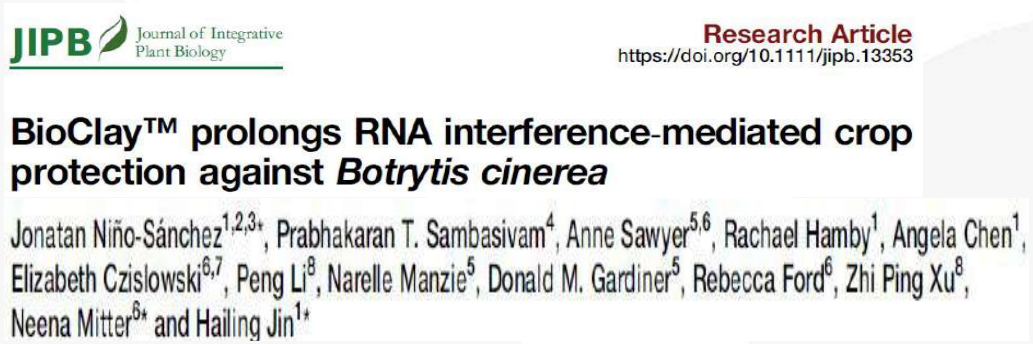

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Double layered hydroxide clay nanosheet (LDH)

Organic nanovesicles

Plant Biotechnology Journal

aab SEB

Plant Biotechnology Journal (2023), pp. 1–12 doi: 10.1111/pbi.14001

Artificial nanovesicles for dsRNA delivery in spray-induced gene silencing for crop protection

Lulu Qiao[1,2,†], Jonatan Niño-Sánchez[1,3,4,†], Rachael Hamby[1,†], Luca Capriotti[5], Angela Chen[1], Bruno Mezzetti[5] and Hailing Jin[1,*]

Artificial vesicles (AV)

SIGS: Spray-Induced Gene Silencing

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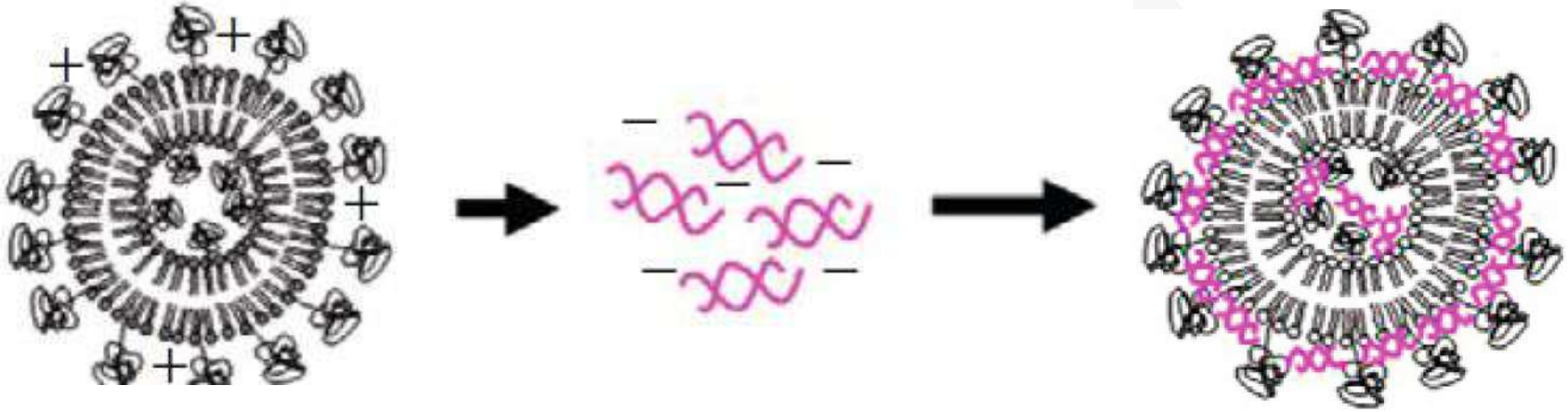
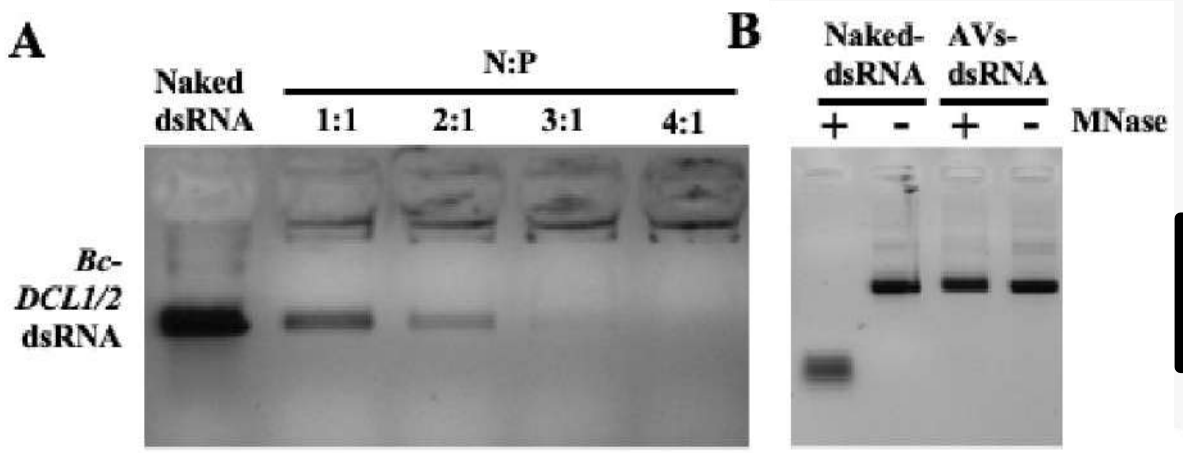
Artificial nanovesicles



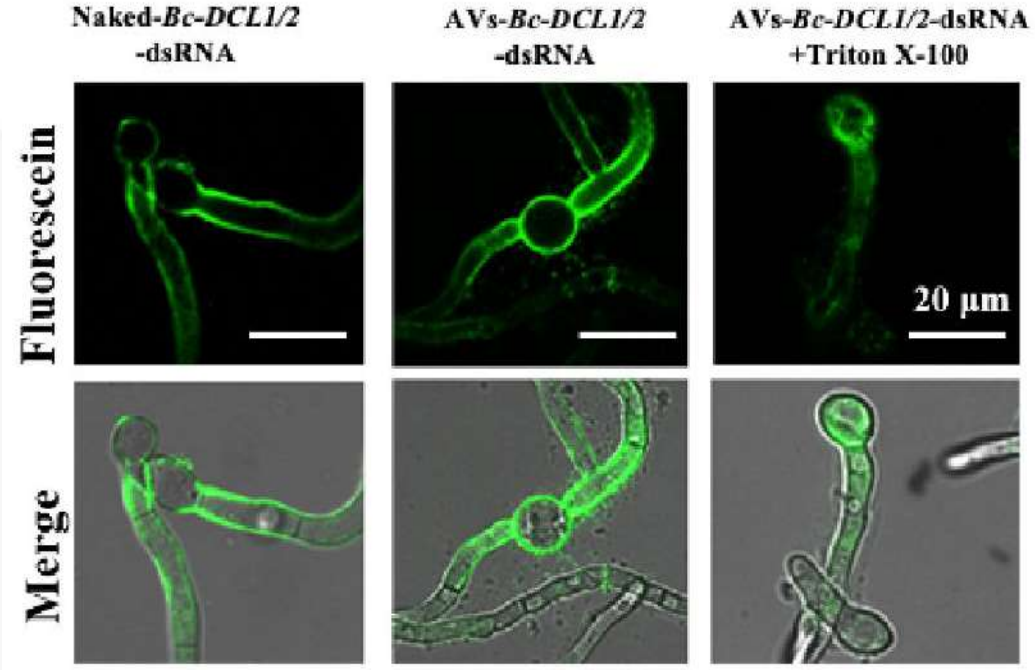
Artificial vesicles | **dsRNA** | **AV-dsRNA**

DOTAP: 1,2-dioleoyl-3-trimethylammonium-propane
Cholesterol
DSPE-PEG2000: 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy(polyethyleneglycol)-2000]

Artificial nanovesicles

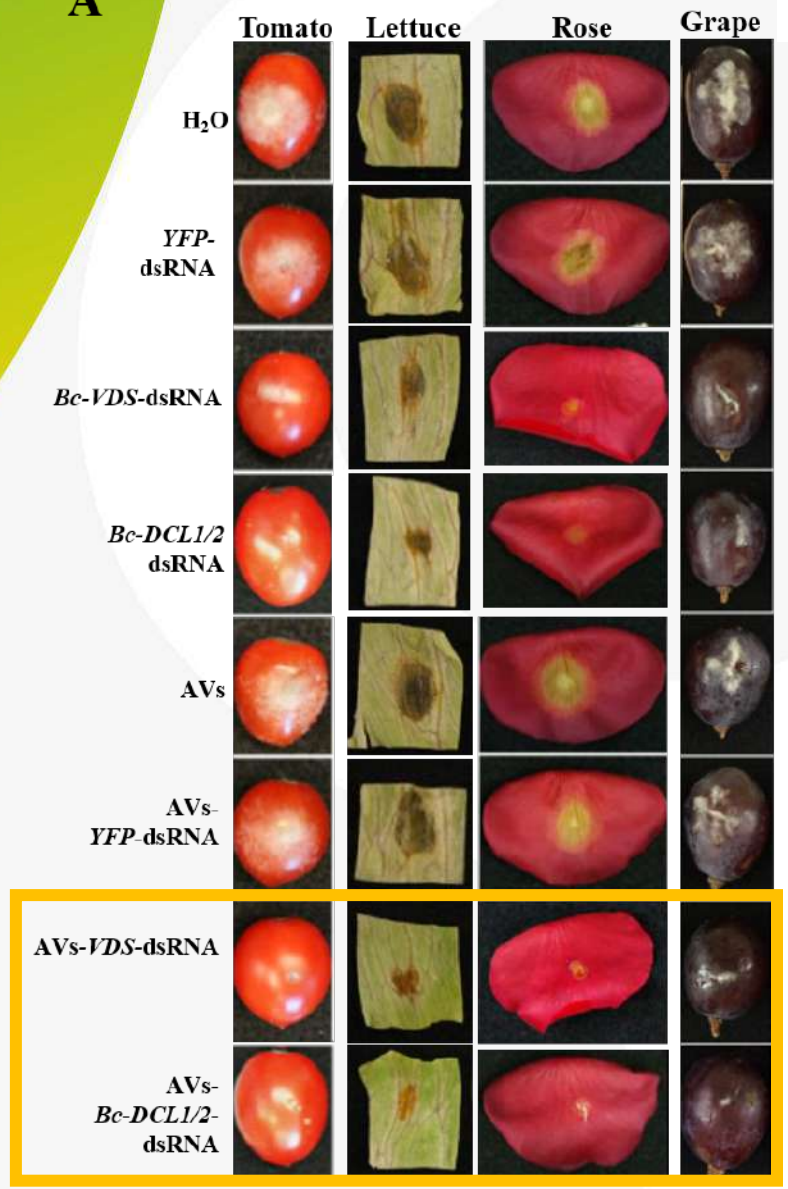


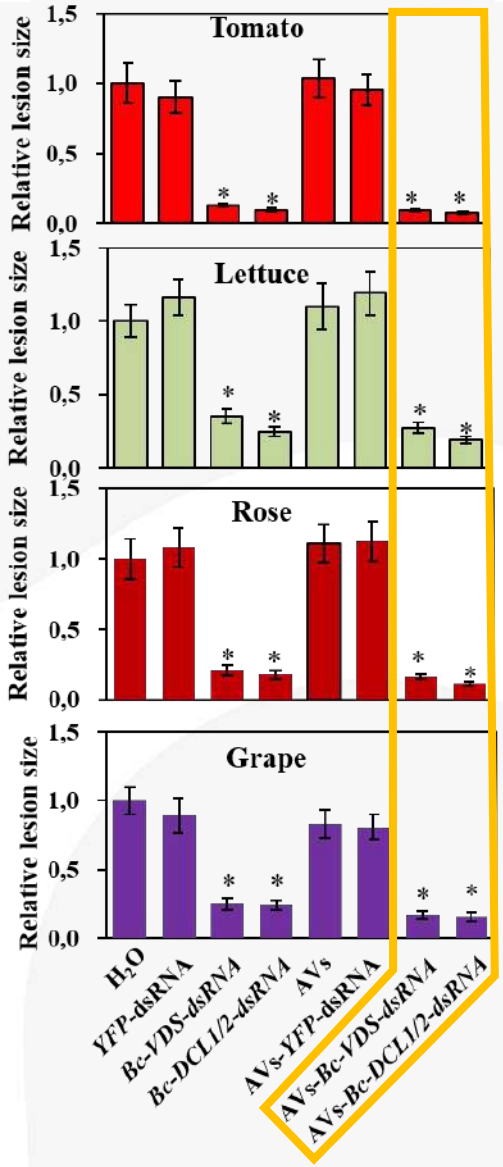
Artificial vesicles provide protection to nucleases.

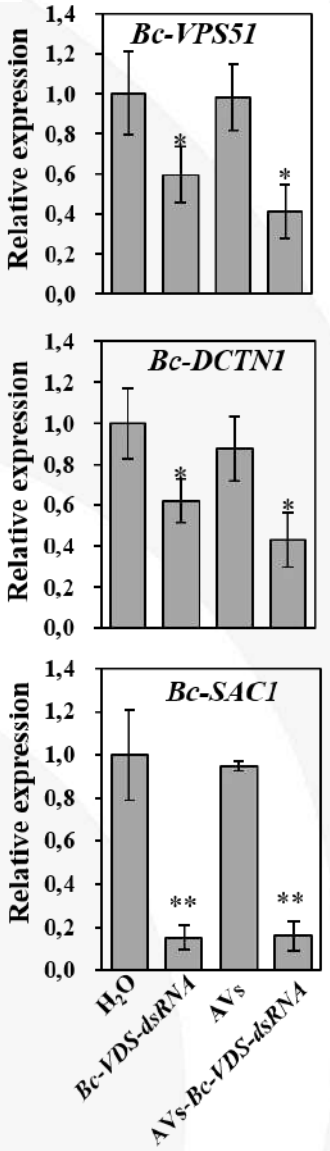


B. cinerea **cells can uptake dsRNA from AV-dsRNA lipoplex**

Artificial nanovesicles

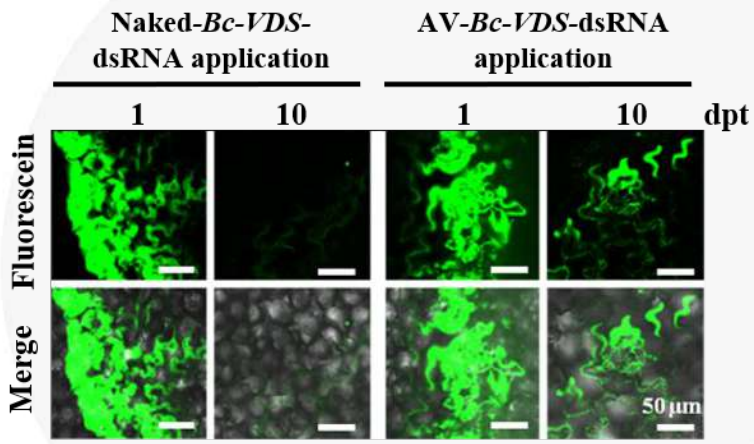


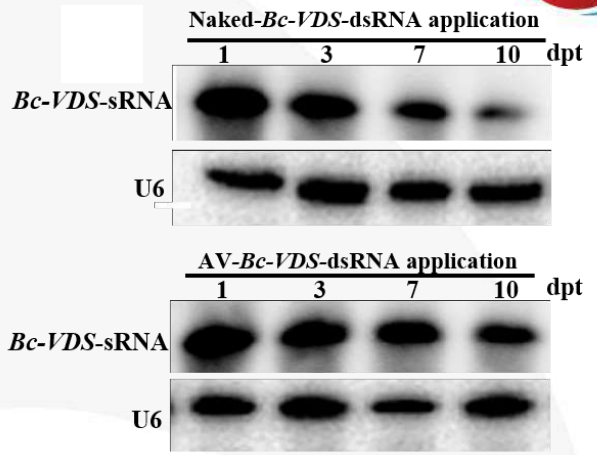


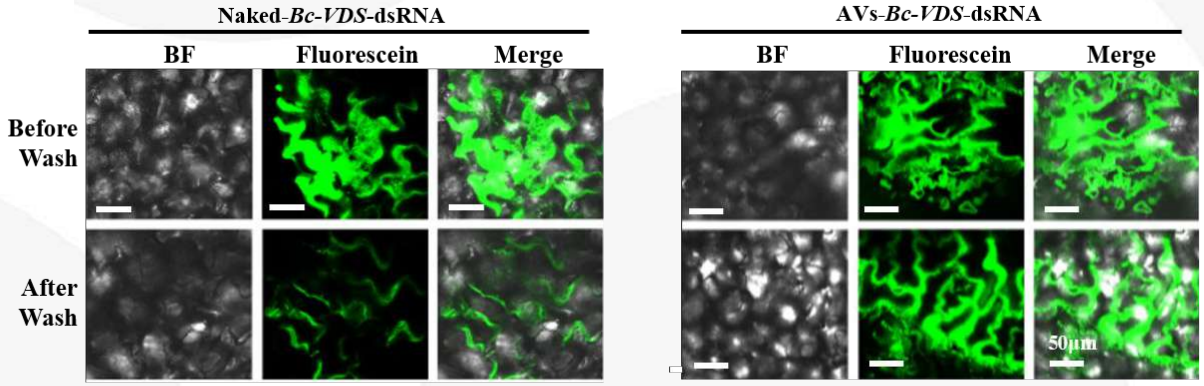


External AV-dsRNA application triggers RNAi in *B. cinerea*

Artificial nanovesicles







due to increase dsRNA stability and durability … ... …

Artificial nanovesicles

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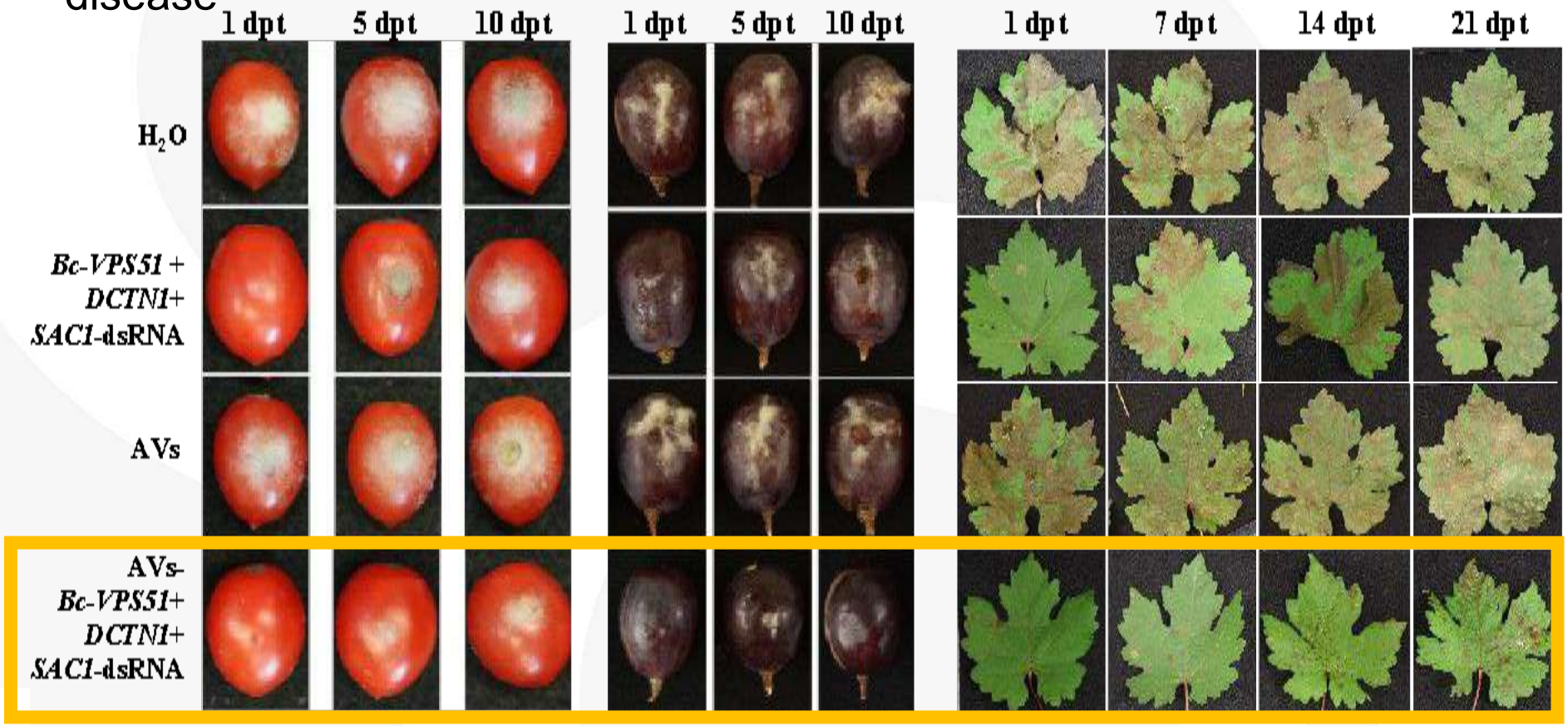
… … … AV-dsRNA extends RNAi-mediated protection against gray mold disease



Artificial nanovesicles

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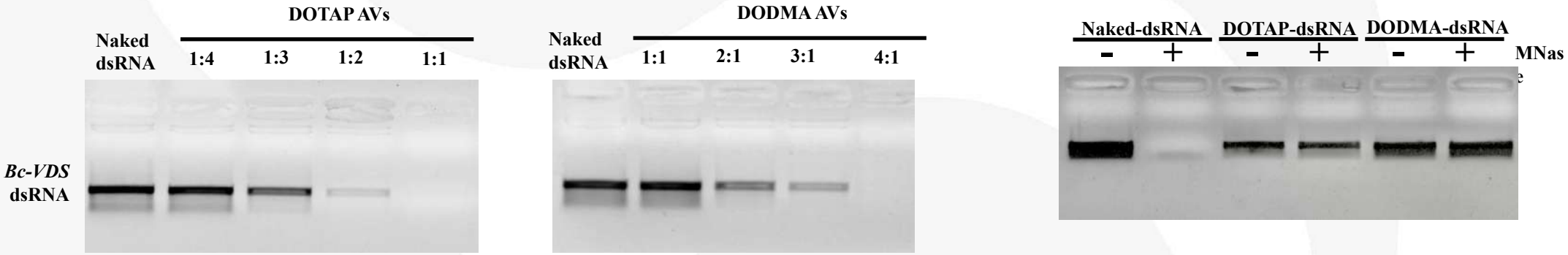
Cost-effective AV formulations also provide strong RNAi activity

Formulation 1	Formulation 2	Formulation 3
DOTAP Cholesterol DSPE-PEG2000	DOTAP Cholesterol	DODMA Cholesterol



Artificial nanovesicles

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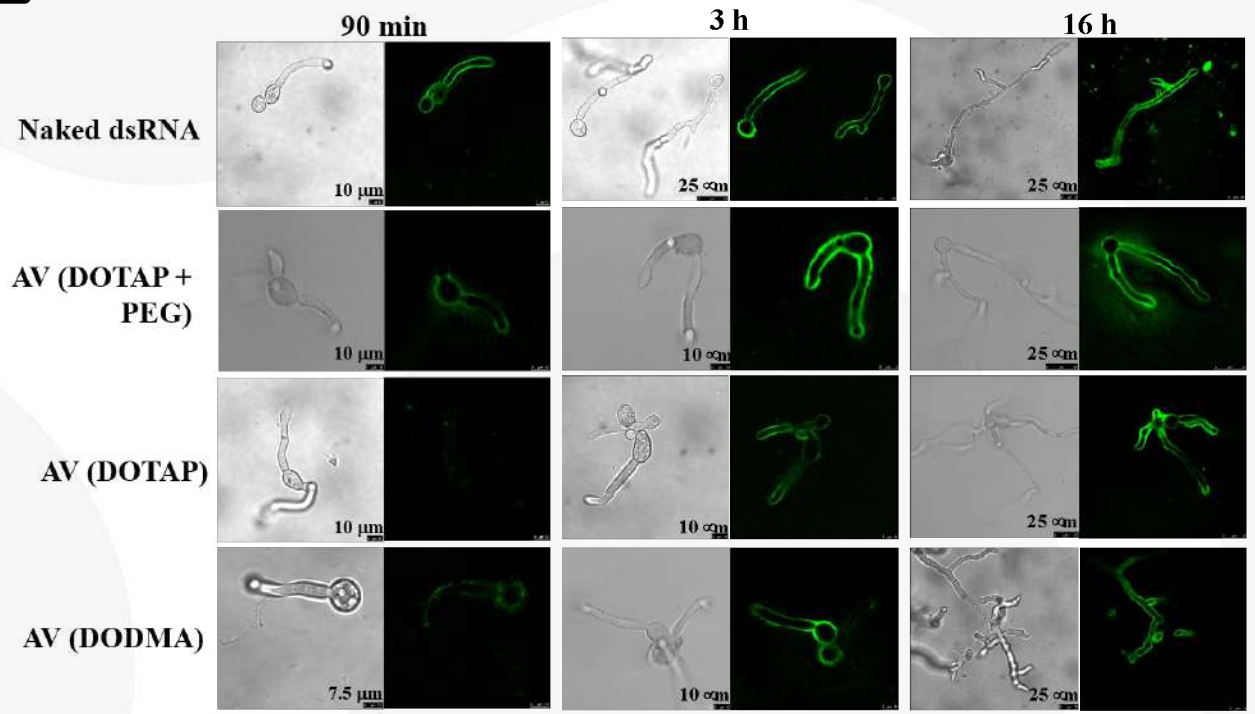
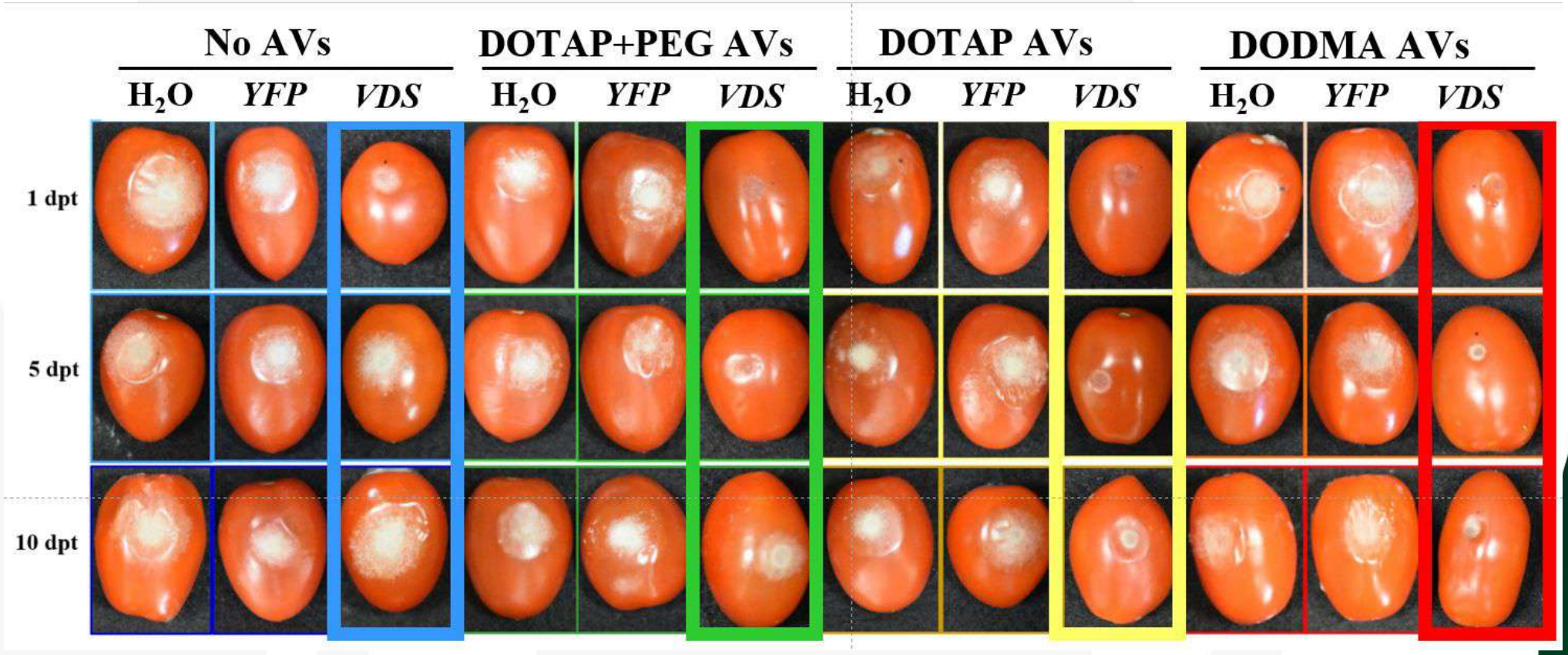


Artificial nanovesicles

Cost-effective AV formulations also provide strong RNAi activity



SIGS: A step-by-step guide. What we know, what we don't

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Nanocarrier		Enhance SIGS	Loading	dsRNA protection	Delivery	Toxicity	Cost	Storage	Production
Organic	Lipid vesicles	↑	●	✓	✓	☠	$	▣	⚙
	Protein-based	?	●	✓	✓	?	$	?	⚙
	Organic polymers	↑	●	✓	✓	☠	$	?	⚙
Inorganic	Layered double hydroxide	↑	●	✓	✓	☠	$	▣	⚙
	Silica	?	●	✓	?	☠	$	▣	⚙
	Metal-based	?	●	✓	?	☠	$	?	?
Carbon-based	Carbon-based	↑	●	✓	✓	☠	$	▣	⚙

Mosquera *et al.*, 2025. J. Integr. Plant Biol. **1**

MIGS: Microbe-Induced Gene Silencing

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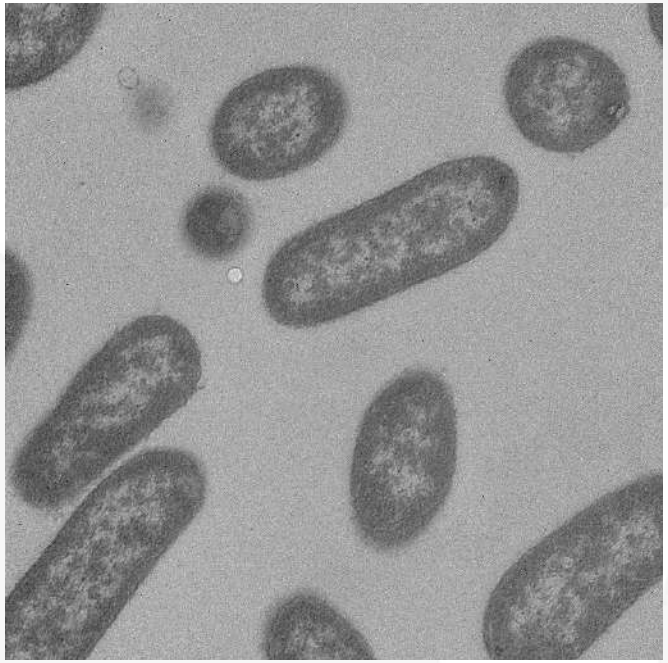
dsRNA-producing Bacteria



Picture: Marles-Wright, Jon (2012)

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Letter | Published: 31 August 2023

Microbe-induced gene silencing boosts crop protection against soil-borne fungal pathogens

Han-Guang Wen, Jian-Hua Zhao, Bo-Sen Zhang, Feng Gao, Xue-Ming Wu, Yong-Sheng Yan, Jie Zhang & Hui-Shan Guo

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TAKE HOME MESSAGE

SIGS technology is innovative, eco-friendly, and versatile but still needs further research to fully understand its biological mechanisms for practical use in agriculture.

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- Irene T. Bocos
- Huma Amin
- Dr. Sandra Mosquera

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- Dr. Angela Chen
- Prof. Hailing Jin

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- Prof. Bruno Mezzetti

The University of Queensland Australia

- Prof. Anne Sawyer
- Prof. Neena Mitter

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