Spray-Induced Gene Silencing: A Promising Strategy for Targeted Plant Disease Control

Katie Stevens
23rd June 2025


FAO
and the
Sustainable
Development
Goals
1 NO POVERTY
Ending rural poverty is critical to ensuring food security
2 ZERO HUNGER
Food should be sufficient, nutritious, safe, and affordable for all
3 GOOD HEALTH AND WELL-BEING
Good health starts with nutrition
4 QUALITY EDUCATION
Nutritious food is critical to learning
5 GENDER EQUALITY
Gender equality and women's empowerment are key to ending hunger
6 CLEAN WATER AND SANITATION
Sustainable agriculture has the potential to address water scarcity
7 AFFORDABLE AND CLEAN ENERGY
Agri-food systems must overcome a dependence on fossil fuels
8 DECENT WORK AND ECONOMIC GROWTH
Agricultural growth in low-income economies can reduce poverty by half
9 INDUSTRY, INNOVATION AND INFRASTRUCTURE
Innovation opens new markets for smallholder farmers
10 REDUCED INEQUALITIES
Land reforms can give fairer access to rural land
11 SUSTAINABLE CITIES AND COMMUNITIES
Rural investment can deter unmanageable urbanization
12 RESPONSIBLE CONSUMPTION AND PRODUCTION
Achieving food security involves reducing food loss and waste
13 CLIMATE ACTION
Agriculture is key in responding to climate change
14 LIFE BELOW WATER
Fish gives 3 bn people 20% of daily animal protein
15 LIFE ON LAND
Forests contain over 80% of the world's terrestrial biodiversity
16 PEACE, JUSTICE AND STRONG INSTITUTIONS
Ending hunger can contribute greatly to peace and stability
17 PARTNERSHIPS FOR THE GOALS
Partnerships help raise the voice of the hungry

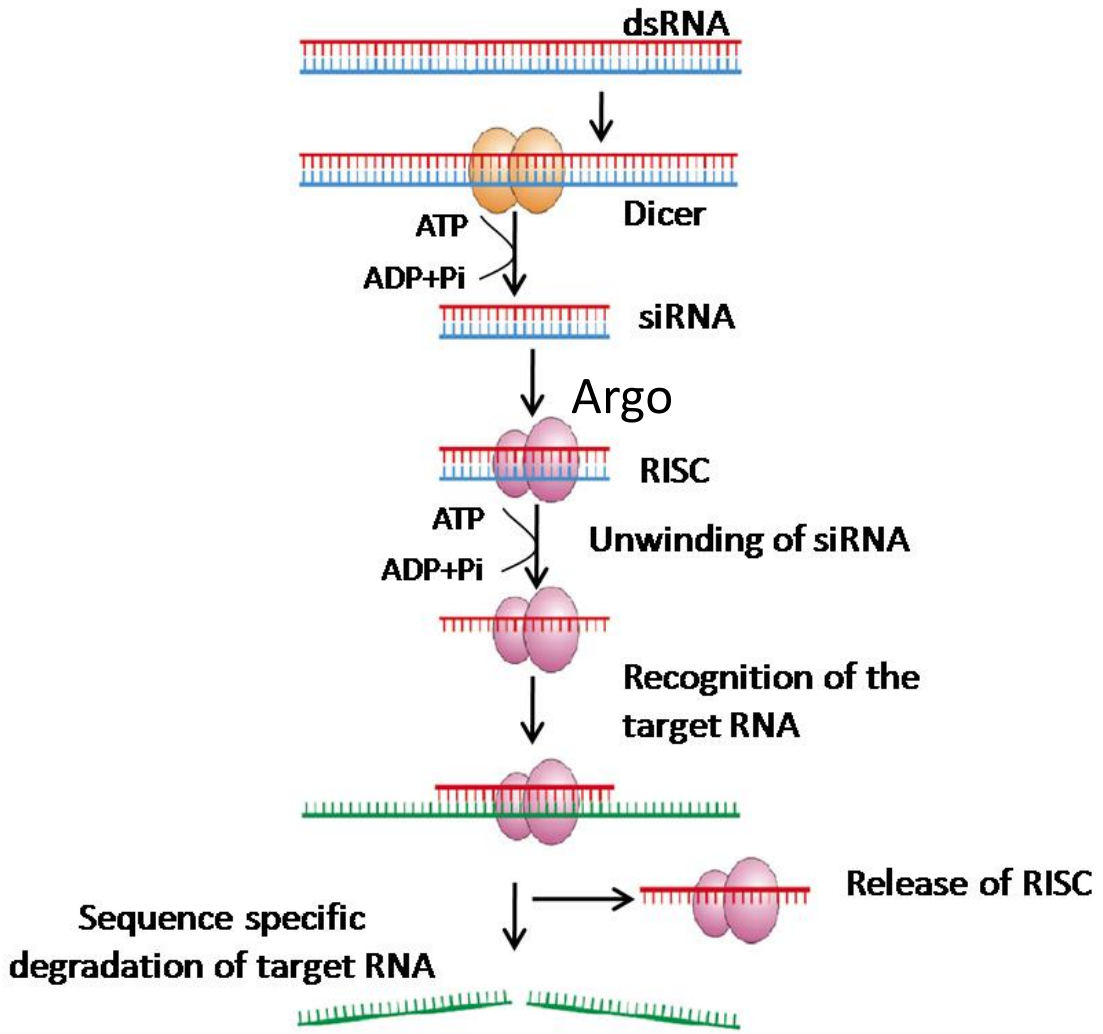
Phytophthora infestans

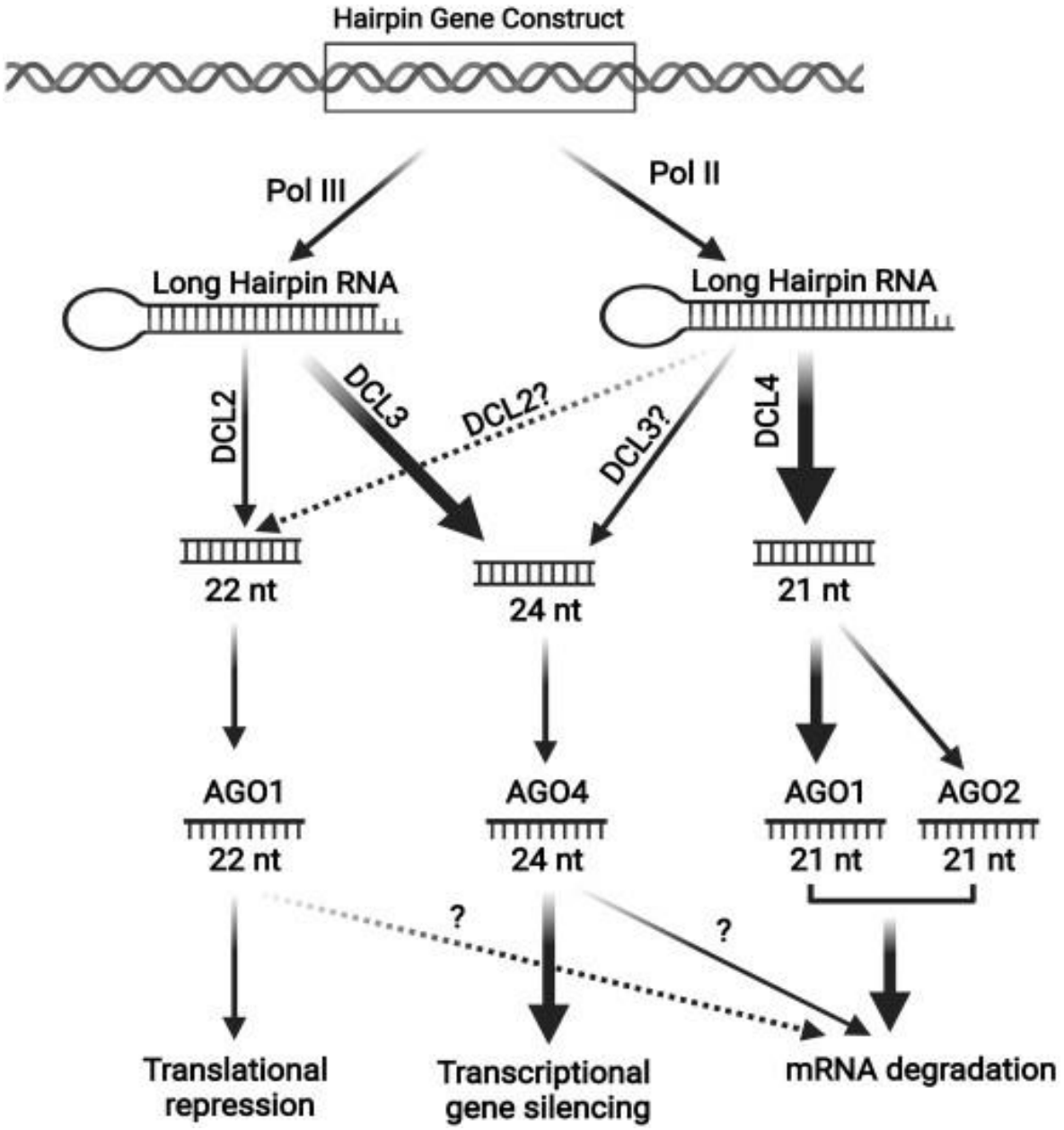
Late blight - The major disease affecting potato and tomato

Major part of applied fungicides

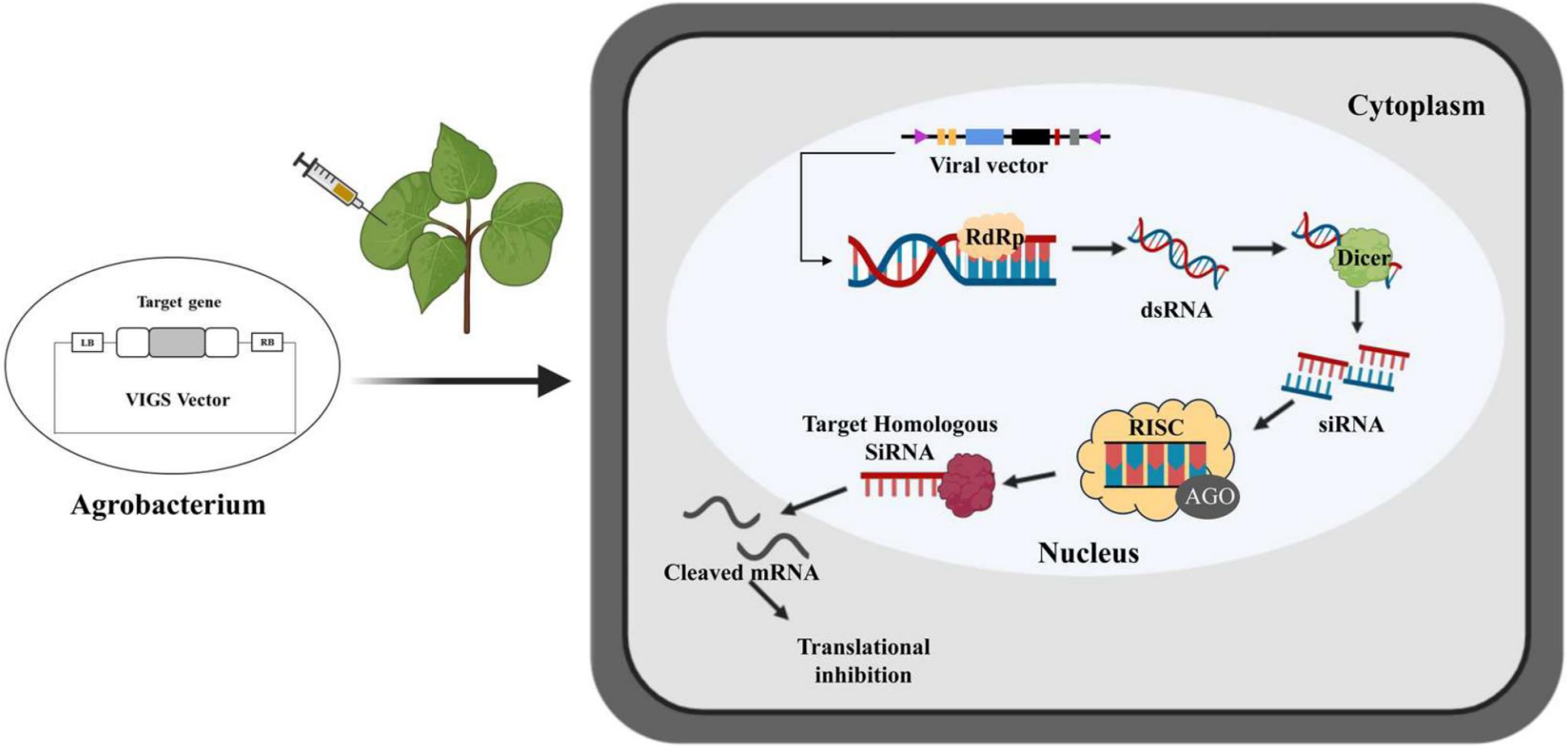
Estimated cost of late blight control measures cost €5 billion/year.

Post-transcriptional gene silencing

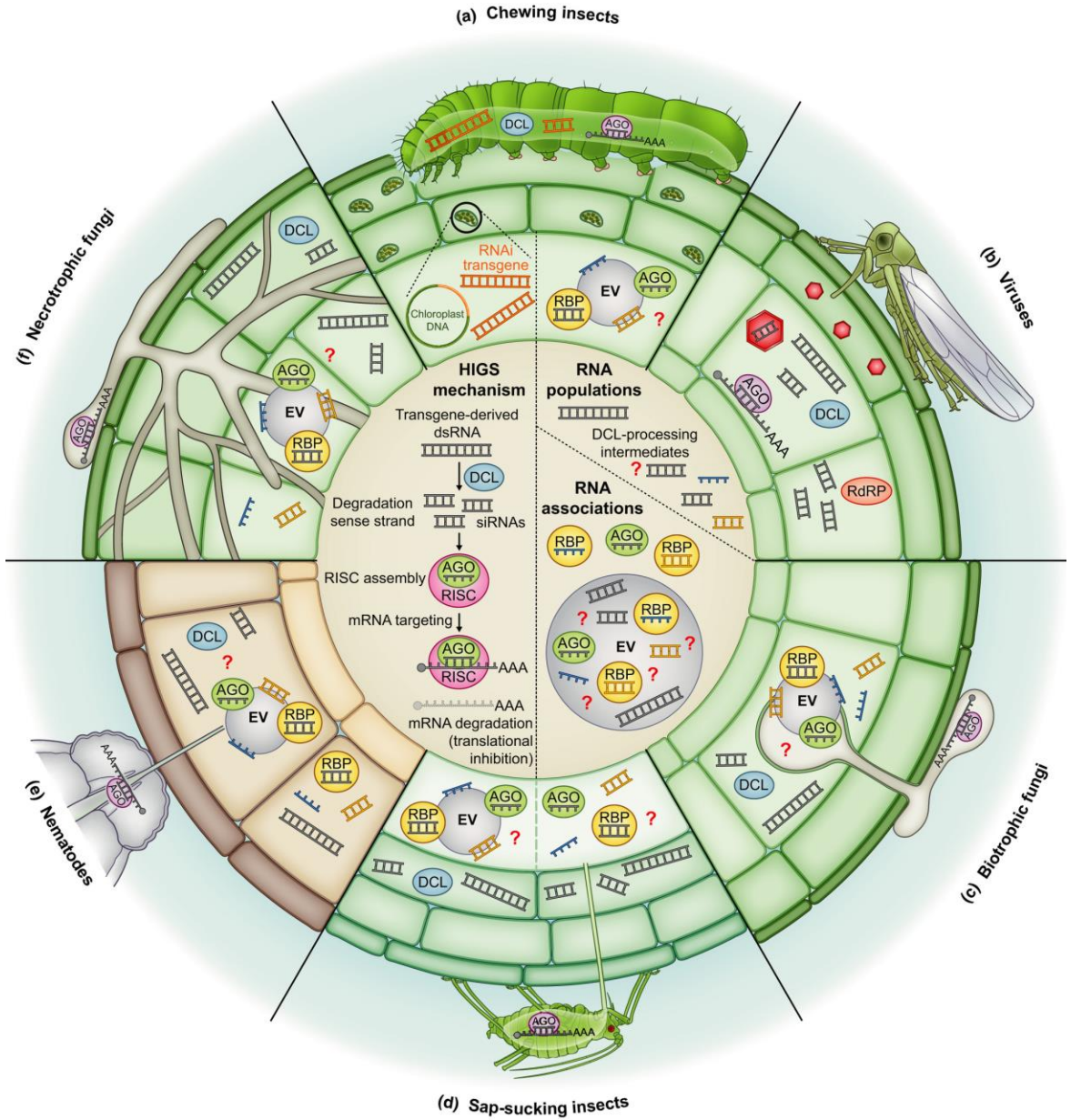



Hairpin Gene Construct
Pol III
Pol II
Long Hairpin RNA
Long Hairpin RNA
DCL2
DCL3
DCL2?
DCL3?
DCL4
22 nt
24 nt
21 nt
AGO1
AGO4
AGO1
AGO2
22 nt
24 nt
21 nt
21 nt
?
?
Translational repression
Transcriptional gene silencing
mRNA degradation

Virus-induced Gene Silencing

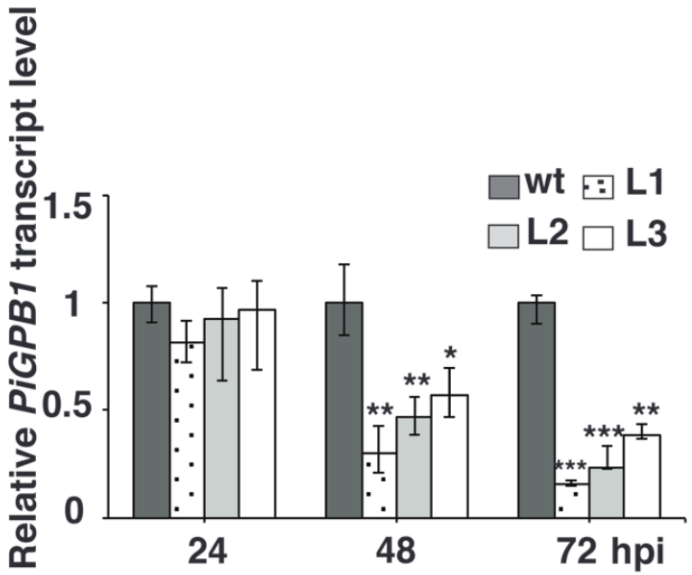


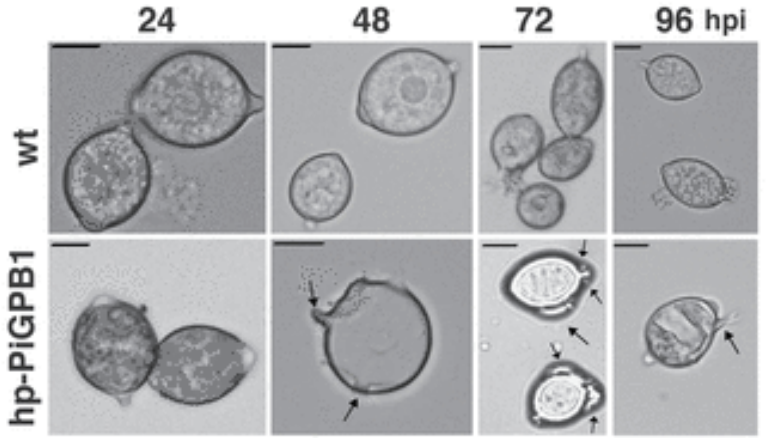
Host-Induced Gene Silencing

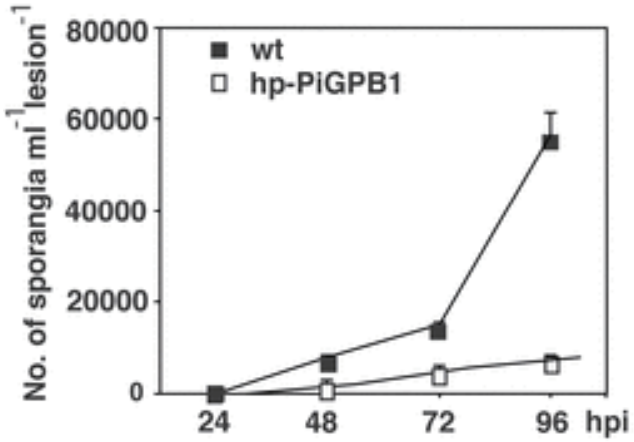


Plant-mediated gene silencing restricts growth of the potato late blight pathogen *Phytophthora infestans*

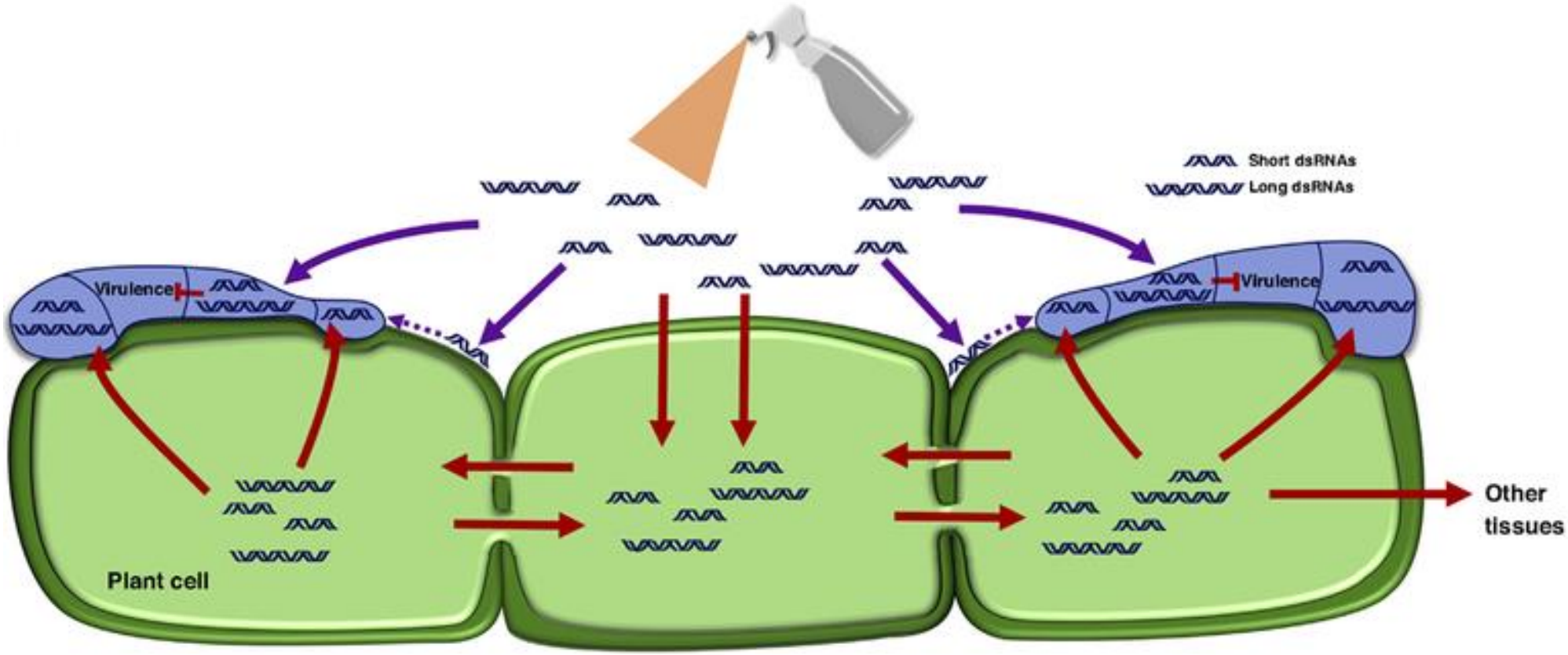
Sultana N. Jahan[1,*], Anna K. M. Åsman[1], Pádraic Corcoran[2], Johan Fogelqvist[1], Ramesh R. Vetukuri[1] and Christina Dixelius[1]







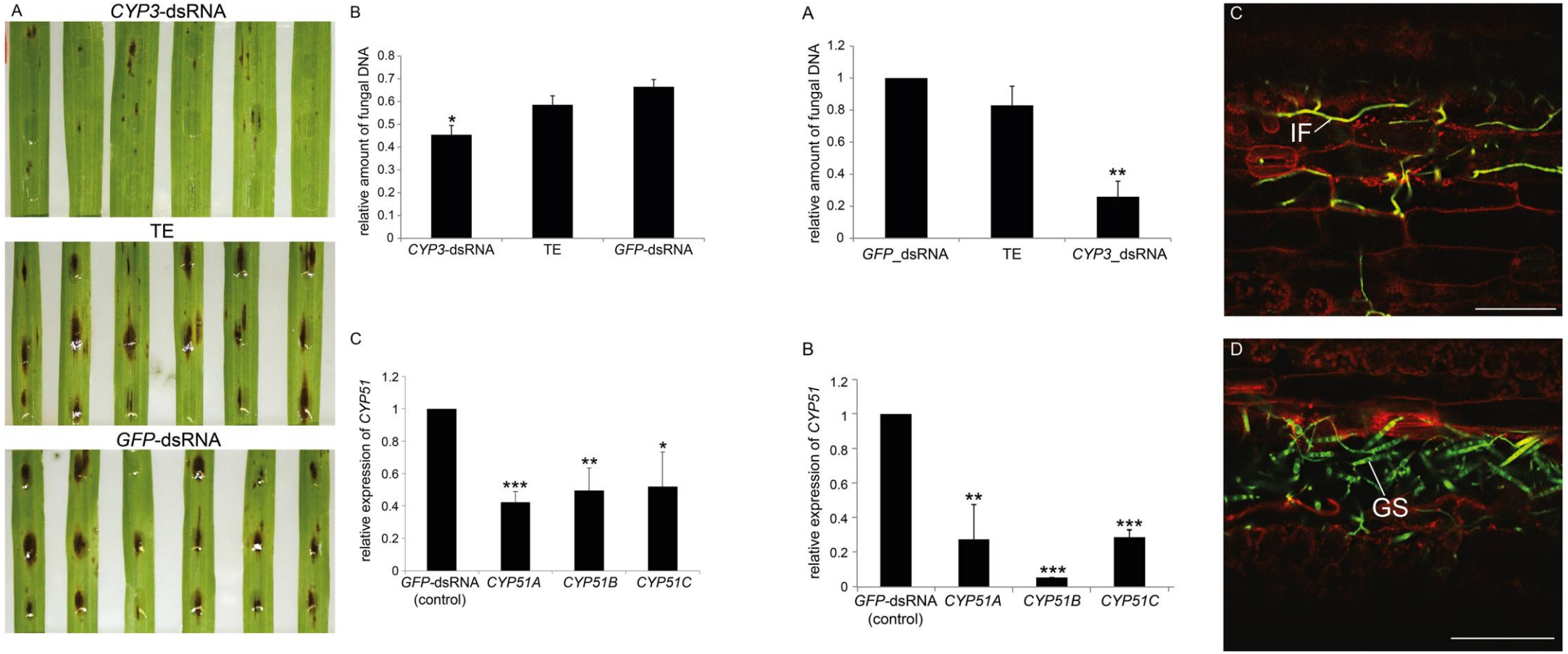
Spray induced gene silencing



An RNAi-Based Control of *Fusarium graminearum* Infections Through Spraying of Long dsRNAs Involves a Plant Passage and Is Controlled by the Fungal Silencing Machinery

Aline Koch, Dagmar Biedenkopf, Alexandra Furch, Lennart Weber, Oliver Rossbach, Eltayb Abdellatef, Lukas Linicus, Jan Johannsmeier, Lukas Jelonek, Alexander Goesmann, Vinitha Cardoza, John McMillan, Tobias Mentzel, Karl-Heinz Kogel

Published: October 13, 2016 • https://doi.org/10.1371/journal.ppat.1005901



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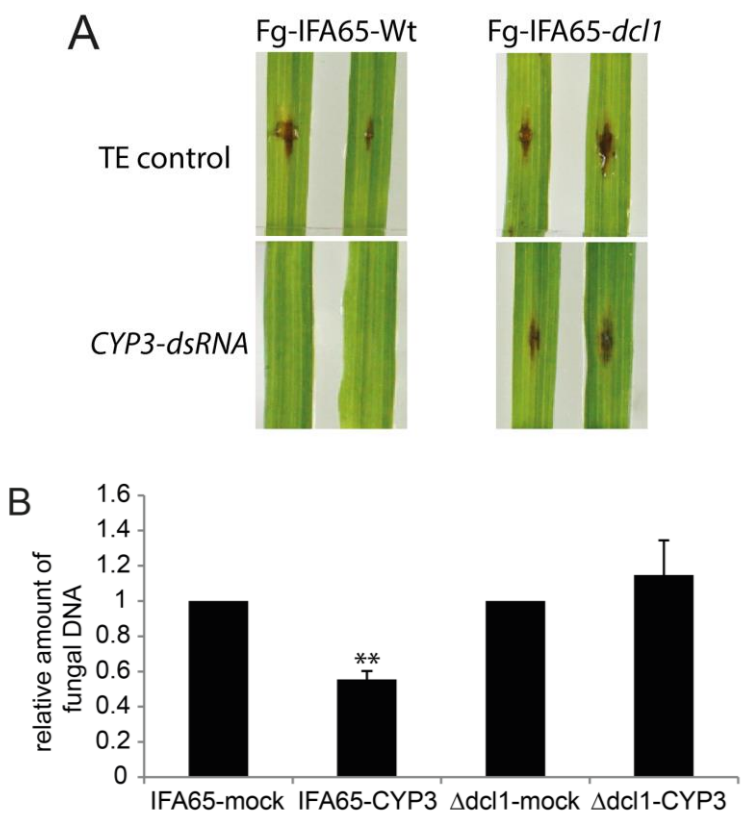
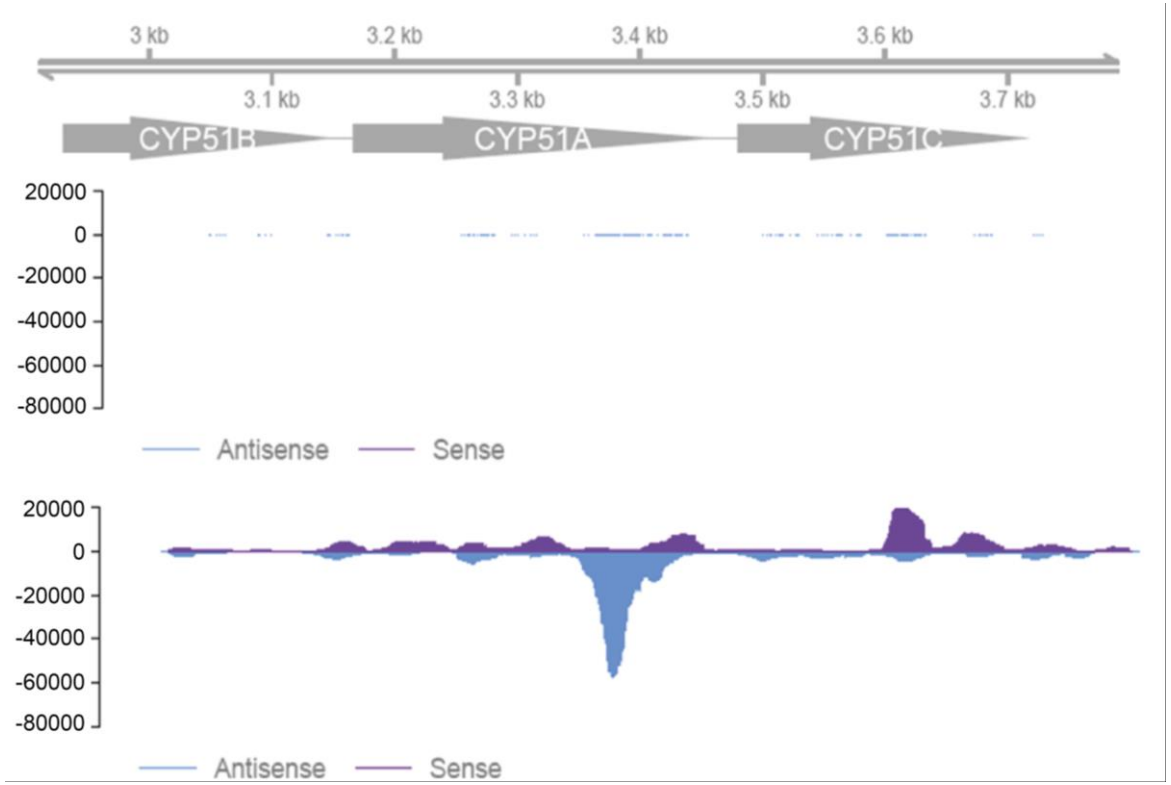
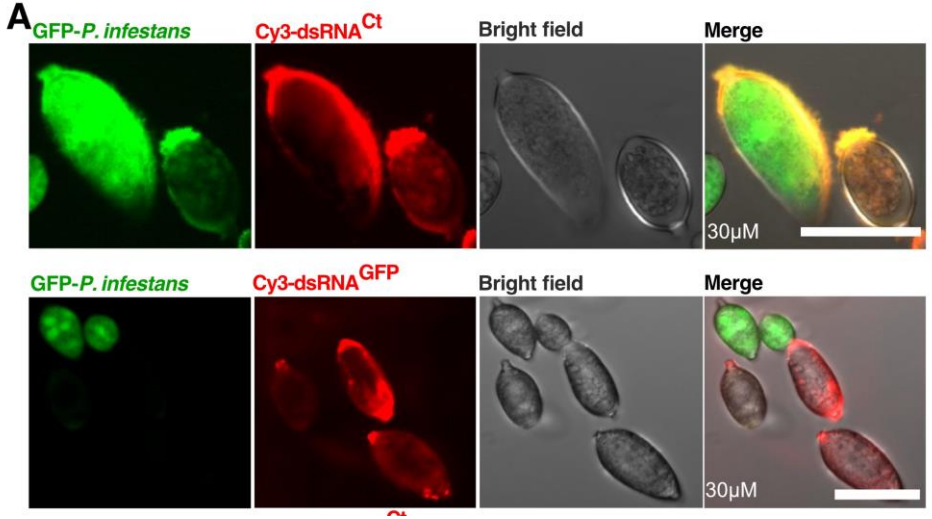
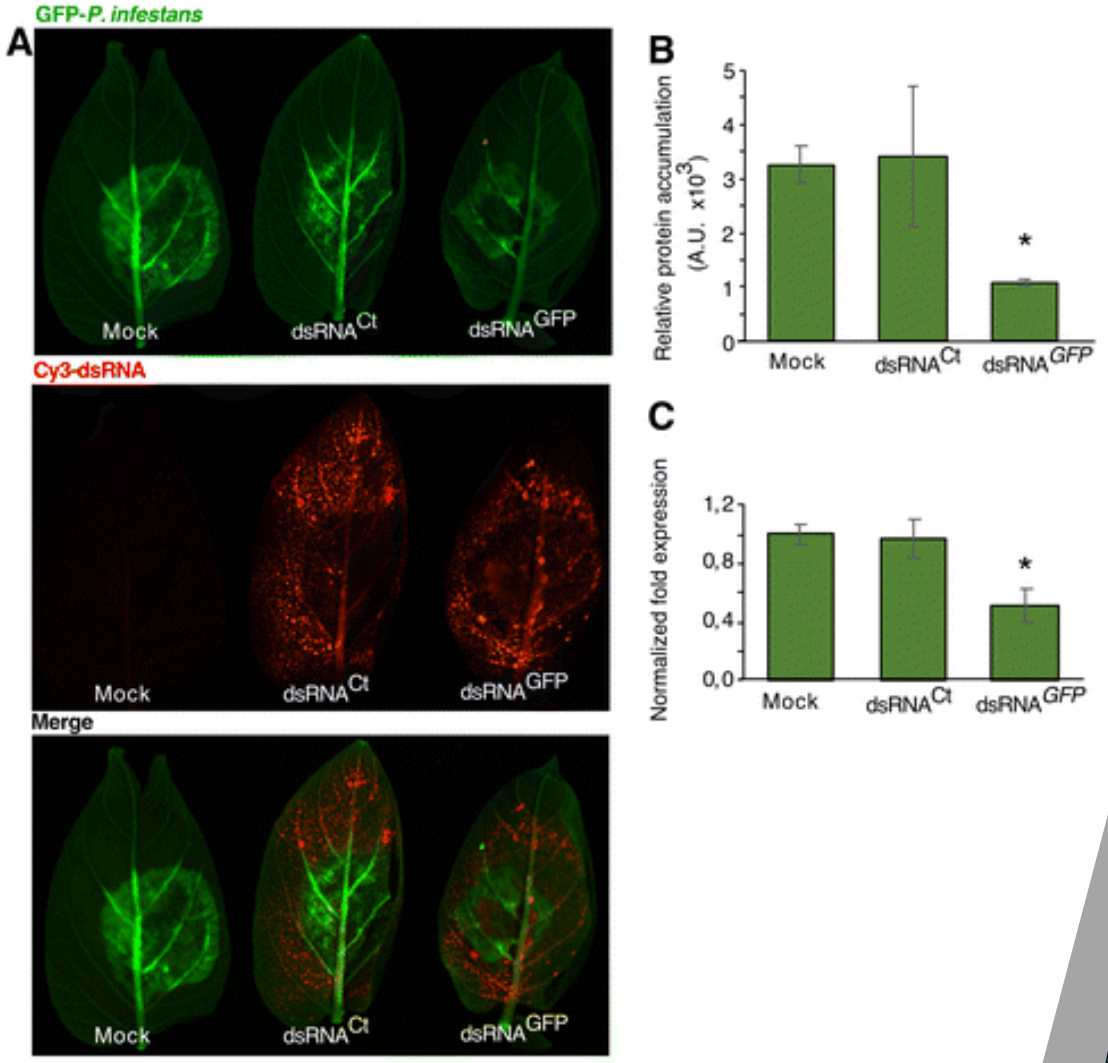




Spray-Induced Gene Silencing as a Potential Tool to Control Potato Late Blight Disease

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,†]





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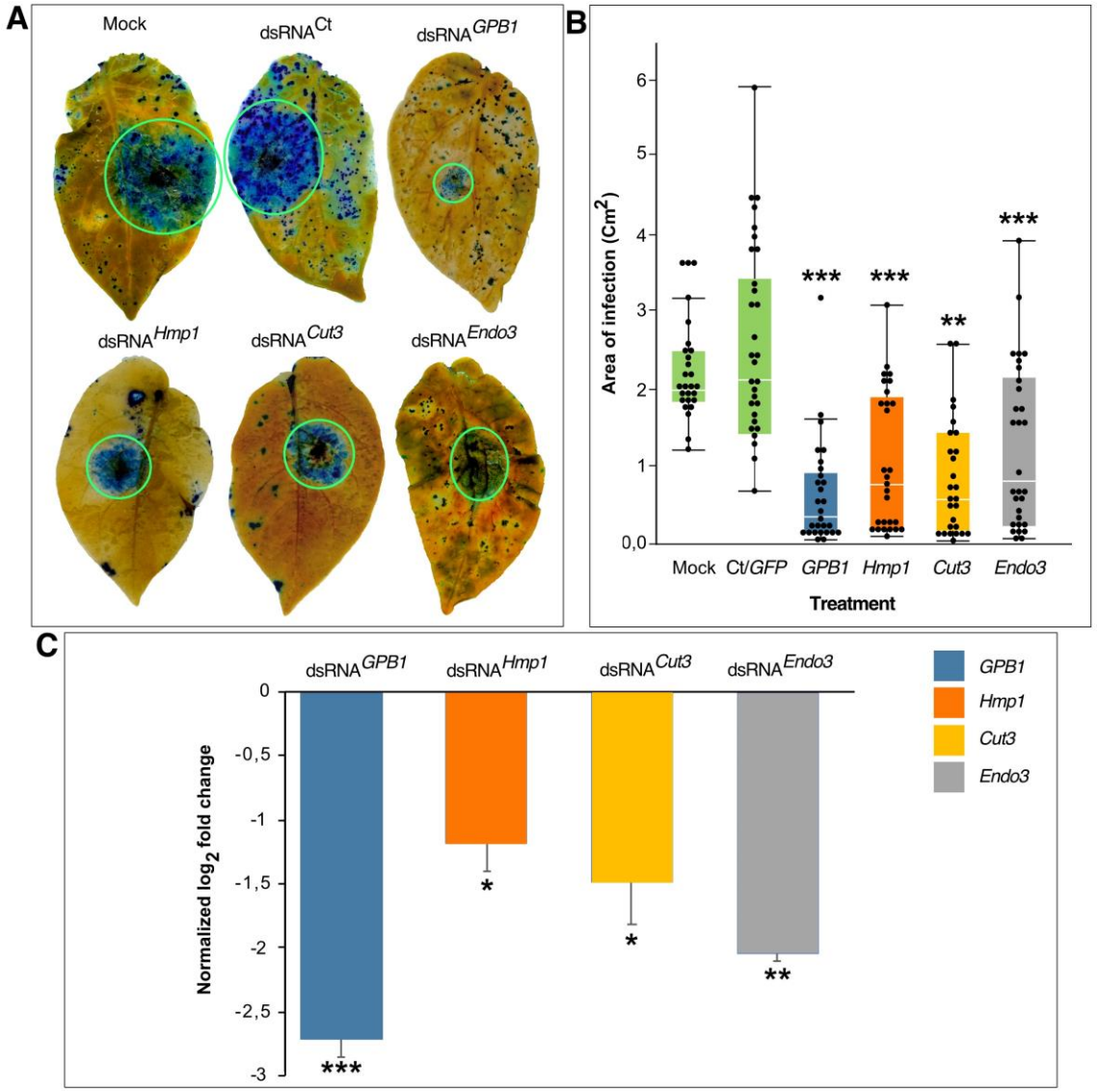
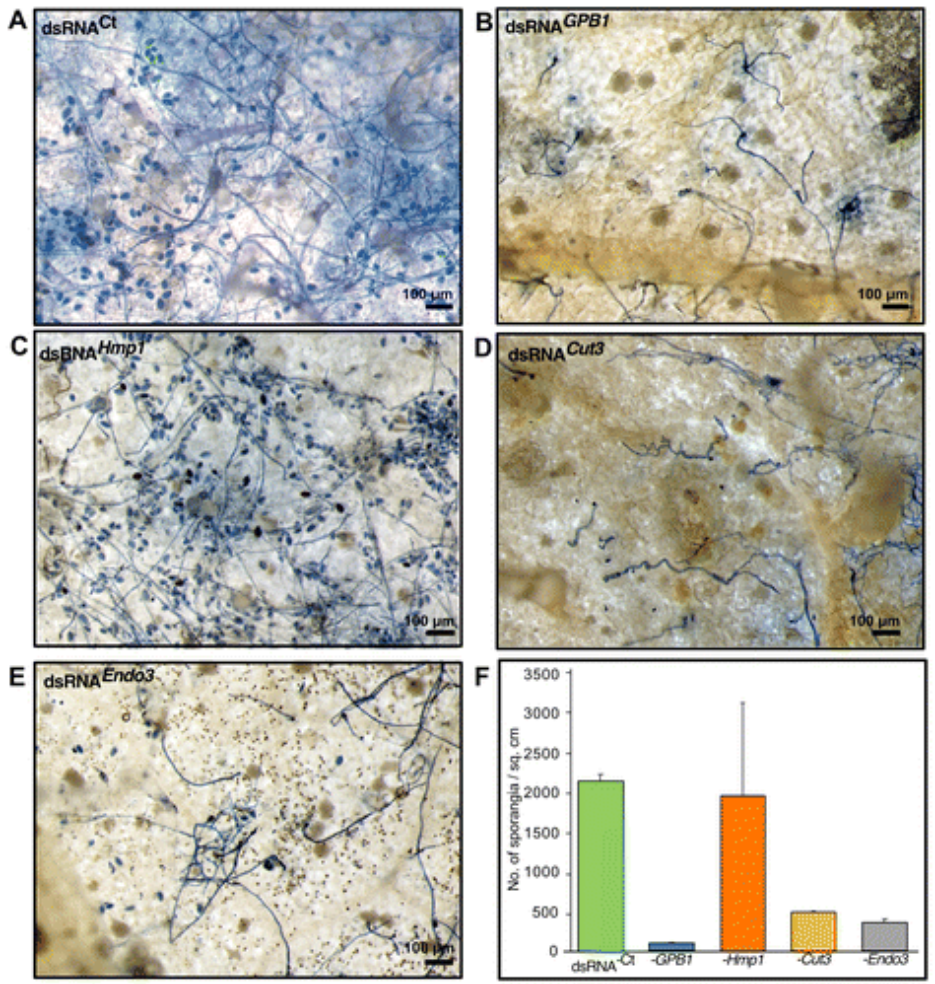
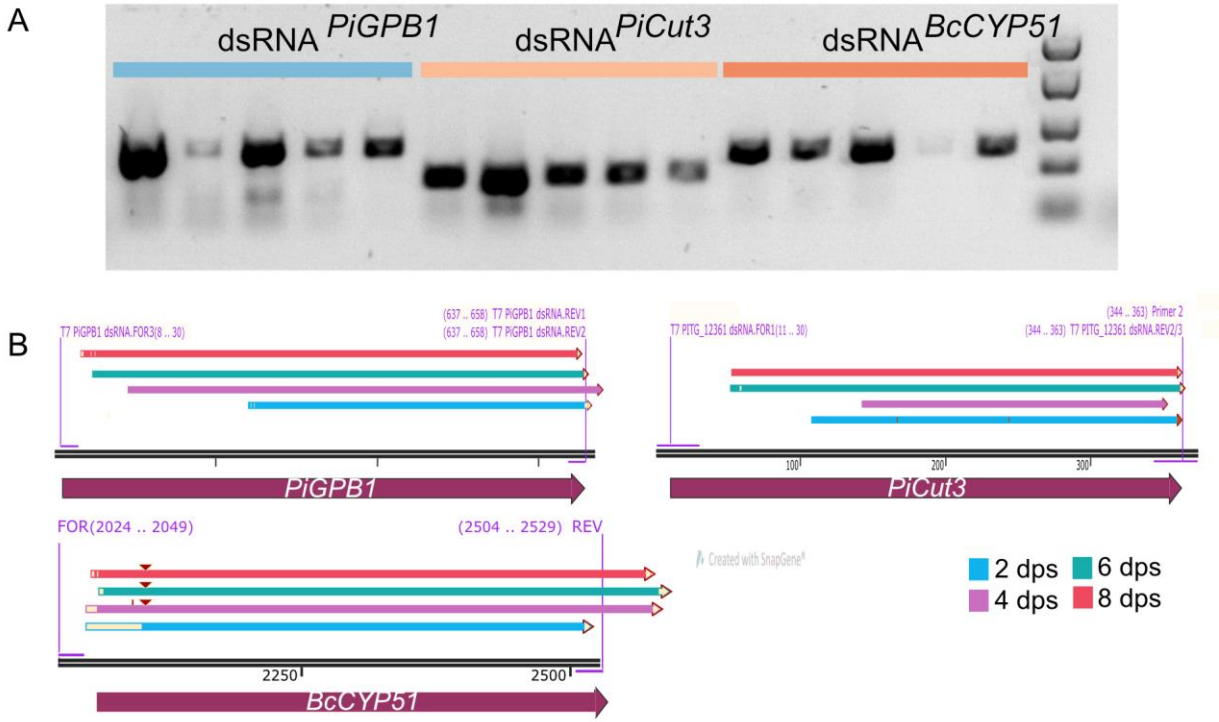
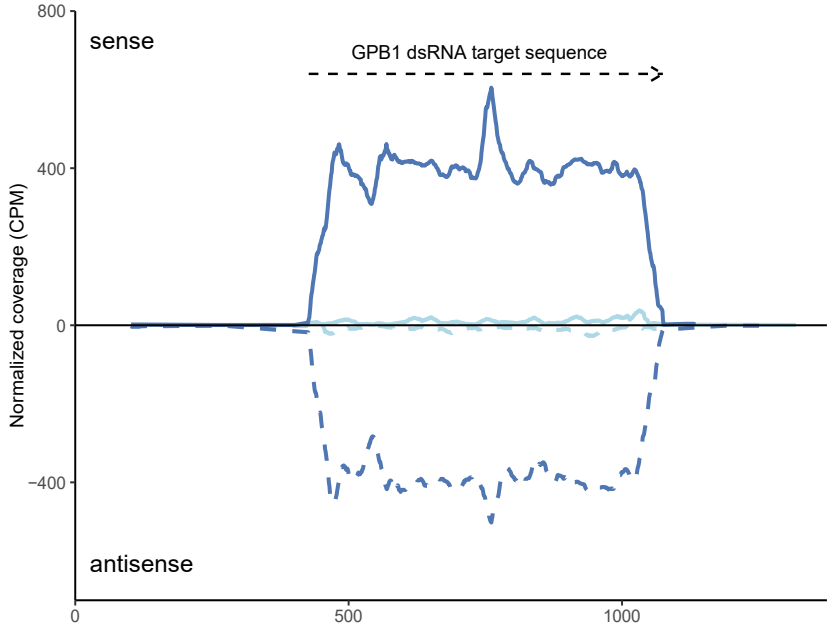


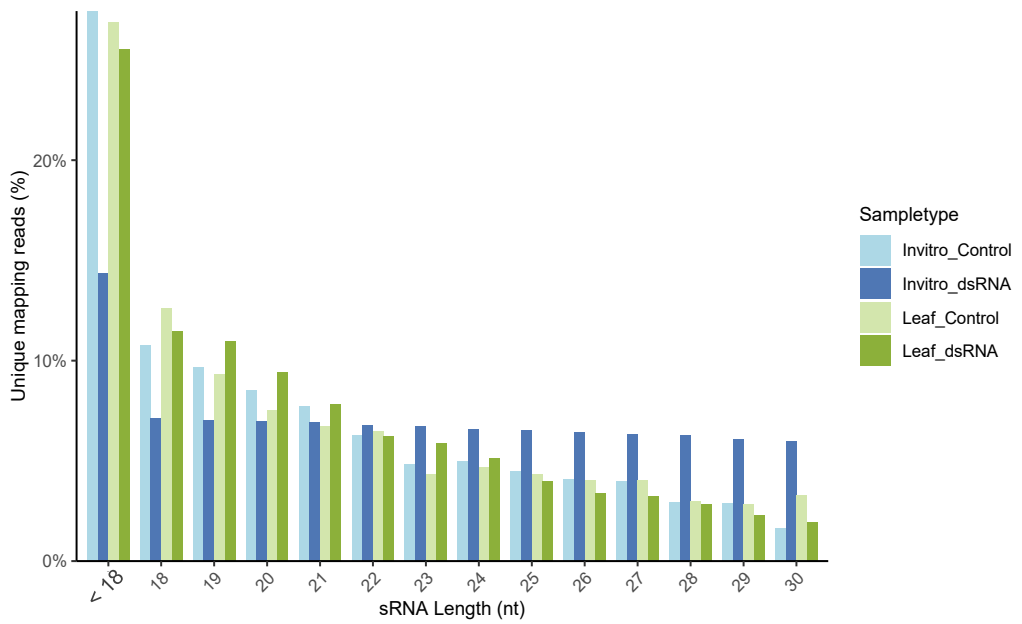


Efficacy and stability of dsRNA upon spraying

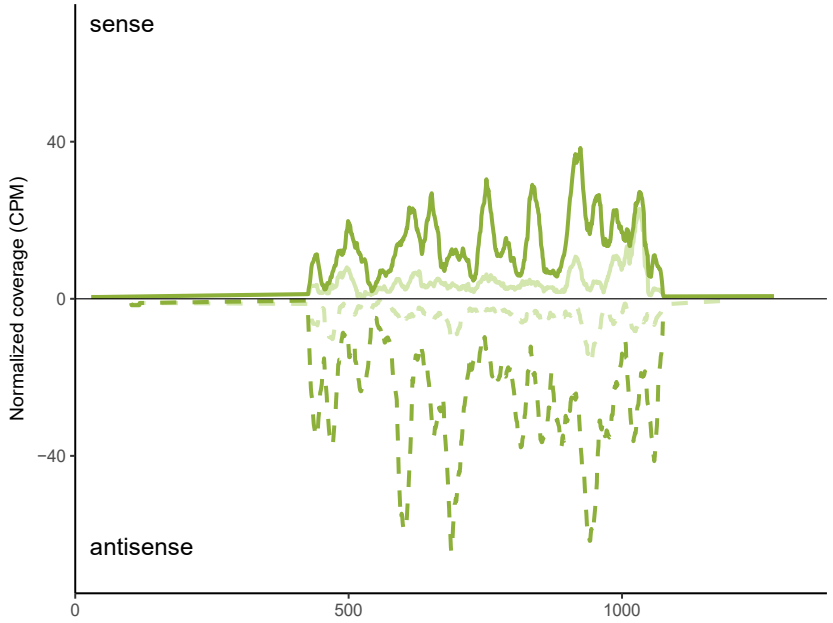


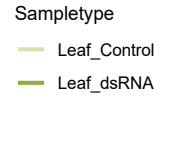

sense
GPB1 dsRNA target sequence
Normalized coverage (CPM)
800
400
0
−400
antisense
0
500
1000

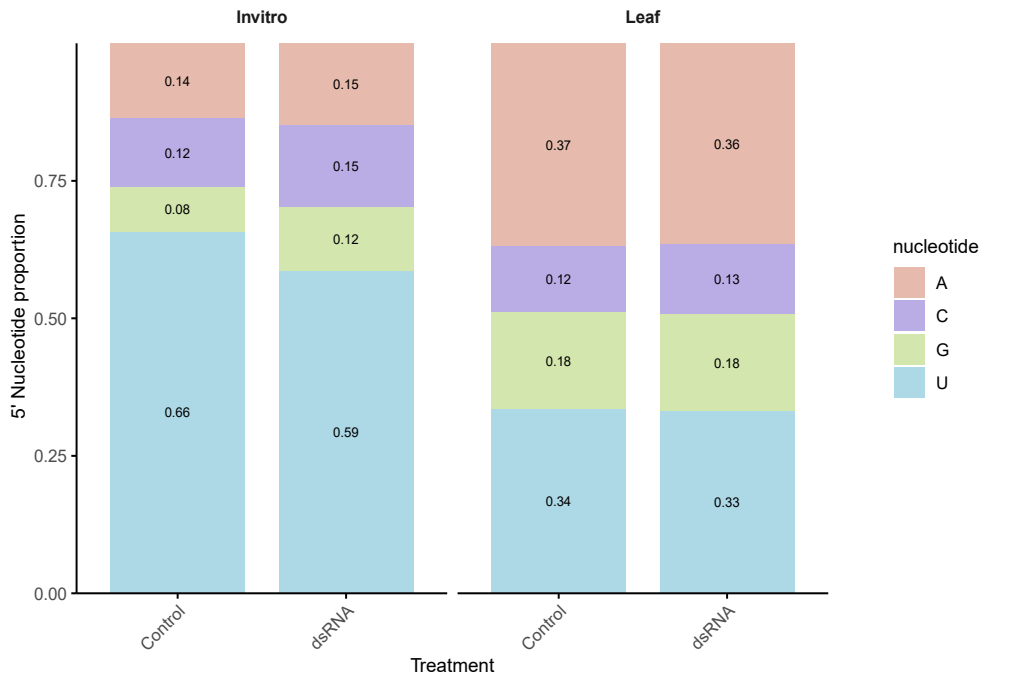

Sampletype
Invitro_Control
Invitro_dsRNA


Unique mapping reads (%)
20%
10%
0%
< 18
18
19
20
21
22
23
24
25
26
27
28
29
30
sRNA Length (nt)

Sampletype
Invitro_Control
Invitro_dsRNA
Leaf_Control
Leaf_dsRNA

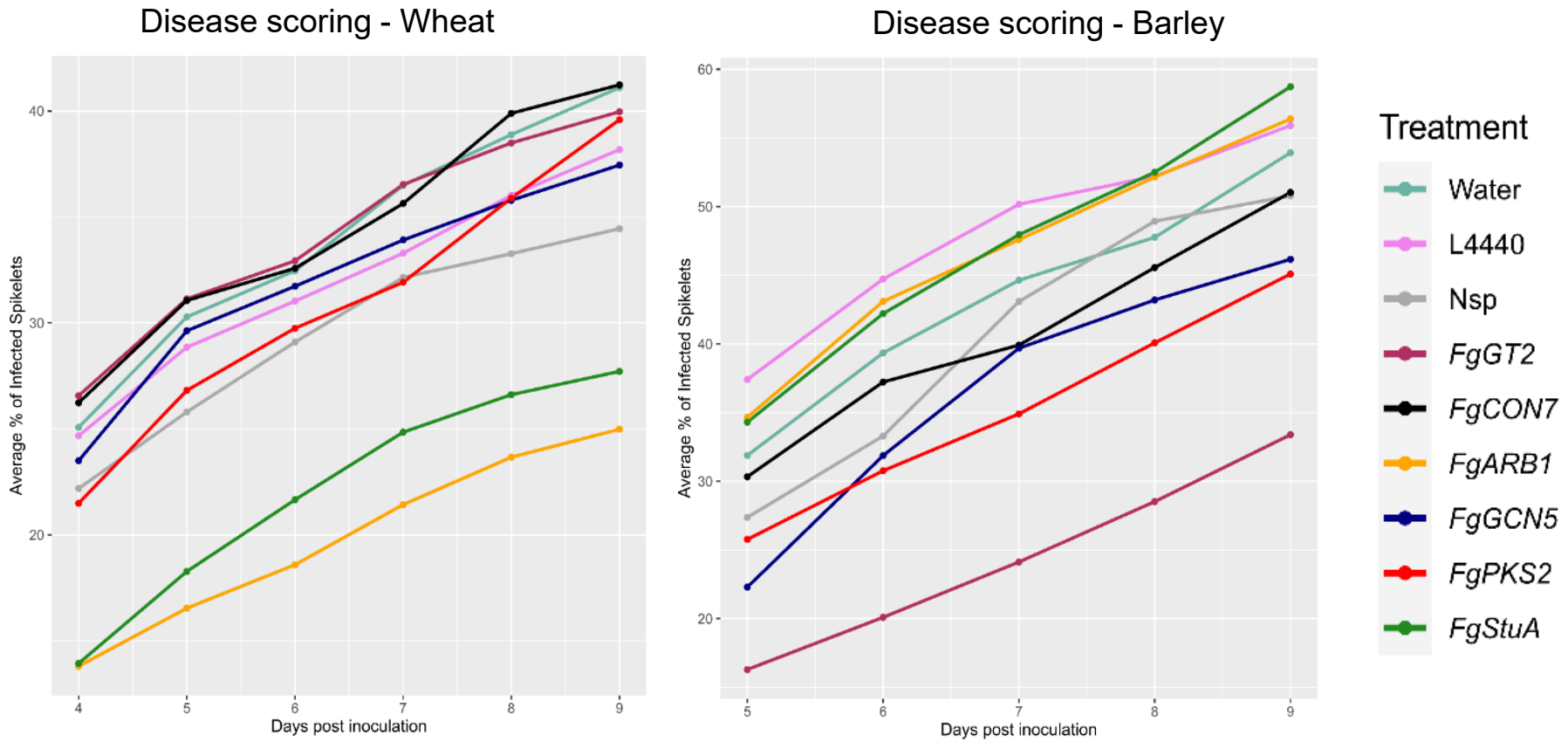

sense
Normalized coverage (CPM)
40
0
−40
antisense
0
500
1000


Sampletype
Leaf_Control
Leaf_dsRNA


Invitro
Leaf
5' Nucleotide proportion
0.00
0.25
0.50
0.75
0.14
0.12
0.08
0.66
0.15
0.15
0.12
0.59
0.37
0.12
0.18
0.34
0.36
0.13
0.18
0.33
Control
dsRNA
Control
dsRNA
Treatment

nucleotide
A
C
G
U

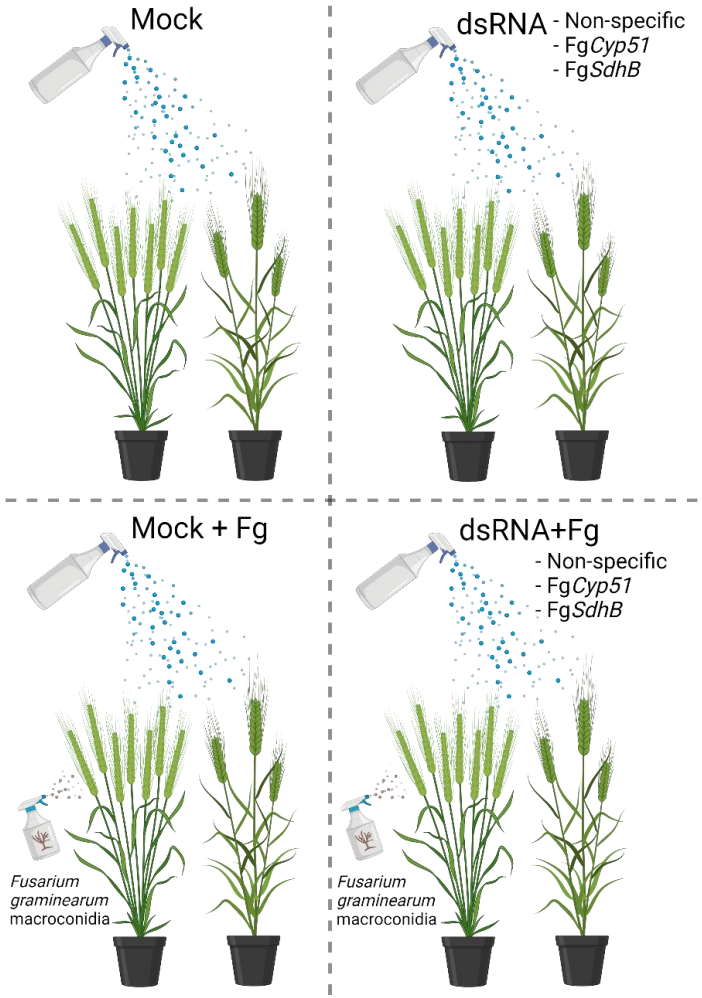
SIGS for protection against *F. graminearum*

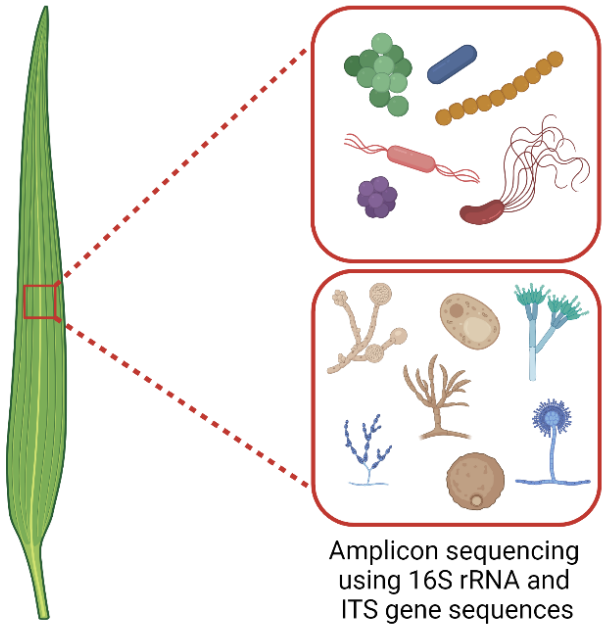


Sundararajan *et al* unpublished

The impact of spray-induced gene silencing on cereal phyllosphere microbiota

Poorva Sundararajan[1†], Samrat Ghosh[1†], Bekele Gelena Kelbessa[1], Stephen C. Whisson[2], Mukesh Dubey[3], Aakash Chawade[1] and Ramesh Raju Vetukuri[1*]



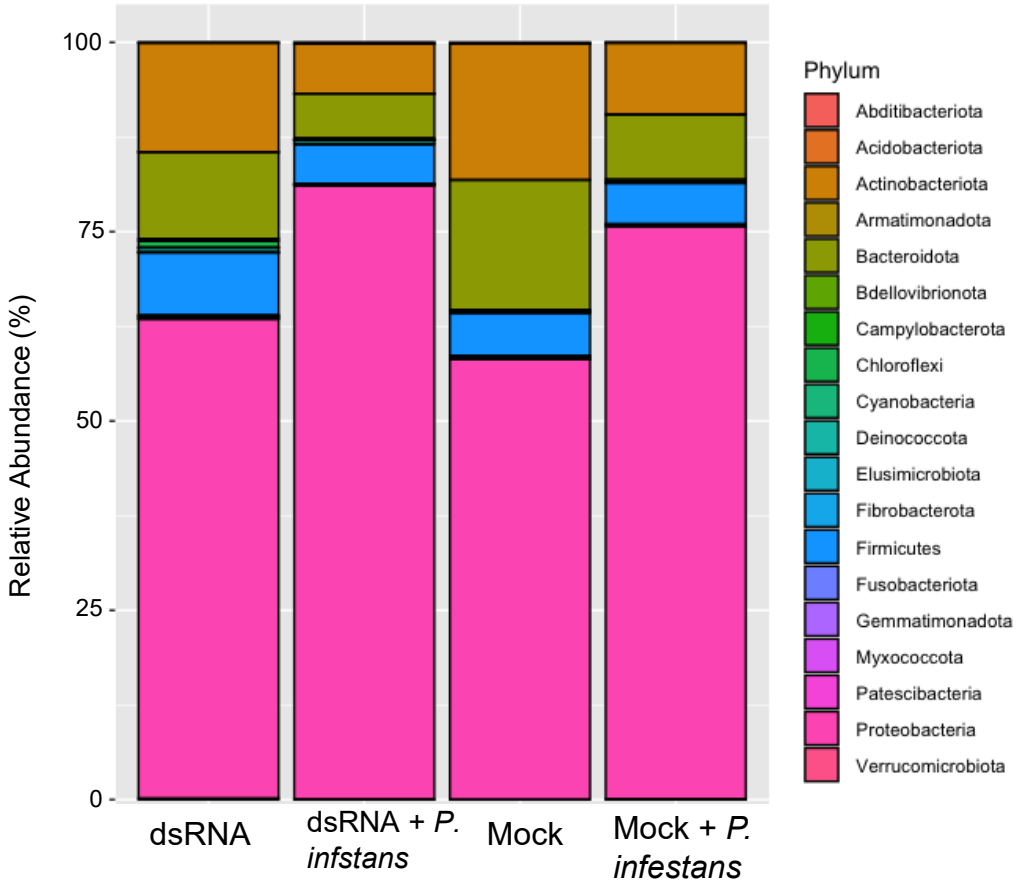


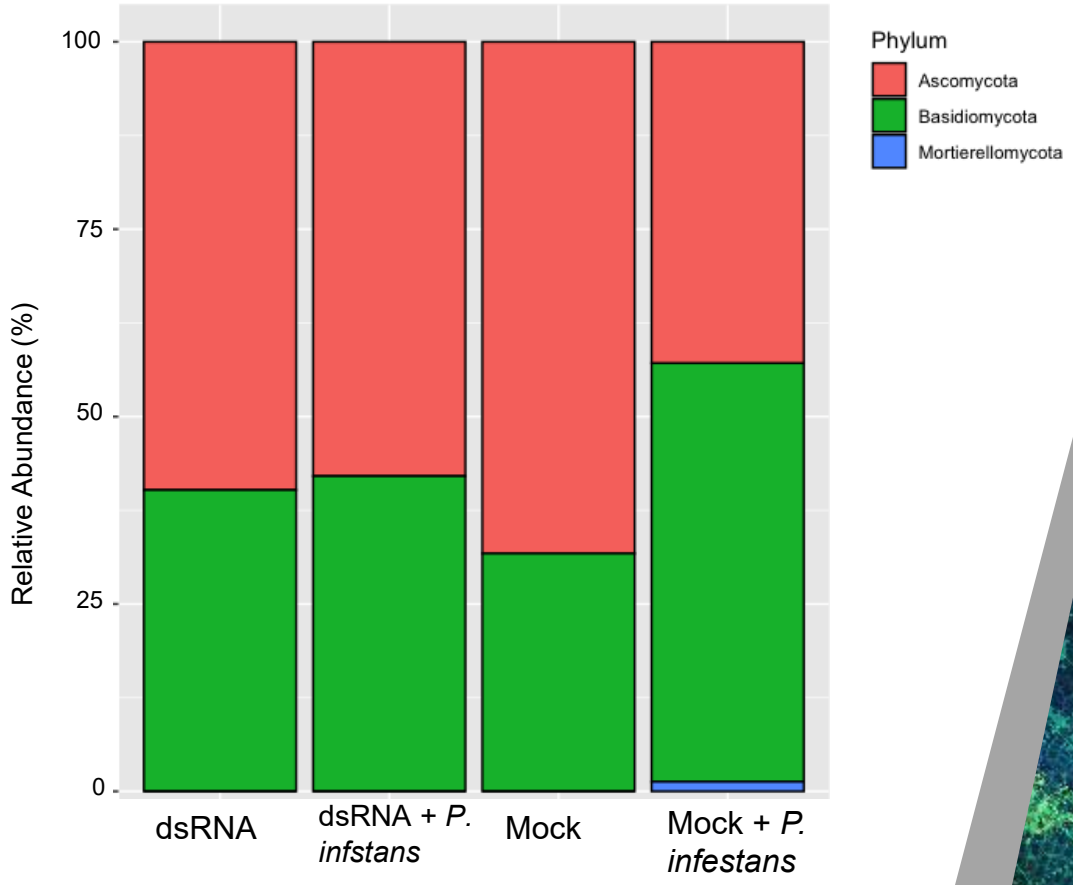
Objectives:

- Assess if dsRNA affects the microbial communities of the phyllosphere
- Assess how these interactions change upon *F. graminearum* infection

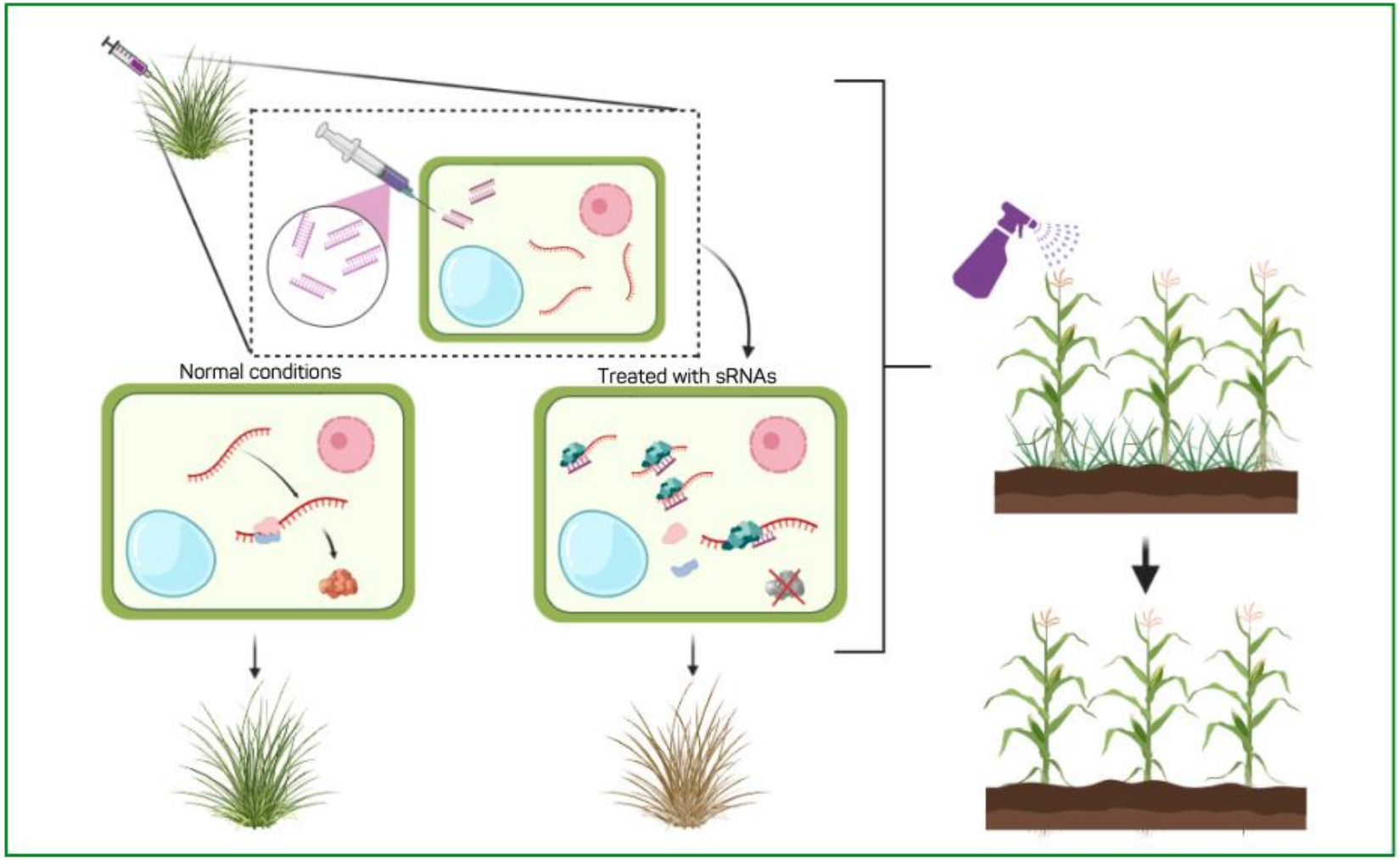
The impact of spray-induced gene silencing on cereal phyllosphere microbiota

Poorva Sundararajan[1†], Samrat Ghosh[1†], Bekele Gelena Kelbessa[1], Stephen C. Whisson[2], Mukesh Dubey[3], Aakash Chawade[1] and Ramesh Raju Vetukuri[1*]





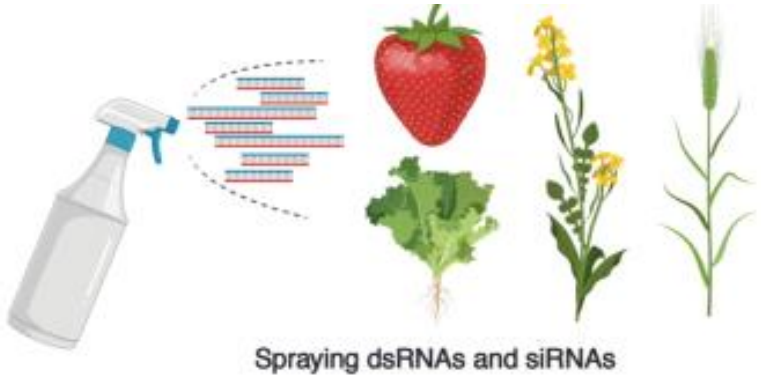
SIGS to target endogenous genes

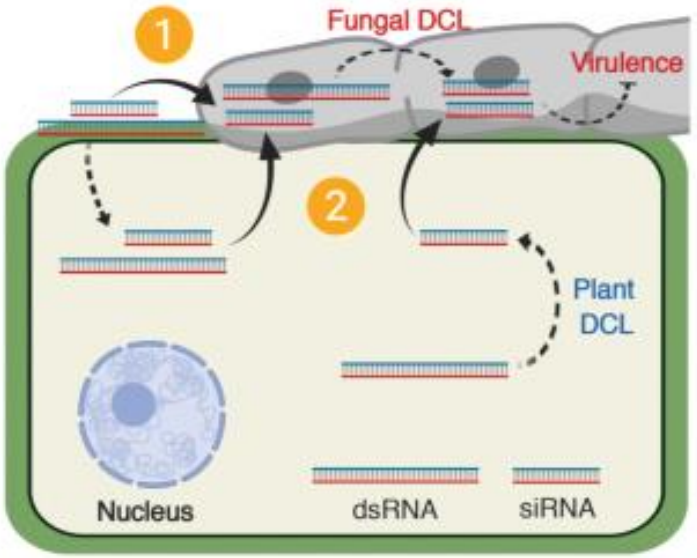


Summary

- Biotechnical exploitation of RNA interference has facilitated VIGS, HIGS and SIGS technology.
- SIGS is a non-transgenic approach to improve plant resistance
- SIGS is effective in a range of plant pathosystems
- SIGS may have reduced environmental impact compared to conventional chemical approaches
- SIGS can be utilised to modulate plant endogenous traits to improve crop growth

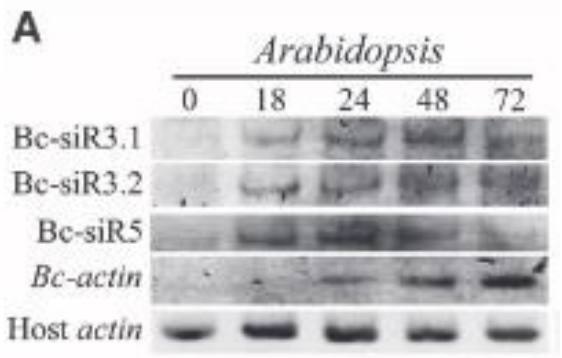
Spray induced gene silencing

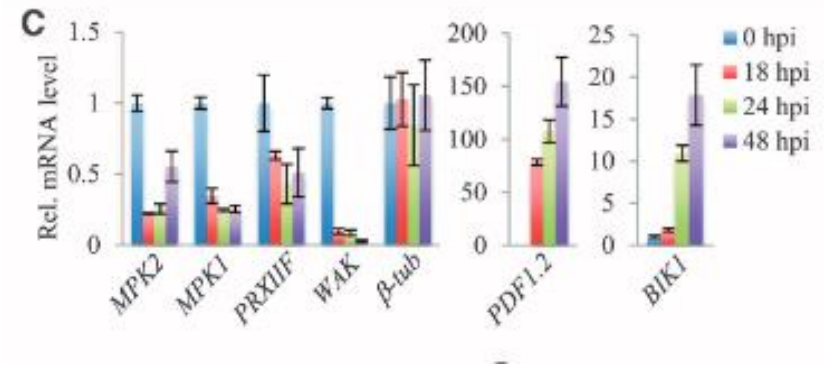




Fungal Small RNAs Suppress Plant Immunity by Hijacking Host RNA Interference Pathways

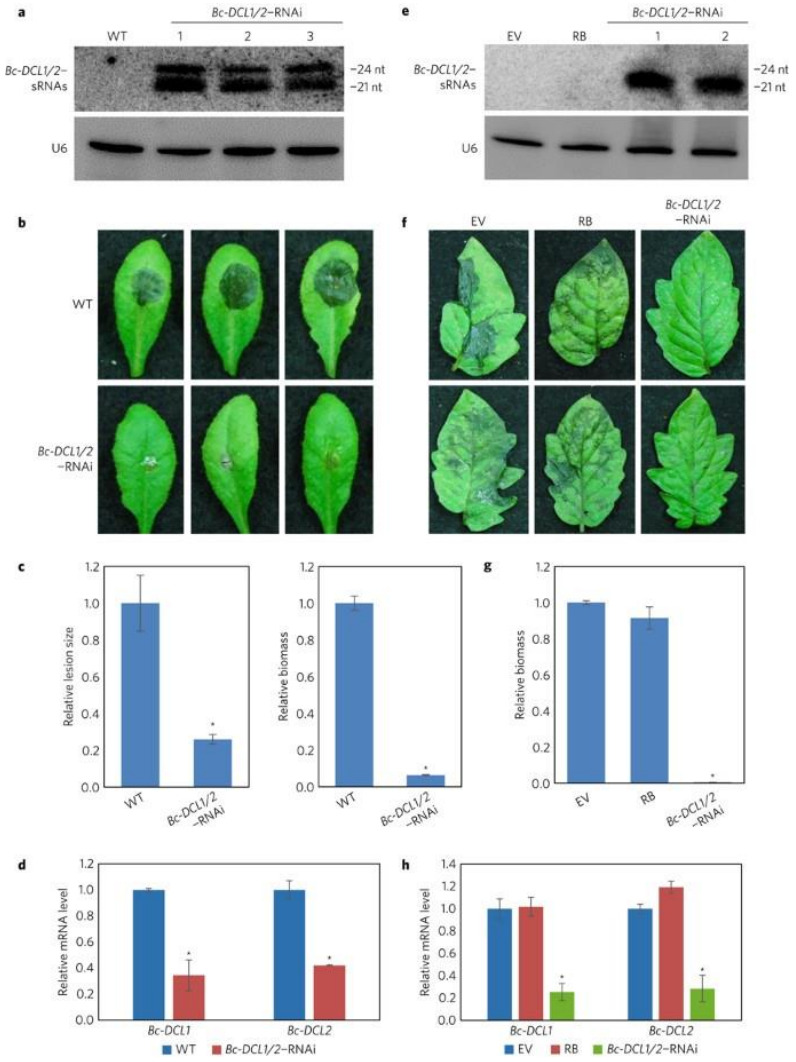
Arne Weiberg,[1,2,3]* Ming Wang,[1,2,3]* Feng-Mao Lin,[4] Hongwei Zhao,[1,2,3]† Zhihong Zhang,[1,2,3,5] Isgouhi Kaloshian,[2,3,6] Hsien-Da Huang,[4,7] Hailing Jin[1,2,3]‡

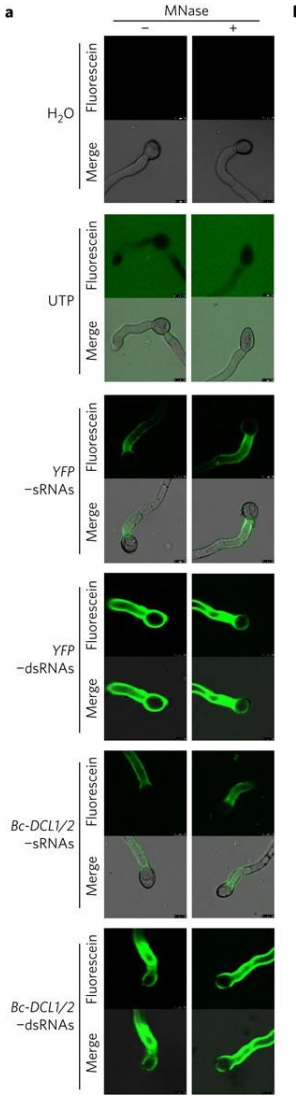


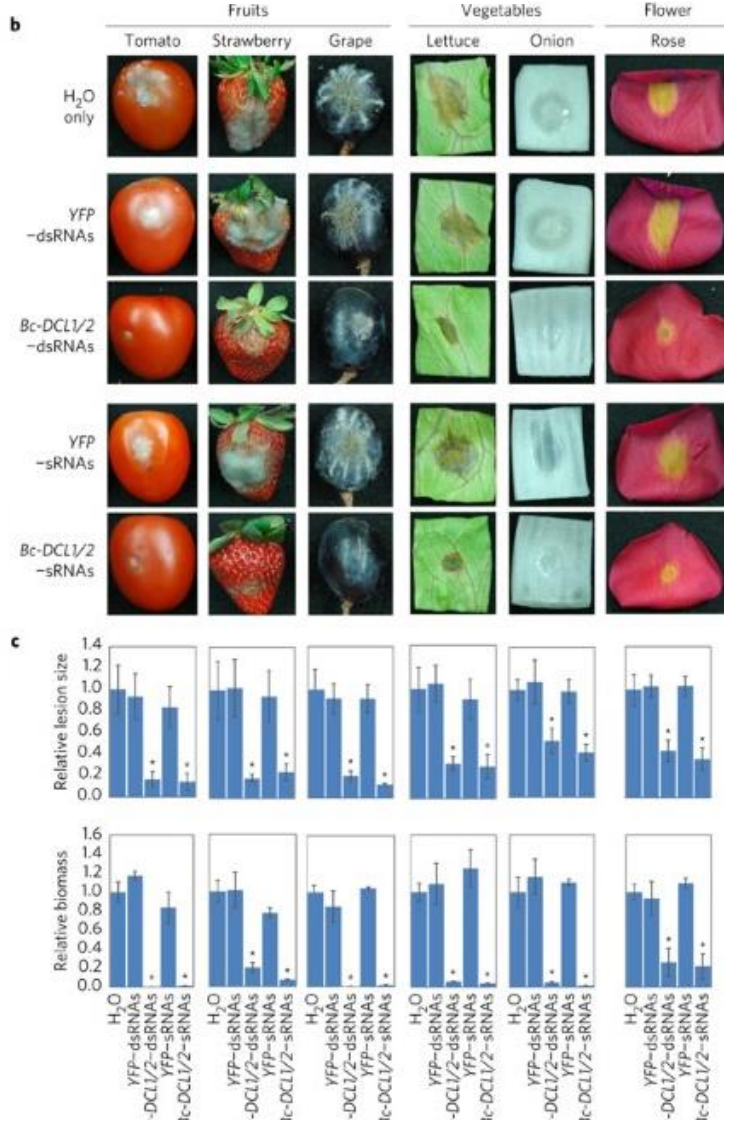


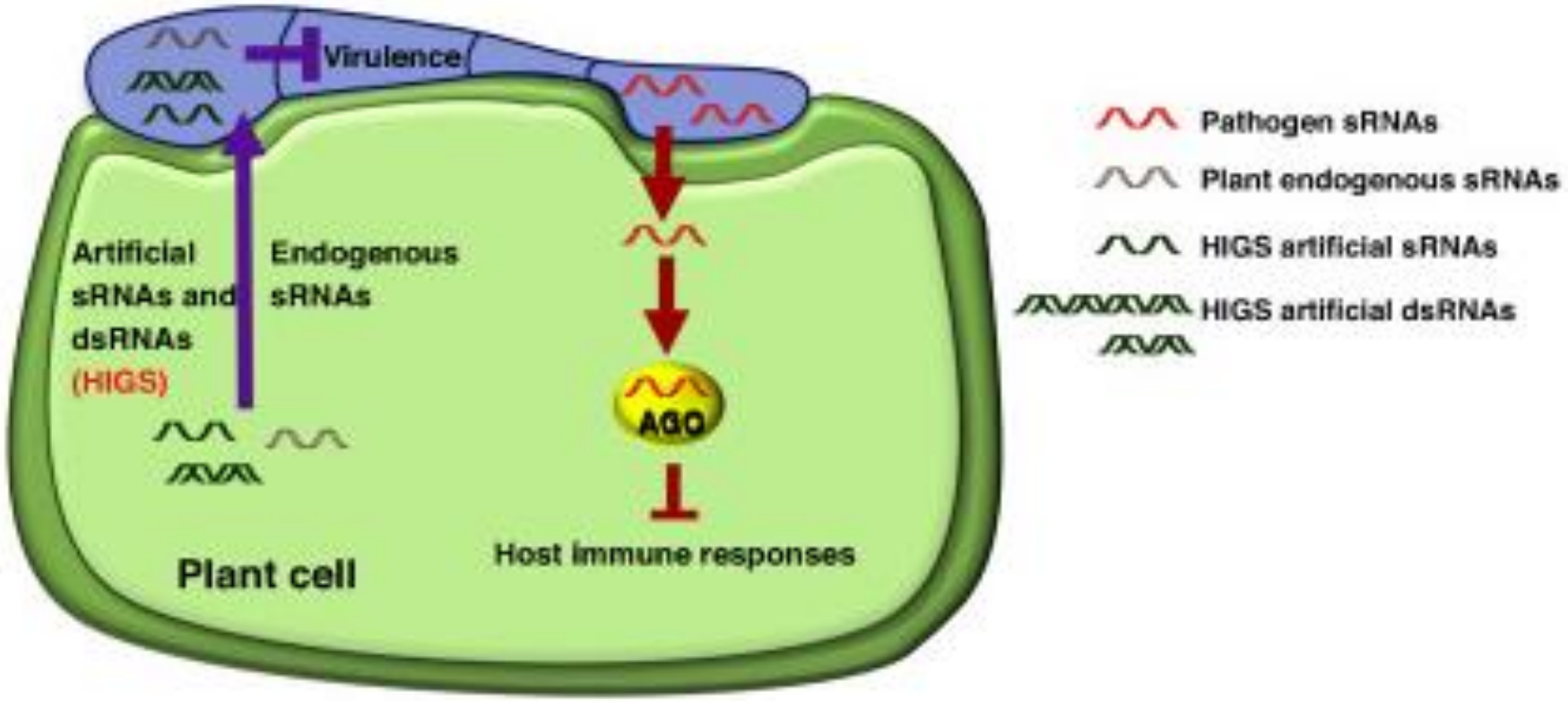
Bidirectional cross-kingdom RNAi and fungal uptake of external RNAs confer plant protection

Ming Wang[1], Arne Weiberg[1†], Feng-Mao Lin[2], Bart P. H. J. Thomma[3], Hsien-Da Huang[2] and Hailing Jin[1*]

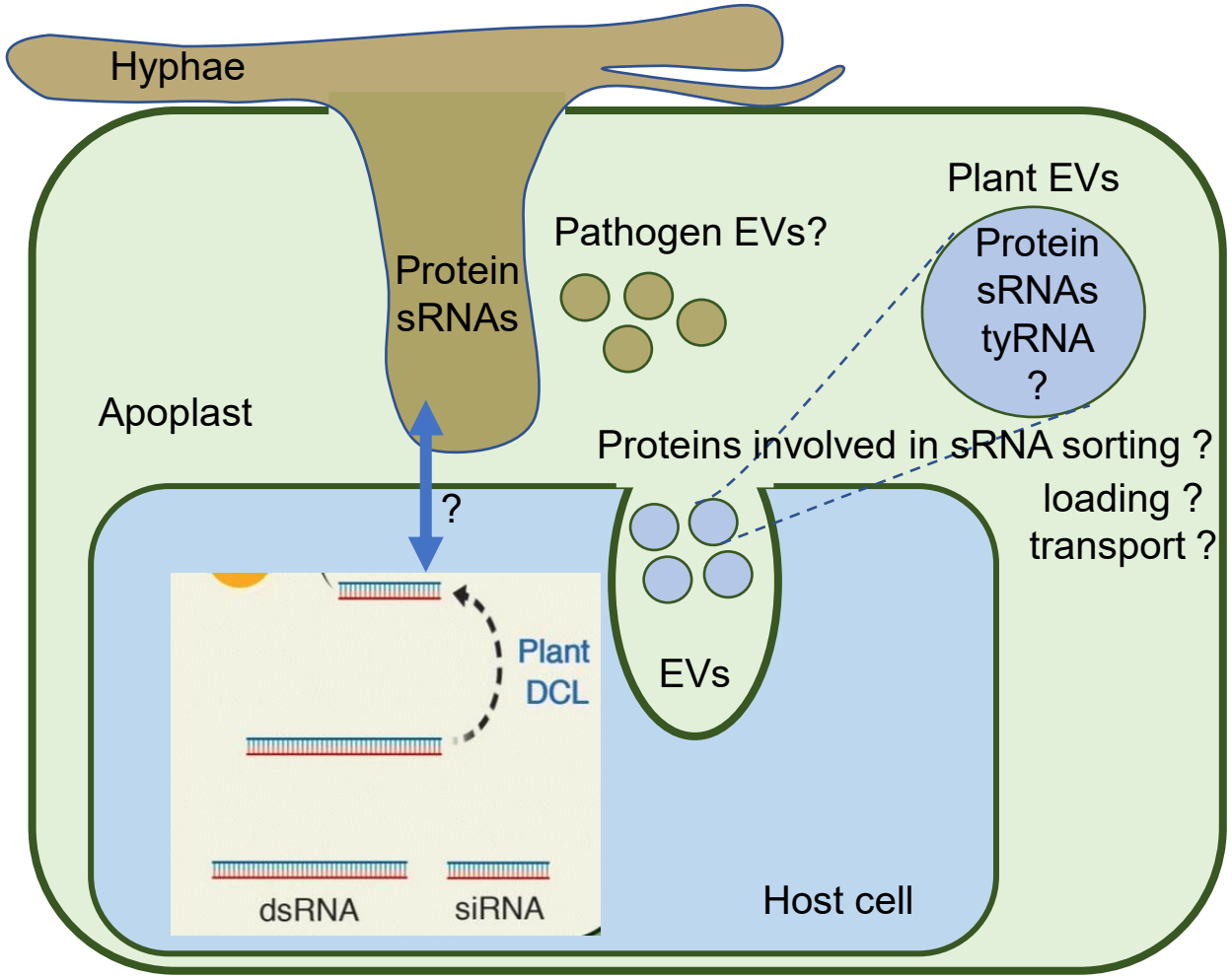







Virulence
Artificial sRNAs and dsRNAs (HIGS)
Endogenous sRNAs
AGO
Host immune responses
Plant cell
Pathogen sRNAs
Plant endogenous sRNAs
HIGS artificial sRNAs
HIGS artificial dsRNAs

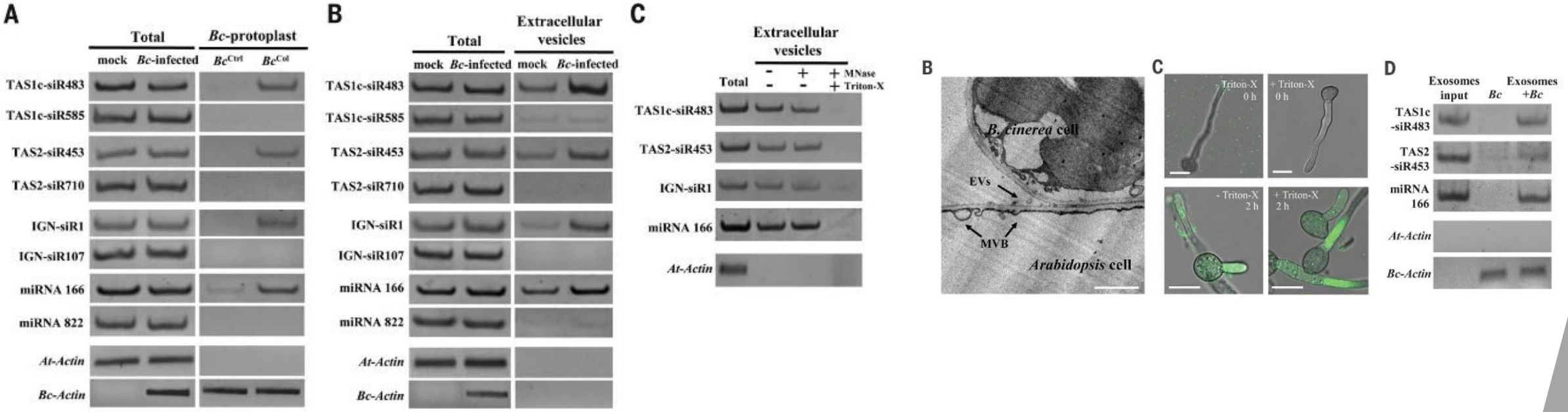
Unrevealing the mechanisms behind SIGS

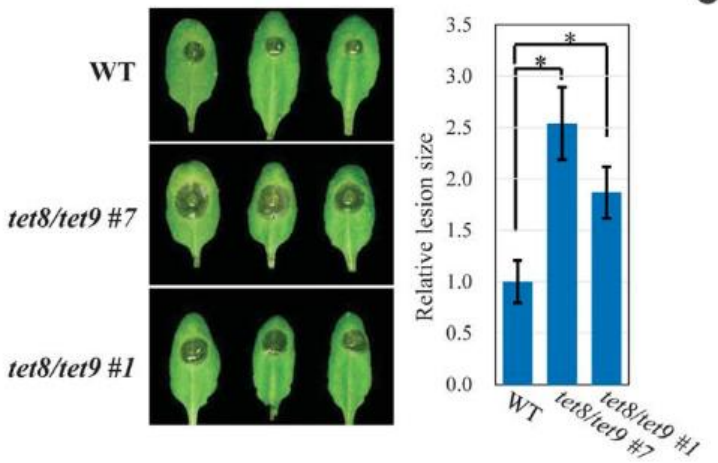


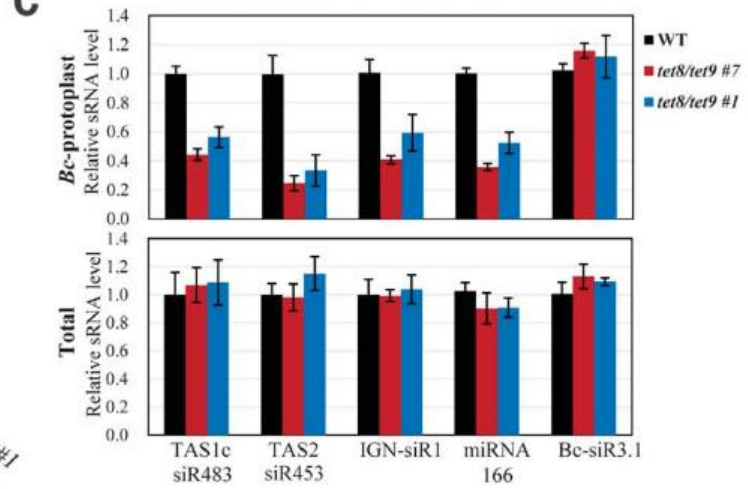
- Cross-kingdom movement of sRNAs and their associated proteins
- Extracellular vesicle (Evs) dependent or independent sRNA transport
- Do sRNAs get loaded into EVs or other protein vehicles?
- Which potato AGOs are responsible for spray induced gene silencing.

Plants send small RNAs in extracellular vesicles to fungal pathogen to silence virulence genes

Qiang Cai[1], Lulu Qiao[1,2], Ming Wang[1], Baoye He[1], Feng-Mao Lin[3], Jared Palmquist[1], Sienna-Da Huang[3], Hailing Jin[1]*

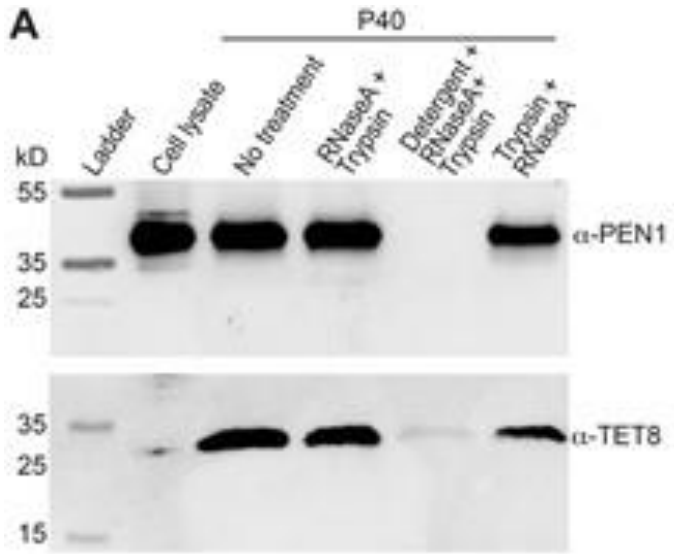


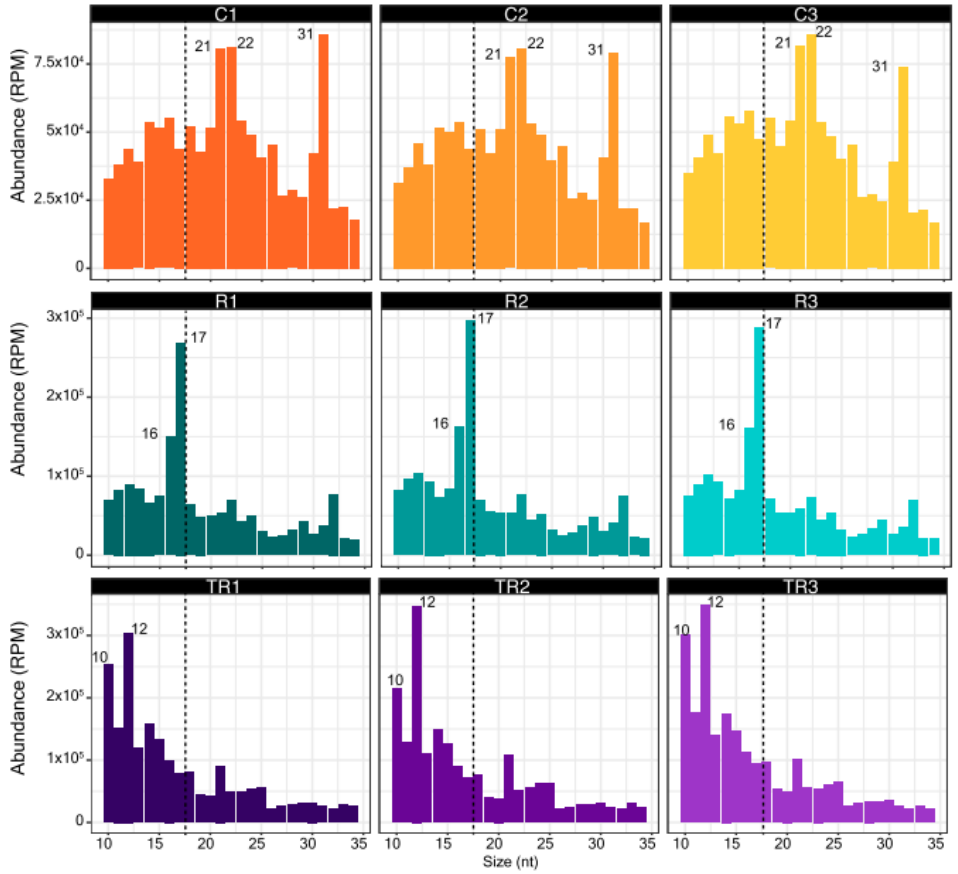




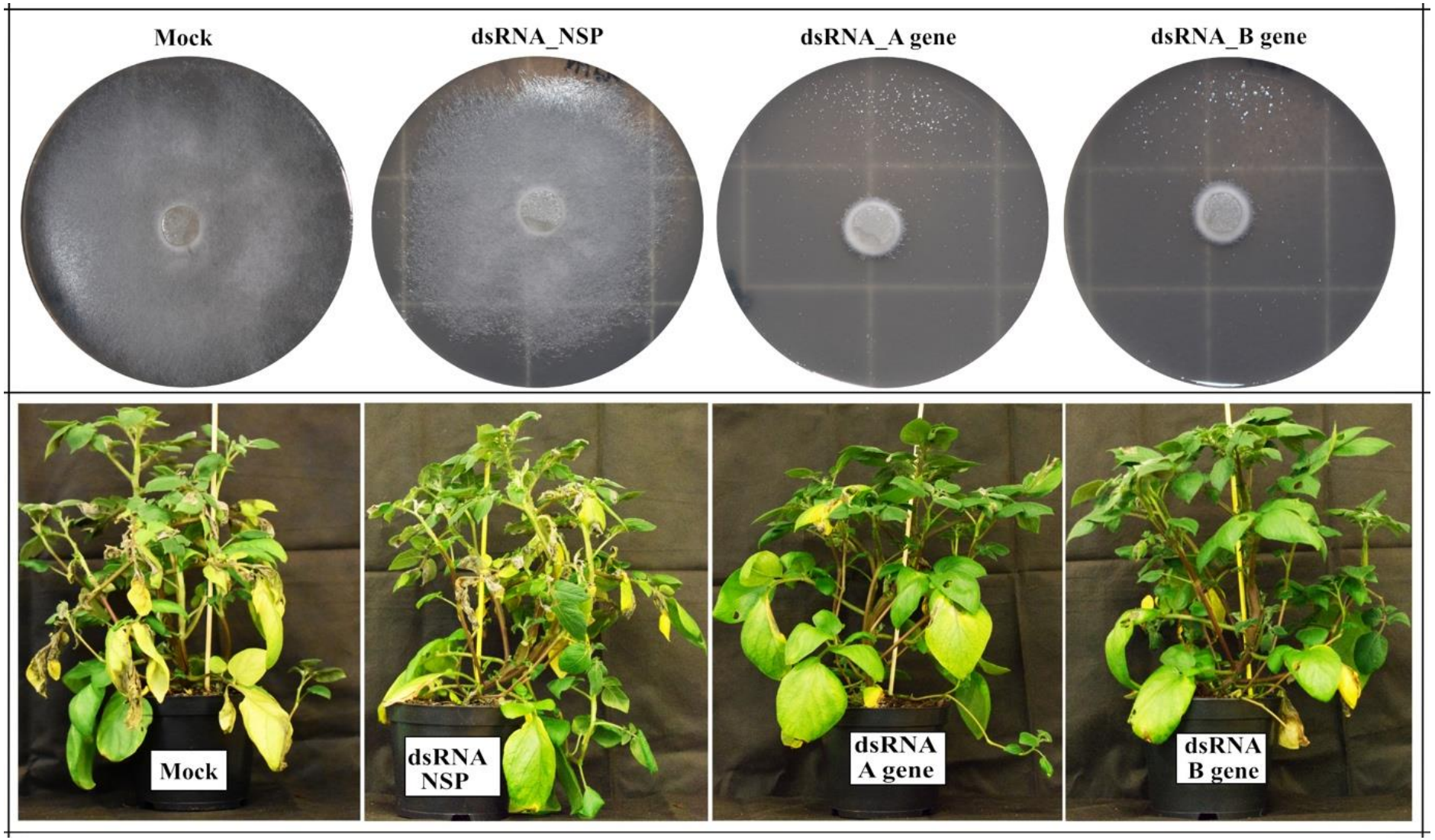
Arabidopsis apoplastic fluid contains sRNA- and circular RNA–protein complexes that are located outside extracellular vesicles

Hana Zand Karimi,[1] Patricia Baldrich,[2] Brian D. Rutter,[1] Lucía Borniego,[1] Kamil K. Zajt,[1] Blake C. Meyers[2,3] and Roger W. Innes[1,*,†]





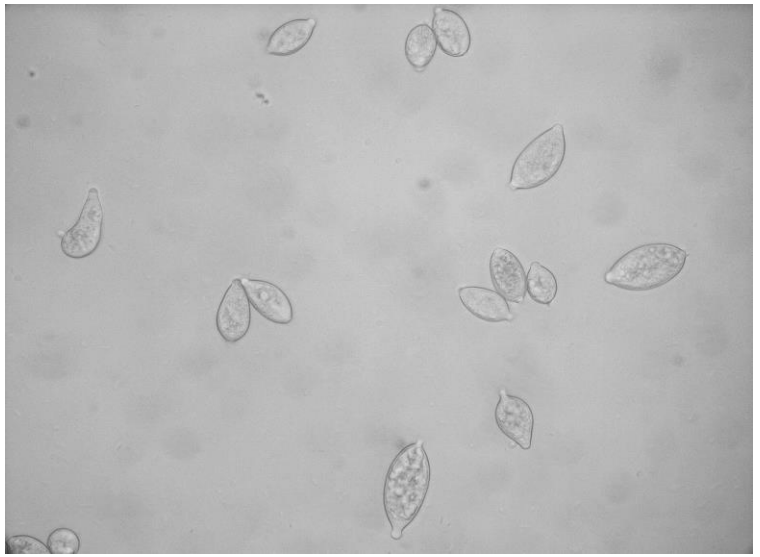
Effective SIGS for protection against *P. infestans*



Konakalla *et al* unpublished

Germinated sporangia_Mock_4 hpi

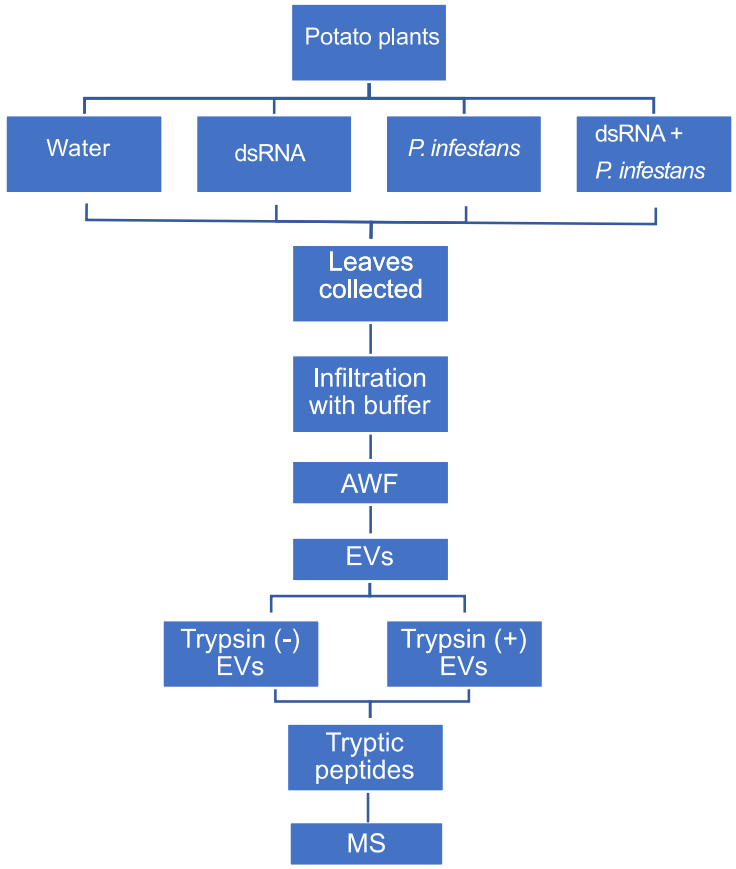
Addition of dsRNA A to the growth medium resulted in deformations of the germ tubes



complete inhibition of germination

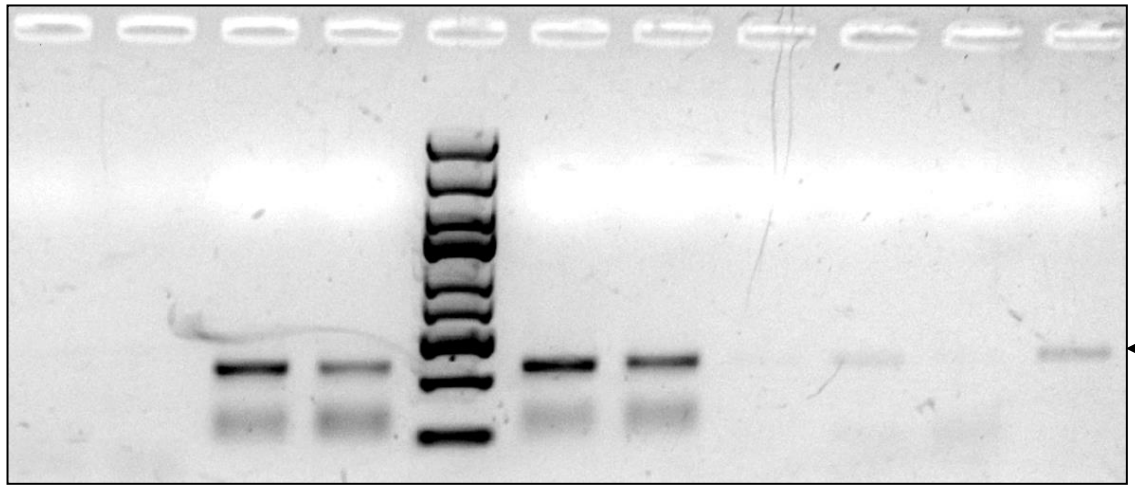
Konakalla *et al* unpublished

dsRNAs are found inside EVs



RT-PCR

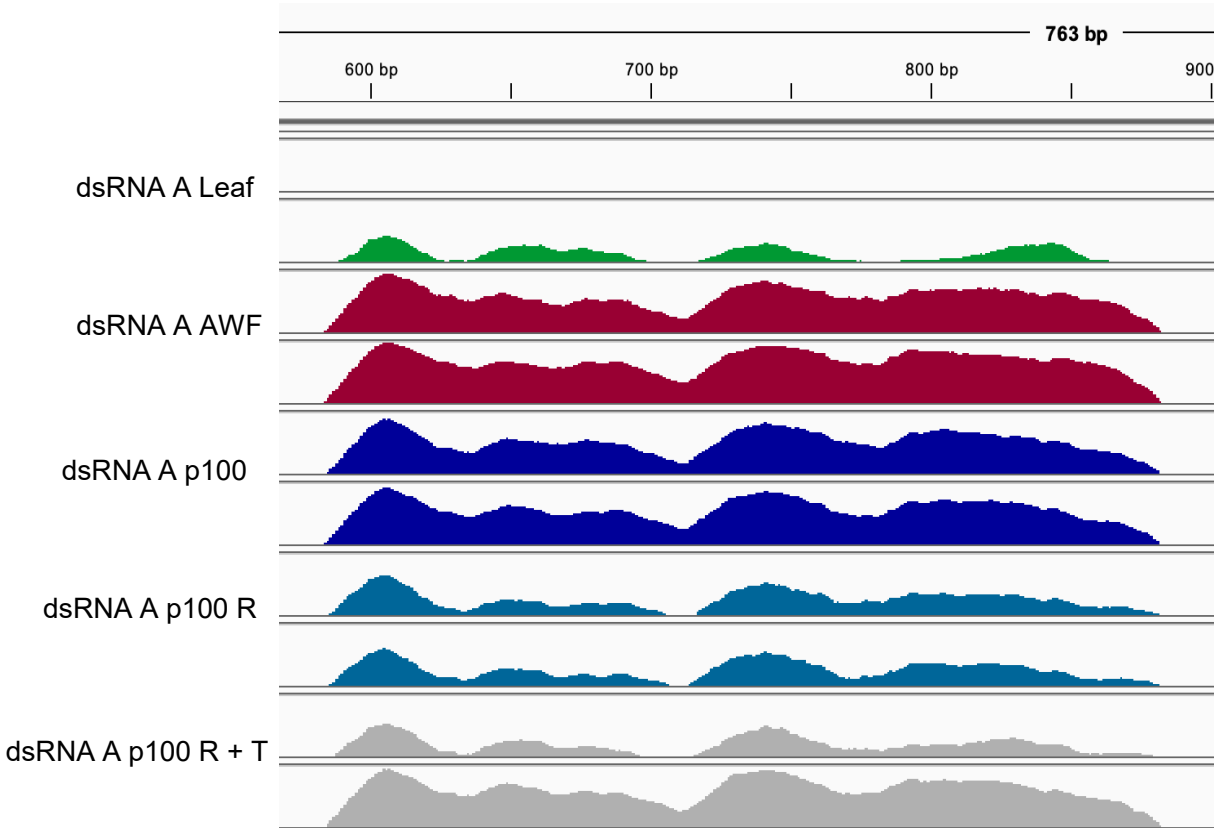
	p100	R+T	p100	p100	p100	p100	R+T	R+T	R+T	R+T
Control	–	–	–	–	–	–	–	–	–	–
dsRNA A	–	–	++++	+++	–	–	+	++	–	–
dsRNA B	–	–	–	–	++++	+++	–	–	+	+

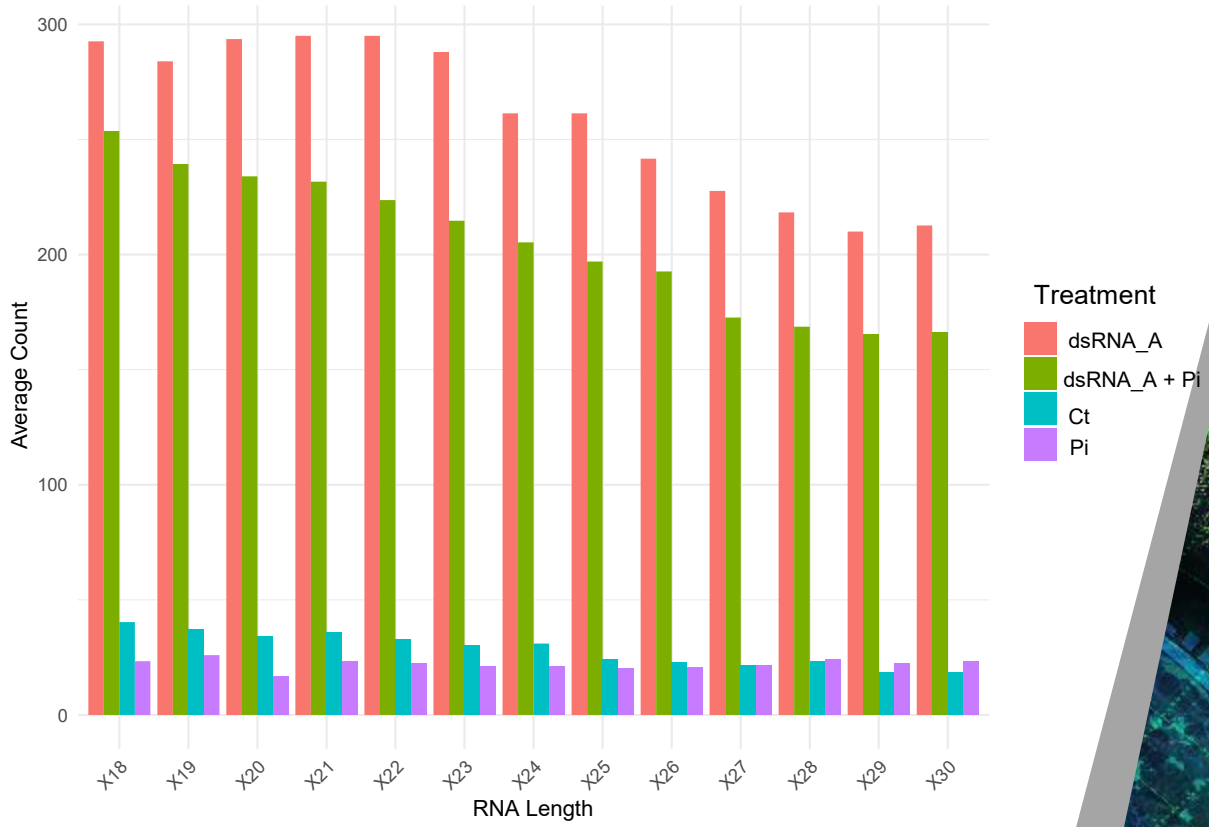


dsRNA A and dsRNA B have been found in *Nicotiana benthamiana* EVs; both p100 fractions have a strong signal (++++), and Rnase I and trypsin (R+T) fractions have a weak signal of dsRNAs.

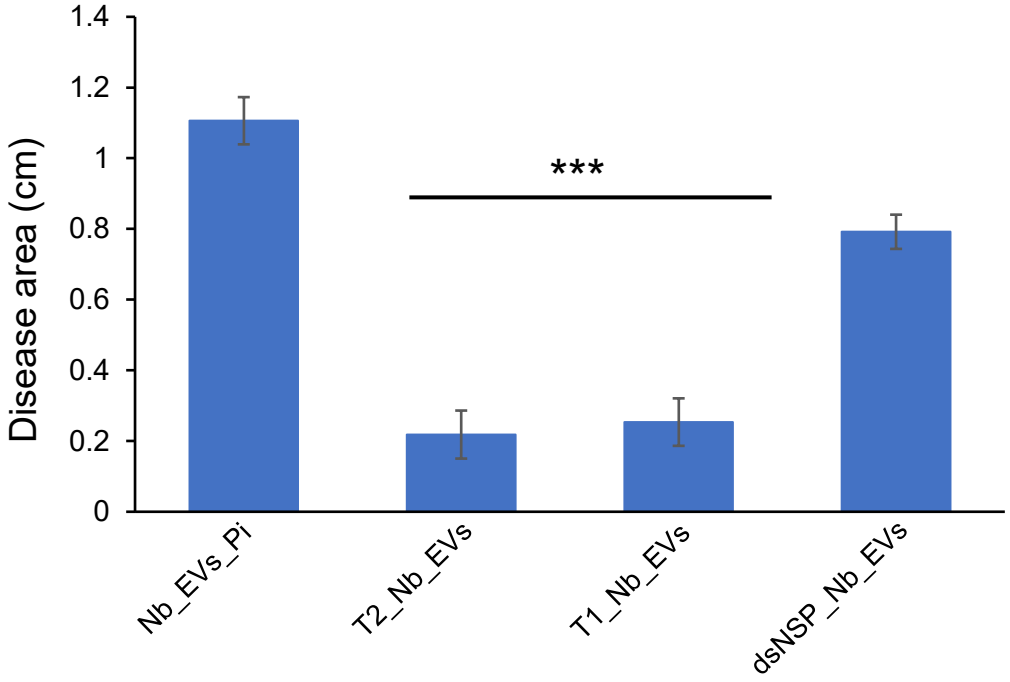
Konakalla *et al* unpublished

dsRNAs are found inside EVs

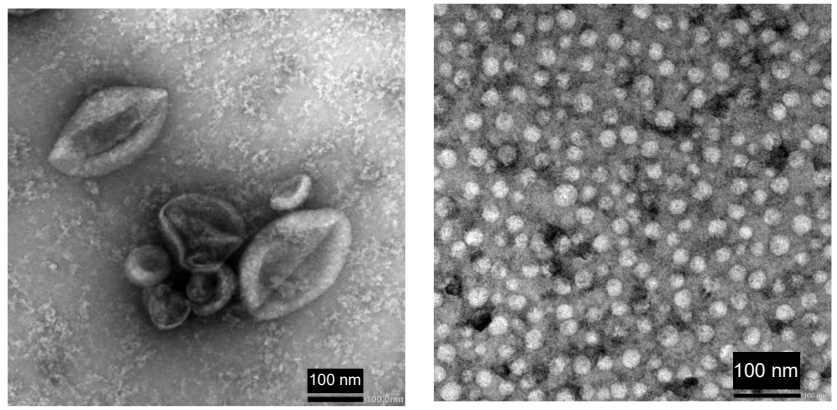




Potential EV transport of dsRNAs



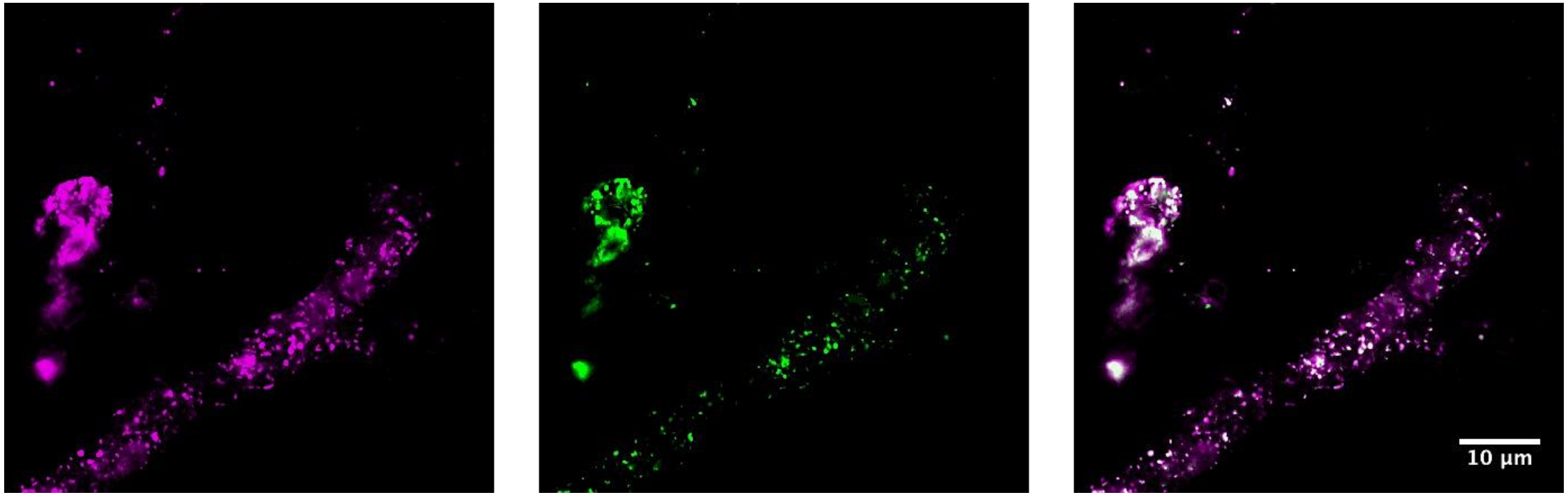
Potato P100 EV - TEM



P. infestans disease severity in potato leaves treated with EVs extracted from dsRNA treated *N. benthamiana,* asterisks denote statistically significant differences between treatment groups

Konakalla *et al* unpublished

Ago4 trafficking into *Phytophthora infestans*$^{Td\text{-}tom}$ mycelia from *N. benthamiana*



Td- Tomato

Ago 4_GFP

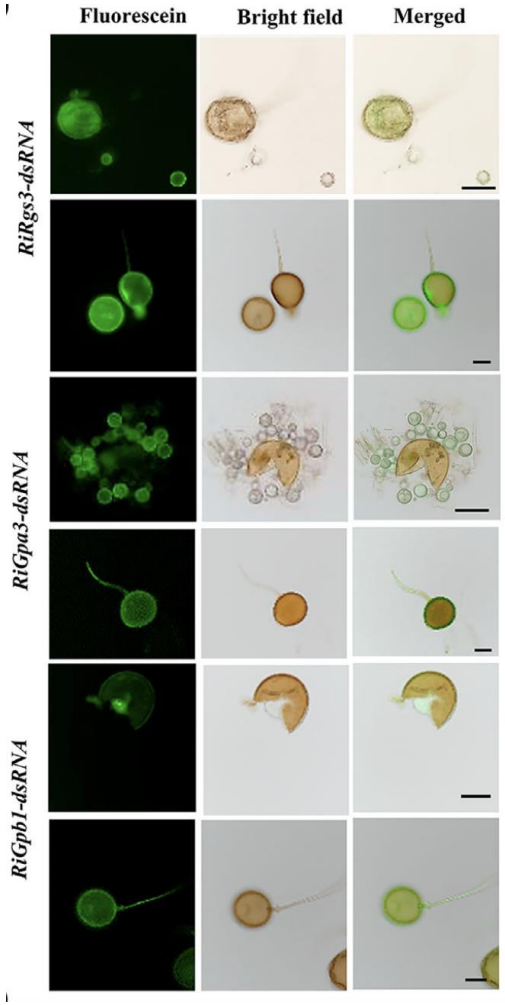
Merge

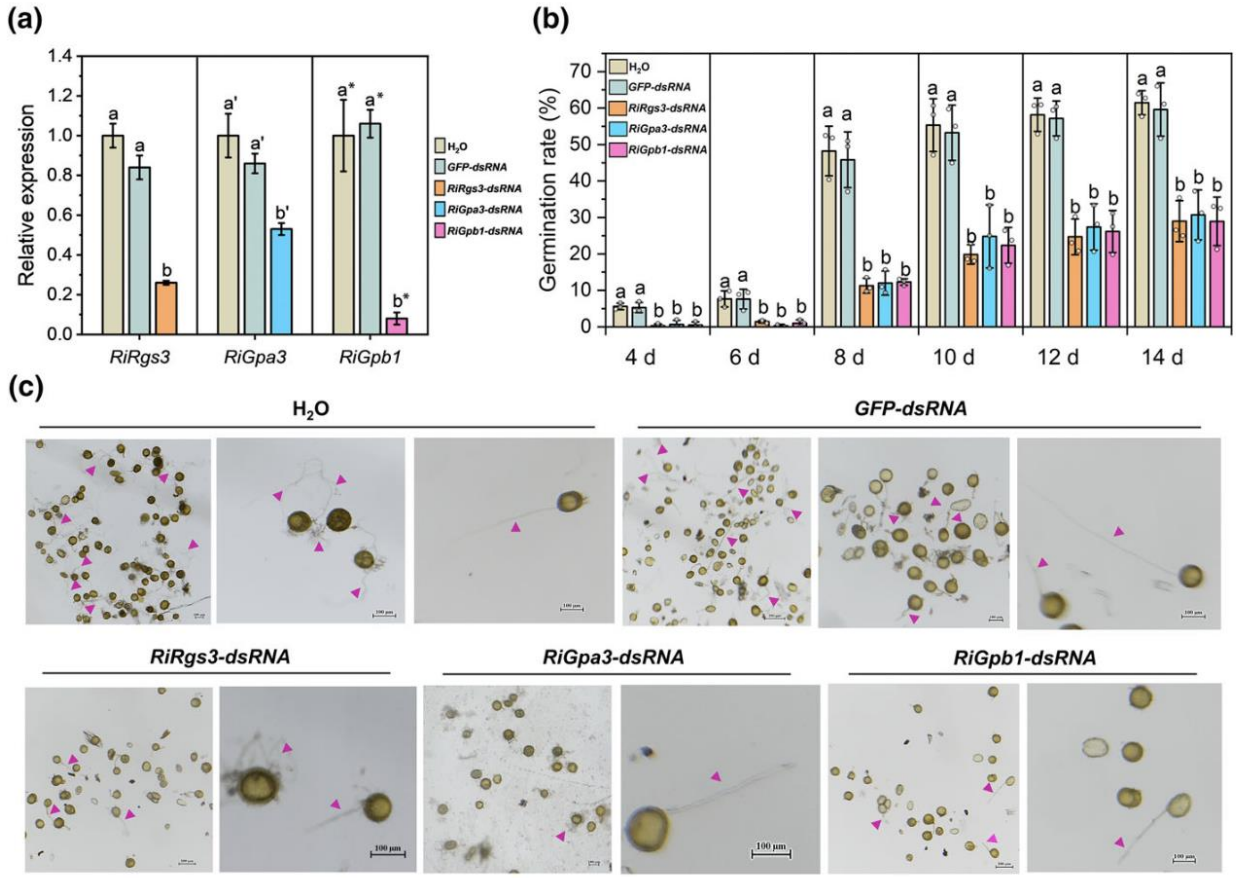
Trafficking of Ago4 GFP tagged protein from *Nicotiana benthamiana* leaves to *Phytophthora infestans*$^{Td\text{-}Tomato}$ mycelia. Representative confocal images showing the co-localization of **a** Td-Tom signal (Magenta), **b** Ago 4-GFP fusion protein (Green) , and **c** merge.

Konakalla *et al* unpublished

Spray-induced gene silencing of three G-protein signaling genes from the arbuscular mycorrhizal fungus *Rhizophagus irregularis* inhibits spore germination and hyphopodium formation

Xiaoning Fan[1]*, Xiaoqin Zhou[1]*, Junliang He[1], Hongyun Xie[1], Nianwu Tang[2], Ming Tang[1] and Xianan Xie[1]





Future SIGS research

How to maximise the durability of SIGS-mediated protection?

What are the long-term evolutionary and epigenetic consequences of (repeated) SIGS?

What are the full impacts of dsRNA on the environment in a field trial setting?

What type of dsRNA provides optimal silencing and robust protection?

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FORMAS (Swedish Research Council)

Crafoord foundation

Carl tryggers foundation

Novo Nordisk foundation – Emerging Investigator grant

Partnerskap Alnarp

SLU Centre for Biological Control

BERTEBOS Foundation

KSLA

SLU holding - SLU innovation prize

VR (Swedish Research Council)

SSF

Royal Physiographic Society of Lund