irc 2023 SYDNEY

16th INTERNATIONAL RAPESEED CONGRESS

24 - 27 September 2023

GLOBAL CROP - GOLDEN OPPORTUNITIES

Using RNA interference to protect *Brassica napus* (canola) against fungal pathogens

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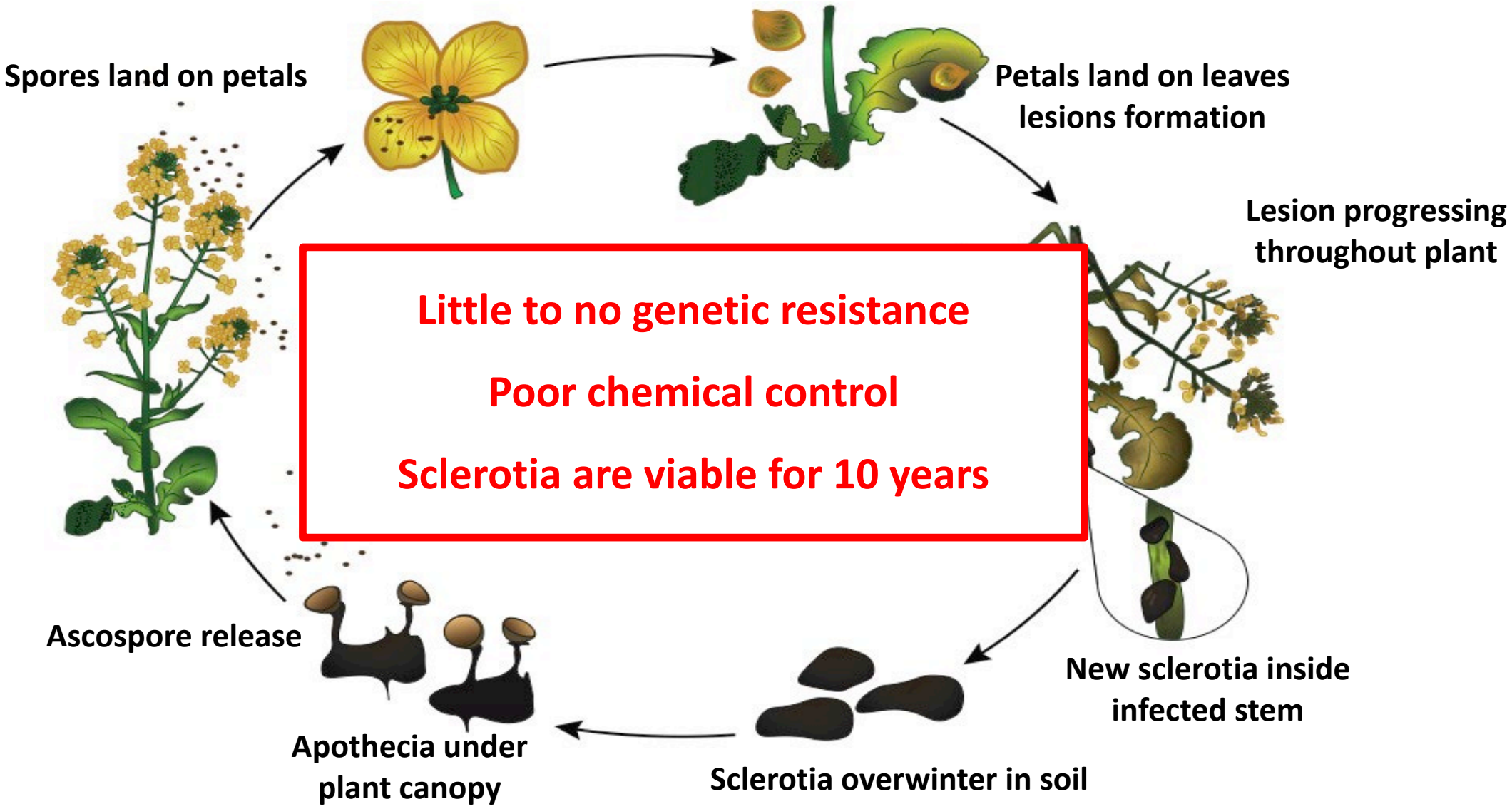
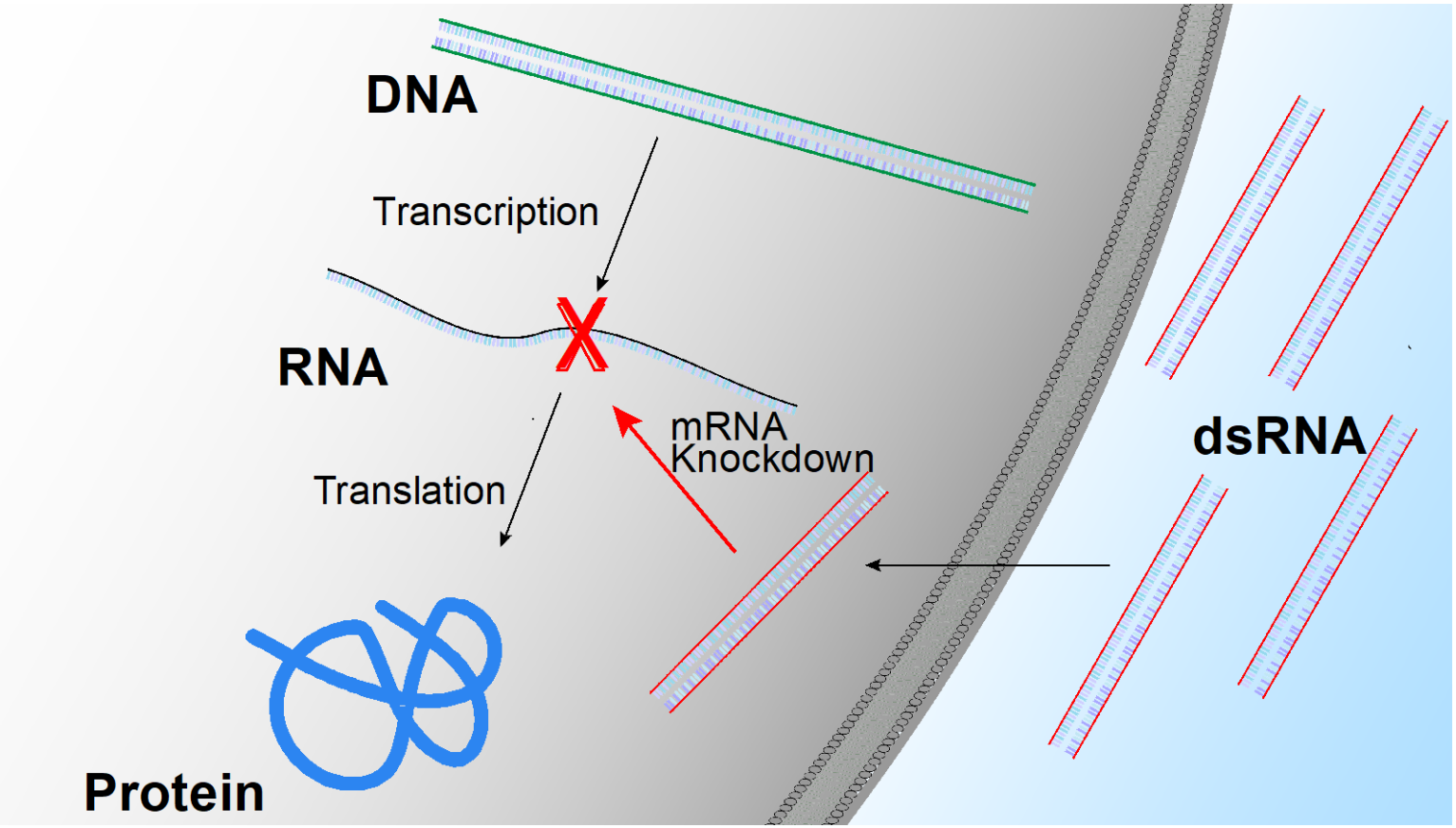
How does **sclerotinia** infect and destroy **canola?**



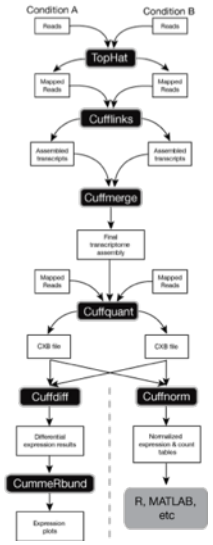
What is our Solution?



With no true resistant cultivars and increasing fungicide resistance, a new control method is needed

Using our knowledge of molecular biology, we can take advantage of RNAi (RNA Interference), an intrinsic cellular defense to viruses

What is our strategy to protect canola using RNAi technology?



Target identification pipeline (TIP)

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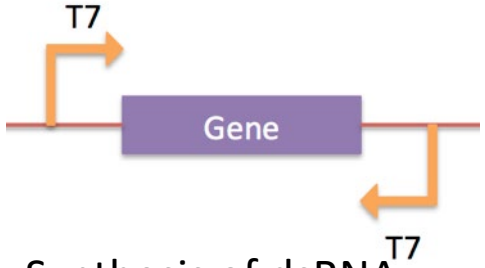
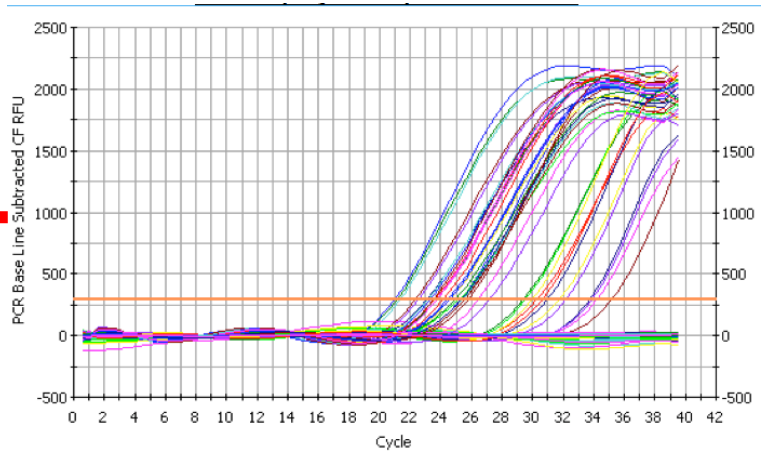
Query  6     TCCTCGTAGCAACCGACGTCGCAGCCCGCGGTCTCGACATCGGAGGCGTCCAATTAGTAA  65
Sbjct  1616  TCCTCGTCGCCACCGACGTCGCAGCCCGCGGGCTCGACATCGGGGGTGTCCAGCTCGTCA  1675
Query  66    TCCATTACCACCTTCCCCGCACCGCAGACATGTATGTGCACCGCTCCGGTCGAACCGCGC  125
Sbjct  1676  TCCACTACCACCTCCCCCGTGCCGCAGACATGTACGTGCACCGCTCCGGCCGTACCGCCC  1735
Query  126   GTGCCGCCGCATCCGGATCCAGTATCCTCCTCTGTGGCCCCGAAGAAGTAGTCGGAACCC  185
Sbjct  1736  GCGGTTCGGCCTCCGGGACCAGCATCCTGATGTGCGCGCCCGAGGAAGTCGTCGGCATGC  1795
Query  186   GCCGCTTGGTAGCTAAGGTCCACGCGCAAAATGCTCTTCACGGAGAAGGCAAAAAATCCA  245
Sbjct  1796  GCCGCCTAGTGGCCAAAGTGCACGCGCAGAACGCGCTCGCGGGCGGGGGCAGCAAATCGA  1855
Query  246   AATTCTACATCCGCTCCCTCGA  267
Sbjct  1856  AATACTACATGCGCTCGCTCGA  1877
```

Identifying off-targets and primer design



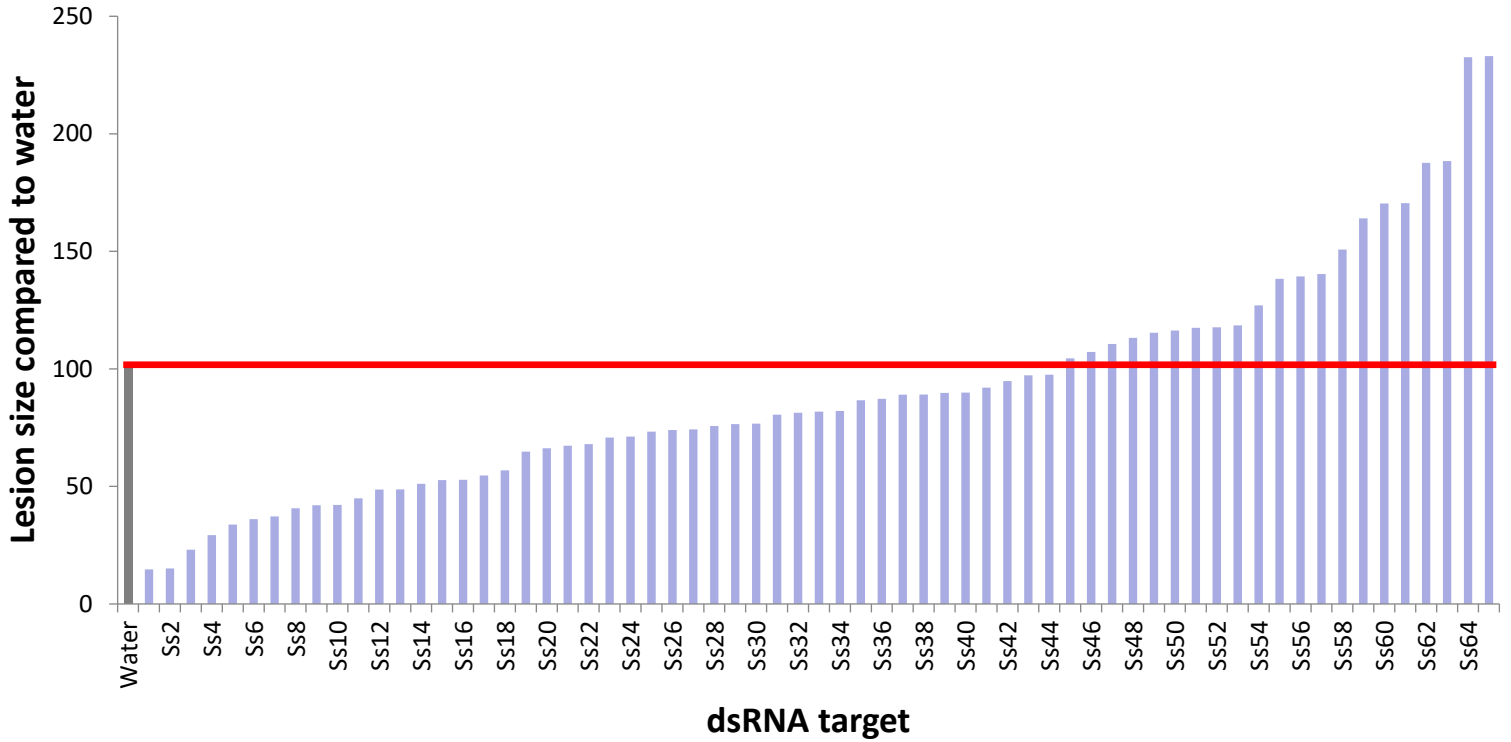
Synthesis of dsRNA

Assessing plant protection (*in planta*)



Quantifying transcript knockdown (*in vitro*)

Can dsRNA molecules protect plants?



- 100+ different *Sclerotinia* genes tested
- 200 ng dsRNA applied to each leaf
- Over 1500 leaves inoculated!
- Lesion sizes 15 – 250% of water.

What does protection look like?

Ss2

SCIENTIFIC REPORTS

OPEN

Identification and application of exogenous dsRNA confers plant protection against *Sclerotinia sclerotiorum* and *Botrytis cinerea*

Received: 3 October 2017
Accepted: 16 April 2018
Published online: 09 May 2018

Austein G. McLoughlin[1], Nick Wytinck[1], Philip L. Walker[1], Ian J. Girard[1], Khalid Y. Rashid[2], Teresa de Kievit[3], W. G. Dilantha Fernando[4], Steve Whyard[1] & Mark F. Belmonte[1]

(Protein synthesis)

(Mitochondrial protein)

Can we protect canola through RNAi using Host Induced Gene Silencing?

- 35 independent T0 insertion lines targeting 5 different Sclerotinia genes
- Assessed each line up to the T3 generation to determine best performing lines
- Over 1000 plants / 3000 lesions were measured
- **RNAi-based transgenics improve plant protection against fungal pathogens**

Selection of transgenic canola

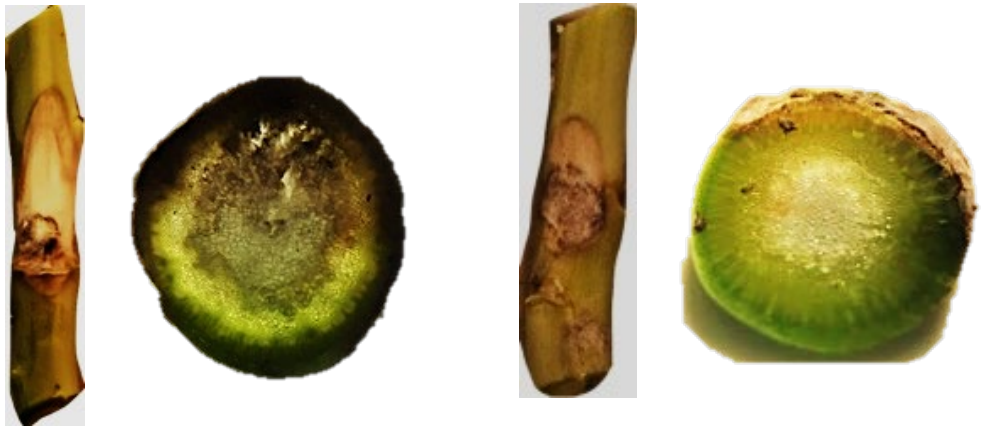
What does protection look like?

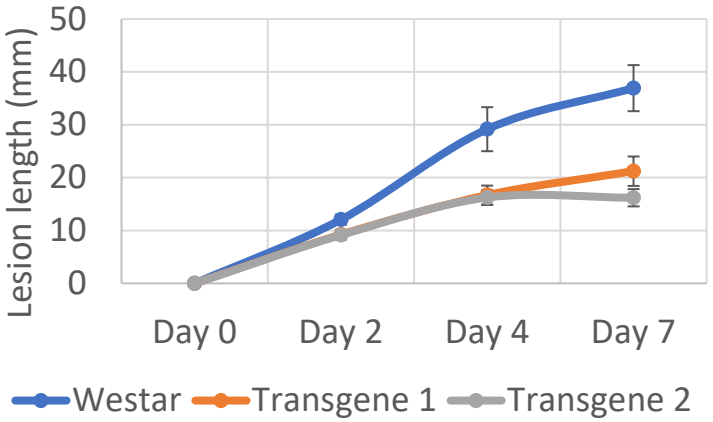
Westar Susceptible

Transgenic (SS1)

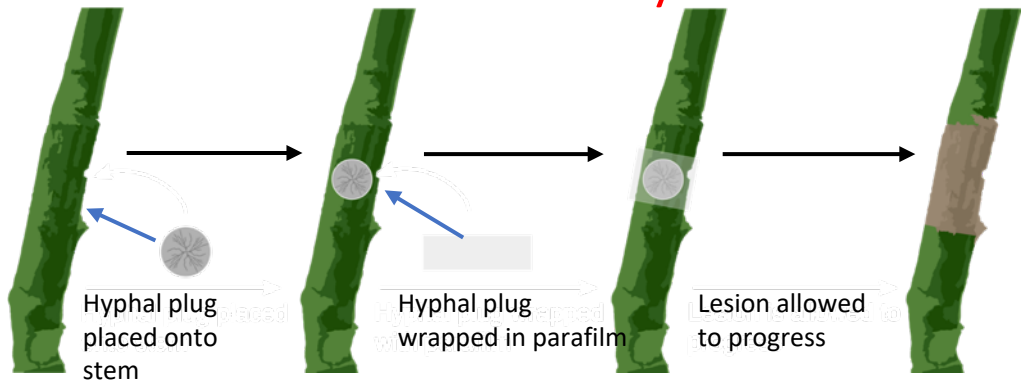
Leaf (3 dpi)

Stem (3 dpi)

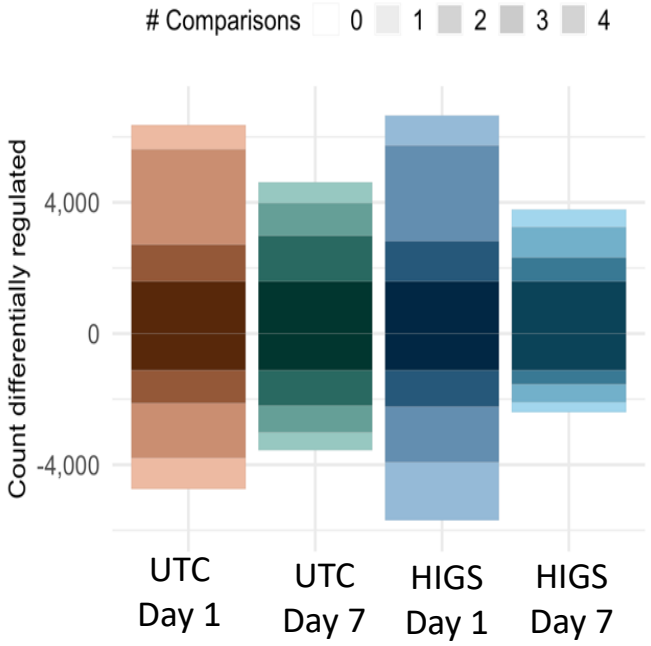




Stem Assay

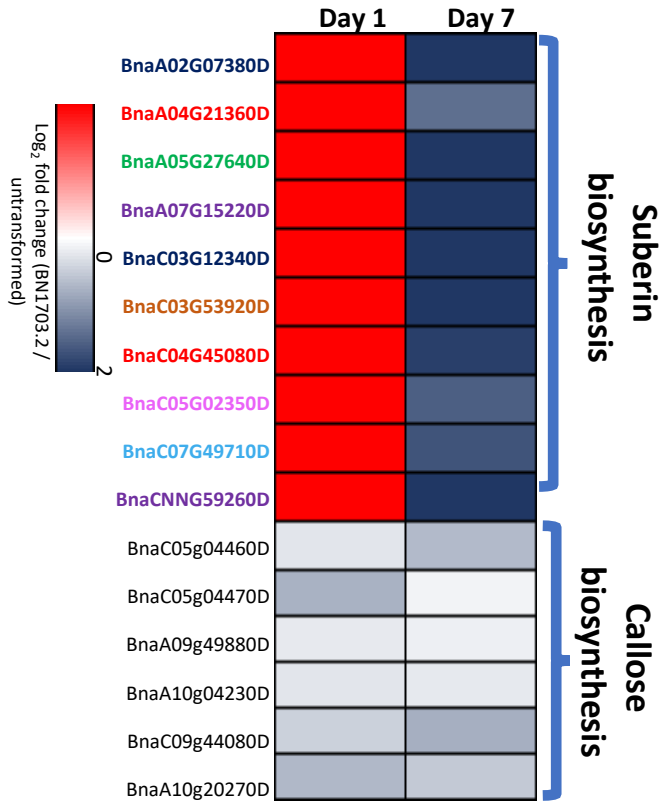


Global RNA sequencing directly at the site of infection of HIGS RNAi lines reveals putative roles for suberin and cell wall modifications in plant-pathogen interactions

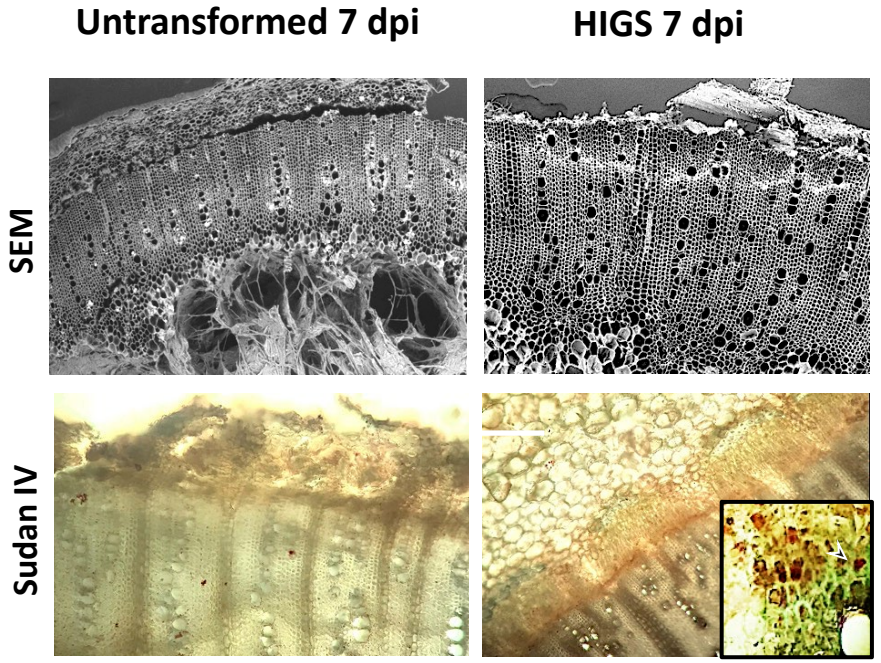


- **RNA-seq** was performed on infected stems at **1 and 7 days post infection**

- **At 1 DPI, HIGS lines** were the most **transcriptionally active**



Gene ontology enrichment reveals **suberin** and **callose biosynthesis** are enriched in infected stems of HIGS lines



- **Electron dense** band detected by SEM in **secondary xylem** of infected **HIGS** stems

- Positive stain for **suberin**, a polyaliphatic polymer that is produced during infection to **block pathogen progression**

What does whole plant protection look like?

SS1 RNAi Line (21 dpi)

Westar – susceptible (21 dpi)

Does increased protection correlate to increased seed production?

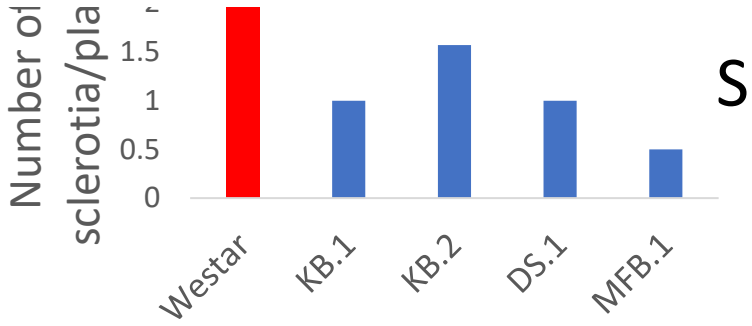
OPEN ACCESS PEER-REVIEWED

RESEARCH ARTICLE

Host induced gene silencing of the *Sclerotinia sclerotiorum ABHYDROLASE-3* gene reduces disease severity in *Brassica napus*

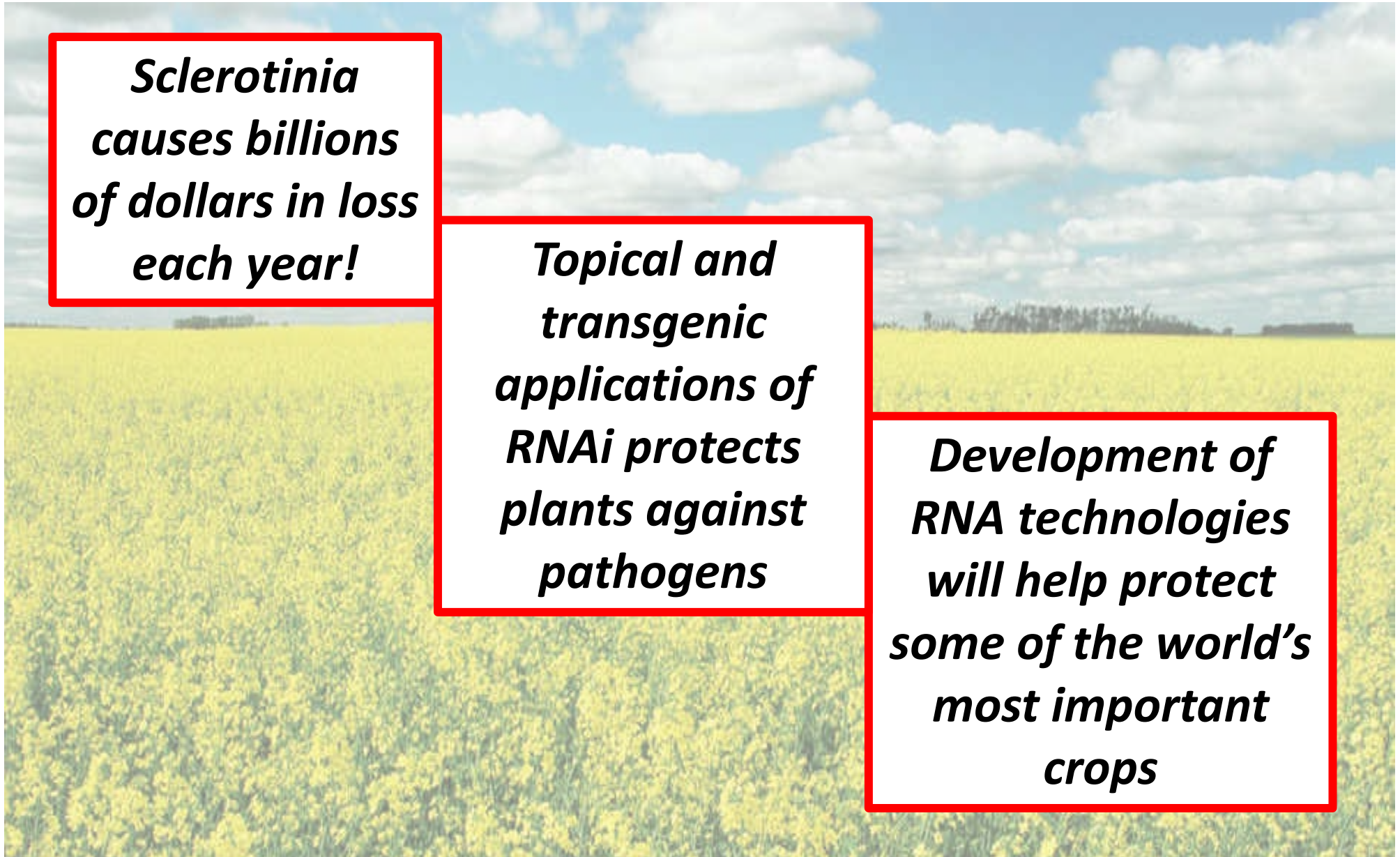
Nick Wytinck, Dylan J. Ziegler, Philip L. Walker, Daniel S. Sullivan, Kirsten T. Biggar, Deirdre Khan, Solihu K. Sakariyahu, Olivia Wilkins, Steve Whyard, Mark F. Belmonte

Published: August 26, 2022 • https://doi.org/10.1371/journal.pone.0261102



Sclerotia

Driving innovation through discovery



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  - Daniel Sullivan
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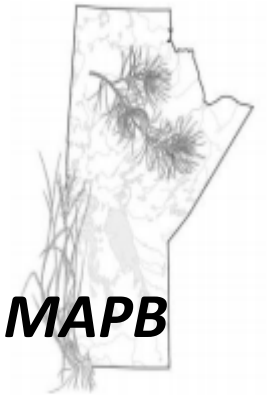
  - Alison Partridge
  - Aditi Singh

ARDI
Agri-Food Research and Development Initiative

University of Manitoba

Western Grains Research Foundation



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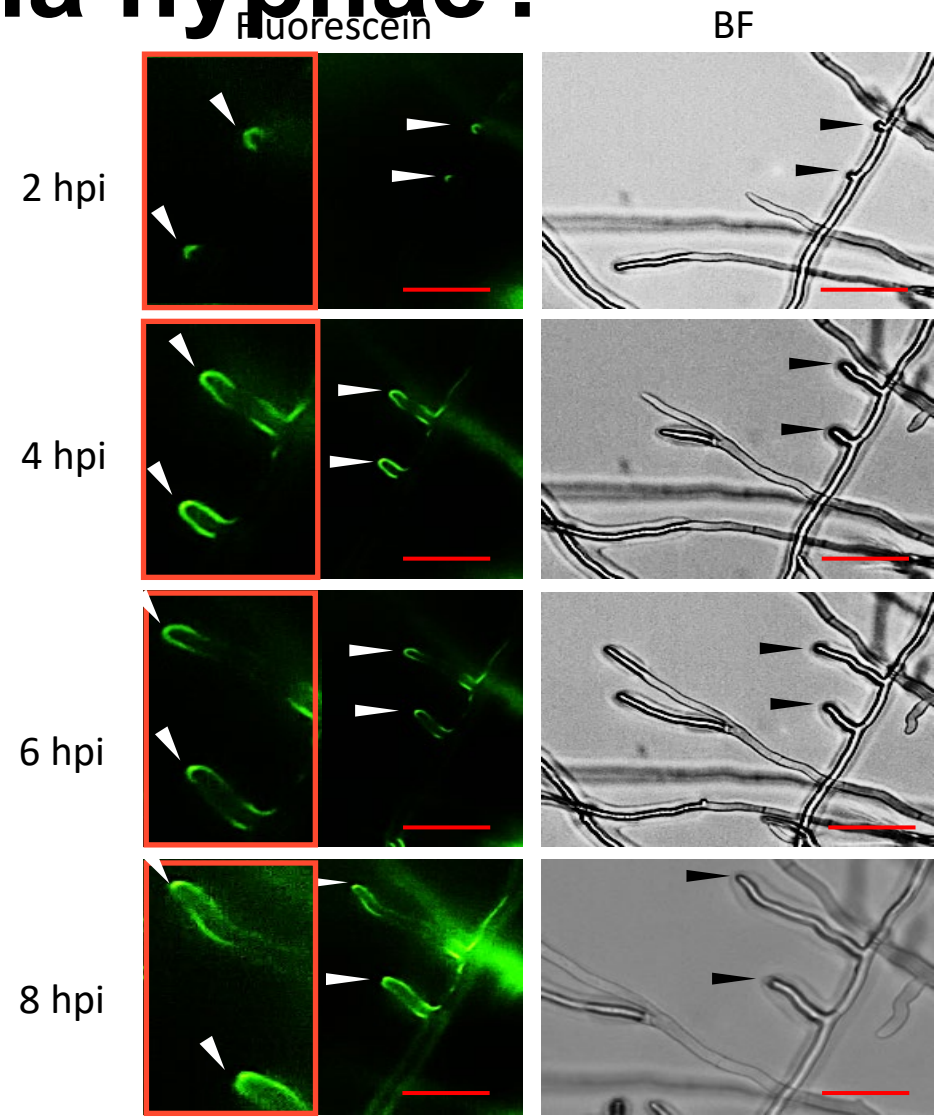
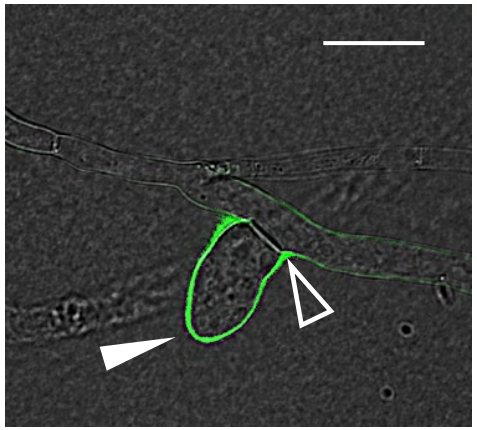
How do dsRNAs enter Sclerotinia hyphae?

- Incubate Sclerotinia with fluorescein-labelled dsRNAs
  - Long dsRNA 250bp sequence
- dsRNAs uptake occurs within hours
- The most actively growing regions (hyphal tips of newly formed branches and septate regions) appear to be the most active sites of dsRNA uptake



Clathrin mediated endocytosis is involved in dsRNA uptake in Sclerotinia

Fluorescent microscopy

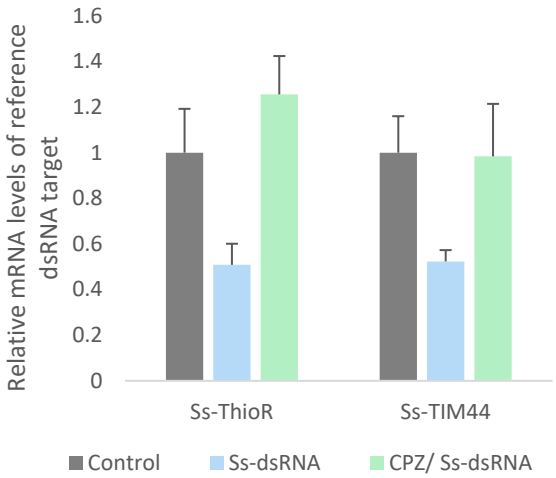


CPZ / F-Ss-dsRNA

Chlorpromazine (CPZ) Chemical inhibitor of clathrin-mediated endocytosis

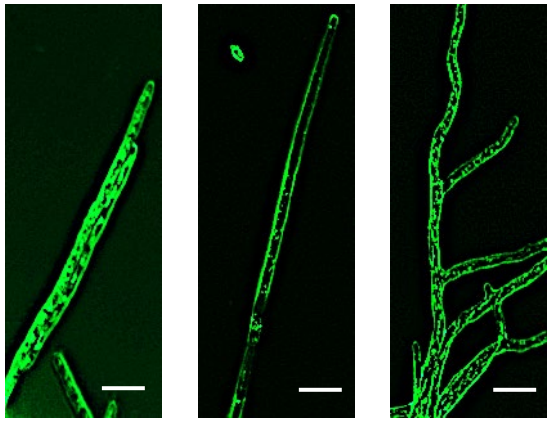
F-Ss-dsRNA accumulates on the outer surface of the cell

In vitro
dsRNA target knockdown



Chlorpromazine (CPZ) interferes with target gene knockdown using two different dsRNAs in vitro

Transgenic Sclerotinia expressing GFP



Ss-gfp

Ss-gfp + Ss-eGFP

Ss-gfp + CPZ / Ss-eGFP

Ss-gfp is used as a tool to study dsRNA uptake

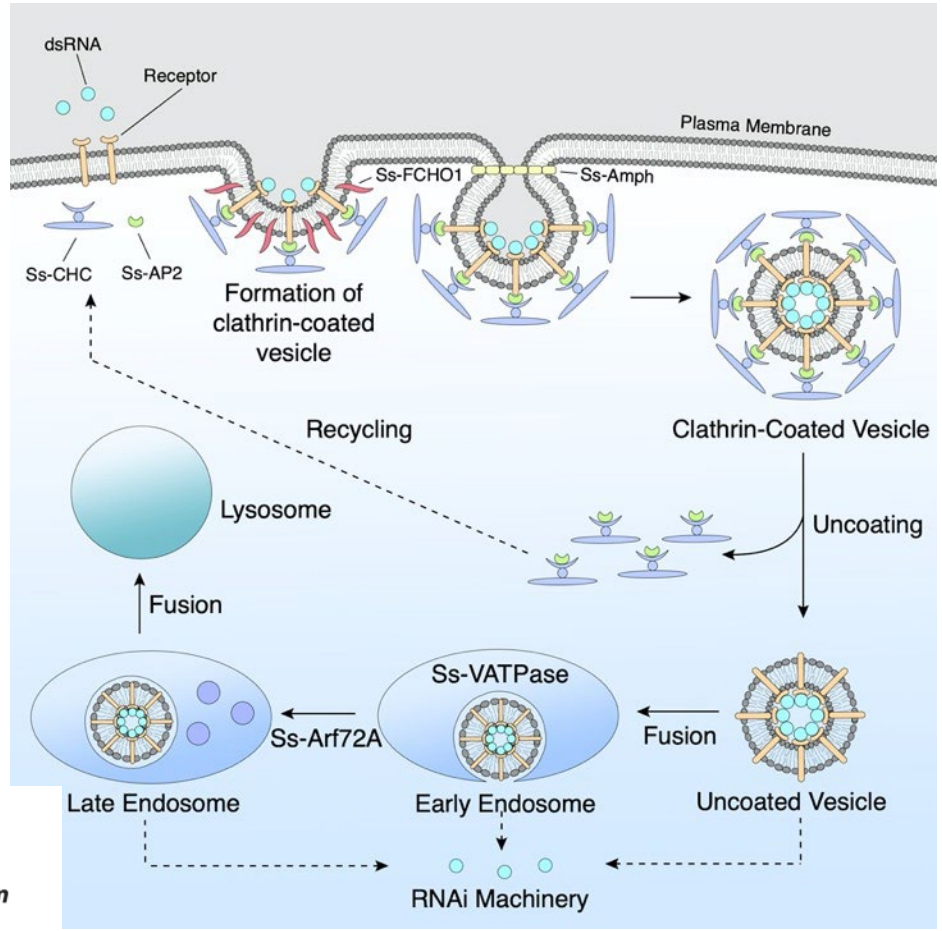
CPZ prevents knockdown of endogenous Ss-GFP

What is our proposed mechanism of dsRNA uptake in Sclerotinia?

- dsRNA uptake occurs through clathrin mediated endocytosis
  - Is there a long dsRNA receptor in Sclerotinia?

- dsRNAs are likely incorporated into vesicles

- dsRNAs are released into the cell resulting in target mRNA degradation
  - How are dsRNAs processed in Sclerotinia at the cellular level?



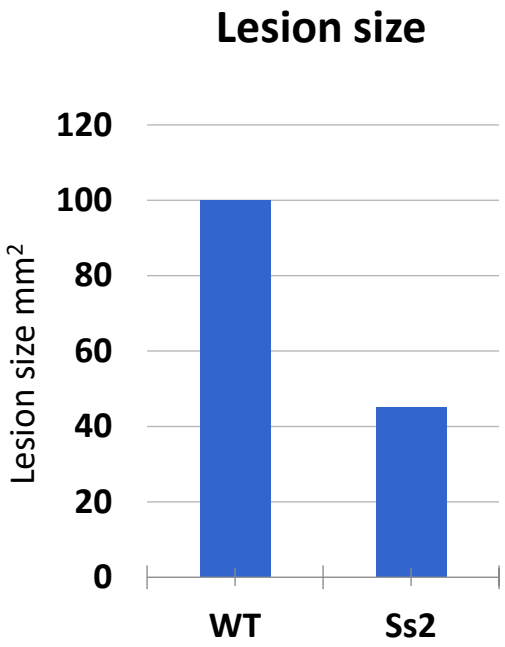
Article | Open Access | Published: 29 July 2020

Clathrin mediated endocytosis is involved in the uptake of exogenous double-stranded RNA in the white mold phytopathogen *Sclerotinia sclerotiorum*

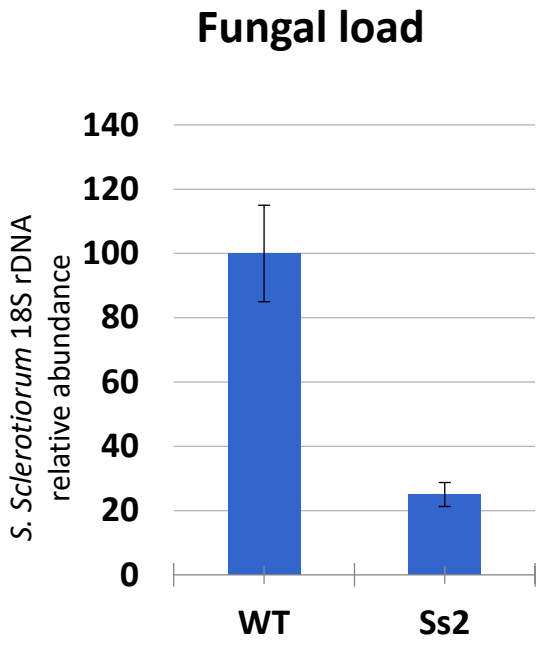
Nick Wytinck, Daniel S. Sullivan, Kirsten T. Biggar, Leandro Crisostomo, Peter Pelka, Mark F. Belmonte & Steve Whyard

Scientific Reports **10**, Article number: 12773 (2020) | Cite this article

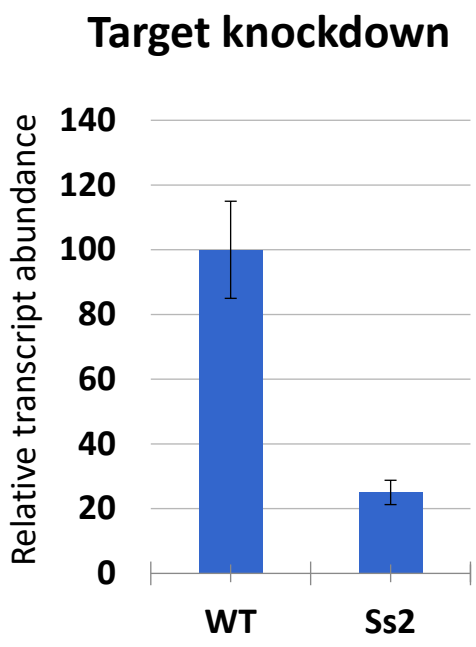
Can we constitutively express dsRNAs to protect plants?



45% reduction



85% reduction



75% reduction

Durable dsRNA expression improves plant health