RNAi technologies to control pests and pathogens in canola

Steve Whyard & Mark Belmonte
Department of Biological Sciences
University of Manitoba

Presentation to: Canola Discovery Forum
Nov. 13, 2019

Two major threats to our canola

Sclerotinia – a fungal pathogen

Flea beetles – a pest insect

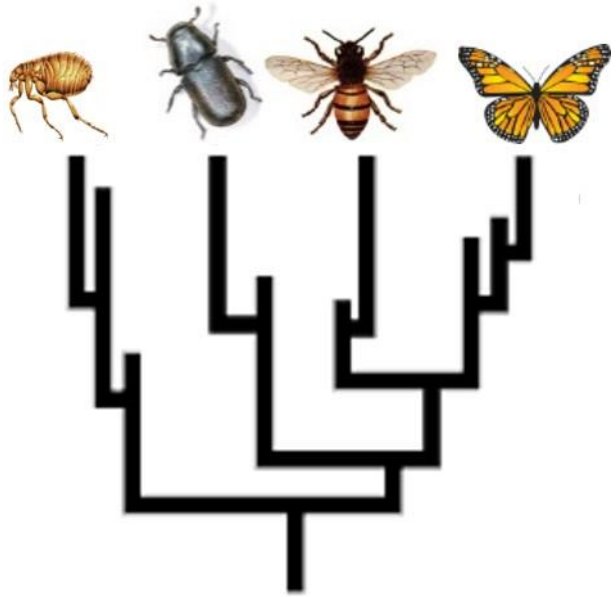
The current approach to protecting canola - chemical pesticides

Two issues of concern about pesticides:

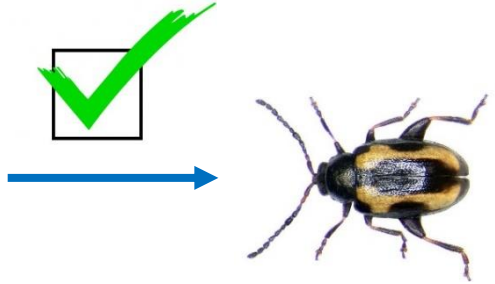
1. Increasing resistance

2. Off-target effects

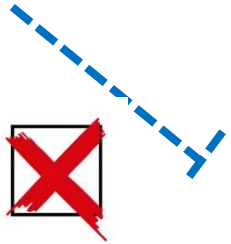
Species-specific dsRNA pesticides/fungicides



gene-specific dsRNAs



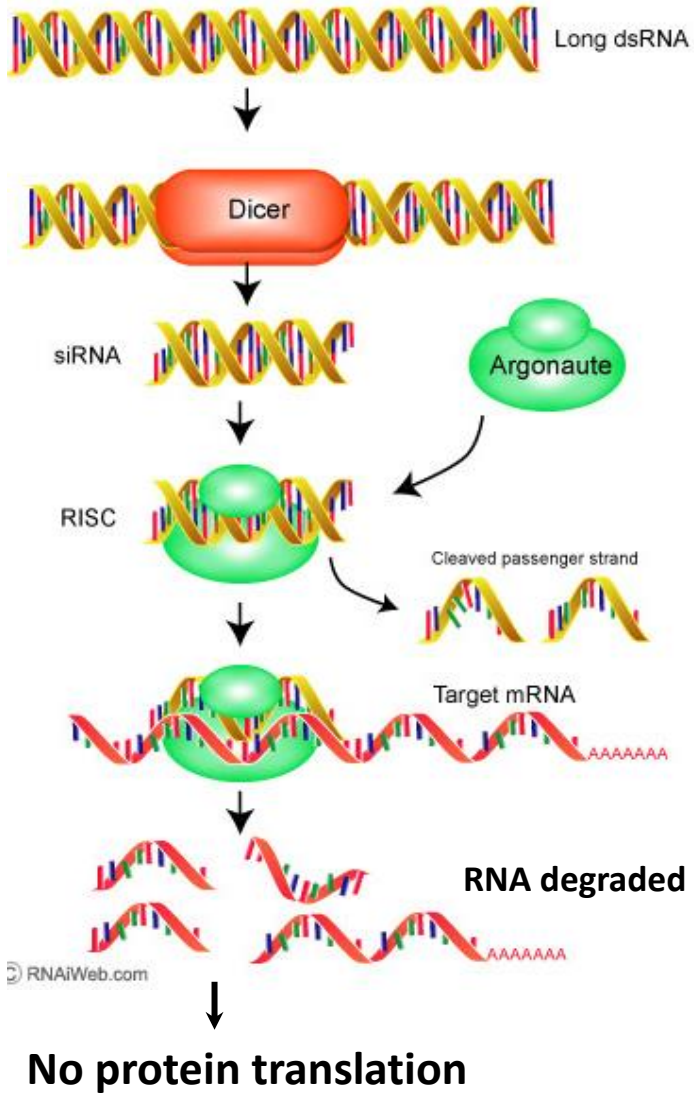
Species-specific pesticides



No harm to beneficial species

RNA-based pesticides – using RNA interference

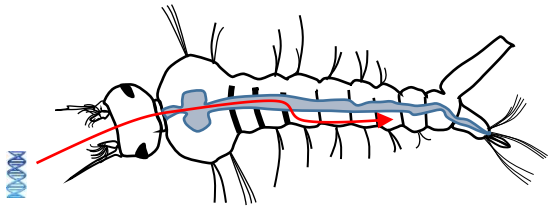
- RNAi = gene silencing using double-stranded RNA
- Useful tool for creating loss-of-function mutants
- Potential to kill pest insects?

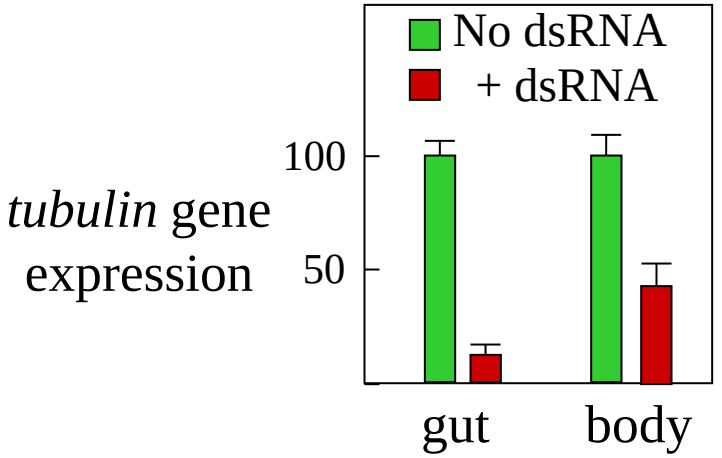


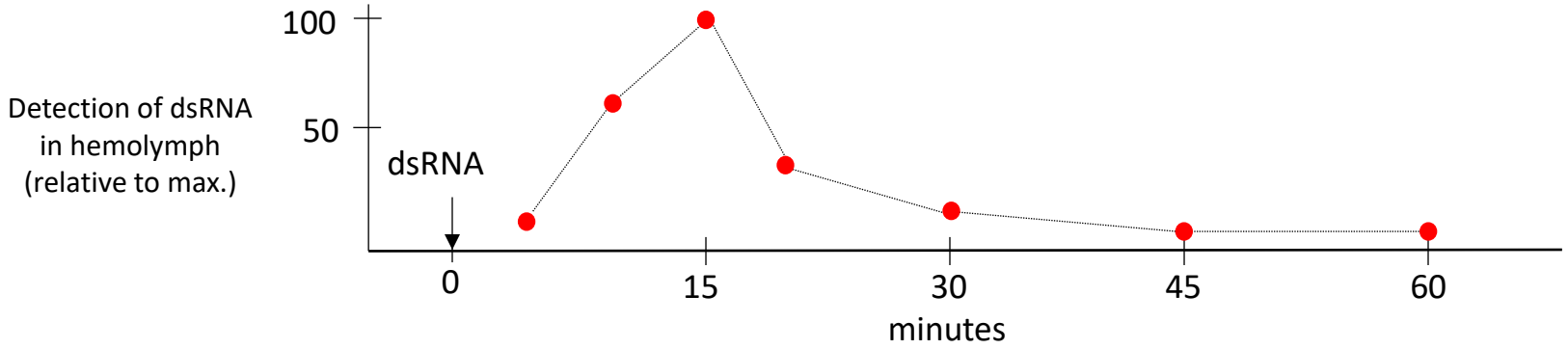
Feeding dsRNA to insects

- Ingested dsRNA can silence genes in the insect gut
- The dsRNA can spread and silence genes in other tissues (systemic RNAi)

mosquito larvae fed dsRNA







Flea beetles

- Pest species in Manitoba:
  - *Phyllotreta cruciferae* (crucifer flea beetle)
  - *Phyllotreta striolata* (striped flea beetle)

- Feed on cruciferous plants
  - including canola

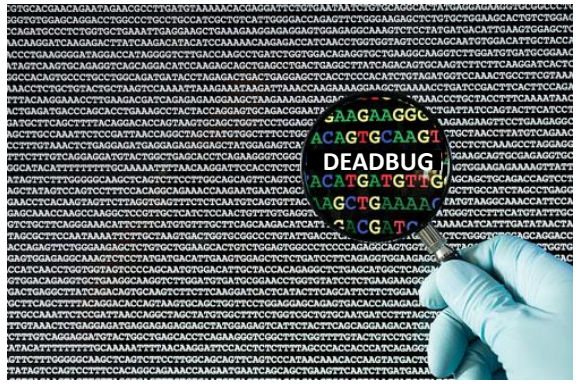
P. cruciferae

P. striolata

Developing RNAi pesticides vs flea beetles

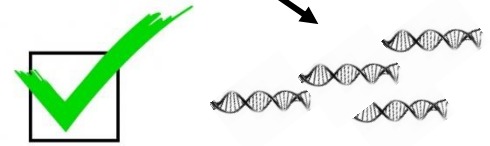
Field collections

Lab colonies

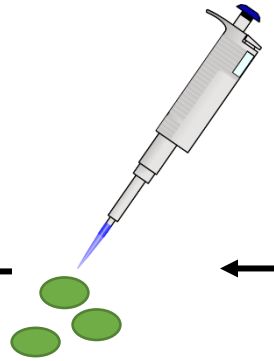


RNA sequencing

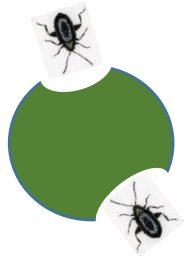
Identify flea beetle-specific gene sequences



DsRNA on leaf discs



Feeding bioassays



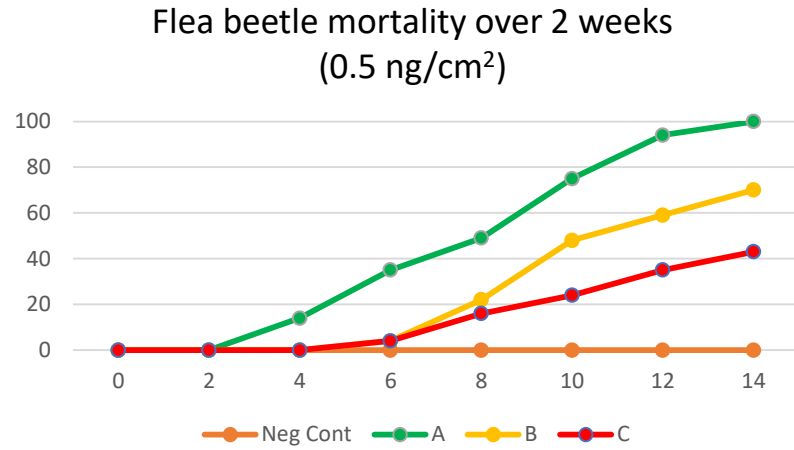
Assess mRNA knockdown

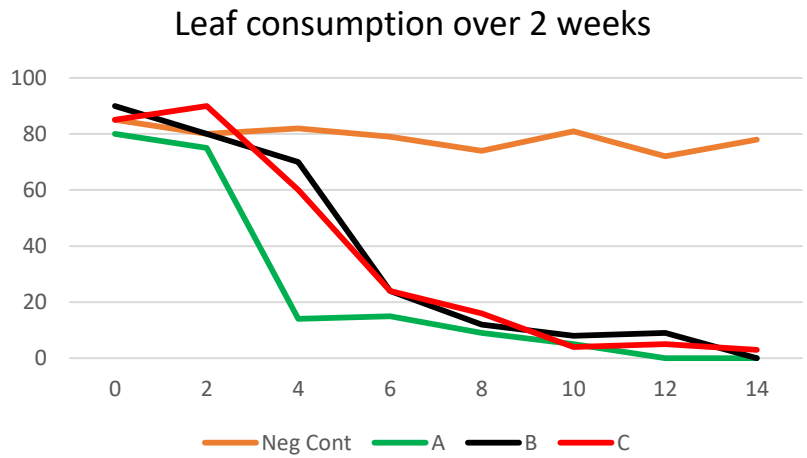
Assess mortality

Assess leaf damage

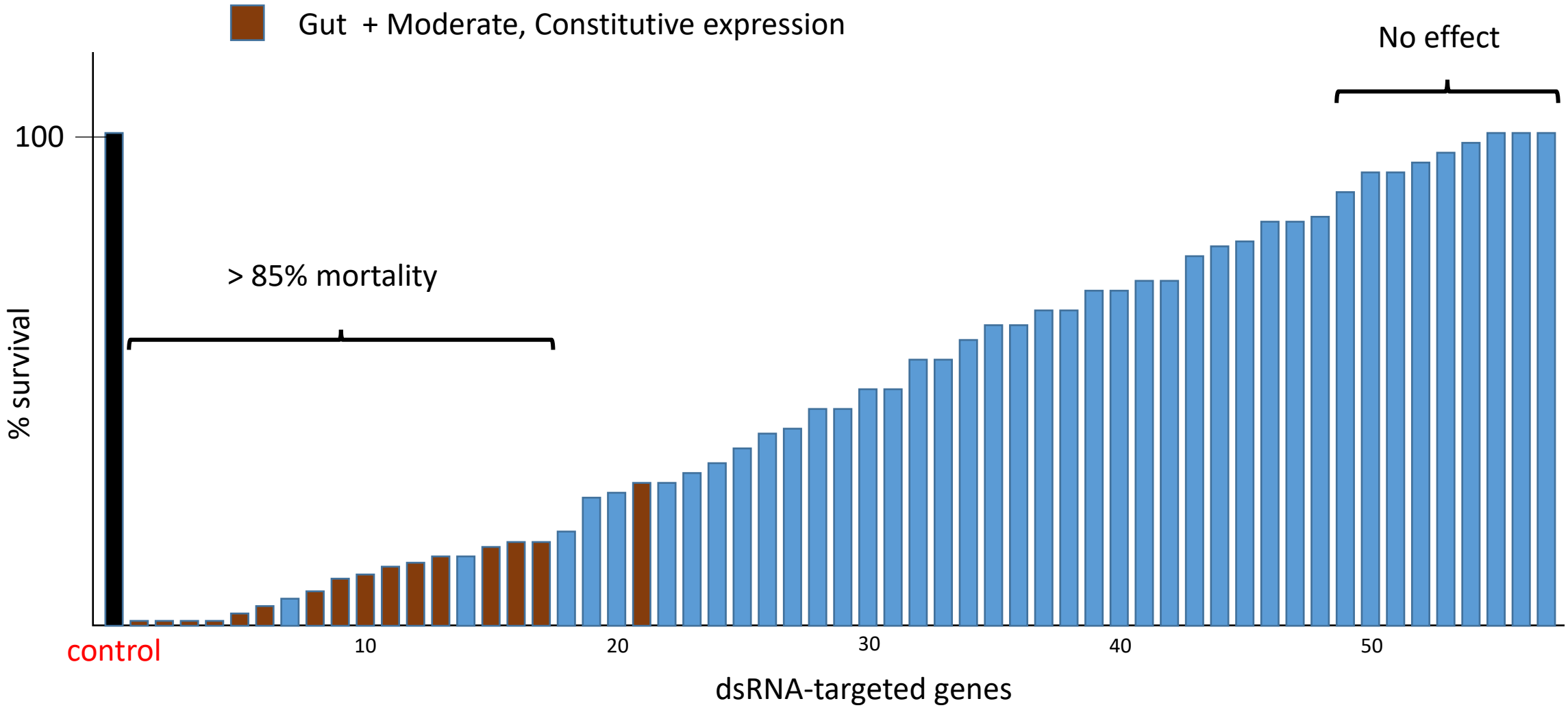
Control of flea beetles on canola

- Transcriptomic data identified >100 target genes tested
- Topical treatment of leaves with dsRNA kills flea beetles
  - But slower than neurotoxic chemicals
- Leaf damage was reduced
  - Insects stopped feeding after 4 days





Identifying ideal RNAi insecticide target genes

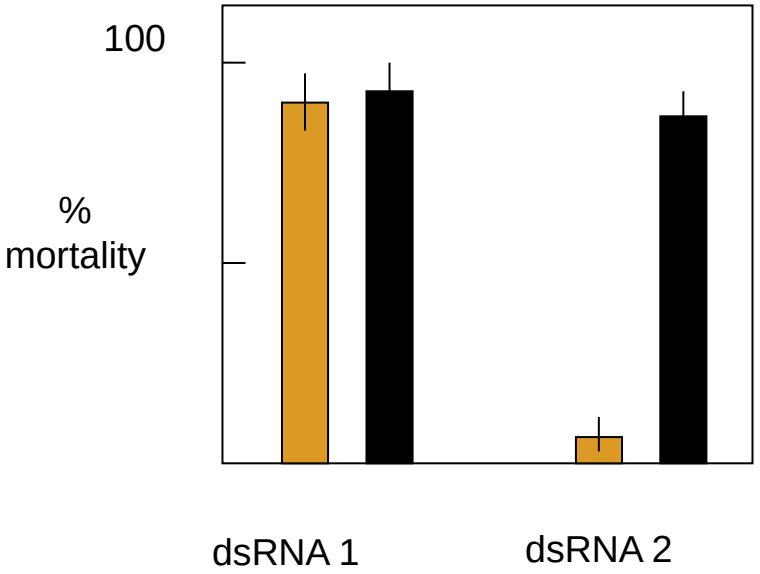


Species-specific RNA insecticides

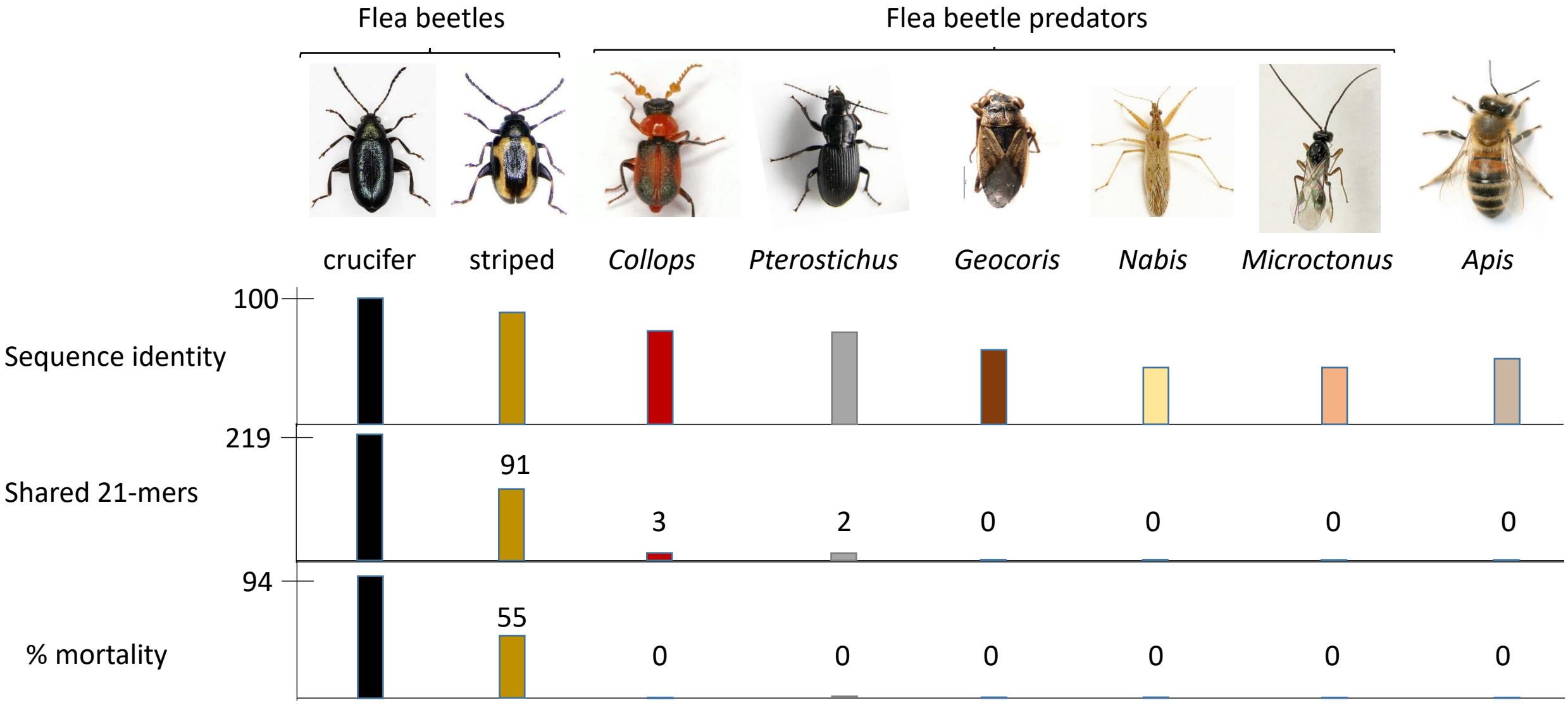
Flea beetle RNAi insecticides

Striped flea beetles

Crucifer flea beetles

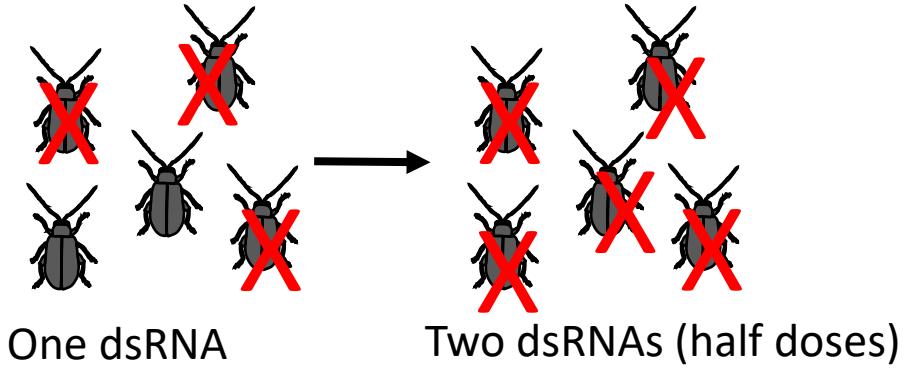


Specificity of a dsRNA pesticide in the canola agrosystem



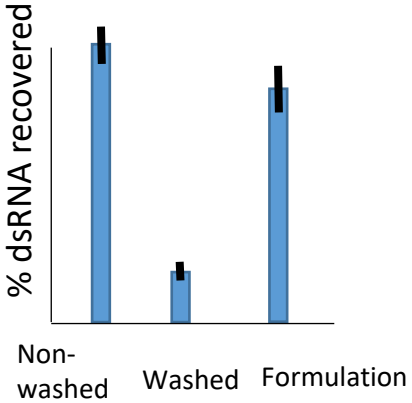
Can we improve RNAi efficiency?

- Easy to combine different dsRNAs
  - Improved percentage kill up to 100%



- Improved RNA stability
  - Reduced dsRNA by >50%

- Improved formulations
  - Did not wash off, remained active

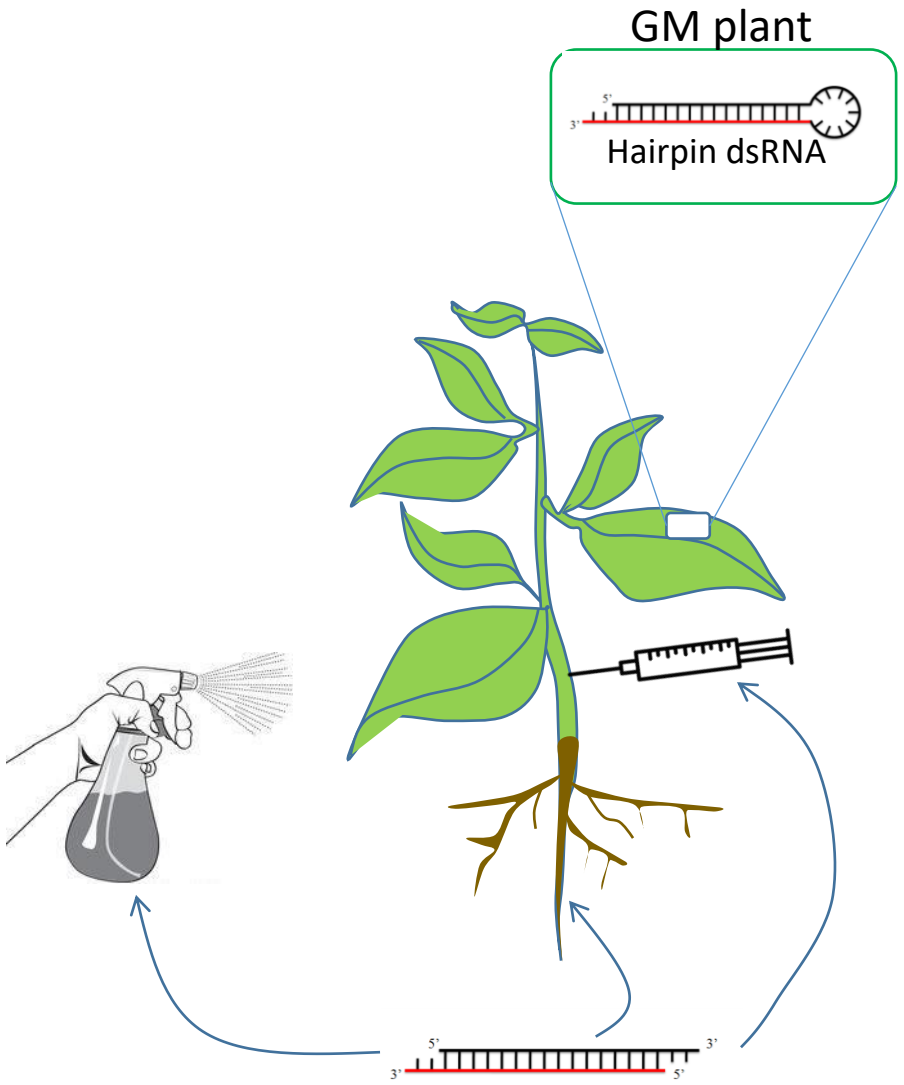


RNAi-based crop protection

Different dsRNA delivery approaches:

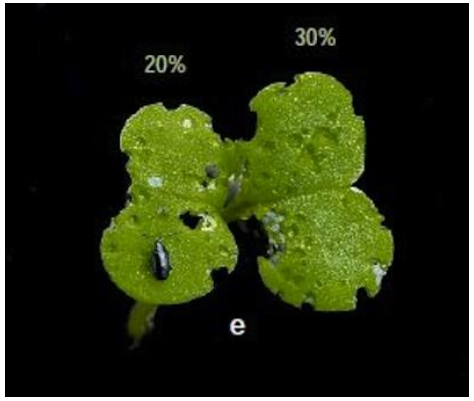
(not just canola!)

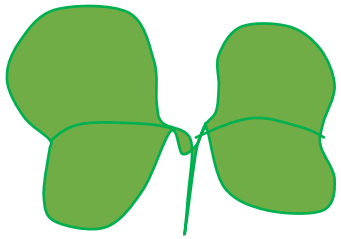
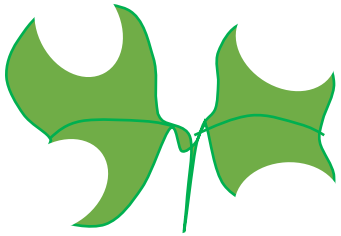
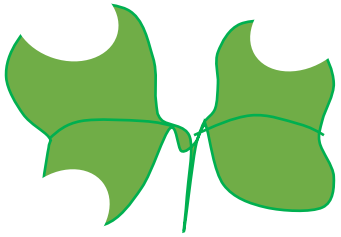
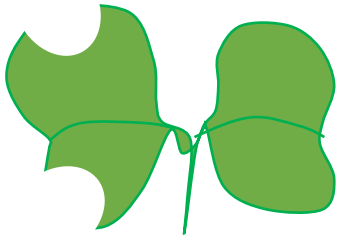
- In-planta (transgenic approach)
- Foliar sprays
- Root delivery
- Stem injections



Comparing transgenic canola vs foliar dsRNA pesticides

- Canola and Arabidopsis transformed with snf7 targeting *Phyllotreta* spp
- Comparing flea beetle damage



	No beetle control	No dsRNA control	Transgenic –hairpin-snf7	Foliar hairpin – snf7
				
% leaf consumption:	0	64 ± 14	24 ± 4	18 + 5
Beetle mortality:	N/A	3 ± 2	38 ± 9	68 ± 12

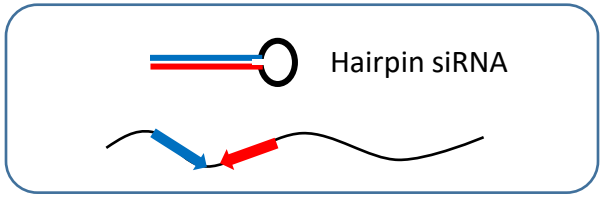
Is the technology affordable?

- In vitro synthesis
- Bacterial production
- Yeast production
- Algae
- Commercial producers

Integrated transgenes

$100/mg

Bacterial cell



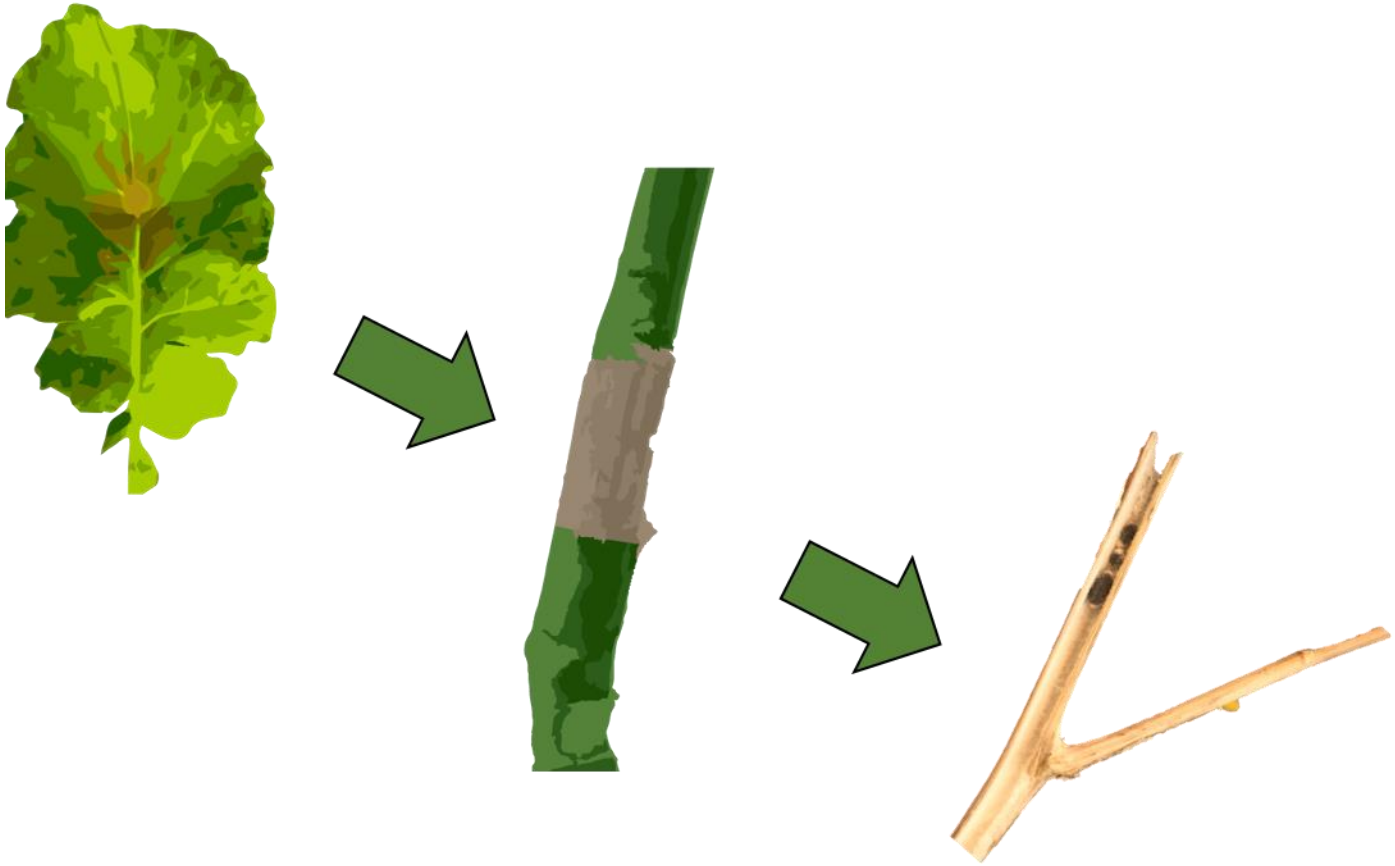
$0.01/mg in our small scale vessels

$0.0005-1/mg

1 mg – can kill about 100,000 flea beetles

Anti-fungal dsRNA: target Sclerotinia

- ***Sclerotinia sclerotiorum***
  - A major cause of yield loss
  - Over 450 host plants
  - 90% of Canadian canola fields show infection
  - Causes millions in lost yields annually
  - Life cycle makes control difficult with conventional methods

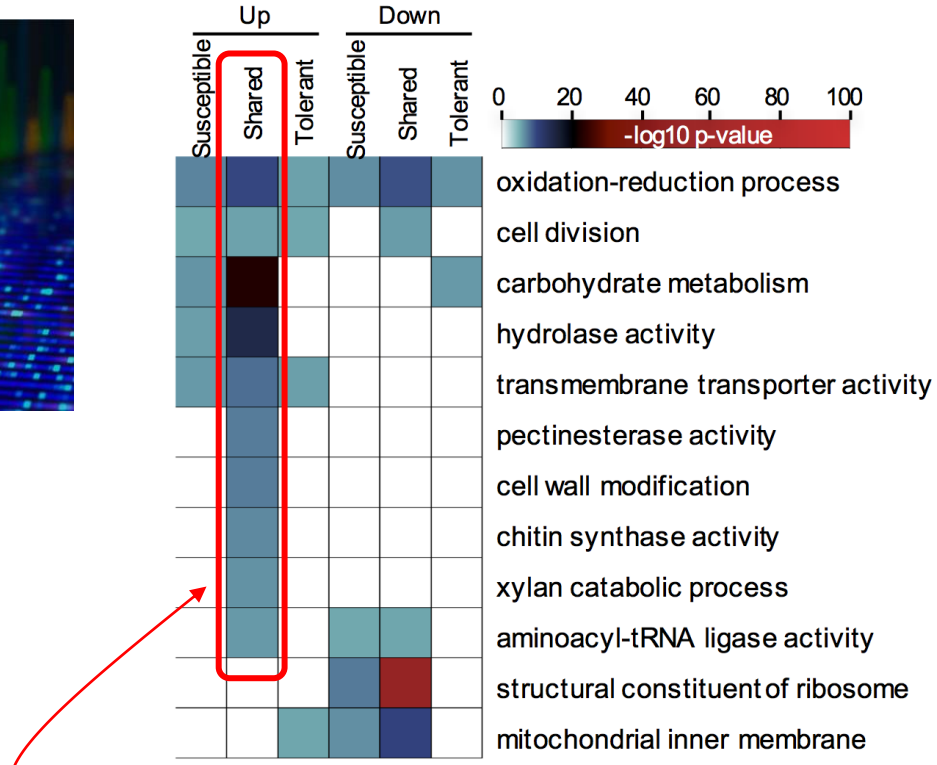


RNA sequencing to identify Sclerotinia infection genes

Susceptible-24h Tolerant-24h

1. Infect different strains of canola

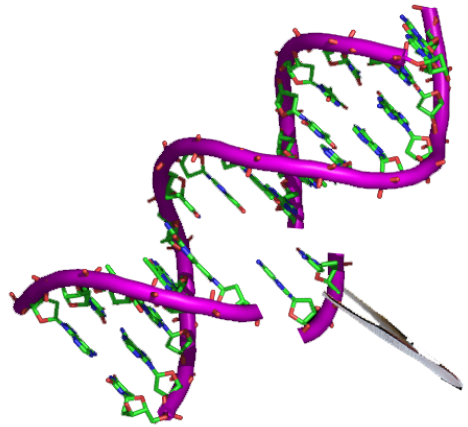
2. RNA sequencing



3. Identify genes up-regulated during infection

Two methods of protection using RNAi

1. Transgenic plants expressing dsRNA



Advantage: Entire plant protected all the time

Disadvantage: Public perception and regulatory delays

2. Foliar dsRNA sprays

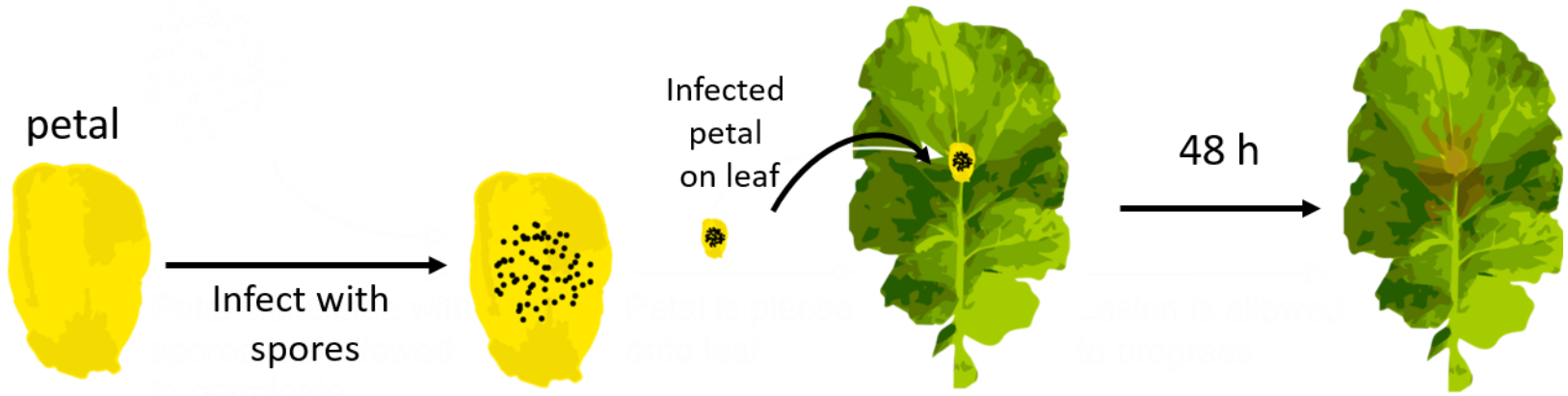
Advantage: Easily transferrable to more crops

Disadvantage: Can require multiple treatments ($)

Two infection assays

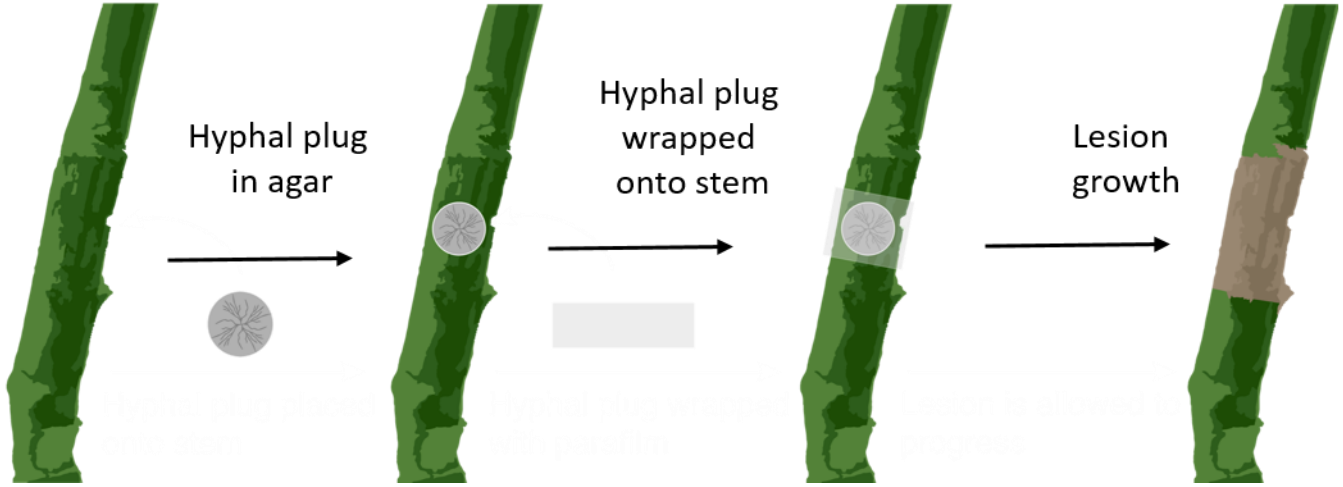
Leaf lesions

- Used mostly for testing foliar dsRNA applications



Stem lesions

- Used mostly for testing transgenic plants

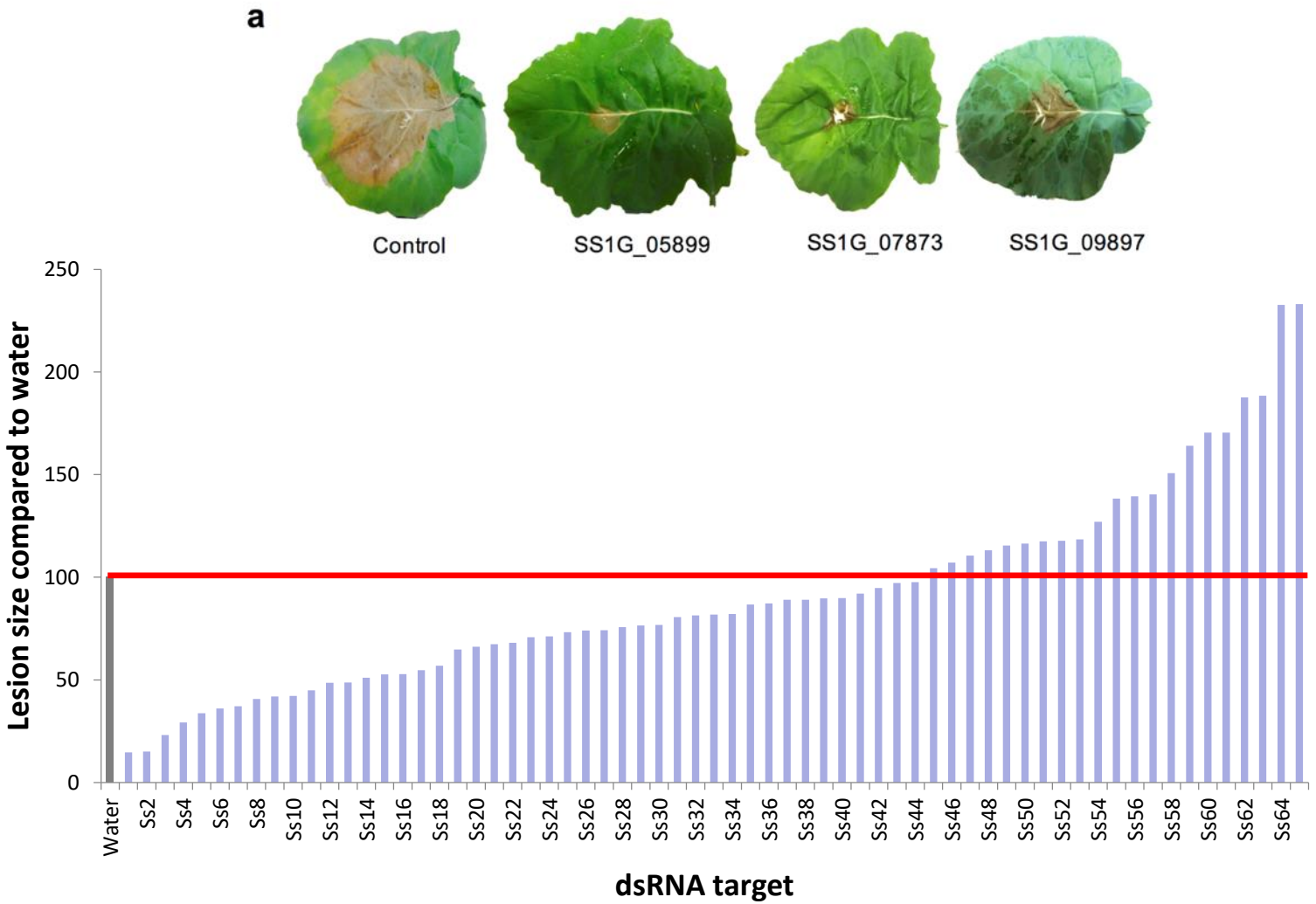


DsRNA screening

Leaf lesion assays

>150 dsRNAs screened

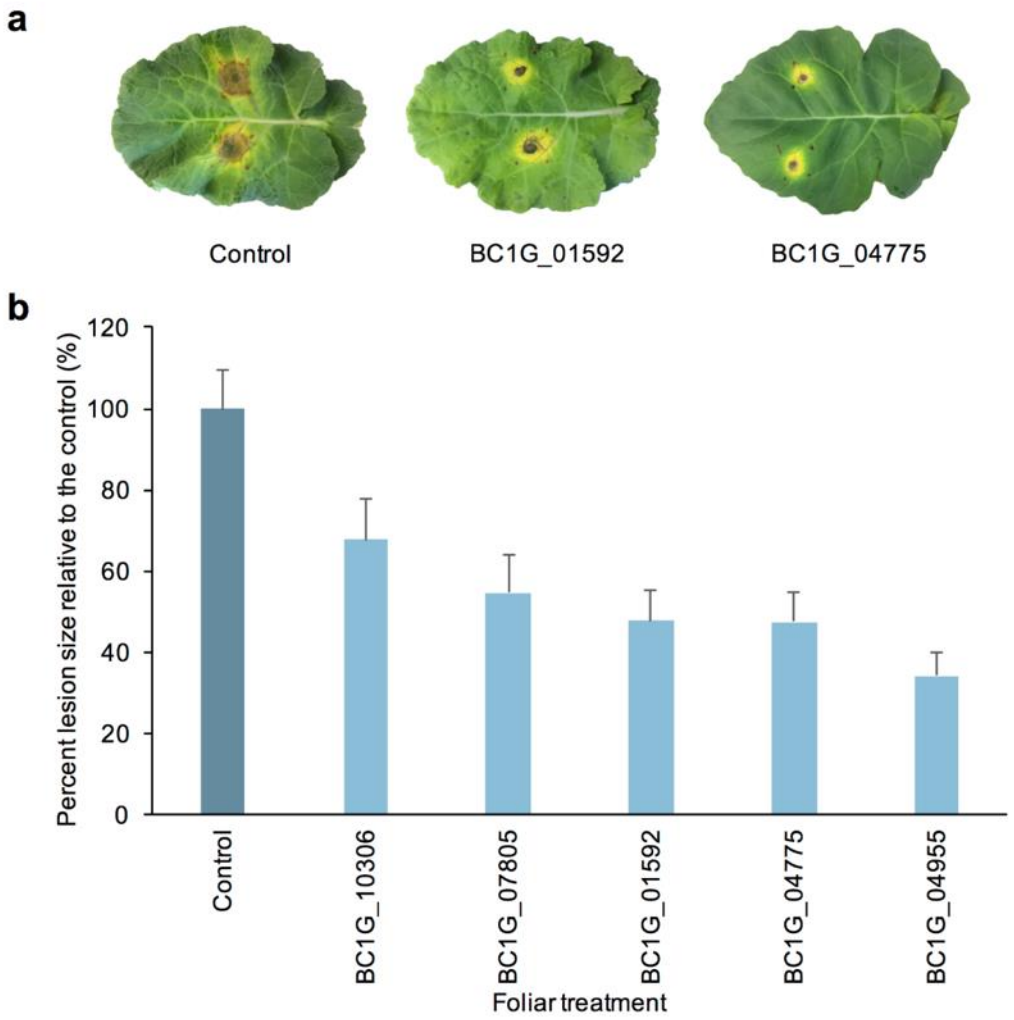
>20 dsRNAs strongly inhibited Sclerotinia infections



Can RNAi be used against other fungal pathogens?

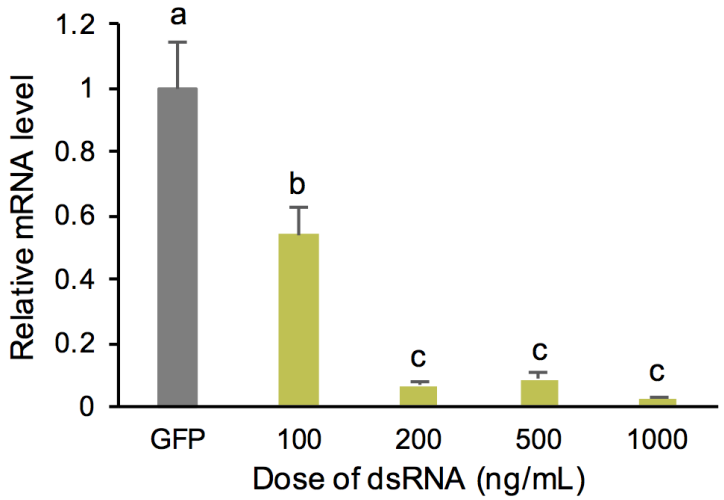
The technology translates to other fungal pathogens

Some of the same genes targeted in Sclerotinia can be effective targets against Botrytis

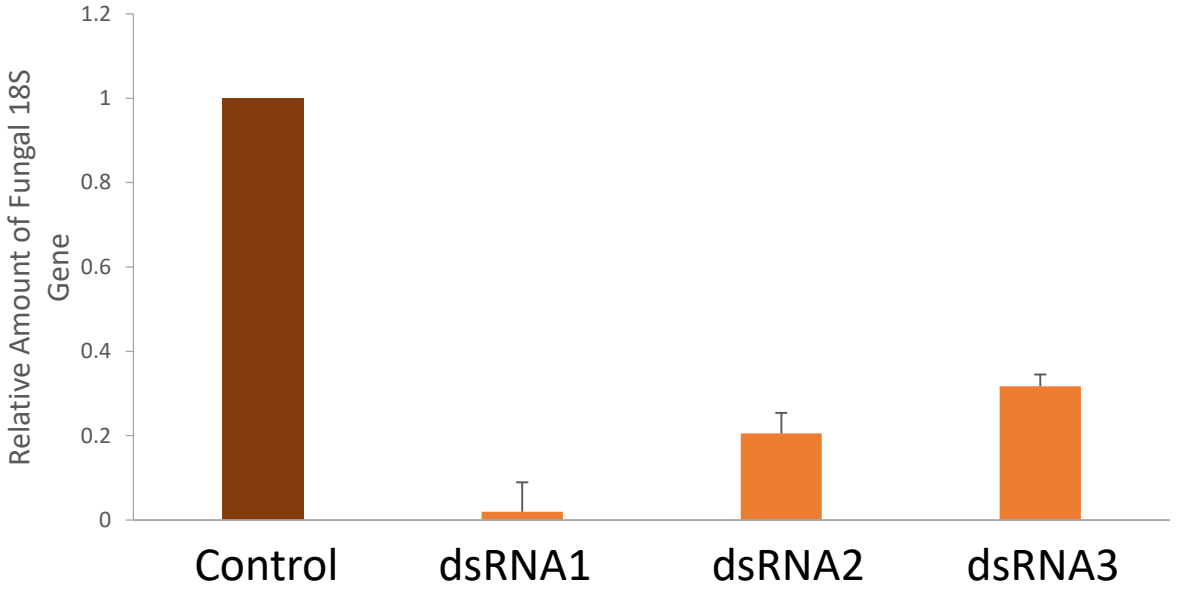


RNAi reduces fungal transcripts and fungal load

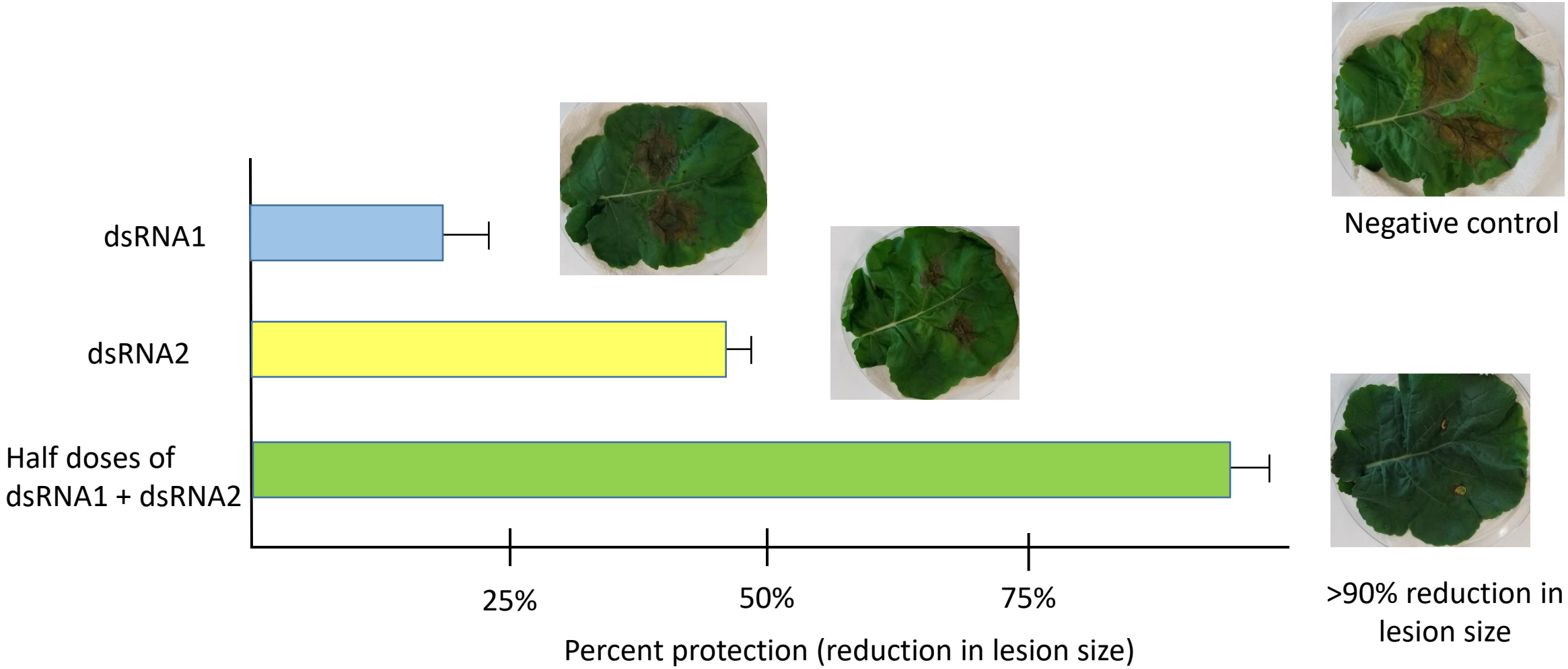
- qRT-PCR confirms that dsRNA is reducing the targeted mRNAs



- qRT-PCR confirms that the amount of fungus in the lesion region is reduced

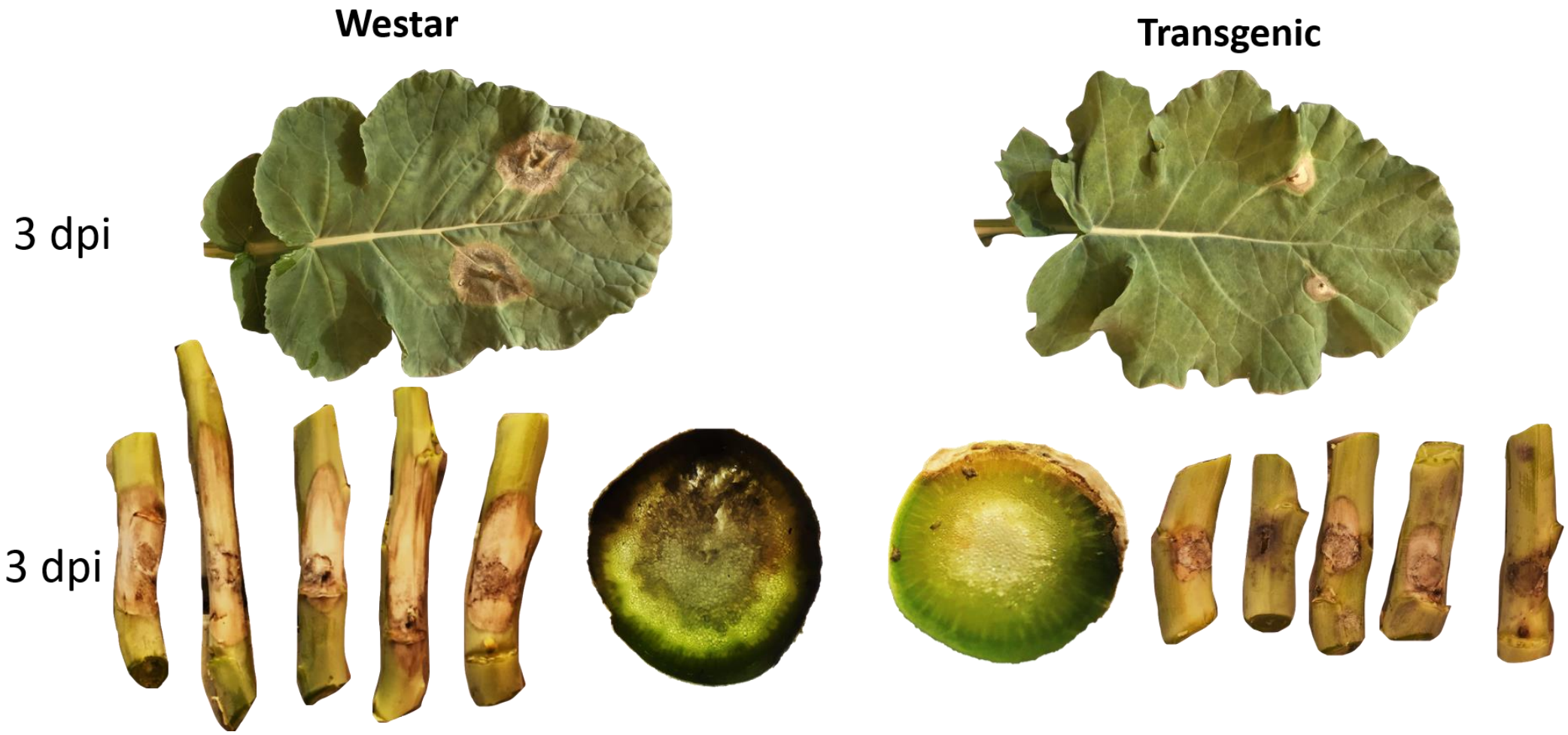


Can combining dsRNAs improve protection?

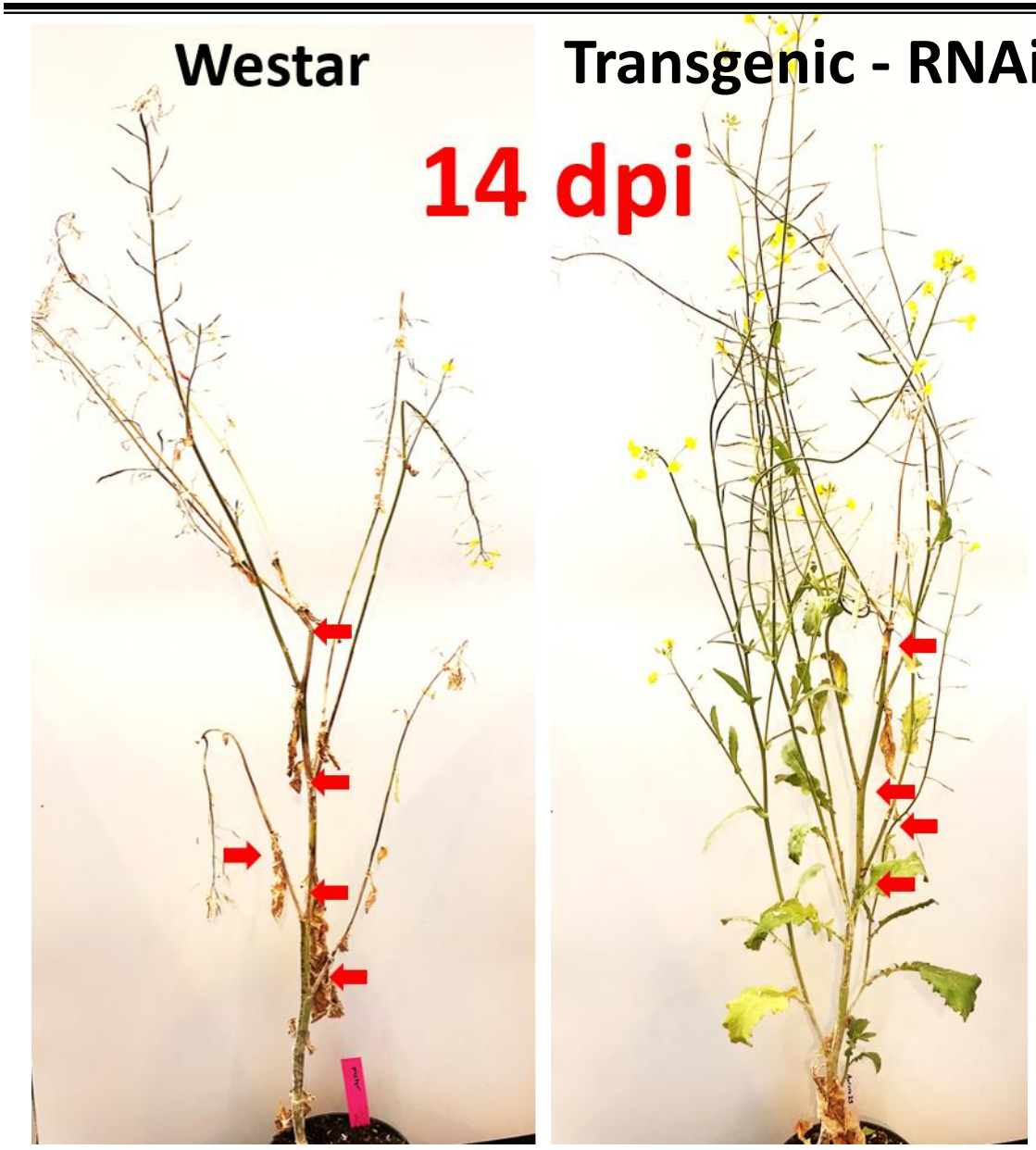


Are transgenic plants protected by RNAi?

What does protection look like?



How about whole-plant protection?



Mature canola plants were sprayed with highly concentrated Sclerotinia spores in high humidity (1 week)

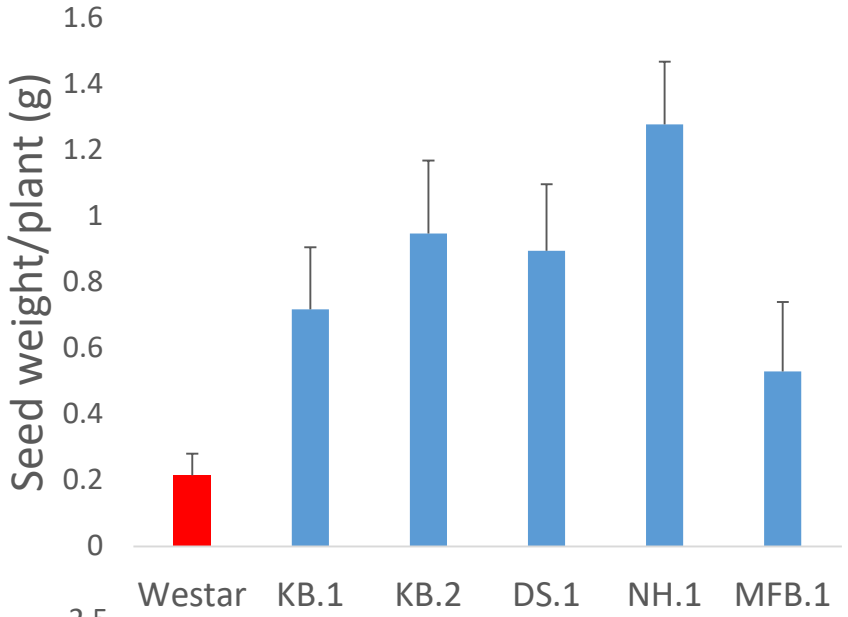
↓

Plants were then removed from chamber and infection continued (1 week)

↓

Control plants dying; transgenic plants producing seed

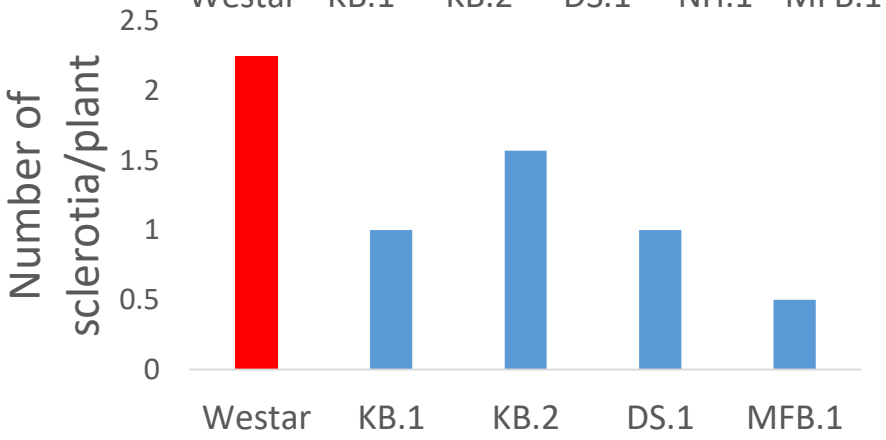
Does increased protection correlate to increased seed production?



Seed

Westar

Transgenic - RNAi



Sclerotia

On-going activities

- Testing foliar formulations to improve field durability (penetrants and adherents)

- Assessing degradation of dsRNA in soil
- Testing off-target effects

- Field tests of the best performing foliar treatments

The main points

- RNAi offers species-specific control of pest insects and fungal pathogens

- Foliar sprays of dsRNA and transgenic plants are both effective at controlling flea beetles and Sclerotinia

- Field trials will provide conclusive evidence of efficacy and affordability of this new technology

Thanks to:

- My research team
  - Aditi Singh
  - Arfa Khan
  - Melissa Rodrigues
  - Alex Fast
  - Satpreet Sandhu
  - Antonio Pellegrino
  - Alana Lamont
  - Dafna Schultz
  - Alison Tayler
  - Suresh Desai
  - Ryan Wahl
  - Alex Zaremba
  - Jaewoo Yang
  - Will Siemen
  - Geoffrey Pagcaliwagan
  - Saeed Farahani
- Mark Belmonte's team
  - Austein McLoughlin
  - Nick Wytinck
  - Phil Walker
  - Vivian Li
  - Deirdre Khan
  - Rachel Robinson
  - Daniel Sullivan
- The Funders:

NSERC CRSNG

canolacouncil OF CANADA

Western Grains Research Foundation

BAYER Crop Science

Agriculture and Agri-Food Canada

ARDI Agri-Food Research and Development Initiative

greenlight BIOSCIENCES