



Agriculture and Agriculture et
Agri-Food Canada Agroalimentaire Canada

Sclerotinia resistance and forecasting in canola

'Things should start to get interestin' right about now'

Lone Buchwaldt

Agriculture and Agri-Food Canada
Saskatoon Research and Development Centre

Canola Industry meeting
December 4, 2017, Saskatoon

AAFC's sclerotinia resistance team



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Missing
Sanjaya Gyawali
Harsh Garg
Brad Hope
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We have developed tools for breeding sclerotinia resistant canola varieties

- ⌋ Sclerotinia screening method - stem test
- ⌋ Seed of resistant lines
- ⌋ Molecular markers linked to sclerotinia resistance
- ⌋ Half way with transfer of sclerotinia resistance to canola quality
- ⌋ Sclerotinia isolates representative of the Canadian population
- ⌋ Cloned defence genes

Contact Todd Olsen for transfer, licencing and/or collaboration

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Sclerotinia screening method – stem test



The five traits are highly correlated

1) Lesion length 7 dai

2) Lesion length 14 dai

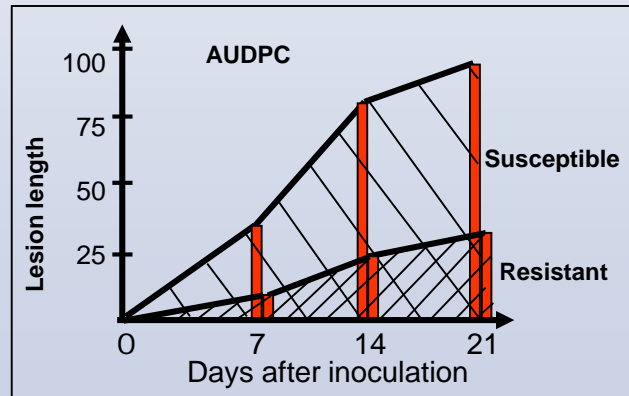
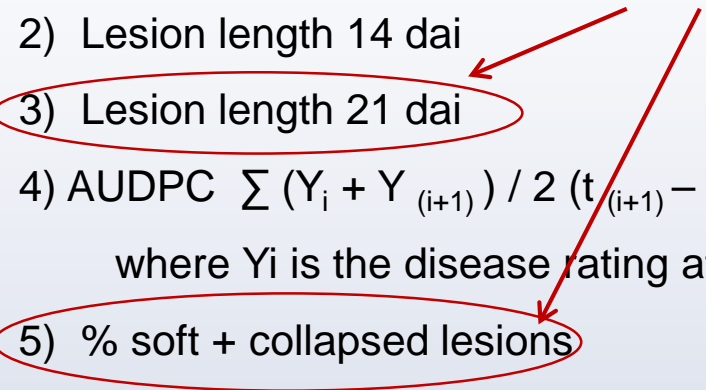
3) Lesion length 21 dai

4) AUDPC $\sum (Y_i + Y_{(i+1)}) / 2 (t_{(i+1)} - t_i)$

where Y_i is the disease rating at time t_i

5) % soft + collapsed lesions

Most important measurements



WCC/RRC adopted the stem test for coop lines with a claim of sclerotinia resistance

Report on stem test evaluation (33 tests) available on Canola Council's web site
http://research.canolacouncil.org/_uploads/documents/CanolaResearchHub_WCC-RRC_2016_Buchwaldt.pdf

Results of 7 sclerotinia stem tests in 2014.

Line	Lesion length 21dai, mm		% of 45H29	% s+c	
45H29	67	BC	100	35	BC
L160S	73	B	109	42	AB
VR9561G	60	CD	90	33	BC
45S52	50	E	74	27	D
LSD =	7			10	

Conclusion:

L160S is non sign.

VR9561G is non sign.

45S52 is significantly better for lesion length (26% reduction) and % soft + collapsed lesions

Results of 8 sclerotinia stem tests in 2015.

Line	Lesion length 21dai, mm		% of 45H29	% s+c	
45H29	69	B	100	32	AB
L160S - late	58	C	84	29	AB
VR9561G	55	CD	80	27	B
45S52	53	CD	77	26	B
LSD =	8			8	

Conclusion:

L160S is sign for lesion length (16% reduction)

VR9561G is sign for lesion length (20% reduction)

45S52 is sign for lesion length (23% reduction)

Sclerotinia reaction in L160S and VR9561G is at the limit of what the stem test can pick up. Higher level of resistance is expected in the next generation of canola varieties. The stem test will be needed in the near future.

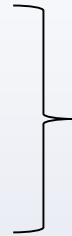
Sclerotinia resistant *Brassica napus* lines

PAK54 (Pakistan)

PAK93 (Pakistan)

DC21 (South Korea)

K22 (Japan)



High glucosinolate and erucic acid

Request the germplasm! *'Don't Think Twice, It's All Right'*

10 Material Transfer Agreements have been signed since 2014

SNP markers linked to sclerotinia resistance

Needed for marker-assisted-selection of progenies in various generations

Bi-parental mapping populations	A-genome	C-genome	Resistance loci
PAK54	A3, A6, A9, A10	C1, C6	7
PAK93	A5, A7	C2, C5, C6, C7	6
DC21	A1, A3	C5	3
K22	In progress		
Zhongyou 821	A1, A3, A5, A6, A7, A9	C1, C2, C3, C6, C9	11
A world collection of 180 <i>B. napus</i> accessions	A1, A2, A3, A6, A7, A8, A9, A10	C1, C2, C3, C4, C5, C6, C7, C9	114

Microsatellite markers are published in Gyawali et al. 2016, Mol Breeding, 36:72-85.

SNP markers will be published in 2018

Transfer of sclerotinia resistance to canola quality background, good agronomy and yield

2013	PAK54 (resistant) x N99-508 (canola quality)
2014	F ₁ backcrossed to N99-508 Plants selected based on SNP markers
2015	1 st DH population
2016	Agronomy, seed quality Sclerotinia test
	1 st inter-cross of DH sister lines based on traits and SNP markers
2017	Agronomy, seed quality, yield Sclerotinia test
	2 nd DH population
2018	Agronomy, seed quality, yield Sclerotinia test
	2 nd inter-cross of DH sister lines based on traits and SNP markers
2019	Agronomy, seed quality, yield Sclerotinia test
	3 rd DH population
2020	Plants selected based on SNP markers Agronomy, seed quality, yield Sclerotinia test
2021	Agronomy, seed quality, yield Sclerotinia test
2022	Agronomy, seed quality, yield Sclerotinia test
2023	Project ends March 31

population development
marker-assisted-selection
field plots
Stem inoculation

Seed of the best lines are available throughout the project

SNP-markers have been validated and used in practise

A successful breeding strategy

Sclerotinia isolates used for inoculation: #321, MB51 and AB29

Sclerotinia isolates for resistance screening

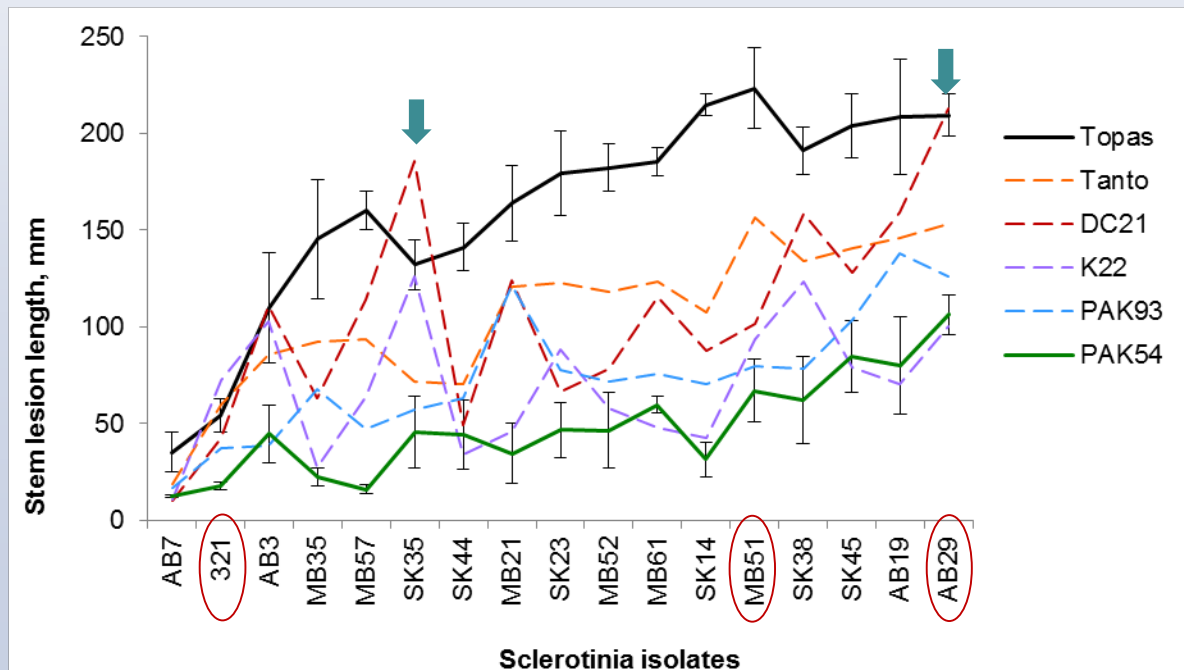
Isolates were collected across the Prairies

DNA fingerprinting identified 17 sub-populations

Inoculation with 17 representative isolates showed PAK54 has the highest level of resistance across isolates


Isolates SK35 and AB29 are more aggressive on some lines (arrows)

The 17 sclerotinia isolates are available from AAFC



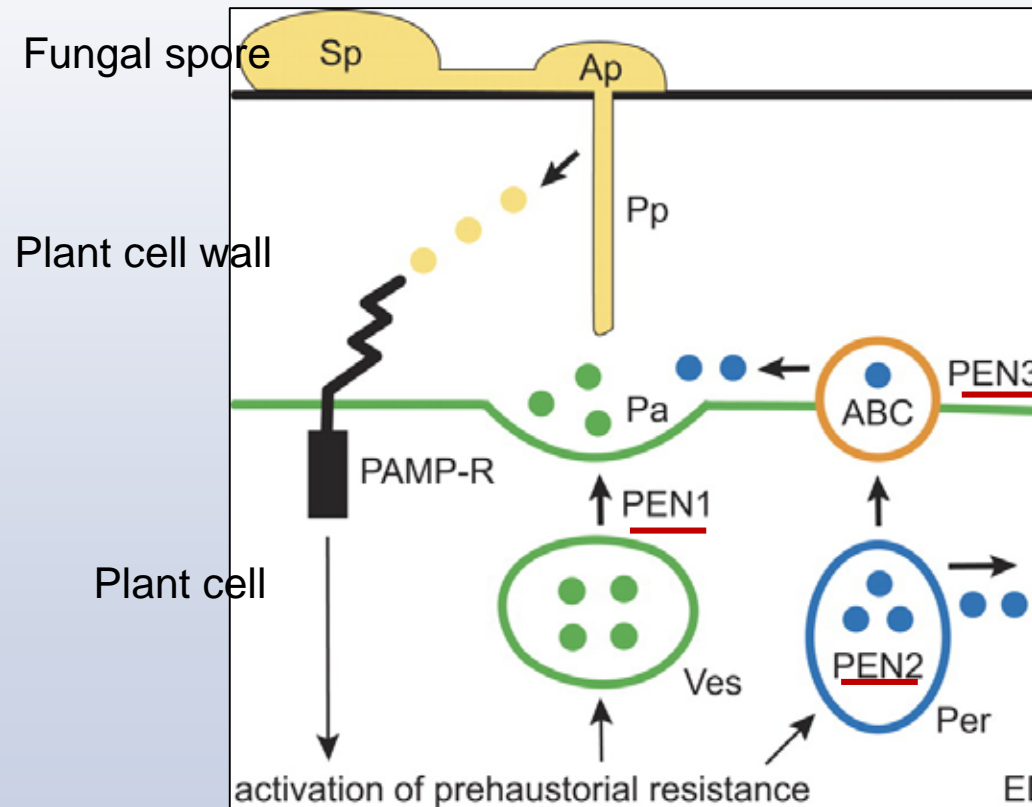
We have identified defense genes that reduce sclerotinia severity - a major step for potential use in GM canola

- Genes were cloned from Zhongyou 821 (China) and transformed into a susceptible *B. napus* line.
- The CaMV 35S promotor ensures the genes are constitutively expressed in all plant tissues all the time.

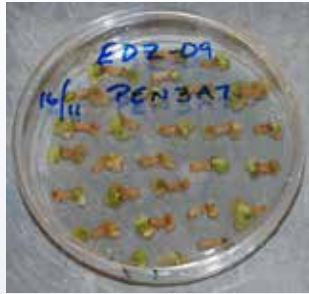
Genes	Function in host resistance	Reduction in sclerotinia severity
Hevein, L	 recognition and binding	40-50%
Lectin, c		20-50%
Lectin, c		40%
Annexin	and organization of vesicles, endocytosis, calcium ion channel formation	30-40%
WRKY33	Regulation of gene transcription	20-30%
O-methyl transferase	Glucosinolate pathway	Small inconsistent effect
PGIP	Inhibitor of fungal polygalacturonase	No effect

Defense genes that prevent fungal penetration of the plant cell, PEN1, PEN2 and PEN3 worthy of investigation in canola

Studied in several plant species such as the model plant *Arabidopsis* and soybean



Does constitutive expression of PEN1, PEN2 and PEN3 in canola reduce sclerotinia severity?



Transformation of *B. napus* DH12075

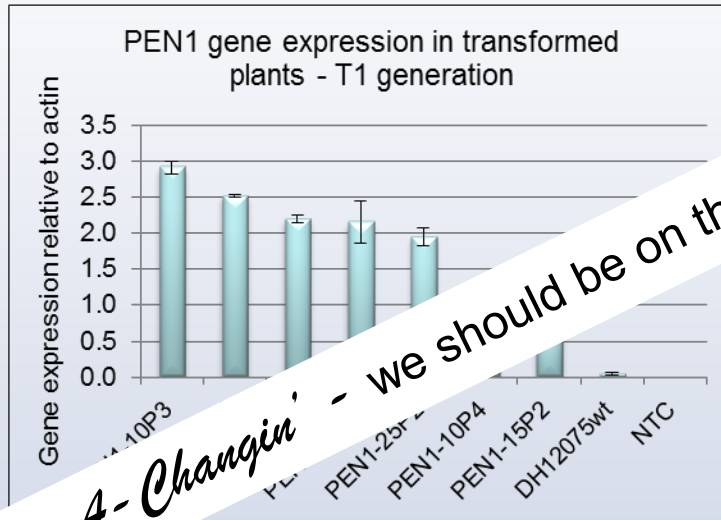


PEN1-10P3
T1 generation

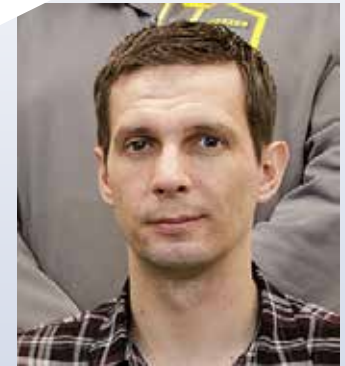
Method

PEN1 cloned from resistant PAK54

Susceptible *B. napus* DH12075 transformed with PEN1 under the control of CaM35S gene promotor



The Times They Are A-Changin' - we should be on the right side of change



Results

A single PEN1 gene copy was inserted in the *B. napus* genome

The particular PEN1 allele was very low in DH12075

Transformants expressed PEN1 0.75 – 2.8 relative to DH12075

Next step

Clone and transform with PEN2 and PEN3

Phenotype PEN1, PEN2 and PEN3 transformants for sclerotinia resistance in the T2 generation



Forecasting - Sclerotinia risk App for smartphones

- Users are canola growers and extension specialists
- Objective is to recommend a fungicide application if an economic return is likely
- Free, fast and user friendly in order to be used on 20 mill acres of canola
- The starting point is a checklist developed in Sweden
- Verified or modified to Canadian canola based on in-depth field surveys
- The App provides real-time and location-specific rainfall data and weather forecast

AAFC's Sclerotinia risk assessment team

AAFC research staff

Erl
Svendsen

Cam
Kenny

Jonathan
Durkin

~60 extension specialists and canola growers reported sclerotia germination in ~200 depots each growing season from 2013 to 2017



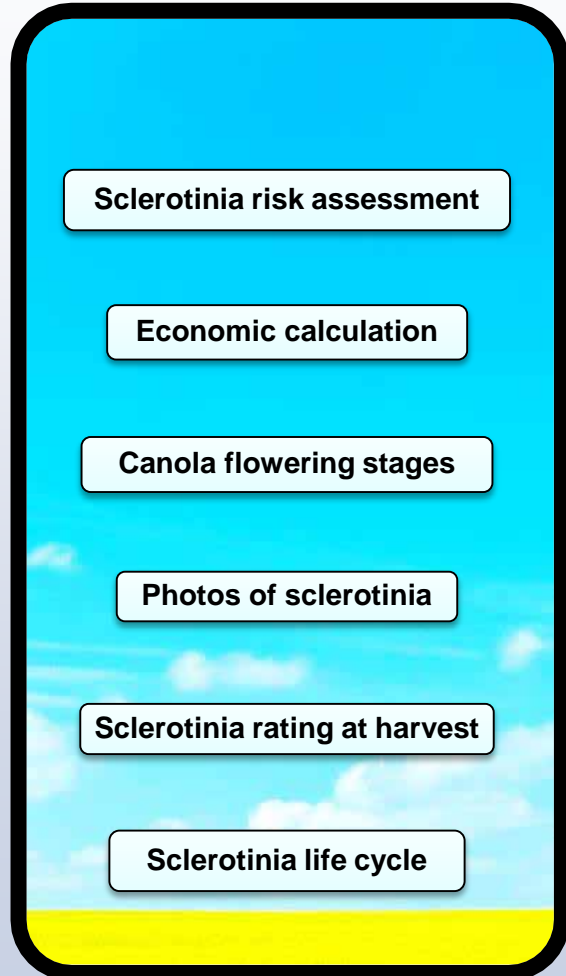
Jackie
Nettleton

Lone
Buchwaldt



'If not for you'

Sclerotinia risk App for smartphones



App programming (a hybrid)

Ready made web applications for the user interface

New programs for user's geolocation (GPS, RM, other names), writing of files such as the check list and economic calculation

Saving and sharing of risk assessment results by texting and email.

Versions for both iPhone and Android phones

The starting point is the Swedish sclerotinia check list

Twengström E, Sigvald R, Svensson C, Yuen J. 1998. Forecasting Sclerotinia stem rot in spring sown oilseed rape. Crop Prot 17:405–411.

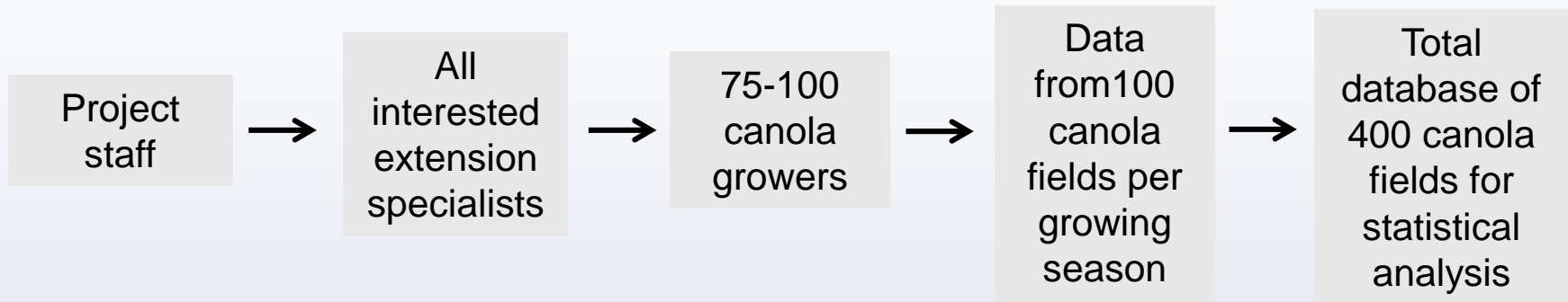
The risk points for each of the six factors were based on data from 384 canola fields.

Risk factor	Risk points
1. Number of years since last canola crop	0, 5 or 10
2. Sclerotinia incidence in last host crop	0, 5, 10 or 15
3. Plant density	0, 5 or 10
4. Rainfall in the last two weeks	0, 5 or 10
5. Weather forecast	0, 10 or 15
6. Regional risk for apothecia development	0, 10 or 15

A similar data set for Canadian canola is needed in order to either verify or modify this risk assessment.

In-depth field survey of Canadian canola to determine the effect of

1. Number of years since last canola crop
2. Sclerotinia incidence in last host crop
3. Plant density



Sclerotinia risk assessment at flowering

Plant density

10-year crop rotation

Sclerotinia disease severity in previous crops

Sclerotinia severity index at harvest (both frequency and severity)

Canola variety (new factor)



Weather Innovations Consulting (WIN) will provide location-specific and real-time rainfall data

4. Rain in the last two weeks

5. Weather forecast

Weather networks

WIN has >1100 weather stations in Alberta, Saskatchewan and Manitoba
Receives data from Environment and Climate Change Canada (Federal Gov.)
Other smaller weather networks

Quality check of data

Monitored daily and adjusted for anomalies (limit tests, other data nearby, radar maps etc.)

Algorithms for calculation of location-specific real-time rainfall

Calculates location-specific rainfall data within a 50 km radius with the closest stations having more influence than more distant ones

Algorithms for local weather forecasts

Weather forecast data from National Oceanic Atmospheric Administration (NOAA)
Environment and Climate Change Canada
A few other sources.

'Come Rain or Come Shine'

Sclerotia depots

6. Regional risk of apothecia development



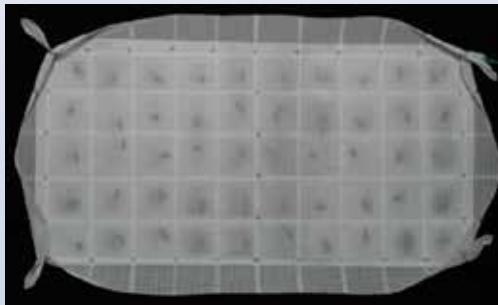
Sclerotia on sterilized barley



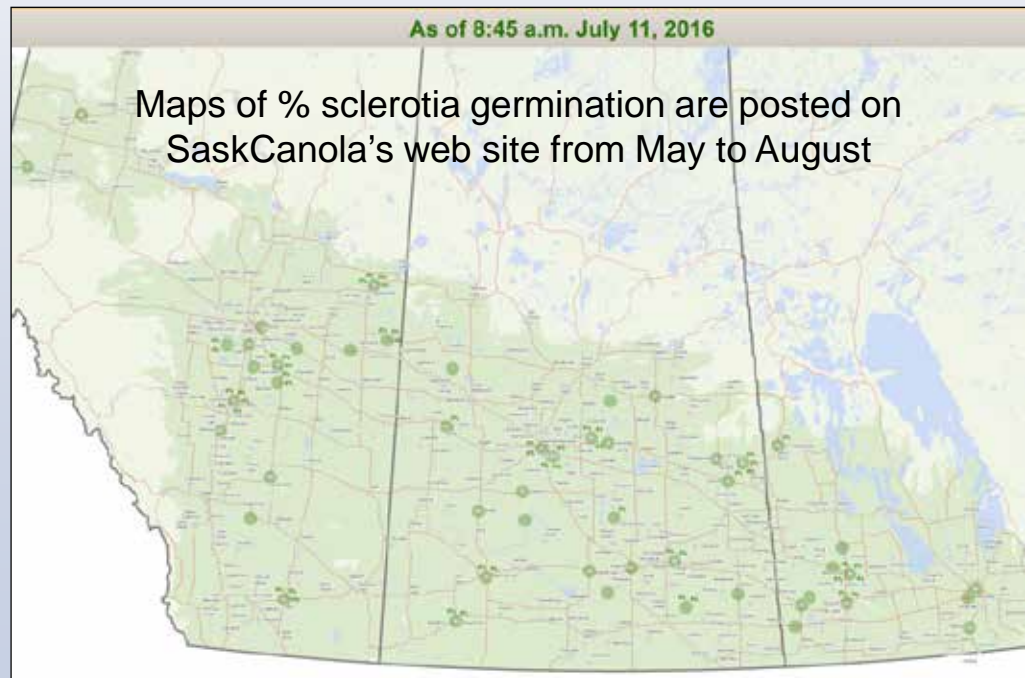
Harvest of sclerotia



Sclerotia readily germinate with apothecia



Volunteers bury depots (50 sclerotia) in canola fields and report % sclerotia germination via smartphones



Economic calculation

The screenshot shows a mobile application interface titled "ECONOMIC CALCULATION". It features five horizontal sliders for adjusting variables: "Expected % infected stems at harvest" (set to 15), "Expected seed sales price" (set to 0.45), "Fungicide costs" (set to 57.90), "Expected yield" (set to 2465), and "All other input costs" (set to 75). A "CALCULATE" button is positioned below the sliders. The results section, titled "Results and fungicide recommendation", contains a table with the following data:

The cost of fungicide application is worth the investment in terms of protecting yield in this scenario	
Estimated value of yield loss due to sclerotinia disease if no fungicide is applied	\$85.19 /hectare
Estimated crop value with fungicide application (assumes fungicide application was 100% effective)	\$976.55 /hectare
Estimated crop value if no fungicide is applied	\$961.06 /hectare

At the bottom of the results section is a "RUN ANOTHER SCENARIO" button.

The following relationship between disease severity and yield loss is built into the calculator:

% infected canola stems = 0.5% yield loss

Example:

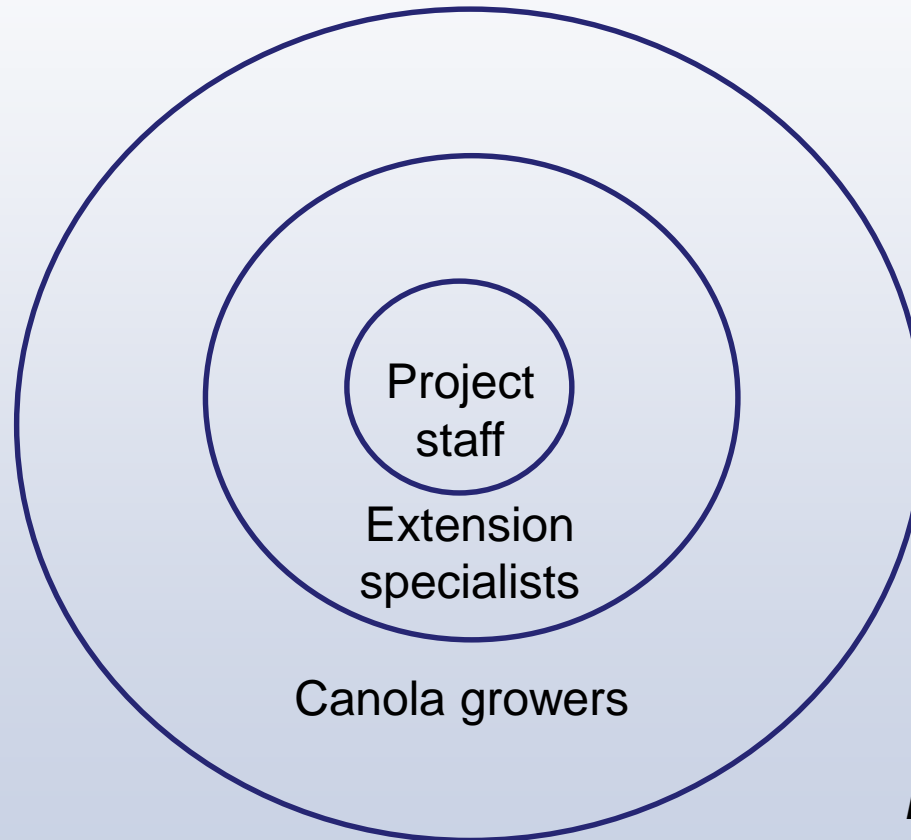
10% infected stems = 5% yield loss

The App will advise growers to follow fungicide manufacturer's instructions for fungicide rate and time of application

'Gonna Change My Way Of Thinking'

Technology transfer & user feed-back

Up-to-date information is easily distributed via App versions



Like rings in water

Future research

- Ü Finish transfer of sclerotinia resistance into canola quality background with good agronomy and yield
- Ü Continue to investigate defence genes that prevent fungal penetration of the plant cell, PEN1, PEN2, PEN3, and identify new defense genes effective against sclerotinia
- Ü Sclerotinia risk App for smartphones

Funding for sclerotinia research is greatly appreciated

Canola Council of Canada

Sclerotinia stem testing of coop lines (via WCC/RRC)

Sclerotia depot project

SaskCanola

Seed of resistant *B. napus* lines

Molecular markers

Transfer of sclerotinia resistance to canola

Identification of defense genes

Sclerotinia isolate survey

Weather Innovations

In-kind via the sclerotia depot project

Agriculture and Agri-Food Canada

All of the above areas