Sclerotinia Stem Rot in Canola: Tales from the Executioner's Handbook

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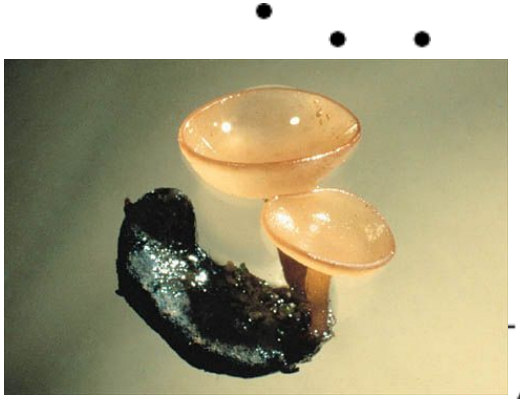
Saskatoon Research Centre

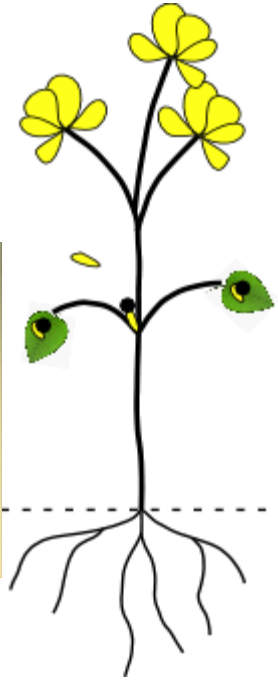
Sclerotinia sclerotiorum

A wide host-range 'necrotic' pathogen – to make dead

Infection at flowering time

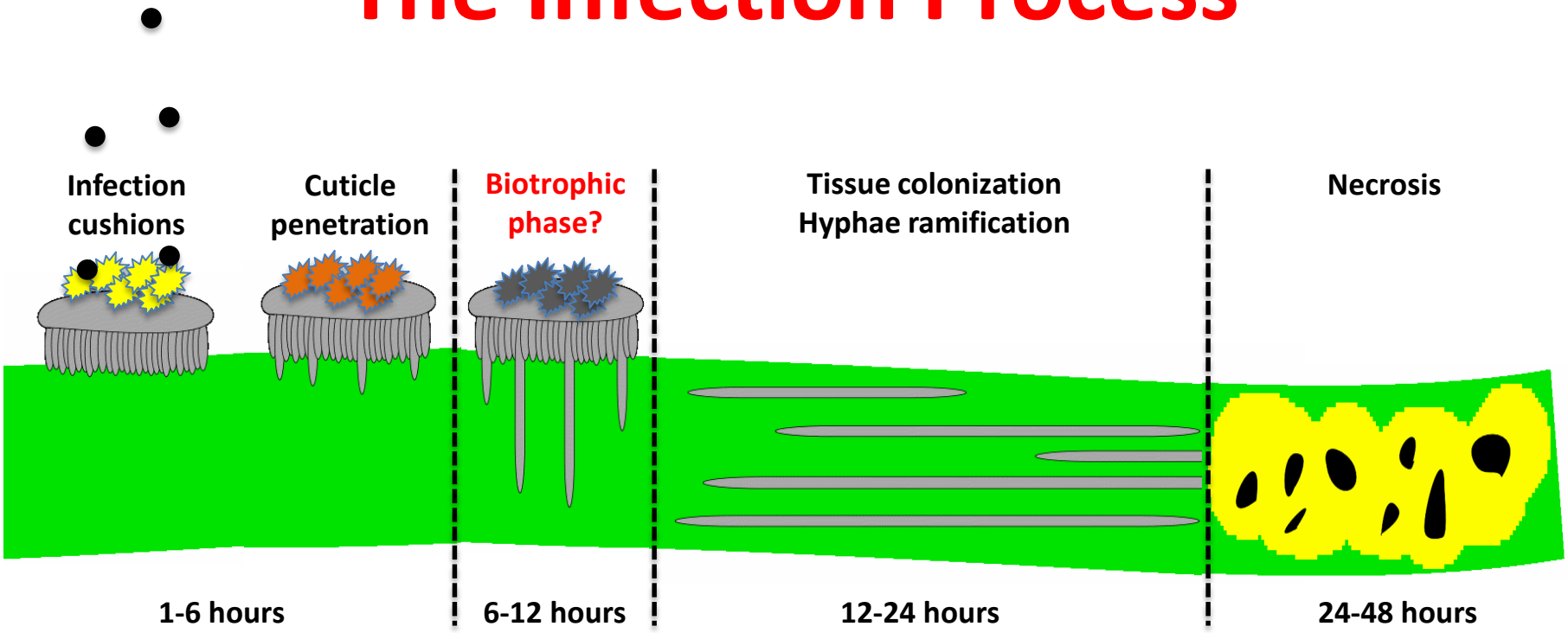


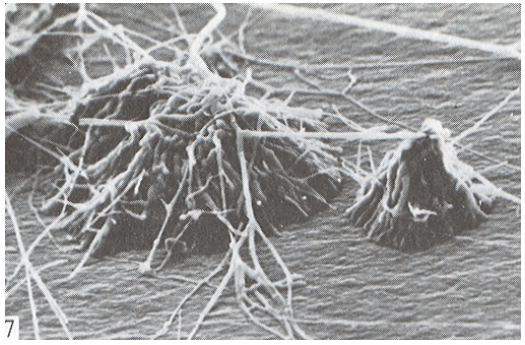
Germinating sclerotia

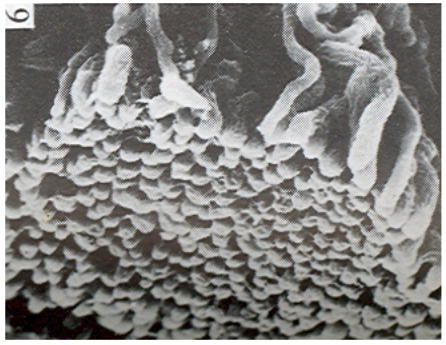


The Executioner

The Infection Process



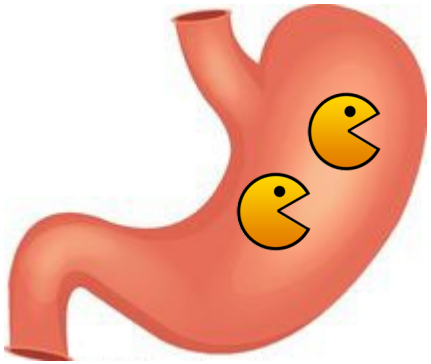




The Executioner's Handbook

Oxalic Acid

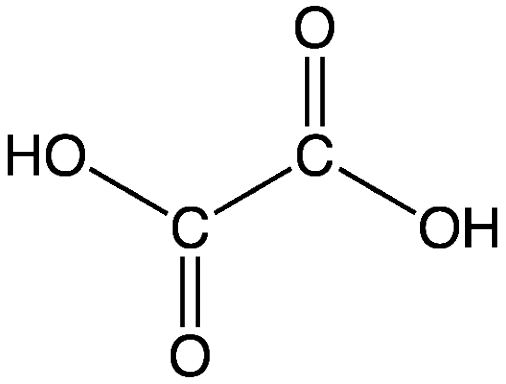
Digestive Enzymes

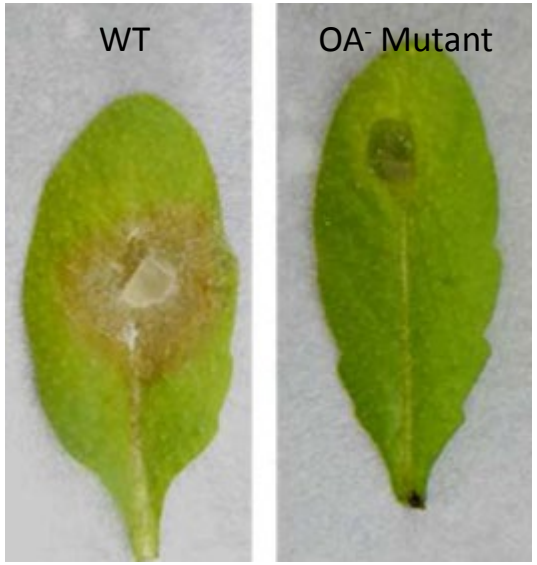


Necrosis Proteins!

Oxalic Acid

- **Simplest dicarboxylic acid**
- **Suppressing early host plant defenses**
  - biotrophic/establishment phase
- **Sclerotinia OA^- mutants are not infective**
- **Soybean producing the enzyme <u>oxalate oxidase</u> is more resistant to Sclerotinia**
  - D. Simmonds (AAFC-Ottawa)

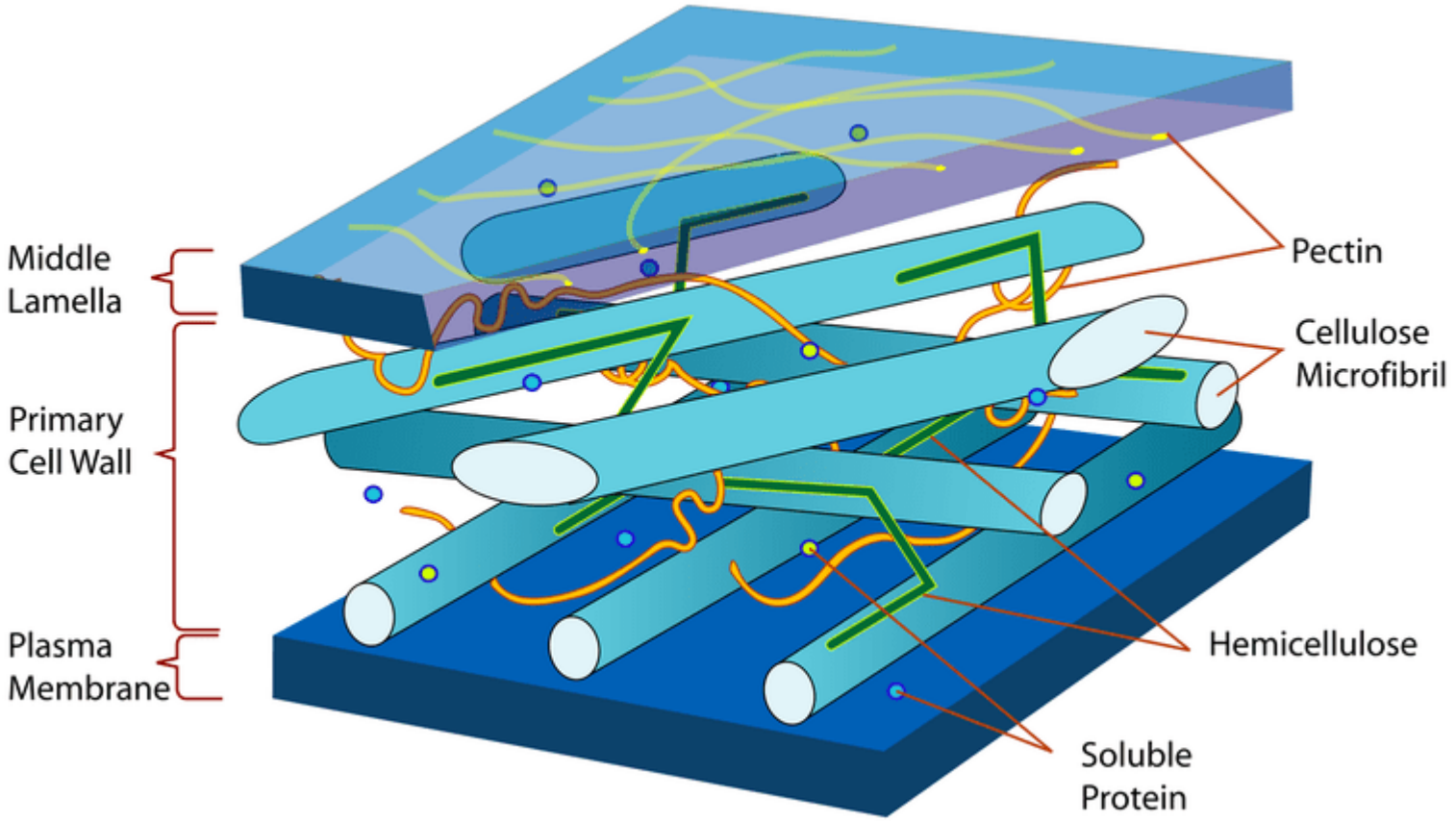




Kabbage PLOS 9:e1003287

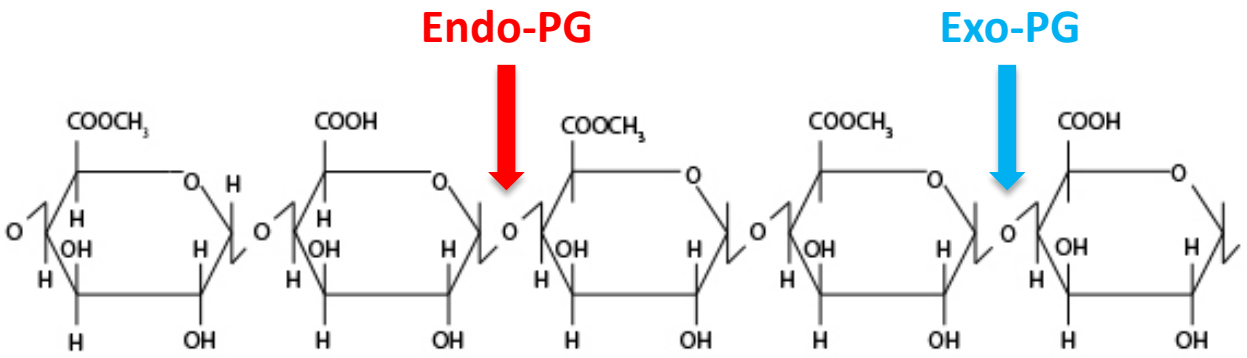
Digestive Enzymes

- **Plants are composed of the sugar polymers cellulose, hemicellulose and pectin**
- **Sclerotinia releases acids that loosen plant structure and enzymes that digest the fibres (sugars > nutrition)**



Sclerotinia Pectinases

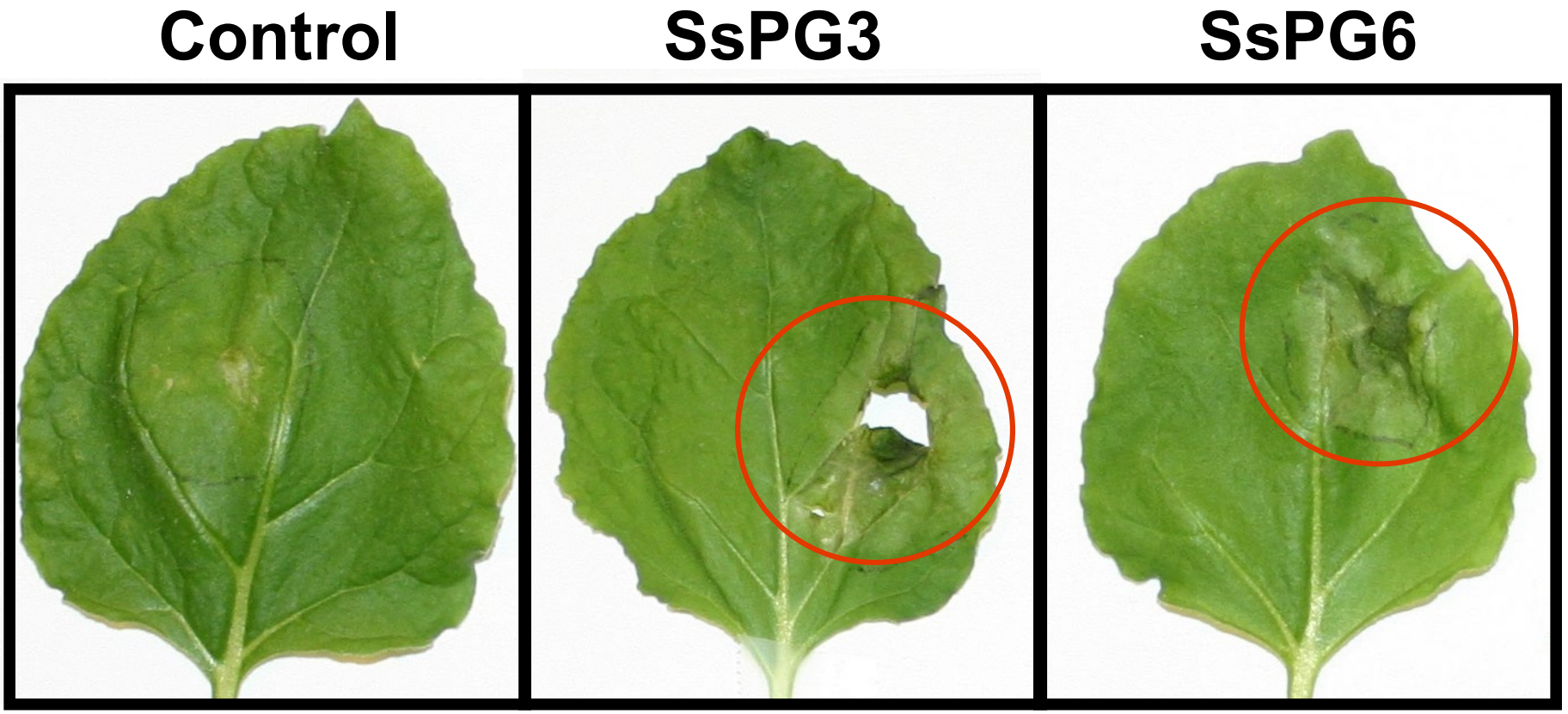
- Pectin is a polymer of galacturonic acid



- Sclerotinia produces 6 pectinases
  - 4 endo-polygalacturonases (SsPG 1, 3, 4 & 6)
  - 2 exo-polygalacturonases (SsXPG1-2)
- Similar sets for cellulose

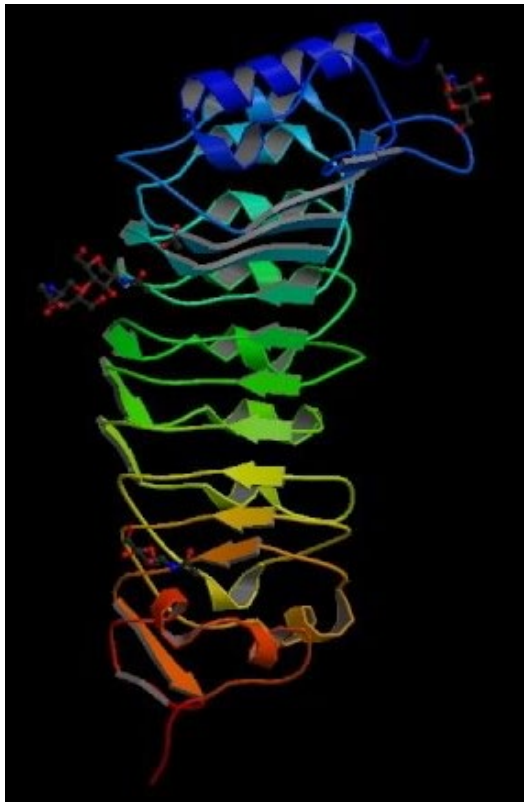
Sclerotinia Pectinases

- Pectinases cause tissue maceration, but not typical necrosis symptoms

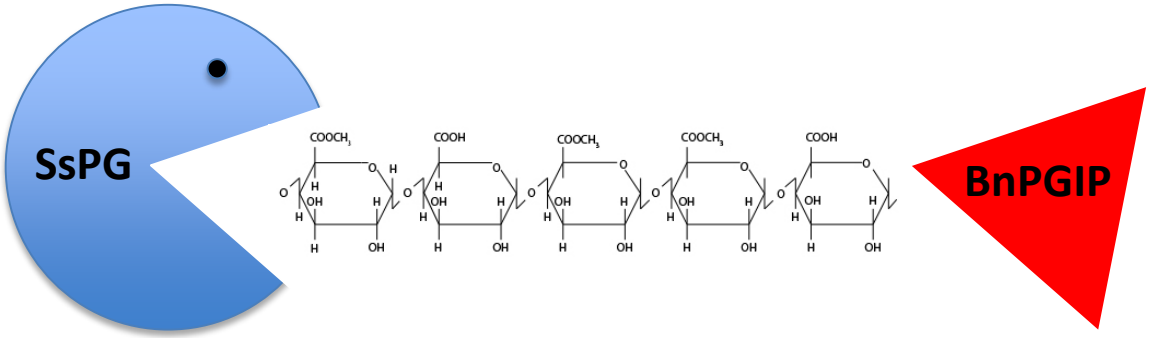


Bashi ZD, Rimmer SR, Khachatourians GG and Hegedus DD (2013) *Brassica napus* polygalacturonase inhibitor proteins inhibit *Sclerotinia sclerotiorum* polygalacturonase enzymatic and necrotizing activities and delay symptoms in transgenic plants. Can. J. Microbiol. 59: 79-86.

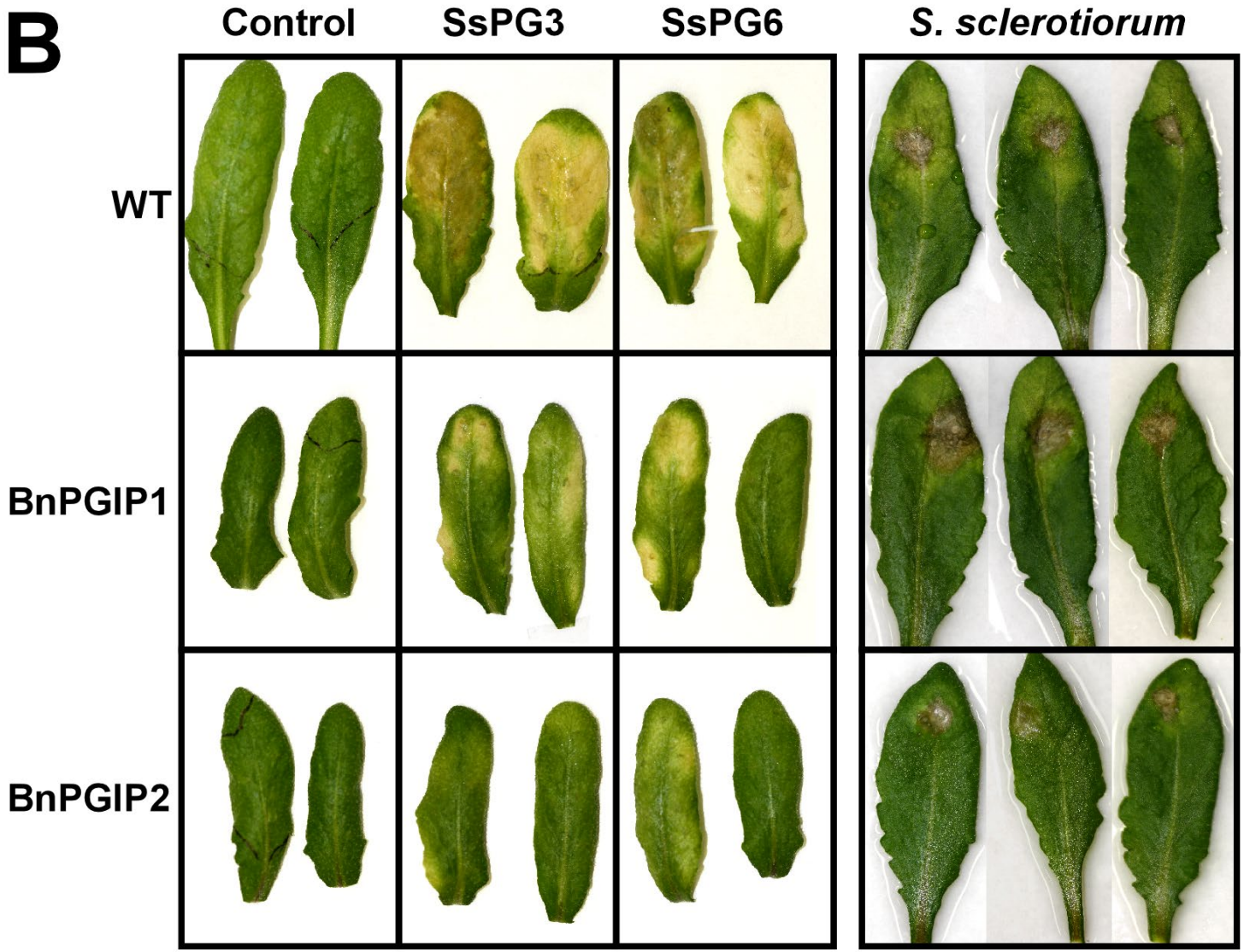
Polygalacturonase Inhibitor Proteins



- PGIPs inhibit the activity of pectinases
- Produced by plant in response to infection
- *B. napus* has at least 21 genes for PGIPs

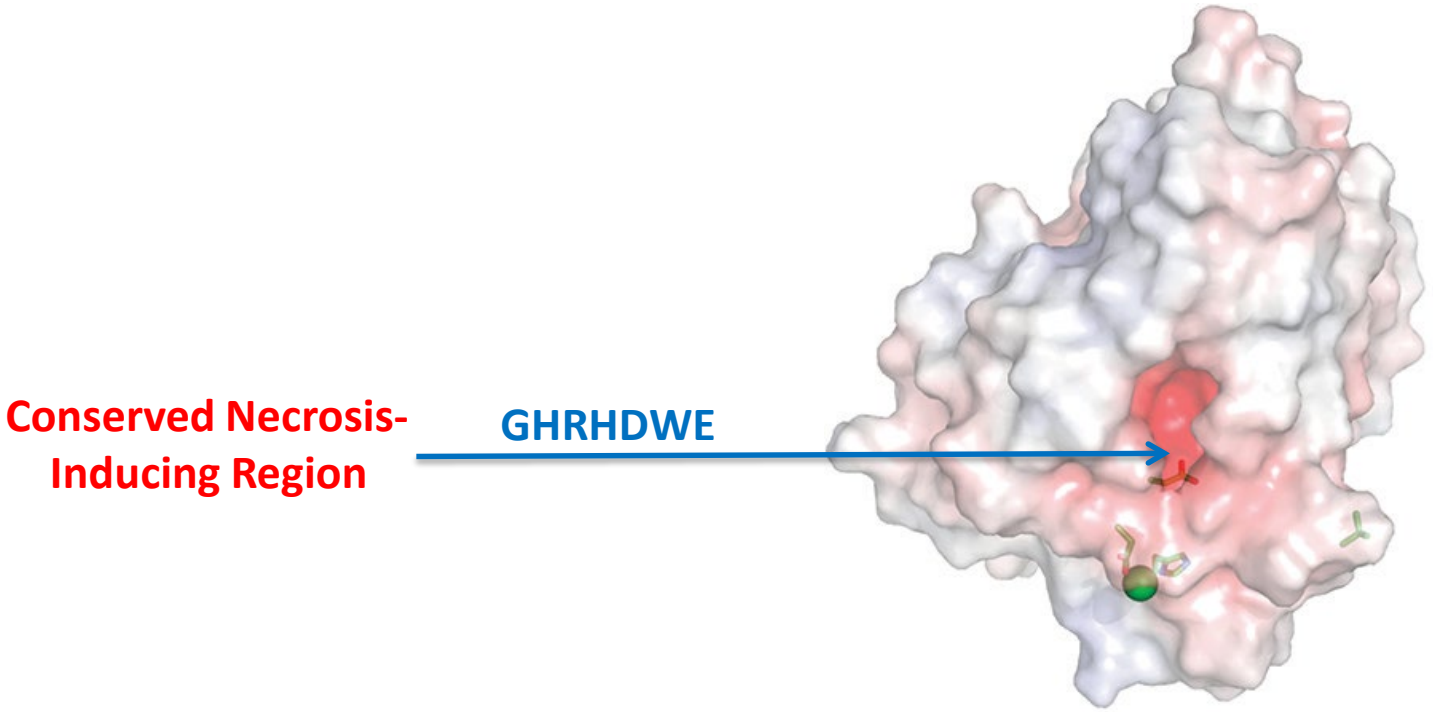


B. napus PGIPs vs Sclerotinia PGs?



Necrosis and Ethylene Inducing Proteins (NEPs)

- Found in bacteria, fungi and oomycete plant pathogens
- Cause necrosis or death of plant tissue
- Ethylene production – induce plant defense response?



Necrosis and Ethylene Inducing Proteins (NEPs)

- Sclerotinia has two NEPs, both cause necrosis
- Only SsNep2 produced during canola infection

Control SsNep1 SsNep2

Bashi ZD, Hegedus DD, Buchwaldt L, Rimmer SR and Borhan MH (2010) Expression and regulation of *Sclerotinia sclerotiorum* necrosis and ethylene inducing peptides (NEPs). Mol. Plant Pathol. 11: 43-53.

Necrosis Protein Discovery in Sclerotinia

1. *S. sclerotiorum* genome sequencing

- International consortium (Australia, Canada, Europe, USA)
- Derbyshire M, Denton-Giles M, Hegedus DD, Seifbarghi S, Rollins J, van Kan J, Seidl, Luigi Faino M, Mbengue M, Navaud O, Raffaele S, Hammond-Kosack K, Heard S, Oliver R (2017) The finished genome of the plant-infecting fungus *Sclerotinia sclerotiorum*: re-visiting the 'two-speed-genome' hypothesis in the context of broad host-range plant pathogenesis. Genome Biology & Evolution 9:593-618.

2. Expression of Ss genes during infection of canola

- Seifbarghi S, Borhan MH, Wei Y, Coutu C, Robinson S and Hegedus DD (2017) Deployment of the *Sclerotinia sclerotiorum* genome during infection of *Brassica napus* as revealed by RNA-Seq analysis. BMC Genomics. 18:e266.

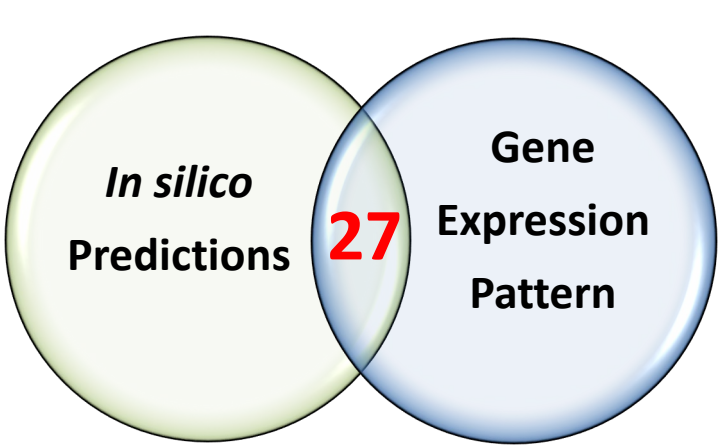
Necrosis Protein Discovery Pipeline

Ss Genome Sequencing
11, 130 Genes

900 Secreted Proteins

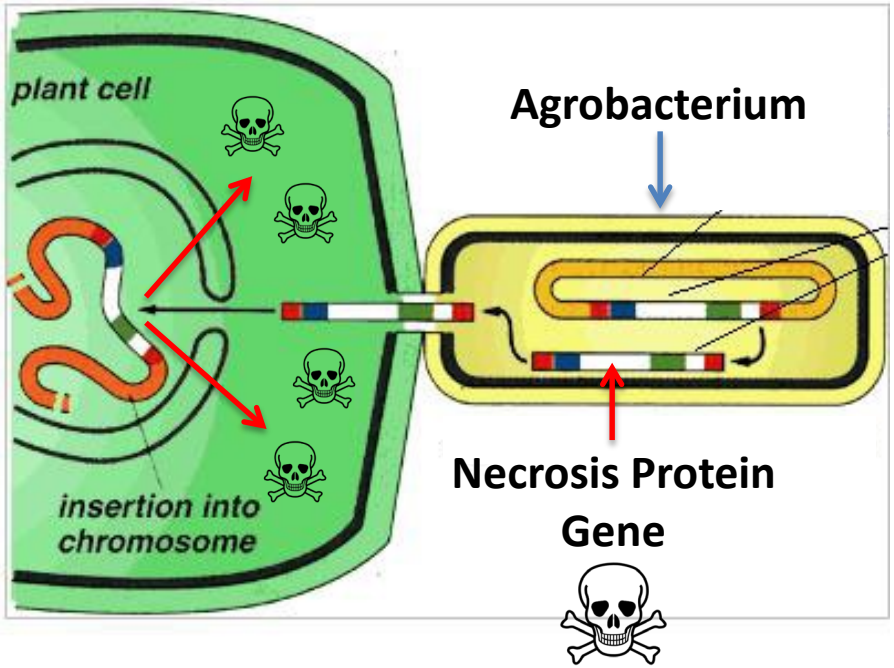
100 Necrosis Proteins?

Different times on canola
1, 3, 6, 12, 24, 48 hours

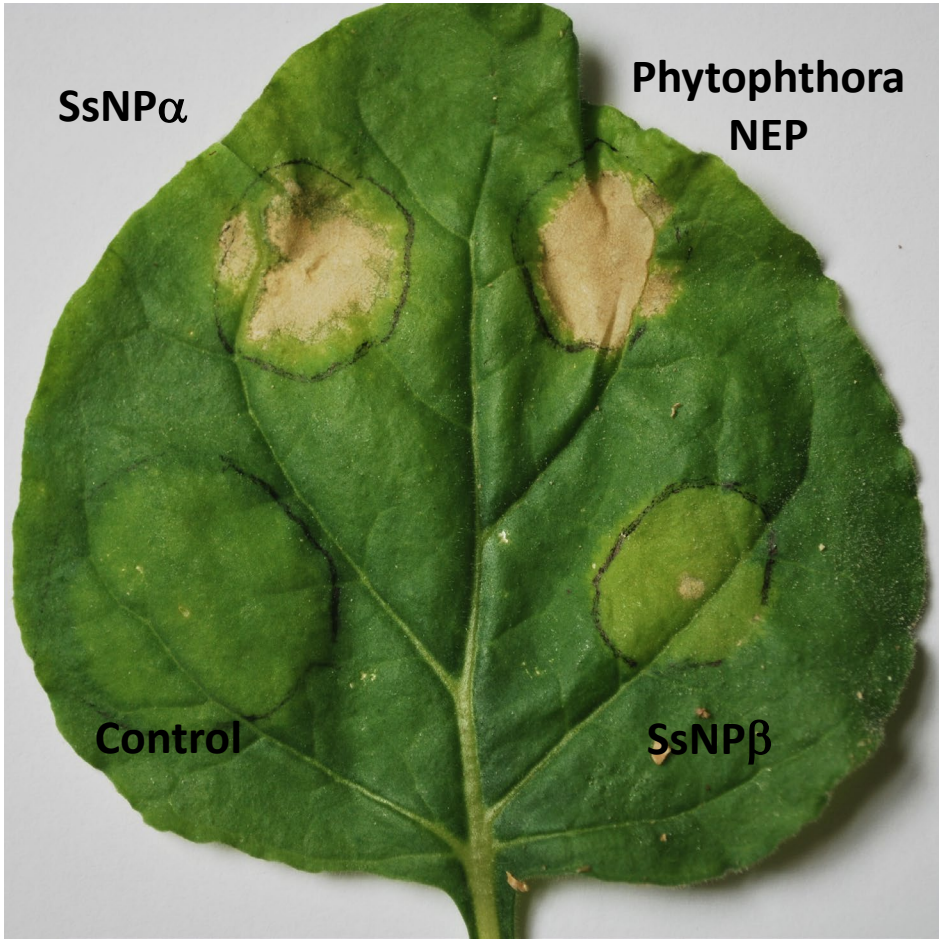


Expressed early or during necrosis

Validation of Necrosis Proteins



Potent New Necrosis Proteins

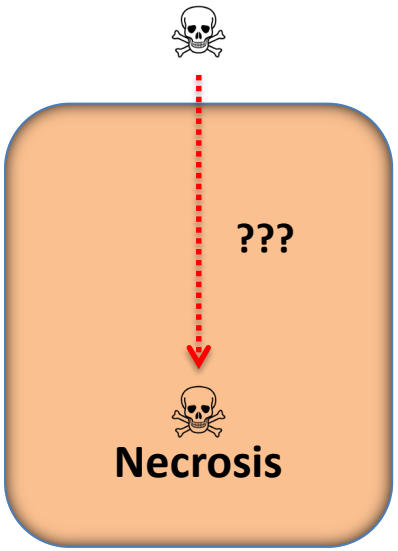


- Six new necrosis Sclerotinia proteins
- No resemblance to known NEPs
- Unique to Sclerotinia and Botrytis

How do Necrosis Proteins Work?

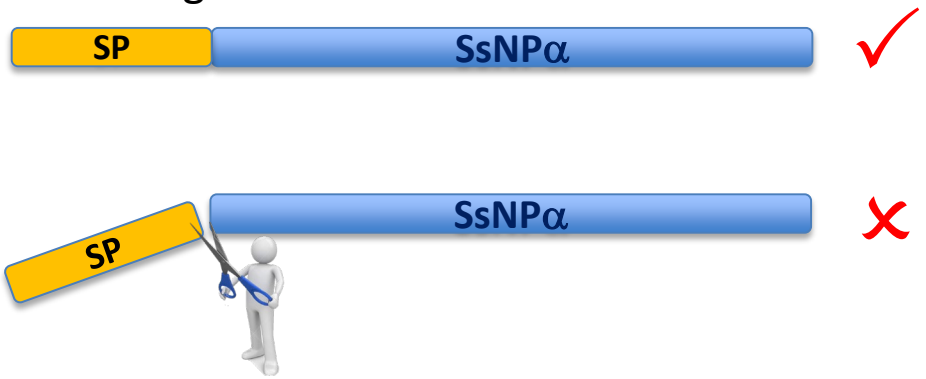
Traditional Model

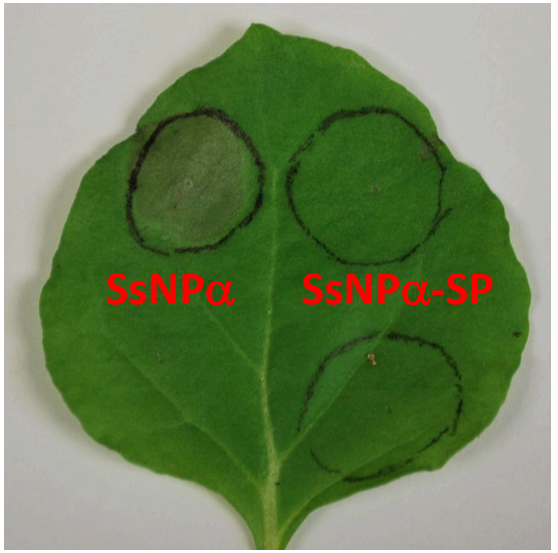
Necrosis Proteins



Necrosis Proteins Act Outside Cell

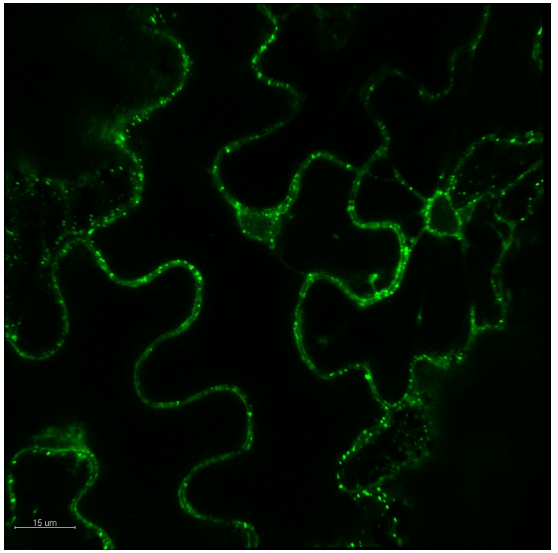
Secretion Signal





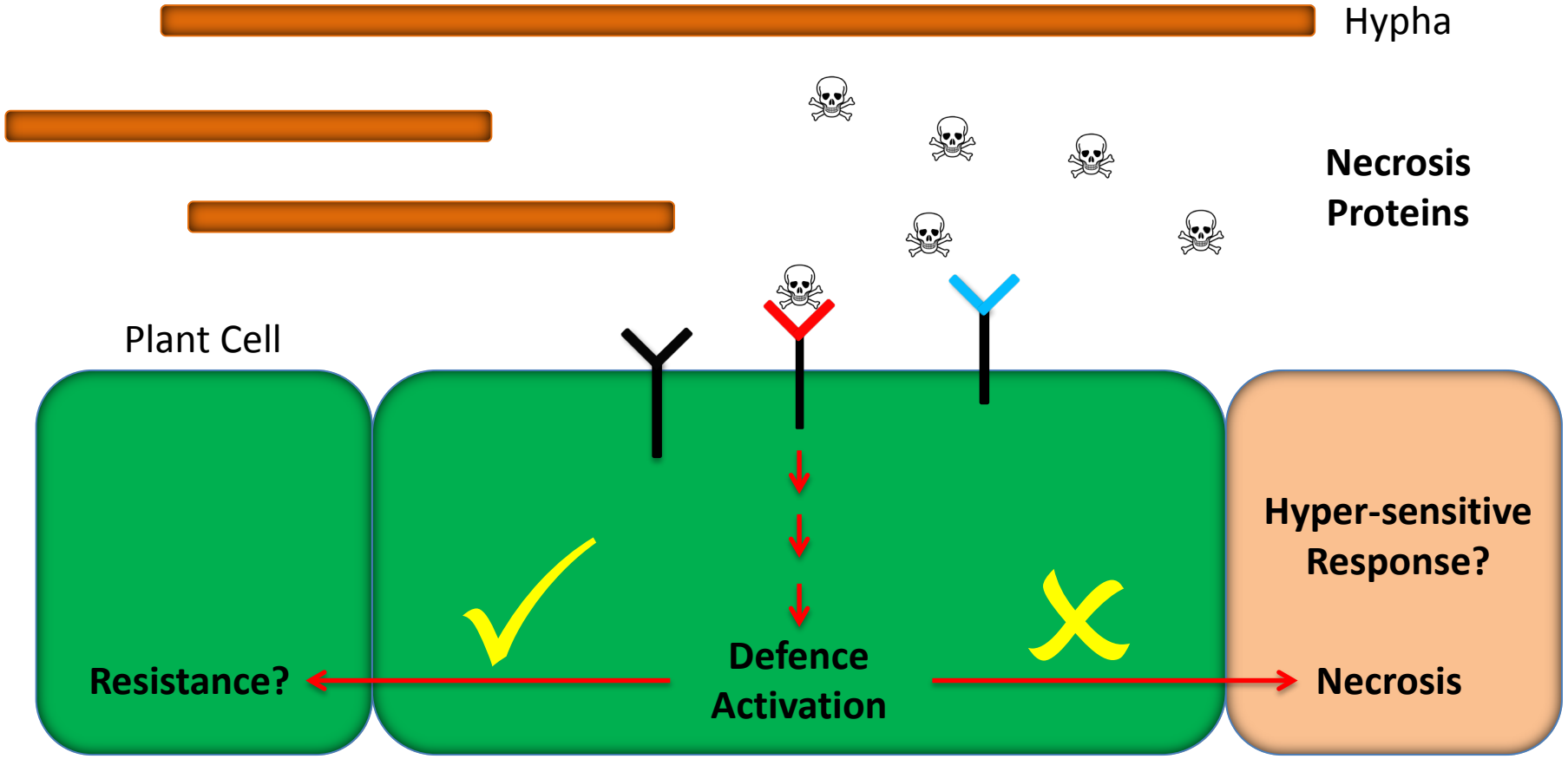
Many necrosis proteins must be secreted

Something is happening outside of the plant cell!



Necrosis Proteins Interact with Receptors

- **Xyloglucanase 1 necrosis protein (Botrytis and Sclerotinia)**



Zhu et al (2017) Plant Physiology 175:438

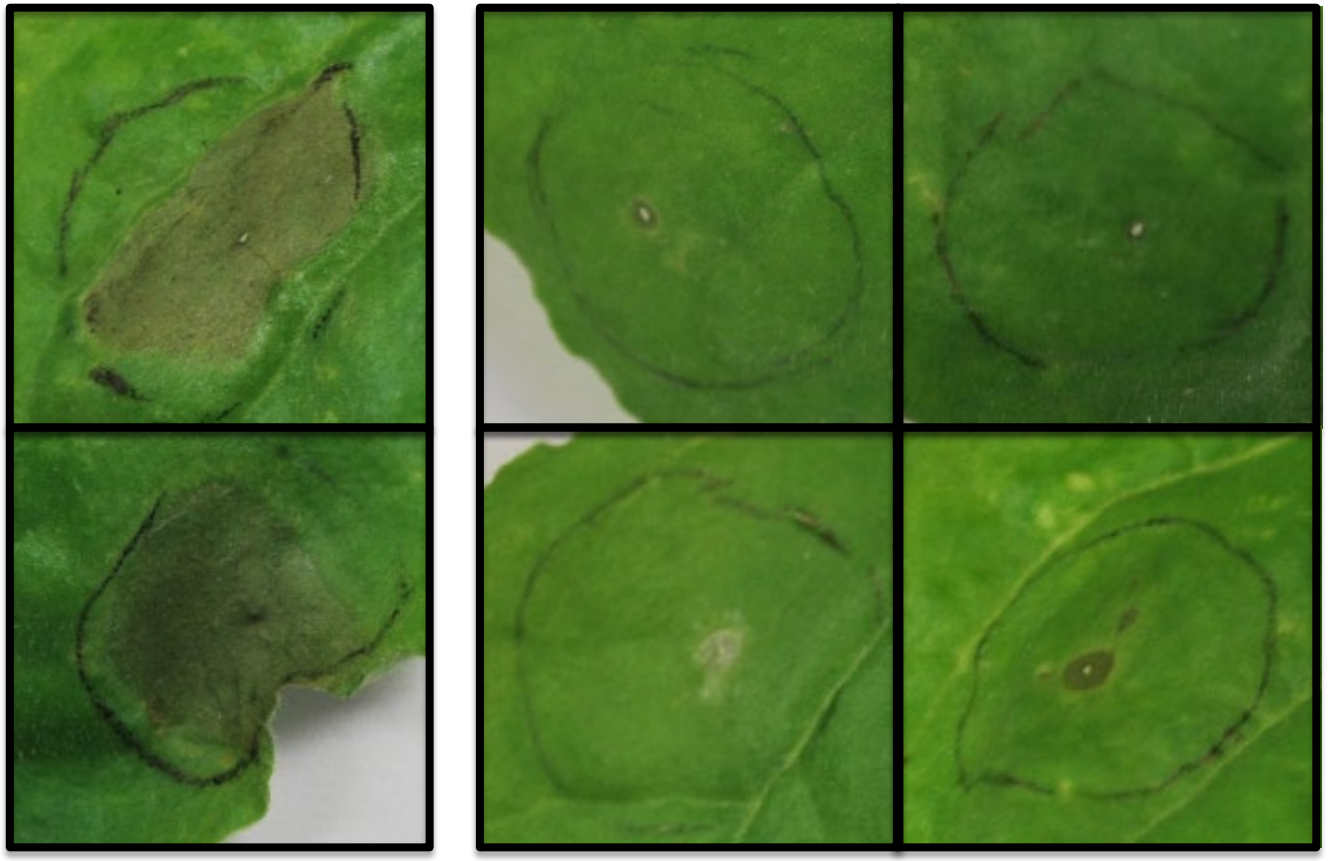
Inactivate Receptor Pathway => No Necrosis

Control

Inactive Receptor Pathway

SsNPα

SsNPγ



No Necrosis!

Why is this Information Useful?

- **Effector-Guided Breeding**

- **Use effectors (necrosis proteins) to screen for resistance to a disease**
  - Wheat tan spot (*Pyrenophora tritici-repentis*) – ToxA protein > *Tsn1* R gene
  - Wheat septoria blotch (*Parastagonospora nodorum*) – SnToxA, SnTox1, SnTox3
    - Kar-Chun Tan and Richard Oliver (Curtin University)

- **Look for lines that are not or are less affected by Ss necrosis proteins**
  - Lack receptor needed for recognition of necrosis proteins
  - Stimulate defense/immune response, but not engage the necrosis response

- **Advantages**
  - Focus on a single and important aspect of the disease
  - Controlled environment (greenhouse) – results often less variable
  - Guide to identify Resistance (R)/Susceptibility(S) genes
  - Coupled with traditional selection and breeding

Effector-Guided Screening

Arabidopsis thaliana

***A. thaliana* leaves exposed to SsNPα**

Path to Stem Rot Resistance?

- **Stay the course!**
- **Agronomic Practices**
  - Monitoring, timely fungicide application, rotations
- **GMO?**
- **Biological Control?**
  - *Coniothyrium minitans* (fungal parasite)
  - Fungal viruses (Daohong Jiang, Huazhong University)
- **Breeding**
  - Quantitative resistance
  - New methods for screening B. napus collections

People

Graduate Students

Zafer Dallal Bashi

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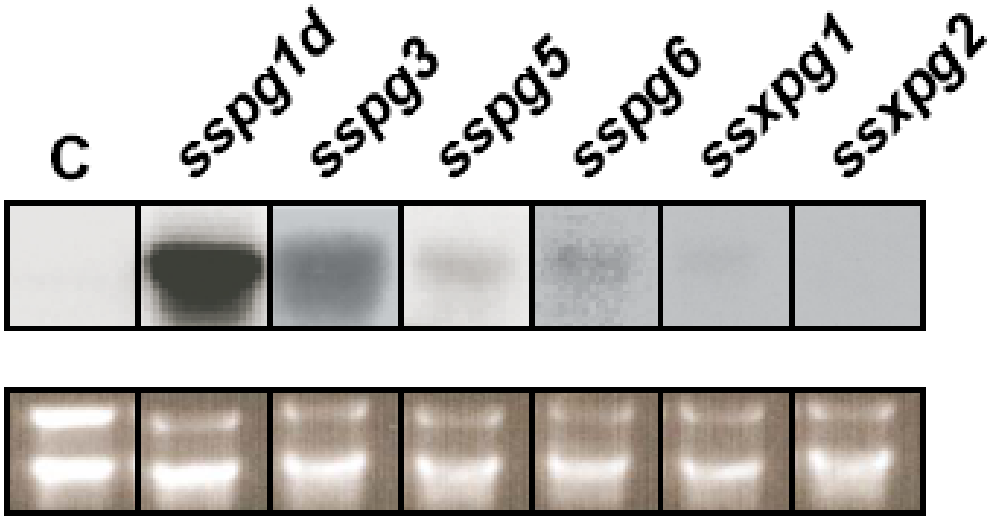
Sclerotinia Pectinases



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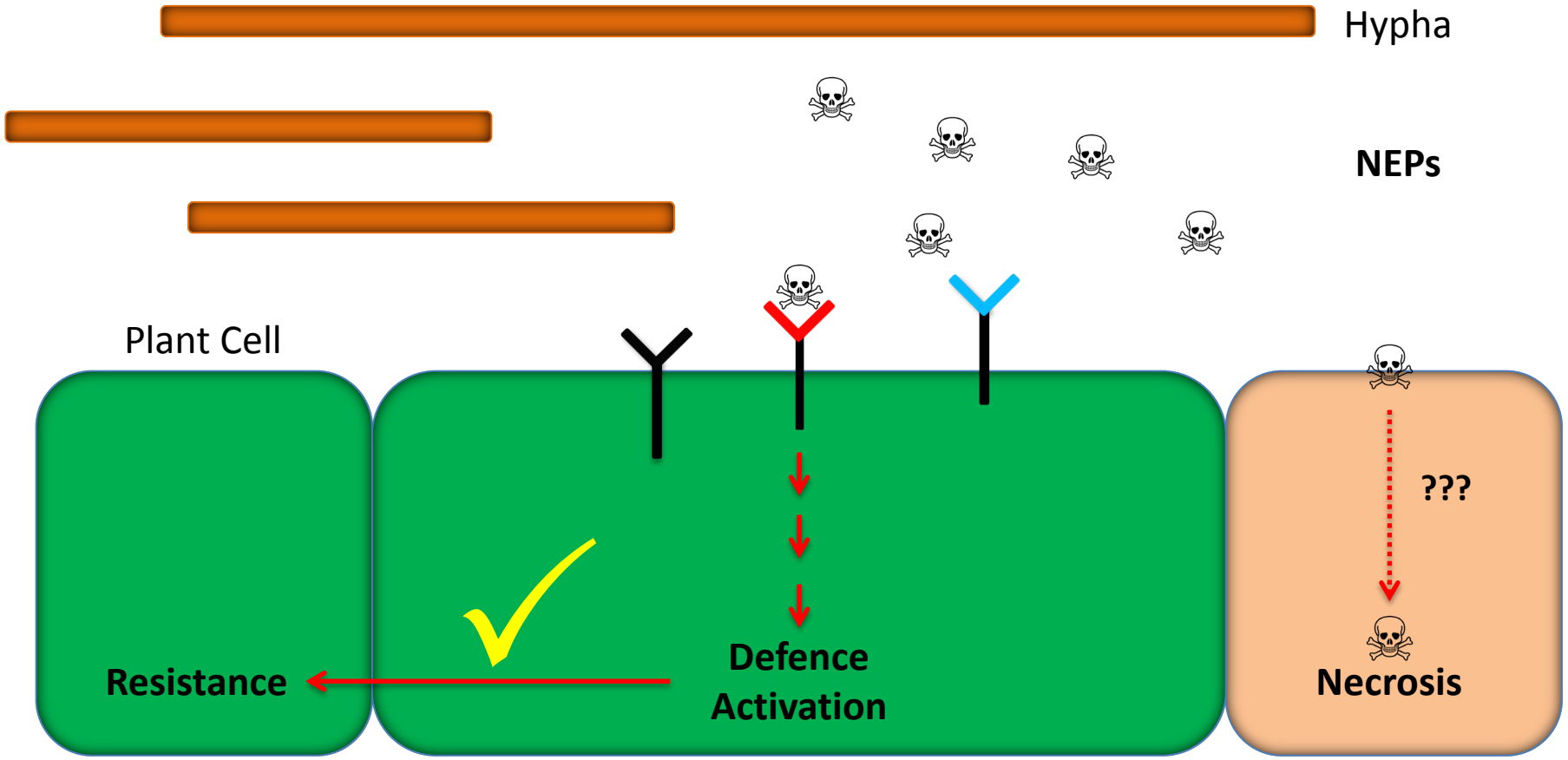
  - 2 exo-polygalacturonases (SsXPG1-2)
- Similar sets for cellulose and hemicelluose

Necrosis Proteins - A Tale of Two Fates

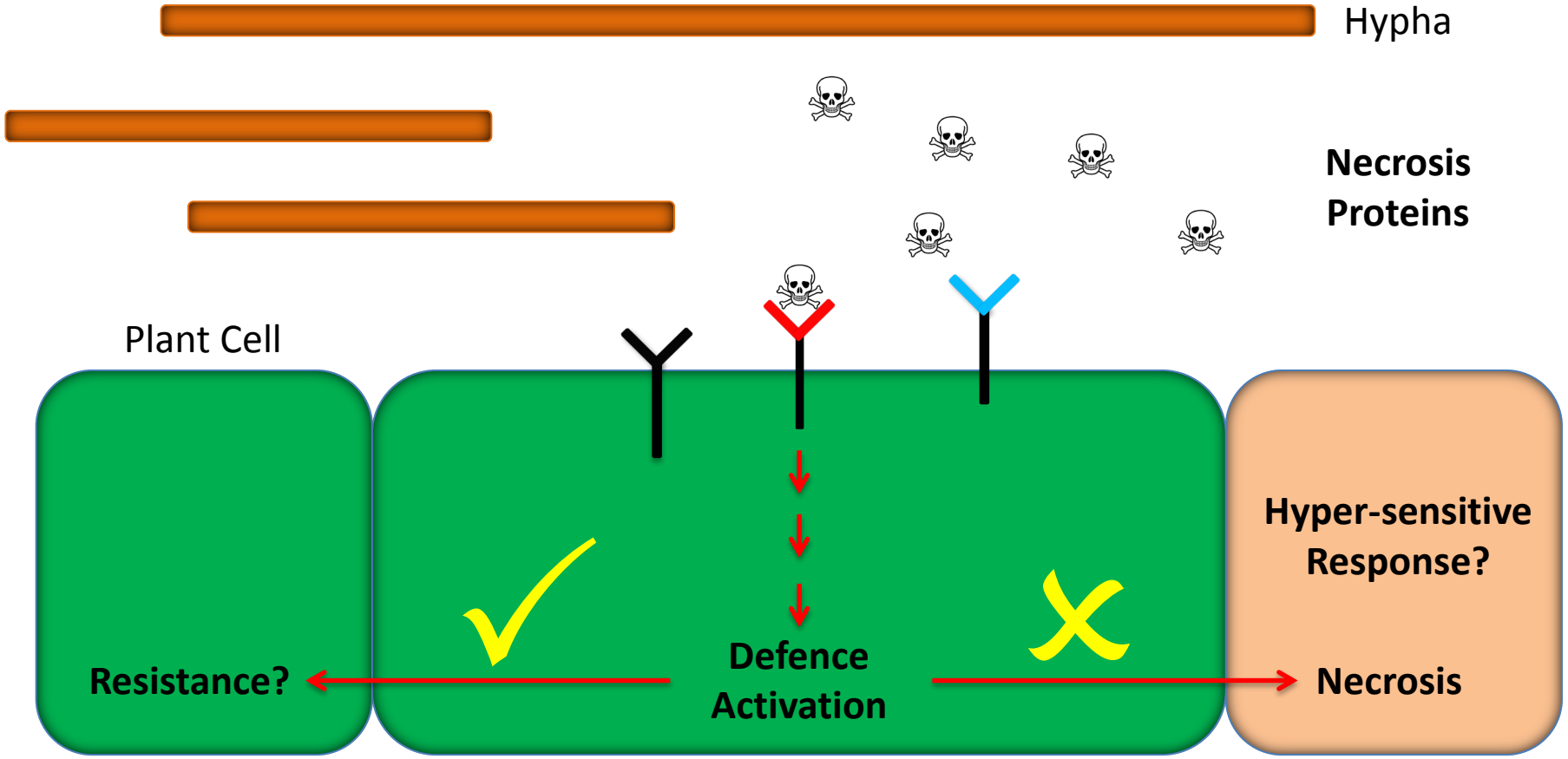
- **Separation of necrosis and induction of immunity activities**
- **Necrosis Ethylene Producing proteins (NEP)**



Albert, Nurnberger et al (2015) Nature Plants 1:15140.
Lenarcic et al (2017) Science 358:1431

Necrosis Proteins - A Tale of Two Fates

- **Necrosis and induction of immunity activities are <u>linked</u>**
- **Xyloglucanase 1 necrosis protein (Botrytis and Sclerotinia)**



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Removal of Receptor Pathway