Characterising fungal proteins that induce or suppress defence in canola

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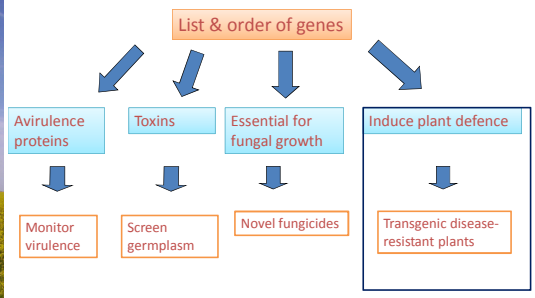
Fungal genome sequences



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How to deliver proteins into plants?

1. Make protein in the lab (using bacteria or yeast), purify and infiltrate into leaf with syringe
2. Clone gene of interest into Agrobacterium vector, infiltrate agro into leaf with syringe.
3. Coat plasmid with gene of interest onto gold particles and shoot into plant tissue

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Make protein *for* the plant

1. Make protein in test tube using bacteria or yeast system, purify and infiltrate into leaf with syringe
   - Purified proteins quite difficult to make
   - May not be accurately made by bacteria/yeast
   - Can be difficult to get large quantities of protein
2. First test: Sclerotinia protein Nlp2 (Necrosis and ethylene inducing protein secreted protein acts in apoplast of dictots)

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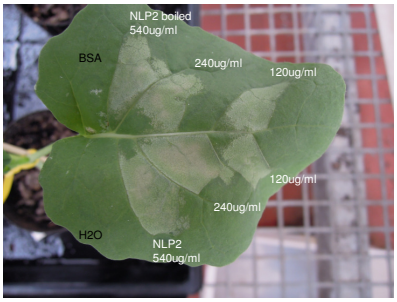
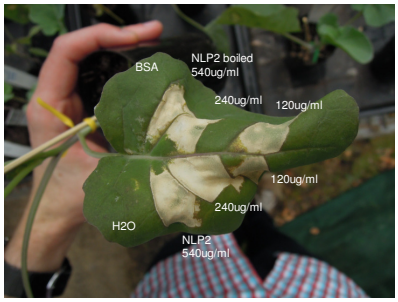
Infiltration of NLP2 after 24 hours



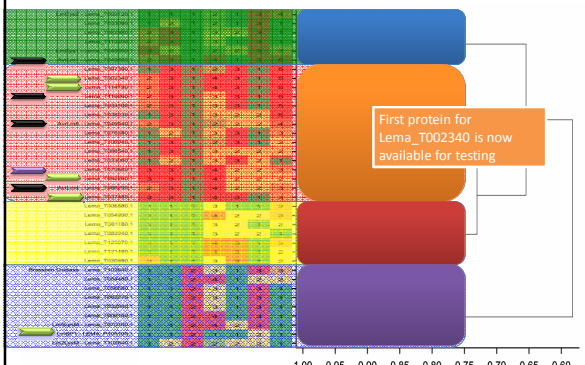
Plant 5
2nd leaf
3 weeks old

Infiltration of NLP2 after 7 days



Plant 5
2nd leaf
3 weeks old

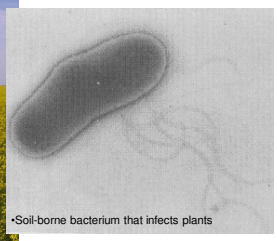
Genes selected for expression in plants



Rohan Lowe

Have *plant* make the protein for itself

Agrobacterium vector integrates DNA into plant genome and causes plant to make large amount of protein



•Soil-borne bacterium that infects plants

1.

2. •Crown Gall disease

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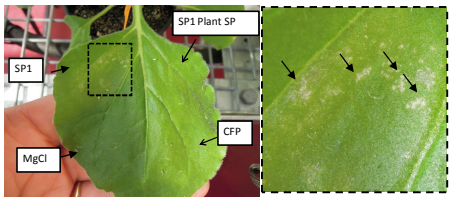
Agrobacterium infiltration method

Identify candidate gene → Clone into Agrobacterium vector → Infiltrate into leaves of canola and model plants → Assess plant reaction after 5 days

Agrobacterium infiltration of *Leptosphaeria maculans* Secreted Protein 1 (SP1) induces cell death in *Nicotiana bethamiana*



5 days

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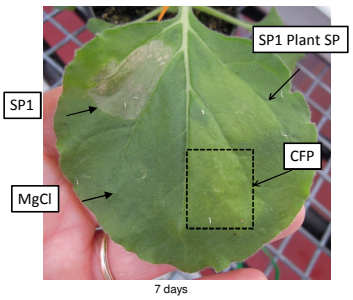
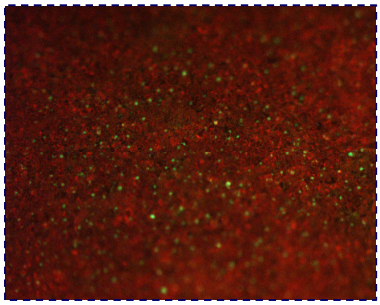


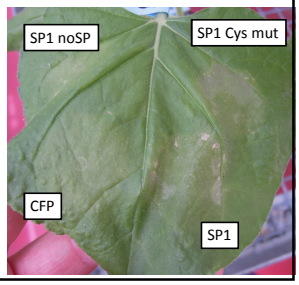
7 days

Nuclear fluorescence of CFP control in Nicotiana benthamiana but not canola



Experimental results with Agro method

- Agro does not work with canola
- Signal peptide is essential for cell death function of SP1
- Fourth cysteine residue is not essential for SP1 function
- Lema_T037480, Lema_T002340 and Lema_T092260 do NOT induce cell death in *N. benthamiana* but they *may suppress cell death*



Engineer *plant* to make the protein for itself

Plasmid vector containing plant promoter is coated onto gold particles and "shot" into leaf

Pros:

1. Simple cloning procedure
2. No agrobacterium to cause reaction in canola

Cons

1. Establishing reliable infiltration of GUS substrate time consuming
2. Cannot make stable transformants

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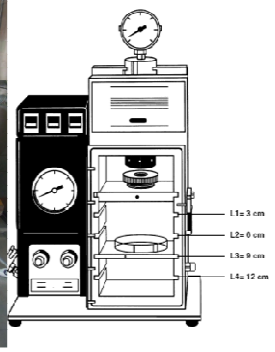
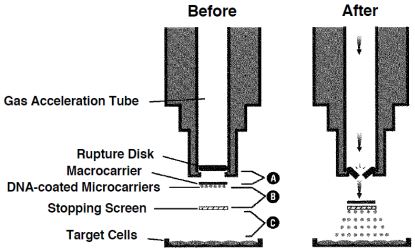
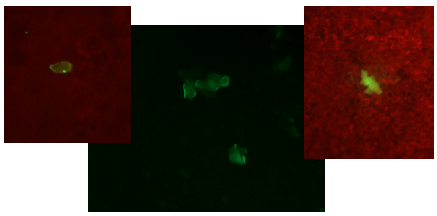
PDS1000



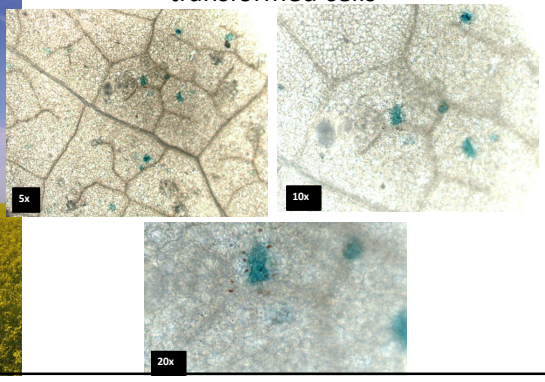
How DNA is delivered by the device



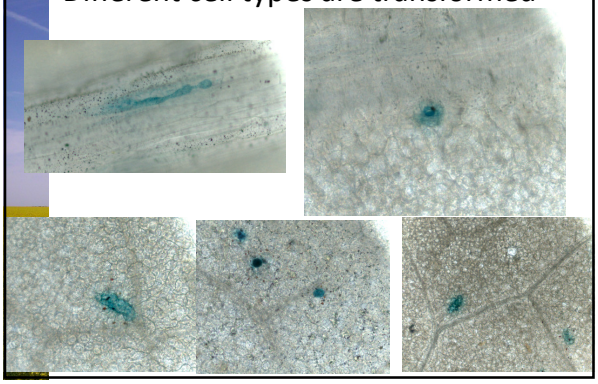
Green Fluorescent protein expressed in epidermal cells of canola leaves



Blue GUS staining on Westar marks transformed cells

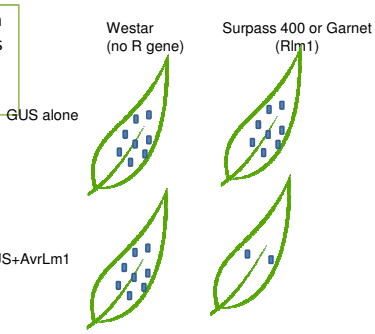


Different cell types are transformed



Proof of concept with known Avirulence gene

AvrLm1 expression in Rlm1 leaf causes cell death (no transformed cells)



Further Experiments with biolistics

- Characterise canola cultivars for presence of resistance genes corresponding to newly uncovered avirulence gene candidates
- Conduct mutational analysis on candidate genes to discover how they work
- Tag candidate proteins with fluorescent tags to track where the fungal proteins go in the plant tissue—mode of action?

Summary

- I have developed three complimentary methods to express fungal proteins in plants
  - Direct injection of proteins is simple but making proteins is difficult and time consuming
  - Agrobacterium-mediated infiltration of *Nicotiana benthamiana* is a robust method to test for induction (and suppression) of cell death
  - Biolistic delivery of fungal genes is a feasible method to test for induction of cell death in canola
- I have confirmed the cell death-inducing phenotype of LmSP1 and SsNLP2
- I have shown Lema_T037480, Lema_T002340 and Lema_T092260 do NOT induce cell death in *N. benthamiana*

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