Phenotypic characterisation of quantitative resistance to blackleg in canola

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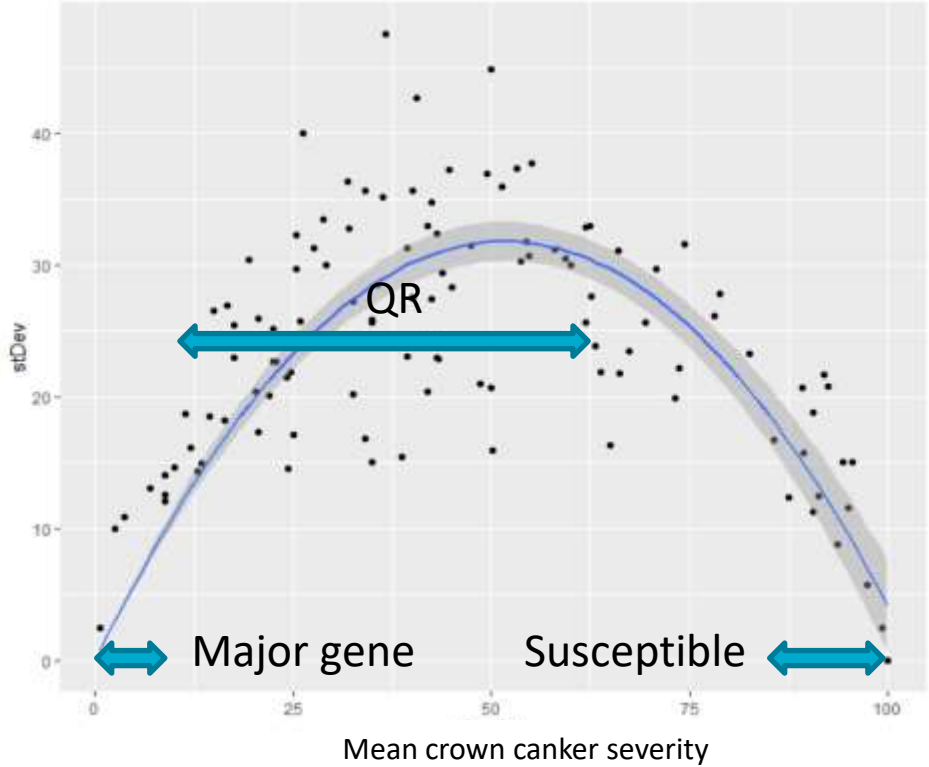
Quantitative resistance (QR)

- Critical for sustainable control of blackleg in Australia

- Difficult to phenotype
  - multiple minor genes involved apparently strong environmental effects (environment and/or pathogen population)
  - masked by effective major genes
  - presumed to be broad-spectrum, race non-specific
  - limited defined germplasm stocks(some consistency between studies)

- Lack fundamental knowledge on interaction & repeatable screening method

High level of variation for QR



Controlled environment experiment with 11 cultivars and 11 isolates.

Crown canker severity measured at maturity following inoculation of cotyledons.

Disease phenotype = Host (G_h) x Pathogen (G_p) x Environment (E) x M

How do we measure disease – visual score, molecular methods?

When/where is resistance expressed?
Influenced by major genes?

Is resistance broad spectrum or isolate-specific?
Pycnidiospores/ascospores

What is the contribution of E?
Controlled environment/field?
Optimised to improve disease expression?

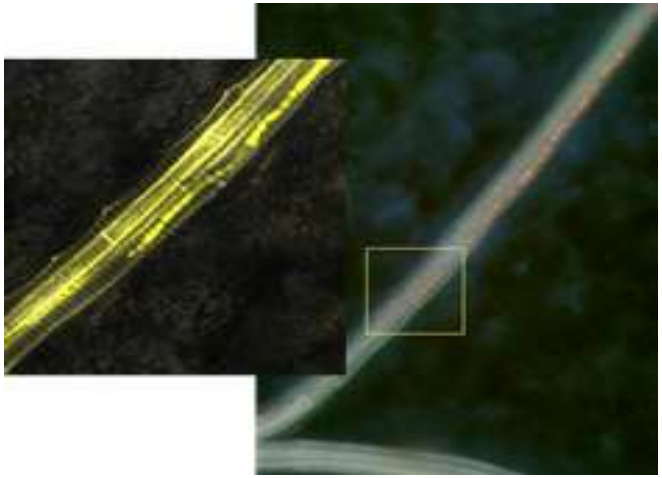
Disease phenotype

- Measurements in different tissues – cotyledons/leaves, petioles, stems

- Quantitative and non-subjective assays
  - PCR – specific to *L. maculans* & high resolution
  - chitin – not specific to blackleg & lower resolution

- Quantification in absence of visual symptoms

- Consistent between assays, sensitive



Schnippenkoetter et al. 2021

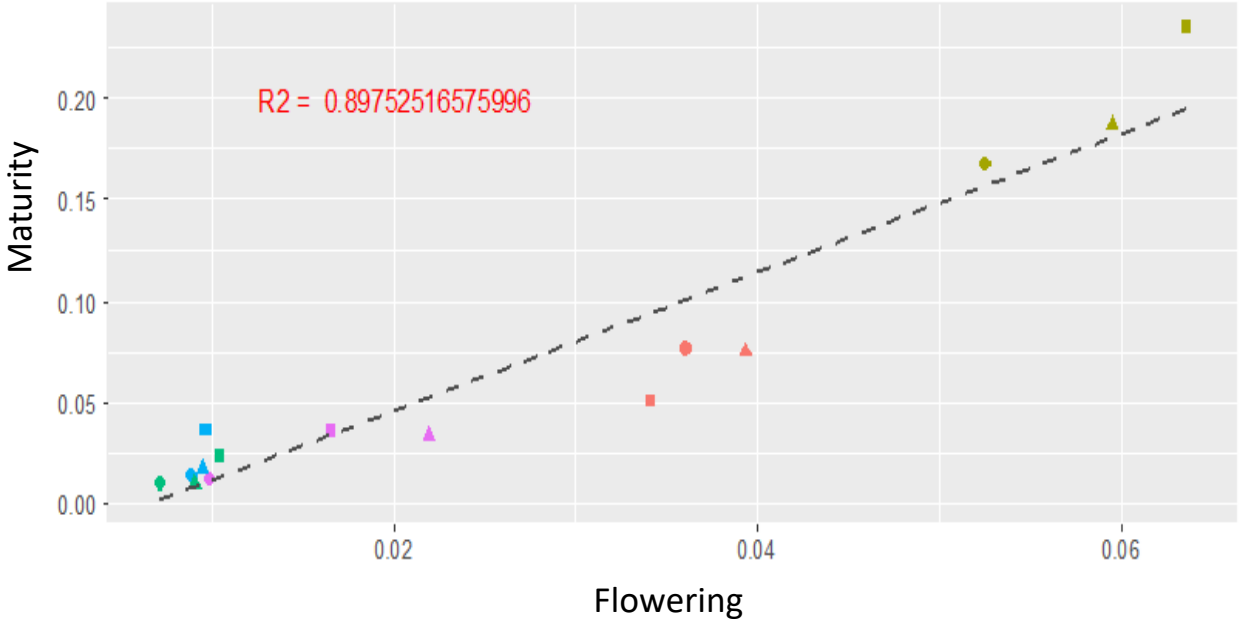
$Genotype_{host}$ (G_h): where is QR expressed?

- fungal biomass using ddPCR – robot
- tissues/timepoints to differentiate QR
- relationship between early and later levels of infection

- 5 host lines with range of QR x 3 isolates x 40 replicate plants
  - ✓ cotyledons
  - x petioles
  - ✓ crowns (6 weeks – maturity)

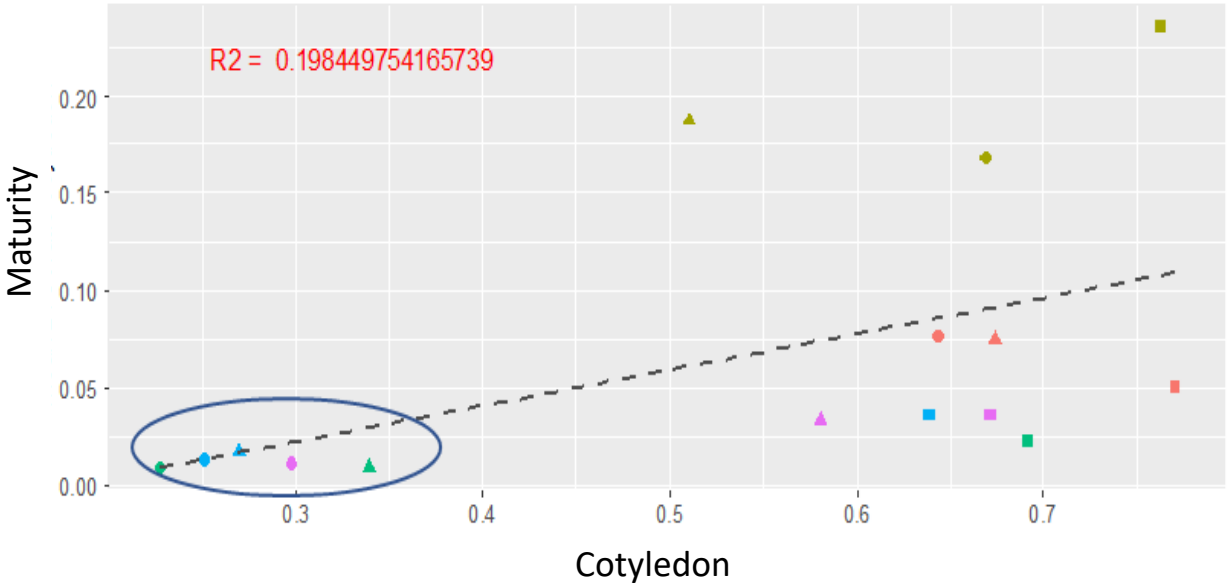
- variability reduced – cotyledon, crown canker

QR identification in the crown prior to maturity



- detection at start of flower predicts maturity

Does cotyledon disease load predict QR?

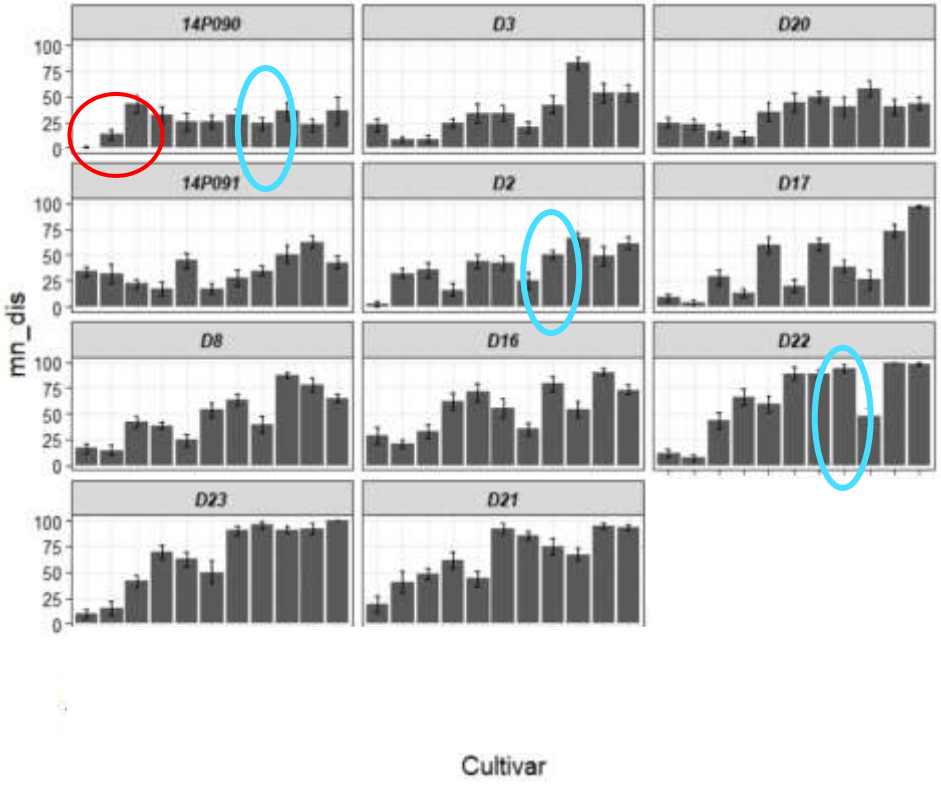


- No but could be used as preliminary screen for susceptibility
- wider screen of gremplasm

G_h: influence of major genes on QR

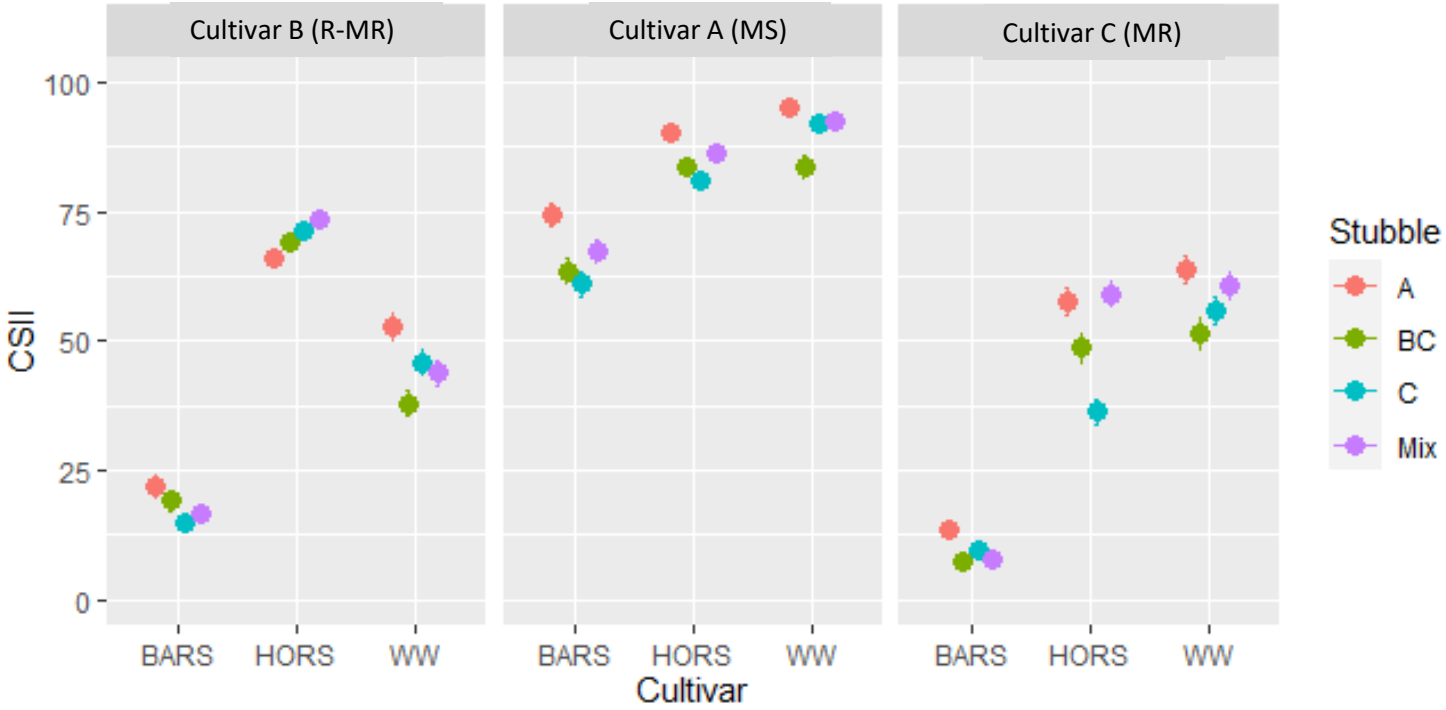
- Larger lesions in presence of Rlm1 & Rlm4 in combination
- Small number of lines tested, requires repeating
- Does this effect persist through to crown canker?

$Genotype_{pathogen}$ (G_p): broad spectrum or isolate specific



- No partial resistance to all isolates, but instead reacts with individual isolates differently
- Some cultivars are resistant or partially resistant to all isolates, suggesting they have broad QR.
- Rank across all isolates reflects blackleg ratings of the cultivars
- Combinations of QTLs or broadspectrum QTLs?
- Screening with mixed inoculum?

Environment (E):



- sig. effects of environment, host genotype

Summary

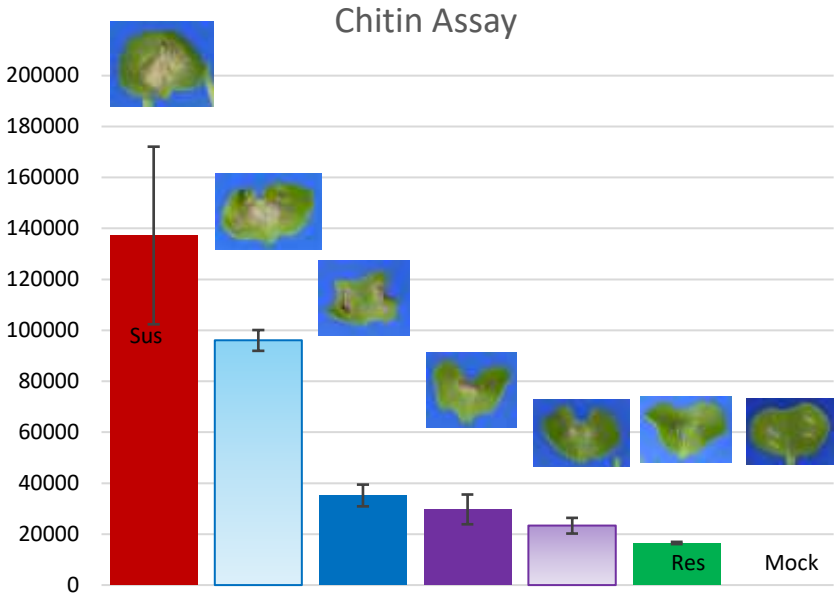
- QR expressed in cotyledons and crown, not petioles
- variability not due to mechanism of inoculation - biological
- Blackleg disease phenotype is extremely complex
- Large environmental effect
- Screening with single/population inoculum for detection of QTL's?

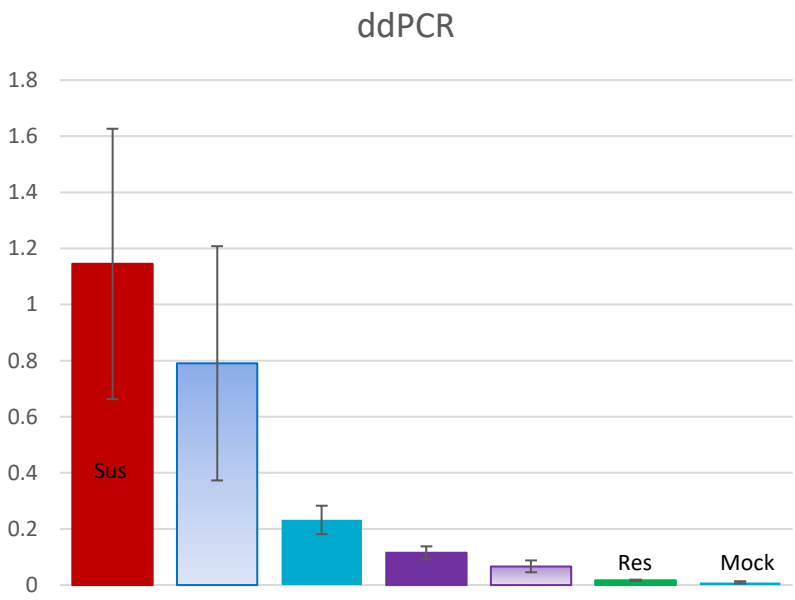
Development of genetic resources

- Westar x Darmor-bzh DH population
  - bulking up
  - phenotyping for blackleg and developmental traits

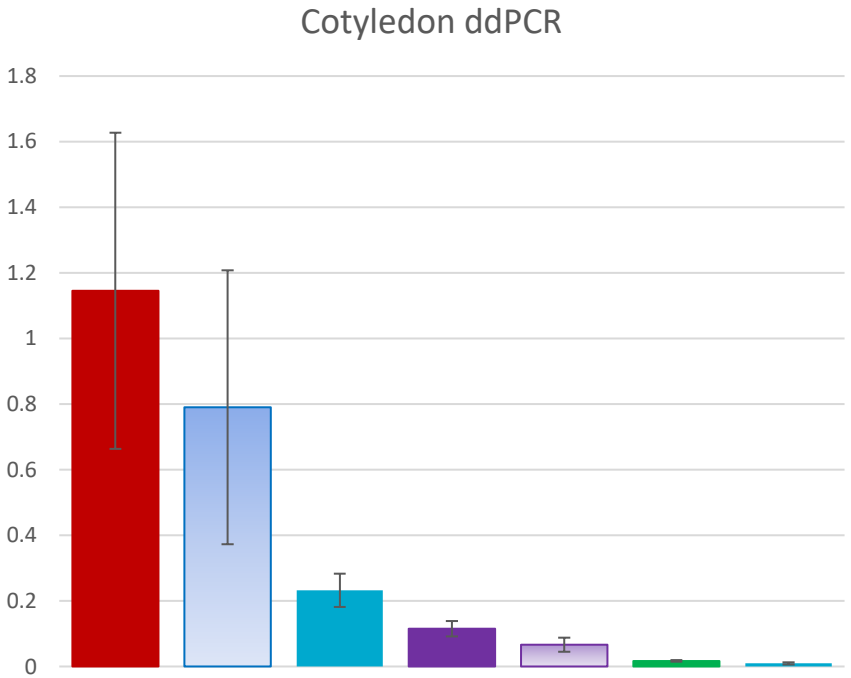
- susceptible background
  - Future resource for introgression of QTLs and major genes

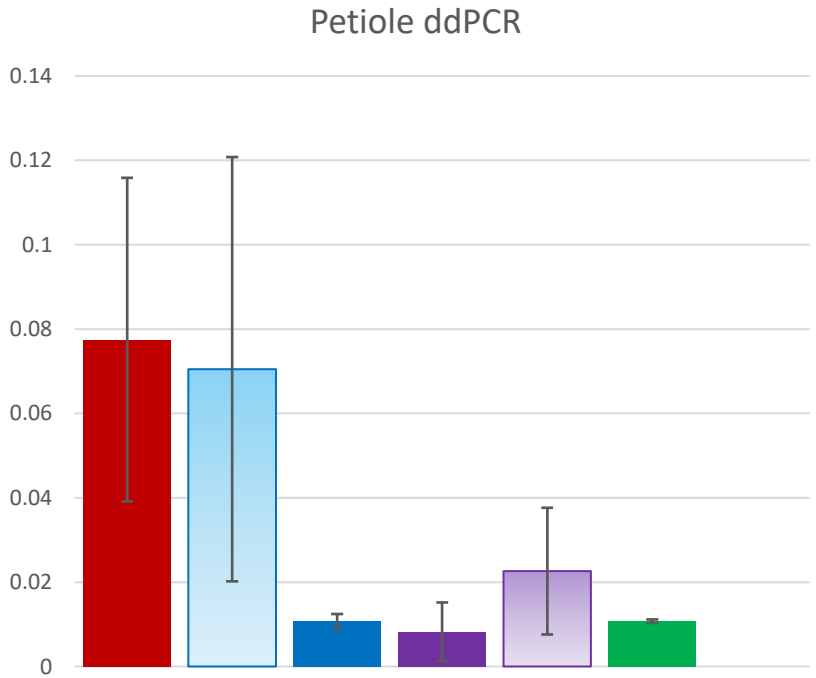
Bioassays detect biomass differences in cotyledons



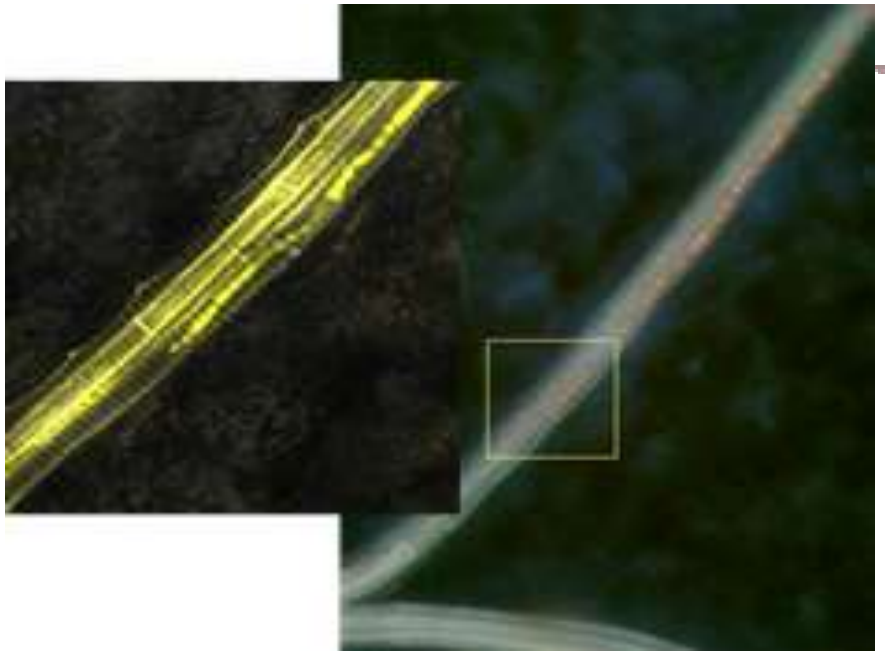


ddPCR detection in different host tissues





Fluorescence microscopy

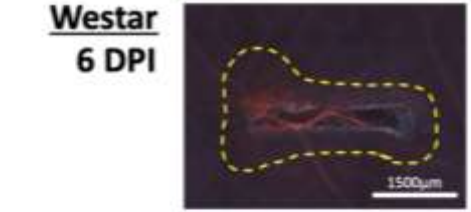


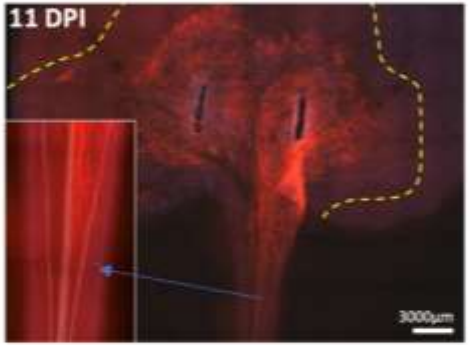
Westar

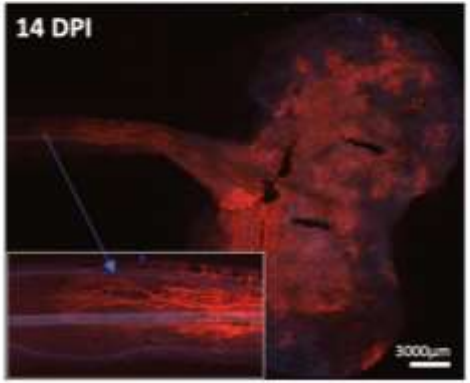
6 DPI

11 DPI

14 DPI

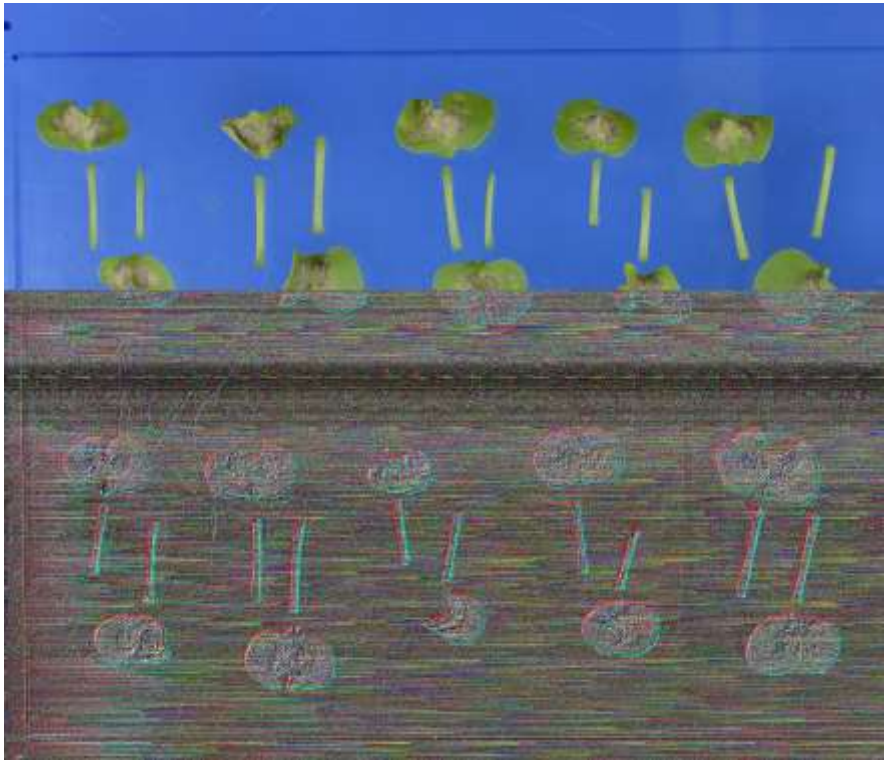


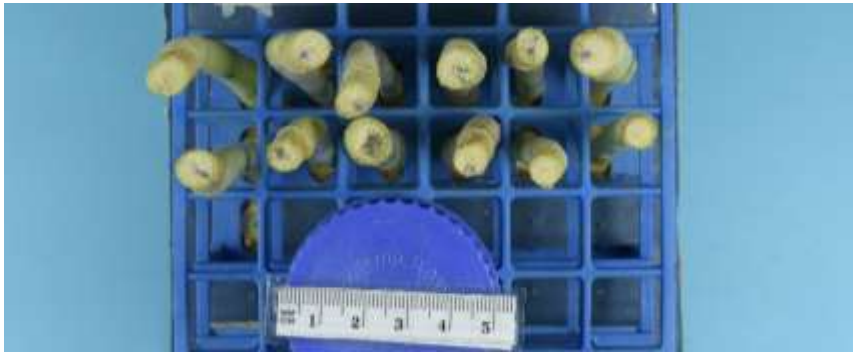




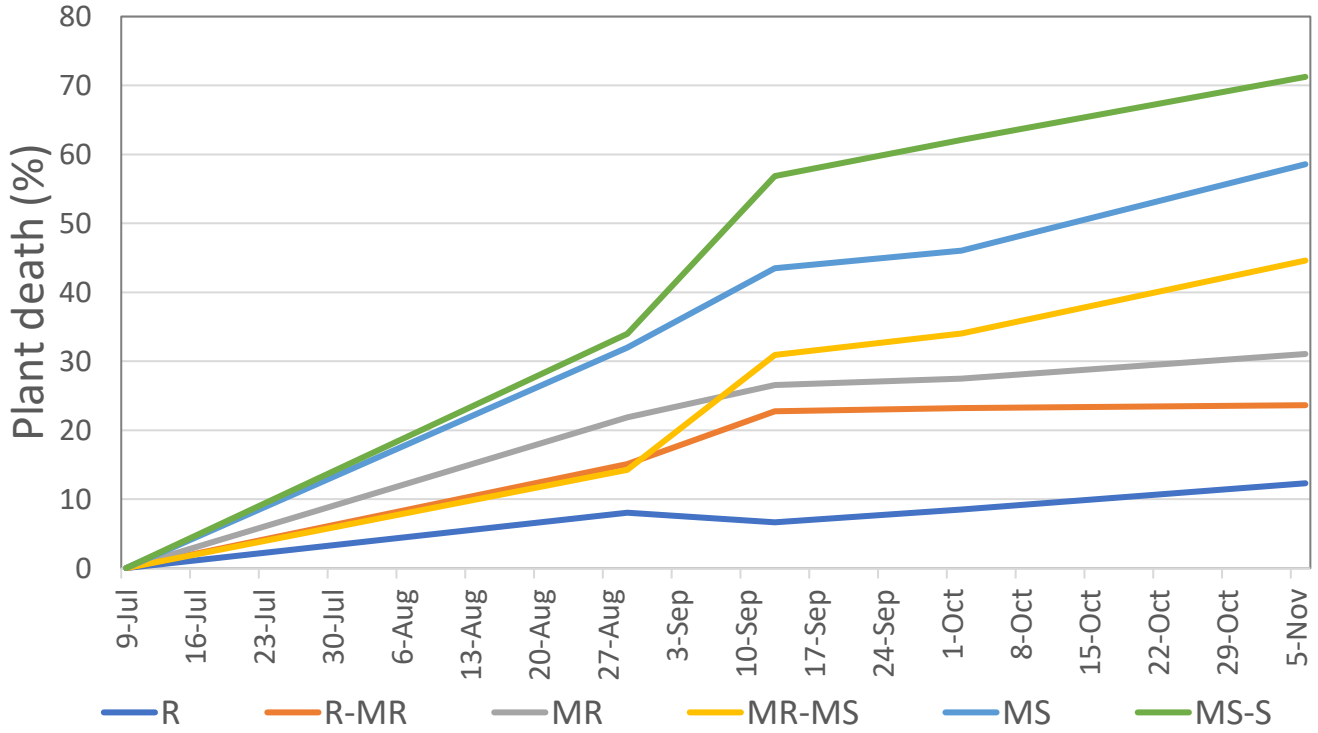
A minor setback!

Greg via Riot-act





2. Higher throughput phenotyping



Wangary, SA 2019

3. Genetic stocks – measurable effects of defined single and multiple QTL's

	QR phenotype components
LepQ-A01	?
LepQ-A08	?
LepQ-C06	?
LepQ-A01+ LepQ-A08	?
LepQ-A01+ LepQ-C06	?
LepQ-A08+ LepQ-C06	?
LepQ-A01+ LepQ-A08 + LepQ-C06	?

- *LepQ-A02* **(ex Darmor) to be added**
- **Available stocks in Topas background**
- **Introduce stocks into susceptible derivative devoid of resistance suppressor**

Collaborators- AgCanada

4. Blackleg adaptation to QR

Row Labels	R gene	Blackleg rating	D2	D3	D8	D16	D17	D20	D21	D22	D23	Average
ATR-Bonito	A	MS	50	42	39	79	39	40	74	94	96	62
ATR-Mako	A	MR	16	24	39	63	13	11	62	66	69	40
AV-Sapphire	B	?	43	34	54	55	21	45	93	89	50	54
BASF3000TR	B	MS-S	62	54	65	73	98	43	94	98	100	76
DG408	AC	MS	25	20	64	35	61	50	86	89	91	58
Hyola575	BF	R	3	23	16	29	9	25	19	12	10	16
Hyola580CT	BC	R-MR	33	9	14	21	4	23	41	7	15	18
Pioneer43Y92	B	R-MR	36	9	42	33	29	17	49	43	41	33
PioneerSturt	C	MS-S	49	53	78	91	74	40	95	99	92	75
Stingray	C	MR-MS	44	34	25	71	60	35	44	59	63	48
Tornado	B	?	66	83	88	55	26	58	67	47	91	65

4. Blackleg adaptation to QR

Blackleg rating	**Average CSII**
R	21
R-MR	18
R-MR	33
MR	40
MR-MS	48
MS	62
MS	58
MS-S	76
MS-S	75

Next steps

1. Finescale phenotyping

- tissues/growth stages to differentiate QR response
- improved disease expression
    - inoculum – pycnidiospores vs ascospores
    - environment

2. Higher throughput phenotyping

- repeat measures in disease nurseries
- exploit under controlled conditions

Next steps

3. Genetic stocks

- Bulk & screen Canadian germplasm – single/multiple QTL NIL's
- Darmor-bzh crosses
- Identifying appropriate susceptible background for crossing

4. Blackleg adaptation

- screen progeny from 'vir' x 'avr' crosses

- Controlled environment screens (pycnidiospore inoculum)
  - literature suggests lesion development & growth through the petiole possible predictors of QR
  - biologically relevant phenotyping method
  - small number of lines
  - repeatable?
  - relevance to field

- Field screen (ascospore inoculum)
  - visual crown canker severity/survival
  - large number of lines
  - diverse environments (E) & blackleg populations (Gh) = high phenotypic variability
  - repeatability?