Phenotyping for quantitative resistance to blackleg crown canker

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The starting position

- Controlled by numerous QTL (some consistency between studies)
- Presumed to be stable, broad-spectrum resistance
- Masked by effective major gene resistance

- Field screen (ascospore inoculum)
  - visual crown canker severity/survival
  - larger number of lines
  - diverse environments (E) & blackleg populations (Gh) = high phenotypic variability
  - not repeatable
- Controlled environment screens (pycnidiospore inoculum)
  - lesion development & growth through the petiole possible predictors of QR
  - phenotyping method?
  - small number of lines
  - repeatable

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

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

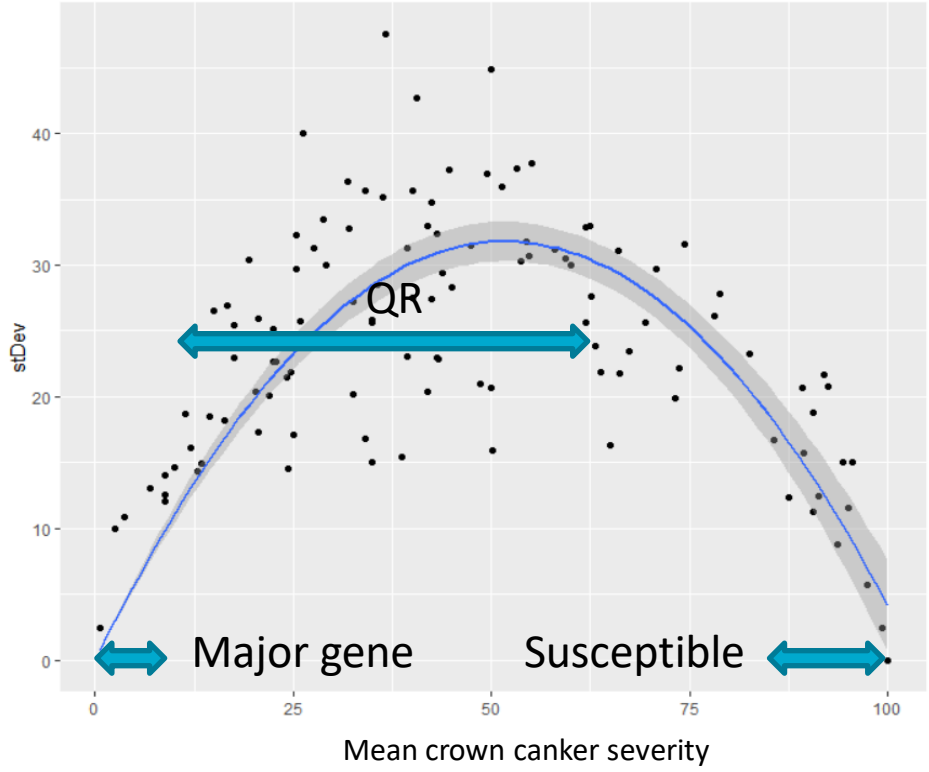
When/where is resistance expressed?

Is resistance broad spectrum or isolate-specific?

Optimised / improve disease expression?

What is the contribution of E?

High level of variation for QR



Controlled environment experiment with 11 cultivars and 11 isolates.

Crown canker severity measured after inoculation of cotyledons.

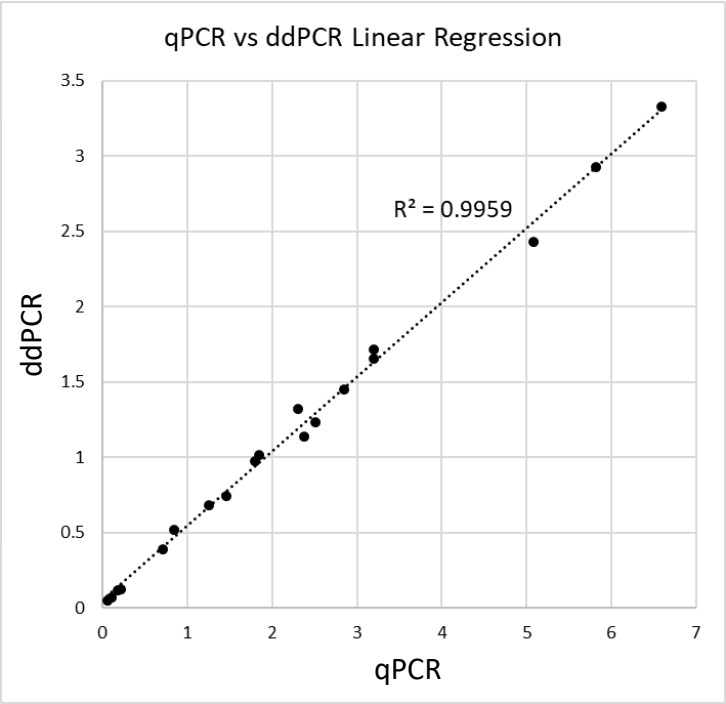
Measuring the phenotype

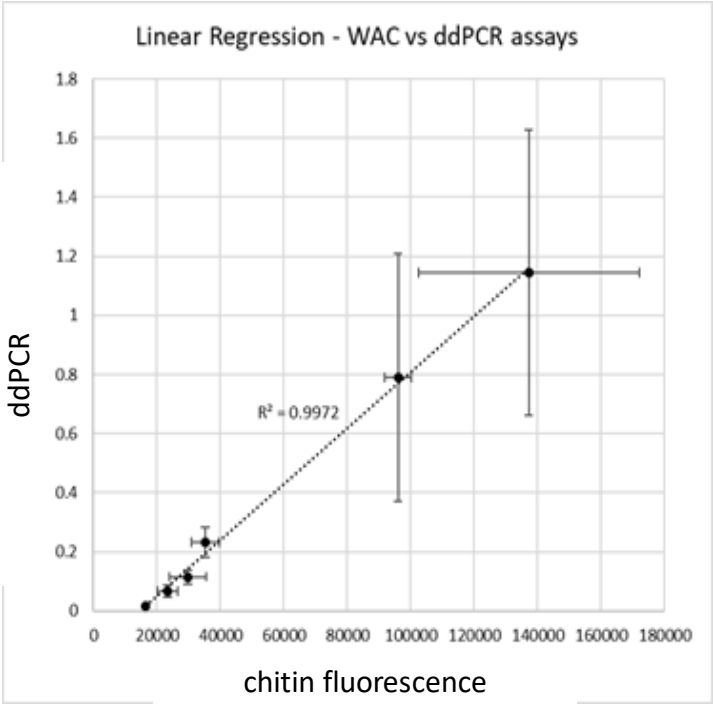
- traditional visual scores – qualitative, assessor bias

- quantitative measure of pathogen load
  - molecular (qPCR, ddPCR): specific to *L. maculans*
  - WAC: chitin-binding fluorescent tag

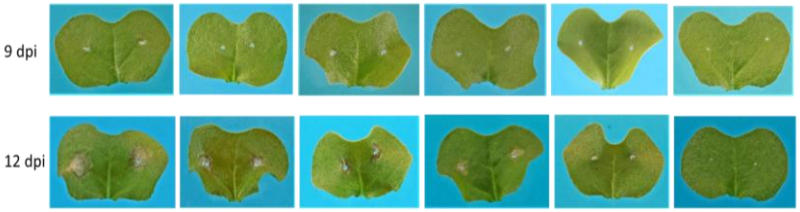
- 5 cultivars x 1 isolate x 14 replicate plants/timepoint - glasshouse

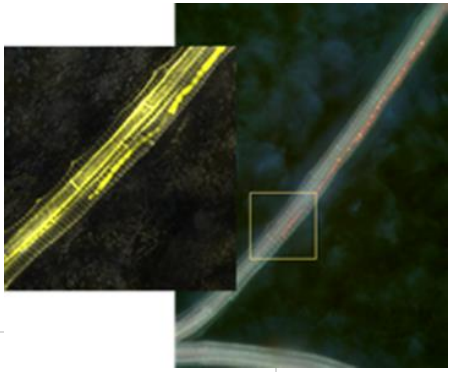
9dpi	cotyledons/petioles
12dpi	cotyledons/petioles
4 weeks	crown
start of flowering	crown
maturity	crown

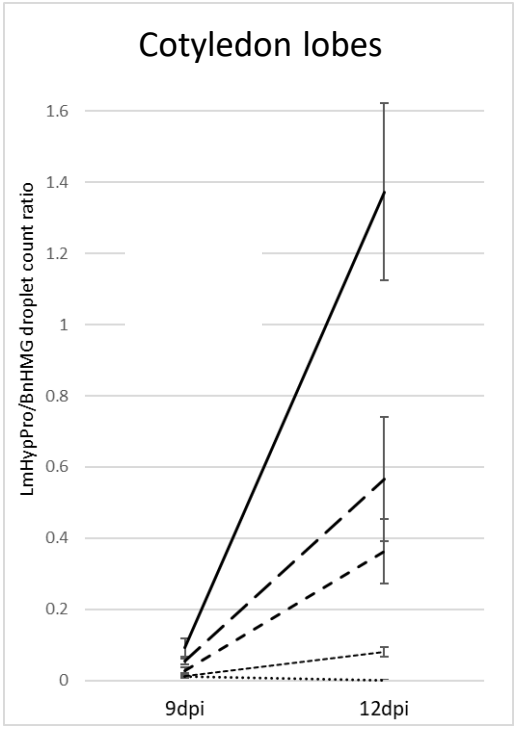


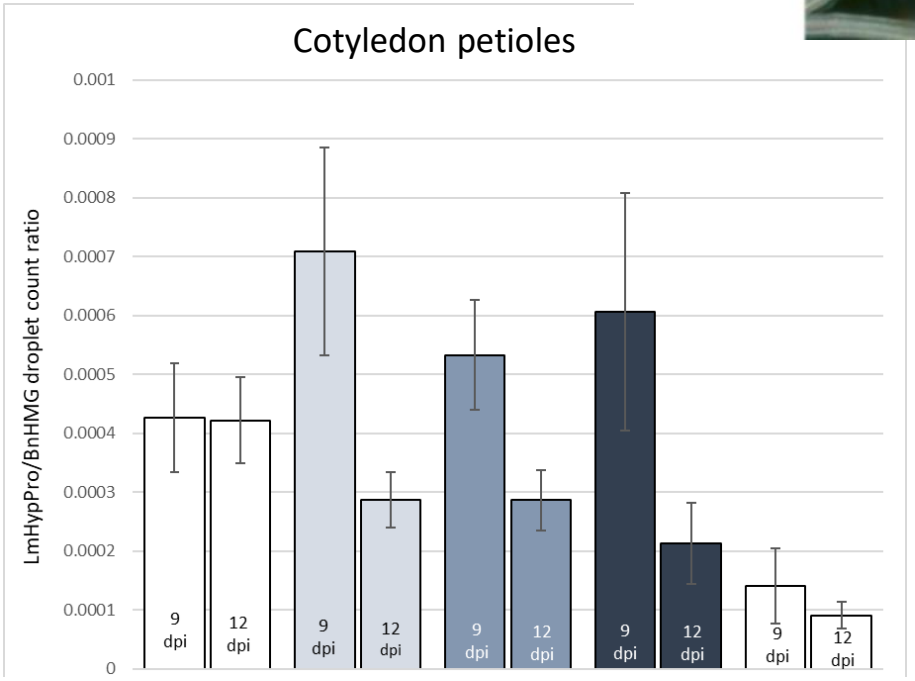


- fungal biomass measurements accurate & repeatable, consistent between assays & across environments
- PCR assays specific and “high throughput”
- chitin assay good for detection at high pathogen loads but not specific, method is laborious

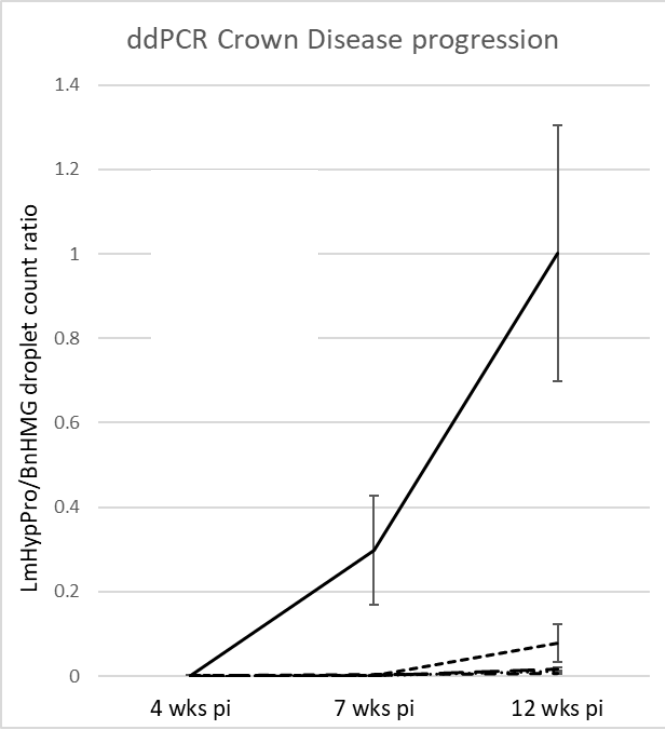

9 dpi
12 dpi




Cotyledon lobes
LmHypPro/BnHMG droplet count ratio
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
9dpi
12dpi


Cotyledon petioles
LmHypPro/BnHMG droplet count ratio
0.001
0.0009
0.0008
0.0007
0.0006
0.0005
0.0004
0.0003
0.0002
0.0001
0
9 dpi
12 dpi
9 dpi
12 dpi
9 dpi
12 dpi
9 dpi
12 dpi
9 dpi
12 dpi

Crown canker



- few strands of hyphae in petiole, unable to detect with chitin assay
- low disease progression at crown (except in Westar) – why?

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

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

When/where is resistance expressed?

Is resistance broad spectrum or isolate-specific?

Optimised / improve disease expression?
What is the contribution of E?

Where is QR expressed *in planta*?

- 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

- inoculations on (1) cotyledons and (2) cut petioles of 1st true leaf

  Sampled:

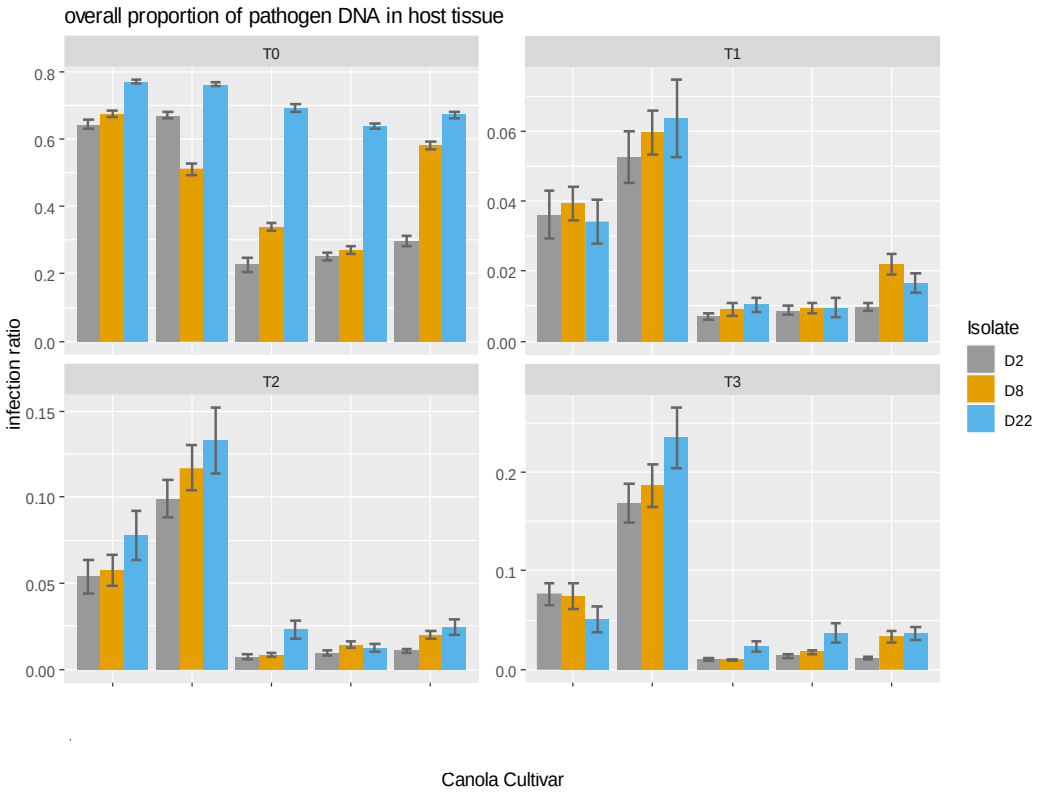
  - T0 = cotyledon (12dpi)
  - T1 – T3 = crowns (6 weeks – maturity)

- fungal biomass using ddPCR – robot

How reliable is infection in the glasshouse?

timepoint	N successful pcr assays	N undetectable infections	Proportion detectable
T0	593	1	0.998
T1	598	122	0.796
T2	597	65	0.891
T3	595	32	0.946

How does pathogen load progress through time?



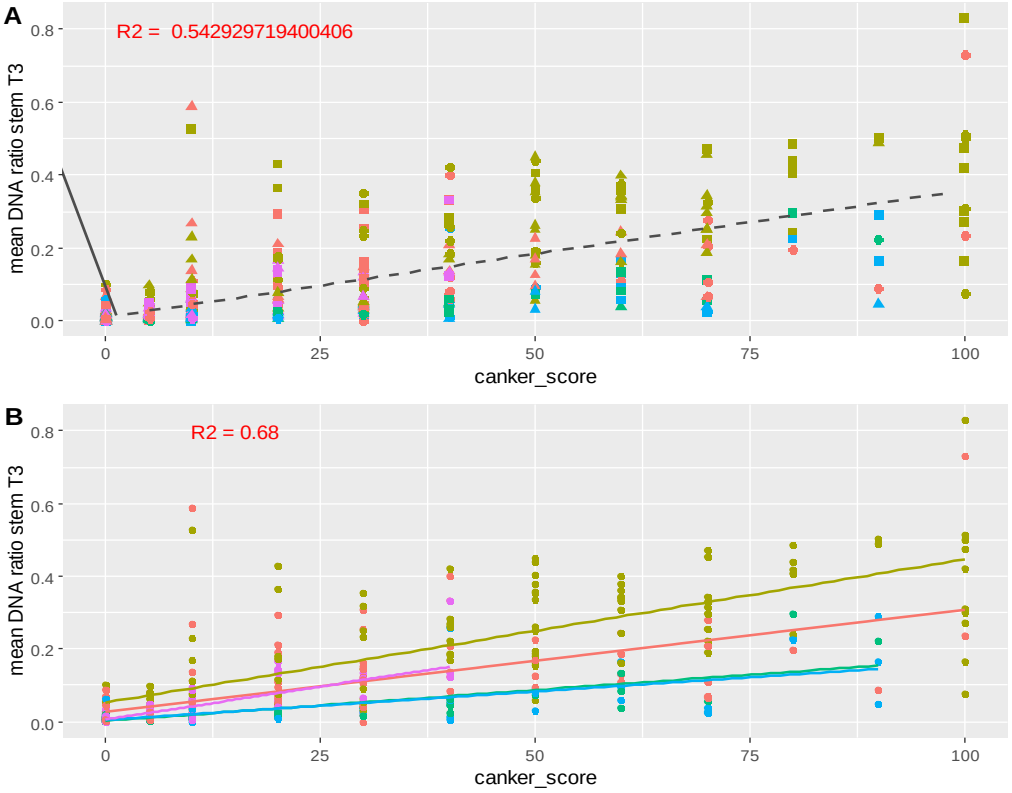
Cotyledons (T0):

- High level of fungus cf. crowns

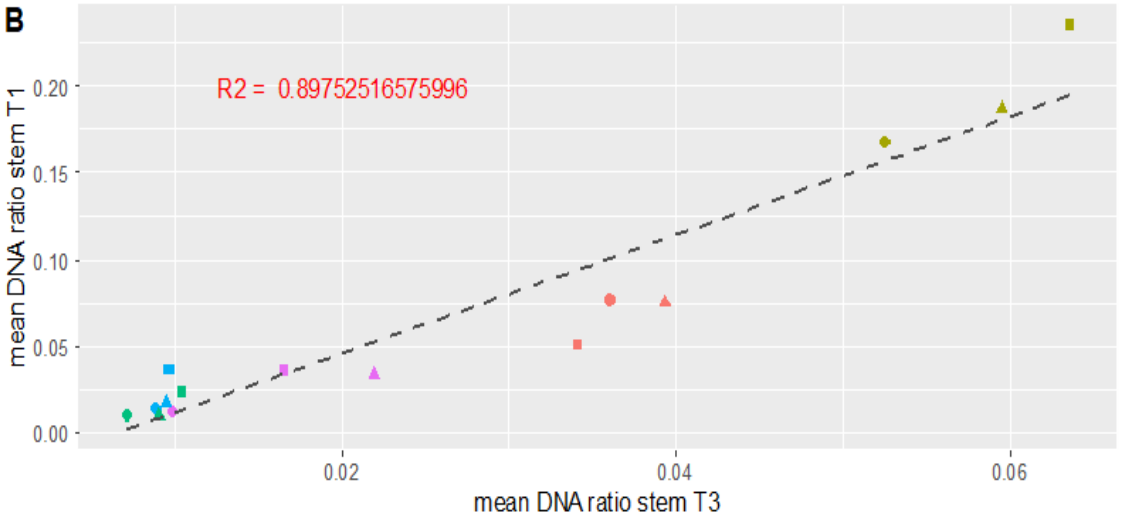
Crowns (T1-T3)

- Disease progresses from T1 – T3

Does fungal biomass relate to visual canker score?

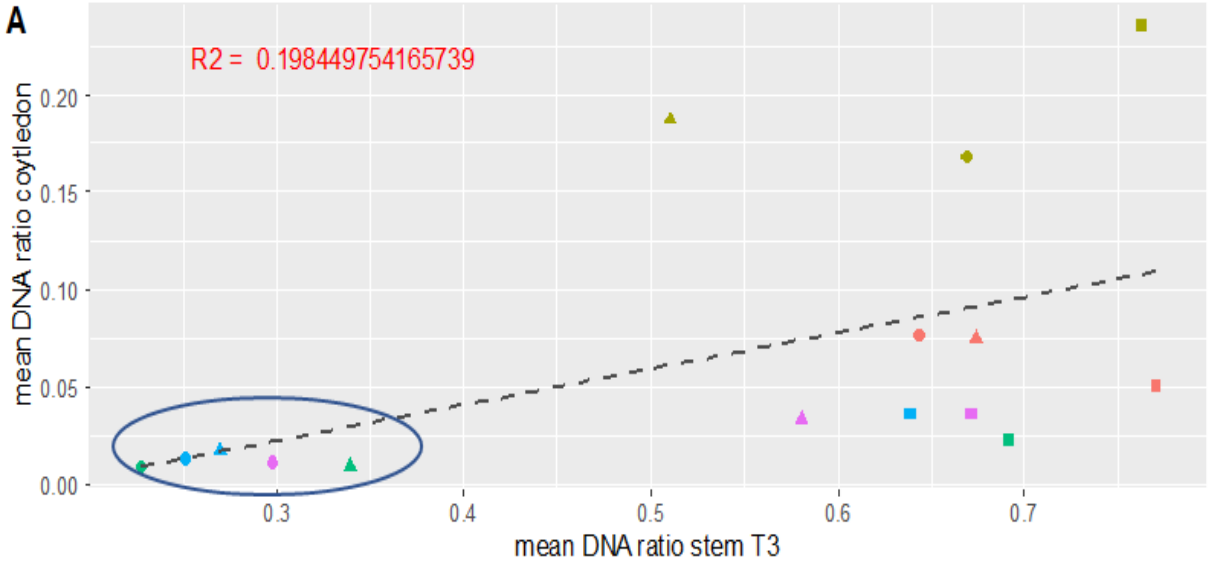


QR identification in the crown prior to maturity



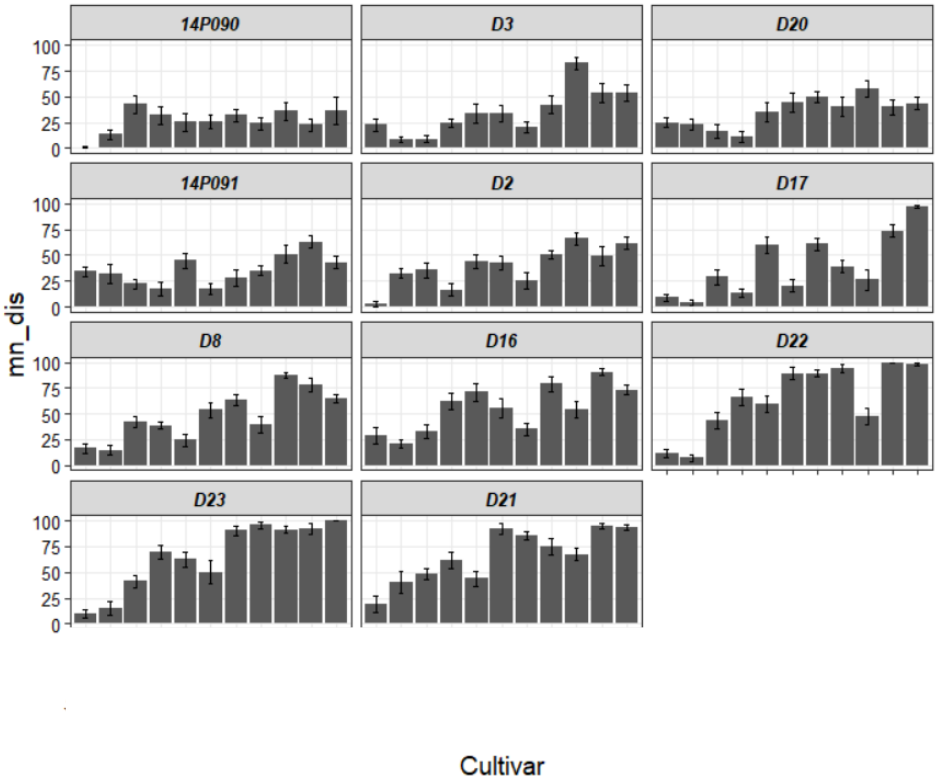
- detection at T1 (start of flower) predicts maturity for the 3 isolates tested

Can cotyledon disease load predict QR?



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

Nature of QR: 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
- Screening with mixed inoculum?

Summary

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Summary

- QR is complex interaction – G x G x E
- Quantitative methods developed
- Early detection possible – shorter screening time
- Cotyledon screen tool to eliminate susceptible lines – wider set of germplasm/isolates required
- High level of variability in phenotype – replication
- Environmental influence