GRDC
GRAINS RESEARCH & DEVELOPMENT CORPORATION

Development of artificial screening protocols for Upper Canopy Infection and what it has taught us so far (Program 1)

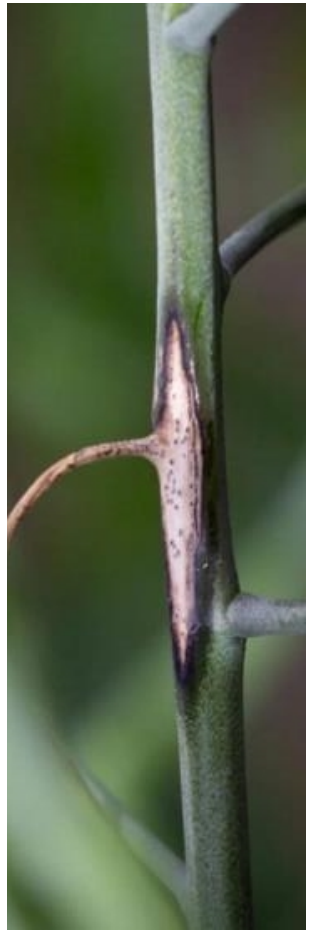
Angela Van de Wouw, Steve Marcroft, Susie Sprague

Upper Canopy Infection – what is it?

- Infection of upper stems, branches, flowers, peduncles
- Caused by *L. maculans*

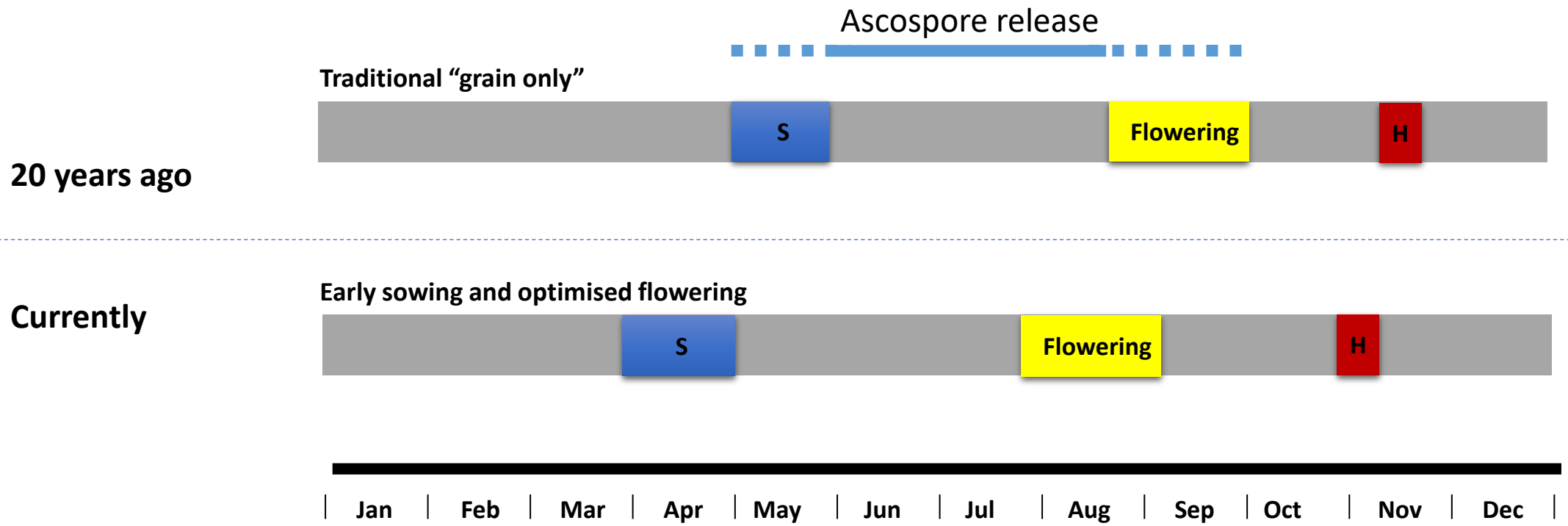




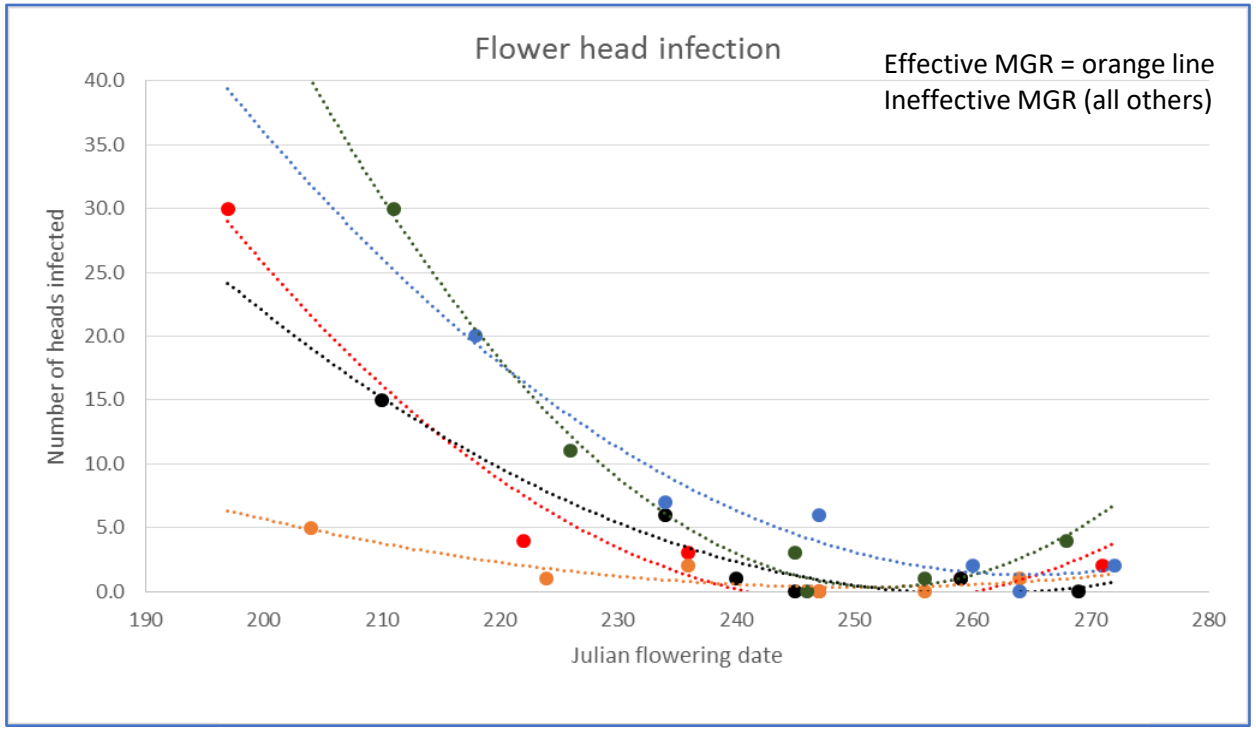

Upper canopy infection – what is the cause?

- Changing farming systems – now flowering during peak disease window



What have we learnt from field trials and observations?

- Flowering time is a major driver
  - Late flowering = fewer disease symptoms
- Effective major gene resistance controls disease
  - Ineffective MGR = no protection to UCI
- Timing of infection important
  - Early infection = time for disease to develop/express
  - Late infection = not enough time for disease to develop



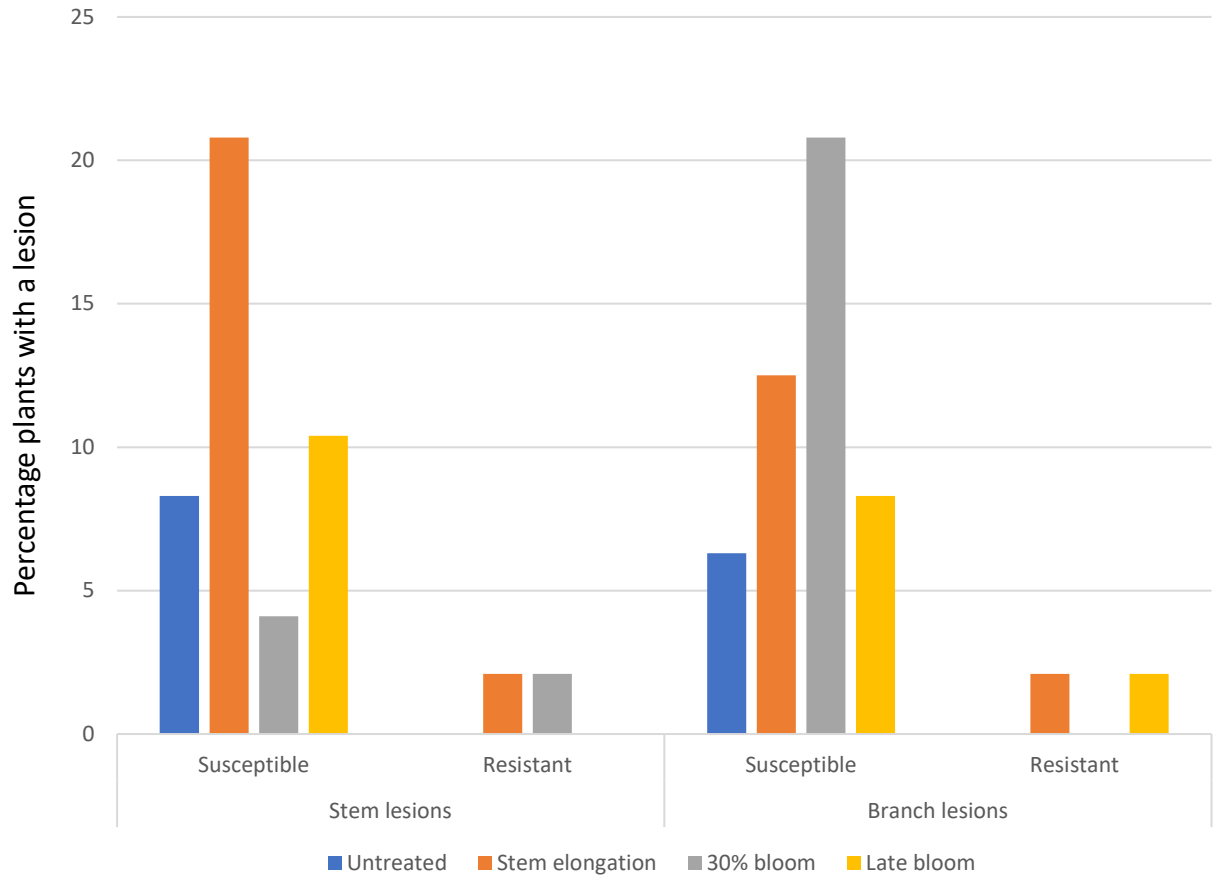
Aim to develop phenotyping system for screening for resistance to UCI

Year 1: Determining appropriate timing of infection and inoculation strategies

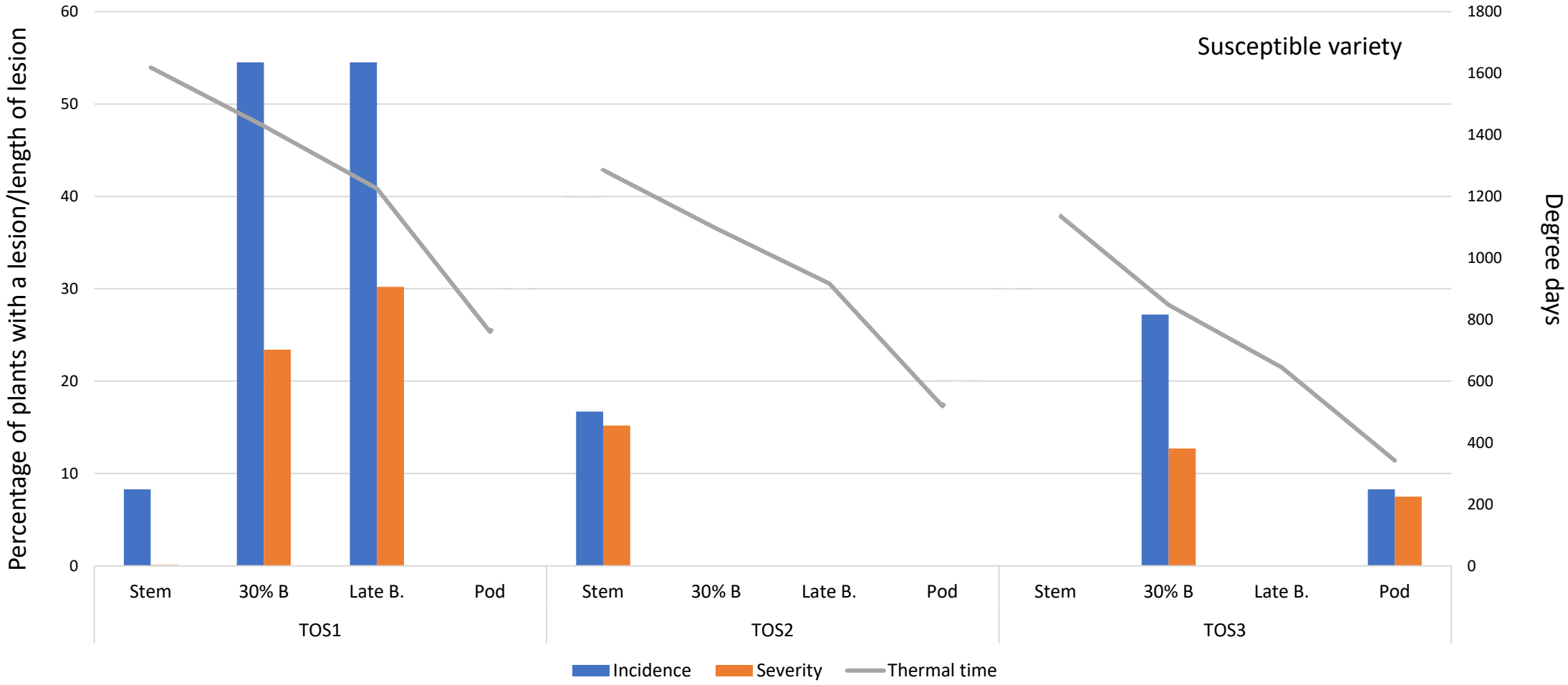
- Used two varieties: one with effective MGR and one without
- Experiments established in glasshouse and shadehouse environments
  - Reduce impact of external inoculum (glasshouse)
- Multiple times of sowing tested
  - Three TOS (March, April and May) in each environment
  - Allows for differing amounts of time for symptom development prior to maturity
- Ascospores versus pycnidiospores
  - Pycnidiospores = clonal and therefore reproducible
  - Ascospores = sexual and therefore populations will change over time
- Multiple growth stages inoculated
  - Stem elongation, 30% bloom, late flower, podding
- Determine use of molecular analysis for phenotyping

Results: Inoculations with pycnidiospores more appropriate than ascospores

- Inoculations with ascospores resulted in infection anywhere on the plant
  - Inoculations with pycnidiospores provided a precise inoculation location that could be tracked.
- External inoculum made assessment difficult



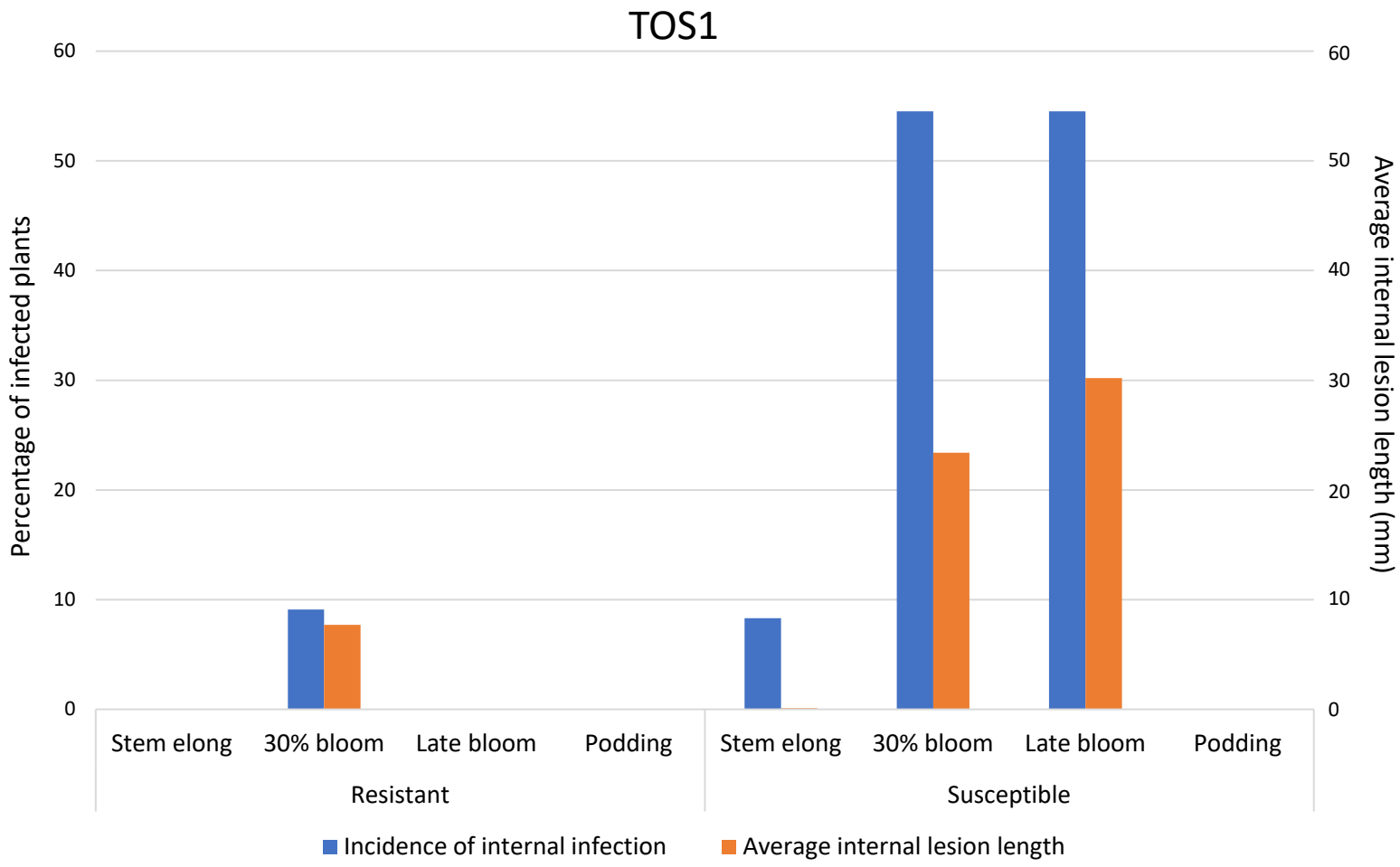
Results: Early sowing required to allow disease development



- Only inoculations carried out on plants from TOS1 reliably produced lesions

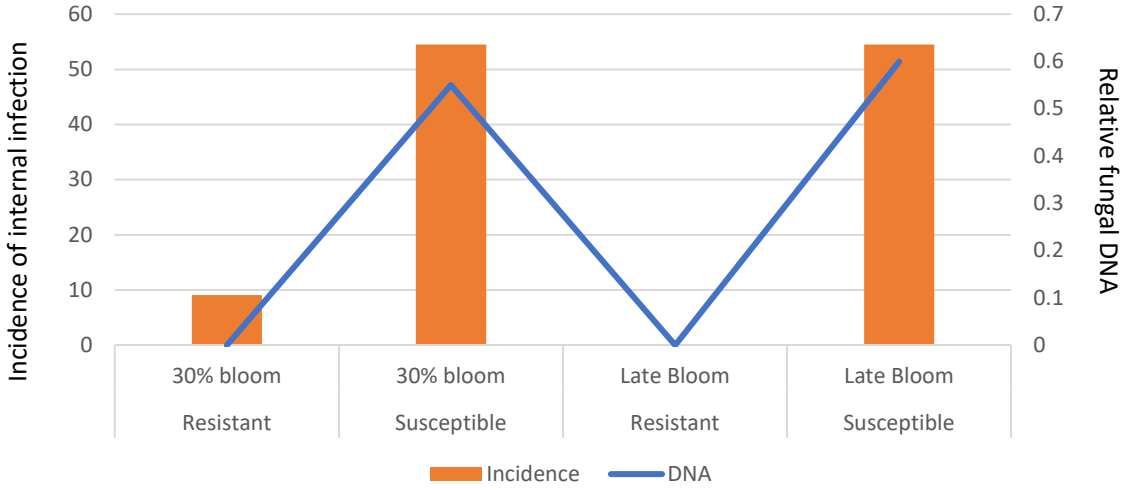
Results: Inoculation at 30% bloom gives most reliable disease severity

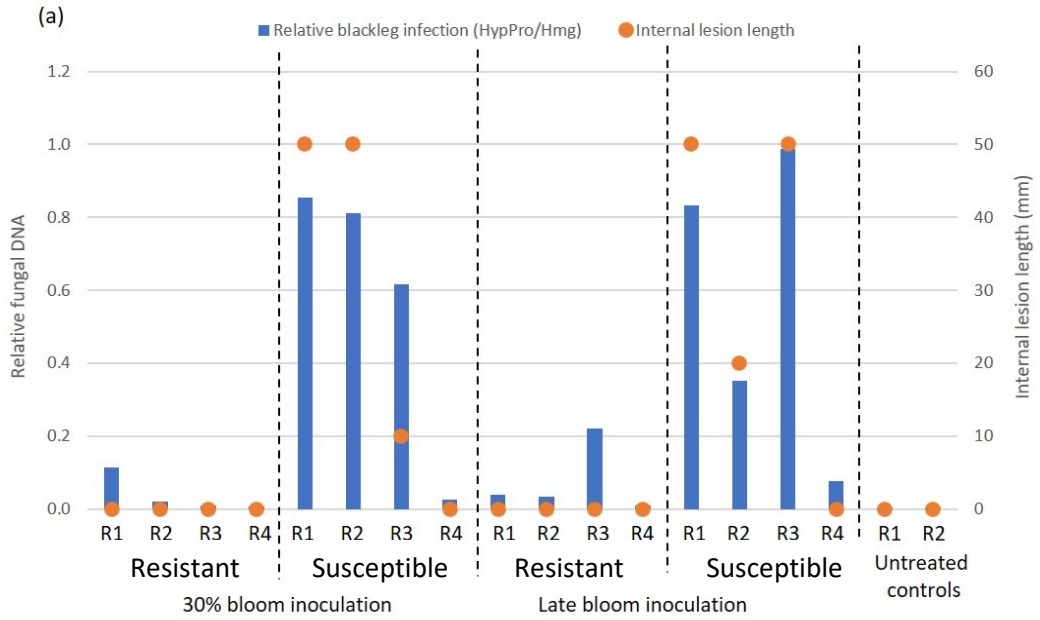
- Resistant cultivar gave a resistant response for all stages inoculated.
  - Used single avirulent isolate
- 30% bloom and late bloom gave most reliable infection



Results: Molecular assays could detect fungal biomass at maturity

- Samples were collected at
  - Inoculation site
  - 5 and 10 cm above and below inoculation site
  - 1 mpi, 2 mpi, 3 mpi and maturity
- Biomass determined by fungal DNA compared to plant DNA using ddPCR
- At maturity, fungal biomass can be detected.
  - Analysis of individual reps shows correlation of R^2=0.90 between fungal biomass and internal infection
- Low or no biomass detected away from wound site or prior to maturity





Findings from Year 1 and plans for Year 2

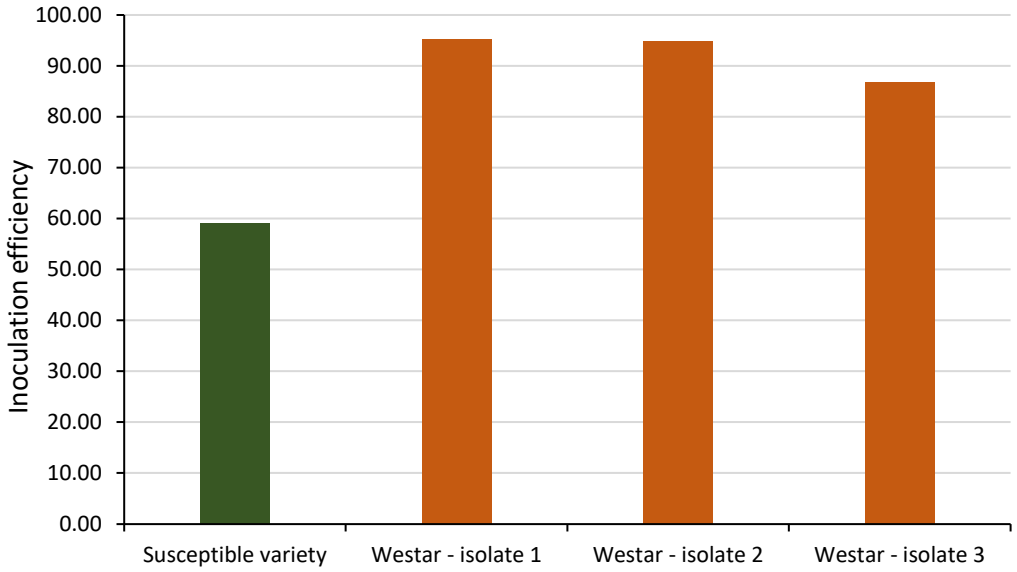
- Best inoculation method:
  - Early sowing time with 30% bloom inoculation timing
    - Allows enough developmental time
  - Pycnidiospores
    - Know where infection should develop

- **Problems: Need to improve infection efficiency**
  - Only obtained 55% disease incidence with the susceptible line
  - Tweak inoculation strategy (add a surfactant, inoculate branch versus junction)

- **Are results consistent across additional isolates and cultivars**

Year 2: Variability in incidence is not due to inoculation problems

- 50-60% infection efficiency in Year 1 suggested problems with inoculation strategy
- Repeated experiments in Year 2 with same susceptible variety and isolate
  - Four different environments
  - Various inoculation strategies (+/- tween, junction versus main stem inoculation, various application methods)
- Best result: 78% efficiency. Average: 59%
- Inoculations with Westar show between 89-96% efficiency suggesting that perhaps something else contributing to low infection efficiency. STAY TUNED!



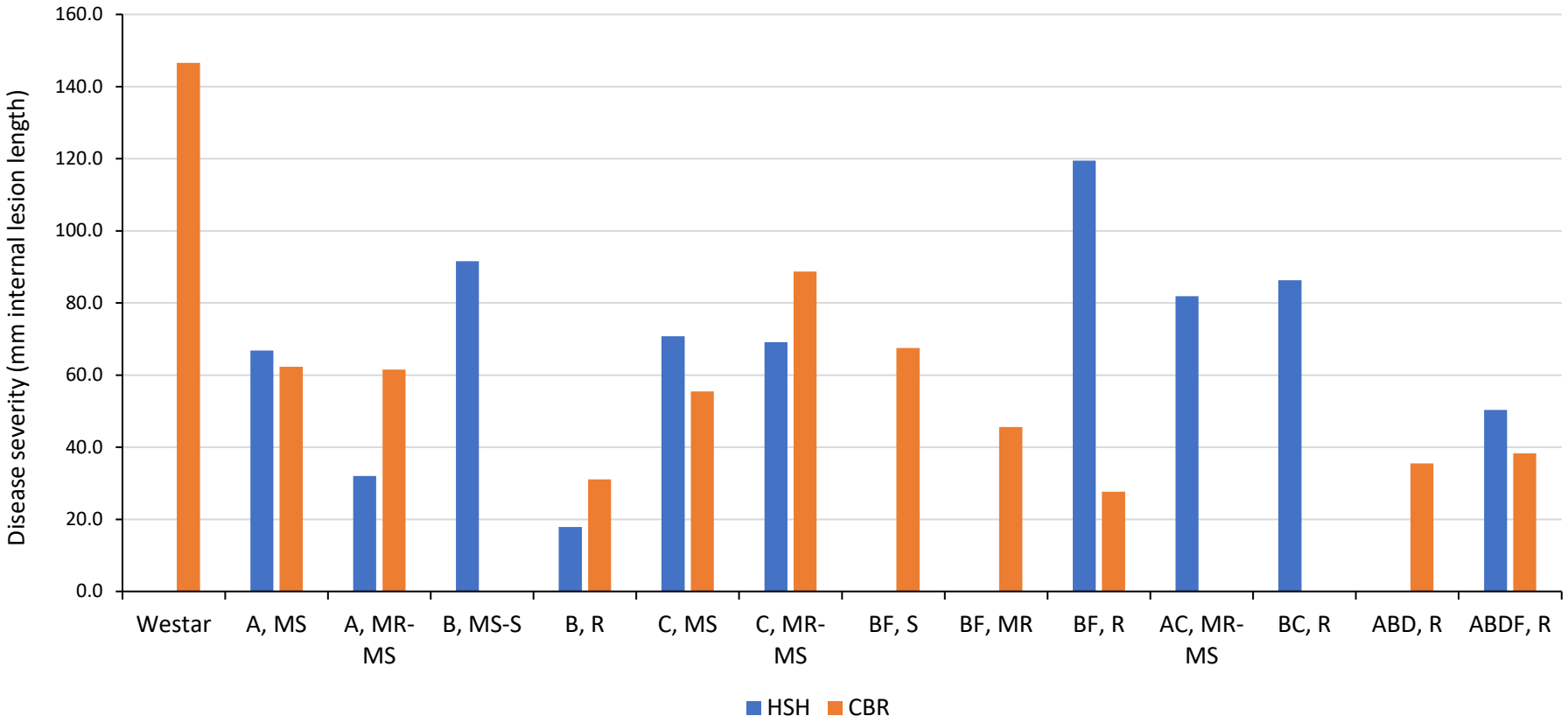
Year 2: Determine reproducibility of screens and evaluate consistency between host genotypes

All cultivars with ineffective major gene resistance are equally susceptible to UCI

- 3 environments tested
  - Outside – Canberra (CBR)
  - Glasshouse – Horsham (GH-HSM)
  - Shadehouse – Horsham (SH – HSM)
    - low disease development – sown later to avoid external inoculum
- Up to ten cultivars screened in each environment. 6 cultivars consistent between all environments.
- Three isolates
- Included isolate and cultivars from Year 1 experiments
- Temperature data recorded

Not all cultivars with ineffective MGR are equally susceptible to UCI

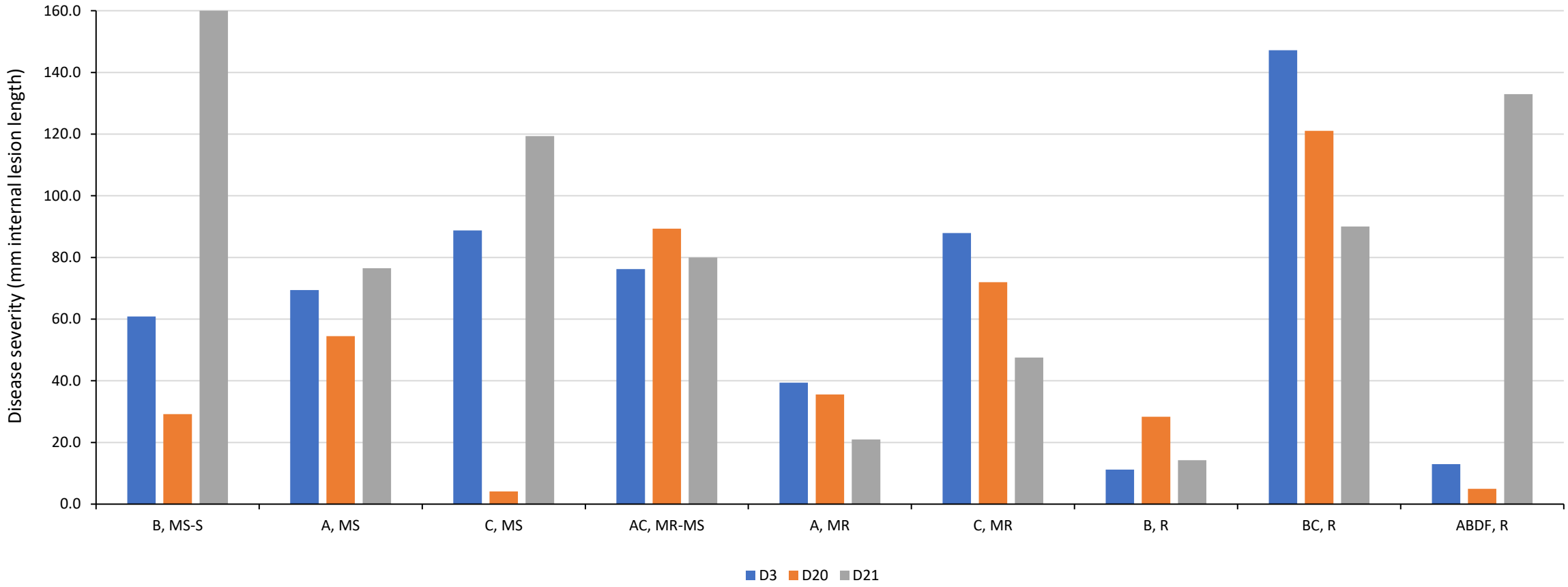
- Average disease severity varies across cultivars



Average disease severity across all three isolates

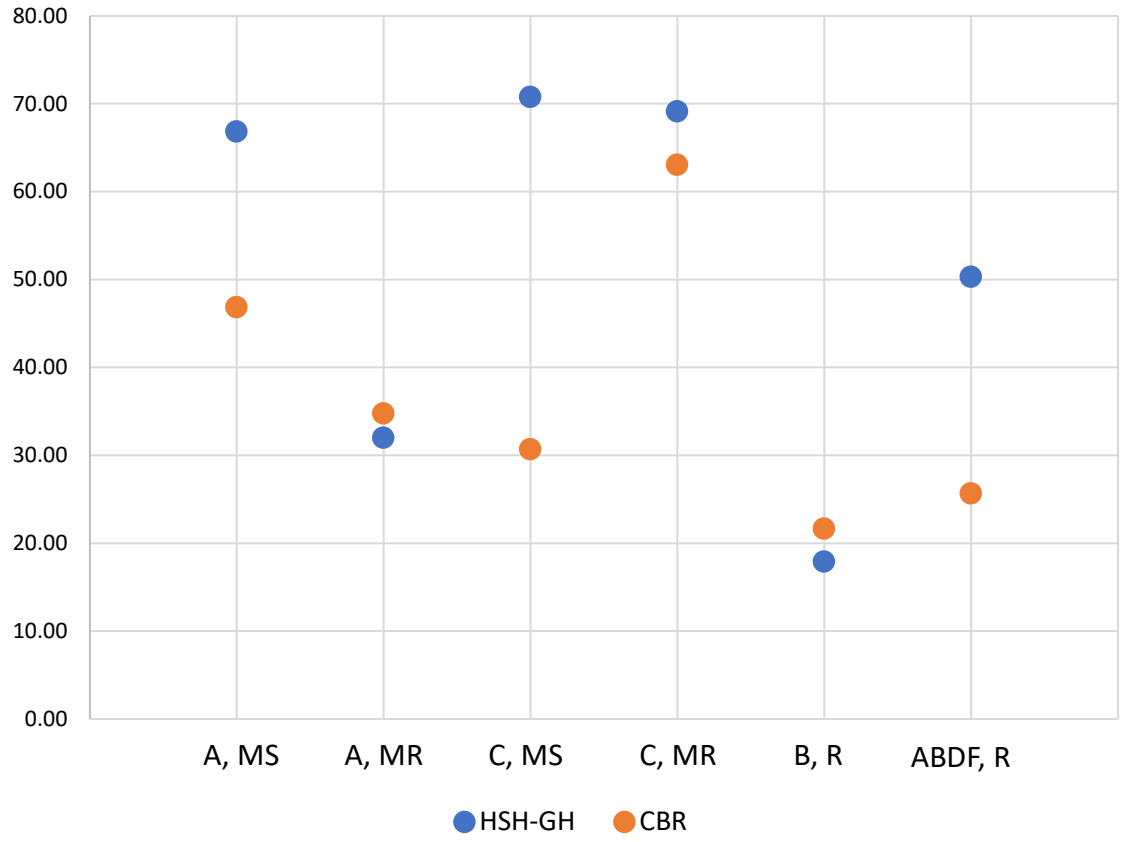
Host genotype x isolate genotype interaction for UCI

- Individual isolates respond differently on individual cultivars suggesting genotypic host x isolate genotypic interaction

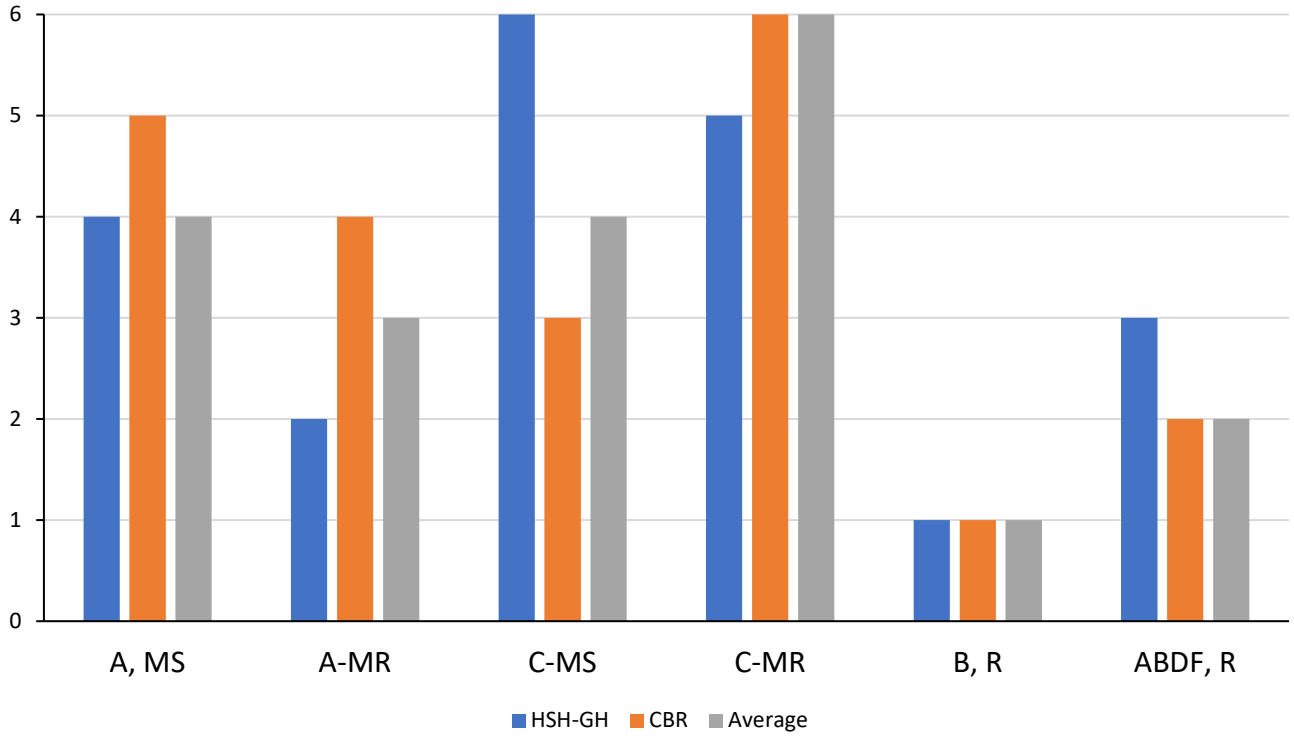


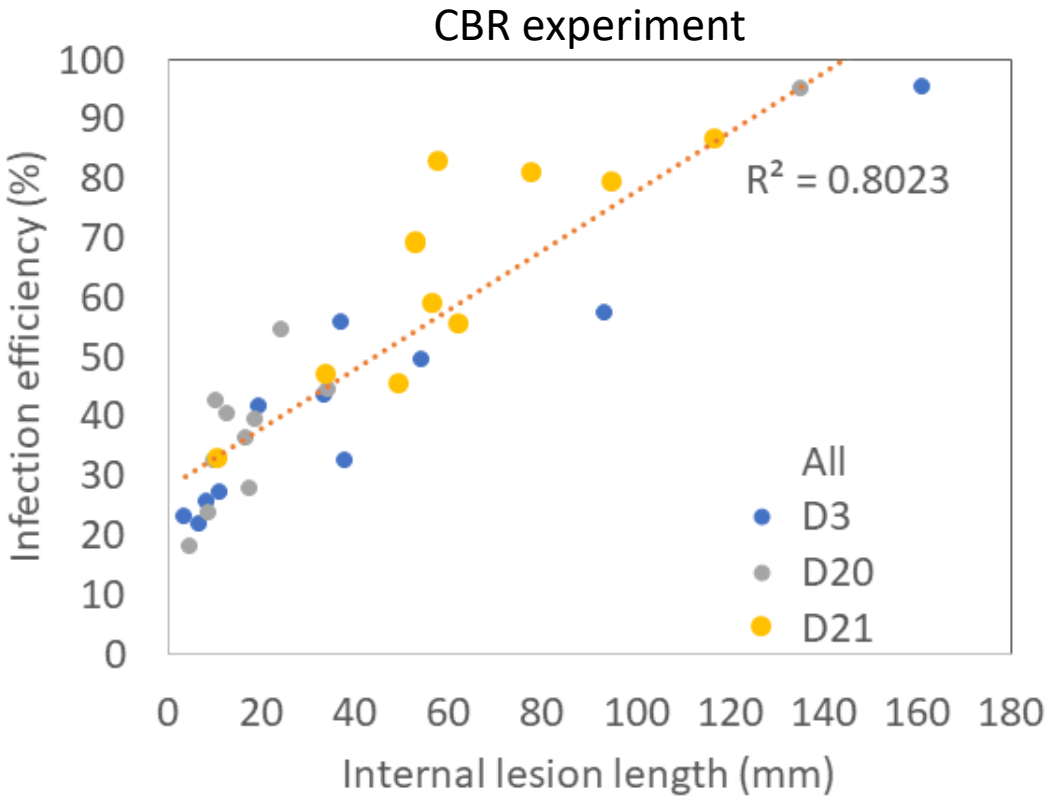
Variation in disease severity between environments

- There is some variation between environments
  - Overall average disease severity higher in HSH exp (51.2 mm) than CBR (37.1 mm)
  - The biggest differences appear to be in the most susceptible cultivars
- Possibly relates to thermal time
  - Average thermal time GH – HSH = 1940.8
  - Average thermal time CBR = 1397.6

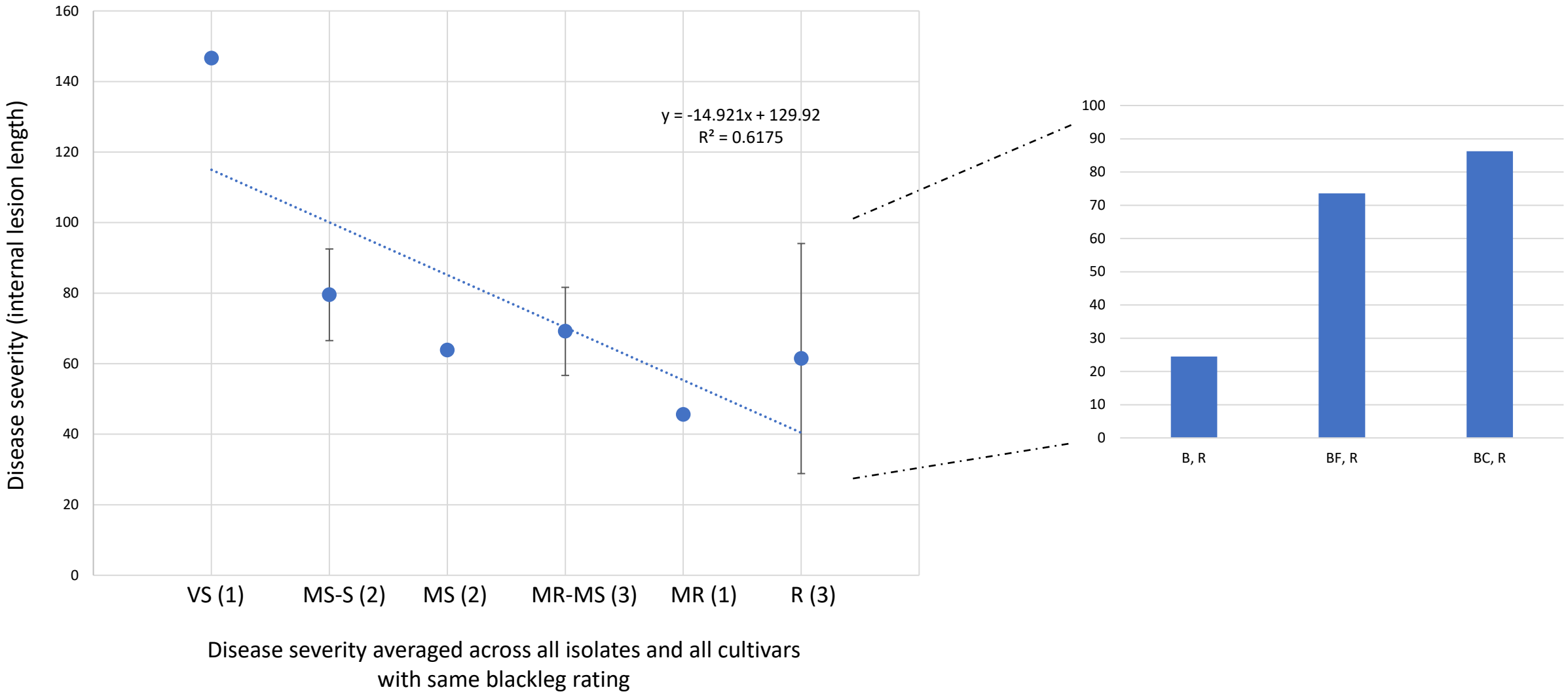


Ranking cultivars, alternative disease measures or standardizing assessment times may reduce impact of environmental differences

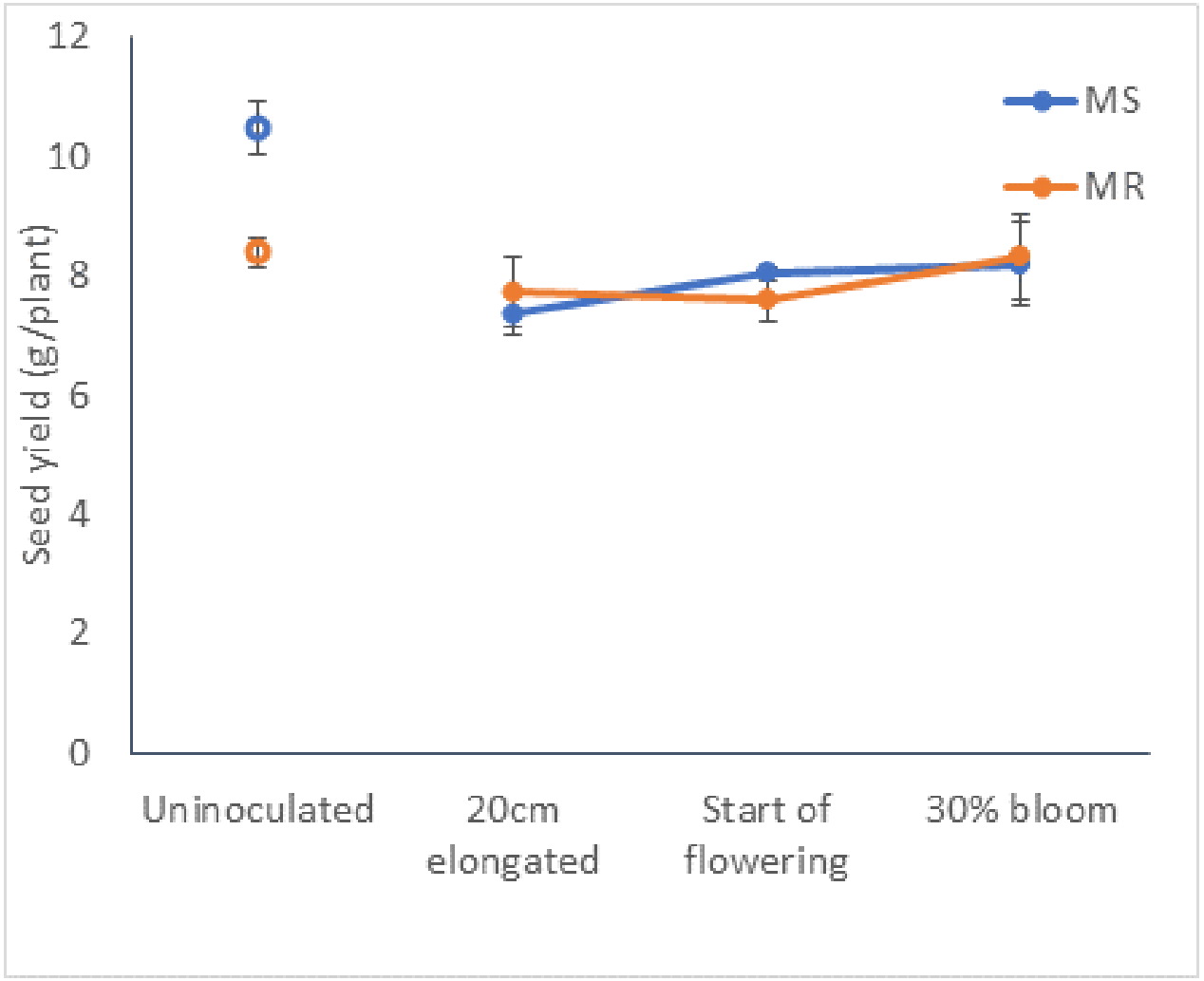




Is quantitative resistance conferring resistance to UCI?



Yield responses also correlate with blackleg rating in preliminary experiment



Yield loss associated with UCI is more significant in an MS cultivar compared to an MR cultivar

UCI phenotyping– not as simple as first thought!

- Preliminary phenotyping strategy developed (30% bloom, early sowing)
  - Need to minimize differences between environment
  - Assess at a specific thermal time rather than maturity?
  - Use infection efficiency rather than disease severity?
- All cultivars are equally susceptible to UCI: BUSTED
  - Host x isolate interactions detected
  - Resistance to UCI potentially correlated with Blackleg rating (or at least sometimes)
  - Similar to QR?
  - Can we inoculate with a mixture of isolates rather than single isolates?
- Is quantitative resistance conferring resistance to UCI?
  - Developed protocol for inoculating plants for both QR and UCI

Acknowledgements

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