Understanding selection of fungicide resistance using blackleg of canola as a model

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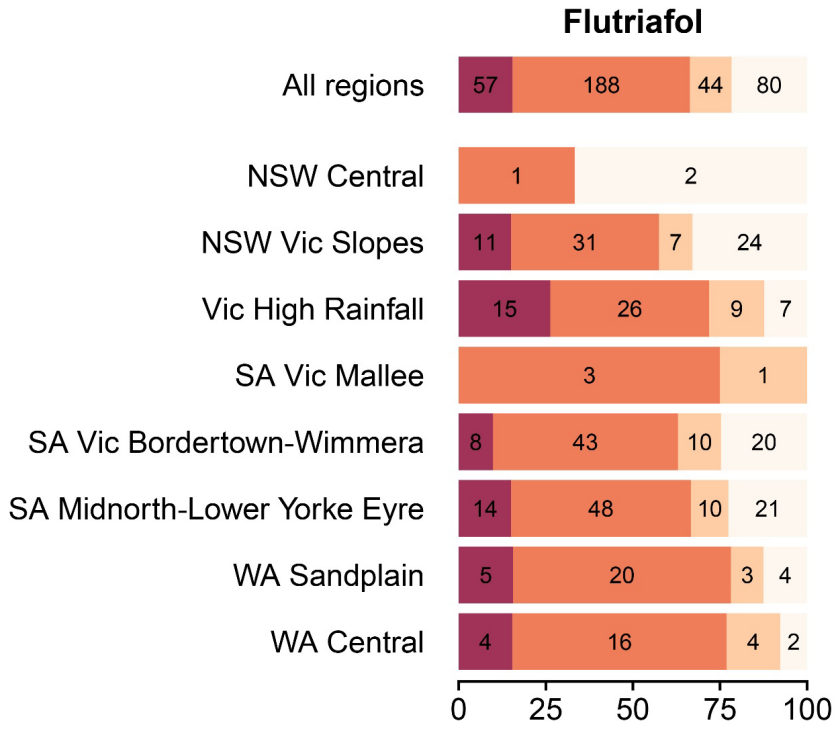
What we do and don't know about fungicide resistance

What we do know:

- Wide-spread DMI resistance across Australia
- Frequency within populations ranges from <0.05-32%

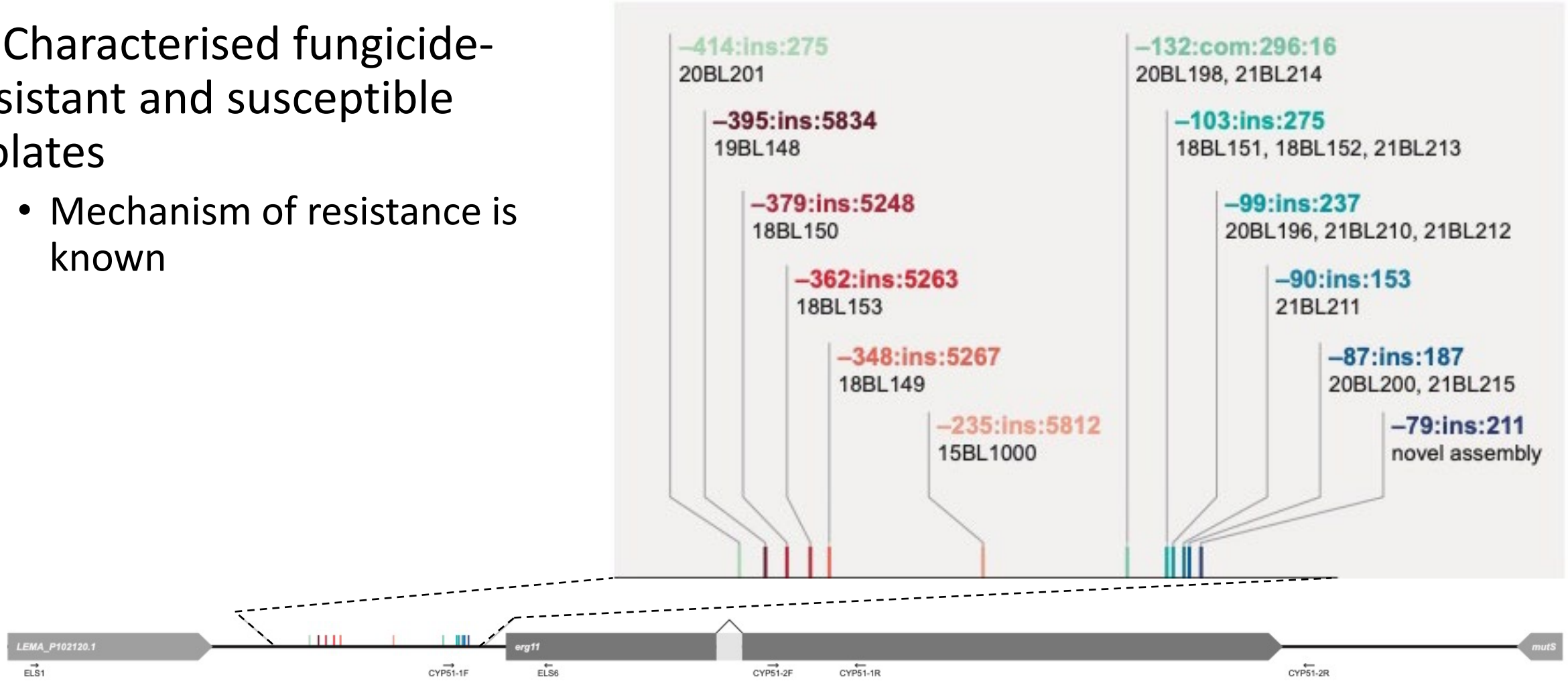


What we don't know:

- What frequency leads to field failure?
- What practices lead to fungicide resistance?

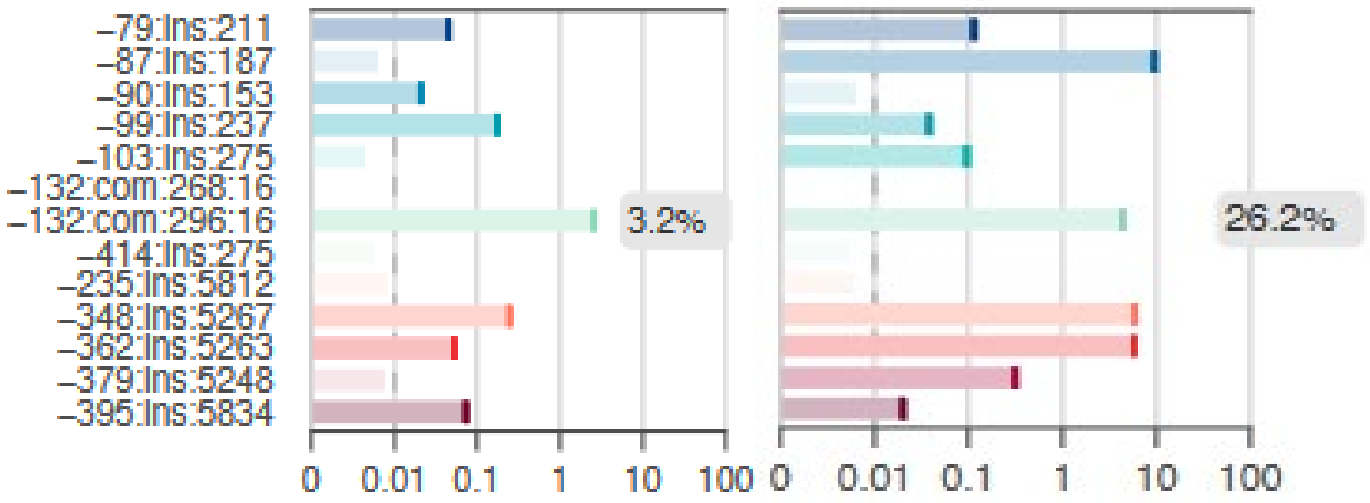
Blackleg as a model for understanding fungicide resistance

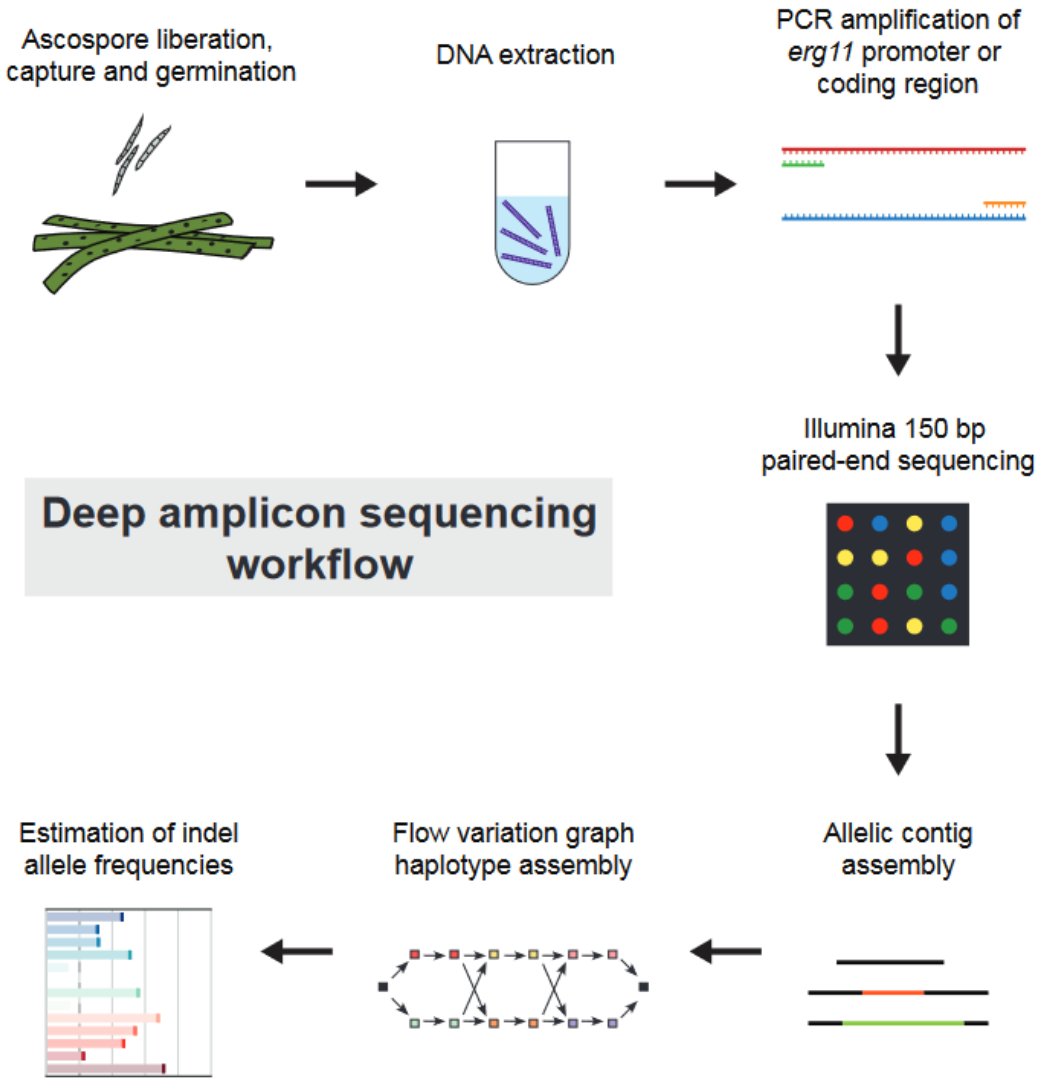
1. Characterised fungicide-resistant and susceptible isolates
    - Mechanism of resistance is known



Blackleg as a model for understanding fungicide resistance

2. Molecular markers for tracking changes in populations
   - Markers are applied to whole populations, not individual isolates
   - Captures all mutations





Blackleg as a model for understanding fungicide resistance

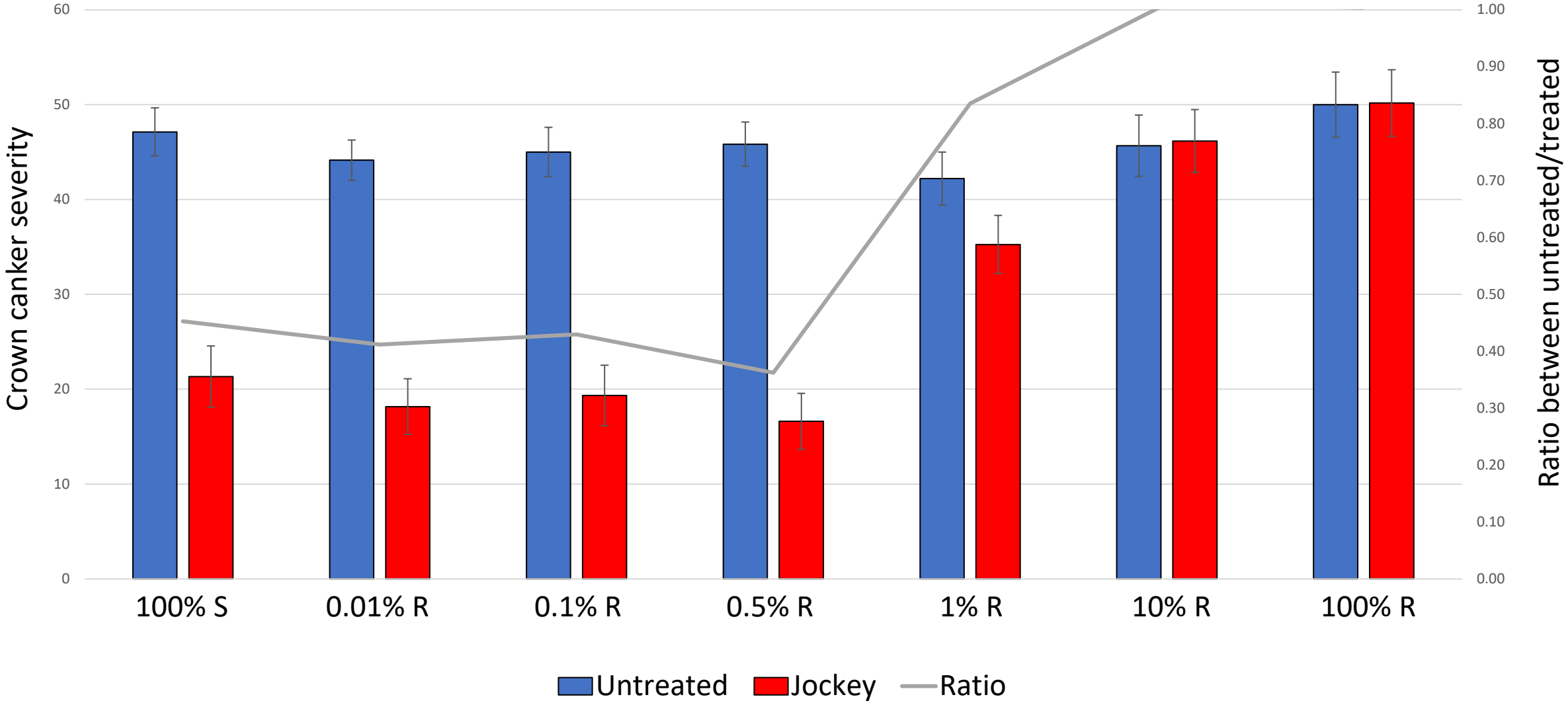
3. *in planta* assays to simulate different selection regimes

- Use ratios of different isolates
- Inoculations at different growth stages
- Grow through to maturity and allow sexual reproduction to occur on stubble

What frequency of resistance is needed to render the fungicide ineffective?

- Untreated and Jockey-treated plants
- Inoculated with populations of isolates with different ratios of fungicide resistance
- Inoculated at multiple growth stages to simulate field conditions
- Assessed disease severity at the end of the year
- Looked at changes in allele frequency following sexual reproduction

Only 1% of the population needs to be resistant for loss of fungicide efficacy



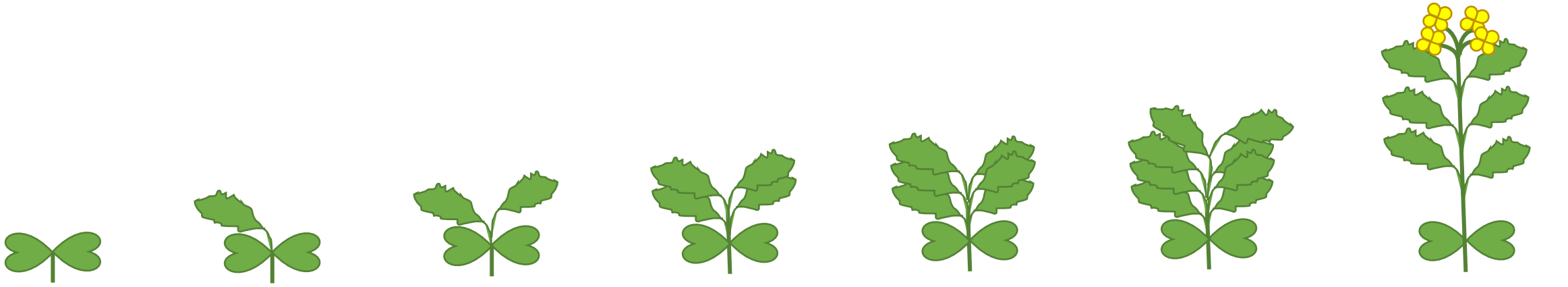
Populations change dramatically after selection

No fungicide		Starting frequency		Plus fungicide
0.1% R	–	0% R	–	0.3% R
0.1% R	–	0.01% R	–	0.7% R
0.2% R	–	0.1% R	–	0.2% R
0.7% R	–	0.5% R	↑	33% R
0.6% R	–	1% R	↑	11% R
6.1% R	–	10% R	↑	58% R
93% R	–	100% R	–	98% R

All above the 10% frequency and therefore the fungicide would be rendered ineffective

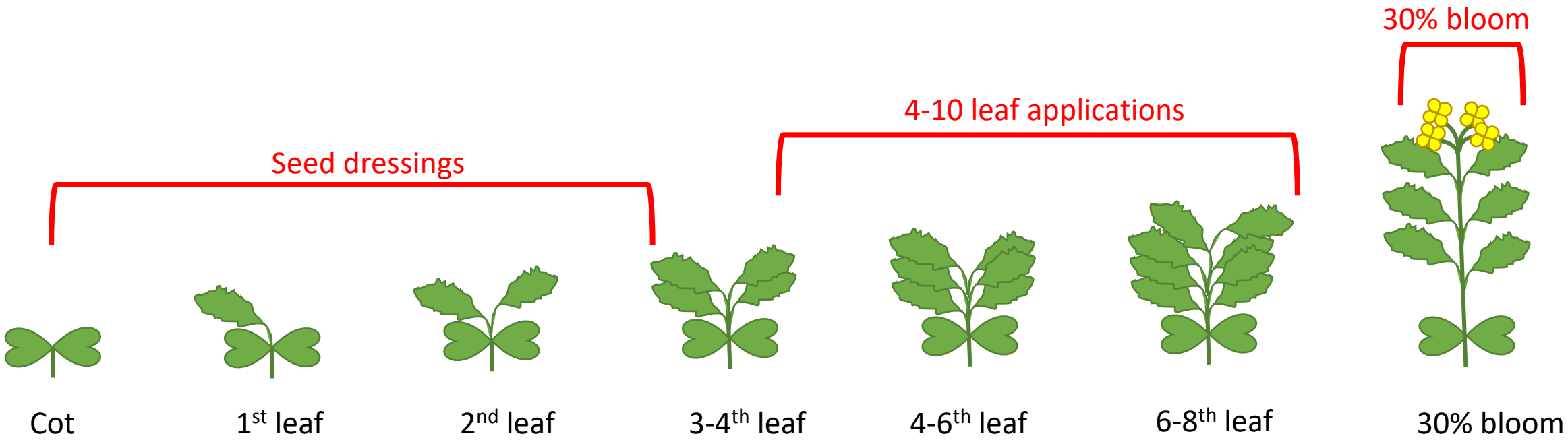
How does timing of infection contribute to fungicide evolution?

- Do later infections have time to grow in the plant and contribute to the next generation?



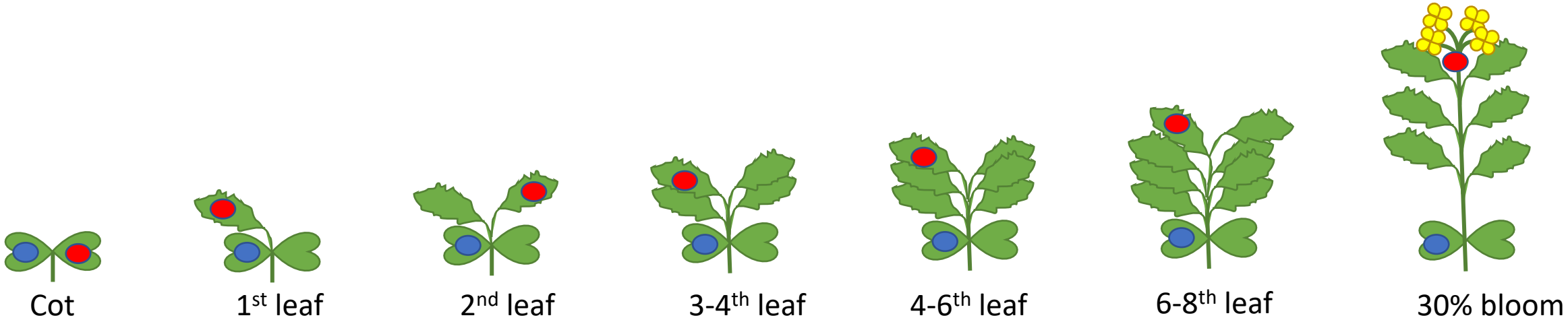
How does timing of infection contribute to fungicide evolution?

- Do later infections have time to grow in the plant and contribute to the next generation?
  - If not, then do later fungicide applications matter for fungicide resistance management?



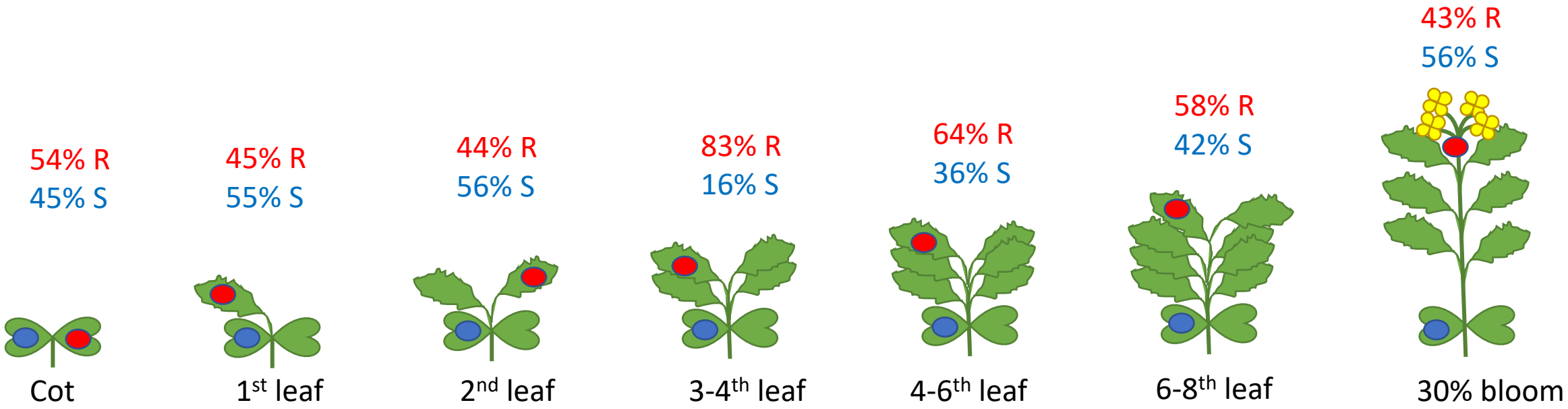
How does timing of infection contribute to fungicide evolution?

- Plants inoculated with two different populations at different growth stages
  - Fungicide susceptible
  - Fungicide resistant
- Plants grown to maturity, assessed for disease and stubble kept



Sexual reproduction detected from all inoculation timings

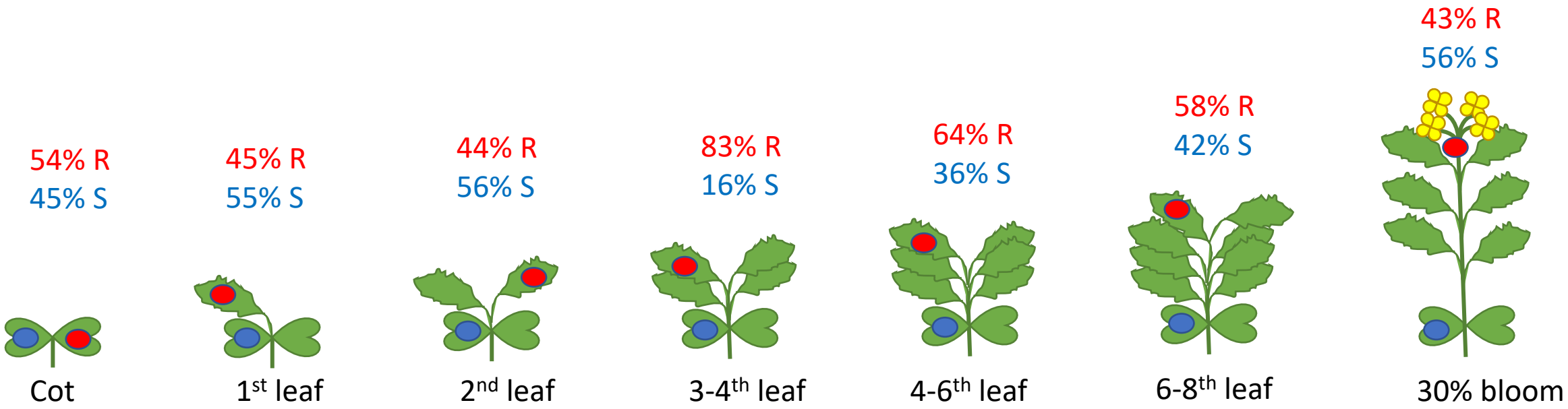
- No fungicides applied to this experiment
  - Fungicide resistance used as a marker for tracking populations



FungS only – 0.5% R, 99% S, FungR only – 80%R, 19%S

Sexual reproduction detected from all inoculation timings

- Populations were designed to be able to have sex within itself
  - Positive control for experimental design

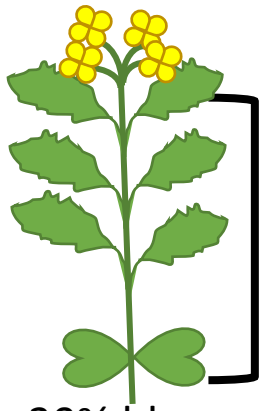


FungS only – 0.5% R, 99% S, FungR only – 80%R, 19%S

Have the populations mated together or individually?

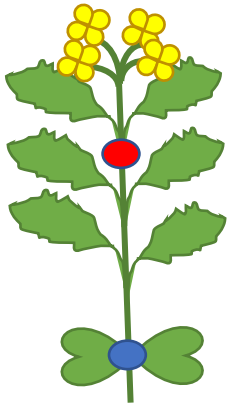
- Capturing of spores was done with entire stem
  - Has the 30% bloom FungR population had sex with itself or with the FungS population?

43% R
56% S

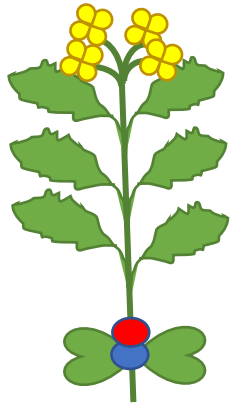


30% bloom

Stubble kept



or



Findings

- Only 1% of the population needs to be resistant for field failure to occur
  - Monitoring strategies need to be sensitive
- One year of selection increases the frequency of resistance dramatically
- More work around timing of infection is required to determine where the sexual reproduction is occurring

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