ALBA
Annual Legume Breeding Australia

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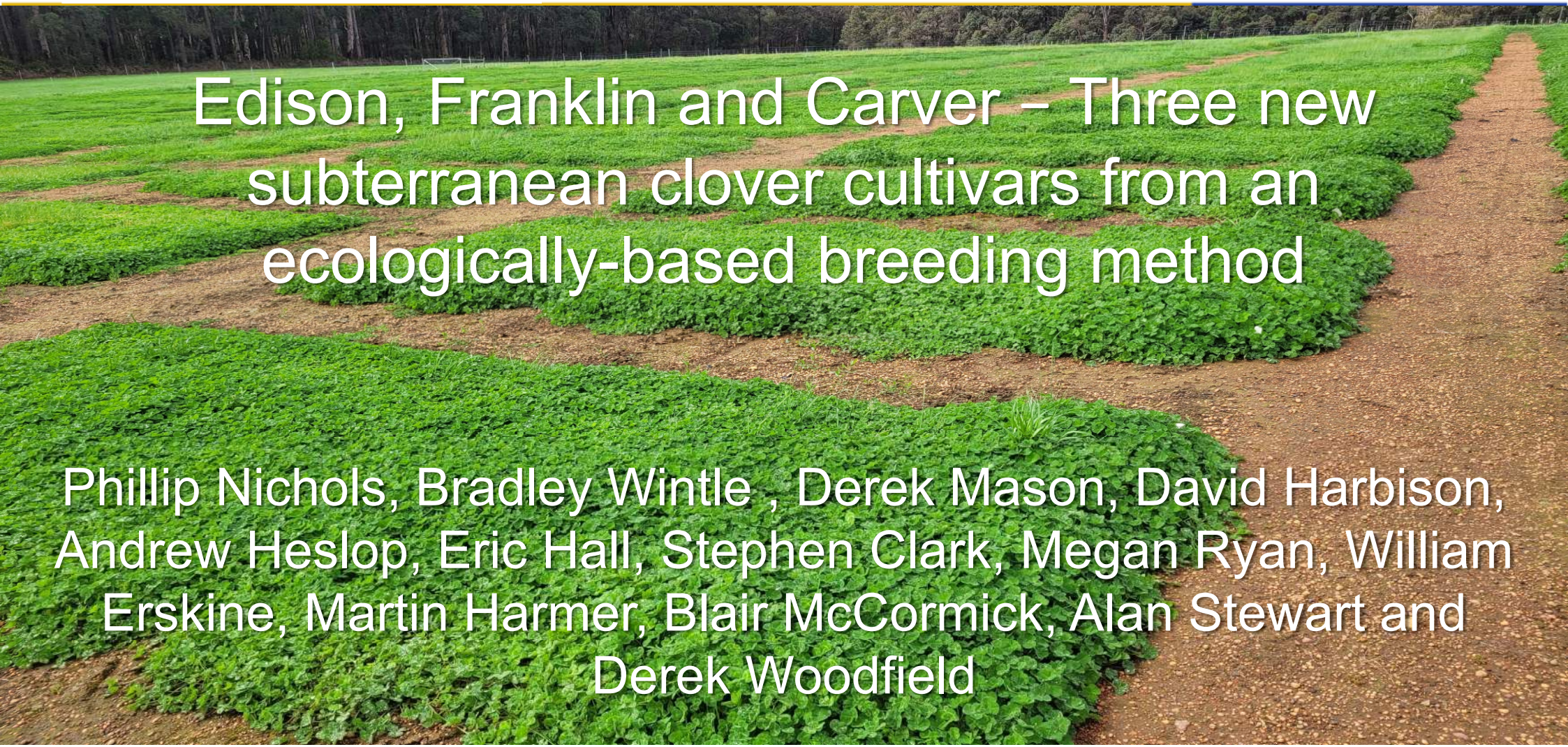

Edison, Franklin and Carver – Three new subterranean clover cultivars from an ecologically-based breeding method

Phillip Nichols, Bradley Wintle , Derek Mason, David Harbison, Andrew Heslop, Eric Hall, Stephen Clark, Megan Ryan, William Erskine, Martin Harmer, Blair McCormick, Alan Stewart and Derek Woodfield

Outline

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- Brief history of sub clover breeding in WA
- Use of an ecologically-based breeding method for sub clover
  - Used to develop Edison, Carver & Franklin
- Origins and field performance of Edison, Carver & Franklin
  - Link back to their breeding history

Subterranean clover (sub clover) (*Trifolium subterraneum* L.)

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- Most widely sown pasture legume in southern Australia – 29 mill ha
  - Noted for persistence under grazing
- Self-pollinated, diploid annual
- Three sub-species
  - ssp. *subterraneum* (for well-drained soils pH 4.5–7.0 in $CaCl_2$)
  - ssp. *yanninicum* (for poorly-drained soils, pH 4.5–7.0 in $CaCl_2$)
  - ssp. *brachycalycinum* (for cracking or stony soils, pH 6.0–8.0 in $CaCl_2$)


ssp. *subterraneum*


ssp. *yanninicum*


ssp. *brachycalycinum*

A (brief) WA sub clover breeding history

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- 1949: Sub clover breeding commenced at the University of Western Australia (UWA)
- 1986: Transferred to the WA State government
  - National Subterranean Clover Improvement Program

A (brief) WA sub clover breeding history

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- 1949: Sub clover breeding commenced at the University of Western Australia (UWA)
- 1986: Transferred to the WA State government
  - National Subterranean Clover Improvement Program
- 1999: Crossing and selection ceased
  - Government and industry funding priorities changed
  - Focus moved to alternative pasture species
  - Much promising breeding material left “on the shelf”

A (brief) WA sub clover breeding history

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- 2015: Expression of Interest for industry ownership
  - Purchased by DLF Seeds
- 2018: Annual Legume Breeding Australia (ALBA) formed
  - Joint Venture between UWA and DLF Seeds
  - Phil Nichols & Brad Wintle transferred to UWA

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Pedigree breeding method used for sub clover

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Early generation selection

Crossing

↓

Grow out F_1 hybrids

↓

F_2 spaced plant selection

↓

F_3 selection between rows

↓

F_4–F_6: Spaced plant/row selection & progression to homozygosity

↓

Selection of elite lines for field trials

↓

Multi-site evaluation in regenerating grazed swards (2 stages – 5 years)

↓

Select best line for seed multiplication & cultivar release

Issues for early generation selection

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- Pedigree method fine for traits with high heritability

Weaknesses

- F_4–F_6 generations mainly to progress towards homozygosity
- Selection conducted under non-grazed conditions
- Unable to incorporate self-regeneration process into selection procedures
  - Key to pasture adaptation and persistence

An alternative F_2-derived, F_4 progeny method

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Early generation selection

Crossing

↓

Grow out F_1 hybrids

↓

F_2 spaced plant selection

↓

F_3 selection between rows

↓

F_4–F_6: Self-regenerating grazed swards in target environments – 3 years

↓

Seeds collected from best lines & selection among them for field trials

↓

Multi-site evaluation in regenerating grazed swards (2 stages – 5 years)

↓

Select best line for seed multiplication & cultivar release

Use of the F_4 progeny method

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- Used for lines from > 300 crosses (1988-1994)
- Selection in F_2 and F_3 generations (Shenton Park)
  - Flowering time
  - Low content of the phyto-oestrogen, formononetin
  - Dense, leafy crowns & vigorous growth
  - Clover scorch (*Kabatiella caulivora*) disease screening
- F_4 lines sown in nursery plots (2 replicates)
  - Self-regenerating swards for 3 years
  - Local management practices & grazing by sheep

Use of the F_4 progeny method

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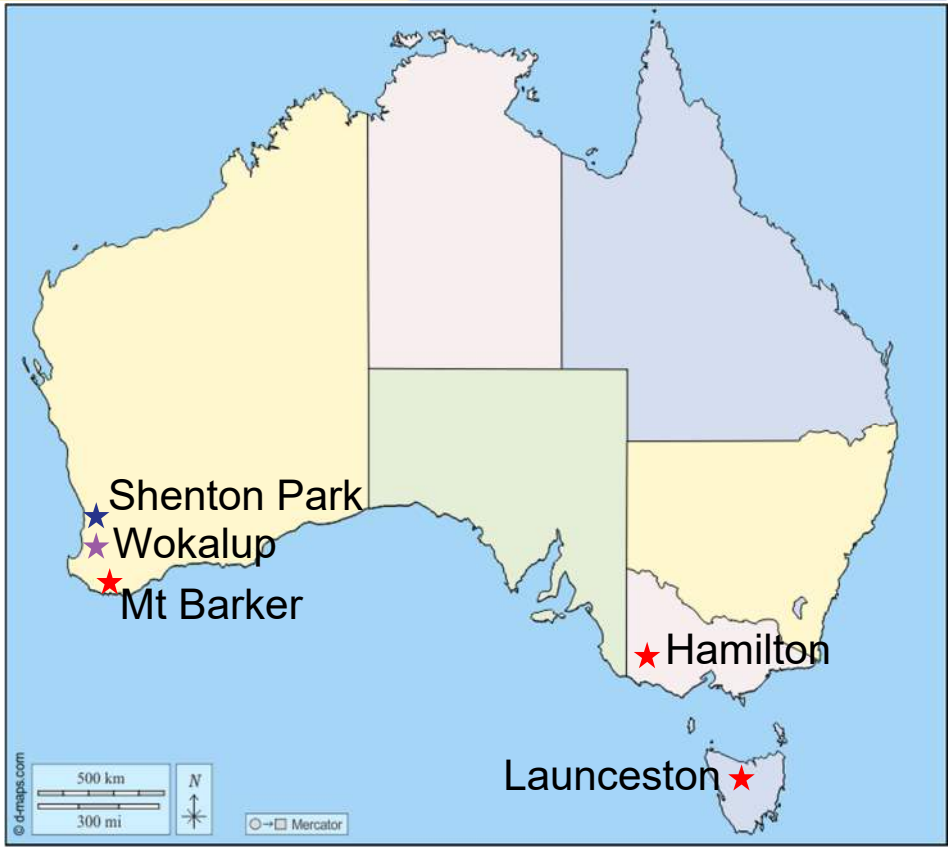
- Used to develop breeding lines that led to Edison, Carver & Franklin
- Late flowering *subterraneum* nurseries
  - Mt Barker (WA), Hamilton (Vic), Launceston (Tas)
- Midseason *yanninicum* nursery
  - Wokalup (WA)
- Seeds harvested from most productive and persistent lines after 3 years
  - Each seed homozygous & genetically different from each other
- Preliminary evaluation of lines (Shenton Park)
- Further work on this material postponed



★ Late flowering *subterraneum* F_4 nurseries
★ Midseason *yanninicum* F_4 nursery

Resurrection with the ALBA Joint Venture

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- Initial DLF Seeds priorities
  - cv. Leura replacement (late flowering *subterraneum*)
  - cv. Goulburn replacement (mid-late flowering *subterraneum*)
  - cv. Riverina replacement (midseason *yanninicum*)
- Evaluated promising lines at Shenton Park (2016)
  - 503 late flowering *subterraneum* lines
  - 119 midseason *yanninicum* lines
- Stage 1 evaluation in 2017-18 (2 replicates)
  - Manjimup


Shenton Park 2016


Manjimup Stage 1 trial 2017

Stage 2 trials of elite lines (2019-2021)

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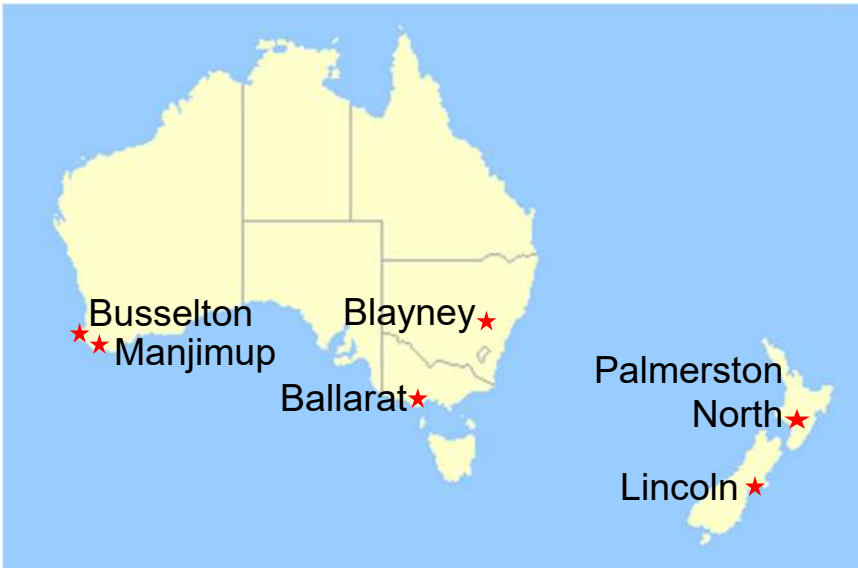
- 18 late flowering *subterraneum* breeding lines + 6 cultivars
- 10 midseason *yanninicum* breeding lines + 6 cultivars
- Sites (4 replicates)
  - Manjimup & Busselton (WA), Ballarat (Victoria) & Blayney (NSW)
  - Extra *subterraneum* sites at Lincoln & Palmerston North (NZ)
- Management
  - Self-regenerating swards
  - Local management practices & grazing by sheep
- Measurements
  - Dry matter yield (DMY) – winter, spring & late spring (3 years)
  - Seedling regeneration densities (Years 2 & 3)
  - Seed yield in Year 1 (WA only)
  - Reaction to pests and diseases



Stage 2 trials (2019-2021)

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Selection of Edison & Carver (late flowering ssp. *subterraneum*)

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Edison

Denmark x Woogenellup cross (1988)

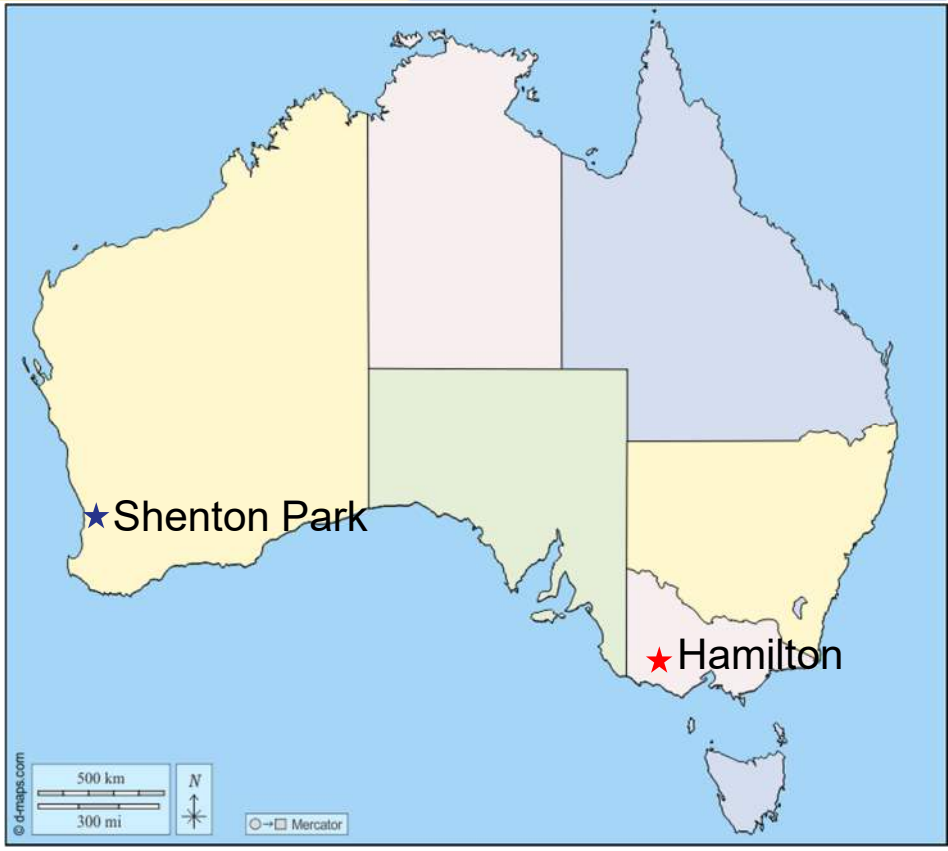

Carver

Goulburn x Woogenellup cross (1989)


- Choice of Denmark and Goulburn parents
  - Late flowering
  - Low formononetin content
  - Resistance to clover scorch (*Kabatiella caulivora*) disease
  - Dense, leafy crowns → Persistence under grazing
- Choice of Woogenellup parent
  - Vigorous winter growth

Edison

- One of 240 F_4 lines sown at 3 selection nurseries
- Derives from a nursery in Hamilton (Vic)



★ Late flowering *subterraneum* F_4 nurseries

Carver

- One of 240 F_4 lines sown at 3 selection nurseries
- Derives from a nursery in Launceston (Tas)

★ Late flowering *subterraneum* F_4 nurseries

Stage 2 late flowering *subterraneum* total dry matter yields (DMY)

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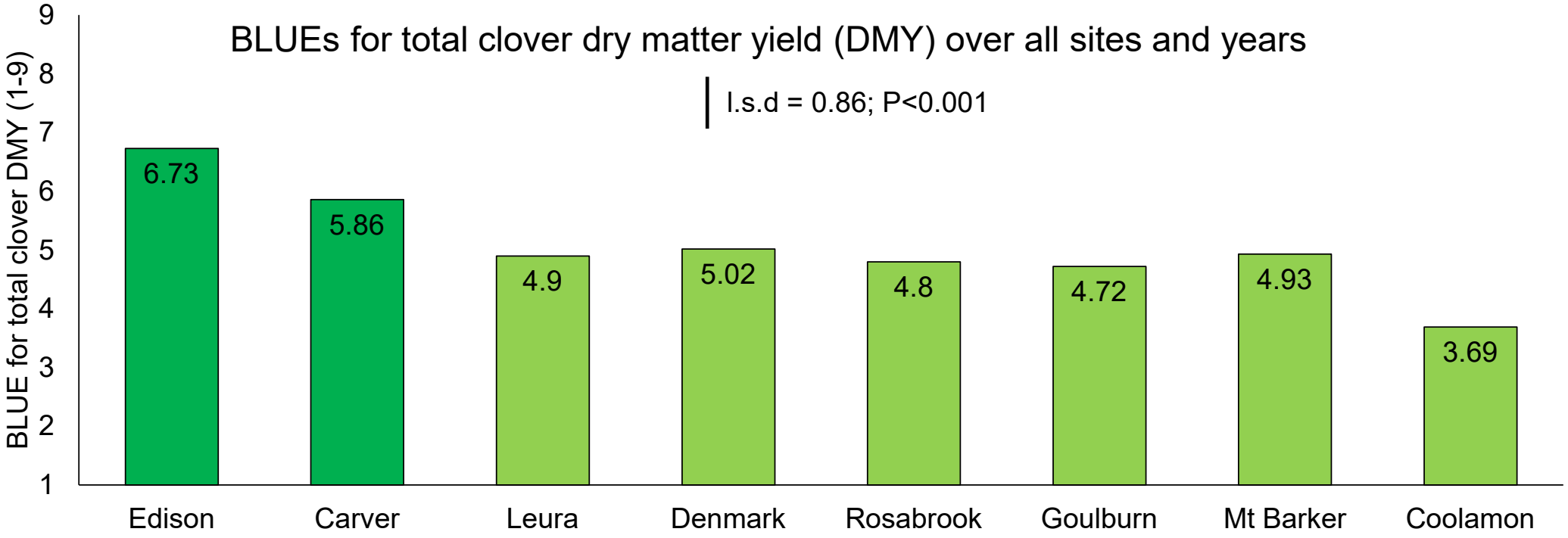
- Best Linear Unbiased Estimates (BLUEs) calculated for DMY over 3 years at 5 sites & 1 year at Palmerston North
  - All clover DMY data first converted to 1-9 scores
- Edison and Carver significantly higher total DMY than all other late flowering cultivars



Days to first flowering at Shenton Park

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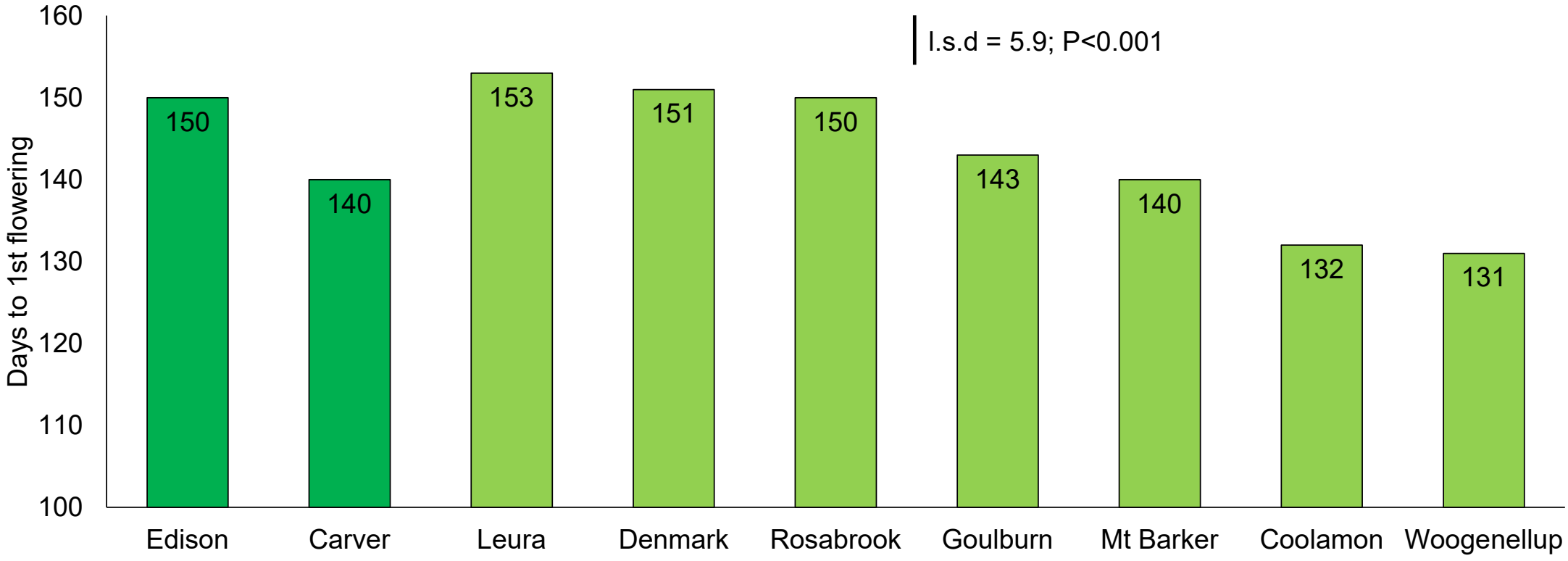
- From an early May sowing; Mean of 8 years
- Edison flowering time similar to Leura, Denmark & Rosabrook
- Carver flowering time similar to Goulburn & Mt Barker



Formononetin content (% of dry matter)

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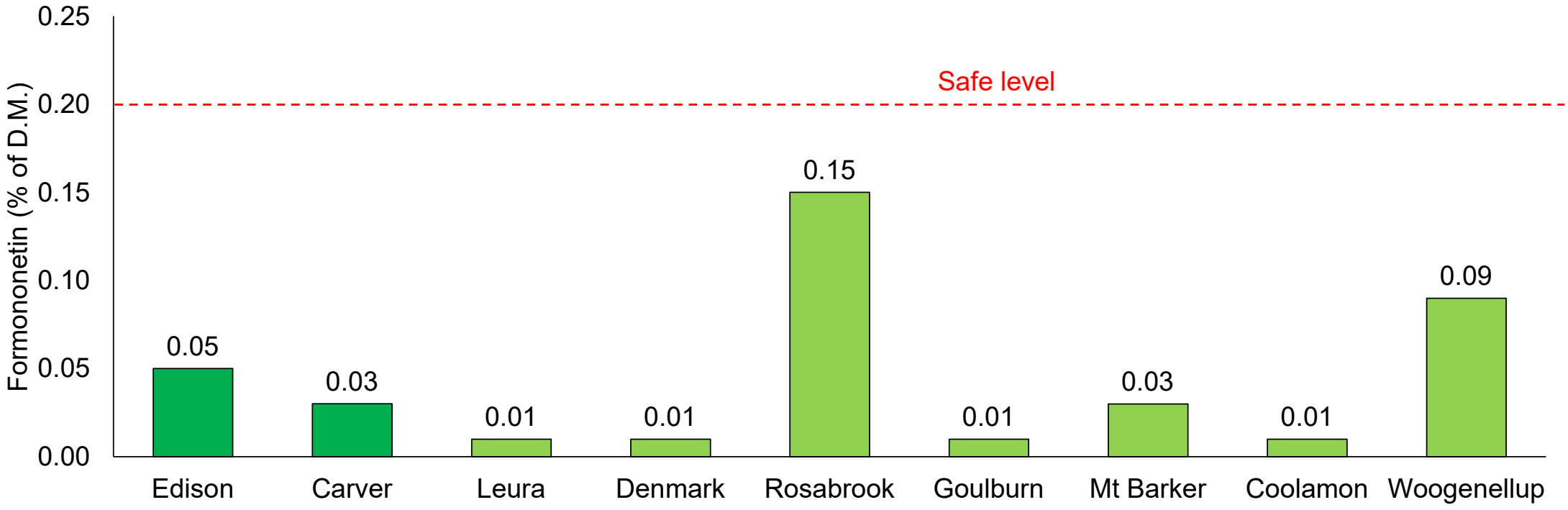
- Edison & Carver have low formononetin content (<0.2% of dry matter)
  - Mean values from 3 years
  - No significant differences between entries



Clover scorch (*Kabatiella caulivora*) resistance

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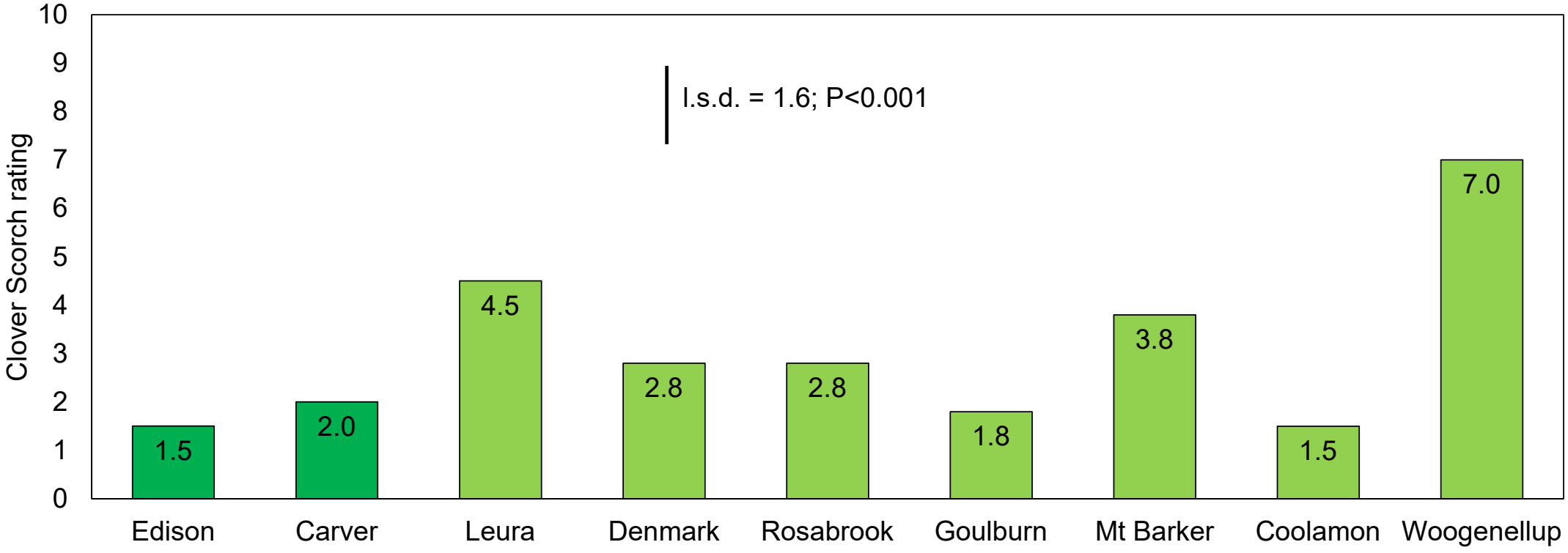
- Field ratings at Manjimup (mean of 2 reps)
  - 0 = no symptoms, 10 = severe symptoms
  - Resistant if rating <4.0
- Edison & Carver have resistance to clover scorch



Selection of Franklin (ssp. *yanninicum*)

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Franklin: Originates from a complex cross (1990)

83Y79.8.3.2
[CPI 39326YA (Greek accession) x (Trikkala x Meteora – a sister line of Riverina)]
X
CPI 103925A (Spanish accession)

- Parents chosen to combine
  - Midseason flowering time
  - Low formononetin content
  - Clover scorch (*Kabatiella caulivora*) resistance
  - High biomass and persistence under grazing

Franklin

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- One of 94 F_4 lines sown in nursery plots
  - Wokalup (WA) – poorly-drained, clay-loam
- Seeds harvested from best lines after 3 years

★ Midseason *yanninicum* F_4 nursery

Stage 2 *yanninicum* total dry matter yields (DMY)

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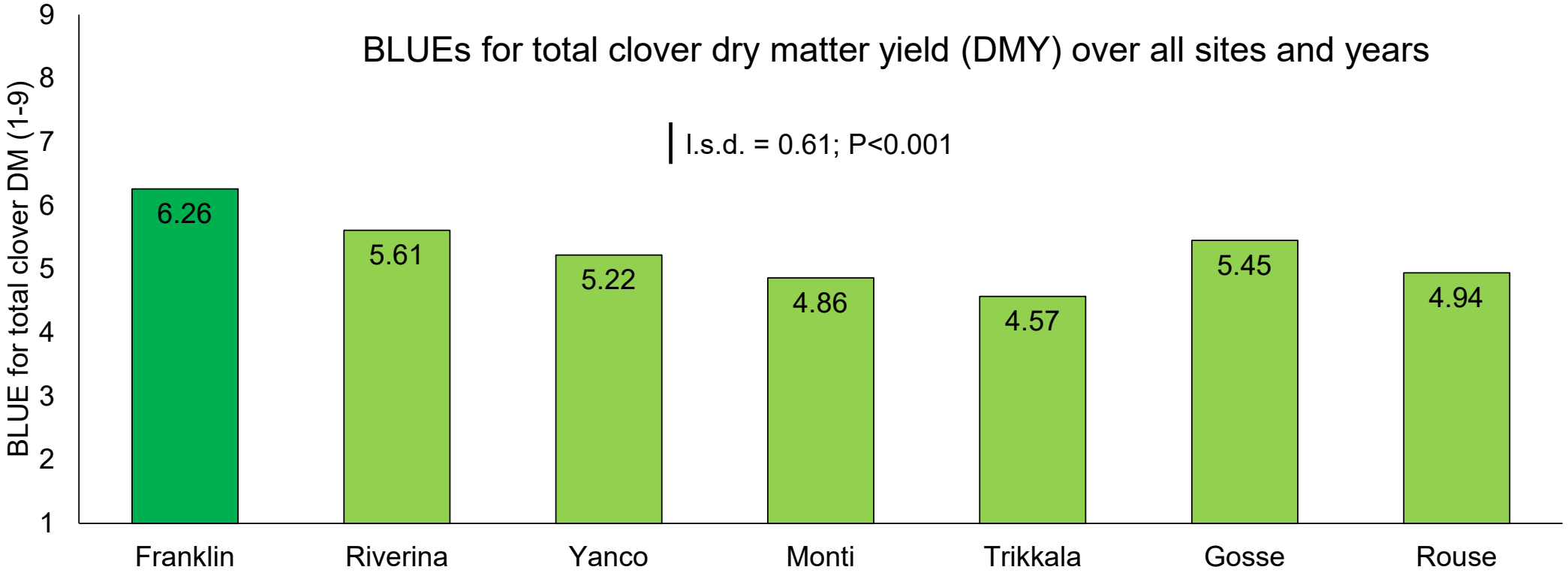
- Best Linear Unbiased Estimates (BLUEs) calculated for DMY over 3 years at 4 sites
  - All clover DMY data first converted to 1-9 scores
- Franklin significantly higher total DMY than all other *yanninicum* cultivars



Days to first flowering at Shenton Park

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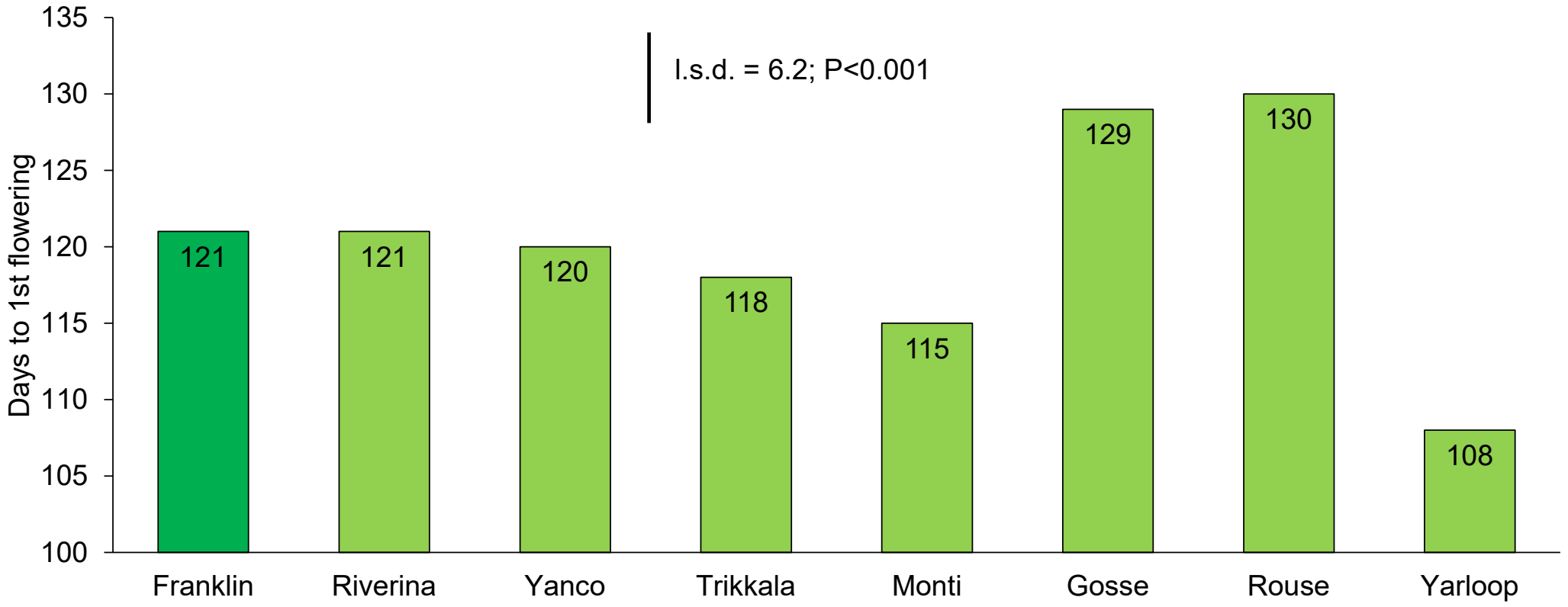
- From an early May sowing; Mean of 8 years
- Franklin flowering similar to Riverina, Yanco, Trikkala & Monti



Formononetin content (% of dry matter)

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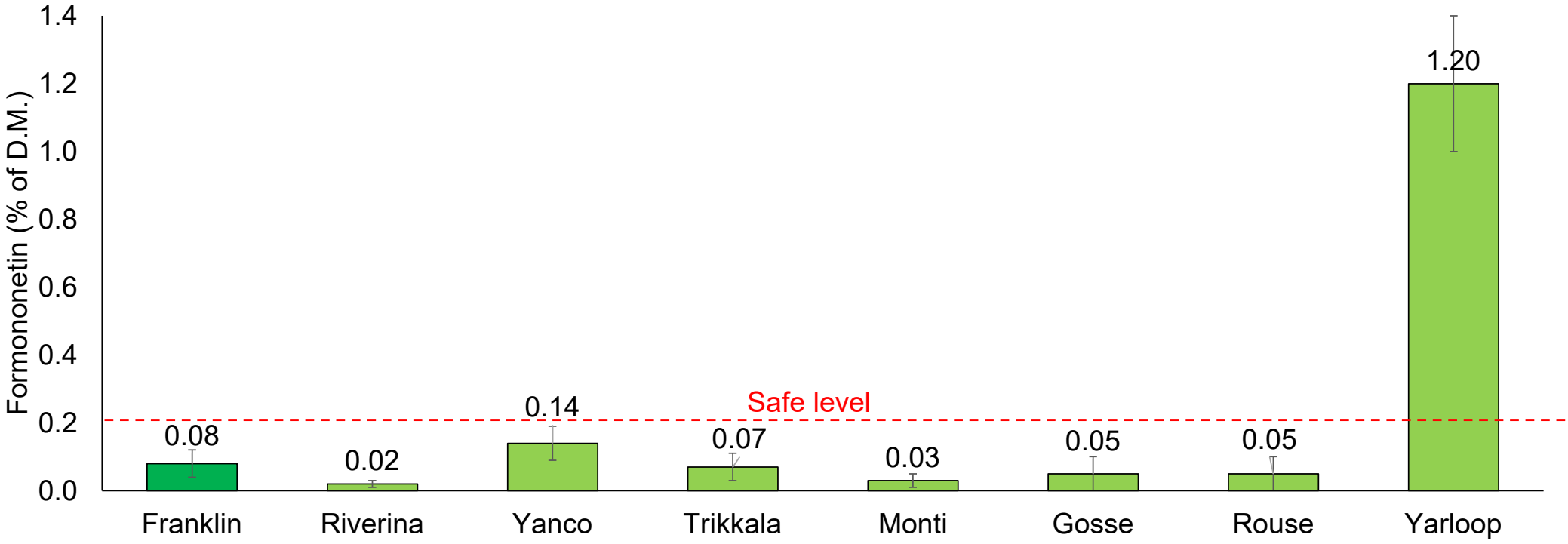
- Franklin has low formononetin content (<0.2% of dry matter) in fresh leaves
  - Mean values from 3 years
- Will not cause infertility or birthing issues for ewes
  - Levels of all newer cultivars way below highly oestrogenic Yarloop



Clover scorch (*Kabatiella caulivora*) resistance

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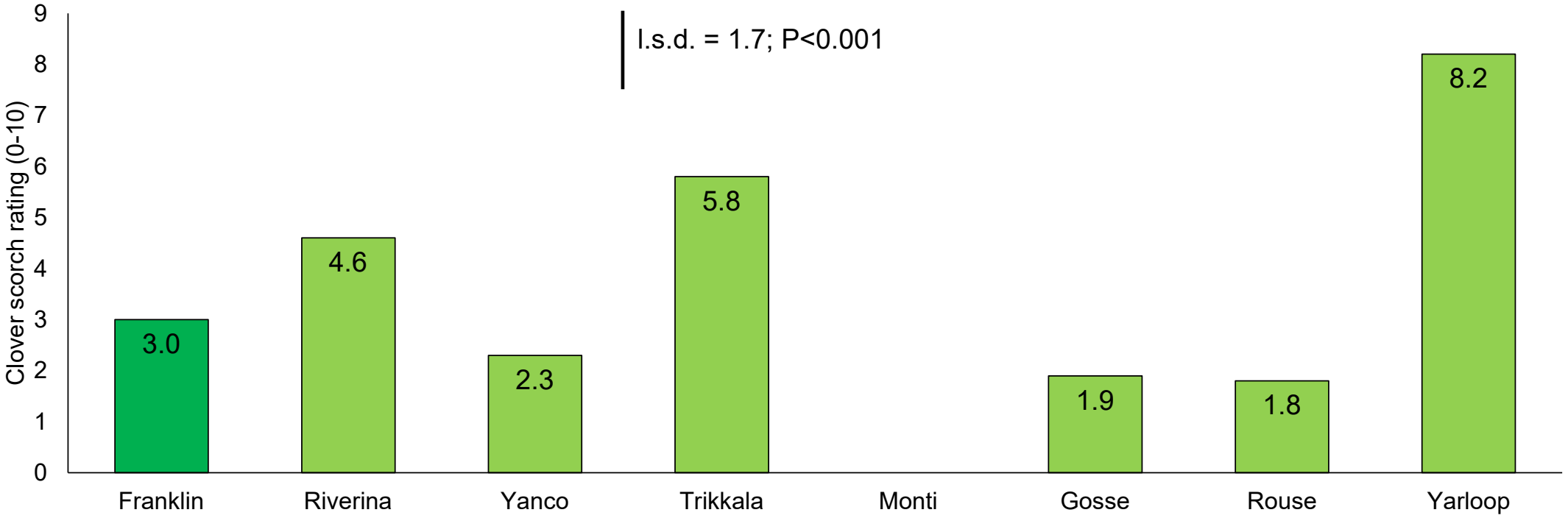
- Field ratings at Denmark, mean of 4 reps
  - 0 = no symptoms, 10 = severe symptoms
  - Resistant if rating <4.0
- Franklin has resistance to clover scorch



Franklin waterlogging tolerance

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- Severe inundation of Busselton *yanninicum* trial in Year 3
  - Plots under water for over 10 weeks in winter-spring
- Franklin performed particularly well

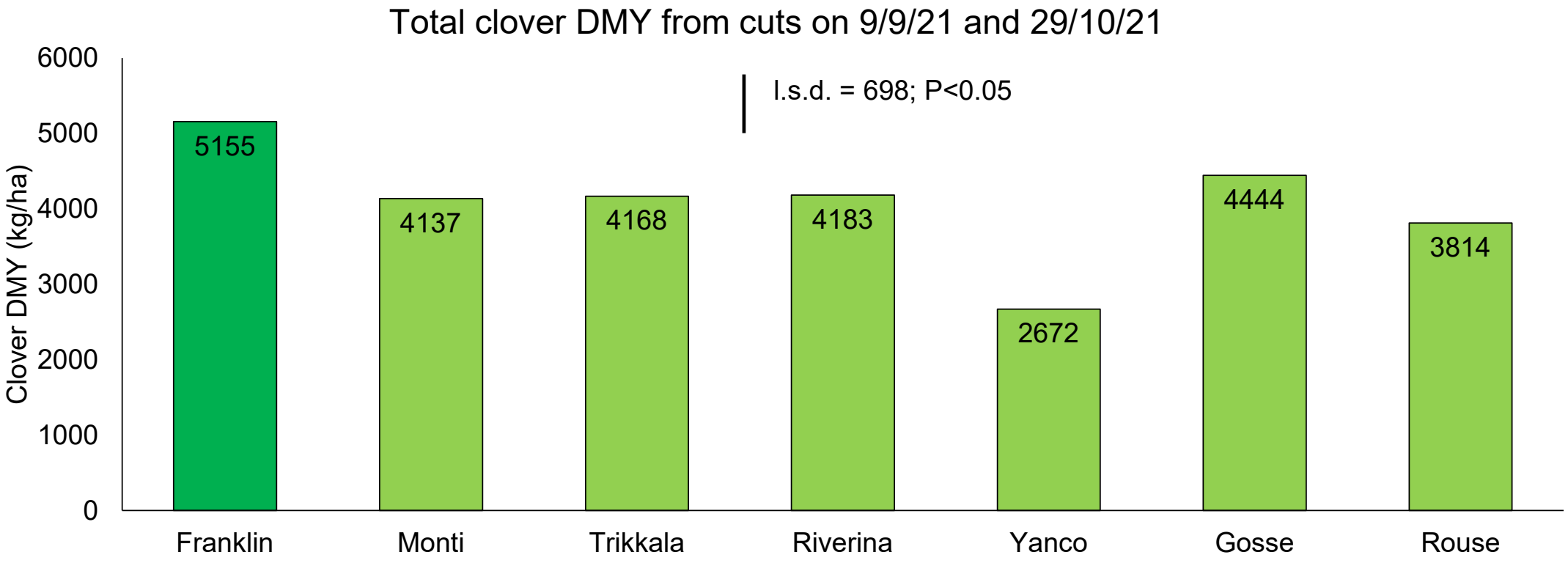

ssp. *yanninicum* plots on July 14


Franklin on September 9


Franklin on October 29

Franklin waterlogging tolerance

- Significantly higher total dry matter yield (DMY) than other cultivars in cuts taken on 9/9 and 29/10 in Year 3



Shoot dry weights after 72 days waterlogging (WL) vs. well-drained (Control) conditions (Enkhbat *et al.* 2023)

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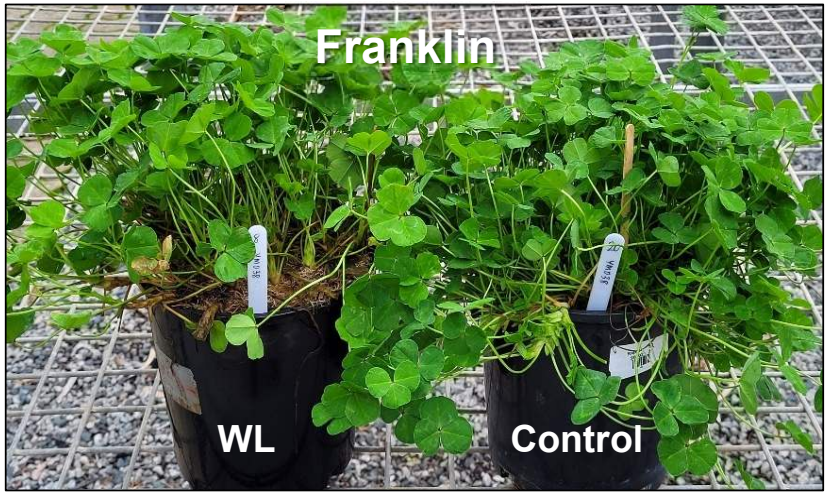
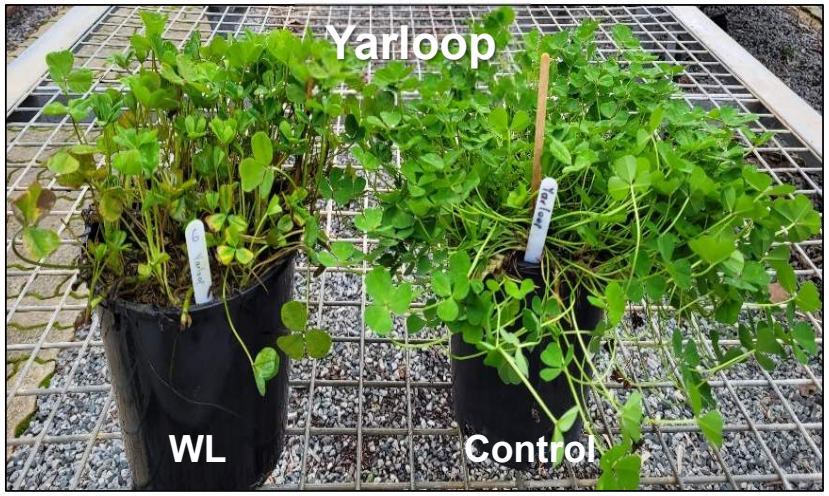


67% of control

47% of control

53% of control



40% of control

Commercialisation

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- Edison, Carver and Franklin selected for cultivar release
  - Edison is a Leura (and Denmark) replacement
  - Carver is a Goulburn replacement
  - Franklin is a Riverina replacement
- Breeders seed produced (>20 kg)
  - Edison & Franklin (2021)
  - Carver (2022)
- Marketed with Plant Breeders Rights protection


Edison Breeders seed increase


Carver Breeders seed increase

Commercialisation

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- Seed multiplication by DLF Seeds
- Certified seed available (limited release)
  - Edison & Franklin (2025)
  - Carver (2026)

Edison Basic seed increase, Kybybolite 2023

Franklin Basic seed increase, Kybybolite 2023

Conclusions

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- The F_4 progeny breeding method led to selection of three cultivars with superior biomass production over 3 years
- Key factors for success are likely to have been:
  - Incorporation of self-regeneration and grazing into early generation selection
  - Selection nursery sites representing key target environments and typical farm management
- Method well-suited to selecting for broad adaptation & good agronomic type
- Less well-suited if the aim is to bring a new trait to market quickly
  - Speed breeding methods (e.g. *in vitro*-assisted single seed descent) are more suited
- These cultivars could have been released >15 years ago if the sub clover breeding program had maintained continuity
  - The ALBA JV has allowed the capture of benefits from prior investments

Acknowledgements

- DLF Seeds for funding the ALBA program
- The Department of Primary Industries and Regional Development (WA) for establishing and providing initial funding for the ALBA program
- Previous funding by GRDC and AWI of the National Subterranean Clover Improvement Program
- The efforts of Brad Wintle and other previous technical staff
- Co-authors for their contributions