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Novel statistical design and analysis that enables valid comparisons of canola varieties across herbicide tolerance groups

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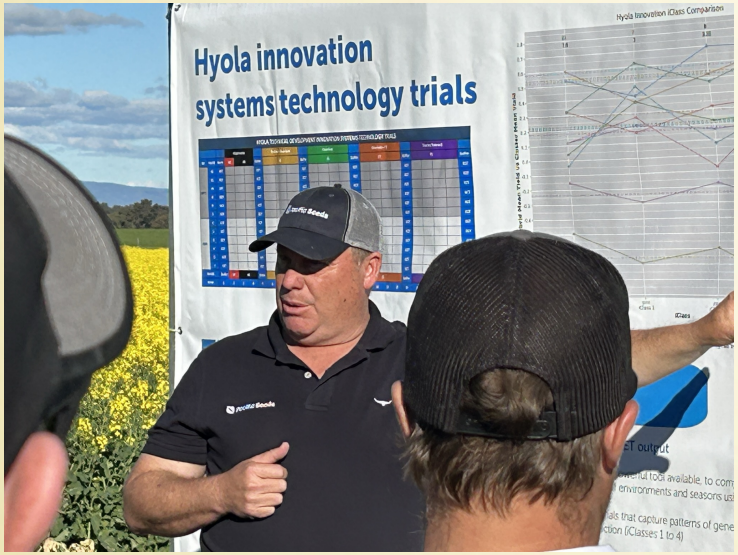
The story
A winning collaboration



"A novel collaboration between the University of Wollongong and Pacific Seeds has demonstrated the ingenuity of two organisations to create an advanced statistical solution to comparing canola varieties across multiple herbicide technologies and environments, ultimately benefitting Australian growers and advisors"†

† Justin Kudnig, National Canola Technical Manager, ADVANTA SEEDS PTY LTD

The context
Variety trials to provide information for growers and agronomists

- Crop variety testing programs aim to provide information for growers and agronomists on the comparative performance of varieties
- This is achieved using a series of field trials conducted in a range of geographic locations and in several seasons (multi-environment trials, METs)
- Each field trial should be (is?) a statistically valid comparative experiment in which a range of varieties is grown

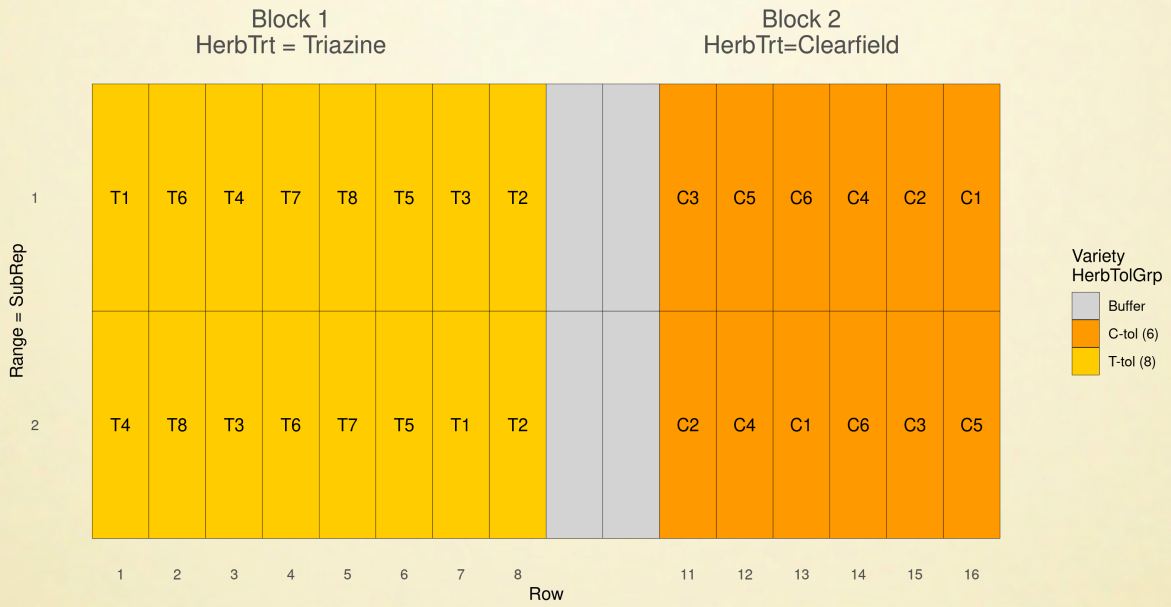
Canola variety trials
Some extra challenges

- Canola poses some additional challenges compared with most other major crops
- Canola variety trials not only involve the growing of a range of varieties but also the application of different herbicide treatments
- So each field trial comprises a factorial experiment where the two treatment factors are herbicide treatments (HerbTrt) and varieties (Variety)
- So how should we design a canola variety trial?
- In constructing any experimental design we need to consider
    - practical constraints and
    - contrasts of interest

- Practical constraints for canola variety trials
  - We cannot randomise herbicide treatments and varieties together to individual plots (so cannot use RCB)
  - Herbicide treatments must be applied to large blocks of plots; varieties can be randomised to plots within blocks (suggests split-plot type design)
  - Canola varieties have tolerance genes for specific herbicides so are classified accordingly into Herbicide Tolerance Groups (HerbTolGrp). They can *only* be treated with associated herbicide treatment.
- Contrasts of interest for canola variety trials
  - We have been told that growers need the ability to compare any pair of varieties irrespective of their herbicide tolerance group
  - We need valid comparisons of varieties both within and across herbicide tolerance groups with all the registered chemicals applied

- Now consider three possible designs:
  - Typical/historical canola variety trial eg. as used in Australian National Variety Trials (NVT) system
  - Hyola Innovation Systems Technology variety trial including stacked varieties (with dual and triple tolerance genes) as recently implemented by Pacific Seeds
  - Hyola Innovation Systems Technology variety trial including stacked varieties plus enhancement suggested by UOW
- Do these designs allow valid comparisons of varieties both within and across herbicide tolerance groups?

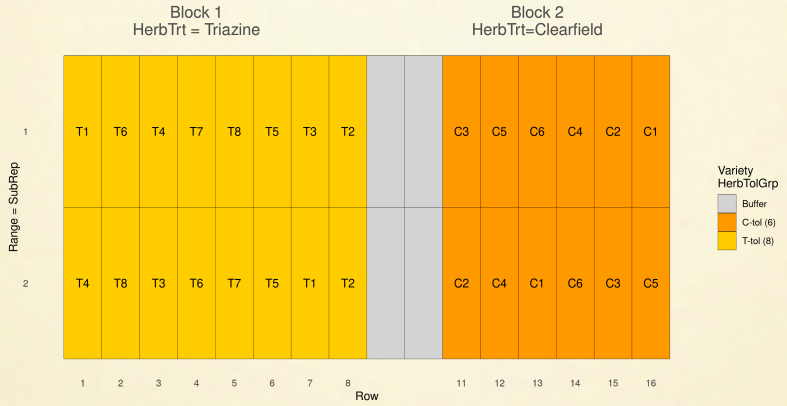
Layout of typical/historical canola variety trial (eg. NVT)
Two herbicide treatments: Triazine and Clearfield



- Two herbicide treatments, each applied to single block of plots: Triazine Rows 1-8; Clearfield Rows 11-16
- Varieties from two herbicide tolerance groups (8 T-tol; 6 C-tol): T-tol varieties in Block 1 only (2 plots); C-tol in Block 2 only (2 plots)

- We've come across many examples in literature of statistical analyses that fail to capture key aspects of the experimental design so lead to invalid inference
- For example, here it is possible to analyse the trial as if it were a RCB with 2 replicates of 14 varieties. A LMM/ANOVA would give a p-value for Variety which is invalid
- Analysis of any designed experiment requires careful consideration of the terms that need to be included in the LMM (or ANOVA)
- We developed the Design Tableau approach to aid with this and (hopefully) ensure valid inference

Typical/historical canola variety trial (eg. NVT)
Abbreviated Design Tableau



- Key elements of Design Tableau are
  - Draw a picture of layout (see above!)
  - Define plot factors: Block (2), SubRep (2) and Plot (8)
  - Define treatment factors: HerbTrt (2) and Variety (14) (and HerbTolGrp (2))
  - Describe design function (earlier slide)

- Key elements of Design Tableau (continued)
  - Define model formula for plot structure:
    Block/SubRep/Plot
    = Block + Block:SubRep + Block:SubRep:Plot
  - Define model formula for treatment structure:
    1 + HerbTrt*Variety
    = 1 + HerbTrt + Variety + HerbTrt:Variety
  - Identify aliasing of factors (these cannot be separately estimated): defer til later

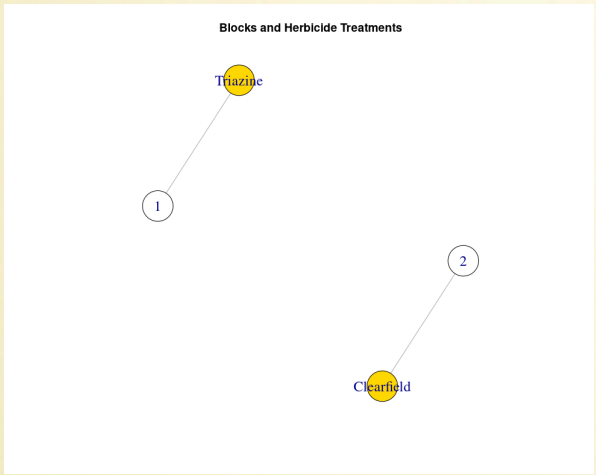
Design Tableau
Aspirational model

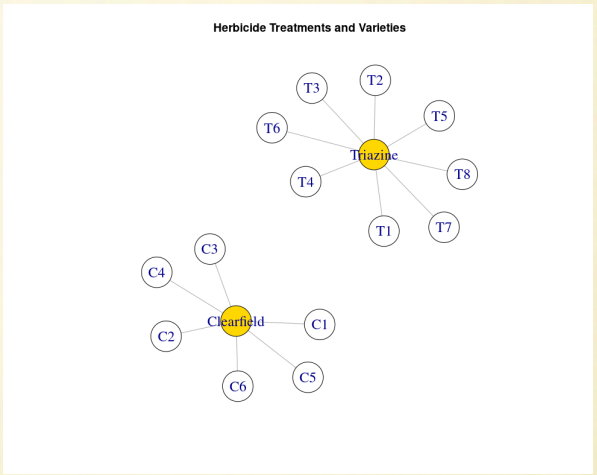
- Specification of plot and treatment structures allows us to draw a table showing terms in the (aspirational) model
- List as treatment terms followed by plot terms

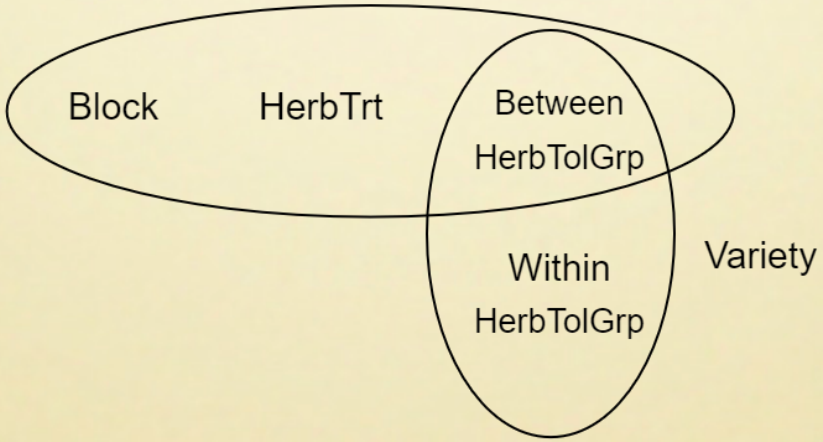
Mean
HerbTrt
Variety
HerbTrt:Variety
Block
Block:SubRep
Block:SubRep:Plot (error)

- Does the experimental design allow us to fit the aspirational model?
- Does the experimental design allow valid inference for each set of treatment effects?
- Answer these questions by investigating aliasing of factors
- May be obvious from working through Design Tableau, perhaps with extra visuals . . .

Typical/historical canola variety trial (eg. NVT)
Aliasing/Connections







Identification of aliasing
A formal approach

- We use Marginality Matrices (Cullis, pers comm) based loosely on ideas in Nelder (1968)
- Developed for a general approach to inference for fixed effects in a LMM (will be implemented in DWReml)
- We use here to identify aliasing of model terms (associated with fixed and/or random effects)
- In our example, the model terms to be assessed are HerbTrt, Variety, HerbTrt:Variety and Block (and we include the Mean)

- First construct design matrix for each model term:
  - Mean: $\boldsymbol{X}_1$ is $n \times 1$ ($\boldsymbol{X}_1 = \mathbf{1}_n$)
  - HerbTrt: $\boldsymbol{X}_2$ is $n \times 2$ indicator matrix
  - Variety: $\boldsymbol{X}_3$ is $n \times 14$ indicator matrix
  - HerbTrt:Variety: $\boldsymbol{X}_4$ is $n \times 28$ indicator matrix
  - Block: $\boldsymbol{X}_5$ is $n \times 2$ indicator matrix
- Then consider

$$\boldsymbol{C} = \boldsymbol{X}^\top \boldsymbol{X} = \begin{bmatrix} \boldsymbol{X}_1^\top \boldsymbol{X}_1 & \boldsymbol{X}_1^\top \boldsymbol{X}_2 & \boldsymbol{X}_1^\top \boldsymbol{X}_3 & \boldsymbol{X}_1^\top \boldsymbol{X}_4 & \boldsymbol{X}_1^\top \boldsymbol{X}_5 \\ \boldsymbol{X}_2^\top \boldsymbol{X}_1 & \boldsymbol{X}_2^\top \boldsymbol{X}_2 & \boldsymbol{X}_2^\top \boldsymbol{X}_3 & \boldsymbol{X}_2^\top \boldsymbol{X}_4 & \boldsymbol{X}_2^\top \boldsymbol{X}_5 \\ \boldsymbol{X}_3^\top \boldsymbol{X}_1 & \boldsymbol{X}_3^\top \boldsymbol{X}_2 & \boldsymbol{X}_3^\top \boldsymbol{X}_3 & \boldsymbol{X}_3^\top \boldsymbol{X}_4 & \boldsymbol{X}_3^\top \boldsymbol{X}_5 \\ \boldsymbol{X}_4^\top \boldsymbol{X}_1 & \boldsymbol{X}_4^\top \boldsymbol{X}_2 & \boldsymbol{X}_4^\top \boldsymbol{X}_3 & \boldsymbol{X}_4^\top \boldsymbol{X}_4 & \boldsymbol{X}_4^\top \boldsymbol{X}_5 \\ \boldsymbol{X}_5^\top \boldsymbol{X}_1 & \boldsymbol{X}_5^\top \boldsymbol{X}_2 & \boldsymbol{X}_5^\top \boldsymbol{X}_3 & \boldsymbol{X}_5^\top \boldsymbol{X}_4 & \boldsymbol{X}_5^\top \boldsymbol{X}_5 \end{bmatrix}$$

- Marginality and aliasing for model terms i and j can be detected by comparing the rank of $\boldsymbol{X}_i^\top \boldsymbol{X}_i$ before and after absorbing $\boldsymbol{X}_j^\top \boldsymbol{X}_j$

- Form Marginality Matrix $\boldsymbol{M} = \{m_{ij}\}$ where

$$m_{ij} = \begin{cases} \text{rank}\,(\boldsymbol{X}_i^\top \boldsymbol{X}_i) & \text{for } i = j \\ \text{rank}\,\left(\boldsymbol{X}_i^\top (\boldsymbol{I}_n - \boldsymbol{X}_j (\boldsymbol{X}_j^\top \boldsymbol{X}_j)^- \boldsymbol{X}_j^\top) \boldsymbol{X}_i\right) & \text{for } i \neq j \end{cases}$$

- Note that if $\boldsymbol{X}_i^\top \boldsymbol{X}_i$ has full column rank we can use standard inverse

Typical/historical canola variety trial (eg. NVT)
Marginality matrix

	Mean	HerbTrt	Variety	Herb:Var	Block
Mean	1	0	0	0	0
HerbTrt	1	2	0	0	0
Variety	13	12	14	0	12
Herb:Var	13	12	0	14	12
Block	1	0	0	0	2

- Note that each $\boldsymbol{X}_i^\top \boldsymbol{X}_i$ apart from $i = 4$ (HerbTrt:Variety) has full column rank
- Typical/historical design therefore does not allow fitting of aspirational model nor inference on effects of interest (*all* pairwise variety comparisons)

Canola variety trials
A short history of BC and AS involvement

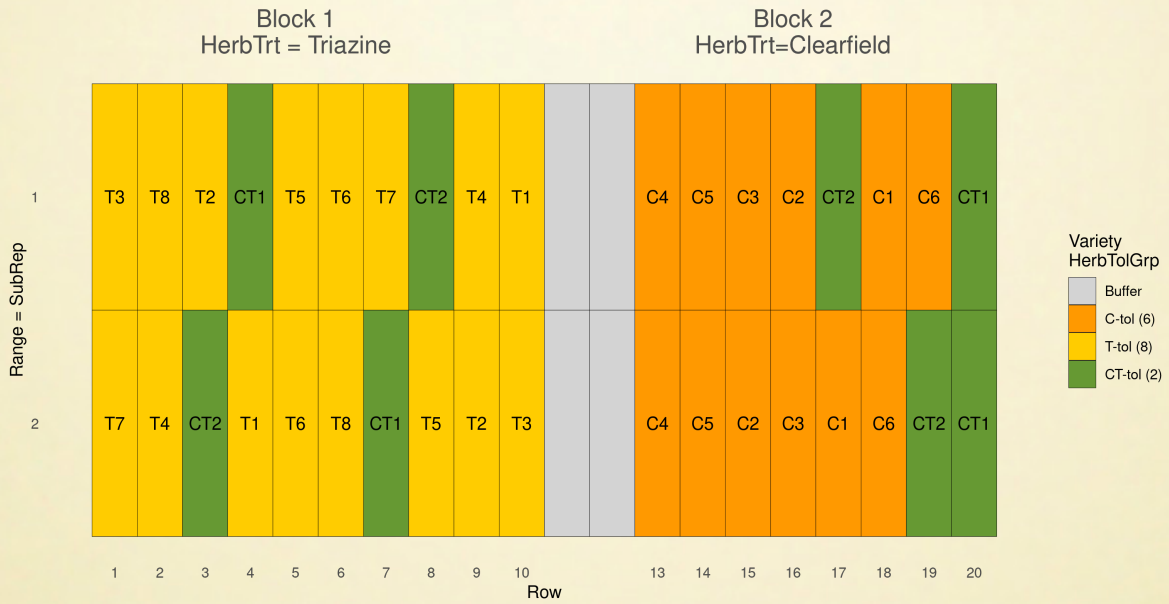
- BC and AS have been involved in the NVT system since its inception in 2005 and until 2023
- Ongoing debate for canola as to whether growers require comparisons across herbicide tolerance groups
- We have always believed yes and have tried to work with NVT to create experimental designs to break aliasing
- Some traction circa 2010 but at that time impossible to break aliasing of HerbTrt and HerbTolGrp
- Game changer has been development of stacked varieties (with multiple tolerance genes)

Canola variety trials
A short history of BC and AS involvement

- Potential to use stacked varieties in canola variety testing to break aliasing of HerbTrt and HerbTolGrp, thence enable comparisons across herbicide tolerance groups
- Proposal made by industry to NVT in 2019 but not adopted
- BC and AS did not engage further on this concept until Pacific Seeds approached them in 2022 to analyse 30 variety trials (Hyola Innovation Systems Technology trials) from 2021 and 2022
- Pacific Seeds had designed these trials using stacked varieties so have broken the aliasing of HerbTrt and HerbTolGrp
- A huge win for growers!
- Conceptually, this is what they did . . .

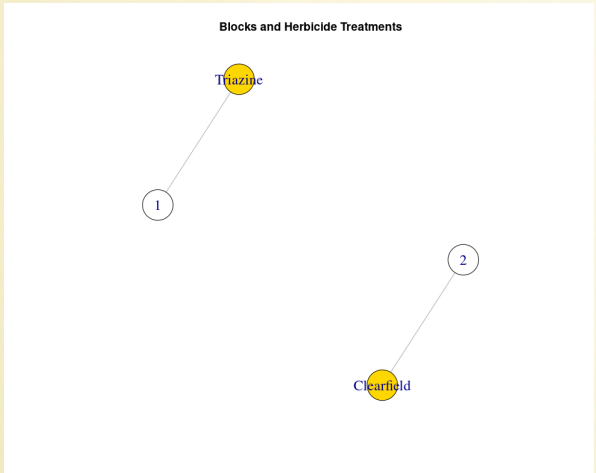
Pacific Seeds trial with stacked varieties

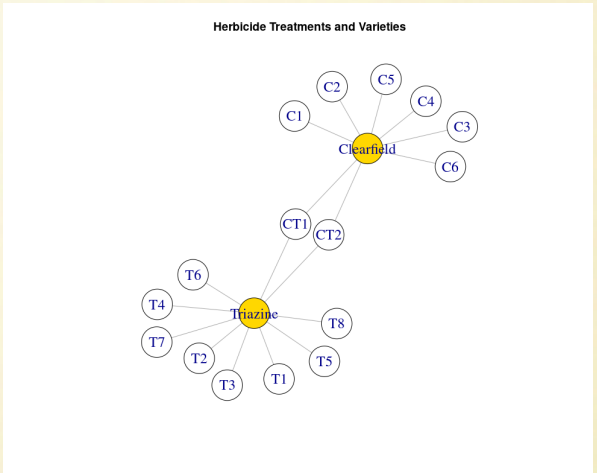
Conceptual layout for two herbicide treatments: Triazine and Clearfield

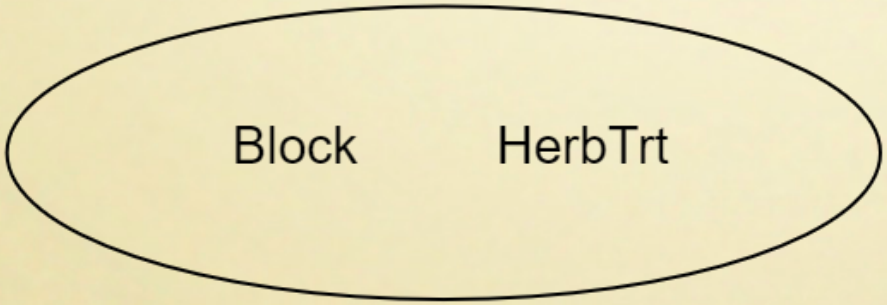


- Two herbicide treatments, each applied to single block of plots: Triazine Rows 1-10; Clearfield Rows 13-20
- Varieties from three herbicide tolerance groups (8 T-tol; 6 C-tol; 2 CT-tol): T-tol varieties in Block 1 only (2 plots); C-tol in Block 2 only (2 plots); CT-tol in both blocks (total of 4 plots)

Pacific Seeds trial with stacked varieties
Aliasing/Connections





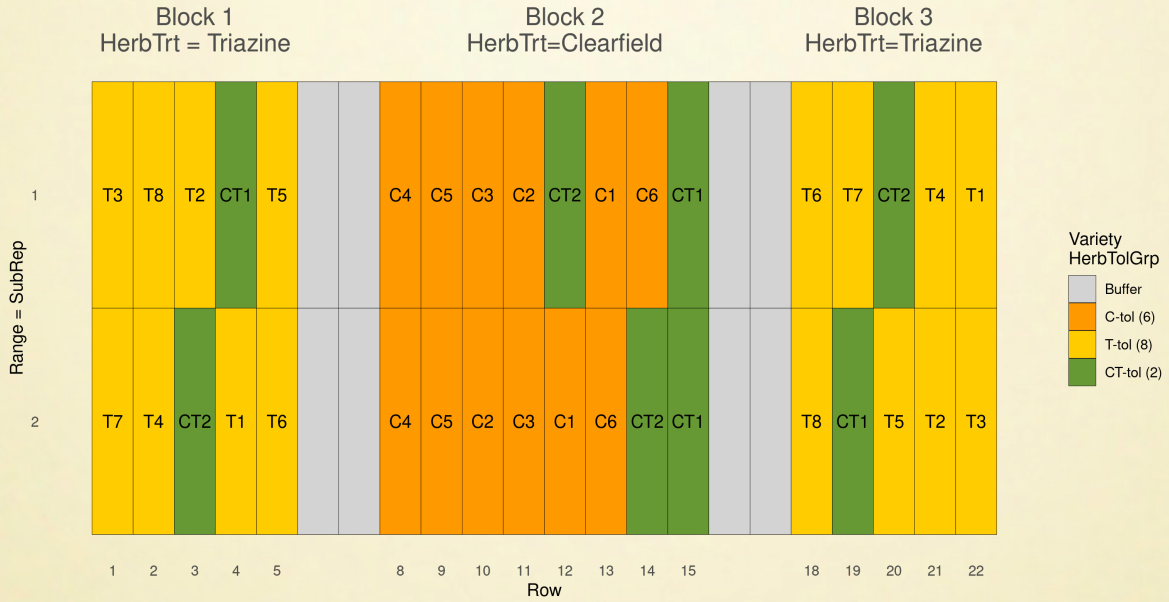


Pacific Seeds trial with stacked varieties
Marginality Matrix

	Mean	HerbTrt	Variety	Herb:Var	Block
Mean	1	0	0	0	0
HerbTrt	1	2	1	0	0
Variety	15	15	16	0	15
Herb:Var	17	16	2	18	16
Block	1	0	1	0	2

- Pacific Seeds designs are a huge improvement over NVT type designs in terms of providing valid comparisons across all canola varieties, irrespective of herbicide tolerance group
- The designs provide 90% of the solution
- Only the aliasing between Block and HerbTrt remains: so if there is Block $\times$ Variety interaction is it simply error (OK) or actually HerbTrt $\times$ Variety interaction (tricky)?
- We have recently devised an enhancement (practically relatively simple and statistically powerful) that Pacific Seeds will be adopting in future trials . . .

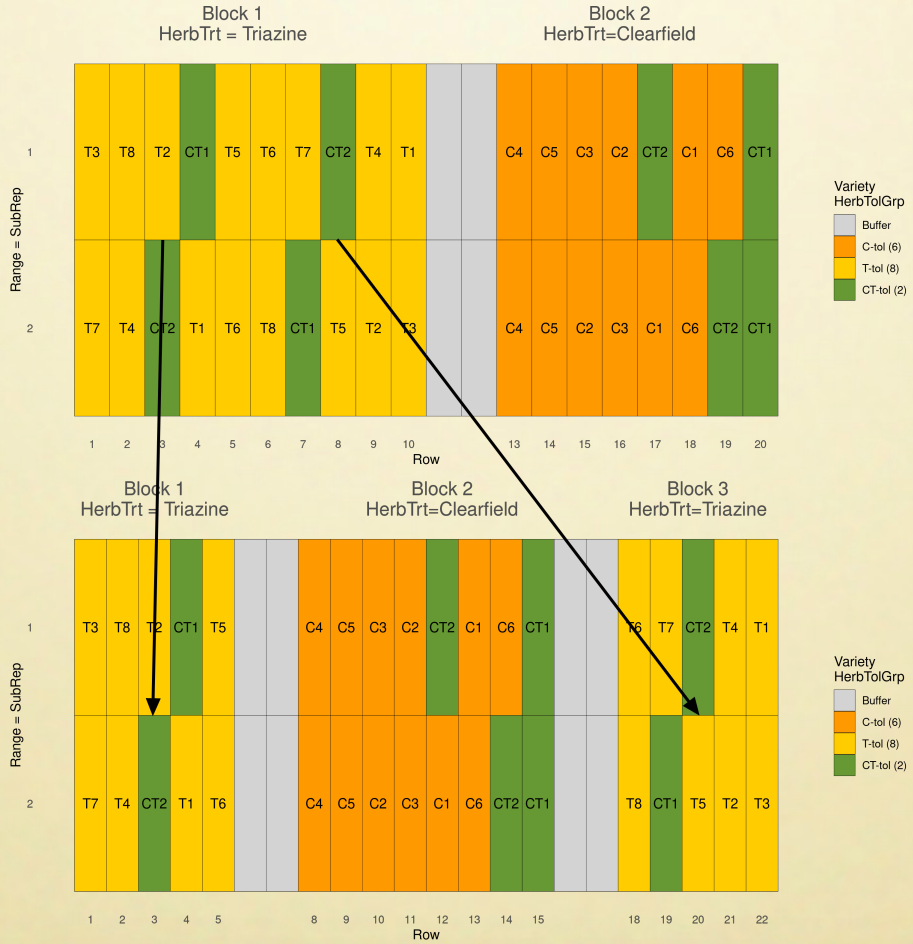
UOW "enhanced" Pacific Seeds trial with stacked varieties
Conceptual layout for two herbicide treatments: Triazine and Clearfield



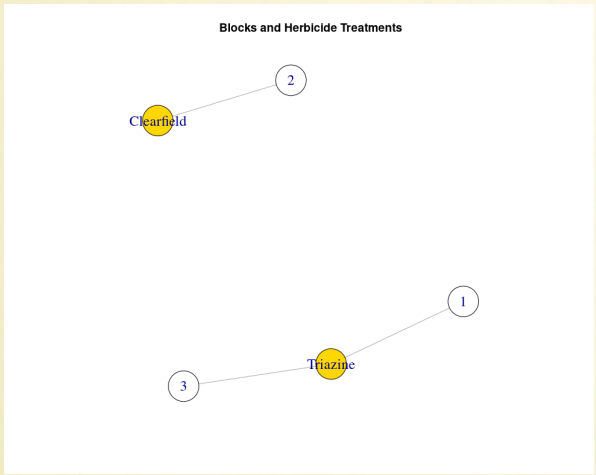
- Two herbicide treatments, with Triazine applied to (split into) two blocks: Triazine Rows 1-5 and 18-22; Clearfield Rows 8-15
- Varieties from three herbicide tolerance groups (8 T-tol; 6 C-tol; 2 CT-tol): T-tol varieties in Blocks 1 and 3 only (total of 2 plots); C-tol in Block 2 only (2 plots); CT-tol in all blocks (total of 4 plots)

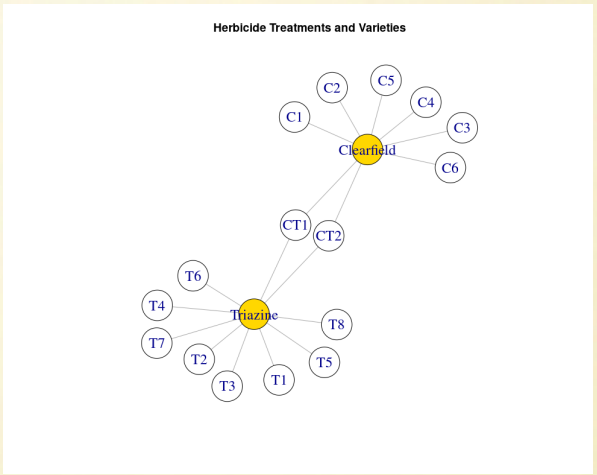
UOW "enhanced" Pacific Seeds trial with stacked varieties
Conceptual layout for two herbicide treatments: Triazine and Clearfield

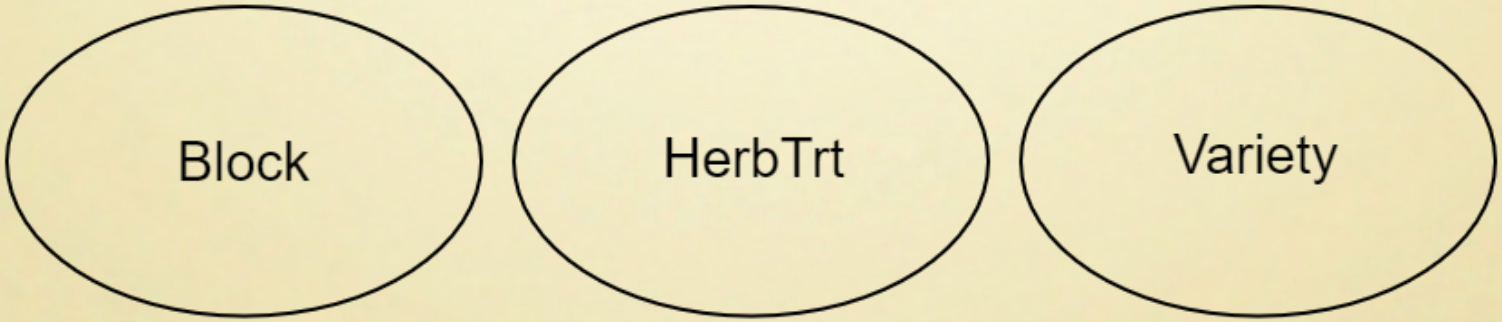
- Proposal involves a simple splitting of original single herbicide treatment block into two smaller blocks located in separate parts of the trial



UOW “enhanced” Pacific Seeds trial with stacked varieties
Aliasing/Connections







UOW “enhanced” Pacific Seeds trial with stacked varieties
Marginality Matrix

	Mean	HerbTrt	Variety	Herb:Var	Block
Mean	1	0	0	0	0
HerbTrt	1	2	1	0	0
Variety	15	15	16	0	15
HerbTrt:Variety	17	16	2	18	16
Block	2	1	2	1	3

Design of canola variety trials
Solved problem of making valid comparisons across all varieties?

Term	0%* NVT	90% Pacific Seeds	100% Pacific Seeds + UOW
Mean	√	√	√
HerbTrt	×[a]	×[a]	√
Variety			
Between HerbTolGrp	×[a]	√	√
Within HerbTolGrp	√	√	√
HerbTrt:Variety	×	?	√
Block	×[a]	×[a]	√
Block:SubRep	√	√	√
Block:SubRep:Plot	√	√	√

[a] aliased terms for this design

* NVT do not claim to allow comparisons across herbicide technology groups; they point out on website (https://nvt.grdc.com.au/trials/frequently-asked-questions) that it is statistically invalid to "compare variety performance across different trials (eg. canola herbicide groups)"

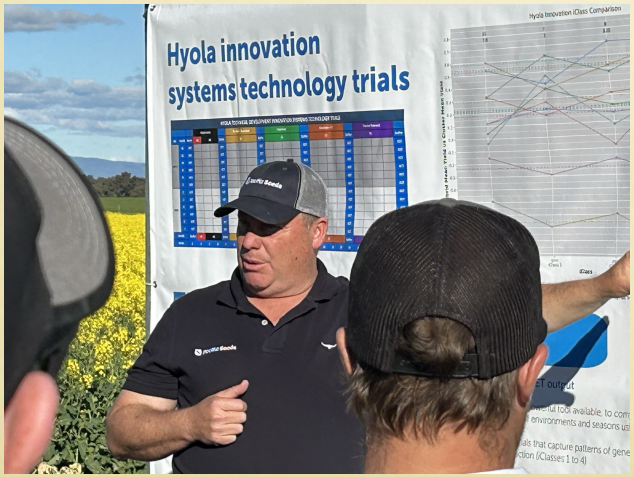
Pacific Seeds trial with stacked varieties
Actual layout

2022 HYOLA TD - SYSTEMS TECHNOLOGY TRIALS (22STSYTL56)																										
			RR		TruFlex			uFlex + Clearfie			Clearfield				Clearfield + TT			Triazine Tolerant								
5m	PLOTS	Buffer		Buffer	XX		Buffer	XC		Buffer	CL			Buffer	CT		Buffer	TT (L4)							Buffer
REP 2	12	XCT		XCT			XCT			XCT				XCT			XCT								XCT
	11	XCT		XCT			XCT			XCT				XCT			XCT								XCT
	10	XCT		XCT			XCT			XCT				XCT			XCT								XCT
	9	XCT		XCT			XCT			XCT				XCT			XCT								XCT
	8	XCT		XCT			XCT			XCT				XCT			XCT								XCT
	7	XCT		XCT			XCT			XCT				XCT			XCT								XCT
REP 1	6	XCT		XCT			XCT			XCT				XCT			XCT								XCT
	5	XCT		XCT			XCT			XCT				XCT			XCT								XCT
	4	XCT		XCT			XCT			XCT				XCT			XCT								XCT
	3	XCT		XCT			XCT			XCT				XCT			XCT								XCT
	2	XCT		XCT			XCT			XCT				XCT			XCT								XCT
	1	XCT		XCT			XCT			XCT				XCT			XCT								XCT
RANGE		Buffer	RR	Buffer	XX		Buffer	XC		Buffer	CL			Buffer	CT		Buffer	TT							Buffer
ROWS		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24

- Blocks are connected using stacked varieties (dual and triple tolerance genes)
- Dual stacked (as illustrated in conceptual layout)
- Pacific Seeds world first triple stacked (XCT) varieties to provide more connections between blocks hence increase reliability of variety comparisons between herbicide tolerance groups

Analysis of canola variety trials
Pacific Seeds multi-environment trial data-set

- UOW has value added further by analysing systems multi-environment trial dataset (30 environments; 7 herbicide treatments; 90 varieties)
  - (bespoke) single stage FALMM analysis
  - probability statements (as graphic) for pairwise comparisons of predictions of (random) variety effects for any given environment
  - variety predictions summarised across environments using interaction class (iClass) technology
  - all embraced by Pacific Seeds and rolled out to growers and agronomists . . .



Acknowledgements

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- David Tabah (currently InterGrain; formerly Pacific Seeds)