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

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Pacific Seeds
Growing possibilities

Scientific endeavour

Information for stakeholders

- Scientific endeavour is only useful if it addresses the needs of stakeholders and does so in a (statistically) rigorous manner so there is confidence in the outcomes
- Today we present a key industry example where we feel this historically has not been achieved
- We present a solution that has been driven by a private company (Pacific Seeds) and enhanced by the statistical expertise of biometricians at UOW (AS and BC)
- The example? Canola variety testing to assist growers in varietal decision making

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)
- BC/AS have a long history in developing and applying statistical methodology for both the design and analysis of variety trials

Variety trials to provide information for growers and agronomists

Key statistical developments

- Single-stage factor analytic linear mixed model (FALMM) analysis of MET data (Smith, Cullis & Thompson, 2001)
  - Fully efficient (uses individual plot data combined across trials)
  - FA model reflects complex patterns of variety by environment interaction (unsupervised machine learning)
  - Used in most major plant breeding programs in Australia and in NVT

- Using FALMM to define groups of environments (iClasses) for summarising variety performance (Smith, Norman, Kuchel & Cullis, 2021; Smith, Hobson, Butler & Cullis submitted)
  - Answers a major challenge ie. providing meaningful and concise information for variety comparisons in the presence of variety by environment interaction
  - Used in many major plant breeding programs in Australia

Canola variety trials

Some extra challenges

- The application of these advanced statistical tools is straightforward for most crops, but canola poses some additional challenges
- Canola variety trials not only involve the growing of a range of varieties but also the application of different combinations of herbicide treatments
- Statistically speaking we have a factorial experiment where the two treatment factors are herbicide treatments (HerbTrt) and varieties (Variety). (Standard factorial analysis would estimate main effects of HerbTrt and Variety, plus HerbTrt × Variety interaction.)
- So how should we design a canola variety trial?
- In constructing any experimental design we need to consider
  - practical constraints and
  - contrasts of interest

Canola variety trials

Experimental design

- 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 as a systems approach

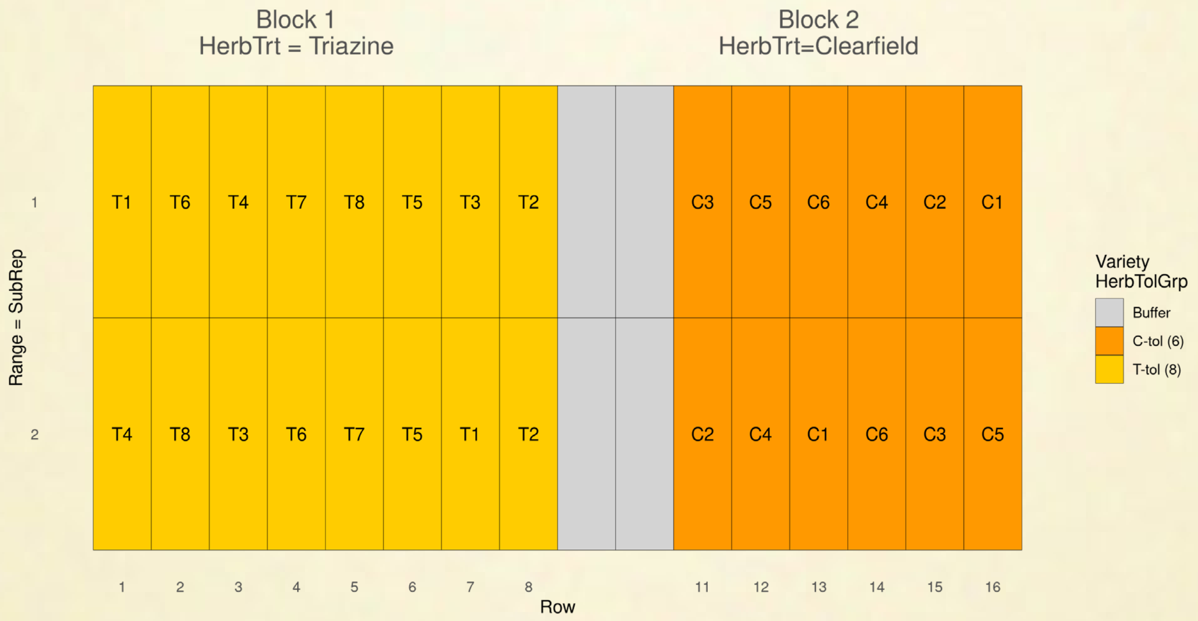
Canola variety trials

Experimental design

- 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 recommended by UOW
- Do these designs allow valid comparisons of varieties both within and across herbicide tolerance groups?

Example layout of typical/historical canola variety trial (eg. NVT)

Two herbicide treatments: Triazine and Clearfield



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2	T4	T8	T3	T6	T7	T5	T1	T2			C2	C4	C1	C6	C3	C5

- 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)

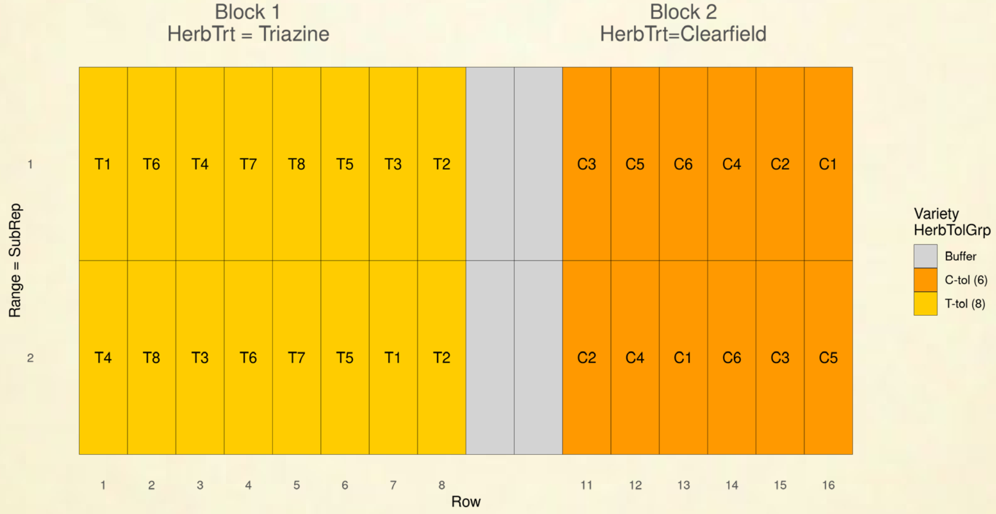
Statistical analysis of comparative experiments

Ensuring valid inference

- 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)

Design Tableau



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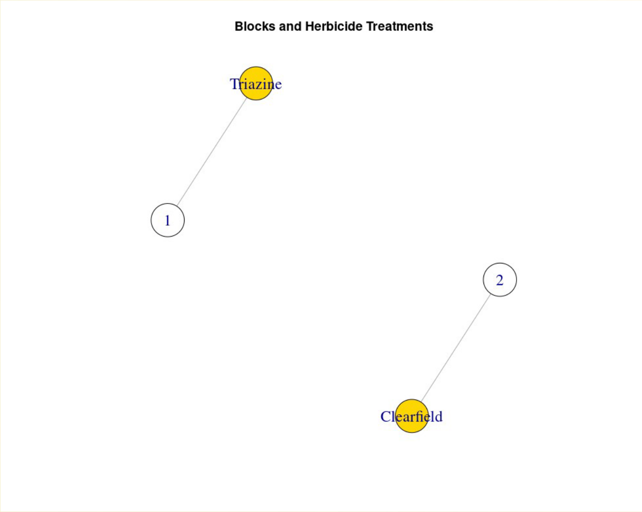
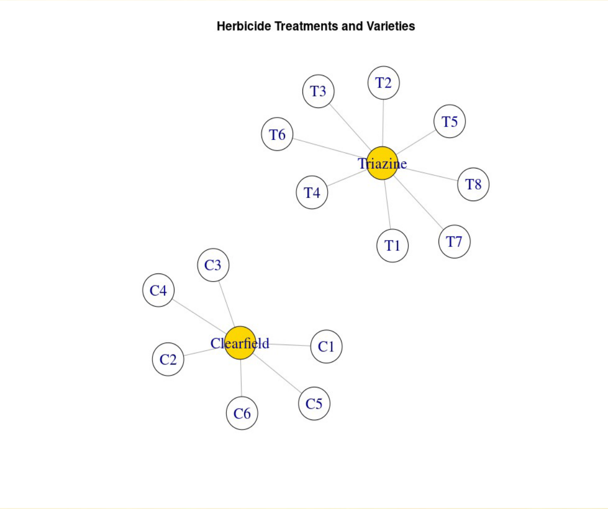
2	T4	T8	T3	T6	T7	T5	T1	T2			C2	C4	C1	C6	C3	C5

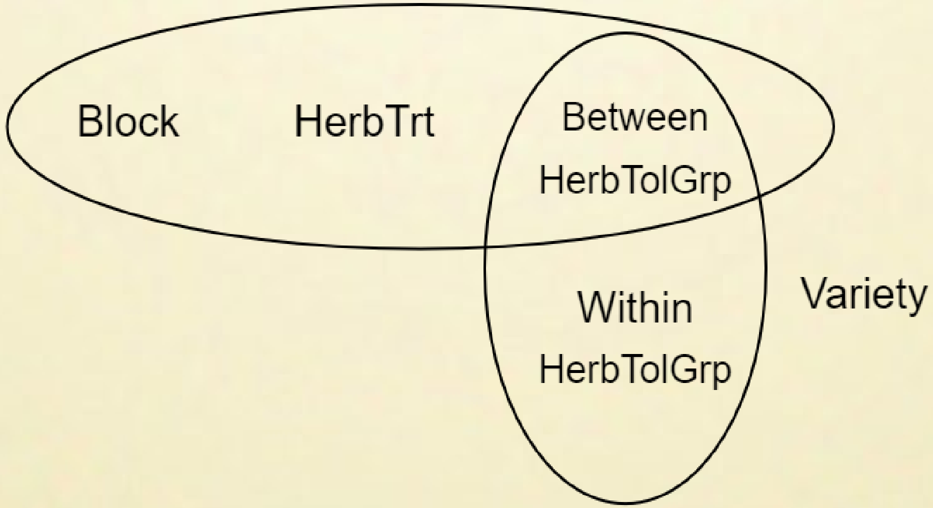
- Key elements of Design Tableau are
  - Define plot factors: here Block, SubRep and Plot
  - Define model formula for plot structure: Block/SubRep/Plot
  - Define treatment factors: here HerbTrt and Variety
  - Define model formula for treatment structure: HerbTrt + Variety + HerbTrt:Variety
  - Define design function (see earlier slide)
  - Identify aliasing of factors: these cannot be separately estimated

Typical/historical canola variety trial (eg. NVT)

Aliasing/Connections


Blocks and Herbicide Treatments


Herbicide Treatments and Varieties


Block

HerbTrt

Between HerbTolGrp

Within HerbTolGrp

Variety

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
- Industry Game changer has been development of innovative stacked varieties (with multiple tolerance genes)

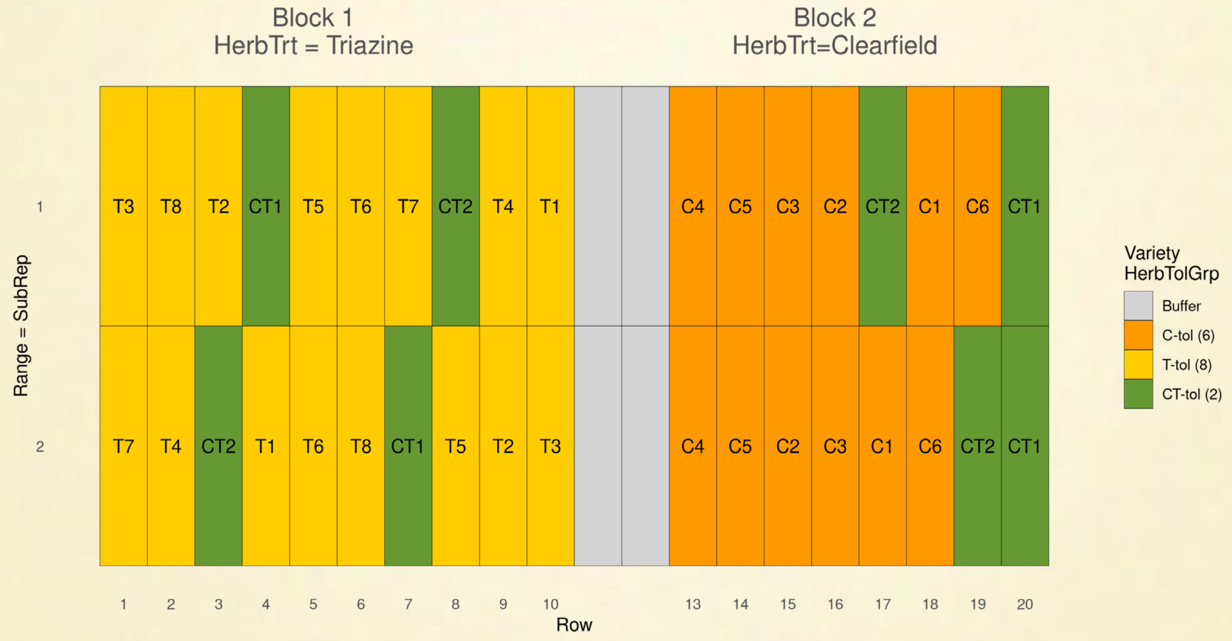
Canola variety trials

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 the whole canola industry and especially growers!
- Conceptually, this is what they did . . .

Pacific Seeds Hyola Innovation Systems Technology trial with stacked varieties

Conceptual layout for two herbicide treatments: Triazine and Clearfield



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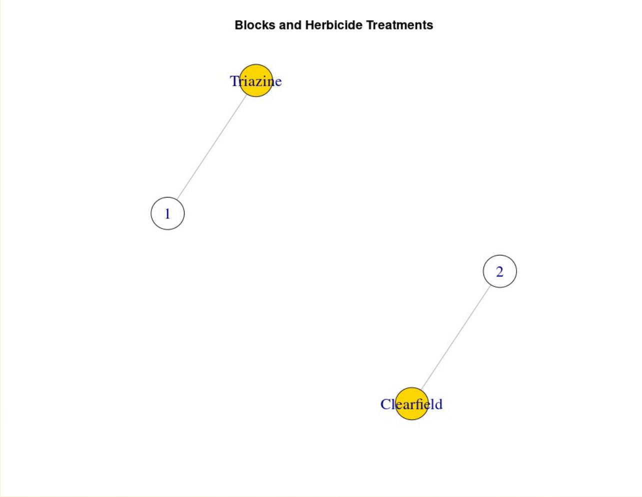
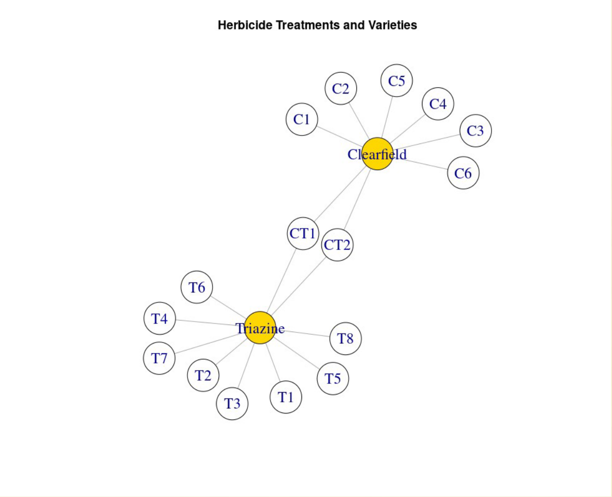
1	T3	T8	T2	CT1	T5	T6	T7	CT2	T4	T1			C4	C5	C3	C2	CT2	C1	C6	CT1
2	T7	T4	CT2	T1	T6	T8	CT1	T5	T2	T3			C4	C5	C2	C3	C1	C6	CT2	CT1

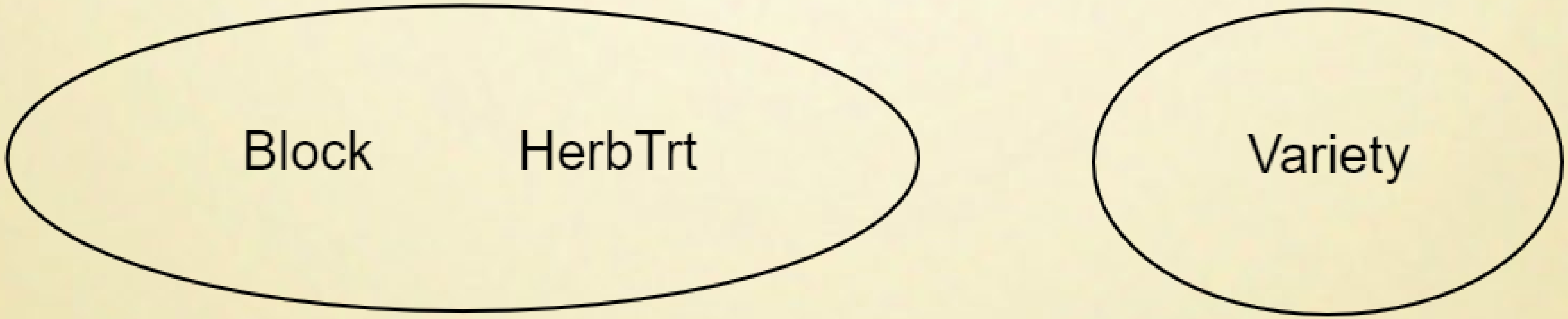
- Two herbicide treatments, each applied to single block of plots:
  - Triazine Rows 1-10; Clearfield Rows 13-20
- Varieties from three herbicide tolerance groups (8 T-tol; 6 C-tol; 2 CT-tol)
  - T-tol varieties in Block 1 only (2 plots); C-tol in Block 2 only (2 plots); CT-tol in both blocks (total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

Pacific Seeds Hyola Innovation Systems Technology trial with stacked varieties

Aliasing/Connections


Blocks and Herbicide Treatments


Herbicide Treatments and Varieties


Block HerbTrt

Variety

Pacific Seeds Hyola Innovation Systems Technology trial with stacked varieties

Design Tableau and aliasing

- 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

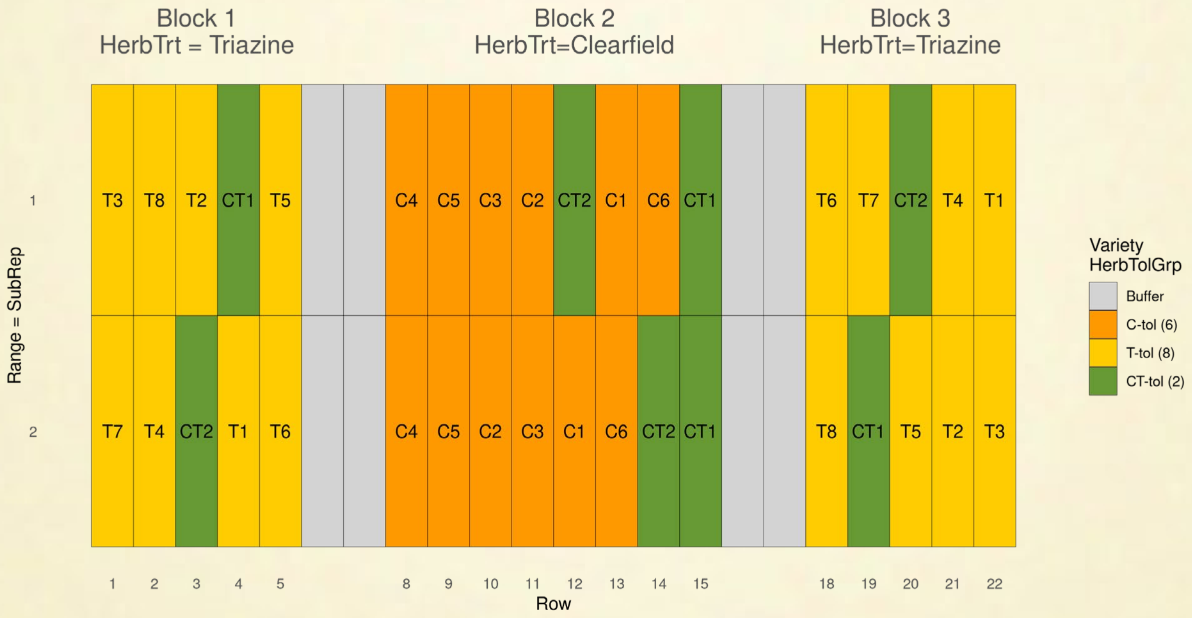
- The designs provide 90% of the solution

- Only the aliasing between Block and HerbTrt remains: so if there is Block × Variety interaction is it simply error (OK) or actually HerbTrt × 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 Hyola Innovation Systems Technology trial

Conceptual layout for two herbicide treatments: Triazine and Clearfield



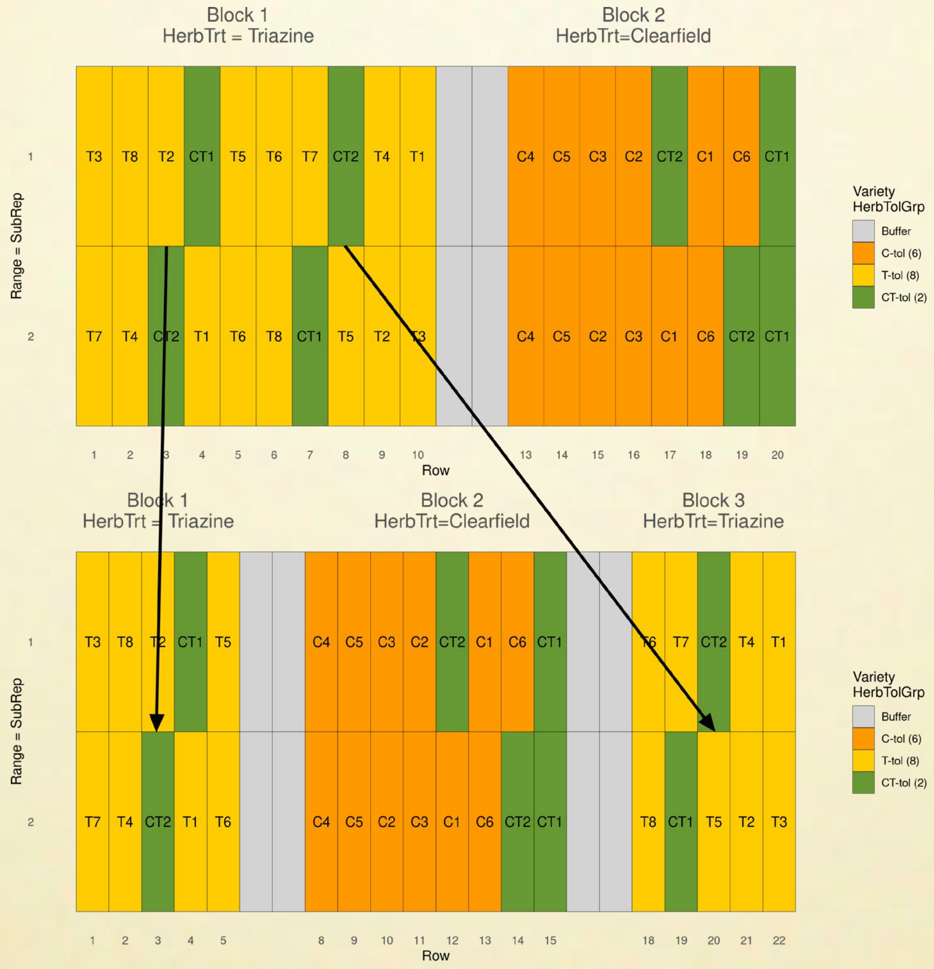
Range \ Row	1	2	3	4	5			8	9	10	11	12	13	14	15			18	19	20	21	22
1	T3	T8	T2	CT1	T5			C4	C5	C3	C2	CT2	C1	C6	CT1			T6	T7	CT2	T4	T1
2	T7	T4	CT2	T1	T6			C4	C5	C2	C3	C1	C6	CT2	CT1			T8	CT1	T5	T2	T3

- 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 Hyola Innovation Systems Technology trial

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



Block 1
HerbTrt = Triazine

Block 2
HerbTrt=Clearfield

Range = SubRep	1	2	3	4	5	6	7	8	9	10			13	14	15	16	17	18	19	20
1	T3	T8	T2	CT1	T5	T6	T7	CT2	T4	T1			C4	C5	C3	C2	CT2	C1	C6	CT1
2	T7	T4	CT2	T1	T6	T8	CT1	T5	T2	T3			C4	C5	C2	C3	C1	C6	CT2	CT1

Row

Variety
HerbTolGrp
- Buffer
- C-tol (6)
- T-tol (8)
- CT-tol (2)

Block 1
HerbTrt = Triazine

Block 2
HerbTrt=Clearfield

Block 3
HerbTrt=Triazine

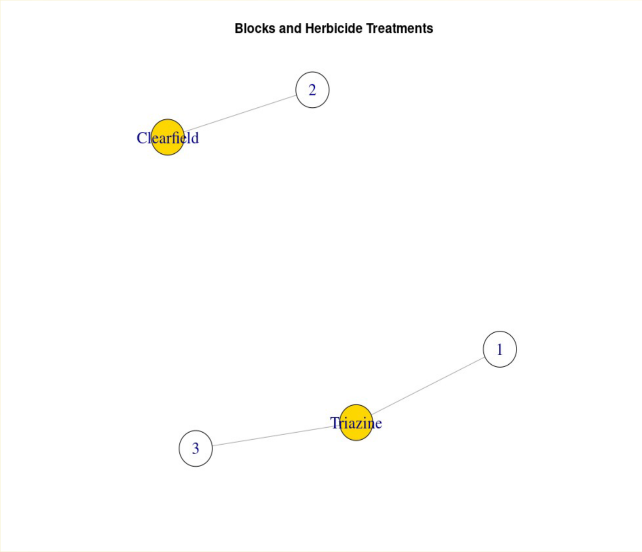
Range = SubRep	1	2	3	4	5			8	9	10	11	12	13	14	15			18	19	20	21	22
1	T3	T8	T2	CT1	T5			C4	C5	C3	C2	CT2	C1	C6	CT1			T6	T7	CT2	T4	T1
2	T7	T4	CT2	T1	T6			C4	C5	C2	C3	C1	C6	CT2	CT1			T8	CT1	T5	T2	T3

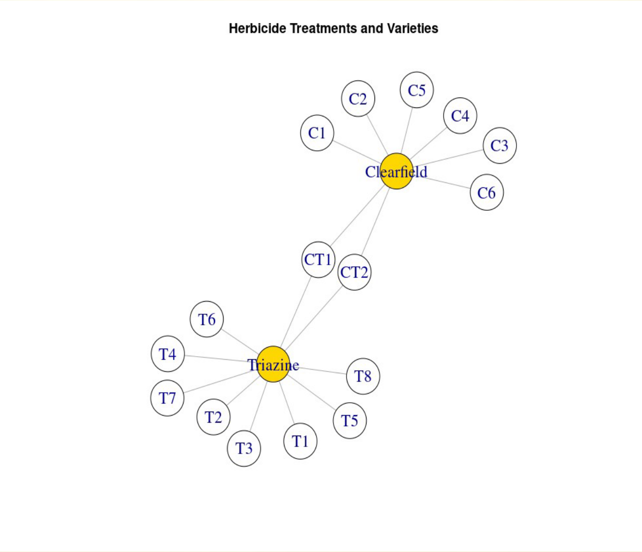
Row

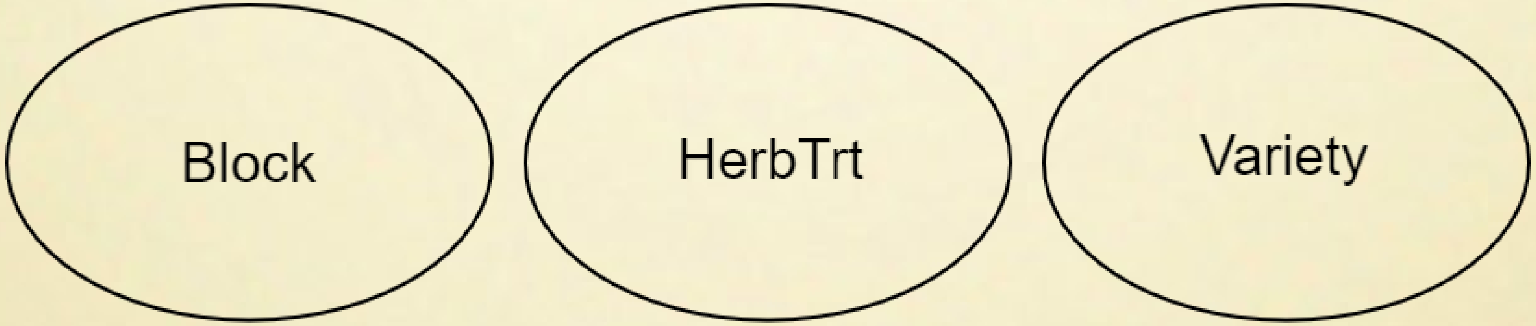
Variety
HerbTolGrp
- Buffer
- C-tol (6)
- T-tol (8)
- CT-tol (2)

UOW ‘enhanced’ Pacific Seeds Hyola Innovation Systems Technology trial

Aliasing/Connections


Blocks and Herbicide Treatments


Herbicide Treatments and Varieties


Block

HerbTrt

Variety

Statistical analysis of canola variety trials

Design Tableau

- Specification of plot and treatment factors allows us to draw a table showing terms to be fitted in the analysis (treatment then plot)

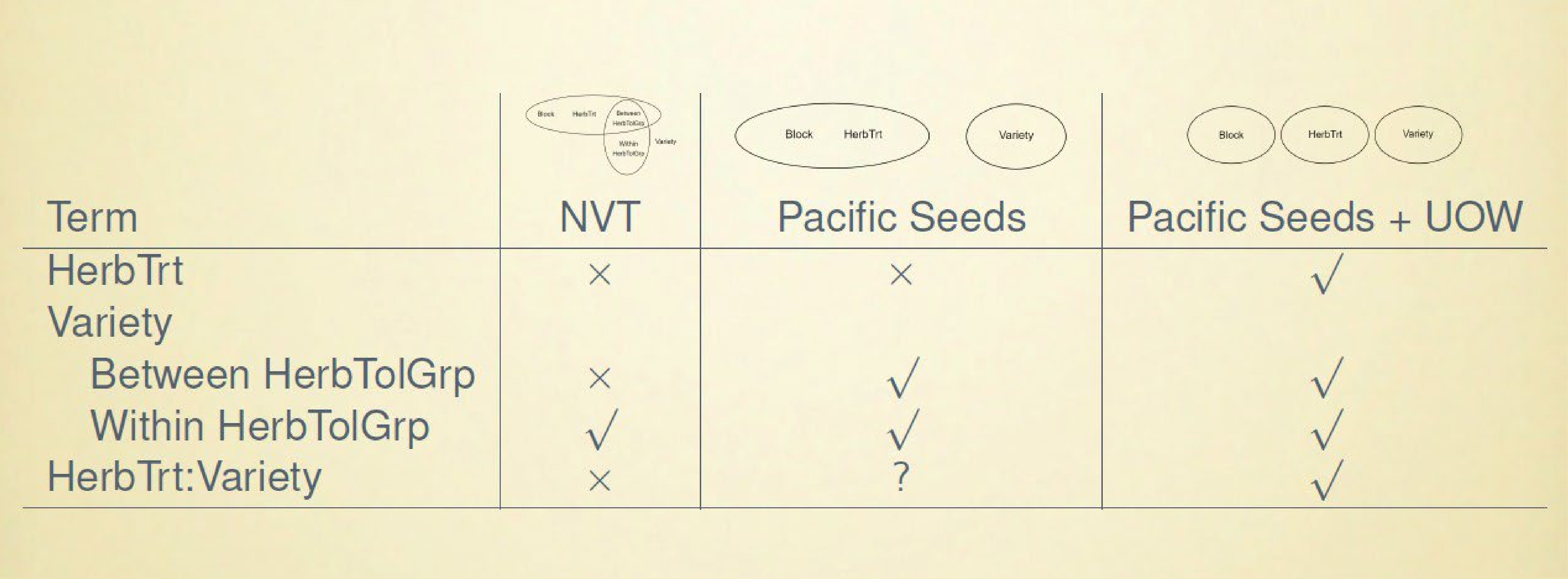
HerbTrt
Variety
HerbTrt:Variety

Block
Block:SubRep
Block:SubRep:Plot

- Specification of aliasing allows us to establish whether there is valid inference for each set of treatment effects . . .

Statistical analysis of canola variety trials

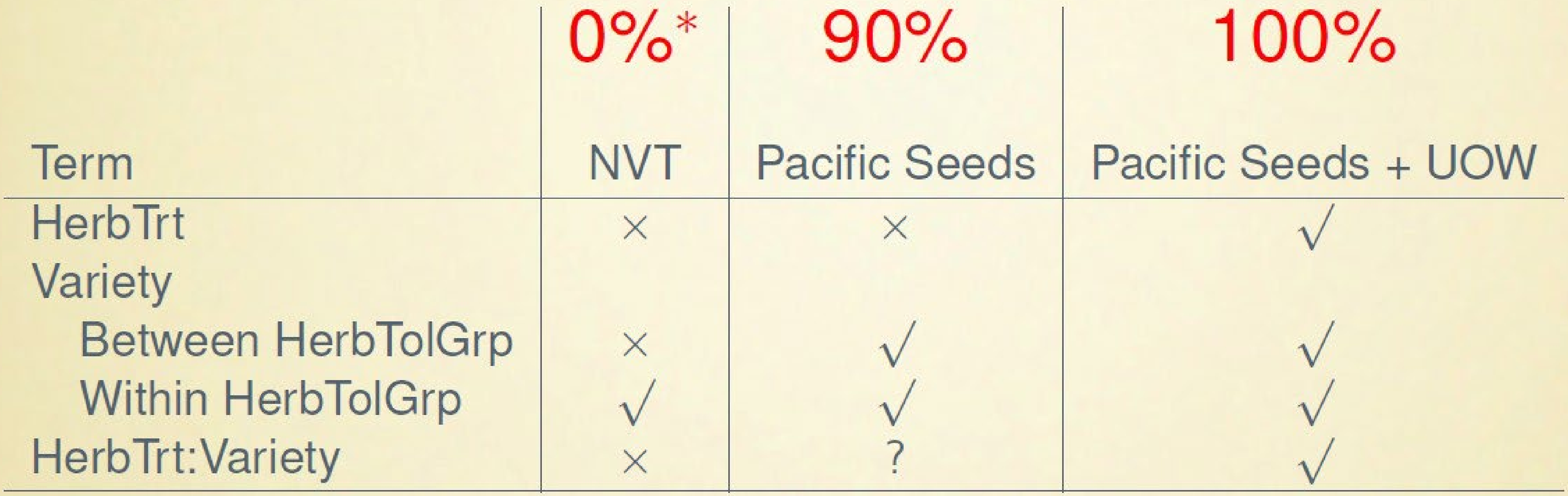
Do we have valid inference for treatment effects?



Term	NVT	Pacific Seeds	Pacific Seeds + UOW
HerbTrt	×	×	√
Variety			
Between HerbTolGrp	×	√	√
Within HerbTolGrp	√	√	√
HerbTrt:Variety	×	?	√

Design of canola variety trials

Have we solved problem of making valid comparisons across all varieties?



	0%*	90%	100%
Term	NVT	Pacific Seeds	Pacific Seeds + UOW
HerbTrt	×	×	√
Variety			
Between HerbTolGrp	×	√	√
Within HerbTolGrp	√	√	√
HerbTrt:Variety	×	?	√

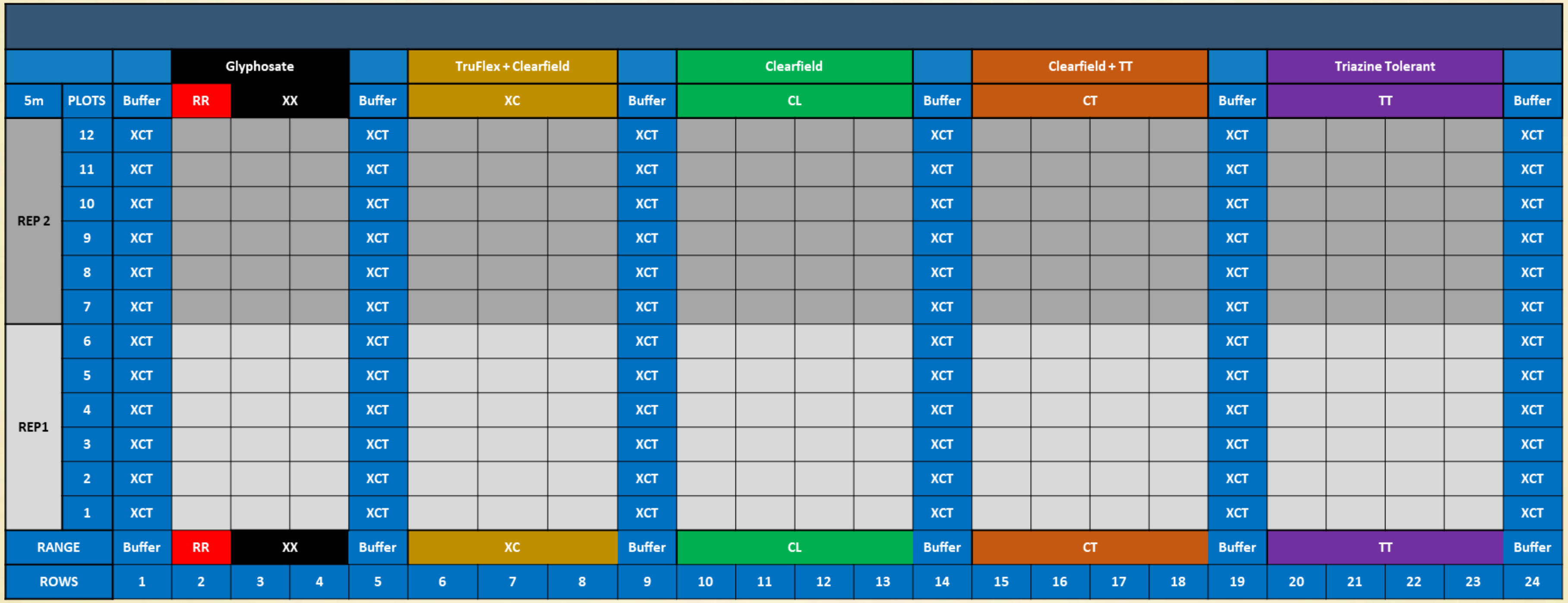
* NVT do not claim to allow comparisons across herbicide technology groups; they point out on website (https://nvt.grdc.com.au/trials/frequently-asked-questions) that it is statistically invalid to "compare variety performance across different trials (eg. canola herbicide groups)"

Analysis of canola variety trials

Pacific Seeds multi-environment trial data-set

- The huge benefits resulting from Pacific Seeds systems trial designs are one side of the story
- UOW has value added by conducting a (bespoke) single stage FALMM analysis of a systems multi-environment trial (MET) dataset
  - 30 environments (trials) in 2021 and 2022 across NSW, Vic, SA and WA
  - Total of 90 varieties across all trials (not all varieties in all trials)
  - Total of 7 herbicide treatments across all trials (not all treatments in all trials)
  - Total of 5133 plots

Actual layout of Pacific Seeds Hyola Innovation Systems Technology trial with



			Glyphosate				TruFlex + Clearfield				Clearfield					Clearfield + TT					Triazine Tolerant				
5m	PLOTS	Buffer	RR	XX		Buffer	XC			Buffer	CL				Buffer	CT				Buffer	TT				Buffer
REP 2	12	XCT				XCT				XCT					XCT					XCT					XCT
	11	XCT				XCT				XCT					XCT					XCT					XCT
	10	XCT				XCT				XCT					XCT					XCT					XCT
	9	XCT				XCT				XCT					XCT					XCT					XCT
	8	XCT				XCT				XCT					XCT					XCT					XCT
	7	XCT				XCT				XCT					XCT					XCT					XCT
REP1	6	XCT				XCT				XCT					XCT					XCT					XCT
	5	XCT				XCT				XCT					XCT					XCT					XCT
	4	XCT				XCT				XCT					XCT					XCT					XCT
	3	XCT				XCT				XCT					XCT					XCT					XCT
	2	XCT				XCT				XCT					XCT					XCT					XCT
	1	XCT				XCT				XCT					XCT					XCT					XCT
RANGE		Buffer	RR	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 deployed to provide more connections between blocks hence increase reliability of variety comparisons between herbicide tolerance groups

Analysis of canola variety trials

Bespoke single stage FALMM

- Fitted an FA model with 4 factors for variety effects in different environments (VE effects): accounted for 95.1% of variation in VE effects

- Included Block[HerbTrt] × Variety interaction effects for individual environments: small for majority of trials (27 out of 30 trials with associated variance < 10% of genetic variance)

- Model provides (yield) predictions for every variety in every environment in data-set: how to use this information?

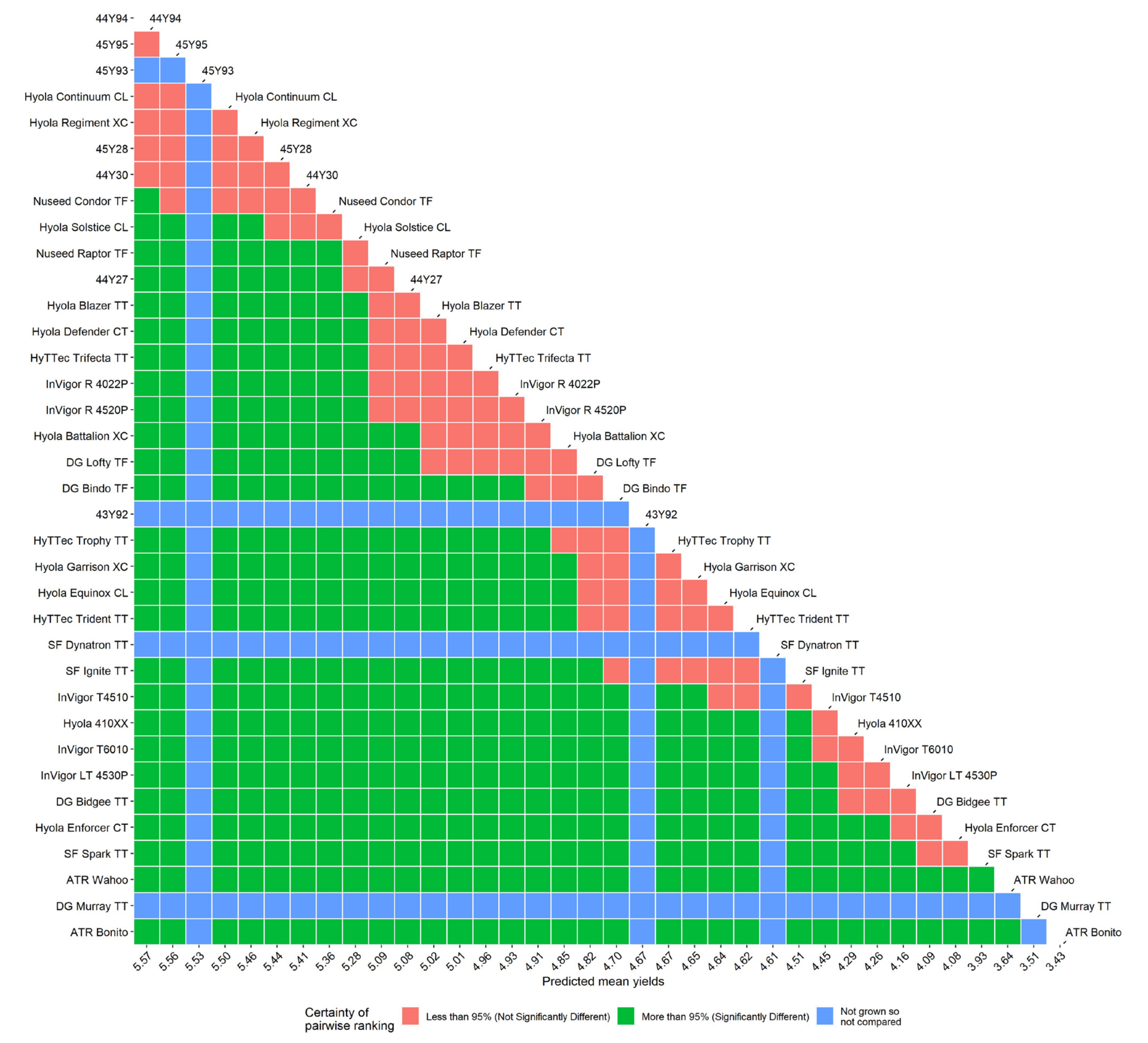
Analysis of canola variety trials

VE predictions for each environment

- We developed methodology (and graphical representation) to provide confidence statements for pairwise comparisons of variety predictions for any given environment
    - Comparisons across all varieties irrespective of herbicide tolerance group
    - Accommodated random rather than fixed variety effects
    - Accommodated range of “SEDs” (between vs within block comparisons; extra replication for stacked varieties)

Variety predictions for Griffith, NSW environment in 2022

Ordered predictions plus measure of certainty of rankings



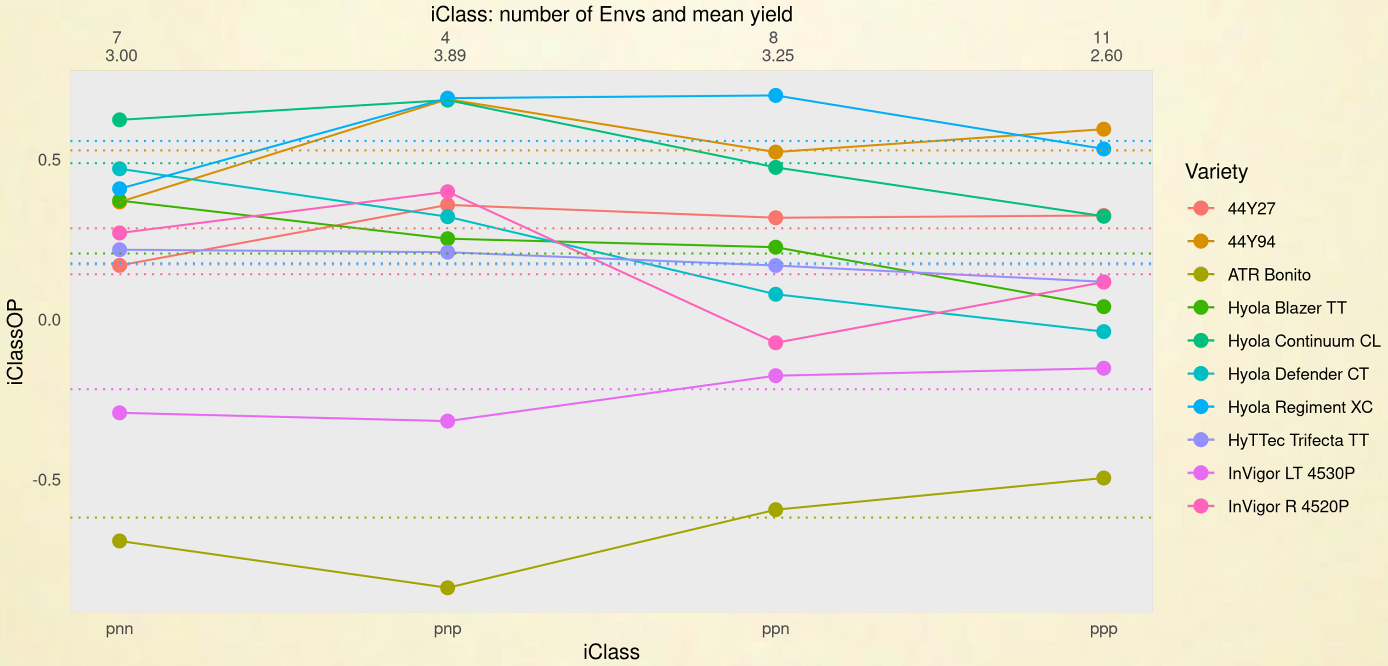
Analysis of canola variety trials

Summarising VE predictions across environments

- Summarised variety predictions for individual environments using interaction class (iClass) technology
  - iClasses are groups of environments that discriminate varieties with different patterns of variety by environment interaction
  - FA model used to form four iClasses labelled as pnn (7 environments); pnp (4); ppn (8); ppp (11)
  - Calculated overall yield performance for each variety in each iClass (mean of VE predictions over environments in iClass)
  - Represent user-specified varieties on iClass interaction plot . . .

Analysis of canola variety trials

iClass interaction plot for 10 varieties across 6 different ‘single’ or ‘stacked’ technologies



Iclass pnn			Iclass pnp			Iclass ppn			Iclass ppp		
Year	State	Site	Year	State	Site	Year	State	Site	Year	State	Site
2021	WA	Nabawa	2022	NSW	Beelbangera	2022	WA	Dalwallinu	2022	SA	Arthurton
2022	SA	Frances	2022	WA	Cranbrook	2022	WA	Corrigin	2022	WA	Dandaragan
2022	VIC	Rupanyup	2021	NSW	Wallendbeen	2021	NSW	Cowra	2022	VIC	Nulawil
2022	VIC	Streatham	2021	NSW	Wallendbeen	2021	WA	Gibson	2021	SA	Yeelanna
2022	NSW	Temora				2022	WA	Dalyup	2021	WA	Cunderdin
2021	VIC	Yarrawonga				2022	WA	Binnu	2022	WA	Gnowangerup
2022	VIC	Yarrawonga				2022	WA	Mingenew	2021	WA	Kojonup
						2021	SA	Tarlee	2021	VIC	Lake Bolac
									2021	WA	Mokine
									2022	WA	Yealering
									2022	WA	York

Concluding remarks – Leading Industry Innovation through Statistical Excellence