Paired plot designs experience and recommendations for in field product evaluation at Syngenta

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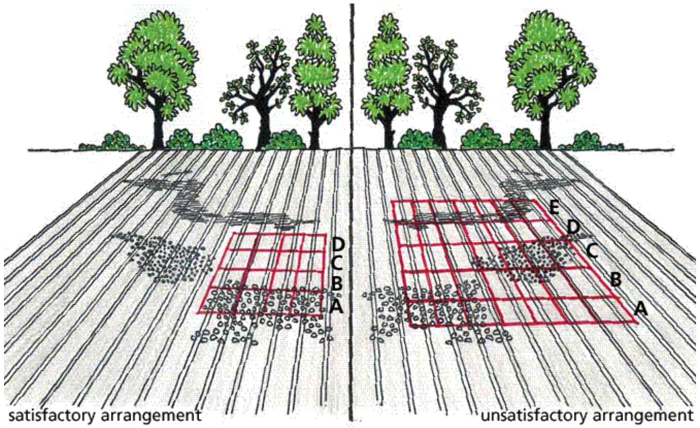
Sommertagung Bad Salzuflen 27/06/2013

Classification: PUBLIC

1. What are paired plot designs ?

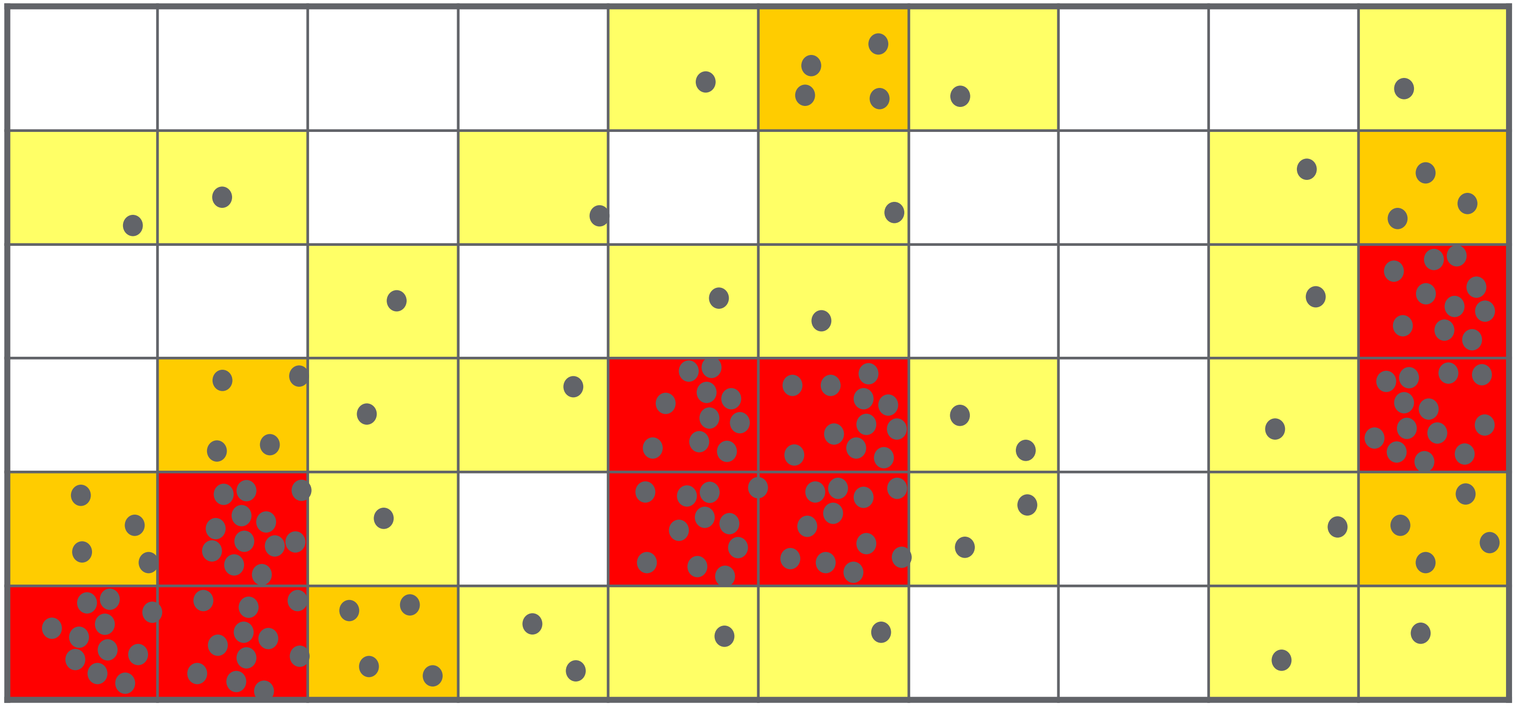
RCB is the most common reference design for product evaluation

- Blocks are typically used to control sources of heterogeneity in field



- Sometimes field variation is unknown or likely to be patchy :
  - do pre-counts of pests or plant sentinel crop and block accordingly
  - use paired plots designs

Take into account pre-counts in the design



Cucurbit – sentinel crop grown for a month and then realize gall ratings
Block by pest pressure level
Difficult : sensitivity of crops are different

High pressure | **Moderate pressure** | **Light pressure** | **No pressure**

Paired plots rationale

- If we expect field variation to be patchy but can't predict it (e.g. via pre-counts) then we can consider using paired plots

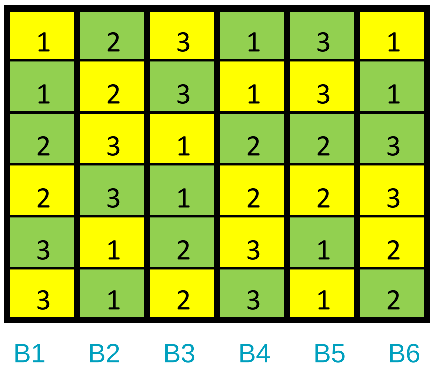
- If a RCB was employed in a patchy environment then it is unlikely that all plots within a block would be homogeneous
  → But adjacent plots are likely to be more homogeneous
  → Better control of field variation and therefore expect increasing power

- Next to every treated plot is a paired check plot which is untreated
  → Data is recorded for all plots

- The rationale is that the data from the paired plots can be used (in one way or another) to influence our interpretation of the data from the treated plots

Paired plot designs concept

Randomised pairs

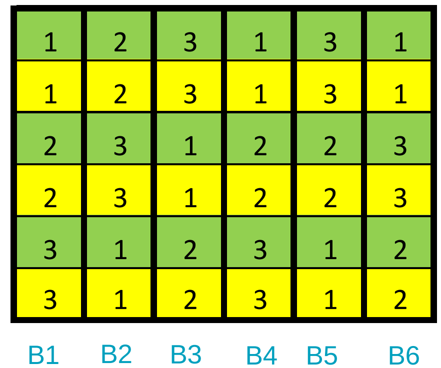


+flexibility ?

Checkerboard

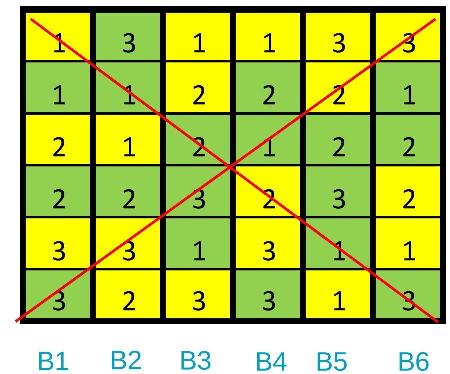
+uniform coverage CHK
+surface analysis possible (>2CHK/TRT)
+control neighbour effect in any direction

Check strip



+practicality
+soil application
-low ctrl gradient along strip
-difficult to assess blindly

Missing pairs...



-missing plots
-unbalanced

Paired plot

Treatment

Paired plot designs expected benefits and drawbacks

- In theory very attractive as it has the potential to reduce experimental error
- Consequently it has the potential to increase power
- Side benefit : assure independence of plots (buffer) in case of checkerboard

- Increase trial area and amount of resources required especially regarding observations done on paired checks which are not of prime interest to the objectives
- Increasing experiment area can lead to increase heterogeneity within trial
- If the trial area is fixed then multiple check plots are included at the expense of more replication of the treatments
- Additional source of complication and potential for errors (randomisation)

2. Analysis and reporting of paired plot designs

Analysis of paired plot designs

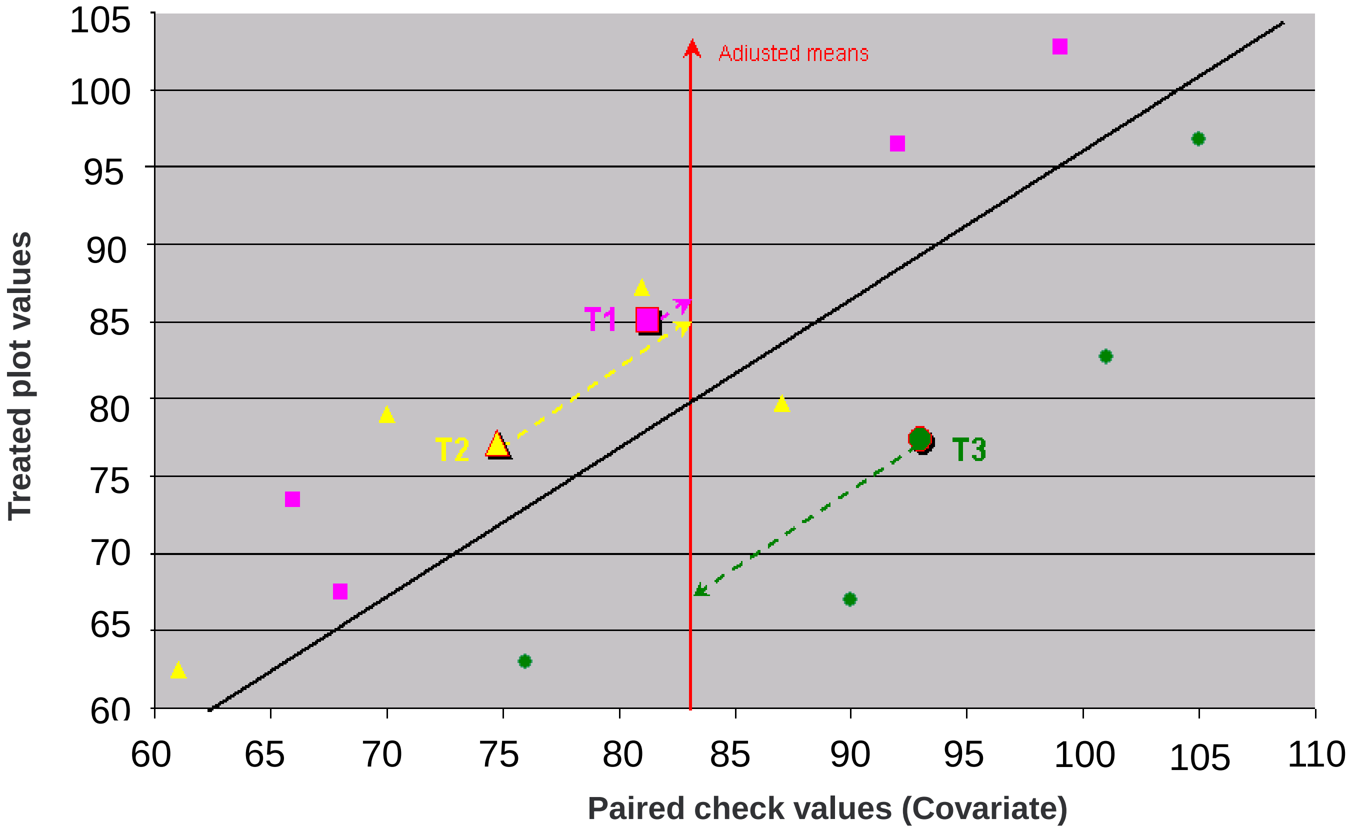
- 3 models will be used in our case studies :
  1) Rcb : Y = block + treatment + error (all UTC excluded from analysis)
  2) RcbOnPct* : $Y_{\%}$ = treatment + error ($Y_{\%} = Y_{trt} / Y_{paired\ UTC} * 100$)
  3) **PairedCheck** : Y = block + treatment + $Y_{paired\ UTC}$ + error (covariance)

- other models possible :
  4) Surface analysis (spatial, row/column)
  5) Nearest neighbour(s) (2D or 4D)
  6) Paired t-test
  7) …

* : a block effect could be added although possibly low (potentially covered by adjacent paired UTC)

UTC : untreated check

Paired plot global adjustment based on covariance analysis



- Generally we assume a linear relationship between treatment and paired check (covariate)
- Same slope for all treatments
- Adjusted Lsmeans with different Standard Errors and consequently different values for mean separation tests (eg LSD) depending on relative distance between covariate mean for that treatment and the overall covariate mean

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Model comparison and reporting of paired plot designs

- Different parameters used to compare models in our case studies :
  - ✓ Coeff Var (CV) : (RootMeanSquareError / Average)*100
  - ✓ PctVarExplByPairedTrt (R^2) : SumSquares $Y_{paired\ UTC}$ / Error SumSquares $_{Rcb}$
  - ✓ Frequency of pairwise treatment means significant differences (based on LSD 5%)
  - ✓ StdErrorPctLsMean : (Standard error of adjusted treatment mean / adjusted treatment mean) * 100
  - ✓ SEM : Standard Error of Means averaged across means

- Reporting :
  - ✓ Display classical anova or ancova results (p-values of F test, estimates parameters)
  - ✓ Emphasis on adjusted treatment Lsmeans

3. Case study 1 : analysis of crop enhancement trials

Context of the study

- Vapor active component for crop heat/drought stress protection
- Compound tested several years in different regions on many crops to assess yield benefit under stressed conditions and to correlate Δ yield with stress indexing
- Yield (Y) for all 280 trials covering 3 years (2008-09-10) is considered in this analysis.

Crop	# Trials
Corn	94
Cotton	34
Rice	26
Soybean	94
Tomatoes	8
Wheat	24

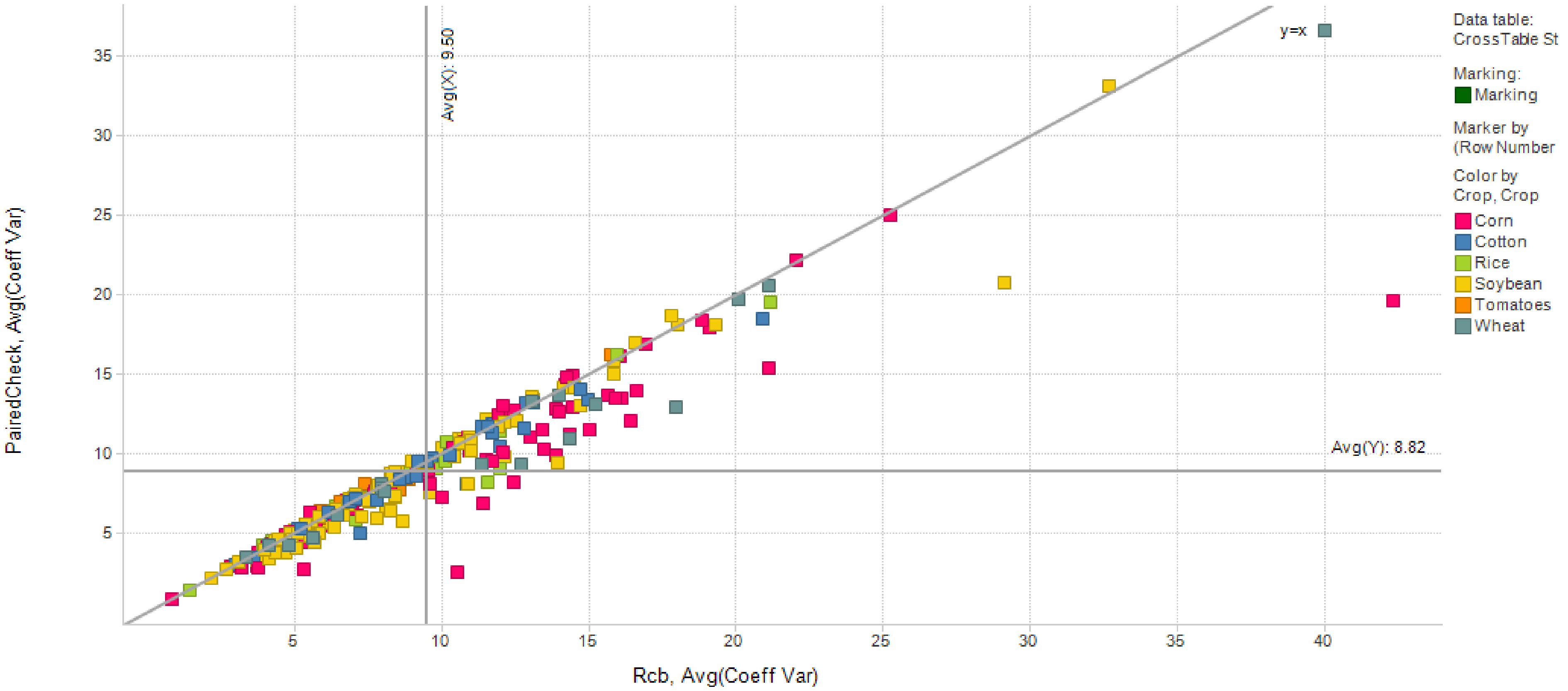
Rationale and drivers behind paired plot design

- Protection against non independence of plots (buffer drift/volatilization)
- Overcome between-plot variability which is likely to be high under stress conditions (cater for microenvironment effects interacting with yield)
- Provide spatially-even means to assess variability among checks expected to respond the same
- Provide adjacent comparison plot for calibrating visual stress assessment and field tour viewing
- Visual row-by-column surface plot analysis possible
- Increase power for treatment comparisons against UTC by reducing background variability and increasing number of replicates for UTC

Paired plot checkerboard design used

	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	
Border	Border	Border	Border	Border	Border	Border	Border
Border	Border	Border	Border	Border	Border	Border	Border
Border	UTC (for trt 6) 101	Trt 10 201	UTC (for trt 10) 301	Trt 4 401	UTC (for trt 2) 501	Trt 2 601	Border
Border	Trt 6 102	UTC (for trt 10) 202	Trt 10 302	UTC (for trt 4) 402	Trt 2 502	UTC (for trt 2) 602	Border
Border	UTC (for trt 8) 103	Trt 2 203	UTC (for trt 6) 303	Trt 8 403	UTC (for trt 12) 503	Trt 4 603	Border
Border	Trt 8 104	UTC (for trt 2) 204	Trt 6 304	UTC (for trt 8) 404	Trt 12 504	UTC (for trt 4) 604	Border
Border	UTC (for trt 2) 105	Trt 8 205	UTC (for trt 2) 305	Trt 10 405	UTC (for trt 6) 505	Trt 10 605	Border
Border	Trt 2 106	UTC (for trt 8) 206	Trt 2 306	UTC (for trt 10) 406	Trt 6 506	UTC (for trt 10) 606	Border
Border	UTC (for trt 10) 107	Trt 4 207	UTC (for trt 4) 307	Trt 12 407	UTC (for trt 10) 507	Trt 12 607	Border
Border	Trt 10 108	UTC (for trt 4) 208	Trt 4 308	UTC (for trt 12) 408	Trt 10 508	UTC (for trt 12) 608	Border
Border	UTC (for trt 12) 109	Trt 6 209	UTC (for trt 8) 309	Trt 2 409	UTC (for trt 4) 509	Trt 8 609	Border
Border	Trt 12 110	UTC (for trt 6) 210	Trt 8 310	UTC (for trt 2) 410	Trt 4 510	UTC (for trt 8) 610	Border
Border	UTC (for trt 4) 111	Trt 12 211	UTC (for trt 12) 311	Trt 6 411	UTC (for trt 8) 511	Trt 6 611	Border
Border	Trt 4 112	UTC (for trt 12) 212	Trt 12 312	UTC (for trt 6) 412	Trt 8 512	UTC (for trt 6) 612	Border
Border	Border	Border	Border	Border	Border	Border	Border
Border	Border	Border	Border	Border	Border	Border	Border

CV comparison for both models (RCB, paired)



On average the CV is slightly improved with PairedCheck analysis compared to Rcb (0.67% difference only)

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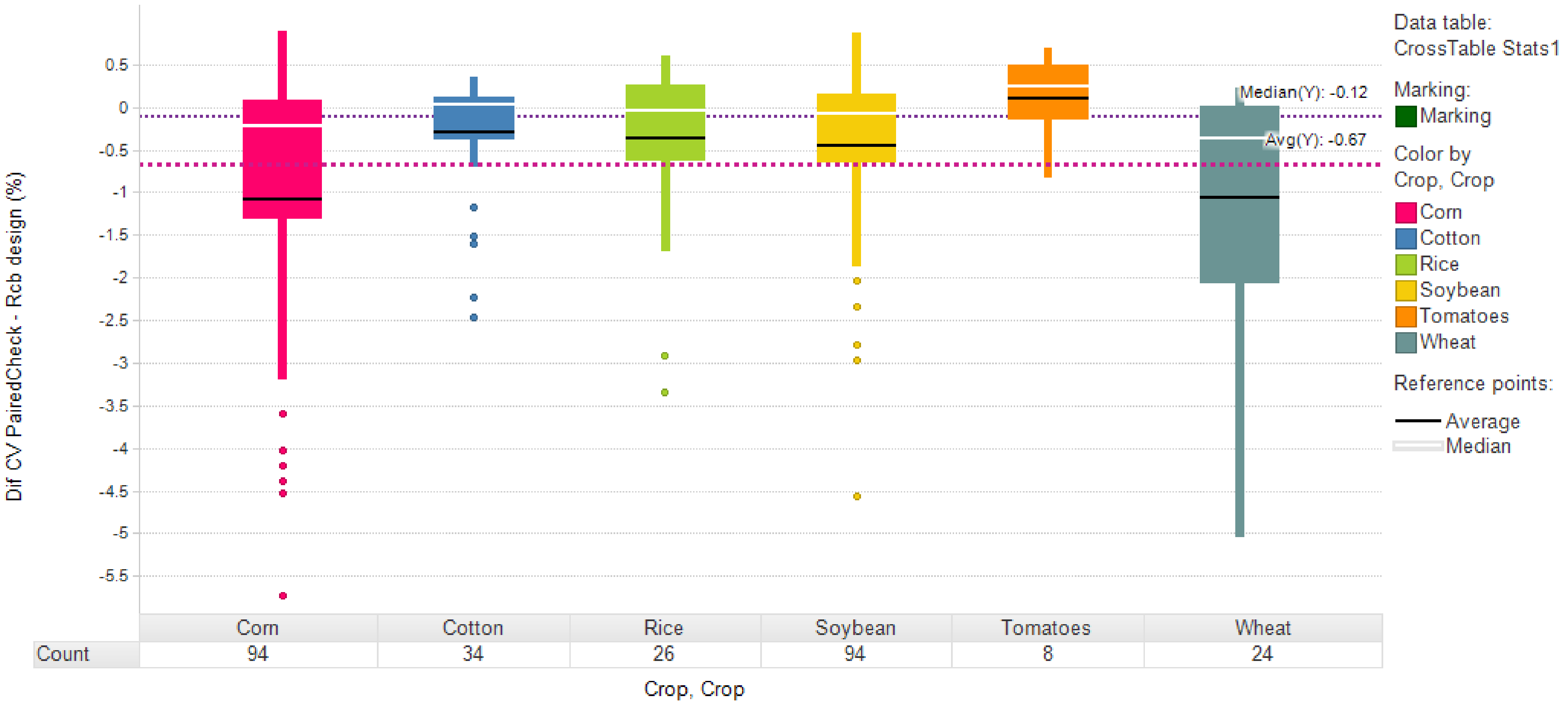
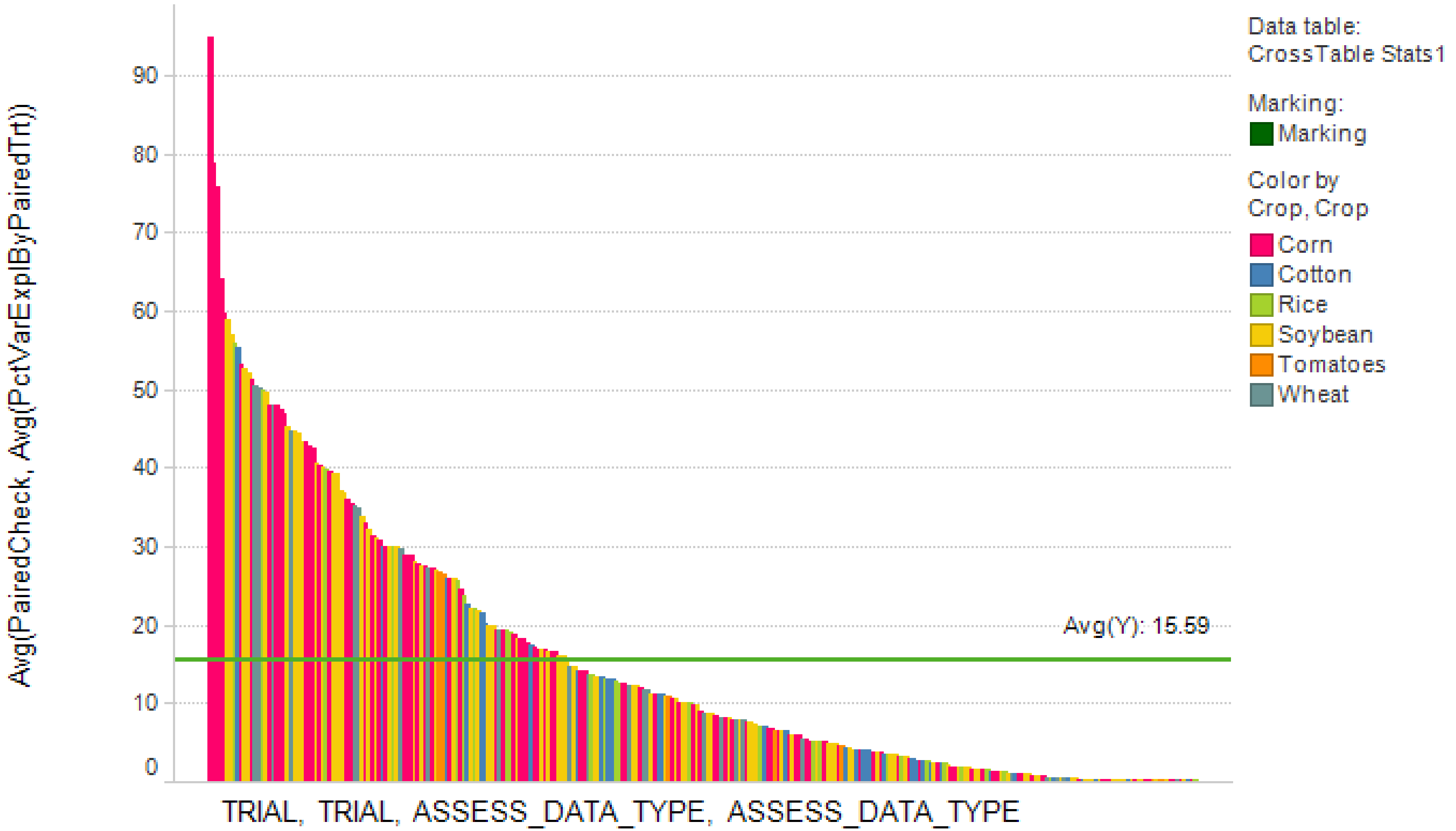
Distribution of CV difference between both models (Rcb, paired)



Distribution of CV differences is highly skewed to the left for most situations and crops with medians close to 0

Percent variability explained by paired check per trial (R^2)



On average the adjustment from the PairedCheck explains only about 15% of the experimental error

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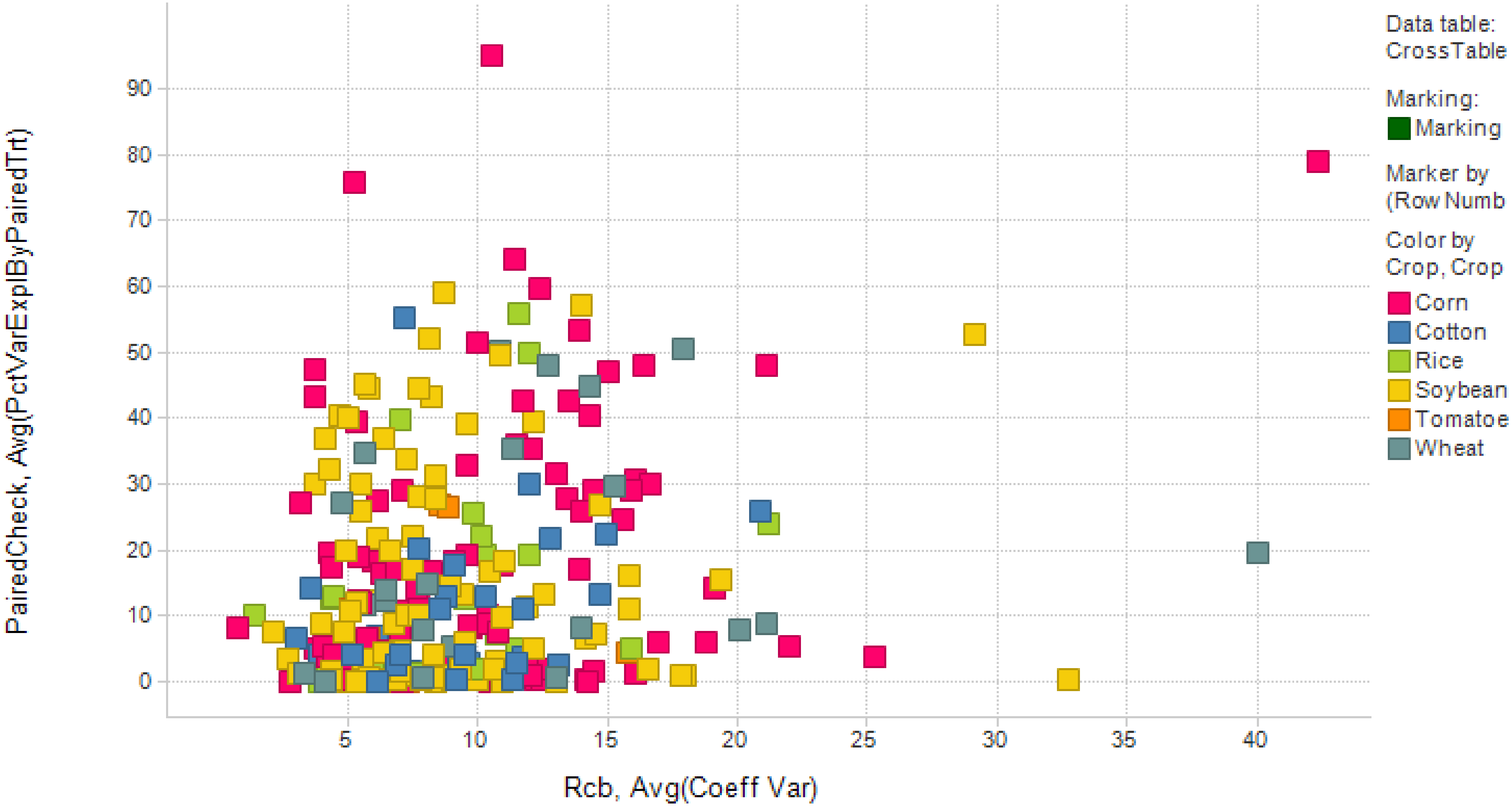
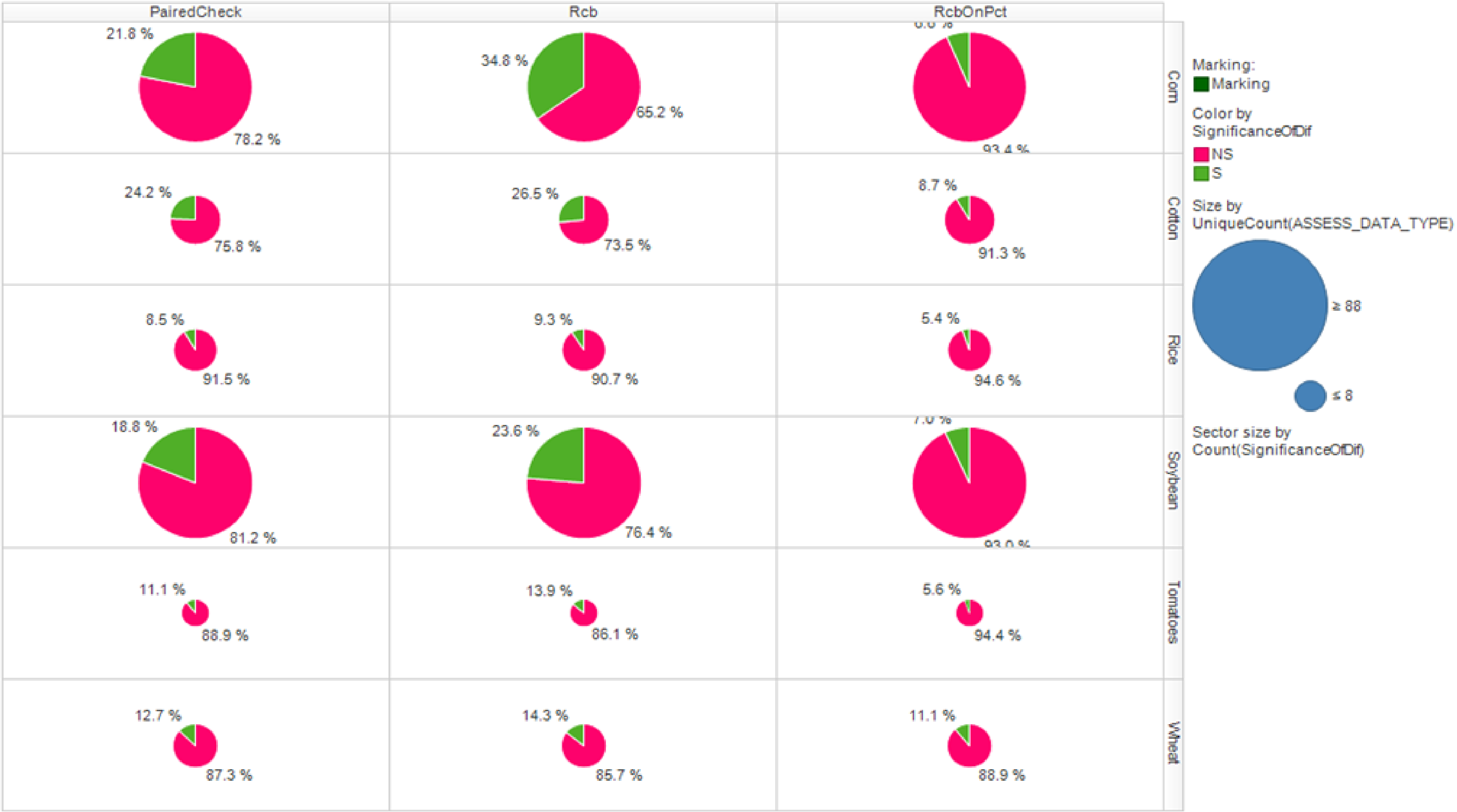
Percent variability explained by paired check per level of trial's precision



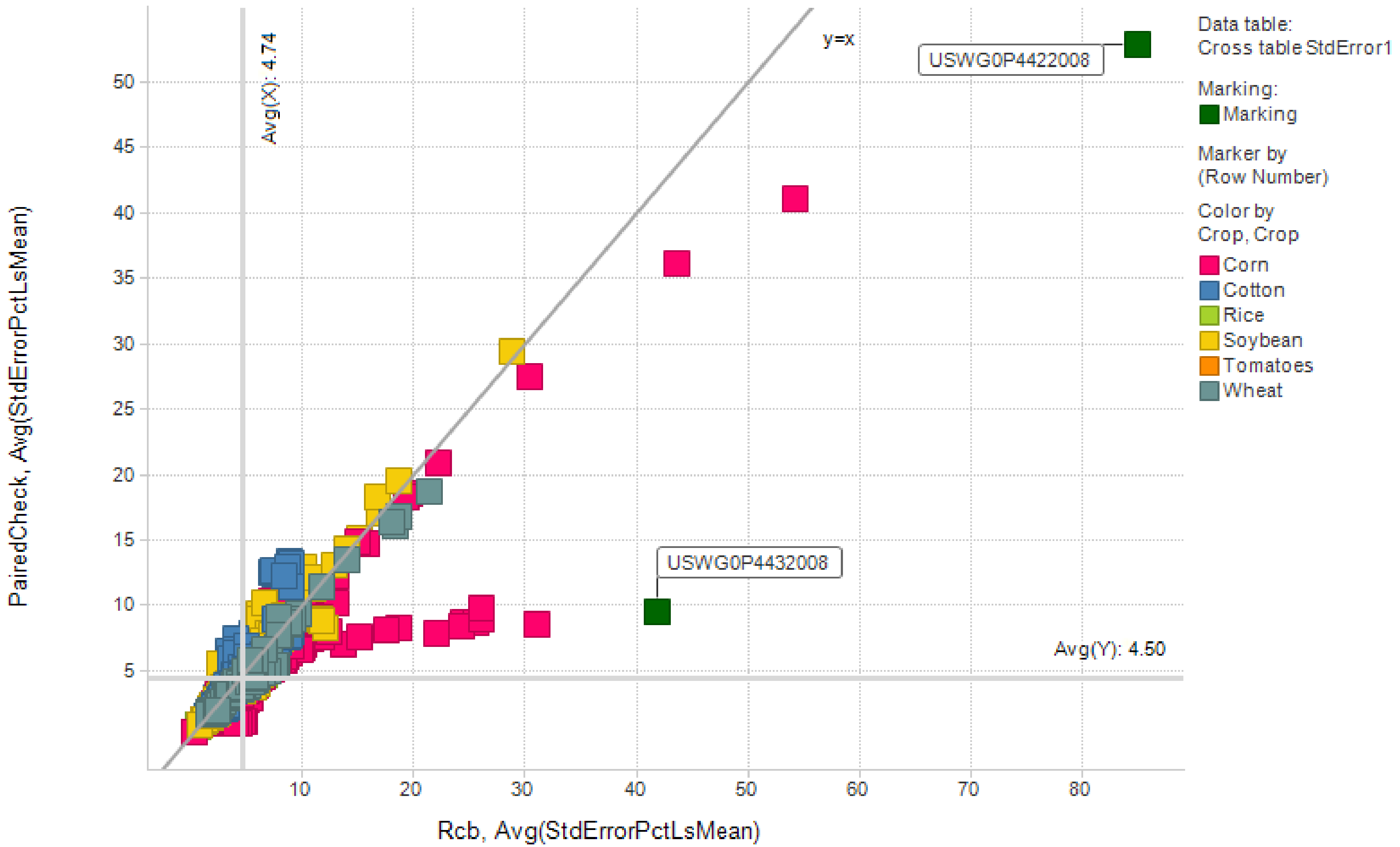
No relationship between coefficient of variation and expected benefit from PairedCheck analysis

Frequency of pairwise lsmeans significant difference



- The frequency of significant pairwise treatment differences tends to be slightly higher with Rcb analysis compared to PairedCheck analysis
- The analysis on the percentages tends to lower the frequency of significant treatment differences
- Means adjustments differ between PairedCheck and RcbOnPct analysis : global versus local trend adjustment ?
- Note that the proportion of significant results is not a measure of power since it includes false positives

Comparison of the size of the confidence intervals for the means (std error in % of mean) for RCB and paired check



The size of the treatment means confidence intervals is comparable for both Rcb and PairedCheck analysis

4. Case study 2 : analysis of sugar beet nematicide trials

Context of the study

- 8 trials from 2012 (CZ, DE, DK) which have used paired plot design (6 rep)
- Treatments : nematicides (products*rates) compared for Sugar Yield
- Nematode susceptible variety used as paired plot
- Design justification : nematode pressure intensity in field is unknown and from experience likely to be very patchy

Findings of the study

- Pertinence and efficiency of paired plot design was derived by calculating the number of reps that would be required for a RCB such that the SEM* of the RCB would equal the SEM of the paired plot design with 6 reps :

$$n = \frac{\sigma^2{}_{RCB}}{SEM_{paired\ plot}{}^2}$$

- For the 6 trials blocked appropriately : 8 replicates of an RCB design would provide equivalent power to a paired plot design with 6 replicates assuming inter-plot correlations are similar in subsequent trials to what was observed in these current trials

- For 2 trials the number of unpaired replicates suggested for the power of an RCB to match that of a 6 replicate paired plot design was around 20. The main obvious reason was wrong direction blocking…

* SEM : Standard Error of the Means

Findings of the study

Field map based on paired checks (adjusted for variety)

DK BHM 200 204																													
Range / Row	**7**	**13**	**19**	**25**	**31**	**37**	**43**	**49**	**55**	**61**	**67**	**73**	**79**	**85**	**91**	**97**	**103**	**109**	**115**	**121**	**127**	**133**	**139**	**145**	**151**	**157**	**163**	**169**	**175**
1	0.2	1.8	0.5	-0.2	-0.4	-0.4	-1.4	-1.5	-0.3	-0.4	-1.3	-0.8	-1.7	-1.3	-1.1	-0.8	-0.5	0.1	-0.2	0.3	1.5	2.3	2.3	1.5	2.2	2.3			
2	-0.5	-0.7	-0.8	-0.5	-0.8	-1.0		-0.4	-0.9	-1.4	-0.6	0.5	0.6	0.8	0.6	0.8	0.2	1.8	1.4		1.3		1.0	0.9	2.0	2.1	1.9	4.5	2.8
3	0.0	1.1	-0.8	-1.1		-0.4	-0.3	-0.1	-1.1	-0.2	-0.2	0.2	1.0	0.6	-0.6	-0.3	0.5	-0.1	0.3	0.6	1.8	2.5		0.8		2.1	1.8	3.8	3.7
4	-0.7	-0.8	-0.6	0.2	-0.9	-0.3	-0.9			0.3	-0.2	-0.3	-0.4	-0.4	-0.8	-1.0	-0.3	0.2	0.2	2.3	1.1	0.0	0.9	2.0	1.9	3.4	1.9		1.6
5	-1.6	-1.3	-2.1	-1.9	-1.4	-1.2	-1.7	-1.4	-1.2	-0.8	-0.7	-0.5	-1.2		-0.6	-0.2	-0.4	-1.3	-1.0	0.4	0.3	0.0	0.7	1.1		1.4	0.2	-0.8	
6		-0.3	-0.1	-0.6		-1.0	-0.4	-2.5	-1.3	-1.2	-1.5		-1.0	-1.4	-1.2	-1.4	-2.1	-3.6	-1.0	-0.3	-0.8	-1.9	-1.5	-1.4	-1.0	-1.6	-0.9		0.9

DK BHM 201 205																													
Range / Row	**7**	**13**	**19**	**25**	**31**	**37**	**43**	**49**	**55**	**61**	**67**	**73**	**79**	**85**	**91**	**97**	**103**	**109**	**115**	**121**	**127**	**133**	**139**	**145**	**151**	**157**	**163**	**169**	**175**
7	0.5	0.7	0.4	0.6	0.5	0.3	0.4	1.0	0.7	-0.5	-0.7	0.1	0.3	0.0	-0.1	-1.0	0.0	-0.6	-0.8	-0.9	-1.2	-1.0	-0.2	-0.1	-0.3	-0.7			
8	0.7	1.4	0.5	0.5	0.5	0.3	0.4	0.9	1.0	-0.2	0.6	0.3	0.2	0.6	-0.3	0.1		0.4	0.1	-0.2	-0.6	-0.2	-0.8	-0.9		-1.2	-0.5		-0.3
9	-0.2	0.0	0.4	0.4	0.3	0.6	-0.1	0.3	0.2	0.2	1.1	0.6	1.1	0.1	-0.5	-0.3	-0.2	-0.5	-1.0	-0.3		-0.5	0.2			-0.3	-0.7	-0.5	0.1
10			-0.6	-0.3	0.0	0.2	0.0	0.2	0.0	0.8	0.1	1.7	0.6	0.6	0.5	-0.5	0.0	-1.4	-0.5	-0.5	-1.4	-0.4	-0.8	-1.3		-0.8	-0.8	-0.1	0.3
11	0.2	-0.8		-0.1	-0.7	0.1	-0.2	0.3	0.2	0.3	0.7	0.7	-0.1		0.7	1.1	0.4	0.0	-0.3	-0.7	-0.9	-0.2	-1.1	-1.2	-0.7	-0.3	-1.2		-0.3
12	0.7	0.2	0.3	0.0	0.4	0.2	-0.5	0.2	-0.2	0.0	0.1	0.5	0.8	1.5	0.3	-0.3	0.0		1.0	0.3		-0.4	-0.2		-0.6	-1.0	-0.6	-0.1	0.1

Values shown are the residuals. Red cells show less favourable parts of the field and green shows more favourable parts of the field. Blocking is by range (shown by black borders).

When blocking is wrong (not controlling gradients) then paired plots can potentially be very useful

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5. Conclusion : learning on paired plots designs

- Build internal Syngenta experience on many trials/crops/assessments where paired plot design was appropriately planned based on solid known rationale at planning phase
- A posteriori, testing at reduced cost the performance of several methods of designing and analyzing experiments
- Benefit of paired check design is likely to be dependent on the assessment type but we don’t have strong evidence on consistency
- If there are treatments within the paired checks (e.g. different varieties in sugar beet nematode trials) it makes the analysis much more complicated
- Various analyzes didn’t show evidence of a real and consistent benefit of paired plots over RCB design
- Technical difficulties and complications to manage paired plots design (increase cost)

5. Conclusion : recommendations on paired plots designs

- Paired plots can help in case of wrong blocking (insurance) but should not replace good blocking practice (including completely randomized designs)
- Whilst paired plot designs may theoretically still be recommended in cases where micro-environment or neighbor effects are very likely to occur and difficult to control…
- In practice, optimization of the use of available resources may rather lead to recommend RCB with increasing # replicates or including some repeated treatments (e.g. controls) in the treatment list, thus increasing power for some comparisons for a fixed amount of resources
- Considering incomplete block designs can also be an alternative to paired plots