ATTACHMENT 3
Analysis of adult plant phenotyping of crown rot symptoms in wheat

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Chapter 1

Field Ring Test 2010

1.1 Introduction

The adult ring test was designed to compare the different screening methods for crown rot in adult plants. Following on from seedling and adult plant trials in 2009, four field trials of the Ring test lines were run in 2010 across three states (NSW (2), Qld & SA). These trials compared inoculated and uninoculated plots for yield loss due to disease. In addition to the SA field trial, the SARDI Terrace trial was conducted again to obtain data for comparison with the other screening methods, due to storm damage to the plots in 2009. The ultimate aim of the project was to compare phenotyping methods and recommend a robust and reliable method for high throughput crown rot screening.

1.2 Experimental method

The four methods in the study each implement a differing trial structure, layout and design, together with method of infection and subsequent scoring technique.

The field trial at Narrabri was designed as 2 replicates of 28 entries, randomly laid out as a 7 bay by 8 run rectangular array in the field. Experiments testing inoculated and uninoculated treatments were designed as separate trials and planted in separate blocks.

The trial at Tamworth was designed as three replicates of a factorial design of two inoculations (plus and minus) by 50 test entries (containing the 28 ring test lines), randomly laid out as a 15 range by 20 row rectangular array.

The Wellcamp trial was designed as three replicates of 30 entries (28 ring test lines plus two standards) in paired disease plots, where inoculation was applied as a strip across columns of the field. The plots were randomly laid out as a 6 column by 30 plot array.

The Terrace trial was designed as part of the routine screening conducted in SA. It included three replicates of 28 entries.

The field trial at Hart was designed as a 4 replicate trial where 26 genotypes were planted with both inoculated and uninoculated plots, and another 8 genotypes were planted with only inoculated plots. The entries were randomly laid out as a 6 column by 40 row array.

All methods used a different design and sampling strategy across plants and across tillers within plants (Table 1.1). One major differentiation between methods is that tillers are bulked across plants in the Wellcamp trial. At Tamworth, Hart and the Terrace trial, average ratings across tillers are recorded, whereas the Narrabri trial records ratings for each tiller on each plant.

Table 1.1: Trial parameters and sampling strategy for the five ring test trials in 2010

Number of	Narrabri	Tamworth	Wellcamp	Hart	SA terrace
Replicates	2	3	3	4	3
Ring test lines	26	28	26	24	28
Plants assessed	5	25		71	25
Tillers per plant	15		40		

Most methods for assessing crown rot in adult plants assign a rating for the extent of browning along the internode according to an underlying scale of 0-4 (Wellcamp, Narrabri), 1-5 (Terrace), 0-3 (Tamworth) and 0-5 for browning up to the first internode (Hart). Thus a maximal rating of 5 for the Hart trial is equivalent to the minimum rating of 1 for Tamworth, Narrabri, Wellcamp .

A simple average of the rating scales for all tillers assessed is used for Narrabri, Hart and the Terrace data, while a weighted average across the number of tillers in each category is formed for the Wellcamp and Tamworth trials. For Wellcamp, the severity index is

$$\mathsf{index} = \frac{\mathsf{cat1} * 1.5 + \mathsf{cat2} * 3.5 + \mathsf{cat3} * 6 + \mathsf{cat4} * 9}{\mathsf{total\ tillers} * 9} \tag{1.1}$$

where $\mathsf{cat}i$ is the number of tillers in category i on the (1-4) scale.

For Tamworth the index is

$$\mathsf{index} = \frac{\mathsf{number\ of\ tillers\ with\ browning}}{\mathsf{total\ tillers}} * \frac{c}{3} * 100 \tag{1.2}$$

where c is the extent of browning on the (0-3) scale.

Disease recordings were taken on uninoculated plots for all trials except the Narrabri trial. In this trial, a quick visual assessment indicated that no uninoculated plots contained diseased plants. This lack of rating is a methodology issue as the uninoculated plots form the control treatment for the experiment and any baseline level of disease *must* be assessed. The measurement is essential to any analysis which attributes yield loss between inoculated and uninoculated plots to disease.

A summary of the raw data is given in Figure 1.1.

1.3 Statistical Analysis

A linear model was fitted to the disease severity data from each trial with terms for genotype, replicate and plot. Data was firstly analysed only over the diseased plots and ring test entries, to assess resistance and compare precision of the methods. Genotypes were fitted as a fixed effect, and best linear unbiased estimates of genotypes were obtained. Two genotypes were excluded from the ring set test due to issues with seed supply and purity (CPI-133814 and Puseas).

The second analysis combined disease severity data for ring test entries in inoculated plots over all seedling and adult ring test trials in 2009 and 2010. The linear mixed model included a fixed term for trial and random terms for both trial by

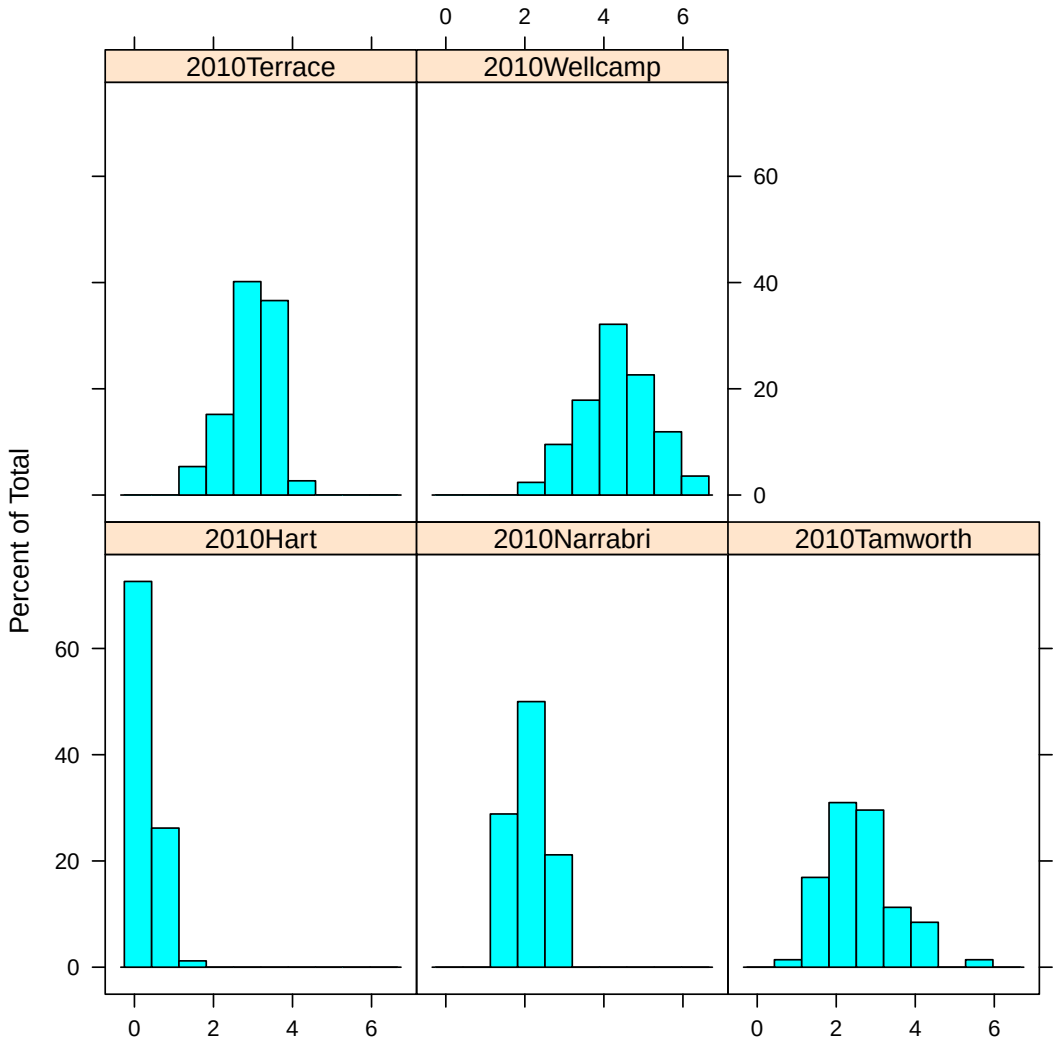

Figure 1.1: Histograms of raw data for five assessment methods of ring test entries in 2010

genotype effects and design factors such as replicate for each trial. A factor analytic form was used for the trial by genotype effects following the methodology of Smith *et al.* (2001).

Yield data was analysed as a joint model across inoculated and uninoculated plots to assess tolerance of genotypes to crown rot disease, following the methodology in Stevens (1999). A bivariate model was fitted in a linear mixed model framework to account for error in both inoculated and uninoculated plots, producing an index of tolerance that is independent of overall yield potential. Best linear unbiased predictions of genotype effects for inoculated and uninoculated treatments were obtained from this analysis, and deviations from the bivariate regression were used as a measure of tolerance.

1.4 Results

1.4.1 Disease severity - resistance

The genotype means for disease severity from individual trial analyses are given in Table 1.2. Results for two lines of the original ring test set are omitted due to issues with seed supply, (CPI-133814 and Puseas). In addition, data for certain genotypes was not available for all trials as shown by gaps in the table.

The best measure of resistance comes solely from the inoculated plots, but in these yield loss trials there is also the opportunity to assess browning on uninoculated plots. Based on the trials this year, it appears that, in practice, it is sometimes difficult to achieve plots free from crown rot. In the trials grown at Hart and Tamworth, uninoculated plots expressed symptoms of browning. Results from Tamworth have been combined across the inoculated and uninoculated plots to determine overall disease severity for each genotype (Figure 1.2). This additional information improves the precision of the severity ranking and, based on the strong correlation (0.98) between treatments, it is obvious in these data that low levels of crown rot give a similar ranking of disease severity for genotypes to high levels of crown rot.

1.4.2 Disease severity - precision of phenotyping methods

The mean crown rot severity and estimated variance components of all ring test trials across the two years of testing are summarised in Table 1.3. Disease severity symptoms in 2010 are only slightly lower than 2009 as shown by mean scores for each trial. Of note is the Hart trial, with an exceedingly low level of crown rot, as a score of 5 for the SA method is equivalent to a score of 1 for the other methods. Genetic variance summarises the variability between genotype predictions for this set of ring test lines. This figure should be interpreted relative to error variance to determine the overall level of genetic discrimination for each trial. Heritability is a measure which combines the information on genetic variance, relative to total variance as a fair means of comparing across trials. Of the field methods under study for two years, it is apparent that the Tamworth and Wellcamp trials give the highest proportion of genetic information relative to total variance, while Narrabri has the lowest heritability.

Sources of variability in phenotyping

Three of the phenotyping methods provided data on the sampling hierarchy for crown rot assessment. The method used in Narrabri has a hierarchy of Plot/Plant/Tiller, assessing up to 15 tillers for each of 5 plants per plot. The Wellcamp method differs in that it mixes tillers from different plants and randomly selects 40 tillers to score, hence the plant strata is lost. Both of these methods show that difference between tillers is the dominant source of variation in the phenotyping method. The Tamworth method is slightly different again in that it visually averages over tillers, but then the variation is carried up to the next level of the hierarchy, as the dominant source of variation for this method is between plants. The percentage of variance for each layer of the sampling hierarchy is given in Table 1.4, and it is obvious that the variation at the lower level dominates variation between genotypes.

To improve the phenotyping methods there must be some control (or measurement) of this variation between tillers. For example, to control the variation, scores may be taken on only the primary tiller. To measure the variation, scores could be given to all tillers, but the *type* of tiller should be recorded and accounted for in the analysis. Either way, it appears crucial to consider this modification to improve the precision of phenotyping.

Table 1.2: Mean crown rot severity rating for genotypes grown in the five ring test trials in 2010

Genotype	Tamworth	Narrabri	SA terrace	Hart	Wellcamp
94		2.28	1.88		28.3
148		1.40	3.18		44.3
2-49	16.7	1.80	1.75	0.03	33.0
AUS29529	20.3	2.04	3.07	0.14	43.3
Batavia	18.9	2.43	3.22		46.0
Bellaroi	45.5	3.11	3.29	0.53	48.7
CIM104	28.7	1.74	3.02	0.15	40.3
Correll	33.4		3.56	0.40	47.7
E17		2.00	2.05		30.7
Gregory	27.2	2.33	2.83	0.31	38.3
Wylie	21.2	2.35	2.32	0.20	44.0
Gladius	37.9	2.20	3.35	0.39	51.0
H45	31.1	2.00	2.94	0.44	42.0
IRN497	18.8	2.43	2.70		30.3
Janz	28.1	1.63	3.48	0.27	48.0
Kalka	41.1	2.66	3.04	0.39	57.3
Kukri	23.0	2.12	2.68	0.14	43.7
Livingston	26.6	1.53	3.68	0.50	42.7
Magenta	20.9	2.23	2.83	0.18	
Sentinel	22.4	2.16	3.35	0.34	44.3
Sunco	18.1	1.76	3.02	0.10	46.7
Sunstate	31.6	2.75	3.52	0.19	40.0
Sunvale	21.6	2.62	3.11		48.3
W21MMT70	16.9	2.07	2.47	0.74	33.0
Wyalkatchem	29.4	2.27	3.27	0.47	41.0
Yitpi	27.5	2.02	3.02	0.38	57.7
LSD(5%)	6.5	0.753	0.5644	0.2542	7.9

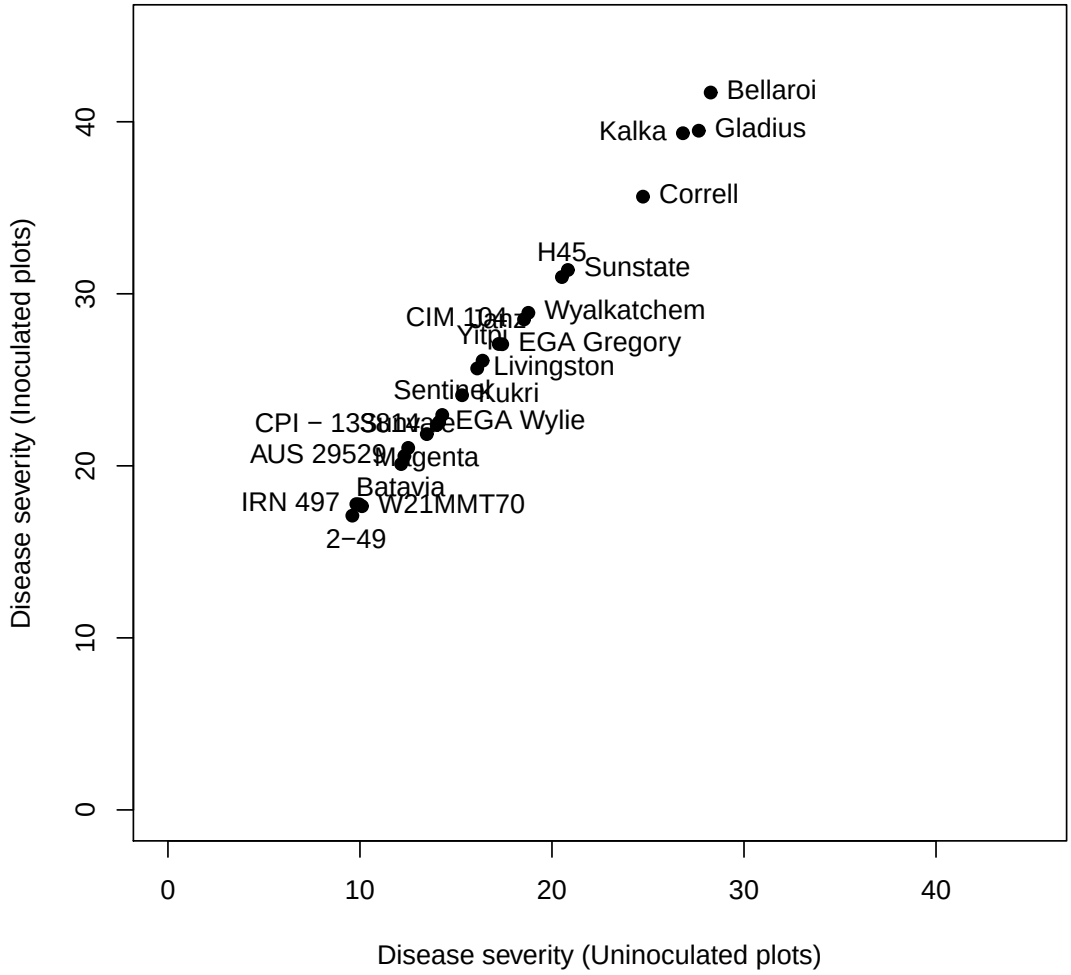

Figure 1.2: Crown rot severity predictions for inoculated and uninoculated plots at the Tamworth field trial

Table 1.3: A summary of mean crown rot severity and variance components for the ring test trials in 2009 and inoculated plots of yield loss trials in 2010

Trial	Mean severity	Genetic variance	Error variance	Heritability
2009				
DEEDI seedling	9.69	7.07	1.53	0.82
SA seedling	4.07	0.67	0.63	0.52
Narrabri	2.70	0.08	0.09	0.47
Terrace	3.12	0.16	0.43	0.28
Wellcamp	4.62	2.11	0.55	0.79
2010				
Hart	0.35	0.06	0.03	0.67
Narrabri	2.15	0.07	0.16	0.30
Tamworth	2.65	0.68	0.16	0.81
Terrace	2.98	0.26	0.16	0.61
Wellcamp	4.24	0.50	0.22	0.69

Table 1.4: Sources of variation in crown rot phenotyping methods based on assessment of ring test lines in 2010

Source	Percentage of variance		
	Tamworth	Wellcamp	Narrabri
Genotype	22	13	3
Replicate	0	2	4
Plot	2	5	24
Plant	77		1
Tiller		80	68

1.4.3 Disease severity - correlation between phenotyping methods

One of the aims of this study was to compare the similarity in ranked genotype performance between each of the methods. Genetic correlations between the adult and seedling methods across years are quantified in Table 1.5. In general there is reasonable agreement between most methods, where the highest correlations tend to occur within years. A notable exception to this is the Narrabri trial in 2010 which is poorly correlated with most other methods, except Tamworth 2010. Also, the 2009 Narrabri trial is not well correlated with most sites in 2010, except for Hart which had relatively low levels of crown rot scored. To a lesser extent, the Wellcamp trial in 2010 shows low correlation with most 2009 trials, although shows reasonable agreement with the 2010 sites.

1.4.4 Tolerance to crown rot - yield loss between inoculated and uninoculated plots -

The yield data from the field trials allows us to assess the ability of the genotype to produce grain in the presence of disease, that is, the tolerance of the genotype to crown rot. If tolerance is to be assessed independently of yield potential, then the deviation from the regression line in Figure 1.3 is the best indicator of tolerance. These deviations are listed in Table 1.6. From the graph and table

it is obvious that W21MMT70 and AUS29529 yield higher than expected in the presence of crown rot, while Bellaroi and CIM104 yield lower than expected in the presence of crown rot.

The benefits of viewing yield loss due to crown rot severity are not obvious for these types of plus and minus inoculated trials as there are only two observations to determine the rate of yield loss per unit change in CR severity for each genotype. However, absolute loss can be calculated from the difference in yield between inoculated and uninoculated field plots, and graphed against CR severity scores in the inoculated plots (Figure 1.4). It is again obvious that W21MMT70 and AUS29529 suffer lower yield loss, but this is also in a background of lower CR severity. Genotypes such as Gladius and Correll suffer a low level of yield loss in the presence of higher CR severity.

Table 1.5: Genetic correlation between methods for the crown rot ring set entries

	2009								2010	
2009										
Seedling										
DEEDI	1									
SA	0.36	1								
Adult										
Narrabri	0.58	0.20	1							
Terrace	0.56	0.30	0.39	1						
Wellcamp	0.74	0.42	0.46	0.60	1					
2010										
Hart	0.84	0.39	0.77	0.63	0.82	1				
Narrabri	0.11	0.16	-0.27	0.16	0.28	0.02	1			
Tamworth	0.62	0.42	0.13	0.55	0.80	0.61	0.51	1		
Terrace	0.65	0.37	0.38	0.53	0.73	0.71	0.27	0.73	1	
Wellcamp	0.34	0.26	-0.03	0.32	0.49	0.31	0.40	0.65	0.45	1
	2009					2010				
	DEEDI seedling	SA seedling	Narrabri	Terrace	Wellcamp	Hart	Narrabri	Tamworth	Terrace	Wellcamp

1.5 Summary

- *Resistance*: The disease severity ratings from inoculated plots can be used to assess the resistance of genotypes to crown rot and are included in the report. Uninoculated plots *must* also be scored for disease as a baseline measure for the tolerance analysis and as additional information on resistance if they are not disease free.

- *Precision of methods*: Based on the trials from the previous two years the field trials with the highest level of genetic discrimination are Tamworth and Wellcamp. The Terrace trial and Hart field trial are the next highest in genetic information relative to error, and the trial with lowest heritability in both years is Narrabri.

- *Improvements to phenotyping*: The precision of testing for all methods is dominated by within field plot variability. The dominant source of this variation is between tillers within a plant and this may be due to the spread of the disease from the main tiller through to ancillary tillers. Sampling should be stratified across tillers and these effects should be adjusted for in the analysis to reduce the residual variance. The option of extending the categorical scale to a continuum should also be explored. Increased measurement detail on fewer homogeneous tillers may improve precision without increasing assessment time.

- *Correlation between trials*: The agreement in ranked genotype performance between methods for disease severity is based on genetic correlations. There was reasonable agreement between all 2009 trials, but less agreement across years. The most striking feature is that the Narrabri and to a lesser extent the Wellcamp and Tamworth trials in 2010 are not strongly correlated with some of the 2009 trials. The DEEDI seedling method appears to be correlated well with all trials except Narrabri 2010 and, to a lesser extent, Wellcamp 2010.

- *Tolerance*: The yield measurements on paired inoculated and uninoculated plots can be used to assess the tolerance of genotypes to crown rot. Some genotypes (AUS29529 and W21MMT70) displayed a higher level of tolerance in the Tamworth trial of 2010. The other trials at Hart and Narrabri showed little change in yield between inoculated and uninoculated plots and were deemed to have insufficient environmental stress to incur yield loss due to crown rot. The magnitude of the genetic correlation between the +,- inoculated plots approaching the boundary valur of 1 supports this observation. This equates to a lack of interaction between inoculation and

Table 1.6: Tolerance of ring test lines to crown rot inoculation at Tamworth in 2010

Genotype	Yield advantage over expected (t/ha)
W21MMT70	0.234
AUS 29529	0.174
Janz	0.074
IRN 497	0.057
Gladius	0.054
CPI 133814	0.052
Livingston	0.039
Sunstate	0.034
Correll	0.009
EGA Wylie	0.008
Sunco	0.005
Yitpi	0.002
Sentinel	-0.001
H45	-0.009
Magenta	-0.015
Kukri	-0.021
EGA Gregory	-0.050
Kalka	-0.051
Wyalkatchem	-0.057
Sunvale	-0.070
Batavia	-0.071
2-49	-0.093
CIM 104	-0.118
Bellaroi	-0.169

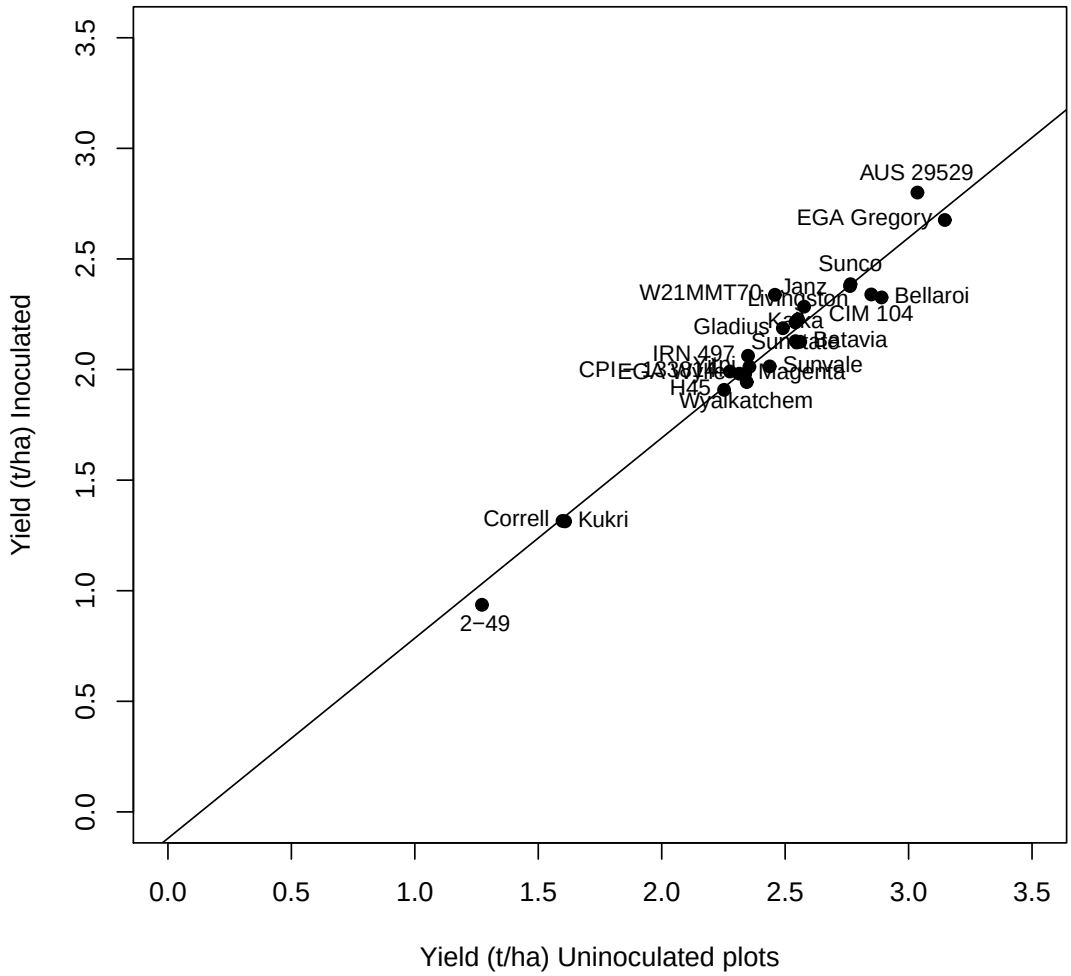

Figure 1.3: Yield predictions for genotypes in inoculated and uninoculated plots at the Tamworth field trial

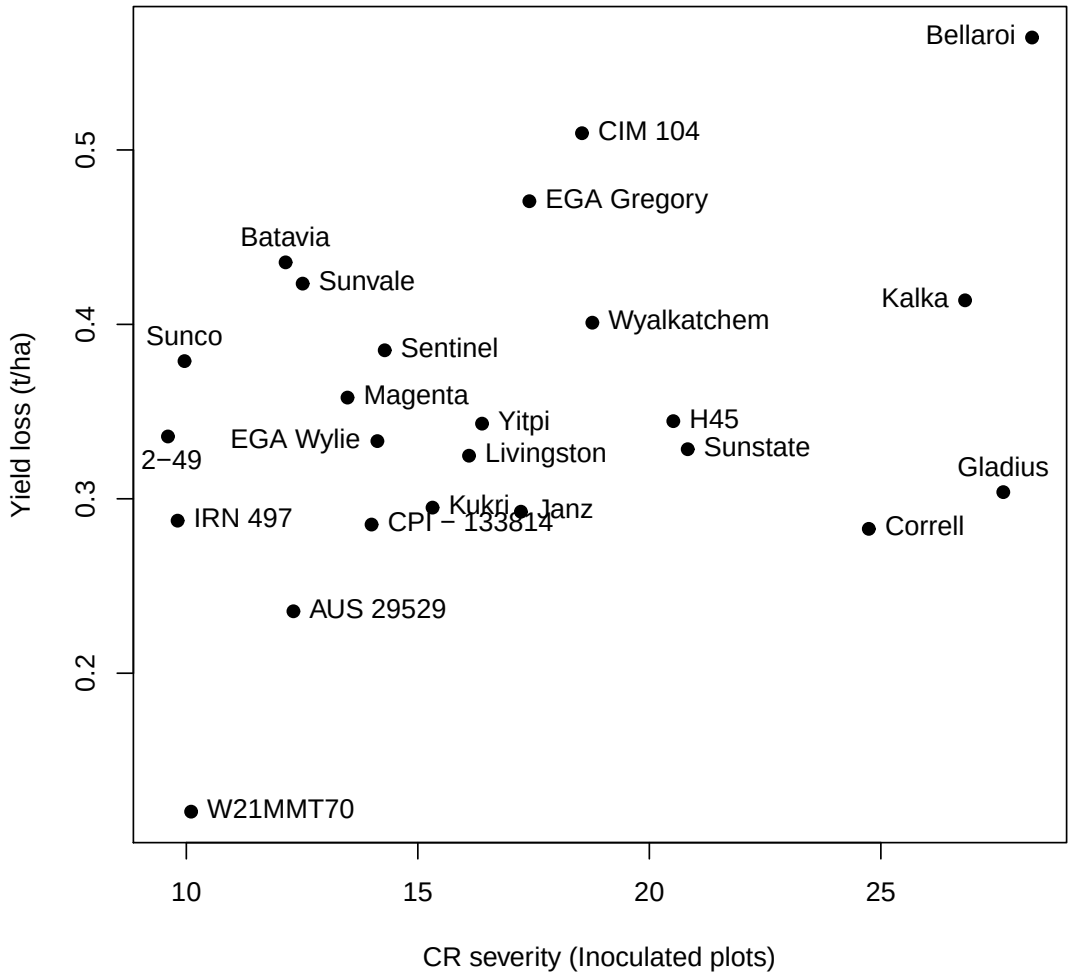

Figure 1.4: Genetic effects of yield difference between inoculated and uninoculated plots against disease severity in inoculated plots for the Tamworth field trial

genotype effects, with an outcome of near identical yield for genotypes regardless of inoculation.