Selection of genotypes for resistance and tolerance to pathogens:

# A bivariate statistical analysis of yield and disease response.

Alison Kelly Bethany Macdonald Susan Fletcher December 2, 2015







- Background to the agricultural research problem
- Linear mixed model formulation
- Interpretation of results



Phenotyping for the effect of disease on genotypes in a plant breeding program requires measurement of

- growth of the pathogen in the plant
- subsequent effect of the pathogen on grain production in the plant





# A bivariate statistical analysis of yield and disease Definitions

# Resistance

- The ability of the host to prevent pathogen entry and control pathogen spread.
- In this application, the ability of the plant to reduce pathogen burden and hence suppress disease expression.

# Tolerance

- The ability of the host to limit the impact of pathogen burden on host health.
- In this application, the ability of the plant to produce grain in the presence of disease.





Uncoupling resistance and tolerance is a challenge, but is desireable for a number of reasons (Kause and Odegard, 2012).

- Each have a different impact on the relationship between the host and the pathogen.
- In plants (and animals) they are weakly genetically correlated, so are dfferent traits.
- Plant breeders should exploit both traits to provide a responsible solution to sustainable farming practices and increased food production.

Host: Wheat Pathogen: Fusarium pseudograminearum



- Experiments to test the resistance and tolerance of genotypes involve a disease treatment with 2 levels: an untreated control (nil) and an imposed disease level (plus).
- The experimental design typically consists of replicated field trials in a strip-plot arrangement of the disease treatment, where genotypes are grown under these two conditions in paired plots.
- Aim: To select genotypes possessing combined traits of resistance and tolerance to disease.



A bivariate statistical analysis of yield and disease Experimental design

Field trial planted as a rectangular array of plots in 25 columns by 44 rows, grown at Narrabri, NSW in 2014







# Treatment allocation for a subset of plots: 25 columns by 4 rows

Inoculum strips (nil,plus)



Genotype mainplots





A bivariate statistical analysis of yield and disease Experimental design

#### Measurements

- Yield of grain (t/ha).
- Disease level of stem browning (cm).



Issues surrounding the methodology

- A baseline treatment of nil disease aims to estimate yield potential.
- Achieving this baseline is difficult in practice due to background levels of disease in the field.
- The severity of disease expression will change with environmental conditions.



The form of the linear mixed model for a single trait (j=Y,D) is

$$\mathbf{y}_j = \mathbf{X}_j \boldsymbol{ au}_j + \mathbf{Z}_{g_j} \mathbf{u}_{g_j} + \mathbf{Z}_{s_j} \mathbf{u}_{s_j} + \mathbf{e}_j$$

 $\mathbf{y}_{j}^{(n\times1)}$  is the vector of responses measured on n plots,  $\boldsymbol{\tau}_{j}^{(t\times1)}$  is a vector of fixed effects for inoculum with design matrix  $\mathbf{X}_{j}^{(n\times t)}$ ,  $\mathbf{u}_{g_{j}}^{(tm\times1)}$  is a vector of random effects for genotype, partitioned for inoculum level, with design matrix  $\mathbf{Z}_{j}^{(n\times m)}$ ,  $\mathbf{u}_{g_{j}}^{(s\times1)}$  is a vector of random effects for structural terms, with design matrix  $\mathbf{Z}_{s_{j}}^{(n\times s)}$ , and  $\mathbf{u}_{s_{j}} = (\mathbf{u}_{r_{j}}', \mathbf{u}_{b_{j}}', \mathbf{u}_{g_{p_{j}}}')'$  and  $\mathbf{e}_{j}^{(n\times1)}$  is the vector of residual errors.



The variance of the random inoculum by genotype effects,  $\mathbf{u}_{g_i}$ , is

$$\mathsf{var}(\mathbf{u}_{g_j}) = \begin{bmatrix} \sigma_{g_{n_j}}^2 & \sigma_{g_{n_{p_j}}} \\ \sigma_{g_{n_{p_j}}} & \sigma_{g_{p_j}}^2 \end{bmatrix} \otimes \mathsf{I}_m$$

The random effects for structural terms and residual errors are assumed to be independent and identically distributed normal variates with mean zero and variance structures,  $var(\mathbf{u}_{s_{j_i}}) = \sigma_{s_{j_i}}^2 \mathbf{I}_{n_{s_i}}$  and  $var(\mathbf{e}) = \sigma_j^2 \mathbf{I}_n$  (where  $n_{s_i}$  is the length of  $\mathbf{u}_{s_{j_i}}$ ).



The form of the linear mixed model for both traits (j=Y,D) is

$$\mathbf{y} = (\mathbf{I}_2 \otimes \mathbf{X}) \mathbf{\tau} + (\mathbf{I}_2 \otimes \mathbf{Z}_g) \mathbf{u}_g + (\mathbf{I}_2 \otimes \mathbf{Z}_s) \mathbf{u}_s + \mathbf{e}$$

where

$$\mathbf{y} = (\mathbf{y}'_Y, \mathbf{y}'_D)', \quad \mathbf{\tau} = (\mathbf{\tau}'_Y, \mathbf{\tau}'_D)', \quad \mathbf{u}_g = (\mathbf{u}'_{g_Y}, \mathbf{u}'_{g_D})', \quad \mathbf{u}_s = (\mathbf{u}'_{s_Y}, \mathbf{u}'_{s_D})' \text{ and } \mathbf{e} = (\mathbf{e}'_Y, \mathbf{e}'_D)'.$$



The variance of the random inoculum by genotype effects,  $\boldsymbol{u}_{g},$  for both traits(Y,D) is

$$\operatorname{var}(\mathbf{u}_g) = \begin{bmatrix} \sigma_{g_{Y_n}}^2 & & & \\ \sigma_{g_{Y_{np}}} & \sigma_{g_{Y_p}}^2 & & \\ \sigma_{g_{Y_nD_n}} & \sigma_{g_{Y_pD_n}} & \sigma_{g_{D_n}}^2 & \\ \sigma_{g_{Y_nD_p}} & \sigma_{g_{Y_p}D_p} & \sigma_{g_{D_{np}}} & \sigma_{g_{D_p}}^2 \end{bmatrix} \otimes \mathbf{I}_m$$

Additionally, the variance of the random structural effects and the residual errors,  ${\bf e},$  for both traits(Y,D) are

$$\mathsf{var}(\mathbf{u}_{s_i}) = egin{bmatrix} \sigma_{s_{Y_i}} & \ \sigma_{s_{YD_i}} & \sigma_{s_{D_i}}^2 \end{bmatrix} \otimes \mathsf{I}_{n_{s_i}}$$

$$\mathsf{var}(\mathbf{e}) = \begin{bmatrix} \sigma_Y^2 & \\ \sigma_{YD} & \sigma_D^2 \end{bmatrix} \otimes \mathsf{I}_n$$



We fit the linear mixed model in ASReml-R (Butler et al., 2013).

- Estimate the variance components using Residual maximum likelihood (REML) (Patterson and Thompson, 1977).
- Produce Best Linear Unbiassed Predictors of the random genotype by inoculum effects.
- Use the inherent regression structure in the bivariate analysis to interpret the results, relating this back to tolerance and resistance in the plant-pathogen context.



#### Trait by Inoculum means and genetic variances estimated from the model.

Trait	Inoculum	Mean	Genetic variance
Disease	nil	0.68	0.084
(sqrt(cm))	plus	2.29	0.128
Yield	nil	3.32	0.197
(kg/ha)	plus	2.88	0.270



#### Results Genotype predictions (BLUPs)



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We focus on the genotype blups, and use the regression structure inherent in the bivariate model to interpret the results.

For Yield, plus vs nil blups

$$\mathbf{u}_{g_{Y_p}} = \beta_Y \mathbf{u}_{g_{Y_n}} + \boldsymbol{\epsilon}_Y, \qquad \beta_Y = \frac{\sigma_{g_{Y_{np}}}}{\sigma_{g_{Y_n}}^2}.$$

Define yield responsiveness as  $\epsilon_Y$ , where responsiveness has zero covariance with yield potential,  $\mathbf{u}_{g_{Y_n}}$ .



# Yield responsiveness, $\epsilon_Y$





Genotypes with high yield potential and responsiveness under disease pressure



Yield-nil

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Again, we use the regression structure inherent in the bivariate model to interpret the results.

For Plus disease plots, yield vs disease blups

$$\mathbf{u}_{g_{Y_p}} = \beta_P \mathbf{u}_{g_{D_p}} + \boldsymbol{\epsilon}_P, \qquad \beta_P = \frac{\sigma_{g_{Y_p D_p}}}{\sigma_{g_{D_p}}^2}.$$

Define yield advantage under disease as  $\epsilon_P$ , where yield advantage has zero covariance with disease severity under inoculation,  $\mathbf{u}_{g_{D_n}}$ .



# Combined selection for tolerance and resistance

Yield advantage under disease,  $\epsilon_D$ 





- We have extended this model to an analysis across multiple trials (environments).
- We have included a genetic covariance relationship through the pedigree of the genotypes.
- This will be extended to a genomic relationship matrix as marker data is available next year.



# The National Crown Rot Initiative team

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