Design and analysis for spatial effects in pasture trials

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Abstract. *Measurements made on pasture plots in field evaluation studies are affected by the spatial heterogeneity of the field due to local influences of paddock history, moisture drying order and fertility gradients. Statistical models to account for these inherent and ephemeral effects can markedly enhance the accuracy of comparisons among test lines or treatments so that interpretation of the results is objective, accurate and reliable. This paper reports on statistical methods for the field evaluation of white clover breeding lines in the development of locally adapted white clover cultivars for New South Wales dry-land pastures.*

Introduction

The breeding process for developing new pasture cultivars includes *in situ* field trials where lines are evaluated under environmental conditions (moisture stress, grass competition, grazing pressure) for which the breeding project is targeted. However, within-site variability of those environmental conditions present at the micro-level in the trial site may contribute to considerable spatial heterogeneity, and these local influences may obscure an objective and fair comparison of test lines.

In the current national white clover breeding program (Jahufer *et al.* 2006), the comparative performance of breeding lines is assessed from longitudinal profiles of agronomic performance (eg. seasonal yield, biotic presence) and plant traits (eg. leaf size stability, stolon survival) relevant to the breeding objectives. A significant component of variation in the data which may affect expression of agronomic performance is the spatial variation due to plot position. As the objective is to evaluate the relative performance of a large number of diverse candidate lines, the germplasm entries are distributed across plots that may vary in paddock history or inherent and ephemeral environmental factors like soil type, soil fertility gradient or patch drying order.

Early field experimenters instituted the tenets of blocking and randomisation as ways to control variation due to spatial influences. Blocking removes large-scale variation and the randomisation allows equal chance for treatment allocation to a particular plot and eliminates latent biases. The early experimenters acknowledged that further spatial effects may remain (Fisher 1959 pp 60–64), but they did not have the statistical tools to further refine the analysis.

The methods developed to correct for these spatial influences have been used in two previous white clover breeding projects. The first was conducted at two sites (Armidale, Glen Innes), and the statistical methods used for those data are explained in Murison *et al.* (2006). In 2005–2008, another experiment was conducted at Glen Innes and Inverell with the aim of using the genetic progress achieved for 'tolerance of moisture-stress' in the previous experiment to develop a white clover cultivar for the New South Wales 'dry margins'.

Methods

In the 2005–2008 breeding project, three blocks (replicates) of 95 breeding lines and five commercial cultivars (as checks) were evaluated in plots of 5 m x 1 m. The plots were laid out in a 15 row by 23 column design so that the experiment occupied an area of 15 m x 46 m. In a field site such as this, uniformity across the trial block is not expected. The experiment was therefore designed so that local influences could be estimated, and the observations corrected, such that comparisons could be made between lines as if they were located on a uniform block.

The treatment of the data is elaborated here using the June 2005 sampling of 'clover presence' scores. The scores are ratings of 0–9 where 1=10%, 2=20% … 9= 90% or greater.

Figure 1(a) shows residuals from the incomplete block analysis of June 2005 presence scores as a 'heat map'. If the field plots were approximately uniform, the residuals would be randomly distributed. This would show in the heat map as a random distribution of dark (negative residuals) and light patches (positive residuals), rather than as clusters.

Gilmour *et al.* (1997) explained how the analysis can be substantially improved by fitting extra terms to the statistical model to account for these spatial effects. The required extra terms can be gauged from the variogram of residuals. The variogram is a plot which relates the change of variance of the residuals across the experiment plots as a function of the distance between plots. If the variance is constant (ideal), there would be no relationship and the graph of the variance with distance would be a flat line. If variance is not constant due to the underlying fertility trends, the variance increases as distance between plots increases and so the variogram reveals the direction and strength of the underlying trends.

Figure 1(b) shows the variograms along four transects across the field for the residuals from an incomplete block analysis and the rising line for the diagonal from bottom left to top right indicates the direction of trend.

Statistical model

The measure (eg. clover presence) from each plot is considered to be due to a combination of (i) the overall mean, (ii) an effect due to the line, (iii) an effect due to the plot position, and (iv) a random unexplained component. The observed data are related to these components by a statistical model which estimates line effect, adjusted for underlying fertility trends across rows and columns. By estimating the fertility trend, the line effect can be calculated for average fertility rather that averaging the three independent measurements for each line. If each line was represented at high, medium and low fertility plots, averaging would be satisfactory but since some lines were allocated (randomly) to only low fertility plots and others to high fertility plots, the naïve estimates for these lines need to be corrected so that the comparison is not influenced by the effect of plot position. The trends are represented by mathematical curves called *splines*, with that name arising from the flexible rod used by draughtsmen to draw asymmetric curves.

Denote y_{ijk} as the response from the ith line whose row-column location is indexed by j and k. The statistical model is of the form

$$y_{ijk} = \mu + \theta i + spl(r_j, c_k) + \varepsilon_{ijk} \quad \varepsilon_{ijk} \sim N(0, \sigma^2)$$

where θ is the effect of Line i, and $spl(r_j, c_k)$ represents *spline* terms of the distances in the row and column directions which account for the trends. The row and column trends are combined to represent the trend as a two-dimensional surface. This model is known as a generalized additive model and fitted in R using the 'mgcv' package (Wood 2008).

Results and discussion

When spatial trends have been removed, the predicted values for each line are collated into a longitudinal profile. Figure 2 compares the profiles of some of the short-listed lines with the best performing check cultivar Tribute. In the figure, the profile for the adjusted mean score for Tribute is plotted as a dotted line, the profile of the unadjusted mean score for each line is plotted in grey, and the adjusted mean scores are plotted in black.

The profile for line 51 shows the greatest effect of being corrected for spatial variation. Line 51 fortuitously occupied positions that were initially favourable positions, but later became adverse. This is a promising line which might not have been selected on the basis of its unadjusted profile because of the 'apparent' poorer performance towards the end of the evaluation. The unadjusted profile for line 69 would give undue optimism. While it is still at least as good as Tribute, expectations of line 69 should be tempered on the basis of the adjusted profile

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(a) (b)

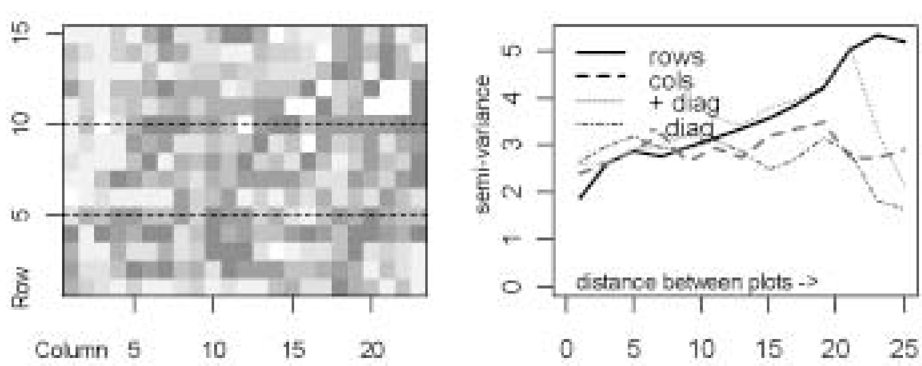

Figure 1. Diagnostic plots for gauging spatial effects: (a) residuals plotted on field plan, and (b) variograms for residuals.

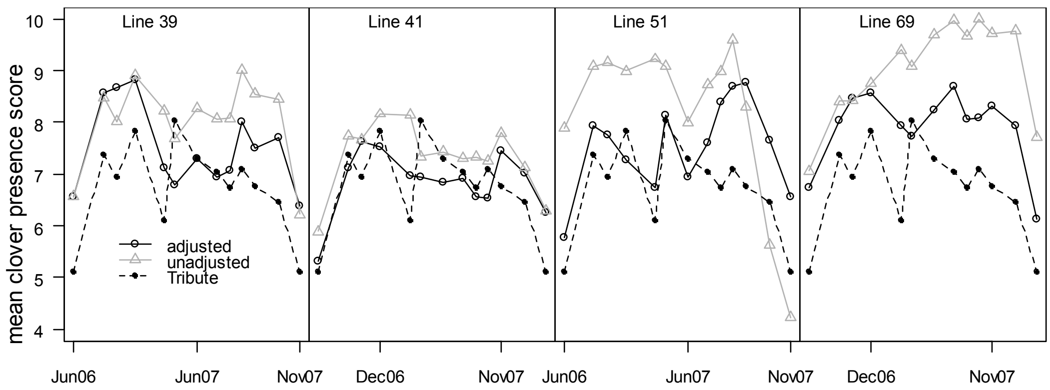

Figure 2. Comparison of the longitudinal profiles of short-listed lines and Tribute

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