



## Statistical methods to address spatial variation in pasture evaluation trials

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**Abstract.** The primary breeding objective for white clover improvement in eastern Australia is more reliable persistence under summer moisture-stress while maintaining herbage production (especially winter growth). To identify elite germplasm, evaluation of candidate lines is done *in situ*, under field conditions for which the prospective cultivar is targeted. These conditions (e.g. moisture stress, nutrient infertility, grass competition) may impose spatial influences within the field site that as artefacts mask or alter the uniform expression of response to environment.

A significant component of artefact variation which affects interpretation of the data is the spatial variation due to plot position. Failure to correct for this may give unreliable comparisons as some lines inadvertently (through randomisation) are allocated to favourable or unfavourable plots.

Our methodology was developed at two sites (Glen Innes, Armidale in northern New South Wales, Australia) in an experiment which sought to identify superior lines among 20 candidate entries. Cultivars of reputed stability and spanning the expected performance range, are replicated at regular intervals through the field site. The difference amongst replicates within cultivar (that is a reduced G X E) is used to estimate spatial trends. Elite lines are identified from temporal profiles of plant performance related to the breeding objectives.

We describe (i) design and analysis which allows estimation of the spatial effects, and (ii) the temporal profiling of plant performance. These statistical procedures are seminal in identifying superior breeding lines that subsequently proceed to cultivar development.

### Introduction

White clover is the main perennial pasture legume for high rainfall temperate regions in eastern Australia (Pearson *et al.* 1997) due to its broad adaptation and high grazing value. The Australian white clover zone encompasses some 8 million hectares (Clements 1987) with potential to extend to 16 million hectares (Ayres and Caradus 2002). However, poor persistence (Robinson and Lazenby 1976, Kenny and Reed 1984, Gillard *et al.* 1989) associated with sensitivity to summer moisture-stress (McCaskill and Blair 1988, Archer and Robinson 1989, Hutchinson *et al.* 1995) is a major limitation of white clover (McDonald 1988). Stolon survival through periods of soil moisture stress is a pre-requisite for persistence (Archer and Robinson 1989); accordingly, improvement of stolon survival is the primary breeding objective for white clover improvement for Australian dryland pastures (Ayres *et al.* 1996).

The experimental aim was to identify at least one white clover line with superior persistence, compared to commercial cultivars, for dryland environments subject to summer moisture-stress. The experiment required measurements over 3 years to reliably assess persistence. Persistence is the primary focus and clover presence (proportion of plot occupied by clover) is a parameter that integrates physiological processes of adaptation, agronomic performance and survival.

The effects of interest ('interest effects') are contrasts amongst cultivar checks and test lines and differences between the checks and lines over time. There is also the possibility of 'nuisance' effects of spatial variability due to differences in potential productivity amongst the plots.

The sampling occasions in a temporal study are chosen to be able to detect change in response over time. Sampling may be more frequent in warmer months than in winter. Sampling occasions may even be chosen *ad hoc* during the experiment when observations indicate that either growth or dormancy phases are changing. However, the allocation of treatments to field plots is determined at the outset of the experiment. A primary concern of field trials is to allow responses to be corrected for local productivity effects and gradients. This is done by (i) blocking and randomisation so that gross influences are removed and minor influences cancel each other and (ii) estimating productivity gradients so that responses can be adjusted and corrected by regression.

The spatial analysis of field experiments is well documented for cereal trials (Gilmour *et al.* 1997, Cullis *et al.* 1998) and the general statistical principles are applicable in the present case. However pasture plant improvement experiments differ from crop experiments because for example, for white clover, (i) lines comprise populations of heterogeneous genotypes, (ii) the plots are not harvested on a single occasion but rather are sampled frequently over a number of years, and (iii) the target plant (white clover) cohabits mixed swards with companion grasses and weeds. These contingencies contribute extra variability to confound comparisons between lines.

## **Materials and methods**

### Measurements

Herbage yield in a temporal study evaluating white clover lines cannot be determined by destructive sampling and so a visual scoring of herbage yield was used to measure performance from each plot. Clover performance was therefore scored visually on a 0-9 scale where 0=no clover, 1=10% clover and 9=90% clover. Data were obtained from two independent observers providing rating values from four quadrants per plot and the plot response was taken as the average of these eight samples. The experiment is required to provide data for the statistical model that will evaluate the test lines over three years.

### Experiment design

There are 25 treatments of interest; six check cultivars (Sustain, Haifa, Waverley, Irrigation, NuSiral and SFU), two other cultivars (El Lucero, Prestige) and 17 test lines. The available area and prospective spatial gradients suggested a row-column layout. Another treatment, the indigenous ecotype, was added so that the treatments could be arranged in 13 columns. There was sufficient seed for five replicates of each test line and the layout was 10 rows of 13 columns. The allocation of treatments to plots was done by constructing an alpha-design (Patterson and Williams, 1976) with block size 13 and five replicates. An entire replicate occupies two rows and each row is an incomplete block of the alpha-design. This ensures that pairs of test lines occur together in the same row as evenly as possible. The alpha-design was constructed using the GENDEX design software (Nguyen, 2003). The check varieties are positioned systematically and the other treatments allocated randomly to the remaining plots. The field plan is shown in Figure 1.

Figure 1. Field plan of treatment allocation to plots. Check cultivar plots (1-6) are shaded, 7-21, 23, 25 are test lines, 22 is El Lucero, 24 is Prestige and 26 is the indigenous ecotype.

	Columns												
Row	A	B	C	D	E	F	G	H	I	J	K	L	M
1	1	14	24	18	12	2	26	19	16	7	3	9	22
2	21	4	20	8	10	11	5	13	15	17	25	6	23
3	15	17	2	21	9	25	12	1	14	23	7	20	6
4	4	22	18	5	24	11	19	13	3	10	16	8	26
5	9	6	21	23	4	26	11	20	7	5	13	17	15
6	3	16	14	10	18	1	8	25	22	24	2	19	12
7	23	5	25	19	8	11	6	9	13	15	22	4	17
8	24	21	1	7	16	12	20	2	18	10	14	26	3
9	11	23	10	6	12	18	14	22	5	26	20	16	1
10	2	9	19	17	3	13	7	15	25	4	24	21	8

### The model

Following Gilmour *et al.* (1997), we build the model stepwise. The initial model has terms to represent the design variables of treatment and row, and the residuals from this model are investigated to see if there are spatial effects. A set of preliminary analyses are done for each sampling occasion to gauge the nature of spatial effects at each occasion. The test lines are assessed on their presence over time, especially their persistence after 2 years. It is not necessary to model the temporal changes as functions of continuous time to discern the elite lines and the temporal profiles are plotted at the discrete sampling times.

The ASREML software (Gilmour *et al.* 2002) was used to analyse the data and graphing was done by the R Statistical and Graphical software (R development team, 2005).

At a particular sampling time  $t$ ,  $\mathbf{y}_t$  denotes the response (viz. yield score) observed at the plot in row  $r$  and column  $c$  as  $y_{rc}$  and the vector of 130 responses from 10 rows and 13 columns. In the following, the subscript  $t$  implies that we are referring to the  $t$ 'th sample time. The initial statistical model is written as:

$$\mathbf{y}_t = \boldsymbol{\mu}_t + \mathbf{X}\boldsymbol{\theta}_t + \mathbf{Z}\mathbf{b}_t + \mathbf{e}_t, \quad \begin{bmatrix} \mathbf{b}_t \\ \mathbf{e}_t \end{bmatrix} \sim \mathbf{N} \left( \begin{bmatrix} \mathbf{0} \\ \mathbf{0} \end{bmatrix}, \sigma^2 \begin{bmatrix} \mathbf{G} & \mathbf{0} \\ \mathbf{0} & \mathbf{R} \end{bmatrix} \right) \quad (1)$$

where

$\mu_t$  is the estimate of the mean of check cultivar 1

$\boldsymbol{\theta}_t$  is the vector of check cultivar and test line effects with  $\theta_{1t} = 0$

$\mathbf{b}_t$  is the vector of incomplete block effects, assumed distributed  $\mathbf{N}(0, \sigma^2)$

$\mathbf{X}$  and  $\mathbf{Z}$  are the design matrices of 1's and 0's which link the data to the treatments and blocks

$\mathbf{e}_t$  is the measurement error

$\mathbf{G}$  is a diagonal matrix with elements  $\sigma_b^2 / \sigma^2$

The correlation matrix  $\mathbf{R}$  is initially taken as the identity matrix under the assumption that the residuals are uncorrelated. After fitting model (1), diagnostic tests are done on the residuals to check the model assumptions. If the incomplete block analysis does not account for across-site gradients, it is replaced by a spatial model. In the spatial model, trends due to plot position are represented as functions of the row and/or column distance from a reference (e.g. a corner of the field) and once again the

residuals examined. Detrending accounts for broad scale gradients but there may remain small scale correlations between adjacent plots. If so, estimates of these correlations are included in the **R** matrix.

### Results and discussion

Yates (1936) explained the roles of check varieties as indicators of fertility in their neighbourhood in experiments involving a large number of varieties. The responses of the experimental plots are corrected by using the fertility measure as a covariate. The check cultivars are assumed to be genetically stable and so can supply the best information upon which to gauge the local productivity gradients. However, Yates also showed that incomplete blocks were more efficient in reducing the standard errors of treatment contrasts than relying solely on check plots.

In our case, check cultivars are included primarily as a reference to which test lines can be compared. Further, the number of test lines is not large (17) and there was sufficient seed to replicate so that productivity gradients can be identified by trends in the residuals after estimating the components of the observed data due to all the lines (checks + tests).

The spatial analysis is illustrated using clover presence data at the Glen Innes site in September 1999. The initial model has only the design factors of line and row. After fitting these terms, the adequacy of the model is reviewed by examining the variogram of the residuals from this model, plotted in the first frame of Figure 2. The variogram is a graph of correlations between field plot residuals at various spatial separations and randomly distributed residuals will give a flat variogram. Departure from “flat” implies that the residuals are not randomly distributed. The dependence of the magnitude of the residual on plot position may be indicative of an underlying fertility gradient. A new model is fitted which includes a spline trend for columns and the 10 random row effects of model 1 are replaced by a spline trend for rows. The variogram of residuals in the second frame of Figure 2 indicates that ‘detrending’ in both directions accounted (largely) for gradient and reduced the measurement error.

Figure 2. The variograms for two models representing the spatial variations in clover presence at Glen Innes from sampling in February 2001.

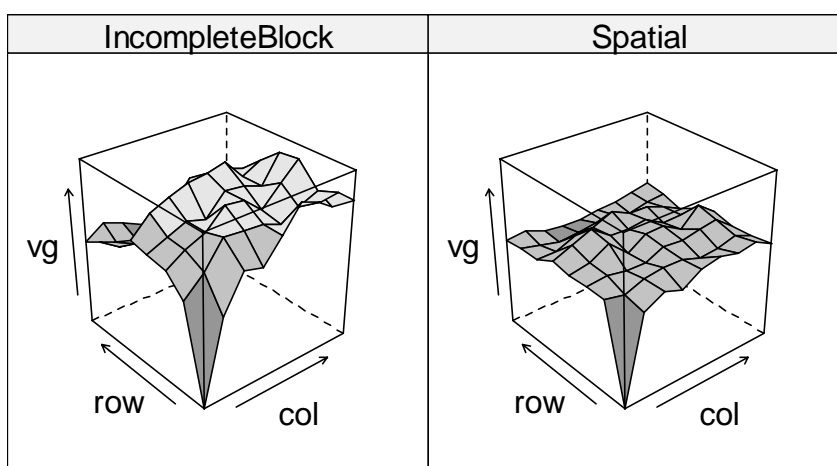


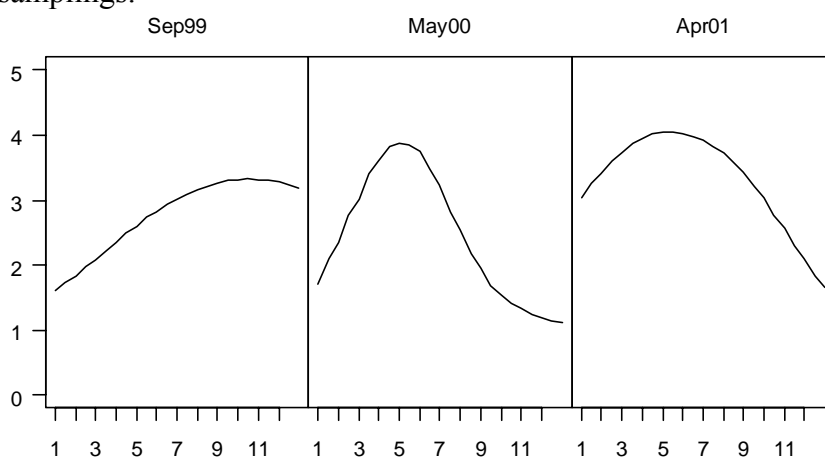
Table 1 shows an example of the effect on parameter estimates from inclusion of terms to account for the spatial variation at the February 2001 sampling. The effect of the spatial analysis is an upwards adjustment on the mean presence scores of these lines.

Table 1. Estimates of mean clover (standard errors in brackets) presence score for Haifa (check cultivar) , Trophy and Saracen (test lines) derived from two models for Glen Innes (February 2001).

<i>Model</i>	Haifa	Trophy	Saracen
Incomplete blocks	0.5 (0.67)	2.1 (0.67)	1.2 (0.67)
Spatial model	2.1 (0.65)	3.5 (0.64)	3.0 (0.67)

After detrending across rows and columns, the errors are inspected to verify (or otherwise) if the assumptions that those components be represented by normal distributions is reasonable. Density curves, q-q plots, box-plots and Kolmogorov-Smirnov tests indicated that the assumption of normality was reasonable. The spatial model for a site may change over time in accordance with the seasonal changes in white clover and data from each sampling are analysed separately. At the Armidale site, incomplete block analysis was satisfactory for most occasions but data from the Glen Innes site showed varying spatial effects with season. The column trends at Glen Innes for three samplings are plotted in Figure 3 to illustrate this.

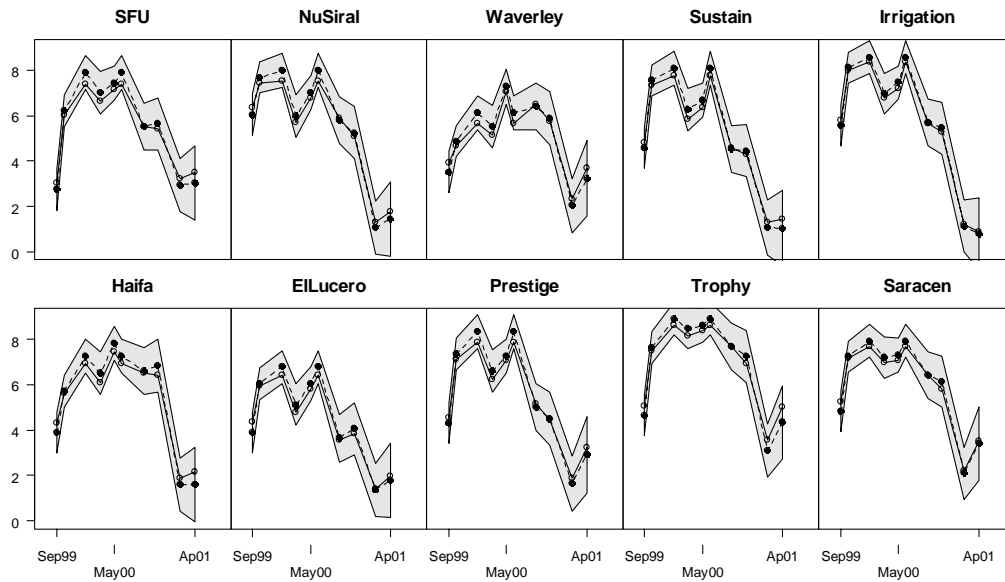
Figure 3. Trends of the mean presence scores across columns at Glen Innes at three samplings.



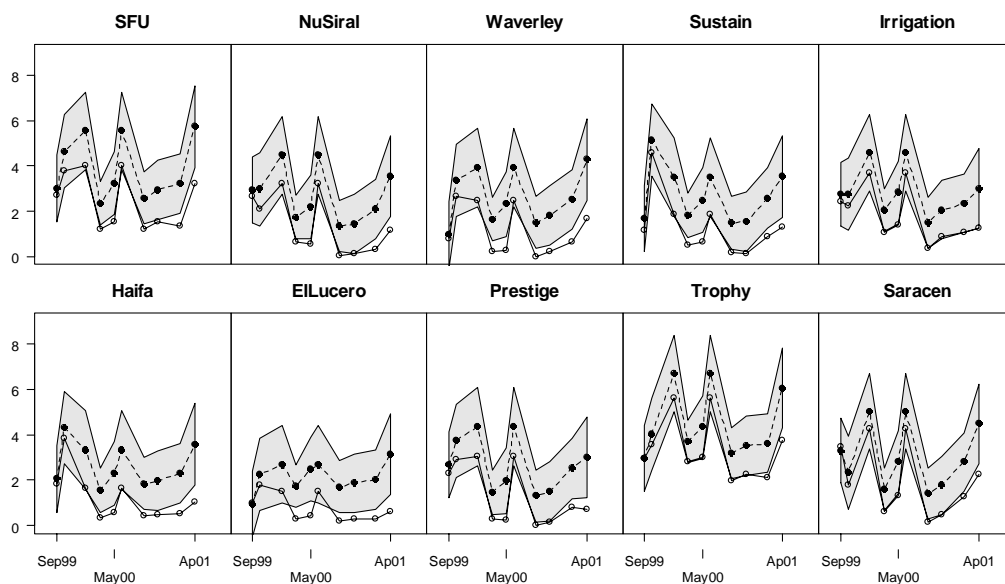
The estimates of mean scores from the straightforward incomplete block analysis and the more complex spatial analysis are compared in profiles of the estimates over time for the six check cultivars and test lines Trophy and Saracen in Figure 4. Both sets of estimates show the overall decline in presence with seasonal variations. There is negligible difference between the sets of estimates at Armidale but substantial difference at Glen Innes where column trends were detected. It is at the end of the experiment ( Autumn 2001) where the data are most informative in assessing persistence. The gains of the spatial analysis for the presence scores of Trophy and Saracen give weight to their nomination as elite lines and support the estimates of their mean presence scores at Armidale.

Figure 4. The temporal profiles of mean presence scores for the check cultivars and test lines Trophy and Saracen at (a) Armidale and (b) Glen Innes. The solid line and open circles denote the estimates from the incomplete block analysis, the dashed lines and dots are estimates from the spatial analysis and the shaded areas denote the 95% confidence intervals for the estimates from the spatial model.

(a)



(b)



The procedures have addressed a previously adverse and challenging characteristic of field sites for pasture variety trials – the ubiquitous presence of spatial heterogeneity, that if not accounted for, produces data confounded and compromised by local influences. With appropriate design and analysis, the artefact responses can be isolated and removed to improve the power of analysis. These procedures have been successfully used by the AgResearch/NSW Department of Primary Industries alliance to develop Grasslands Trophy and Grasslands Saracen (Jahufer *et al.* 2005) as new white clover cultivars for dryland environments where summer moisture-stress limits persistence and are presently being used in a new breeding project to develop cultivars for low rainfall environments in Australia (Ayres 2005).

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