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Experimental Designs for Alley Cropping to Estimate Shrub × Grass Interaction

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Abstract

An alley cropping comprises rows of perennial shrubs/trees bordering the alleys of grasses/crops. An appropriately chosen alley cropping provides improvement in feeds for small ruminants, food for human consumption, and contributes to economic security and environmental sustainability. Several experimental designs and statistical models are presented. The experimental/environment designs considered are the complete block with or without split-plot frames for the self-borders and partial diallel borders in shrubs and alley experimental units. The treatment designs include a factorial structure of shrub-borders and grasses. The linear models consisting of shrubs effects, grasses effects and their interaction with different structures are considered with parameters defined for shrub × grass interaction. A statistical analysis of the alley- responses will be illustrated with a simulated dataset.

Key words: Alley cropping; Shrub and grass effects and interaction; Self and diallel designs; Blocks; Split plots.

1. Introduction

An alley cropping, an agroforestry system, comprises rows of perennial shrubs or trees bordering the alleys of grasses/crops, is a low input system for forage and food production and serves as a mechanism for sustainable agriculture. With suitable choice of crop, shrub or tree species in the system it supports diverse needs of human and other domestic animals, arrest the land degradation and soil erosion, and plays a major role in mitigating climate change. Alley cropping manages the soil nutrients more effectively between the species, *e.g.*, perennial trees/shrubs and annual crops, and different layers of soil depth. A wide range of references are available on various types of crop production systems including alley cropping (Solaimalai *et al.*, 2005; AFNTA 1992a, b). Rangeland and forage development studies aim at evaluation of interference of shrubs (*e.g.*, saltbush Atriplex) with the grasses or fodder/forage crops (*e.g.,* vetch/barley).

Experimental designs and data analysis for evaluating shrub × grass interaction are presented here. These designs can also be used to estimate main effects and interaction of crops involved in inter-cropping systems. Section 2 presents the construction of experimental

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designs and proposed linear models for estimation of effects and interactions. For one of the experimental designs discussed here, the proposed method of estimation has been illustrated with simulated data.

2. Experimental Designs and Models for Statistical Analysis

Consider a set of s shrubs denoted by $S_1, \ldots, S_i, \ldots, S_s$ for planting as the borders and a set of g grasses/crops $G_1, \ldots, G_j, \ldots, G_g$ for the alleys. The following frames of experimental units, or shrub–grass plots will be considered. Experimental units receive: (1) combinations of shrubs and grasses, or (2) shrubs with long borders and all grasses in smaller alleys within these borders. The following two treatment designs, (1) self-borders and grasses combination, and (2) diallel-borders and grasses combinations, can be implemented with any one of the above two frames of the experimental units. The resulting designs may or may not share borders between two alleys. In case they do, search for appropriate covariance structures for grass plot errors would be needed. Examples of such designs are given in the following schemas along with models for data analysis.

3. Designs for Non-Shared Borders

Consider the case where the borders are not shared between the alleys, *i.e.*, same shrub does not affect the grasses on its opposite sides of alleys.

a. Self- borders

In the self-border situation is defined here as the one where the same shrubs serve as the border of a grass plot on its both sides.

Design 1. Self-borders of shrubs and grasses combinations in Randomized Complete Block (RCB) design

Method of construction: Get all the possible combinations of shrubs ($S_1, \ldots, S_i, \ldots, S_s$) and grasses ($G_1, \ldots, G_j, \ldots, G_g$). For a combination ($S_iG_j$), the grass G_j will have shrub S_i on both (left and right) borders. These sg combinations are randomized independently within each of the r complete blocks. Figure 1 shows an example of randomized plan for s = 4 shrubs ($S_1, \ldots, S_4$), g = 3 grasses (G_1, G_2, G_3), for one replicate.

Replicate	1											
Left border	S_1	S_1	S_2	S_2	S_1	S_3	S_4	S_4	S_3	S_2	S_4	S_3
Alley	G_2	G_3	G_2	G_3	G_1	G_3	G_2	G_1	G_2	G_1	G_3	G_1
Right border	S_1	S_1	S_2	S_2	S_1	S_3	S_4	S_4	S_3	S_2	S_4	S_3
Plots	101	102	103	104	105	106	107	108	109	110	111	112

Figure 1: Schema of a randomized plan for 4 shrubs (S_1, S_2, S_3, S_4), 3 grasses (G_1, G_2, G_3), self-borders, factorial in RCB design, one replicate shown.

Statistical model for response of grasses (under Design 1)

Let $y_{i,jj,l}$ = response from the alley under grass G_i or i, self-borders (left, right): (S_j, S_j) or jj, block/replicate l, μ = general mean; β_l = Effect of block l; γ_i = effect of grass i; ψ_j =

effect of borders, jj, under shrub j from both sides; δ_{ij} = interaction between grass i and shrub borders jj; $i = 1,\ldots,g$; $j = 1,\ldots,s$; and $l = 1,\ldots,r$.

The following response model can be assumed:

Response = general mean + block effect + grass effect + shrub-effect + shrub × grass interaction + Error, or,

$$y_{i,jj,l} = \mu + \beta_l + \gamma_i + \psi_j + \delta_{ij} + \varepsilon_{i,jj,l}$$

where independently distributed errors $\varepsilon_{i,jj,l} \sim N(0,\sigma^2)$.

For generating this class of experimental design and carrying out data analysis, modify the "Randomize" directive in the Genstat software (VSN Inc., 2015) codes given in the Appendix.

Design 2. Self-borders of shrubs in main plots in RCB design and grasses in sub-plots.

Method of construction: Get the each of shrubs (S_1, …, S_i, …, S_s) as both the borders long enough to accommodate the plots of all the grasses (G_1, …, G_j, …, G_g). Randomize the shrubs within each block. Randomize the grasses within each shrub border. In this way shrubs form main-plots and grasses subplot within each of the r complete blocks. Figure 2 shows an example of randomized plan for s = 4 shrubs (S_1, S_2, S_3, S_4) and g = 3 grasses (G_1, G_2, G_3), for one replicate.

Replicate	1											
Left border	S_2	S_2	S_2	S_3	S_3	S_3	S_1	S_1	S_1	S_4	S_4	S_4
Alley	G_1	G_3	G_2	G_2	G_3	G_1	G_1	G_2	G_3	G_1	G_2	G_3
Right border	S_2	S_2	S_2	S_3	S_3	S_3	S_1	S_1	S_1	S_4	S_4	S_4
Plots	101	102	103	104	105	106	107	108	109	110	111	112

Figure 2: Schema for a randomized plan for 4 shrubs (S_1, S_2, S_3, S_4), 3 grasses (G_1, G_2, G_3), self-borders, split-plot (Shrub-borders main plot) in RCB design, one replicate.

Statistical model for response of grasses (under Design 2)

Response = general mean + block effect + shrub-effect + Error (a) [Block × Shrub interaction] + grass effect + Shrub × grass interaction + Error(b), or,

$$y_{i,jj,l} = \mu + \beta_l + \psi_j + (\beta\psi)_{jl}[= \mathrm{E}rror(a)] \; + \gamma_i + \delta_{ij} + \varepsilon_{i,jj,l}[= Error(b)]$$

For generating this class of experimental design and carrying out data analysis, modify the "Randomize" directive in the Genstat software codes given in the Appendix.

b. Diallel- borders:

Different shrubs on the borders will be used in the following two designs.

Design 3. Combinations of shrub diallel-borders and grasses in RCB design

Method of construction. We create borders of shrubs (S_1, …, S_i, …, S_s), by selecting them from a diallel crosses plan in s lines, say, $S_iS_{i'}$. It may be noted that there are no genetic crosses between two shrubs being made, but the combinations of lines that would have been in a cross are used for the borders. Such combinations of shrubs will be called diallel-borders. The all possible cominations of the diallel- border and grasses are randomized within each of the r blocks. Figure 3 shows an example of randomized plan for s = 4 shrubs (S_1, S_2, S_3, S_4), with dillel-borders (S_1S_3, S_3S_4, S_4S_2, S_2S_1), and g = 3 grasses (G_1, G_2, G_3), for one replicate.

Replicate	1											
Left border	S_1	S_4	S_2	S_2	S_1	S_3	S_4	S_1	S_3	S_2	S_3	S_4
Alley	G_2	G_1	G_1	G_3	G_3	G_3	G_2	G_1	G_1	G_2	G_2	G_3
Right border	S_3	S_2	S_1	S_1	S_3	S_4	S_2	S_3	S_4	S_1	S_4	S_2
Plots	101	102	103	104	105	106	107	108	109	110	111	112

Figure 3: Schema for a randomized plan for diallel-borders in 4 shrubs, and 3 grasses as factorial combinations in RCB design, one replicate.

In case of diallel-borders, the number of borders (shrub pairs) p say, may not necessarily be equal to s , the number of shrubs. For generating this class of experimental designs based on diallel boders, we may use the partial crosses designs presented in Curnow and Kempthorne (1961), Curnow (1963), Arya (1983), Singh and Hinkelmann (1990) among other papers, and also reviewed in Singh *et al.* (2012). These designs are constructed for estimation of general combining ability (gca) effects while specific combining ability (sca) effects are assumed absent or can be ignored. In case of the complete diallel crosses, sca effects are also estimable.

Statistical model for response of grasses (under Design 3)

Let $y_{i,jk,l}$= response from the alley under grass i , diallel-borders (left, right): (S_j, S_k) or jk (shrub j left border and shrub k on the right) and block/replicate l

A statistical model for the response is

$$y_{i,jk,l} = \mu + \beta_l + \gamma_i + \psi_j + \psi_k + \psi_{jk} + \delta_{ij} + \delta_{ik} + \delta_{ijk} + \varepsilon_{i,jk,l}$$

In the above model, the parameters ψ_j in the alley cropping design is the general effect of shrub S_j (irrespective of border direction) on the grasses (gesg) and is equivalent to the gca in the case of partial dial crosses. The ψ_{jk}, is the specific effect of the shrub borders (S_j, S_k) on the grasses (sesg) and would be equivalent to the sca in the diallel crosses situation. The quantity δ_{ij} is the interaction between shrub effect ψ_j and grass effect γ_i and may be termed as grass-specific general effect of shrub S_j (irrespective of border direction) on the grass (gs-gseg), and δ_{ijk} is grass-specific specific effect of the shrub borders (S_j, S_k) on the grass (gs-sesg). Errors $\varepsilon_{i,jk,l} \sim N(0, \sigma^2)$.

There may be situations where the following assumption may apply.

Assumption: sesg ψ_{jk} and gs-sesg δ_{ijk} may be absent or negligible

In this case the model reduces to

$$y_{i,jk,l} = \mu + \beta_l + \gamma_i + \psi_j + \psi_k + \delta_{ij} + \delta_{ik} + \varepsilon_{i,jk,l}$$

Further, ψ_j's under the designs 1 and 2 (self-borders) would be different from those under the diallel borders. However, in case ψ_{jk} (specific border combination effects) are absent, then ψ_j's under Designs 1 and 2 would be twice of those under Design 3 and Design 4.

Design 4. Diallel-borders in main plots in RCB design and grasses in sub-plots

Method of construction: Get the each of diallel combinations of shrubs ($S_iS_{i'}$) as the borders long enough to accommodate the plots of all the grasses (G_1, ..., G_s). Randomize theses diallel borders shrubs within each block. Randomize the grasses within each diallel-borders of the shrubs. In this way pair of shrubs (diallel-borders) form main-plots and grasses subplot within each of the r complete blocks. Figure 4 shows an example of randomized plan for s = 4 shrubs (S_1, S_2, S_3, S_4) with dillel-borders (S_1S_3, S_3S_2, S_2S_4, S_4S_1) in main-plots, and g = 3 grasses (G_1, G_2, G_3), for one replicate.

Replicate	1											
Left border	S_2	S_2	S_2	S_4	S_4	S_4	S_1	S_1	S_1	S_3	S_3	S_3
Alley	G_2	G_3	G_1	G_3	G_1	G_2	G_3	G_2	G_1	G_2	G_3	G_1
Right border	S_4	S_4	S_4	S_1	S_1	S_1	S_3	S_3	S_3	S_2	S_2	S_2
Plots	101	102	103	104	105	106	107	108	109	110	111	112

Figure 4: Schema for a randomized plan for 4 shrubs, 3 grasses, diallel-borders, split-plot (Shrub-borders main plot) in RCBD.

Statistical model for response of grasses (under Design 4)

$$y_{i,jk,l} = \mu + \beta_l + \psi_j + \psi_k + \psi_{jk} + (\beta\psi)_{jk,l}[= \mathrm{E}rror(a)] \ + \gamma_i + \delta_{ij} + \delta_{ik} + \delta_{ijk} + \varepsilon_{i,jk,l}[= Error(b)]$$

Assumption: sesg ψ_{jk} and gs-sesg δ_{ijk} may be absent or negligible

$$y_{i,jk,l} = \mu + \beta_l + \psi_j + \psi_k + (\beta\psi)_{jk,l}[= \mathrm{E}rror(a)] \ + \gamma_i + \delta_{ij} + \delta_{ik} + \varepsilon_{i,jk,l}[= Error(b)]$$

Design for diallel boders as discussed in Design 3 can be used for conducting the trial in split-plots with diallel-borders in mainplots and grasses in sub-plots. The codes for generating the Design 4 are given in the Appendix.

Estimation of the effects and interactions

A practical approach would be to estimate the response of the combinations of shrub-borders and gasses with adjustment for block differences, covariates for slope and fertility trend in the alleys, spatial error structures. Let the adjusted mean for the treatment combination: grass

i and diallel-border (left, right) (j,k) be denoted by $\bar{y}_{i,jk}$. In vector notation, we can use $\bar{y} = (\bar{y}_{1,12}, \bar{y}_{1,13}, \bar{y}_{1,1s}, ..., \bar{y}_{g,s-1s})'$. One may use all pairs of shrubs (S_j, S_k), equivalent to (S_k, S_j) as borders, but limited resouces may lead to the choice of partial diallel-borders. Based on a simple cyclic structure in shrubs may give a set of diallel-borders as: $(S_1,S_2),(S_2,S_3),...,(S_s,S_1)$, which could be chosen for all the replicates, or even a better spread could be carried over the replication by using a different spacing between the shrub numbers, *e.g.*, $(S_1,S_3),(S_3,S_5),...,(S_\_,S_\_)$ in replicate 2, where the subscripts "_" stand for appropriate shrub numbers, *etc*. Let the estimated variance covariance of vector $\bar{y}$ be denoted by $\hat{\Sigma}$. For the full factorial of border and alley treatment factors in an RCB design with r replicates and estimated residual mean square $\hat{\sigma}^2$, $\hat{\Sigma} = (\hat{\sigma}^2 / r)I$. Let the grass effects, shrub effects and their interaction be represented in vector form respectively as:

$$\gamma = (\gamma_1,...,\gamma_g)',\ \psi = (\psi_1,...,\psi_s)' \text{ and } \delta = (\delta_{11}, \delta_{12}..., \delta_{1s},..., \delta_{g1}, \delta_{g2},..., \delta_{gs})'.$$

Let the interaction between grass and border combinations (not the shrubs) be denoted by $\phi = (\phi_{11}, \phi_{12}..., \phi_{1p},..., \phi_{g1}, \phi_{g2},..., \phi_{gp})'$.

Thus $\phi_{im} = \delta_{ij} + \delta_{ik}$ where m stands for the border comprising of the shrubs S_j and S_k; $m = 1,..., p$.

A model for estimation of γ, ψ and ϕ may be wriiten as

$$\bar{y} = \mu J + X_1\gamma + X_2\psi + X_3\phi + \bar{\varepsilon}$$

where μ is general mean, J a vector of 1s and length of $\bar{y}$, and vector of mean errors with $\bar{\varepsilon} \sim \text{MVN}(0, \hat{\Sigma})$.

Conditions on the vectors of effects are: $\gamma' J = 0$, $\psi' J = 0$ and more than one conditions on the interaction vector: $(I_p \otimes J_g')\phi = 0_{p,1}$ and $(J_p' \otimes I_g)\phi = 0_{1,g}$.

The estimation can have one of the several approaches, particularly in case of orthogonal structure between grasses and diallel-borders.

Approach 1: One can estimate grasses and borders effects and interaction using ANOVA directives. The border effects overall the grasses or for individual grasses data can be modelled by fitting columns of X_2 (no intercept) to estimate ψ s and δ s respectively.

Approach 2: Another could be based on matrices but still using the ANOVA estimates of border effects with variance-covariance matrix or ignoring the covariances. This may be completed in the following two stages:

Stage 1: Estimate γ gamma and ψ, we can fit the general model, ignoring ϕs and fitting a reduced model for $\bar{y} \sim MVN(X\beta, \hat{\Sigma})$, where $X = [J : X_1 : X_2]$ of order $(p, 1+g+s)$ and $\beta = (\mu, \gamma', \psi')'$.

Using Rao (1973), $\hat{\beta} = (\hat{\mu}, \hat{\gamma}', \hat{\psi}')' = S^{-1}Q$ where,

$S = X'\hat{\Sigma}^{-1}X$ and $Q = X'\hat{\Sigma}^{-1}\bar{y}$, assuming that the design keeps matrix S non-singular, otherwise replace S^{-1} by its Moore-Penrose psuedoinverse denoted by S^{+}.

Estimated variance-covariance matrix of $\hat{\beta}$ is $D(\hat{\beta}) = S^{-1}$.

Borders × grass interaction vector ϕ can be estimated as the residual vector

$$\hat{\phi} = \bar{y} - X\hat{\beta} \text{ with } \mathrm{D}(\hat{\phi}) = \Sigma - XS^{-1}X' = \Sigma^{*}, \text{ say.}$$

Actually, the variance-covariance matrix $D(\hat{\phi})$ may be available along with $\hat{\phi}$ while using ANOVA in any software, *e.g.*, Genstat (VSN Inc. 2015).

Stage 2: Next step would be to partition $\hat{\phi}$ into δ's estimates as follows. Obtain a matrix Z with its column number $i_j = j + (i-1)s$ obtained by element-wise multiplication of ith column of X_1 and jth column of X_2, *i.e.* Schur multiplication of all possible cross combinations between columns of X_1 and X_2. The order of Z is $p \times gs$. We can obtain gs parameters in δs by solving the equation:

$$\hat{\phi} = Z\delta, \text{ where } D(\hat{\phi}) = \Sigma^{*}$$

to obtain

$$\hat{\delta} = (Z'\Sigma^{*+}Z)^{+}Z'\Sigma^{*+}\hat{\phi} \text{ and } D(\hat{\delta}) = (Z'\Sigma^{*+}Z)^{+}$$

where for a matrix A, A^{+} denotes its Moore-Penrose psuedoinverse.

Optimal design: Optimality and efficiency of the design can be studied in terms of the respective covariance matrices for $\hat{\gamma}$ gamma, $\hat{\psi}$s and $\hat{\delta}$'s. There could be alternative options to estimate the effects using a software. Genstat codes are given on the set of data generated for illustration in the following section.

4. Shared Borders

Design 5. Sharing of borders between the alleys would lead to a resource saving design. However, data analysis may be based on a relatively more complex model due to the feature that the same shrub may affect grasses on its opposite sides of alleys. Self-borders or diallel-boders can be used. Due to sharing of the same border between the alleys the randomization of the shrubs as borders would become quite restricted.

Method of construction: The construction can be easily explained using an example, which requires that one has a partial diallel design. Let the partial diallel considered for the borders be written as S_1S_3, S_3S_2, S_2S_4, S_4S_3, S_3S_1…, while the grasses (G_1, G_2, G_3) are in alleys, that is, the sub-plots. We will have much restricted randomization as these partial diallels in shrubs now follow a sequence where the the shrub on right of a diallel is same the left of the diallel that follows in the sequence. Hence the randomization can be done within the set of all the shrubs. In the present case, the shrubs pair (S_1, S_3) from the partial diallel, one border, say left-border is S_1 while the right-border is S_3. This right-border S_3 is same as the left border in S_3S_2, therefore S_3 is shared border. Similarly, S_2 in (S_3S_2) is shared border with (S_2S_4), and so on (Figure 5).

Left border	S_1	S_1	S_1
Alley	G_1	G_3	G_2
Shared border	S_3	S_3	S_3
Alley	G_2	G_3	G_1
Shared border	S_2	S_2	S_2
Alley	G_1	G_2	G_3
Shared border	S_4	S_4	S_4
Alley	G_3	G_1	G_2
Shared border	S_3	S_3	S_3
Alley	G_2	G_3	G_1
Shared border	S_1	S_1	S_1
Alley	G_3	G_2	G_1
Shared border		.	
Alley		.	

Figure 5. Schema for a randomized plan for 4 shrubs (S_1, S_2, S_3, S_4) using the partial diallel (S_1S_3, S_3S_2, S_2S_4, S_4S_3, S_3S_1... more) and 3 grasses with shared diallel-borders in a split-plot (Shrub-borders main plot) in RCB design.

Statistical model for response of grasses (under Design 5)

In this case correlated responses may be assumed, and covariance modelling would a worthy exercise to induct in the analysis. Model:

$$y_{i,jk,l} = \mu + \beta_l + \psi_j + \psi_k + (\beta\psi)_{jk,l}[= \mathrm{E}rror(a)] \;\; + \gamma_i + \delta_{ij} + \delta_{ik} + \varepsilon_{i,jk,l}[= Error(b)]$$

Correlated model structures:

$\mathrm{Cov}((\beta\psi)_{jk,l}, (\beta\psi)_{km,l})$ and $Cov(\varepsilon_{i,jk,l}, \varepsilon_{i,km,l})$ may need to be simplified using a criterion such as Akaike Information Criterion (AIC) (Akaike, 1974). The selected covariance structure(s) can then be used for estimation of the effects and interaction.

5. An Illustration

A dataset was generated for experimental design situation, Design 4 using the following set of values of effects taken for random generation of data (Table 1).

Table 1: **Values of parameters to model the response from Design 4**

General mean: $\mu = 5$

Coefficient of variation based on main-plot error = 10%

Coefficient of variation based on subplot error = 15%

Block effects: β_l (l=1…3) = –1.0, –0.5, 0.0

Grasses effects: γ_i (i=1…3) = –2, –1, 3

Shrubs effects: ψ_j (j) = –1., –.5, 1., 0.5, 0.0

Interactions δ_{ij}	Shrubs				
Grasses	S_1	S_2	S_3	S_4	S_5
G_1	0.2	–0.4	–0.2	0.0	0.4
G_2	–0.3	0.2	0.4	0.1	–0.4
G_3	0.1	0.2	–0.2	-0.1	0.0

Genstat codes for generating a randomized plan and data analysis (Design 4) are given in Appendix. The statistical analysis was repeated 100 randomly generated data sets. Table 2 gives average, over the simulation runs, of each of the effects and interactions parameters set in Table 1. It may be observed that, the displayed averages of gesg (ψ_j s general effects of shrubs on the grasses) and δ_{ij} is the interaction (or gs-gseg, the grass-specific general effect of shrub S_j on the grass G_i) are very close to the values of the respective parameters. GenStat codes for construction and analysis of data using the other experimental designs are given in Singh (2017).

Table 2: Mean of 100 simulations of estimates of shrub effects and interaction with grasses

	A. Shrub Effects		
	Shrub S_j	True value (ψ_j)	Average of 100 simulations
	S_1	–1.0	–0.997
	S_2	–0.5	–0.518
	S_3	1.0	1.068
	S_4	0.5	0.478
	S_5	0.0	–0.031
SE			±0.325

B. Shrub × Grass Interaction

Grass i	Shrub S_j	True value (δ_{ij})	Average of 100 simulations
1	S_1	0.2	0.230
	S_2	–0.4	–0.447
	S_3	–0.2	–0.200
	S_4	0	–0.018
	S_5	0.4	0.435
2	S_1	–0.3	–0.344
	S_2	0.2	0.248
	S_3	0.4	0.372
	S_4	0.1	0.122
	S_5	–0.4	–0.399
3	S_1	0.1	0.114
	S_2	0.2	0.199
	S_3	–0.2	–0.172
	S_4	–0.1	–0.104
	S_5	0.0	–0.037
SE			±0.455

SE = Estimated standard error

6. Conclusions

Alley cropping with shrubs as borders or hedges and crops/grasses in the alleys are often agroforestry practices for sustainable crop production. The experimental designs and statistical models for data analyses are discussed for commonly occurring situations. These designs are recommended for conducting alley cropping trials. Once the real data become available, the steps presented here may be used for analysis. These designs and the approach of analysis can also be adapted for examining interactions or interference in intercropping experiments, which would need further extension to analyze two or more correlated responses on the component crops.

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Appendix

a. GenStat Codes for generation of Design 4

Let NGrass, NShrub and NRep be the number of grasses, shrubs and replications respectively. The GenStat codes:

```
"Generate Design 4.............. Diallel-borders in main-plots of a split plot design"
Scal NRep, NGrass, NBorder, NPlots; 3, 3, 5,*
Calc NPlots=NRep*NGrass*NBorder

Unit[NPlots]
Factor[Levels=NRep] Rep  : &[Levels=NGrass] Grass : &[Levels=NBorder; labels=!t('S5S4',
'S4S1', 'S1S2','S2S3', 'S3S5')]Border
Generate Rep,  Border, Grass

Randomize[Block=Rep/Border/Grass; Seed=130572] Border, Grass
Prin Rep,  Border, Grass
```

b. GenStat Codes for analysis of data from Design 4

Let Yield be the vector of plot yields. The codes for statistical analysis of variance and estimation of means, effects and interaction are:

```
"............................. Analysis part............"
"Genstat codes for estimating the effects and interactions using ANOVA and linear model fitting directives
............................. Analysis part............"
"Stage 1: Estimate borders [shrub combinations] and grasses effect and their interactions"
```

```
Dele[rede=y] Borders0, LeftBorder0, RightBorder0,Grass0,Sh[1...NShr], yMeans, Weight, tbEff,
yEff,tbMn, tbSeMn
            Block Rep/Border/Grass
            Treat Border*Grass
            Anova[print=a,%cv, eff, mean;pse=m;fpro=y]Yield
"Get the Grasses effects from above or below: Gammas"
            Akeep  Grass; means=tbMn ; SEmeans=tbSe; Effect=tbEff
Prin tbMn, tbEff, tbSe

 "Stage 2:...............To get direct effect of Shrubs (saai s) on grasses"

            Akeep  Border; means=tbMn ; SEmeans=tbSe; Effect=tbEff
" Get error mean squre, weight of means "
Vtable Table=tbMn, tbSe, tbEff; Vari=yMeans, ySeMn, yEff; Class=!P(Borders0)
Vari [nval=NShr]Sh[1...NShr], Weight
Calc Weight=1/ySeMn**2
"Decode Borders into left and right border shrubs: S5S4, S4S1, S1S2,S2S3, S3S5"
Vari[Values=5,4,1,2,3]LeftB
Vari[Values=4,1,2,3,5]RightB
For i=1...NShr;dd=Sh[1...NShr]
  Calc dd=(LeftB.eq.i.or.RightB.eq.i)
Endf
"Print Borders0, LeftB, RightB,Sh[1...NShr], yMeans, ySeMn,yEff, Weight; field=6"

" Regression Model/Fit to estimate Shrub direct effects: saai s "
Print '   ***** Saai s and their standard errors for shrubs *****'
Model[Weight=Weight; disp=1] yEff
Terms [Full=Y] Sh[1...NShr]
Fit[Prin=m,s,e; cons=o; fpro=y; tpro=y] Sh[1...NShr]

"Estimate Shrub X Grass interaction delta s"
Dele[rede=y] GrassBorders0, GrassLeftBorder0, GrassRightBorder0,Grass0,Sh[1...NShr], yMeans,
Weight, tbMn, tbSe,tbEff, yEff
            Akeep  Grass.Border; means=tbMn ; SEmeans=tbSe; Effects=tbEff
" Get error mean squre, weight of means "
Vtable Table=tbMn, tbSe, tbEff; Vari=yMeans, ySeMn, yEff; Class=!P(Grass0,Borders0)

Scal NGrassXNShrub : Calc NGrassXNShrub=NShr*NGrass
Scal NGrassXNBorder : Calc NGrassXNBorder=NBorder*NGrass
Vari [nval=NGrassXNBorder]Sh[1...NShr], Grs[1...NGrass], Weight
Calc Weight=1/ySeMn**2
" Decode Borders into left and right border shrubs: S5S4, S4S1, S1S2,S2S3, S3S5 for each grass
Borders0  Boders and grasses:
  G1        G1        G1 G1     G1        / G2        G2        G2        G2  G2    / G3
            G3  G3    G3        G3
 S5S4, S4S1, S1S2,S2S3, S3S5   S5S4, S4S1, S1S2,S2S3, S3S5   S5S4, S4S1, S1S2,S2S3, S3S5
"
Vari[Values=(5,4,1,2,3)3]LeftBG
Vari[Values=(4,1,2,3,5)3]RightBG
For i=1...NShr;dd=Sh[1...NShr]
  Calc dd=(LeftBG.eq.i.or.RightBG.eq.i)
Endf
For i=1...NGrass; dd=Grs[1...NGrass]
  Calc dd=(Grass0==i)
```

```
Endf
Print Grass0, Borders0, LeftBG, RightBG,Sh[1...NShr], Grs[1...NGrass],yMeans, ySeMn,yEff,
Weight ; field=6
" Shrubs x Grass interaction: deltas and SE for each grass"
For i=1...NGrass
Print '   ***** Deltas and their standard errors for Grass = ', i, '  *****'
Rest Sh[1...NShr], yEff ; Grass0==i
Model[Weight=Weight; disp=1] yEff
Terms [Full=Y] Sh[1...NShr]
Fit[Prin=m,s,e; cons=o; fpro=y; tpro=y] Sh[1...NShr]
Rest Sh[1...NShr], yEff
Endf
 STOP
```