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ORIGINAL PAPER

Mixed spatial models for data analysis of yield on large grapevine selection field trials

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Abstract In large field trials, it may be desirable to adjust for spatial correlation due to variation in soil fertility and in other environmental factors. Spatial correlation within a field trial can mask differences in the genotypic values of clones, consequently reducing the possibility of identifying superior genotypes. This paper describes a strategy to improve the precision of statistical data analysis of grapevine selection trials through the use of mixed spatial models. The efficiency of mixed spatial models was compared with that of a classical randomized complete block model (with independent and identically distributed errors). The comparisons were based on yield data from three large experimental populations of clones of the Arinto, Aragonez (Tempranillo) and Viosinho grapevine varieties. The fit of the spatial mixed models applied to yield data was significantly better than that of the classical approach, resulting in a positive impact on selection decisions and increasing the accuracy of genetic gain prediction.

Introduction

Most grapevine varieties used today originated many hundreds or thousands of years ago, probably as genetically homogenous clones. With the accumulation of mutations, these ancient varieties became heterogeneous. They now consist of mixtures of genetically distinct clones (genotypes) with different agronomic and quality traits (Rives 1961).

Quantification of variability within ancient varieties is very useful, not only because it provides the raw material for selection, but also because it allows for understanding of the antiquity of varieties as well as providing good strategies for the conservation of genetic resources. Consequently, in the last 25 years, innovative methods for grapevine selection have been developed in Portugal (Martins et al. 1987, 1990) involving quantification of genetic variability and mass selection of genotypes from within varieties.

An efficient study of the genetic variability within ancient varieties requires sampling of mother plants representative of that variability, and grafting them in large field trials, providing experimental populations of clones, preferably with homogeneous environmental conditions. In Portugal, however, vineyards are markedly small and geometrically irregular, with high soil heterogeneity. To overcome these difficulties and effectively quantify genetic variability and select superior clones, we should use appropriate experimental designs, such as alpha designs and resolvable row–column designs (at the beginning of the experimental process), together with appropriate statistical models for data analysis, such as mixed spatial models (at the end).

Plant breeding researchers have faced these practical field experimentation problems for many years, and great efforts have been made to develop theoretical tools to overcome them, including experimental designs and models for data

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analysis. Since the introduction of randomized complete block (RCB) designs by Fisher (1935), many alternative experimental designs have been developed, especially in the area of plant breeding initial trials. Among these are the categories of incomplete block designs (Yates 1936; Patterson and Williams 1976; Patterson et al. 1978), row–column designs (Nguyen and Williams 1993) and augmented designs (Federer 1961, 2002; Federer and Raghavarao 1975). In the field of statistical models, new solutions have emerged in the literature based upon the principles of neighbour analysis or spatial analysis. These are models that take advantage of the assumption that neighbouring individuals will share a similar environment, i.e., models that take into account the spatial correlation between neighbour observations (Papadakis 1937, 1984; Bartlett 1978; Wilkinson et al. 1983; Besag and Kempton 1986; Cullis and Gleeson 1991; Zimmerman and Harville 1991; Grondona and Cressie 1991). Spatial analysis is now widely used in breeding programs of annual crops (Stroup et al. 1994; Grondona et al. 1996; Cullis et al. 1998; Smith et al. 2001, 2005) and forest species (Kusnandar and Galwey 2000; Costa e Silva et al. 2001; Dutkowski et al. 2002; Joyce et al. 2002), but there are no published reports of its use in grapevine selection.

The present work aims to demonstrate the utility of mixed spatial models through the following steps: (1) characterization of spatial dependence of yield in neighbouring plots in large field populations of grapevine clones, (2) use of mixed spatial models for yield data analysis of experimental populations of grapevine clones and, finally, (3) selection of clones with and without inclusion of spatial autocorrelation in the model.

Materials and methods

Field trials and data collection

Mixed spatial models were applied to yield data from three experimental populations of clones from the grapevine varieties Arinto, Aragonez (Tempranillo) and Viosinho. Data analysis was based on the average yield observed over several years. These trials were laid out in balanced RCB designs. Since control of heterogeneity within complete blocks is best accomplished with a row–column arrangement within each complete block, the plots were located on a grid (not always regular) of columns by rows. Information about the field trials and the yield data collected over several years is shown in Table 1. Given that we detected clones which were not of the Aragonez and Viosinho varieties in their respective experimental populations, only 255 Aragonez clones and 199 Viosinho clones were utilized for data analysis of these two varieties.

The statistical model

The cases studied can be described according to the general linear mixed model

$$y = X\beta + Zu + e$$

where $y_{n \times 1}$ denotes the vector of observations (phenotypic values) for yield, $\beta_{p \times 1}$ is a vector of fixed effects, $u_{q \times 1}$ is a vector of random effects, $X_{n \times p}$ and $Z_{n \times q}$ are design matrices for the fixed and random effects, respectively, and $e_{n \times 1}$ is a vector of errors. It is assumed that the joint distribution of (u,e) is multivariate normal, with 0 expectation and

$$\mathrm{Var}\begin{bmatrix} u \\ e \end{bmatrix} = \begin{bmatrix} G & 0 \\ 0 & R \end{bmatrix}$$

where 0 are null matrices and $G_{q \times q}$ and $R_{n \times n}$ are symmetric positive definite matrices and correspond to variance–covariance matrices of u and e, respectively. The distribution of the vector y is multivariate normal, with expectation $X\beta$ and variance matrix $V = ZGZ^{\mathrm{T}} + R$ where Z^{T} denotes the transpose of Z.

The vector $u_{q \times 1}$ of random effects consists of subvectors $u_{q_i \times 1}$ $(i = 1, 2)$, each one associated to one random factor. These subvectors are assumed mutually independent with variance–covariance matrix $G_i = \sigma_i^2 I_{q_i}$. Therefore, the matrix G is the direct sum of the variance–covariance

Table 1 Description of field trials

Trial	Location	No. of clones	No. of plants per plot	No. of complete blocks	No. of rows	No. of columns	Distance between centers of adjacent plots in the		Yield data are the average of the years
							Row (m)	Column (m)	
Arinto[a]	Sesimbra	247	4	4	61	19	2.75	3.2	1995, 1998, 1999, 2000
Aragonez	Reguengos de M.	260	3	5	26	50	2.25	3.75	1999, 2001, 2002
Viosinho	Palmela	204	3	5	17	60	2.80	3.60	1992, 1993, 1994, 1996, 1997, 1999, 2000

[a] The Arinto trial was laid out in an irregular shape (see Fig. 1a) within a rectangle of 61 rows by 19 columns

matrices of each of the random terms $(G = G_1 \oplus G_2)$. Thus, in all models the vector u includes the genotypic effects (with variance component denoted by σ_{g}^2) and the block effects (with variance component denoted by σ_{b}^2). In the traditional data analysis of grapevine initial trials, the block effects are usually considered as fixed. However, because our purpose is to compare non-spatial with spatial models, blocks are contiguous and the spatial correlation extends across the whole field, we considered block effects as random. Hence, in these studied cases β is a scalar (the overall mean).

In the classical mixed model R is a diagonal matrix defined as $R = \sigma_{\mathrm{e}}^2 I_n$, where σ_{e}^2 is the error variance and I_n denotes the $n \times n$ identity matrix. In the spatial analysis approach, matrix R assumes a different structure based on a decomposition of e into $e = \varepsilon + \eta$, where the vectors ε and η refer to spatially dependent (ε) and spatially independent (η) errors. Furthermore, ε is assumed as second-order stationary so that the correlation between the two plots depends only on the distance between them, and its structure can be modeled in various ways. Thus, matrix R is defined as $R = \sigma^2 \sum + \sigma_\eta^2 I_n$, where σ^2 is the spatial error variance (also called partial sill, $\sigma^2 > 0$), Σ is the $n \times n$ error correlation matrix and σ_η^2 is the independent error variance (also called nugget effect, $\sigma_\eta^2 \geq 0$).

In this study, three isotropic spatial correlation functions were considered: spherical, Gaussian and power (a reparameterization of the exponential correlation model). We also considered the anisotropic power correlation model, which depends on two parameters, one representing the correlation between plots in the direction of rows and the other representing the correlation in the direction of columns. Many authors express the distance between plots as the difference in the number of rows (or columns) (Gilmour et al. 1997; Smith et al. 2001). However, we thought it would be better to express the distance between plots as the Euclidean distance, in meters. This is a more acceptable option in isotropic processes. The anisotropic power model, which uses the Euclidean distance between plots (in meters), and the AR(1) × AR(1) model (Gilmour et al. 1997), which express the distance between plots as the difference in the number of rows (or columns), give similar results. Table 2 describes the five models fitted to the yield data of the experimental populations of clones under study.

Tools for error model identification

The diagnostic of spatial correlation in the errors was based on the model 1 fit residuals. The plots of residuals against row and column positions and the plots of the sample semivariogram [a tool that is widely used in geostatistical analysis to visualize spatial dependence (Matheron 1963)] were used. Besides being a good tool for spatial correlation diagnosis, the sample semivariogram provides the starting parameter values for the mixed model procedure.

Parameter estimation

Model parameters were estimated by the residual or restricted maximum likelihood method (REML, Patterson and Thompson 1971), using the Fisher-Scoring algorithm (Jennrich and Sampson 1976). With residual maximum likelihood estimates $\hat{G}$ and $\hat{R}, \beta$ and u were estimated by solving the mixed model equations (Henderson 1975; Searle et al. 1992). The final solutions can be written as: $\hat{\beta} = (X^{\mathrm{T}}\hat{V}^{-1}X)^{-}X^{\mathrm{T}}\hat{V}^{-1}y$, where $\hat{V} = Z\hat{G}Z^{\mathrm{T}} + \hat{R}$ and $(X^{\mathrm{T}}\hat{V}^{-1}X)^{-}$ is the generalized inverse of $(X^{\mathrm{T}}\hat{V}^{-1}X)$, and $\tilde{u} = GZ^{\mathrm{T}}\hat{V}^{-1}(y - X\hat{\beta})$. In those solutions, $\hat{\beta}$ is the empirical best linear unbiased estimator of β, and $\tilde{u}$ is the empirical best linear unbiased predictor of u (Littell et al. 1996).

Table 2 Description of models 1–5

Models	Non-diagonal elements of $\sum^{\mathrm{a}}$
Model 1—classical RCB (classical randomized complete block analysis)	0
Model 2—power (RCB with isotropic power function for spatial correlated errors)	$\rho^{\lVert h_{ab} \rVert}$
Model 3—anisotropic power (RCB with anisotropic power function for spatial correlated errors)	$\rho_{\mathrm{row}}^{\lVert h_{ab} \rVert_{\mathrm{row}}} \rho_{\mathrm{col}}^{\lVert h_{ab} \rVert_{\mathrm{col}}}$
Model 4—Gaussian (RCB with Gaussian function for spatial correlated errors)	$\exp^{\left(-\frac{\lVert h_{ab} \rVert}{\theta}\right)^2}$
Model 5—spherical (RCB with spherical function for spatial correlated errors)	$1 - 1.5\frac{\lVert h_{ab} \rVert}{\theta} + 0.5\left(\frac{\lVert h_{ab} \rVert^3}{\theta}\right)$

[a] Matrix Σ is the error correlation matrix. ρ is the coefficient of autocorrelation, and must satisfy $|\rho| < 1$ for stationarity. ρ_{row} is the coefficient of autocorrelation between adjacent plots in a row, and must satisfy $|\rho_{\mathrm{row}}| < 1$ for stationarity. ρ_{col} is the coefficient of autocorrelation between adjacent plots in a column, and must satisfy $|\rho_{\mathrm{col}}| < 1$ for stationarity. $\lVert h_{ab} \rVert = \lVert s_a - s_b \rVert$ is the Euclidean distance between the center of the plot located at s_a and the center of the plot located at s_b, $\lVert h_{ab} \rVert_{\mathrm{row}} = \lVert s_a - s_b \rVert_{\mathrm{row}}$ is the Euclidean distance between the center of the plot located at s_a and the center of the plot located at s_b in row direction, $\lVert h_{ab} \rVert_{\mathrm{col}} = \lVert s_a - s_b \rVert_{\mathrm{col}}$ is the Euclidean distance between the center of the plot located at s_a and the center of the plot located at s_b in column direction. θ is the range (distance within which observations are spatially dependent, $\theta \geq 0$)

Genetic selection

Spearman's rank correlation coefficient was used to compare predicted genotypic effects rankings between the classical RCB and spatial models. For genotypic selection, the ranking of best linear unbiased predictors of genotypic effects of the clones obtained from the solutions of the mixed model equations was used. The average of the best linear unbiased predictors of the corresponding selected group provided the predicted genetic gain of yield.

Inference for variance parameters and model selection

For each model, the residual or restricted likelihood ratio test (REMLRT) was used to test the hypothesis that the genotypic variance component was equal to 0 and the *P*-value of the test was half of the reported *P*-value from the χ^2 distribution with one degree of freedom (Self and Liang 1987; Stram and Lee 1994).

When comparing the relative goodness-of-fit among non-nested models (i.e., among spatial models with different spatial covariance structures and the same matrix X), the Akaike Information Criterion (AIC) (Sakamoto et al. 1986) and the Bayesian Information Criterion (BIC) (Schwarz 1978) were used. Smaller AIC and BIC values indicate a better fit.

Isotropic and anisotropic power models are nested and may be also compared using the REMLRT. The asymptotic distribution of the residual likelihood ratio test statistic (λ), under the null hypothesis $\rho_{\text{row}} = \rho_{\text{col}}$, is a χ^2 distribution with one degree of freedom.

With the best error spatial covariance structure chosen, the next step consisted in testing the spatial correlation. The simplest model (model 1) is a reduced form of the mixed spatial models. Therefore, the models are nested and can be compared using not only the AIC and BIC criteria already referred to, but also the residual likelihood ratio test. The distribution of the residual likelihood ratio test statistic (λ) consists of mixtures of χ^2 distributions because the tested parameters were in the boundary of parameter space. More precisely, testing *k* parameters in these conditions involves a mixture of χ^2 distributions from 0 to *k* degrees of freedom (Self and Liang 1987; Stram and Lee 1994; Verbeke and Molenberghs 2000). Consequently, for the different situations of nested models being compared we will have the following cases.

- Case 1, to test model 1 vs. model 2 (i.e., testing the null hypothesis $\sigma_\eta^2 = 0$ and $\rho = 0$). One parameter is on the boundary of parameter space (σ_η^2) and the other (ρ) is not. The asymptotic distribution of λ is a mixture of χ^2 distributions with unknown degrees of freedom (certainly less than two).
- Case 2, to test model 1 vs. model 3 (i.e., testing the null hypothesis $\sigma_\eta^2 = 0$ and $\rho_{\text{row}} = 0$ and $\rho_{\text{col}} = 0$). One parameter is on the boundary of parameter space (σ_η^2) and two (ρ_{row} and ρ_{col}) are not. The asymptotic distribution of λ is a mixture of χ^2 distributions with unknown degrees of freedom (certainly less than three).
- Case 3, to test model 1 vs. model 4, or model 1 vs. model 5 (i.e., testing the null hypothesis $\sigma_\eta^2 = 0$ and $\theta = 0$). The two parameters are on the boundary of parameter space. The asymptotic distribution of λ involves a mixture of χ^2 distributions with unknown degrees of freedom (certainly less than two).

Determining the correct asymptotic null distribution for the likelihood test statistic in this type of nested mixed models is not straightforward and requires simulation studies. Thus, we decided to use a conservative solution as a reference: the naive approach of using a Chi-squared distribution with the number of degrees of freedom equal to the increase in the number of parameters between the two models. Consequently, for the different situations described above, the naive asymptotic null distribution of λ is a χ^2 distribution with two degrees of freedom, for cases 1 and 3, and with three degrees of freedom, for case 2.

Defining $\Delta\text{AIC}_\text{m} = \text{AIC}_\text{m} - \text{AIC}_\text{min}$ as the difference between the AIC value for model m and the minimum AIC value of the two models under comparison and, similarly, $\Delta\text{BIC}_\text{m} = \text{BIC}_\text{m} - \text{BIC}_\text{min}$ as the difference between the BIC value for model m and the minimum BIC value of the two models under comparison, a common question is to know how these differences in adjustment can be considered relevant. To find a solution for this question, we followed guidelines suggested by Burnham and Anderson (2002), for $\Delta\text{AIC}_\text{m}$, and by Kass and Raftery (1995), for $\Delta\text{BIC}_\text{m}$. For $\Delta\text{AIC}_\text{m}$, a model m with $\Delta\text{AIC}_\text{m} \leq 2$ has substantial support. With $4 \leq \Delta\text{AIC}_\text{m} \leq 7$ it has considerably less support, while with $\Delta\text{AIC}_\text{m} > 10$ it has essentially no support (Burnham and Anderson 2002). For $\Delta\text{BIC}_\text{m}$, the evidence against model m is almost negligible when $\Delta\text{BIC}_\text{m} \leq 2$, positive when $2 < \Delta\text{BIC}_\text{m} \leq 6$, strong when $6 < \Delta\text{BIC}_\text{m} \leq 10$ and very strong when $\Delta\text{BIC}_\text{m} > 10$ (Kass and Raftery 1995).

Statistical software

All models were fitted in Proc Mixed (Littell et al. 1996) of SAS version 9.1 (SAS Institute 2003). For graphical tools, R version 2.2.1 ("Comprehensive R Archive Network", http://www.cran.r-project.org) and its packages Fields version 2.3 (Nychka 2006) and GeoR version 1.6-5 (Ribeiro Jr and Diggle 2006) were used.

Results

Spatial correlation diagnosis

The initial strategy used to investigate spatial correlation within the yield data of the three experimental populations of clones shows the disposition of model 1-fit residuals in a grid of row × column positions (Fig. 1). It can be seen that the residuals are not randomly distributed in the experimental field. Instead, there is clear evidence of some local trends, showing that the residuals from plots that are closer together tend to be more similar than those which are farther apart.

To provide a description of how the residuals are correlated with distance, directional empirical semivariograms of the model 1-fit residuals were obtained for distances less than 45 m (Fig. 2). In a preliminary diagnosis, there is no strong evidence of anisotropy, i.e., the correlation between plots in the direction of rows is similar to the correlation in the direction of columns. It can be seen that the semivariance between residuals increases as lag distance increases, and this is observed within a range of more or less 30 m. At distances greater than this range, the variance between observations has a tendency to stabilize and spatial correlation tends to be zero. This is particularly obvious in Fig. 2a, c, e and f, and not as clear in Fig. 2b and d (column direction in Arinto and Aragonez, respectively). Furthermore, all semivariograms reveal an evident nugget effect or independent variance (semivariance remained quite high as the lag distance approached zero).

The best mixed linear model

According to the shape of the exploratory semivariograms, several candidate spatial models were fitted to the collected yield data, as summarized in Tables 3, 4 and 5.

All spatial models fitted yield data better than the classical RCB approach for the three varieties (smaller values of AIC and BIC criteria for the spatial models). There were not large differences amongst isotropic spatial models, especially between isotropic power and spherical correlation functions. However, the power function seemed to be the most appropriate to model the error correlation structure. The models with this correlation function showed the smallest AIC and BIC values when compared to those obtained with the Gaussian and spherical models. The Gaussian model was the least appropriate.

Comparing between isotropic and anisotropic power functions, the anisotropic power model (model 3) showed a significantly better fit than model 2 for the Aragonez field trial ($P < 0.05$), but comparative criteria (AIC and BIC) penalized this more highly parameterized model for the Arinto and Viosinho. However, advantage of isotropic power model in these latter trials is misleading because the REML estimates of the spatial variance converged to high values suggesting that too much weight was given to more distant pairs (particularly for Arinto and not as clearly for Viosinho). On the contrary, the REML estimates obtained with the anisotropic power model for this parameter were plausible, indicating a better fit for short lag distances, which is the most important for our objectives.

To model spatial correlation, we therefore chose the anisotropic power model (model 3) for Arinto, Aragonez and Viosinho. The AIC and BIC values obtained with this mixed spatial model were much lower than those from the classical RCB model (model 1). The differences in AIC and BIC values were $\Delta\text{AIC} = 173.5$ and $\Delta\text{BIC} = 162.9$ for Arinto, $\Delta\text{AIC} = 207.5$ and $\Delta\text{BIC} = 196.9$ for Aragonez and $\Delta\text{AIC} = 100.8$ and $\Delta\text{BIC} = 90.9$ for Viosinho, suggesting considerable differences in adjustment between the two models. These results were supported by testing the significance of this mixed spatial model through the residual

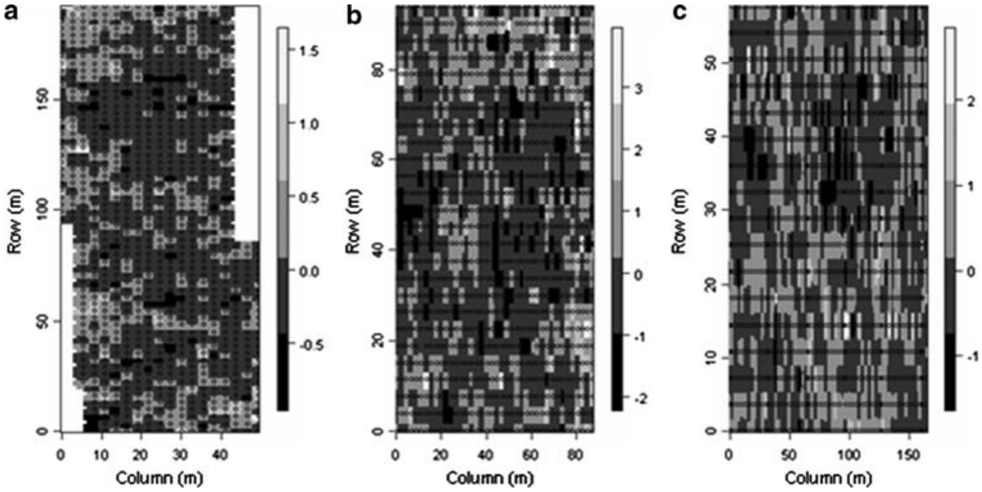

Fig. 1 Image of spatial patterns of the model 1 fit residuals in the Arinto **(a)**, Aragonez **(b)** and Viosinho **(c)** trials

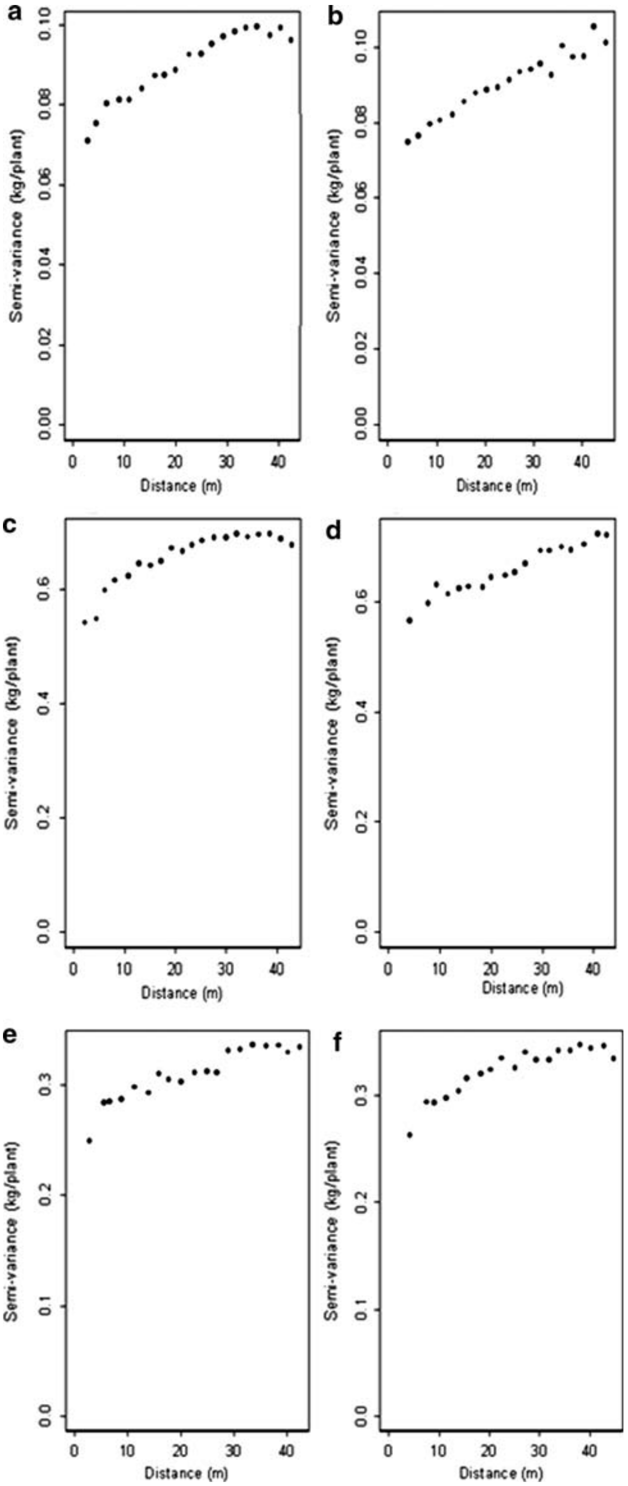

Fig. 2 Directional empirical semivariograms for model 1 fit residuals in the experimental populations of Arinto, Aragonez and Viosinho (**a**, **c**, **e**—row direction; **b**, **d**, **f**—column direction)

likelihood ratio test (see considerations in Sect. “Materials and methods”), which allowed us to reject the null hypothesis ($\sigma_\eta^2 = 0$ and $\rho_{\mathrm{row}} = 0$ and $\rho_{\mathrm{col}} = 0$) ($P < 0.0001$).

According to anisotropic power model (model 3), the residual maximum likelihood estimates for the spatial variance were 0.0581, 0.3780 and 0.1128 for Arinto, Aragonez and Viosinho, respectively. The spatial autocorrelation coefficients for rows and columns were 0.9506 and 0.9497 for Arinto, 0.9583 and 0.9442 for Aragonez, and 0.9423 and 0.9419 for Viosinho. The independent error variances were 0.0754, 0.5498 and 0.2788, for Arinto, Aragonez and Viosinho, respectively. As shown in Tables 3, 4 and 5, in all cases there was a tendency of the correlated error term to retain some of the block variance. In model 3, the sum of spatial and independent error variances ($\sigma^2 + \sigma_\eta^2$) was higher than the error variance (σ_{e}^2) in model 1. Compared with model 1, the independent error variance was always smaller in model 3, but always higher than spatial dependent variance. As already mentioned, the block effects were substantially reduced, particularly in the Aragonez trial. However, in the Viosinho trial, the block effects remained significant ($P < 0.01$) in the presence of spatial correlation terms, demonstrating the importance of preserving the design effects in spatial models. Since the spatial variability of each trial is unique, the possibility of retaining design information together with spatial adjustment should be considered when searching for the best model.

Genotypic variance

The residual maximum likelihood estimates of genotypic variance (σ_{g}^2) components were almost identical among the several fitted models for each population, as well as highly statistically significant ($P < 0.001$) in all the models and for all trials (Tables 3, 4 and 5). This indicated the presence of substantial raw material for genetic selection.

Ranking of predicted genotypic effects and genetic selection

Spearman’s rank correlation coefficient was used to compare predicted genotypic effects rankings between the best spatial model (model 3, anisotropic power correlation function) and classical RCB model (model 1). The results are shown in the Table 6. The correlations were 0.964, 0.946 and 0.979 for Arinto, Aragonez and Viosinho, respectively. This indicates that spatial analysis changed the ranking of the predicted genotypic effects, which will have consequences in selection decisions. In order to visualize these ranking changes, the clones with the highest predicted genotypic effect for yield resulting from the classical RCB analysis (model 1) were compared to those obtained with mixed spatial anisotropic power model (model 3). It can be seen (Table 6) that spatial analysis

Table 3 Models fitted to yield data in the Arinto variety (kg/plant)

Models	$\hat{\sigma}_g^2$ (SE)	$\hat{\sigma}_b^2$ (SE)	$\hat{\sigma}_e^2$ (SE)	$\hat{\sigma}^2$ (SE)	$\hat{\theta}$ (SE)	$\hat{\rho}$ (SE)	$\hat{\rho}_{row}$ (SE)	$\hat{\rho}_{col}$ (SE)	$\hat{\sigma}_\eta^2$ (SE)	$-2l_R^a$	λ^b	AIC[c]	BIC[d]
Model 1—classical RCB	0.1234 (0.0140)	0.0609 (0.0502)	0.1217 (0.0063)							1142.1		1148.1	1158.6
Model 2—power	0.1266 (0.0135)	0		0.1680 (0.1414)		0.9824 (0.0164)			0.0746 (0.0052)	954.2	187.9	962.2	976.2
Model 3—anisotropic power	0.1283 (0.0136)	0.0223 (0.0238)		0.0581 (0.0152)			0.9506 (0.0174)	0.9497 (0.0189)	0.0754 (0.0052)	962.6	179.5	974.6	995.7
Model 4—Gaussian	0.1256 (0.0134)	0.0364 (0.0330)		0.0440 (0.0103)	11.4903 (1.3418)				0.0830 (0.0047)	973.6	168.5	983.6	1001.1
Model 5—spherical	0.1259 (0.0134)	0.0180 (0.0198)		0.0786 (0.0187)	38.0842 (3.7541)				0.0741 (0.0051)	959.4	182.7	969.4	987.0

SE asymptotic standard error

[a] Minus twice the residual log-likelihood

[b] Residual likelihood ratio test statistic [$\lambda = (-2l_R$ of model 1) – ($-2l_R$ of model 2, or 3, or 4, or 5)]

[c] Akaike Information Criterion (AIC = $-2l_R + 2d$), where d is the number of parameters in the covariance structure

[d] Bayesian Information Criterion [BIC = $-2l_R + d\ln(n^* - p)$], where d is the number of parameters in the covariance structure, n^* is the sample size (in PROC MIXED, version 9.1, is defined as the number of levels of the first random factor declared in the random statement, which corresponded to the number of clones, in all models) and p is the rank of matrix X

Table 4 Models fitted to yield data in the Aragonez variety (kg/plant)

Models	$\hat{\sigma}_g^2$ (SE)	$\hat{\sigma}_b^2$ (SE)	$\hat{\sigma}_e^2$ (SE)	$\hat{\sigma}^2$ (SE)	$\hat{\theta}$ (SE)	$\hat{\rho}$ (SE)	$\hat{\rho}_{row}$ (SE)	$\hat{\rho}_{col}$ (SE)	$\hat{\sigma}_\eta^2$ (SE)	$-2l_R^a$	λ^b	AIC[c]	BIC[d]
Model 1—classical RCB	0.3106 (0.0427)	0.1962 (0.1410)	0.8179 (0.0363)							3653.4		3659.4	3670.0
Model 2—power	0.2969 (0.0383)	0.0088 (0.0303)		0.5117 (0.1955)		0.9633 (0.0175)			0.5544 (0.0325)	3445.3	208.1	3455.3	3473.0
Model 3—anisotropic power	0.3008 (0.0384)	0.0162 (0.0389)		0.3780 (0.0863)			0.9583 (0.0129)	0.9442 (0.0174)	0.5498 (0.0308)	3439.9	213.5	3451.9	3473.1
Model 4—Gaussian	0.3054 (0.0391)	0.1190 (0.0966)		0.2508 (0.0440)	8.5302 (0.8863)				0.5790 (0.0295)	3469.0	184.4	3479.0	3496.7
Model 5—spherical	0.2968 (0.0383)	0.0269 (0.0436)		0.4889 (0.1065)	42.6318 (3.7702)				0.5600 (0.0308)	3451.3	202.1	3461.3	3479.0

SE asymptotic standard error

[a] Minus twice the residual log-likelihood

[b] Residual likelihood ratio test statistic [$\lambda = (-2l_R$ of model 1) – ($-2l_R$ of model 2, or 3, or 4, or 5)]

[c] Akaike Information Criterion (AIC = $-2l_R + 2d$), where d is the number of parameters in the covariance structure

[d] Bayesian Information Criterion [BIC = $-2l_R + d\ln(n^* - p)$], where d is the number of parameters in the covariance structure, n^* is the sample size (in PROC MIXED, version 9.1, is defined as the number of levels of the first random factor declared in the random statement, which corresponded to the number of clones, in all models) and p is the rank of matrix X

Table 5 Models fitted to yield data in the Viosinho variety (kg/plant)

Models	$\hat{\sigma}^2_g$ (SE)	$\hat{\sigma}^2_b$ (SE)	$\hat{\sigma}^2_e$ (SE)	$\hat{\sigma}^2$ (SE)	$\hat{\theta}$ (SE)	$\hat{\rho}$ (SE)	$\hat{\rho}_{row}$ (SE)	$\hat{\rho}_{col}$ (SE)	$\hat{\sigma}^2_\eta$ (SE)	$-2l_R^a$	λ^b	AIC[c]	BIC[d]
Model 1—classical RCB	0.3319 (0.0411)	0.1842 (0.1316)	0.3751 (0.0189)							2206.1		2212.1	2222.0
Model 2—power	0.3298 (0.0396)	0.1020 (0.0838)		0.1453 (0.0457)		0.9417 (0.0280)			0.2692 (0.0207)	2094.6	111.5	2104.6	2121.1
Model 3—anisotropic power	0.3272 (0.0394)	0.1134 (0.0916)		0.1128 (0.0279)			0.9423 (0.0279)	0.9419 (0.0222)	0.2788 (0.0193)	2099.3	106.8	2111.3	2131.1
Model 4—Gaussian	0.3262 (0.0394)	0.1072 (0.0854)		0.0882 (0.0285)	18.0936 (5.1630)				0.3089 (0.0174)	2104.1	102.0	2114.1	2130.6
Model 5—spherical	0.3291 (0.0396)	0.0991 (0.0816)		0.1448 (0.0474)	43.7126 (4.1087)				0.2841 (0.0178)	2095.4	110.7	2105.4	2121.8

SE asymptotic standard error

[a] Minus twice the residual log-likelihood

[b] Residual likelihood ratio test statistic [λ = ($-2l_R$ of model 1) – ($-2l_R$ of model 2, or 3, or 4, or 5)]

[c] Akaike Information Criterion (AIC = $-2l_R + 2d$), where *d* is the number of parameters in the covariance structure

[d] Bayesian Information Criterion [BIC = $-2l_R + d\ln(n^* - p)$], where *d* is the number of parameters in the covariance structure, n^* is the sample size (in PROC MIXED, version 9.1, is defined as the number of levels of the first random factor declared in the random statement, which corresponded to the number of clones, in all models) and *p* is the rank of matrix *X*

provided smaller standard errors associated with the predicted genotypic effects (i.e., more accurate predictions) and produced a different ranking of clones than that obtained with non-spatial analysis (model 1). This means that some of the clones, ranked as amongst the most productive in the classical RCB analysis, could be discarded using the spatial analysis. For example, spatial modulation in Arinto moved clone 9604 from the 9th to 19th, clone 3704 from the 12th to 28th and clone 9609 from the 10th to 35th positions. In Aragonez, clone 0707 dropped from the 1st to 13th, clone 1704 from the 6th to 18th and clone 0524 from the 10th to 36th positions. In Viosinho, clone 1225 dropped from the 9th to 20th, clone 0923 from the 12th to 17th, and clone 1236 from the 14th to 29th positions. These results will have important consequences in the final composition of the group of selected clones (i.e., for the final mass genotypic selection). More precisely, when a 15% proportion of clones are selected as the most productive from the entire population (37 of 247 clones in Arinto, 38 of 255 clones in Aragonez and 30 of 199 clones in Viosinho), there is a marked difference in the group of selected clones between the two statistical analyses. This difference was found to be in the order of 29.7% (11 of 37), 21.1% (8 of 38) and 10.0% (3 of 30) for Arinto, Aragonez and Viosinho, respectively.

Assuming the same proportion of clones for selection (15% of the population), the predicted yield genetic gain (the average of the best linear unbiased predictors of genotypic effects of selected genotypes) was compared between the two types of analyses. For Arinto, 0.439 kg/plant was obtained with the classical RCB analysis and 0.457 kg/plant with spatial analysis. For Aragonez, 0.674 kg/plant was obtained with classical RCB analysis and 0.695 kg/plant with spatial analysis. For the Viosinho variety, 0.700 kg/plant was obtained with classical RCB analysis and 0.695 kg/plant with spatial analysis.

Finally, the predicted genetic gains obtained with the spatial mixed models showed smaller predicted average standard errors associated with them than those obtained with the classical RCB model (decreasing by 11.4, 8.5, 6.4%, for Arinto, Aragonez and Viosinho, respectively). As a consequence, considering the same level of significance, the spatial models allowed for narrower intervals of prediction, and therefore more accurate prediction of genetic gains.

Discussion

Yield data collected from the three large grapevine field trials show the existence of spatial correlation and therefore accentuate the importance of mixed spatial models to improve efficiency of grapevine selection. In this paper, only

Table 6 Empirical best linear unbiased predictors of genotypic effects ($\tilde{g}$) of clones within selected groups, predicted genetic gains obtained with classical RCB and anisotropic power spatial models and Spearman's rank correlation coefficient between the rankings of all predicted genotypic effects of the two models

Ranking	**Arinto**				**Aragonez**				**Viosinho**			
	Classical model		**Anisotropic power model**		**Classical model**		**Anisotropic power model**		**Classical model**		**Anisotropic power model**	
	Selected clones	**$\tilde{g}$ (kg/plant)**	**Selected clones**	**$\tilde{g}$ (kg/plant)**	**Selected clones**	**$\tilde{g}$ (kg/plant)**	**Selected clones**	**$\tilde{g}$ (kg/plant)**	**Selected clones**	**$\tilde{g}$ (kg/plant)**	**Selected clones**	**$\tilde{g}$ (kg/plant)**
1	**4108**	0.811	**4108**	0.808	**0707**	1.001	**6505**	1.023	**1634**	1.050	**1612**	1.001
2	**3605**	0.670	**3605**	0.735	**7810**	0.984	**4201**	0.998	**1310**	0.976	**1326**	0.947
3	**3502**	0.658	**3903**	0.638	**6505**	0.983	**6112**	0.877	**1612**	0.958	**1634**	0.899
4	**3903**	0.642	**0310**	0.616	**1178**	0.923	**8302**	0.861	**1431**	0.909	**1510**	0.891
5	**0310**	0.615	**2404**	0.553	**4201**	0.873	**1703**	0.857	**1510**	0.907	**1310**	0.874
6	**2404**	0.598	**3502**	0.551	**1704**	0.826	**6709**	0.852	**1508**	0.877	**1508**	0.820
7	**3113**	0.578	**4101**	0.522	**6709**	0.801	**1605**	0.851	**1504**	0.868	**1706**	0.798
8	**4101**	0.544	**3902**	0.510	**1302**	0.788	**1302**	0.847	**1326**	0.807	**1431**	0.783
9	**9604**	0.500	**3113**	0.494	**7207**	0.780	**7810**	0.841	**1225**	0.760	**1741**	0.779
10	**9609**	0.498	**8201**	0.492	**0524**	0.772	**0386**	0.838	**1706**	0.732	**1504**	0.768
11	**8201**	0.469	**0664**	0.484	**0136**	0.770	**1178**	0.833	**1741**	0.729	**1521**	0.705
12	**3704**	0.465	**4110**	0.477	**6112**	0.769	**0136**	0.790	**0923**	0.711	**0201**	0.685
13	**0664**	0.459	**3404**	0.474	**0452**	0.714	**0707**	0.790	**1735**	0.703	**1735**	0.672
14	**4110**	0.446	**1501**	0.472	**1124**	0.713	**7207**	0.774	**1236**	0.692	**1412**	0.652
15	**3902**	0.434	**8801**	0.455	**1502**	0.695	**1110**	0.758	**1410**	0.673	**1601**	0.641
16	**3504**	0.432	**0344**	0.453	**8601**	0.687	**4504**	0.684	**1330**	0.668	**1410**	0.635
17	**0640**	0.430	**3905**	0.442	**0386**	0.674	**1124**	0.667	**1521**	0.635	**0923**	0.634
18	**6707**	0.429	**6707**	0.436	**1703**	0.649	**1704**	0.667	**1325**	0.635	**1611**	0.634
19	**8801**	0.386	**9604**	0.430	**1243**	0.631	**1158**	0.658	**1305**	0.626	**1728**	0.628
20	**3403**	0.376	**0640**	0.427	**1605**	0.626	**7311**	0.658	**1412**	0.625	**1225**	0.626
21	**9603**	0.374	**2401**	0.409	**1601**	0.626	**1603**	0.626	**1206**	0.609	**0226**	0.626
22	**0503**	0.373	**3504**	0.409	**1334**	0.622	**1243**	0.625	**1313**	0.565	**0235**	0.620
23	**3404**	0.360	**9210**	0.407	**0618**	0.619	**7801**	0.616	**1243**	0.563	**1243**	0.620
24	**3204**	0.352	**6116**	0.406	**1158**	0.616	**1336**	0.612	**0201**	0.559	**1313**	0.600
25	**0517**	0.350	**0498**	0.392	**7801**	0.597	**0314**	0.609	**0235**	0.540	**1325**	0.593
26	**2019**	0.348	**8807**	0.390	**6210**	0.584	**1218**	0.605	**1328**	0.536	**1330**	0.568
27	**6116**	0.348	**0223**	0.388	**6309**	0.567	**8601**	0.601	**1411**	0.528	**1206**	0.541
28	**8807**	0.347	**3704**	0.387	**9208**	0.554	**1334**	0.590	**1245**	0.523	**1305**	0.541
29	**9005**	0.344	**9610**	0.384	**8306**	0.554	**8809**	0.584	**1702**	0.521	**1236**	0.528
30	**2403**	0.338	**9606**	0.383	**8302**	0.542	**6510**	0.575	**1611**	0.518	**1328**	0.527
31	**8007**	0.333	**3901**	0.378	**0714**	0.530	**6210**	0.569				
32	**1501**	0.333	**3910**	0.363	**1117**	0.522	**1601**	0.566				
33	**8204**	0.333	**4107**	0.355	**0513**	0.521	**0452**	0.547				
34	**3905**	0.319	**0244**	0.352	**1511**	0.518	**7502**	0.520				
35	**0344**	0.318	**9609**	0.348	**1336**	0.506	**7907**	0.518				
36	**0315**	0.316	**1632**	0.343	**7502**	0.502	**0524**	0.517				
37	**0498**	0.316	**0483**	0.340	**7907**	0.498	**1502**	0.517				
38					**1276**	0.495	**9208**	0.506				
Genetic gain (kg/plant) (SE)		0.439 (0.158)		0.457 (0.140)		0.674 (0.329)		0.695 (0.301)		0.700 (0.250)		0.695 (0.234)
Spearman's rank correlation, classical RCB: anisotropic power				0.964				0.946				0.979

SE asymptotic standard error of prediction

The underline identifies some clones that change their ranking according to the fitted model

yield data were analyzed because this agronomical trait was considered to be of utmost importance for selection priorities and was therefore the most studied in initial trials. However, viticulture priorities have recently changed and selection objectives now include quality traits of the must, such as sugar content, acidity and anthocyanines. Although the evaluation of traits in the must is laborious in large experimental populations, collection of data is currently underway. In the future, the adoption of mixed spatial models should also be tested and applied to those traits.

Given that the objective of these trials was to select groups of clones to be grown as mixtures (mass genotypic selection), we considered it adequate to base our analysis on the average yield observed over several years, without taking into account the repeated measures nature of the data. In the next cycle of selection (clonal selection), the chosen groups of clones will be submitted to further analyses, involving plantation and data collection in several multi-environment trials. In this case, individual clones will be selected and recommended for specific regions or conditions and therefore patterns of behavior over years and locations will be extremely important to consider.

In respect to adjustment for spatial variability, the anisotropic power function was chosen for all the three field trials. When compared to what is currently observed with other crops, where the modulation of ε as a first order separable autoregressive process [AR(1) × AR(1)] is recommended (Gilmour et al. 1997; Cullis et al. 1998; Smith et al. 2001), our results reinforce the broad adequacy of this type of spatial structure in field experiments.

Contrary to what is usually described for annual crops, where most (or all) of the error component is spatially dependent (only ε is included and η corresponds to measurements errors and is ignored), our grapevine selection trials show a strong nugget component. Taking into consideration the residual maximum likelihood estimates for model 3 in the Arinto, Aragonez and Viosinho trials, this independent error component represents 56.5, 59.3 and 71.2% of the total error variance. This is in close agreement with previous results obtained in forest trials (Kusnandar and Galwey 2000; Costa e Silva et al. 2001; Dutkowski et al. 2002) and can be justified by specific conditions associated with the cultivation of vines, some of which are also observed in forests: marginal soils as well as highly heterogeneous topographical, physical and chemical conditions; artificial plant management, including grafting, intense pruning and recurrent treatments for fungi, insects and weeds; and average plot yield based on few plants. In sum, the strong non-spatial component of the error variance is essentially due to these conditions, with measurement errors being a negligible part of this component.

When judging the significance of the anisotropic power model, the high values of the test statistic (λ = 179.5, λ = 213.5, λ = 106.8, for Arinto, Aragonez and Viosinho, respectively) clearly show that the null hypothesis (H_0: $\sigma_\eta^2 = 0$ and $\rho_{\text{row}} = 0$ and $\rho_{\text{col}} = 0$) must be rejected ($P < 0.0001$). Furthermore, such high values of the test statistic confirm that the naive approach (i.e., considering the asymptotic distribution of the likelihood ratio test statistic as a Chi-squared distribution with three degrees of freedom) is applicable and does not cause misinterpretation in the present case. However, this application may be problematic in cases where the test statistic results in low values, since small values of λ could cause the non-rejection of the classical RCB model.

As expected, the mass genotypic selection and the differences in the reduction of predicted standard error between the classical RCB and spatial analyses are more discrepant when the effects of spatial variability are higher, as seen for Arinto and Aragonez. Consequently, failure to account for spatial correlation and ignoring some inherent structure in the error covariance matrix can increase the risk of confounding genotypic effects of individual clones and hinder genetic selection.

The simple RCB designs used in our experiments are typical of the designs used in Portugal some years ago. As is well known, RCB designs are not well suited to experiments with many genotypes. There are other more sophisticated designs, such as incomplete block designs and resolvable row–column designs that can successfully account for much of the field spatial heterogeneity. Their application in grapevine initial trials is desirable and studies for demonstrating their efficiency in these experiments are clearly opportune. In the meantime, there are many RCB-design grapevine trials in the field, from which data continue to be collected. Spatial modeling provides an important approach for the analysis of such data.

In conclusion, we hope that the results presented here encourage the exploration and the usage of mixed spatial models for data analysis, as a general complement of good experimental design strategies, in order to improve the efficiency of all processes of grapevine selection.

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