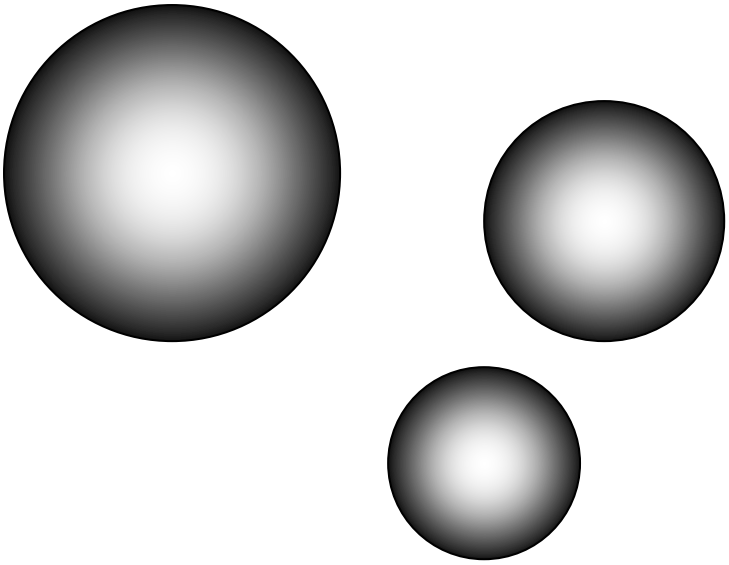

# Linear Mixed Models

## Applications in

# InfoStat

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June, 2017

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## Introduction

InfoStat implements a friendly interface of the **R** platform to estimate extended and mixed linear models through the **gls** and **lme** procedures of the **nlme** library. The reference bibliography for this implementation is Pinheiro & Bates (2004), and some of the examples used come from this book. InfoStat communicates with R by its own communication technology (developed by Eng. Mauricio Di Rienzo, 2016).

## Requirements

To let InfoStat to have full access to R, it must be installed on your system and an updated version of R. To perform the installation process correctly, consult the online help in the InfoStat *Help* menu, submenu *How to install R?* and follow the instructions given without omitting any steps.

## Extended and mixed linear models

In the S*tatistics* menu, select the *Extended and mixed linear models* submenu, here you will find three options. The first option, with the heading *Model estimation* activates the dialogue window for the specification of the model structure. The second option, with the heading *Model exploration*, is activated when a model has been previously estimated, and it contains a group of tools for diagnostic analysis. The third option links to the *Tutorial* for mixed model analysis and estimation.

## Specification of fixed effects

Let us begin by indicating how to adjust a fixed effects model using the *Atriplex.IDB2* file located in InfoStat test datasets (*File*, *open test data*). Once this file is open, activate the *Statistics* menu, the *Extended and mixed linear models* submenu, *Model estimation* option. In the variables selection window, the dependent variables (*Variables*), classification factors (*Class variables*) and *covariates* can be specified as in an analysis of variance for fixed effects. For the data in the *Atriplex.IDB2* file, *Germination* should be specified as a response variable, and *Size* and *Color* as classification variables. Once the selection is accepted, the principal window of the interface for mixed models will appear. This window contains five tabs (Figure 1).

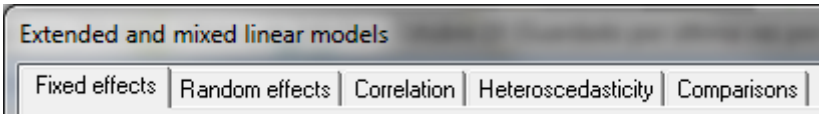



***Figure 1: Tabs with the options for the specification of an extended and mixed linear model.***

The first tab allows the user to specify the fixed effects of the model, to select options for the presentation of results and the generation of predictions, to obtain residuals for the model, and to specify the estimation method. The default estimation method is restricted maximum likelihood (REML).

To the right of the window, a list containing the classification variables and covariates declared in the variables selection window will appear. To include a factor (classification variable) or a covariate in the fixed part of the model, the user needs only to double click on the name of the factor o covariate that he/she wishes to include. This action will add a line to the fixed effects list. Additional double clicks on a factor or a covariate will successively add linear terms that are implicitly separated by a "+" sign (additive model). By selecting the main factors and activating the "*" button, the user may add a term that specifies an interaction between factors. For the data set in the *Atriplex.IDB2* file, include in the fixed effects model the factors *Size*, *Color* and their interaction (Figure 2). Some of the fonts in this window have been increased in size to improve their visualization (this is done by moving the mouse roller while pressing the *Ctrl* key).

If we accept this specification, this will generate an output in the InfoStat results window, shown below Figure 2. This is the simplest output because neither additional model characteristics nor other analysis options have been specified. The first part

contains the specification of the way the estimation model was invoked in the R syntax, and it indicates the name of the R object containing the model and its estimation, in this case, `model000_Germination_REML`. This specification is of interest only to those users who are familiar with R commands.

The second part shows measures of fit that are useful in comparing different models fitted to a data set. *AIC* refers to the Akaike's criterion, *BIC* to Schwarz' Bayesian information criterion, *logLik* to the logarithm of the likelihood, and *Sigma* to the residual standard deviation. The third part of this output presents an analysis of variance table and shows sequential-type hypothesis testing.

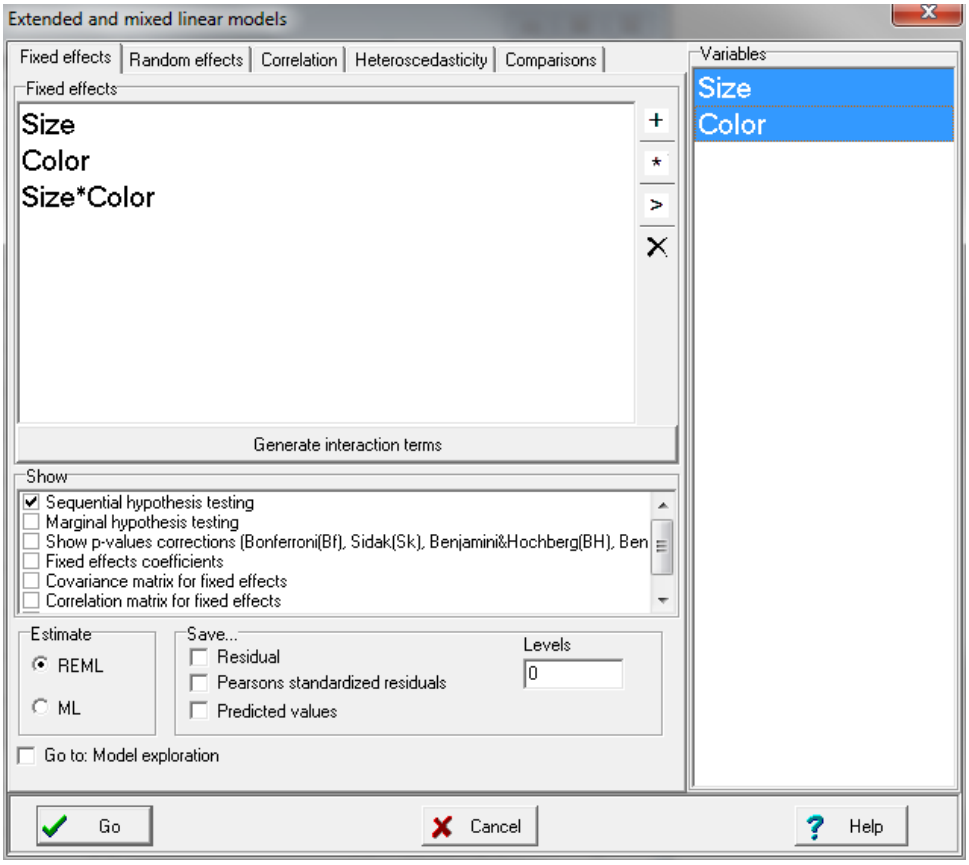



***Figure 2: Window displaying the Fixed effects tab (Atriplex.IDB2 file).***

```
Extended and mixed linear models

R specification of the model

model000_Germination_REML<-gls(Germination~1+Size+Color+Size:Color
,method="REML"
,na.action=na.omit
,data=R.data00)
```

**Results for model: model000_Germination_REML**

*Dependent variable:Germination*

**Fit measurements**

| N | AIC | BIC | logLik | Sigma | R2_0 |
|---|---|---|---|---|---|
| 27 | 160.36 | 169.26 | -70.18 | 9.07 | 0.92 |

*Smaller AIC and BIC is better*

**Sequential hypothesis testing**

| | numDF | F-value | p-value |
|---|---|---|---|
| (Intercept) | 1 | 1409.95 | <0.0001 |
| Size | 2 | 10.49 | 0.0010 |
| Color | 2 | 90.53 | <0.0001 |
| Size:Color | 4 | 2.29 | 0.0994 |

## Specification of random effects

Random effects are associated with groups of observations. Typical examples are repeated measurements on the same individual or the observed responses for a group of homogeneous experimental units (blocks) or for the individuals in the same family group, etc. These random effects are "added" to the fixed effects in a selective manner. Because of this, in the specification of random effects it is necessary to have one or more grouping or stratification criteria, and to choose the fixed effects to which the associated random effects should be added. In the R *lme* procedure on which this implementation is based, when more than one grouping criterion is acceptable, these are nested or hierarchical. However, it is possible to use crossed random effects. In the *Extended and mixed linear models* submenu, *Random effects* tab, the symbol > is used to denote a nested factor (A>B indicates that B is nested within A); the symbol + is used to denote crossed factors (A+B indicates that A and B are crossed factors); the symbol * is used to denote interactions (A*B indicates the interaction between A and B). These symbols can be written directly in the window, or, by clicking the mouse right button on two or more previously selected factors, a window with these options appears.

In the second tab of the model specification dialogue, we can choose the stratification or grouping criteria and the way these incorporate random effects to fixed components. To exemplify the specification of the random effects, let us consider the *Block.IDB2* data file. This file contains three columns: *Block*, *Treatment* and *Yield*. In this example, we

will indicate that the blocks were selected in a random manner or that they produce a random effect (for example, if the blocks are a set of plots, their effect could be considered random, because their response will depend on environmental conditions that are not predictable, among other things), whereas the treatments add fixed effects. To specify this model, the first two columns of the data file *Block.IDB2* (*Block* and *Treatment*) should be introduced as classification criteria and the last one (*Yield*) as a dependent variable. The *Treatment* factor should be included in the *Fixed effects* tab as the only component of that part of the model. To include the random effect of the blocks, the *Random effects* tab should be selected. When this tab is selected, the *Stratification criteria* list is empty. Double clicking on *Block* in the variables list adds this classification factor as a grouping criterion. The inclusion of a stratification criterion activates a device in the inferior panel that allows the user to specify the way in which the random effect enters the model. In this device, there is a list of components for the fixed part of the model. The first component refers to the *Constant* and the other components refer to the remaining terms, in this case *Treatment* (Figure 3).

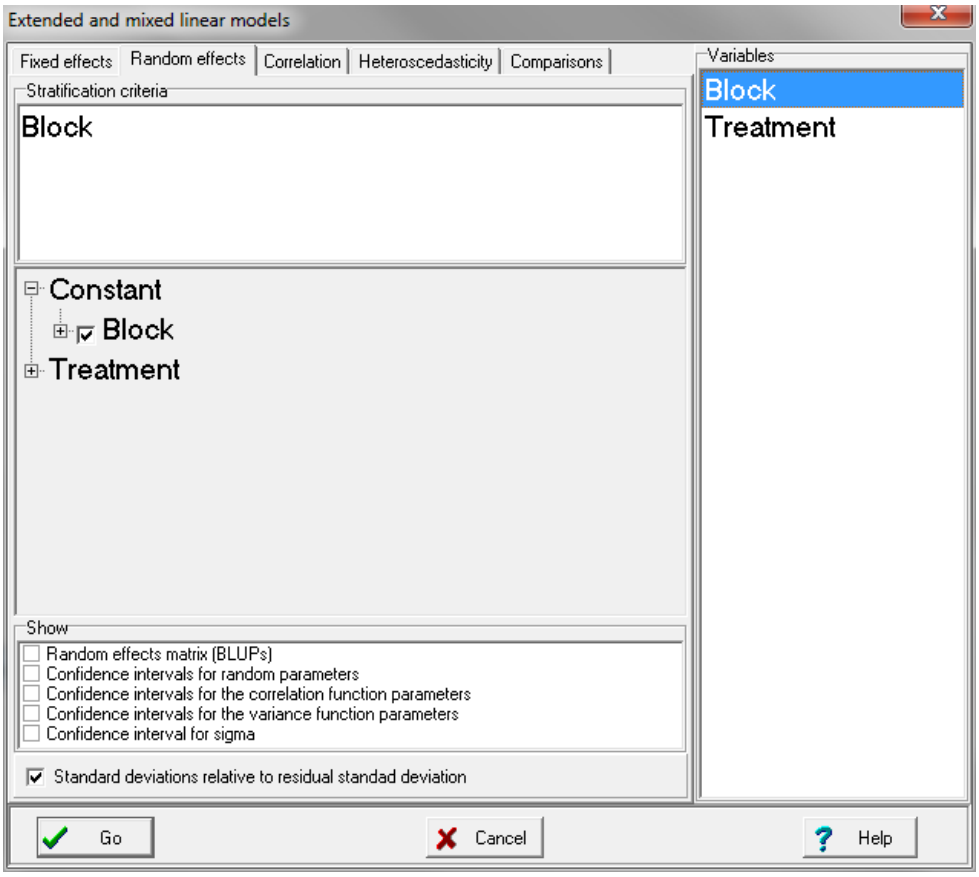



***Figure 3: Window displaying the Random effects tab (Block.IDB2 file).***

The previously specified stratification criteria appear in the list of fixed terms. The combination of both lists defines the random effects. For this, every stratification criterion within each fixed effect is associated with a *check box*. When the *check box* is checked, this indicates that there is a group of random effects associated with a corresponding fixed effect. The number of random effects is equal to the number of levels of the fixed term of the model, or equal to 1 in the case of the constant or the covariates. The illustrated example includes a random effect induced by the blocks on the constant.

This specification represents the following model:

$$y_{ij} = \mu + \tau_i + b_j + \varepsilon_{ij};\ i = 1,..,T;\ j = 1,...,B \qquad (1)$$

where $y_{ij}$ is the response to the *i*-th treatment in the *j*-th block; $\mu$ is the general mean of yield; $\tau_i$ is the fixed effects of the treatments; $b_j$ is the middle level change of $y_{ij}$ associated with the *j*-th block; and $\varepsilon_{ij}$ is the error term associated with observation $y_{ij}$. *T* and *B* are the number of levels of the classification factor that correspond to the *Treatment* fixed effect and to the number of blocks, respectively. The nature of these effects is different from the fixed effects: the $b_j$'s are considered identically distributed $N\left(0,\sigma_b^2\right)$ random variables whose realizations are interpreted as the effects of the different groups or strata (blocks in this example). In these models, the $b_j$'s are not estimated; instead, the $\sigma_b^2$ parameter that characterizes its distribution is estimated. The $\varepsilon_{ij}$'s are also interpreted as identically distributed $N\left(0,\sigma_\varepsilon^2\right)$ random variables, and they describe the random error associated with each observation. Moreover, the random variables $b_j$ and $\varepsilon_{ij}$ are assumed to be independent.

The output for this example is shown below. The new part of this output, with respect to the example for the fixed effects linear model, is a section of parameters for the random effects.

```
Extended and mixed linear models

R specification of the model

model001_Yield_REML<-lme(Yield~1+Treatment
.random=list(Block=pdIdent(~1))
.method="REML"
.na.action=na.omit
.data=R.data01
.keep.data=FALSE)
```

**Results for model: model001_Yield_REML**

*Dependent variable:Yield*

**Fit measurements**

| N | AIC | BIC | logLik | Sigma | R2_0 | R2_1 |
|---|---|---|---|---|---|---|
| 20 | 218.77 | 223.73 | -102.39 | 160.65 | 0.89 | 0.93 |

*Smaller AIC and BIC is better*

**Sequential hypothesis testing**

| | numDF | denDF | F-value | p-value |
|---|---|---|---|---|
| (Intercept) | 1 | 12 | 2240.00 | <0.0001 |
| Treatment | 4 | 12 | 41.57 | <0.0001 |

**Random effects parameters**

*Covariance model for random effects: pdIdent*
*Formula: ~1|Block*

*Standard deviations relative to residual standard deviation and correlation*

| | (const) |
|---|---|
| (const) | 0.57 |

In this case the estimation of $\sigma_b$ (the standard deviation of the $b_j$'s relative to the residual) is 0.57. At the beginning of the output, the estimation of $\sigma_\varepsilon$, the standard deviation of the $\varepsilon_{ij}$'s, is presented as 160.65. Thus, the variance of the blocks can be calculated as: $\sigma_b^2 = (0.57 \times 160.65)^2 = 8385.15$

## Comparison of treatment means

Continuing with the Comparisons tab (Figure 4), if one of the fixed terms of the model is checked in the panel list, a means and standard errors table is obtained, as well as a the Fisher's LSD-type multiple comparison test (this is based on a Wald test) or a cluster-based DGC test (Di Rienzo et al. 2002). Various corrections options for multiple comparisons are also presented.

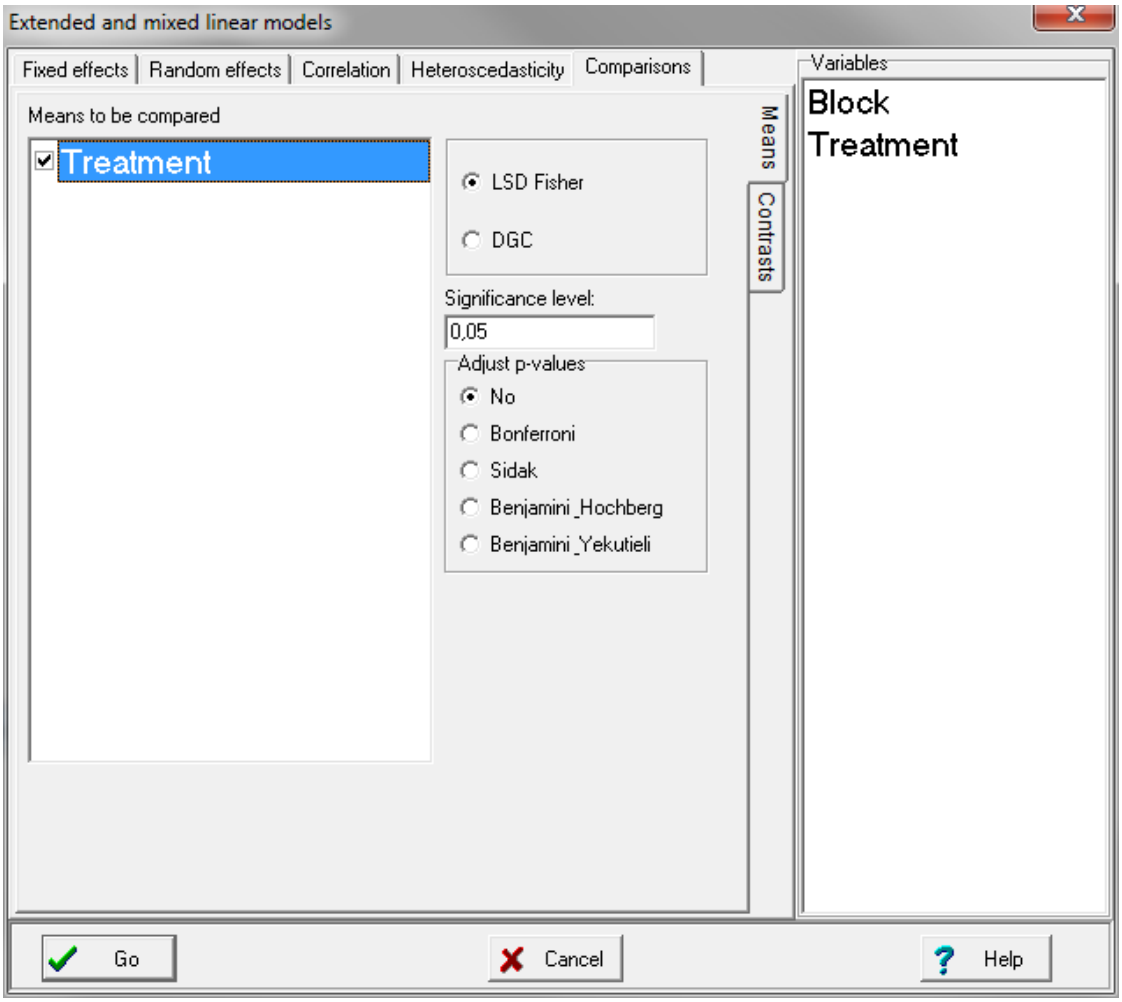



***Figure 4: Window displaying the Comparisons tab (Block.IDB2 file).***

The output corresponding to the treatment means comparisons is shown below.

**Adjusted means and standard error for Treatment**

*LSD Fisher (alpha=0.05)*

*p-value correction procedure: No*

| Treatment | Means | S.E. | | | | |
|---|---|---|---|---|---|---|
| 300 | 3237.75 | 92.47 | A | | | |
| 225 | 3093.50 | 92.47 | A | B | | |
| 150 | 2973.00 | 92.47 | | B | | |
| 75 | 2498.50 | 92.47 | | | C | |
| 0 | 1972.75 | 92.47 | | | | D |

*Means with a common letter are not significantly different (p<= 0.05)*

The treatments mean comparison is shown in the classic form as a list arranged in a decreasing order.

If the user wishes to control type I error for the family of all paired comparisons, he can opt for one of the four implemented criteria: Bonferroni (Hsu 1996), Sidak (Hsu 1996), Benjamini-Hochberg (Benjamini & Hochberg 1995) o Benjamini-Yekutieli (Benjamini

& Yekutieli 2001). If the Bonferroni option is selected for this same data set, the following result is obtained:

**Adjusted means and standard error for Treatment**
*LSD Fisher (alpha=0.05)*
*p-value correction procedure: Bonferroni*

| Treatment | Means | S.E. | | |
|---|---|---|---|---|
| 300 | 3237.75 | 92.47 | A | |
| 225 | 3093.50 | 92.47 | A | B |
| 150 | 2973.00 | 92.47 | A | B |
| 75 | 2498.50 | 92.47 | | B |
| 0 | 1972.75 | 92.47 | | B |

*Means with a common letter are not significantly different (p<= 0.05)*

If there is more than random effect, InfoStat allows the specification of complex structures: hierarchical and/or crossed, with and without interaction. Suppose that there is one fixed factor (A) and three random factors (B, C, y D). In order to specify nested random terms (the default option), the factors are listed in hierarchical order in the *Random effects* tab (Figure 5)

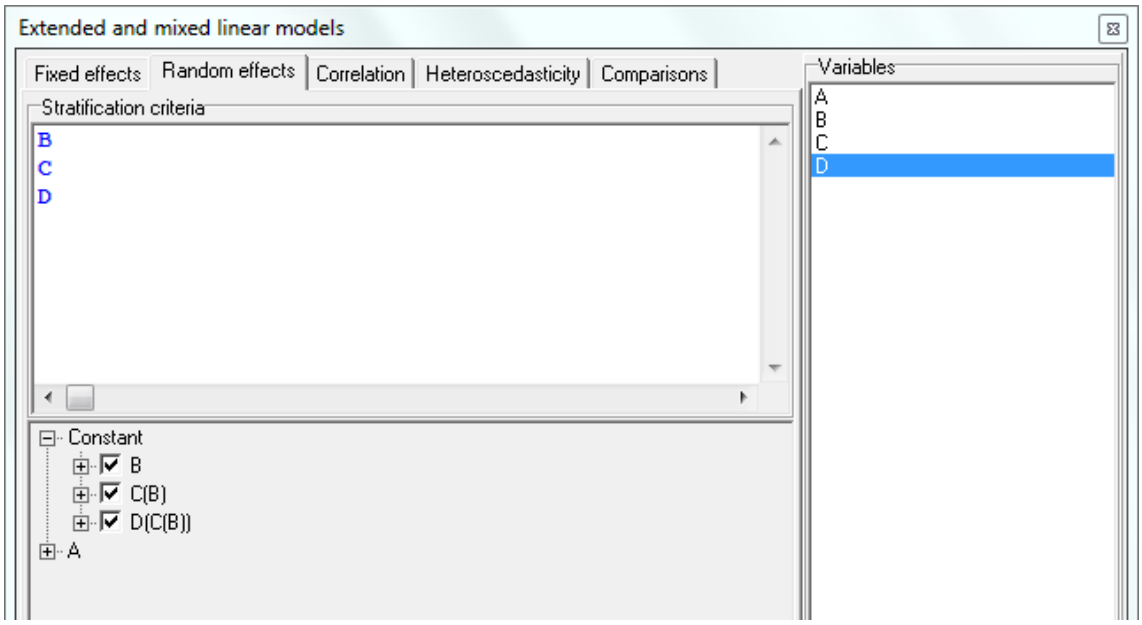



***Figure 5: Window with the Random effects tab for a hypothetical example with four classification factors: A, B, C, and D (A is fixed; B, C, and D are random). In this case B, C, and D are included as nested random effects.***

This formulation is equivalent to the following one (Figure 6)

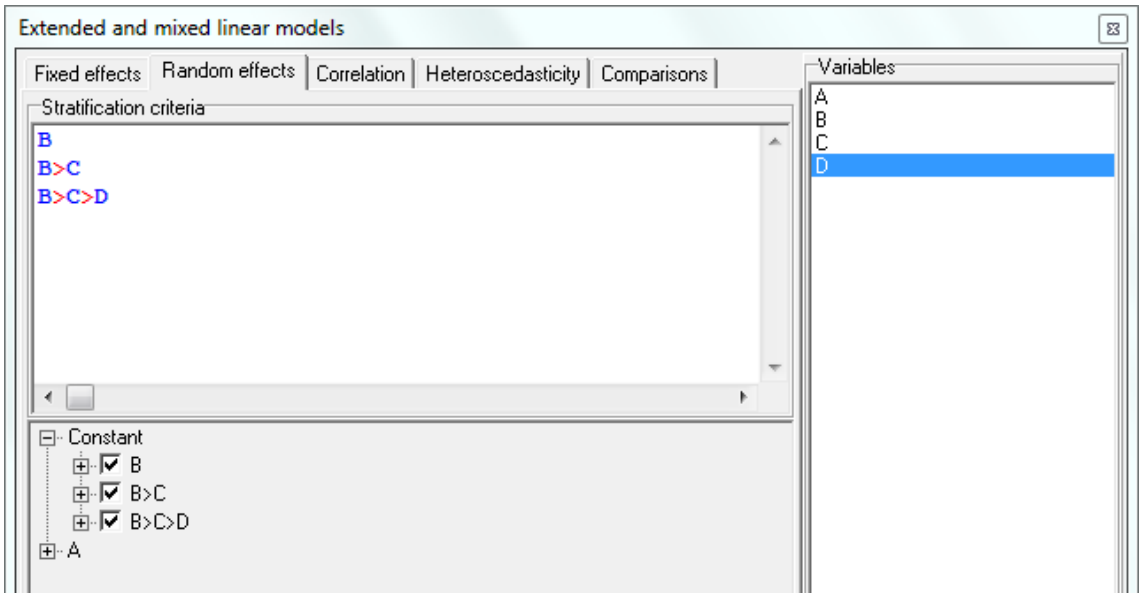



***Figure 6: Window with the Random effects tab for a hypothetical example with four classification factors: A, B, C, and D (A is fixed; B, C, and D are random). In this case B, C, and D are included as nested random effects (explicit form).***

The specification of crossed effects with no interaction is done by selecting all factors to be declared as crossed in the *Variables* tab, and then clicking the right mouse to add the crossed effects in the *Stratification criteria* window (Figure 7).

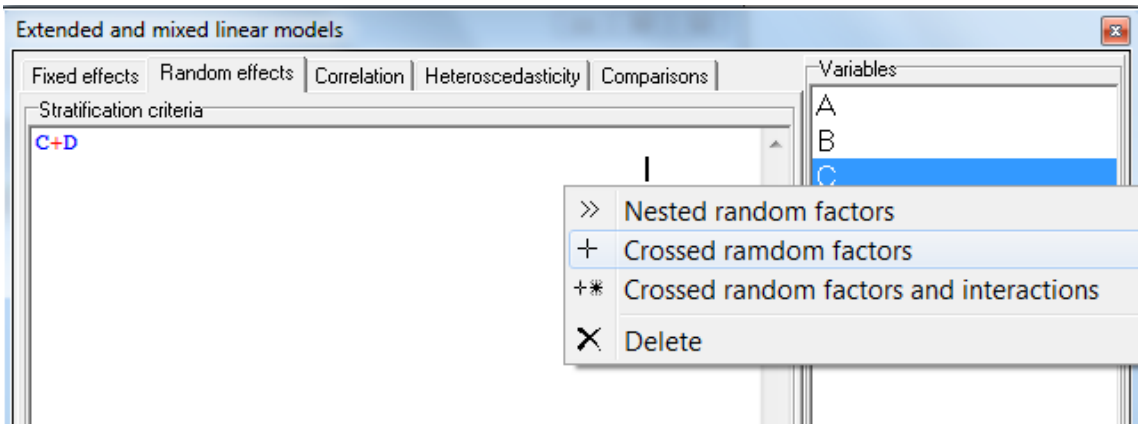



***Figure 7: Window with the Random effects tab for a hypothetical example with four classification factors: A, B, C, and D (A is fixed; B, C, and D are random.) In this case D and C are included as crossed random effects.***

The specification of crossed effects with no interaction is done by adding the desired interaction term(s) to the previous specification (Figure 8)

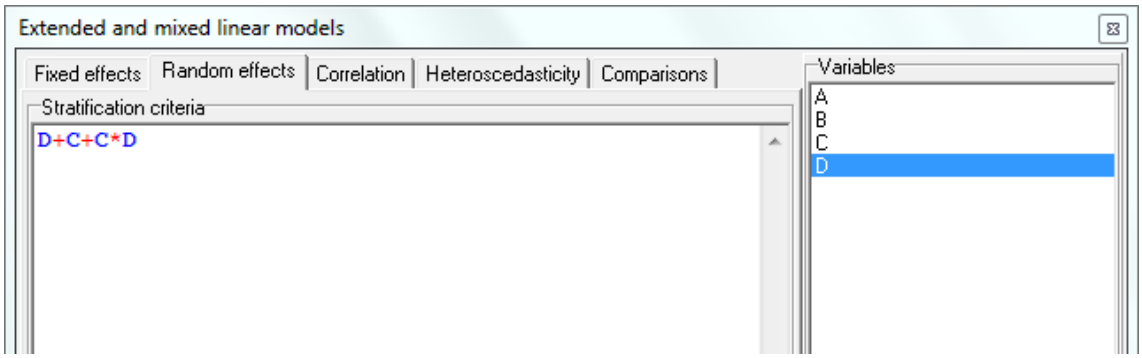



***Figure 8: Window with the Random effects tab for a hypothetical example with four classification factors: A, B, C, and D (A is fixed; B, C, and D are random.) In this case D and C are included as crossed random effects with interaction.***

In order to combine nested and crossed random effects, different lines can be used in the *Stratification criteria* window. For example, to specify a model with C and D crossed with interaction, and B nested in the C main effect we can write this as in Figure 9.

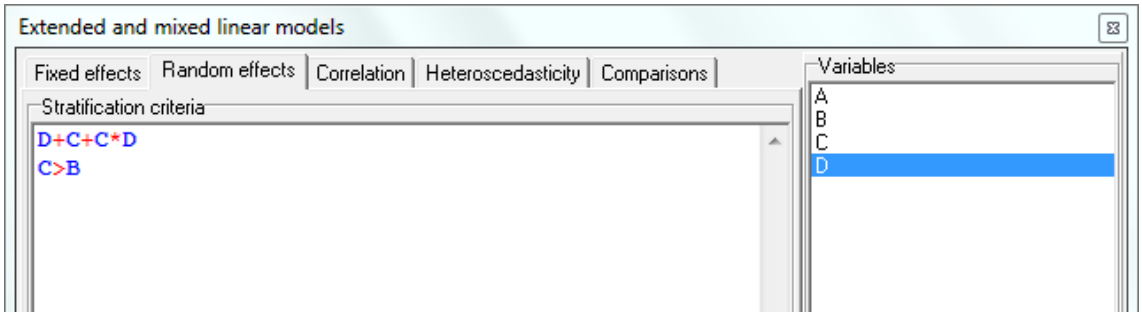



***Figure 9: Window with the Random effects tab for a hypothetical example with four classification factors: A, B, C, and D (A is fixed; B, C, and D are random.) In this case D and C are included as crossed random effects with interaction, and B is nested in C.***

In order to specify B and C effects nested within A (remember that A is fixed), we can write in the *Stratification criteria* window as shown in Figure 10.

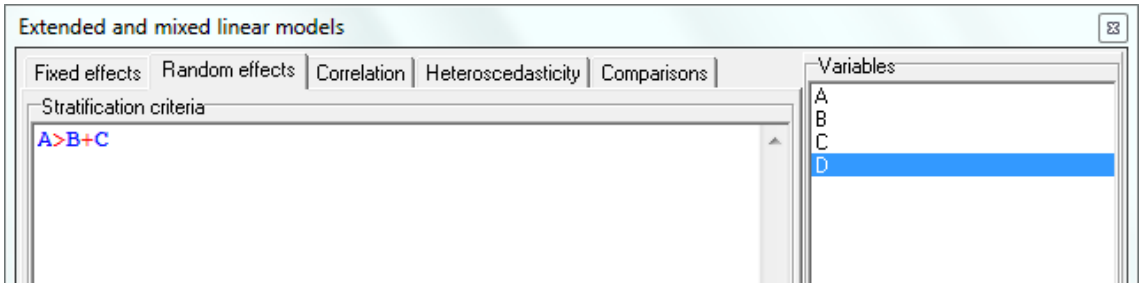



***Figure 10: Window with the Random effects tab for a hypothetical example with four classification factors: A, B, C, and D (A is fixed; B, C, and D are random.) In this case B and C are included as crossed random effects, both nested within the fixed factor A.***

In order to specify the B random effect, and the C and D crossed random effects (both nested within B) we can write in the *Stratification criteria* window as shown in Figure 11.

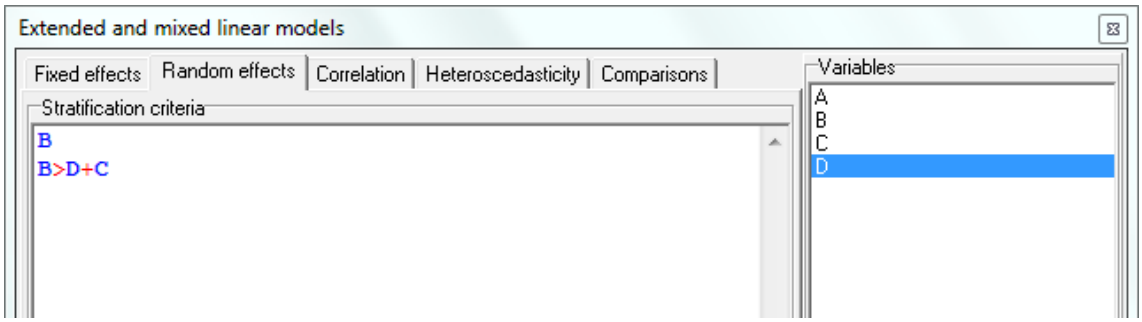



***Figure 11: Window with the Random effects tab for a hypothetical example with four classification factors: A, B, C, and D (A is fixed; B, C, and D are random.) In this case C are D are included as crossed random effects, both nested within the random effect B.***

In all cases including non-nested random effects, the only covariance structure available is the independence among random effects and equal variances for realizations of the same effect. One can also specify random coefficient (regression) models, but the sintaxis differs. See an example of random coefficient model in Applications in linear regression.

## Specification of the correlation and error variance structures

The variance and covariance structures can be modeled separately. For this, InfoStat presents two tabs: in the *Correlation* tab two options are found to specify the error correlation structure, and the *Heteroscedasticity* tab allows the user to select different models for the variance function. The contents of these tabs are described below.

### *Specification of the correlation structure*

To exemplify the use of this tool we will use an example cited in Pinheiro & Bates (2004). The example corresponds to the "Ovary" file, which contains the data from a study by Pierson & Ginther (1987) on the number of follicles bigger than 10 mm in mare ovaries. These numbers were recorded through time 3 days before ovulation and up to 3 days after the next ovulation. The data can be downloaded from the *nlme* library using the menu item *Applications>> Open R-data set*. When this option is activated the

following dialogue window opens, which can differ in the number of libraries that are installed in the user's local R configuration (Figure 12).

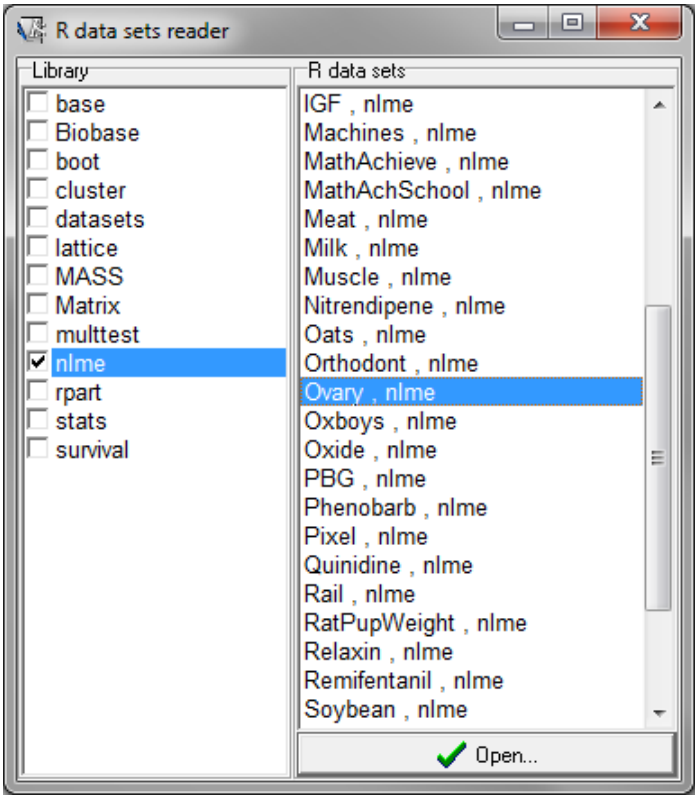



***Figure 12: Dialogue window for importing data from R libraries.***

In this window the *nlme* library is checked, and to the right is the list of data files of this library. Double clicking on "Ovary, nlme" will open an InfoStat data table containing the corresponding data. The heading of the open table is shown below (Figure 13).

Ovary

| case | Mare | Time | follicles |
|---|---|---|---|
| 1 | 1.00 | -0.14 | 20.00 |
| 2 | 1.00 | -0.09 | 15.00 |
| 3 | 1.00 | -0.05 | 19.00 |
| 4 | 1.00 | 0.00 | 16.00 |
| 5 | 1.00 | 0.05 | 13.00 |
| 6 | 1.00 | 0.09 | 10.00 |
| 7 | 1.00 | 0.14 | 12.00 |

Real | records: 308*3

***Figure 13: Heading of the data table (Ovary file).***

A graph of the relation between the number of follicles and time is shown below (Figure 14).

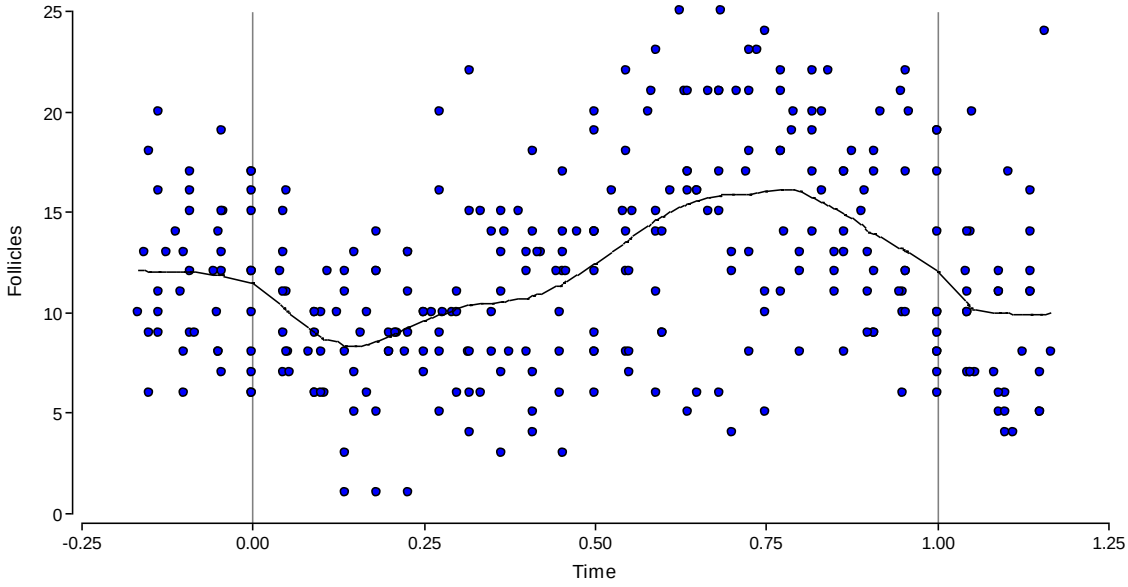



***Figure 14. Relationship between the number of follicles and time.***

Pinheiro & Bates (2004) propose to fit a model where the number of follicles depends linearly on sine (2*pi**Time*) and cosine (2*pi**Time*). This model tries to reflect the cyclical variations of the number of follicles through the inclusion of trigonometric functions. They also propose the inclusion of a random effect, *Mare*, on the constant of the model and a first-order autocorrelation of the errors within each mare. A random

effect was included to model the lack of independence that results from subject-dependent effects expressed as parallel profiles of the number of follicles through time. The proposed model would have the following general form:

$$y_{it} = \beta_0 + \beta_1 sin\left(2 * pi * Time\right) + \beta_2 cos\left(2 * pi * Time\right) + b_{0i} + \varepsilon_{it} \qquad (2)$$

where the random components are $b_{0i} \sim N\left(0, \sigma^2_{bo}\right)$ and $\varepsilon_{it} \sim N\left(0, \sigma^2\right)$, and are supposed to be independent.

On the other hand, the inclusion of a first-order autocorrelation within each mare allows the modeling of an eventual serial correlation. To specify this model in InfoStat, we will indicate that *follicles* is the dependent variable, that *Mare* is the classification criterion, and that *Time* is a covariate.

### *Specification of the fixed part*

The fixed part of the model will be indicated as shown in Figure 15. InfoStat verifies that the elements in this window correspond to the factors and covariates listed on the right-hand side of the window.

If this is not the case, because lowercase and uppercase letters have not been used consistently (R is sensitive to typography), then InfoStat substitutes those terms for the appropriate ones. If there are words that InfoStat cannot interpret (such as *sin*, *cos* and *pi*, in this case), then the line is marked in red when the user press <Enter>. This does not necessarily mean that they are incorrect, but that they could be, and warns the user to verify them.

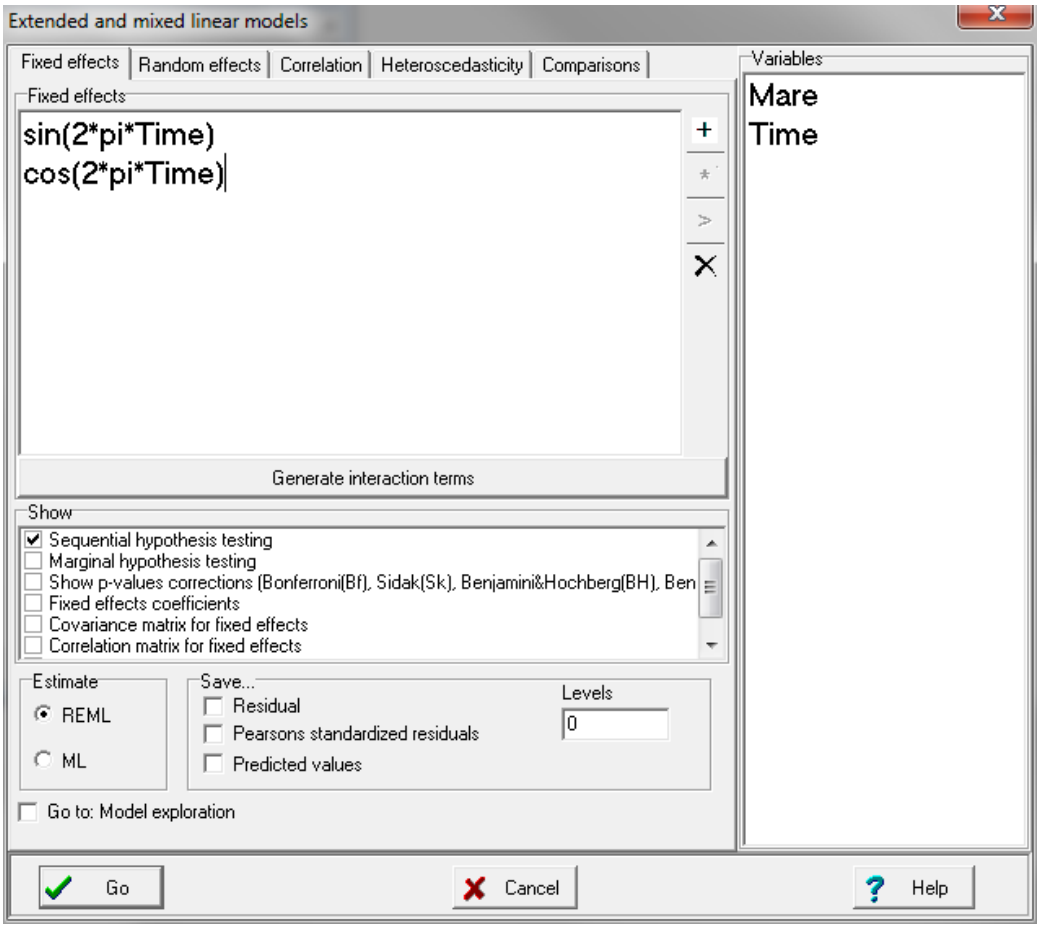



***Figure 15: Window displaying the Fixed effects tab (Ovary file).***

## *Specification of the random part*

The random part is added to the model by including the *Mare* factor to the stratification criteria list in the Random tab. In this way, the *Mare* random factor is automatically associated to the constant term of the model as shown in Figure 16. This way of including the ramdom factor introduces a subject-specific effect on the overall level of the response (follicles). Thus, the predicted profiles at subject level (Mare) of the number of follicles along time are parallels. There are other terms in the model: *sin*(2**pi***Time*) and *cos*(2**pi***Time*) that have not yet been associated with any random effects.

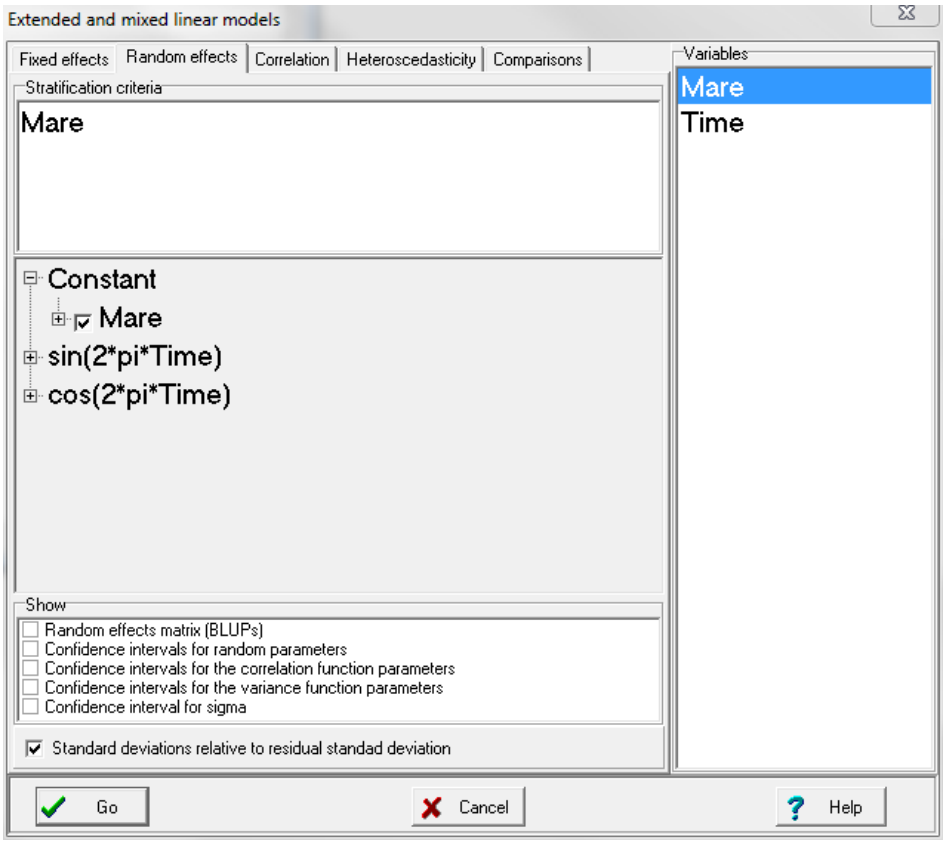



***Figure 16: Window displaying the Random effects tab (Ovary file).***

## *Specification of the correlation of errors*

The specification of the first-order autoregressive correlation of the errors within each mare is indicated in the *Correlation*[1] tab, as illustrated in Figure 17. In R, there are two groups of correlation functions. The first corresponds to serial correlation functions, where data are assumed to be acquired in a sequence, and the second group models spatial correlations and the data have to be spatially referenced. In the first group we find the following functions: *compound symmetry*, *without structure*, *first-order autoregressive*, *first-order continuous autoregressive*, and *ARMA* (*p*,*q*), where *p* indicates the number of autoregressive terms and *q* indicates the number of moving average terms. All of these models assume that data are ordered in a sequence. By default, InfoStat assumes the sequence in which the data are arranged in the file, but if

[1] If the errors are assumed to be independent (not correlated), then the first option of the correlation structure list should be selected (selected by default).

there is a variable that indexes the order of the data in a different manner, this should be indicated in the *Variable that indicates the order of observations* box (to activate this box, one of the correlation structures should be selected). This variable must be an integer for the autoregressive option. Because of this, in the sentence translated to R language, InfoStat adds an indication so that the variable is interpreted as an integer. In the illustrated example, the variable *Time* is a real number that encodes relative time to a reference point, and it is in an inappropriate scale to be used as an ordering criterion. However, because the data are arranged by time within each *Mare*, this specification can be omitted (Figure 17).

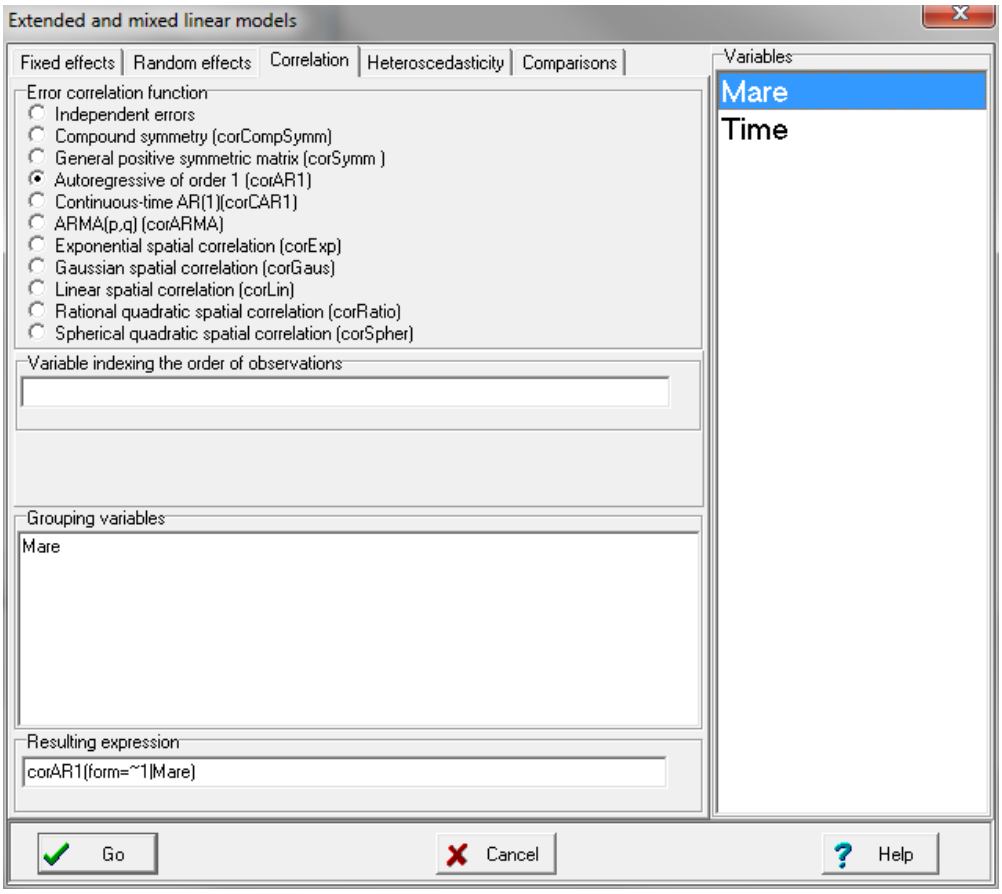



***Figure 17: Window displaying the Correlation tab (Ovary file).***

If the data are not organized in ascending order within the grouping criterion (*Mare*), a variable that indicates the order must be added. To add an ordering variable to the *Variable that indicates the order of observations* box, its name can be written, or dragged with the mouse, from the variables list. It is common for the correlation structure to be associated to a grouping criterion, *Mare* in this case. This is indicated in the panel labeled *Grouping variables* (to activate this text box one of the correlation

structures must to be selected). If more than one criterion is included, InfoStat constructs as many groups as there are combination levels in the specified classification factors. At the bottom of the window labeled *Resulting expression,* the R expression that is being specified for the component "corr=" of *gls* or *lme* is shown. This expression is only informative and cannot be edited.

Below we present the complete output for the fitted model containing an analysis of variance table for the fixed effects, which in this case are sequential tests on the slopes associated with the covariates *sin*(2**pi***Time*) and *cos*(2**pi***Time*). Note that the standard deviation of the random component of the constant is 0.77 times the residual standard deviation and that the parameter *phi* of the autoregressive model is 0.61.

**Extended and mixed linear models**

**R specification of the model**

```
model006_follicles_REML<-lme(follicles~1+sin(2*pi*Time)+cos(2*pi*Time)
,random=list(Mare=pdIdent(~1))
,correlation=corAR1(form=~1|Mare)
,method="REML"
,na.action=na.omit
,data=R.data06
,keep.data=FALSE)
```

**Results for model: model006_follicles_REML**

*Dependent variable:follicles*

**Fit measurements**

| N | AIC | BIC | logLik | Sigma | R2_0 | R2_1 |
|---|---|---|---|---|---|---|
| 308 | 1562.45 | 1584.77 | -775.22 | 3.67 | 0.21 | 0.56 |

*Smaller AIC and BIC is better*

**Sequential hypothesis testing**

| | numDF | denDF | F-value | p-value |
|---|---|---|---|---|
| (Intercept) | 1 | 295 | 163.29 | <0.0001 |
| sin(2 * pi * Time) | 1 | 295 | 34.39 | <0.0001 |
| cos(2 * pi * Time) | 1 | 295 | 2.94 | 0.0877 |

**Random effects parameters**

*Covariance model for random effects: pdIdent*
*Formula: ~1|Mare*

*Standard deviations relative to residual standard deviation and correlation*

| | (const) |
|---|---|
| (const) | 0.77 |

**Correlation structure**

*Correlation model: AR(1)*
*Formula: ~ 1 | Mare*

*Model parameters*

| Parameter | Estim. |
|---|---|
| Phi | 0.61 |

The predicted values by the fitted model versus time are shown in Figure 18. The black solid line represents the estimation of the population average and corresponds to the estimations based on the fixed part of the model. To obtain the estimates to draw this curve, the user must indicate in the *Fixed effect tab* that the *Predicted value*s are requested. By default the *Level* of *Predicted values* is zero (indicated in the *Levels* edit-box), which indicates that predictions are based only on the fixed part of the model.

The dotted curves parallel to the population average curve (solid line) are the predictions for each mare profile derived from the inclusion of the random effect (subject-specific) on the constant. To obtain the predictions to draw these curves the user must indicate in the *Fixed effect tab* that the *Predicted value*s of level 1 are also requested. To do this the user must type: 0;1 in the *Levels* edit-box.

To check the adequacy of the model we identified the points corresponding to each mare in Figure 14 and draw a smooth curve for each one as shown in Figure 19. Comparing Figure 18 and Figure 19 it is clear that each mare has its own biological timing that is over-simplified by the model we have just fitted. How do we include in the model the subject-specific variability observed in Figure 19? The simplest way to include this subject-specific behavior is to add more random effects to model of equation (2). As result, we have the following model:

$$\begin{aligned} y_{it} &= \beta_0 + \beta_1 \sin\left(2 * pi * Time\right) + \beta_2 \sin\left(2 * pi * Time\right) \\ &+ b_{0i} + b_{1i} \sin\left(2 * pi * Time\right) + b_{2i} \cos\left(2 * pi * Time\right) + \varepsilon_{it} \end{aligned} \tag{3}$$

where the random components are $b_{0i} \sim N\left(0, \sigma^2_{bo}\right)$, $b_{1i} \sim N\left(0, \sigma^2_{b1}\right)$, $b_{2i} \sim N\left(0, \sigma^2_{b2}\right)$ and $\varepsilon_{it} \sim N\left(0, \sigma^2\right)$ and, as a first approximation they are supposed to be mutually independent.

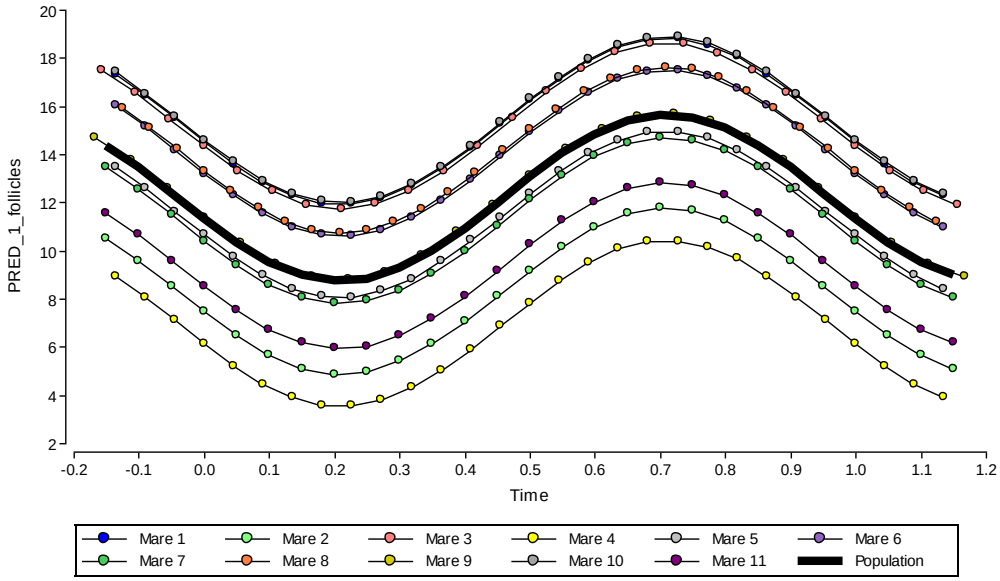



***Figure 18: Fitted functions for the population number of follicles (solid black line) and for each mare generated by the random effect on the constant (Ovary file).***

To fit model (3) we need to make some changes to the data set because of some restrictions in the use of formulas in the Random effects tab. Therefore, we calculated $\sin T = \sin(2 * pi * Time)$ and $\cos T = \cos(2 * pi * Time)$ as new variables in the dataset. In the fixed part of the model, instead of specifying a list of covariables, we specify in a single line: $1 + \sin T + \cos T$, as shown in Figure 20. This way of specifying the fixed part of the model does not affct the fixed effects estimations but allows us to easily introduce the random effects: $b_{0i}, b_{1i}, b_{2i}$. Then, in the *Random effects* tab, we especify the random effecs as shown in Figure 21. Note that the covariance structure assumed for these random has been specified as pdDiag, which means that the variances of each random component is different and that these components are not correlated. The results of fitting this model are shown in Figure 22.

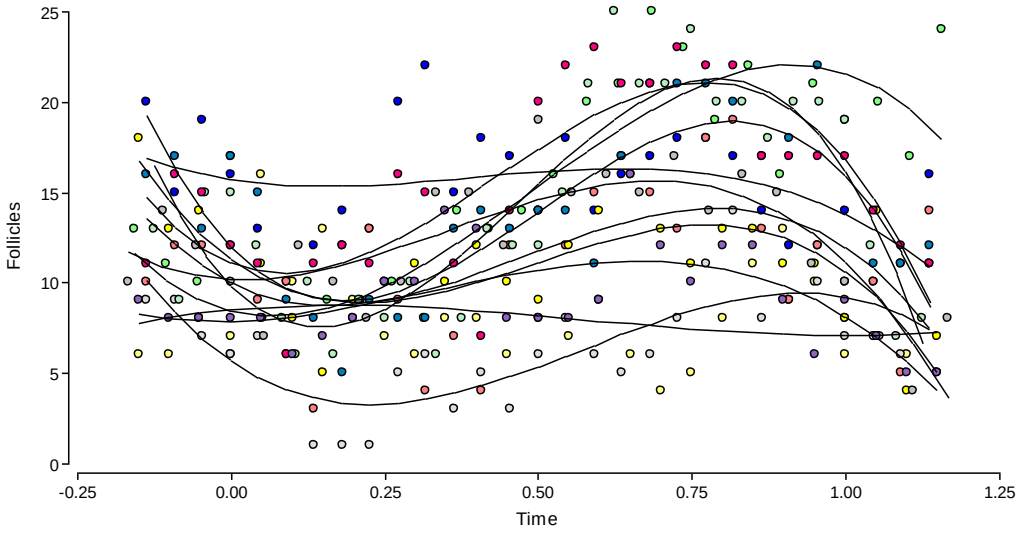



***Figure 19: Smooth functions (third order polynomial) for the number of follicles (solid black lines) for each mare generated by the random effect on the constant (Ovary file).***

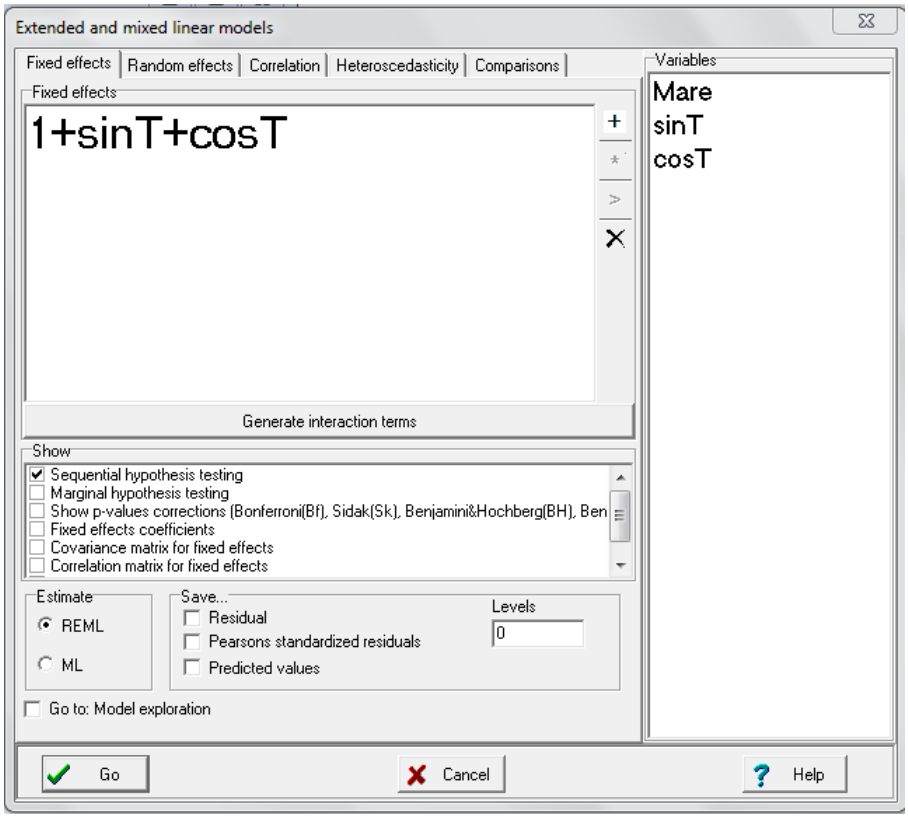



***Figure 20: Specification of the fixed part of model (3)***

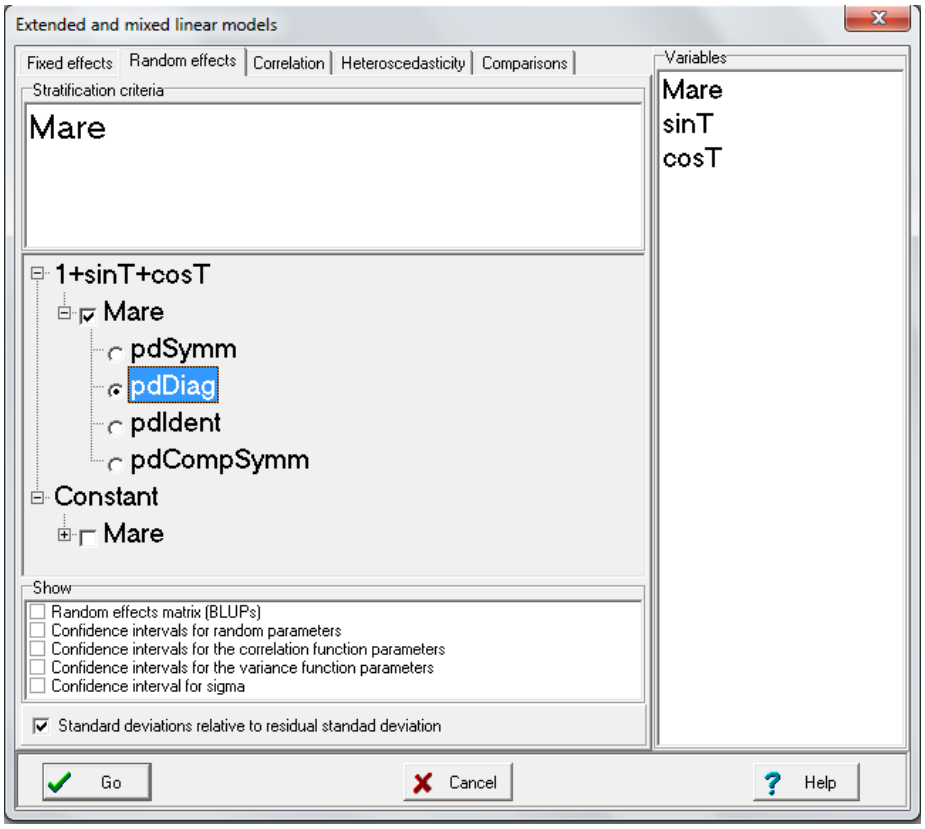



***Figure 21: Specification of the random part of model (3). Different variances for each random effect.***

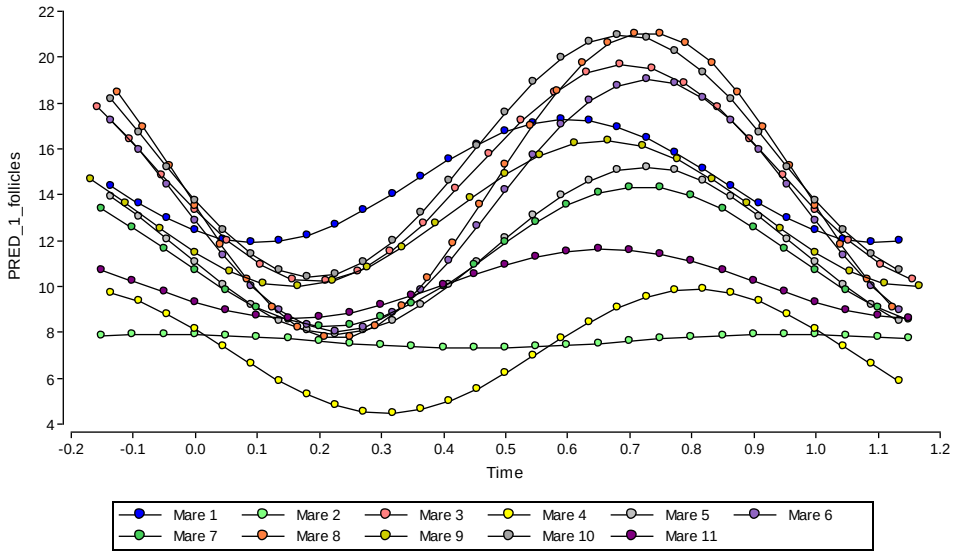



***Figure 22: Predicted values for the number of follicles of each mare generated by the inclusion of random effects on all parameters of the fixed part of the model (pdDiag covariance structure) (Ovary file).***

In Figure 22 we can see the effect of adjusting subject-specific curves for each mare, which permits more realistic representation of the mare individual profiles. Nevertheless, from a statistical point of view, it is not appropriate to assume independence among random effects on the parameters of a regression model. To

specify correlation among random effects, we specify the covariance structure as pdSymm. This is shown in Figure 23. The results of this fit are shown in Figure 24.

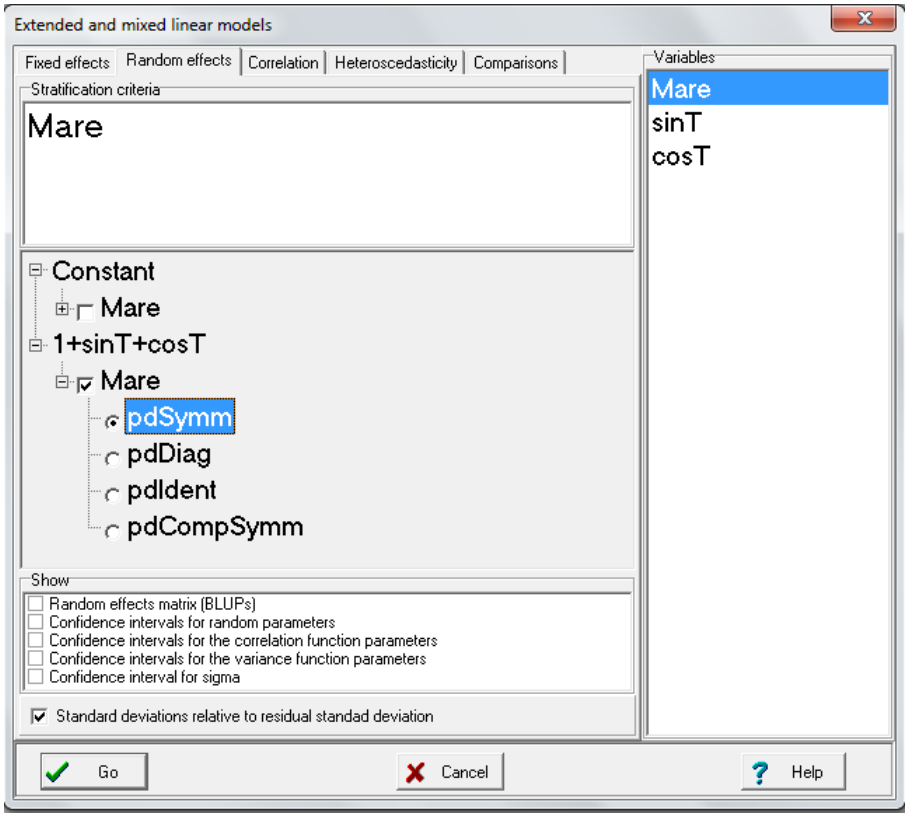



***Figure 23: Specification of the random part of model (3), with different variances of each random effect and random effects correlated .***

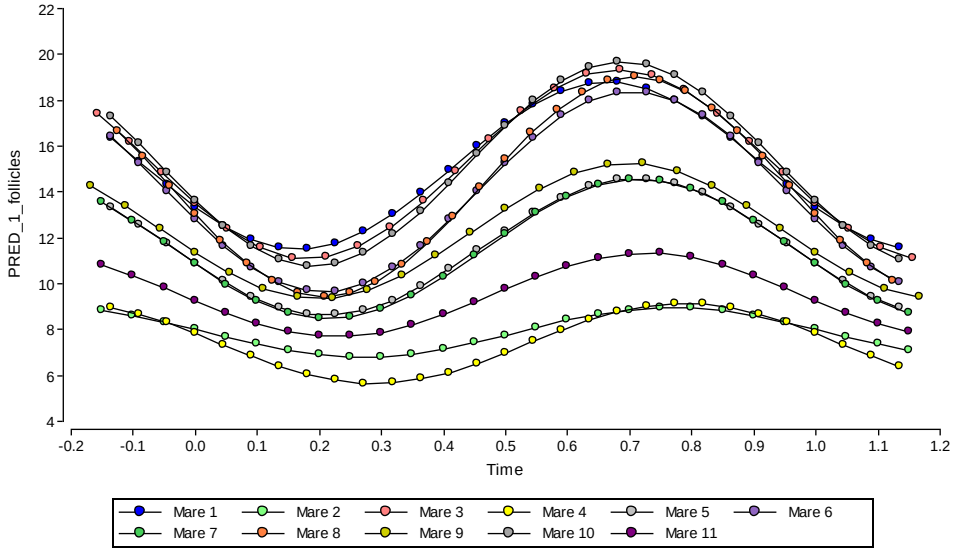



***Figure 24: Predicted values for the number of follicles of each mare generated by the inclusion of random effects on all parameters of the fixed part of the model (pdSym covariance structure) (Ovary file).***

### *Specification of the error variance structure*

This module allows the estimation of heteroscedastic models. However, heteroscedasticity does not have a single origin and hence it can be modeled in the same way that the correlation of errors can be modeled. The errors variance model can be specified in the following way: $\text{var}(\varepsilon_i) = \sigma^2 g^2(\mu_i, \mathbf{z}_i, \boldsymbol{\delta})$ where $g(.)$ is known as the *variance function*. This function can depend on the expected value $(\mu_i)$ of $Y_i$ (the response variable), a set of explanatory variables $(\mathbf{z}_i)$, and a parameters vector $(\boldsymbol{\delta})$. Through R, InfoStat estimates the parameters $(\boldsymbol{\delta})$ according to the selected variance function. The *Heteroscedasticity* tab is shown in Figure 25. The following variance functions are permitted: (*varIdent*), exponential (*varExp*), power (*varPower*), power shifted by a constant (*varConstPower*), or fixed (*varFixed*). R allows that various models to be overlapped, in other words, that for certain part of the dataset the variance can be associated with one covariate, and for other part with another covariate. The simultaneous specification of various models for the variance function is obtained by simply marking and specifying each of the components and adding them to the variance functions list. InfoStat constructs the appropriate sentence for R.

In the *Heteroscedasticity* tab for the follicles example, we have indicated that the errors variance is different for each mare, by selecting *varIdent* as the variance function model and writing *Mare* in *Grouping variables*.

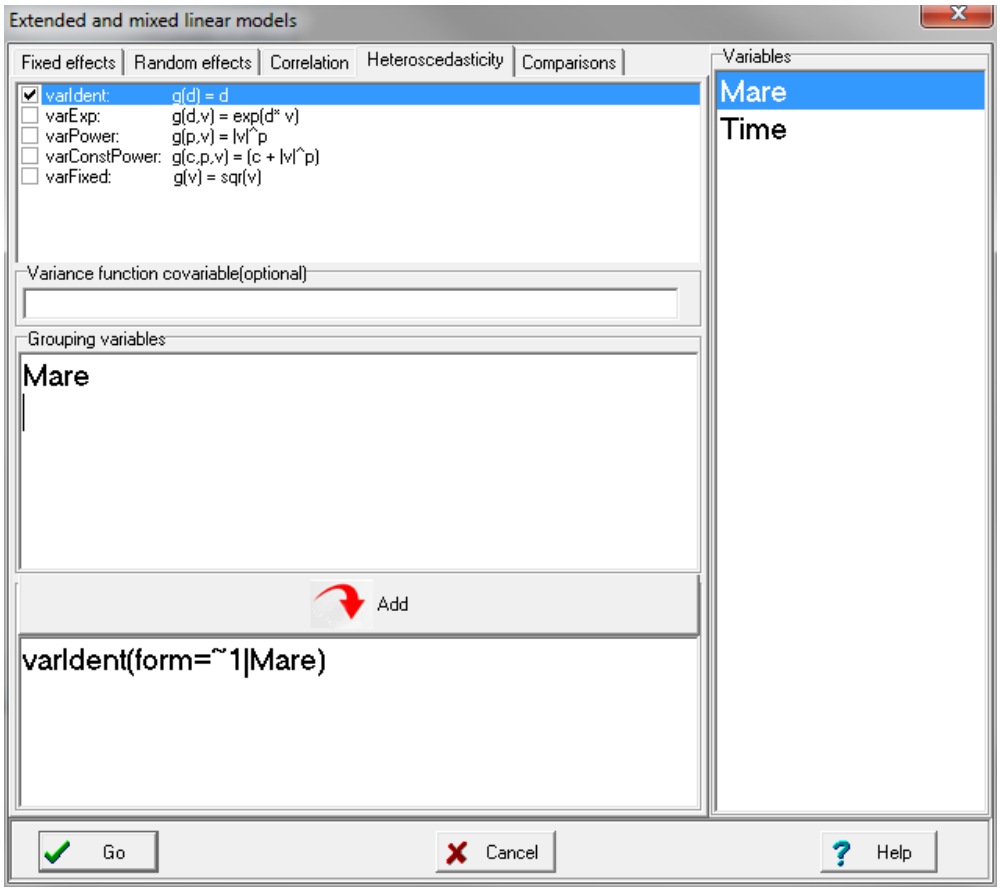



***Figure 25: Window displaying the Heteroscedasticity tab (Ovary file).***

Below is the output for the fitted model, including estimations of the standard deviation of the error for each mare. The standard deviations are also expressed relative to the residual standard deviation. Moreover, the first level of the specified grouping variable used to calculate these differential standard deviations always starts with 1, otherwise the model would not be identifiable. In the output you can see that, compared to the other females, female 5 has a larger variability in the number of follicles.

The model considered in Equation (4) with heterogeneous residual variances would be:

$$y_{it} = \beta_0 + \beta_1 sin\left(2 * pi * Time\right) + \beta_2 cos\left(2 * pi * Time\right) + b_{0i} + \varepsilon_{it} \qquad (5)$$

where the random components are now $b_{0i} \sim N\left(0, \sigma^2_{bo}\right)$ and $\varepsilon_{it} \sim N\left(0, \sigma^2_{i}\right)$. Note that the residual variance is indexed with the mare identifier.

As usual, the random components of the model are assumed to be independent. Next, if we take a mare at random, the variance of the response will be the sum of the variances of the random part, in other words $\text{var}(y_{it}) = \sigma^2_{b0} + \sigma^2_{i}$, that is $(3.57*0.8)^2 + (3.57*g_i)^2$,

where $g_i$ is the variance function for a mare selected at random. Now then, conditional to a given mare (eg., 5), the individual effect ($b_{0i}$) is fixed, so the variance of mare 5 is only associated with the residual part, and furthermore the variance function is specified (in other words, we need to use $g_5$) and the conditional variance would be $(3.57*1.34)^2$.

**Extended and mixed linear models**

**R specification of the model**

```
model012_follicles_REML<-lme(follicles~1+sin(2*pi*Time)+cos(2*pi*Time)
,random=list(Mare=pdIdent(~1))
,weight=varComb(varIdent(form=~1|Mare))
,correlation=corAR1(form=~1|Mare)
,method="REML"
,na.action=na.omit
,data=R.data12
,keep.data=FALSE)
```

**Results for model: model012_follicles_REML**

*Dependent variable:follicles*

**Fit measurements**

| N | AIC | BIC | logLik | Sigma | R2_0 | R2_1 |
|---|---|---|---|---|---|---|
| 308 | 1569.02 | 1628.55 | -768.51 | 3.57 | 0.21 | 0.56 |

*Smaller AIC and BIC is better*

**Sequential hypothesis testing**

| | numDF | denDF | F-value | p-value |
|---|---|---|---|---|
| (Intercept) | 1 | 295 | 156.36 | <0.0001 |
| sin(2 * pi * Time) | 1 | 295 | 34.22 | <0.0001 |
| cos(2 * pi * Time) | 1 | 295 | 3.18 | 0.0756 |

**Random effects parameters**

*Covariance model for random effects: pdIdent*
*Formula: ~1|Mare*

*Standard deviations relative to residual standard deviation and correlation*

| | (const) |
|---|---|
| (const) | 0.80 |

**Correlation structure**

*Correlation model: AR(1)*
*Formula: ~ 1 | Mare*

*Model parameters*

| Parameter | Estim. |
|---|---|
| Phi | 0.61 |

**Variance structure**

*Variance model: varIdent*
*Formula: ~ 1 | Mare*

*Variance-function parameters*

| Parameter | Estim. |
|---|---|
| 1 | 1.00 |
| 2 | 1.01 |
| 3 | 1.20 |
| 4 | 0.82 |
| 5 | 1.34 |
| 6 | 1.05 |
| 7 | 0.92 |
| 8 | 1.06 |
| 9 | 0.93 |
| 10 | 0.99 |
| 11 | 0.77 |

### Analysis of a fitted model

When InfoStat fits an extended or mixed linear model with the *Estimation* menu, the *Analysis-exploration of the estimated models* menu is activated. In this dialogue, various tabs are shown, as seen in Figure 26.

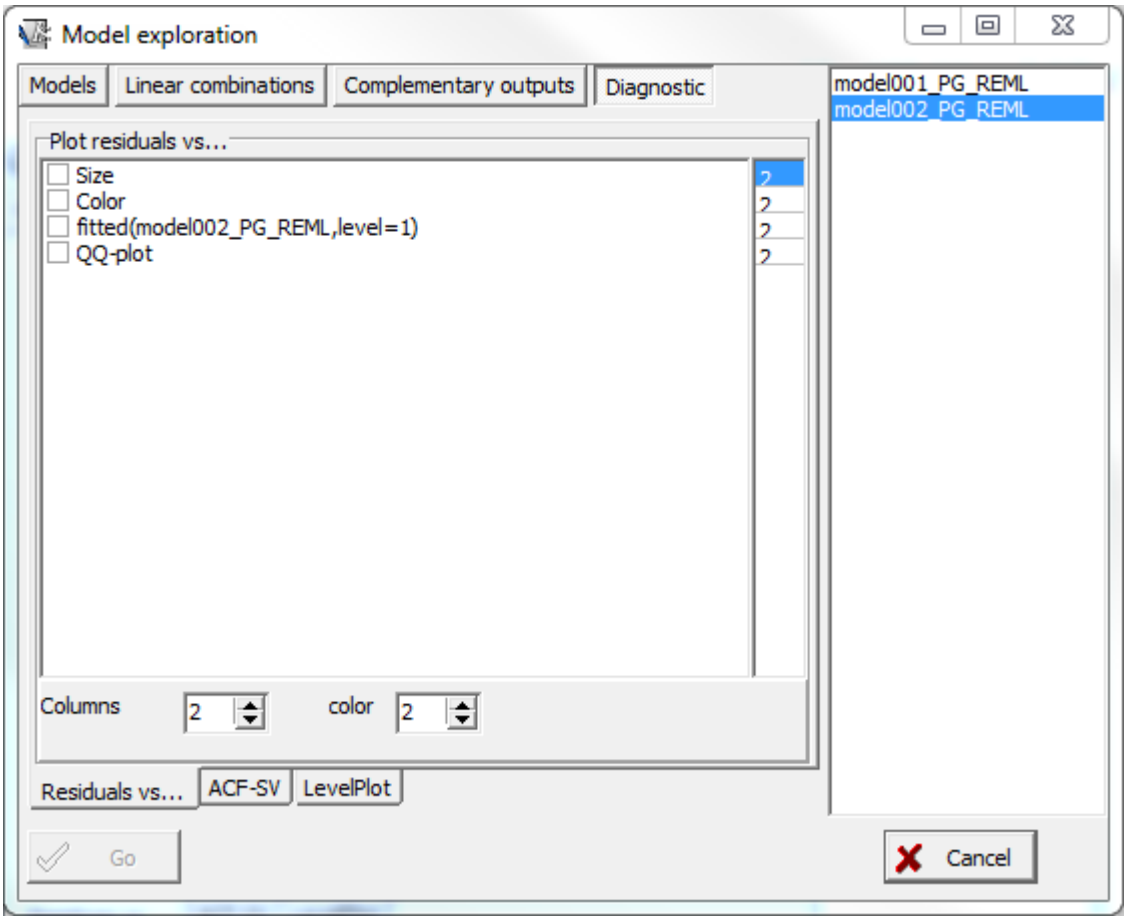



***Figure 26: Model exploration window displaying the Diagnostic tab (Atriplex.IDB2 file).***

The example used in this case is from the *Atriplex.IDB2* file, with which two fixed effects models where estimated: `model000_PG_REML`, which contains the effects *Size*, *Color* and their interaction, and `model001_PG_REML`, which only contains the main effects *Size* and *Color*.

The *Models* tab only appears in the case that there is more than one estimated model and shows a list of the evaluated models in a check-list. The selected models are listed along with their respective summary statistics and a hypothesis test of model equality; the applicability of the latter should be interpreted with caution, since not all of the models are strictly comparable. In any case, the AIC and BIC criteria are good indicators for the selection of a more parsimonious model.

The purpose of the *Linear combinations* tab is to test linear combinations hypotheses. The null hypothesis is that the expected value of the linear combination is zero. This dialogue window lists the fixed parameters of the model that were selected from the list shown on the right-hand side of the screen (Important: the last one on the list is always selected by default). At the bottom of the screen, there is an edition field where the constants of the linear combination can be specified. As the coefficients are added, the corresponding parameters are colored to facilitate the specification of the constants, as shown in Figure 27.

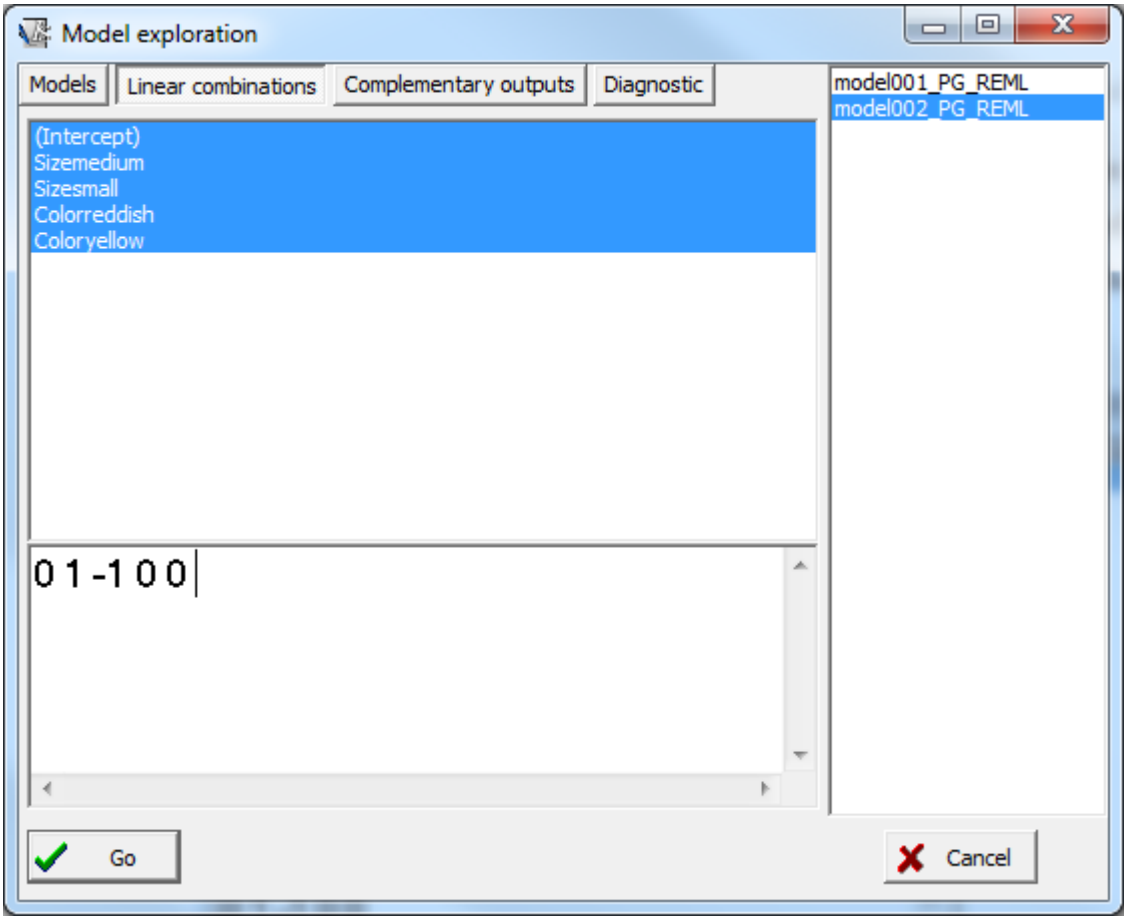



***Figure 27: Model exploration window displaying the Linear combination tab (Atriplex.IDB2 file).***

Finally, the *Diagnostic* tab has three subtabs (Figure 26). The first, identified as "*Residuals vs*…" has devices to easy generate *boxplot* graphs for the standardized residuals vs. each of the fixed factors of the model, or scatter plots of the standardized residuals and the covariates of the model, and scatter plots of the standardized residuals vs. the fitted values. In the same way, it is possible to obtain the normal *Q-Q plot*. The second tab, identified as "*ACF-SV"*, allows the user to generate a graph of the

autocorrelation function (useful for the diagnosis of serial correlations), and the third one, identified as *LevelPlot*, allows the user to generate residuals vs. spatial correlations graphs to construct a map of the directions and intensity of the residuals. This tool is useful in spatial correlation diagnostics.

To exemplify the use of the *ACF-FV* tab, let us consider the follicles example (*Ovary* file). In this example it is argued that the purpose of including the first-order autoregressive term was to correct a lack of independence generated by the discrepancies between the individual cycles of every mare with respect to the individual cycles that only differed from the average population by a constant. The serial autocorrelation graph of the residuals that corresponds to a model without the inclusion of the first-order autocorrelation shows a clear autoregressive pattern (Figure 28). On the other hand, the residual autocorrelation graph for the model that includes the autocorrelation through a first-order autoregressive term corrects the lack of independence (Figure 29).

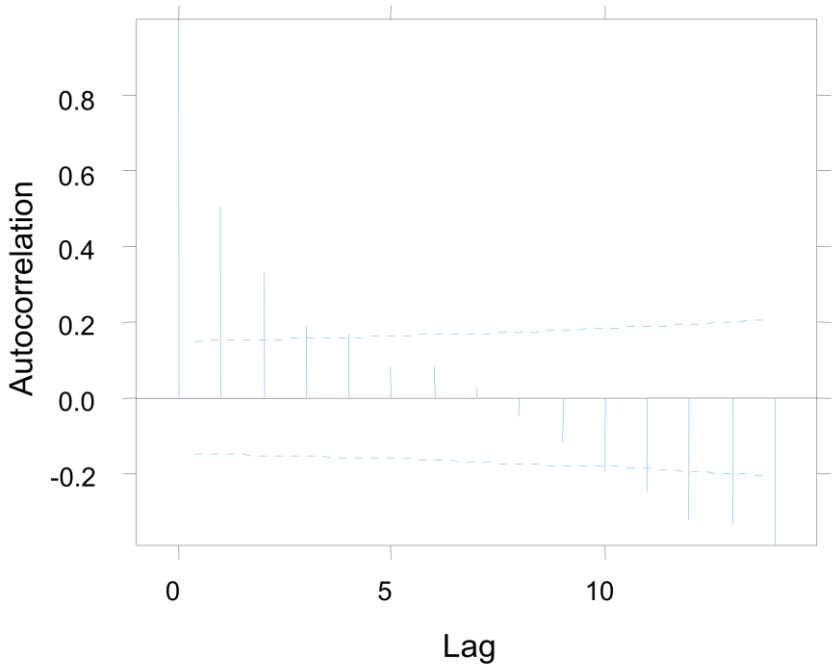



***Figure 28: Residual autocorrelation function of the model shown in Equation, excluding serial autocorrelation.***

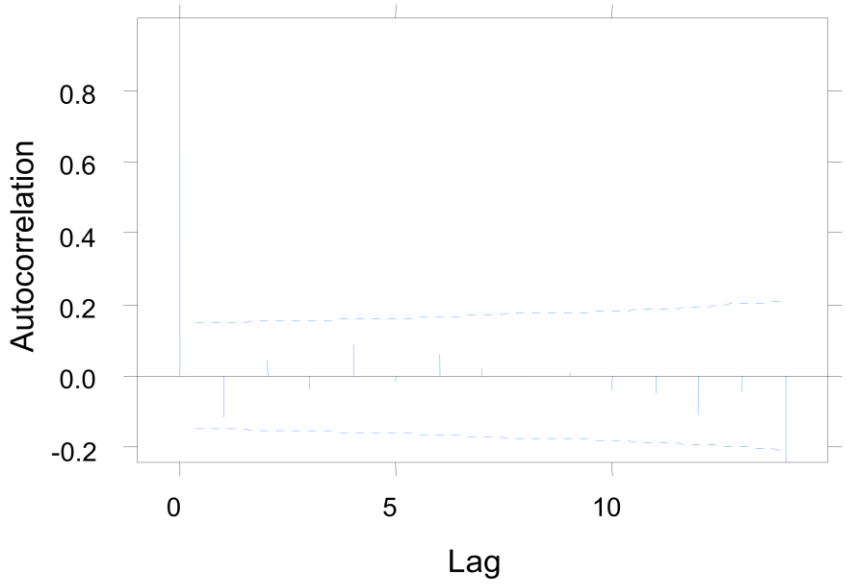



***Figure 29: Residual autocorrelation function of the model presented in Equation (2), including serial autocorrelation.***

The devices on the *Diagnostic* tab allow the researcher to quickly diagnose any eventual problem with the fit of the fixed part as well as for the random part of the model. The next section provides examples illustrating the use of these tools more extensively.

# Examples of Applications of Extended and Mixed Linear Models

### *Estimation of variance components*

In research areas such as animal or plant breeding, the estimation of the variance components it is of particular interest. These are used to obtain heritability, response to the selection, additive genetic variance, genetic differentiation coefficients, etc. The mixed linear models can be used to estimate the variance components using restricted maximum likelihood (REML) estimators.

In many genetic studies, several populations are used which are represented by one or more individuals of different families. In this case we have two factors in the model: the populations and the families within each population. To exemplify the use of variance components, the data file *VarCom.IDB2* (Navarro et al. 2005) is used. These data come from a trial with seven cedar populations (*Cedrela odorata* L.) with a total of 115 families. Some families have repetitions available while others do not. Moreover, the number of families within each population is not the same. The registered variables are average seed length (*length*), stem diameter (*diameter*), *stem length*, and *number of leaves* in cedar seedlings.

In addition to estimating the variance components, the researchers are also interested in comparing the population means. We can study various inference spaces, according to the design and the interests of the researchers. If the populations are a random sample of a large set of populations, then the inference will be aimed at this large set of populations. The effect of the studied populations is random, and the interest will be the estimation of the variance components due to the variance among populations and among the families within the populations. Another point of interest will be the BLUP predictors of the random effects (especially those of the population effects).

If the inference is oriented only toward the studied populations, the population effect is fixed, and the main interest is to estimate and compare the population means. If the population mean is interpreted as an average throughout all possible families of that particular population (not only those studied), then the family effect is random. In this case, it would be of interest to estimate the variance component due to variance among families within the populations, and to predict the effects of the studied families (BLUP).

A third inference space is when the interest resides only in the studied populations and families. In this case both effects are fixed. This kind of model has several limitations, both in its interpretation and in its implementation. Due to this, we do not study this model in this tutorial.

For the analysis of the *VarCom.IDB2* data file, the first two discussed cases will be fitted:

Model 1: Random populations and random families

Model 2: Fixed populations and random families

First, we select the *Statistics* menu; then the *Extended and mixed linear models* submenu, and then *Model estimation*. When the selection is done, the variables selection window will show, where we specify *Length*, *Diameter*, *Stem length* and *number of leaves* as dependent variables, and *Population* and *Family* as classification variables (Figure 30).

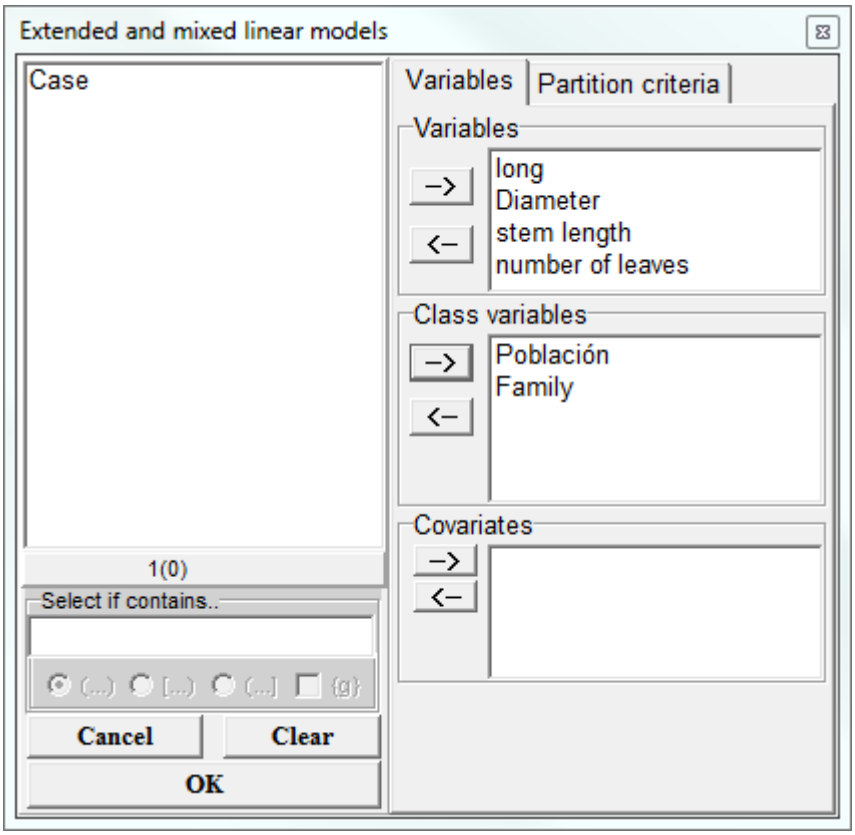



***Figure 30: Variables selection window for extended and mixed linear models (VarCom.IDB2 file).***

Model 1: For the estimation of the variance components, the variables should be specified as in Figure 30. Afterwards, in the *Random effects* tab, indicate first *Population* and then *Family*, since R assumes that the different random components that

are being sequentially added are nested in the previously declared factors. In the *Show* sub-window, the options shown in Figure 31 are checked and the default option for the *Standard deviations relative to residual standard deviation* is unchecked.

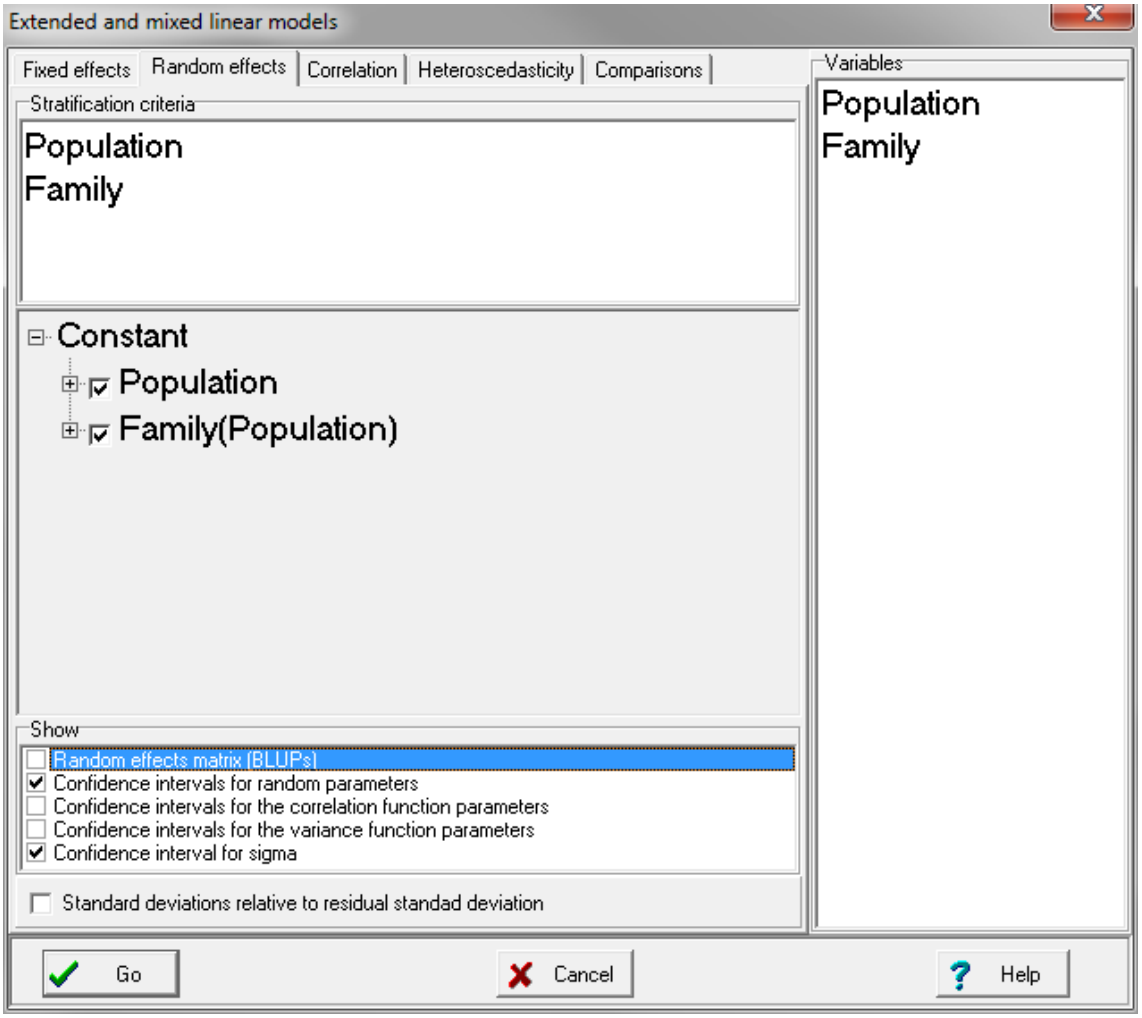



***Figure 31: Window displaying the Random effects tab for Model 1(VarCom.IDB2 file).***

In the *Fixed effects* tab no effect should appear, and the estimation method should be that of restricted maximum likelihood (REML), which is the default option. Note that the default option *Standard deviations relative to residual standard deviation* is deactivated, therefore the estimations shown will be the absolute standard deviations. The output obtained with these specifications only for the *length* variable is shown below.

**Extended and mixed linear models**

**R specification of the model**

```
model006_length_REML<-lme(length~1
,random=list(Population=pdIdent(~1)
,Family=pdIdent(~1))
,method="REML"
,na.action=na.omit
,data=R.data06
,keep.data=FALSE)
```

**Results for model: model006_length_REML**

*Dependent variable:length*

**Fit measurements**

| N | AIC | BIC | logLik | Sigma | R2_0 | R2_1 | R2_2 |
|---|---|---|---|---|---|---|---|
| 214 | 2016.47 | 2029.91 | -1004.23 | 21.53 | | 0.51 | 0.76 |

*Smaller AIC and BIC is better*

**Sequential hypothesis testing**

| | numDF | denDF | F-value | p-value |
|---|---|---|---|---|
| (Intercept) | 1 | 108 | 22.68 | <0.0001 |

**Random effects parameters**

*Covariance model for random effects: pdIdent*
*Formula: ~1|Population*

*Standard deviations and correlations*

| | (const) |
|---|---|
| (const) | 27.16 |

*Covariance model for random effects: pdIdent*
*Formula: ~1|Family in Population*

*Standard deviations and correlations*

| | (const) |
|---|---|
| (const) | 14.80 |

**Confidece intervals (95%) for the random effects parameters**

*Formula: ~1|Population*

| | LB(95%) | Est. | UB(95%) |
|---|---|---|---|
| sd(const) | 15.09 | 27.16 | 48.89 |

*Formula: ~1|Family in Population*

| | LB(95%) | Est. | UB(95%) |
|---|---|---|---|
| sd(const) | 10.72 | 14.80 | 20.43 |

**Confidece interval (95%) for sigma**

| | lower | est. | upper |
|---|---|---|---|
| sigma | 18.77 | 21.53 | 24.70 |

From the standard deviation estimates and confidence intervals, the variance components and their confidence intervals are obtained (Table 1).

***Table 1. Estimated variance components (VarCom.IDB2 file)***

| Component | Estimated variance | CI for the variance | Relative variability with respect to the total (%) |
|---|---|---|---|
| Population | $\sigma^2_{pob} = 27.16^2 = 737.66$ | $(15.09^2, 48.88^2)$ | 52.0 |
| Family within population | $\sigma^2_{fam(pob)} = 14.80^2 = 219.04$ | $(10.72^2, 20.43^2)$ | 15.4 |
| Residual | $\sigma^2_{res} = 21.53^2 = 463.54$ | $(18.77^2, 24.70^2)$ | 32.6 |

According to the results shown in Table 1, it is interesting to note that the variability of the families within populations is lower than the residual variability, which implies that there is no differentiation among families within a population. Meanwhile, the higher variation is attributable to differences among populations.

Now we will see the diagnostics for Model 1, with random effects for both family and population. To do so, we go to the *Model exploration* submenu and request the diagnostic graphs (Figure 32). The diagnostic analysis of this model shows that there is a strong lack of homogeneity of residual variances (Figure 33).

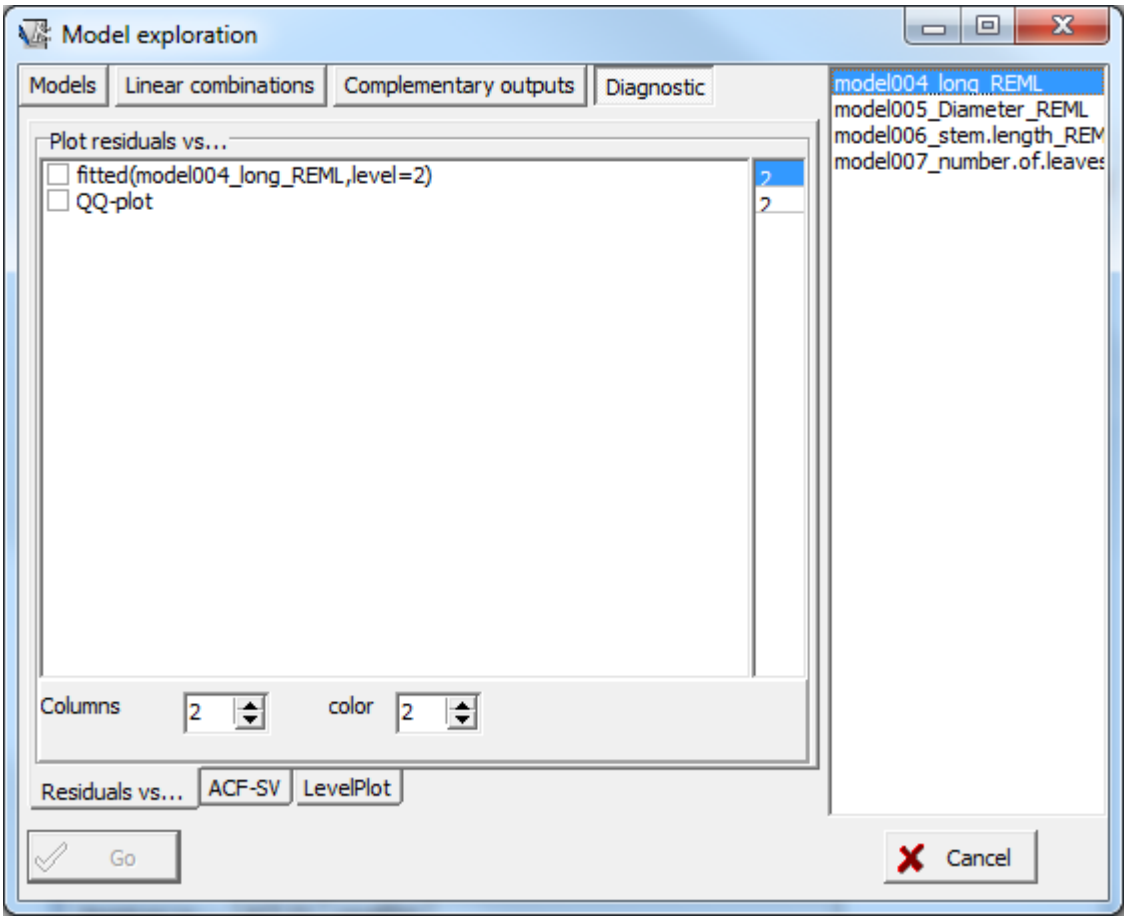



***Figure 32: Model exploration window displaying the Diagnostic tab for Model 1 (VarCom.IDB2 file).***

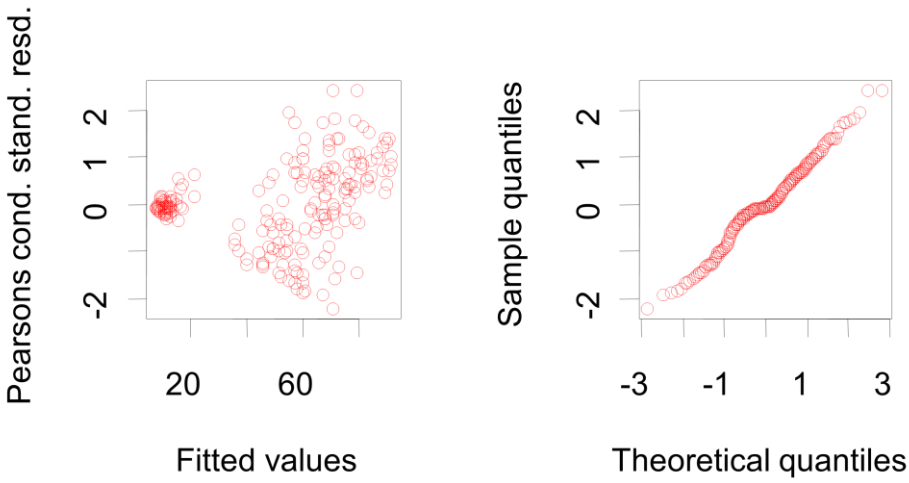



***Figure 33: Diagnostic graphs obtained for the variable Length, Model 1 (VarCom.IDB2 file).***

In Figure 33, the standardized Pearson residuals are approximations of the errors, and because of this, the heteroscedasticity observed should be modeled at this level.

To correct the lack of homogeneity at this level, fit Model 1 (*Population* and *Family* as random factors) with heterogeneous residual variances. . To incorporate the residual variances that are eventually different for each *Population* level, the population factor should be specified in the *Heteroscedasticity* tab, as shown Figure 34.

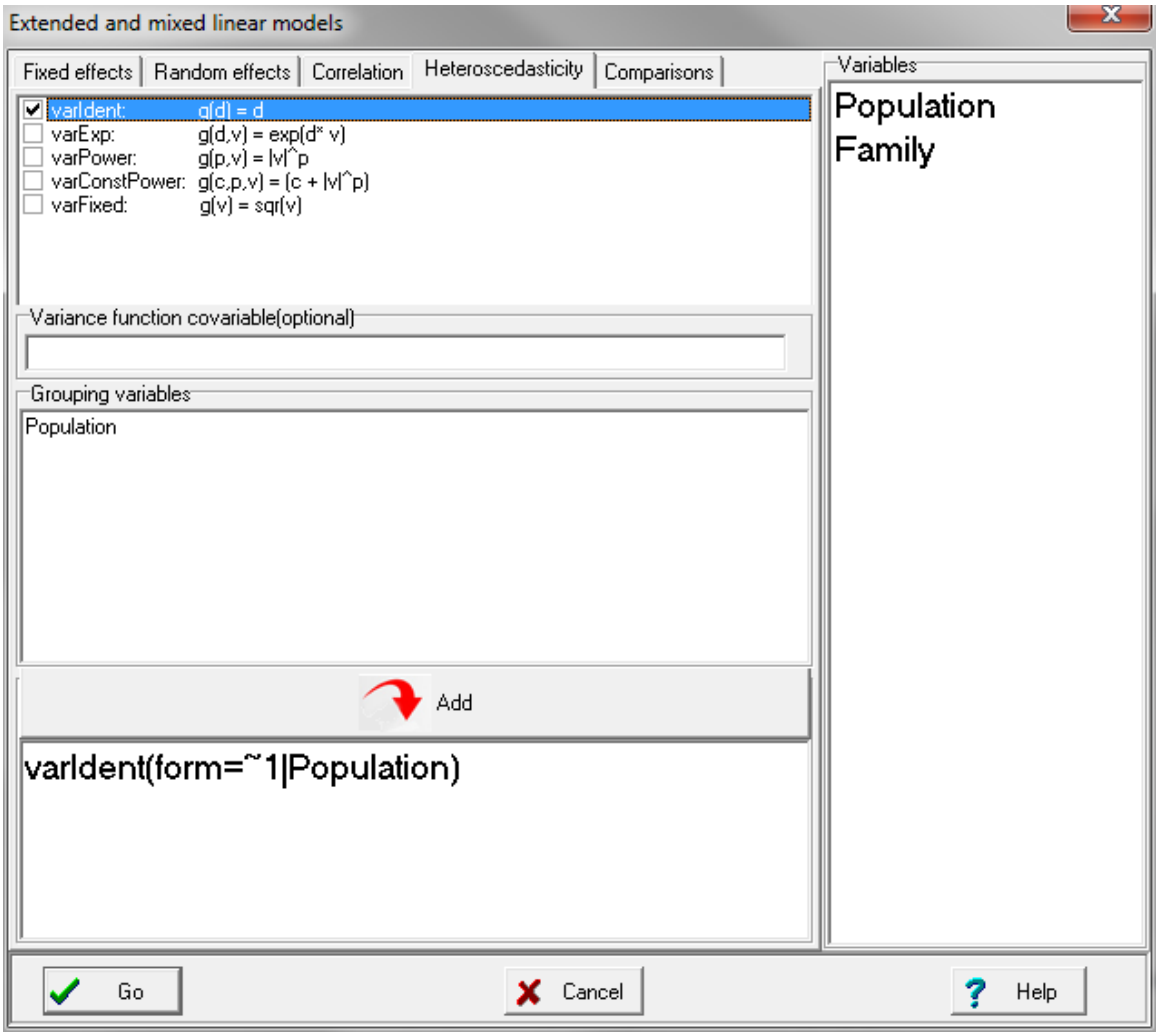



***Figure 34: Window displaying the Heteroscedasticity tab for the specification of heterogeneous variables for populations (VarCom.IDB2 file).***

Below is the output for Model 1 with heterogeneous residual variances for *Population* and the *Random effects matrix* option selected in the *Random effects* tab, in order to obtain the BLUP estimators.

```
Extended and mixed linear models

R specification of the model

model002_length_REML<-lme(length~1
```

```
,random=list(Population=pdIdent(~1)
,Family=pdIdent(~1))
,weight=varComb(varIdent(form=~1|Population))
,method="REML"
,na.action=na.omit
,data=R.data02
,keep.data=FALSE)
```

**Results for model: model002_length_REML**

*Dependent variable:length*

**Fit measurements**

| N | AIC | BIC | logLik | Sigma | R2_0 | R2_1 | R2_2 |
|---|---|---|---|---|---|---|---|
| 214 | 1872.14 | 1905.75 | -926.07 | 2.32 | | 0.51 | 0.51 |

*Smaller AIC and BIC is better*

**Sequential hypothesis testing**

| | numDF | denDF | F-value | p-value |
|---|---|---|---|---|
| (Intercept) | 1 | 108 | 21.59 | <0.0001 |

**Random effects parameters**

*Covariance model for random effects: pdIdent*
*Formula: ~1|Population*

*Standard deviations and correlations*

| | (const) |
|---|---|
| (const) | 27.72 |

*Covariance model for random effects: pdIdent*
*Formula: ~1|Family in Population*

*Standard deviations and correlations*

| | (const) |
|---|---|
| (const) | 1.56 |

**Confidece intervals (95%) for the random effects parameters**

*Formula: ~1|Population*

| | LB(95%) | Est. | UB(95%) |
|---|---|---|---|
| sd(const) | 15.61 | 27.72 | 49.24 |

*Formula: ~1|Family in Population*

| | LB(95%) | Est. | UB(95%) |
|---|---|---|---|
| sd(const) | 0.47 | 1.56 | 5.14 |

**Variance structure**

*Variance model: varIdent*
*Formula: ~ 1 | Population*

*Variance-function parameters*

| Parameter | Estim. |
|---|---|
| Charagre | 1.00 |
| Escarcega | 13.09 |
| Esclavos | 11.64 |
| La Paz | 15.94 |
| Pacífico Sur | 2.81 |
| Xpujil | 13.38 |
| Yucatán | 12.54 |

**Random effects coefficients (BLUP) (~1|Population)**

| | const |
|---|---|
| Charagre | -41.20 |
| Escarcega | 15.42 |
| Esclavos | 16.12 |
| La Paz | 19.80 |
| Pacífico Sur | -36.51 |
| Xpujil | 23.29 |
| Yucatán | 3.08 |

**Random effects coefficients (BLUP) (~1|Family in Population)**

| | const |
|---|---|
| Charagre/Ch_71 | -1.07 |
| Charagre/Ch_710 | 0.59 |
| Charagre/Ch_711 | 1.31 |
| Charagre/Ch_712 | 1.42 |
| Charagre/Ch_713 | -0.95 |
| Charagre/Ch_714 | -1.07 |
| Charagre/Ch_715 | -0.70 |
| Charagre/Ch_72 | 0.70 |
| Charagre/Ch_73 | -0.83 |
| Charagre/Ch_74 | -0.35 |
| Charagre/Ch_75 | -0.59 |
| Charagre/Ch_76 | -0.08 |
| Charagre/Ch_77 | -0.47 |
| Charagre/Ch_78 | 0.48 |
| Charagre/Ch_79 | 1.48 |
| Escarcega/Es_1126 | 7.2E-04 |
| Escarcega/Es_1127 | 0.18 |
| Escarcega/Es_1128 | 0.14 |
| Escarcega/Es_1129 | 0.07 |
| Escarcega/Es_1130 | 3.6E-04 |
| Escarcega/Es_1131 | -0.06 |
| Escarcega/Es_1132 | 0.21 |
| Escarcega/Es_1133 | 0.01 |
| Escarcega/Es_1134 | -0.11 |
| Escarcega/Es_1135 | -0.09 |
| Escarcega/Es_1136 | -0.08 |
| Escarcega/Es_1137 | -0.17 |
| Escarcega/Es_1138 | 0.16 |
| Escarcega/Es_1139 | -0.08 |
| Escarcega/Es_1142 | 0.08 |
| Escarcega/Es_1148 | -0.20 |
| Esclavos/Ec_31 | -0.08 |
| Esclavos/Ec_310 | 0.08 |
| Esclavos/Ec_311 | -0.07 |
| Esclavos/Ec_312 | -0.03 |

| | |
|---|---|
| Esclavos/Ec_313 | -0.22 |
| Esclavos/Ec_314 | 0.28 |
| Esclavos/Ec_315 | -0.34 |
| Esclavos/Ec_316 | 0.15 |
| Esclavos/Ec_317 | 0.04 |
| Esclavos/Ec_318 | -0.08 |
| Esclavos/Ec_319 | 0.04 |
| Esclavos/Ec_32 | -0.07 |
| Esclavos/Ec_320 | 0.18 |
| Esclavos/Ec_33 | -3.7E-03 |
| Esclavos/Ec_34 | -0.11 |
| Esclavos/Ec_35 | 0.15 |
| Esclavos/Ec_36 | -0.17 |
| Esclavos/Ec_37 | 0.18 |
| Esclavos/Ec_38 | 0.08 |
| Esclavos/Ec_39 | 0.05 |
| La Paz/LP_41 | -0.13 |
| La Paz/LP_410 | 0.14 |
| La Paz/LP_411 | 0.11 |
| La Paz/LP_412 | 0.16 |
| La Paz/LP_413 | -0.08 |
| La Paz/LP_414 | -0.01 |
| La Paz/LP_415 | -0.13 |
| La Paz/LP_42 | 0.01 |
| La Paz/LP_43 | -0.01 |
| La Paz/LP_44 | -0.01 |
| La Paz/LP_45 | 0.02 |
| La Paz/LP_46 | -0.07 |
| La Paz/LP_48 | -0.01 |
| La Paz/LP_49 | 0.07 |
| Pacífico Sur/PS_6204 | -0.46 |
| Pacífico Sur/PS_6206 | -0.58 |
| Pacífico Sur/PS_6207 | 0.52 |
| Pacífico Sur/PS_6208 | -0.33 |
| Pacífico Sur/PS_6209 | -0.15 |
| Pacífico Sur/PS_6210 | 0.31 |
| Pacífico Sur/PS_6211 | -0.22 |
| Pacífico Sur/PS_6212 | -0.43 |
| Pacífico Sur/PS_6213 | 0.03 |
| Pacífico Sur/PS_6214 | -0.56 |
| Pacífico Sur/PS_6215 | -0.07 |
| Pacífico Sur/PS_6216 | 1.80 |
| Pacífico Sur/PS_6217 | -0.12 |
| Pacífico Sur/PS_6218 | 0.88 |
| Pacífico Sur/PS_6219 | -0.35 |
| Pacífico Sur/PS_6220 | -0.51 |
| Pacífico Sur/PS_6221 | -0.12 |
| Pacífico Sur/PS_6222 | -0.48 |
| Pacífico Sur/PS_660 | 0.72 |
| Xpujil/Xp_11 | -0.12 |
| Xpujil/Xp_110 | 0.02 |
| Xpujil/Xp_112 | 3.8E-03 |
| Xpujil/Xp_113 | -0.07 |
| Xpujil/Xp_114 | 0.02 |
| Xpujil/Xp_115 | -0.12 |
| Xpujil/Xp_116 | 0.17 |
| Xpujil/Xp_117 | 0.11 |
| Xpujil/Xp_118 | 0.08 |
| Xpujil/Xp_119 | 0.18 |
| Xpujil/Xp_12 | -0.01 |
| Xpujil/Xp_120 | 0.19 |

| | |
|---|---|
| Xpujil/Xp_122 | -0.21 |
| Xpujil/Xp_123 | -0.27 |
| Xpujil/Xp_15 | 0.02 |
| Xpujil/Xp_16 | 0.03 |
| Xpujil/Xp_17 | 0.03 |
| Xpujil/Xp_18 | -0.05 |
| Xpujil/Xp_19 | 0.07 |
| Yucatán/Yu_1111 | -0.17 |
| Yucatán/Yu_1114 | -0.19 |
| Yucatán/Yu_1115 | -0.04 |
| Yucatán/Yu_1116 | 0.02 |
| Yucatán/Yu_1117 | 0.05 |
| Yucatán/Yu_1118 | 0.03 |
| Yucatán/Yu_1119 | 0.10 |
| Yucatán/Yu_1121 | -0.06 |
| Yucatán/Yu_1122 | 0.20 |
| Yucatán/Yu_1123 | -0.09 |
| Yucatán/Yu_1124 | -0.05 |
| Yucatán/Yu_1125 | 0.20 |

**Confidence interval (95%) for sigma**

| | lower | est. | upper |
|---|---|---|---|
| sigma | 1.59 | 2.32 | 3.38 |

This model shows lower AIC and BIC values than the model without heterogeneous variances for *Population* and *Family* within *Population*. Note that the population variances are very different: the *La Paz* population has the highest estimated variance, $(15.94*2.32)^2 = 1367.57$, while the population with the lowest variance has a variance of $(1*2.32)^2 = 5.38$. When we compare the models with heterogeneous and homogeneous variances by means of a likelihood ratio test, we confirm that the model with heterogeneous variances is best ($p<0.0001$), as shown in the following output.

**Comparison of models**

| | Call | Model | df | AIC | BIC | logLik | Test | L.Ratio | p-value |
|---|---|---|---|---|---|---|---|---|---|
| Model000_Long_REML | 1 | 1 | 4 | 2016.47 | 2029.91 | -1004.23 | | | |
| Model001_Long_REML | 2 | 2 | 10 | 1872.14 | 1905.75 | -926.07 | 1 vs 2 | 156.33 | <0.0001 |

The residuals obtained for Model 1 with different residual variances in each population do not show heteroscedasticity problems, and they show an improvement in the distributional assumptions (Q-Q plot) with respect to Model 1 with homogeneous variances (Figure 35).

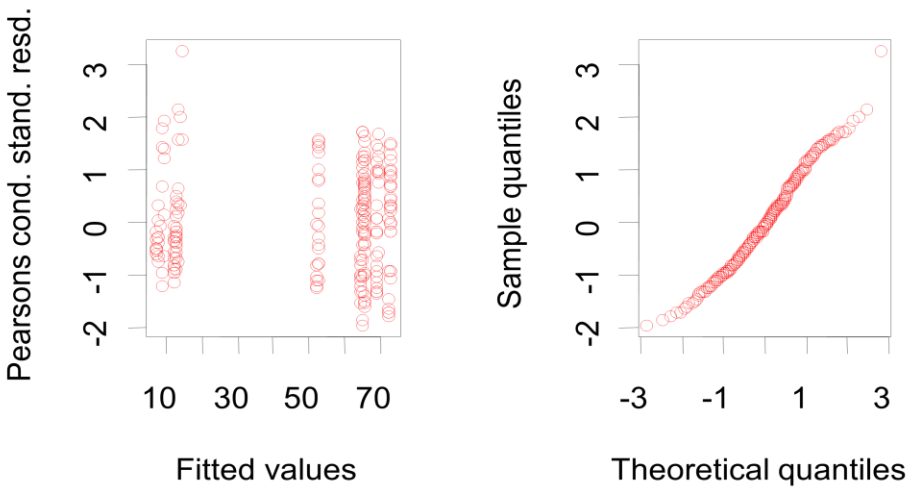



***Figure 35: Diagnostic graphs obtained for the variable Length, Model 1 with heterogeneous residual variables for populations (VarCom.IDB2 file).***

Model 2: For this model, *Population* should be declared in the *Fixed effects* tab. Note that *Fixed effects coefficients* has also been selected in this tab (Figure 36). In the *Random effects* tab, *Family* has been declared as random, the default option of *Family* as an effect on the *Constant* (intercept) has been deselected, and *Family* as affecting the parameters of the *Population* effect has been selected. The covariance matrix of random effects assigned to populations is assumed independent (*pdIdent*). The *Random effects matrix (BLUP's)*, *Confidence intervals for random parameters* and *Confidence interval for sigma* options (Figure 37) have also been selected. In the *Comparisons* tab the DGC option is selected for *Population* (Figure 38).

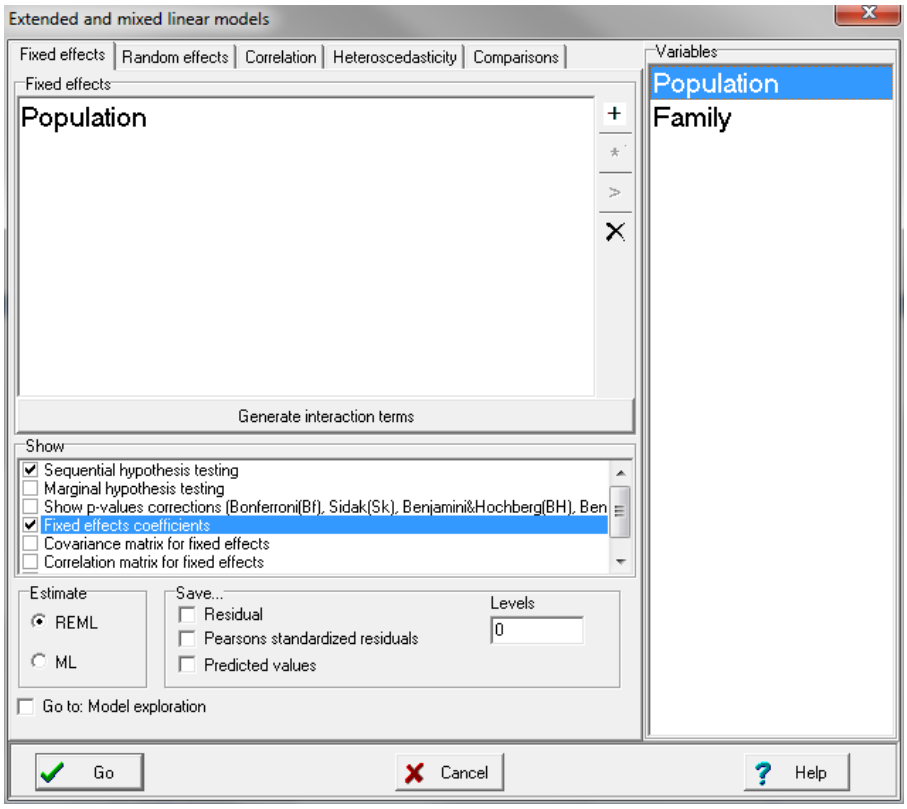



***Figure 36: Window displaying the Fixed effects tab, Model 2 (VarCom.IDB2 file).***

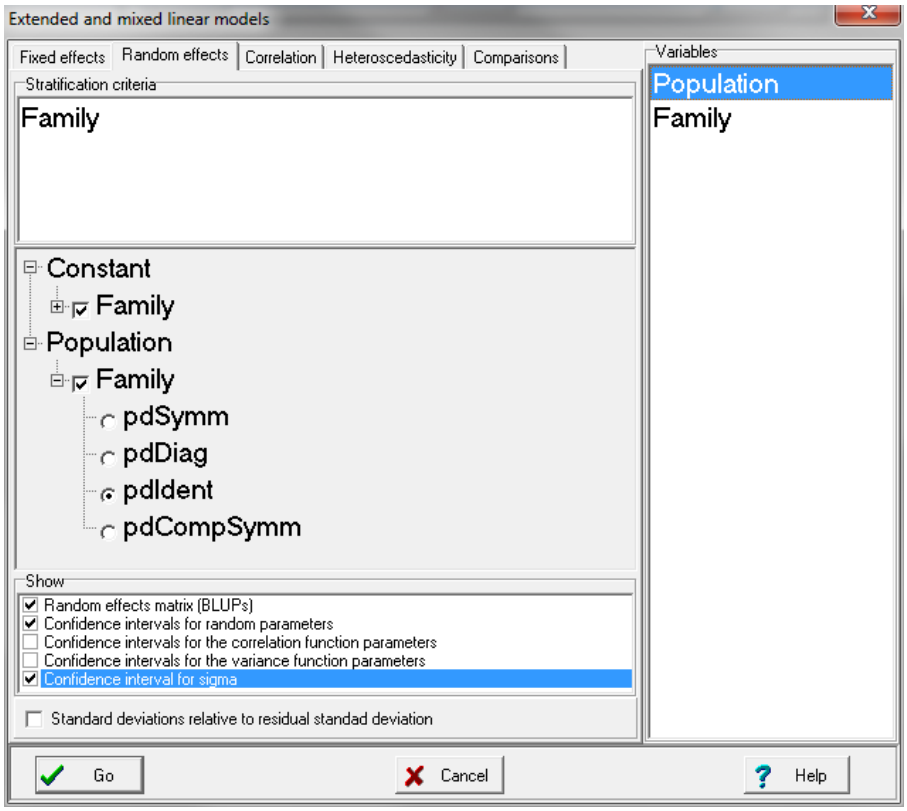



***Figure 37: Window displaying the Random effects tab, Model 2 (VarCom.IDB2 file).***

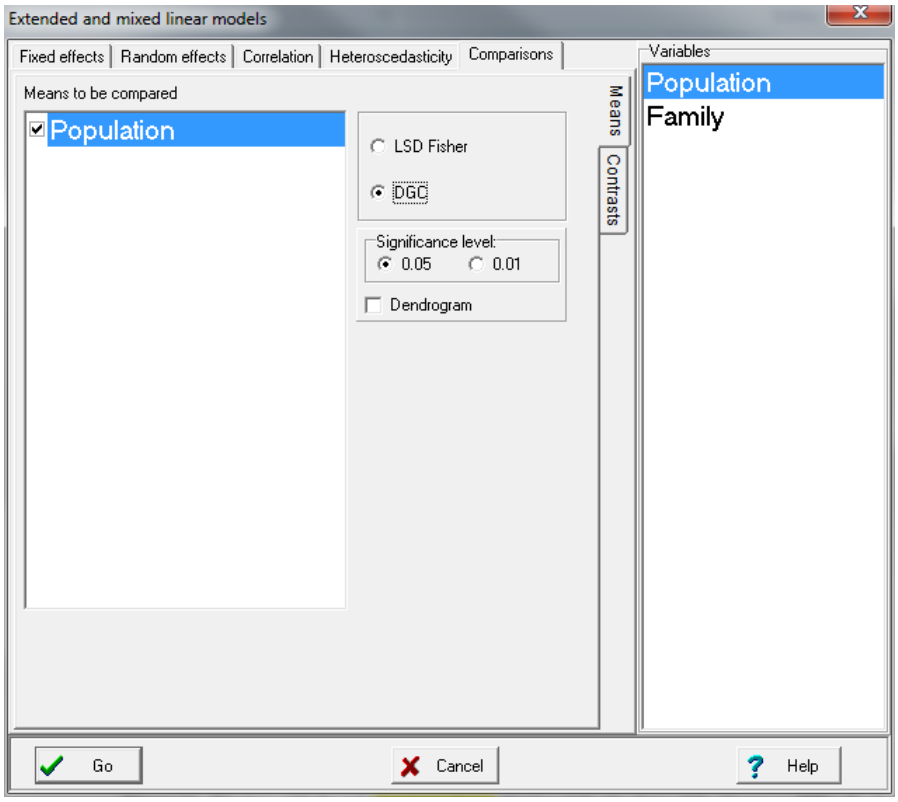



***Figure 38: Window displaying the Comparisons tab, Model 2 (VarCom.IDB2 file)..***

The output corresponding to these specifications is shown below:

**Extended and mixed linear models**

**R specification of the model**

```
model001_length_REML<-lme(length~1+Population
,random=list(Family=pdIdent(~Population-1))
,method="REML"
,na.action=na.omit
,data=R.data00
,keep.data=FALSE)
```

**Results for model: model001_length_REML**

*Dependent variable:length*

**Fit measurements**

| N | AIC | BIC | logLik | Sigma | R2_0 | R2_1 |
|---|---|---|---|---|---|---|
| 214 | 1967.65 | 1997.64 | -974.82 | 21.54 | 0.51 | 0.75 |

*Smaller AIC and BIC is better*

**Sequential hypothesis testing**

| | numDF | denDF | F-value | p-value |
|---|---|---|---|---|
| (Intercept) | 1 | 108 | 601.79 | <0.0001 |
| Population | 6 | 108 | 27.23 | <0.0001 |

**Fixed effects**

| | Value | Std.Error | DF | t-value | p-value |
|---|---|---|---|---|---|
| (Intercept) | 8.23 | 5.75 | 108 | 1.43 | 0.1551 |
| PopulationEscarcega | 56.89 | 8.03 | 108 | 7.08 | <0.0001 |
| PopulationEsclavos | 57.72 | 7.46 | 108 | 7.74 | <0.0001 |
| PopulationLa Paz | 62.24 | 8.13 | 108 | 7.66 | <0.0001 |
| PopulationPacífico Sur | 4.65 | 7.53 | 108 | 0.62 | 0.5382 |
| PopulationXpujil | 65.45 | 7.72 | 108 | 8.48 | <0.0001 |
| PopulationYucatán | 44.44 | 8.40 | 108 | 5.29 | <0.0001 |

**Random effects parameters**

*Covariance model for random effects: pdIdent*
*Formula: ~Population - 1|Family*

*Standard deviations and correlations*

| | Charagre | Escarcega | Esclavos | La Paz | Pacífico Sur | Xpujil | Yucatán |
|---|---|---|---|---|---|---|---|
| Charagre | 14.79 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Escarcega | 0.00 | 14.79 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Esclavos | 0.00 | 0.00 | 14.79 | 0.00 | 0.00 | 0.00 | 0.00 |
| La Paz | 0.00 | 0.00 | 0.00 | 14.79 | 0.00 | 0.00 | 0.00 |
| Pacífico Sur | 0.00 | 0.00 | 0.00 | 0.00 | 14.79 | 0.00 | 0.00 |
| Xpujil | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 14.79 | 0.00 |
| Yucatán | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 14.79 |

**Confidece intervals (95%) for the random effects parameters**

*Formula: ~Population - 1|Family*

| | LB(95%) | Est. | UB(95%) |
|---|---|---|---|
| sd( - 1) | 10.71 | 14.79 | 20.42 |

**Random effects coefficients (BLUP) (~Population - 1|Family)**

| | LI(95%) | est. | LS(95%) |
|---|---|---|---|
| sd( - 1) | 10.71 | 14.79 | 20.42 |

**Random effects coefficients (BLUP) (~Population - 1|Family)**

| | Charagre | Escarcega | Esclavos | La Paz | Pacífico Sur | Xpujil | Yucatán |
|---|---|---|---|---|---|---|---|
| Ch_71 | -1.08 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Ch_710 | 0.62 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Ch_711 | 1.34 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Ch_712 | 1.47 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Ch_713 | -0.96 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Ch_714 | -1.08 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Ch_715 | -0.71 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Ch_72 | 0.73 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Ch_73 | -0.84 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Ch_74 | -0.35 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Ch_75 | -0.60 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Ch_76 | -0.07 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Ch_77 | -0.48 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Ch_78 | 0.50 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Ch_79 | 1.53 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Ec_31 | 0.00 | 0.00 | -6.04 | 0.00 | 0.00 | 0.00 | 0.00 |
| Ec_310 | 0.00 | 0.00 | 5.36 | 0.00 | 0.00 | 0.00 | 0.00 |
| Ec_311 | 0.00 | 0.00 | -5.56 | 0.00 | 0.00 | 0.00 | 0.00 |
| Ec_312 | 0.00 | 0.00 | -2.65 | 0.00 | 0.00 | 0.00 | 0.00 |
| Ec_313 | 0.00 | 0.00 | -16.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Ec_314 | 0.00 | 0.00 | 20.66 | 0.00 | 0.00 | 0.00 | 0.00 |
| Ec_315 | 0.00 | 0.00 | -25.46 | 0.00 | 0.00 | 0.00 | 0.00 |
| Ec_316 | 0.00 | 0.00 | 10.95 | 0.00 | 0.00 | 0.00 | 0.00 |
| Ec_317 | 0.00 | 0.00 | 2.69 | 0.00 | 0.00 | 0.00 | 0.00 |
| Ec_318 | 0.00 | 0.00 | -5.80 | 0.00 | 0.00 | 0.00 | 0.00 |
| Ec_319 | 0.00 | 0.00 | 2.94 | 0.00 | 0.00 | 0.00 | 0.00 |
| Ec_32 | 0.00 | 0.00 | -5.56 | 0.00 | 0.00 | 0.00 | 0.00 |
| Ec_320 | 0.00 | 0.00 | 12.89 | 0.00 | 0.00 | 0.00 | 0.00 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Ec_33 | 0.00 | 0.00 | -0.46 | 0.00 | 0.00 | 0.00 | 0.00 |
| Ec_34 | 0.00 | 0.00 | -7.99 | 0.00 | 0.00 | 0.00 | 0.00 |
| Ec_35 | 0.00 | 0.00 | 10.95 | 0.00 | 0.00 | 0.00 | 0.00 |
| Ec_36 | 0.00 | 0.00 | -12.84 | 0.00 | 0.00 | 0.00 | 0.00 |
| Ec_37 | 0.00 | 0.00 | 12.89 | 0.00 | 0.00 | 0.00 | 0.00 |
| Ec_38 | 0.00 | 0.00 | 5.36 | 0.00 | 0.00 | 0.00 | 0.00 |
| Ec_39 | 0.00 | 0.00 | 3.67 | 0.00 | 0.00 | 0.00 | 0.00 |
| Es_1126 | 0.00 | -0.06 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Es_1127 | 0.00 | 16.20 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Es_1128 | 0.00 | 16.63 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Es_1129 | 0.00 | 6.49 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Es_1130 | 0.00 | -0.04 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Es_1131 | 0.00 | -7.09 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Es_1132 | 0.00 | 19.12 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Es_1133 | 0.00 | 1.15 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Es_1134 | 0.00 | -10.25 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Es_1135 | 0.00 | -10.94 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Es_1136 | 0.00 | -7.58 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Es_1137 | 0.00 | -16.32 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Es_1138 | 0.00 | 14.26 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Es_1139 | 0.00 | -10.30 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Es_1142 | 0.00 | 7.71 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Es_1148 | 0.00 | -18.99 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| LP_41 | 0.00 | 0.00 | 0.00 | -18.43 | 0.00 | 0.00 | 0.00 |
| LP_410 | 0.00 | 0.00 | 0.00 | 18.95 | 0.00 | 0.00 | 0.00 |
| LP_411 | 0.00 | 0.00 | 0.00 | 14.82 | 0.00 | 0.00 | 0.00 |
| LP_412 | 0.00 | 0.00 | 0.00 | 20.89 | 0.00 | 0.00 | 0.00 |
| LP_413 | 0.00 | 0.00 | 0.00 | -12.12 | 0.00 | 0.00 | 0.00 |
| LP_414 | 0.00 | 0.00 | 0.00 | -2.41 | 0.00 | 0.00 | 0.00 |
| LP_415 | 0.00 | 0.00 | 0.00 | -18.67 | 0.00 | 0.00 | 0.00 |
| LP_42 | 0.00 | 0.00 | 0.00 | 1.23 | 0.00 | 0.00 | 0.00 |
| LP_43 | 0.00 | 0.00 | 0.00 | -1.93 | 0.00 | 0.00 | 0.00 |
| LP_44 | 0.00 | 0.00 | 0.00 | -1.68 | 0.00 | 0.00 | 0.00 |
| LP_45 | 0.00 | 0.00 | 0.00 | 1.96 | 0.00 | 0.00 | 0.00 |
| LP_46 | 0.00 | 0.00 | 0.00 | -9.69 | 0.00 | 0.00 | 0.00 |
| LP_48 | 0.00 | 0.00 | 0.00 | -2.39 | 0.00 | 0.00 | 0.00 |
| LP_49 | 0.00 | 0.00 | 0.00 | 9.48 | 0.00 | 0.00 | 0.00 |
| PS_6204 | 0.00 | 0.00 | 0.00 | 0.00 | -2.13 | 0.00 | 0.00 |
| PS_6206 | 0.00 | 0.00 | 0.00 | 0.00 | -2.73 | 0.00 | 0.00 |
| PS_6207 | 0.00 | 0.00 | 0.00 | 0.00 | 2.48 | 0.00 | 0.00 |
| PS_6208 | 0.00 | 0.00 | 0.00 | 0.00 | -1.52 | 0.00 | 0.00 |
| PS_6209 | 0.00 | 0.00 | 0.00 | 0.00 | -0.67 | 0.00 | 0.00 |
| PS_6210 | 0.00 | 0.00 | 0.00 | 0.00 | 1.51 | 0.00 | 0.00 |
| PS_6211 | 0.00 | 0.00 | 0.00 | 0.00 | -1.03 | 0.00 | 0.00 |
| PS_6212 | 0.00 | 0.00 | 0.00 | 0.00 | -2.01 | 0.00 | 0.00 |
| PS_6213 | 0.00 | 0.00 | 0.00 | 0.00 | 0.18 | 0.00 | 0.00 |
| PS_6214 | 0.00 | 0.00 | 0.00 | 0.00 | -2.61 | 0.00 | 0.00 |
| PS_6215 | 0.00 | 0.00 | 0.00 | 0.00 | -0.31 | 0.00 | 0.00 |
| PS_6216 | 0.00 | 0.00 | 0.00 | 0.00 | 8.55 | 0.00 | 0.00 |
| PS_6217 | 0.00 | 0.00 | 0.00 | 0.00 | -0.55 | 0.00 | 0.00 |
| PS_6218 | 0.00 | 0.00 | 0.00 | 0.00 | 4.18 | 0.00 | 0.00 |
| PS_6219 | 0.00 | 0.00 | 0.00 | 0.00 | -1.64 | 0.00 | 0.00 |
| PS_6220 | 0.00 | 0.00 | 0.00 | 0.00 | -2.37 | 0.00 | 0.00 |
| PS_6221 | 0.00 | 0.00 | 0.00 | 0.00 | -0.55 | 0.00 | 0.00 |
| PS_6222 | 0.00 | 0.00 | 0.00 | 0.00 | -2.25 | 0.00 | 0.00 |
| PS_660 | 0.00 | 0.00 | 0.00 | 0.00 | 3.46 | 0.00 | 0.00 |
| Xp_11 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | -14.96 | 0.00 |
| Xp_110 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 2.35 | 0.00 |
| Xp_112 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | -0.09 | 0.00 |
| Xp_113 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | -7.12 | 0.00 |
| Xp_114 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 1.61 | 0.00 |
| Xp_115 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | -12.46 | 0.00 |
| Xp_116 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 15.93 | 0.00 |
| Xp_117 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 10.11 | 0.00 |
| Xp_118 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 6.95 | 0.00 |
| Xp_119 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 16.91 | 0.00 |
| Xp_12 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | -2.14 | 0.00 |
| Xp_120 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 18.36 | 0.00 |
| Xp_122 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | -20.72 | 0.00 |
| Xp_123 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | -27.03 | 0.00 |
| Xp_15 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 1.86 | 0.00 |
| Xp_16 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 2.99 | 0.00 |
| Xp_17 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 3.95 | 0.00 |
| Xp_18 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | -4.94 | 0.00 |
| Xp_19 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 8.44 | 0.00 |
| Yu_1111 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | -14.89 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Yu_1114 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | -16.59 |
| Yu_1115 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | -3.24 |
| Yu_1116 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 1.86 |
| Yu_1117 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 4.53 |
| Yu_1118 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 2.83 |
| Yu_1119 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 8.66 |
| Yu_1121 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | -5.18 |
| Yu_1122 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 17.15 |
| Yu_1123 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | -7.85 |
| Yu_1124 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | -4.45 |
| Yu_1125 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 17.15 |

**Confidece interval (95%) for sigma**

| | lower | est. | upper |
|---|---|---|---|
| sigma | 18.77 | 21.54 | 24.71 |

**Adjusted means and standard error for Population**

*DGC (alpha=0.05)*

| Population | Means | S.E. | | |
|---|---|---|---|---|
| Xpujil | 73.68 | 5.16 | A | |
| La Paz | 70.47 | 5.74 | A | |
| Esclavos | 65.95 | 4.75 | A | |
| Escarcega | 65.12 | 5.61 | A | |
| Yucatán | 52.67 | 6.13 | A | |
| Pacífico Sur | 12.88 | 4.87 | | B |
| Charagre | 8.23 | 5.75 | | B |

*Means with a common letter are not significantly different (p<= 0.05)*

The following example is an estimation of the BLUP for some families of the population *Charagre*:

$$\hat{Y}_{cha,71} = \hat{\mu} + \hat{\alpha}_{cha} + \hat{\beta}_{71(cha)} = 8.2296 + 0 + (-1.0823) = 7.1473$$

$$\hat{Y}_{cha,72} = \hat{\mu} + \hat{\alpha}_{cha} + \hat{\beta}_{72(cha)} = 8.2296 + 0 + 0.7277 = 8.9573$$

$$\hat{Y}_{cha,73} = \hat{\mu} + \hat{\alpha}_{cha} + \hat{\beta}_{73(cha)} = 8.2296 + 0 + (-0.8396) = 7.3900$$

$$\hat{Y}_{cha,74} = \hat{\mu} + \hat{\alpha}_{cha} + \hat{\beta}_{74(cha)} = 8.2296 + 0 + (-0.3542) = 7.8754$$

The BLUP for family 42 of La Paz population is:

$$\hat{Y}_{lpaz,42} = \hat{\mu} + \hat{\alpha}_{lpaz} + \hat{\beta}_{42(lpaz)} = 8.2296 + 62.2374 + 1.2297 = 71.6967$$

Now we will conduct the fitness analysis for Model 2. In the *Model exploration* submenu the diagnostic graphs are requested (Figure 39).

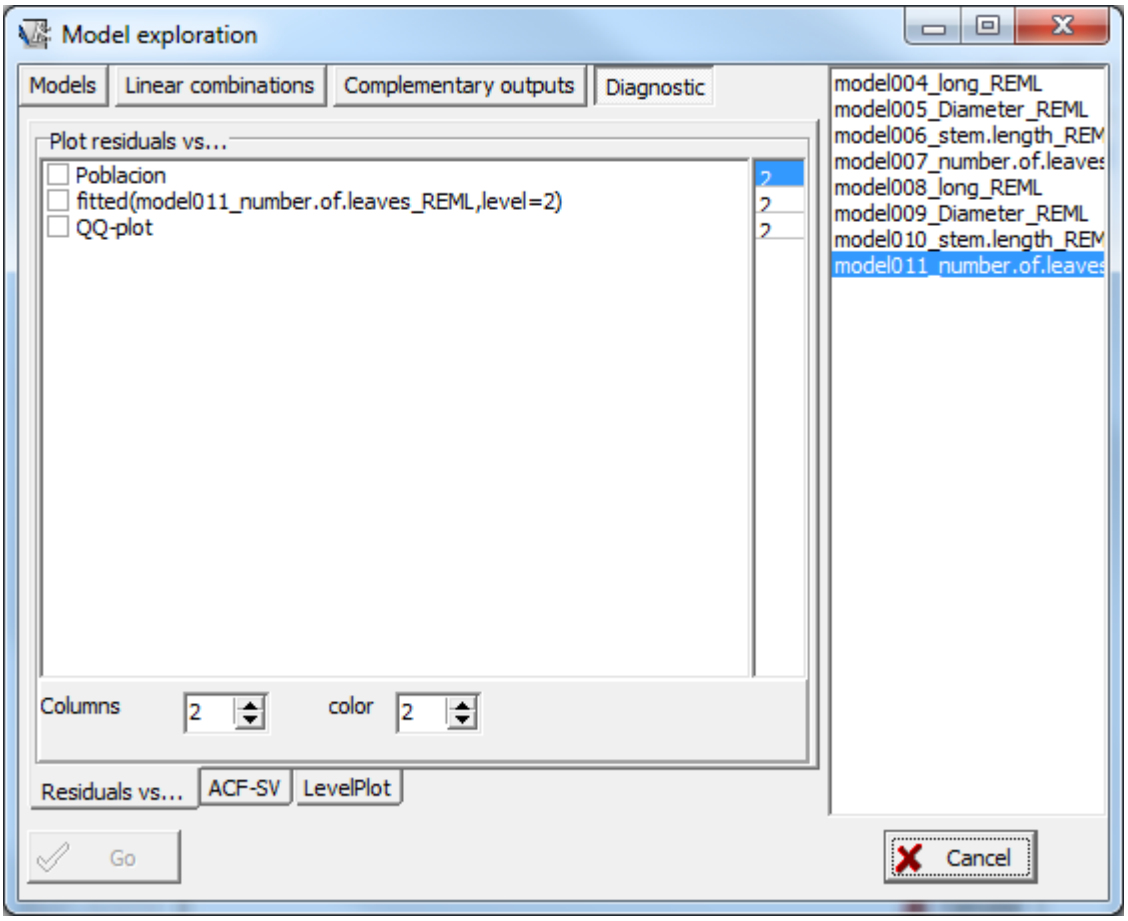



***Figure 39: Model exploration window displaying the Diagnostic tab, Model 2 (VarCom.IDB2 file).***

The Pearson's standardized conditional residuals vs. fitted values graph (Figure 40) shows heterogeneous residual variances for the Length variable.

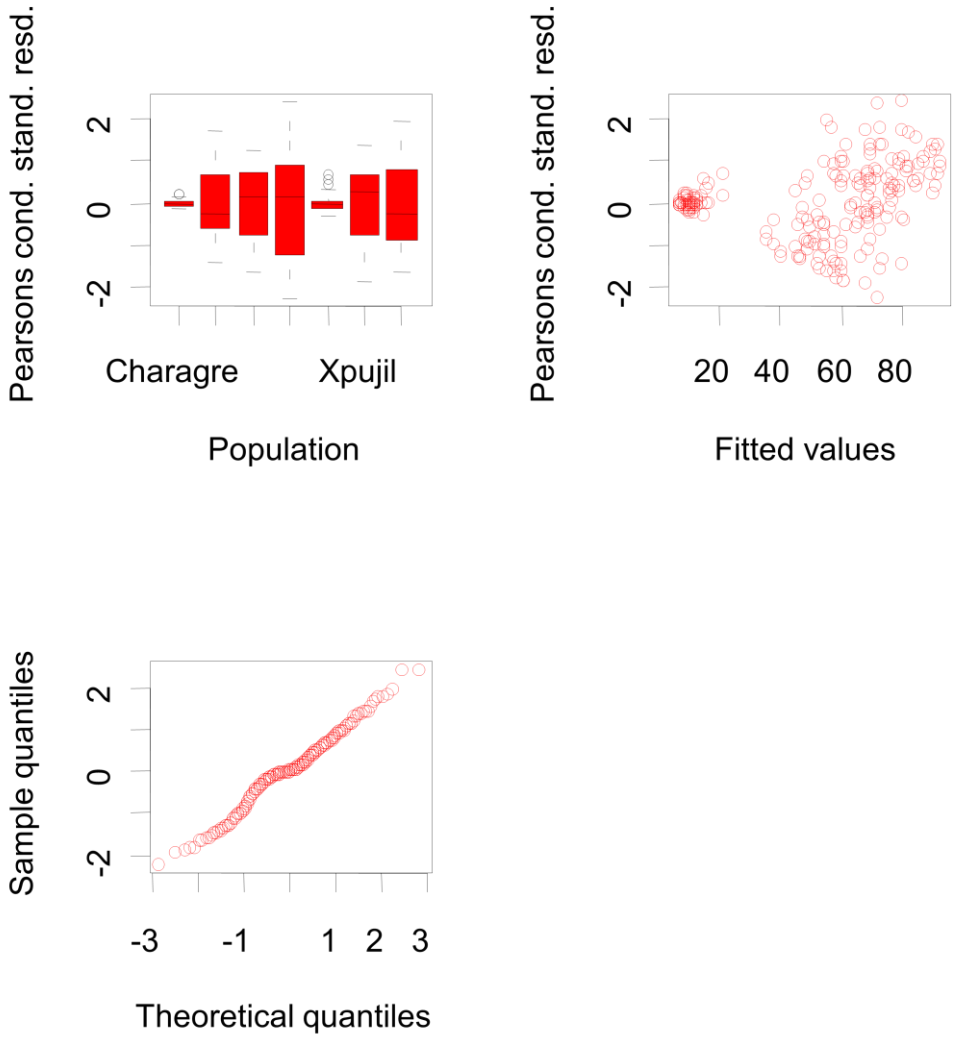



***Figure 40: Diagnostic graphs obtained for the variable Length, Model 2 (VarCom.IDB2 file).***

With respect to the distributional assumptions, it is important to emphasize that, when heteroscedasticity exists, the Q-Q plot should not be interpreted until this problem is solved. To incorporate heterogeneous variables of the *Population* effect, the *Population* factor should be specified in the *Heterogeneity* tab, as shown in Figure 34.

This model has lower AIC and BIC values than does the model without heterogeneous variances for *Population*. Note that the variances of the populations are very different: The population *La Paz* has a highest estimated variance, $(15.94*2.32)^2 = 1367.57$, while the lowest variance, for *Charagre*, is $(1*2.32)^2 = 5.38$.

**Extended and mixed linear models**

**R specification of the model**

```
model001_length_REML<-lme(length~1+Population
,random=list(Family=pdIdent(~Population-1))
,weight=varComb(varIdent(form=~1|Population))
,method="REML"
,na.action=na.omit
,data=R.data00
,keep.data=FALSE)
```

**Results for model: model001_length_REML**

*Dependent variable:length*

**Fit measurements**

| N | AIC | BIC | logLik | Sigma | R2_0 | R2_1 |
|---|---|---|---|---|---|---|
| 214 | 1823.20 | 1873.20 | -896.60 | 2.32 | 0.51 | 0.51 |

*Smaller AIC and BIC is better*

**Sequential hypothesis testing**

| | numDF | denDF | F-value | p-value |
|---|---|---|---|---|
| (Intercept) | 1 | 108 | 509.60 | <0.0001 |
| Population | 6 | 108 | 86.55 | <0.0001 |

**Fixed effects**

| | Value | Std.Error | DF | t-value | p-value |
|---|---|---|---|---|---|
| (Intercept) | 8.23 | 0.61 | 108 | 13.42 | <0.0001 |
| PoblacionEscarcega | 57.32 | 5.90 | 108 | 9.72 | <0.0001 |
| PoblacionEsclavos | 57.72 | 4.33 | 108 | 13.33 | <0.0001 |
| PoblacionLa Paz | 62.33 | 7.16 | 108 | 8.70 | <0.0001 |
| PoblacionPacífico Sur | 4.65 | 1.28 | 108 | 3.65 | 0.0004 |
| PoblacionXpujil | 65.43 | 5.54 | 108 | 11.81 | <0.0001 |
| PoblacionYucatán | 44.44 | 6.00 | 108 | 7.41 | <0.0001 |

**Random effects parameters**

*Covariance model for random effects: pdIdent*
*Formula: ~Population - 1|Family*

*Standard deviations relative to residual standard deviation and correlation*

| | Charagre | Escarcega | Esclavos | La Paz | Pacífico Sur | Xpujil | Yucatán |
|---|---|---|---|---|---|---|---|
| Charagre | 1.56 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Escarcega | 0.00 | 1.56 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Esclavos | 0.00 | 0.00 | 1.56 | 0.00 | 0.00 | 0.00 | 0.00 |
| La Paz | 0.00 | 0.00 | 0.00 | 1.56 | 0.00 | 0.00 | 0.00 |
| Pacífico Sur | 0.00 | 0.00 | 0.00 | 0.00 | 1.56 | 0.00 | 0.00 |
| Xpujil | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 1.56 | 0.00 |
| Yucatán | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 1.56 |

**Confidece intervals (95%) for the random effects parameters**

*Formula: ~Population - 1|Family*

| | LB(95%) | Est. | UB(95%) |
|---|---|---|---|
| sd( - 1) | 0.45 | 1.56 | 5.38 |

**Variance structure**

*Variance model: varIdent*
*Formula: ~ 1 | Population*

*Variance-function parameters*

| Parameter | Estim. |
|---|---|
| Charagre | 1.00 |
| Esclavos | 11.64 |
| Escarcega | 13.09 |
| La Paz | 15.94 |
| Pacífico Sur | 2.81 |
| Xpujil | 13.38 |
| Yucatán | 12.55 |

To prove that this less parsimonious model is the one with the better fit, we conducted a likelihood ratio test, and the output is shown below.

**Comparison of models**

| Model | df | AIC | BIC | logLik | Test | L.Ratio | p-value |
|---|---|---|---|---|---|---|---|
| 001 | 9 | 1967.65 | 1997.64 | -974.82 | | | |
| 002 | 15 | 1823.20 | 1873.20 | -896.60 | 1 vs 2 | 156.44 | <0.0001 |

The model with heterogeneous variances for the different populations is better than the one with homogeneous variances ($p<0.0001$). Note that with the inclusion of the heterogeneous variances for the different populations, the fit has improved with respect to the previous fits (

Figure 41). In the box-plot of Pearson Studentized Conditional residuals and in the scatter plot of the Pearson Studentized Conditional residuals versus fitted values, lack of homogeneity of the variances is no longer a serious problem. The Q-Q plot shows an improvement in the distributional assumption.

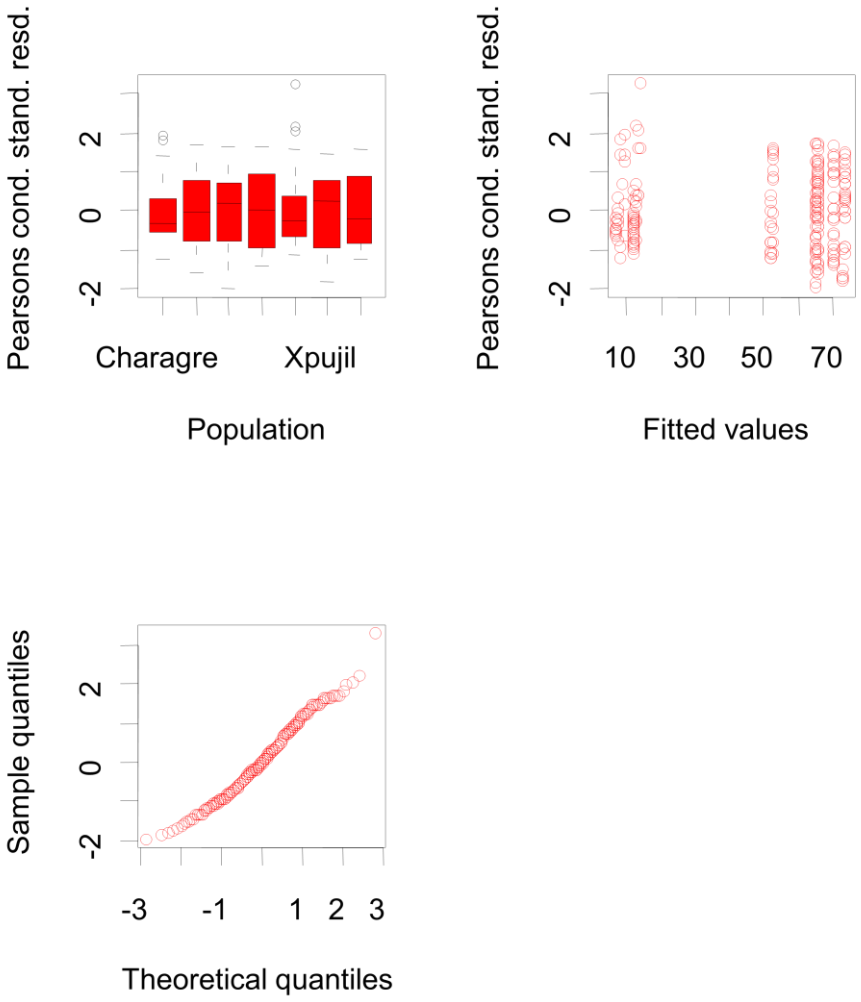



***Figure 41: Diagnostic graphs obtained for the variable Length once the different residual variances for each population were declared, Model 2 (VarCom.IDB2 file).***

### *Crossed random effects with interaction*

There are many situations in which the interest lies in estimating variance components associated with two crossed factors and their interaction. Milliken and Johnson (1992, p. 265) present an example of efficiency in three production lines (randomly chosen in a factory). Four operators were randomly chosen, and these operators worked in each of the production lines. Originally each operator was supposed to work on each production line five times, but for different reasons there are combinations which were repeated fewer times (there are between one and five efficiency data for each operator-production line combination).

Since both the production line and the operator are random, and we are also interested in the additional variability generated by each specific combination, we are going to use a model with two random effects and their interaction:

$$\begin{aligned} &Y_{ijk} = \mu + a_i + b_j + ab_{ij} + e_{ijk} \\ &a_i \sim N\left(0, \sigma_a^2\right), b_j \sim N\left(0, \sigma_b^2\right) \\ &ab_{ij} \sim N\left(0, \sigma_{ab}^2\right), e_{ijk} \sim N\left(0, \sigma_e^2\right) \end{aligned} \tag{6}$$

where all the random effects are mutually independent.

In order to fit this model we will use the dataset *Production.IDB2* (Milliken and Johnson, 1992). *Efficiency* is indicated in the *Variables* window, *Production Line* and *Operator* are selected in the *Class variables* window. Since there is no fixed effect (except the general mean), nothing is selected in the *Fixed effects* tab. In the *Random effects* tab we select *Production Line* and *Operator*, and the option *Crossed random factors and interactions* appears after clicking the right mouse with both variables selected. In order to simplify the interpretation of the output (remember that the main goal in this type of models is the estimation of variance components), we have unchecked the option *Standard deviations relative to residual standard deviation* Figure 42.

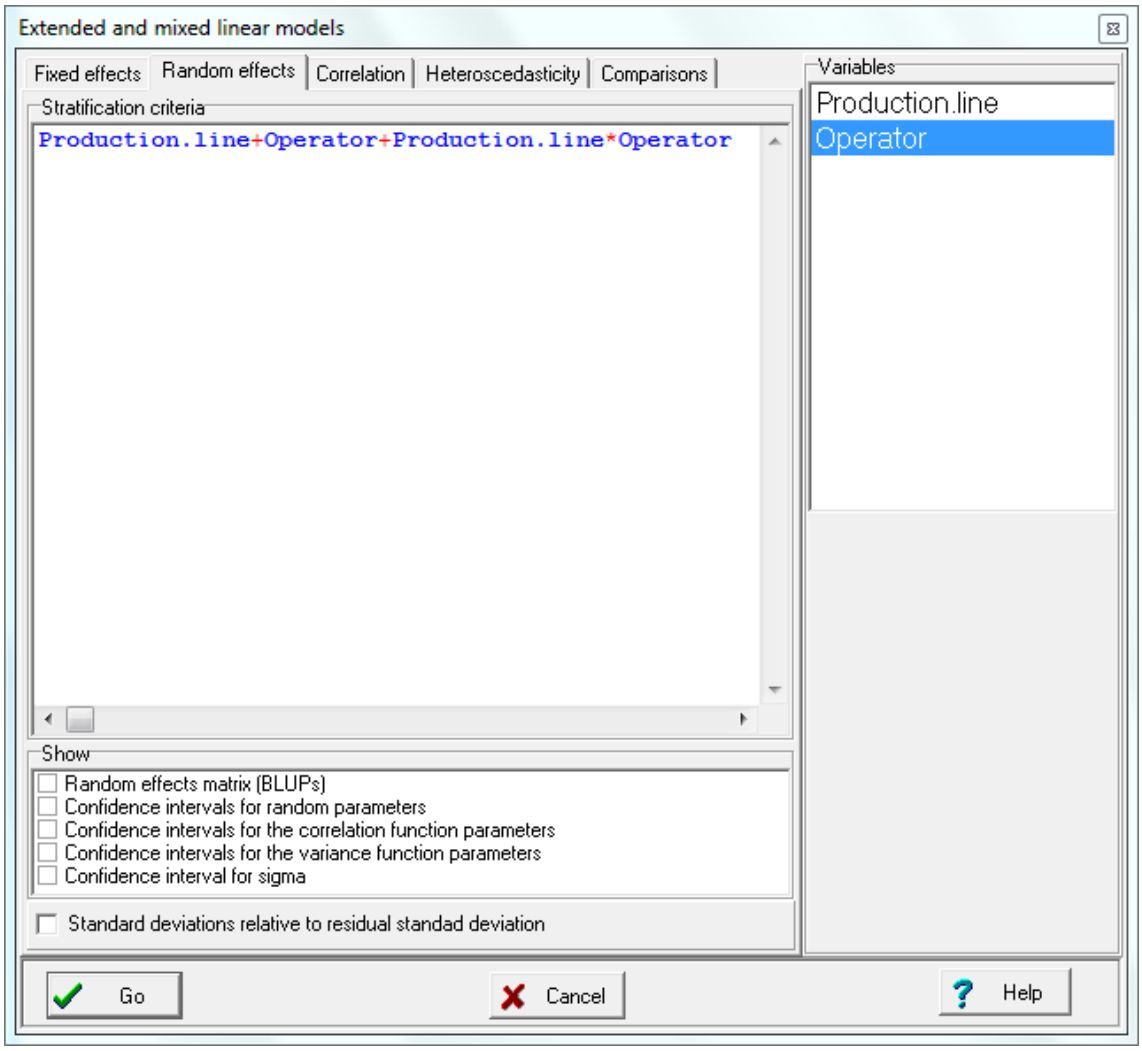



***Figure 42: Window displaying the Random effects tab with Line and Operator effects crossed and their interaction, file Production.IDB2.***

```
Extended and mixed linear models

R specification of the model

model.000_Efficiency_REML<-lme(Efficiency~1
,random=list(.U.=pdBlocked(list(pdIdent(~Production.line-1)
,pdIdent(~Operator-1)
,pdIdent(~Production.line:Operator-1))))
,method="REML"
,control=lmeControl(msMaxIter=200)
,na.action=na.omit
,data=R.data00
,keep.data=FALSE)

Results for model: model.000_Efficiency_REML

Dependent variable: Efficiency
Fit measurements

N   AIC    BIC    logLik  Sigma R2_0 R2_1
47 249.64 258.78 -119.82  1.99       0.95
Smaller AIC and BIC is better
```

**Sequential hypothesis testing**

| | numDF | denDF | F-value | p-value |
|---|---|---|---|---|
| (Intercept) | 1 | 46 | 478.29 | <0.0001 |

**Fixed effects**

| | Value | Std.Error | DF | t-value | p-value |
|---|---|---|---|---|---|
| (Intercept) | 83.38 | 3.81 | 46 | 21.87 | <0.0001 |

**Random effects parameters**

*Covariance model for random effects: pdBlocked*
*Formula: ~Production.line + Operator + Production.line:Operator - 1*

*Standard deviations and correlations*

| | S.D. |
|---|---|
| Production.line1 | 5.67 |
| Production.line2 | 5.67 |
| Production.line3 | 5.67 |
| Operator1 | 1.74 |
| Operator2 | 1.74 |
| Operator3 | 1.74 |
| Operator4 | 1.74 |
| Production.line1:Operator1.. | 5.96 |
| Production.line2:Operator1.. | 5.96 |
| Production.line3:Operator1.. | 5.96 |
| Production.line1:Operator2.. | 5.96 |
| Production.line2:Operator2.. | 5.96 |
| Production.line3:Operator2.. | 5.96 |
| Production.line1:Operator3.. | 5.96 |
| Production.line2:Operator3.. | 5.96 |
| Production.line3:Operator3.. | 5.96 |
| Production.line1:Operator4.. | 5.96 |
| Production.line2:Operator4.. | 5.96 |
| Production.line3:Operator4.. | 5.96 |

From this output we note that the standard deviation estimates for each random effect are:

$$\hat{\sigma}_a = 5.6672, \hat{\sigma}_b = 1.7353, \hat{\sigma}_{ab} = 5.9618, \hat{\sigma}_e = 1.9947$$

This information can be used, for example, to estimate different types of intraclass correlations. For example, the correlation between two observations from the same production line and operator is:

$$\text{corr}\left(Y_{ijk}, Y_{ijk'}\right) = \frac{\text{cov}\left(Y_{ijk}, Y_{ijk'}\right)}{\text{var}\left(Y_{ijk}\right)} = \frac{\hat{\sigma}_a^2 + \hat{\sigma}_b^2 + \hat{\sigma}_{ab}^2}{\hat{\sigma}_a^2 + \hat{\sigma}_b^2 + \hat{\sigma}_{ab}^2 + \hat{\sigma}_e^2}$$

$$= \frac{5.6672^2 + 1.7353^2 + 5.9618^2}{5.6672^2 + 1.7353^2 + 5.9618^2 + 1.9947^2} = 0.9467$$

On the other hand, the correlation between two observations from the same operator but in different production lines is much smaller:

$$\text{corr}\left(Y_{ijk}, Y_{i'jk'}\right) = \frac{\text{cov}\left(Y_{ijk}, Y_{i'jk'}\right)}{\text{var}\left(Y_{ijk}\right)}$$

$$= \frac{\hat{\sigma}_b^2}{\hat{\sigma}_a^2 + \hat{\sigma}_b^2 + \hat{\sigma}_{ab}^2 + \hat{\sigma}_e^2} = \frac{1.7353^2}{5.6672^2 + 1.7353^2 + 5.9618^2 + 1.9947^2} = 0.0403.$$

## *Application of mixed models for hierarchical data*

### *Split plots*

Let us suppose a two-factor experiment is performed, in which it is not possible to randomly assign the combinations of both factors to the experimental plots (EP). In some cases, groups of EP randomly receive the different levels of one of the classification factors, and within these plot groups, the levels of the second factors are randomly assigned.

The experiment previously described differs from a conventional two-factor experiment in that, although the levels of the factors are randomly assigned to the EP, the treatments (i.e., the combinations of the factors levels) are not the assigned in this way.

This particular way of assigning the different levels of the factors to the plots represents a restriction to the randomization, and it induces correlation structures that should be taken into account in the analysis. This is known as the *split plot* design.

The name emerges from the idea that the *main* PLOTS receive the levels of a factor (sometimes also called *main factor*) and that these plots are SPLIT into SUBPLOTS, which receive the levels of the second classification factor.

Although in the split plots the factors of a level are assigned within the levels of another factor, this is NOT a nested design. It consists of a typical factorial experiment in which the factors are crossed. It is only the randomization that has been done in a sequential way.

Depending on the arrangement of the main plots, the design can be of:

- Split plots arranged in a randomized complete block design (RCBD).
- Split plots in a completely randomized design
- Split plots arranged in other designs

### *Split plots arranged in a RCBD*

The classical analysis of a split plots design with the main plots arranged in complete blocks includes the following terms in the model:

| |
|---|
| *Factor associated with the main plot (MPF)* |
| *Block* |
| *Block*MPF (main plot error)* |
| *Factor associated with the subplot (SPF)* |
| *MPF*SPF* |
| *Error (error for the subplot)* |

The key point to perform the analysis of this model is to understand that the experimental error for MPF is different from the terms of the model that include the SPF. The experimental error of the main plots is generally larger than that of the subplots.

The experimental error variance of the main plots in a split-plot design with main plots arranged in a randomized complete block design, is estimated as the mean square (MS) of the interaction Block*MPF (assuming that there is no interaction Block*MPF, and consequently this MS estimates the error between main plots treated in the same way). The MS of this "interaction" is used as a reference point to calculate the F statistic of the hypothesis test for the main factor. For the rest of the tests, the residual MS is used to construct the F statistic.

The analysis of this design by means of a linear mixed model is based on the identification of two grouping levels for the response variable. The first level is given by the blocks, and the second level is given by the main plots within the blocks. Each one of these grouping levels generates a correlation, known as intra-class correlation, among the observations it contains.

The mixed linear model for this design is the following:

$$y_{ijk} = \mu + \tau_i + \gamma_j + \delta_{ij} + b_k + p_{ik} + \varepsilon_{ijk};\ i = 1,..,T;\ j = 1,...,G; k = 1,...,B \qquad (7)$$

where $y_{ijk}$ represents the response variable in the *k*-th block, in the *i*-th level of the principal factor, and in the *j*-th level of the factor associated with the sub-plots; $\mu$ represents the general mean of the response; $\tau_i$ represents the effect of the *i*-th level of the factor associated with the main plot; $\gamma_j$ represents the effect of the *j*-th level of the

factor associated with the subplots; and $\delta_{ij}$ represents the effect of the interaction of the *ij*-th treatment. On the other hand $b_k$, $p_{ik}$ and $\varepsilon_{ijk}$ correspond to the random effects of the blocks, the plots within the blocks and the experimental errors, respectively. The assumptions for these random components are as follows: $b_k \sim N\left(0, \sigma_b^2\right)$, $p_{ik} \sim N\left(0, \sigma_p^2\right)$, $\varepsilon_{ijk} \sim N\left(0, \sigma_\varepsilon^2\right)$, and that these three random components are independent. Below we exemplify the analysis of a split plot design with blocks through the use of a mixed linear model.

In this example (Di Rienzo 2007), four varieties of wheat are evaluated: BUCK-Charrua (BC), Las Rosas-INTA (LI), Pigué (Pe), and Pro-INTA Puntal (PP), irrigated and rain-fed with the field design shown in Figure 43.

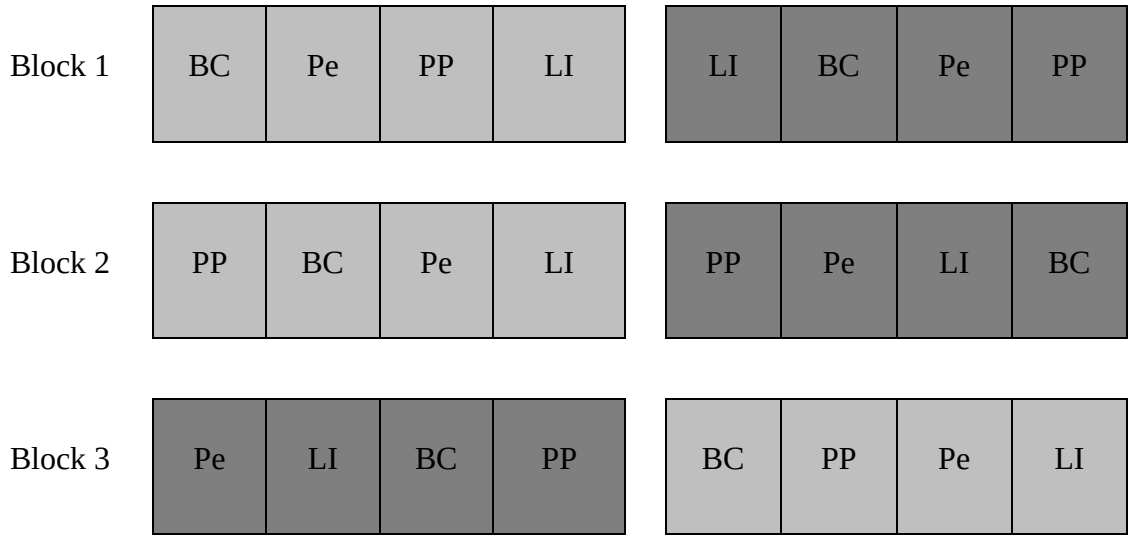



***Figure 43: Scheme of the split plots design for the example (Wheat.IDB2 file, light gray =irrigation, dark gray=rainfed).***

The data in this example can be found in the Wheat.IDB2 file. The following is the heading for the data table (Figure 44).

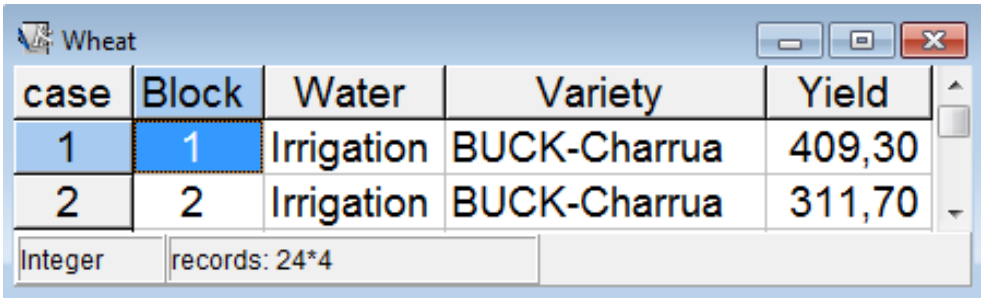



***Figure 44: Heading of the data table (Wheat.IDB2 file).***

The factor in the main plot is *Water*, the factor associated with the subplots is *Variety*, and the response variable is *Yield*. The blocks are clearly identified, but the main plots are not shown explicitly. This is so because in a split-plot design, the main plots within a block are confounded with the main factor. Thus, the observations under *Irrigation* in block 1 represent the observations of one of the main plots of this block.

To analyze this example we request the estimation of a mixed linear model. This generates the variables selection window. Figure 45 shows the appropriate selection of response variables and factors

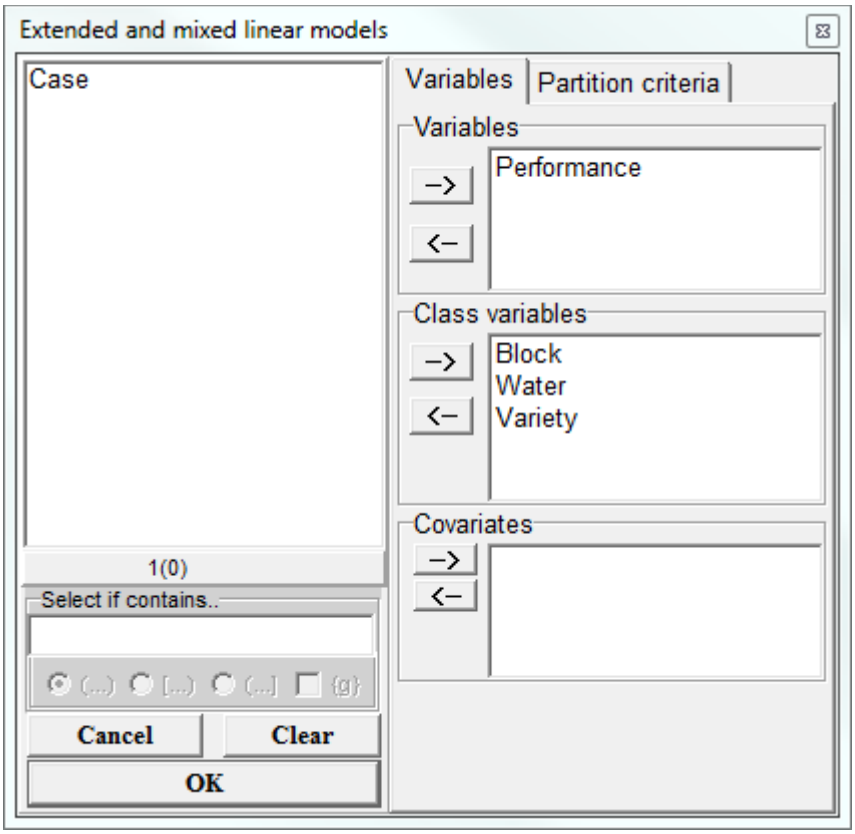



***Figure 45: Variables selection window for Extended and mixed linear models (Wheat.IDB2 file).***

Upon accepting this specification, the dialogue to specify the model appears. The tab for the fixed part, which has already been specified, is shown in Figure 46. Here the main effects *Water*, *Variety* and the interaction *Water*Variety* appear.

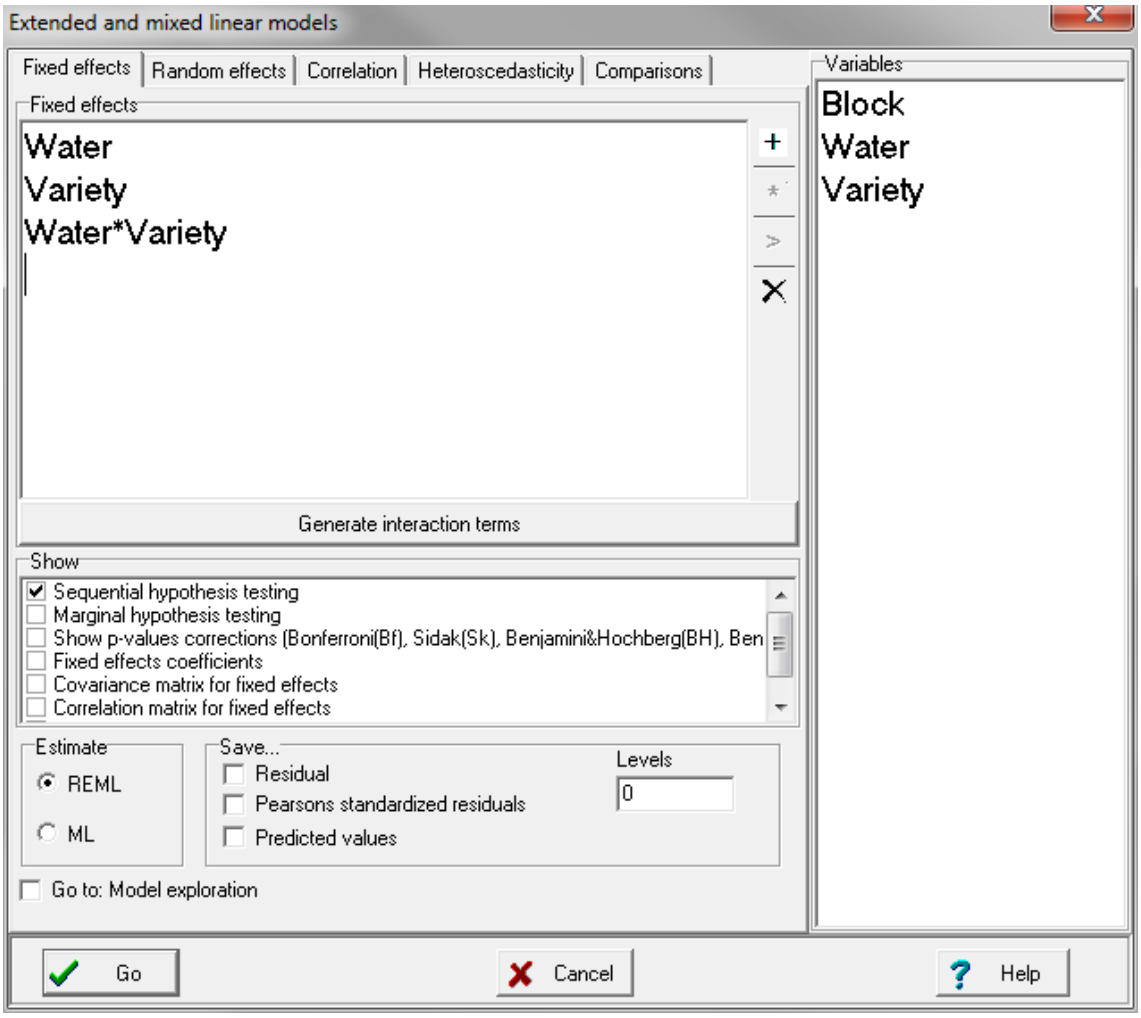



***Figure 46: Window displaying the Fixed effects tab (Wheat.IDB2 file).***

For the specification of the random part, first the random factor *Block* and then the factor *Water* are incorporated into the *Random effects* tab. This is how to indicate that *Water* is within *Block*. The specification of the random part should be as shown in Figure 47.

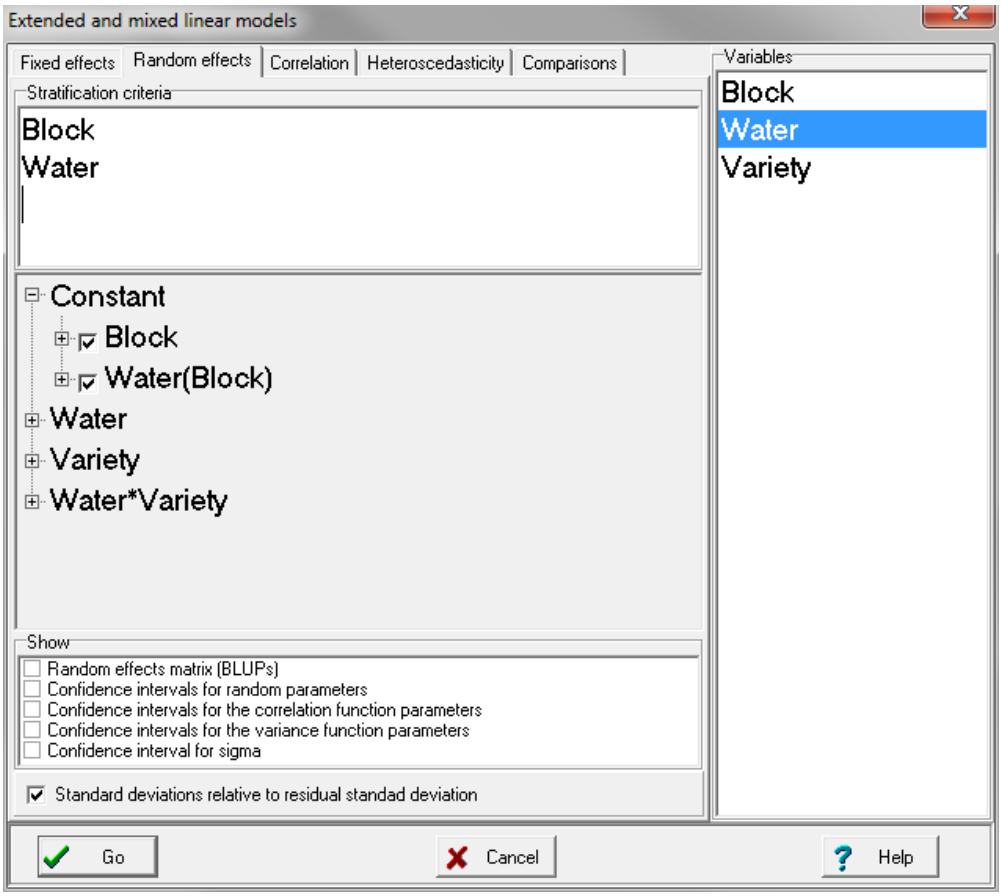



***Figure 47: Window displaying the Random effects tab, with Block and Water as stratification criteria (Wheat.IDB2 file).***

The following output corresponds to this estimation:

**Extended and mixed linear models**

**R specification of the model**

```
model000_Performance_REML<-
lme(Performance~1+Water+Variety+Water:Variety
,random=list(Block=pdIdent(~1)
,Water=pdIdent(~1))
,method="REML"
,na.action=na.omit
,data=R.data00
,keep.data=FALSE)
```

**Results for model: model000_Performance_REML**

*Dependent variable:Performance*

**Fit measurements**

| N | AIC | BIC | logLik | Sigma | R2_0 | R2_1 | R2_2 |
|---|---|---|---|---|---|---|---|
| 24 | 206.59 | 215.09 | -92.30 | 51.65 | 0.84 | 0.89 | 0.91 |

*Smaller AIC and BIC is better*

**Sequential hypothesis testing**

| | numDF | denDF | F-value | p-value |
|---|---|---|---|---|
| (Intercept) | 1 | 12 | 363.93 | <0.0001 |
| Water | 1 | 2 | 55.24 | 0.0176 |
| Variety | 3 | 12 | 6.38 | 0.0078 |
| Water:Variety | 3 | 12 | 2.36 | 0.1223 |

**Random effects parameters**

*Covariance model for random effects: pdIdent*
*Formula: ~1|Block*

*Standard deviations relative to residual standard deviation and correlation*

| | (const) |
|---|---|
| (const) | 0.55 |

*Covariance model for random effects: pdIdent*
*Formula: ~1|Water in Block*

*Standard deviations relative to residual standard deviation and correlation*

| | (const) |
|---|---|
| (const) | 0.47 |

In this problem, sequential hypothesis tests are equivalent to marginal tests because the data are balanced.

Before continuing with our analysis, we will validate some of the assumptions of the models by reviewing standardized residuals vs. predicted values and other classification criteria as well as the normal Q-Q plot for standardized residuals. These residuals are conditional on the random effects (in other words, they approximate the errors). To do so, we will select the *Model exploration* submenu. In the dialogue window, we will select the *Diagnostic* tab, and then select the *Residuals vs.* subtab (Figure 48).

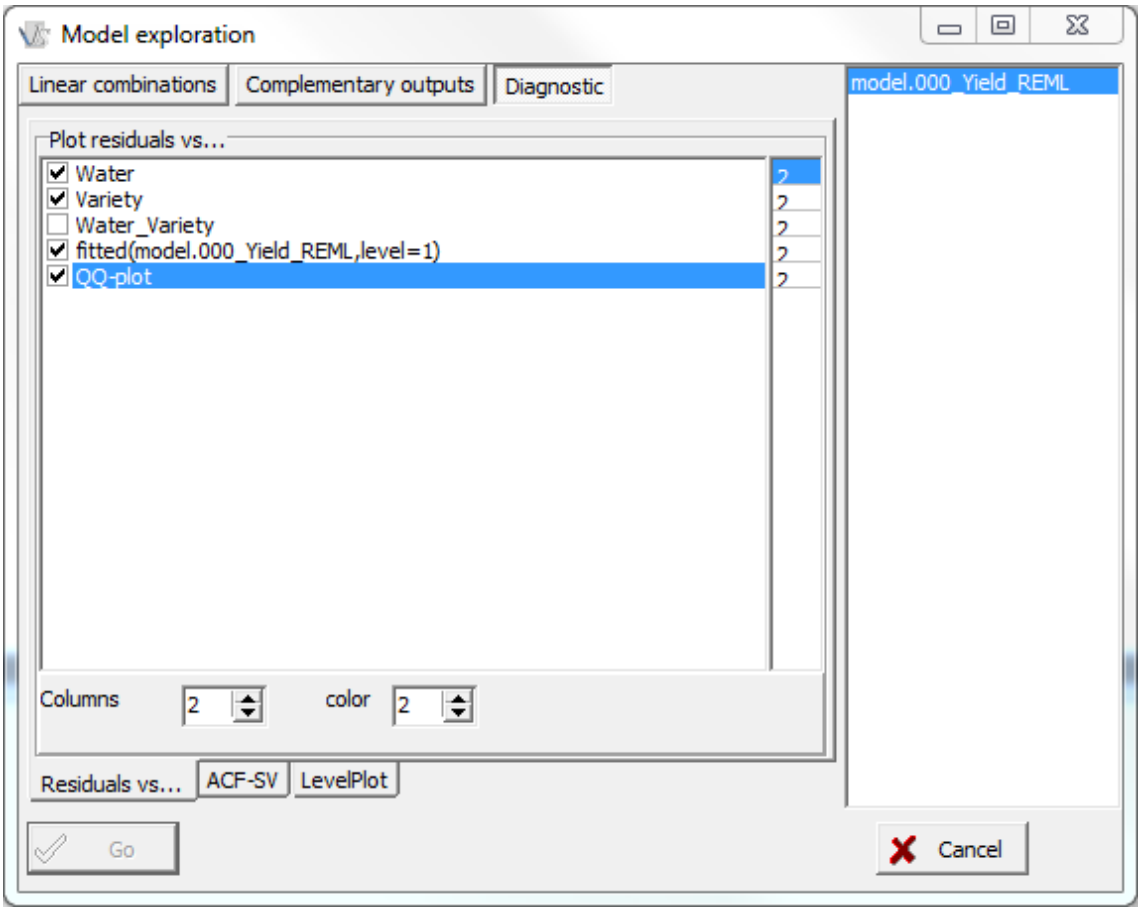



***Figure 48: Model exploration window for the comparison of extended and mixed models displaying the Diagnostic tab (Wheat.IDB2 file).***

If the items are selected from the available list, as shown in Figure 48, the graph shown below will be obtained (Figure 49). This is shown in a new window that R generates, and its content can be copied by right clicking on the image. In the displayed menu, the options “Copy as metafile” or “Copy as bitmap” are available.

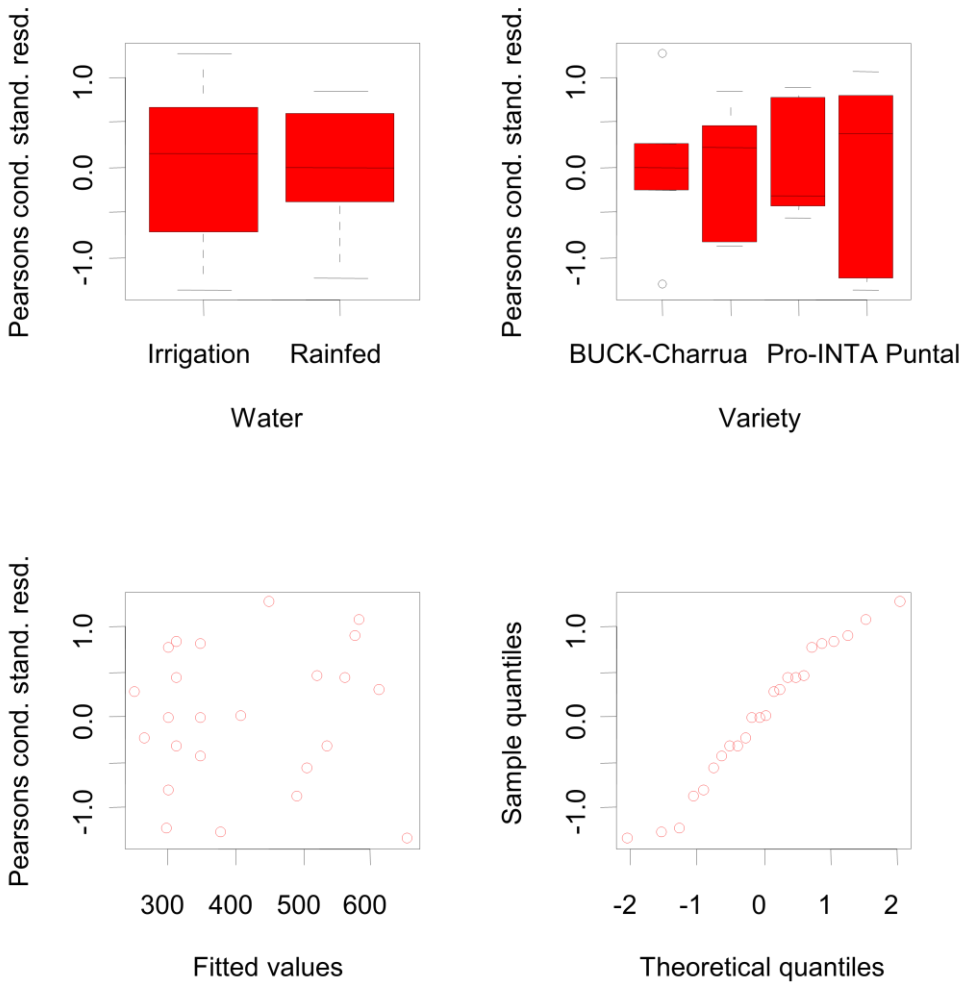



***Figure 49: Diagnostic graphs (Wheat.IDB2 file).***

A quick overview of the figure suggests that there may be heterogeneity of variances among varieties. In order to test if the inclusion of the different residual variance estimations for each variety is necessary, a heteroscedastic model should be fit and compared to a homoscedastic model, using some criterion such as AIC or BIC (or a likelihood ratio test, since the homoscedastic model is a particular case of the heteroscedastic one).

To fit the heteroscedastic model we again select the estimation module for the mixed models, and in the *Heteroscedasticity* tab we select the *varIdent* model; once selected, we double click on *Variety* (in the list on the right-hand side of the window) to specify this variable as the grouping variable (Figure 50). Then we activate the *Add* button to

make the incorporation of this model specification effective. If for some reason the specification is not wanted, the user can erase it by double clicking on it.

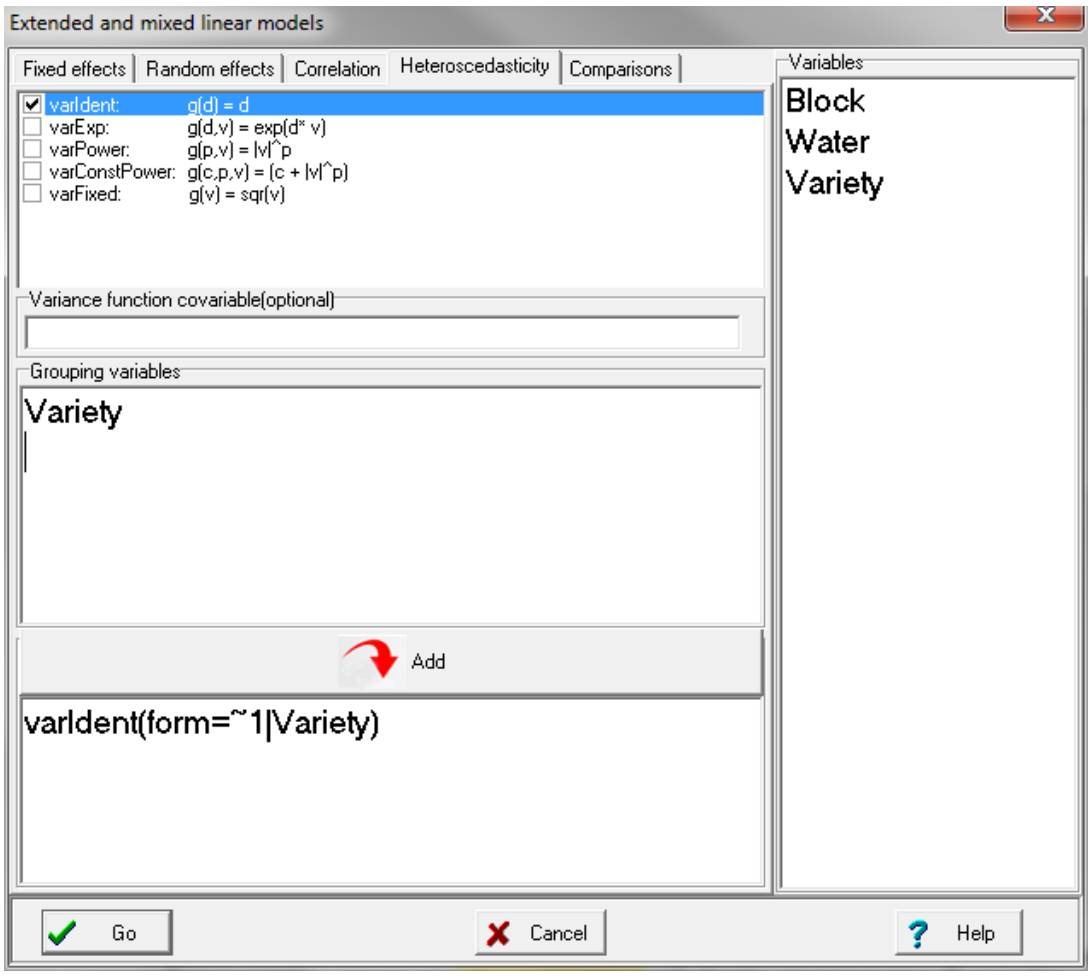



***Figure 50: Window displaying the Heteroscedasticity tab, with the varIdent function with Variety selected as a grouping variable (Wheat.IDB2 file).***

The fitted measures for the specified model are as follows

**Measures of model fit**

| N | AIC | BIC | logLik | Sigma | R2_0 | R2_1 | R2_2 |
|---|---|---|---|---|---|---|---|
| 24 | 209.47 | 220.28 | -90.73 | 24.49 | 0.84 | 0.89 | 0.90 |

*AIC and BIC smaller means better*

Compared to the fitted measures for the homoscedastic model an improvement is not observed; on the other hand, both AIC and BIC increased. Therefore, the heteroscedastic model is rejected.

Returning to the homoscedastic model, we will make multiple comparisons of Fisher's LSD in order to evaluate the differences between the varieties. To do so, in the *Comparisons* tab, in the *Means* subtab, we check the *Variety* option, as shown in Figure 51.

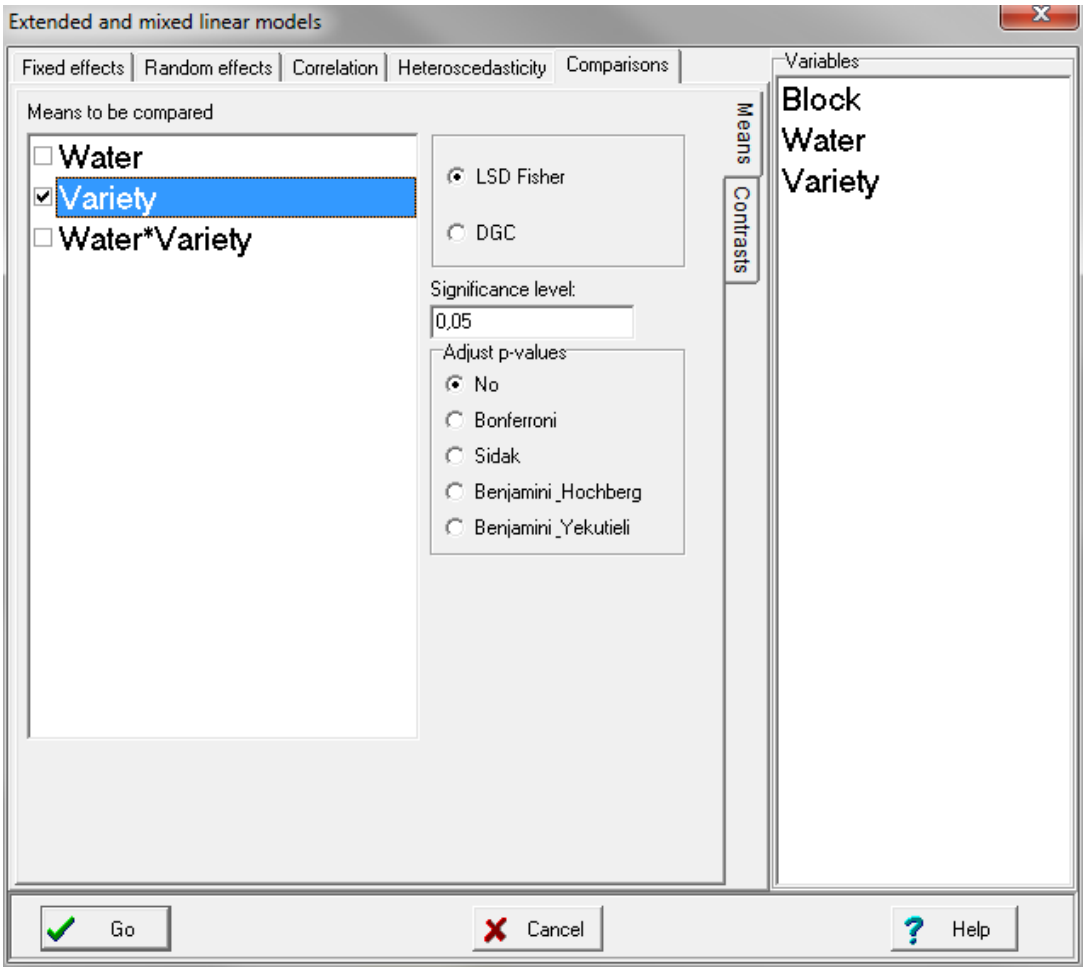



***Figure 51: Window displaying the Comparisons tab, and the selection of the Means subtab (Wheat.IDB2 file).***

At the end of the output there is a means comparison. Note that only *BUCK-Charrua* had the lowest yields, and this occurred independently of whether it had irrigation. Meanwhile, other varieties had statistically indistinguishable yields.

**Adjusted means and standard error for Variety**

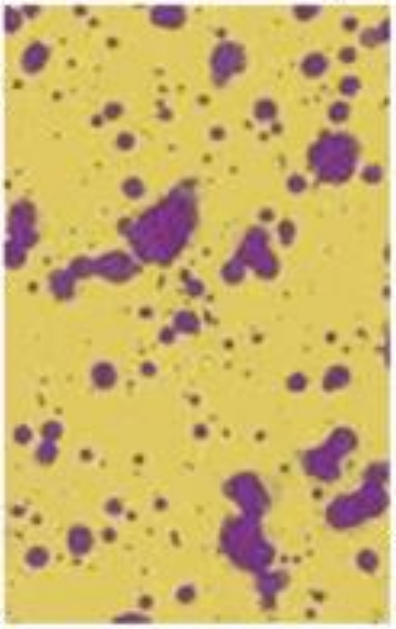
*LSD Fisher (alpha=0.05)*
*p-value correction procedure: No*

| Variety | Means | S.E. | |
|---|---|---|---|
| Pro-INTA Puntal | 469.50 | 46.28 | A |
| Pigue | 430.98 | 28.77 | A |
| LasRosas-INTA | 423.98 | 33.74 | A |
| BUCK-Charrua | 342.73 | 29.38 | B |

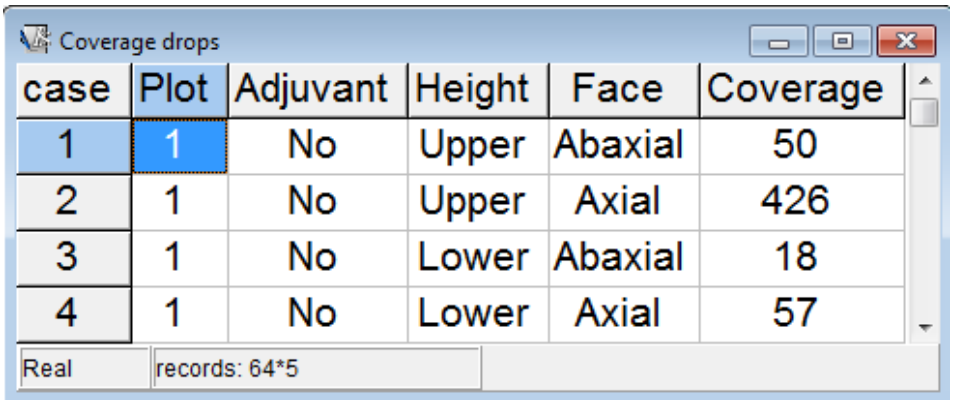
*Means with a common letter are not significantly different (p<= 0.05)*

*Split plots in a completely randomized design*

The following example comes from an experiment whose objective was to evaluate the effect of an adjuvant on the drops coverage and on the uniformity of application on different locations of the leaves in the canopy of a soy crop (Di Rienzo 2007). Sixteen sites where selected, in each of which 4 hydro-sensitive cards where located at two heights of the canopy (*upper* and *lower*), with their sensitive face pointing in one of two directions (*abaxial* and *axial*). The hydro-sensitive cards show a spot on the location where a drop of water falls. The stained surface of these cards is a measure of how much water penetrates and disperses in a given zone of the canopy. In 8 of the 16 sites, an adjuvant was added to the pulverized water (to diminish the surface water tension and improve the dispersion of the drops), and in the remaining 8 no adjuvant was added. Thus, at each pulverization site 4 records are obtained that correspond to the combination of heights (*upper* and *lower*) and the direction of the sensitive face of the cards (*abaxial* and *axial*). Then at each site there is a complete repetition of an experiment with 4 treatments (*upper-abaxial*, *lower-abaxial*, *upper-axial* and *lower-axial*), combined with the presence or absence of the adjuvant in the sprayed solution.



The resulting experiment is tri-factorial, with a main factor (adjuvant) associated with the main plots (sites where the spraying is done), and two factors (height and direction of the sensitive side of the card) associated with the subplots (cards within the site). The file containing the data is called *Coverage drops.IDB2* and the heading of the data table is shown in Figure 52.


Coverage drops

| case | Plot | Adjuvant | Height | Face | Coverage |
|---|---|---|---|---|---|
| 1 | 1 | No | Upper | Abaxial | 50 |
| 2 | 1 | No | Upper | Axial | 426 |
| 3 | 1 | No | Lower | Abaxial | 18 |
| 4 | 1 | No | Lower | Axial | 57 |

Real records: 64*5

***Figure 52: Heading of the data table (Coverage drops.IDB2 file).***

In the data table there is a column that identifies the plot; it is numbered from 1 to 16. This is going to be the only random effect in our model (besides the error term, which is always present).

The linear model for the observations of this experiment is as follows:

$$y_{ijkl} = \mu + \tau_i + \gamma_j + \eta_k + \delta_{ij} + \varphi_{ik} + \lambda_{jk} + \theta_{ijk} + p_l + \varepsilon_{ijkl};$$
$$i = 1,..,2;\ j = 1,...,2;\ k = 1,...,2;\ l = 1,...,16 \qquad (8)$$

where $y_{ijkl}$ represents the response variable (*Coverage*) in the *i*-th level of the *Adjuvant* factor, in the *j*-th level of the *Height* factor, in the *k-th* level of the *Face* factor, and in the *l*-th *Plot*; $\mu$ represents the general mean of the response; $\tau_i$ represents the effect of the *i*-th level of the factor associated with the main plots (*Adyuvant*); $\gamma_j$ represents the effect of the *j*-th level of the *Height* factor; and $\eta_k$ represents the *k*-th level of the *Face* factor, both of which are associated with the subplots; and $\delta_{ij}, \varphi_{ik}, \lambda_{jk}$ y $\theta_{ijk}$ are the interactions of the second and third order corresponding to the *Adjuvant*, *Height* and *Face* factors. Furthermore, $b_l$ and $\varepsilon_{ijkl}$ represent the random effect of the plots and experimental errors, respectively. The assumptions about these random components are that $b_l \sim N\left(0, \sigma_b^2\right)$, that $\varepsilon_{ijkl} \sim N\left(0, \sigma_\varepsilon^2\right)$, and that these two random components are independent. Next we show how the previous model is specified in InfoStat, the output, an interpretation and some complementary actions for the validation of the model. For this, we select the *Extended and mixed linear models >>Model estimation* menu. The variable selection dialogue for this case is shown in Figure 53.

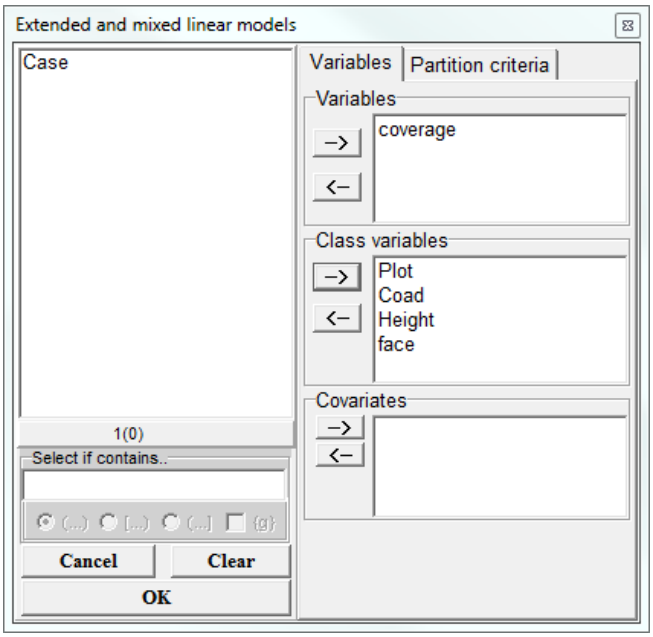



***Figure 53: Variables selection window for Extended and mixed linear models (Coverage drops.IDB2 file).***

The specification of the fixed part of the model in this example contains the three factors and the double and triple interactions (Figure 54).

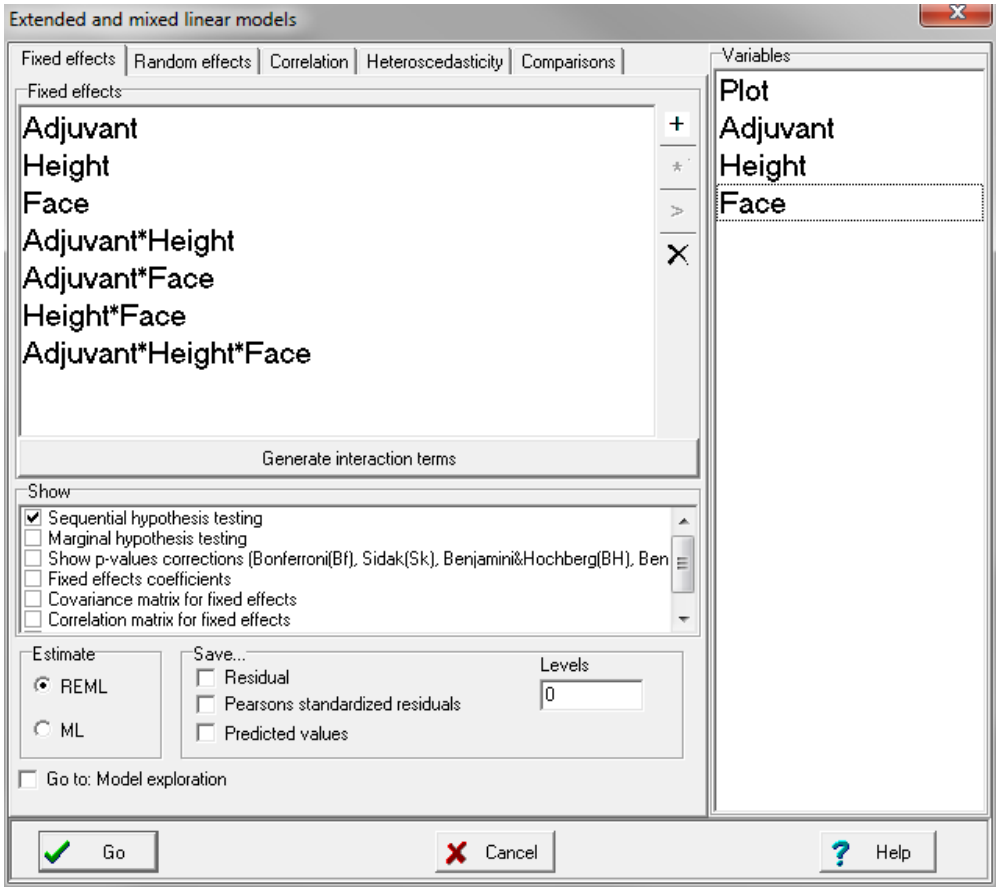



***Figure 54: Window displaying the Fixed effects tab (Coverage drops.IDB2 file).***

The random effect considered in this example is *Plot* (Figure 55).

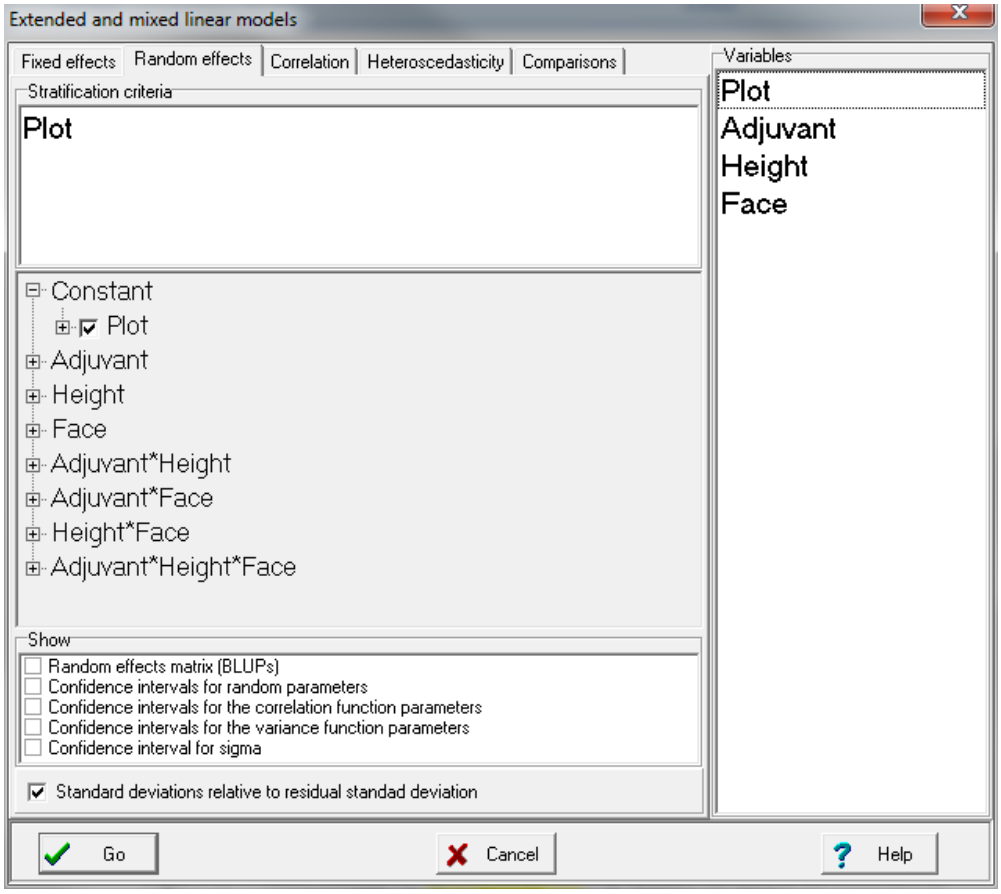



***Figure 55: Window displaying the Random effects tab, with Plot as the stratification criterion (Coverage drops.IDB2 file).***

After accepting the previous specifications, we obtain the following output:

**Extended and mixed linear models**

**R specification of the model**

```
model000_Coverage_REML<-
lme(Coverage~1+Adjuvant+Height+Face+Adjuvant:Height+Adjuvant:Face+Heig
ht:Face+Adjuvant:Height:Face
,random=list(Plot=pdIdent(~1))
,method="REML"
,na.action=na.omit
,data=R.data00
,keep.data=FALSE)
```

**Results for model: model000_Coverage_REML**

*Dependent variable:Coverage*

**Fit measurements**

| N | AIC | BIC | logLik | Sigma | R2_0 | R2_1 |
|---|---|---|---|---|---|---|
| 64 | 670.38 | 690.63 | -325.19 | 65.17 | 0.76 | 0.82 |

*Smaller AIC and BIC is better*

**Sequential hypothesis testing**

| | numDF | denDF | F-value | p-value |
|---|---|---|---|---|
| (Intercept) | 1 | 42 | 233.37 | <0.0001 |
| Adjuvant | 1 | 14 | 1.89 | 0.1909 |
| Height | 1 | 42 | 72.86 | <0.0001 |
| Face | 1 | 42 | 95.32 | <0.0001 |
| Adjuvant:Height | 1 | 42 | 1.58 | 0.2152 |
| Adjuvant:Face | 1 | 42 | 0.01 | 0.9271 |
| Height:Face | 1 | 42 | 34.77 | <0.0001 |
| Adjuvant:Height:Face | 1 | 42 | 0.21 | 0.6476 |

**Random effects parameters**

*Covariance model for random effects: pdIdent*
*Formula: ~1|Plot*

*Standard deviations relative to residual standard deviation and correlation*

| | (const) |
|---|---|
| (const) | 0.40 |

A revision of the standardized residuals of this model using the diagnostic tools in the *Extended and mixed linear models >> Model exploration* menu shows that heterogeneity of variances may exist when we compare the residuals for both faces (abaxial and axial) of the hydro-sensitive card (Figure 56).

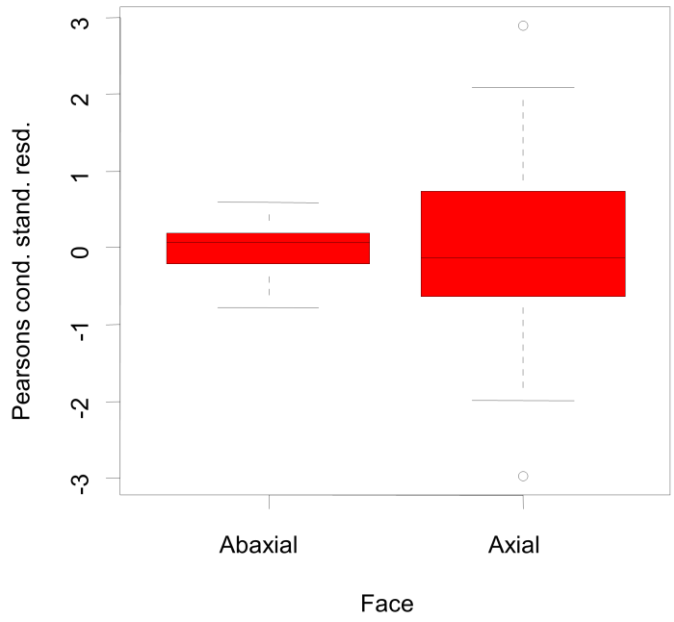



***Figure 56: Box plots for the Pearson standardized residuals for the levels of the factor Face.***

In order to take into account the heterogeneity of variances, we will again select the model estimation menu. All of the previous specifications have been kept, which is why we need only concentrate on the specification of the variance model. To do so, we use the *Heteroscedasticity* tab as shown in Figure 57.

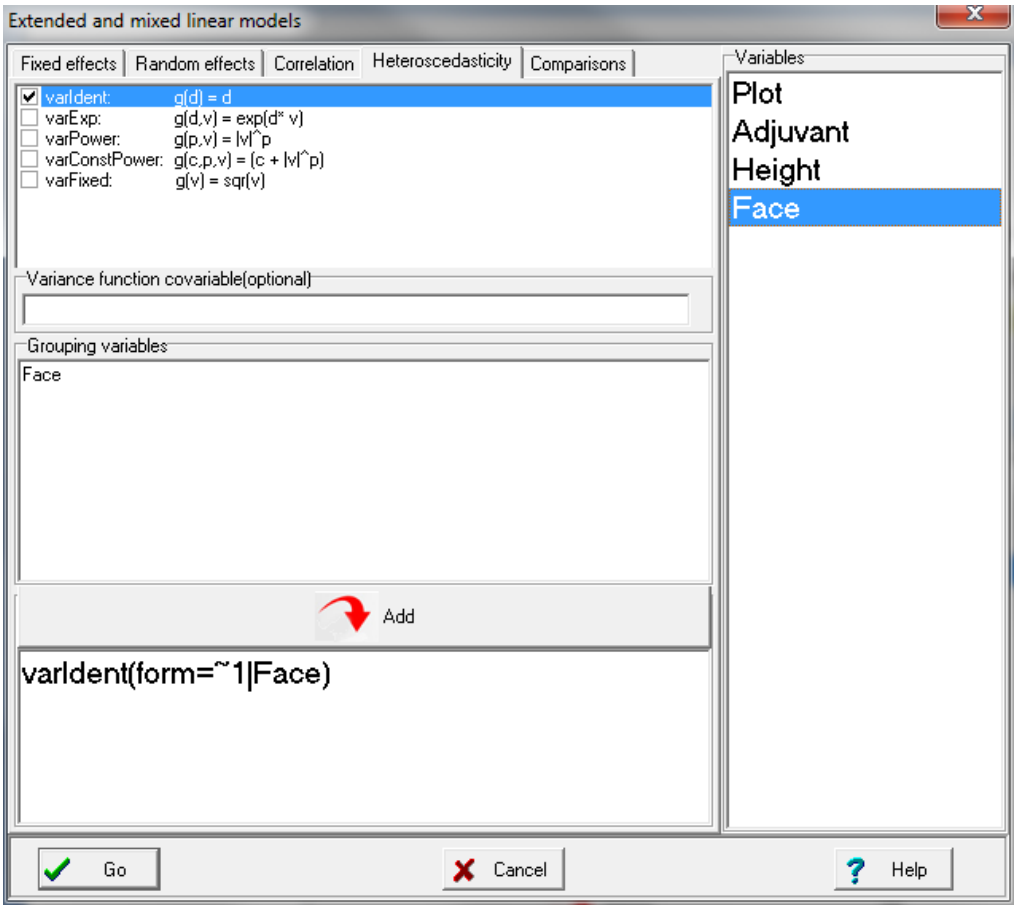



***Figure 57: Window displaying the Heteroscedasticity tab, with Face as a grouping variable (Coverage drops.IDB2 file).***

The resulting output is as follows:

**Extended and mixed linear models**

**R specification of the model**

```
model002_Coverage_REML<-
lme(Coverage~1+Adjuvant+Height+Face+Adjuvant:Height+Adjuvant:Face+Heig
ht:Face+Adjuvant:Height:Face
,random=list(Plot=pdIdent(~1))
,weight=varComb(varIdent(form=~1|Face))
,method="REML"
,na.action=na.omit
,data=R.data00
,keep.data=FALSE)
```

**Results for model: model002_Coverage_REML**

*Dependent variable:Coverage*

**Fit measurements**

| N | AIC | BIC | logLik | Sigma | R2_0 | R2_1 |
|---|---|---|---|---|---|---|
| 64 | 636.54 | 658.82 | -307.27 | 21.26 | 0.76 | 0.81 |

*Smaller AIC and BIC is better*

**Sequential hypothesis testing**

| | numDF | denDF | F-value | p-value |
|---|---|---|---|---|
| (Intercept) | 1 | 42 | 176.66 | <0.0001 |
| Adjuvant | 1 | 14 | 4.19 | 0.0599 |
| Height | 1 | 42 | 53.72 | <0.0001 |
| Face | 1 | 42 | 98.43 | <0.0001 |
| Adjuvant:Height | 1 | 42 | 13.83 | 0.0006 |
| Adjuvant:Face | 1 | 42 | 0.01 | 0.9259 |
| Height:Face | 1 | 42 | 35.90 | <0.0001 |
| Adjuvant:Height:Face | 1 | 42 | 0.22 | 0.6423 |

**Random effects parameters**

*Covariance model for random effects: pdIdent*
*Formula: ~1|Plot*

*Standard deviations relative to residual standard deviation and correlation*

| | (const) |
|---|---|
| (const) | 1.06 |

**Variance structure**

*Variance model: varIdent*
*Formula: ~ 1 | Face*

*Variance-function parameters*

| Parameter | Estim. |
|---|---|
| Abaxial | 1.00 |
| Axial | 4.15 |

The model for this data would be $y_{ijkl} = \mu_{i\,jk} + p_l + \varepsilon_{ijkl}$, where $\mu_{i\,jk}$ represents the fixed effect of the *i*-th Adjuvant, *j*-th Height and *k*-th *Face* (*Axial* or *Abaxial*), $b_l$ is the random effect of *l*-th experimental plot experimental that is assumed $N\left(0,\sigma_p^2\right)$, and $\varepsilon_{ijkl} \sim N\left(0,\sigma_k^2\right)$. The variance of an observation taken from a randomly selected plot will depend on whether the observation is taken from a card facing the *Axial* or *Abaxial* direction. Thus, if we take an observation from an *Abaxial* face, the variance is $(21.26*1.06)^2 + (21.26*1)^2$, and if we take the observation from an *Axial* face it is $(21.26*1.06)^2 + (21.26*4.15)^2$.

The summary statistics for the homoscedastic and heteroscedastic models are shown below.

**Measures for homoscedastic model fits**

| N | AIC | BIC | logLik | Sigma | R2_0 | R2_1 |
|---|---|---|---|---|---|---|
| 64 | 670.38 | 690.63 | -325.19 | 65.17 | 0.76 | 0.82 |

*AIC and BIC smaller means better*

**Measures heteroscedastic model fits**

| N | AIC | BIC | logLik | Sigma | R2_0 | R2_1 |
|---|---|---|---|---|---|---|
| 64 | 636.54 | 658.82 | -307.27 | 21.26 | 0.76 | 0.81 |

*AIC and BIC smaller means better*

Upon comparing the AIC and BIC we can see that the last fitted model is better, and therefore the interpretation of the hypothesis tests should be based on the second model.

Note that in the variance structure, the residual standard deviation of the observations taken from the cards facing in the *Axial* direction is 4.15 times higher than that of the observations taken from the cards facing in the *Abaxial* direction.

In studying the results of the hypothesis tests it turns out that the `Adjuvant:Height:Face` interaction is not significant, which is why double interactions can be studied (Figure 58). Among these, `Adjuvant:Height` and `Height:Face` are significant. These interactions are analyzed by using the *Comparisons* tab of the *Extended and mixed linear models* window and by selecting the corresponding interactions in the list of model terms shown in this window. This procedure will create a table with the means of all the resulting combinations of the factor levels involved in the interaction. The result, shown at the end of the output, displays the following tables.

**Adjusted means and standard error for Adjuvant*Height**

*LSD Fisher (alpha=0.05)*
*p-value correction procedure: No*

| Adjuvant | Height | Means | S.E. | | |
|---|---|---|---|---|---|
| Yes | Upper | 253.94 | 17.89 | A | |
| No | Upper | 204.69 | 17.89 | A | |
| Yes | Lower | 94.38 | 17.89 | | B |
| No | Lower | 86.13 | 17.89 | | B |

*Means with a common letter are not significantly different (p<= 0.05)*

**Adjusted means and standard error for Height*Face**

*LSD Fisher (alpha=0.05)*
*p-value correction procedure: No*

| Height | Face | Means | S.E. | | | |
|---|---|---|---|---|---|---|
| Upper | Axial | 356.88 | 22.74 | A | | |
| Lower | Axial | 121.75 | 22.74 | | B | |
| Upper | Abaxial | 101.75 | 7.73 | | B | |
| Lower | Abaxial | 58.75 | 7.73 | | | C |

*Means with a common letter are not significantly different (p<= 0.05)*

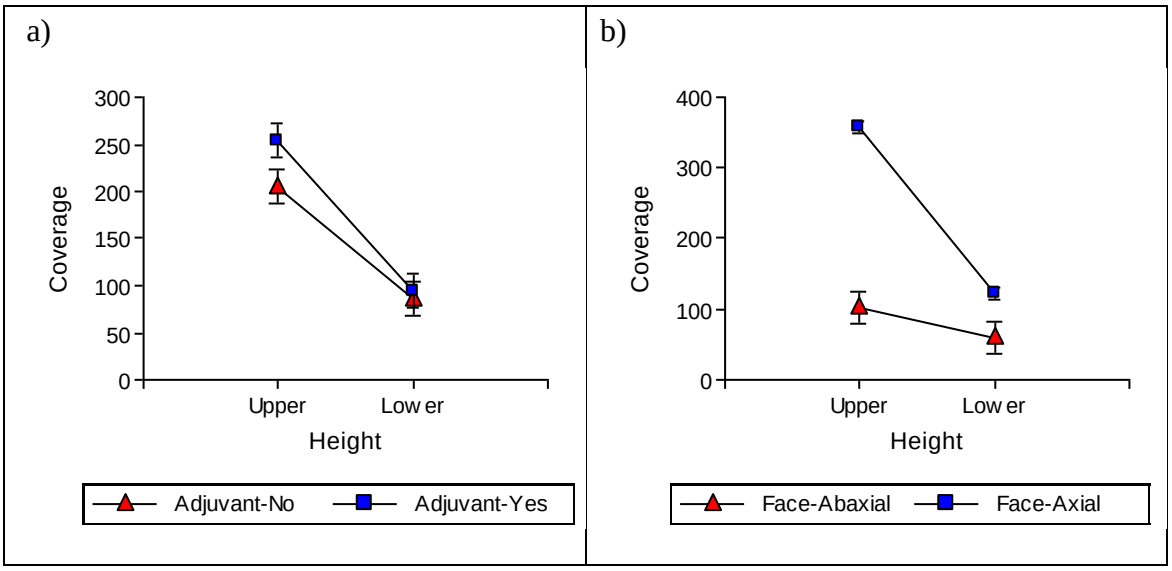



***Figure 58: Dot plot for the study of the interaction between Adjuvant and Height (a) and between Face and Height (b).***

## *Split-split plot*

This design is based on the same principle as the split plots, but it expands it a step further. The principle can be arbitrarily extended to deeper levels of division. The linear model for this design, assuming that the main plots are grouped in randomized complete blocks, is the following:

$$y_{ijkl} = \mu + \alpha_i + \beta_j + \chi_k + \delta_{ij} + \phi_{ik} + \gamma_{jk} + \eta_{ijk} + b_l + p_{il} + sp_{jil} + \varepsilon_{ijkl} \qquad (9)$$

In the previous expression $\mu$ represents the general mean; $\alpha_i$ represents the *i*-th level of the factor associated with the main plots; $\beta_j$ represents the *j*-th level of the factor associated with the subplots within the main plots; $\chi_k$ represents the *k*-th level of the factor associated with the subplots (within the subplots); and $\delta_{ij}$, $\phi_{ik}$, $\gamma_{jk}$ y $\eta_{ijk}$ represents the corresponding interactions. The random terms of this model correspond to the effects of the blocks, $b_l \sim N\left(0, \sigma_b^2\right)$, the effects of the plots, $p_{il} \sim N\left(0, \sigma_p^2\right)$, the effects of the sub-plots, $sp_{jil} \sim N\left(0, \sigma_{sp}^2\right)$, and the experimental error, $\varepsilon_{ijkl} \sim N\left(0, \sigma_\varepsilon^2\right)$. All of them, as usual, are assumed to be independent.

Let us now consider an example. The data are in the *Starch quality.IDB2* file (Di Rienzo 2007). In this experiment, we evaluate the water absorption index (WAI) of cooked and raw starch of two genotypes of Quinoa (Chenopodium quinoa) cultivated under 4 levels

of nitrogen fertilization. The varieties are *Faro* and *UDEC10*. These are assigned to main plots divided into 3 blocks. The plots where the varieties were sown were split into 4 plots, to which 4 doses of fertilization were assigned: 0, 75, 150 y 225 kg/ha. The subplots were split again in two to assign the Cooked or Raw treatment. The diagram for this experimental design is shown in Figure 59.

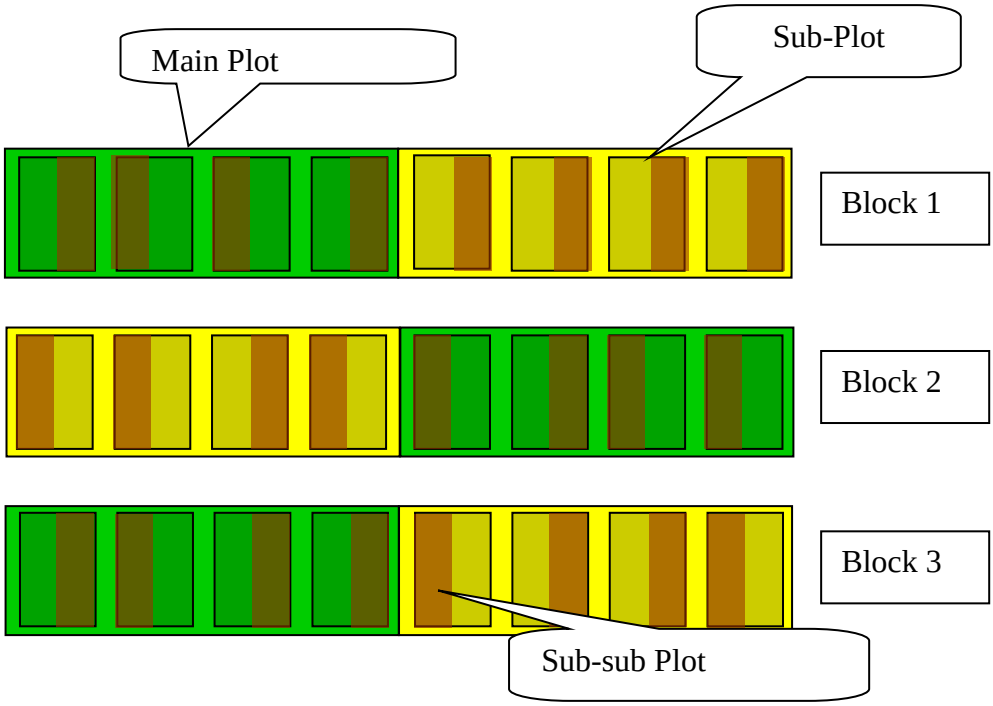



***Figure 59: Diagram of the split-split plot design for the example (Starch quality.IDB2 file).***

For the analysis of this design with a mixed model, in addition to the tri-factorial specification of the fixed part (as is the case for a classical three-factor experiment), we need only to specify the random part to include the random effect of the *Blocks*, of the *Main Plots* within the *Blocks* and of the *Subplots* within the *Plots*. The heading of the *Starch quality.IDB2* file is shown in the Figure 60.

Starch quality

| case | Block | Genotype | Nitrogen | Cooking | WAI |
|---|---|---|---|---|---|
| 1 | 1 | Faro | 0 | Cooked | 3,97 |
| 2 | 1 | Faro | 0 | Raw | 1,96 |
| 3 | 1 | Faro | 75 | Cooked | 2,88 |

Real | records: 48*5

***Figure 60: Heading of the data table (Starch quality.IDB2).***

The variables selection window for this example must contain the information shown in Figure 61.

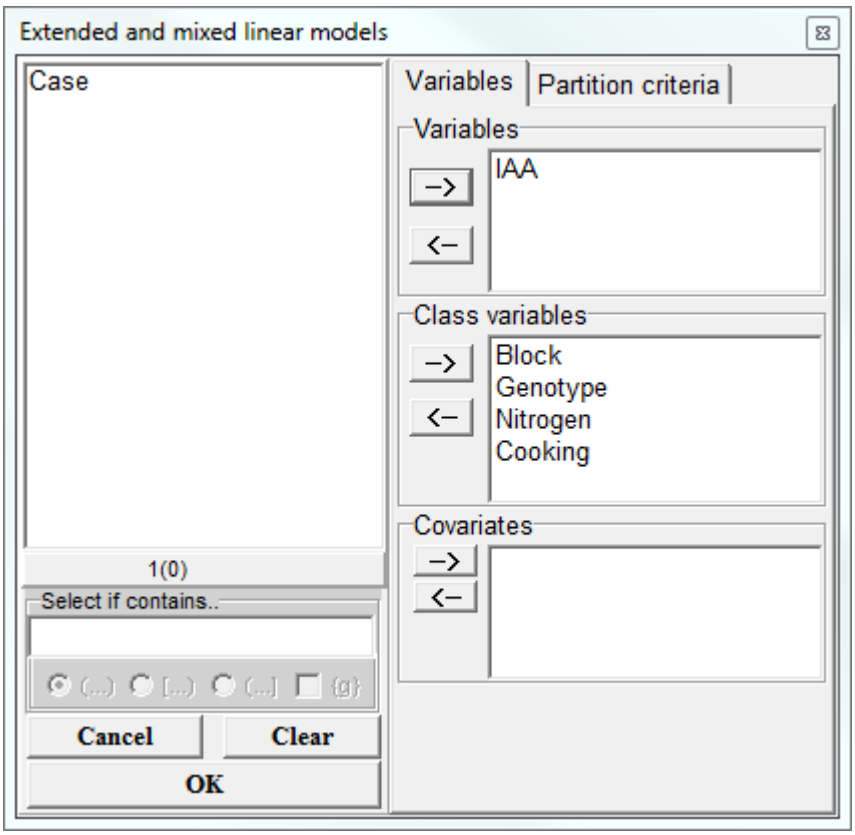



***Figure 61: Variables selection window for the Extended and mixed linear models (Starch quality.IDB2 file).***

The specification of the fixed part should contain the factors and interactions as shown in

Figure 62.

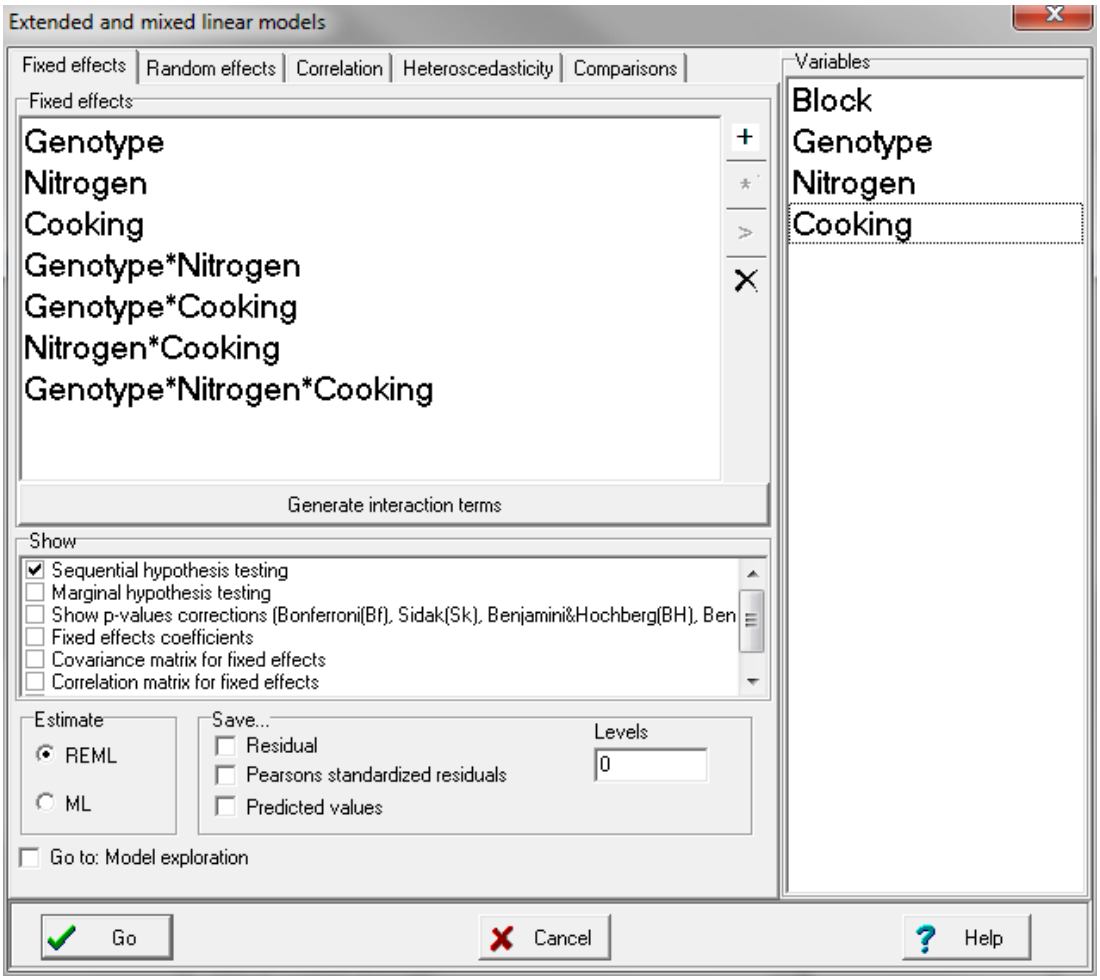



***Figure 62: Window with the tab Fixed effects displayed for the data in the file Starch quality.IDB2.***

The random part should have the blocks (*Block*), the main plots within the Blocks (*Genotype*), and the subplots within the main plots (*Nitrogen*) declared (Figure 63).

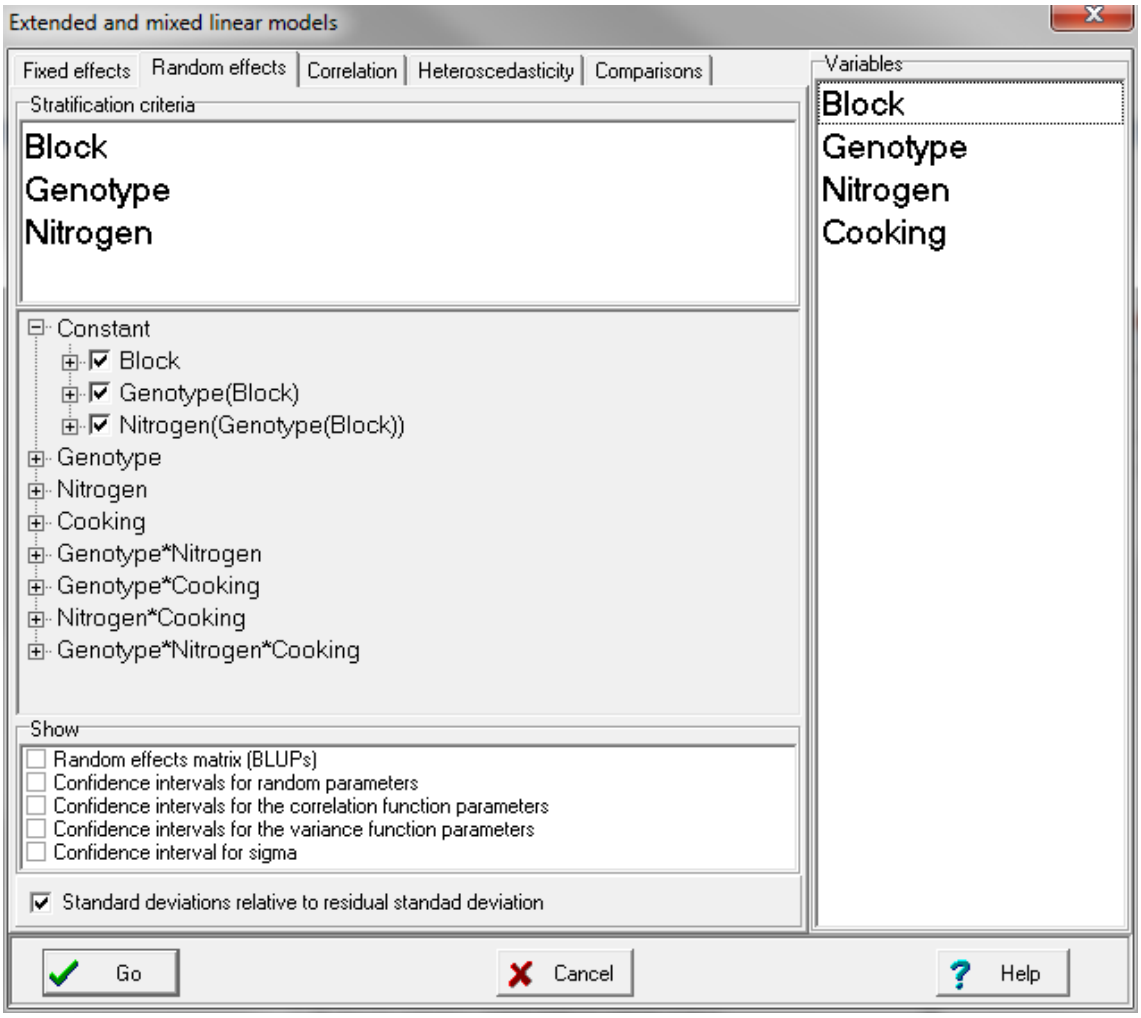



***Figure 63: Window displaying the Random effects tab (Starch quality.IDB2 file).***

The corresponding output is as follows:

```
Extended and mixed linear models

R specification of the model

model000_WAI_REML<-
lme(WAI~1+Genotype+Nitrogen+Cooking+Genotype:Nitrogen+Genotype:Cooking
+Nitrogen:Cooking+Genotype:Nitrogen:Cooking
,random=list(Block=pdIdent(~1)
,Genotype=pdIdent(~1)
,Nitrogen=pdIdent(~1))
,method="REML"
,na.action=na.omit
,data=R.data00
,keep.data=FALSE)

Results for model: model000_WAI_REML

Dependent variable:WAI
```

**Fit measurements**

| N | AIC | BIC | logLik | Sigma | R2_0 | R2_1 | R2_2 | R2_3 |
|---|---|---|---|---|---|---|---|---|
| 48 | 116.45 | 145.76 | -38.22 | 0.61 | 0.75 | 0.75 | 0.75 | 0.75 |

*Smaller AIC and BIC is better*

**Sequential hypothesis testing**

| | numDF | denDF | F-value | p-value |
|---|---|---|---|---|
| (Intercept) | 1 | 16 | 1389.20 | <0.0001 |
| Genotype | 1 | 2 | 14.49 | 0.0626 |
| Nitrogen | 3 | 12 | 0.78 | 0.5287 |
| Cooking | 1 | 16 | 32.90 | <0.0001 |
| Genotype:Nitrogen | 3 | 12 | 0.88 | 0.4769 |
| Genotype:Cooking | 1 | 16 | 37.67 | <0.0001 |
| Nitrogen:Cooking | 3 | 16 | 1.74 | 0.1998 |
| Genotype:Nitrogen:Cooking | 3 | 16 | 0.46 | 0.7108 |

**Random effects parameters**

*Covariance model for random effects: pdIdent*
*Formula: ~1|Block*

*Standard deviations relative to residual standard deviation and correlation*

| | (const) |
|---|---|
| (const) | 1.3E-05 |

*Covariance model for random effects: pdIdent*
*Formula: ~1|Genotype in Block*

*Standard deviations relative to residual standard deviation and correlation*

| | (const) |
|---|---|
| (const) | 5.0E-06 |

*Covariance model for random effects: pdIdent*
*Formula: ~1|Nitrogen in Genotype in Block*

*Standard deviations relative to residual standard deviation and correlation*

| | (const) |
|---|---|
| (const) | 1.8E-05 |

We could continue to conduct diagnostic tests, but we will assume that the model is correct. The interpretation of the hypothesis tests indicates that only the Genotype:Cooking interaction is significant. The multiple comparisons for the means of the corresponding treatments for this interaction are shown below. In these tests, note that only the cooked starch of the *UDEC10* genotype shows a WAI significantly higher than that of the rest of the combinations of *Genotype* and *Cooking*.

**Adjusted means and standard error for Genotype*Cooking**

*LSD Fisher (alpha=0.05)*
*p-value correction procedure: No*

| Genotype | Cooking | Means | S.E. | | |
|---|---|---|---|---|---|
| UDEC10 | Cooked | 4.64 | 0.18 | A | |
| Faro | Raw | 2.97 | 0.18 | | B |
| Faro | Cooked | 2.90 | 0.18 | | B |
| UDEC10 | Raw | 2.56 | 0.18 | | B |

*Means with a common letter are not significantly different (p<= 0.05)*

***An alternative way to formulate the previous model consists of leaving the fixed effects as shown in*** Figure 62 and specifying the random effects as shown in Figure 64. The results are exactly the same as before, except for the calculation of the degrees of freedom of the denominator; therefore, the probability values are not the same. This approximation is also valid, although the previous version is in line with the traditional analysis for balanced data based on fixed effects. Note that the variance estimations are presented differently.

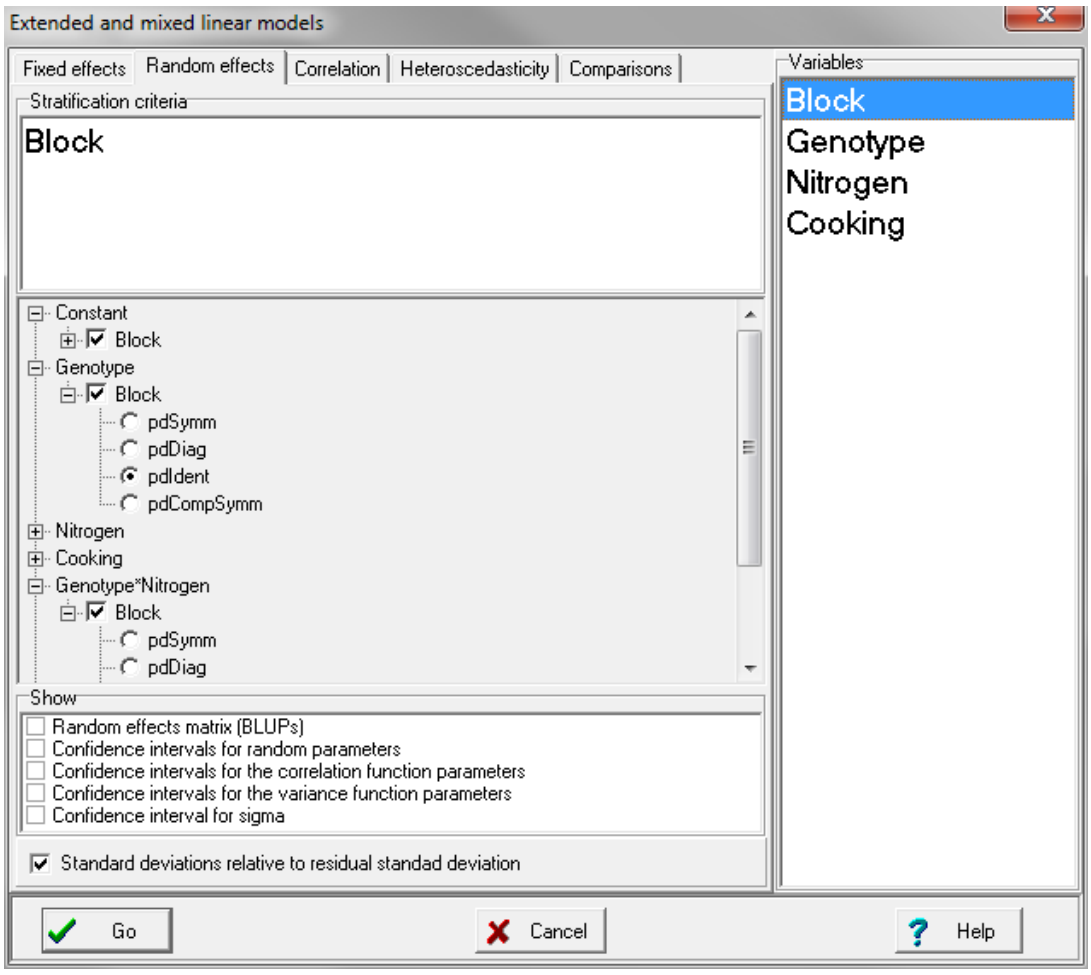



***Figure 64: Window displaying the Random effects tab that shows another way to specify the random part (Starch quality.IDB2 file).***

**Extended and mixed linear models**

**R specification of the model**

```
model003_WAI_REML<-
lme(WAI~1+Genotype+Nitrogen+Cooking+Genotype:Nitrogen+Genotype:Cooking
+Nitrogen:Cooking+Genotype:Nitrogen:Cooking
,random=list(Block=pdIdent(~1)
,Block=pdIdent(~Genotype-1)
,Block=pdIdent(~Genotype:Nitrogen-1))
,method="REML"
,na.action=na.omit
,data=R.data00
,keep.data=FALSE)
```

**Results for model: model003_WAI_REML**

*Dependent variable:WAI*

**Fit measurements**

| N | AIC | BIC | logLik | Sigma | R2_0 | R2_1 | R2_2 | R2_3 |
|---|---|---|---|---|---|---|---|---|
| 48 | 116.45 | 145.76 | -38.22 | 0.61 | 0.75 | 0.75 | 0.75 | 0.75 |

*Smaller AIC and BIC is better*

**Sequential hypothesis testing**

| | numDF | denDF | F-value | p-value |
|---|---|---|---|---|
| (Intercept) | 1 | 30 | 1389.20 | <0.0001 |
| Genotype | 1 | 30 | 14.49 | 0.0006 |
| Nitrogen | 3 | 30 | 0.78 | 0.5157 |
| Cooking | 1 | 30 | 32.90 | <0.0001 |
| Genotype:Nitrogen | 3 | 30 | 0.88 | 0.4605 |
| Genotype:Cooking | 1 | 30 | 37.67 | <0.0001 |
| Nitrogen:Cooking | 3 | 30 | 1.74 | 0.1807 |
| Genotype:Nitrogen:Cooking | 3 | 30 | 0.46 | 0.7089 |

**Random effects parameters**

*Covariance model for random effects: pdIdent*
*Formula: ~1|Block*

*Standard deviations relative to residual standard deviation and correlation*

| | (const) |
|---|---|
| (const) | 1.3E-05 |

*Covariance model for random effects: pdIdent*
*Formula: ~Genotype - 1|Block*

*Standard deviations relative to residual standard deviation and correlation*

| | Faro | UDEC10 |
|---|---|---|
| Faro | 5.0E-06 | 0.00 |
| UDEC10 | 0.00 | 5.0E-06 |

*Covariance model for random effects: pdIdent*
*Formula: ~Genotype:Nitrogen - 1|Block*

*Standard deviations relative to residual standard deviation and correlation*

| | Faro:0 | UDEC10:0 | Faro:75 | UDEC10:75 | Faro:150 | UDEC10:150 | Faro:225 | UDEC10:225 |
|---|---|---|---|---|---|---|---|---|
| Faro:0 | 1.8E-05 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| UDEC10:0 | 0 | 1.8E-05 | 0 | 0 | 0 | 0 | 0 | 0 |
| Faro:75 | 0 | 0 | 1.8E-05 | 0 | 0 | 0 | 0 | |
| UDEC10:75 | 0 | 0 | 0 | 1.8E-05 | 0 | 0 | 0 | 0 |
| Faro:150 | 0 | 0 | 0 | 0 | 1.8E-05 | 0 | 0 | 0 |
| UDEC10:150 | 0 | 0 | 0 | 0 | 0 | 1.8E-05 | 0 | 0 |
| Faro:225 | 0 | 0 | 0 | 0 | 0 | 0 | 1.8E-05 | 0 |
| UDEC10:225 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1.8E-05 |

## *Application of mixed models for repeated measures in time*

### *Longitudinal data*

When modeling longitudinal data, the most important aspect to consider is the structure of the residual covariance matrix, which can be modeled by specifying the correlation matrix. In some cases, the variances can also be different for some grouping criterion and heteroscedasticity should be modeled. Let us recall that there is a residual correlation between observations that share the same value of the stratification criterion, also known as subject (for example, observations taken over the same person, same plot, same animal, same tree, etc.). Thus, the residual covariance matrix for all the observations will be a block diagonal matrix by blocks, and in each block the desired structure will be reflected, *i.e.* compound symmetry, first-order autoregressive, etc.

To specify this, InfoStat has two tabs. In the *Correlation* tab, options that allow the specification of the error correlation structure can be found, and in the *Heteroscedasticity* tab different variance models can be selected. Thus, the different possible structures of the residual covariance matrix that can be fitted result from the combination of the different correlation structures with potential heteroscedasticity in time. If the researcher also wishes to specify a random effect, it is also possible to do so by using the corresponding tab. In this case much caution should be taken to avoid combining random effects, correlation structures and heteroscedasticity such that the final model is not identifiable. This occurs when there is an infinite set of values for the parameters for which the model is indistinguishable, and therefore the solutions to the likelihood equations are not unique.

Examples of these situations occur when the user specifies both a compound symmetry correlation structure with a stratification criterion (for example, the plot) and a random effect of the same stratification criterion on the constant. In this case the covariance structure of the observations will be a diagonal matrix by blocks, and each block will have a compound symmetry structure. Therefore, this structure intrinsically has two parameters. However, because of the way in which we have specified the structure, three parameters are shown (random effect variance, intra-class correlation of the residual correlation structure, and residual variance). This overparameterization creates infinite solutions, and consequently the estimators cannot be interpreted (and in many cases the numerical algorithm does not converge). Another common phenomenon is a

correlation without structure (*corSymm*) with a given stratification criterion (for example, plot) and a random effect of that same stratification criteria on the constant (*intercept*).

### *Analysis of a forage establishment experiment*

Next is an example of a model for repeated measures in time. The data come from a trial for forage establishment that compares five tilling methods (minimal tilling, minimal tilling with herbicide, minimal tilling with herbicide and disc plow on day 45, no tilling, and conventional tilling, here after T1, T2, T3, T4 and T5, respectively) in the humid central region of Puerto Rico. The species used was *Brachiaria decumbens* cv. Basilik. The experimental was conducted under a randomized complete blocks design with three repetitions; here we analyzed the coverage measurements (estimated percent coverage in each plot). There are 5 repeated measurements, taken at one-month intervals between August and December 2001 (Moser & Macchiavelli 2002). The data can be found in the *Forage Coverage.IDB2* file in InfoStat test datasets. Figure 65 shows the average profile of the coverage observed during the 5 measurements for each treatment.

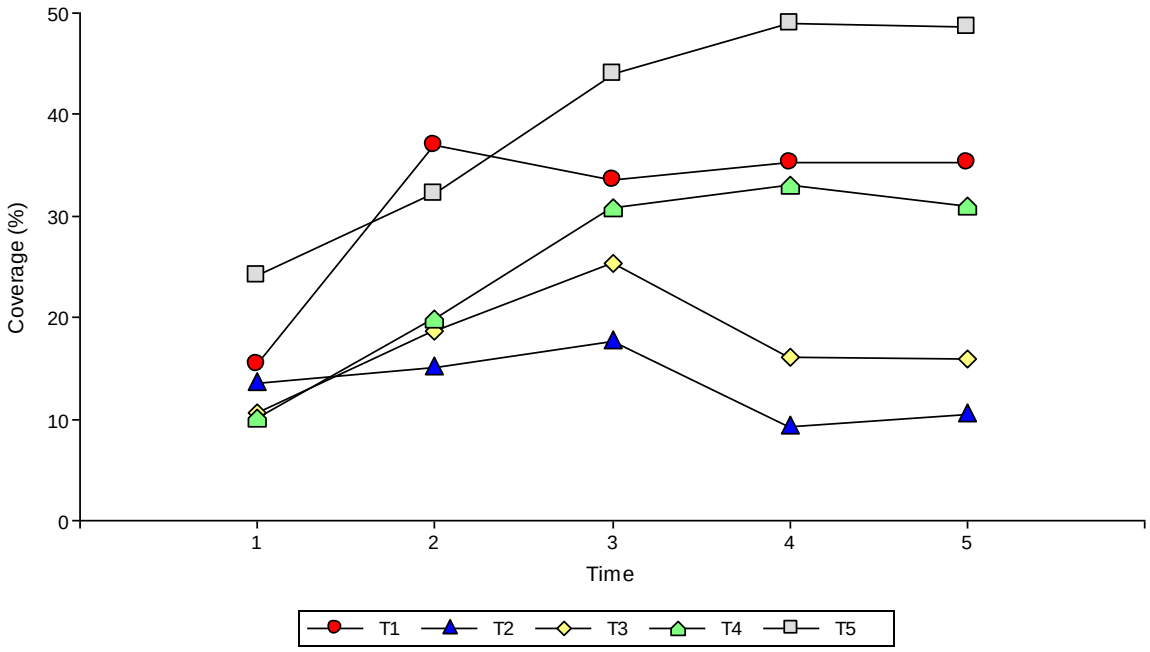



***Figure 65: Relationship between Coverage and Time for five treatments (Forage Coverage.IDB2 file).***

As a general strategy to analyze this data, models with different covariance structures will be adjusted first, appropriately combining residual correlation structures, residual heteroscedasticity, and random effects. The model that best describes the data will be selected using penalized likelihood criteria (AIC and BIC), and inferences regarding the

means (comparing treatments, studying the effect of time, analyzing whether the average profiles vary in time, whether they are parallel, etc.) will be based on this model.

To choose the best model we will start by proposing a simple model with few parameters to estimate (i.e., parsimonious), and then we will add parameters until finally considering the model without structure, which is the least parsimonious.

The following covariance structures will be used for the data (marginal covariance):

1. *Random block effects and independent homoscedastic errors.*
2. *Random block and plot effects, and independent homoscedastic errors.*
3. *Random block effects and independent heteroscedastic errors.*
4. *Random block and plot effects, and independent heteroscedastic errors.*
5. *Random block effects, constant correlation within plots, and homoscedastic errors (equivalent to model 2).*
6. *Random block effects, constant correlation within plots, and heteroscedastic errors.*
7. *Random block effects, first-order autoregressive structure between errors of the same plot and homoscedastic errors.*
8. *Random block and plot effects, first-order autoregressive structure between errors of the same plot and homoscedastic errors.*
9. *Random block effects, first-order autoregressive structure between errors of the same plot and heteroscedastic errors.*
10. *Random block and plot effects, first-order autoregressive structure between errors of the same plot and heteroscedastic errors.*
11. *Random block effects, unstructured correlations between errors from the same plot and time-varying residual variances.*

To fit these models, the variables should be specified as shown in Figure 66.

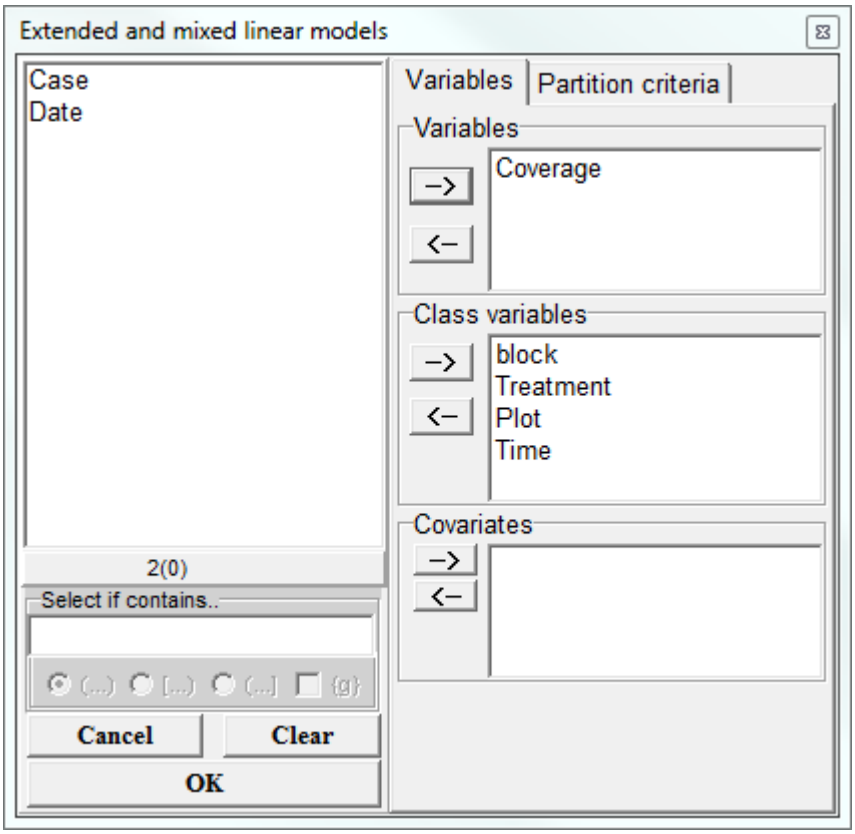



***Figure 66: Variables selection window for Extended and mixed linear models (Forage Coverage.IDB2 file).***

The same means model was used in every case, because the fixed part of the model did not change (this is essential in order to compare covariance structures using REML, and thus AIC and BIC criteria) (Figure 67). A way to declare each one of the models to be evaluated is shown below, followed by the InfoStat output with the corresponding fitness measures.

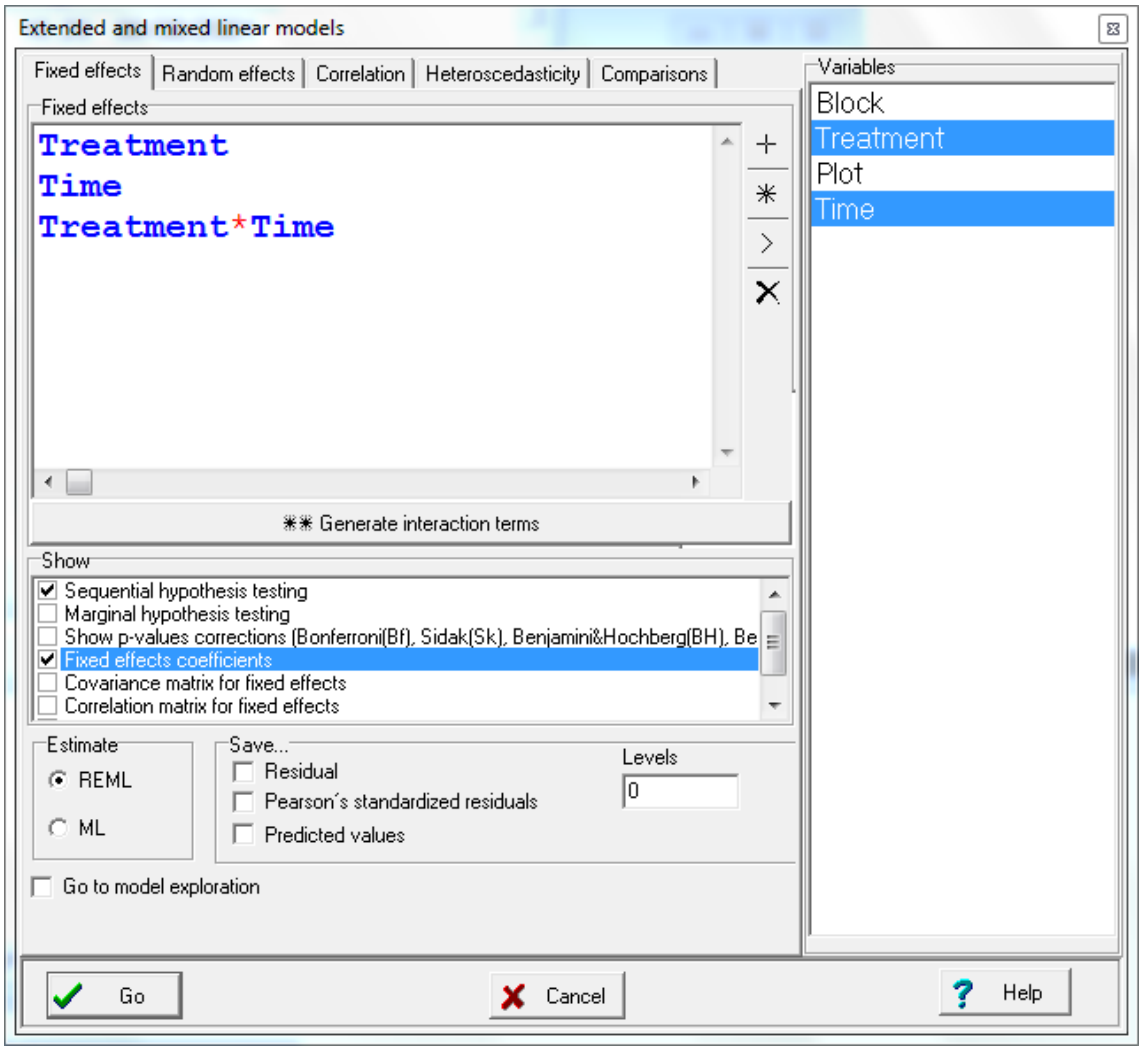



***Figure 67: Window displaying the Fixed effects tab (Forage Coverage.IDB2 file).***

Model 1: *Random block effects and independent homoscedastic errors.*

Select *Block* in the *Random effects* tab (Figure 68). In the *Correlation* tab the *Independent errors* should be declared (Figure 69), which is the default option, and nothing should be declared in the *Heteroscedasticity* tab.

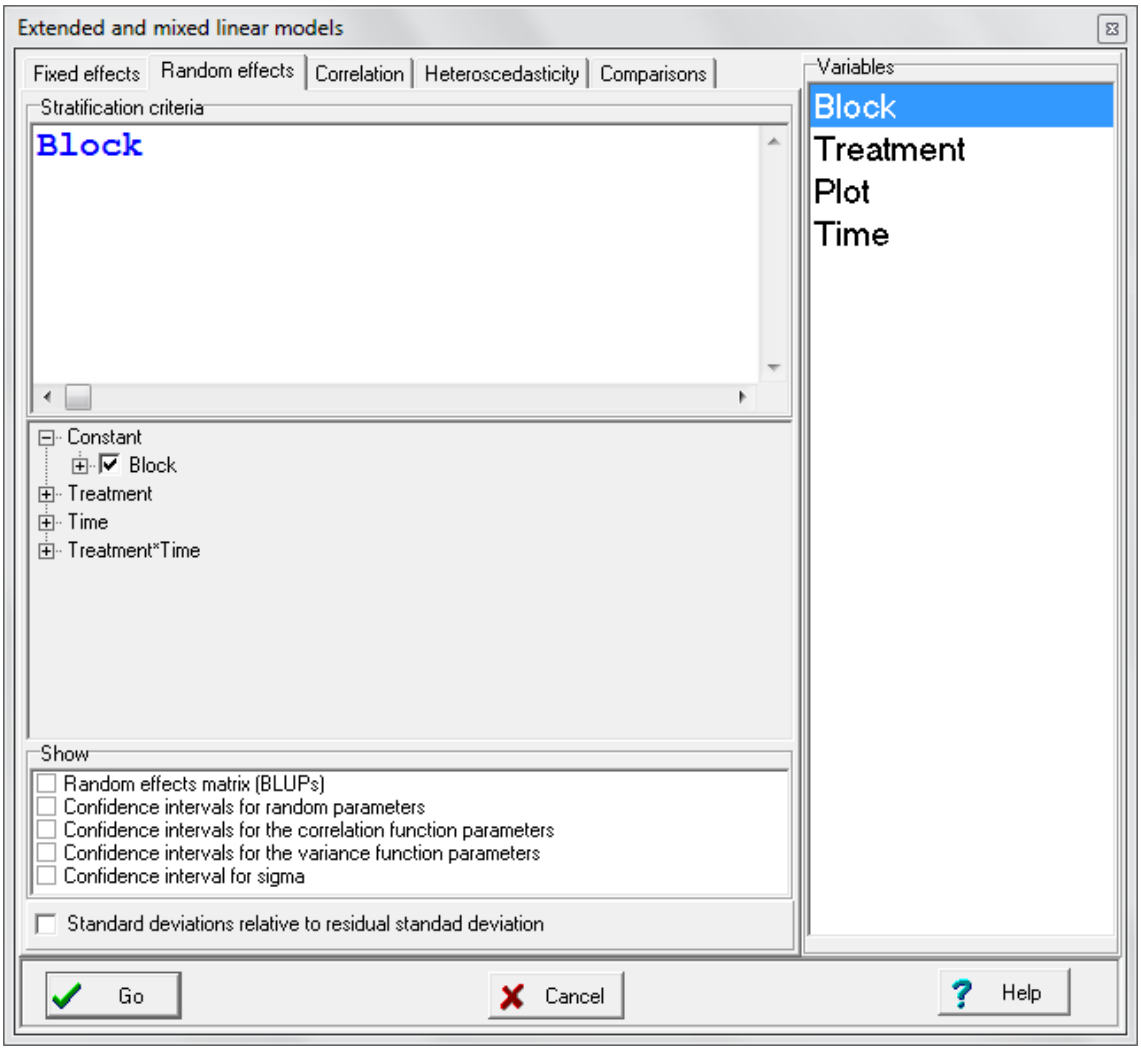



***Figure 68: Window displaying the Random effect tab for model 1(Forage Coverage.IDB2 file).***

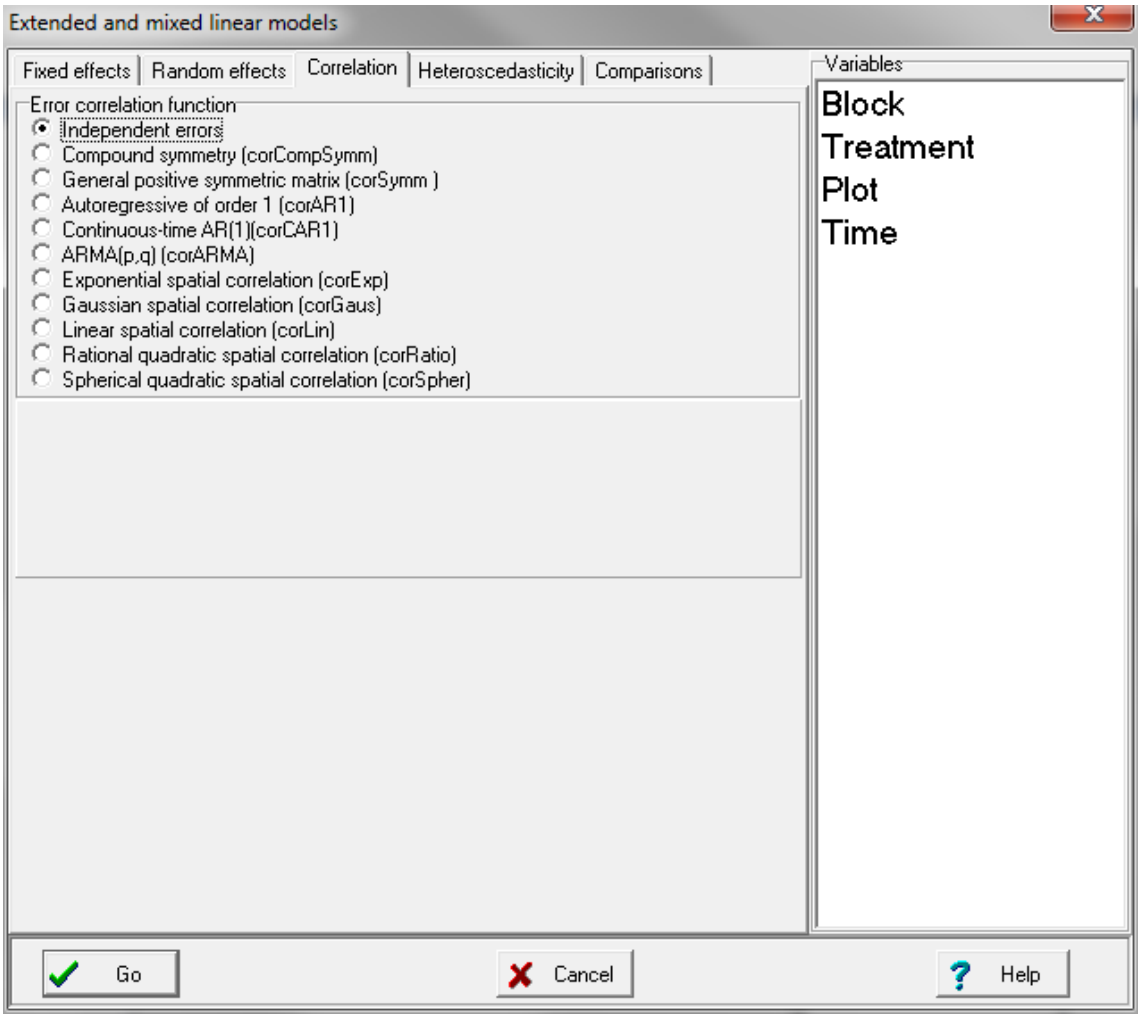



***Figure 69: Window displaying the Correlation tab and the selection of Independent errors (Model 1) (Forage Coverage.IDB2 file).***

**Fit measurements**

| N | AIC | BIC | logLik | Sigma | R2_0 | R2_1 |
|---|---|---|---|---|---|---|
| 75 | 476.39 | 528.01 | -211.19 | 12.19 | 0.56 | 0.63 |

*Smaller AIC and BIC is better*

Model 2: *Random block and plot effects, and independent homoscedastic errors.*

Select *Block* and Plot in the *Random effects* tab (Figure 70). In the *Correlation* tab the *Independent errors* should be declared (Figure 69), which is the default option, and nothing should be declared in the *Heteroscedasticity* tab.

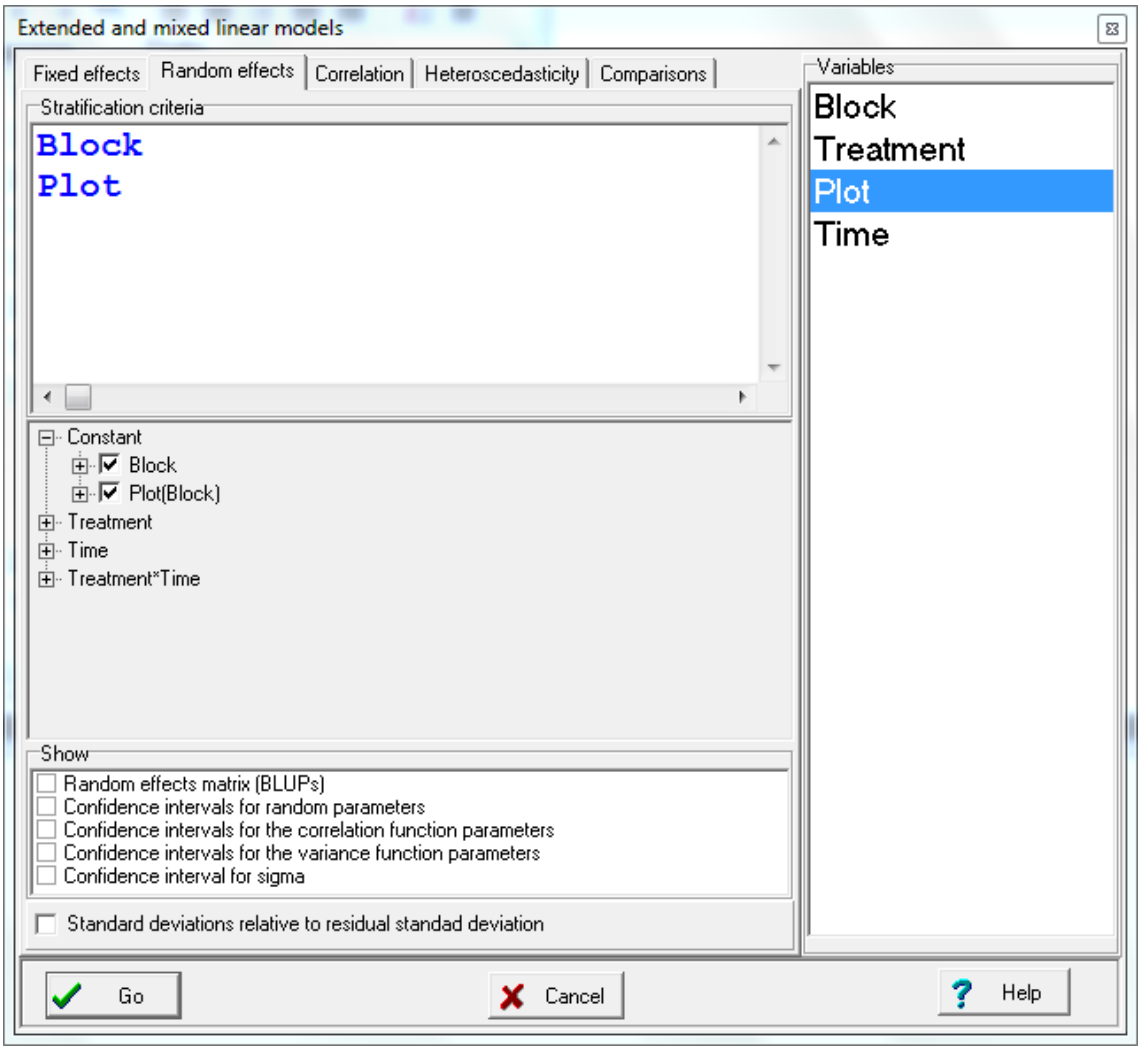



***Figure 70: Window displaying the Random Effect tab, with Block and Plot selected (Model 2) (Forage Coverage.IDB2 file).***

**Fit measurements**

| N | AIC | BIC | logLik | Sigma | R2_0 | R2_1 | R2_2 |
|---|---|---|---|---|---|---|---|
| 75 | 470.00 | 523.54 | -207.00 | 9.95 | 0.56 | 0.61 | 0.78 |

*Smaller AIC and BIC is better*

Model 3: *Random block effects and independent heteroscedastic errors*

The Random effect and Correlation tabs are specified as in model 1 (Figure 68 y Figure 69); in the *Heteroscedasticity* tab *varIdent* is specified; and *Time* is dragged in the *Grouping variables* window (Figure 71).

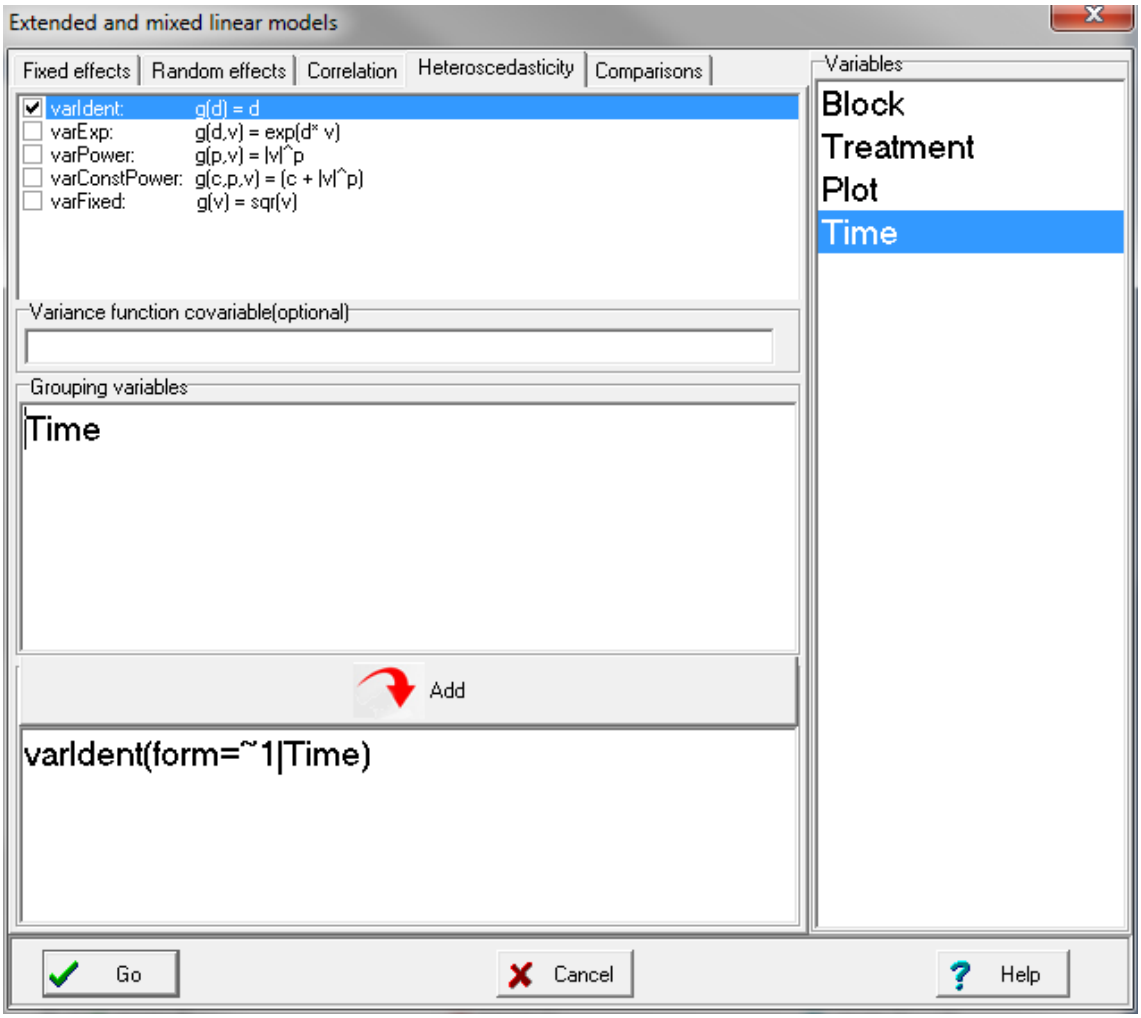



***Figure 71: Window displaying the Heteroscedasticity tab, with the varIdent function selected and Time as the grouping variable (Model 2) (Forage Coverage.IDB2 file).***

**Fit measurements**

| N | AIC | BIC | logLik | Sigma | R2_0 | R2_1 |
|---|---|---|---|---|---|---|
| 75 | 475.13 | 534.40 | -206.57 | 6.50 | 0.56 | 0.59 |

*Smaller AIC and BIC is better*

Model 4: *Random block and plot effects, and independent heteroscedastic errors*

Select *Block* and Plot in the *Random effects* tab (Figure 70). In the *Correlation* tab the *Independent errors* should be declared (Figure 69), which is the default option; in the *Heteroscedasticity* tab *varIdent* is selected; and *Time* is declared in the *Grouping variables* window (Figure 71).

**Fit measurements**

| N | AIC | BIC | logLik | Sigma | R2_0 | R2_1 | R2_2 |
|---|---|---|---|---|---|---|---|
| 75 | 470.54 | 531.73 | -203.27 | 4.20 | 0.56 | 0.56 | 0.70 |

*Smaller AIC and BIC is better*

Model 5: *Random block effects, constant correlation within plots, and homoscedastic errors*

In the *Random effects* tab *Block* is declared; in the *Correlation* tab the *Compound symmetry* option is selected. In the *Grouping variables* window we should also specify *Block* and *Plot*, so that it is explicitly stated the correlation of data coming from the same plot and block is being modeled (Figure 72). In the *Heteroscedasticity* tab the default option was kept, in other words, no criteria were selected (to do this, go to the *Heteroscedasticity* tab and erase the previous selection by deactivating all the options and erasing *varIdent(form=~1)* with a double click).

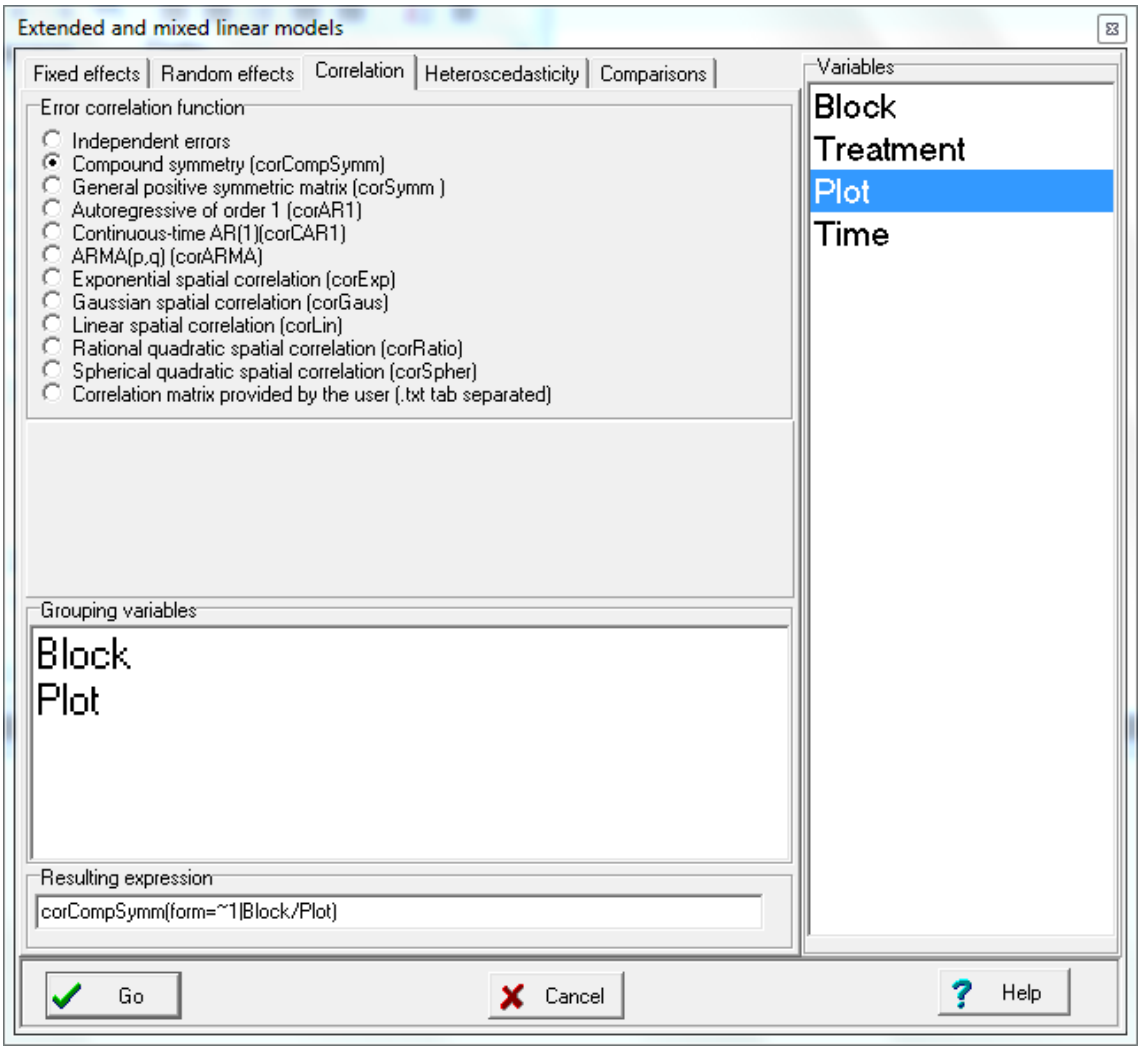



***Figure 72: Window displaying the Correlation tab, and selection of Compound symmetry for data grouped block and plot (Forage Coverage.IDB2 file).***

**Fit measurements**

| N | AIC | BIC | logLik | Sigma | R2_0 | R2_1 |
|---|---|---|---|---|---|---|
| 75 | 470.00 | 523.54 | -207.00 | 12.59 | 0.56 | 0.61 |

*Smaller AIC and BIC is better*

Notice that this model yields the same value of –loglik, AIC, and BIC, because it is essentially the same model (except in the case in which the constant correlation is negative). Model 2 incorporates the correlation of observations in the same plot through the random plot effect, while model 5 does this through the compound symmetry structure. Due to this fact, it is not possible to fit a model including both random plot effect and compound symmetry correlation at the same grouping level. This model would be unidentifiable, and its estimates not valid (although the program may show an output, this is not correct).

Model 6: *Random block effects, constant correlation within plots, and heteroscedastic errors.*

In the *Random effects* tab *Block* is declared; in the *Correlation* tab the *Compound symmetry* option is selected as in Figure 72. In the *Heteroscedasticity* tab *varIdent* is declared; and *Time* is declared in the *Grouping variables* window (Figure 71).

**Fit measurements**

| N | AIC | BIC | logLik | Sigma | R2_0 | R2_1 |
|---|---|---|---|---|---|---|
| 75 | 466.67 | 527.85 | -201.33 | 6.77 | 0.56 | 0.56 |

*Smaller AIC and BIC is better*

Model 7: *Random block effects, first-order autoregressive structure between errors of the same plot and homoscedastic errors*

In the *Correlation* tab the *Autoregressive of order 1* option was selected (Figure 73), and in the *Heteroscedasticity* tab the default option was kept. In other words, no criterion was selected and the previously made selections were erased. In the *Random effects* tab, *Block* was declared as *Stratification criteria*. Since this model takes into account the order in which observations are taken, a variable indicating this ordering should be declared in the corresponding window (*Variable indexing the order of observations*). To select this variable (*Time* in this example) pick drag it to the window. If the times are not equidistant, the structure corAR1 is not applicable, and its continuous analogue should be used (corCAR1). In this example both structures are equivalent because the times are equidistant.

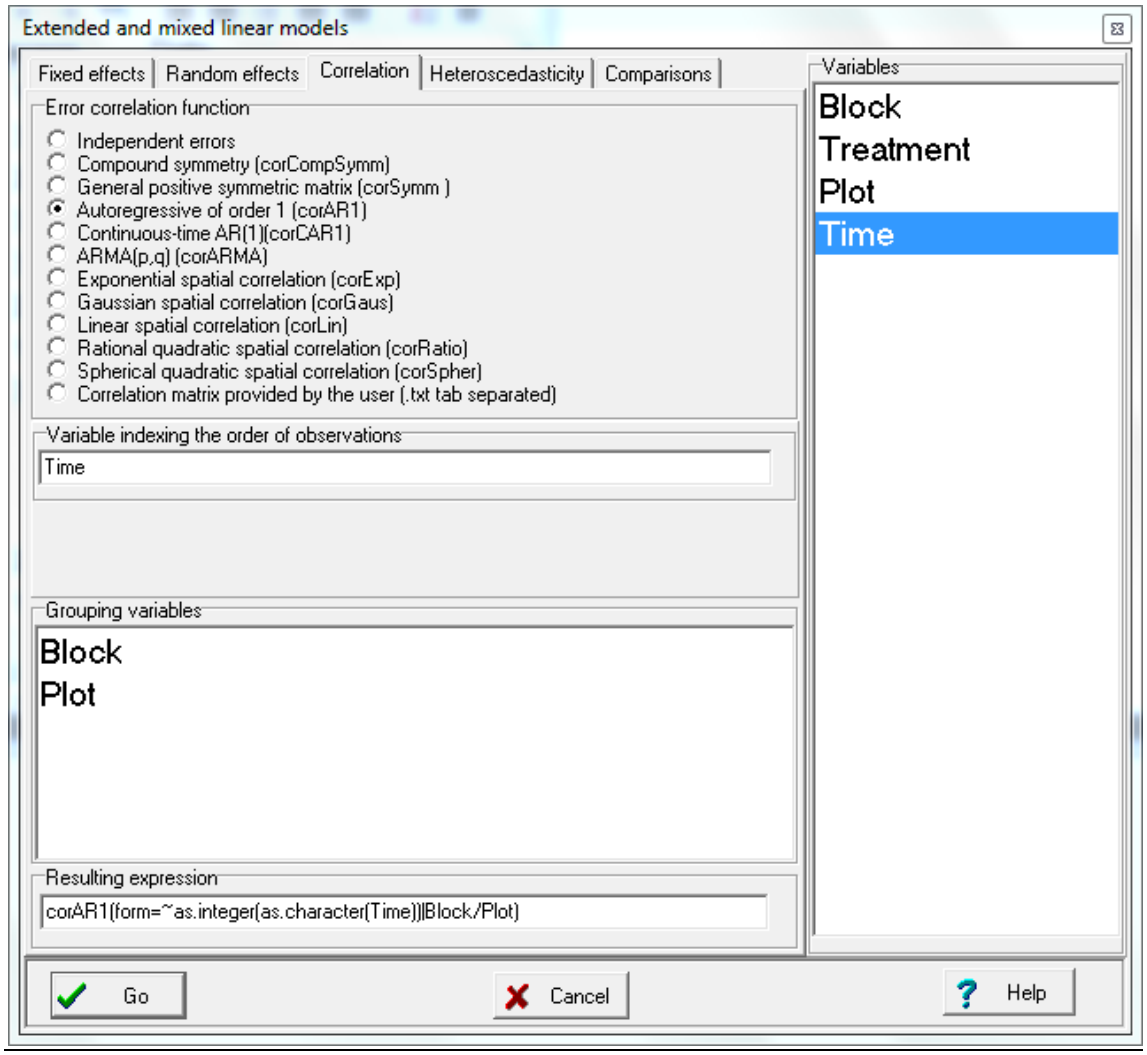



***Figure 73: Window displaying the Correlation tab, and selection of the Autoregressive of order 1. Data grouped by Block and Plot, and the order of the observations indicated by the variable Time (Forage Coverage.IDB2 file).***

**Fit measurements**

| N | AIC | BIC | logLik | Sigma | R2_0 | R2_1 |
|---|---|---|---|---|---|---|
| 75 | 460.93 | 514.47 | -202.47 | 12.36 | 0.56 | 0.62 |

*Smaller AIC and BIC is better*

**Fit measurements**

| N | AIC | BIC | logLik | Sigma | R2_0 | R2_1 |
|---|---|---|---|---|---|---|
| 75 | 460.93 | 514.47 | -202.47 | 12.36 | 0.56 | 0.62 |

*Smaller AIC and BIC is better*

Model 8: *Random block and plot effects, first-order autoregressive structure between errors of the same plot and homoscedastic errors.*

This model is like model 7 but in the *Random effects* tab, *Block* and *Plot* were declared in *Stratification criteria*. The results are very similar, although some differences can be appreciated if we increased the number of decimal positions.

**Fit measurements**

| N | AIC | BIC | logLik | Sigma | R2_0 | R2_1 | R2_2 |
|---|---|---|---|---|---|---|---|
| 75 | 462.93 | 518.38 | -202.47 | 12.36 | 0.56 | 0.62 | 0.62 |

*Smaller AIC and BIC is better*

Model 9: *Random block effects, first-order autoregressive structure between errors of the same plot and heteroscedastic errors*

This model is specified as model 7. In the *Heteroscedasticity* tab the *varIdent* option is selected, and *Time* declared in the *Grouping variables* window.

**Fit measurements**

| N | AIC | BIC | logLik | Sigma | R2_0 | R2_1 |
|---|---|---|---|---|---|---|
| 75 | 462.70 | 523.89 | -199.35 | 7.49 | 0.56 | 0.58 |

*Smaller AIC and BIC is better*

Model 10: *Random block and plot effects, first-order autoregressive structure between errors of the same plot and heteroscedastic errors*

This model is specified as model 8. In the *Heteroscedasticity* tab the *varIdent* option is selected, and *Time* declared in the *Grouping variables* window.

**Fit measurements**

| N | AIC | BIC | logLik | Sigma | R2_0 | R2_1 | R2_2 |
|---|---|---|---|---|---|---|---|
| 75 | 464.70 | 527.80 | -199.35 | 7.49 | 0.56 | 0.58 | 0.58 |

*Smaller AIC and BIC is better*

Model 11: *Random block effects, unstructured correlations between errors from the same plot and time-varying residual variances.*

In the *Correlation* tab the *General positive symmetric matrix* option was selected (Figure 74) and in the *Heteroscedasticity* tab the *varIdent* option was kept (as in Figure 71). In the *Random effects* tab, only *Block* was declared in *Stratification criteria* (it is not possible to fit a model including both random plot effect and general correlation at the same grouping level. This model would be unidentifiable, and its estimates not valid. Although the program may show an output, this is not correct).

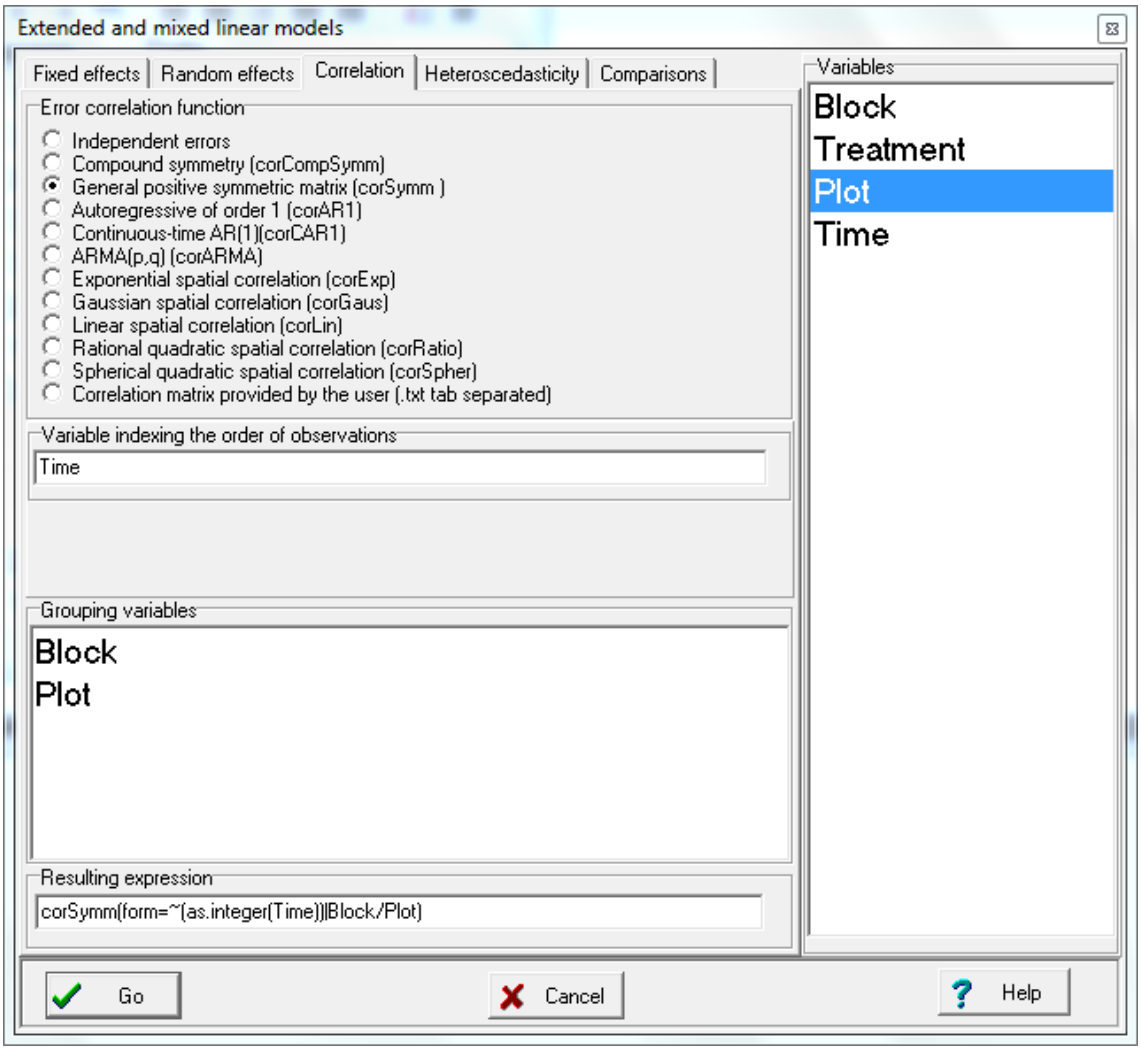



***Figure 74: Window displaying the Correlation tab, and the selection of the General positive symmetric matrix Model for the data grouped by plot, and the order of the observations indicated by the variable Time (Forage Coverage.IDB2 file).***

**Fit measurements**

| N | AIC | BIC | logLik | Sigma | R2_0 | R2_1 |
|---|---|---|---|---|---|---|
| 75 | 434.74 | 513.14 | -176.37 | 6.48 | 0.56 | 0.59 |

*Smaller AIC and BIC is better*

*Selection of the covariance structure*

Upon comparing the values of AIC (or BIC) for the structures that we have fitted, note that that *Model 11* (AIC = 434.74, BIC = 513.14) produces the lowest value. Because of this, we select the unstructured covariance matrix (*General positive symmetric matrix*). The following are the parameters estimated by this model:

**Fit measurements**

| N | AIC | BIC | logLik | Sigma | R2_0 | R2_1 |
|---|---|---|---|---|---|---|
| 75 | 434.74 | 513.14 | -176.37 | 6.48 | 0.56 | 0.59 |

*Smaller AIC and BIC is better*

**Random effects parameters**

*Covariance model for random effects: pdIdent*
*Formula: ~1|Block*

*Standard deviations and correlations*

| | (const) |
|---|---|
| (const) | 2.55 |

**Correlation structure**

*Correlation model: General correlation*
*Formula: ~ (as.integer(Time)) | Block/Plot*

| 0 | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| 1.00 | 0.29 | 0.70 | 0.12 | 0.10 |
| 0.29 | 1.00 | 0.25 | 0.15 | 0.18 |
| 0.70 | 0.25 | 1.00 | 0.48 | 0.47 |
| 0.12 | 0.15 | 0.48 | 1.00 | 1.00 |
| 0.10 | 0.18 | 0.47 | 1.00 | 1.00 |

**Variance structure**

*Variance model: varIdent*
*Formula: ~ 1 | Time*

*Variance-function parameters*

| Parameter | Estim. |
|---|---|
| 1 | 1.00 |
| 2 | 1.24 |
| 3 | 2.03 |
| 4 | 2.58 |
| 5 | 2.46 |

The estimated residual variances for each of the five times are calculated in the following way:

$$\hat{\sigma}_1^2 = 6.4813^2 = 42.0072$$

$$\hat{\sigma}_2^2 = \left(1.2400 \times 6.4813\right)^2 = 64.5903$$

$$\hat{\sigma}_3^2 = \left(2.0278 \times 6.4813\right)^2 = 172.7327$$

$$\hat{\sigma}_4^2 = \left(2.5759 \times 6.4813\right)^2 = 278.7291$$

$$\hat{\sigma}_5^2 = \left(2.4614 \times 6.4813\right)^2 = 254.5005$$

The 10 estimated correlations appear directly as a matrix in the *Correlation Structure.* To obtain the variance of an observation, we need to add the block variance to the residual variance shown above:

$$\hat{\sigma}^2_{bloque} = \left(0.3942 \times 6.4813\right)^2 = 6.5277$$

*Inference regarding the means*

Once the covariance structure of the data is chosen (in this case the model with no structure), we can proceed to make inferences about the means. The average observed profiles for every treatment are shown in Figure 65.

In a factorial experiment such as this one, where we have a treatment factor and a time factor, the first thing we should do is verify whether an interaction between the treatments and time exists. To do so, we can conduct a Wald test, which in InfoStat appears directly as Treatment:Time in the marginal tests. Another option would be to conduct a likelihood ratio test (LRT). For this test we cannot use REML, since we are testing models with different fixed effects, and therefore the REML estimators are not comparable. Instead, the maximum likelihood (ML) estimator is used.

**Marginal hypothesis testing (Type III SS)**

| | numDF | denDF | F-value | p-value |
|---|---|---|---|---|
| (Intercept) | 1 | 48 | 82.60 | <0.0001 |
| Treatment | 4 | 48 | 4.05 | 0.0065 |
| Time | 4 | 48 | 16.77 | <0.0001 |
| Treatment:Time | 16 | 48 | 1.49 | 0.1417 |

To carry out a likelihood ratio test we can fit (with ML) two models with the same covariance structure (in this example the model without structure) but that differ in their fixed part: one contains the interaction term (complete model) and the other one does not (reduced model):

Complete model:

**Fit measurements**

| N | AIC | BIC | logLik | Sigma | R2_0 | R2_1 |
|---|---|---|---|---|---|---|
| 75 | 539.51 | 634.52 | -228.75 | 5.29 | 0.56 | 0.59 |

*Smaller AIC and BIC is better*

Reduced Model:

**Fit measurements**

| N | AIC | BIC | logLik | Sigma | R2_0 | R2_1 |
|---|---|---|---|---|---|---|
| 75 | 531.19 | 589.12 | -240.59 | 5.52 | 0.33 | 0.35 |

*Smaller AIC and BIC is better*

Although the LRT can be obtained directly from the *Model* tab of the *Model exploration* menu, another calculation method is shown below. In the first place, the statistic for the LRT test is obtained:

$$G = 2\log \text{lik}_{completo} - 2\log \text{lik}_{reducido} = 2(-240.59) - 2(-228.75) = 23.68\,.$$

This has 42-26=16 degrees of freedom, and generates a p-value = 0.0967, which is why we can say that there is no interaction, with a 5% significance level. This probability value is obtained from a chi-square distribution with 16 degrees of freedom, and it can be calculated with the *Probability and quantile calculator* tool available in InfoStat's *Statistics* menu (Figure 75).

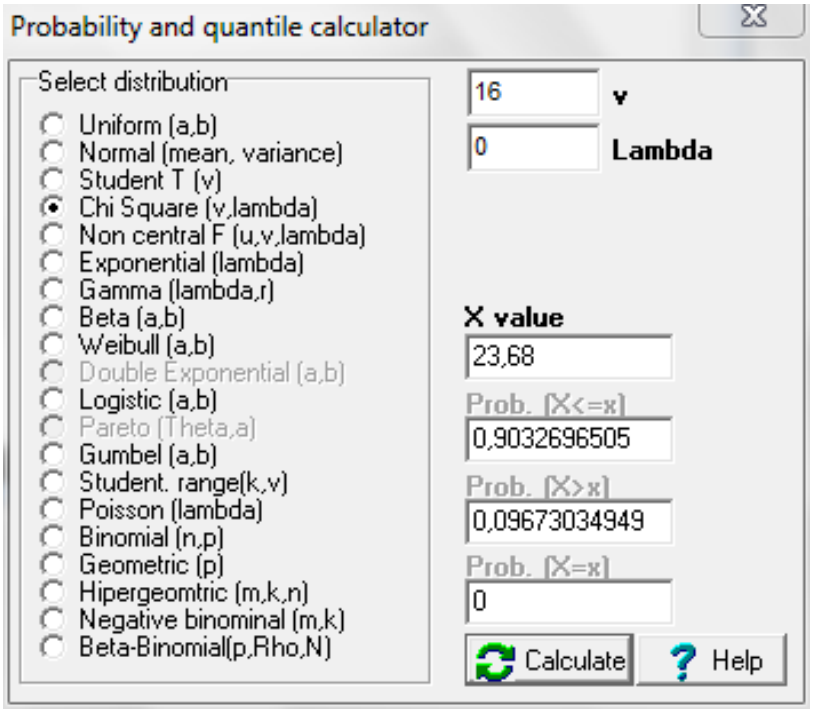



***Figure 75: InfoStat Probability and quantile calculator window.***

Both tests (Wald and LRT) indicate that the interaction is not significant (even though the p-values are not too high, p=0.1417 and p=0.0967, respectively), which is why we can (with precaution) conduct tests of treatments effects and time separately.

*Contrasting successive times*

To compare the successive times, in other words time 1 with time 2, time 2 with time 3, and so on, the *Comparisons* tab should be activated and within this tab the *Contrasts* subtab; and the effect *Time* should be selected (Figure 76). The rest of the windows should be kept as in Model 9, which was chosen as the model with the best correlation structure to explain the behavior of these data through time.

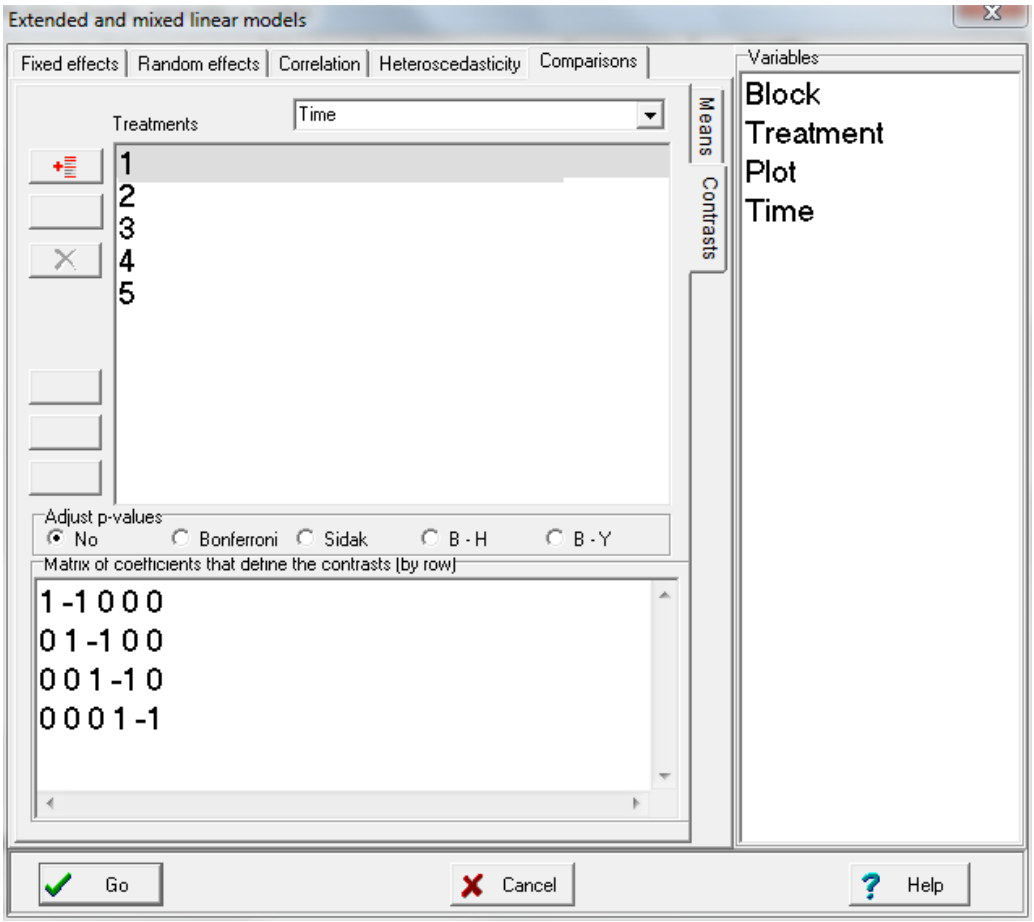



***Figure 76: Window displaying the Comparisons tab, with the Contrasts subtab selected (Forage Coverage.IDB2 file).***

**Hypothesis testing for contrasts**

| Time | Contrast | S.E. | F | df(num) | df(den) | p-value |
|---|---|---|---|---|---|---|
| Ct.1 | -9.80 | 1.84 | 28.41 | 1 | 48 | <0.0001 |
| Ct.2 | -5.77 | 2.87 | 4.05 | 1 | 48 | 0.0499 |
| Ct.3 | 1.76 | 3.28 | 0.29 | 1 | 48 | 0.5949 |
| Ct.4 | 0.26 | 0.37 | 0.51 | 1 | 48 | 0.4807 |
| Total | | | 25.15 | 4 | 48 | <0.0001 |

The outputs shown here correspond to the REML estimations. It is clear from these results that, on average for the four treatments, a significant change is observed between times 1 and 2, but in subsequent times the average coverage does not change significantly. The same conclusions are obtained by conducting a means comparison for each time (LSD):

**Adjusted means and standard error for Time**

*LSD Fisher (alpha=0.05)*
*p-value correction procedure: No*

| Time | Means | S.E. | | |
|---|---|---|---|---|
| 3 | 30.29 | 3.70 | A | |
| 4 | 28.53 | 4.56 | A | |
| 5 | 28.27 | 4.38 | A | |
| 2 | 24.53 | 2.55 | A | |
| 1 | 14.73 | 2.23 | | B |

*Different letters indicate significant difference between location parameters (p<= 0,05)*

*Comparison of treatments*

***Adjusted means and standard error for Treatment***

*LSD Fisher (alpha=0.05)*
*p-value correction procedure: No*

| Treatment | Means | S.E. | | | |
|---|---|---|---|---|---|
| 5 | 39.60 | 5.47 | A | | |
| 1 | 31.27 | 5.47 | A | B | |
| 4 | 24.96 | 5.47 | A | B | C |
| 3 | 17.33 | 5.47 | | B | C |
| 2 | 13.19 | 5.47 | | | C |

*Means with a common letter are not significantly different (p<= 0.05)*

Based on this adjusted means comparison we conclude that treatments 5, 1 and 4 provide the highest coverage and are not significantly different among each other.

*Analysis of a trial for asthma drugs*

A pharmaceutical company has examined the effects of two drugs (*A* and *B*) on the breathing capacity of asthma patients (Littell et al. 2002, 2006). Both drugs and a placebo (*P*) were randomly administered to a group of patients. Each of the three treatments had 24 patients. The basal respiratory capacity (*Bas _Resp _Cap*) of each patient was measured immediately before the treatment was applied and once an hour during the 8 hours following treatment application (*Res_ Cap)*. The data are in the *Respiratory capacity.IDB2* file.

Using the strategy defined in the previous examples, first models with different covariance structures will be fitted by appropriately combining residual correlation structures, residual heteroscedasticity, and random effects. The model that best describes the data will be selected by using penalized likelihood criteria (AIC and BIC) and likelihood ratio tests. Once the adequate variance structure coefficient is selected, inferences will be made about the means (compare drugs means, study the effect of time, analyze whether the average profiles vary through time, whether they are parallel, etc.). It is important to emphasize that all the inferences regarding the means will be based on the model with the selected covariance structure.

Since the variable that identifies the patient (*Patient*) in the database takes on equal values for each drug, in order to identify the 72 patients in this study a new variable had to be created (*Patient_drugs*) that completely identifies the patient. To do so, use the *Data* menu, select the *Make a new column by merging categorical variables* submenu (selecting *Patient* and *Drugs* in the variables selector window). This is a two-factor experiment, and the model used takes into account the factors *Drugs*, *Hours* and their interaction, and the covariate *Basal _Resp _Cap* (all fixed effects). To conduct the analysis for this model, the variables need to be declared in the following way (Figure 77).

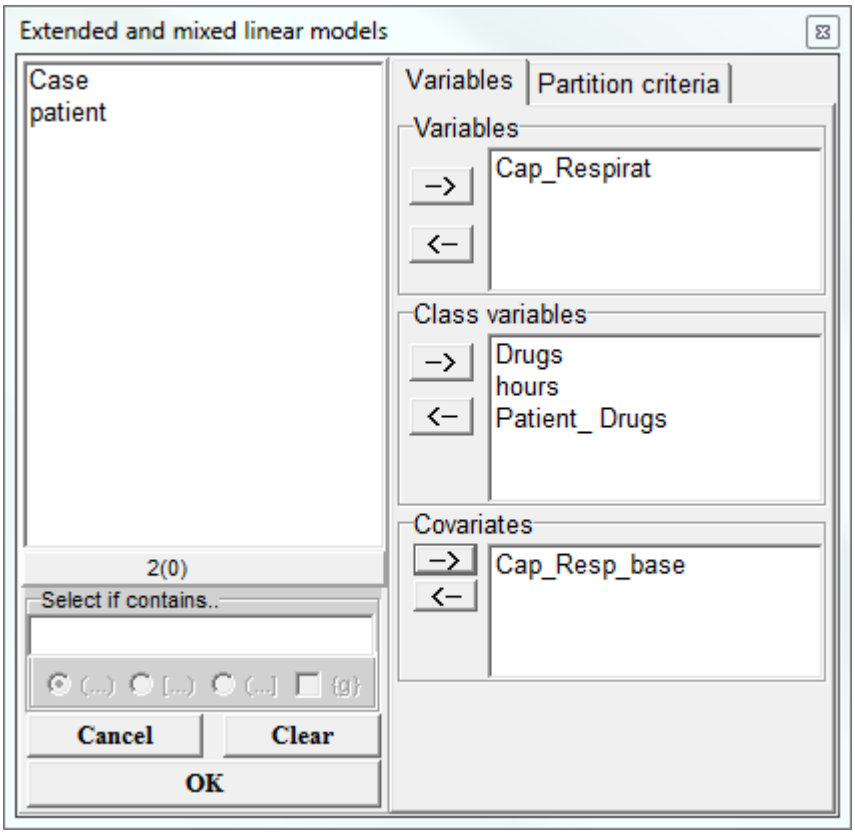



***Figure 77: Variable selector window (Respiratory capacity.IDB2 file).***

First, a group of models will be evaluated to determine which one has the better fit. The following models are evaluated:

1. *Independent errors and homoscedastic residual variances.*
2. *Compound symmetry and homoscedastic residual variances.*
3. *First-order autoregressive and homoscedastic residual variances.*
4. *First-order autoregressive and heteroscedastic residual variances.*
5. *First-order autoregressive, homoscedastic residual variances, and random patient effect.*
6. *First-order autoregressive, heteroscedastic residual variances, and random patient effect.*
7. *Variances matrix and covariance without structure and heteroscedastic residual variances.*

The specification of the fixed part is the same for the seven evaluated models (Figure 78). To fit Model 1, simply activate the *Go* button with the fixed effects model presented below: button with fixed effects model present below:

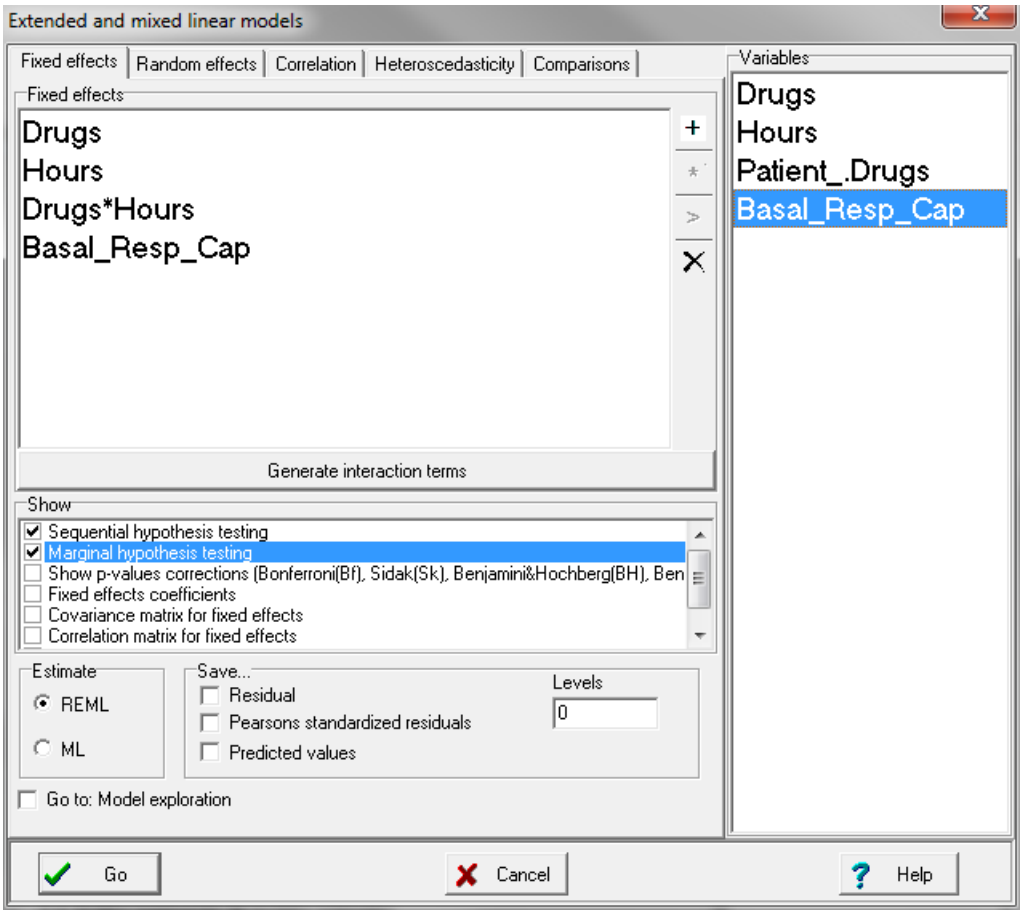



***Figure 78: Window displaying the Fixed effects tab (Respiratory capacity.IDB2 file).***

To fit Model 2, the windows should be specified as in Figure 78 and Figure 79. Model 3 is specified as in Figure 78 and Figure 80. Model 4 is specified as in the preceding model but including heterogenious residual variances, as shown in Figure 81. Model 5 is specified with the windows as shown in Figure 78, Figure 80 and Figure 82. Model 6 is like Model 5 but incldues the specification of the heterogeneous residual variances (Figure 81). Model 7 is specified as shown in Figure 78, Figure 81 and Figure 83).

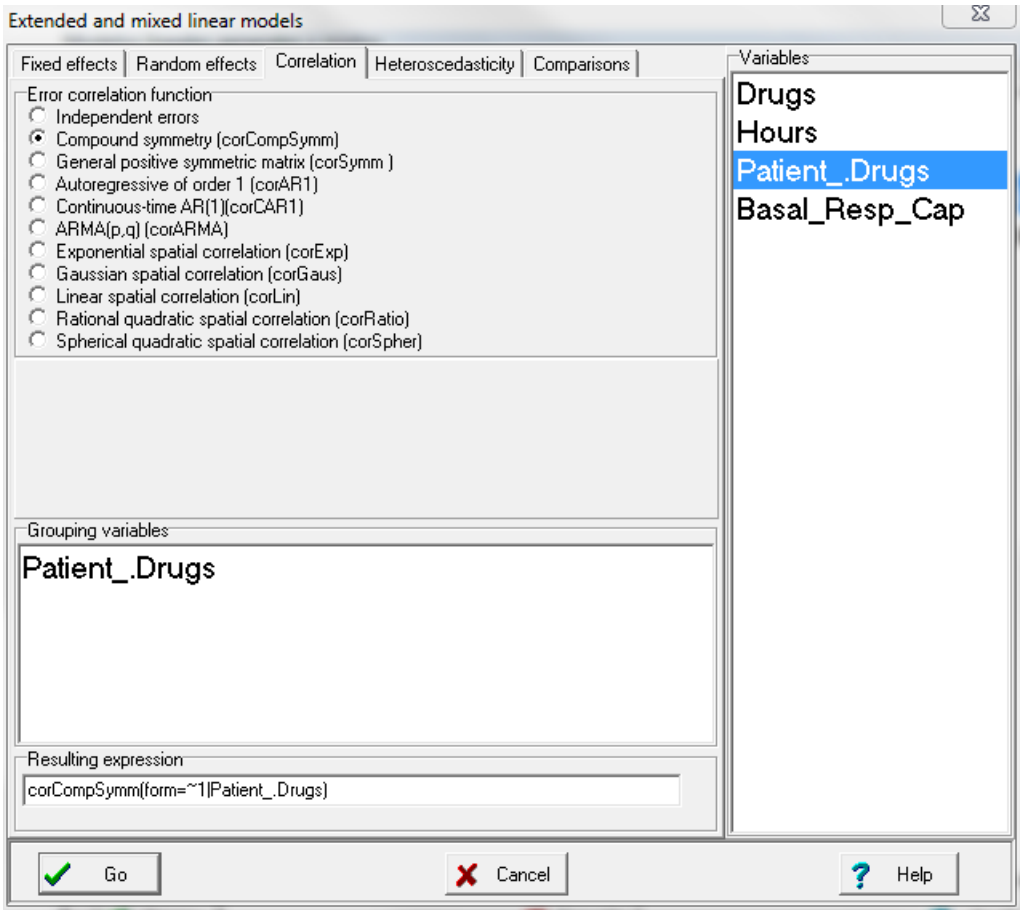



***Figure 79: Window displaying the Correlation tab, with the Compound symmetry option selected (Respiratory capacity.IDB2 file).***

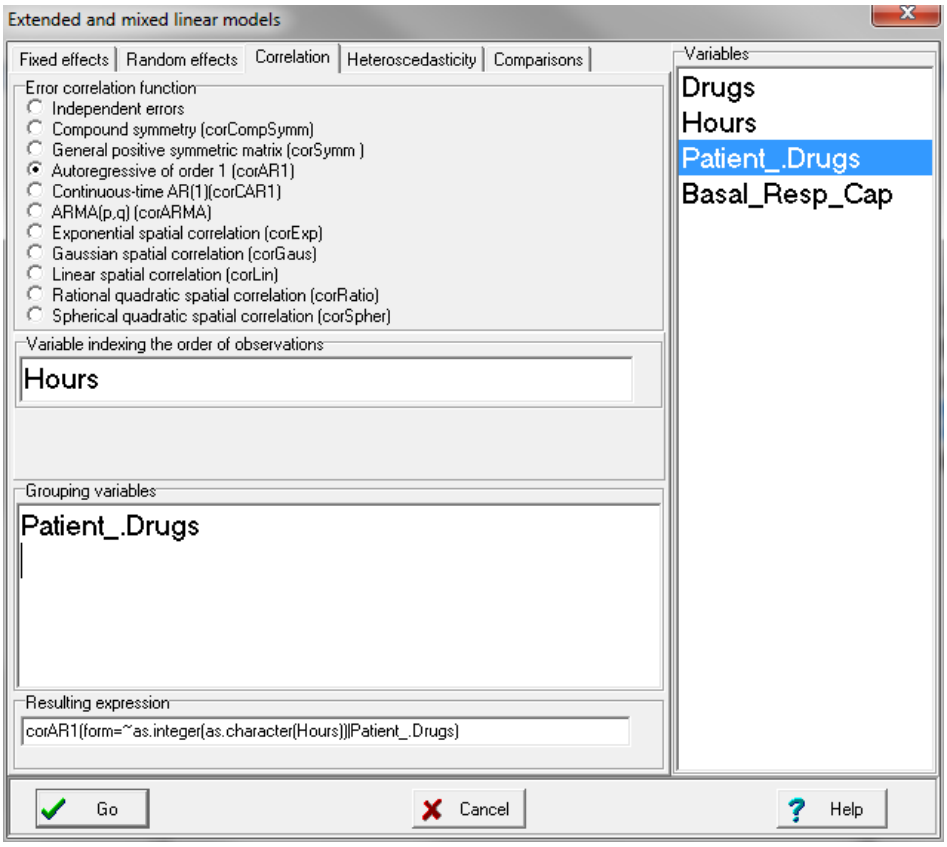



***Figure 80: Window displaying the Correlation tab, with the Autoregressive of order 1 option selected (Respiratory capacity.IDB2 file).***

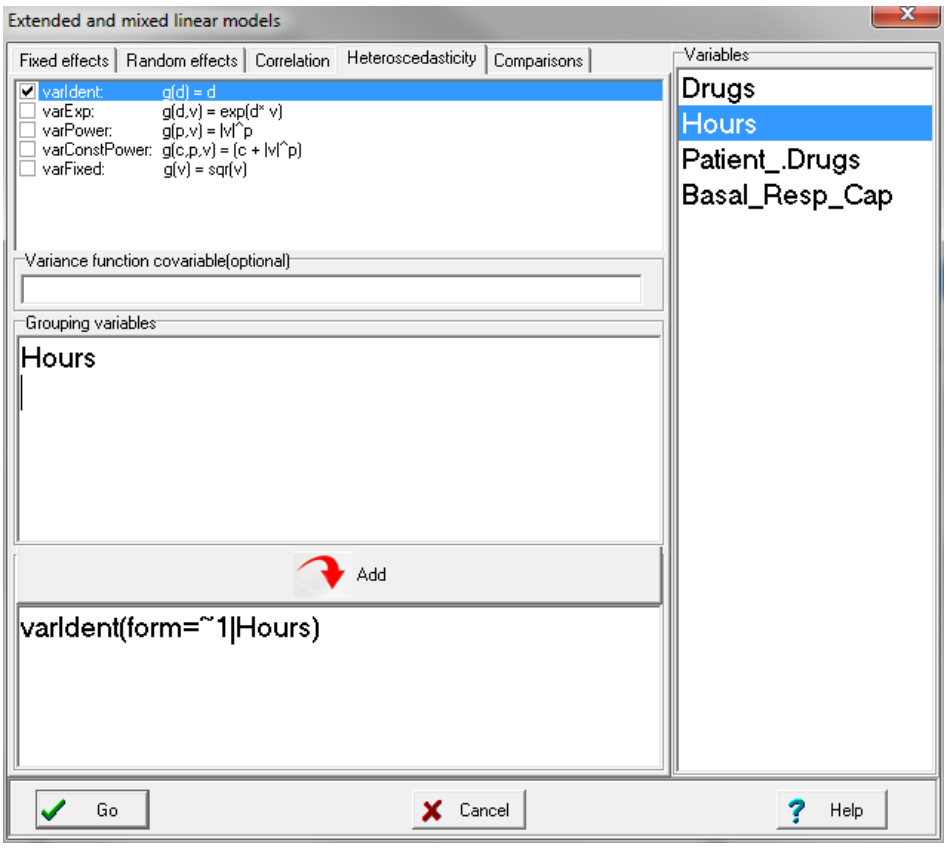



***Figure 81: Window displaying the Heteroscedasticity tab (Respiratory capacity.IDB2 file).***

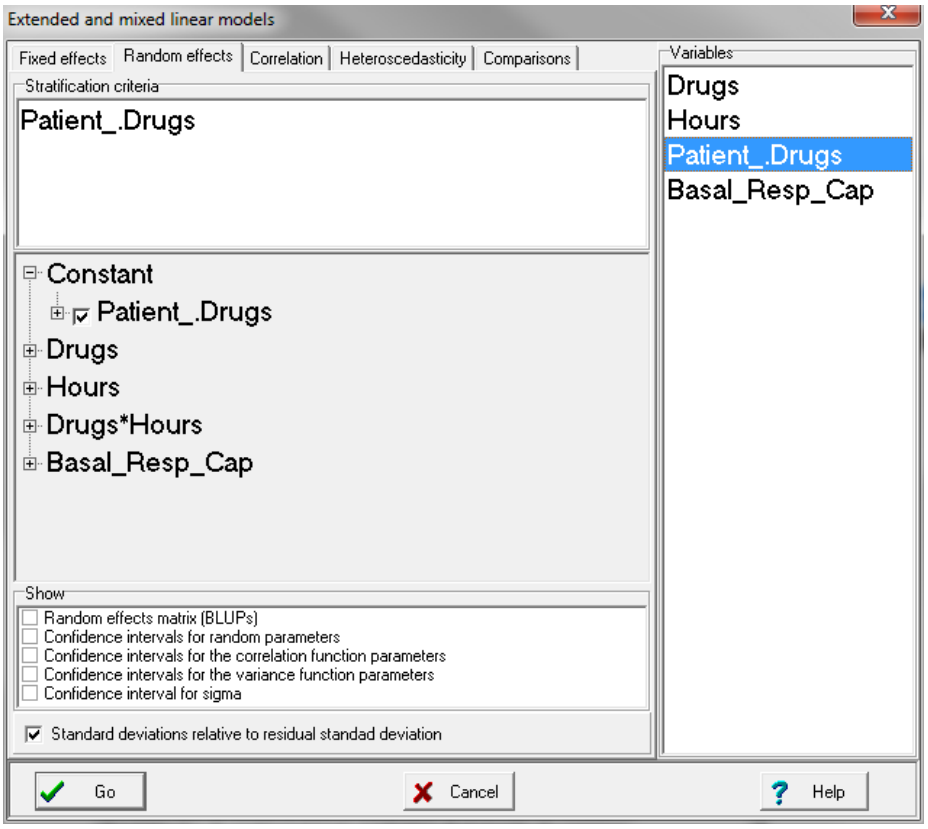



***Figure 82: Window displaying the Random effects tab (Respiratory capacity.IDB2 file).***

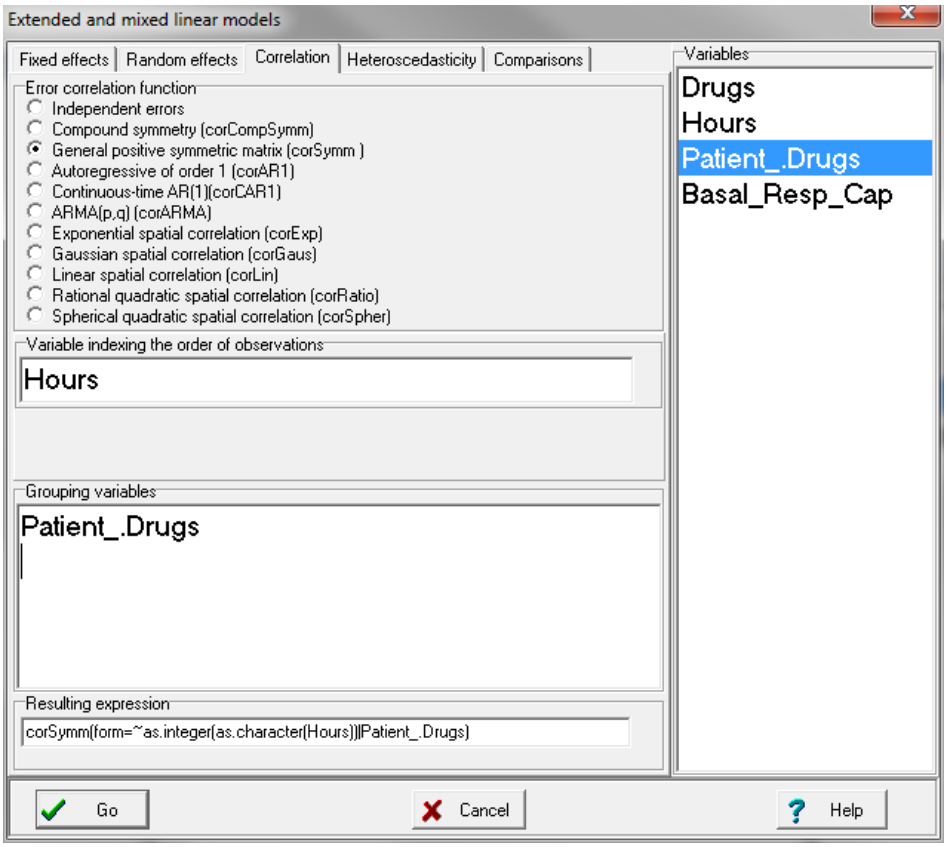



***Figure 83: Window displaying the Correlation tab, with the General positive symmetric matrix option selected (Respiratory capacity.IDB2 file).***

After fitting the following models these are the results:

***Table 2. Characteristics and fitted measures of the evaluated model (Respiratory capacity.IDB2 file).***

| Model | Patient random effect | Residual correlation | Heterogeneous residual variances in Time | AIC | BIC | log lik |
|---|---|---|---|---|---|---|
| 1 | Yes | No | No | 968.94 | 1081.04 | -458.47 |
| 2 | No | Compound Simmetry | No | 401.29 | 517.71 | -173.65 |
| 3 | No | AR1 | No | 329.04 | 445.45 | -137.52 |
| 4 | No | AR1 | Yes | 324.57 | 471.17 | -128.28 |
| 5 | Yes | AR1 | No | 303.03 | 423.76 | -123.52 |
| 6 | Yes | AR1 | Yes | 287.80 | 438.71 | -108.90 |
| 7 | No | General positive symmetric matrix | Yes | 270.27 | 533.29 | -74.14 |

From Table 2 we can observe that models 6 and 7 have the lowest AIC values, while models 5 and 6 have the lowest BIC values. A formal likelihood ratio test used to compare models 5 and 6 can be obtained by means of the following equation:

$$X^2 = -2(loglink\ reduced\ model - loglink\ full\ full\ model\ )$$
$$= -2(-123.52 + 108.90) = 29.24$$

Because both models differ in 7 parameters (Model 5 has a single residual variance and Model 6 has 8 residual variances), the likelihood ratio test statistic is compared to a critical value of a chi-square distribution with 7 degrees of freedom. When this is done with InfoStat's *Probabilities and quantiles calculator* we obtain a p-value of 0.0001, which leads us to choose the complete model (Model 6).

The same test can be done with the *Statistics>> Extended and mixed linear models >> Model exploration* menu. To compare both models we select the *Models* tab and obtain the following results:

**Comparison of models**

| Model | df | AIC | BIC | logLik | Test | L.Ratio | p-value |
|---|---|---|---|---|---|---|---|
| 5 | 28 | 303.03 | 423.76 | -123.52 | | | |
| 6 | 35 | 287.80 | 438.71 | -108.90 | 1 vs 2 | 29.23 | 0.0001 |

The results from the likelihood ratio test indicate that Model 6 is the better model of the two. Now, we only need to compare Model 6 to Model 7. In this case the reduced model is 6 and the complete model is 7. The results for this comparison are following:

**Comparison of models**

| Model | df | AIC | BIC | logLik | Test | L.Ratio | p-value |
|---|---|---|---|---|---|---|---|
| 7 | 61 | 270.27 | 533.29 | -74.14 | | | |
| 6 | 35 | 287.80 | 438.71 | -108.90 | 1 vs 2 | 69.53 | <0.0001 |

The complete output for this model indicates that Model 7 is the best one. Therefore the selected model has a residual correlation without structure and heterogeneous residual variances in time. The complete output for this model is shown below:

**Extended and mixed linear models**
**R specification of the model**

```
model001_Res_Cap_REML<-
gls(Res_Cap~1+Drugs+Hours+Drugs:Hours+Basal_Resp_Cap
,weight=varComb(varIdent(form=~1|Hours))
,correlation=corSymm(form=~as.integer(as.character(Hours))|Patient_.Dr
ugs)
,method="REML"
,na.action=na.omit
,data=R.data00)
```

**Results for model: model001_Res_Cap_REML**

*Dependent variable:Res_Cap*

**Fit measurements**

| N | AIC | BIC | logLik | Sigma | R2_0 |
|---|---|---|---|---|---|
| 576 | 270.27 | 533.29 | -74.14 | 0.48 | 0.55 |

*Smaller AIC and BIC is better*

**Marginal hypothesis testing(Type III SS)**

| | numDF | F-value | p-value |
|---|---|---|---|
| (Intercept) | 1 | 6.49 | 0.0111 |
| Drugs | 2 | 7.25 | 0.0008 |
| Hours | 7 | 13.72 | <0.0001 |
| Basal_Resp_Cap | 1 | 92.57 | <0.0001 |
| Drugs:Hours | 14 | 4.06 | <0.0001 |

**Sequential hypothesis testing**

| | numDF | F-value | p-value |
|---|---|---|---|
| (Intercept) | 1 | 3936.01 | <0.0001 |
| Drugs | 2 | 13.87 | <0.0001 |
| Hours | 7 | 13.72 | <0.0001 |
| Basal_Resp_Cap | 1 | 92.57 | <0.0001 |
| Drugs:Hours | 14 | 4.06 | <0.0001 |

**Correlation structure**

*Correlation model: General correlation*
*Formula: ~ as.integer(as.character(Hours)) | Patient_.Drugs*

*Common correlation matrix*

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|---|
| 1 | 1.00 | 0.89 | 0.88 | 0.78 | 0.69 | 0.67 | 0.52 | 0.65 |
| 2 | 0.89 | 1.00 | 0.91 | 0.87 | 0.81 | 0.70 | 0.59 | 0.70 |
| 3 | 0.88 | 0.91 | 1.00 | 0.91 | 0.81 | 0.75 | 0.64 | 0.74 |
| 4 | 0.78 | 0.87 | 0.91 | 1.00 | 0.82 | 0.73 | 0.67 | 0.75 |
| 5 | 0.69 | 0.81 | 0.81 | 0.82 | 1.00 | 0.85 | 0.73 | 0.84 |
| 6 | 0.67 | 0.70 | 0.75 | 0.73 | 0.85 | 1.00 | 0.81 | 0.88 |
| 7 | 0.52 | 0.59 | 0.64 | 0.67 | 0.73 | 0.81 | 1.00 | 0.82 |
| 8 | 0.65 | 0.70 | 0.74 | 0.75 | 0.84 | 0.88 | 0.82 | 1.00 |

**Variance structure**

*Variance model: varIdent*
*Formula: ~ 1 | Hours*

*Variance-function parameters*

| Parameter | Estim. |
|---|---|
| 1 | 1.00 |
| 2 | 1.07 |
| 3 | 1.06 |
| 4 | 1.15 |
| 5 | 1.12 |
| 6 | 1.07 |
| 7 | 1.09 |
| 8 | 1.15 |

**Adjusted means and standard error for Drugs**
*LSD Fisher (alpha=0.05)*
*p-value correction procedure: No*

| Drugs | Means | S.E. | | |
|---|---|---|---|---|
| B | 3.33 | 0.09 | A | |
| A | 3.11 | 0.09 | A | |
| Placebo | 2.82 | 0.09 | | B |

*Means with a common letter are not significantly different (p<= 0.05)*

**Adjusted means and standard error for Hours**
*LSD Fisher (alpha=0.05)*
*p-value correction procedure: No*

| Hours | Means | S.E. | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 1 | 3.33 | 0.06 | A | | | | | |
| 2 | 3.30 | 0.06 | A | | | | | |
| 3 | 3.22 | 0.06 | | B | | | | |
| 4 | 3.12 | 0.06 | | | C | | | |
| 5 | 3.02 | 0.06 | | | | D | | |
| 6 | 2.96 | 0.06 | | | | D | | |
| 7 | 2.88 | 0.06 | | | | | E | |
| 8 | 2.87 | 0.06 | | | | | E | |

*Means with a common letter are not significantly different (p<= 0.05)*

**Adjusted means and standard error for Drugs*Hours**

*LSD Fisher (alpha=0.05)*

*p-value correction procedure: No*

| Drug | Hours | Means | S.E. | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| B | 1 | 3.69 | 0.10 | A | | | | | | |
| B | 2 | 3.63 | 0.10 | A | B | | | | | |
| B | 3 | 3.58 | 0.10 | A | B | | | | | |
| A | 1 | 3.47 | 0.10 | A | B | C | | | | |
| B | 4 | 3.44 | 0.11 | | B | C | D | | | |
| A | 2 | 3.39 | 0.10 | | B | C | D | | | |
| B | 5 | 3.25 | 0.11 | | | C | D | E | | |
| A | 3 | 3.18 | 0.10 | | | | D | E | F | |
| B | 6 | 3.08 | 0.10 | | | | | E | F | G |
| A | 5 | 3.05 | 0.11 | | | | | E | F | G |
| A | 4 | 3.04 | 0.11 | | | | | E | F | G |
| B | 8 | 3.01 | 0.11 | | | | | E | F | G |
| A | 6 | 2.98 | 0.10 | | | | | E | F | G |
| B | 7 | 2.98 | 0.11 | | | | | | F | G |
| P | 3 | 2.90 | 0.10 | | | | | | F | G |
| P | 2 | 2.89 | 0.10 | | | | | | | G |
| P | 4 | 2.87 | 0.11 | | | | | | | G |
| A | 7 | 2.87 | 0.11 | | | | | | | G |
| A | 8 | 2.86 | 0.11 | | | | | | | G |
| P | 1 | 2.83 | 0.10 | | | | | | | G |
| P | 6 | 2.82 | 0.10 | | | | | | | G |
| P | 7 | 2.79 | 0.11 | | | | | | | |
| P | 5 | 2.77 | 0.11 | | | | | | | |
| P | 8 | 2.73 | 0.11 | | | | | | | |

| H | I |
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| | I |

*Means with a common letter are not significantly different (p<= 0.05)*

Note that there is a significant interaction between the drug and time ($p<0.0001$), so we proceed to carry out an interaction graph. To make this graph, first the *Adjusted means and standard errors* for *Drugs*Hours* were copied and pasted in a new InfoStat table. This table was saved as *Respiratory capacity average.IDB2*. Then in the *Graphs>>Dot plot* menu the variables were declared as shown below (Figure 84 and Figure 85):

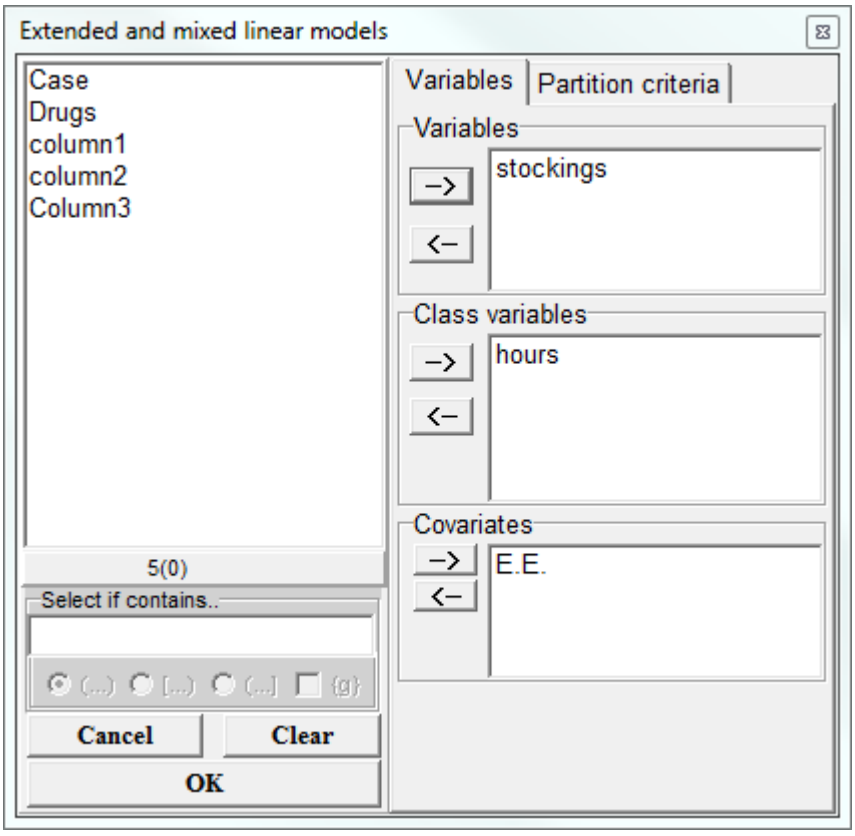



***Figure 84: Variables selector window (Respiratory capacity average.IDB2 file).***

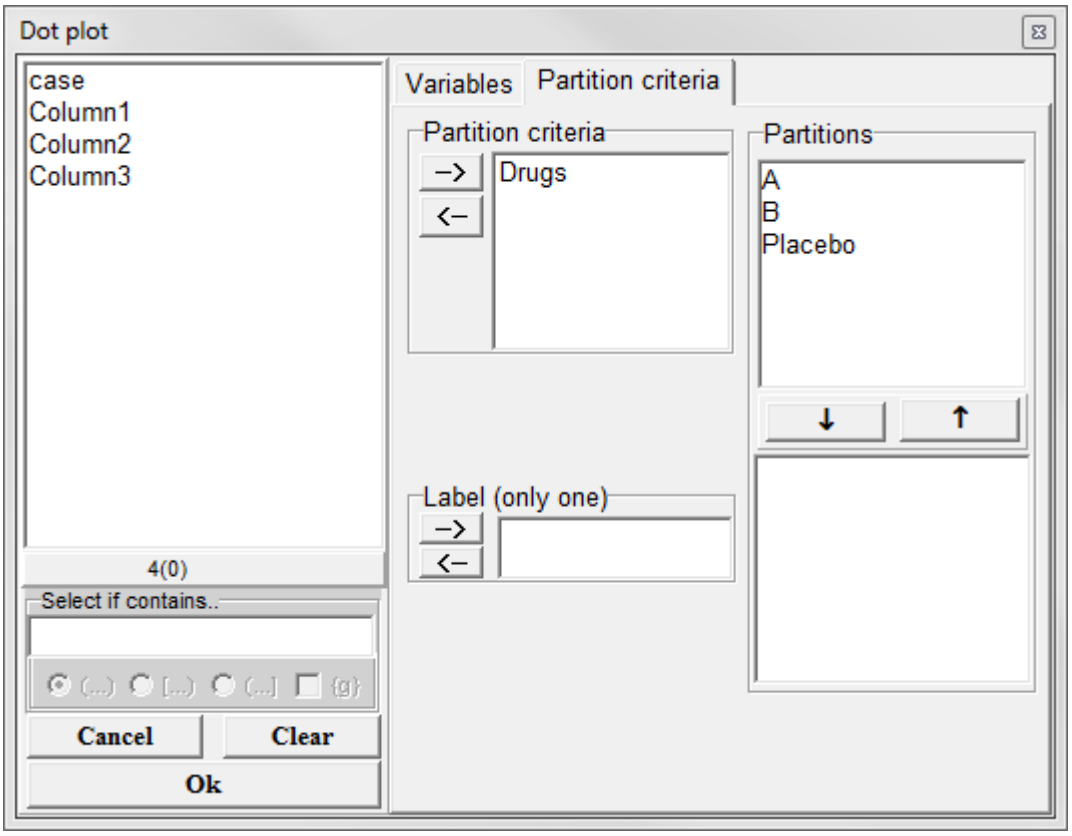



***Figure 85: Variables selector window displaying the activated Partitions tab (Respiratory capacity average.IDB2 file).***

It is important to emphasize that because the standard errors of each of the combinations of treatments and hours are different, these should be taken into account when requesting the graph. This is achieved by declaring the error measure in the *Error* sub-window. With these specifications a graph is obtained to the study of the interaction (Figure 86).

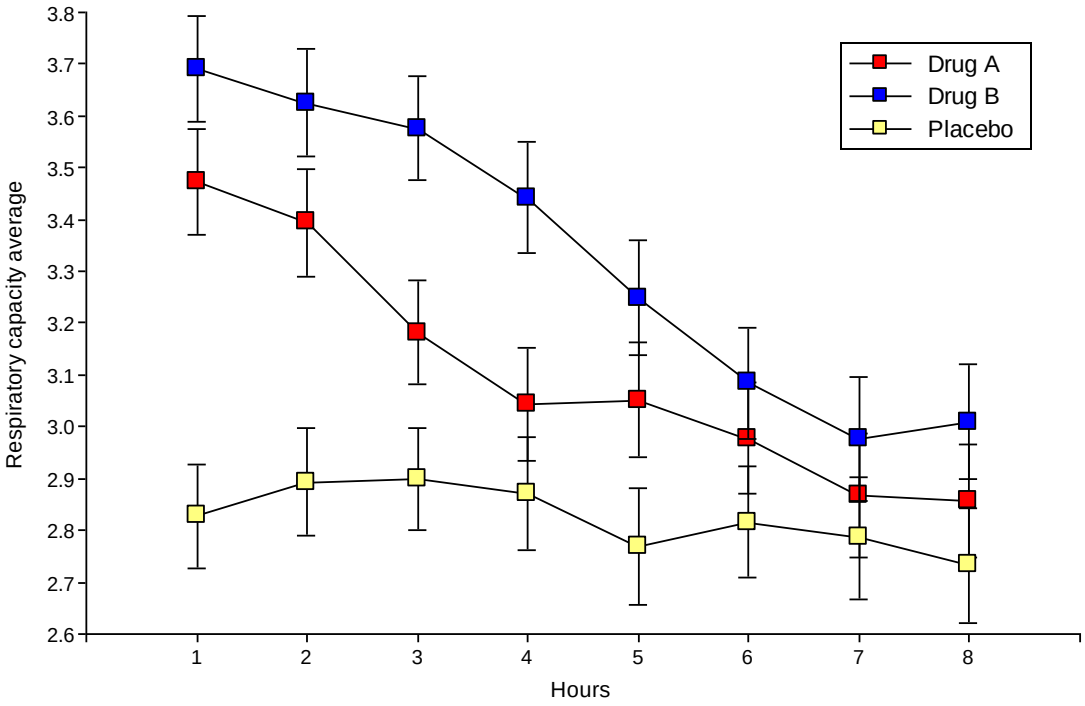



***Figure 86: Box plot to study the interactions between treatments and time with the data from the Respiratory capacity.IDB2 file.***

We can observe that while the placebo has a practically constant response, drugs A and B increase the respiratory capacity after their application. This capacity is going to decrease with time, and the mean value of drug B is always greater than that of drug A. In order to find significant differences between the treatments, contrasts can be conducted within each hour. In this case, at each hour we can test hypotheses regarding the equality of means between the drugs and the placebo, and between the two drugs. To obtain the contrasts (in this case orthogonal) these should be declared in the *Comparisons* tab, in the *Contrasts* subtab, as shown in Figure 87.

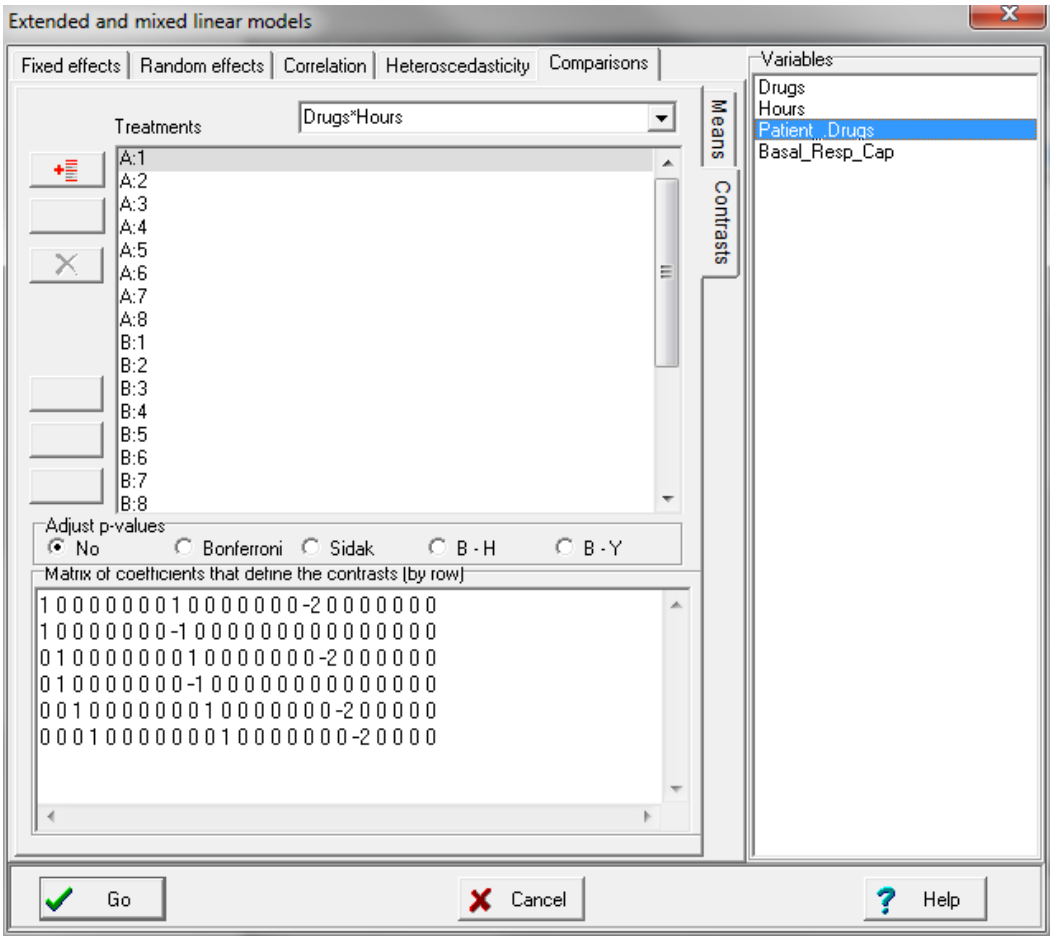



***Figure 87: Window displaying the Comparisons tab, Contrasts subtab (Respiratory capacity.IDB2 file).***

The p-values for the requested contrasts are shown below:

**hypothesis testing for contrasts**

| Drugs*Hours | F | df(num) | df(den) | p-value |
|---|---|---|---|---|
| Ct.1 | 40.08 | 1 | 551 | <0.0001 |
| Ct.2 | 2.54 | 1 | 551 | 0.1119 |
| Ct.3 | 23.46 | 1 | 551 | <0.0001 |
| Ct.4 | 2.46 | 1 | 551 | 0.1170 |
| Ct.5 | 14.55 | 1 | 551 | 0.0002 |
| Ct.6 | 7.36 | 1 | 551 | 0.0069 |
| Ct.7 | 7.39 | 1 | 551 | 0.0068 |
| Ct.8 | 6.37 | 1 | 551 | 0.0119 |
| Ct.9 | 8.11 | 1 | 551 | 0.0046 |
| Ct.10 | 1.63 | 1 | 551 | 0.2022 |
| Ct.11 | 2.86 | 1 | 551 | 0.0914 |
| Ct.12 | 0.53 | 1 | 551 | 0.4651 |
| Ct.13 | 1.09 | 1 | 551 | 0.2965 |
| Ct.14 | 0.53 | 1 | 551 | 0.4656 |
| Ct.15 | 2.13 | 1 | 551 | 0.1446 |
| Ct.16 | 0.94 | 1 | 551 | 0.3319 |
| Total | 5.19 | 16 | 551 | <0.0001 |

Contrasts 1, 3, 5, 7 and 9 compare the placebo to the average of the drugs for hours 1, 2, 3, 4 and 5 respectively. Seeing that all of these are significant ($p<0.05$) we can say that at hour 6 after the drugs were administered, the drugs lose their effect, since contrasts 11, 13 and 15 are not significant. Regarding the drug comparisons among each other, the superiority of B over A is manifested ($p<0.05$) only in hours 3 and 4 (contrasts 6 and 8 respectively).

### *Analysis of litter decomposition bags*

In the leaf litter decomposition trials the remnant dry matter at each time is generally analyzed through ANCOVA, using time as a covariate and a logarithmic transformation of the response, or ANOVA for a split-plot design, when the evaluation periods are equidistant. The experimental units consist of bags containing the vegetative material. Usually, these bags are grouped to form a repetition that can be evaluated through time, and the contents of each bag are evaluated at different times. Even though each time the evaluated bags are different, often the correlation structure that assumes independence or compound symmetry (induced by grouping of bags that represent a repetition) is not enough to explain the observed correlations. Observations closer in time tend to be more correlated than those distant in time, or correlations between observations from earlier times are different from correlations between observations from later times. The use of mixed models allows us not only to manage more complex correlation structures but also makes it possible to model heterogeneous variances. In these models, the treatments can be included as classification factors and time can be model as a covariate or as a factor. This last case produces models that are less parsimonious but more flexible in terms of their ability to model different trends through time. On the other hand, the introduction of random effects on the parameters that involve time can be used to correct the lack of fit.

In the example shown below, a data set generated by a decomposition trial conducted in a tropical aquatic environment is analyzed (Martinez 2006). The compared treatments consist of the following: two Species (*Guadua* sp. and *Ficus* sp.) of which vegetative material is obtained, and two types of Bag in which the material is placed (Fine and Coarse). The four treatments had 5 repetitions (with 7 bags each), and they were evaluated at 7 times. The purpose of this trial was to establish the effect of Species, Bag and Time on the rate of decomposition. The data can be found in the Decomposition.IDB2 file.

The original data (remnant dry matter) were transformed to logarithms. The graph showing the logarithm of the remnant dry matter (from here on, the *response*) as a function of time and for each treatment (Figure 88) shows a decrease of the remnant dry weight as a function of time. The graph also suggests the existence of heteroscedasticity that is a function of time, which depends on the species and the type of bag. An initial

approximation to model these data could be to fit a regression model with different intercept and slopes. To perform this fit we select the mixed models module, indicating *LnDryWeight* as the dependent variable, *Species* and *Bag* as classification factors, and *Time* as a covariate. Then, in the tab for the fixed part of the model, terms are indicated as shown in

Figure 89. The graph for the adjusted model is shown in Figure 90.

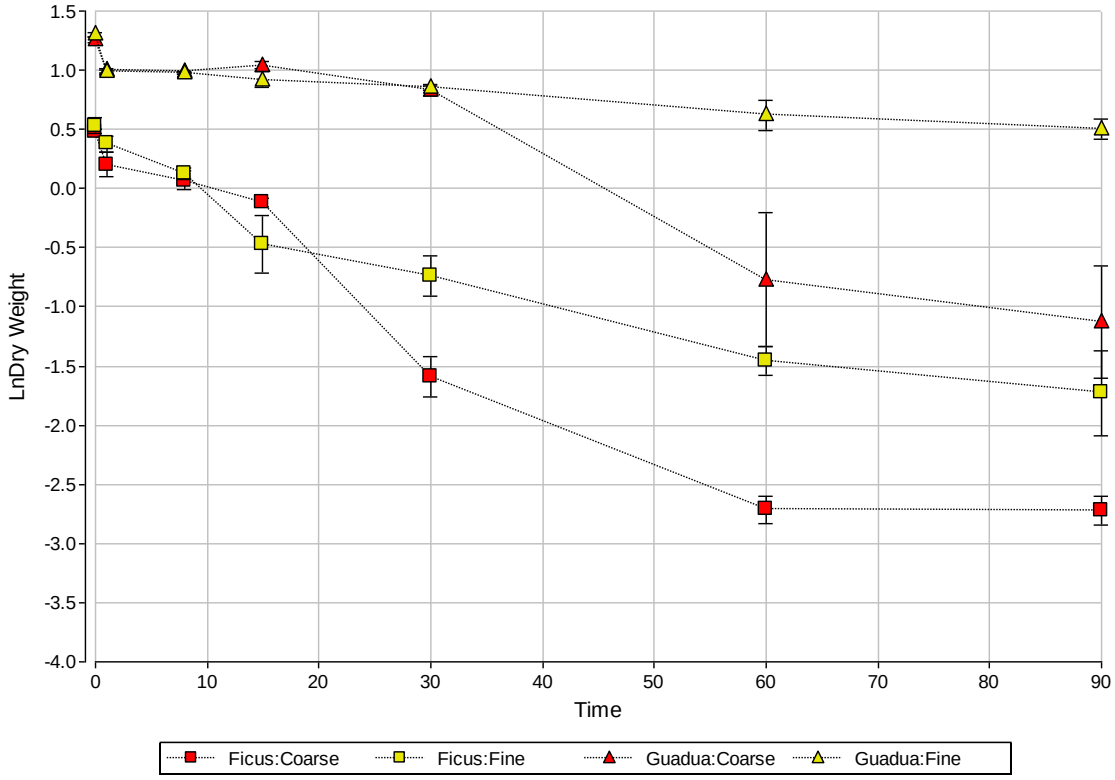



***Figure 88: Dot plot for the logarithm of remnant dry weight as a function of Time for the four treatments (Species-Bag) (Decomposition.IDB2 file).***

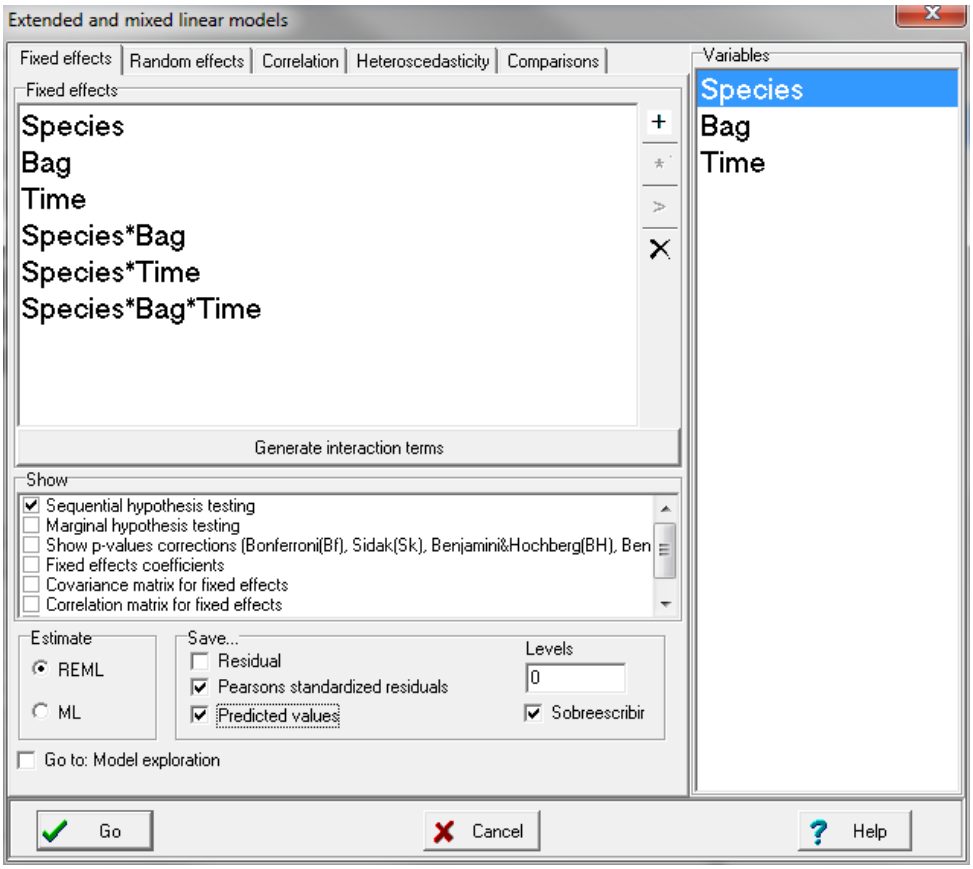



***Figure 89: Specification of the linear regression model with different intercepts and slopes for the logarithm of remnant dry matter as a function of Time for the four treatments determined by the combination of species and bag type (Species-Bag) (Decomposition.IDB2 file).***

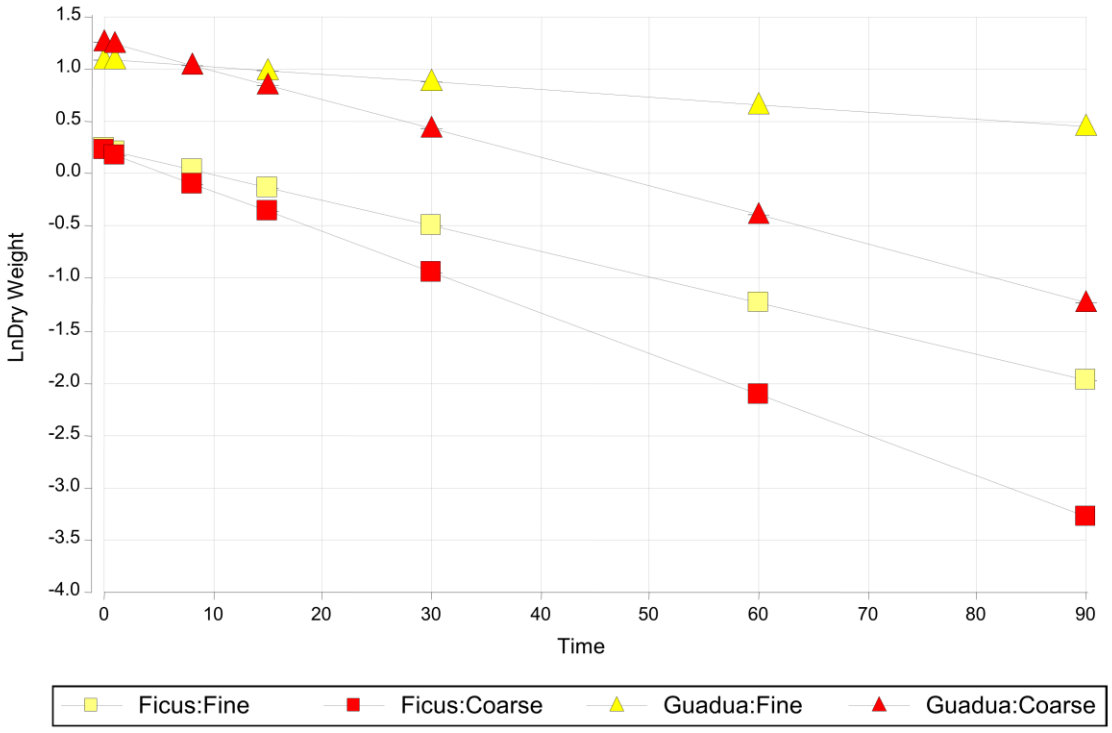



***Figure 90: Dot plot for the Predicted value (logarithm of remnant dry weight) as a function of Time for the four treatments (Species-Bag) (Decomposition.IDB2 file).***

Figure 90 shows that the fit of different straight lines by treatment is an approximation that, although plausible, does not account for some of the particularities of the loss of dry weight. This is reflected by the presence of curvatures in the residuals (Figure 91). A way to resolve the problem of the presence of curvature is the specification of a model that includes quadratic terms for time. For this, we must extend the model proposed in

Figure 89 to include all the terms that correspond to squared time. To simplify the notation, we have created three variables, *T1* and *T2* represent time and time squared, respectively. *T1* is time centered with respect to the value 30 (days) and *T2* is the square of *T1*.The covariates are centered in order to eliminate the colinearity that results from using a regressor and its square, and hence to improve the condition of the X'X matrix. The variables *T1* and *T2* as well as *Species_Bag* are included in the *Decomposition.IDB2* file. In the specification of the mixed model, *Species_Bag* should be included as a classification factor and *T1* and *T2* should be included as covariates. The *Fixed effects* tab should look as shown in Figure 92.

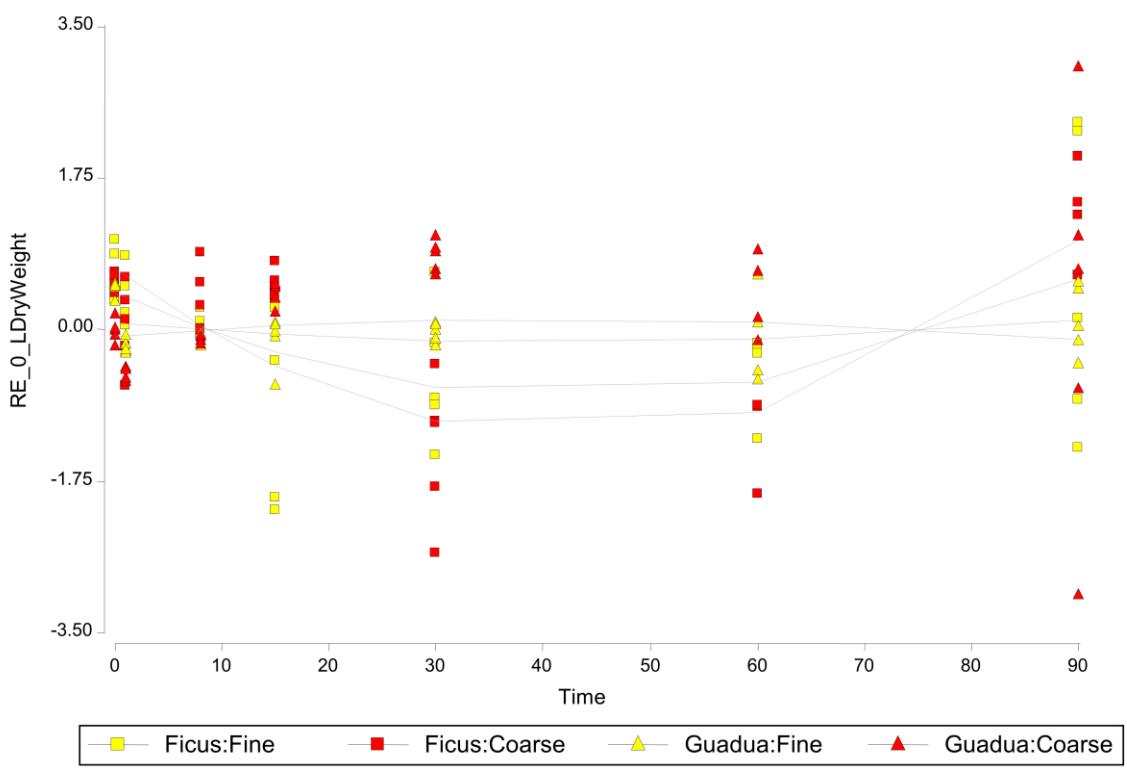



***Figure 91: Graph of Pearson studentized residuals vs. Time for a regression model of remnant dry matter as a function of Time for the four treatments (Species-Bag) with different intercepts and slopes, (Decomposition.IDB2 file).***

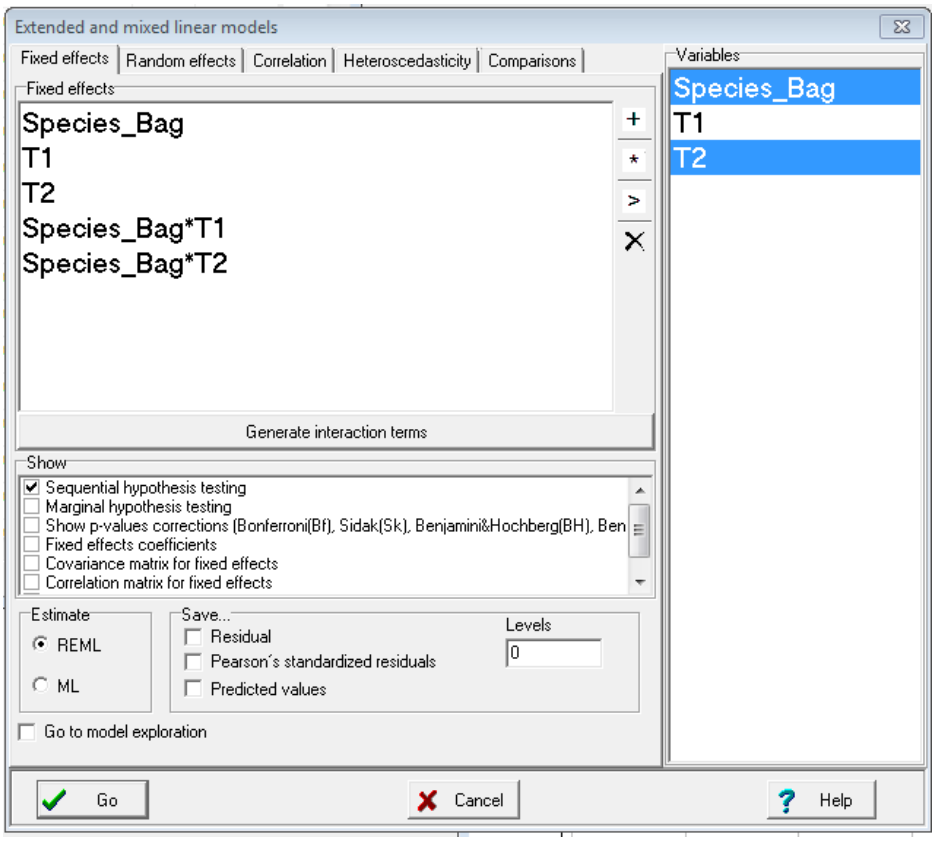



***Figure 92: Specification of the linear regression model with different intercepts and slopes for the logarithm of remnant dry matter as a function of Time for the four Species-Bag treatments (Descomposition.IDB file2).***

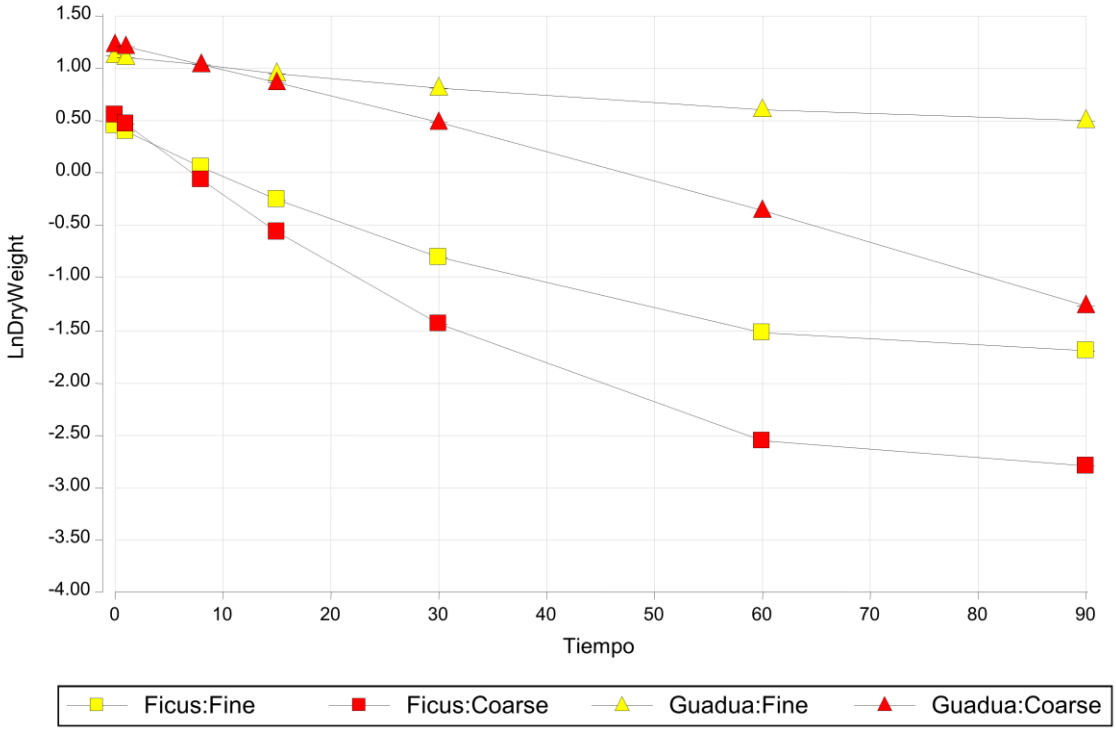



***Figure 93: Fits for the second-order polynomial regression model with different intercepts and slopes for the logarithm of remnant dry matter as a function of Time^2 (centered) for four Species-Bag treatments (Decomposition.IDB2 file).***

The residuals of the fitted model according to Figure 92, show two problems: heteroscedasticity (that depends on time and treatments) and lack of fit, seeing that for some treatments and times, the Pearson residuals appear on top or under the zero line (enveloped by a circle, Figure 94).

At this point, we will opt to model first the problem of heteroscedasticity using an identity variance function. For this, we will leave the fixed part of the specification window of the model just as indicated in Figure 92, but in the *Heteroscedasticity* tab we will indicate that the variance should be estimated in a different way for the combination of time and treatment as shown in Figure 95. Pearson's studentized residuals vs. time for this model are shown in Figure 96. Even if the problem of heteroscedasticity seems mostly solved, problems of lack of fit continue to exist, and these are visualized in groups of residuals of a single treatment in a given time that are either all positive or all negative.

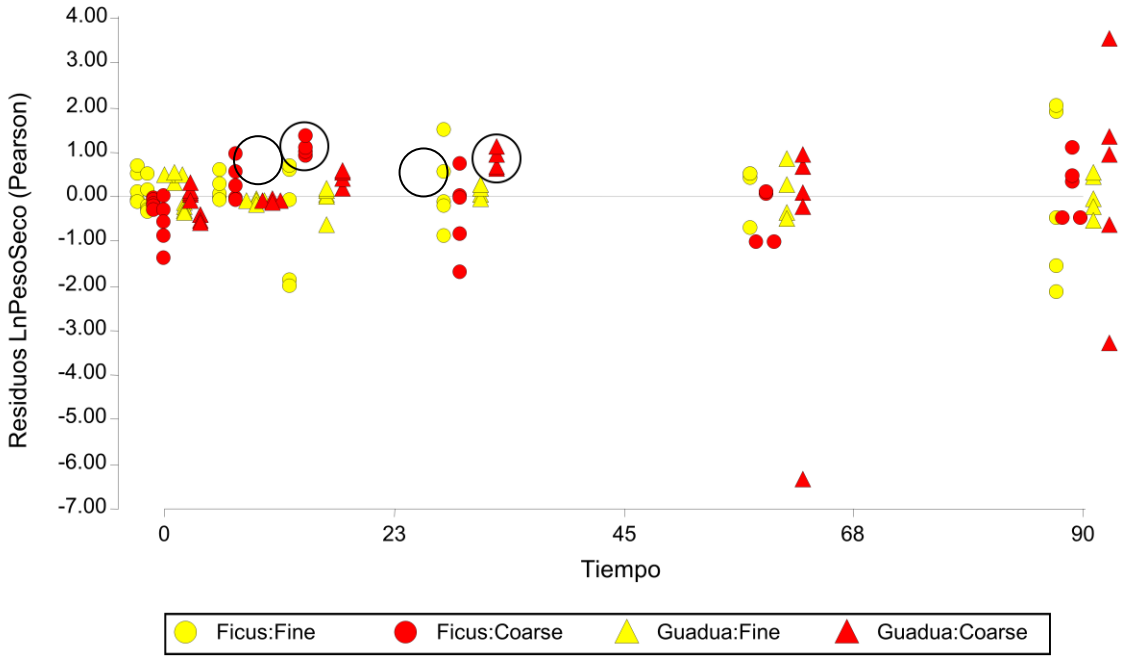



***Figure 94: Studentized residuals (Pearson) vs. Time for the second-order polynomial regression model with different intercept and slopes for the logarithm of remnant dry weight as a function of Time and Time^2 (centered) for four Species-Bag treatments (Decomposition.IDB2 file).***

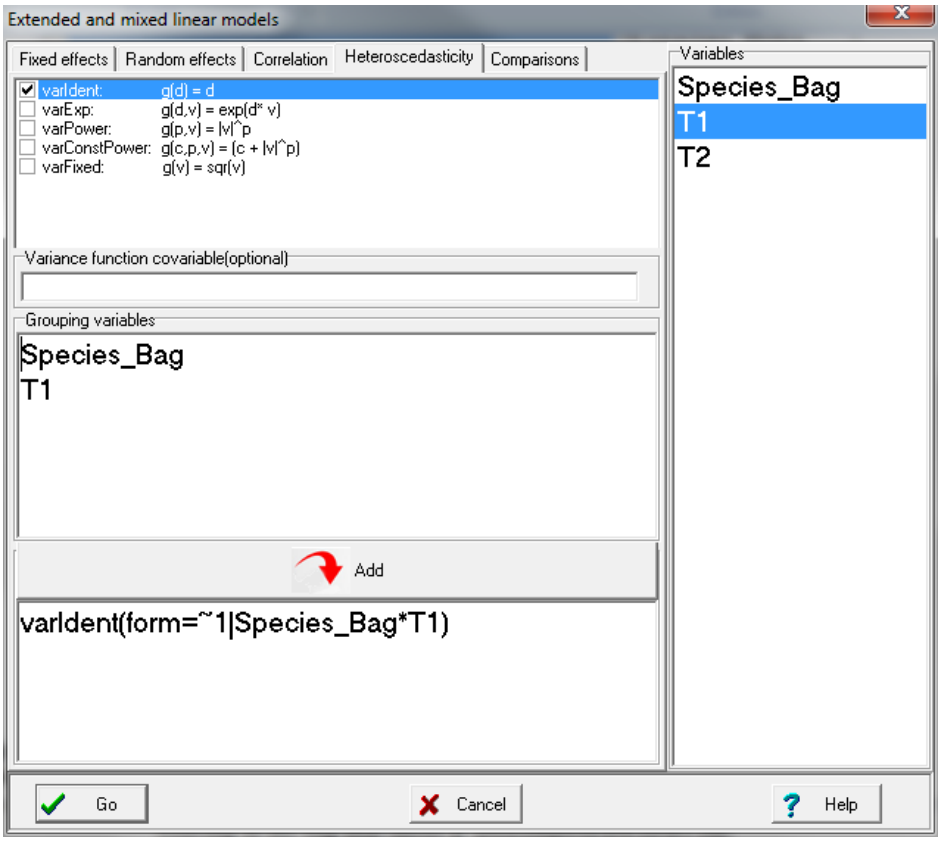



***Figure 95: Specification of the heteroscedastic part of the second-order polynomial regression model with different intercepts and slopes for the logarithm of remnant dry weight as a function of Time and Time^2 (centered) for four Species-Bag treatments (Decomposition.IDB2 file).***

A way to resolve this lack of fit is to add random effects to the average level of each combination of time and treatment. If in the *Random effects* tab we add *Time_Species_Bag* and keep the box that corresponds to the *Constant* checked, we are indicating that we are dealing with a random displacement that affects the expected value for the combination of *Time Species* and *Bag under the fitted model* (Figure 97). Finally, the studentized residuals graph for this model shows an image in which there is no evidence of lack of fit or presence of heteroscedasticity (Figure 98).

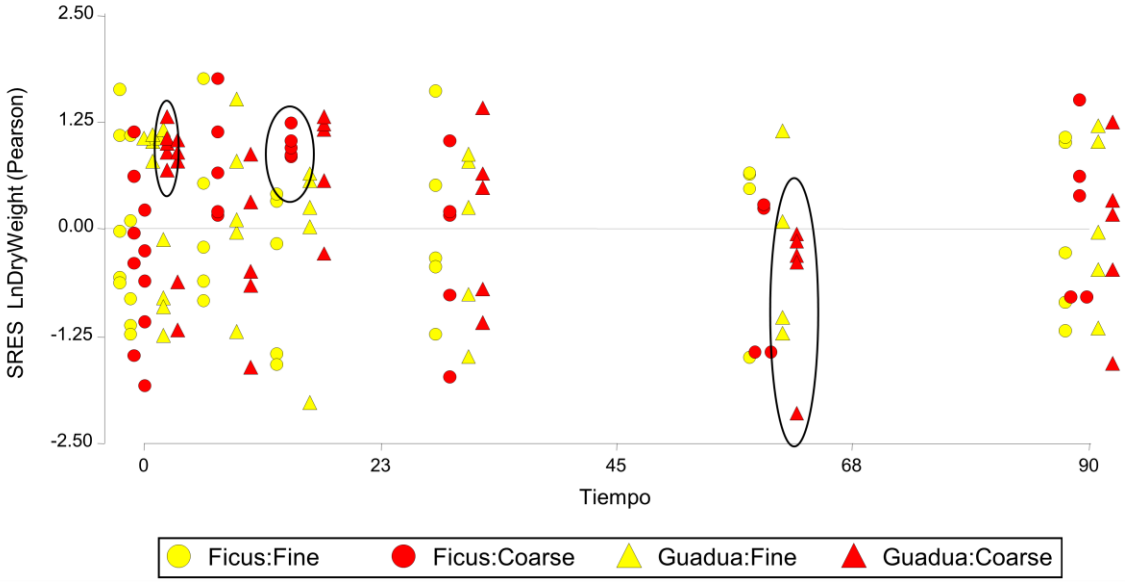



***Figure 96: Studentized residuals (Pearson) vs. Time for the heteroscedastic regression model with different intercepts and slopes by treatment for the logarithm of Remnant dry weight as a function of Time and Time^2 (centered) for the four treatments (Species-Bag) (Decomposition.IDB2 file).***

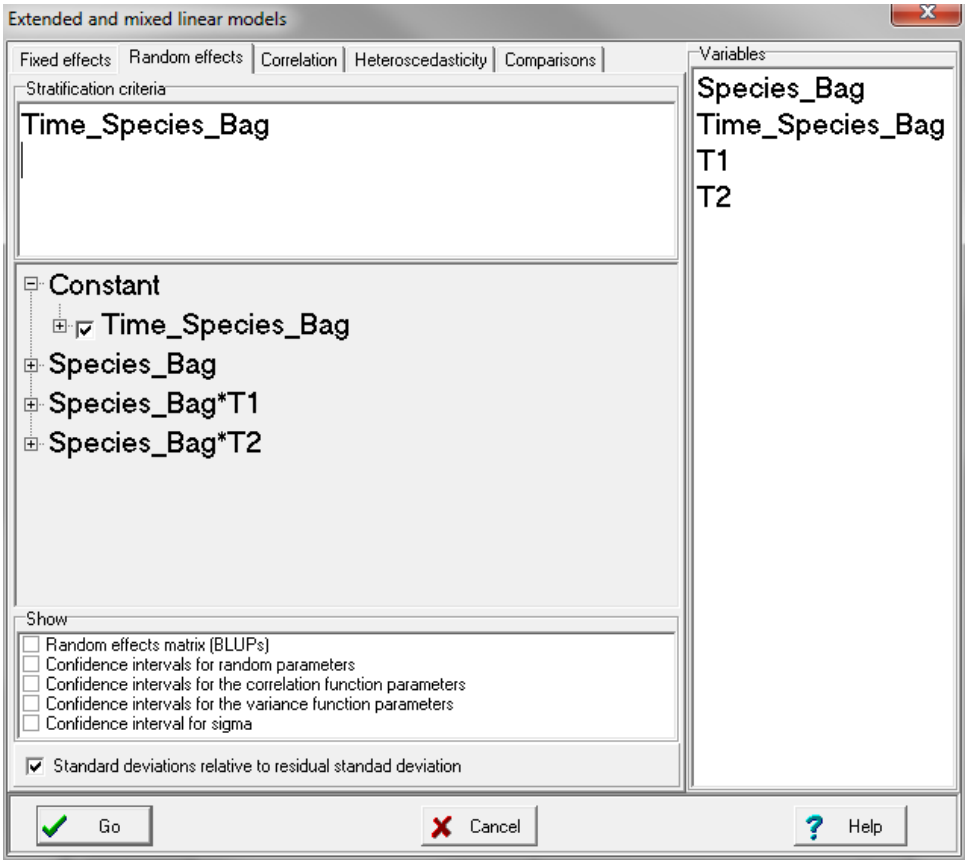



***Figure 97: Specification of the random part of the second-order heteroscedastic regression model with different intercepts and slopes for the logarithm of Remnant dry weight as a function of Time and Time^2 (centered) for the four treatments (Species-Bag) (Decomposition.IDB2 file).***

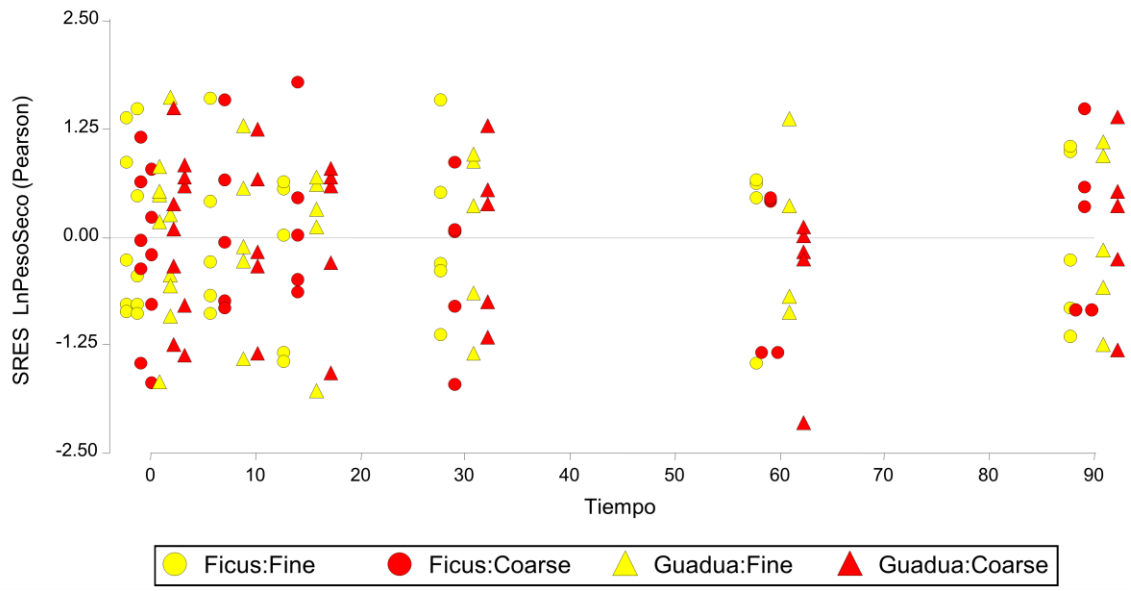



***Figure 98: Studentized residuals (Pearson) vs. Time for the heteroscedastic regression model with different intercept and slopes by treatment and the addition of a random effect on the constant that is particular for each combination of Time and Treatment, for the logarithm of remnant dry weight as a function of Time and Time^2 (centered) for the four treatments (Species-Bag) (Decomposition.IDB2 file).***

Finally, since the purpose of this trial was to calculate the decomposition rate, and we have fitted a linear model for the logarithm of the remnant dry matter weight, we can estimate the decomposition rate as the derivative of *-exp(fitted model).* We will use the interface with R to obtain these derivatives. Pressing F9 the interpreter window of R is called (Figure 99). To the right of the window a list will appear. It contains the R objects that have been created during the work session. In this list, the fitted models using extended and mixed linear models should appear as a string composed of the word *"model"+ correlative number _name of the dependent variable_ Estimation method.* In our example they should appear as follows: `model#_LnDryWeight _REML` (in position # there should be a number dependent on the number of times that the model was fit for the same dependent variable). The example shows the model `model001_LnDryWeight _REML`.

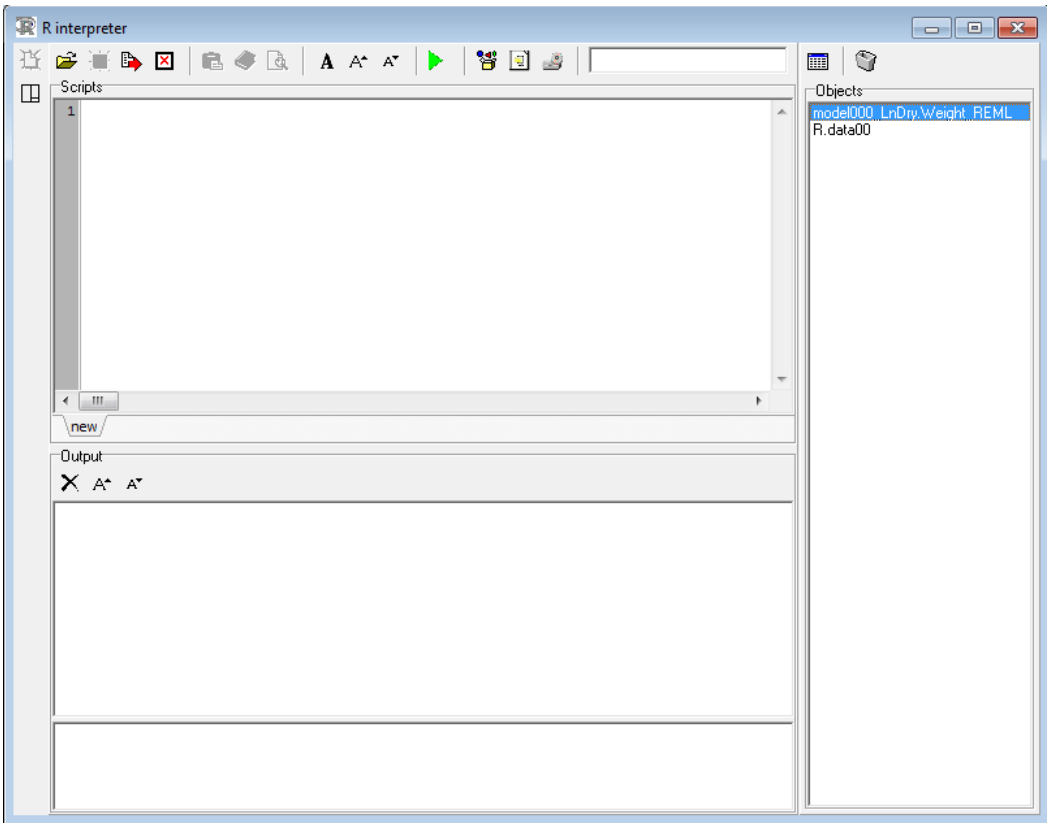



***Figure 99: R interface with four panels: Script contains the R programs to be executed, Output shows the executed script, Objects shows the list of objects retained in R's memory, and the bottom panel shows messages and error reports sent by R to the console.***

To calculate the decomposition rates, we must first understand what we have fitted with the estimated linear model. The fixed part of the proposed model is as follows:

| |
|---|
| *Species_Bag*<br>*T1*<br>*T2*<br>*Species_Bag*T1*<br>*Sspecies_Bag*T2* |

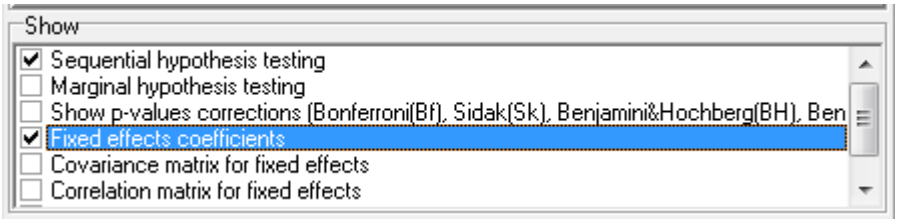
Este modelo es equivalente a:

| |
|---|
| *Species_Bag-**1***<br>*Species_Bag*T1*<br>*Species_Bag*T2* |

The advantage of the previous specification is that the coefficients of the fixed part of the model correspond with those in (10). This model specifies a second-order polynomial regression in time (centered around 30 days) for each of the combinations of *Species* and *Bag*. Thus, we are estimating a function of the following form:

$$LnDryWeight = \beta_{i0} + \beta_{i1}(T-30) + \beta_{i2}(T-30)^2 \qquad (10)$$

where the *i* index indicates the treatment (in this case *i* identifies the four combinations of *Species* and *Bag*). This means that we have an equation like (7) specific for each condition. The estimated coefficients for the fixed part can be obtained during the model estimation by checking the *Fixed effects coefficients* option in the *Fixed effects* tab.




Since we will use R to calculate the derivatives of equation (7), we will review these coefficients from R. If we write `Model004_LnDryWeight _REML$coefficients$fixed` in the *Script* window, and then at the end of the line press `Shift Enter`, the following output will appear:

```
     Species_BagFicus_Fine     Species_BagFicus_Coarse
                -0.7738921650                 -1.3680878569
   Species_BagGuadua_Fine    Species_BagGuadua_Coarse
                 0.8162357629                  0.7630705376
 Species_BagFicus_Fine:T1  Species_BagFicus_Coarse:T1
                -0.0326126598                 -0.0508364778
Species_BagGuadua_Fine:T1 Species_BagGuadua_Coarse:T1
                -0.0086055613                 -0.0192635993
 Species_BagFicus_Fine:T2  Species_BagFicus_Coarse:T2
                 0.0002938702                  0.0004422140
Species_BagGuadua_Fine:T2 Species_BagGuadua_Coarse:T2
                 0.0000571603                 -0.0002451274
```

The first four coefficients (from left to right), correspond to the constants $(\beta_{i0})$ of the following variables: Ficus_Fine, Ficus_Coarse, Guadua_Fine and Guadua_Coarse. The Ficus_Fine constant does not appear explicitly, because it is confounded with the intercept (*Intercept*), which is assumed present by default.

The second group of four coefficients (0.0326126598, ... ,-0.0192635993) are the coefficients $(\beta_{i1})$ of the linear term of (10), and the last group of four (0.0002938702, … , -0.0002451274) are the coefficients $(\beta_{i2})$ of the quadratic term in (10). The equation for dry remnant weight for *Species Ficus* with *Fine Bag* is as follows:

$$\ln DryWeight = -0.7738921651 - 0.0326126598\,(T-30) + 0.0002938702(T-30)^2$$

Since function (7) represents remnant dry weight, decomposed dry weight should be calculated as follows:

$$DryWeightConsumed = InitialWeight - \exp(\beta_{i0} + \beta_{i1}(T-30) + \beta_{i2}(T-30)^2)$$

With respect to the rate of decomposition, this would be the derivative of the function, namely:

$$RateDecomp = -\exp(\beta_{i0} + \beta_{i1}(T-30) + \beta_{i2}(T-30)^2)(\beta_{i1} + 2\beta_{i2}(T-30))$$

The following *script* generates a table whose first column is Time, and the subsequent columns are the rates of decomposition for each of the treatments. Note that the model with the best fit should be specified (in our case, model004):

```
a=model004_LnDry Weight_REML$coefficients$fixed
T=seq(0,90,1)
dFF = -exp(a[1]+(T-30)*a[5]+(T-30)*(T-30)*a[9]) *(a[5]+2*(a[9] *(T-30)))
dFC = -exp(a[2]+(T-30)*a[6]+(T-30)*(T-30)*a[10])*(a[6]+2*(a[10]*(T-30)))
dGF = -exp(a[3]+(T-30)*a[7]+(T-30)*(T-30)*a[11])*(a[7]+2*(a[11]*(T-30)))
dGC = -exp(a[4]+(T-30)*a[8]+(T-30)*(T-30)*a[12])*(a[8]+2*(a[12]*(T-30)))
Tasas=as.data.frame=cbind(T,dFF,dFC,dGF,dGC)
```

The following objects will appear in the list of objects: *a*, *T*, *dFF*, *dFC*, *dGF*, *dGC* and *Rates.* Right clicking on *Rates* will make the *Action* menu appear, which includes the following options: *Convert matrix, dataframe or vector to InfoStat table*. By selecting this option, we will obtain a new table in InfoStat like the one shown to the right of this paragraph.

New table

| Caso | T | dFF | dFG | dGF | dGG |
|---|---|---|---|---|---|
| 1 | 0.00 | 0.08 | 0.13 | 0.04 | 0.01 |
| 2 | 1.00 | 0.08 | 0.12 | 0.04 | 0.02 |
| 3 | 2.00 | 0.07 | 0.11 | 0.04 | 0.02 |
| 4 | 3.00 | 0.07 | 0.10 | 0.03 | 0.02 |
| 5 | 4.00 | 0.06 | 0.10 | 0.03 | 0.02 |
| 6 | 5.00 | 0.06 | 0.09 | 0.03 | 0.02 |
| 7 | 6.00 | 0.06 | 0.08 | 0.03 | 0.02 |
| 8 | 7.00 | 0.05 | 0.07 | 0.03 | 0.02 |
| 9 | 8.00 | 0.05 | 0.07 | 0.03 | 0.02 |
| 10 | 9.00 | 0.05 | 0.06 | 0.03 | 0.03 |
| 11 | 10.00 | 0.04 | 0.06 | 0.03 | 0.03 |
| 12 | 11.00 | 0.04 | 0.05 | 0.03 | 0.03 |
| 13 | 12.00 | 0.04 | 0.05 | 0.03 | 0.03 |
| 14 | 13.00 | 0.04 | 0.05 | 0.03 | 0.03 |
| 15 | 14.00 | 0.04 | 0.04 | 0.03 | 0.03 |
| 16 | 15.00 | 0.03 | 0.04 | 0.03 | 0.03 |

Real | registros: 91*5

Using the *Scatterplot diagram* submenu in the *Graphics* menu, we can obtain the following representation of the decomposition rates (Figure 100). In the emerging dialogue window of the Scatter plot diagram, the following variables were assigned for this purpose: *dFF, dFC, dGF* and *dGC* on the y-axis and *T* on the x-axis.

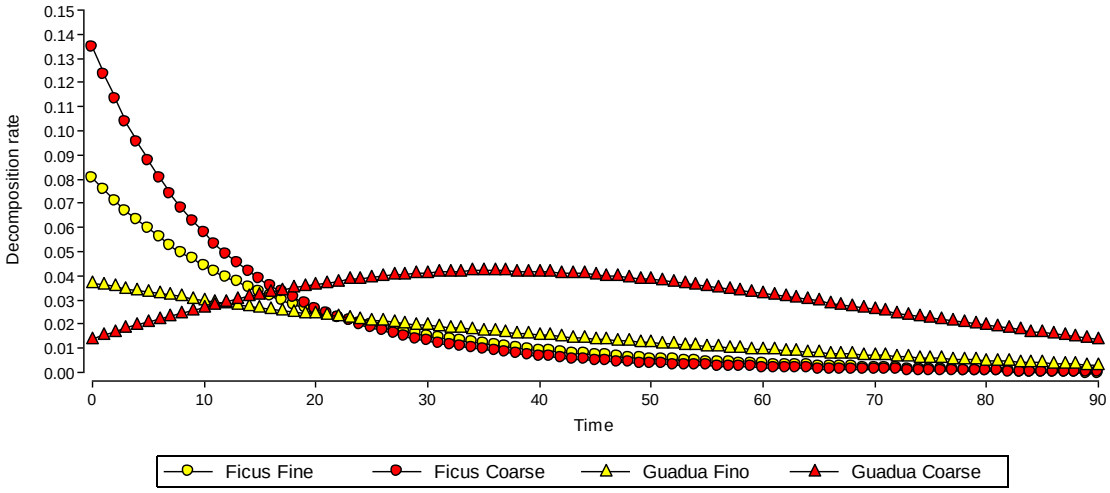



***Figure 100: Decomposition curves, by species and bag type.***

## *Use of mixed models to control spatial variability in agricultural experiments*

### *Spatial correlation*

Stratification, or blocking, is a technique used to control the effects of variation in experimental units. Blocks are groups of experimental units formed in such a way that plots within blocks are as homogenous as possible. Designs with plot stratification, such as randomized complete block designs (RCBD), incomplete block designs and lattices, are more efficient than a completely randomized design when differences between experimental units that make up a single stratum (block) are minimal and differences between strata are large. When this condition is not met, the error term can be overestimated, and if the data are unbalanced, treatment effect estimations can also be biased. When many treatment plots are evaluated in the field, the size of the blocks needed to obtain a repetition of the experiment is large, and consequently it is difficult to ensure homogeneity of the plots that make up the block: plots that are closer to each other can be more similar than plots that are farther away from each other, which generates spatial variation (Casanoves et al. 2005). Spatial variability refers to variation between observations obtained from plots with spatial arrangements on the field. Due to the existence of spatial variation within blocks, standard analysis of variance for designs that involve blocks of experimental unit does not always eliminate bias in the comparison of treatment effects. Variation from plot to plot within a single block can be caused by competition, heterogeneity in soil fertility, insect dispersion, weeds, crop illness, or cultivation practices, among others. For this reason, statistical procedures that account for spatial variation among plots and that adjust treatment means as a function of the observations in close neighboring plots have been proposed (Papadakis 1937); as well as models that account for spatial correlation in the error term and that also adjust treatment means (Mead 1971, Besag 1974, 1977, Ripley 1981). Gilmour et al. (1997) partition spatial variability among plots from an experiment in local and global spatial variability. Local spatial variability refers to small-scale difference between plots, where intra-block variations are considered. Local spatial trends and residual heterogeneity are modeled with the variance and covariance residual matrix. A bi-dimensional coordinate system allows the definition of plot location in the field. The modeling of plot spatial structure based on distance functions can be done in the context of linear mixed models

(Zimmerman & Harville 1991, Gilmour et al. 1997, Cullis et al. 1998), where in addition to accounting for the correlation structure among observations from different plots, it is possible to model heterogeneity of residual variance. This is very useful in comparative production experiments, since these are conducted in different environments. If the correlation depends only on the distance (magnitude and/or direction of the distance), models that estimate covariance among observations are called *stationary*. Correlation functions for stationary models can be isotropic or anisotropic. The former are identical in any direction (they only depend on the magnitude of the distances) while the latter allow different parameter values in different directions (i.e., they also depend on the direction in which the distance is calculated).

### *Analysis of a comparative yield trials for peanuts*

To provide examples of the alternative analyses, we use data from the *Peanut MET.IDB2* file, which come from a comparative yield trial (CYT) for one agricultural year for experimental lines (genotypes) of peanuts (*Arachis hypogaea* L.) from the EEA-Manfredi, INTA, Peanut Improvement Program in Argentina. In each year, CYTs were conducted in three locations of the cultivation area in the province of Córdoba: Manfredi, General Cabrera and Río Tercero. The group of genotypes evaluated was the same for each location. At each of the three locations, the experiments were conducted according to a RCBD with four repetitions, and grain yield values were recorded (kg/plot).

Yield data were analyzed using different modifications of the following model:

$$y_{ijk} = \mu + \tau_i + \gamma_j + \eta_k + \delta_{jk} + \varphi_{ik} + \varepsilon_{ijk};\ i = 1,..,16;\ j = 1,...,4; k = 1,...,3 \qquad (11)$$

where $y_{ijk}$ represents the observed response in the *i*-th level of the *Genotype* factor, in the *j*-th level of the *Block* factor, and in the *k*-th level of the *Location* factor; $\mu$ represents the general mean of the response; $\tau_i$ represents the effect of the *i*-th level of the *Genotype* factor; $\gamma_j$ represents the effect of the *j*-th level of the *Block* factor; $\eta_k$ is the *k*-th level of the *Location* factor; $\varphi_{ik}$ is the interaction between *Genotype* and *Location*; $\delta_{ik}$ is the effect of the *Block* within the *Location*; and $\varepsilon_{ijkl}$ represents the experimental error. The usual assumption is that $\varepsilon_{ijkl} \sim N\left(0, \sigma^2_{\varepsilon}\right)$.

In most cases, all factors in the model are considered fixed, except for $\varepsilon_{ijk}$ and block effects (the latter are in some cases considered random). This has the effect of restricting the comparison of models to their own plot structure. Different plot structures induce a correlation structure between observations that can be understood within the framework of mixed models, including analytical techniques for the control of spatial variability.

The following covariance structures are used for the data (marginal covariance):

1. *FB Model: Fixed block effect, independent errors, constant variance between locations.*
2. *RB Model: Random block effect, independent errors, constant variance between locations.*
3. *FBE Model: Fixed block effect, independent errors, different variances between locations.*
4. *RBE Model: Random block effect, independent errors, different variances between locations.*
5. *Exp Model: Exponential spatial correlation, no block effect, constant variance between locations.*
6. *FBExp Model: Exponential spatial correlation, fixed block effect, constant variance between locations.*
7. *ExpH Model: Exponential spatial correlation, no block effect, different variances between locations.*
8. *Gau Model: Guassian spatial correlation, no block effect, constant variance between locations.*
9. *Sph Model: Spherical spatial correlation, no block effect, constant variance between locations.*

In the first two models, $\varepsilon_{ijk}$ is assumed to be independent with constant variance, $\sigma^2$, i.e., the assumption is that spatial variation does not exist (intra-block) and, furthermore, that homogeneity of residual variance exists between locations. The effects of the block are considered fixed or random, denoted as *FB Model* or *RB Model*, respectively.

The procedures denoted as *FBE Model* and *RBE Model* are also based on a RCBD model, but they consider possible heterogeneous residual variances depending on the different levels of the location factor.

The fifth procedure consists in adjusting an isotropic spatial correlation model with a correlation power function (*Exp Model*) for each location, without specifying a block effect. This model assumes that an exponential function accounts for intra-block variation as well as variation among blocks.

The sixth procedure is the same as the previous one, but with a fixed block effect (*FBExp Model*).

The seventh model consists of a model similar to the *Exp Model*, but allows for the possibility of different variances (and correlations) for each location.

The last two procedures consist in adjusting an isotropic spatial correlation model with a function for Gaussian correlation (*Gau Model*) and a spherical correlation function, without specifying a fixed block effect.

REML estimation is used in all cases. In the variables selector, *Yield* is indicated as a dependent variable and *Block*, *Location* and *Geno* are indicated as classification variables.

To adjust the *FB Model*, effects should be specified in the fixed effects tab as shown in Figure 101.

To adjust the *RB Model*, factors should be specified in the fixed effects and random effects tabs, as shown in Figure 102 andFigure 103 respectively. Nothing should be specified in the remaining tabs.

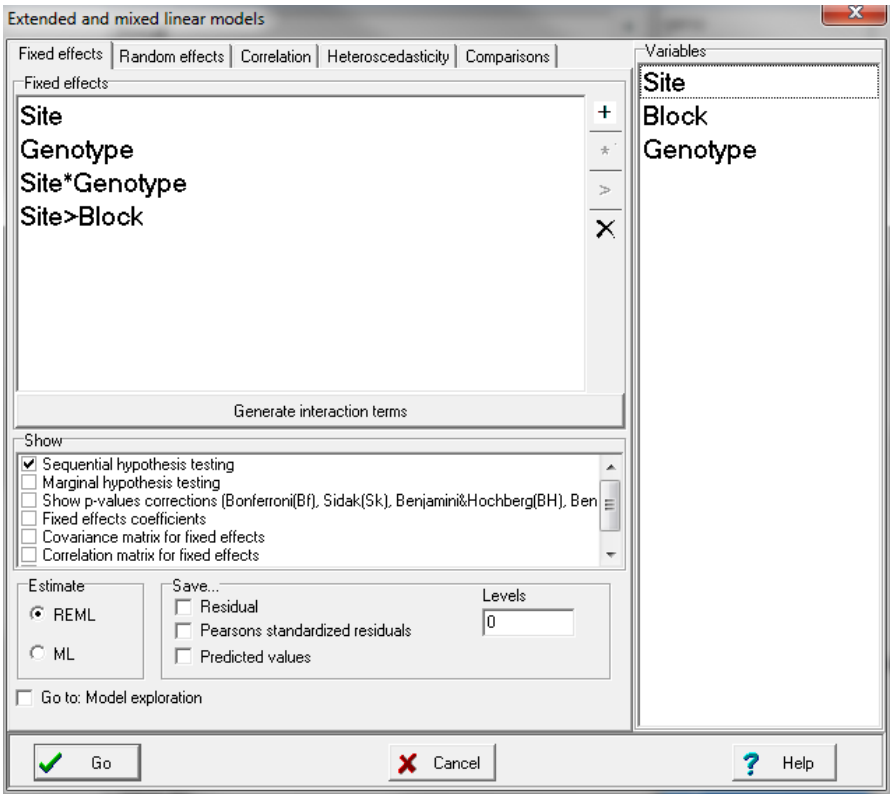



***Figure 101: Window displaying the Fixed effects tab, FB Model (Peanut MET.IDB2 file).***

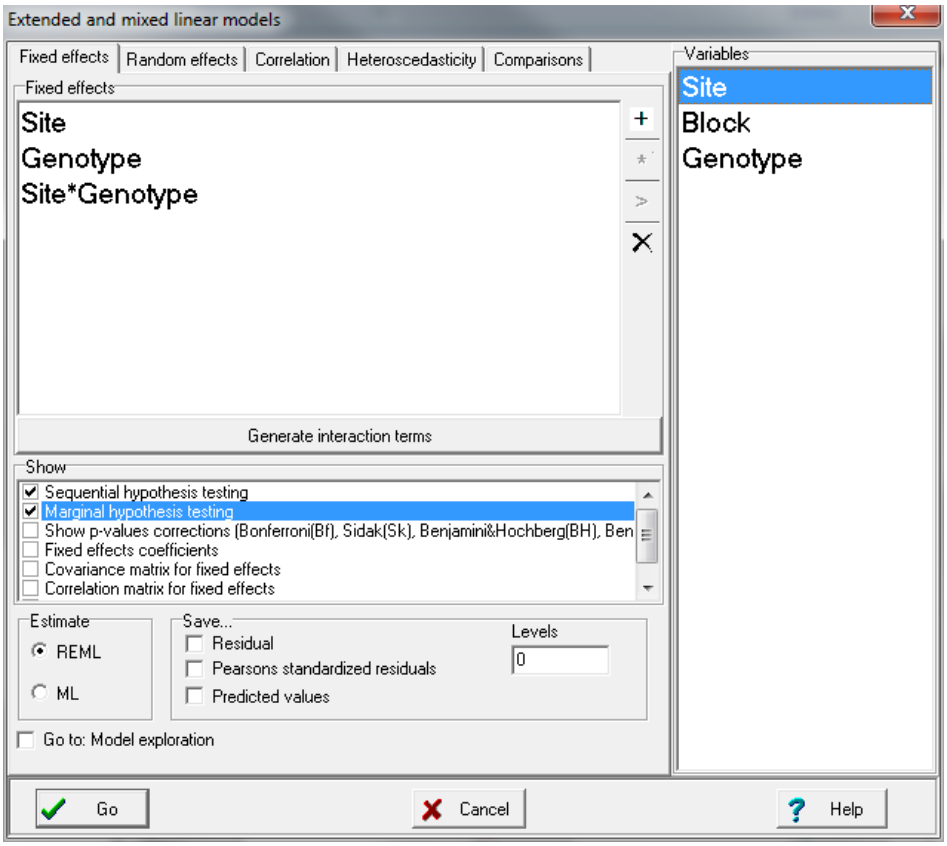



***Figure 102: Window displaying the Fixed effects tab, RB Model (Peanut MET.IDB2 file).***

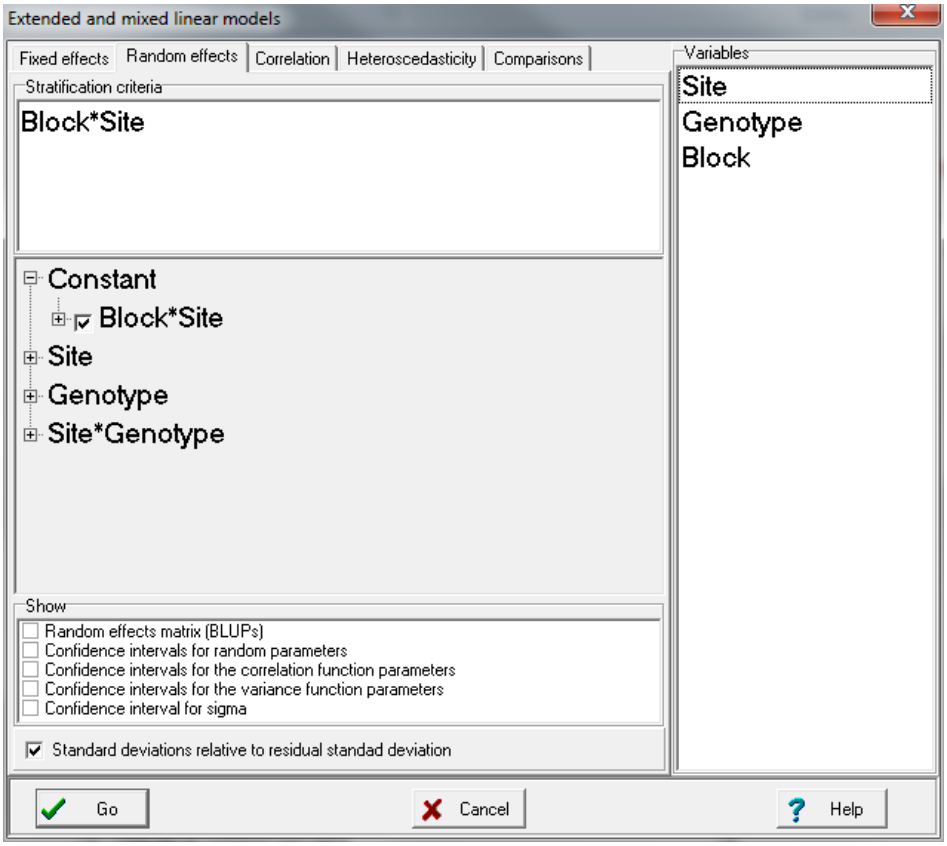



***Figure 103: Window displaying the Random effects tab, RB Model (Peanut MET.IDB2 file).***

The FBE and RBE models consider independent errors and different variance between locations. To specify these models, proceed in the same way as in the two previous cases (i.e., FB and RB), but add a *varIdent* function in the *Heteroscedasticity* tab and indicate *Location* as the grouping variable. Once the function and grouping criteria are specified, click on *Add* (Figure 104).

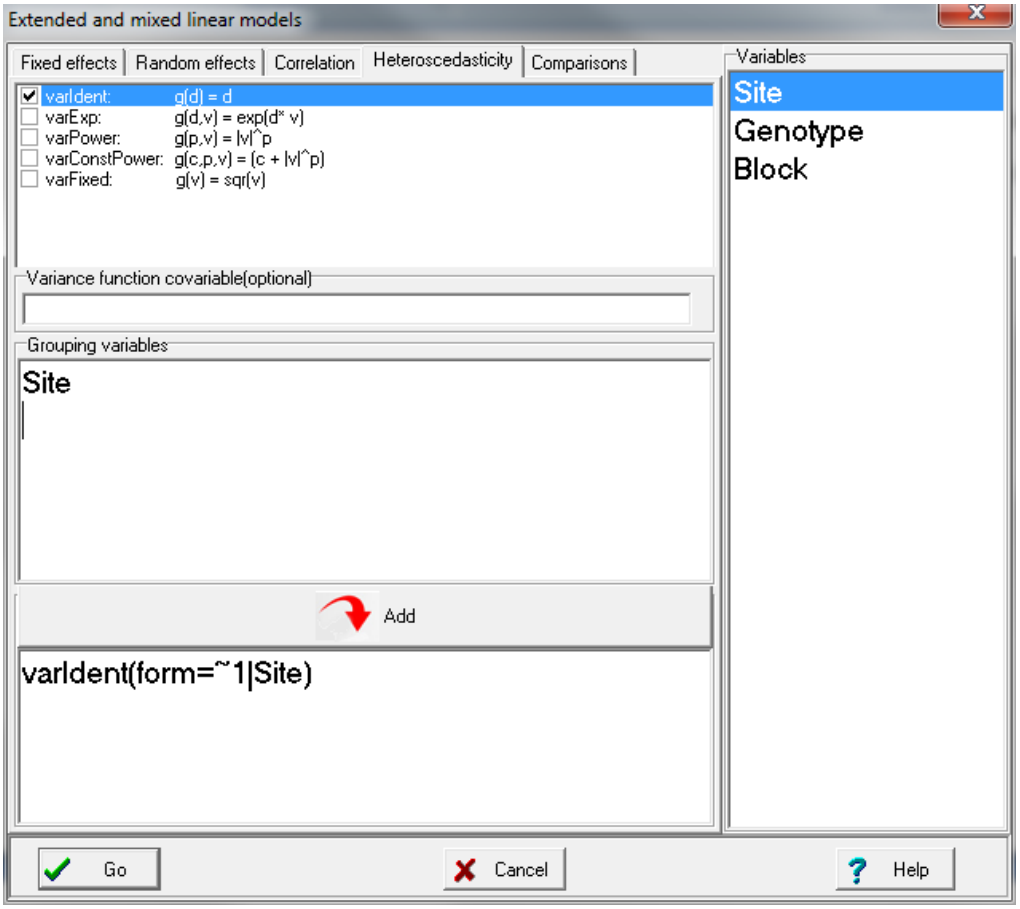



***Figure 104: Window displaying the Heteroscedasticity tab, with Location as the grouping variable, FBE and RBE models (Peanut MET.IDB2 file).***

The fifth model does not include block effects, and it models variability within and between blocks by means of an exponential isotropic function (Exp Model) with constant variance between locations. To use the exponential function, we should add variables that denote spatial coordinates to the model. To do so, we should add the variables *la* and *lon* in *Covariates*. In the fixed effects tab, we keep *geno*, *location* and *geno*location*, and in the random effects tab no factor is specified. In the *Hetersdedasticity* tab, no function should be specified. To specify exponential spatial correlation, the corresponding function should be selected in the *Correlations* tab, the X

and Y coordinates should be declared as well as the grouping variables (in this case is *Location*), because there is a coordinate system within each site (Figure 105).

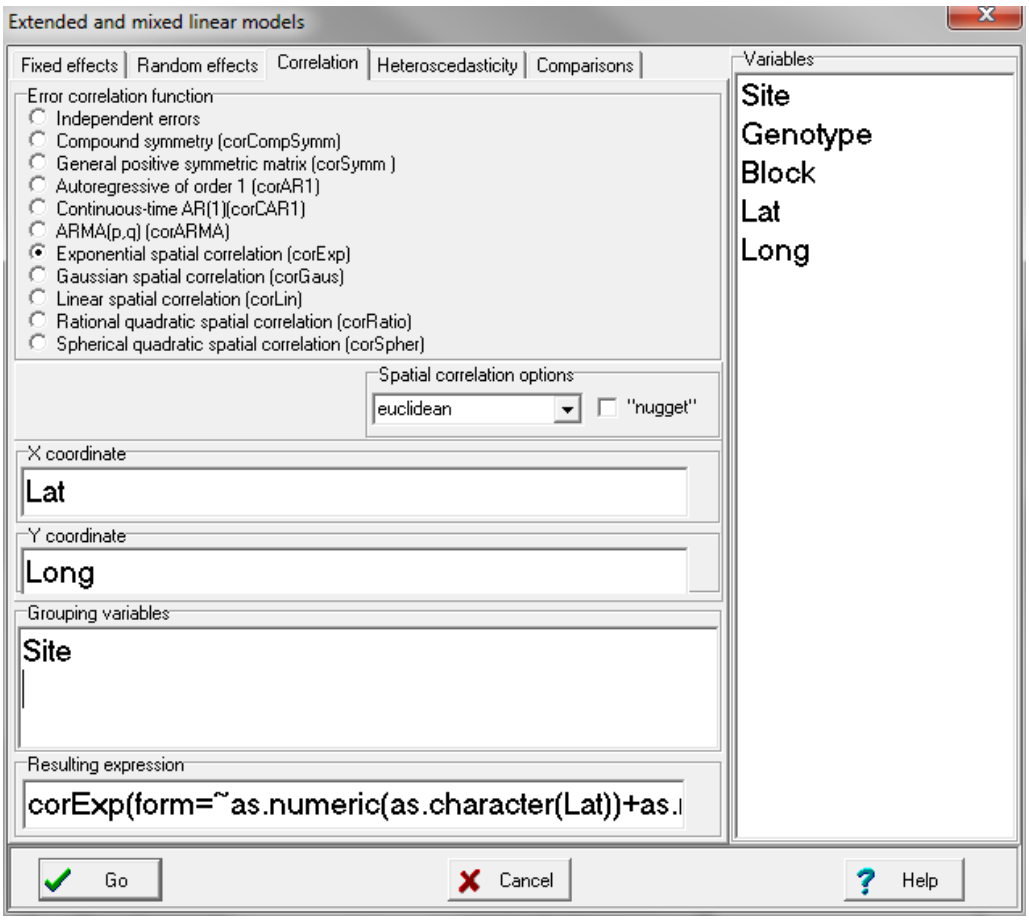



***Figure 105: Window displaying the Correlation tab using the la and lon variables as X and Y coordinates, respectively, and Site as the grouping variable, Exp and FBExp Models (Peanut MET.IDB2 file).***

The sixth model (*FBExp Model*) is the same as the previous one, but it specifies fixed block effects within each location (as shown in Figure 101). The inclusion of fixed blocks restricts the modeling of spatial variation to variation within the block. The variation among blocks is considered, in the classical sense, through the inclusion of blocks in the fixed part. Thus, specifying *la* and *lon* as coordinates of the spatial coordinate model is redundant, since it would be sufficient to declare only *lon* (the coordinate that varies within the block). Nevertheless, in order to omit the *la* coordinate, it would be necessary to declare a new stratification criterion that is consistent in the combination of the levels of location and block. This procedure generates identical results to those generated by the *FBExp Model*.

The seventh model (*ExpH Model*) is similar the *Exp Model*, but it allows for heterogeneous variances between locations (as shown in Figure 104). The *Gau* and *Sph* models are fitted just like the *Exp Model* without a block effect, as shown in Figure 105, but the Guassian and Spherical spatial correlation functions are selected, respectively. In the *Heteroscedasticity* tab, nothing should be specified.

The results of the different fits for the different models are shown below.

**FB Model**

**Extended and mixed linear models**

**R specification of the model**

```
model000_Yield_REML<-
gls(Yield~1+Site+Genotype+Site:Genotype+Site:Block
,method="REML"
,na.action=na.omit
,data=R.data00)
```

**Results for model: model000_Yield_REML**

*Dependent variable:Yield*

**Fit measurements**

| N | AIC | BIC | logLik | Sigma | R2_0 |
|---|---|---|---|---|---|
| 192 | 299.71 | 468.22 | -91.86 | 0.35 | 0.86 |

*Smaller AIC and BIC is better*

**Sequential hypothesis testing**

| | numDF | F-value | p-value |
|---|---|---|---|
| (Intercept) | 1 | 8372.75 | <0.0001 |
| Site | 2 | 280.56 | <0.0001 |
| Genotype | 15 | 6.02 | <0.0001 |
| Site:Genotype | 30 | 4.32 | <0.0001 |
| Site:Block | 9 | 4.77 | <0.0001 |

**RB Model**

**Extended and mixed linear models**

**R specification of the model**

```
model002_Yield_REML<-lme(Yield~1+Site+Genotype+Site:Genotype
,random=list(Block_Site=pdIdent(~1))
,method="REML"
,na.action=na.omit
,data=R.data00
,keep.data=FALSE)
```

**Results for model: model002_Yield_REML**

*Dependent variable:Yield*

**Fit measurements**

| N | AIC | BIC | logLik | Sigma | R2_0 | R2_1 |
|---|---|---|---|---|---|---|
| 192 | 283.41 | 431.90 | -91.71 | 0.35 | 0.81 | 0.86 |

*Smaller AIC and BIC is better*

**Sequential hypothesis testing**

| | numDF | denDF | F-value | p-value |
|---|---|---|---|---|
| (Intercept) | 1 | 135 | 1754.21 | <0.0001 |
| Site | 2 | 9 | 58.78 | <0.0001 |
| Genotype | 15 | 135 | 6.02 | <0.0001 |
| Site:Genotype | 30 | 135 | 4.32 | <0.0001 |

**Random effects parameters**

*Covariance model for random effects: pdIdent*
*Formula: ~1|Block_Site*

*Standard deviations relative to residual standard deviation and correlation*

| | (const) |
|---|---|
| (const) | 0.49 |

## FBE Model

**Extended and mixed linear models**

**R specification of the model**

```
model011_Yield_REML<-
gls(Yield~1+Site+Genotype+Site:Genotype+Site:Block
,weight=varComb(varIdent(form=~1|Site))
,method="REML"
,na.action=na.omit
,data=R.data00)
```

**Results for model: model011_Yield_REML**

*Dependent variable:Yield*

**Fit measurements**

| N | AIC | BIC | logLik | Sigma | R2_0 |
|---|---|---|---|---|---|
| 192 | 303.44 | 477.75 | -91.72 | 0.36 | 0.86 |

*Smaller AIC and BIC is better*

**Sequential hypothesis testing**

| | numDF | F-value | p-value |
|---|---|---|---|
| (Intercept) | 1 | 8547.37 | <0.0001 |
| Site | 2 | 292.67 | <0.0001 |
| Genotype | 15 | 6.02 | <0.0001 |
| Site:Genotype | 30 | 4.36 | <0.0001 |
| Site:Block | 9 | 4.76 | <0.0001 |

**Variance structure**

*Variance model: varIdent*
*Formula: ~ 1 | Site*

*Variance-function parameters*

| Parámeter | Estim |
|---|---|
| gralcabr | 1.00 |
| manf | 0.92 |
| rio3 | 0.96 |

**RBE Model**

**Extended and mixed linear models**

**R specification of the model**

```
model013_Yield_REML<-
lme(Yield~1+Site+Genotype+Site:Genotype+Site:Block
,random=list(Block_Site=pdIdent(~1))
,weight=varComb(varIdent(form=~1|Site))
,method="REML"
,na.action=na.omit
,data=R.data00
,keep.data=FALSE)
```

**Results for model: model013_Yield_REML**

*Dependent variable:Yield*

**Fit measurements**

| N | AIC | BIC | logLik | Sigma | R2_0 | R2_1 |
|---|---|---|---|---|---|---|
| 192 | 287.12 | 441.55 | -91.56 | 0.36 | 0.81 | 0.86 |

*Smaller AIC and BIC is better*

**Sequential hypothesis testing**

| | numDF | denDF | F-value | p-value |
|---|---|---|---|---|
| (Intercept) | 1 | 135 | 1765.74 | <0.0001 |
| Site | 2 | 9 | 59.53 | <0.0001 |
| Genotype | 15 | 135 | 6.01 | <0.0001 |
| Site:Genotype | 30 | 135 | 4.36 | <0.0001 |

**Random effects parameters**

*Covariance model for random effects: pdIdent*
*Formula: ~1|Block_Site*

*Standard deviations relative to residual standard deviation and correlation*

| | (const) |
|---|---|
| (const) | 0.46 |

**Variance structure**

*Variance model: varIdent*
*Formula: ~ 1 | Site*

*Variance-function parameters*

| Parameter | Estim. |
|---|---|
| gralcabr | 1.00 |
| manf | 0.92 |
| rio3 | 0.95 |

## Exp Model

**Extended and mixed linear models**

**R specification of the model**

```
model002_Yield_REML<-gls(Yield~1+Genotype+Site+Site:Genotype
,correlation=corExp(form=~as.numeric(as.character(Lat))+as.numeric(as.
character(Long))|Site
,metric="euclidean"
,nugget=FALSE)
,method="REML"
,na.action=na.omit
,data=R.data00)
```

**Results for model: model002_Yield_REML**

*Dependent variable:Yield*
**Fit measurements**

| N | AIC | BIC | logLik | Sigma | R2_0 |
|---|---|---|---|---|---|
| 192 | 273.43 | 421.92 | -86.72 | 0.39 | 0.81 |

*Smaller AIC and BIC is better*

**Sequential hypothesis testing**

| | numDF | F-value | p-value |
|---|---|---|---|
| (Intercept) | 1 | 1687.54 | <0.0001 |
| Genotype | 15 | 7.27 | <0.0001 |
| Site | 2 | 56.18 | <0.0001 |
| Genotype:Site | 30 | 5.33 | <0.0001 |

**Correlation structure**

*Correlation model: Exponential spatial correlation*
*Formula: ~ as.numeric(as.character(Lat)) +*
*as.numeric(as.character(Long)) | Site*
*Metric: euclidean*

*Model parameters*

| Parameter | Estim |
|---|---|
| range | 0.96 |

## FBExp Model

**Extended and mixed linear models**

**R specification of the model**

```
model001_Yield_REML<-
gls(Yield~1+Genotype+Site+Site:Genotype+Site/Block
,correlation=corExp(form=~as.numeric(as.character(Lat))+as.numeric(as.
character(Long))|Site
,metric="euclidean"
,nugget=FALSE)
,method="REML"
,na.action=na.omit
,data=R.data00)
```

**Results for model: model001_Yield_REML**

*Dependent variable:Yield*

**Fit measurements**

| N | AIC | BIC | logLik | Sigma | R2_0 |
|---|---|---|---|---|---|
| 192 | 284.85 | 456.26 | -83.42 | 0.35 | 0.86 |

*Smaller AIC and BIC is better*

**Sequential hypothesis testing**

| | numDF | F-value | p-value |
|---|---|---|---|
| (Intercept) | 1 | 2785.57 | <0.0001 |
| Genotype | 15 | 7.86 | <0.0001 |
| Site | 2 | 92.79 | <0.0001 |
| Genotype:Site | 30 | 5.74 | <0.0001 |
| Site:Block | 9 | 3.46 | 0.0007 |

**Correlation structure**

*Correlation model: Exponential spatial correlation*
*Formula: ~ as.numeric(as.character(Lat)) +*
*as.numeric(as.character(Long)) | Site*
*Metric: euclidean*

*Model parameters*

| Parameter | Estim |
|---|---|
| range | 0.78 |

## ExpH Model

**Extended and mixed linear models**

**R specification of the model**

```
model002_Yield_REML<-gls(Yield~1+Genotype+Site+Site:Genotype
,weight=varComb(varIdent(form=~1|Site))
,correlation=corExp(form=~as.numeric(as.character(Lat))+as.numeric(as.
character(Long))|Site
,metric="euclidean"
,nugget=FALSE)
,method="REML"
,na.action=na.omit
,data=R.data02)
```

**Results for model: model002_Yield_REML**

*Dependent variable:Yield*

**Fit measurements**

| N | AIC | BIC | logLik | Sigma | R2_0 |
|---|---|---|---|---|---|
| 192 | 275.01 | 429.44 | -85.50 | 0.43 | 0.81 |

*Smaller AIC and BIC is better*

**Sequential hypothesis testing**

| | numDF | F-value | p-value |
|---|---|---|---|
| (Intercept) | 1 | 1633.46 | <0.0001 |
| Genotype | 15 | 7.15 | <0.0001 |
| Site | 2 | 61.51 | <0.0001 |
| Genotype:Sit | 30 | 5.53 | <0.0001 |

**Correlation structure**

*Correlation model: Exponential spatial correlation*
*Formula: ~ as.numeric(as.character(Lat)) +*
*as.numeric(as.character(Long)) | Site*
*Metric: euclidean*

*Model parameters*

| Parameter | Estim |
|---|---|
| range | 0.99 |

**Variance structure**

*Variance model: varIdent*
*Formula: ~ 1 | Site*

*Variance-function parameters*

| Parameter | Estim |
|---|---|
| gralcabr | 1.00 |
| manf | 0.85 |
| rio3 | 0.81 |

## Gau Model

**Extended and mixed linear models**

**R specification of the model**

```
model004_Yield_REML<-gls(Yield~1+Genotype+Site+Site:Genotype
,correlation=corGaus(form=~as.numeric(as.character(Lat))+as.numeric(as
.character(Long))|Site
,metric="euclidean"
,nugget=FALSE)
,method="REML"
,na.action=na.omit
,data=R.data04)
```

**Results for model: model004_Yield_REML**

*Dependent variable:Yield*

**Fit measurements**

| N | AIC | BIC | logLik | Sigma | R2_0 |
|---|---|---|---|---|---|
| 192 | 277.81 | 426.30 | -88.90 | 0.37 | 0.81 |

*Smaller AIC and BIC is better*

**Sequential hypothesis testing**

| | numDF | F-value | p-value |
|---|---|---|---|
| (Intercept) | 1 | 3399.06 | <0.0001 |
| Genotype | 15 | 7.36 | <0.0001 |
| Site | 2 | 113.57 | <0.0001 |
| Genotype:Site | 30 | 4.97 | <0.0001 |

**Correlation structure**

*Correlation model: Gaussian spatial correlation*
*Formula: ~ as.numeric(as.character(Lat)) +*
*as.numeric(as.character(Long)) | Site*
*Metric: euclidean*

*Model parameters*

| Parameter | Estim |
|---|---|
| range | 0.87 |

**Sph Model**

**Extended and mixed linear models**

**R specification of the model**

```
model005_Yield_REML<-gls(Yield~1+Genotype+Site+Site:Genotype
,correlation=corSpher(form=~as.numeric(as.character(Lat))+as.numeric(a
s.character(Long))|Site
,metric="euclidean"
,nugget=FALSE)
,method="REML"
,na.action=na.omit
,data=R.data05)
```

**Results for model: model005_Yield_REML**

*Dependent variable:Yield*

**Fit measurements**

| N | AIC | BIC | logLik | Sigma | R2_0 |
|---|---|---|---|---|---|
| 192 | 277.72 | 426.21 | -88.86 | 0.38 | 0.81 |

*Smaller AIC and BIC is better*

**Sequential hypothesis testing**

| | numDF | F-value | p-value |
|---|---|---|---|
| (Intercept) | 1 | 3170.04 | <0.0001 |
| Genotype | 15 | 7.61 | <0.0001 |
| Site | 2 | 105.96 | <0.0001 |
| Genotype:Site | 30 | 5.15 | <0.0001 |

**Correlation structure**

*Correlation model: Spherical spatial correlation*
*Formula: ~ as.numeric(as.character(Lat)) +*
*as.numeric(as.character(Long)) | Site*
*Metric: euclidean*

*Model parameters*

| Parameter | Estim |
|---|---|
| range | 1.91 |

*Comparison of fitted models*

Due to the fact that the fitted models have different components in their fixed part, those that share the same fixed effects are compared by means of their AIC and BIC criteria. First, the FB, FBE and FBExp models (Table 3) are compared.

***Table 3. Goodness of fit criteria for the adjusted models with a fixed block effect (Peanut MET.IDB2 file).***

| Model | AIC | BIC |
|---|---|---|
| FB | 299.72 | 468.22 |
| FBE | 303.44 | 477.75 |
| FBExp | 284.85 | 456.26 |

For this group of models that consider the fixed block effect, we can observe that the fixed block model plus an exponential correlation function provides the best fit. This implies that intra-block correlation exists, and it is removed by the exponential correlation function. Note also that there is no improvement in the models when heterogeneous variances between locations are allowed (FB with respect to FBE). If the variances are calculated from the coefficients of the different locations, we can also observe that these are very similar:

Variance of gralcabr = $(1*0.36)^2 = 0.129$

Variance of manf = $(0.92*0.36)^2 = 0.109$

Variance of rio3 = $(0.96*0.36)^2 = 0.119$

The remaining 6 models can be compared among each other, given that they all share the same fixed effects, i.e., *Geno*, *Location* and *Geno*Location* (Table 4).

***Table 4. Goodness of fit criteria for the adjusted models without a fixed block effect (Peanut MET.IDB2 file).***

| Model | AIC | BIC |
|---|---|---|
| RB | 283.41 | 431.90 |
| RBE | 287.12 | 441.55 |
| Exp | 273.43 | 421.92 |
| ExpH | 275.01 | 429.44 |
| Gau | 277.81 | 426.30 |

| | | |
|---|---|---|
| Sph | 277.72 | 426.21 |

Among the models that consider random block effects, we can observe that allowing for heterogenous variances between locations does not improve the model, since AIC and BIC are smaller in *RB* than in *RBE*. The same occurs when spatial variability is modeled only through an exponential correlation function, because allowing for heterogeneous variances (*ExpH*) does not improve the *Exp Model*.

Compared to different spatial correlation models, no important differences were found for AIC and BIC between the *Gau* and *Sph* models, however these criteria had lower values for the exponential spatial correlation function. This last model had the best fit among the models without a fixed block effect.

Even if the first group of models (*FB*, *FBH* and *FBExp*) are not comparable through AIC and BIC with this last group, the researcher should be able to determine if the blocks should be considered fixed or random. The selection of the model group will have an effect on the inferences that can be made. This is easily visualized by observing that the standard errors used for the comparison of means change depending on the model. A more detailed discussion on the selection of fixed or random blocks is provided by Casanoves et al. (2007).

In this example, the best models within each group (i.e., *FBExp* and *Exp* for the first and second group of models, respectively) have the same covariance structure but differ in their fixed part: some have a block effect and others do not. In order to decide which of the two models is best, a likelihood ratio test using ML estimations for the models with and without block effect should be conducted (remember that ML should be used to compare models with different fixed effects).

Model with blocks (complete *FBExp*):

**Fit measurements**

| N | AIC | BIC | logLik | Sigma | R2_0 |
|---|---|---|---|---|---|
| 192 | 163.82 | 356.01 | -22.91 | 0.29 | 0.86 |

*Smaller AIC and BIC is better*

Model without blocks (reduced *Exp*):

**Fit measurements**

| N | AIC | BIC | logLik | Sigma | R2_0 |
|---|---|---|---|---|---|
| 192 | 182.85 | 345.73 | -41.43 | 0.34 | 0.81 |

*Smaller AIC and BIC is better*

Thus, the statistic $G = 2\log \text{lik}_{completo} - 2\log \text{lik}_{reducido} = 2(-22.91 + 41.43) = 37.04$ with 9 degrees of freedom and a p-value < 0.0001. Thus we can say with a 5% confidence level that it is best to keep the fixed block effect and the exponential correlation function. The comparison can be done manually, or by using the module for *Exploratory analysis of an estimated model*. The following result is obtained by selecting the *Models* tab and checking the estimated models that correspond to *FBExp* and *Exp*.

**Comparison of models**

| | Model | df | logLik | Test | L.Ratio | p-value |
|---|---|---|---|---|---|---|
| model009_rendim_ML | 1 | 59 | -22.91 | | | |
| model010_rendim_ML | 2 | 50 | -41.43 | 1 vs 2 | 37.04 | <0.0001 |

The complete result corresponding to the *FBExp Model* is shown below. The hypothesis tests for the interaction between *Genotype* and *Location* are significant (p<0.0001), thus the recommended *Genotype* can change depending on location. Note that due to the fit of the the spatial correlation function, the standard errors of the *Genotypes* are not constant. The multiple comparisons shown are generated through the application of the DGC procedure (Di Rienzo et al. 2002). This procedure was adapted to account for the particularities of the correlation structure among estimates in mixed models. The application of this procedure is recommended because of the large number of means to be compared, since it ensures a simpler interpretation than that which can be given by an LSD or Fisher's test. One can use the means of the combinations of locations and Genotypes as well as the interaction graph (Figure 106) to make recommendations.

**Extended and mixed linear models**

**R specification of the model**

```
model007_Yield_REML<-
gls(Yield~1+Genotype+Site+Site:Genotype+Site/Block
,correlation=corExp(form=~as.numeric(as.character(Lat))+as.numeric(as.
character(Long))|Site
,metric="euclidean"
,nugget=FALSE)
,method="REML"
,na.action=na.omit
,data=R.data07
```

**Results for model: model007_Yield_REML**

*Dependent variable:Yield*

**Fit measurements**

| N | AIC | BIC | logLik | Sigma | R2_0 |
|---|---|---|---|---|---|
| 192 | 284.85 | 456.26 | -83.42 | 0.35 | 0.86 |

*Smaller AIC and BIC is better*

**Sequential hypothesis testing**

| | numDF | F-value | p-value |
|---|---|---|---|
| (Intercept) | 1 | 2785.57 | <0.0001 |
| Site | 2 | 92.79 | <0.0001 |
| Genotype | 15 | 7.86 | <0.0001 |
| Site:Genotype | 30 | 5.74 | <0.0001 |
| Site:Block | 9 | 3.46 | 0.0007 |

**Correlation structure**

*Correlation model: Exponential spatial correlation*
*Formula: ~ as.numeric(as.character(Lat)) + as.numeric(as.character(Long)) | Site*
*Metric: euclidean*

*Model parameters*

| Parameter | Estim |
|---|---|
| range | 0.78 |

**Adjusted means and standard error for Site**

*DGC (alpha=0.05)*

| Site | Means | E.E. | | | |
|---|---|---|---|---|---|
| manf | 3.00 | 0.08 | A | | |
| gralcabr | 2.27 | 0.08 | | B | |
| rio3 | 1.56 | 0.08 | | | C |

*Different letters indicate significant difference between location parameters (p<= 0,05)*

**Adjusted means and standard error for Genotype**

*DGC (alpha=0.05)*

| Genotype | Means | S.E. | | | |
|---|---|---|---|---|---|
| mf435 | 2.73 | 0.10 | A | | |
| mf407 | 2.59 | 0.10 | A | | |
| mf429 | 2.51 | 0.10 | A | | |
| mf415 | 2.49 | 0.10 | A | | |
| mf420 | 2.38 | 0.10 | | B | |
| mf421 | 2.36 | 0.10 | | B | |
| mf431 | 2.34 | 0.10 | | B | |
| mf405 | 2.31 | 0.10 | | B | |
| manf68 | 2.24 | 0.10 | | B | |
| mf408 | 2.22 | 0.10 | | B | |
| manf393 | 2.22 | 0.10 | | B | |
| colirrad | 2.21 | 0.10 | | B | |
| mf404 | 2.14 | 0.10 | | B | |
| mf433 | 1.96 | 0.10 | | | C |
| mf432 | 1.96 | 0.10 | | | C |
| mf410 | 1.78 | 0.10 | | | C |

*Different letters indicate significant difference between location parameters (p<= 0,05)*

**Adjusted means and standard error for Site*Genotype**

*DGC (alpha=0.05)*

| Site | Genotype | Means | E.E. | | | | | |
|---|---|---|---|---|---|---|---|---|
| manf | mf407 | 3.67 | 0.17 | A | | | | |
| manf | mf421 | 3.54 | 0.17 | A | | | | |
| manf | mf405 | 3.38 | 0.17 | | B | | | |
| manf | mf431 | 3.28 | 0.17 | | B | | | |
| manf | mf435 | 3.24 | 0.17 | | B | | | |
| manf | manf68 | 3.23 | 0.17 | | B | | | |
| manf | mf420 | 3.17 | 0.17 | | B | | | |
| manf | mf429 | 3.08 | 0.17 | | B | | | |
| manf | colirrad | 3.05 | 0.17 | | B | | | |
| manf | manf393 | 3.02 | 0.17 | | B | | | |
| gralcabr | mf435 | 2.96 | 0.17 | | B | | | |
| manf | mf408 | 2.90 | 0.17 | | B | | | |
| manf | mf415 | 2.90 | 0.17 | | B | | | |
| gralcabr | mf420 | 2.82 | 0.17 | | B | | | |
| gralcabr | mf404 | 2.71 | 0.17 | | | C | | |
| manf | mf433 | 2.64 | 0.17 | | | C | | |
| gralcabr | mf415 | 2.61 | 0.17 | | | C | | |
| manf | mf410 | 2.53 | 0.17 | | | C | | |
| gralcabr | mf429 | 2.52 | 0.17 | | | C | | |
| manf | mf432 | 2.48 | 0.17 | | | C | | |
| gralcabr | mf421 | 2.42 | 0.17 | | | C | | |
| gralcabr | mf408 | 2.32 | 0.17 | | | C | | |
| gralcabr | manf393 | 2.30 | 0.17 | | | C | | |
| gralcabr | mf407 | 2.30 | 0.17 | | | C | | |
| gralcabr | mf405 | 2.25 | 0.17 | | | C | | |
| gralcabr | mf431 | 2.05 | 0.17 | | | | D | |
| gralcabr | manf68 | 2.04 | 0.17 | | | | D | |
| rio3 | mf435 | 1.99 | 0.17 | | | | D | |
| rio3 | mf415 | 1.98 | 0.17 | | | | D | |
| manf | mf404 | 1.97 | 0.17 | | | | D | |
| rio3 | mf429 | 1.93 | 0.17 | | | | D | |
| gralcabr | colirrad | 1.92 | 0.17 | | | | D | |
| rio3 | mf432 | 1.89 | 0.17 | | | | D | |
| rio3 | mf407 | 1.81 | 0.17 | | | | D | |
| gralcabr | mf410 | 1.79 | 0.17 | | | | D | |
| gralcabr | mf433 | 1.77 | 0.17 | | | | D | |
| rio3 | mf404 | 1.74 | 0.17 | | | | D | |
| rio3 | mf431 | 1.70 | 0.17 | | | | D | |
| rio3 | colirrad | 1.64 | 0.17 | | | | D | |
| gralcabr | mf432 | 1.50 | 0.17 | | | | | E |
| rio3 | mf433 | 1.47 | 0.17 | | | | | E |
| rio3 | manf68 | 1.45 | 0.17 | | | | | E |
| rio3 | mf408 | 1.44 | 0.17 | | | | | E |
| rio3 | manf393 | 1.33 | 0.17 | | | | | E |
| rio3 | mf405 | 1.32 | 0.17 | | | | | E |
| rio3 | mf420 | 1.16 | 0.17 | | | | | E |
| rio3 | mf421 | 1.14 | 0.17 | | | | | E |
| rio3 | mf410 | 1.02 | 0.17 | | | | | E |

*Means with a common letter are not significantly different (p<= 0.05)*

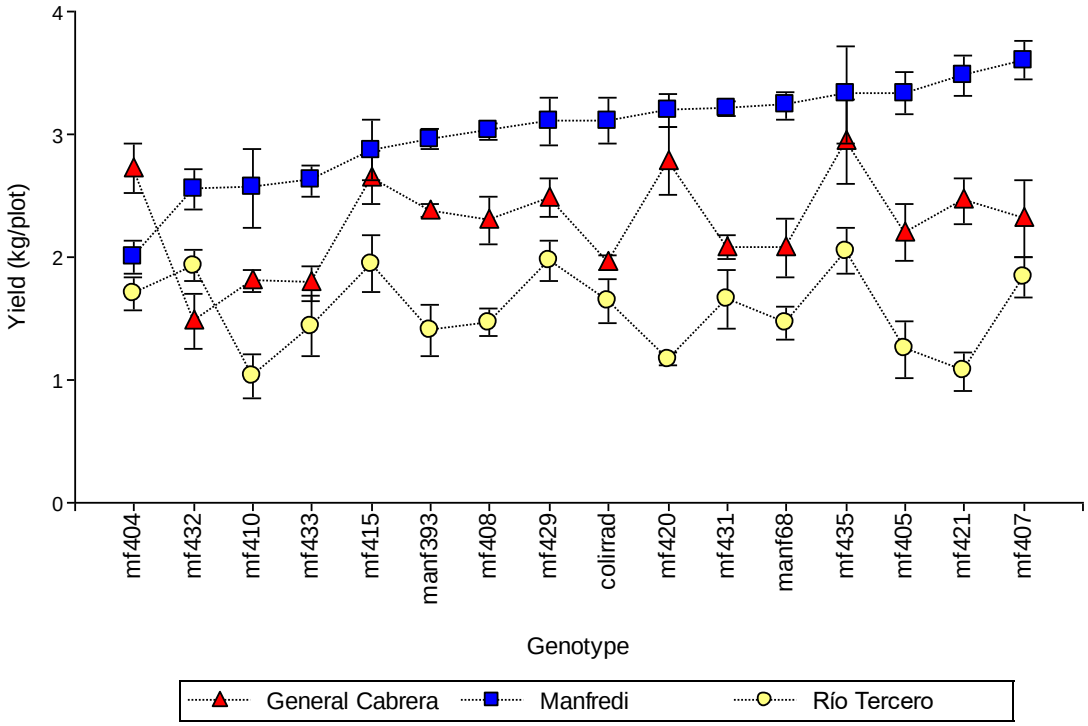



***Figure 106: Dot plot used to study the interaction between Location and Genotype for the variable Yield (Peanut MET.IDB).***

## *Applications of mixed models to other experimental designs*

### *Strip-plot design*

The strip-plot design is the result of restrictions in the randomization. Like the split-plot design, the strip-plot design results from an experiment that involves two or more factors. These factors (or their combinations) are applied to different levels, usually 2, and the randomization restrictions produce experimental units of different sizes and thus produce different error terms for each of the factors or combinations thereof (Milliken and Johnson 1992).

Consider an example in which we wish to evaluate three levels of fertilization with N (0, 50 and 100 kg N/ha) and two irrigation levels (low and high) on corn yields (data in Strip-plot.IDB2). The experiment was conducted under a randomized complete block design with four blocks.

Due to restrictions in the application of treatments, in the first stage, the three levels of nitrogen are randomized within each block; in the second stage, the irrigation levels are randomized, within each block and transversely with respect to the application of nitrogen.

Although in the following diagram (Figure 107) the randomization is shown within a specific block, the experiment was repeated in various blocks, as needed in order to obtain different error terms and for the resulting model to make sense. If each stage of the design has more than one factor, and these do interact with each other, one could use the interactions of highest order as error terms and thus obtain F tests without the need for repetitions.

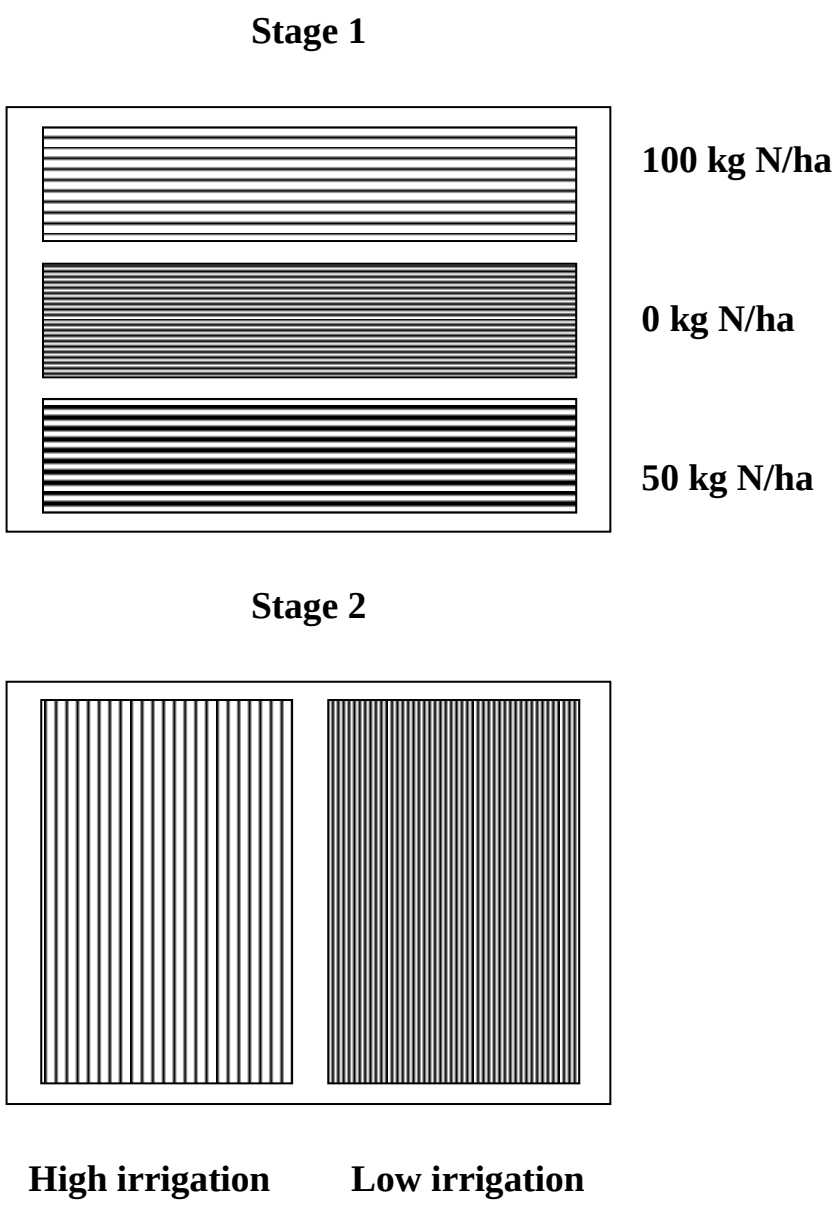



***Figure 107: Outline of an experiment conducted under a strip-plot design, repeated in completely randomized blocks with the randomization of the factors Nitrogen and Amount of irrigation for a particular block (StripPlot.IDB2 file).***

Yield data were analyzed using the following model:

$$y_{ijk} = \mu + \tau_i + \gamma_j + \lambda_{ij} + b_k + f_{ki} + c_{kj} + e_{kij};\ i = 1,..,3;\ j = 1,2;\ k = 1,...,4 \qquad (12)$$

where $y_{ijk}$ is the observed response in the *i*-th level of the *nitrogen* factor, the *j*-th level of the irrigation factor, and the *k*-th level of the block factor; $\mu$ is the general mean of the response; $\tau_i$ is the effect of the *i*-th level of the nitrogen factor; $\gamma_j$ is the effect of the *j*-th level of the *irrigation* factor; $b_k$ is the *k*-th level of the random *Block* factor; $f_{ik}$

is the effect of *Block k* on level *i* of *nitrogen* (random effect); $c_{jk}$ is the effect of block *k* on level *j* of irrigation (random effect); $\lambda_{ij}$ is the interaction between the factors *nitrogen* and *irrigation*; and $e_{ijk}$ is the residual error. The usual assumption is that $b_k \sim N\left(0,\sigma_b^2\right)$, $f_{ki} \sim N\left(0,\sigma_f^2\right)$, $c_{kj} \sim N\left(0,\sigma_c^2\right)$, and $e_{kij} \sim N\left(0,\sigma_e^2\right)$, where all are independent.

A dot plot (Figure 108) is used to explore the observed means:

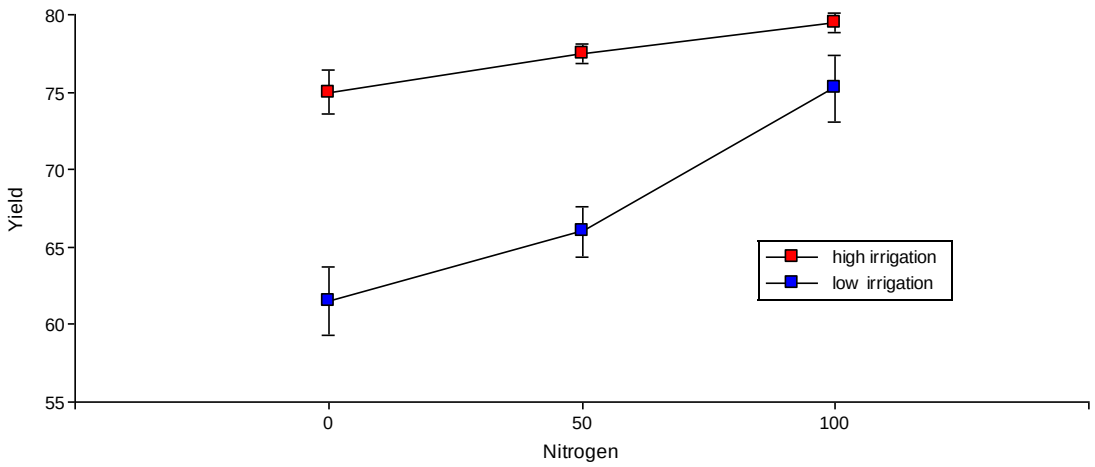



***Figure 108: Dot plot used to explore the means of Irrigation and Nitrogen (StripPlot.IDB2 file).***

This model can be fitted in InfoStat in the menu *Extended mixed and linear models*, selecting *Yield* as variable, and *Irrigation, Nitrogen*, and *Block* as class variables. Then in the Fixed effects tab the variables are specified as shown in Figure 109.

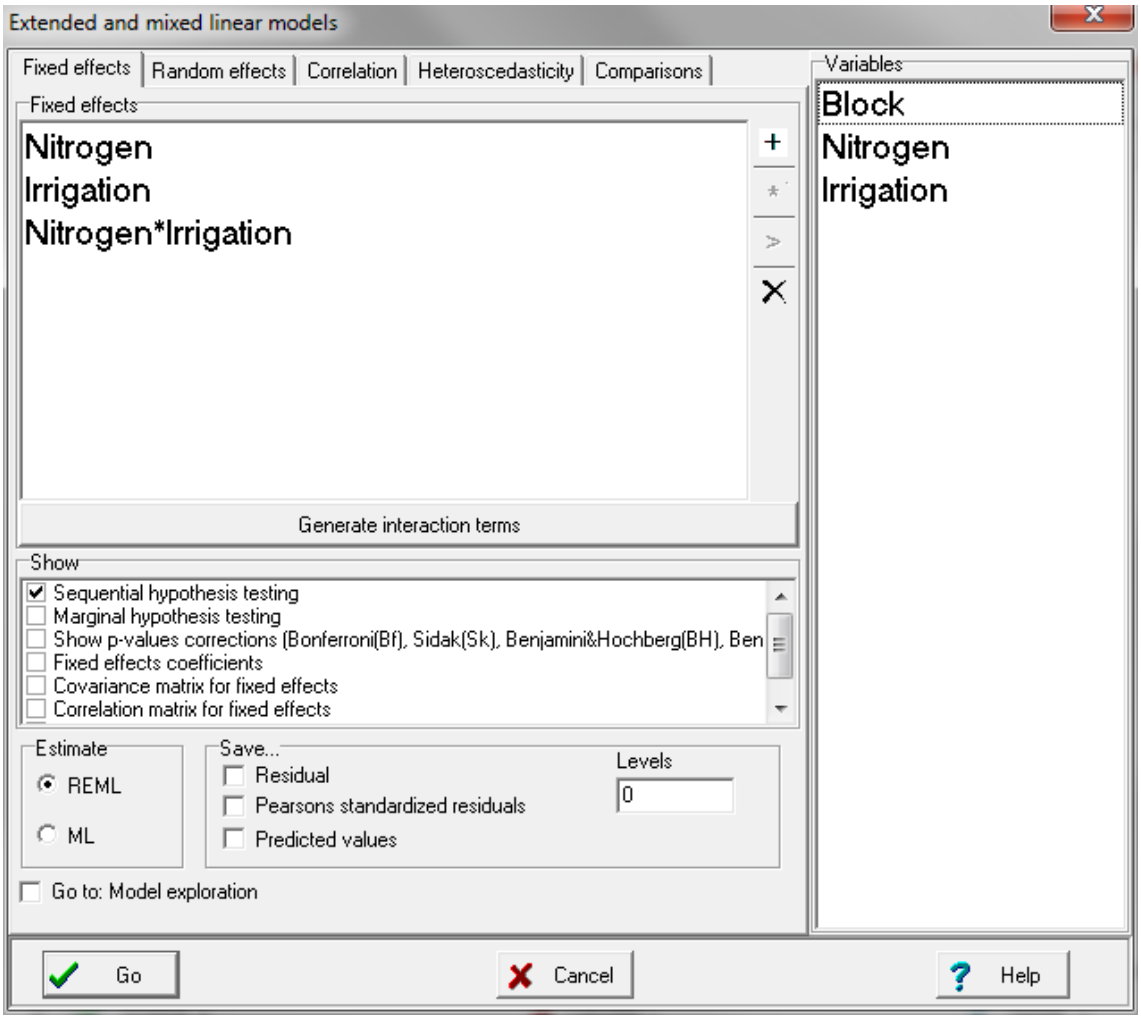



***Figure 109: Window displaying the Fixed effects tab to evaluate a mixed model (StripPlot.IDB2 file).***

In the *Random effects* tab, the *block* effect should be specified for the constant ($b_k$) as well as for the fixed factors *Nitrogen* and *Irrigation* ($f_{ik}$ y $c_{jk}$, respectively) (Figure 110).

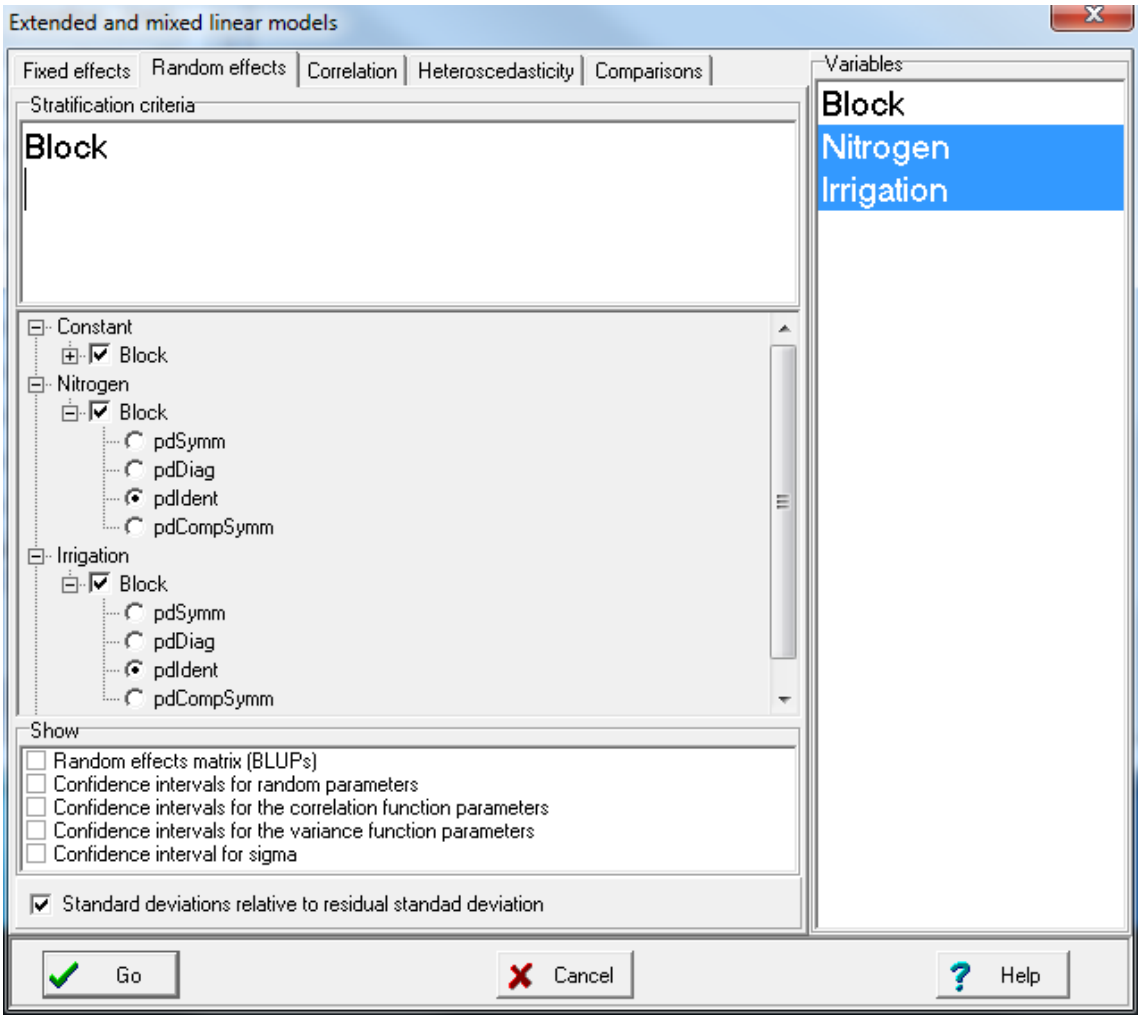



***Figure 110: Window displaying the Random effects tab for mixed model (StripPlot.IDB2 file).***

**Extended and mixed linear models**

**R specification of the model**

```
model000_Yield_REML<-
lme(Yield~1+Nitrogen+Irrigation+Nitrogen:Irrigation
,random=list(Block=pdIdent(~1)
,Block=pdIdent(~Nitrogen-1)
,Block=pdIdent(~Irrigation-1))
,method="REML"
,na.action=na.omit
,data=R.data00
,keep.data=FALSE)
```

**Results for model: model000_Yield_REML**

*Dependent variable:Yield*

**Fit measurements**

| N | AIC | BIC | logLik | Sigma | R2_0 | R2_1 | R2_2 | R2_3 |
|---|---|---|---|---|---|---|---|---|
| 24 | 106.09 | 115.00 | -43.05 | 1.20 | 0.85 | 0.94 | 0.95 | 0.99 |

*Smaller AIC and BIC is better*

**Sequential hypothesis testing**

| | numDF | denDF | F-value | p-value |
|---|---|---|---|---|
| (Intercept) | 1 | 15 | 3061.88 | <0.0001 |
| Nitrogen | 2 | 15 | 60.13 | <0.0001 |
| Irrigation | 1 | 15 | 52.18 | <0.0001 |
| Nitrogen:Irrigation | 2 | 15 | 33.12 | <0.0001 |

**Random effects parameters**

*Covariance model for random effects: pdIdent*
*Formula: ~1|Block*

*Standard deviations relative to residual standard deviation and correlation*

| | (const) |
|---|---|
| (const) | 1.83 |

*Covariance model for random effects: pdIdent*
*Formula: ~Nitrogen - 1|Block*

*Standard deviations relative to residual standard deviation and correlation*

| | 0 | 100 | 50 |
|---|---|---|---|
| 0 | 0.70 | 0.00 | 0.00 |
| 100 | 0.00 | 0.70 | 0.00 |
| 50 | 0.00 | 0.00 | 0.70 |

*Covariance model for random effects: pdIdent*
*Formula: ~Irrigation - 1|Block*

*Standard deviations relative to residual standard deviation and correlation*

| | high | low |
|---|---|---|
| high | 1.49 | 0.00 |
| low | 0.00 | 1.49 |

**Adjusted means and standard error for Nitrogen**
*LSD Fisher (alpha=0.05)*
*p-value correction procedure: No*

| Nitrogen | Means | S.E. | | | |
|---|---|---|---|---|---|
| 100 | 77.38 | 1.40 | A | | |
| 50 | 71.75 | 1.40 | | B | |
| 0 | 68.25 | 1.40 | | | C |

*Means with a common letter are not significantly different (p<= 0.05)*

**Adjusted means and standard error for Irrigation**
*LSD Fisher (alpha=0.05)*
*p-value correction procedure: No*

| Irrigation | Means | S.E. | | |
|---|---|---|---|---|
| high | 77.33 | 1.47 | A | |
| low | 67.58 | 1.47 | | B |

*Means with a common letter are not significantly different (p<= 0.05)*

**Adjusted means and standard error for Nitrogen*Irrigation**

*LSD Fisher (alpha=0.05)*

*p-value correction procedure: No*

| Nitrogen | Irrigation | Means | S.E. | | | | | |
|---|---|---|---|---|---|---|---|---|
| 100 | high | 79.50 | 1.59 | A | | | | |
| 50 | high | 77.50 | 1.59 | A | B | | | |
| 100 | low | 75.25 | 1.59 | | B | C | | |
| 0 | high | 75.00 | 1.59 | | | C | | |
| 50 | low | 66.00 | 1.59 | | | | D | |
| 0 | low | 61.50 | 1.59 | | | | | E |

*Means with a common letter are not significantly different (p<= 0.05)*

An alternative equivalent formulation of this model is presented in Figure 111.

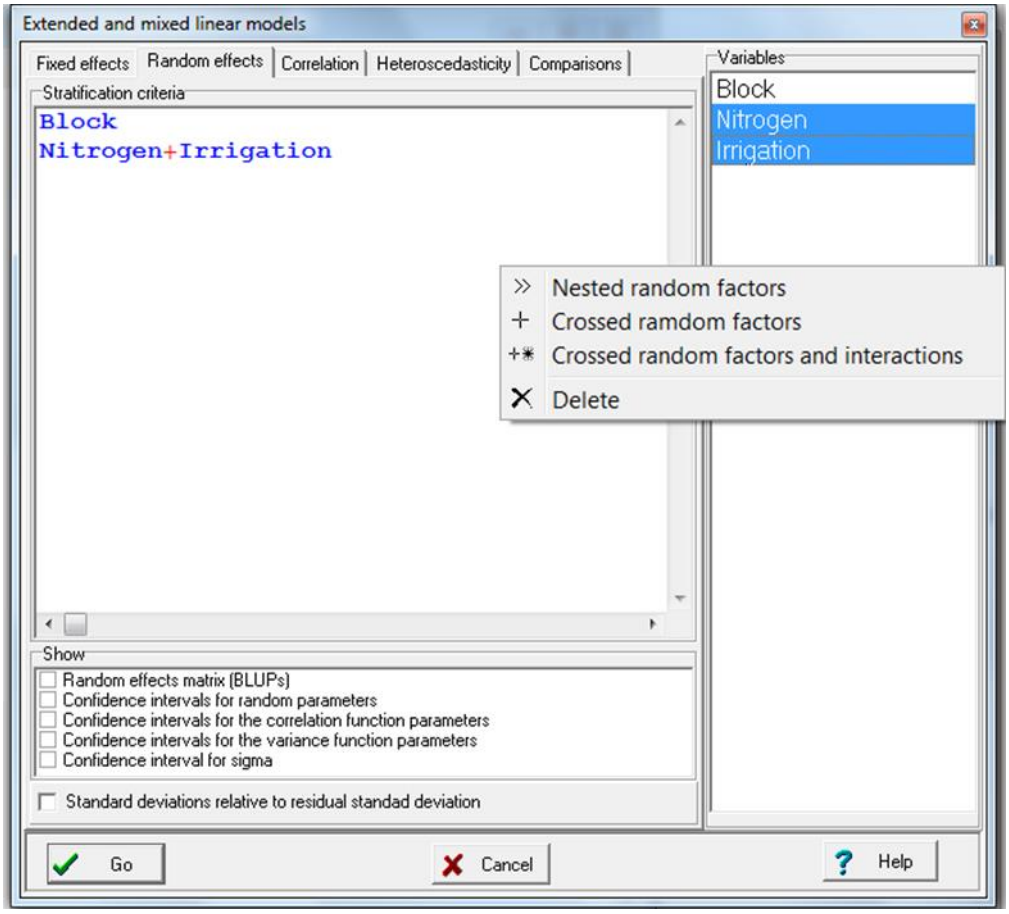



***Figure 111: Window displaying the Random effects tab to evaluate a mixed model with Nitrogen and Irrigation as cross factors (StripPlot.IDB2 file).***

**Extended and mixed linear models**

**R specification of the model**

```
model.019_Yield_REML<-
lme(Yield~1+Nitrogen+Irrigation+Nitrogen:Irrigation
,random=list(Block=pdIdent(~1)
,Block=pdBlocked(list(pdIdent(~1)
,pdIdent(~Nitrogen-1)
,pdIdent(~Irrigation-1))))
,method="REML"
,control=lmeControl(msMaxIter=200)
,na.action=na.omit
,data=R.data19
,keep.data=FALSE)
```

**Results for model: model.019_Yield_REML**

*Dependent variable: Yield*

**Fit measurements**

| N | AIC | BIC | logLik | Sigma | R2_0 | R2_1 | R2_2 |
|---|---|---|---|---|---|---|---|
| 24 | 108.09 | 117.89 | -43.05 | 1.20 | 0.85 | 0.91 | 0.99 |

*Smaller AIC and BIC is better*

**Marginal hypothesis testing (Type III SS)**

| | numDF | denDF | F-value | p-value |
|---|---|---|---|---|
| (Intercept) | 1 | 15 | 3061.88 | <0.0001 |
| Nitrogen | 2 | 15 | 60.13 | <0.0001 |
| Irrigation | 1 | 15 | 52.18 | <0.0001 |
| Nitrogen:Irrigation | 2 | 15 | 33.12 | <0.0001 |

**Sequential hypothesis testing**

| | numDF | denDF | F-value | p-value |
|---|---|---|---|---|
| (Intercept) | 1 | 15 | 3061.88 | <0.0001 |
| Nitrogen | 2 | 15 | 60.13 | <0.0001 |
| Irrigation | 1 | 15 | 52.18 | <0.0001 |
| Nitrogen:Irrigation | 2 | 15 | 33.12 | <0.0001 |

**Random effects parameters**

*Covariance model for random effects: pdIdent*
*Formula: ~1|Block*

*Standard deviations and correlations*

| | (const) |
|---|---|
| (const) | 1.67 |

*Covariance model for random effects: pdBlocked*
*Formula: ~Nitrogen + Irrigation|Block*

*Standard deviations and correlations*

| | S.D. |
|---|---|
| (const) | 1.42 |
| 0 | 0.83 |
| 100 | 0.83 |
| 50 | 0.83 |
| high | 1.78 |
| low | 1.78 |

*Experimental design with two factors and spatial dependence*

There are often situations in which levels of a factor of interest cannot be assigned randomly, due to their nature. This is the case when taking water samples in a river, when evaluating effects at different distances in a forest, or when taking soil samples at different depths.

The fact that the factor levels cannot be randomized creates a spatial dependence that must be taken into account. Here we present an example (*Earthworms.IDB2* file), where four types of shade in coffee crops are evaluated: control with sun (sun), legume 1 (ShadeL1), legume 2 (ShadeL2) and non legume (ShadeNL) at three depths (1=0-10 cm, 2=10-20 cm y 3=20-30 cm). In each of these experimental units (combination of treatments and repetitions), samples of 30×30 cm and 10 cm-deep were taken at each of the three depths. Worms were collected from each sample, and their live weight (biomass) was recorded. The experimental units were arranged in a completely randomized design with three repetitions. The variable *treatment_rep* identifies the experimental units in which the different depths are measured, and it was generated from the *Data* menu, from the *Cross categories to form a new variable* submenu (in the variables selection window, *Treatment* and *replication* were declared as variables).

To analyze the data from the *Earthworms.IDB2* file, the variables should be specified in the following way (Figure 112).

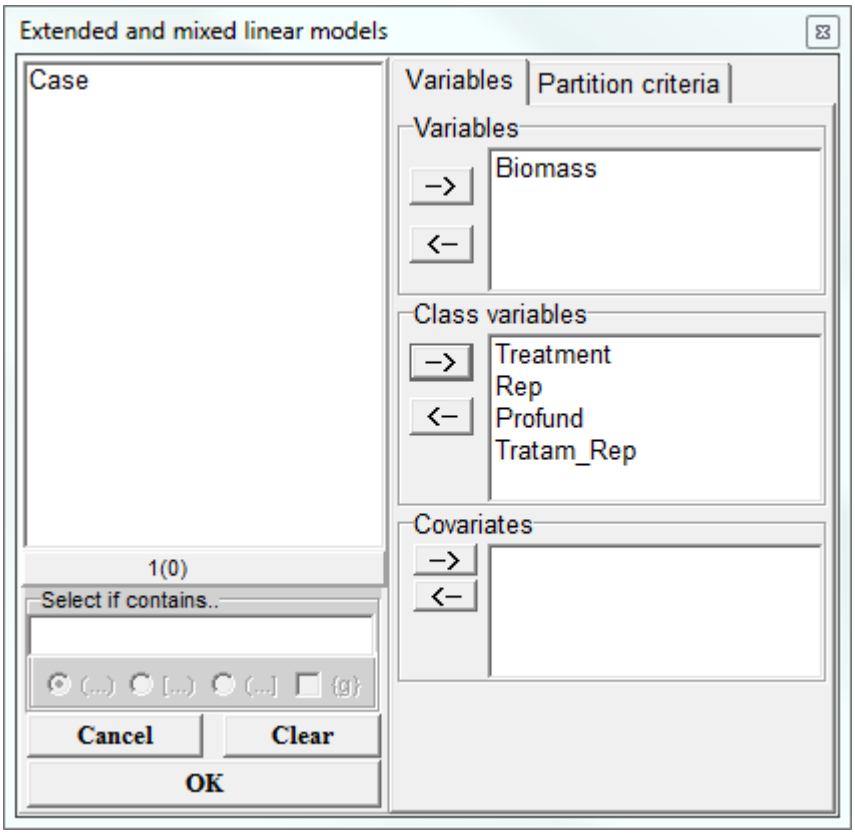



***Figure 112: Variables selector window for the Extended and mixed linear model (Earthworms.IDB2 file).***

Then, in the *Fixed effects* tab, the variables should be specified as shown in the following figure (Figure 113).

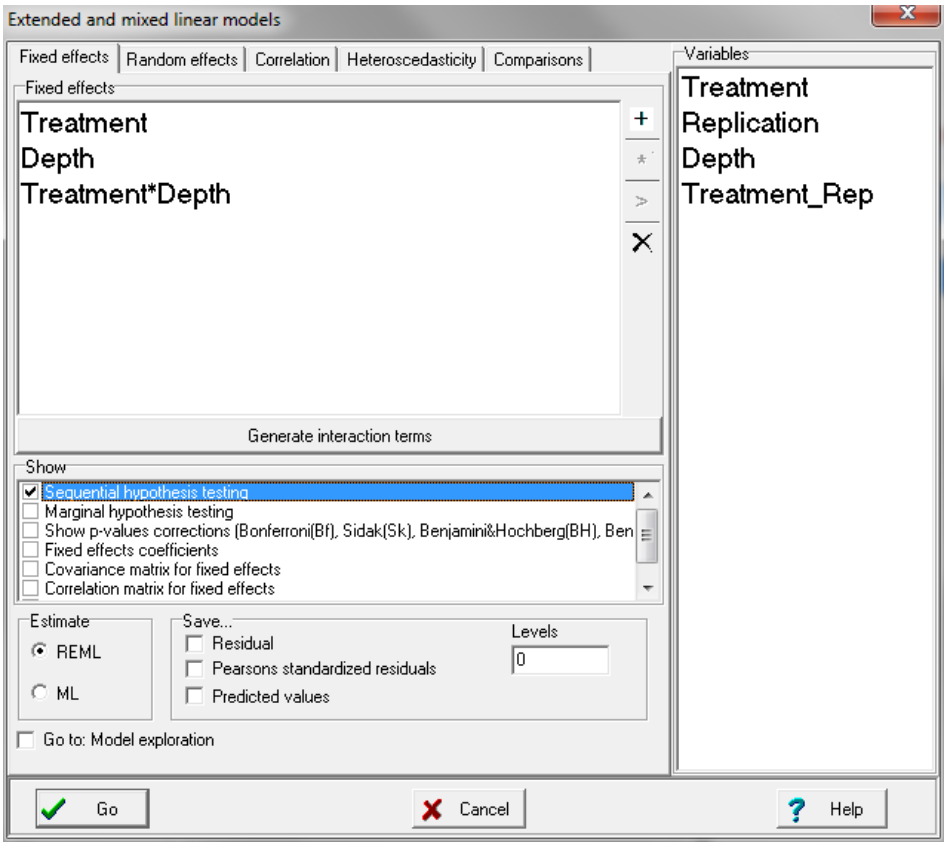



***Figure 113: Window displaying the Fixed effects tab for evaluating a mixed model (Earthworms.IDB2 file).***

Finally, the exponential spatial correlation model should be specified in the *Correlation* tab; *depth* should be identified as the X coordinate and *Treatment_rep* as the grouping variable (Figure 114).

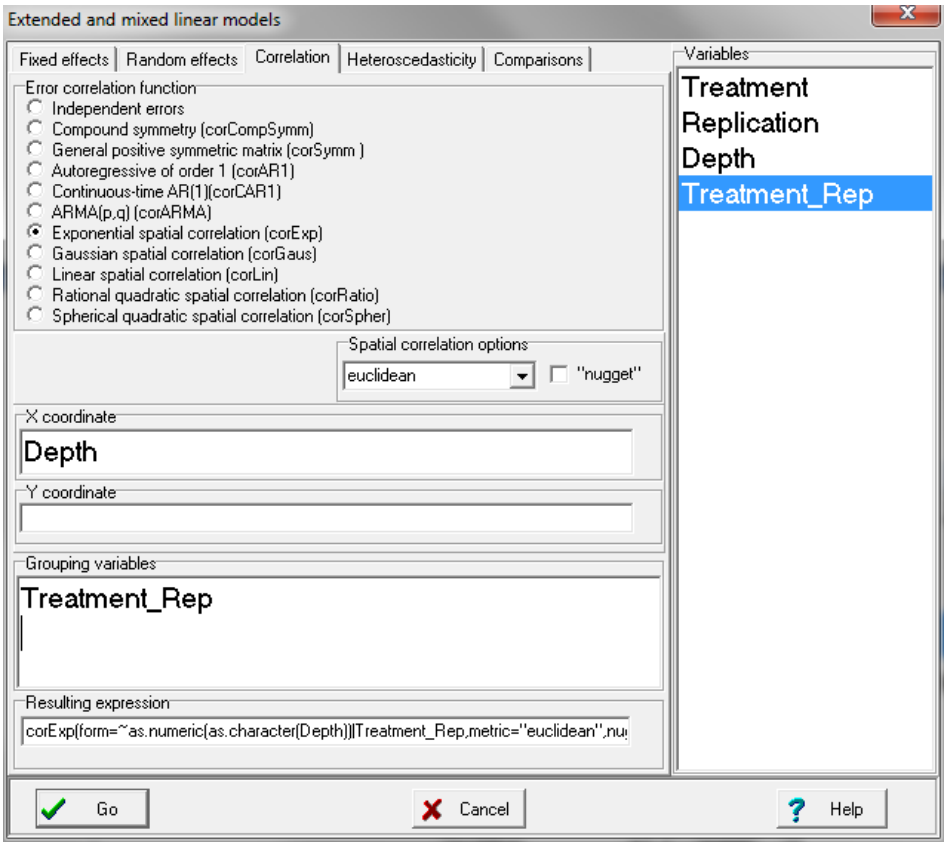



***Figure 114: Window displaying the Correlation tab for evaluating a mixed model with exponential spatial correlation (Earthworms.IDB2 file).***

The corresponding result is shown below.

**Extended and mixed linear models**

**R specification of the model**

```
model001_Biomass_REML<-gls(Biomass~1+Treatment+Depth+Treatment:Depth
,correlation=corExp(form=~as.numeric(as.character(Depth))|Treatment_Re
p
,metric="euclidean"
,nugget=FALSE)
,method="REML"
,na.action=na.omit
,data=R.data01)
```

**Results for model: model001_Biomass_REML**

*Dependent variable:Biomass*

**Fit measurements**

| N | AIC | BIC | logLik | Sigma | R2_0 |
|---|---|---|---|---|---|
| 36 | 161.03 | 177.52 | -66.52 | 3.46 | 0.97 |

*Smaller AIC and BIC is better*

**Sequential hypothesis testing**

| | numDF | F-value | p-value |
|---|---|---|---|
| (Intercept) | 1 | 3725.04 | <0.0001 |
| Treatment | 3 | 66.75 | <0.0001 |
| Depth | 2 | 303.14 | <0.0001 |
| Treatment:Depth | 6 | 4.86 | 0.0022 |

**Correlation structure**

*Correlation model: Exponential spatial correlation*
*Formula: ~ as.numeric(as.character(Depth)) | Treatment_Rep*
*Metric: euclidean*

*Model parameters*

| Parameter | Estim. |
|---|---|
| range | 2.12 |

All the resulting factors are significant, and there is an interaction between *Treatment* and *Depth* (p=0.0022). The range parameter has an estimated value of 2.12. This parameter should be interpreted with care, depending on the spatial correlation model that is used. In the geostatistical bibliography, *Range*, for second-order stationary spatial processes, is defined as the distance at which observations can be considered independent. The *Range* parameter shown in the results is related to this definition, but it is not the distance at which there is no more correlation (except for the spherical and linear models). In the spatial correlation models for which the covariance only asymptotically reaches zero (all except for spherical and linear), there is no distance at which spatial correlation equals zero; thus, the concept is of a *practical range* (distance at which covariance is reduced to 5%, or, equivalently, the distance at which the semivariogram reaches 95% of its maximum). This distance depends on the model used: for exponential spatial correlation it is equal to 3 times the estimated range; for the Gaussian spatial correlation it equals √3 times the estimated range (Littel et al. 2006).

In this example, an exponential spatial correlation model is used. *Depth 1* is between 0 and 10 cm, *Depth 2* is between 10 and 20 cm, and *Depth 3* is between 20 and 30 cm; in other words, according to the way in which they were declared, the difference between depths 1 and 2 is 1, however in the original scale, this difference equals 10. Thus, the *practical range* of the original scale equals 3×21.2 cm=63.6 cm. This implies that the worm biomass observations will never be independent (for the observations to be considered practically independent they should be more than 63.6 cm apart, which is impossible for these data).

The exponential isotropic spatial correlation model shown here is equivalent to a first-order autoregressive model (Casanoves et al. 2005). If we apply a first-order autoregressive model (Figure 115) to this same dataset, the following results are obtained:

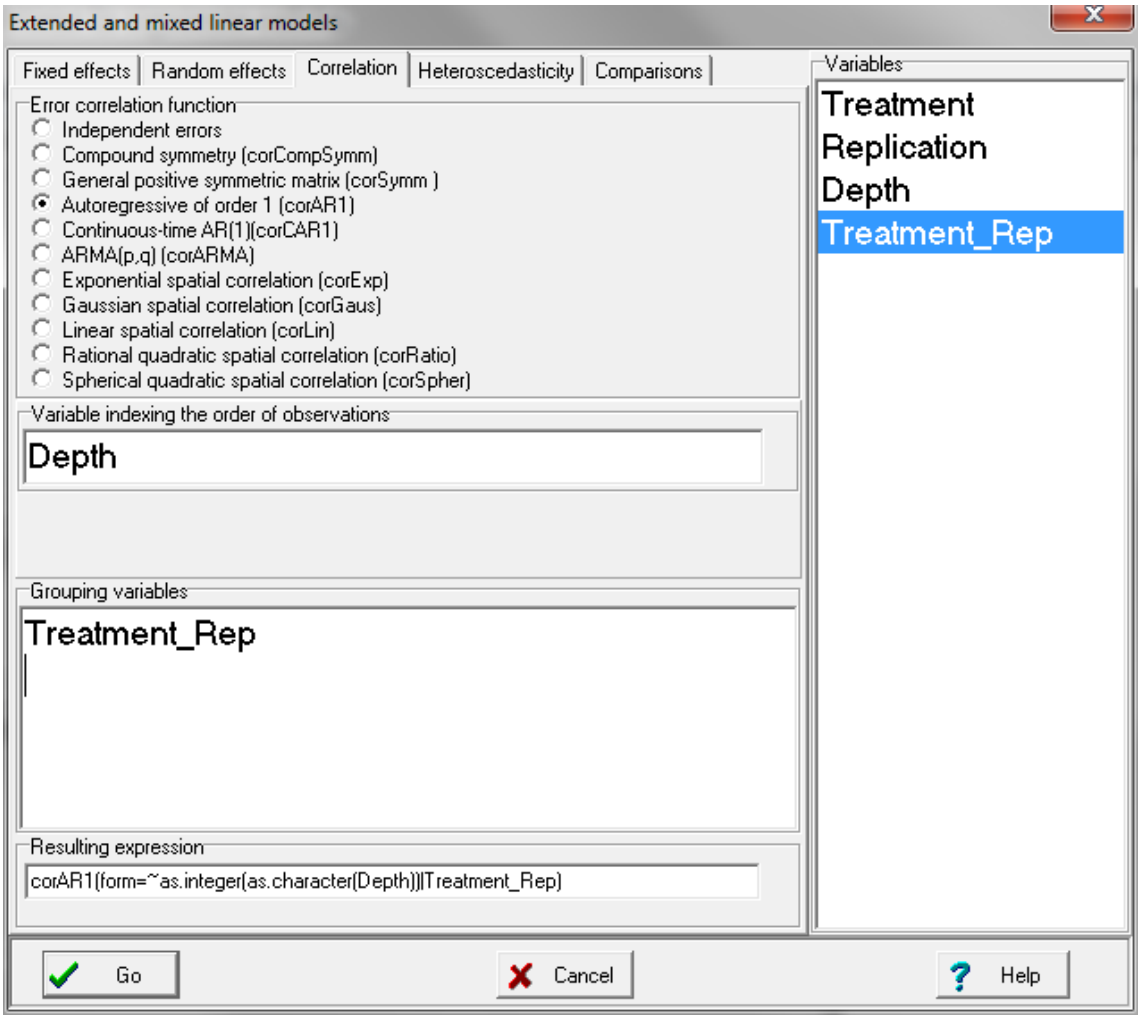



***Figure 115: Window displaying the Correlation tab for evaluating a mixed model with first-order autoregressive correlation (Earthworms.IDB2 file).***

```
Extended and mixed linear models

R specification of the model

model002_Biomass_REML<-gls(Biomass~1+Treatment+Depth+Treatment:Depth
,correlation=corAR1(form=~as.integer(as.character(Depth))|Treatment_Re
p)
,method="REML"
,na.action=na.omit
,data=R.data01)

Results for model: model002_Biomass_REML

Dependent variable:Biomass
```

**Fit measurements**

| N | AIC | BIC | logLik | Sigma | R2_0 |
|---|---|---|---|---|---|
| 36 | 161.03 | 177.52 | -66.52 | 3.46 | 0.97 |

*Smaller AIC and BIC is better*

**Sequential hypothesis testing**

| | numDF | F-value | p-value |
|---|---|---|---|
| (Intercept) | 1 | 3725.05 | <0.0001 |
| Treatment | 3 | 66.75 | <0.0001 |
| Depth | 2 | 303.14 | <0.0001 |
| Treatment:Depth | 6 | 4.86 | 0.0022 |

**Correlation structure**

*Correlation model: AR(1)*
*Formula: ~ as.integer(as.character(Depth)) | Treatment_Rep*

*Model parameters*

| Parameter | Estim. |
|---|---|
| Phi | 0.62 |

The only difference between these results and the previous ones is that these show the Phi correlation parameter (0.62) instead of the range parameter.

We will now study the validity of the assumptions of this model. For this, we requested the diagnostic graphs from the *Analysis-exploration of estimated models* submenu, shown below (Figure 116).

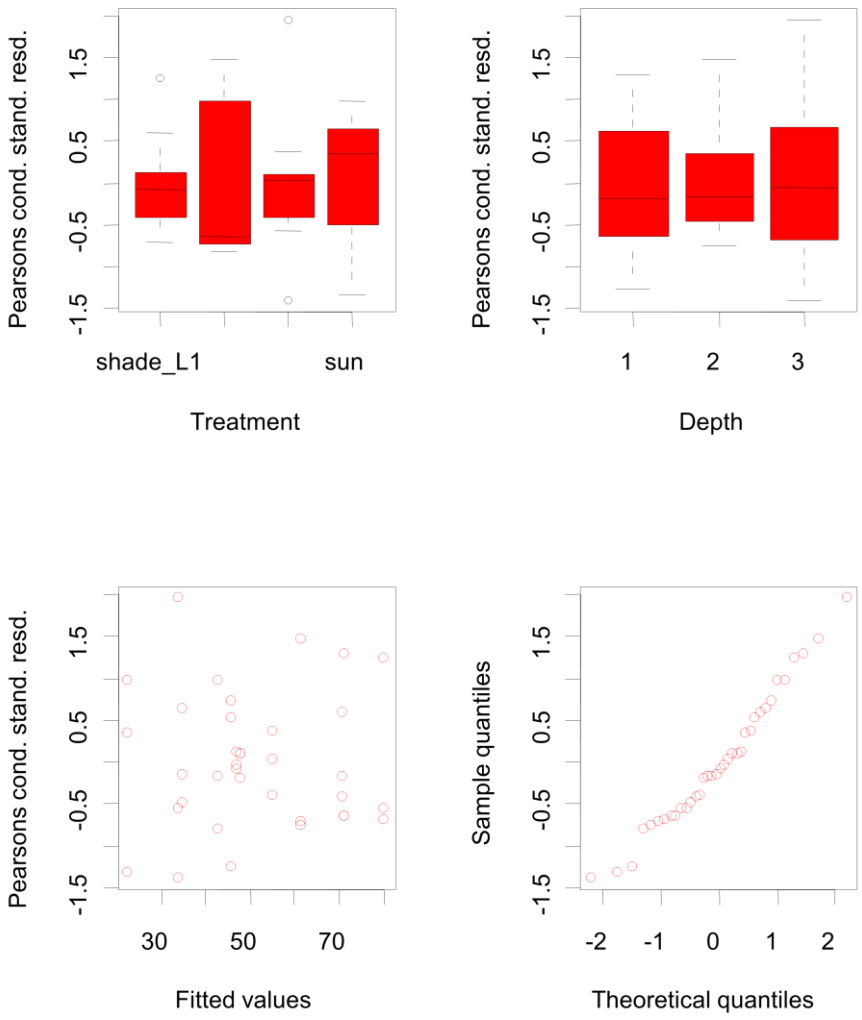



***Figure 116: Graphic diagnostic tools (Earthworms.IDB2 file).***

As shown in these graphs, the variability of the residuals apparently differs for the different treatments. In order to evaluate a heteroscedastic model by treatment, variables were specified in the *Heteroscedasticity* tab, as shown in Figure 117, and the following results were obtained.

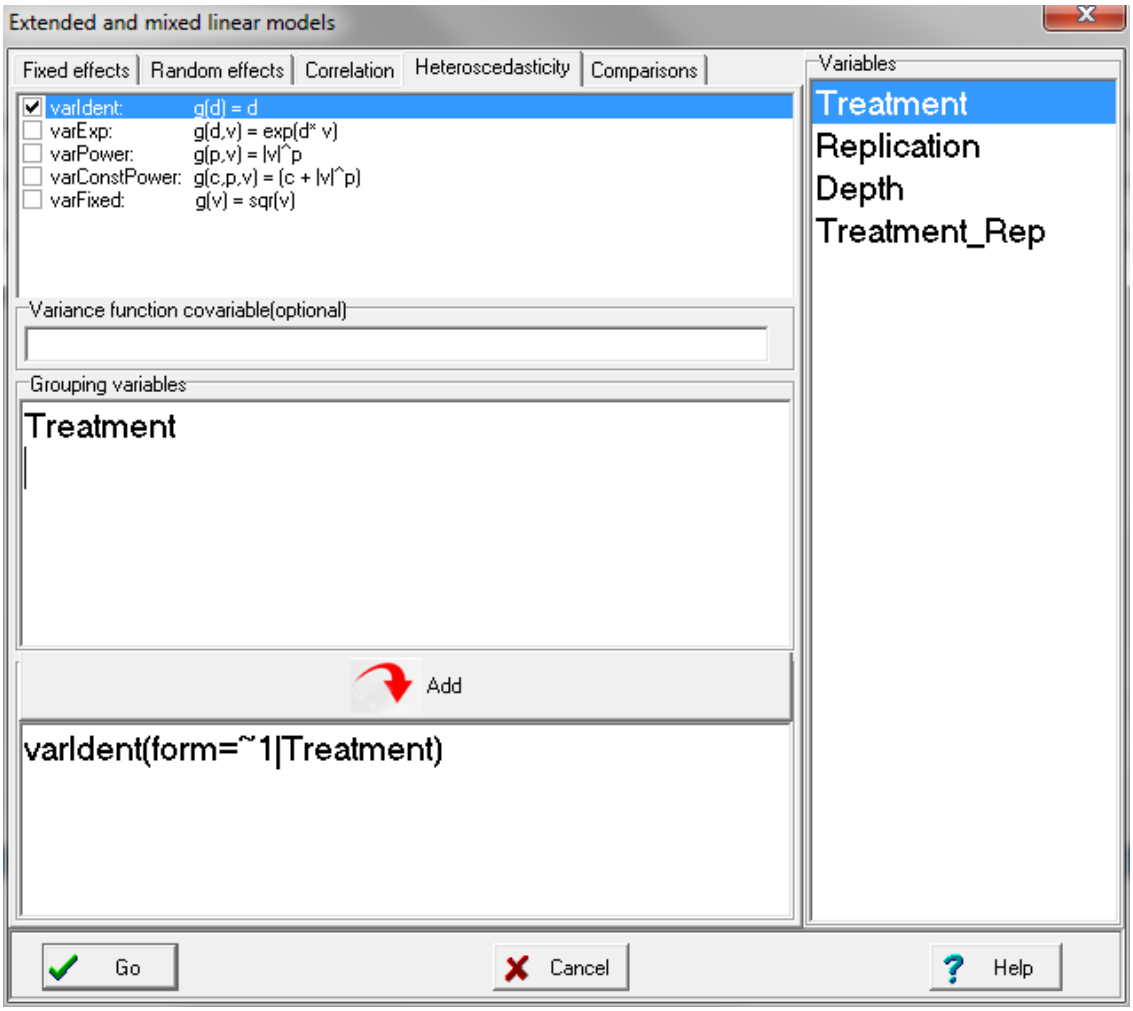



***Figure 117: Window displaying the Heteroscedasticity tab for evaluating a mixed model (Earthworms.IDB2 file).***

```
Extended and mixed linear models


R specification of the model


model005_Biomass_REML<-gls(Biomass~1+Treatment+Depth+Treatment:Depth
,weight=varComb(varIdent(form=~1|Treatment))
,correlation=corAR1(form=~as.integer(as.character(Depth))|Treatment_Re
p)
,method="REML"
,na.action=na.omit
,data=R.data01)

Results for model: model005_Biomass_REML

Dependent variable:Biomass
```

**Fit measurements**

| N | AIC | BIC | logLik | Sigma | R2_0 |
|---|---|---|---|---|---|
| 36 | 164.03 | 184.06 | -65.02 | 4.20 | 0.97 |

*Smaller AIC and BIC is better*

**Sequential hypothesis testing**

| | numDF | F-value | p-value |
|---|---|---|---|
| (Intercept) | 1 | 4300.37 | <0.0001 |
| Treatment | 3 | 54.19 | <0.0001 |
| Depth | 2 | 511.72 | <0.0001 |
| Treatment:Depth | 6 | 6.32 | 0.0004 |

**Correlation structure**

*Correlation model: AR(1)*
*Formula: ~ as.integer(as.character(Depth)) | Treatment_Rep*

*Model parameters*

| Parameter | Estim. |
|---|---|
| Phi | 0.73 |

**Variance structure**

*Variance model: varIdent*
*Formula: ~ 1 | Treatment*

*Variance-function parameters*

| Parameter | Estim |
|---|---|
| sun | 1.00 |
| shadeL1 | 0.65 |
| shadeL2 | 0.66 |
| shadeNL | 1.22 |

The AIC and BIC criteria are larger for the heteroscedastic model than for the homoscedastic model, indicating that the latter is better. A similar conclusion is obtained by using the likelihood ratio test (p=0.3916) when comparing the models as shown in the section *Analysis of fitted models.*

**Comparison of models**

| | df | AIC | BIC | logLik | Test | L.Ratio | p-value |
|---|---|---|---|---|---|---|---|
| model001_Biomass_REML | 14 | 161.03 | 177.52 | -66.52 | | | |
| model002_Biomass_REML | 17 | 164.03 | 184.06 | -65.02 | 1 vs 2 | 3.00 | 0.3916 |

For this reason, we selected the homoscedastic model, and because there is interaction between the two factors, we generate a scatter plot to visualize the behavior of the worm biomass means (Figure 118).

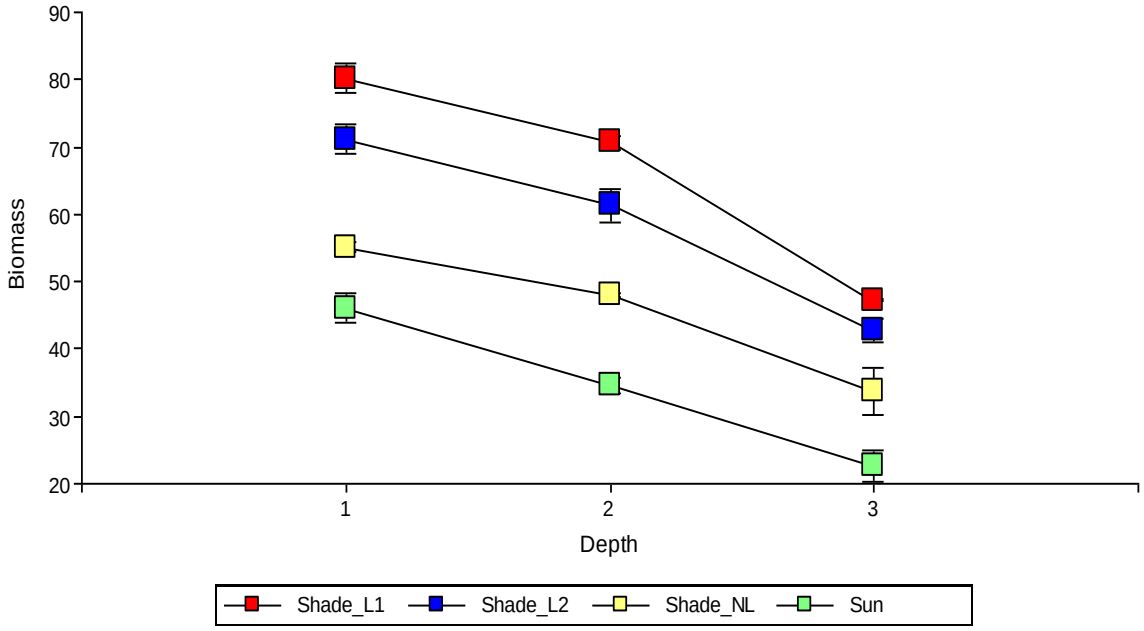



***Figure 118: Dot plot used to study the interaction between Treatments and Depth and its effect on biomass (Earthworms.IDB2 file).***

As shown, this graph suggests the presence of linear behavior for *sun* and quadratic behavior for the other treatments. To test these hypotheses, orthogonal and polynomial contrasts are conducted, from the *Comparison* tab, *Contrasts* subtab (Figure 119).

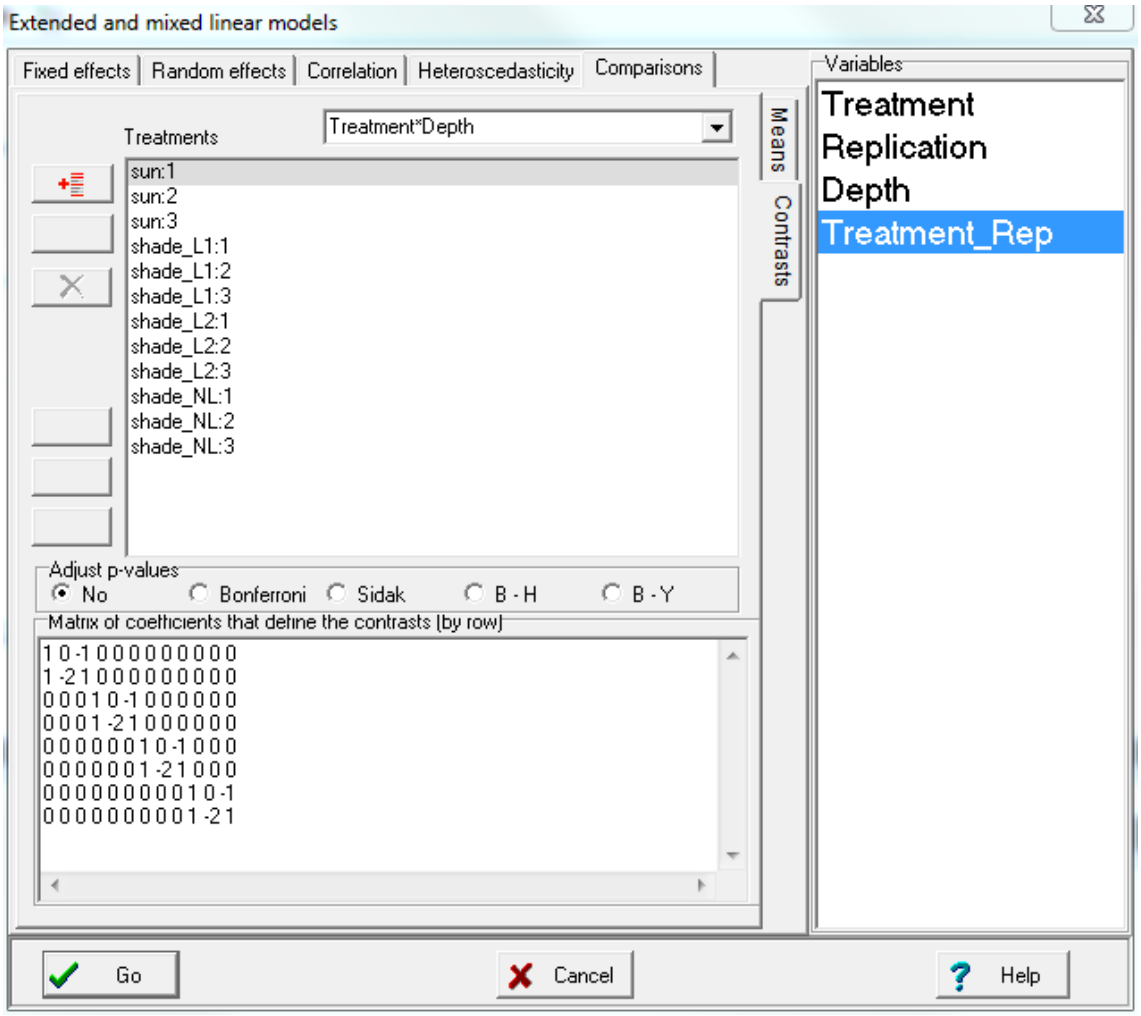



***Figure 119: Window displaying the Comparisons tab and Contrasts subtab for evaluating a mixed model (Earthworms.IDB2 file).***

The results of the contrasts are shown below. The only treatment that shows only a linear trend, and no quadratic trend, is the sun treatment ($p<0.0001$ and $p=0.8147$ respectively). The rest of the treatments show a quadratic trend in addition to a linear trend.

**hypothesis testing for contrasts**

| Treatment*Depth | F | df(num) | df(den) | p-value |
|---|---|---|---|---|
| Ct.1 | 111.81 | 1 | 24 | <0.0001 |
| Ct.2 | 0.06 | 1 | 24 | 0.8147 |
| Ct.3 | 222.11 | 1 | 24 | <0.0001 |
| Ct.4 | 26.66 | 1 | 24 | <0.0001 |
| Ct.5 | 164.40 | 1 | 24 | <0.0001 |
| Ct.6 | 10.52 | 1 | 24 | 0.0035 |
| Ct.7 | 92.62 | 1 | 24 | <0.0001 |
| Ct.8 | 7.26 | 1 | 24 | 0.0127 |
| Total | 79.43 | 8 | 24 | <0.0001 |

**Contrasts coefficients**

| Treatment | Depth | Ct.1 | Ct.2 | Ct.3 | Ct.4 | Ct.5 | Ct.6 | Ct.7 | Ct.8 |
|---|---|---|---|---|---|---|---|---|---|
| sun | 1 | -1.00 | 1.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| sun | 2 | 0.00 | -2.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| sun | 3 | 1.00 | 1.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| shadeL1 | 1 | 0.00 | 0.00 | -1.00 | 1.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| shadeL1 | 2 | 0.00 | 0.00 | 0.00 | -2.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| shadeL1 | 3 | 0.00 | 0.00 | 1.00 | 1.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| shadeL2 | 1 | 0.00 | 0.00 | 0.00 | 0.00 | -1.00 | 1.00 | 0.00 | 0.00 |
| shadeL2 | 2 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | -2.00 | 0.00 | 0.00 |
| shadeL2 | 3 | 0.00 | 0.00 | 0.00 | 0.00 | 1.00 | 1.00 | 0.00 | 0.00 |
| shadeNL | 1 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | -1.00 | 1.00 |
| shadeNL | 2 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | -2.00 |
| shadeNL | 3 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 1.00 | 1.00 |

### *Augmented design with replicated checks*

This type of treatment arrangement is common in the evaluation of new cultivars (varieties, hybrids, etc.) in genetic plant breeding. Essentially, it consists of randomly locating the group of cultivars to be evaluated and always inserting a common control between the groups. The presence of this control allows us to model the systematic effects of soil quality where the experimental plots are located. An example with 16 hybrids (H1, ..., H16) and one control, for a total of 32 experimental units, is presented. The data are in the file *Matched checks.IDB2*.

A basic alternative for analyzing these data (that is not very efficient) is to conduct a one-way ANOVA in order to compare treatments using an estimation of the error term based on the variance between the controls (the only levels of the treatment factor that are repeated). This model is not able to take into account the bias produced by the systematic difference between experimental units. In order to fit this model, specify *Yield* as the dependent variable in the variables selector and *Hybrid* as the classification variable.

In the *Fixed effects* tab, *Hybrid* was specified as shown in Figure 120. Then, in the *Comparisons* tab, Fisher's LSD test for *Hybrid* was requested.

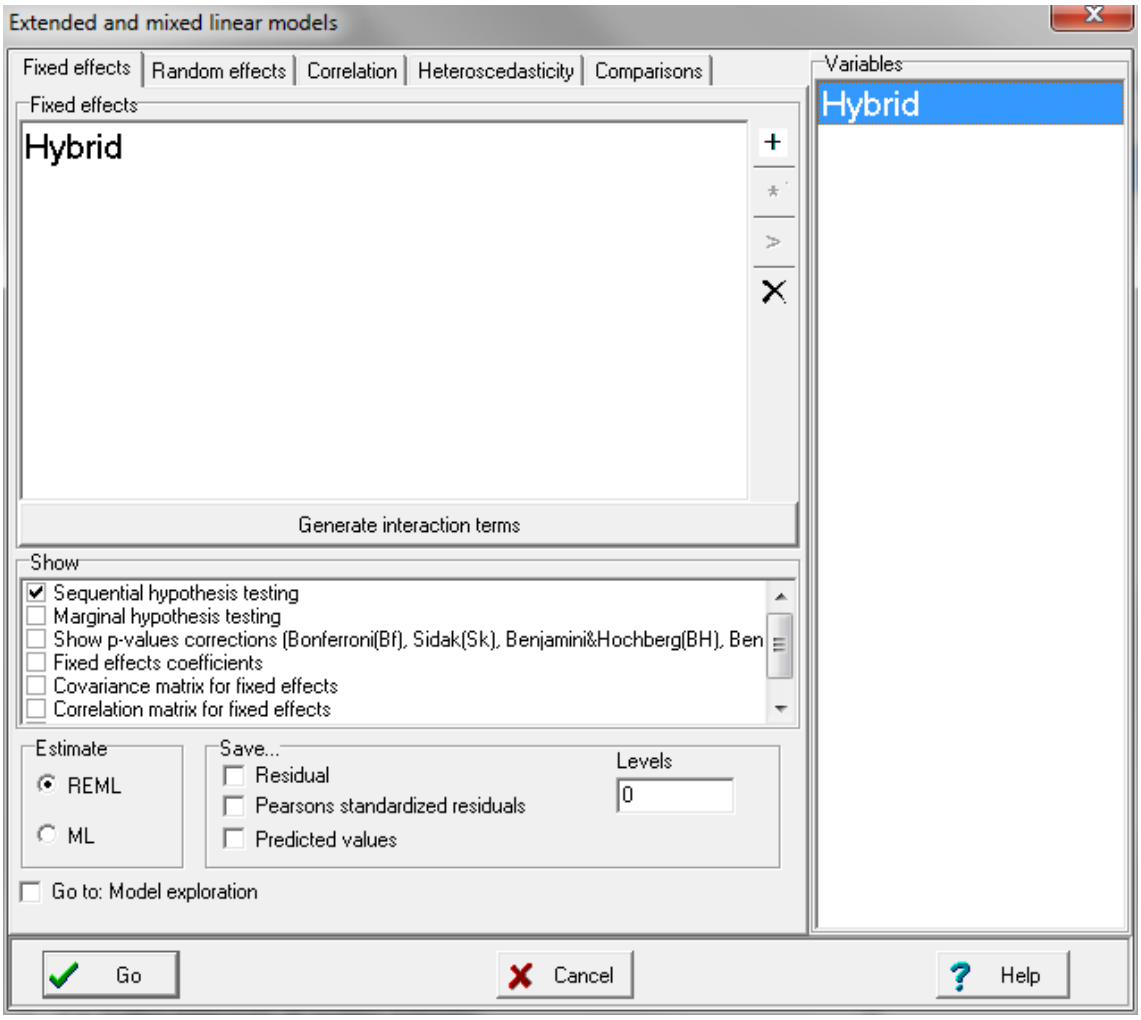



***Figure 120: Window displaying the Fixed effects tab (Matched checks.IDB2 file).***

The corresponding results are shown below.

**Extended and mixed linear models**

**R specification of the model**

```
model001_Yield_REML<-gls(Yield~1+Hybrid
,method="REML"
,na.action=na.omit
,data=R.data01)
```

**Results for model: model001_Yield_REML**

*Dependent variable:Yield*

**Fit measurements**

| N | AIC | BIC | logLik | Sigma | R2_0 |
|---|---|---|---|---|---|
| 32 | 219.90 | 232.64 | -91.95 | 101.35 | 0.69 |

*Smaller AIC and BIC is better*

**Sequential hypothesis testing**

| | numDF | F-value | p-value |
|---|---|---|---|
| (Intercept) | 1 | 3580.56 | <0.0001 |
| Hybrid | 16 | 2.12 | 0.0763 |

**Adjusted means and standard error for Hybrid**

*LSD Fisher (alpha=0.05)*
*p-value correction procedure: No*

| Hybrid | Means | S.E. | | | | |
|---|---|---|---|---|---|---|
| H4 | 1230.00 | 101.35 | A | | | |
| H3 | 1222.00 | 101.35 | A | | | |
| H14 | 1193.00 | 101.35 | A | B | | |
| H10 | 1168.00 | 101.35 | A | B | C | |
| H11 | 1116.00 | 101.35 | A | B | C | |
| Witness | 1115.81 | 25.34 | A | B | C | |
| H5 | 1099.00 | 101.35 | A | B | C | |
| H9 | 1063.00 | 101.35 | A | B | C | |
| H2 | 1037.00 | 101.35 | A | B | C | D |
| H12 | 1033.00 | 101.35 | A | B | C | D |
| H8 | 975.00 | 101.35 | A | B | C | D |
| H7 | 966.00 | 101.35 | A | B | C | D |
| H16 | 928.00 | 101.35 | A | B | C | D |
| H6 | 907.00 | 101.35 | | B | C | D |
| H1 | 886.00 | 101.35 | | | C | D |
| H13 | 876.00 | 101.35 | | | C | D |
| H15 | 756.00 | 101.35 | | | | D |

*Means with a common letter are not significantly different (p<= 0.05)*

The F trial for *Hybrid* was not significant (p = 0.0763), thus the differences between means presented by Fisher's LSD test should not be interpreted.

Alternatively, it is possible to use spatial correlations to correct the means of each hybrid for the "site effect" of the location to which they were randomly assigned. To do this, we proceed to specify plot *Position* as a covariate.

The *Fixed effects* tab should look as in Figure 120. In the *Correlation* tab, the following different models should be specified:

Model 1: *Exponential spatial correlation* (Figure 121).

Model 2: *Gaussian spatial correlation* (Figure 122).

Model 3: *Linear spatial correlation* (Figure 123).

Model 4: "*Rational quadratic" spatial correlation* (Figure 124).

Model 5: *Spherical spatial correlation* (Figure 125).

The spatial correlation selection windows and the fit measures for each of the estimated models are shown below.

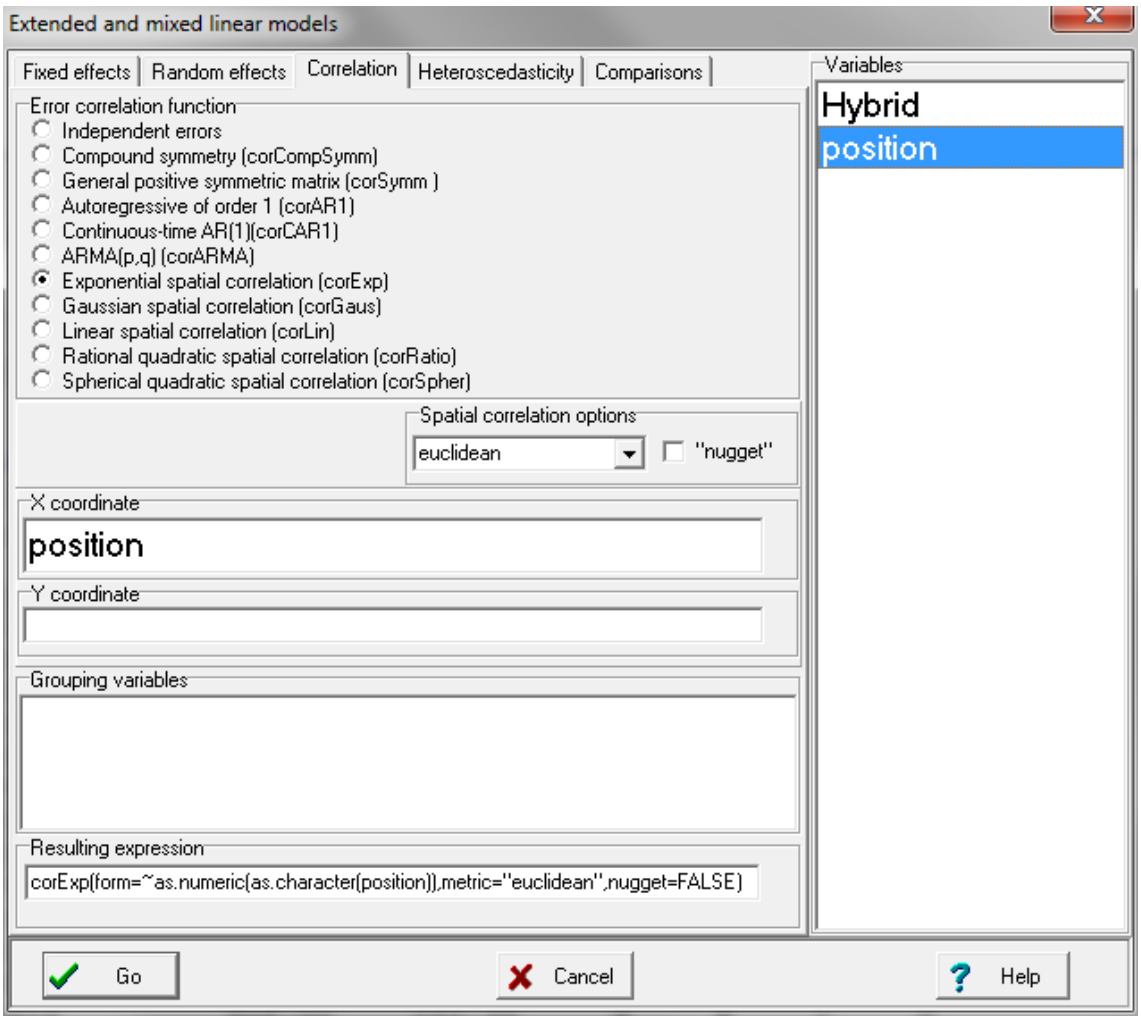



***Figure 121: Window displaying the Correlation tab, and selection of Exponential spatial correlation (Matched checks.IDB2 file).***

**Fit measurements**

| N | AIC | BIC | logLik | Sigma | R2_0 |
|---|---|---|---|---|---|
| 32 | 218.62 | 232.08 | -90.31 | 112.79 | 0.58 |

*Smaller AIC and BIC is better*

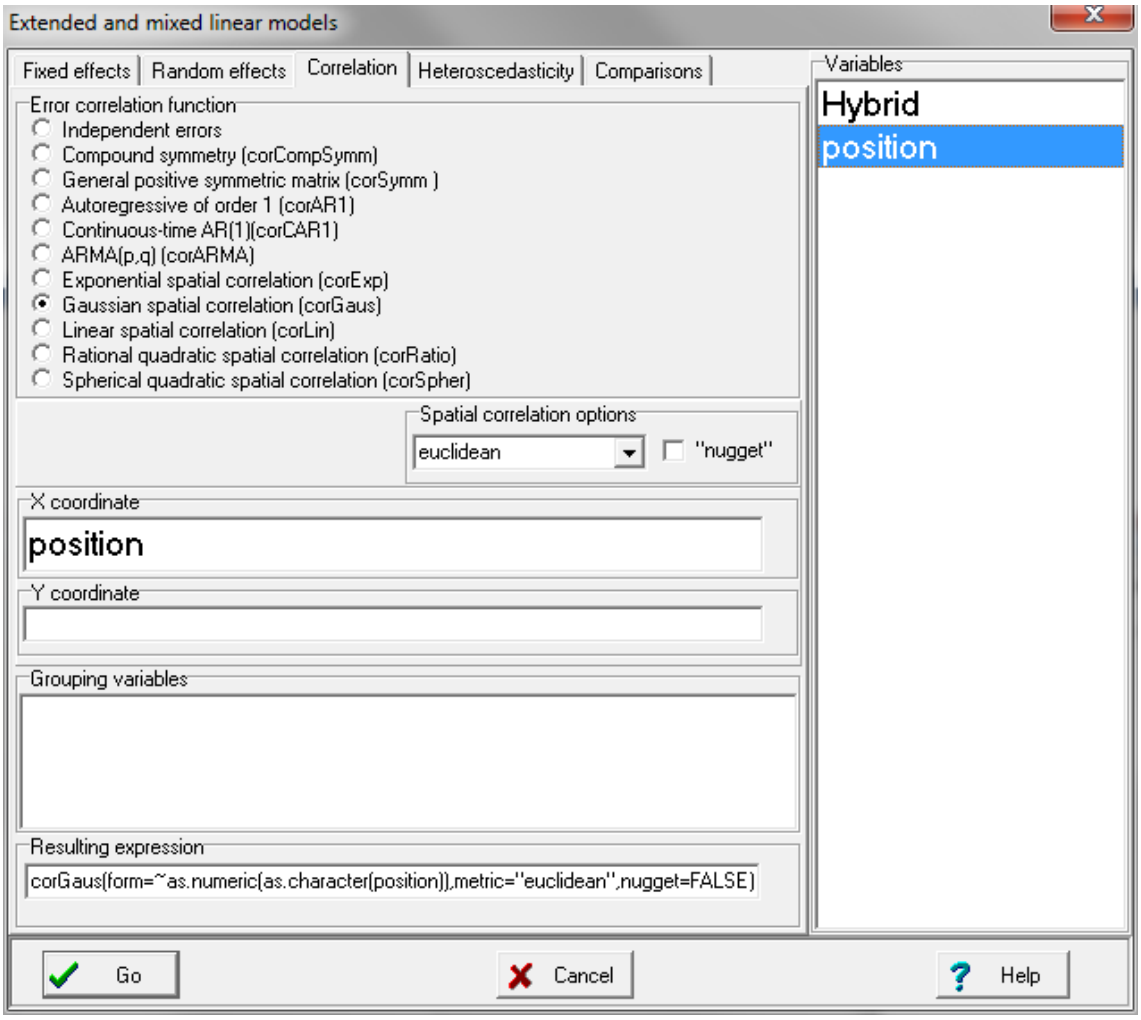



***Figure 122: Window displaying the Correlation tab, and selection of Guassian spatial correlation (Matched checks.IDB2 file).***

**Fit measurements**

| N | AIC | BIC | logLik | Sigma | R2_0 |
|---|---|---|---|---|---|
| 32 | 219.17 | 232.62 | -90.58 | 106.78 | 0.58 |

*Smaller AIC and BIC is better*

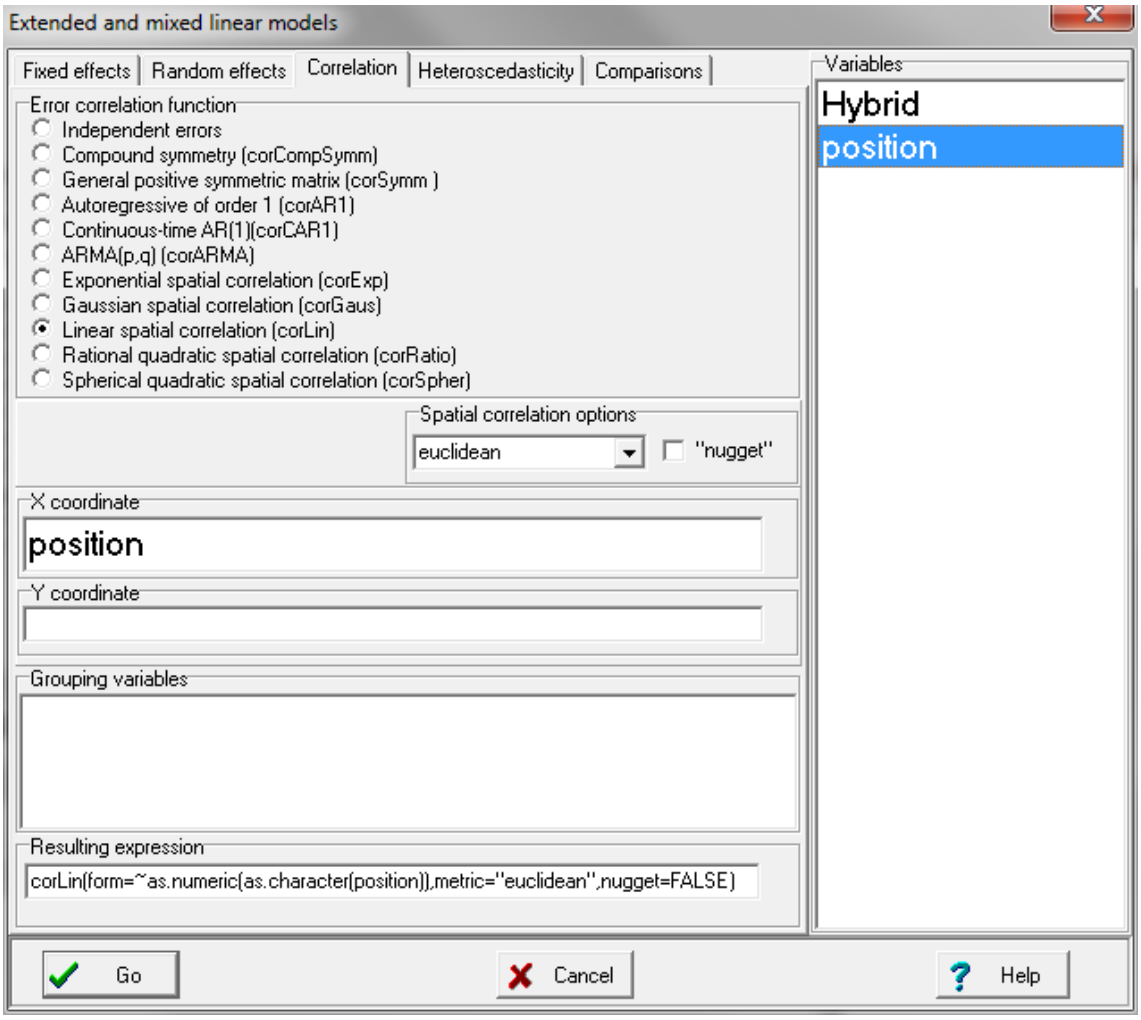



***Figure 123: Window displaying the Correlation tab, and selection of Linear spatial correlation (Matched checks.IDB2 file).***

**Fit measurements**

| N | AIC | BIC | logLik | Sigma | R2_0 |
|---|---|---|---|---|---|
| 32 | 219.13 | 232.58 | -90.56 | 107.52 | 0.56 |

*Smaller AIC and BIC is better*

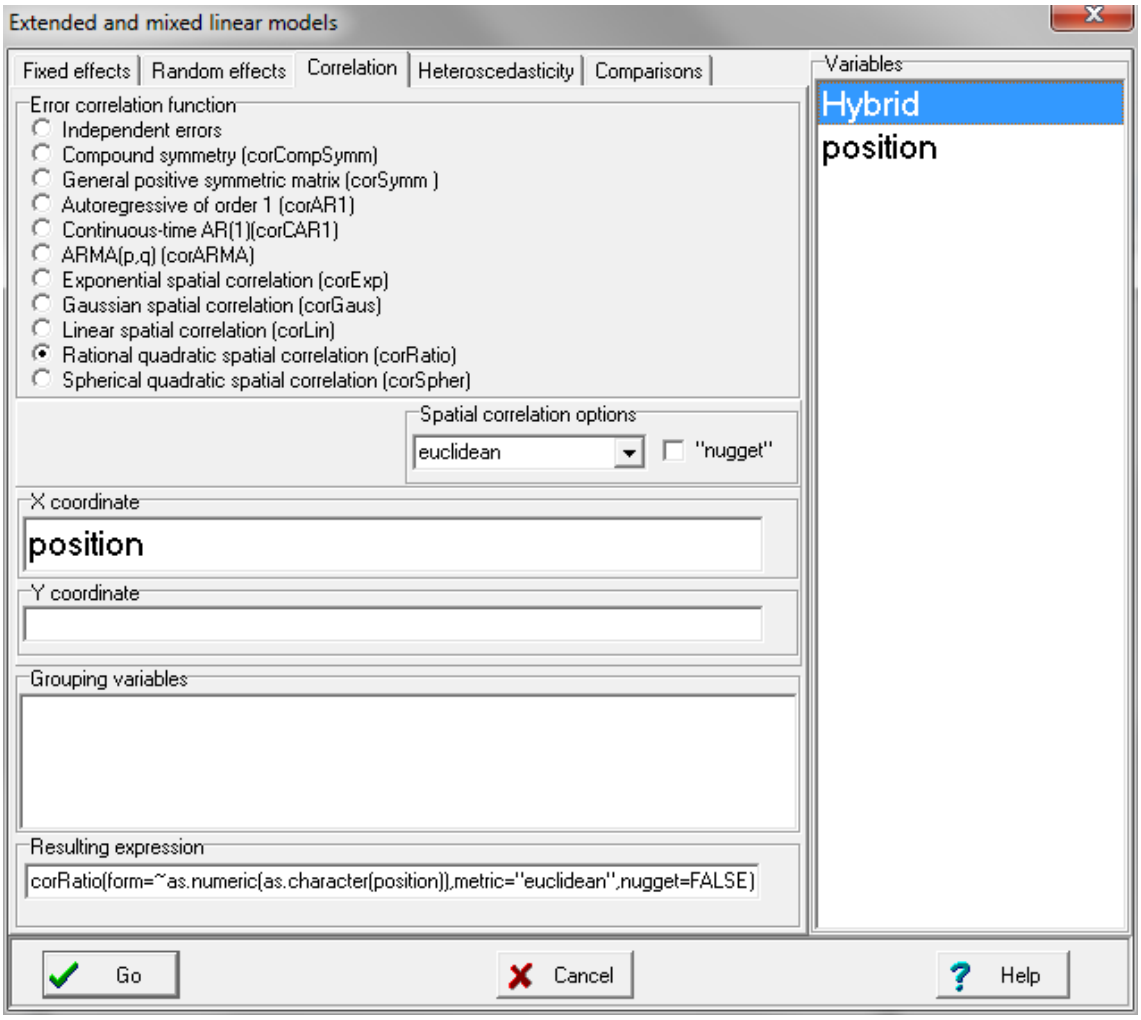



***Figure 124: Window displaying the Correlation tab, and selection of "Rational quadratic" spatial correlation (Matched checks.IDB2 file).***

**Fit measurements**

| N | AIC | BIC | logLik | Sigma | R2_0 |
|---|---|---|---|---|---|
| 32 | 218.81 | 232.26 | -90.40 | 106.92 | 0.59 |

*Smaller AIC and BIC is better*

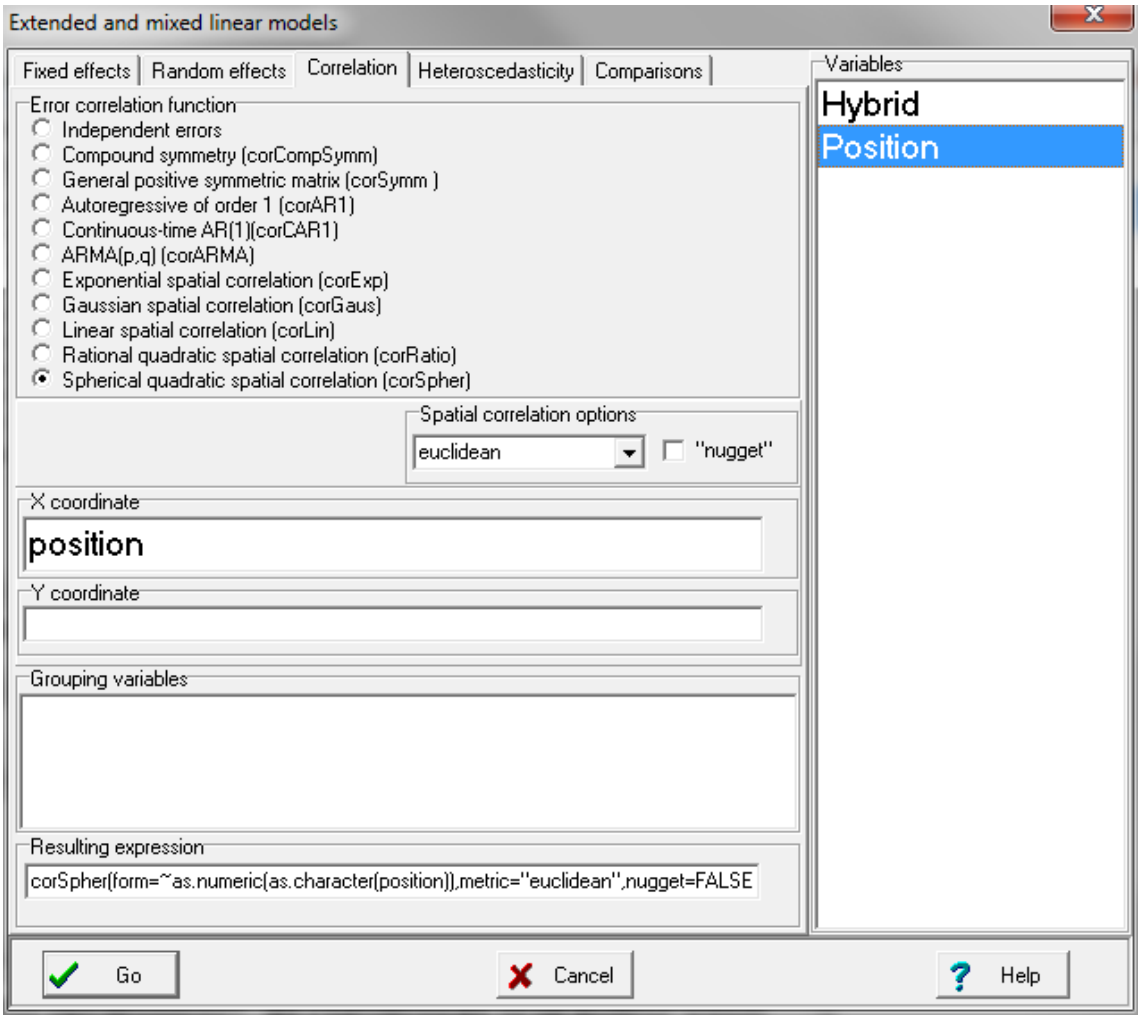



***Figure 125: Window displaying the Correlation tab, and selection of Spherical spatial correlation (Matched checks.IDB2 file).***

**Fit measurements**

| N | AIC | BIC | logLik | Sigma | R2_0 |
|---|---|---|---|---|---|
| 32 | 219.21 | 232.66 | -90.60 | 137.39 | 0.56 |

*Smaller AIC and BIC is better*

All the models have a good fit, as shown by the similarity between their AIC and BIC values. The model with the lowest values is the exponential spatial correlation model (AIC=218.62, BIC=232.08). The results for that model are shown below.

**Extended and mixed linear models**

**R specification of the model**

```
model003_Yield_REML<-gls(Yield~1+Hybrid
,correlation=corExp(form=~as.numeric(as.character(position))
,metric="euclidean"
,nugget=FALSE)
,method="REML"
,na.action=na.omit
,data=R.data03)
```

**Results for model: model003_Yield_REML**

*Dependent variable:Yield*

**Fit measurements**

| N | AIC | BIC | logLik | Sigma | R2_0 |
|---|---|---|---|---|---|
| 32 | 218.62 | 232.08 | -90.31 | 112.79 | 0.58 |

*Smaller AIC and BIC is better*

**Sequential hypothesis testing**

| | numDF | F-value | p-value |
|---|---|---|---|
| (Intercept) | 1 | 582.79 | <0.0001 |
| Hybrid | 16 | 5.27 | 0.0012 |

**Correlation structure**

*Correlation model: Exponential spatial correlation*
*Formula: ~ as.numeric(as.character(position))*
*Metric: euclidean*

*Model parameters*

| Parameter | Estim. |
|---|---|
| range | 2.74 |

**Adjusted means and standard error for Hybrid**
*LSD Fisher (alpha=0.05)*
*p-value correction procedure: No*

| Hybrid | Means | S.E. | | | | | |
|---|---|---|---|---|---|---|---|
| H3 | 1248.31 | 85.33 | A | | | | |
| H4 | 1244.19 | 85.33 | A | | | | |
| H10 | 1145.64 | 85.33 | A | B | | | |
| H5 | 1128.65 | 85.33 | A | B | C | | |
| Witness | 1096.98 | 45.09 | A | B | C | | |
| H2 | 1091.07 | 85.33 | A | B | C | | |
| H11 | 1078.43 | 85.33 | A | B | C | | |
| H9 | 1078.28 | 85.33 | A | B | C | | |
| H14 | 1070.07 | 85.33 | A | B | C | | |
| H1 | 1005.46 | 85.33 | | B | C | | |
| H12 | 979.80 | 85.33 | | B | C | | |
| H6 | 966.31 | 85.33 | | B | C | | |
| H7 | 936.21 | 85.33 | | B | C | D | |
| H8 | 933.40 | 85.33 | | B | C | D | |
| H16 | 902.87 | 85.33 | | | C | D | |
| H13 | 727.55 | 85.33 | | | | D | E |
| H15 | 653.36 | 85.33 | | | | | E |

*Means with a common letter are not significantly different (p<= 0.05)*

Differences between hybrids were found (p = 0.0012). Fisher's LSD means comparison shows that the hybrids with the greatest yield are H2, H3, H4, H5, H9, H10, H11, H14, and that these do not differ from the control.

Alternatively, the problem can be thought of as it might have been during the early days of spatial correlation modeling (Papadakis 1937), and one might use covariance analysis to adjust the means of the hybrids in the different positions. In order to approximate this type of analysis, we created a new variable called *Tes*, which contains the yields corresponding to the hybrid closest to each control. Then, the difference between the control yield and the hybrid yield (*Dif*) was calculated.

We then conducted a linear regression analysis, with *Dif* as the dependent variable and *position* as the regressor. The predicted values of this model were saved with the intention of using them as covariates in the analysis of the hybrid means. Then, in the *Extended and mixed linear models* variables selector window, variables are specified as shown in Figure 126.

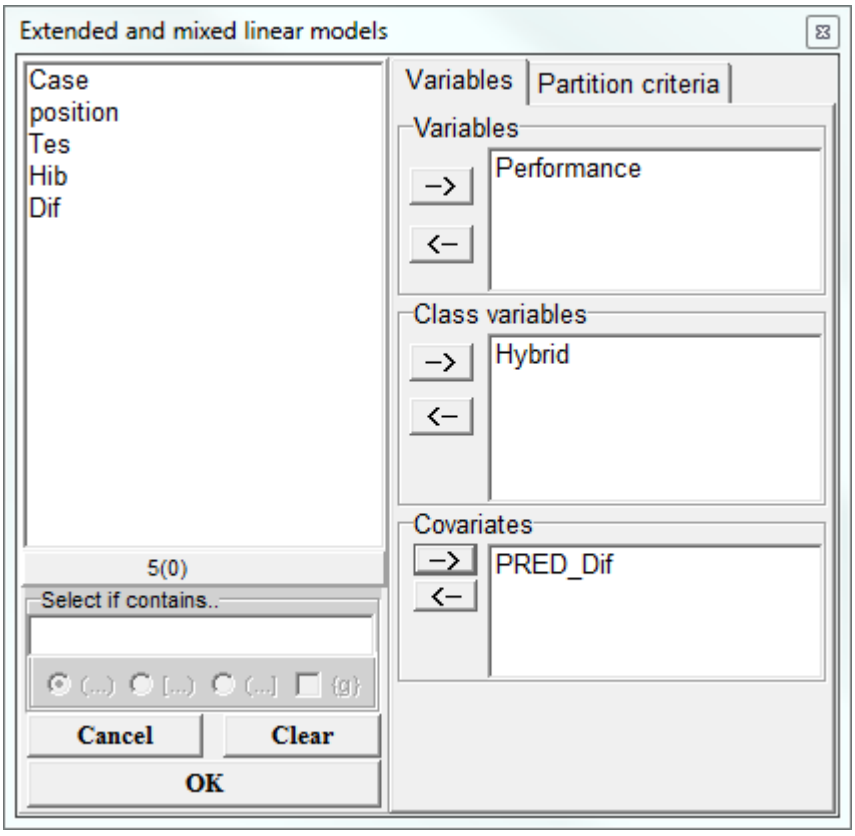



***Figure 126: Variables selector window for the Extended and mixed linear models (Matched checks.IDB2 file).***

In the fixed effects window, declare *PRED_Dif* and *Hybrid*. The corresponding output is shown below.

**Extended and mixed linear models**

**R specification of the model**

```
model004_Yield_REML<-gls(Yield~1+Hybrid+PRED_Dif
.method="REML"
.na.action=na.omit
.data=R.data04)
```

**Results for model: model004_Yield_REML**

*Dependent variable:Yield*

**Fit measurements**

| N | AIC | BIC | logLik | Sigma | R2_0 |
|---|---|---|---|---|---|
| 32 | 215.09 | 227.23 | -88.54 | 79.89 | 0.82 |

*Smaller AIC and BIC is better*

**Sequential hypothesis testing**

| | numDF | F-value | p-value |
|---|---|---|---|
| (Intercept) | 1 | 5763.58 | <0.0001 |
| Hybrid | 16 | 3.42 | 0.0129 |
| PRED_Dif | 1 | 10.15 | 0.0066 |

**Adjusted means and standard error for Hybrid**

*LSD Fisher (alpha=0.05)*

*p-value correction procedure: No*

| Hybrid | Means | S.E. | | | | |
|---|---|---|---|---|---|---|
| H4 | 1295.07 | 82.46 | A | | | |
| H3 | 1293.92 | 83.02 | A | | | |
| H10 | 1150.88 | 80.07 | A | B | | |
| H5 | 1143.52 | 81.10 | A | B | | |
| H2 | 1129.47 | 85.00 | A | B | | |
| H14 | 1121.08 | 83.02 | A | B | | |
| Witness | 1115.81 | 19.97 | A | B | | |
| H11 | 1078.33 | 80.76 | A | B | | |
| H9 | 1052.73 | 79.95 | A | B | C | |
| H12 | 988.48 | 81.10 | | B | C | |
| H1 | 985.32 | 85.76 | | B | C | |
| H8 | 985.27 | 79.95 | | B | C | |
| H7 | 983.12 | 80.07 | | B | C | |
| H6 | 944.67 | 80.76 | | B | C | |
| H16 | 828.68 | 85.76 | | | C | D |
| H13 | 810.93 | 82.46 | | | C | D |
| H15 | 663.53 | 85.00 | | | | D |

*Means with a common letter are not significantly different (p<= 0.05)*

Although the conclusion regarding the cultivars is the same as that of the exponential spatial correlation analysis, we can observe that the adjusted means and the standard errors are different. The order, or ranking, among the hybrids that generated the greatest yields is also different. Furthermore, studying spatial correlation is a much simpler alternative to conduct this type of analysis.

## *Applications in linear regression*

### *Random coefficient regression*

In this example, we are evaluating the academic performance of sixth grade Mathematics students. Eight teachers were randomly selected to participate in the study. At the beginning of the academic year, the students from the participant teachers were administered a diagnostic test (pre-test) with sixth grade mathematical contents. At the end of the year, the same students were administered a post-test assessing the same content (Cáceres et al., 2011).

Each teacher had between 10 and 30 students, and some students completed the pre-test but not the post-test. We wish to assess whether there is a relationship between learning gain (difference between pre- and post-test score) and the pre-test score. If we plot this relationship using the data in the file Learning.IDB2, we observe a negative relationship between gain and pre-test score. Furthermore, adding a smooth line to each teacher's data we can notice that the trends are approximately linear, and that the parameters of these lines vary from teacher to teacher (Figure 127):

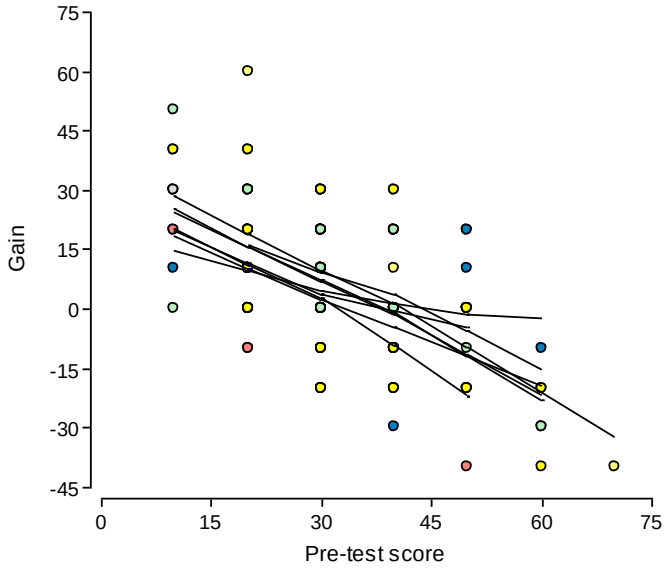



***Figure 127: Relationship between Learning gain and Pre-test score, smoothed separately for each teacher. File Learning.idb2.***

In order to fit a model in InfoStat to describe these data one must consider that the teachers are randomly selected, and therefore the variability between lines is random. An appropriate model is a simple linear regression with random intercept and slope.

These random effects must clearly be correlated (in general, if the slope increases the intercept should decrease for the data to stay in the same observed area). Hence, the model must be specified in InfoStat in such a way that correlated intercept and slope random effects can be incorporated. To do this, in the *Variables* window we need to select *Gain* as variable, *Teacher* as class variable, and *Pre-test score* as covariate. Then, in the *Fixed effect* tab, *Pre-test* score is selected, and we need to add the explicit specification of the intercept (to be able to declare both as random effects later). This is done adding 1 in the *Fixed effects* tab (Figure 128). We also need to check the option *Fixed effects coefficients* to obtain the equation of the average line.

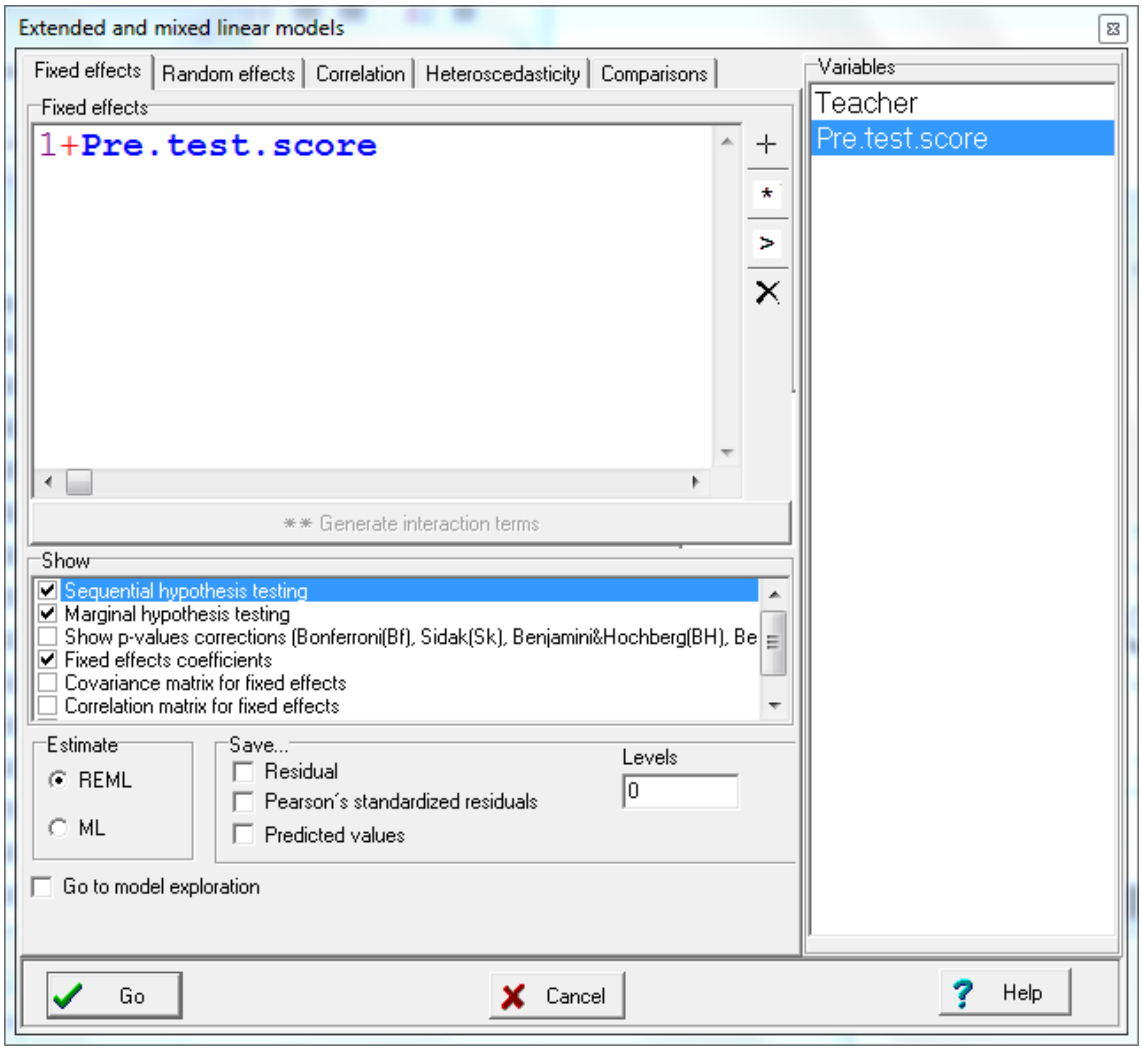



***Figure 128: Window to select fixed effects in the Extended and mixed models for the data in the file LearningIDB2.***

The specification of the random effects is done by adding *Teacher* as stratification criterion, and indicating that this effects acts on *1+Pre.test* (i.e., there are random teacher effects on the intercept and on the slope) (Figure 129). Furthermore, it is necessary to indicate *pdSymm* as the covariance structure of these random effects in order to have different variances for intercept and slope (obvious given the different nature of both parameters), and correlation between both random effects

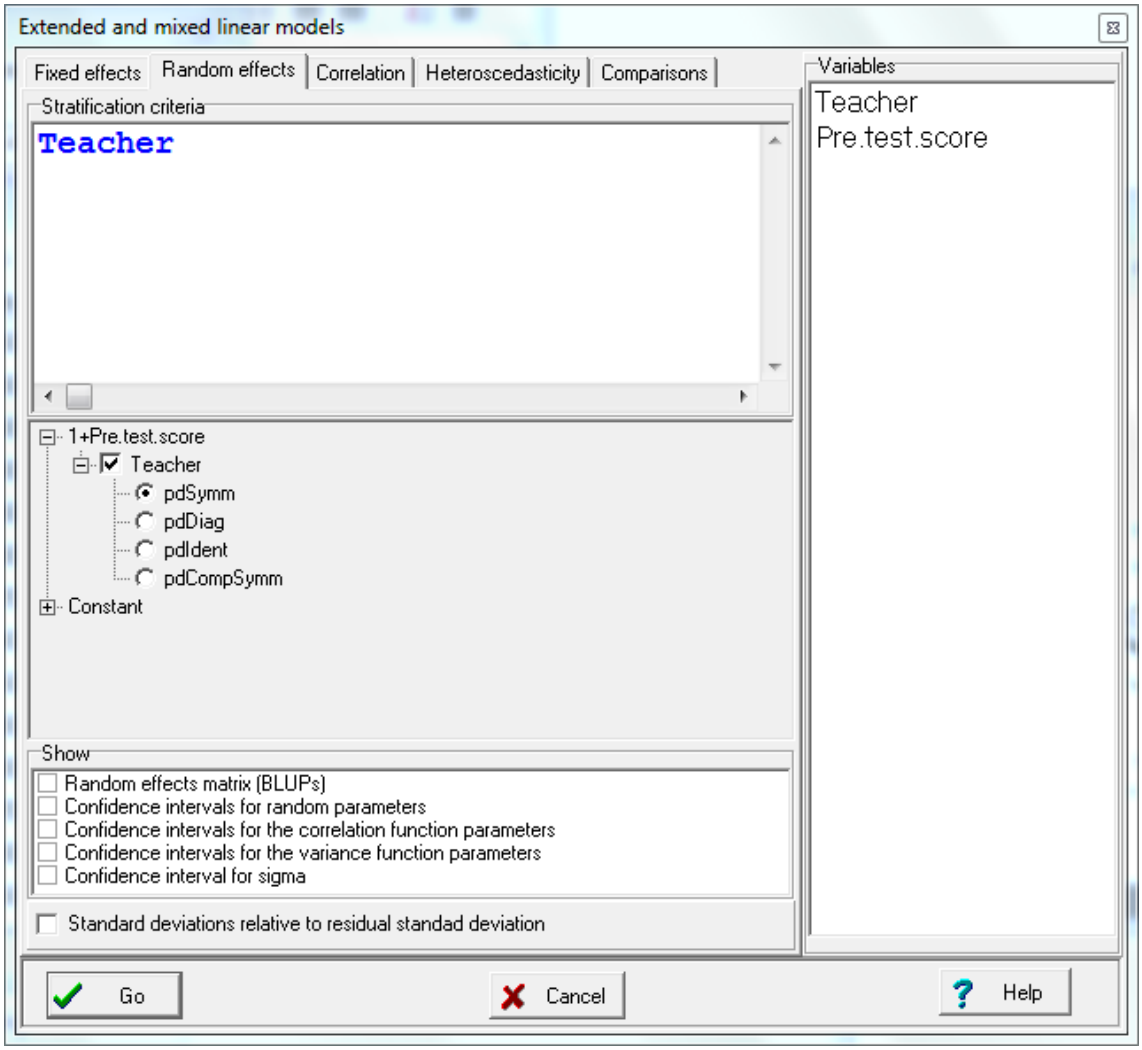



***Figure 129: Extended and mixed linear models with the Random effects tab for the data in the file Learning.IDB2.***

The output for this model shows, in addition to the usual parts in mixed models, the coefficients of the average straigth line, $\hat{Y} = 30.13 - 0.81x$. As expected, as the pre-test score is larger the gain decreases. One can also notice the large correlation between intercept and slope (-0.876), which confirms the need to incorporate this parameter in the model.

**Extended and mixed linear models**

**R specification of the model**

```
model.008_Gain_REML<-lme(Gain~1+Pre.test.score
,random=list(Teacher=pdSymm(~1+Pre.test.score))
,method="REML"
,control=lmeControl(msMaxIter=200)
,na.action=na.omit
,data=R.data08
,keep.data=FALSE)
```

**Results for model: model.008_Gain_REML**

*Dependent variable: Gain*

**Fit measurements**

| N | AIC | BIC | logLik | Sigma | R2_0 | R2_1 |
|---|---|---|---|---|---|---|
| 184 | 1485.954 | 1505.178 | -736.977 | 13.174 | 0.339 | 0.366 |

*Smaller AIC and BIC is better*

**Marginal hypothesis testingmodel.008_Gain_REML**

| | numDF | denDF | F-value | p-value |
|---|---|---|---|---|
| (Intercept) | 1 | 175 | 104.797 | <0.0001 |
| Pre.test.score | 1 | 175 | 78.129 | <0.0001 |

**Sequential hypothesis testing**

| | numDF | denDF | F-value | p-value |
|---|---|---|---|---|
| (Intercept) | 1 | 175 | 29.007 | <0.0001 |
| Pre.test.score | 1 | 175 | 78.129 | <0.0001 |

**Fixed effects**

| | Value | Std.Error | DF | t-value | p-value |
|---|---|---|---|---|---|
| (Intercept) | 30.133 | 2.944 | 175 | 10.237 | <0.0001 |
| Pre.test.score | -0.811 | 0.092 | 175 | -8.839 | <0.0001 |

**Random effects parameters**

*Covariance model for random effects: pdSymm*
*Formula: ~1 + Pre.test.score|Teacher*

*Standard deviations and correlations*

| | (const) | Pre.test.score |
|---|---|---|
| (const) | 2.645 | -0.876 |
| Pre.test.score | -0.876 | 0.111 |

### *Heteroscedastic regression*

In a research to evaluate primary productivity in pastures and its relationship with precipitation, nine plots were evaluated (five with native pasture and four with sown pastures). The primary productivity for 22-day periods was measured several times during the year in each plot (most plots were measures 12 times). Simultaneously, rainfall (mm) during the 22-days growing period was recorded (Ospina 2011, Ospina et al. 2012). The data are in the file Primary productivity.IDB2. In order to make a regression analysis including pasture as a classification variable, we select the variables in the Extended and mixed linear models module as shown in Figure 130.

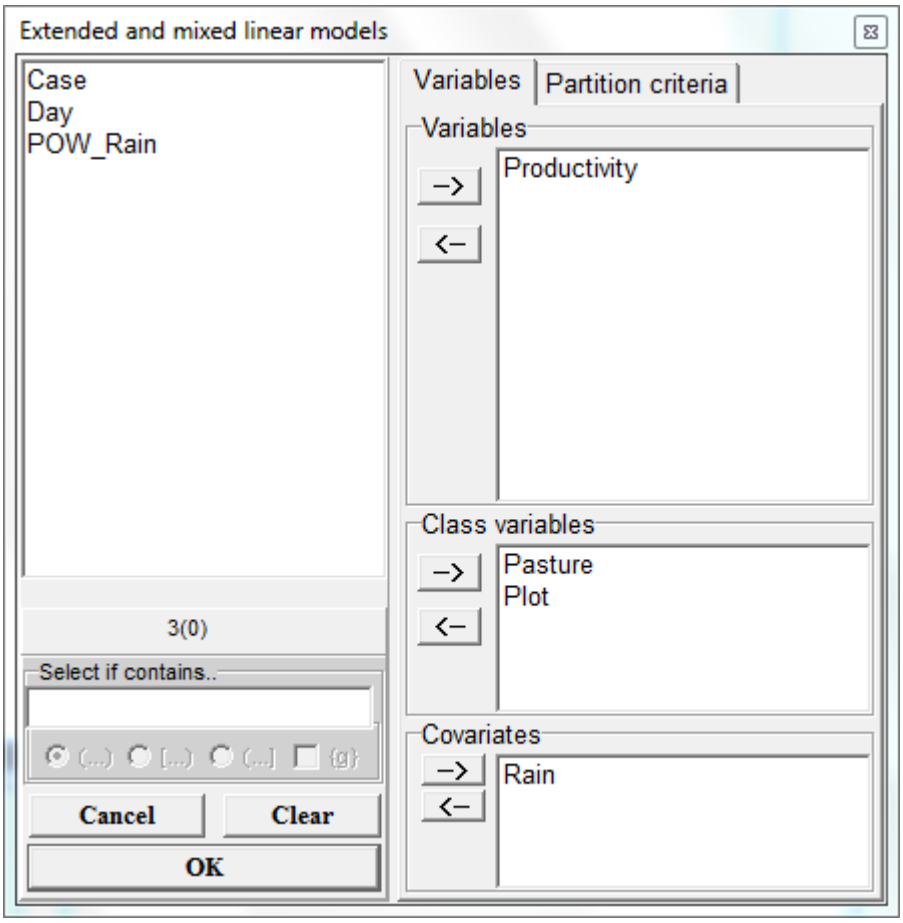



***Figure 130: Variable selection window in the Extended and mixed linear models module for the data in file Primary productivity.IDB2.***

In the *Fixed effects* tab we select the variables as in Figure 131 and in the *Random effects* tab we select *Plot* (Figure 132). With these specifications we are fitting a linear regression model with two intercepts (one for native and one for sown pastures), and a random plot effect. The residuals obtained from fitting this model can be used to

diagnose possible problems. In Figure 133 the q-q plot shows that the residuals distribution is approximately normal, but the scatterplot of Pearson conditional residuals vs. Rain shows a runs of negative residual values beyond 300 mm. This same run is observed in the scatterplot of Pearson conditional residual vs. fitted values, in this case beyond 110 g of primary productivity. This suggests the need to add a quadratic term for the regression *Rain*. To do this, in the *Data* menu, *Transformations* submenu, select *Rain* as variable. Then request a power transformation (in this case of order 2), and this generates a new variable in the dataset call *POW_Rain*.

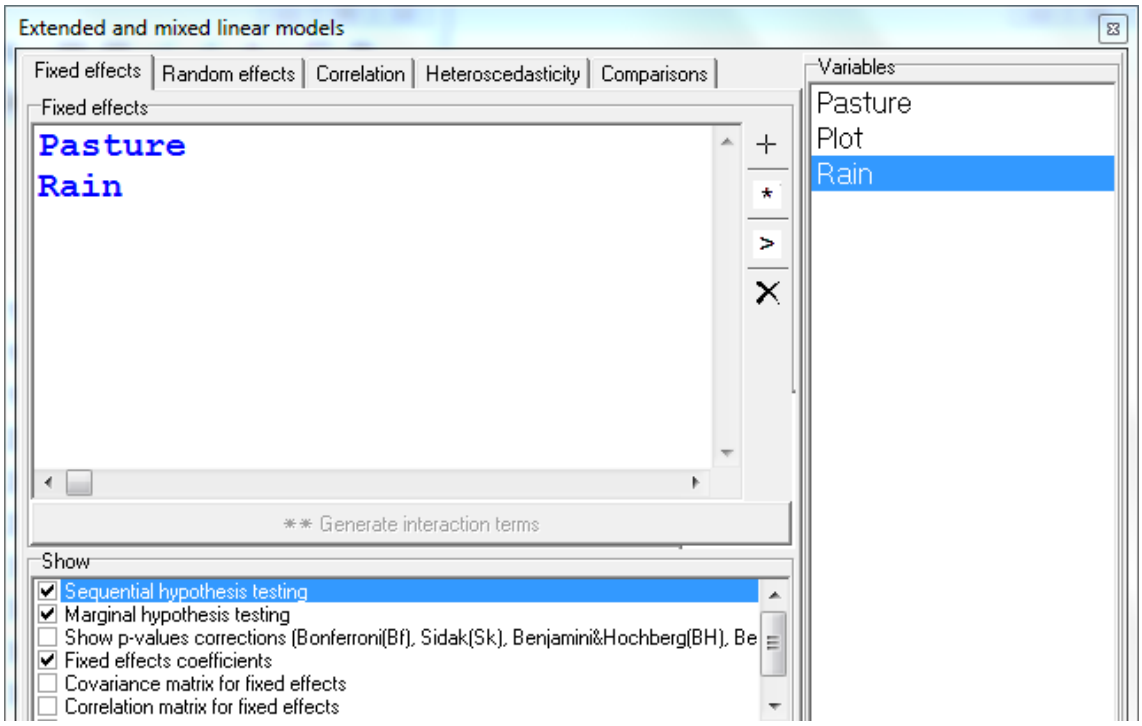



***Figure 131: Window from the Extended and mixed linear model with the Fixed effects tab for the data in file Primary productivity.IDB2.***

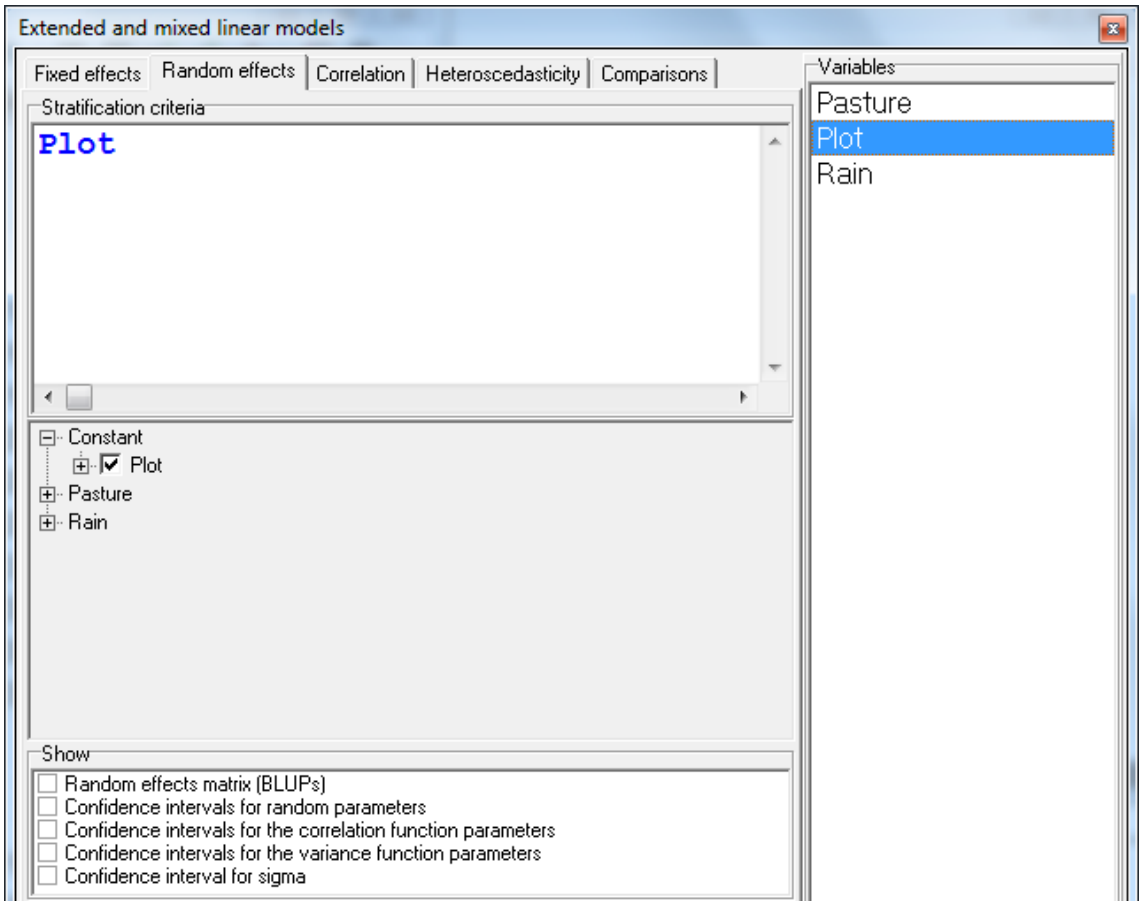



***Figure 132: Window from the Extended and mixed linear model with the Random effects tab for the data in file Primary productivity.IDB2.***

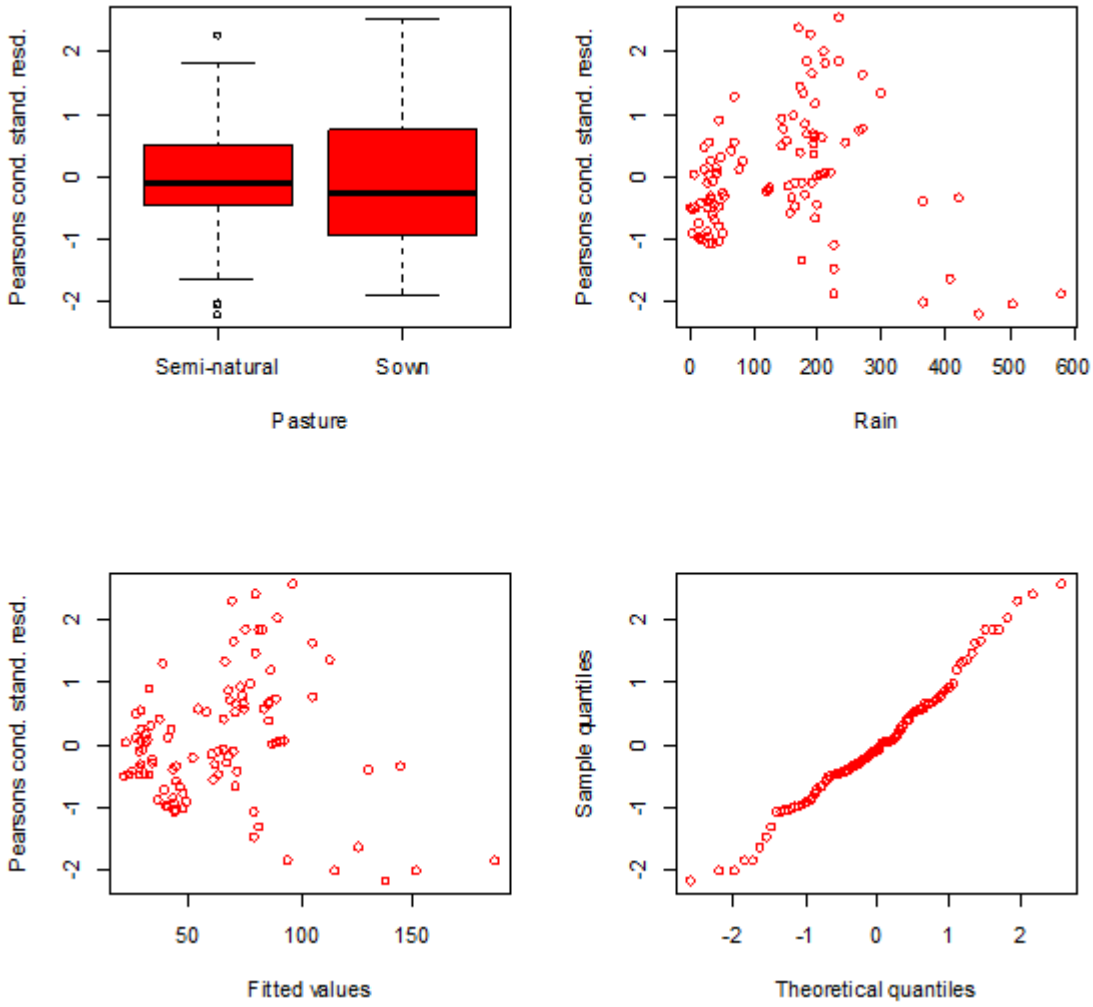



***Figure 133: Graphical tools for diagnostic obtained from the data in file Primary productivity.IDB2 with Rain as regressor and Pasture as fixed factor.***

The variable *POW_Rain* was added as covariate in the variable selector window in *Extended and mixed linear models*. Then it was included in the *Fixed effects* tab in the model, together with the other variables already entered as shown in Figure 142. The random effects tab is left unchanged as in Figure 132. Once this model is fitted and the residual diagnostic is requested, we obtain the plots shown in Figure 134.

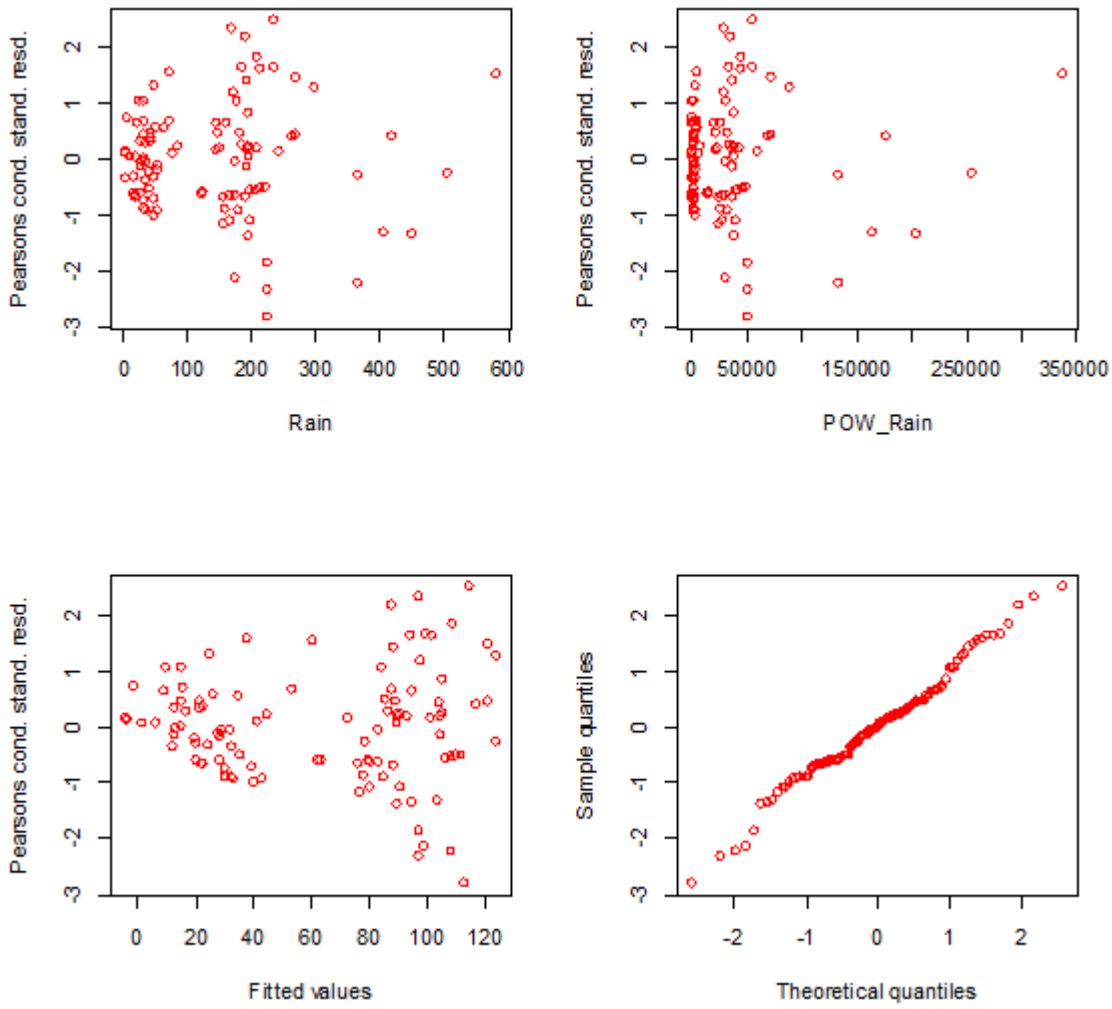



***Figure 134: Graphical tools for diagnostic obtained from the data in file Primary productivity.IDB2 with Rain and POW_Rain as regressors and Pasture as fixed factor.***

Analyzing the scatter plot of Pearson conditional residuals vs. fitted values, we notice a clear trend for the residuals to increase their variance as their mean value increases. This suggests the need to model the variance heterogeneity with a function relating the residual variances with the mean. To use this function, we run the analysis again (remember that CTRL + r repeats the last command in InfoStat), and in the Heteroscedasticity tab we add *VarPower* as shown in Figure 135.

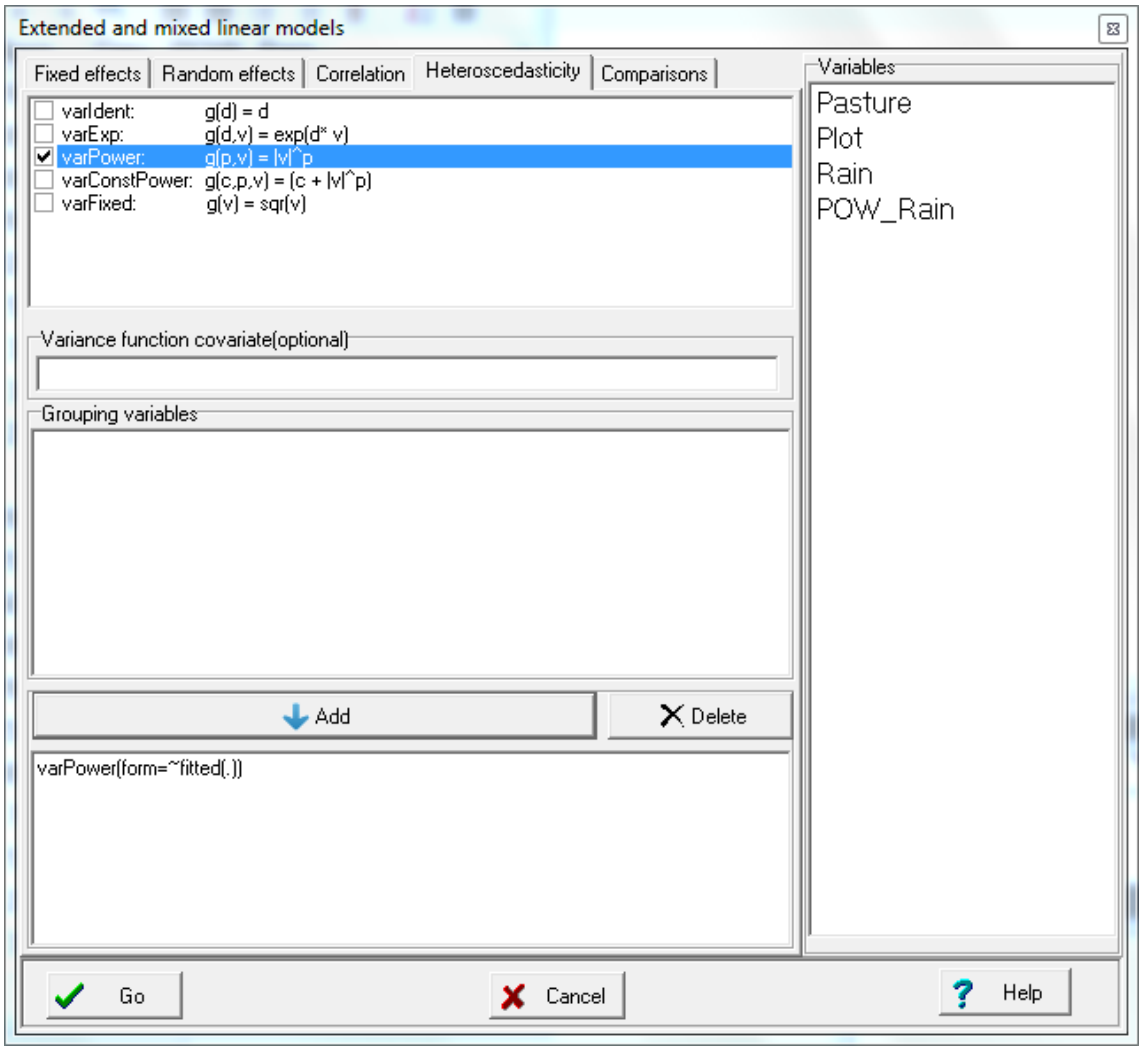



***Figure 135: Window from the Extended and mixed linear model with the Heteroscedasticity tab for the data in file Primary productivity.IDB2, and the selection of the function VarPower.***

This analysis was repeated with two other variance functions: *VarExp* y *VarConstPower*. Here are the fit statistics for the three models:

VarExp

**Fit measurements**

| N | AIC | BIC | logLik | Sigma | R2_0 | R2_1 |
|---|---|---|---|---|---|---|
| 104 | 1002.64 | 1020.87 | -494.32 | 9.89 | 0.52 | 0.52 |

*Smaller AIC and BIC is better*

VarPower

**Fit measurements**

| N | AIC | BIC | logLik | Sigma | R2_0 | R2_1 |
|---|---|---|---|---|---|---|
| 104 | 1001.07 | 1019.31 | -493.54 | 2.16 | 0.53 | 0.53 |

*Smaller AIC and BIC is better*

VarConstPower

**Fit measurements**

| N | AIC | BIC | logLik | Sigma | R2_0 | R2_1 |
|---|---|---|---|---|---|---|
| 104 | 1001.85 | 1022.69 | -492.92 | 0.62 | 0.52 | 0.52 |

*Smaller AIC and BIC is better*

We cannot compare these models using likelihood ratio tests because they do not form a nested set of hypotheses (except *VarPower* and *VarConstPower*). In these cases only the AIC and BIC criteria are useful. The model *VarPower* results the best alternative to model the variance heterogeneity. After fitting this model, residuals do not show any trend (Figure 136).

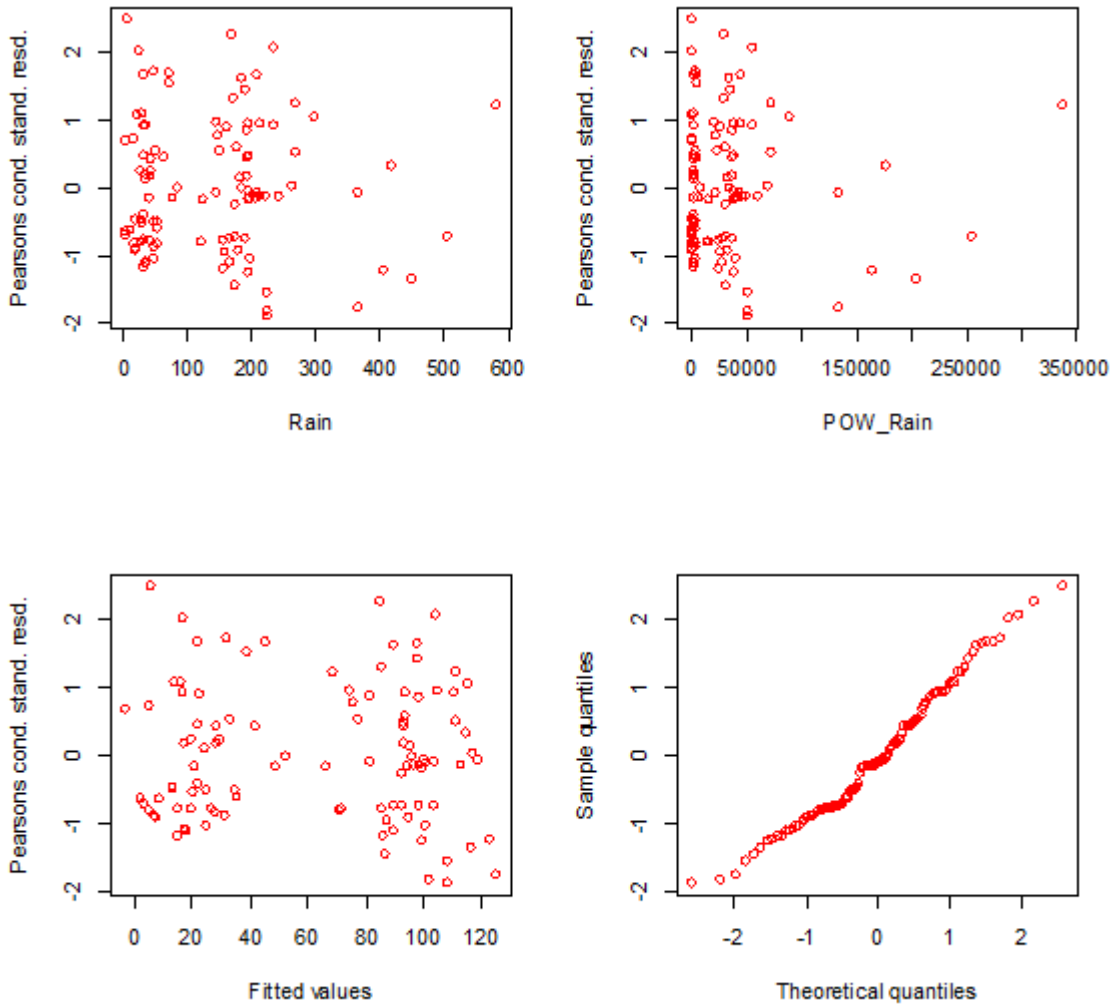



***Figure 136: Graphical tools for diagnostic obtained from the data in file Primary productivity.IDB2 with Rain and POW.Rain as regressors, Pasture as fixed factor, and a VarPower function to model variance heterogeneity.***

To test the hypothesis of equal linear and quadratic trends for both pastures, we included in the model the interactions between pasture type and the two regressors (Figure 137). The random plot effect was added in the *Random effects* tab (Figure 138) and the heteroscedasticity was specified as in Figure 135.

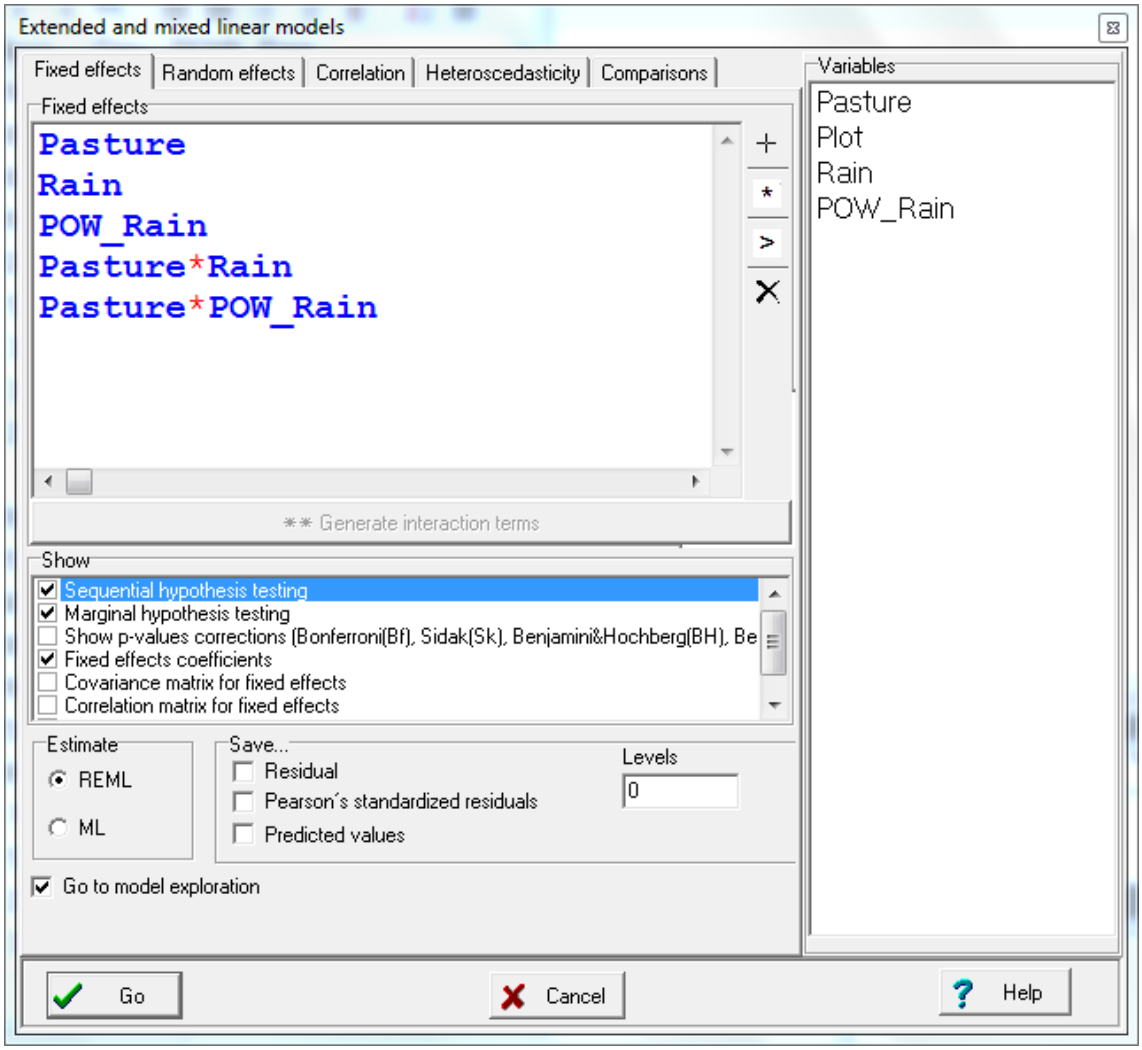



***Figure 137: Window from the Extended and mixed linear model for the data in file Primary productivity.IDB2, and the specification of the model with interaction.***

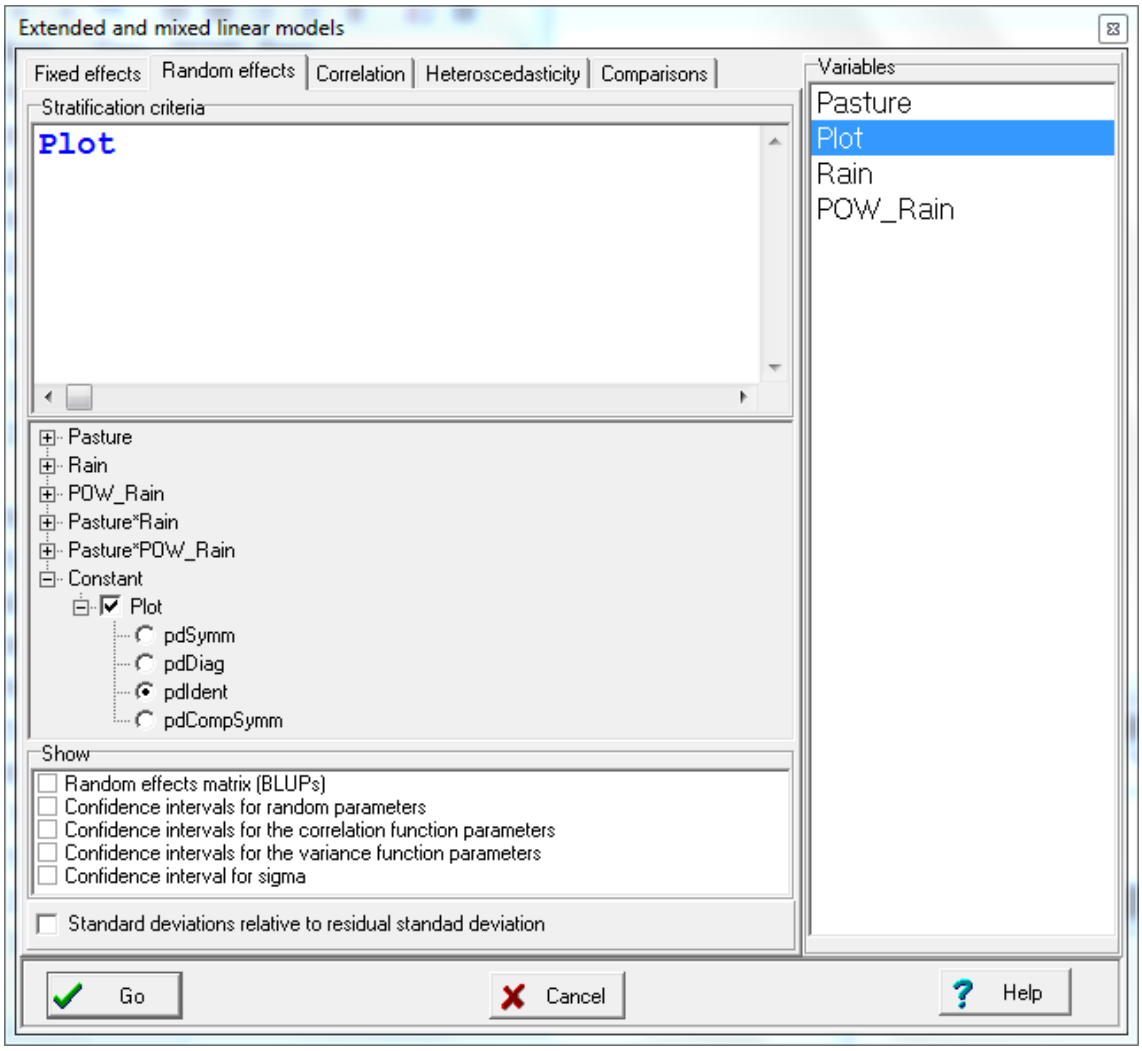



***Figure 138: Window from the Extended and mixed linear model module with the Random effects tab for the data in file Primary productivity.IDB2.***

With these specifications we obtained the following output:

```
Extended and mixed linear models

R specification of the model

model.014_Productivity_REML<-
lme(Productivity~1+Pasture+Rain+POW_Rain+Pasture:Rain+Pasture:POW_Rain
,random=list(Plot=pdIdent(~1))
,weights=varComb(varPower(form=~fitted(.)))
,method="REML"
,control=lmeControl(msMaxIter=200)
,na.action=na.omit
,data=R.data14
,keep.data=FALSE)

Results for model: model.014_Productivity_REML

Dependent variable: Productivity
```

**Fit measurements**

| N | AIC | BIC | logLik | Sigma | R2_0 | R2_1 |
|---|---|---|---|---|---|---|
| 104 | 1004.89 | 1028.15 | -493.45 | 2.46 | 0.65 | 0.65 |

*Smaller AIC and BIC is better*

**Marginal hypothesis testing (Type III SS)**

| | numDF | denDF | F-value | p-value |
|---|---|---|---|---|
| (Intercept) | 1 | 91 | 0.32 | 0.5703 |
| Pasture | 1 | 7 | 12.62 | 0.0093 |
| Rain | 1 | 91 | 167.15 | <0.0001 |
| POW_Rain | 1 | 91 | 55.66 | <0.0001 |
| Pasture:Rain | 1 | 91 | 4.19 | 0.0435 |
| Pasture:POW_Rain | 1 | 91 | 0.01 | 0.9179 |

**Sequential hypothesis testing**

| | numDF | denDF | F-value | p-value |
|---|---|---|---|---|
| (Intercept) | 1 | 91 | 152.56 | <0.0001 |
| Pasture | 1 | 7 | 34.31 | 0.0006 |
| Rain | 1 | 91 | 184.34 | <0.0001 |
| POW_Rain | 1 | 91 | 62.10 | <0.0001 |
| Pasture:Rain | 1 | 91 | 21.04 | <0.0001 |
| Pasture:POW_Rain | 1 | 91 | 0.01 | 0.9179 |

**Fixed effects**

| | Value | Std.Error | DF | t-value | p-value |
|---|---|---|---|---|---|
| (Intercept) | 6.83 | 3.67 | 91 | 1.86 | 0.0660 |
| PastureSown | -16.27 | 4.58 | 7 | -3.55 | 0.0093 |
| Rain | 0.60 | 0.07 | 91 | 8.32 | <0.0001 |
| POW_Rain | -1.0E-03 | 1.8E-04 | 91 | -5.74 | <0.0001 |
| PastureSown:Rain | 0.23 | 0.11 | 91 | 2.05 | 0.0435 |
| PastureSown:POW_Rain | -2.9E-05 | 2.8E-04 | 91 | -0.10 | 0.9179 |

**Random effects parameters**

*Covariance model for random effects: pdIdent*
*Formula: ~1|Plot*

*Standard deviations and correlations*

| | (const) |
|---|---|
| (const) | 1.3E-03 |

**Variance structure**

*Variance model: varPower*
*Formula: ~ fitted(.)*

*Variance-function parameters*

| Parameter | Estim. |
|---|---|
| power | 0.60 |

As can be seen from the previous output, there is interaction of pasture with rain, which indicates that the linear component is different in both pastures. On the other hand, there

are no differences in the quadratic component and hence it is the same in both pastures. The final model eliminated the interaction *Pasture*POW_Rain*, and the resulting output follows.

**Extended and mixed linear models**

**R specification of the model**

```
model.015_Productivity_REML<-
lme(Productivity~1+Pasture+Rain+POW_Rain+Pasture:Rain
,random=list(Plot=pdIdent(~1))
,weights=varComb(varPower(form=~fitted(.)))
,method="REML"
,control=lmeControl(msMaxIter=200)
,na.action=na.omit
,data=R.data15
,keep.data=FALSE)
```

**Results for model: model.015_Productivity_REML**

*Dependent variable: Productivity*

**Fit measurements**

| N | AIC | BIC | logLik | Sigma | R2_0 | R2_1 |
|---|---|---|---|---|---|---|
| 104 | 988.37 | 1009.14 | -486.19 | 2.46 | 0.65 | 0.65 |

*Smaller AIC and BIC is better*

**Marginal hypothesis testing (Type III SS)**

| | numDF | denDF | F-value | p-value |
|---|---|---|---|---|
| (Intercept) | 1 | 92 | 0.35 | 0.5579 |
| Pasture | 1 | 7 | 17.01 | 0.0044 |
| Rain | 1 | 92 | 171.84 | <0.0001 |
| POW_Rain | 1 | 92 | 57.67 | <0.0001 |
| Pasture:Rain | 1 | 92 | 21.17 | <0.0001 |

**Sequential hypothesis testing**

| | numDF | denDF | F-value | p-value |
|---|---|---|---|---|
| (Intercept) | 1 | 92 | 154.21 | <0.0001 |
| Pasture | 1 | 7 | 33.77 | 0.0007 |
| Rain | 1 | 92 | 186.37 | <0.0001 |
| POW_Rain | 1 | 92 | 63.32 | <0.0001 |
| Pasture:Rain | 1 | 92 | 21.17 | <0.0001 |

**Fixed effects**

| | Value | Std.Error | DF | t-value | p-value |
|---|---|---|---|---|---|
| (Intercept) | 6.66 | 3.43 | 92 | 1.94 | 0.0553 |
| PastureSown | -16.00 | 3.88 | 7 | -4.12 | 0.0044 |
| Rain | 0.60 | 0.06 | 92 | 10.42 | <0.0001 |
| POW_Rain | -1.0E-03 | 1.4E-04 | 92 | -7.59 | <0.0001 |
| PastureSown:Rain | 0.21 | 0.05 | 92 | 4.60 | <0.0001 |

**Random effects parameters**

*Covariance model for random effects: pdIdent*
*Formula: ~1|Plot*

*Standard deviations and correlations*

| | (const) |
|---|---|
| (const) | 2.2E-03 |

**Variance structure**

*Variance model: varPower*
*Formula: ~ fitted(.)*

*Variance-function parameters*

| Parameter | Estim. |
|---|---|
| power | 0.60 |

**Adjusted means and standard error for Pasture**
*LSD Fisher (Alpha:=0.05)*
*p-value correction procedure: No*

| Pasture | Means | S.E. | |
|---|---|---|---|
| Sown | 70.01 | 4.58 | A |
| Semi-natural | 56.17 | 3.52 | B |

*Means with a common letter are not significantly different (p<= 0.05)*

Due to the presence of a polynomial regression, it is more appropriate to report sequential tests (type I). We can conclude that there is a pasture effect (p=0.0007), a rain quadratic effect (p<0.0001), and a difference in linear trend between pastures (p<0.0001). Figure 139 shows the estimated average lines and the observations at individual plots.

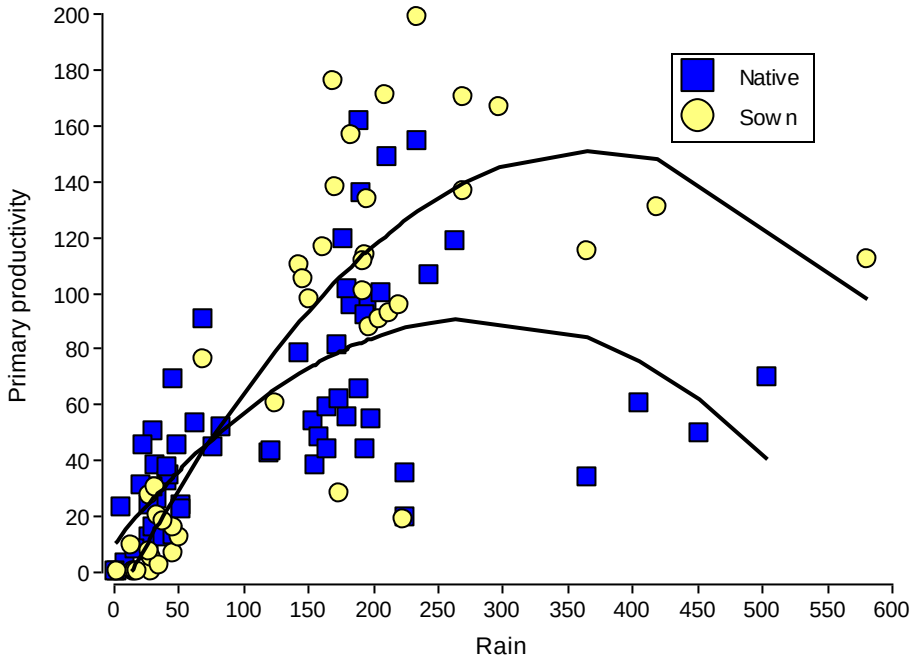



***Figure 139: Scatterplot showing the relationship between primary productivity and rain for each of the two pastures. File Primary productivity.IDB2.***

Since there is interaction, it is not possible to interpret the adjusted means. In order to compare both pastures at a given level of rain we can use the menu *Model Exploration*, *Linear combinations* tab. To determine the coefficients to be used, consider the following hypothesis (which tests if there are differences in primary productivity of both pastures for a cumulative rain of 100 mm):

$$H_0 : \beta_0 + 100\beta_1 + 10000\beta_2 = \beta_0 + \alpha + 100\beta_1 + 10000\beta_2 + 100\alpha\beta_1$$

This hypothesis is equivalent to $H_0 : \alpha + 100\alpha\beta_1 = 0$, which is expressed as a linear combination of parameters in the model . Similarly, it is possible to contrast pastures at other rain levels. The coefficients to do for 100 mm, 300 mm and 500 mm are shown in Figure 140.

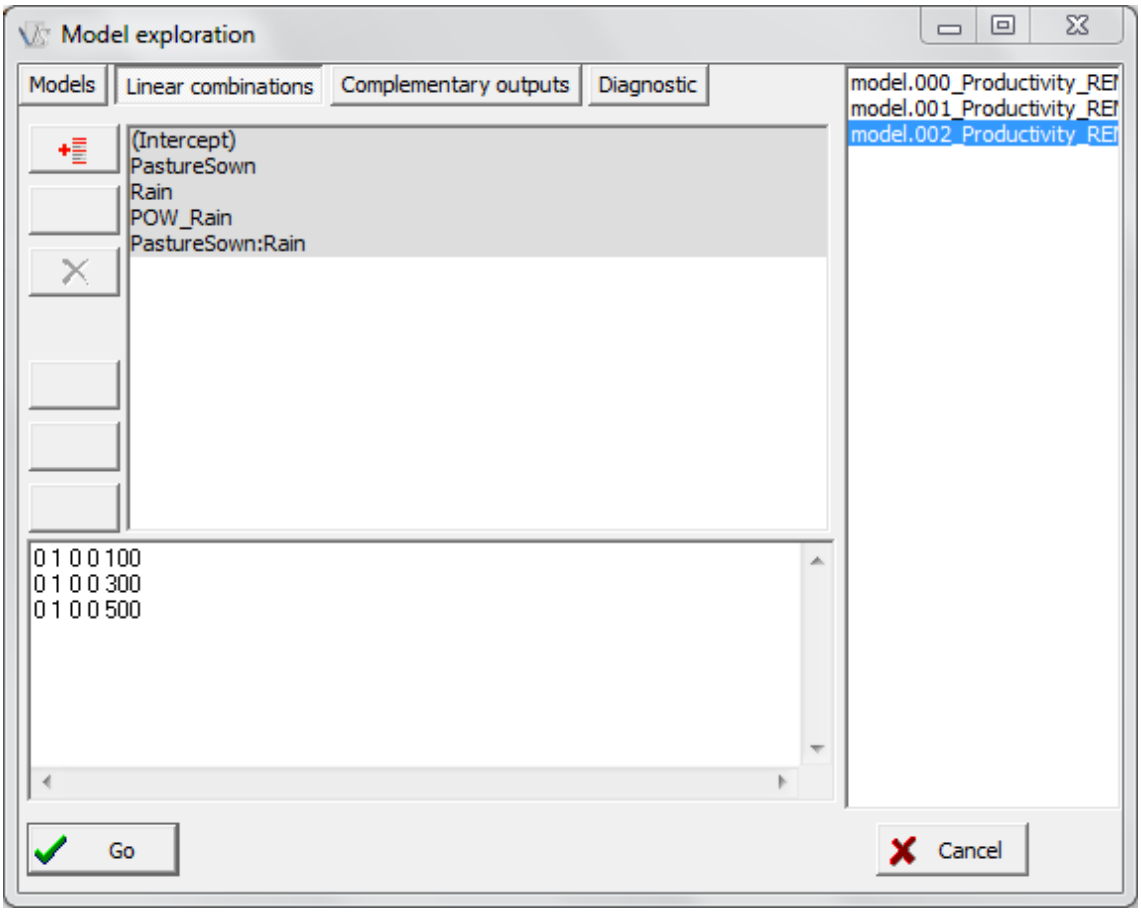



***Figure 140: Model exploration window with Linear combination tab for the data in file Primary productivity.IDB2.***

The results indicate that there are no significant differences between pastures when the cumulative rain is 100 mm (p=0.2008), but when the cumulative rain is 300 mm or 500 mm the sown pasture has a significantly larger primary productivity (P=0.0002 and p=0.0001 respectively).

**Hypothesis testing for linear combinations**

| Linear comb. | Estimate | S.E. | df | F | p-value |
|---|---|---|---|---|---|
| Comb.1 | 5.48 | 4.25 | 1 | 1.66 | 0.2008 |
| Comb.2 | 48.44 | 12.45 | 1 | 15.14 | 0.0002 |
| Comb.3 | 91.40 | 21.59 | 1 | 17.92 | 0.0001 |
| Total | | | | nd | nd |

**Linear combination coefficients**

| Parameters | Comb.1 | Comb.2 | Comb.3 |
|---|---|---|---|
| (Intercept) | 0.00 | 0.00 | 0.00 |
| PastureSown | 1.00 | 1.00 | 1.00 |
| Rain | 0.00 | 0.00 | 0.00 |
| POW_Rain | 0.00 | 0.00 | 0.00 |
| PastureSown:Rain | 100.00 | 300.00 | 500.00 |

## *Incomplete blocks and related designs*

### *Alpha lattice designs*

The data from this example come from an 18-variety barley trial conducted in Scotland (Patterson et al., 1989). Due to the large number of treatments, it was impossible to find blocks with 18 homogenous experimental units, and hence the blocks were incomplete (Data: Alpha lattice.IDB2). A complete replication for this experiment consists of 3 incomplete blocks with 4 experimental units each, and 2 blocks with 3 units each. There was a total of 4 replications (Figure 141).

| | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1<br>11 | 1<br>3 | 1<br>8 | 1<br>10 | 2<br>13 | 2<br>17 | 2<br>16 | 2<br>5 | 3<br>14 | 3<br>18 | 3<br>4 | 4<br>6 | 4<br>1 | 4<br>7 | 4<br>12 | 5<br>9 | 5<br>2 | 5<br>15 | **I** |
| 1<br>15 | 1<br>4 | 1<br>11 | 1<br>17 | 2<br>5 | 2<br>12 | 2<br>10 | 3<br>9 | 3<br>1 | 3<br>13 | 4<br>2 | 4<br>14 | 4<br>7 | 4<br>8 | 5<br>16 | 5<br>6 | 5<br>3 | 5<br>18 | **II** |
| 1<br>12 | 1<br>15 | 1<br>3 | 2<br>4 | 2<br>16 | 2<br>7 | 3<br>8 | 3<br>4 | 3<br>12 | 3<br>13 | 4<br>18 | 4<br>8 | 4<br>5 | 4<br>9 | 5<br>10 | 5<br>17 | 5<br>14 | 5<br>1 | **III** |
| 1<br>10 | 1<br>2 | 1<br>16 | 2<br>9 | 2<br>17 | 2<br>7 | 2<br>3 | 3<br>8 | 3<br>4 | 3<br>12 | 3<br>13 | 4<br>15 | 4<br>6 | 4<br>5 | 4<br>14 | 5<br>11 | 5<br>1 | 5<br>18 | **IV** |

***Figure 141: Layout of the design for the 18-variety barley trial conducted in an alpha-lattice. Roman numbers at the right indicate replicates, arabic numbers at the top of each cell indicate the incomplete block (of sizes 4 and 3), and numbers at the bottom of each cell represent the varieties.***

The first analysis will be done considering only the repetitions as whole (complete) blocks. Since there are 4 repetitions, an error term for the comparisons can be estimated.

In the variable selection window in *Extended and mixed linear models* we select *Yield* as variable, *Variety*, *Incomplete block* and *Repetition* as class variables. After this, we select *Variety* in the *Fixed effects* tab (Figure 142), and *Repetition* in the *Random effect* tab (Figure 143). Since all the varieties are present in each repetition, this form corrects for possible repetition bias, although it ignores the incomplete block effects (and hence the possible bias due to incomplete blocks).

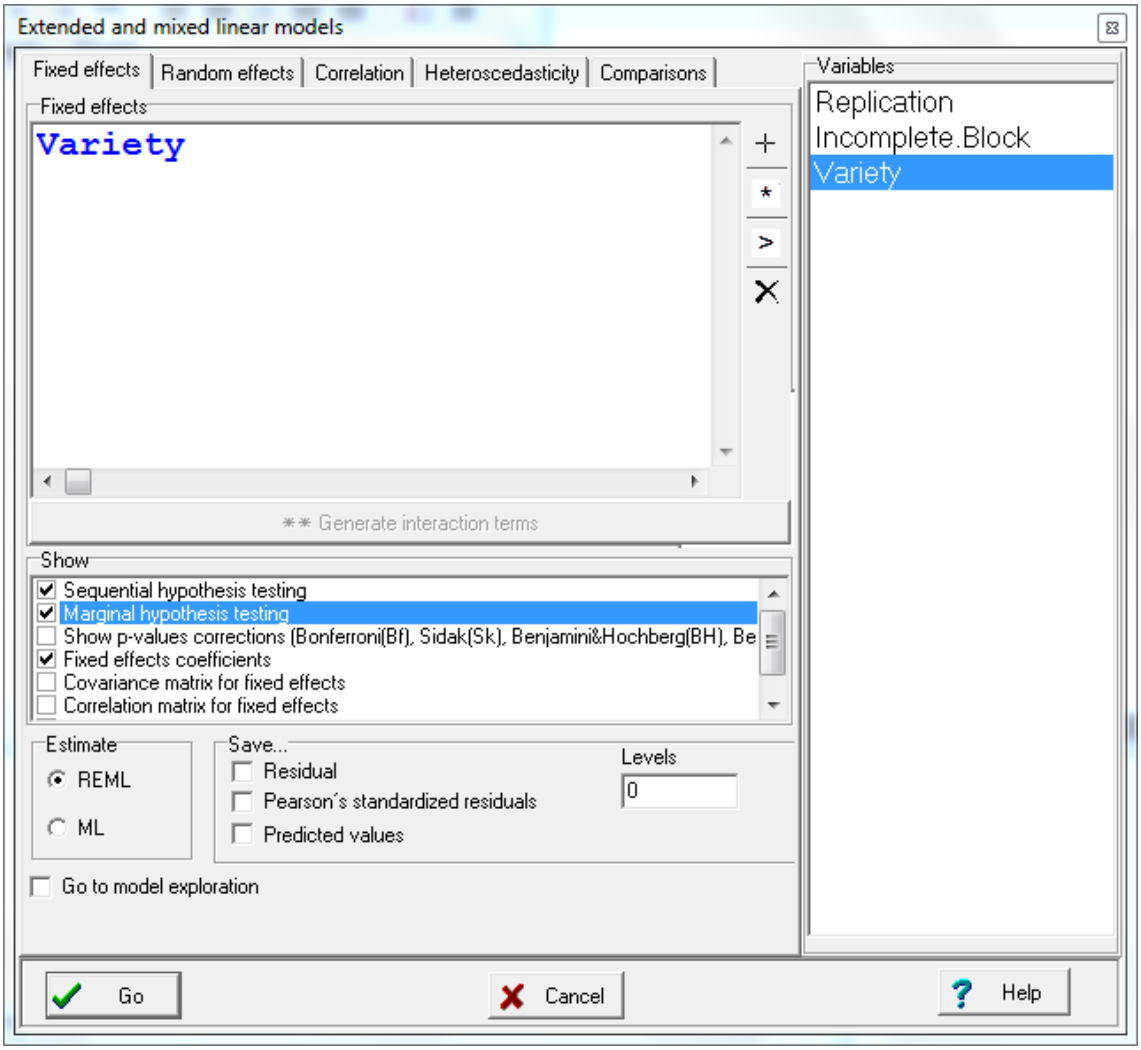



***Figure 142: Window from the Extended and mixed linear model with the Fixed effects tab for the data in file Alpha lattice.IDB2.***

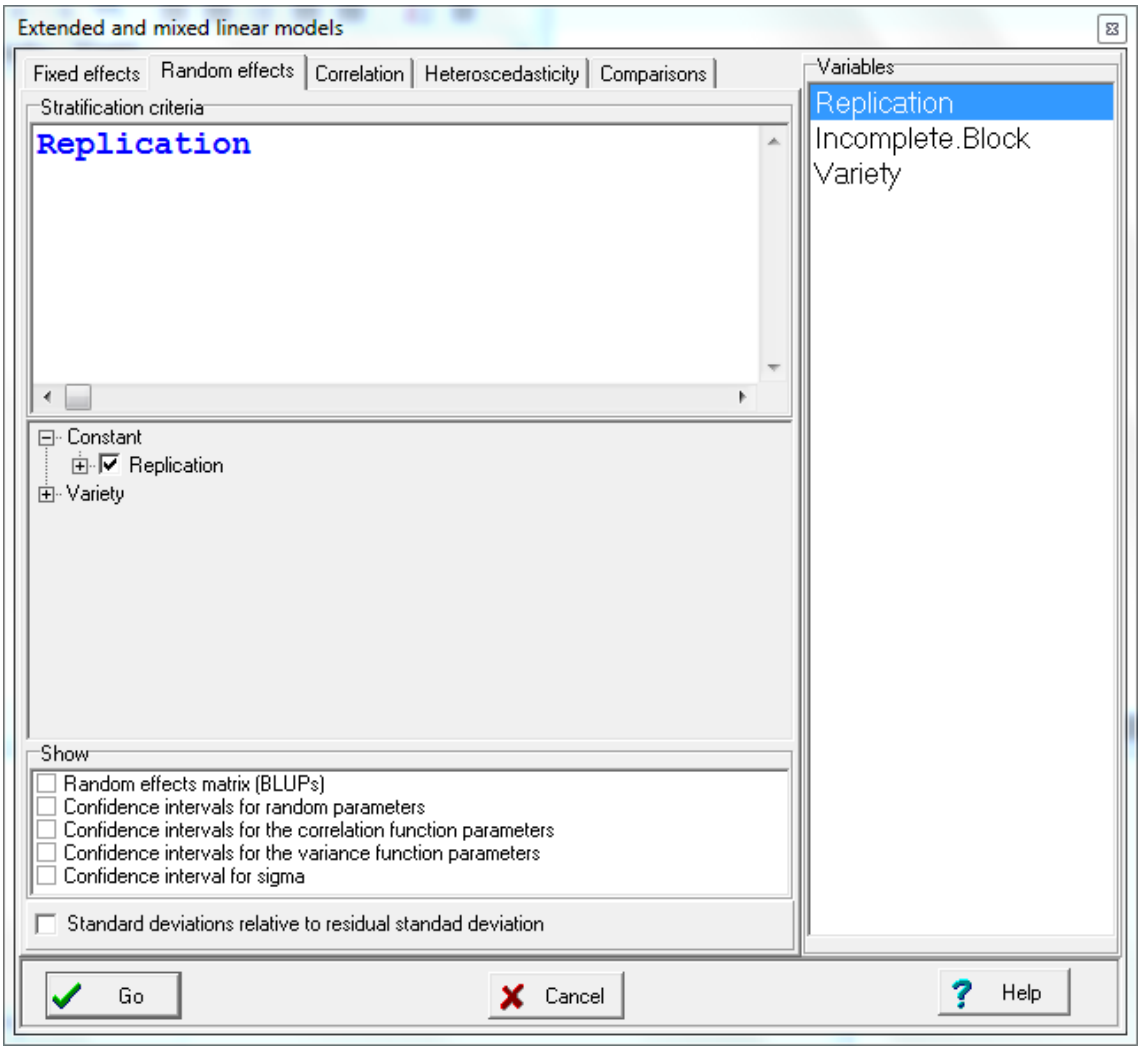



***Figure 143: Window from the Extended and mixed linear model with the Random effects tab and the Replication effect for the data in file Alpha lattice.IDB2.***

The following output shows the result from this model.

```
Extended and mixed linear models

R specification of the model

model.002_Yield_REML<-lme(Yield~1+Variety
,random=list(Replication=pdIdent(~1))
,method="REML"
,control=lmeControl(msMaxIter=200)
,na.action=na.omit
,data=R.data02
,keep.data=FALSE)

Results for model: model.002_Yield_REML

Dependent variable: Yield

Fit measurements
N   AIC   BIC   logLik Sigma R2_0 R2_1
72 65.28 105.06 -12.64  0.22 0.37 0.70
Smaller AIC and BIC is better
```

**Marginal hypothesis testing (Type III SS)**

| | numDF | denDF | F-value | p-value |
|---|---|---|---|---|
| (Intercept) | 1 | 51 | 1977.36 | <0.0001 |
| Variety | 17 | 51 | 3.74 | 0.0001 |

**Sequential hypothesis testing**

| | numDF | denDF | F-value | p-value |
|---|---|---|---|---|
| (Intercept) | 1 | 51 | 1977.36 | <0.0001 |
| Variety | 17 | 51 | 3.74 | 0.0001 |

**Fixed effects**

| | Value | Std.Error | DF | t-value | p-value |
|---|---|---|---|---|---|
| (Intercept) | 5.33 | 0.16 | 51 | 33.73 | <0.0001 |
| Variety10 | -0.68 | 0.16 | 51 | -4.27 | 0.0001 |
| Variety11 | -0.20 | 0.16 | 51 | -1.26 | 0.2118 |
| Variety12 | -0.48 | 0.16 | 51 | -3.00 | 0.0041 |
| Variety13 | -0.33 | 0.16 | 51 | -2.05 | 0.0451 |
| Variety14 | 0.00 | 0.16 | 51 | 0.00 | >0.9999 |
| Variety15 | -0.05 | 0.16 | 51 | -0.32 | 0.7532 |
| Variety16 | -0.23 | 0.16 | 51 | -1.42 | 0.1610 |
| Variety17 | 0.18 | 0.16 | 51 | 1.11 | 0.2738 |
| Variety18 | -0.48 | 0.16 | 51 | -3.00 | 0.0041 |
| Variety2 | -0.43 | 0.16 | 51 | -2.69 | 0.0097 |
| Variety3 | -0.08 | 0.16 | 51 | -0.47 | 0.6374 |
| Variety4 | -0.05 | 0.16 | 51 | -0.32 | 0.7532 |
| Variety5 | -0.38 | 0.16 | 51 | -2.37 | 0.0216 |
| Variety6 | -0.33 | 0.16 | 51 | -2.05 | 0.0451 |
| Variety7 | -0.20 | 0.16 | 51 | -1.26 | 0.2118 |
| Variety8 | -0.40 | 0.16 | 51 | -2.53 | 0.0146 |
| Variety9 | -0.13 | 0.16 | 51 | -0.79 | 0.4330 |

**Random effects parameters**

*Covariance model for random effects: pdIdent*
*Formula: ~1|Replication*

*Standard deviations and correlations*

| | (const) |
|---|---|
| (const) | 0.22 |

**Adjusted means and standard error for Variety**
*LSD Fisher (Alpha:=0.05)*
*p-value correction procedure: No*

| Variety | Means | S.E. | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 17 | 5.50 | 0.16 | A | | | | | |
| 14 | 5.33 | 0.16 | A | B | | | | |
| 1 | 5.33 | 0.16 | A | B | | | | |
| 15 | 5.28 | 0.16 | A | B | C | | | |
| 4 | 5.28 | 0.16 | A | B | C | | | |
| 3 | 5.25 | 0.16 | A | B | C | D | | |
| 9 | 5.20 | 0.16 | A | B | C | D | E | |
| 11 | 5.13 | 0.16 | | B | C | D | E | F |
| 7 | 5.13 | 0.16 | | B | C | D | E | F |
| 16 | 5.10 | 0.16 | | B | C | D | E | F |
| 6 | 5.00 | 0.16 | | | C | D | E | F |
| 13 | 5.00 | 0.16 | | | C | D | E | F |

```
5         4.95 0.16           D  E  F  G
8         4.93 0.16              E  F  G
2         4.90 0.16              E  F  G
18        4.85 0.16                 F  G
12        4.85 0.16                 F  G
10        4.65 0.16                    G
Means with a common letter are not significantly different (p<= 0.05)
```

As we can see, the mean comparisons are done with a single standard error (the standard error of the estimated treatment effects, 0.1582, represent the standard error of the difference between each treatment and the reference treatment). Now we will conduct a new analysis incorporating the *Incomplete Block* effects. To do this, we leave the Fixed effects tab as in Figure 142 (*Variety*) and in the Random effects tab we select *Repeticion* and *Incomplete Block* as shown in Figure 144.

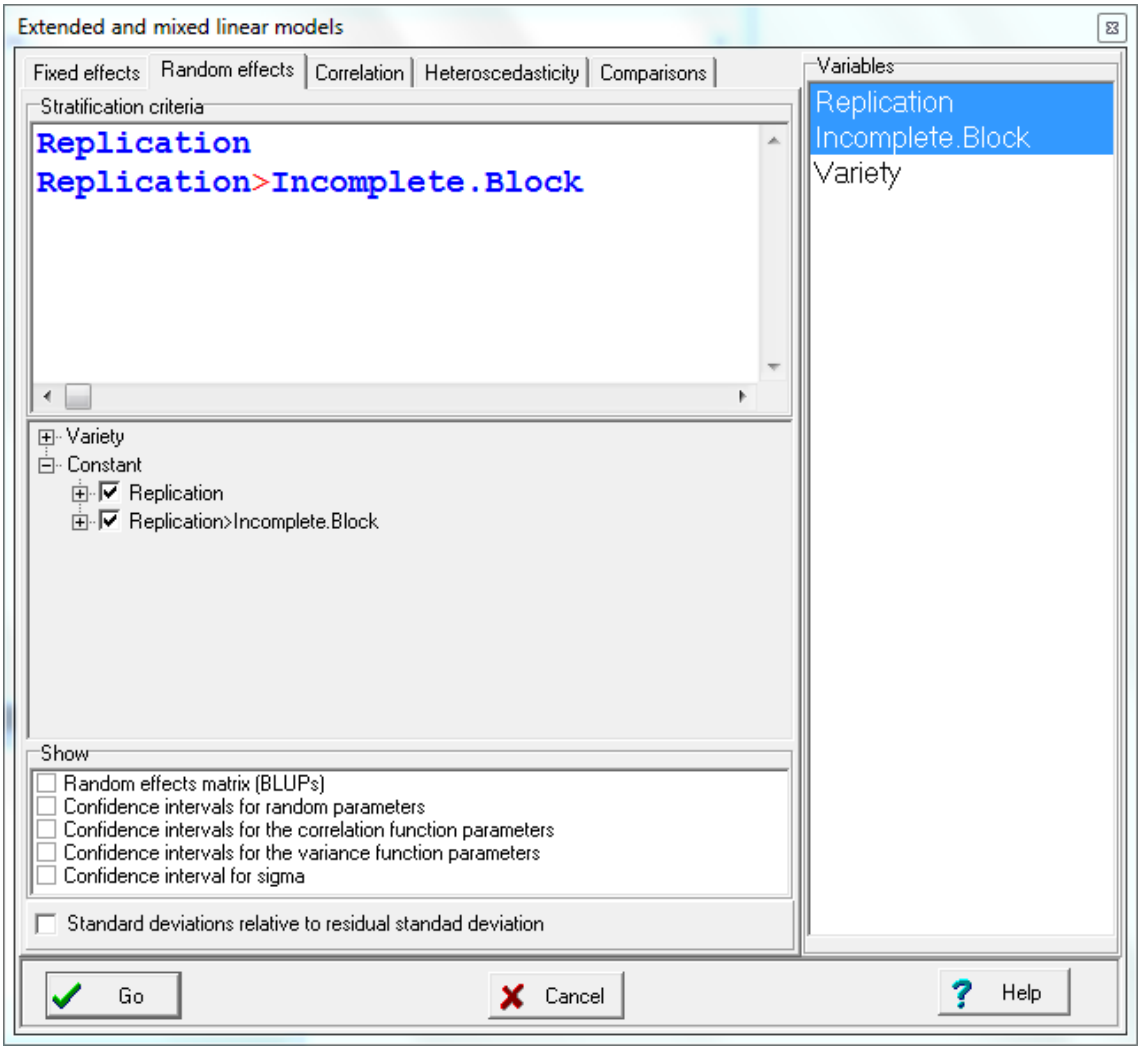



***Figure 144: Window from the Extended and mixed linear model with the Random effects tab and the Replication and Incomplete block effect for the data in file Alpha lattice.IDB2.***

The following is the output corresponding to this model.

**Extended and mixed linear models**

**R specification of the model**

```
model.003_Yield_REML<-lme(Yield~1+Variety
,random=list(Replication=pdIdent(~1)
,Replication=pdIdent(~Incomplete.Block-1))
,method="REML"
,control=lmeControl(msMaxIter=200)
,na.action=na.omit
,data=R.data03
,keep.data=FALSE)
```

**Results for model: model.003_Yield_REML**

*Dependent variable: Yield*

**Fit measurements**

| N | AIC | BIC | logLik | Sigma | R2_0 | R2_1 | R2_2 |
|---|---|---|---|---|---|---|---|
| 72 | 52.35 | 94.12 | -5.17 | 0.15 | 0.34 | 0.66 | 0.90 |

*Smaller AIC and BIC is better*

**Marginal hypothesis testing (Type III SS)**

| | numDF | denDF | F-value | p-value |
|---|---|---|---|---|
| (Intercept) | 1 | 51 | 2154.51 | <0.0001 |
| Variety | 17 | 51 | 6.54 | <0.0001 |

**Sequential hypothesis testing**

| | numDF | denDF | F-value | p-value |
|---|---|---|---|---|
| (Intercept) | 1 | 51 | 2153.76 | <0.0001 |
| Variety | 17 | 51 | 6.54 | <0.0001 |

**Fixed effects**

| | Value | Std.Error | DF | t-value | p-value |
|---|---|---|---|---|---|
| (Intercept) | 5.36 | 0.14 | 51 | 38.82 | <0.0001 |
| Variety10 | -0.74 | 0.12 | 51 | -6.09 | <0.0001 |
| Variety11 | -0.16 | 0.12 | 51 | -1.35 | 0.1824 |
| Variety12 | -0.43 | 0.12 | 51 | -3.55 | 0.0008 |
| Variety13 | -0.39 | 0.12 | 51 | -3.23 | 0.0022 |
| Variety14 | -0.13 | 0.12 | 51 | -1.04 | 0.3050 |
| Variety15 | -0.07 | 0.13 | 51 | -0.54 | 0.5892 |
| Variety16 | -0.22 | 0.13 | 51 | -1.75 | 0.0861 |
| Variety17 | 0.11 | 0.12 | 51 | 0.94 | 0.3517 |
| Variety18 | -0.52 | 0.12 | 51 | -4.35 | 0.0001 |
| Variety2 | -0.45 | 0.13 | 51 | -3.57 | 0.0008 |
| Variety3 | -0.09 | 0.12 | 51 | -0.73 | 0.4664 |
| Variety4 | -0.11 | 0.13 | 51 | -0.84 | 0.4027 |
| Variety5 | -0.46 | 0.12 | 51 | -3.74 | 0.0005 |
| Variety6 | -0.34 | 0.12 | 51 | -2.83 | 0.0067 |
| Variety7 | -0.19 | 0.12 | 51 | -1.57 | 0.1233 |
| Variety8 | -0.59 | 0.12 | 51 | -4.76 | <0.0001 |
| Variety9 | -0.27 | 0.12 | 51 | -2.24 | 0.0292 |

**Random effects parameters**

*Covariance model for random effects: pdIdent*
*Formula: ~1|Replication*

*Standard deviations and correlations*

| | (const) |
|---|---|
| (const) | 0.20 |

*Covariance model for random effects: pdIdent*
*Formula: ~Incomplete.Block - 1|Replication*

*Standard deviations and correlations*

| | S.D. |
|---|---|
| 1 | 0.18 |
| 2 | 0.18 |
| 3 | 0.18 |
| 4 | 0.18 |
| 5 | 0.18 |

**Adjusted means and standard error for Variety**
*LSD Fisher (Alpha:=0.05)*
*p-value correction procedure: No*

| Variety | Means | S.E. | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 17 | 5.47 | 0.14 | A | | | | | | | | |
| 1 | 5.36 | 0.14 | A | B | | | | | | | |
| 15 | 5.29 | 0.14 | A | B | C | | | | | | |
| 3 | 5.27 | 0.14 | A | B | C | | | | | | |
| 4 | 5.26 | 0.14 | A | B | C | D | | | | | |
| 14 | 5.24 | 0.14 | A | B | C | D | | | | | |
| 11 | 5.20 | 0.14 | | B | C | D | E | | | | |
| 7 | 5.17 | 0.14 | | B | C | D | E | F | | | |
| 16 | 5.14 | 0.14 | | B | C | D | E | F | G | | |
| 9 | 5.09 | 0.14 | | | C | D | E | F | G | H | |
| 6 | 5.02 | 0.14 | | | | D | E | F | G | H | I |
| 13 | 4.98 | 0.14 | | | | | E | F | G | H | I |
| 12 | 4.93 | 0.14 | | | | | | F | G | H | I |
| 2 | 4.91 | 0.14 | | | | | | | G | H | I |
| 5 | 4.90 | 0.14 | | | | | | | | H | I |
| 18 | 4.84 | 0.14 | | | | | | | | | I |
| 8 | 4.77 | 0.14 | | | | | | | | | |
| 10 | 4.62 | 0.14 | | | | | | | | | |

| | | |
|---|---|---|
| | J | K |
| | J | K |
| | | K |

*Means with a common letter are not significantly different (p<= 0.05)*

In this output we can notice that the standard errors of the differences (=standard error of the variety effect) are still similar for each variety, although not equal, since although all varieties have the same number of repetitions ($n$=4), the incomplete blocks are of different size (3 or 4 experimental units). Also, these standard errors are smaller than in the previous model, since now we have eliminated the variance of blocks within each

replicate. The means in this last analysis are also corrected for the block effects (adjusted means), and therefore this is a better analysis: the adjusted means are unbiased (conditionally on the observed blocks). The mean ranking has changed: in the whole-block-only analysis the top three means are 17, 14, and 1 respectively; while in the incomplete block analysis the top three means were 17, 1, and 15 respectively. The standard error of the difference of two means in the first analysis was 0.158 and in the second analysis was 0.122 on average (in order to compute an average standard error of differences, the usual procedure is to square each S.E., average these values, and then take the square root of the average). The efficiency of the second analysis with respect to the first one was:

$$E = \left( \frac{SEdiff_{WholeBlocksOnly}}{SEdiff_{IncompleteBlocks}} \right)^2 = \left( \frac{0.158}{0.122} \right)^2 = 1.68$$

The analysis including the incomplete blocks is 68% more efficient than the one not including this effect.

A summary of the fit measures of the two models follows. Using AIC and BIC criteria the incomplete block analysis is better.

**Incomplete block analysis**

| N | AIC | BIC | logLik | Sigma | R2_0 | R2_1 | R2_2 |
|---|---|---|---|---|---|---|---|
| 72 | 52.3497 | 94.1184 | -5.1749 | 0.1532 | 0.3431 | 0.6595 | 0.8961 |

*Smaller AIC and BIC is better*

**Whole blocks only analysis**

| N | AIC | BIC | logLik | Sigma | R2_0 | R2_1 |
|---|---|---|---|---|---|---|
| 72 | 65.2834 | 105.0631 | -12.6417 | 0.2237 | 0.3714 | 0.7015 |

*Smaller AIC and BIC is better*

## *Latinized row-column design*

The data in this example come from a trial to evaluate 30 cotton varieties. Each variety was repeated 5 times (William 1986). Figure 145 shows the field arrangement of the design. Each variety is present only once in each column (latinized columns). At the

same time, there are groups of six rows each, containing all 30 varieties (complete replication). These two are the randomization restrictions which must be taken into account in the design. Notice that the rows within each repetition constitute incomplete blocks (Lattice row column.IDB2).

To conduct the analysis in *Extended and mixed linear models*, select *Yield* as variable and *Variety*, *Replication*, *Row* and *Column* as class variables. Then we select *Variety* in the *Fixed effects* tab (Figure 146) and the rest of the variables in the *Random effects* tab, as shown in Figure 147.

| Repetition | Row | Col 1 | Col 2 | Col 3 | Col 4 | Col 5 |
|---|---|---|---|---|---|---|
| I | 1 | 21 | 20 | 25 | 14 | 1 |
| | 2 | 10 | 3 | 29 | 28 | 13 |
| | 3 | 11 | 24 | 26 | 5 | 15 |
| | 4 | 16 | 7 | 22 | 19 | 17 |
| | 5 | 30 | 2 | 27 | 9 | 6 |
| | 6 | 4 | 8 | 18 | 23 | 12 |
| II | 7 | 2 | 17 | 14 | 15 | 23 |
| | 8 | 27 | 18 | 24 | 29 | 25 |
| | 9 | 6 | 21 | 10 | 12 | 7 |
| | 10 | 13 | 9 | 20 | 26 | 16 |
| | 11 | 8 | 19 | 3 | 30 | 5 |
| | 12 | 28 | 1 | 11 | 4 | 22 |
| III | 13 | 9 | 29 | 15 | 1 | 8 |
| | 14 | 18 | 14 | 5 | 22 | 10 |
| | 15 | 7 | 27 | 23 | 20 | 11 |
| | 16 | 26 | 25 | 17 | 6 | 3 |
| | 17 | 12 | 30 | 16 | 24 | 28 |
| | 18 | 19 | 4 | 13 | 21 | 2 |
| IV | 19 | 1 | 26 | 2 | 7 | 18 |
| | 20 | 15 | 16 | 21 | 3 | 27 |
| | 21 | 29 | 12 | 19 | 11 | 14 |
| | 22 | 23 | 5 | 28 | 25 | 9 |
| | 23 | 20 | 10 | 30 | 17 | 4 |
| | 24 | 22 | 13 | 6 | 8 | 24 |
| V | 25 | 5 | 6 | 4 | 16 | 29 |
| | 26 | 24 | 23 | 1 | 10 | 19 |
| | 27 | 25 | 15 | 7 | 13 | 30 |
| | 28 | 17 | 11 | 9 | 18 | 21 |
| | 29 | 14 | 28 | 8 | 27 | 26 |
| | 30 | 3 | 22 | 12 | 2 | 20 |

***Figure 145: Field layout for a 30-variety cotton trial conducted as a latinized row-column design with five replication.***

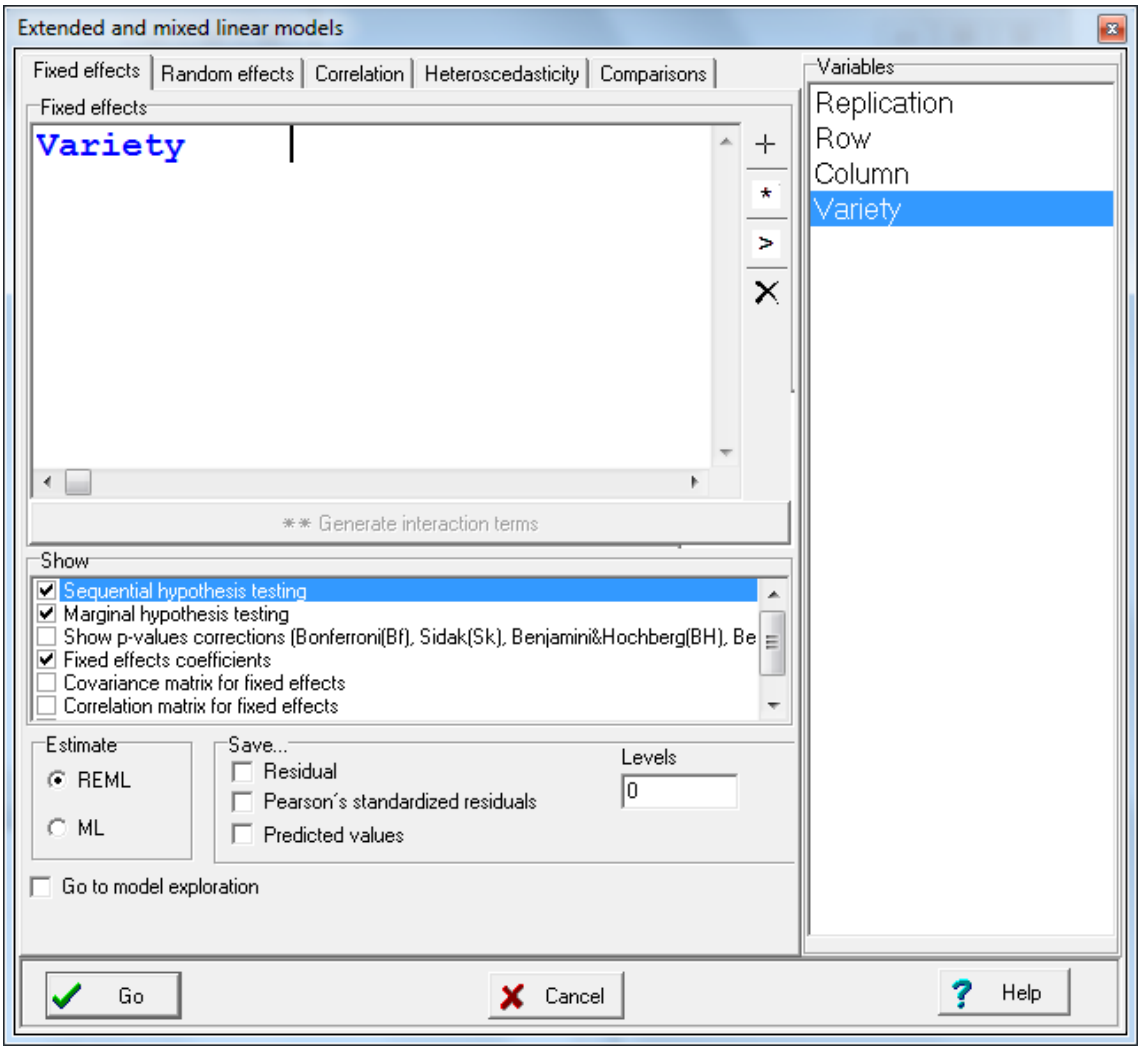



***Figure 146: Window from the Extended and mixed linear model with the Fixed effects tab and the Variety effect for the data in file Lattice row column.IDB2.***

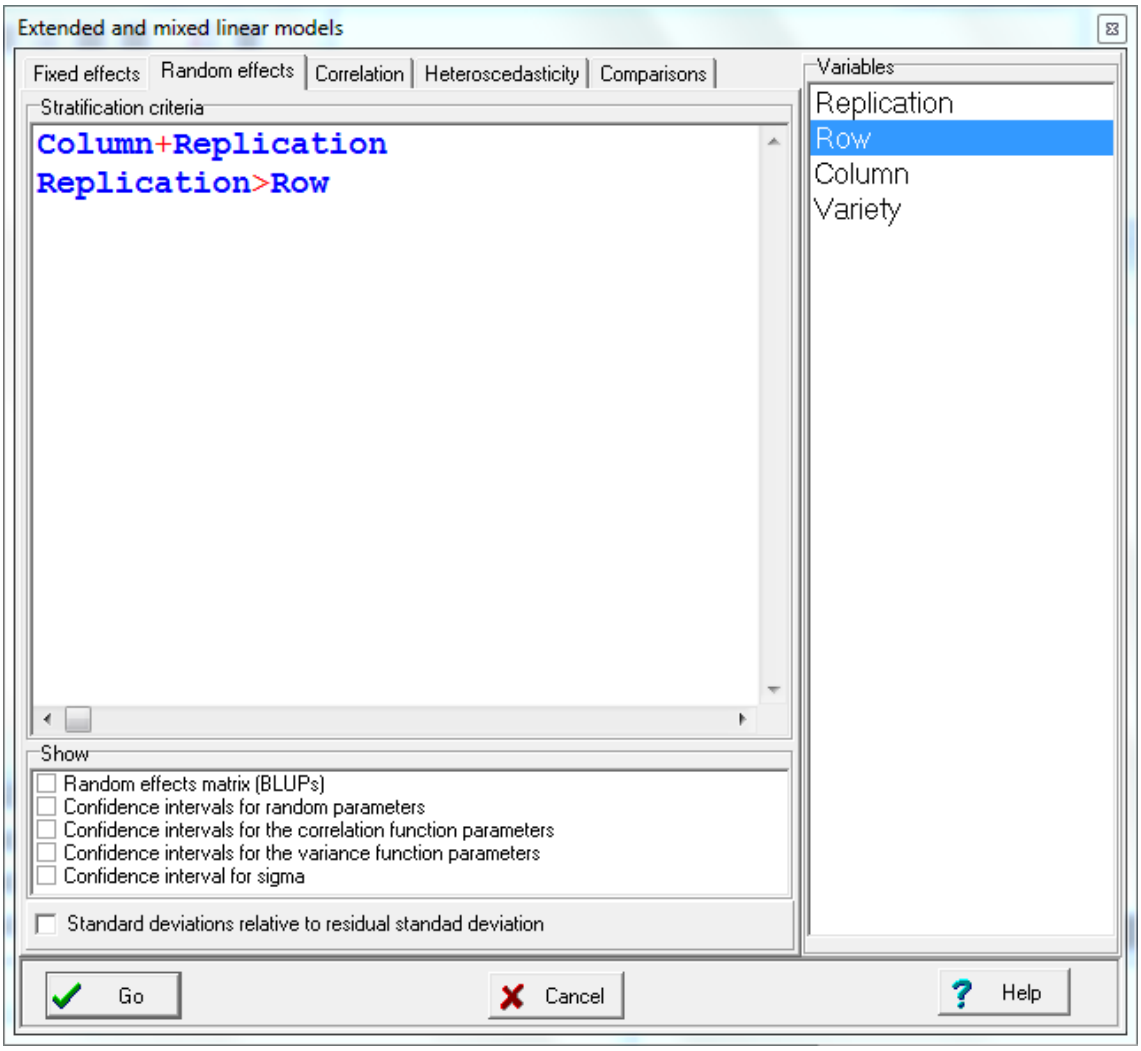



***Figure 147: Window from the Extended and mixed linear model with the Random effects tab and the Column, Row and Replication effects for the data in file Lattice row column.IDB2.***

The following is the output corresponding to this model.

```
Extended and mixed linear models



R specification of the model

model.016_Yield_REML<-lme(Yield~1+Variety
,random=list(.U.=pdBlocked(list(pdIdent(~Column-1)
,pdIdent(~Replication-1)))
,Replication=pdIdent(~Row-1))
,method="REML"
,control=lmeControl(msMaxIter=200)
,na.action=na.omit
,data=R.data16
,keep.data=FALSE)

Results for model: model.016_Yield_REML

Dependent variable: Yield
```

**Fit measurements**

| N | AIC | BIC | logLik | Sigma | R2_0 | R2_1 | R2_2 |
|---|---|---|---|---|---|---|---|
| 150 | 1673.82 | 1768.59 | -802.91 | 140.59 | 0.32 | 0.63 | 0.70 |

*Smaller AIC and BIC is better*

**Marginal hypothesis testing (Type III SS)**

| | numDF | denDF | F-value | p-value |
|---|---|---|---|---|
| (Intercept) | 1 | 116 | 1285.12 | <0.0001 |
| Variety | 29 | 116 | 3.25 | <0.0001 |

**Sequential hypothesis testing**

| | numDF | denDF | F-value | p-value |
|---|---|---|---|---|
| (Intercept) | 1 | 116 | 1285.12 | <0.0001 |
| Variety | 29 | 116 | 3.25 | <0.0001 |

**Fixed effects**

| | Value | Std.Error | DF | t-value | p-value |
|---|---|---|---|---|---|
| (Intercept) | 1929.15 | 86.43 | 116 | 22.32 | <0.0001 |
| Variety10 | 271.61 | 91.94 | 116 | 2.95 | 0.0038 |
| Variety11 | 100.51 | 91.90 | 116 | 1.09 | 0.2763 |
| Variety12 | -12.83 | 92.68 | 116 | -0.14 | 0.8902 |
| Variety13 | 339.68 | 92.68 | 116 | 3.66 | 0.0004 |
| Variety14 | 97.63 | 91.90 | 116 | 1.06 | 0.2903 |
| Variety15 | 295.71 | 91.94 | 116 | 3.22 | 0.0017 |
| Variety16 | 15.66 | 92.73 | 116 | 0.17 | 0.8662 |
| Variety17 | 14.84 | 92.68 | 116 | 0.16 | 0.8730 |
| Variety18 | 165.33 | 91.90 | 116 | 1.80 | 0.0746 |
| Variety19 | 161.42 | 91.94 | 116 | 1.76 | 0.0818 |
| Variety2 | 48.63 | 91.94 | 116 | 0.53 | 0.5979 |
| Variety20 | 185.26 | 91.94 | 116 | 2.01 | 0.0462 |
| Variety21 | -126.88 | 91.94 | 116 | -1.38 | 0.1702 |
| Variety22 | 113.80 | 91.94 | 116 | 1.24 | 0.2183 |
| Variety23 | 142.67 | 91.90 | 116 | 1.55 | 0.1233 |
| Variety24 | 64.77 | 91.94 | 116 | 0.70 | 0.4826 |
| Variety25 | 288.17 | 91.94 | 116 | 3.13 | 0.0022 |
| Variety26 | 232.68 | 91.94 | 116 | 2.53 | 0.0127 |
| Variety27 | 347.24 | 92.68 | 116 | 3.75 | 0.0003 |
| Variety28 | 234.60 | 91.94 | 116 | 2.55 | 0.0120 |
| Variety29 | 232.48 | 91.94 | 116 | 2.53 | 0.0128 |
| Variety3 | 18.77 | 92.73 | 116 | 0.20 | 0.8400 |
| Variety30 | 196.12 | 92.73 | 116 | 2.11 | 0.0366 |
| Variety4 | 63.15 | 91.94 | 116 | 0.69 | 0.4936 |
| Variety5 | 173.95 | 92.68 | 116 | 1.88 | 0.0630 |
| Variety6 | 290.18 | 92.73 | 116 | 3.13 | 0.0022 |
| Variety7 | 149.96 | 91.94 | 116 | 1.63 | 0.1056 |
| Variety8 | 281.10 | 91.94 | 116 | 3.06 | 0.0028 |
| Variety9 | 115.42 | 91.94 | 116 | 1.26 | 0.2119 |

**Random effects parameters**

*Covariance model for random effects: pdBlocked*
*Formula: ~Column + Replication - 1*

*Standard deviations and correlations*

| | S.D. |
|---|---|
| Column1 | 111.22 |
| Column2 | 111.22 |
| Column3 | 111.22 |
| Column4 | 111.22 |
| Column5 | 111.22 |
| Replication1 | 58.01 |
| Replication2 | 58.01 |
| Replication3 | 58.01 |
| Replication4 | 58.01 |
| Replication5 | 58.01 |

*Covariance model for random effects: pdIdent*
*Formula: ~Row - 1|Replication*

*Standard deviations and correlations*

| | S.D. |
|---|---|
| 1 | 50.57 |
| 10 | 50.57 |
| 11 | 50.57 |
| 12 | 50.57 |
| 13 | 50.57 |
| 14 | 50.57 |
| 15 | 50.57 |
| 16 | 50.57 |
| 17 | 50.57 |
| 18 | 50.57 |
| 19 | 50.57 |
| 2 | 50.57 |
| 20 | 50.57 |
| 21 | 50.57 |
| 22 | 50.57 |
| 23 | 50.57 |
| 24 | 50.57 |
| 25 | 50.57 |
| 26 | 50.57 |
| 27 | 50.57 |
| 28 | 50.57 |
| 29 | 50.57 |
| 3 | 50.57 |
| 30 | 50.57 |
| 4 | 50.57 |
| 5 | 50.57 |
| 6 | 50.57 |
| 7 | 50.57 |
| 8 | 50.57 |
| 9 | 50.57 |

**Adjusted means and standard error for Variety**
*LSD Fisher (Alpha:=0.05)*
*p-value correction procedure: No*

| Variety | Means | S.E. | | | |
|---|---|---|---|---|---|
| 27 | 2276.39 | 86.43 | A | | |
| 13 | 2268.83 | 86.43 | A | B | |
| 15 | 2224.86 | 86.43 | A | B | C |
| 6 | 2219.33 | 86.43 | A | B | C |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 25 | 2217.33 | 86.43 | A | B | C | | | | | | |
| 8 | 2210.25 | 86.43 | A | B | C | D | | | | | |
| 10 | 2200.76 | 86.43 | A | B | C | D | E | | | | |
| 28 | 2163.75 | 86.43 | A | B | C | D | E | F | | | |
| 26 | 2161.84 | 86.43 | A | B | C | D | E | F | | | |
| 29 | 2161.63 | 86.43 | A | B | C | D | E | F | | | |
| 30 | 2125.28 | 86.43 | A | B | C | D | E | F | G | | |
| 20 | 2114.41 | 86.43 | A | B | C | D | E | F | G | | |
| 5 | 2103.10 | 86.43 | A | B | C | D | E | F | G | H | |
| 18 | 2094.48 | 86.43 | A | B | C | D | E | F | G | H | I |
| 19 | 2090.57 | 86.43 | | B | C | D | E | F | G | H | I |
| 7 | 2079.11 | 86.43 | | | C | D | E | F | G | H | I |
| 23 | 2071.82 | 86.43 | | | C | D | E | F | G | H | I |
| 9 | 2044.57 | 86.43 | | | C | D | E | F | G | H | I |
| 22 | 2042.96 | 86.43 | | | C | D | E | F | G | H | I |
| 11 | 2029.67 | 86.43 | | | | D | E | F | G | H | I |
| 14 | 2026.78 | 86.43 | | | | | E | F | G | H | I |
| 24 | 1993.92 | 86.43 | | | | | | F | G | H | I |
| 4 | 1992.30 | 86.43 | | | | | | F | G | H | I |
| 2 | 1977.78 | 86.43 | | | | | | | G | H | I |
| 3 | 1947.92 | 86.43 | | | | | | | G | H | I |
| 16 | 1944.82 | 86.43 | | | | | | | G | H | I |
| 17 | 1944.00 | 86.43 | | | | | | | G | H | I |
| 1 | 1929.15 | 86.43 | | | | | | | | H | I |
| 12 | 1916.32 | 86.43 | | | | | | | | | I |
| 21 | 1802.28 | 86.43 | | | | | | | | | |

*Means with a common letter are not significantly different (p<= 0.05)*

Residual analysis suggests no evidence of violations to assumptions of homoscedasticity or normality (Figure 147), and hence we can recommend the best varieties according to the ranking shown by the LSD test using this model. Varieties 27, 15, 13, 6, 25, 8, 10, 28, 26, 29, 30, 20, 5, and 18 are the ones with the highest yield, and there are no significant differences among them.

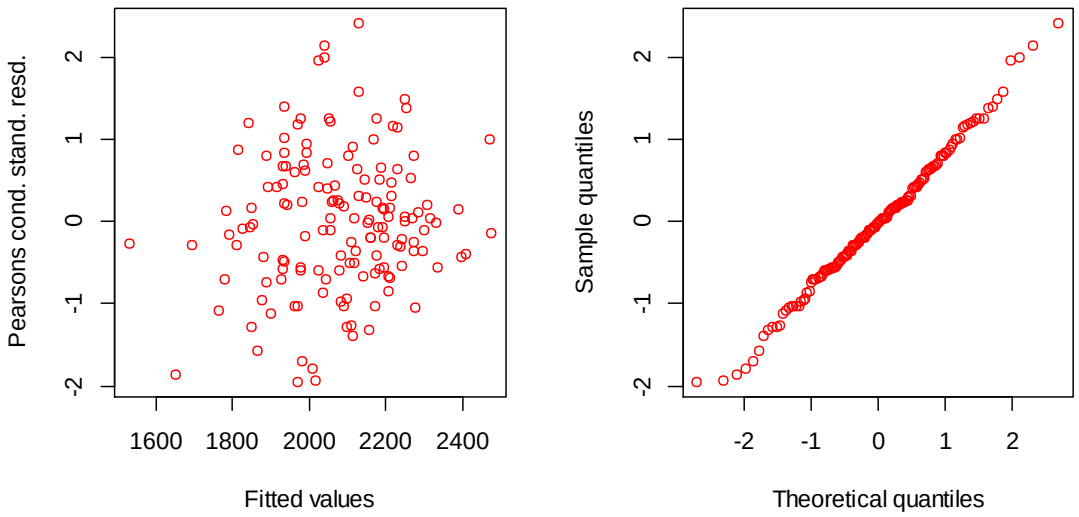



***Figure 148: Graphical tools for diagnostic obtained from the data in file Lattice row column.IDB2.***

### *Balanced squared lattice design*

This example shows data from a trial with 25-wheat variety in 6 repetitions. Rows and columns effects will be accounted for within each square (repetition). Rows and columns constitute incomplete blocks. This trial was conducted in 1976 in Slate Hall Farm, Cambridgeshire, UK (Gleason, 1997). Figure 149 shows the field arrangement. The data are in the file Square lattice.IDB2. In order to analyze these data we first use the variable selector window in *Extended and mixed linear models*. *Yield* is selected as variable, and *Replication*, *Row*, and *Column* as class variables (Figure 150). Then we select *Variety* in the *Fixed effects* tab (Figure 151). The *Random effects* tab is completed as follows: first we include *Repetition*, and then we need to indicate that both *Row* and *Column* are nested within *Repetition* (Figure 152).

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ***1*** | ***2*** | ***4*** | ***3*** | ***5*** | ***19*** | ***23*** | ***2*** | ***6*** | ***15*** | ***18*** | ***25*** | ***9*** | ***11*** | ***2*** |
| ***6*** | ***7*** | ***9*** | ***8*** | ***10*** | ***8*** | ***12*** | ***16*** | ***25*** | ***4*** | ***5*** | ***7*** | ***16*** | ***23*** | ***14*** |
| ***21*** | ***22*** | ***24*** | ***23*** | ***25*** | ***11*** | ***20*** | ***24*** | ***3*** | ***7*** | ***6*** | ***13*** | ***22*** | ***4*** | ***20*** |
| ***11*** | ***12*** | ***14*** | ***13*** | ***15*** | ***22*** | ***1*** | ***10*** | ***14*** | ***18*** | ***24*** | ***1*** | ***15*** | ***17*** | ***8*** |
| ***16*** | ***17*** | ***19*** | ***18*** | ***9*** | ***5*** | ***9*** | ***13*** | ***17*** | ***21*** | ***12*** | ***19*** | ***3*** | ***10*** | ***21*** |
| ***3*** | ***18*** | ***8*** | ***13*** | ***23*** | ***16*** | ***24*** | ***10*** | ***13*** | ***2*** | ***10*** | ***4*** | ***17*** | ***11*** | ***23*** |
| ***1*** | ***16*** | ***6*** | ***11*** | ***21*** | ***12*** | ***20*** | ***1*** | ***9*** | ***23*** | ***12*** | ***6*** | ***24*** | ***18*** | ***5*** |
| ***5*** | ***20*** | ***10*** | ***15*** | ***25*** | ***4*** | ***7*** | ***18*** | ***21*** | ***15*** | ***19*** | ***13*** | ***1*** | ***25*** | ***7*** |
| ***2*** | ***17*** | ***7*** | ***12*** | ***22*** | ***25*** | ***2*** | ***14*** | ***17*** | ***6*** | ***21*** | ***20*** | ***8*** | ***2*** | ***14*** |
| ***4*** | ***19*** | ***9*** | ***14*** | ***24*** | ***8*** | ***11*** | ***22*** | ***5*** | ***19*** | ***3*** | ***22*** | ***15*** | ***9*** | ***16*** |

***Figure 149: Layout of the design for the 25-variety wheat trial conducted in a square lattice whit six replications (squared) and numbers of each cell represent the varieties.***

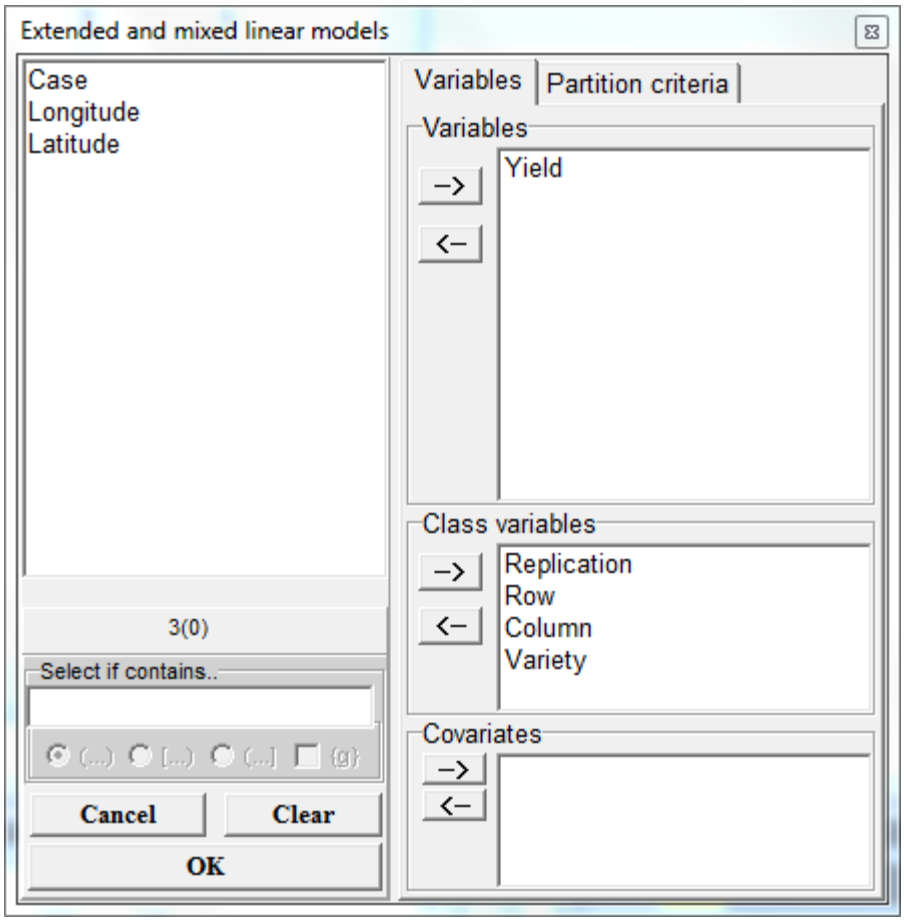



***Figure 150: Window from the Extended and mixed linear model with the variable selection for data in file Square lattice.IDB2.***

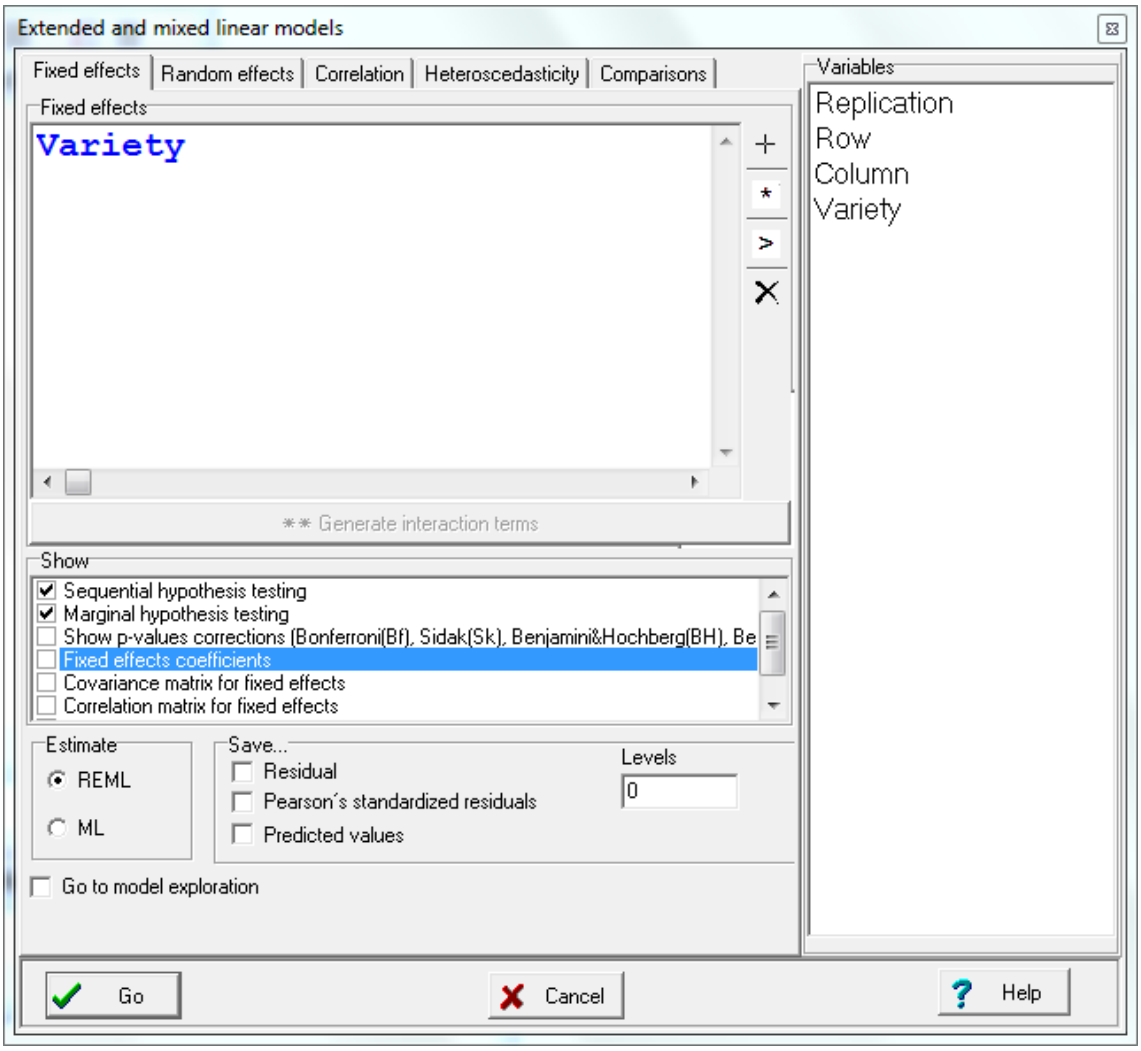



***Figure 151: Window from the Extended and mixed linear model with the Fixed effect tab for data in file Square lattice.IDB2.***

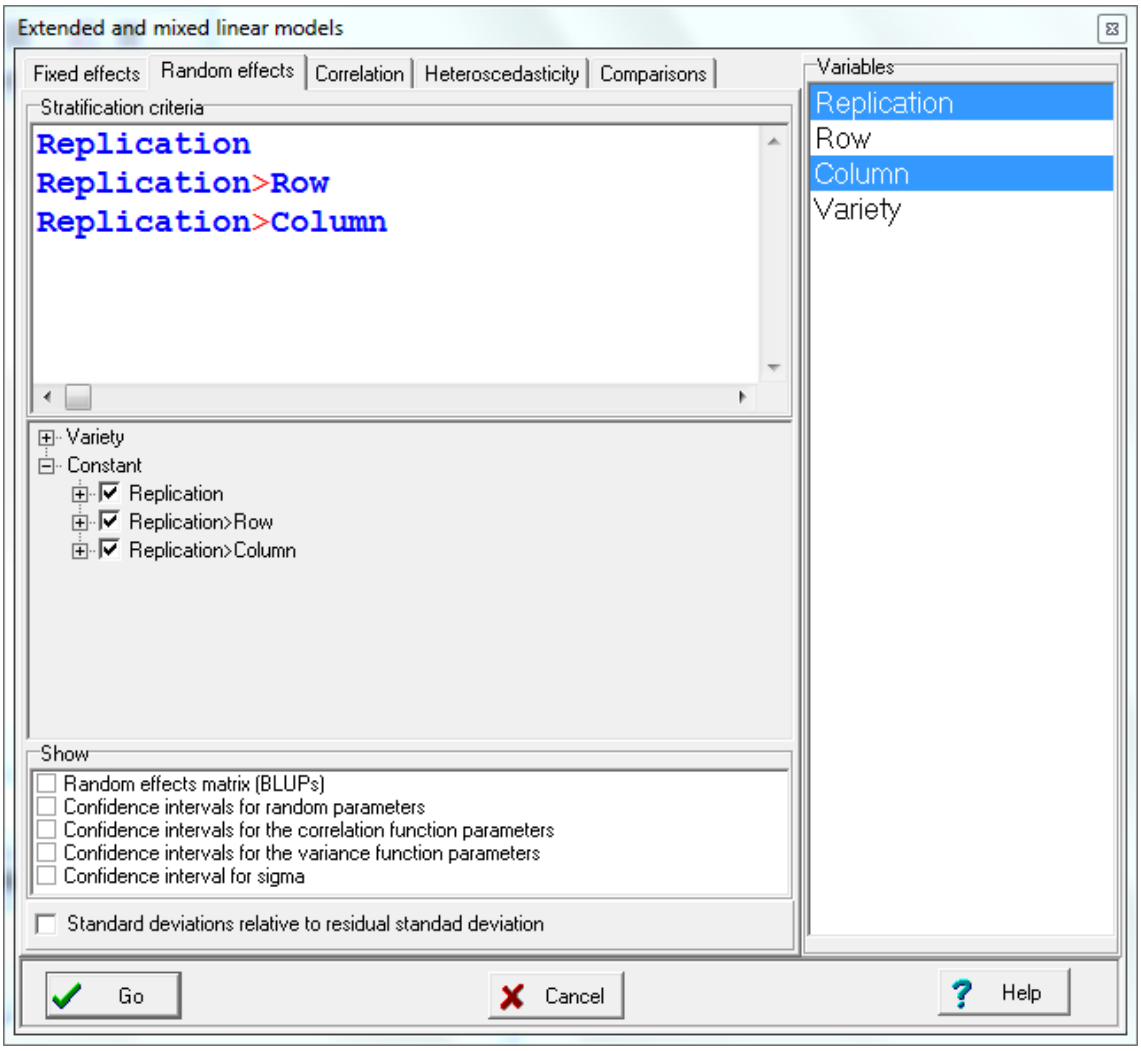



***Figure 152: Window from the Extended and mixed linear model with the Random effect tab for data in file Square lattice.IDB2.***

```
Extended and mixed linear models



R specification of the model

model.017_Yield_REML<-lme(Yield~1+Variety
,random=list(Replication=pdIdent(~1)
,Replication=pdIdent(~Row-1)
,Replication=pdIdent(~Column-1))
,method="REML"
,control=lmeControl(msMaxIter=200)
,na.action=na.omit
,data=R.data17
,keep.data=FALSE)

Results for model: model.017_Yield_REML

Dependent variable: Yield
```

**Fit measurements**

| N | AIC | BIC | logLik | Sigma | R2_0 | R2_1 | R2_2 | R2_3 |
|---|---|---|---|---|---|---|---|---|
| 150 | 1703.31 | 1785.33 | -822.65 | 89.79 | 0.27 | 0.38 | 0.67 | 0.92 |

*Smaller AIC and BIC is better*

**Marginal hypothesis testing (Type III SS)**

| | numDF | denDF | F-value | p-value |
|---|---|---|---|---|
| (Intercept) | 1 | 120 | 1216.28 | <0.0001 |
| Variety | 24 | 120 | 8.84 | <0.0001 |

**Sequential hypothesis testing**

| | numDF | denDF | F-value | p-value |
|---|---|---|---|---|
| (Intercept) | 1 | 120 | 1216.28 | <0.0001 |
| Variety | 24 | 120 | 8.84 | <0.0001 |

**Random effects parameters**

*Covariance model for random effects: pdIdent*
*Formula: ~1|Replication*

*Standard deviations and correlations*

| | (const) |
|---|---|
| (const) | 65.29 |

*Covariance model for random effects: pdIdent*
*Formula: ~Row - 1|Replication*

*Standard deviations and correlations*

| | S.D. |
|---|---|
| 1 | 124.88 |
| 2 | 124.88 |
| 3 | 124.88 |
| 4 | 124.88 |
| 5 | 124.88 |

*Covariance model for random effects: pdIdent*
*Formula: ~Column - 1|Replication*

*Standard deviations and correlations*

| | S.D. |
|---|---|
| 1 | 121.70 |
| 2 | 121.70 |
| 3 | 121.70 |
| 4 | 121.70 |
| 5 | 121.70 |

**Adjusted means and standard error for Variety**
*LSD Fisher (Alpha:=0.05)*
*p-value correction procedure: No*

| Variety | Means | S.E. | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Var19 | 1669.55 | 60.20 | A | | | | | | | | | |
| Var22 | 1644.38 | 60.20 | A | B | | | | | | | | |
| Var20 | 1639.95 | 60.20 | A | B | | | | | | | | |
| Var25 | 1630.63 | 60.20 | A | B | | | | | | | | |
| Var13 | 1619.04 | 60.20 | A | B | C | | | | | | | |
| Var18 | 1592.18 | 60.20 | A | B | C | D | | | | | | |
| Var02 | 1549.01 | 60.20 | A | B | C | D | E | | | | | |
| Var24 | 1546.47 | 60.20 | | B | C | D | E | | | | | |
| Var05 | 1533.27 | 60.20 | | B | C | D | E | F | | | | |
| Var06 | 1527.41 | 60.20 | | B | C | D | E | F | | | | |
| Var17 | 1498.17 | 60.20 | | | C | D | E | F | G | | | |
| Var15 | 1498.01 | 60.20 | | | C | D | E | F | G | | | |
| Var21 | 1493.44 | 60.20 | | | | D | E | F | G | | | |
| Var12 | 1483.79 | 60.20 | | | | D | E | F | G | | | |
| Var08 | 1457.37 | 60.20 | | | | | E | F | G | H | | |
| Var04 | 1451.86 | 60.20 | | | | | E | F | G | H | I | |
| Var03 | 1420.93 | 60.20 | | | | | | F | G | H | I | J |
| Var07 | 1400.73 | 60.20 | | | | | | | G | H | I | J |
| Var16 | 1346.15 | 60.20 | | | | | | | | H | I | J |
| Var23 | 1329.11 | 60.20 | | | | | | | | | I | J |
| Var11 | 1327.25 | 60.20 | | | | | | | | | | J |
| Var14 | 1326.65 | 60.20 | | | | | | | | | | J |
| Var09 | 1298.86 | 60.20 | | | | | | | | | | J |
| Var01 | 1283.59 | 60.20 | | | | | | | | | | |
| Var10 | 1193.22 | 60.20 | | | | | | | | | | |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | K | L | |
| | | | | | | | | | | K | L | |
| | | | | | | | | | | | | L |

*Means with a common letter are not significantly different (p<= 0.05)*

As shown in the section *Use of mixed models to control spatial variability in agricultural experiments*, an alternative way of modelling this type of trials is to use the location in the plot space (in all lattice designs plots are of identical size and in a rectangular array) as covariates to adjust a spatial correlation function. Assuming that the field arrangement of the trial is as shown in Figure 149, the file Square lattice.IDB2 contains two variables, *Latitude* and *Longitude*, that can be used for this. In order to fit this alternative model, variables are selected as shown in Figure 153. Nothing is selected in the *Random effects* tab, and *Latitude* and *Longitude* are entered in the *Correlations* tab, as shown in Use of mixed models to control spatial variability in agricultural experiments, page 136.

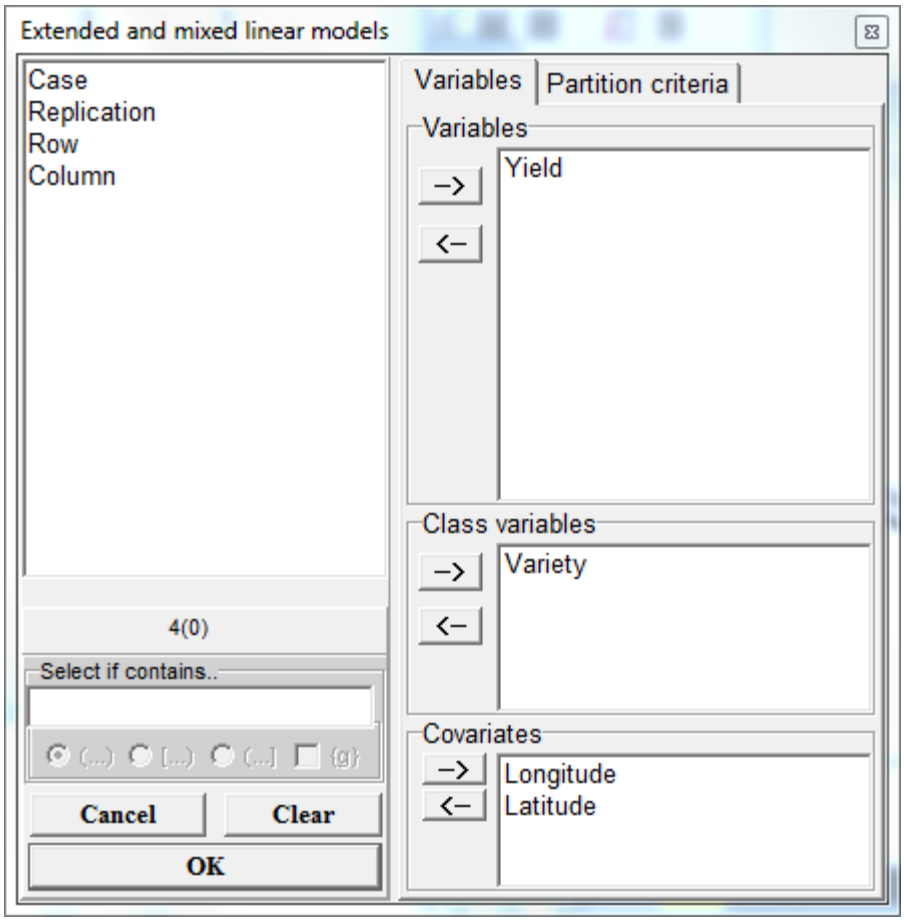



***Figure 153: Window from the Extended and mixed linear model with the selected variables for spatial analysis for data in file Square lattice.IDB2.***

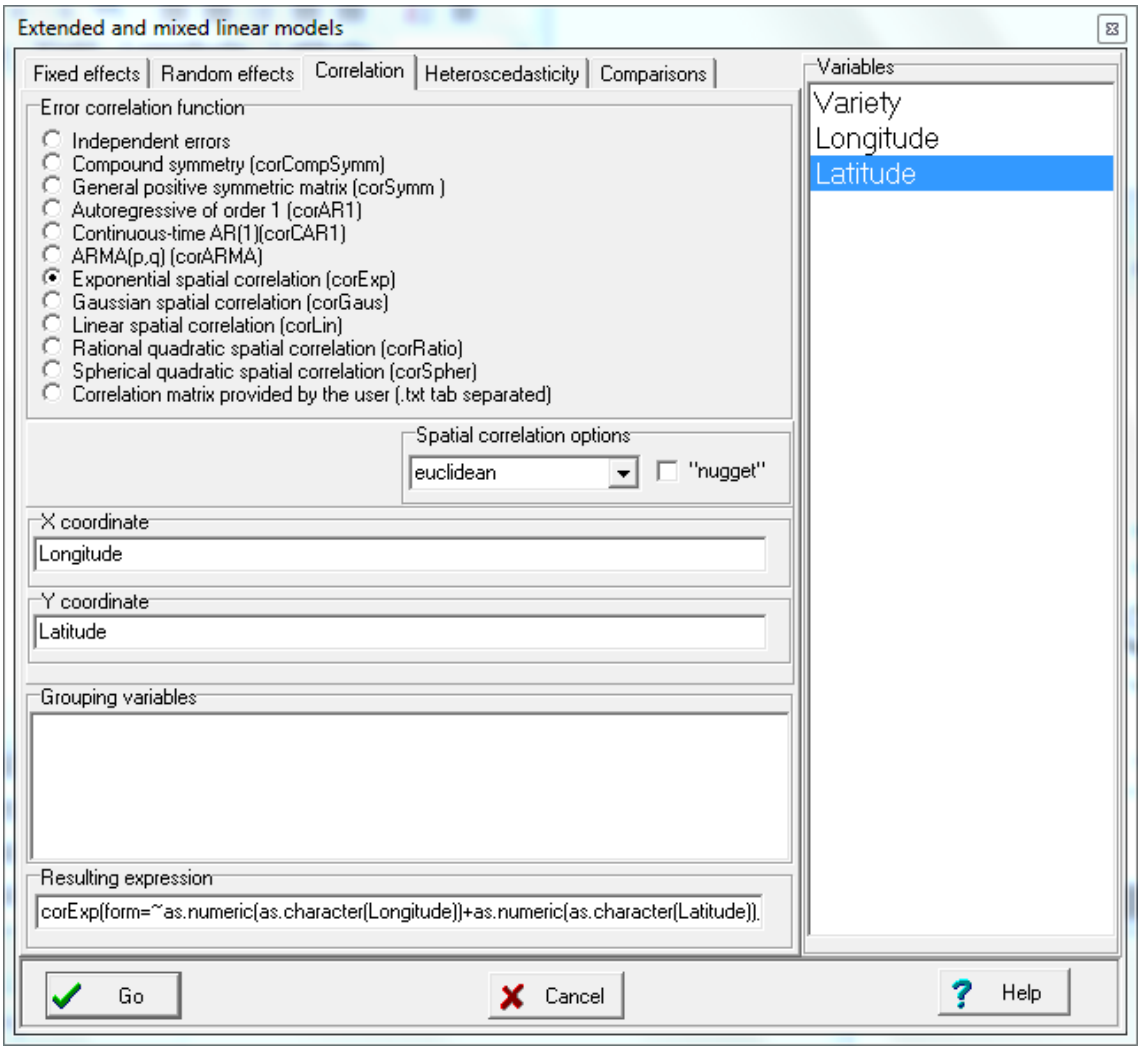



***Figure 154: Window from the Extended and mixed linear model with the Correlation tab and the inclusion of Latitude and Longitude for data in file Square lattice.IDB2.***

```
Extended and mixed linear models


R specification of the model

model.018_Yield_REML<-gls(Yield~1+Variety
,correlation=corExp(form=~as.numeric(as.character(Longitude))+as.numer
ic(as.character(Latitude))
,metric="euclidean"
,nugget=FALSE)
,method="REML"
,na.action=na.omit
,data=R.data18)

Results for model: model.018_Yield_REML

Dependent variable: Yield

Fit measurements
```

| N | AIC | BIC | logLik | Sigma | R2_0 |
|---|---|---|---|---|---|
| 150 | 1692.55 | 1768.92 | -819.28 | 212.96 | 0.27 |

*Smaller AIC and BIC is better*

**Marginal hypothesis testing (Type III SS)**

| | numDF | F-value | p-value |
|---|---|---|---|
| (Intercept) | 1 | 399.80 | <0.0001 |
| Variety | 24 | 7.70 | <0.0001 |

**Sequential hypothesis testing**

| | numDF | F-value | p-value |
|---|---|---|---|
| (Intercept) | 1 | 393.70 | <0.0001 |
| Variety | 24 | 7.70 | <0.0001 |

**Correlation structure**

*Correlation model: Exponential spatial correlation*
*Formula: ~ as.numeric(as.character(Longitude)) + as.numeric(as.character(Latitude))*
*Metric: euclidean*

*Model parameters*

| Parameter | Estim. |
|---|---|
| range | 2.45 |

**Adjusted means and standard error for Variety**

*LSD Fisher (Alpha:=0.05)*
*p-value correction procedure: No*

| Variety | Means | S.E. | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Var19 | 1664.57 | 86.82 | A | | | | | | |
| Var20 | 1659.01 | 87.25 | A | | | | | | |
| Var13 | 1626.37 | 87.32 | A | B | | | | | |
| Var22 | 1589.59 | 87.21 | A | B | C | | | | |
| Var06 | 1552.97 | 86.99 | A | B | C | D | | | |
| Var24 | 1550.00 | 87.19 | A | B | C | D | | | |
| Var25 | 1544.63 | 87.50 | A | B | C | D | | | |
| Var18 | 1537.52 | 87.20 | A | B | C | D | E | | |
| Var17 | 1535.60 | 87.60 | A | B | C | D | E | | |
| Var02 | 1531.18 | 86.48 | A | B | C | D | E | | |
| Var21 | 1510.73 | 87.08 | | B | C | D | E | F | |
| Var05 | 1477.87 | 86.64 | | | C | D | E | F | |
| Var08 | 1473.21 | 87.17 | | | C | D | E | F | |
| Var12 | 1456.69 | 87.40 | | | C | D | E | F | G |
| Var15 | 1422.74 | 87.02 | | | | D | E | F | G | H |
| Var03 | 1407.62 | 86.78 | | | | | E | F | G | H |
| Var04 | 1399.11 | 86.90 | | | | | E | F | G | H |
| Var07 | 1389.06 | 87.46 | | | | | | F | G | H |
| Var16 | 1332.43 | 86.72 | | | | | | | G | H | I |
| Var14 | 1327.22 | 87.19 | | | | | | | G | H | I |
| Var11 | 1326.39 | 87.04 | | | | | | | G | H | I |
| Var23 | 1306.36 | 87.02 | | | | | | | | H | I |
| Var09 | 1289.71 | 86.66 | | | | | | | | H | I |
| Var01 | 1231.80 | 86.54 | | | | | | | | | I |
| Var10 | 1201.09 | 87.28 | | | | | | | | | I |

*Means with a common letter are not significantly different (p<= 0.05)*

In order to compare the fit of both models, we can compare their AICs or BICs (since one of the models is not a special case of the other, we cannot conduct a likelihood ratio test to compare them). The following are the values of AIC and BIC:

| Model | AIC | BIC |
|---|---|---|
| IBD | 1703.31 | 1785.33 |
| Spatial correlation | 1692.55 | 1768.92 |

From these results, the spatial correlation model fits the data better. Nevertheless, if we compute the average standard errors of the difference of means for both models we find that the spatial correlation model yields a value of 69.118, while the model considering the design structure yields a value of 62.019. Hence if the goal is to compare variety means, the model considering the design structure is more appropriate.

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