Adequacy of Different Experimental Designs for Eucalyptus Spacing Trials in Portuguese Environmental Conditions

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Abstract.—In Portugal, several eucalyptus spacing trials cover a relatively broad range of experimental designs: trials with a non-randomized block design with plots of different size and number of trees per plot; trials based on a non-systematic design in which spacings were randomized resulting in a factorial arrangement with plots of different size and shape and equal number of trees per plot; trials based on a completely randomized block design with different plot sizes and different numbers of trees per plot. Limitations and advantages of these experimental designs, when applied to Portuguese climatic and edaphic conditions, are discussed based on theoretical principles and empirical observations. This analysis suggests that, when the main objectives are to improve databases and to sample extremes of stand conditions for modeling purposes, more importance should be placed on the practical consequences in the prediction of stand characteristics than on the theoretical aspects.

Portugal, located between the parallels 42º and 37º north and the meridians 9º and 6º west of Greenwich, is the most occidental country of the European Continent. Continental Portugal has an area of 8.900×103 ha. The Atlantic Ocean limits Portugal along the west coast at an extent of 848 km, while the terrestrial frontier with Spain is 1,232 km long. The Portuguese climate can be considered Mediterranean tempered by oceanic influence, with long hot and dry summers; the Mediterranean influence is particularly dominant in the southern half of the country. Portugal presents an evident contrast between the north and the south of the Tagus River (Alves *et al.* 1990): to the north, the average altitude is 421 m and increases to more than 400 m in 47.6 percent of the area; to the south, the average altitude is only 169 m and it is lower than 400 m in 97 percent of the area. Portugal also spans a great diversity of geological formations. The half-arid and dry sub-humid areas present, in general, soils with medium to strong slopes that are poor in organic matter and have a low capacity to retain and to store water. Low to medium fertility and a high probability of erosion normally characterize these Leptosols, Cambiosols, and Luvisols. Soils with high potential production and great resistance (Fluvisols, Vertisols, Calcisols, and Podzols) occur only to a small extent in Portugal (DGF 1998).

Eucalyptus globulus, a fast-growing tree species with free growth without pre-formed overwintering buds, was introduced in Portugal 150 years ago. At present, it is the third most represented forest species in Portugal, covering 20.7 percent of the total forestland and occupying an area of 3,358.8×103 ha (DGF 1998). The success of eucalyptus was a consequence, in part, of the adequacy of good environmental conditions in a substantial part of the country for eucalyptus growth. In fact, eucalyptus species are highly productive even in areas where drought and nutrient stress occur, in spite of the fact that their productivity is strongly dependent on soil water and nutrient availability (Florence 1996, Pereira 1989). In Portugal, eucalyptus plantations are mainly used by the pulp industry and are intensively managed as a short-rotation coppice system, in which the first cycle of planted seedlings (single stem) is followed by two or three coppiced stands, with an average cutting cycle of 10 years. To be able to make accurate growth predictions, data from permanent plots and experimental trials (where there is some control of the factors influencing tree growth) are needed. The spacing trials allow for the study of the relation between stand development and stand density. This knowledge is crucial for the silviculture of short-rotation crops, in which the main purpose is the quick occupation of the site and exploitation of its available resources to maximize production, within the limits of sustainable forest management. The Stora Celbi Company established the first spacing trial in 1975 at the Furadouro site (center of Portugal and close to the sea). In 1993, the Department of Forestry initiated a research project mainly to study the influence of spacing on the growth and productivity of eucalyptus stands on different sites. Seven spacing trials were installed in different

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regions and over different time periods (only four survived!). Problems finding an adequate experimental design began. Different experimental designs were tried. In the following paragraphs, we present some of the problems and/or benefits that were found in relation to each one of the experimental designs that were tried.

REFLECTIONS

What is Desirable in a Spacing Trial?

To be able to estimate the behavior and the production of future stands, it is necessary to have both stand simulators and silviculture experiments that will allow for the definition of the relationships between growth, stand conditions, and stand treatments. These experiments help to improve databases and to sample extremes of stand conditions that are essential even if they will never be applied; they are fundamental to properly define the response surface for growth models (Vanclay 1994). The analysis of tree growth response to stand density level implies, compulsorily, the installation of spacing trials and therefore the selection of an experimental design. Questions arise that are related not only to the experimental design but also to the number of trees per plot and the plot size: (1) different number of plants per plot in plots with the same size; (2) equal number of measured plants per plot in plots with the same size; (3) equal number of plants in plots with different sizes. Lin and Morse (1975) stated that, from the statistical point of view, a choice must be made between (1) and (2), but from the economic point of view, option (3) is much more attractive. If the main objective is to analyze the influence of spacing in stand yield, the quality of the yield estimation per hectare will be better when based on plots of the same size. When plots of different sizes are selected, the statistical analysis can become complex if the variance components cannot be assumed to be homogeneous.

The definition of the plot size is an ambiguous point: plots cannot be excessively small, because that results in biased estimates of the stand parameters, but they must be sufficiently small to guarantee homogeneity within the block. Very small plot sizes are undesirable and sometimes unusable as a basis for growth models and for estimations of mortality and diameter distributions (Curtis 1983). Small plots can also result in biased site index estimates, especially when the dominant height is based on a number of the largest dbh trees per unit area. Curtis (1983) recommended that a site index estimate should not be based on fewer than four trees per plot. However, to minimize the experimental error, homogeneous plots are required and that means minimizing within-plot variance (Vanclay 1994). This homogeneity is related not only to the natural site conditions but also to the site preparation methods. Site preparation machines can restrict the distances between rows especially if deep mobilizations or subsoil plowing are the options; the minimum distance in which they normally can operate is 3 m between rows. Experimental designs that require a large number of plots within homogeneous stand conditions must be avoided because that forces the use of small plots. The plots must be subjectively located with subsequent random assignment of treatments and must be installed with buffer strips between adjacent treatments.

The experimental designs normally used in spacing trials can be systematic (e.g., Nelder 1963), non-systematic with a factorial base (Lin and Morse 1975), or non-systematic with randomized blocks (e.g., Montgomery 1991). However, the validity of a certain experimental design can only be assessed in relation to the uses to which it is put (Mead 1979, Cousens 1996). Systematic designs allow the use of a wide range of spacings in a single experimental trial, which is most adequate if the space and the plant material are limited. They can be used to define the optimal spacing or the effect of rectangularity, but presumably they are not the most adequate because of the asymmetric density around each tree (Amateis *et al.* 1988). The area per plant and/or the rectangularity of the space available to a plant changes in some consistent fashion over the different parts of the grid of plants (Nelder 1962). Systematic designs are efficient when the explanation of the relationships is of primary importance using regression techniques (Amateis *et al.* 1988). One advantage of the randomized block design is that randomized designs provide, when the plot-treatment condition holds, an unbiased estimate of the variance of treatments effects, also giving unbiased estimates of the mean (Nelder 1962). The randomization allows for the use of analysis of variance techniques as well as the fitting of response surfaces with regression methods (Amateis *et al.* 1988). Nelder (1962) referred to some disadvantages of randomization when applied to spacing trials: the difficulty to put together all the plots if a square plot pattern and an equal number of trees per plot were selected; the large number of trees associated with the close spacings when plots of the same size were selected. He also noted the difficulty of maintaining small blocks when using buffer trees between treatments.

The Spacing Trials in Portugal

Several eucalyptus spacing trials in Portugal were installed by pulp companies or by the Department of Forestry in collaboration with these companies (table 1). Except for the Alto do Vilão trial, an equal number of trees per plot was used within a trial; the number of measured trees per plot ranged from 16 to 360. This work focuses on some of the spacing trials installed by the Department of Forestry and by the Stora Celbi Company.

Table 1.—*Principal characteristics of the eucalyptus spacing trials in Portugal*

Designation	Responsible	Year of plantation	Number blocks	Number plots/block	Nº of measured trees/plot	Spacings (m×m)
Alto Vilão	Stora Celbi	1975	2	5	80-360	3×2 3×3 4×3 4×4 4×5
Qta Paço	Stora Celbi	1990	2	5	196	2×1 3×1 2×2 3×2 3×3
Cabroeira	Silvicaima	1990	2	5	16	3×1.5 3×2 3×2.5 3×3 3×3.5
Oliveirinha	Silvicaima	1990	4	6	25	3×1.5 3×2 3×2.5 3×3 3×3.5 3×4
P. Cavaleira	Silvicaima	1990	4	5	16	3×1.5 3×2 3×2.5 3×3 3×3.5
Qta Laurentim	Silvicaima	1992	4	6	25	3×1.5 3×2 3×2.5 3×3 3×3.5 3×4
Vilar Luz	DEF[1]	1993	3	12	64	2×1 2×2 2×3 2×4 3×1 3×2 3×3 3×4 4×1 4×2 4×3 4×4
Ribeiro Seixo	DEF[1]	1994	2	12	64	2×1 2×2 2×3 2×4 3×1 3×2 3×3 3×4 4×1 4×2 4×3 4×4
Vale Bezerra	DEF[1]	1994	2	8	64	3.5×1 3.5×2 3.5×3 3.5×4 4×1 4×2 4×3 4×4
Agolada	DEF[1]	1994	2	7	3×15[2]	1×1 2×1 2×2 3×3 4×1.5 4×2 4×4
Nicolaus	DEF[1]	1996	3	5	4×15[2]	3×0.5 3×1 3×2 3×3 3×4

[1] DEF, Department of Forestry; 2 were established with clonal material, with the clone as a split inside each plot (3 and 4 clones, respectively, in Agolada and Nicolaus).

The experimental designs of the spacing trials listed in table 1 differ (fig. 1):

a) Alto do Vilão: each block is a combination of five plots with different sizes and shapes that were distributed without previous randomization; the blocks are located side by side and the spacings were distributed according to decreasing density in one of the blocks and increasing density in the other block.
b) Quinta do Paço: each block is a combination of five plots with different sizes and shapes that were randomly distributed but restricted by the position of the 2×2 and 2×1 spacings that were placed side by side.
c) Ribeiro do Seixo, Vale da Bezerra, and Vilar de Luz: randomized block design, with three spacing factors between rows and four spacing factors within rows, resulting in factorial combinations of spacings occupying plots of unequal size with the same number of trees.
d) Agolada: randomized block design with plots of unequal size with the same number of trees.
e) Nicolaus: randomized block design with plots of unequal size with the same number of treesÑthe distance between rows is constant.

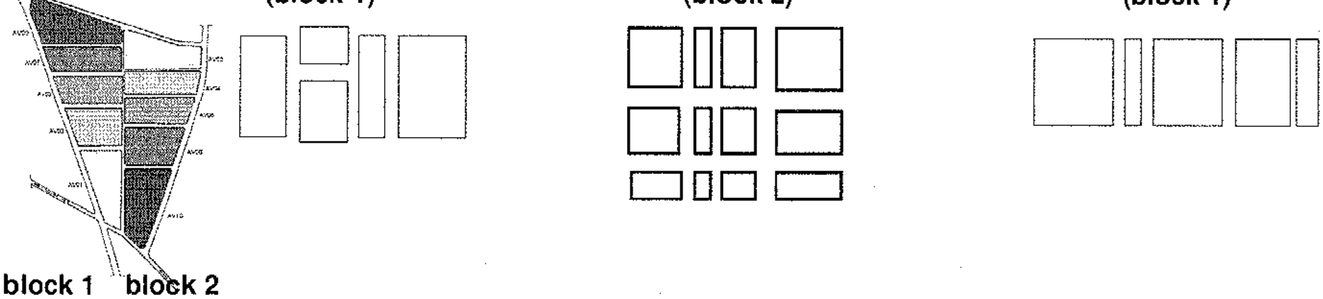


Figure 1.—*Some of the spacing trials in Portugal (all the plots are surrounded by at least two buffer rows).*

The experimental design used in Ribeiro do Seixo, Vale da Bezerra, and Vilar de Luz (randomized block design with factorial combination of row and between-row spacings resulting in plots of unequal size with the same number of trees) was not the most adequate for Portuguese environmental conditions. In fact, the advantages of this design (e.g., the high number of initial densities tested and the opportunity to test the rectangularity effects) are offset by the difficulty of finding a homogeneous block of such dimensions (approximately 1.2 ha). The Vilar de Luz spacing trial is a good example (table 2). It is located on the north coast of Portugal in a region where the water is not a serious restriction during the summer and where the soil presents good conditions for forest growth. When the total block is considered, the dominant trees, defined as the 100 largest trees in dbh per hectare, are located mainly in the plots corresponding to spacings 4×4 and 3×2; some plots have a no or only a small number of dominant trees (4×2 and 3×4). Note that this analysis is based on measurements at a very young age (21 months), before competition is evident. The difficulty of finding homogeneous areas restricts the number of measured trees in each plot. In the closest spacings of these trials, site index estimates are based on only one dominant tree, making it difficult to study the effect of spacing on dominant height, one of the main questions in relation to the effect of spacing on stand parameters.

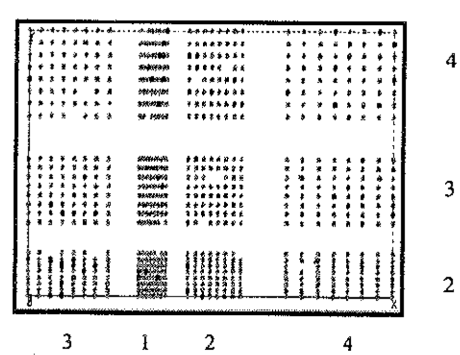
The problem of soil heterogeneity is also evident in the Quinta do Paço trial at a very young age (31 months). When the total area of the plot is considered, in the widest spacing, three of the four dominant trees are located in the same quarter of the plot (table 3). In this trial the influence of the lack of homogeneity is also evident in the mortality. Westoby (1984) noted that a stand with a natural mortality greater than 20 percent can be considered self-thinning. If only a quarter of the plot (III), corresponding to the 2x1 spacing of the Quinta do Paço trial, was considered, it could be concluded that the stand was self-thinning. However, only at 67 months, natural mortality greater than 20 percent was detected for the first time in the whole plot. The influence of soil heterogeneity is also evident in basal area, although less so. In fact, the fewer number of living trees per hectare associated with subplot III was offset by a slight increase in the quadratic mean diameter resulting from the lower intraspecific competition intensity or from the higher site productivity (or a mix of both) (table 3). However, at that age, the effects of the intraspecific competition were not yet evident in the plot.

The soil heterogeneity at a small scale is also evident in the Agolada spacing trial located in the center-south of Portugal. The soils are Luvic Arenosols with several layers of sandy texture intermixed with layers that have a higher content of lime and clay with a high proportion of coarse material of natural quartz (Pacheco et al. 1997), a common soil type in Portugal. This trial has been the object of intensive monitoring, including not only biometric measurements, but also measurement of physiological parameters and soil water. Each block has

Table 2.—*Values of dominant height when the total block and each plot are considered (dominant trees are the 100 largest trees in dbh per hectare)–Vilar de Luz spacing trial at 21 months*

Spacing (m×m)	h_{dom} (m)	$S_{h,t}$ [1]	Number of dominant trees
1x2	6.8	*20.0*	1
1x3	6.3	19.2	2
1x4	5.9	18.4	3
2x2	6.0	18.6	3
2x3	4.6	15.7	4
3x2	5.6	17.8	4
4x2	5.3	17.1	5
2x4	5.5	17.6	5
3x3	4.8	16.1	6
4x3	5.3	17.1	8
3x4	5.9	18.4	8
4x4	5.6	17.8	10
all block	6.6	19.7	58

Position of the dominant trees when the total block is considered

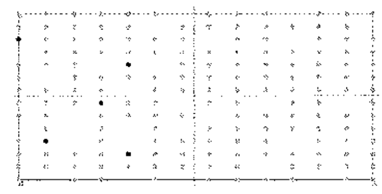


[1] Site index defined as the mean height of the dominant trees, 100 largest trees in dbh per hectare, at 10 years - estimated according to Tomé (1990) and based on the measurement of July 1995 (21 months).

Table 3.—*Values of dominant height when all the plot and fractions of the plot are considered (dominant trees are the 100 largest trees in dbh per hectare)–spacing 2x1 of the Quinta do Paço spacing trial at 31 months*

Spacing 2×1	h_{dom} (m)	$S_{h,t}$ [1]	Number of dominant trees	Mortality (%)	BA[2] (m^2ha^{-1})
I	12.0	24.6	1	14.3	1.91
II	11.0	23.1	1	8.2	1.79
III	13.3	26.4	1	322.4	1.87
IV	11.1	23.3	1	12.2	1.77
all plot	12.3	25.0	4	9.7	1.83

Position of the dominant trees in a plot of 392 m^2



[1] Site index defined as the mean height of the dominant trees, 100 largest trees in dbh per hectare, at 10 years - estimated according to Tomé (1990) and based on the measurement of November 1992 (31 months); 2 basal area; five trees were marked because the fourth and fifth largest trees presented the same dbh.

an area of 0.5 ha and, in spite of the fact that the blocks were established along the contour lines to minimize the heterogeneity inside the blocks, much variability was still found inside each block. Soil heterogeneity effects were particularly evident in block 2 during May and October 1996. In this block, dbh and height growth were higher in the 3×3 spacing than in the 4×4 spacing. In fact, the dbh increment was approximately 8 mm for the 4×4 spacing and 11.5 mm for the 3×3 spacing; the height increment was approximately 20 cm for the 4×4 spacing and 150 cm for the 3×3 spacing. Different soil water profiles and different soil water extraction patterns were the main cause for this unexpected behavior. Figure 2 represents the evolution of the soil water extraction patterns by spacing during the growing season (May until October) up to 2.6 m deep. The pattern associated with the first measurement corresponds to the soil water profile. It was observed that, at 16 months, the volumetric water content along the total profile was different for both spacings, reflecting different growth conditions. An accurate observation showed that plot 3×3 is located above a sandstone layer with very low permeability, maybe with a physical obstacle hindering the lateral movement of water originating from a zone of water accumulation highly favorable to plant growth (Pacheco *et al.* 1997).

The Alto do Vilão trial, established with a non-randomized design, resulted in a good tool to use in analyzing the effect of spacing on dominant height growth. It was decided to install a spacing trial with two blocks located side by side where the spacings were distributed according to decreasing density in one of the blocks and increasing density in the other block (fig. 1). A different relationship between dominant height and spacing was observed in both blocks (fig. 3). In fact, a north/south productivity gradient was detected. When the dominant

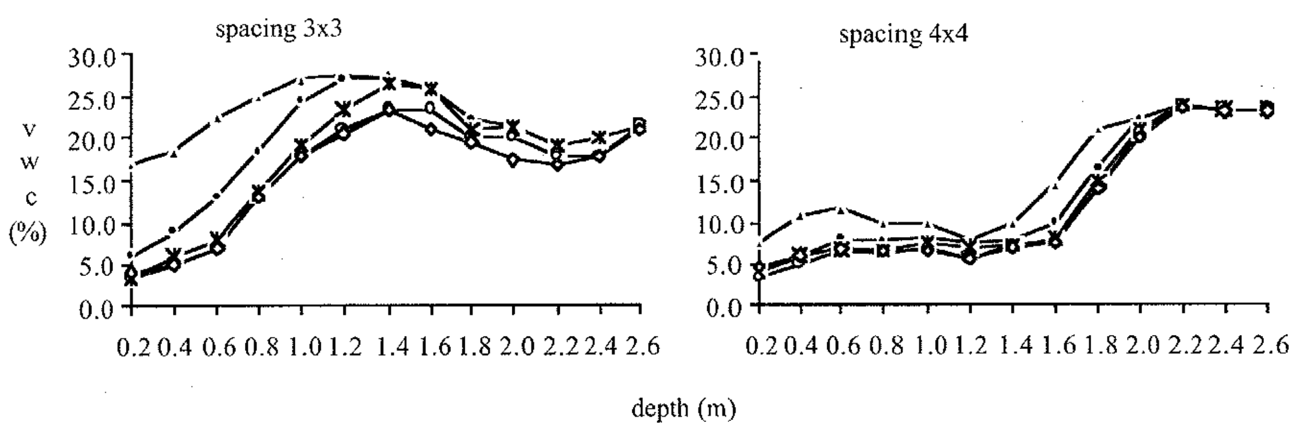


Figure 2.—*Evolution of the pattern of water extraction by spacing during the growing season (May Å until October Ø) up to 2.6 m deep; vwc, volumetric water content (in: Pacheco* et al. *1997).*

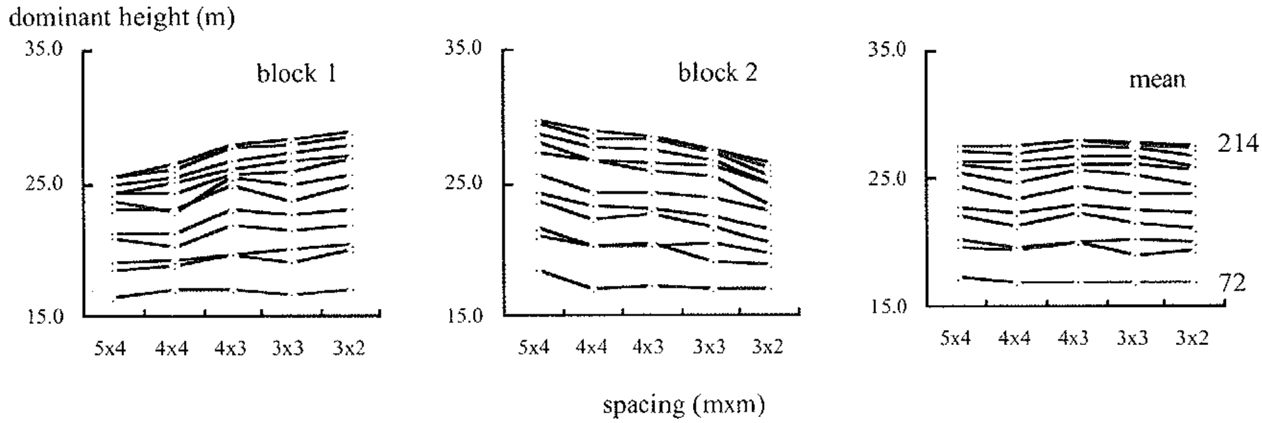


Figure 3.—*Relation between dominant height and spacing at the Alto do Vilão trial (72-214 months).*

height associated with the spacing is estimated by the mean of both blocks, the effect of the productivity gradient is eliminated. The third graph in figure 3 suggests that dominant height is independent of the planting density.

FINAL CONSIDERATIONS

In Portugal, the edaphic and the climatic conditions limit the dimension of the blocks, mainly when the homogeneity within the plots and blocks needs to be guaranteed. This need of homogeneity is particularly important for eucalyptus because it is very sensitive to soil water content. The non-systematic design with a factorial base proposed by Lin and Morse (1975) was the first design selected by the team from the Department of Forestry responsible for the installation of some of the spacing trials. The selection was based on the opportunity to test, in the same trial, a great number of spacings. Four spacing trials were installed, but this design was abandoned after the first measurements. The team found it impossible to guarantee homogeneity in 1.2-ha blocks. Also during this period, strong night-frosts affected the initial development of these trials, and the mortality, although not related to spacing, was not constant within the blocks.

After trying other experimental designs, it was found that a good alternative could be an experimental design based on plots of unequal size but with the same number of measured trees. However, in the closest spacing, more plants should be planted to provide an opportunity to measure at least four dominant trees. Twelve spacing designs should be rejected and instead only five spacings considered. The distance between rows should be fixed within the trial and defined considering the fact that the pulp companies normally weed the stands with mechanical equipment to control weed competition and to prevent forest fires. Three meters between rows is the suggested minimum distance to test. At least two guard rows must buffer each plot.

The choice of the experimental design most adequate to Portuguese conditions cannot be unique. However, when the main objectives are to improve databases and to sample extremes of stand conditions for modeling purposes, more importance should be placed on the practical consequences of predicting stand characteristics than on the theoretical aspects.

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