# Analysis of variance of primary data on plant growth analysis[1]

Adelson Paulo Araújo[2]

Abstract – Plant growth analysis presents difficulties related to statistical comparison of growth rates, and the analysis of variance of primary data could guide the interpretation of results. The objective of this work was to evaluate the analysis of variance of data from distinct harvests of an experiment, focusing especially on the homogeneity of variances and the choice of an adequate ANOVA model. Data from five experiments covering different crops and growth conditions were used. From the total number of variables, 19% were originally homoscedastic, 60% became homoscedastic after logarithmic transformation, and 21% remained heteroscedastic after transformation. Data transformation did not affect the F test in one experiment, whereas in the other experiments transformation modified the F test usually reducing the number of significant effects. Even when transformation has not altered the F test, mean comparisons led to divergent interpretations. The mixed ANOVA model, considering harvest as a random effect, reduced the number of significant effects of every factor which had the F test modified by this model. Examples illustrated that analysis of variance of primary variables provides a tool for identifying significant differences in growth rates. The analysis of variance imposes restrictions to experimental design thereby eliminating some advantages of the functional growth analysis.

Index terms: ANOVA model, statistic, phosphorus, common bean, rice.

**Análise de variância dos dados primários na análise de crescimento vegetal**

Resumo – A análise de crescimento vegetal apresenta dificuldades relacionadas à comparação estatística das curvas de crescimento, e a análise de variância dos dados primários pode orientar a interpretação dos resultados. Este trabalho objetivou avaliar a análise de variância de dados de distintas coletas de um experimento, abordando particularmente a homogeneidade das variâncias e a escolha do modelo adequado de ANOVA. Foram utilizados dados de cinco experimentos com diferentes culturas e condições de crescimento. Do total de variáveis, 19% foram originalmente homocedásticas, 60% tornaram-se homocedásticas após transformação logarítmica, e 21% mantiveram-se heterocedásticas após transformação. A transformação dos dados não afetou o teste F em um experimento, enquanto nos demais experimentos a transformação o modificou geralmente com redução dos efeitos significativos. Mesmo quando a transformação não afetou o teste F, comparações de médias induziram a diferentes interpretações. O modelo de ANOVA misto, considerando a coleta como um efeito aleatório, reduziu o número de efeitos significativos de todos os fatores modificados por este modelo. Alguns exemplos ilustram que a análise de variância dos dados primários constitui uma ferramenta na identificação de efeitos significativos nas taxas de crescimento. A análise de variância impõe restrições ao delineamento experimental, eliminando algumas vantagens da análise funcional de crescimento.

Termos para indexação: modelo de ANOVA, estatística, fósforo, feijão, arroz.

## Introduction

In plant growth analysis, data are usually obtained from successive destructive harvests performed within the plant growth cycle, from which the growth rates are calculated. Two main approaches have been used toward estimating growth rates: in the classical approach, mean values of growth rates are calculated by formulae previously derived, using data of two consecutive harvests, whereas in the functional approach, mathematical functions are fitted throughout the growth data over time, their differentiation providing instantaneous values of growth rates.

[1] Accepted for publication on August 14, 2002. Sponsored by a grant of CNPq.

[2] Universidade Federal Rural do Rio de Janeiro, Dep. de Solos, BR-465, km 7, CEP 23890-000 Seropédica, RJ, Brazil. E-mail: aparaujo@ufrrj.br

The classical method requires that individual replicates of one harvest must be paired with those of another, which may result in an overestimation of the variances of the growth rates (Causton, 1994). The functional approach has some problems related to the choice of the appropriate function and to the statistical comparison of growth rates (Hunt, 1982; Poorter, 1989). Although the statistical comparison of curves fitted to the primary data is feasible (Neter et al., 1990), the statistical comparison of derived rates is a complex task, which has not been solved satisfactorily by some attempts (Hughes & Freeman, 1967; Hunt & Parsons, 1974; Hunt & Evans, 1980; Keuls & Garretsen, 1982; Garretsen & Keuls, 1986; Poorter & Lewis, 1986).

As the statistical comparison of the growth rates presents strong difficulties, both for the classical and functional approaches, the analysis of variance of the primary data could identify differences in the treatments under investigation, guiding the interpretation of the results. However, the analysis of variance of data obtained from different harvests faces inexorably the heterogeneity of the variances, since, in general, plant size increases as the successive measurements are performed. Although the analysis of variance of each separately harvest could be performed, only the analysis of overall data can improve the comprehension of plant growth, identifying the interactions between time and the treatments under study.

One basic assumption of the analysis of variance is that the experimental errors are random, independently and normally distributed, and with a common variance (Steel & Torrie, 1980). The lack of normality is not an important matter, provided the departure from normality is not of extreme form (Neter et al., 1990). Otherwise, when the population variances are unequal, characterizing the heteroscedasticity, size and power of the F test are affected especially under a high variance ratio, demanding some transformation of the data (Zimmermann, 1987). When standard deviations are proportional to the means, the logarithmic transformation usually stabilizes the variances (Neter et al., 1990). Since natural logarithms are necessary for the calculation of the growth rates, they are conventionally used in growth analysis experiments (Hunt, 1982).

Another subject is concerned to the choice of the appropriate model of analysis of variance (ANOVA model). When the factor levels under study are chosen because of intrinsic interest in them and they are not considered as a sample from a larger population, such factor is considered as a fixed effect, and the conclusions will pertain to just those factor levels included in the study; when the factor levels constitute a sample from a larger population and interest is in the larger population, such factor is considered as a random effect, and the conclusions can be extended to the population (Neter et al., 1990). ANOVA model I includes only fixed effects, model II only random effects, and model III both fixed and random effects. In functional growth analysis studies, time is widespreadly considered as an independent variable for fitting experimental data to mathematical models, and the analysis of variance is usually restricted to evaluate the adequacy of these models (Hunt & Parsons, 1974; Hunt, 1982; Bullock et al., 1993). Alternatively, when harvest is intended to be included as a factor in the analysis of variance of functional growth analysis experiments, time should be considered as a random effect, since it is implicit that harvests represent continuously the growth within the period of investigation. Such concept of a random effect can not be confounded with the variables that have random measures, which deserves a specific statistical approach for regression analysis (Neter et al., 1990), i. e, time is a random (or continuous) effect but not a random variable.

The objective of this work was to evaluate the analysis of variance of data obtained from distinct harvests of an experiment, focusing especially on the homogeneity of variances and the choice of an adequate ANOVA model.

## Material and Methods

### Description of the experiments

Data from five experiments were studied, which are succinctly described as follow.

Tomato plants (*Lycopersicon esculentum* Mill.) were cultivated in greenhouse in pots with 13 kg of soil, in a 2x2x9 factorial randomized block design with four replicates, i. e, two levels of applied P (60 and 120 mg $kg^{-1}$), either inoculated or not with arbuscular mycorrhizal fungus, and nine weekly harvests between 34 and 90 days after

transplant of plantlets (Araújo et al., 1996). At each harvest, leaf area and root area were measured. Leaves, stems, fruits and roots were separately dried, weighed and ground, and P concentration was determined in each plant portion.

In a field experiment, eight common bean (*Phaseolus vulgaris* L.) cultivars were grown at two levels of applied P (12 and 50 kg $ha^{-1}$), in an 8x2 factorial randomized block design with four replicates (Araújo et al., 2000). Each plot had four rows 6 m long and 0.5 m apart, and biomass was sampled at three growth stages (third trifoliate fully expanded, plentiful flowering, pod setting), when six plants were harvested from each plot. Shoots, roots and nodules were separately dried and weighed, and N and P concentrations were measured in roots and shoots.

In a pot experiment, eight common bean cultivars were grown in pots with 10 kg of soil at two levels of applied P (20 and 80 mg $kg^{-1}$) and harvested at three growth stages (third trifoliate fully expanded, plentiful flowering, pod setting), in an 8x2x3 factorial randomized block design with four replicates (Araújo & Teixeira, 2000). At each harvest, roots and nodules were recovered, leaves and nodules were counted, and leaf area and root area were measured. Leaves, stems, pods, roots, and nodules were separately dried, weighed, and ground, and P concentration was determined.

Four pearl millet (*Pennisetum glaucum* (L.) R. Brown) cultivars were compared in a field experiment in a randomized block design with three replicates (Geraldo et al., 2000). Each plot had six rows 5.5 m long and 1 m apart with plants 0.5 m spaced. Biomass was sampled weekly at 11 stages, between 30 and 100 days after planting, when two plants were harvested from each plot. Leaf area was measured, and leaf sheets, sheaths, stems and panicles were separately dried and weighed. Data were converted to leaf area index and biomass per land area.

Two rice (*Oryza sativa* L.) cultivars were grown in greenhouse in pots with 12 L of nutrient solution, and they were harvested between 25 and 85 days after planting, in a 2x7 completely randomized design with four replicates (França et al., 1999). At each harvest, leaf area and root area were measured. Roots, leaf sheets, and stems plus sheaths, were separately dried, weighed, and ground, and N concentration was determined.

For each experiment, the usual allometric ratios of growth analysis (as specific leaf area, leaf area ratio, and root:shoot ratio) were calculated whenever possible. Nutrient content was obtained by the product of nutrient concentration and dry mass.

**Evaluation of heteroscedasticity**

Among the tests available to verify the equality of variances from some populations, the Hartley test identifies the maximal ratio between the largest and the smallest population variances (Neter et al., 1990), and it was used to ascertain the homogeneity of the variances of data obtained at each harvest, as much for the original data as for natural logarithmic transformed data. Hence the variables measured in each experiment were classified as originally homoscedastic, transformed homoscedastic (originally heteroscedastic, homoscedastic after transformation), or transformed heteroscedastic (originally heteroscedastic, heteroscedastic after transformation). The variables were also grouped according to their nature, as variables related to accumulation of biomass and nutrients, and variables related to allometric ratios and nutrient concentration in plant portions.

**Analysis of variance**

In the experiments mycorrhiza on tomato and P on bean cultivars in pots, the analysis of variance was performed as a three crossed factors design. In the experiment rice cultivars, the analysis was performed as a two-crossed factors in a completely randomized design. The experiment millet cultivars had a split-plot design, with cultivar as main plot and harvest as subplot. In the experiment P on bean cultivars in the field, the analysis comprised two-crossed factors for P and cultivar with harvest as subplot. The analysis of variance was performed on original and transformed data, evaluating the impact of transformation on the F test.

The experiments P on bean cultivars in the field and P on bean cultivars in pots were planned as a classical growth analysis, with only three harvests at specific stages of crop development. Therefore, harvest was considered as a fixed effect, and the ANOVA model I (fixed effects) is adequate. The other three experiments were planned as a functional growth analysis, with several harvests intending to represent growth continuously. Thus harvests should be considered as a random effect, and the ANOVA model III (fixed and random effects) may be used. In these functional growth analysis experiments, the analysis of variance was performed for ANOVA models I and III, examining the effect of the ANOVA model on the F test. Such analysis were performed only for originally or transformed homoscedastic variables.

The fixed and mixed ANOVA models have identical sums of squares, but they differ in the expected mean squares and the choice of the appropriate statistical test (Neter et al., 1990). For the fixed model, the error mean square is an appropriate term for testing hypotheses about any source of variation, but for the mixed model the choice of a suitable error term is more difficult particularly when main effects are tested (Steel & Torrie, 1980). Table 1 presents the appropriate F test for each experiment of

functional growth analysis considering ANOVA model III. In factorial experiments with three or more factors involving a random or mixed model, and in other more complex designs, there are frequently no exact test for certain effects (Montgomery, 1991). For tests of main effects in such situations, the approximate Satterthwaite F test may be employed (Montgomery, 1991), and it was used to evaluate the effect of cultivars in experiment millet cultivars (Table 1).

**Examples**

The analysis of variance of some variables was presented to illustrate the effects of data transformation and the type of ANOVA model on the interpretation of results. Some data were fitted over time by mathematical functions, and instantaneous values of growth rates were calculated by differentiation of these functions (Hunt, 1982). The significance of treatment x harvest interaction for natural logarithmic transformed data indicated differences in relative growth rates, as proposed by Poorter & Lewis (1986).

**Table 1.** Appropriate F test for each experiment of functional growth analysis, considering harvest as a random effect (ANOVA model III); F tests built with the mean squares of each source of variation.

| Source of variation | Appropriate F test for ANOVA model III |
|---|---|
| Experiment mycorrhiza on tomato | |
| Harvest (H) | H/E |
| Phosphorus (P) | P/HxP |
| HxP | HxP/E |
| Mycorrhiza (M) | M/HxM |
| HxM | HxM/E |
| PxM | PxM/HxPxM |
| HxPxM | HxPxM/E |
| Error (E) | |
| Experiment millet cultivars | |
| Cultivar (C) | $C/(E_A + CxH - E_B)$ |
| Error A ($E_A$) | |
| Harvest (H) | $H/E_B$ |
| CxH | $CxH/E_B$ |
| Error B ($E_B$) | |
| Experiment rice cultivars | |
| Harvest (H) | H/E |
| Cultivar (C) | C/HxC |
| HxC | HxC/E |
| Error (E) | |

## Results and Discussion

### Evaluation of heteroscedasticity

The tests for homogeneity of variances are sensitive to non-normality of data, and they may detect non-normality rather than heteroscedasticity (Steel & Torrie, 1980). Most data presented some deviations from normality as detected by Lilliefors test, but the Hartley test had different results from the Lilliefors test; thus it was assumed that the Hartley test can be used to identify the homogeneity of variances.

Data from variables of biomass and nutrient accumulation increased as plant aged, except in few instances when leaf senescence induced some biomass decrease at the end of experiments. The respective variances also increased with time, and the high variance ratio between the end and the beginning of the experiment characterized the heteroscedasticity. For most of these variables, the standard deviation increased proportionally with the mean, like total P content of tomato plants (Figure 1); thus the logarithmic transformation equalized the variances (Steel & Torrie, 1980). Therefore, only 8% of these variables were originally homoscedastic and 73% were heteroscedastic but became homoscedastic after transformation (Table 2). The great variation in the magnitude of biomass values as plant ages is a general growth pattern among annual species (Hunt, 1982). The variances of plant mass traits are expected to increase concomitantly, and the heterogeneity of variances due to sampling effects is a foreseeable occurrence in growth analysis studies (Carter Junior et al., 1983).

On the other hand, data from variables related to allometric ratios and nutrient concentration usually decreased with the ontogenetic drift, as illustrated by leaf P concentration of tomato plants (Figure 1). Many of these variables (39%) were originally homoscedastic (Table 2). Some of these variables had the standard deviation decreasing proportionally with the mean, like tomato leaf P concentration (Figure 1), and logarithmic transformation homogenize the variances; thus 36% of these variables were heteroscedastic but became homoscedastic after transformation (Table 2).

Some variables remained heteroscedastic even after logarithmic transformation (Table 2), denoting an independent variation of means and variances or a so high variance ratio that can not be stabilized by this transformation. In these cases, one possible procedure is to omit certain portions of the data from the analysis (Steel & Torrie, 1980). In the experiment millet cultivars, the exclusion of an initial or final harvest presenting an anomalous variance achieved homoscedasticity for the remaining data. Another solution is to perform the analysis of variance for each harvest separately, though losing much

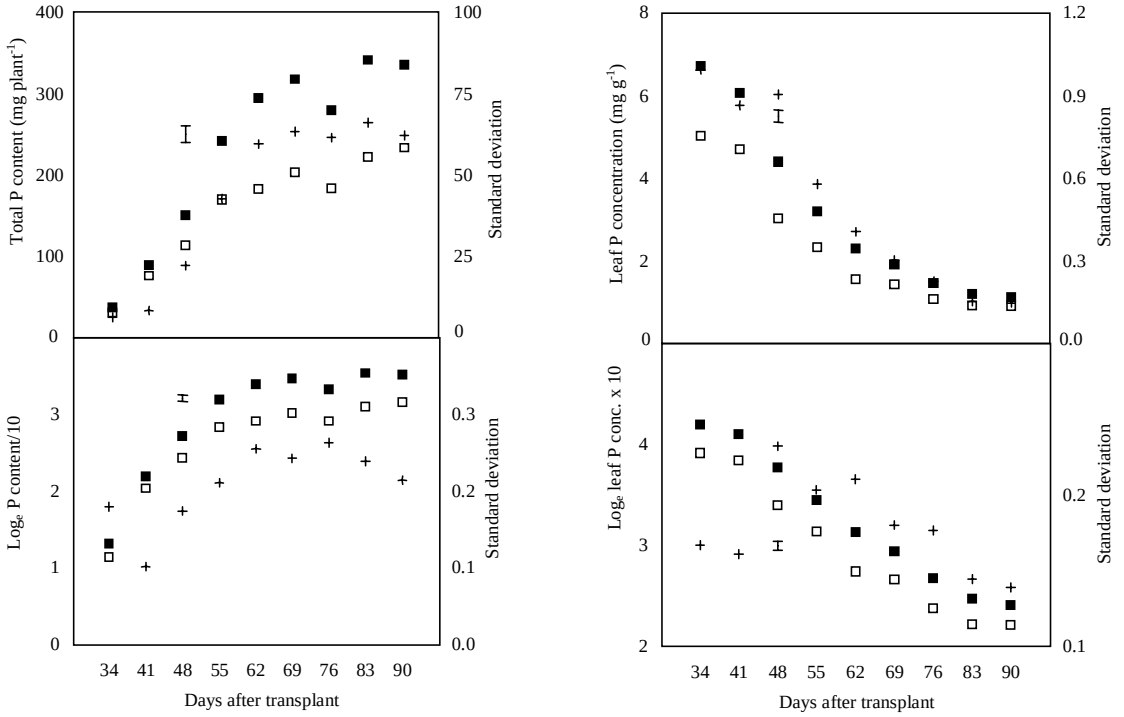


**Figure 1.** Original and natural logarithmic transformed data of total P content and leaf P concentration of tomato plants grown at the levels of P applied to the soil: (□) 60 mg $kg^{-1}$; (■) 120 mg $kg^{-1}$; + standard deviation; means of plants with or without mycorrhizal inoculation. Vertical bars represent the least significant difference (Tukey test $p<0.05$), and compare P levels within each harvest.

**Table 2.** Number of homoscedastic and heteroscedastic variables as verified by Hartley test ($p<0.01$), grouped according to the experiment or to the kind of variable.

| Group | Total | Originally homoscedastic | Transformed homoscedastic | Transformed heteroscedastic |
|---|---|---|---|---|
| Experiment | | | | |
| Mycorrhiza on tomato | 25 | 3 | 20 | 2 |
| Phosphorus on bean cultivars in the field | 14 | 4 | 8 | 2 |
| Phosphorus on bean cultivars in pots | 30 | 6 | 14 | 10 |
| Millet cultivars | 8 | 1 | 4 | 3 |
| Rice cultivars | 21 | 5 | 12 | 4 |
| Kind of variable | | | | |
| Biomass and nutrient accumulation | 62 | 5 | 45 | 12 |
| Allometric ratio and nutrient concentration | 36 | 14 | 13 | 9 |

information about the variations of the treatments over time, besides turning the analysis less sensible owing to the consequent decreased error degrees of freedom.

**Analysis of variance**

Data transformation of transformed homoscedastic variables affected the F test in different ways (Table 3). In the experiment millet cultivars, the F test was not affected whether the analysis was performed on original or transformed data. In the other experiments, transformation modified the F test usually reducing the number of significant effects (Table 3). A chi square test ($p<0.05$) confirmed that data transformation modified the number of significant effects of the F test for transformed homoscedastic variables. In general, data transformation little affected the number of significant effects of the main factors, whereas the significance of the harvest x treatment interaction was broadly reduced (Table 3). Hence, the heteroscedasticity of the original data affected the size of the F test, as verified by Zimmermann (1987), increasing the probability of false rejection of the null hypothesis of no significant effect. Nevertheless, transformation increased the number of significant effects of the triple interaction in the experiment mycorrhiza on tomato and of the cultivar x harvest interaction in the experiment P on bean cultivars in the field (Table 3), such increment in latter experiment partially due to the concomitant strong reduction in the coefficient of variation.

Mean comparisons of original data of the experiment mycorrhiza on tomato indicated that the higher soil P level increased significantly P content of tomato plants only after 48 days after transplant (Figure 1). Contrariwise, the effect of soil P supply on leaf P concentration was significant since the beginning of the experiment but disappeared after 76 days after transplant. Actually, mean comparisons of original data did not identify significant differences for values with small magnitude irrespective of plant growth stage, as at the beginning of the experiment for total P content or at the end of the experiment for leaf P concentration. Mean comparisons of transformed data denoted that the higher soil P supply increased significantly leaf P concentration and total P content of tomato plants during the entire experiment (Figure 1), a more meaningful biological interpretation.

The analysis of original data of the experiment rice cultivars showed that cultivars differed intrinsically for production of dry matter (Table 4), but the analysis of transformed data indicated that distinctions between cultivars depended on growth stage (significant harvest x cultivar interaction and no significant cultivar effect). Although both analysis presented significant harvest x cultivar interaction (Table 4), further mean comparisons led to divergent interpretations. The original data showed that cultivars did not differ up to 55 days after planting, and Comum Branco was superior to

**Table 3.** Number of variables with significant F test ($p<0.05$) in the analysis of variance, considering all effects as fixed; analysis performed for variables originally heteroscedastic that became homoscedastic after logarithmic transformation.

| Source of variation | Original data | Transformed data |
|---|---|---|
| Experiment mycorrhiza on tomato | | |
| Harvest (H) | 20 | 20 |
| Phosphorus (P) | 16 | 15 |
| HxP | 11 | 9 |
| Mycorrhiza (M) | 9 | 7 |
| HxM | 8 | 3 |
| PxM | 7 | 8 |
| HxPxM | 1 | 6 |
| Experiment phosphorus on bean cultivars in the field | | |
| Cultivar (C) | 7 | 7 |
| Phosphorus (P) | 8 | 8 |
| CxP | 0 | 0 |
| Harvest (H) | 8 | 8 |
| CxH | 2 | 6 |
| PxH | 4 | 2 |
| CxPxH | 0 | 0 |
| Experiment phosphorus on bean cultivars in pots | | |
| Harvest (H) | 14 | 14 |
| Cultivar (C) | 13 | 14 |
| HxC | 12 | 12 |
| Phosphorus (P) | 14 | 14 |
| HxP | 14 | 6 |
| CxP | 12 | 6 |
| HxCxP | 3 | 4 |
| Experiment millet cultivars | | |
| Cultivar (C) | 4 | 4 |
| Harvest (H) | 4 | 4 |
| CxH | 4 | 4 |
| Experiment rice cultivars | | |
| Harvest (H) | 12 | 12 |
| Cultivar (C) | 9 | 8 |
| HxC | 9 | 7 |

IAC4440 thereafter (Table 5). Yet transformed data showed that IAC4440 produced more dry matter at 35 days after planting but was inferior to Comum Branco after 75 days after planting. Hence, the modern cultivar IAC4440 possesses an earlier growth suitable for a high planting density associated to an intense tillering, whereas the traditional cultivar Comum Branco has a greater final growth adapted to a low stand (França et al., 1999). It confirms that although the F test can be robust against unequal variances when the sample sizes are equal, comparisons between factor levels means can be substantially affected by unequal variances (Neter et al., 1990).

## Type of ANOVA model

The ANOVA model III, as compared to model I, reduced the number of significant effects of every factor which had the F test changed by this type of ANOVA model (Table 6). In the mixed ANOVA model some simple effects are tested against interactions and some duple interactions are tested against triple interaction (Table 1), making the F test more conservative due to the reduced denominator degrees of freedom. The components of variance of the expected mean squares in the mixed models illustrate that some simple effects actually include an algebraic term related to the interaction (Steel & Torrie, 1980), the appropriate F test controlling this type of error.

In the experiment millet cultivars, the analysis of variance considering ANOVA model I (Table 7) indicated an intrinsic distinction among millet

**Table 4.** Analysis of variance of original and natural logarithmic transformed data of total dry mass of rice cultivars, considering all effects as fixed (ANOVA model I); values of mean squares.

| Source of variation | d.f. | Original data | Transformed data |
|---|---|---|---|
| Harvest (H) | 6 | 11,477*** | 37.264*** |
| Cultivar (C) | 1 | 288*** | 0.013 |
| HxC | 6 | 79*** | 0.049*** |
| Error | 42 | 5.8 | 0.010 |
| CV (%) | | 6.92 | 2.10 |

***Significant at 0.001 probability level by the F test.

**Table 5.** Original or natural logarithmic transformed data of total dry mass of two rice cultivars at seven times of harvesting.

| Days after planting | Total dry mass (g plant$^{-1}$) | | | |
|---|---|---|---|---|
| | Original data | | Transformed data | |
| | Comum Branco | IAC4440 | Comum Branco | IAC4440 |
| 25 | 0.26 | 0.25 | 0.95 | 0.88 |
| 35 | 1.69 | 2.16 | 2.83 | 3.07* |
| 45 | 7.74 | 8.74 | 4.34 | 4.47 |
| 55 | 22.40 | 21.10 | 5.41 | 5.35 |
| 65 | 45.60 | 40.10* | 6.12 | 5.99 |
| 75 | 73.60 | 61.50* | 6.60 | 6.42* |
| 85 | 107.6 | 93.50* | 6.98 | 6.84* |

*Significant difference between cultivars by Tukey test ($p<0.05$).

**Table 6.** Number of variables with significant F test ($p<0.05$) in the analysis of variance, considering all effects as fixed (ANOVA model I) or harvest as a random effect (ANOVA model III); analysis performed only for originally or transformed homoscedastic variables; only the effects which could be changed by the type of ANOVA model are presented (see Table 1).

| Number of variables | Source of variation | Model I | Model III |
|---|---|---|---|
| | Experiment mycorrhiza on tomato | | |
| 23 | Phosphorus (P) | 17 | 12 |
| | Mycorrhiza (M) | 8 | 4 |
| | PxM | 9 | 3 |
| | Experiment millet cultivars | | |
| 5 | Cultivar | 4 | 2 |
| | Experiment rice cultivars | | |
| 17 | Cultivar | 13 | 6 |

**Table 7.** Analysis of variance of shoot biomass and leaf area index of pearl millet cultivars, considering all effects as fixed (ANOVA model I) or harvest as a random effect (ANOVA model III); values of mean squares of natural logarithmic transformed data. In model III cultivar was tested by Satterthwaite F test (see Table 1).

| Source of variation | d.f. | Shoot biomass | | Leaf area index | |
|---|---|---|---|---|---|
| | | Model I | Model III | Model I | Model III |
| Block | 2 | 1.472** | 1.472** | 0.992** | 0.992** |
| Cultivar (C) | 3 | 0.737* | 0.737 | 0.311* | 0.311 |
| Error A | 6 | 0.133 | 0.133 | 0.049 | 0.049 |
| Harvest (H) | 10 | 27.762*** | 27.762*** | 6.045*** | 6.045*** |
| CxH | 30 | 0.187*** | 0.187*** | 0.223*** | 0.223*** |
| Error B | 80 | 0.072 | 0.072 | 0.065 | 0.065 |

*, **, ***Significant at 0.05, 0.01, and 0.001 probability levels by the F test, respectively.

cultivars for shoot biomass and leaf area index (significant cultivar effect). However, the ANOVA model III showed that differences among cultivars depended on time of harvesting (no significant cultivar effect and significant harvest x cultivar interaction) (Table 7). Therefore, the selection of pearl millet cultivars for production of biomass and leaf area must consider the crop growth stage, as discussed by Geraldo et al. (2000).

**Comparing growth rates**

In the experiment rice cultivars, data of total dry mass and leaf area of each cultivar were fitted over time, by the Gompertz model and by the third degree exponential polynomial, respectively. Considering that the significant harvest x cultivar interaction for natural logarithm of dry mass (Table 4) indicates significant differences in relative growth rates (Poorter & Lewis, 1986), it is assured that IAC4440 had a greater relative growth rate than Comum Branco in the beginning of the experiment, whereas Comum Branco was superior at the end of the experiment (Figure 2). Significant differences in net assimilation rate remain uncertain, since this rate includes two primary variables, but it is possible to admit that, by the end of the experiment, Comum Branco showed a higher net assimilation rate due to its higher growth rate concomitant with a smaller leaf area.

The analysis of variance of primary data arises as a useful tool for identifying significant effects in growth rates, enlightening the interpretation of the results. Since the significant treatment x harvest interaction for natural logarithm of the primary data has been identified, the growth stages presenting significant differences between treatments can also be considered as differing in relative growth rates (Poorter & Lewis, 1986; Table 4 and Figure 2). Moreover, data from equally spaced harvests in time allows the use of orthogonal polynomials, which permits the partitioning of the interactions into single degree of freedom components, indicating whether differences in relative growth rates are maintained or not throughout the whole period examined (Poorter & Lewis, 1986).

The analysis of variance imposes some restrictions to the experimental design toward increasing its accuracy. The equal number of replicates per treatment is required if all factor levels have equal importance, maximizing the precision of mean comparisons (Neter et al., 1990). Furthermore, the F test is relatively insensitive to small departures from the assumption of equal sample variances if the sample sizes are equal (Montgomery, 1991). To avoid bias in comparisons among treatment means, it is important to have uniformity to guarantee that all treatments will produce their effects under comparable conditions (Steel & Torrie, 1980); therefore, the treatments under investigation should be harvested simultaneously at least within each block. Since systematic designs, where the treatments are applied to the experimental units in a selected fashion, often result in erroneous estimation of experimental error (Steel & Torrie, 1980), harvests should be done almost equally spaced in time, rather than gathered within a specific plant growth stage.

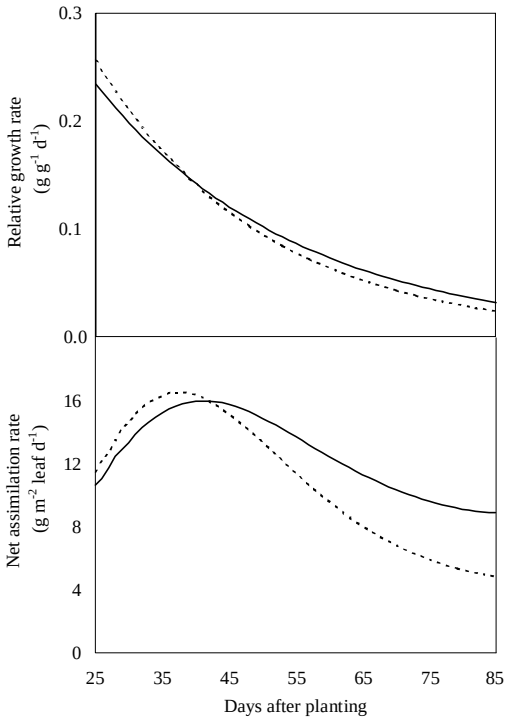


**Figure 2.** Time variation of relative growth rate and net assimilation rate of the rice cultivars Comum Branco (——) and IAC4440 (····) grown in nutrient solution, estimated from the mathematical functions fitted to data of total dry mass and leaf area.

These restrictions to experimental design eliminate some advantages of the functional approach of growth analysis such as the interval between harvests can be altered for any individual treatment (Hughes & Freeman, 1967); the harvesting of different treatments need not be simultaneous; the number of replicates per treatment or harvests need not be equal (Hunt, 1982). A more rigid experimental design is required when the analysis of variance of primary data is presumed even for the functional approach (Hughes & Freeman, 1967). On the other hand, such strictness permits the calculation of the growth rates by the classical and functional methods simultaneously (Poorter, 1989), improving the interpretation of the results when the functional approach itself is insufficient (França et al., 1999) or contradictory (Wickens & Cheeseman, 1988).

## Conclusions

1. Most data obtained from experiments with several harvests is heteroscedastic, but the majority of such data became homoscedastic after logarithmic transformation.

2. Logarithmic data transformation affects the analysis of variance of experiments with several harvests, but in a different way depending on the experiment.

3. The mixed ANOVA model, considering harvest as a random effect, reduces the number of significant effects as compared to the fixed ANOVA model.

4. The analysis of variance of primary variables provides a tool for identifying significant effects in growth rates.

## References

ARAÚJO, A. P.; ROSSIELLO, R. O. P.; SILVA, E. M. R.; ALMEIDA, D. L. Growth analysis of tomato colonized with arbuscular mycorrhizal fungi. **Revista Brasileira de Ciência do Solo**, Campinas, v. 20, n. 2, p. 233-240, 1996.

ARAÚJO, A. P.; TEIXEIRA, M. G. Ontogenetic variations on absorption and utilization of phosphorus in common bean cultivars under biological nitrogen fixation. **Plant and Soil**, Dordrecht, v. 225, n. 1/2, p. 1-10, 2000.

ARAÚJO, A. P.; TEIXEIRA, M. G.; ALMEIDA, D. L. Growth and yield of common bean cultivars at two soil phosphorus levels under biological nitrogen fixation. **Pesquisa Agropecuária Brasileira**, Brasília, v. 35, n. 4, p. 809-817, abr. 2000.

BULLOCK, D. G.; SIMMONS, F. W.; CHUNG, I. M.; JOHNSON, G. I. Growth analysis of corn grown with or without starter fertilizer. **Crop Science**, Madison, v. 33, p. 112-117, 1993.

CARTER JUNIOR, T. E.; BURTON, J. W.; CAPPY, J. J.; ISRAEL, D. W.; BOERMA, H. R. Coefficients of variation, error variances, and resource allocation in soybean growth analysis experiments. **Agronomy Journal**, Madison, v. 75, p. 691-696, 1983.

CAUSTON, D. R. Plant growth analysis: a note on the variability of unit leaf rate (net assimilation rate) within a sample. **Annals of Botany**, London, v. 74, p. 513-518, 1994.

FRANÇA, M. G. C.; ROSSIELLO, R. O. P.; ZONTA, E.; ARAÚJO, A. P.; RAMOS, F. T. Desenvolvimento radicular e influxo de nitrogênio em duas cultivares de arroz. **Pesquisa Agropecuária Brasileira**, Brasília, v. 34, n. 10, p. 1845-1853, out. 1999.

GARRETSEN, F.; KEULS, M. Functions of time for growth characters, their evaluation and approximation to examine differences between genotypes. **Euphytica**, Wageningen, v. 35, p. 11-15, 1986.

GERALDO, J.; ROSSIELLO, R. O. P.; ARAÚJO, A. P.; PIMENTEL, C. Diferenças em crescimento e produção de grãos entre quatro cultivares de milheto pérola. **Pesquisa Agropecuária Brasileira**, Brasília, v. 35, n. 7, p. 1367-1376, jul. 2000.

HUGHES, A. P.; FREEMAN, P. R. Growth analysis using frequent small harvests. **Journal of Applied Ecology**, Oxford, v. 4, p. 553-560, 1967.

HUNT, R. **Plant growth curves**: the functional approach to plant growth analysis. London: E. Arnold, 1982. 248 p.

HUNT, R.; EVANS, G. C. Classical data on the growth of maize: curve fitting with statistical analysis. **New Phytologist**, Cambridge, England, v. 86, p. 155-180, 1980.

HUNT, R.; PARSONS, I. T. A computer program for deriving growth-functions in plant growth-analysis. **Journal of Applied Ecology**, Oxford, v. 11, p. 297-307, 1974.

KEULS, M.; GARRETSEN, F. Statistical analysis of growth curves in plant breeding. **Euphytica**, Wageningen, v. 31, p. 51-64, 1982.

MONTGOMERY, D. C. **Design and analysis of experiments**. 3rd ed. New York: Wiley, 1991. 649 p.

NETER, J.; WASSERMAN, W.; KUTNER, M. H. **Applied linear statistical models**. 3rd ed. Burr Ridge: R. D. Irwin, 1990. 1181 p.

POORTER, H. Plant growth analysis: towards a synthesis of the classical and the functional approach. **Physiologia Plantarum**, Copenhagen, v. 75, p. 237-244, 1989.

POORTER, H.; LEWIS, C. Testing differences in relative growth rate: a method avoiding curve fitting and pairing. **Physiologia Plantarum**, Copenhagen, v. 67, p. 223-226, 1986.

STEEL, R. G. D.; TORRIE, J. H. **Principles and procedures of statistics**: a biometrical approach. 2nd ed. New York: McGraw-Hill, 1980. 633 p.

WICKENS, L. K.; CHEESEMAN, J. M. Application of growth analysis to physiological studies involving environmental discontinuities. **Physiologia Plantarum**, Copenhagen, v. 73, p. 271-277, 1988.

ZIMMERMANN, F. J. P. Efeito de heterogeneidade de variância e distribuição de probabilidade dos dados sobre o poder e tamanho do teste F. **Pesquisa Agropecuária Brasileira**, Brasília, v. 22, n. 11/12, p. 1209-1213, nov./dez. 1987.