Journal of Experimental Botany, Vol. 47, No. 302, pp. 1343-1351, September 1996

Journal of Experimental Botany

Plant growth analysis: an evaluation of experimental design and computational methods

Hendrik Poorter[1,3] and Eric Garnier[2]

[1] *Department of Plant Ecology and Evolutionary Biology, University of Utrecht, PO Box 800.84, 3508 TB Utrecht, The Netherlands*

[2] *Centre d'Ecologie Fonctionnelle et Evolutive, CNRS, BP 5051, F-34033 Montpellier Cedex 1, France*

Received 20 October 1995; Accepted 26 April 1996

Abstract

Various aspects of the experimental design and computational methods used in plant growth analysis were investigated. This was done either analytically, or by repeatedly simulating harvests from theoretical populations upon which were imposed the underlying growth curves as well as the variability in plant material.

In the first part the consequences of neglecting an ln-transformation of the primary weight data were considered. *T*-tests are affected in such a way that significant differences between treatments show up less readily than in transformed data. A more fundamental point is that most hypotheses on plant weight concern proportional effects rather than absolute ones. In these cases, an ln-transformation prior to a statistical test is required anyway. Secondly, the accuracy of average *RGR* estimates was evaluated. Variability in *RGR* estimation increases linearly with variability in the plant material. It is also strongly dependent on the time interval between harvests and the number of replicates per harvest. Even with conservative values for plant weight variability, the chances of arriving at aberrant *RGR* estimates are rather high. Therefore, it is suggested that the variability in the population is decreased deliberately, unless variability within the population is itself of biological interest. Thirdly, three computational methods to fit dry weight progressions and describe time trends in *RGR* and related growth parameters were evaluated. Although complicated to calculate, the Richards function was superior to polynomials fitted through either the weight data ('polynomial' approach), or the classically derived RGR values ('combined' approach).

Key words: Plant growth analysis, experimental design, computational methods, relative growth rate, net assimilation rate.

Introduction

Plant growth analysis is now a widely used tool in such different fields as plant breeding (Wilson and Cooper, 1970; Spitters and Kramer, 1986), plant physiology (Clarkson *et al.,* 1986; Rodgers and Barneix, 1988) and plant ecology (Grime and Hunt, 1975; Tilman, 1988). Its methodology started to evolve in the 1920s (Blackman, 1919; West *et al.,* 1920) with what is now called the 'classical' approach. In this method, the relative growth rate (*RGR*) is calculated by dividing the difference in ln-transformed plant weight between two harvests by the time interval between those harvests. Compound rates, like the net assimilation rate (*NAR;* increase in weight per unit of leaf area and time), are computed in a similar, discrete, way (Evans, 1972). The classical method was challenged in the 1960s, when increased computational power enabled curve-fitting procedures with polynomial equations on progressions of plant weight and leaf area with time (Vernon and Allison, 1963; Hughes and Freeman, 1967). Although this method, which will be called the 'polynomial' approach, evaded some of the problems encountered in the classical approach, its application is not always satisfactory. For example, choice of the degree of the polynomial carries large consequences for growth parameter estimates (Nicholls and Calder, 1973; Hunt and Parsons, 1974; Elias and Causton, 1976; Hurd, 1977). Lately, these problems have led to a reappraisal of the classical approach (Wickens and Cheeseman, 1988; Causton, 1991) and to the introduction

[3]To whom correspondence should be addressed. Fax: +31 30 251 8366. E-mail: H.Poorter@boev.biol.ruu.nl

of a method combining the classical approach and curve fitting ('combined' approach; Poorter, 1989). Alternatively, it has been suggested that specific growth equations like the Richards function, in which the parameters have a biologically more relevant meaning, are used (Venus and Causton, 1979*a*).

Notwithstanding the large body of literature on the methodology of growth analysis (Evans, 1972; Causton and Venus, 1981; Hunt, 1982), little attention has been paid to such practical problems as how the experimental design used affects growth parameter estimates and how adequately different methods describe time trends in *RGR* and *NAR*. The first aspect was only touched upon by Causton and Venus (1981). Comparisons between methods have been made more frequently, using experimentally derived data (Buttery and Buzzell, 1974; Hunt and Parsons, 1977; Sivakumar and Shaw, 1978; Venus and Causton, 1979*a*; Whale *et al.,* 1985; Poorter, 1989). However, since underlying trends in *RGR* and its components are not known, these comparisons can never yield a conclusive answer to the question of which method is the most accurate.

In the present paper three problems encountered when using growth analysis are considered, by constructing hypothetical populations of plants for which were imposed variability in dry weight as well as time trends of growth rates. Firstly, some consequences of transforming or not transforming plant weights to log-normal values will be examined. Secondly, the consequences of the choice of different experimental designs on the reliability of the mean *RGR* estimate will be explored. Thirdly, the three different curve-fitting methods will be compared for their adequacy in describing time trends in *RGR, NAR* and leaf area ratio (*LAR,* leaf area/total plant weight). For other aspects of growth analyses, like testing differences in *RGR* and *NAR,* or the application of growth functions designed for plants grown in competition, reference is made to papers such as those by Venus and Causton (1979*b*), Poorter and Lewis (1986), Goudriaan and Monteith (1990), and Causton (1991, 1994).

Methods

1 or 2 harvests

Where possible, the above-mentioned problems were solved analytically. For more complicated cases, a simulation model was developed in which hypothetical populations of plants were constructed by imposing a population mean and a standard deviation ($\mu_{\ln W}$ and $\sigma_{\ln W}$, respectively, in the case of ln-transformed plant weights). A harvest is then simulated by drawing randomly a number of replicates from the hypothetical population, characterized by $\mu_{\ln W}$ and $\sigma_{\ln W}$. The mean of these replicates is the estimator E_{lnW} of the population mean $\mu_{\ln W}$ at a given time t. Performing these random drawings for a sequence of 'harvest days' enables us to calculate E_{RGR}, an estimate of the true relative growth rate, μ_{RGR}. In the case of an experimental design with two harvests, the *RGR* estimate is calculated as:

$$E_{RGR} = \frac{E_{\ln W_2} - E_{\ln W_1}}{t_2 - t_1} \quad (1)$$

where $E_{\ln Wi}$ is the estimated population mean of the ln-transformed total plant weights at time t_i. By repeating this procedure, say 1000 times, a frequency distribution of E_{RGR} is generated. The influence of the number of harvests, the number of replicates per harvest and the variability of the population on the *RGR* estimate can thus be assessed.

In those cases where *NARs* are of interest as well, not only the weight but also the leaf area of the plant has to be known. To justify the close correlation between these two, leaf areas were simulated by imposing for each harvest date a certain *LAR* with a given standard deviation (1.5 m^2 kg^{-1}, average of a range of harvest data of several experiments) upon the simulated weight.

There are no absolute criteria by how much E_{RGR} may differ from μ_{RGR}. The following procedure was applied. As *RGR* is a parameter that integrates growth over a period of time, the criterion used here is how well E_{RGR} will predict $\mu_{\ln W}$ at the end of the growth period (t_2). Therefore, $E_{\ln W2}$ is calculated from the real, parametric value of biomass at the beginning of the growth period ($\mu_{\ln W}$), and E_{RGR}. The maximum absolute difference between $E_{\ln W2}$ and $\mu_{\ln W2}$ which is considered acceptable in this paper is 0.223. This corresponds to an estimate of the total dry weight (E_{W2}) which, at most, is 25% higher or 20% lower than its actual value (μ_{W2}).

What are the consequences of this criterion for the *RGR* estimate? Following equation 1, it can be calculated that for a 24 d experiment, deviations from the true *RGR* are acceptable, if they are less than 9.3 mg g^{-1} d^{-1}. As the differences are related to the final dry weight of the experiment, the acceptable deviations will be larger for shorter experiments, and smaller for longer ones. From a biological point of view, the applied limits are not very strict. For example, one of the interests in the literature at the moment is how plant growth is affected by elevated CO_2 concentrations. On average, the increase in total plant weight due to a doubling of CO_2 was 47% (Poorter *et al.,* 1996). Assuming a true final weight of 1 g for the control plants, any estimate of *RGR* that will lead to a final weight between 0.8 g and 1.25 g for plants grown at control levels and 1.18 g to 1.84 g for the CO_2-enriched plants will be accepted. Consequently, with the above criteria, *RGR* estimates which lead to a ratio of final dry weights (high CO_2 : control CO_2) between 0.95 and 2.3 will be considered 'acceptable'.

More than 2 harvests

The impact of an experimental design with more than two harvests on the *RGR* estimate was subsequently assessed. This was done by calculating a linear regression for all the data of each of 2000 simulated experiments. The slope of each of the lines then gives the mean *RGR* over the experimental period. The standard deviations for the *RGR* were calculated from the 2000 estimates.

Finally, to compare time trends in *RGR* and *NAR,* polynomial functions were fitted either to ln-transformed dry weights and areas (the 'polynomial' approach) or to the classically-derived *RGR* and *NAR* values (the 'combined' approach) with procedure REGRESSION of the SPSS statistical program (SPSS Inc., Chicago, USA). Richards functions were fitted with the non-linear regression procedure of the same package. Comparison of the fitted functions with real values were made by calculating the sum of squares of the difference between the estimated and true values.

Results and discussion

Transforming or not transforming the data?

Generally, dry weights of a population of individually grown plants are distributed non-normally (Koyama and Kira, 1956; cf. Weiner and Thomas, 1986; Biere, 1987), with a standard deviation which increases with the mean. In such cases, testing differences in dry weight by using parametric tests such as an analysis of variance or Student's *t*-test is allowed only after ln-transformation of the data to homogenize the variances of the compared means. However, especially in reports where only final dry weights are measured, ln-transformation is often neglected.

What are the consequences of not-transforming? Do conclusions drawn from *t*-tests for data not transformed differ substantially from *t*-tests performed on ln-transformed values? An elaboration of this problem is given in the Appendix. The conclusions are that (1) a *t*-test on ln-transformed data is always more sensitive than one on non-transformed data, (2) the difference between the two *t*-tests increases with increasing variability in the original data, and (3) this difference does not depend on the sample size. However, only for plant populations with a high $\sigma_{\ln W}$ (i.e. > 0.6, see next section), differences caused by transformation are substantial.

A second and more important reason to use ln-transformation lies in the multiplicative nature of treatment effects. In general, different treatments are not supposed to have the same absolute effect on (say) small and large plants, rather they are supposed to increase or decrease plant weight by the same percentage (Galton, 1879). An analysis of variance on non-transformed data testing the effect of a treatment on small and large plants, could lead to the conclusion that there was a significant interaction between plant size and treatment, based on the wrong assumption of additivity of the independent factors. After ln-transformation, an interaction between the main factors would no longer be found, as would be expected from the multiplicative nature of the treatment effect (Slob, 1987).

A final point is that the log-normal nature of the distribution of plant weights also has consequences for the computation of the mean dry weight of a harvest. Instead of calculating a normal arithmetic mean, a geometric mean is preferred. This is the back-transformed value of the arithmetic mean of the ln-transformed original data. Such a geometric mean, which is an unbiased estimator of the median of a log-normal distribution, is a better reflection of the average weight in such a population (Slob, 1987). A consequence of this computation is that confidence intervals will be unequal, and indicate ratios rather than absolute values of confidence.

Mean RGR

A second problem addressed in this paper is how the choice of experimental design affects the precision of the mean *RGR* estimate. In its simplest form, a growth analysis consists of two harvests, from which a mean *RGR* can be calculated. Especially for larger screening programmes (Elias and Chadwick, 1979; Shipley and Peters, 1990) such a mean *RGR* provides a valuable parameter to characterize different species and/or populations. But what is the reliability of the RGR-estimates?

Given that $\ln W_1$ and $\ln W_2$, as defined in the previous section, are normally distributed variables with standard deviation $\sigma_{\ln W}$, and assuming similar numbers of replicates (n) per harvest, then, by the standard statistical theorem of a linear combination of variables, the *RGR* estimate will also be normally distributed, with a standard error of the mean given by:

$$S_{\overline{RGR}} = \frac{\sqrt{2} \times \sigma_{\ln W}}{(t_2 - t_1) \times \sqrt{n}} \qquad (2)$$

(cf. Venus and Causton, 1979b). From this parameter, the distribution of observed *RGRs* can be derived. Subsequently, it can be calculated what proportion of the values will deviate by more than 9.3 mg g^{-1} d^{-1}.

In a survey of 100 literature sources, the median duration of growth experiments was found to be 24 d. It is difficult to determine $\sigma_{\ln W}$, as it is the variability in weight of the entire population. However, an estimate of $\sigma_{\ln W}$ can be obtained by calculating $s_{\ln W}$, the standard deviation of a sample of harvested plants. Published information on $s_{\ln W}$ is scarce. Various colleagues were asked to calculate $s_{\ln W}$ for a number of their growth experiments. These experiments deal with a variety of species (trees, crop plants, wild herbs), treatments (light, CO_2, nutrients, O_3) and plants grown for a period from 7 d to more than a year. The distribution of these data is given in Fig. 1. Values of $s_{\ln W}$ range from less than 0.1 to over 1.0, with a median value of 0.30. From the 25th and 75th percentile of this distribution (0.21 and 0.46, respectively) a $\sigma_{\ln W}$ smaller than 0.2 is considered to be low, and a value over 0.5 to be high. By doing so, it is assumed that the observed $s_{\ln W}$'s are reasonably good estimates of $\sigma_{\ln W}$.

Given a simple growth experiment, with one harvest of six plants at day 0 and another harvest of six plants 24 d later, an acceptable estimate of *RGR* is obtained in more than 99% of the experiments, when the population has a $\sigma_{\ln W}$ of 0.15. However, by taking a $\sigma_{\ln W}$ value of 0.6, the percentage of experiments that falls within the limits outlined in the Methods (maximal deviation 9.3 mg g^{-1} d^{-1}) drops to 35%. This example illustrates that reducing the variability of dry weight data is of prime importance in increasing the reliability of *RGR* estimates.

A more elaborate analysis of the effect of experimental design on variability in *RGR* estimates is presented in Fig. 2. According to equation 2, both variability in plant weight, the duration of the experiment and the number

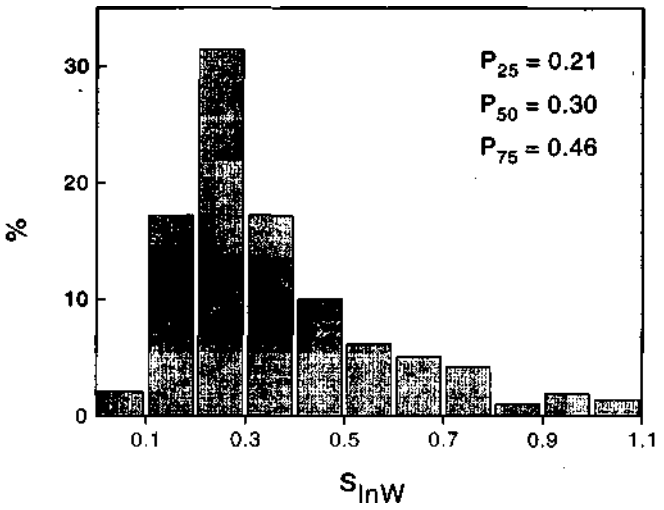

Fig. 1. Distribution of observed s_{lnW} (n = 569) from a wide range of growth experiments with a variety of plant species (trees, wild herbs, crop species). The 25th, 50th and 75th percentile of the distribution are indicated in the top right part of the graph. Values were taken from the publication of Shipley and Peters (1990), and supplied by A Biere, H Olff, S Osunkaya, K Reiling, J Roy, B Shipley, J Virgona, and J Weiner, as well as calculated from our own experiments.

of plants per harvest may affect the standard error of the mean *RGR,* and thus the precision of the *RGR* estimate. Therefore, the influences of varying these three parameters on E_{RGR} were explored. First, the duration of the experiment was fixed at 24 d, and both σ_{lnW} and the number of replicates were changed (Fig. 2). On the left *y*-axis the resulting standard error of the mean *RGR* is given; on the right *y*-axis was plotted the chance of a strongly deviating *RGR* value, as outlined in the Methods. The probability of such a deviation in *RGR* estimate increases sharply with increasing σ_{lnW} values when a limited number of plants are harvested and more gently with larger replication (Fig. 2A). When variability is really high, it requires unrealistic numbers of replicates (48 or more) to keep the chance of an outlying *RGR* estimate below 10%. The number of 'non-acceptable' results appears to be independent of the duration of the experiment. This is because boundaries are defined with respect to the last harvest day within an experiment (see Methods). However, it is obvious that an aberrant *RGR* estimate, which causes a large deviation in the calculated weight after just a few days will cause even stronger deviations

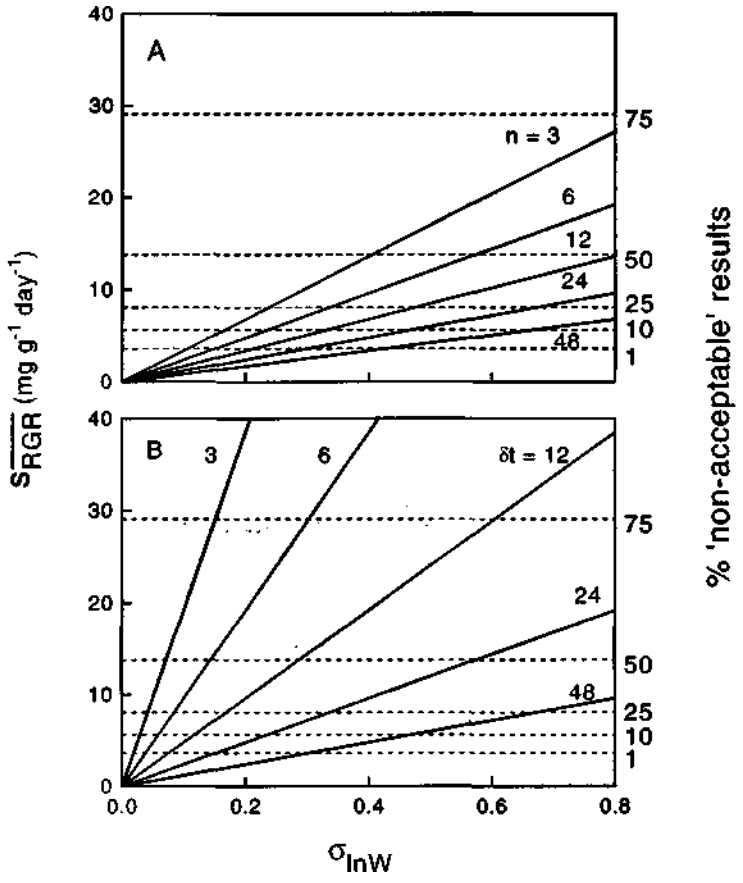

Fig. 2. The standard error of the *RGR* estimate as dependent on the standard deviation in ln-transformed dry weight of the populations that are sampled (left y-axis), and the corresponding percentage of RGR-observations that are beyond the limit, which is considered to be acceptable (cf. Methods); (A) for a range of replicates harvested on each of the two harvests, 24 d apart; and (B) as related to the time interval between the first and the second harvest. In (B) the limit for all calculations is taken to be that of a 24 d experiment (9.3 mg g^{-1} d^{-1}) and the number of replicates fixed to 6 plants per harvest. In all cases s_{RGR} was calculated according to equation 2.

when considered over a longer time span. Accordingly, the effect of harvest interval on the percentage of non-acceptable results for *RGR* by extra- or interpolating deviations in dry weight in all cases to day 24 was calculated. The shorter the harvest time interval the less precise the *RGR* estimate (Fig. 2B). This is easily understood: the closer two points on a line are, each with a certain variability, the less accurate is the estimate of the slope of the line between them. Especially for short-term experiments (less than a week), 6 plants is clearly not enough to arrive at a good *RGR* estimate.

Finally, the duration of the experiment was fixed at 24 d and the number of harvested plants at 24 as well, but this number of plants was divided over a different number of harvest days. This simulated various experimental designs, ranging from the classical approach of a few harvests and many plants to the design advocated when using fitting procedures (many harvests, few plants). For each of these experimental designs mean *RGR* was calculated over the 24 d period. Deviations from the 'true' mean *RGR* increased with number of harvests (Fig. 3), with increases being particularly strong for populations with a high $\sigma_{\ln W}$. Again this has to do with the estimate of the slope of the line describing the progression of ln-transformed dry weights with time: the more information at the outer parts of the curve, the higher the precision with regard to the estimate of the slope of the line. A third harvest in the middle of the experiment will have no effect at all on the estimate of the overall slope. Thus, an extra harvest at this point in the experiment is actually a wasted effort, if only mean *RGR* is of interest. The problem becomes slightly more complicated if mean *NAR* is of concern as well. Formulas for *NAR* depend on the actual relationship between leaf area and plant weight (Radford, 1967). Deviations between the different calculations are small when leaf area has not more than doubled between the two harvests (Evans, 1972). However, if mean *NAR* has to be known over longer periods of time, the advice is to harvest some plants in between the first and second harvest, to enable assesment of the exact allometry between leaf area and plant weight.

What implications can be inferred from the results? Firstly, it is extremely important to diminish the size variability in the population. Rigorous trimming of the plant population before the start of the experiment is advised, if variation within the population is not of interest *per se* (Poorter, 1989). Otherwise, one should subdivide the plants into smaller and more homogeneous subpopulations before the start of the experiment (Whale *et al.,* 1985). Another possibility to reduce variability in $\sigma_{\ln W}$ might be to trim the observed data afterwards by systematically removing the smallest and largest individual of the sample of plants of one harvest. In such cases, procedures for statistical testing have to be adjusted (Barnett and Lewis, 1978). Secondly, the shorter the experiment, the more plants have to be harvested to obtain a reliable *RGR* estimate. Thirdly, if only mean *RGR* is of interest, it is better not to use the technique of frequent small harvests, as it reduces the precision of the *RGR* estimation. Finally, it is suggested that in reports on plant growth analysis, average $s_{\ln W}$ is given in the results section, to enable evaluation of variability in the plant material as well as in estimated growth rates.

In all the above calculations the focus has been on the variability in *RGR* of one population. However, often a comparison between treatments or species is of interest. In comparing *RGR* values, one should be aware of the additive nature of this parameter. That is, for a given starting weight and period of time, an increase in *RGR* from 100 to 110 mg g^{-1} d^{-1} will result in the same

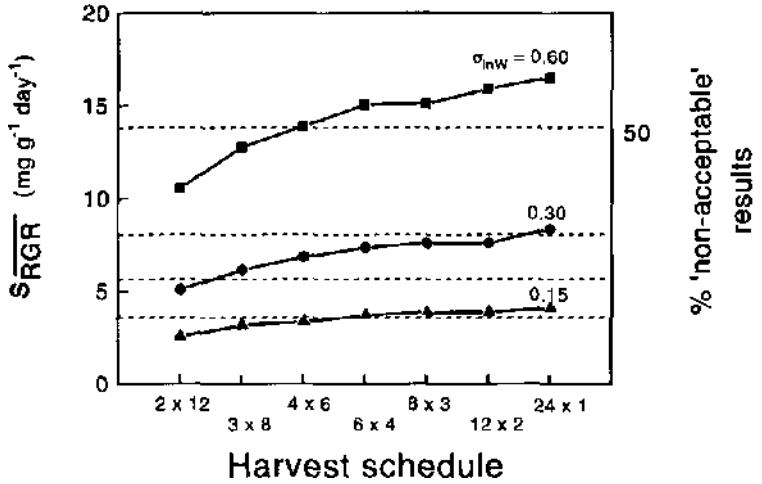

Fig. 3. The standard error of the *RGR* estimate (left *y*-axis), and the corresponding percentage of *RGR* observations that are beyond the limit, which is considered to be acceptable (right axis; cf. Methods), for a range of experimental designs and for a low, intermediate and high variability of dry weight in the plant population. The total number of plants harvested throughout the experiment was fixed at 24, but the way they were divided over the experimental period of 24 d varied. Values below the *x*-axis give the number of harvests times the number of replicates. The limit for an acceptable estimate of mean *RGR* over the whole experimental period was set at 9.3 mg g^{-1} d^{-1} (cf. Methods).

stimulation of final dry weight as an increase from 300 to 310 mg g^{-1} d^{-1} (Poorter *et al.,* 1996). Consequently, when testing differences in *RGR,* an additive rather than a multiplicative model should be applied.

Time trends

The third point addressed is the adequacy of different smoothing methods to produce reliable time trends in *RGR* and *NAR.* Given wide fluctuations in these parameters in the classical approach (Evans, 1972; Poorter and Lewis, 1986), the smoothing of curve-fitting procedures with polynomial exponentials was felt to be a considerable improvement. However, as stated in the introduction, results are rather dependent on the degree of the polynomial used in fitting the original data. Causton and Venus (1981) advocated the use of the Richards function instead of a polynomial exponential, as this would reflect the biological nature of plant growth much better. Recently, Poorter (1989) claimed that it would be better to fit the classically-derived *RGR* and *NAR* values, as it avoided the rather critical step of selecting the proper polynomial to fit ln*W* and dividing its derivative *(RGR)* by the ratio of the fitted leaf area and plant weight (for *NAR).* The evaluation for most of these claims was made by comparing different techniques using experimental data. As the real underlying trend in *RGR* and *NAR* for such data is not known, this approach

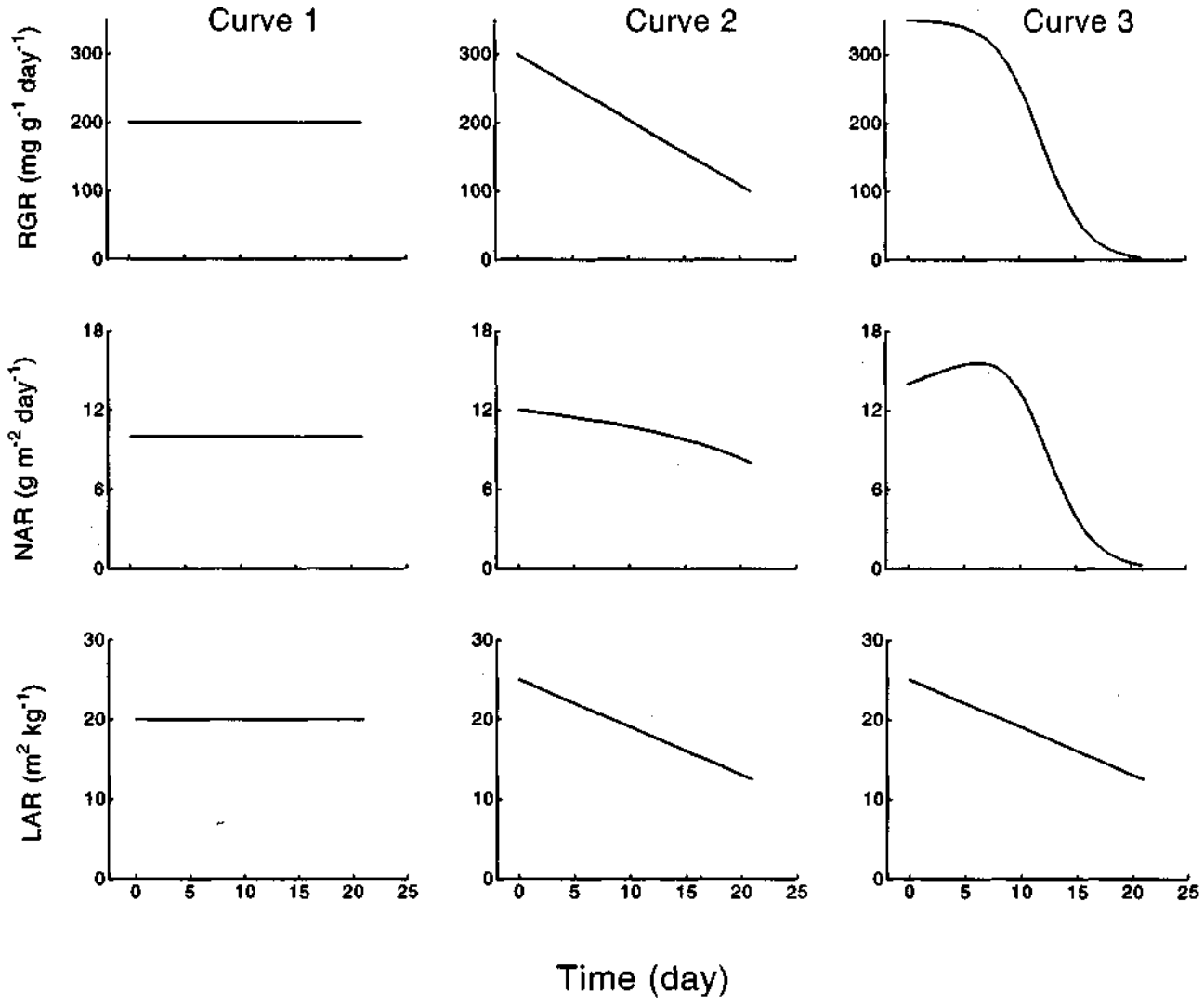

Fig. 4. The three different growth curves which were analysed with the various growth analysis methods. Curve 1: a constant *RGR, NAR* and *LAR* (C1). Curve 2: *RGR* and *LAR* decreasing monotonously from 300 to 100 mg g^{-1} d^{-1}, and 25 to 12.5 m^2 kg^{-1}, respectively (C2). Curve 3: a last one which followed a Richards curve with parameters a = 67.2, b = 6, k = 0.5, and n = 1.426 (Causton, 1981; C3). In all cases the average *RGR* over the 21 d period was 200 mg g^{-1} d^{-1}, the simulated harvest days were 0, 3, 6, 9, 12, 15, 18, and 21 with double harvests at the beginning and the end, and the number of replicates per harvest was 6.

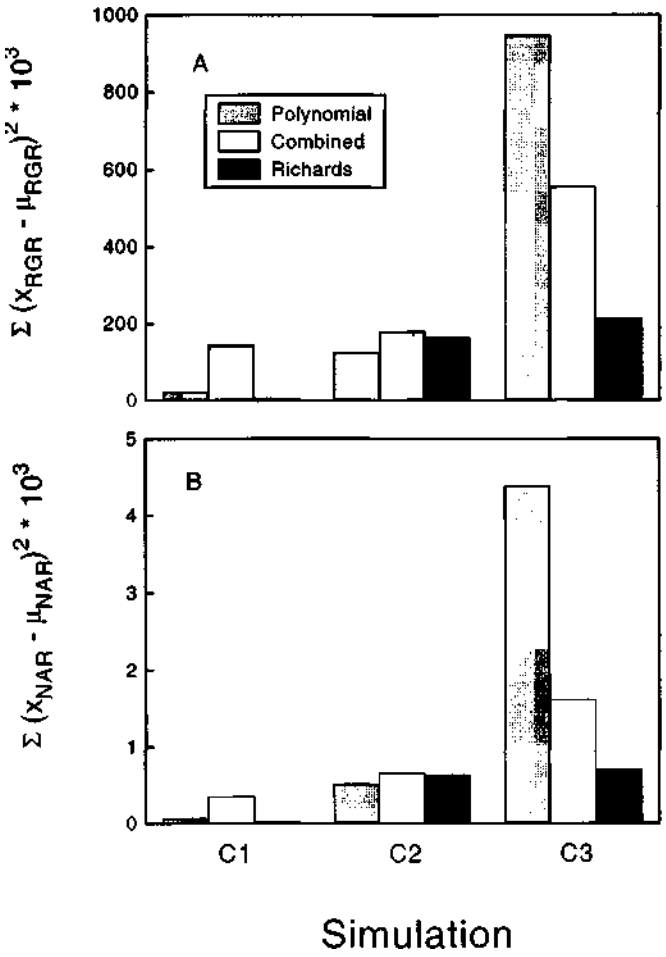

Fig. 5. The sum of squares of the difference between calculated and real values of *RGR* (A) and *NAR* (B) for various simulated plant growth curves. For each growth curve 50 experiments were simulated and the data fitted with the procedures for the 'polynomial' approach (Hunt, 1982), the 'combined' approach (Poorter, 1989) and the Richards function (Causton, 1981). The difference between the resulting curves and the 'real curves' were squared and summed over the 7 harvest days and the 50 experiments.

provides a weak basis for any claim that one method is better than another. Therefore, it was decided to compare these three methods by simulating experiments as described above. To this end three hypothetical plant populations were constructed, the first following a linear trend of ln*W* and ln*A* with time. Consequently, this theoretical population had a constant *RGR, LAR* and *NAR* (Fig. 4; curve 1), a trend that corresponds with the most simple equation in the polynomial approach. A second population followed a second order polynomial, with *RGR* and *LAR* decreasing linearly (curve 2). The third one followed the Richards equation, with a linearly decreasing *LAR* (curve 3). In all cases, the average *RGR* of plants was 200 mg g^{-1} d^{-1}. For each of the growth curves 50 experiments were simulated in which 8 harvests of 6 plants were taken from populations with a median σ_{lnW} (0.3). Following Poorter (1989) two extra sets of 6 plants were harvested at the beginning and at the end of the experiment. Such an experiment is considered a good average of the usual type of experiment carried out to follow time curves of growth. For each growth curve, 'observed' values of *RGR* and *NAR* were calculated over time using the three fitting methods. The accuracy of the lines predicted by the different methods was evaluated by calculating the total sum of squares of differences between observed and real values of *RGR* and *NAR* over all simulated harvest dates and all 50 experiments (Fig. 5). Which fitting method performs best? For plants with simple linear growth the 'combined' approach behaves worst. The 'polynomial' method approaches the theoretical trend very well, as could be expected. For a population following a quadratic growth curve all of the methods are about equally accurate. In a more complicated case, the 'polynomial' approach has the largest deviations, and the Richards function the smallest. Thus, the fit for each growth curve was relatively good when plants were simulated to grow according to that growth curve. Surprisingly, fitted values of the Richards function did very well in describing the linear growth model, even better than the functional approach, although confidence limits around the parameter estimates were extremely wide. Other observations from these simulations are that deviations are generally larger at the end of the growth curve than in the beginning, and that the relative precision of *NAR* is smaller than that of *RGR* (data not shown). If the results are representative for the majority of growth curves, it would be concluded that, notwithstanding its complicated calculation, the Richards function performs better than any of the more simple polynomial formulas to fit the growth progressions of plants.

Conclusions

The conclusions are summarized in Table 1. Variability in plant weight, expressed as standard deviation of the ln-transformed dry weight of plants, is a valuable parameter with which to evaluate growth experiments. High variability, short harvest intervals and low numbers of replicates all increase the chance for *RGR* estimates to deviate largely from true values. The protocol of many small harvests should not be used if the average *RGR* over the experimental period is the only parameter of interest. In a comparison of different fit methods the Richards function generally provided the best estimates.

Acknowledgements

We thank Arjen Biere, Han Olff, Segun Osunkaya, Kevin Reiling, Jacques Roy, Bill Shipley, Jim Virgona, and Jacob Weiner for generously providing some of their experimental data on plant weight variability. Adrie van der Werf, Herman Wijnne, David Causton, Hans Lambers, Hermann Heilmeier, and Jacques Roy

Table 1. *List of main conclusions*

Parameter of interest	Conclusions
1. Final plant weight	Harvest at least 8 plants per treatment Ln-transform data prior to testing
2a. Mean *RGR*	Plan 2 harvests with many plants The amount of plants per harvest depends on $\sigma_{\ln W}$ 8 is a minimum
2b. Mean *NAR*	Follow the mean *RGR* design, but also harvest some plants in between to assess the leaf area:dry weight relationship
3. Time trends in *RGR* and *NAR*	Plan at least 6 harvests with a few plants Use the Richard function to fit the data
4. Any growth parameter	Report on the average $s_{\ln W}$

made helpful comments on a previous version of this manuscript. HP acknowledges financial support from the European Science Foundation and from the CNRS at Montpellier.

Appendix

In this appendix, the ratio of *t*-values obtained with and without ln-transformation of the data is derived. Let $\ln W_c$ and $\ln W_t$ be the means of the ln-transformed dry weight of control and treated plants, respectively, and σ the standard deviation of these means (assumed to be equal). The *t*-value computed in a *t*-test to estimate the significance level of the difference between the two ln-transformed means is (Sokal and Rohlf, 1969):

$$t_\delta = \frac{(\overline{\ln W}_c - \overline{\ln W}_t) \times \sqrt{n}}{\sigma \times \sqrt{2}} = \frac{\delta \times \sqrt{n}}{\sigma \times \sqrt{2}} \qquad \text{(A1)}$$

where *n* is the sample size (assumed to be equal for the two treatments). The *t*-value for non-transformed data is:

$$t_d = \frac{(\bar{W}_c - \bar{W}_t) \times \sqrt{n}}{\sqrt{\sigma_c^2 + \sigma_t^2}} = \frac{d \times \sqrt{n}}{\sqrt{\sigma_c^2 + \sigma_t^2}} \qquad \text{(A2)}$$

where W_c and W_t are the means, and σ^2_c and σ^2_t the variances of the means for the non-transformed dry weights of control and treated plants, respectively. Even though σ is assumed to be equal for the two treatments, this is not necessarily the case for σ_c and σ_t.

To compare t_d with t_δ, we need to express t_d as a function of δ and σ. The relationships between the means and variances of non-transformed and ln-transformed data are (Aitchison and Brown, 1966):

$$\bar{W} = e^{\overline{\ln W} + 0.5\sigma^2} \qquad \text{(A3)}$$

and

$$\sigma^2_W = (e^{\ln W + 0.5\sigma^2})^2 \times (e^{\sigma^2} - 1) \qquad \text{(A4)}$$

where σ^2 without subscript indicates the variance in the ln-transformed data. From equations A2, A3 and A4, t_d may be written as:

$$t_d = \frac{(e^{\overline{\ln W_c} + 0.5\sigma^2} - e^{\overline{\ln W_t} + 0.5\sigma^2}) \times \sqrt{n}}{\sqrt{(e^{\overline{\ln W_c} + 0.5\sigma^2})^2 + (e^{\overline{\ln W_t} + 0.5\sigma^2})^2} \times \sqrt{e^{\sigma^2} - 1}} \qquad \text{(A5)}$$

After re-arranging:

$$t_d = \frac{(e^{\ln W_c} - e^{\ln W_t}) \times \sqrt{n}}{\sqrt{e^{2\overline{\ln W_c}} + e^{2\overline{\ln W_t}}} \times \sqrt{e^{\sigma^2} - 1}} \qquad \text{(A6)}$$

but $\ln W_t = \ln W_c - \delta$. Then:

$$t_d = \frac{(1 - e^{-\delta}) \times \sqrt{n}}{\sqrt{1 + e^{-2\delta}} \times \sqrt{e^{\sigma^2} - 1}} \qquad \text{(A7)}$$

To investigate the relationships between t_δ and t_d, the ratio between these two values is calculated as:

$$\frac{t_\delta}{t_d} = \frac{\delta \times \sqrt{1 + e^{-2\delta}}}{1 - e^{-\delta}} \times \frac{\sqrt{e^{\sigma^2} - 1}}{\sigma \times \sqrt{2}} \qquad \text{(A8)}$$

Thus, an estimate of the ratio between the *t*-value of transformed (t_δ) and non-transformed (t_d) data has been arrived at. This ratio is independent of sample size. Figure 6 shows the ratio as dependent on δ and σ. Both at high δ and σ or a combination of those two, the ratio is larger than 1. At low values of δ and σ, the ratio

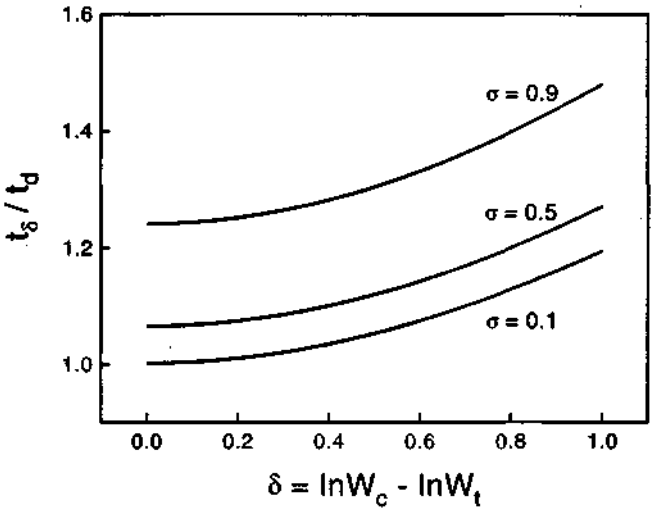

Fig. 6. The ratio of *t*-values for testing differences in plant weight after ln-transformation (t_δ) and without transformation (t_d). This ratio is plotted as a function of δ, which is the difference in ln-transformed biomass between the two treatments, for selected values of σ. Lines are derived from equation A8 of the Appendix.

approaches unity. From these results it is concluded that a *t*-test on ln-transformed data is generally more sensitive and, therefore, will reach significance at a smaller value of δ. However, unless σ reaches really high values (> 0.6) the difference between the two approaches is not very large.

References

Aitchison J, Brown JAC. 1966. *The lognormal distribution with special reference to its use in economics.* Cambridge University Press.

Barnett V, Lewis T. 1978. *Outliers in statistical data.* Chichester: Wiley and Sons.

Biere A. 1987. Ecological significance of size variation within populations. In: van Andel J, Bakker JP, Snaydon RW, eds. *Disturbance in grasslands.* Dordrecht: Junk Publishers, 253-63.

Blackman VH. 1919. The compound interest law and plant growth. *Annals of Botany* **33**, 353-60.

Buttery BR, Buzzell RI. 1974. Evaluation of methods used in computing net assimilation rates of soybeans (*Glycine max* (L.) Merrill). *Crop Science* **14,** 41-4.

Causton DR. 1991. Plant growth analysis: the variability of relative growth rate within a sample. *Annals of Botany* **67**, 137-44.

Causton DR. 1994. Plant growth analysis: a note on the variability of unit leaf rate (net assimilation rate) within a sample. *Annals of Botany* **74**, 513-18.

Causton DR, Venus JC. 1981. *The biometry of plant growth.* London: Edward Arnold.

Clarkson DT, Hopper MJ, Jones LHP. 1986. The effect of root temperature on the uptake of nitrogen and the relative size of the root system in *Lolium perenne.* I. Solutions containing both NH_4^+ and NO_3^-. *Plant, Cell and Environment* 9, 535-45.

Elias CO, Causton DR. 1976. Studies in data variability and the use of polynomials to describe plant growth. *New Phytologist* **77**, 421-30.

Elias CO, Chadwick MJ. 1979. Growth characteristics of grass and legume cultivars and their potential for land reclamation. *Journal of Applied Ecology* **16,** 537-44.

Evans GC. 1972. *The quantitative analysis of plant growth.* Oxford: Blackwell Scientific Publications.

Galton F. 1879. The geometric mean in vital and social statistics. *Proceedings of the Royal Society of London* **29**, 365-7.

Goudriaan J, Monteith JL. 1990. A mathematical function for crop growth based on light interception and leaf area expansion. *Annals of Botany* **66**, 695-701.

Grime JP, Hunt R. 1975. Relative growth rate: its range and adaptive significance in a local flora. *Journal of Ecology* **63**, 393-422.

Hughes AP, Freeman PR. 1967. Growth analysis using frequent small harvests. *Journal of Applied Ecology* **4**, 553-60.

Hunt R. 1982. *Plant growth curves.* London: Edward Arnold.

Hunt R, Parsons IT. 1974. A computer program for deriving growth-functions in plant growth-analysis. *Journal of Applied Ecology* **11,** 297-307.

Hunt R, Parsons IT. 1977. Plant growth analysis: further applications of a recent curve-fitting program. *Journal of Applied Ecology* **14**, 965-8.

Hurd RG. 1977. Vegetative plant growth analysis in controlled environments. *Annals of Botany* **41**, 779-87.

Koyoma H, Kira T. 1956. Intraspecific competition among higher plants. VIII. Frequency distribution of individual weight as affected by the interaction between plants. *Journal of the Institute of Polytechnics of the Osaka City University, Series D* **7**, 73-94.

Nicholls AD, Calder DM. 1973. Comments on the use of regression analysis for the study of plant growth. *New Phytologist* **72**, 571-81.

Poorter H. 1989. Plant growth analysis: towards a synthesis of the classical and the functional approach. *Physiologia Plantarum* **75**, 237-44.

Poorter H, Lewis C. 1986. Testing differences in relative growth rate: a method avoiding curve-fitting and pairing. *Physiologia Plantarum* **67**, 223-6.

Poorter H, Roumet C, Campbell B. 1996. Interspecific variation in the growth response of plants to elevated CO_2: a search for functional types. In: Körner C, Bazzaz FA, eds. *Biological diversity in a CO_2-rich world.* Physiological Ecology Series. San Diego, Academic Press, 375-412.

Radford PJ. 1967. Growth analysis formulae - their use and abuse. *Crop Science* **7**, 171-5.

Rodgers CO, Barneix AJ. 1988. Cultivar differences in the rate of nitrate intake by intact wheat plants as related to growth rate. *Physiologia Plantarum* **72**, 121-6.

Shipley B, Peters RH. 1990. A test of the Tilman model of plant strategies: relative growth rate and biomass partitioning. *American Naturalist* **136**, 139-53.

Sivakumar VK, Shaw RH. 1978. Methods of growth analysis in field grown soya beans (*Glycine max* (L.) Merrill). *Annals of Botany* **42**, 213-22.

Slob W. 1987. Strategies in applying statistics in ecological research. PhD thesis, Free University, Amsterdam.

Sokal RR, Rohlf FJ. 1969. *Biometry. The principles and practice of statistics in biological research.* San Francisco: WH Freeman and Company.

Spitters CJT, Kramer T. 1986. Differences between spring wheat cultivars in early growth. *Euphytica* **35**, 273-92.

Tilman D. 1988. *Plant strategies and the dynamics and structure of plant communities.* Princeton University Press.

Venus JC, Causton DR. 1979*a*. Plant growth analysis: the use of the Richards function as an alternative to polynomial exponentials. *Annals of Botany* **43**, 623-32.

Venus JC, Causton DR. 1979*b*. Plant growth analysis: a re-examination of the methods of calculation of relative growth and net assimilation rates without using fitted functions. *Annals of Botany* **43**, 633-8.

Vernon AJ, Allison JCS. 1963. A method of calculating net assimilation rate. *Nature* **200,** 814.

Weiner J, Thomas SC. 1986. Size variability and competition in plant monocultures. *Oikos* **47**, 211-22.

West C, Briggs GE, Kidd F. 1920. Methods and significant relations in the quantitative analysis of plant growth. *New Phytologist* **19,** 200-7.

Whale DM, Heilmeier H, Milbrodt H. 1985. The application of growth analysis to structured experimental designs and a new procedure. *Annals of Botany* **56**, 631-50.

Wickens LK, Cheeseman JM. 1988. Application of growth analysis to physiological studies involving environmental discontinuities. *Physiologia Plantarum* **73**, 271-7.

Wilson D, Cooper JP. 1970. Effect of selection for mesophyll cell size on growth and assimilation in *Lolium perenne* L. *New Phytologist* **69**, 233-45.