

Plant growth analysis: towards a synthesis of the classical and the functional approach

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A method of calculating relative growth rates (RGR) and net assimilation rates is presented. The method is based on the fitting of a polynomial through the relative growth rate values calculated by the 'classical' approach rather than through the ln-transformed plant weights as in the 'functional' method. Additional ways of reducing the harvest-to-harvest variation characteristic of the classical approach are discussed. The main advantages of the present approach over the functional one are: (1) The degree of the polynomial can be increased (within certain limits) without inducing spurious fluctuations in RGR. Thus, quite complex trends in RGR can be described. (2) There is little interference between RGR values in different parts of the experiment. The main advantages over the classical approach are: (1) The erratic fluctuations in RGR are dampened. (2) As frequent small harvests are allowed, the workload at each harvest can be diminished and a more reliable impression of ontogenetic drift in RGR can be obtained. (3) RGR is described by a continuous function, thus facilitating further calculations and compilations.

Key words - Growth analysis, relative growth rate.

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Introduction

One of the oldest methods in plant growth analysis is the 'classical' approach. In this method, introduced in the beginning of this century (Blackman 1919, West et al. 1920), a relative growth rate (RGR) is calculated by dividing the difference in ln-transformed plant weight at two harvests by the time difference between those harvests. Although straightforward, this approach has been considered unsatisfactory. Firstly, the time course of the growth rate may be severely obscured by fluctuations in RGR between adjacent harvest intervals (Causton and Venus 1981). Secondly, in the calculation of the net assimilation rate (NAR), a fixed relation between leaf area and plant weight has to be assumed (Evans 1972). Third, it is difficult to statistically evaluate differences in RGR (Poorter and Lewis 1986).

The 'functional' approach, developed in the 1960's (Vernon and Allison 1963, Hughes and Freeman 1967), has been presented as a solution to these problems. In

this approach a polynomial of the form $Y = b_0 + b_1X + b_2X^2 + \dots + b_nX^n$ is fitted through the growth data. The dependent variable Y is the ln-transformed weight of the plant, the independent variable X is the time of harvest. By differentiating this equation, an equation for RGR is obtained. NAR may also be computed fairly easily.

In his extensive work on the functional approach, Hunt (1982) mentions 12 advantages of this method. Among them are the following: (1) The functional approach provides a clearer perception of ontogenetic drift; (2) Assumptions involved in the calculation of mean values of NAR are avoided; (3) Statistical analyses may be integrated into the same analytical procedure as the calculation of the derived quantities. Despite these valid claims the functional approach does not necessarily result in correct values for RGR, NAR and the confidence limits of these parameters. Poorter and Lewis (1986) showed that the testing of differences in RGR had only limited biological meaning. Wickens and

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Cheeseman (1988) argued that the functional approach is of limited value if plants are subjected to short-term environmental changes. But even when plants are grown in a constant environment, the functional approach has some pitfalls.

In the present paper I will summarize some of the problems and propose an alternative method which combines the advantages of both the classical and the functional approach.

Abbreviations - NAR, net assimilation rate; RGR, relative growth rate.

The functional approach

The main problem of the functional approach is the choice of the appropriate degree of the polynomial to fit the data. Selecting a first degree polynomial will inevitably result in a constant RGR, whereas a quadratic function will invariably lead to a linearly increasing or decreasing value of RGR with time. This implies that complex growth patterns are 'underfitted' if a too low-order polynomial is used. Hughes and Freeman (1967) proposed fitting all data with a third degree polynomial to be able to describe both simple and more complicated growth curves. However, Nicholls and Calder (1973) showed that a high degree of the polynomial may lead to 'overfitting', resulting in an RGR with spurious upward or downward trends, especially at the ends of the curve. To prevent underfitting and overfitting, Hunt and Parsons (1974) proposed using a stepwise method, determining at each step whether an extra term is significant or not. However, this solution does not always work satisfactorily, as can be illustrated by data from Sivakumar and Shaw (1978). Growth of soybeans for 12 weeks (Fig. 1A) was typical of that of a crop plant, with first a decline in plant weight, followed by a steady growth and finally a decrease in leaf area and a slightly diminishing dry weight. However, the time course of RGR calculated by the authors according to the functional approach (Fig. 1B) shows that the fastest growth was at the beginning of the experiment and that the last period of 2 weeks was still characterized by considerable growth. Due to these anomalies the curve showing NAR (Fig. 1C) is even more unrealistic, as the highest values are found in the periods when the plants are actually losing weight. Presumably, a cubic function should have been used here to fit the plant dry weight data, whereas the authors used a quadratic equation, finding only the linear and quadratic terms to be significant in their statistical test.

The rigidity of the equation describing the course of the RGR, combined with the risk of underfit or overfit, leads to a second problem. What would happen if the growth of plants exposed to two almost identical treatments is compared, in which the quadratic term of the growth curve for treatment A is barely significant and

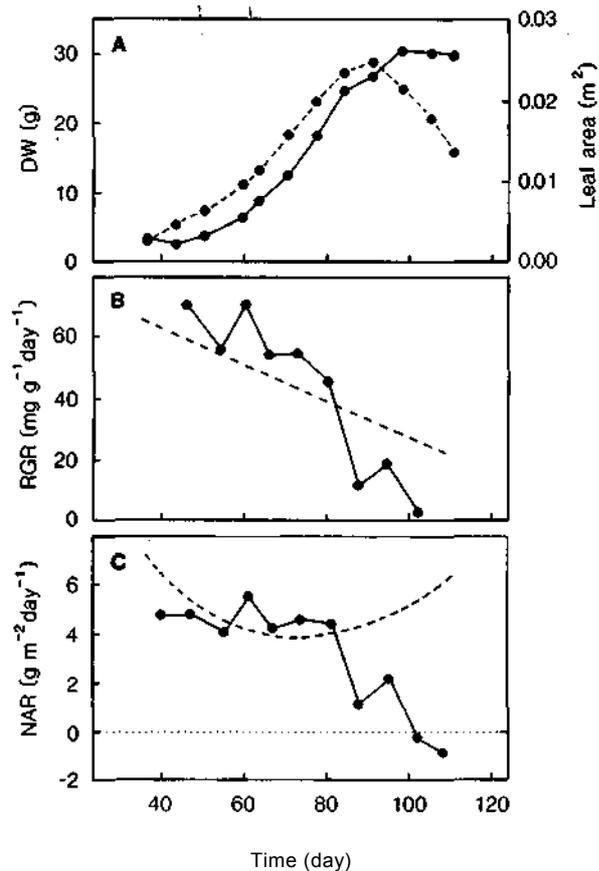


Fig. 1. A. Time course of total dry weight (●—●) and total leaf area (●- - -●) of soybean. B. Time course of RGR calculated according to the classical (●—●) and the functional approach (— — —). C. Time course of NAR: (●—●), classical approach; (— — —), functional approach. Redrawn from Sivakumar and Shaw (1978). For unknown reasons, the authors omitted the classically derived RGR values for the first and the last harvest period. Time is indicated as days after start of the experiment.

the quadratic term for treatment B is barely not? Assuming that the mean RGR for both treatments is equal, RGR in treatment B is then found to be constant during the entire experiment and to decrease continuously in treatment A. The conclusion is thus that treatment A causes plants to grow faster than B in the first period and slower by exactly the same amount in the second period, although in reality the pattern of growth for plants of both treatments was virtually the same. Hurd (1977) encountered this problem, finding significant first, second and third order polynomials for treatments in one experiment. He decided to use a second degree polynomial in all cases. Hunt (1982) on the other hand advised to stiffen the requirements of acceptance of higher degree polynomials in this case. Both solutions will introduce deflections in the time course of the RGR. In the solution of Hurd, some growth curves are overfitted and some underfitted. In the solution of Hunt

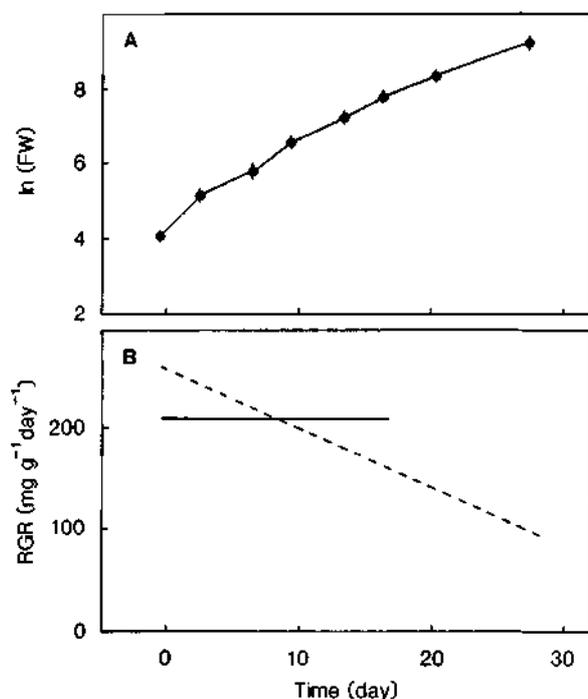


Fig. 2. A. Time course of total dry weight of *Lolium perenne* (previously unpublished data of M. Bergkotte). Mean values \pm SE ($n = 6$). B. Time course of RGR calculated according to the functional approach. (----), RGR calculated for the whole experimental period; (—), RGR calculated over the first 18 days.

a number of curves are described by a constant RGR, whereas the RGR was actually time-dependent.

A third point, also a consequence of the rigidity of this method, is illustrated in Fig. 2. Hunt (1979) states as an advantage of the functional approach: 'Information from all sampling occasions is used in determining each value of the derived quantities'. However, this may actually be a serious disadvantage. In an experiment with *Lolium perenne*, plants were harvested 8 times during 28 days (Fig. 2A). Fitting according to the stepwise procedure of Hunt and Parsons (1974) results in a quadratic polynomial and, consequently, an RGR is found which continuously decreases with time. However, if the experiment had been stopped after the 6th harvest, only the linear term would have been significant, resulting in a constant RGR during that period (Fig. 2B). Apparently, the time course of the RGR for the first period depended on the last 2 harvests, an undesirable situation.

The functional approach is based only on the assumption (Hunt 1982) that the fitted growth function adequately describes the primary data. The above-mentioned examples show that this assumption is not necessarily correct in all cases. On the contrary, authors have repeatedly stated that the results of the functional approach (and especially the stepwise procedure of selecting the degree of the polynomial) do not fit their bi-

ological expectations and that they decide to neglect the statistical considerations (e.g. Hurd 1977, Spitters and Kramer 1985, Wickens and Cheeseman 1988, see also Buttery and Buzzell 1974). The above considerations prompted a search for an alternative method in which these problems are avoided.

The alternative method

Wickens and Cheeseman (1988) showed that the functional approach did not work properly if short-term changes in the environment were considered. They proposed a return to the classical approach. However, as mentioned in the introduction, the classical approach also has some shortcomings, the main one being that there may be considerable fluctuation in RGR from harvest interval to harvest interval. This is caused by deviations between the sample mean and the true population mean. As each harvest is used twice to calculate RGR, a slightly underestimated plant weight in the second harvest will result not only in a low RGR in the first interval but also in a high RGR in the second. The smaller the number of plants in each sample and the smaller the time between two harvests, the worse this autocorrelation will be. Therefore, it is often advised to use few harvests and many plants per harvest (Evans 1972). However, this results in a considerable loss of information about the exact time course of growth.

A second disadvantage of the classical approach compared to the functional one is that information on the time trend of RGR is discontinuous. If one would like to make further calculations, as in the nutrient uptake studies of Wickens and Cheeseman (1988), or if one would like to compare the RGR of different treatments at several plant sizes rather than on a time-based scale (cf. Poorter et al. 1988), the evaluation is hampered if the values of only a few intervals are known. Such calculations are more straightforward with a continuous function. If we want to pursue with the classical method, the above-mentioned problems have to be solved.

The first step in improving the classical approach is to reduce the difference between the sample mean and the population mean. A few possibilities exist. Firstly, it is important to start with a homogeneous population. Even in genetic homogeneous populations, the coefficient of variation of plant dry weight may exceed 30% (H. Poorter, unpublished results for an inbred line of *Plantago major* ssp. *major*). If possible, and if plant-to-plant variation is not of interest, one should minimize this variation by removing the smallest and largest plants before the start of the experiment. Secondly, one could divide the population by eye in, say, two groups just before each harvest, with all small plants in one and all large plants in the other group, and with the intermediate plants randomly assigned to both groups, so that each group has the same number of plants. Sampling plants at random from each size class ensures that not

only small or only large plants are harvested by chance. This is a conservative method, enlarging the variation within a harvest but diminishing the difference between the sample mean and the true population mean. Thirdly, after the harvests have been carried out, the largest and the smallest plant of each harvest can be removed from the analysis, a method termed 'trimming' (Barnett and Lewis 1978). This method minimizes the influence of outliers and reduces both the within-harvest-variation and the degrees of freedom but is not necessarily conservative.

The next improvement of the classical approach can be achieved in the computation of RGR itself. To minimize autocorrelation, RGR may be calculated by skipping one harvest each time, rather than deriving values from adjacent harvests. Thus, harvest 1 is compared with harvest 3, harvest 2 with harvest 4, etc. If the RGR value of an interval is assigned to each day in that interval, 2 or 3 values for each day are obtained which may then be averaged (Wickens and Cheeseman 1988). However, this method will leave single values before the second and after the penultimate harvest and thus a greater uncertainty about the real value of RGR for these periods. This can be prevented by taking a double harvest at the first and last day of an experiment, combining one of the two harvests of the first day with harvest 2 and the other with harvest 3. Similarly, one of the two harvests at the last day is compared with the penultimate and the other with the last-but-two. Also, in the case of a sudden change in the environment, as in the experiments of Wickens and Cheeseman, 2 double harvests are to be recommended, as information on these points can not be leveled out with a harvest at the other side of the change.

Up till now, there will still be variation from interval to interval that one would like to see reduced. Wickens and Cheeseman (1988) suggest extending their method by skipping 2, 3 or more harvests each time if a higher degree of smoothing is desired. However, this will also obscure possible time trends, as all differences in RGR are leveled off. Another problem of this approach is that no formalized criteria can be given for the number of harvests to be skipped each time. Finally, this method results in a discontinuous function, whereas a continuous one is preferred.

The essence of the method proposed here is to smooth the interval-to-interval fluctuations with a polynomial. This combines the advantages of the functional and the classical approach: Trends in RGR are smoothed with a continuous function but not necessarily to the same extent as in the functional approach. As the differentiation of a polynomial is avoided, the danger of erratic fluctuations is considerably diminished. This enables the experimenter, if necessary, to describe ontogenetic trends in RGR with a more complex polynomial than a quadratic equation.

However, one has to be aware that the choice of the polynomial is more or less subjective, depending among

other things on the degree of smoothing one wishes to obtain. Selecting a high degree of the polynomial results in a good fit of the data (a high proportion of the variance is explained by the polynomial) but also in very little smoothing. A low degree polynomial will not fit the data as well, but will smooth small random fluctuations. In principle the simplest equation that adequately describes the data should be used (cf. Hunt 1982). Thus, an algorithm should be applied that (a) obtains a minimum of unexplained residual variance with (b) the smallest number of independent variables and (c) avoids too high order terms in the equation. The following procedure is suggested. First, start with a stepwise regression with the averaged values of RGR for each day as dependent variable and time (T) as independent variable: $RGR = b_0 + b_1T$. The independent variable is removed if a non-significant part of the variance in RGR data is explained by the linear term and a mean value remains. If the independent variable contributes significantly, the proportion of variance explained by the equation (r^2) can be calculated. The second step is to consider whether addition of a quadratic term would improve the description of RGR. Thus, a stepwise multiple regression is performed on the equation $RGR = b_0 + b_1T + b_2T^2$. All the non-significant terms are removed and the r^2 of the remaining equation is calculated. Addition of the quadratic term would only appear useful if r^2 is substantially increased. A value of 0.05 is considered to be reasonable in most cases. If the increase is less than 0.05 the analysis could be terminated and one could use the result of the first stepwise regression. If the increase is more than 0.05, the procedure could be repeated by testing the performance of an equation with a third, cubic, term. In exceptional cases this may lead to the inclusion of very high-order terms. As a rule of thumb, the highest order term should not exceed half the number of harvest days and will, except for complex growth patterns, not go beyond the third degree. Thus, with 5 harvests, at most a second degree polynomial is selected. With 12 harvests, performed in a relatively short time period, a third degree term is generally sufficient. Note that this method is at variance with the stepwise method of Hunt and Parsons (1974).

An optimal use of the present method requires an appropriate experimental design. The protocol of frequent small harvests (Hunt 1982) can also be applied in this case. This divides the amount of work and will improve the impression of the ontogenetic drift in RGR. Maximal benefits of the analysis are obtained if harvests are about equally spaced in time and if each harvest includes the same number of plants. Typically, plants are harvested 2 or 3 times per week and 5-10 plants are measured on each occasion. Such a design has two attendant assets. The first concerns the calculation of NAR, for which the procedure for RGR may be applied. In calculating NAR in the classical way, one has to be aware of the assumption that there is a linear

relation between leaf area and plant weight. This may not be the case. However, as Evans (1972) pointed out, if the leaf area has not more than doubled between two harvests, this deviation will be small (<5%). By harvesting frequently, this condition is generally met. A second advantage of the proposed experimental setup concerns the statistical evaluation of differences in RGR between treatments (or species). By using the advised orthogonal experimental design, these differences may be evaluated as a Treatment \times Time interaction in an analysis of variance with ln-transformed plant weight as dependent variable (Poorter and Lewis 1986).

Materials and methods

Experimental design

To evaluate the proposed method two experiments were carried out. In the first experiment *Geum urbanum* L. plants were grown in a growth chamber in nutrient solution. The solution was replenished each week to prevent nutrient depletion. Before the start of the harvest period (day 0) the largest and the smallest plants were removed (ca 40% of the population). The growth of the plants was followed for 38 days. During this period 24 harvests were performed, with either 1 or 2 days between each harvest. Each harvest day the fresh weight of 5 plants was measured, except for days 0 and 37 when 10 plants were measured.

In the second experiment the ontogenetic drift of *Carex flacca* Schreb. was studied (A. van der Werf and D. Raaimakers, personal communication). Plants were grown as described above. A growth analysis was carried out for 38 days with a total of 18 harvests of 8 plants. A double harvest was performed at the beginning and the end of the experiment. For each plant the total dry weight was determined.

Statistics

Prior to statistical analysis, data were trimmed by removing the smallest and the largest plant from each harvest (Barnett and Lewis 1978). Data were analysed using the stepwise multiple regression procedure of the SAS program (Joyner 1985). Terms were considered significant at the 5% level.

Results and discussion

Three examples are given to illustrate the proposed method. In the first experiment the growth of *Geum urbanum* plants was followed. Although the harvests were densely spaced and only 5 plants were measured each time, the curve of the ln-transformed plant weight does not show considerable fluctuations (Fig. 3). The RGR calculated according to the classical approach and averaged by the running mean method decreased with the age of the plants. (Note that this method is sensitive

enough to detect a possible flaw in the scheme of the replenishment of the nutrient solution. During the last 2 weeks of the experiment the RGR increased immediately after each change of the nutrient solution, which may indicate that these large plants had exhausted the solution already after a few days.) The functional and the 'combined' approach yield in this case the same time course, i.e. a linear decrease in RGR.

In the second example, data on growth of *Carex flacca* are analyzed (Fig. 4). Tested with a multiple regression, a quadratic polynomial was found to adequately fit the means of the ln-transformed plant weights. Thus, the RGR calculated according to the functional approach decreases linearly with time. When the classically derived RGR values are fitted, a curvilinear time trend is found. This function explains 48% of the total variation of the running mean RGR's, whereas the straight line of the functional approach could account for only 23% of this variation.

The last example are the complicated growth data of Kreuzler et al. (1879) for maize, used among others by Hunt (1982) for illustrational purposes. The results of the classical approach and the functional approach are shown in Fig. 5A. Using the criteria of Parsons and Hunt (1974), a third order polynomial was used to fit the ln-transformed plant weight data. Apparently this

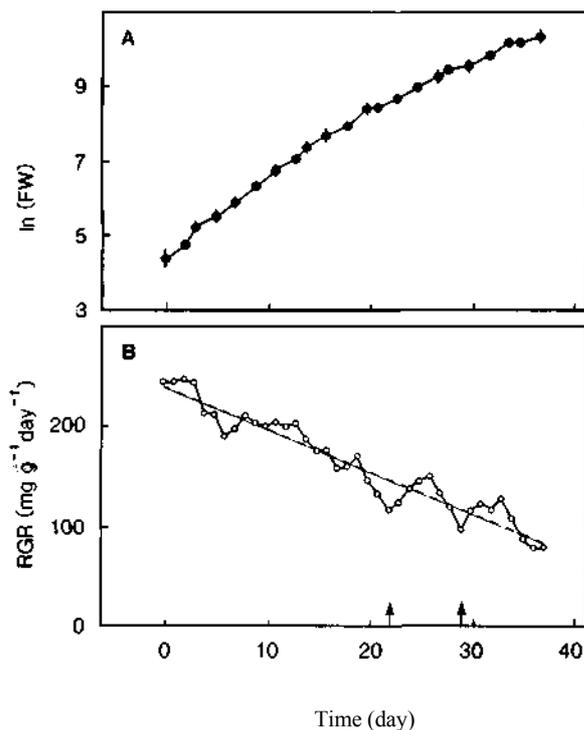


Fig. 3. A. Time course of total fresh weight of *Geum urbanum*. Mean value \pm SE ($n = 3$). Time course of RGR. (o—o), Averaged over two periods; (---), result of the functional approach; (—), result of the 'combined' approach. Arrows indicate the last two times that the nutrient solution was replenished.

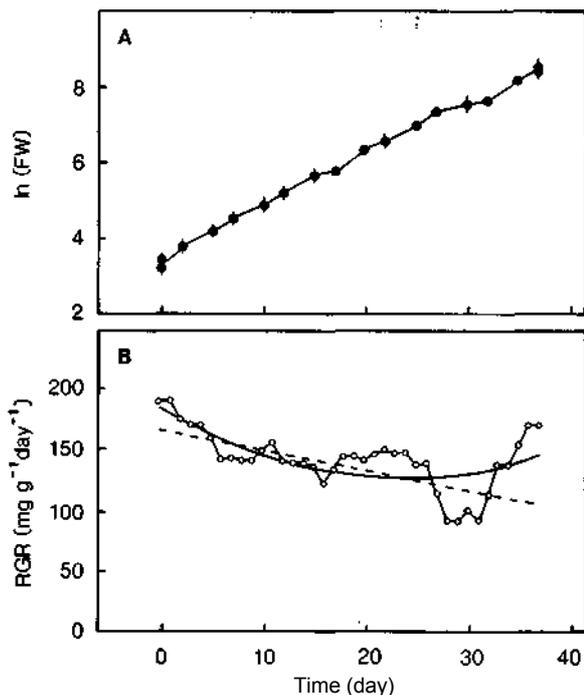


Fig. 4. A. Time course of total dry weight of *Carex flacca* (previously unpublished data of A. van der Werf and D. Raai-makers). Mean value \pm SE ($n = 6$). B. Time course of RGR. (o—o), Averaged over two periods; (----), result of the functional approach; (—), result of the 'combined' approach.

leads to considerable underfitting of RGR. Increasing the degree of the polynomial would result in spurious fluctuations, as was shown by Hunt (1982). The running mean values of the RGR are dampened compared to the classical approach (Fig. 5B). This trend could not be described by a simple polynomial. Up to 8 terms were calculated; 6 of them appeared to be significant (5 terms were significant at the 0.1% level, 1 at the 5% level) in the regression procedure. The calculated curve describes the data better than the equation calculated by the functional approach. Parsons and Hunt (1981) could find a comparable trend by applying the technique of splined cubic polynomial exponentials using 4 different cubic equations to fit different parts of the growth curve. However, this technique is not simple, requires several subjective decisions (is a normal polynomial insufficient, how many exponentials are used to fit the data, where are the connecting points between the different equations) and a complicated statistical program and may also yield erratic time trends (Wickens and Cheeseman 1988). It is thought that the 'combined' approach described here is more straightforward and easier to use with standard available computational power.

What is the effect of data collected in one part of the experiment on the RGR in another part of the experimental period? By omitting the 3 first and the 3 last

harvests in the maize growth experiment of Kreuzler et al. (1879), an impression of the stability of the two methods may be obtained. As expected, the fit of the functional approach is better when analyzing the smaller data set (Fig. 6A) but is still rather poor. The curves for the entire data set and the restricted one differ considerably from each other ($r^2 = 0.68$ in a linear regression of the estimates of each day in both data sets), indicating a rather low stability. The 'combined' approach yields a far better result, as the RGR values for the restricted period hardly differ from the whole curve ($r^2 = 0.95$).

The data of Kreuzler et al. (1879) may also be used to illustrate the difference between the method of Wickens and Cheeseman (1988) and the 'combined' approach. If no skipping is applied, the normal classically derived time course of RGR is obtained (Fig. 6B). Skipping 2 harvests each time results in considerable smoothing, but the cessation of growth at the end of the experiment is overlooked. By skipping 4 harvests the whole time course is strongly influenced. The decrease in weight at the beginning has vanished as the whole time trend is dampened. The more harvests are skipped, the stronger this dampening will be. As the choice of the interval to be skipped influences the resulting time course, a formalized decision rule is essential. Wickens and Cheese-

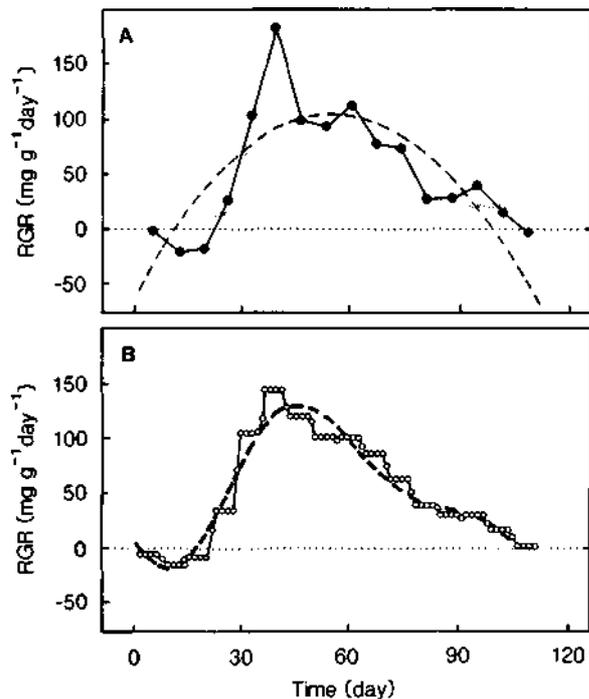


Fig. 5. A. Time course of RGR of *Zea mays* (Kreuzler et al. 1879). (●—●), Calculated according to the classical approach; (----), result of the functional approach (3rd order polynomial). B. Time course of RGR. (o—o), Averaged for each day over two adjacent harvest intervals; (----), result of the 'combined' approach.

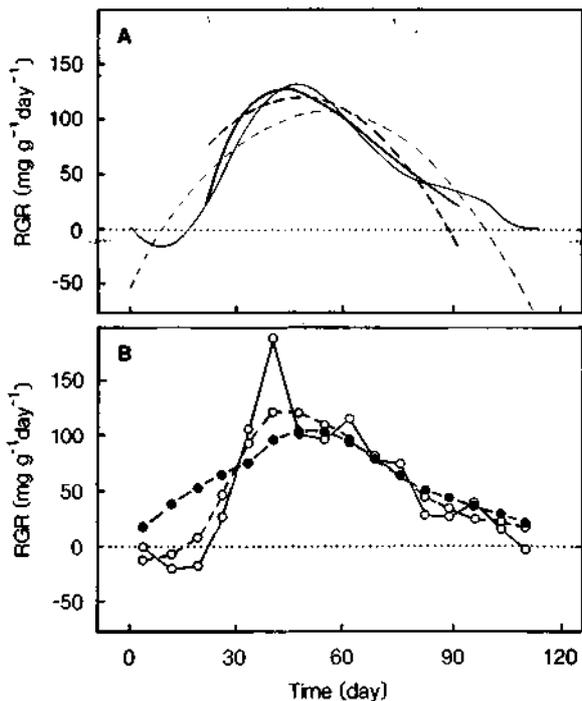


Fig. 6. A. Time course of the RGR of *Zea mays* (Kreusler et al. 1879). (---), calculated according to the functional approach for day 0-113; (-.-.-), the same for day 22-92; (—), calculated according to the 'combined' approach for day 0-113; (.....), the same for day 22-92. B. Time course of RGR, calculated according to Wickens and Cheeseman (1988). (o—o), no skipping of harvests; (o-----o), 2 harvests skipped each time; (•---•), 4 harvests skipped each time. For visual clarity only the values at the midpoint between two harvests are given.

man (1988) do not pay any attention to this point. The proposed method also uses the technique of skipping but as this skipping is restricted to one harvest each time, short-term fluctuations are minimized without strong dampening of the overall time trend.

A prerequisite for a correct use of a method is knowledge of its weak points. Whereas curve fitting of plant weight data may result in very rigid time courses of RGR, one should be aware that fitting RGR values could lead to time courses which are rather flexible. If such time courses are not justified from a biological point of view, it is advised to avoid too complex polynomials. The extremes of the curve also require attention. In contrast to the middle region of a curve, where deviating values are averaged with neighbouring points, an aberrant point at one of the extremes may exert a decisive influence on the course of the polynomial. Using double harvests at the start and the end of the experiment may mitigate against serious deflections. An alternative could be to use the first and last harvest in the calculations but to consider only the values between the second and the penultimate harvest day.

Conclusions

The method described above, where RGR values derived in the classical way are fitted by a polynomial, combines the advantages of the functional and the classical approach while avoiding some of their pitfalls and disadvantages. Complicated ontogenetic trends may be described with a smoothed curve, with minimal danger of over- and underfitting. Also, values in one part of the experiment exert little influence on values elsewhere. The method is straightforward and requires only standard statistical procedures.

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