Growth Analysis Techniques: An Overview

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Abstract

The rate at which new plant material is accumulated is an integrated measure of plant responses to the environment and is a useful character when comparing either genetic differences between populations or the effects of different environmental factors on populations. There are two approaches to analyse growth of plant quantitatively-either to derive growth attributes in the form of mean values over the intervening period of time between two harvests (classical approach) or to fit smooth curves to experimental data and then to derive fitted values for the growth attributes which may be plotted continuously (functional approach). This article provides an overview on the development of the concept of both the techniques of growth analysis.

INTRODUCTION

All living organisms, at various stages in their life history, are capable of change in size, change in form and change in number, given suitable conditions. These three processes together form an important part of the phenomenon of life itself and among natural systems help to distinguish the living from the non-living. The three are strongly interlinked and the term 'growth' may be applied to any or all of them. For these reasons a precise definition of what is meant by growth is not at all easy (Hunt, 1978).

Analysis of quantitative aspects of growth of whole plants can be effectively conducted using growth analysis techniques. Growth analysis represents the first step in the analysis of primary production, being a link between recording plant production and analyzing it by means of physiological methods. Using growth analysis techniques it is possible to follow the formation and accumulation of plant biomass as determined by either environmental or internal factors or both. In practical terms, the ultimate aim of the plant physiologist is to understand the factors which contribute to plant growth may be eliminated, and those promoting growth may be encouraged.

The advantage of growth analysis is that the primary measurements required in the technique are relatively easy to obtain without great demands on laboratory equipment. These primary measurements are usually the dry weight of the whole plant and its components (stems, leaves, roots etc.), and the dimensions of the assimilatory apparatus (leaf area, chlorophyll content, protein content etc.). These primary measurements are made on matched pairs of plants at two harvests separated by appropriate time intervals. From these measurements various indexes and characteristics are calculated which describe the growth of the plants and their parts as well as the relationship between the assimilatory apparatus and dry matter production. Growth analysis can be regarded as a

useful approach to analyzing net photosynthetic production by plants. Net production being defined as the net result of the assimilatory work taking place in a plat during a certain period. In photosynthetic terminology, this means that net production is equal to net assimilation (gross assimilation minus respiration) minus the losses of dead plant parts over a certain period of time.

Growth analysis techniques can be divided into two groups: (i) In the 'classical' analysis of growth, the primary measurements are collected through a series of relatively infrequent, large harvest. Mean growth characteristics are calculated between two harvests.

(ii) In the 'functional' analysis of growth, the harvests are smaller with less replication of measurement but they are more frequent. Growth characteristics are calculated from growth curves fitted through all the primary values. Such an approach offers great advantages for investigating general trends rather than single values which may be affected by short-term variations in environmental conditions. It also allows an entirely different design of experiment through the experiment instead of being concentrated at peak periods (Hughes and Freeman, 1967; Radford, 1967).

CLASSICAL GROWTH ANALYSIS

The early investigators of the quantitative relationships involved in the growth of plants were following the classical pathway when they tried to find simple empirical relationships which would, in a similar way, sum up many individual measurements of attributes of growing plants. The ideas underlying some of these attempts proved to be of such limited application that they are now almost forgotten. An example is afforded by the attempts of Brailsford (1908a, b) to demonstrate that plant growth follows the time course of an autocatalytic chemical reaction in a closed system. He had an initial success in fitting an autocatalytic curve to the latter part of the growth curve of an organ of limited growth-a fruit of *Cucurbita pepo*. But even here there were large deviations in the early part of the fruits' development. However, this line of approach subsequently led to the development of more complex formulae with increasing numbers of arbitrary constants, without even reaching the point where the quantitative relationship of a plant's growth could be predicted.

Modern concepts on the growth of whole plants can be traced in unbroken sequence to a paper by Noll in 1906, unfortunately preserved only in title in the proceedings of

the society to which it was read. There is, however, an almost contemporary reference, apparently written in the following year, by Noll's pupil Hackenberg (1909). The same period also saw the publication of the other researches by Noll's students all making use of the same data: Gressler (1907) on the growth of a number of species of *Helianthus*; Gericke (1908) on the effect of removal of the cotyledons on the growth of *Helianthus annus*; and Kiltz (1909) on *Nicotiana tabacum*.

In 1919, V. H. Blackman, stimulated by the first investigation of one of his students, F. G. Gregory, on leaf expansion of cucumber, pointed out that the growth of an intact plant, as exemplified by the gain in weight, was a continuous process. He drew attention to the fact that the changes in weight with time obeyed the compound-interest law, that is to say, the gain at any time was proportional to the amount of biological capital. On this basis he criticized the previous proposals put forward by Noll in 1906 that the best measure of the rate of change in plant weight was the ratio that relates the dry weights of two given time intervals. Such a proposal erroneously implied that the relationship between time and weight was linear.

Examining the field data on the growth of *Helianthus annus* contained in a doctoral thesis of Gresslee, one of Noll's students, Blackman demonstrated that for a considerable part of the growth cycle the calculated rate was relatively constant. This rate, therefore, reflected the performance of the plant under a given set of, conditions, and Blackman contended that this 'efficiency index' could be employed as a critical yardstick for comparisons between either species or habitats.

Brechley (1920-21) published an account of her investigations on the pattern of change in weight of *Pisum sativum* grown under conditions of water culture in a greenhouse at different seasons of the year. In each experiment, the efficiency index between consecutive weekly sample was calculated as recommended by Blackman, and an appraisal was made of the influence of temperature and hours of bright sunshine on the variation of the index. The statistical treatment of the data was undertaken by R. A. Fisher, and he was the first to calculate multiple regressions linking the index with other factors.

The next year saw the publication of three papers by Briggs *et al*. (1920-21a,b,c). Like Blackman, for their basic field data they drew on the extensive records accumulated, but not interpreted, by German Botanists in

the last quarter of the nineteenth century, particularly the findings of Kreusler *et al.* (1877) on *Zea mays* and on Hornberger (1885) on *Sinapis alba*. From the seedling stage to maturity and to compare the seasonal trends with those derived for *H. annus* from the data of Gressler cited by Blackman. These authors observed that the efficiency index, or the relative growth, as they preferred to call it, fluctuated considerably from week to week. They were also critical of Blackman and Brenchley for their assumption that growth was a continuous exponential process and that the best method of estimating the rate over a given time interval was obtained by the change in weight on a logarithmic scale. Contributing to this argument from a mathematical point of view, Fisher (1921) pointed out that the relative growth rate was most simply expressed as an instantaneous value. In calculus notation this reads

$$\frac{1}{W}\frac{dW}{dt} = \frac{d}{dt}\log_e W$$

Integration of both sides between the limit of t_1 and t_2, the times of harvests in question, yields the required formula for the mean relative growth rate as follows:

$$\bar{R} = \frac{\log_e W_2 - \log_e W_1}{t_2 - t_1}$$

Gregory (1918) was the first to conceive the idea of net assimilation rate (NAR) and to apply it to growing plants. He calculated mean net assimilation rate between two harvests by dividing the differences in the total plant weight at each harvest by the mean leaf area between those harvests, i. e.

$$\bar{E} = \frac{W_2 - W_1}{LA}$$

Briggs *et al.* (1920-21b) termed it 'unit leaf rate' (ULR) and defined it as the increase in dry weight per square centimeter of leaf per week, taking as the leaf area the average of the area at the beginning and end of the week. This quantity is conventionally given the symbol E and the expression for its instantaneous value is

$$E = \frac{1}{LA} \cdot \frac{dW}{dt},$$

Where LA is the total leaf area present on the plant. Williams (1946) provided a convenient formula for the estimation of mean NAR over a period of time:

$$\bar{E} = \frac{W_2 - W_1}{t_2 - t_1} \cdot \frac{\log_e LA_2 - \log_e LA_1}{LA_2 - LA_1}$$

Briggs *et al.* (1920-21b) also calculated the leaf area ratio (LAR) i. e. the total leaf area divided by the plant weight. Lastly, Briggs *et al.* (1920-21b,c) made an important contribution to the quantitative analysis of growth. They pointed out that if the changes in plat weight and leaf area are on an exponential basis, it can be shown mathematically that the relative growth rate is the product of the unit leaf rate and leaf area ratio.

The next 15 years saw the further application of these concepts to the study of plant development under either field or glasshouse condition. Crowther, one of Gregory`s students, carried out pioneering investigations on cotton in the Sudan and in Egypt, and by subsampling techniques he obtained comprehensive records of the leaf production and flower and ball formation. In two papers (Crowther, 1934; 1937) he was concerned with the changes in the relative growth rate and net assimilation rate induced by internal and external factors.

Between 1936 and 1946, a number of Australian workers examined the effect of such factors as nitrogen, phosphorus and water supply on the growth of a number of crop species. These results, together with further information derived from data published by workers outside Australia, were reviewed from the point of view of growth analysis by Williams (1946). One aspect he discussed was the magntitude of the ontogenetic drift in net assimilation rate and the most appropriate index of the internal factors governing the efficiency of the leaves. Comparisons of the change in the rate were made when the rate was expressed in terms of leaf weight, leaf area and leaf protein.

Further evidence for the relative constancy of the net assimilation rate on an area basis for sugar beet and mangolds is provided by the data of Watson and Baptiste (1938) from Rothamsted Experimental Station, England. A notable feature of this investigation was that it was the first occasion on which a detailed growth analysis of the relative performance of two species has been made in the field.

FUNCTIONAL GROWTH ANALYSIS

The calculation of the various growth analysis parameters as mean values over the period of time between two harvests has been the standard approach for most of the sixty years that have elapsed since the early origins of the

subject. More recently, however, many workers have taken advantage of high-speed computing facilities to fit mathematical functions to experimental data. From these functions (growth curves), fitted values of data are extracted and then used to derive the various growth parameters which may subseqnently be plotted as fitted instantaneous values.

Some of the advantages of the functional approach over the classical approach to plant growth analysis are listed below (Hunt, 1978).

1. Many of the assumptions involved in the calculation of mean values of parameters such as NAR and LAR are avoided completely. The only necessary assumption being that the fitted growth functions adequately describe the primary data.

2. Information from all sampling occasions is used in determining each value of the derived parameters.

3. The difficulties involved in the pairing of plants prior to applying the classical formulae are avoided.

4. The procedure does not depend upon large harvests and the labour is spread evenly through the experiment.

5. Provided that plants in different experimental treatments are grown simultaneously, the harvesting of these treatments need not be synchronous; interpolated comparisons are feasible.

6. Replication at different points in time need not be equal.

7. Small deviations from the overall trend may be smoothed to gain an impression of growth which is free from random fluctuations.

8. Statistical analyses may be integrated into the same analytical procedure as the calculation of the derived parameters.

9. The approach provides the clearest possible perception of time-dependent phenomena such as ontogenetic drift.

Historically, there has been considerable interest in the idea of fitting mathematical functions to plant growth data. A good historical review of growth functions has been given by Richards (1969). In the early days the logistic was almost universally used (Reed and Holland, 1919; Reed, 1920a, b; Robertson, 1923), although Reed (1920c) used the monomolecular and Pearl and Reed (1925) employed an empirical generalization of the logistic. Very often, a hypothesis was postulated to explain the application of a particular function: Robertson (1923), for example, explained the suitability of the logistic function by suggesting that growth was regulated by an autocatalytic 'master reaction' . Some workers, like Mithorpe (1959), have used the logistic empirically as a smoothing device, whereas others, e.g. Amer and Williams (1957) in attempting to compare parameter values of Gompertz functions fitted to *Pelargonium* leaf data, have endowed the function with a degree of biological validity. A major disadvantage of the three functions described above is that each has a particular and unalterable shape.

Richards (1959) developed a generalized logistic function which includes the monomolecular, Gompertz, and logistic function as special cases. The Richards' function, through the introduction of an extra parameter, interpreted in a biologically meaningful way.

Since the derivation of the Richards' function in 1959, it has been employed extensively in growth studies (Friend *et al*., 1962; Voldeng and Blackman, 1973; Hackett and Rawson, 1974; Williams, 1975; Woodward, 1976). These workers either stated that they fitted the function to data using the method originally suggested by Richards, or no method was described at all. Richards' (1959) method is not statistically efficient, but it was the best method that could be used before the widespread availability of electronic computers. The statistical procedures for Richards' function involve the approximate methods of non-linear regression. A reliable method is now available for estimating the parameters of Richards, function together with the standard errors of the estimates (Causton *et al*., 1978). The problem of the confidence band has been solved (Venus and Causton, 1979). Therefore, the full potential of the function to describe quantitatively growth and growth characteristics can be exploited now.

Because of the difficulties involved in the estimation of variances, both for the function and its first differential coefficient, logistic and Gompertz functions have not been adopted widely, until recently. Therefore, considerably more use had been made of polynomial functions, which are linear from the statistical point of view since the coefficients are linearly related.

Vernon and Allison (1963) reported the successful use of polynomial regressions for estimating net assimilation rate (NAR). Presenting data on the growth of *Zea mays* these authors fitted second order polynomials (quadratic

curves) to total dry weight (W) and leaf area (LA) as follows:

$$W = a + bt + ct^2 \quad \text{........(i)}$$
$$LA = a' + b't + c't^2 \quad \text{........(ii)}$$

Where a, a', b, b', b, c and c' are constants.

Differentiating equation (i) they obtained the absolute growth rate as follows:

$$\frac{dW}{dt} = b + 2ct$$

from which instantaneous values of net assimilation rate (E) were derived in this manner,

$$E = \frac{1}{LA} \cdot \frac{dW}{dt} = \frac{b + 2ct}{a' + b't + c't^2}$$

Solving the above equation for different values of t enabled Vernon and Allison to plot E vs. t on a continuous basis throughout the period studied. They found that the use of regression analysis substantially smoothed the fluctuations is estimates of NAR over a period of time compared with the estimates derived from the harvest interval approach. Although straightforward in its approach, and making an important step forward, method suffers from the serious statistical drawback that progressions of W and LA against time (as opposed to those of $\log_e$ W and $\log_e$ LA) seldom show a uniform variability with increasing t, which they must do if they are to be subjected to regression analysis.

Hughes and Freeman (1967) have improved Vernon and Allison`s application of regression analysis to growth studies, firstly by transforming the primary data (total dry weight and leaf area) to natural logarithms, and secondly, by calculating standard errors for estimates of the derived functions. Using data on the growth of *Callistephus chinensis* under controlled-environment conditions, they developed the following third-order (cubic) regression:

$$\log_e W = a + bt + ct^2 + dt^3$$
$$\log_e LA = a' + b't + c't^2 + d't^3$$

Hughes and Freeman (1967) derived relative growth rate (R), leaf area ratio (F) and net assimilation rate (E) in this manner:

$$R = \frac{d\log_e W}{dt} = b + 2ct + 3dt^2 \quad \text{.........(i)}$$

$$F = \frac{LA}{W} = \text{antilog}_e (\log_e LA - \log_e W) \quad \text{....... (ii)}$$

$$E = R/F \quad \text{....................(iii)}$$

However, one disadvantage of Hughes and Freeman's (1967) approach was that their computer programme fitted cubic polynomials to all log transformed data in every case, whether or not a real cubic relationship existed. This had the effect of unnecessarily increasing the S. E.' s of the derived attributes in cases where the quadratic or cubic parameters of regression were not significant.

Nicholls and calder (1973) have published a general discussion on the use of regression analysis for the study of plant growth. In worked examples on the growth of *Atriplex spongiosa* and *A. lindleyi*, they demonstrated that increasing the complexity of the regressions used to describe the changes with time in log transformed plant variables increases the S. E.' s of the derived growth attributes. It may also lead to spurious values of these attributes themselves. They summarized their approach by suggesting that each set of data should be considered on its own for selection of the appropriate regression model, over-fitting is a real trap.

Working independently, but along similar lines to Nicholls and Calder (1973), Hunt and Parsons (1974) developed Hughes and Freeman's (1967) approach a stage further. A step-wise regression procedure was incorporated in which polynomials of the first to third order were fitted to $\log_e$ W and $\log_e$ LA. Tests were made to determine the best fit in each case. A disadvantage of Hunt and Parsons' (1974) approach is that difficulties sometimes occur when trying to interpret a whole body of data in which regressions of varying order have been applied objectively, according to the needs of individual sets of data (Hurd, 1977). Because the form of the progressions of the derived attributes on time depends immutably on the nature of the original regressions, and because small differences among the latter are often reflected in large differences among the former, comparisons between species or treatments are sometimes puzzling when quite distinct patterns in the behavior of the growth attributes emerge from sets of raw data which differ only slightly from one another, but nevertheless enough to cause the selection of different growth attributes.

Another problem in the application of polynomials to biological data is whether to use harvest mean or individual data points. Nicholls and Calder (1973) criticized Eagles (1969) for his use of harvest means in fitting polynomials to *Dactylis glomerata* data, saying that the use of the mean values represents a serious loss of a major portion of the raw data. By artificially manipulating data on the growth of several species, Elias and Causton (1976) showed that when growth curves were fitted to data of low variability, unrealistically high degrees of polynomial were quite likely to be selected if purely objective criteria alone were used. When variability was high, however, the best fit was more likely to be a lower-order polynomial. They concluded that regressions should be fitted using harvest mean values where the test of adequacy of fit is independent of the underlying population variability.

Richards (1969) in reviewing the quantitative analysis of growth, considered that polynomial equations when fitted to the basic data were inappropriate because they were not based on biological concepts and the concepts have no biological meaning. Fitting polynomial to log transformed data partly overcomes this objection.

In spite of the above objection, the use of polynomial curves had great potential in a purely empirical approach to the study of plant growth, where the objective is to assess and compare either genetic of environmental influences. Because $\log_e$ W and $\log_e$ LA are related to time, polynomial curves have an added advantage, viz. the regression equations are linear models in the statistical sense, and thus allow the commonly used statistical tests to be applied, simplifying the provision of errors to the estimate of growth attributes.

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