

Computer analysis of N. crassa  
growth curves.

The determination of the best fit line to a growth curve has been difficult because of its sigmoidal nature. A number of equations have been developed which attempt to describe growth curves. These include the logistic, Gompertz, von Bertalanffy, and Richardson equations (Ricklieds, 1967, Ecology 48:978-983; Richardson, 1984, Bull. Southern Calif. Acad. Sci. 83:101-115). Several statistical packages are available which allow the use of these equations for computer analysis of the data, including the SAS system; BMDP (Bio-Medical Data Processing), and Systat (IBM). All packages can be run on an IBM microcomputer, but require a hard disk drive. We describe here the use of the SAS system for the analysis of N. crassa growth curve data.

The SAS system is an all-purpose system designed for data analysis and is available through SAS institute, Inc. Box 8000; Cary, NC 27511-8000 (919-467-8000). The software is compatible with IBM 370/30XX/43XX; Digital Corporation VAX 11/7XX; and Data General ECLIPSE series to name a few. We used the Data General System available through the Academic Computer Center at SUNY-Plattsburgh.

The particular procedure used was PROC NLIN and is written as shown in Table I. This is a least-squares procedure for estimating parameters for non-linear models. Data is entered into the program using the DATA statement. This is accomplished by retrieving data stored in a separate file or by typing the data directly into the program as was done here. PROC NLIN invokes the SAS procedure. BEST = 10 requests that the residual sums of squares for only the best iterations are printed. PARMS sets the starting values for the parameters. In this case, BO = asymptote and is set at 50 mg dry weight; B1 = growth rate constant: the program will iterate from a value of 0 to 0.99 in increments of 0.05; B2 = inflection point: the program will iterate from a value of 15 to 30 in increments of 1.0. The MODEL statement is the equation for the logistic curve written in SAS nomenclature. The remaining statements involve instructions for output of the analysis. Since no method was chosen, the default method DUD was used as the iterative procedure. Several other methods of iteration are possible. The above procedures and statements can be modified for use with the Gompertz, von Bertalanffy, and Richardson equations. As a result, one can determine the equation which best describes the data.

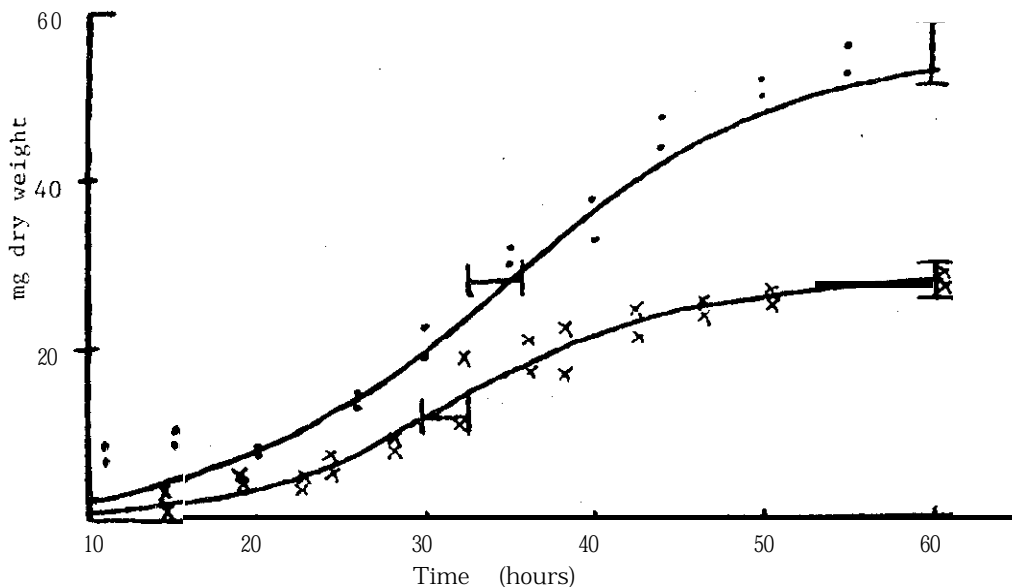


FIGURE 1 - Growth of Wild-Type N. crassa at 30°C (.) and 25°C (X).  
 Lines are drawn through the values predicted by computer analysis.  
 Bars indicate the 95% confidence intervals for the asymptote and inflection points.

Table 1 - Program for Data Analysis

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DATA A
INPUT X Y @@;
CARDS;
13.0 08.0 13.0 08.7 17.0 09.0 etc.

PROC NLIN BEST = 10;
PARMS BO = 50.0
      B1 = 0 to 0.99 by 0.05
      B2 = 15 to 30 by 1.0;

MODEL Y = BO/(1 + EXP (-B1*(X-B2)));

OUTPUT OUT = B P = YHAT R = YRESID;

PROC PLOT DATA = B;
PLOT Y*X = 'A' YHAT *X = 'P'/OVERLAY VPOS = 25;
PLOT YRESID * X/VREF = 0 VPOS = 25;

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Table 2 - Summary of Computed Variables for Growth at 30°C (A) and 25°C (B). All values are rounded off to two decimal places.

Parameter	Estimate	Std. Error	95% Confidence Interval	
			Lower	Upper
A				
asymptote	53.69	1.65	50.41	56.97
growth rate	0.12	0.01	0.10	0.14
constant				
inflection	34.25	0.77	32.72	35.78
B				
asymptote	26.94	0.70	25.51	28.37
growth rate	0.15	0.01	0.12	0.17
constant				
inflection	31.37	0.70	29.93	32.80

This program calculates predicted values from the equation which describes the best fit line through the actual data (Figure 1). In addition, the output includes a table of values for the asymptote, growth rate constant, and inflection point (Table 2). Note that for growth at 30° C the asymptote is estimated to be 53.69 mg dry wt with a standard error of 1.65. The 95% confidence interval falls between values of 50.41 and 56.97 mg dry weight. For growth at 25° C, the asymptote is 26.94 with a standard error of 0.70 and a 95% confidence interval of 25.51 to 28.37. The inflection points can be compared in a similar manner.

One advantage of using this method of analysis is that it allows a comparison of different growth curves. Due to the absence of overlap between the values for the 95% confidence intervals for both asymptote and inflection point, we can conclude that the curves are significantly different. The program also provides an analysis of variance from which the coefficient of determination ( $r^2$ ) can be calculated. In this case,  $r^2$  for the growth curve at 30° C was 98.9%, while  $r^2$  for the curve at 25° C was 99.2%. It can therefore be concluded that the logistic equation accurately describes the data. Furthermore, once the equation has been defined, additional techniques for comparison among growth curves can be used (Ricklefs, 1967). - - - Dept. of Biological Sciences, State University College at Plattsburgh, Plattsburgh, NY 12901