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NONLINEAR ESTIMATION OF GROWTH CURVE MODELS FOR GERMINATION DATA ANALYSIS

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ABSTRACT

Logistic, Gompertz, Richards and Weibull growth curves were evaluated for their suitability as mathematical and empirical models to represent cumulative germination. By avoiding the limitations associated with the method of moments and single-value germination indices, the fitted models provided superior description of the time course of germination. The four-parameter Weibull model gave the best fit across a relatively wide range of seed species and germination conditions, and the resulting parameter estimates reflected identifiable aspects of the germination process. The nonlinear estimation of the germination response included a parameter summary, together with their asymptotic standard errors and correlation matrix, along with an approximate band for the expectation function, pairwise plots of the parameter inference region, and profile t plots. Evaluation of the fitted models also included information on lack of fit and residual structure. Empirical results and hypothesis testing were demonstrated with reference to a replicated experiment designed to determine the effects of reduced water potential on germination of onion seeds.

Keywords: nonlinear regression, cumulative germination, growth curves, Weibull model.

I. INTRODUCTION

Seed germination is a complex biological process beginning with water uptake by the seed and culminating in emergence of the embryo from the seed coat (radical or hypocotyl emergence). Many researchers have attempted to quantify this process which is influenced by various environmental as well as genetic factors. The effect of specific factors is typically presented in terms of an S-shaped germination curve, relating the cumulative percentage of germination to time. The ideal description of germination should be complete, concise, unambiguous, and amenable to statistical analysis (Brown and Meyer, 1988a). It should also provide information concerning
location (lag), rates (speed), and extent of germination.

Traditional methods used in germination data analysis include single-value indices and the method of moments. Single-value indices have been used extensively in order to summarize the time course of germination with a few coefficients (germination indices). They have often been utilized to emphasize independent aspects of germination, or enable distinguishing superior germination from inferior germination (e.g. Kotowski, 1926; Macguire, 1962; Timson, 1965; Gordon, 1971; Lehle and Putnam, 1982). Similarly, in the method of moments, statistics such as total, mean, and variance of time to germination, quartiles, percentiles, time to 50% germination, etc., are used to represent the germination process and assimilate final germination (e.g., Tucker and Wright, 1965; Nichols and Heydecker, 1968; Thompson, 1970; Orchard, 1977).

Limitations associated with moments and indices in describing the germination process are: i) they are insensitive, ambiguous, and incomplete, ii) they do not supply essential information about location, acceleration, dispersion in time, and extent of germination, iii) they assume a normal distribution for the frequency of germination (i.e., method of quantiles, probit analysis, polynomial regression) whereas the frequency distribution of germination time is skewed, and iv) they do not describe the germination process, they simply represent it.

An alternative to using an index number for defining the processes of germination is the use of growth models. Given the correct mathematical specification along with the appropriate statistical estimation, this approach can provide considerable information resulting in parameter estimates with meaningful and relevant biological interpretations. Many mathematical models have been proposed to describe germination curves including (but not limited to) logistic (Janssen, 1973; Hsu, Nelson and Chow, 1984; Torres and Frutos, 1990), Gompertz (Lapp and Skoropad, 1976; Tipton, 1984), Richards (Berry, Cawood and Flood, 1988), and Weibull (Bonner, and Dell, 1976; Brown and Mayer, 1988b) functions.

The purpose of this study was to evaluate the suitability of the specified growth models to describe germination data using nonlinear regression. Additionally, statistical criteria pertinent to presenting nonlinear estimation results are discussed. Empirical applications are demonstrated using seeds from a wide range of both crop and weed species (onion, rapeseed, sugarbeet, kochia, matgrass, and medusa head) under a variety of germination conditions. Specific references are made to a replicated experiment designed to investigate the effects of reduced water potential on germination behavior of onion seeds.

II. METHODS

Four common nonlinear asymptotic growth models were used for evaluation, including two three-parameter and two four-parameter models. These were:

(i) logistic

\[ y = M[1 + \exp(L - Kt)]^{-1} \]

(ii) Gompertz

\[ y = M[\exp(-\exp(L - Kt))] \]
(iii) Richards
\[ y = M[1 - \exp(-K(t - L))]^{(1-C)}; \]
and
(iv) Weibull
\[ y = M[1 - \exp(-K(t - L))^C], \]
where
- \( y \) = cumulative percentage germination at time \( t \),
- \( M \) = asymptote (theoretical maximum for \( y \)),
- \( L \) = time scale (lag related) constant,
- \( K \) = rate of increase, and
- \( C \) = shape parameter.

The logistic function is sigmoid and symmetrical about the inflection point. This function is very similar to the cumulative normal distribution. (unskewed and perfectly symmetrical). In spite of its popularity however, it can impose a limitation in fitting germination data, since cumulative germination curves are nearly always skewed (Moore and Ross, 1982; Brown and Mayer, 1988b). The Gompertz function is sigmoid and asymmetrical about the inflection point with a fixed skewness of approximately 1.3. Like the logistic, the Gompertz function starts with a lower asymptote, making the estimation of lag time (start of germination) rather arbitrary.

The Richards model is based on a four-parameter sigmoid function, with \( C \), the shape parameter, measuring the various patterns and indicating alternative functional forms (i.e. monomolecular, logistic, Gompertz) that may be generated by this model. While flexible, the function has significant curvature in the solution locus of all four of its parameters (Ratkowsky, 1983), lacks desirable properties associated with the estimated parameters (Ratkowsky, 1990), and demonstrates some degree of nonconvergence in empirical estimation. The Weibull model is a flexible and simple function with great potential for application to biological data, particularly if the system to be modeled can exist in either of two states (e.g., germinated or ungerminated). It is often suitable where conditions of strict randomness of the exponential distribution are not satisfied (Brown, 1987). The Weibull has been used by several authors for analyzing and describing seed germination (Bonner and Dell, 1976; Brown and Mayer, 1988b; Bridges, et al, 1989). The function's parameters are biologically interpretable, reflecting maximum germination (M), germination rate (K), lag in onset of germination (L), and the shape of the cumulative distribution (C).

The estimation of specified growth curve models was accomplished using the univariate nonlinear regression and the Gauss-Newton algorithm. Theoretical developments concerning the iterative estimation procedure and linear approximation along with practical considerations are reviewed by Bard (1971), Gallant (1987), and Bates and Watts (1988). Details of alternative estimation methods are given in Kennedy and Gentle (1980).

Statistical computations were carried out using SAS/STAT,IML (1989, 1985). Program codes required for the Gauss-Newton algorithm, alternative functional forms and their respective derivatives, as well as other statistical criteria presented in this section are given in the Appendix.

Presenting the statistical results of nonlinear estimation, as in all statistical analyses, is an important consideration. Regardless of the specific area of research to
which they may apply, results from a nonlinear regression analysis should be reported clearly and succinctly to ensure provision of meaningful and valid conclusions. The following statistical criteria may be considered in presenting nonlinear regression results, particularly when analyzing germination growth curves:

(i) Parameter estimates along with parameter approximate standard errors, t-ratios, and corresponding p-values,

(ii) Asymptotic correlation matrix of the parameters,

(iii) RMS/PRESS,

(iv) If the experiment includes replications:
   - Lack of fit analysis
   - Plot of replication standard deviations vs. replication averages,

(v) Pairwise plots of parameter inference regions and associated marginal intervals,

(vi) Plot of data, fitted expectation function and approximate confidence bands,

(vii) Residual plots,

(viii) If appropriate, results of single and joint hypothesis testing,

(ix) Profile t plots.

As in linear regression, the assessment of any fitted model should begin with careful consideration of parameter estimates, both in sign and magnitude. Given convergence to reasonable values has been reached, the parameter approximate standard errors and t-ratios must be checked. Insignificant t-ratios must be investigated and if necessary, the model should be modified or refitted deleting the corresponding parameter(s) from the expectation function. The parameter approximate correlation matrix should be checked for excessively high correlation (> .99 in many cases). High correlations indicate overparameterization and a need for either simplifying the expectation function or transforming the variables or parameters to reduce collinearities (Bates and Watts, 1988).

Statistics such as Residual Mean Squares (RMS) and Prediction Sum of Squares (PRESS) help in assessing the overall fit, and if non-nested models are considered may be used to select among candidate models (aside from biological considerations, one might favor the model with the smallest RMS/PRESS and the most random looking residuals).

When the experiment includes replications, tests for Lack of Fit (LOF) of the expectation function may be performed, which involves decomposing the residual sum of squares (SS) with n-p degrees of freedom into replication SS with r degrees of freedom and LOF SS with n-p-r degrees of freedom. The ratio MS(LOF)/MS(Rep) is then compared to the critical value of F(n-p-r, r; α) to determine the significance of LOF tests. In addition, plot of replication standard deviations against replication averages (even prior to specifying an expectation function) may be used to check for systematic relationships and to determine whether a variance-stabilizing transformation is necessary.

Approximate inference regions for parameters of the nonlinear model

\[
y = F(X_1, X_2, \ldots, X_n; \theta_1, \theta_2, \ldots, \theta_p) + \epsilon \\
= F(\theta) + \epsilon,
\]  

(1)
is given by
\[ (\theta - \hat{\theta})' V (\theta - \hat{\theta}) \leq ps^2 F(p, n-p; \alpha), \]  
(2)

where \( V \), the derivative matrix is evaluated at \( \hat{\theta} \) (Bates and Watts, 1988). Note that \( V = Q'R \) following a QR decomposition which involves decomposing \( V \) into the product of an orthogonal matrix, \( Q \) (i.e.: \( Q'Q = QQ' = I \)) and an upper triangular, easily inverted matrix, \( R = \begin{pmatrix} \hat{R}_1 \\ 0 \end{pmatrix} \). Hence,

\[ (\theta - \hat{\theta})' \hat{R}_1 (\theta - \hat{\theta}) \leq ps^2 F(p, n-p; \alpha) \]  
(3)

The inference region associated with parameter values is a disk centered at \( \hat{R}_1 \hat{\theta} \) on the expectation plane and is an ellipse centered at \( \hat{\theta} \) in the parameter space. Plots of parameter inference regions contain least squares estimates of the parameters, marginal intervals and a joint inference region, and could help visualize the direction of correlation between the specified parameters.

Plot of data along with the fitted expectation function and an approximate confidence band, is an excellent way to assess fit. The approximate inference band for the expected response is given by:

\[ f(X, \hat{\theta}) \pm s||U'\hat{R}^{-1}||\sqrt{pF(p, n-p; \alpha)} \]  
(4)

where \( U \) is the derivative vector,

\[ U = \partial f(X, \theta)/\partial \theta |_{\theta}. \]

Plotting of residuals against predicted values and other lurking factors or control variables (e.g. time, in germination analysis) is a simple and effective method of detecting violations of underlying assumptions and highlighting model inadequacies. Given the nonlinear regression model in (1), where disturbances are assumed to have a spherical normal distribution, i.e. \( E(\varepsilon) = 0; \ \text{var}(\varepsilon) = E(\varepsilon\varepsilon') = \sigma^2 I \), it is important that residuals are uniformly spread and random looking (around zero) with no detectable trend. Probability plots of the residuals should also be made to verify the normality assumption [see Draper and Smith (1988), Myers (1986), and Bates and Watts (1988) for details].

Results of single and joint hypothesis testing should also be provided, particularly if various biological or environmental factors (treatments) are present in the experiment. This may include results of single and multiple degree of freedom contrasts on parameter estimates as well as curve comparisons. Let \( H \) and \( \theta \) represent the hypothesis (contrast) and parameter vectors, respectively. define the matrix

\[ C = (R_1' R_1)^{-1} \]

where \( C \) represents the inverse of the derivative crossproduct matrix. The statistic
W = \((H\theta)'(HCH')^{-1}(H\theta)/qs^2\)
follows the F distribution with q (numerator) and n-p (denominator) degrees of freedom (Gallant, 1987). That is,

\[
\frac{SS(\text{contrast})}{q} \sim F(q, n-p; \alpha).
\]

Note that q is the number of restrictions on \(\theta\) which is the row rank of \(H\).

For a nonlinear model, the profile t function (Bliss and James, 1966; Bates and Watts, 1988), \(\tau(\theta_p)\), is defined as

\[
\tau(\theta_p) = \text{sign}(\theta_p - \hat{\theta}_p) \sqrt{\frac{SS_2 - SS_1}{MS_1}}
\]

where \(SS_2\) is the profile sum of squares (achieved by constraining an individual parameter to a constant, \(\theta_p\), and obtaining least squares estimates for the remaining parameters) and \(SS_1\) and \(MS_1\) represent the sum of squares and mean square error from the final unconstrained estimation of \(\theta\), and \(\hat{\theta}_p\) is the unconstrained estimate of the \(p^{th}\) parameter. The plot of \(\tau(\theta_p)\) versus the studentized parameter, \(\delta(\theta_p) = (\theta_p - \hat{\theta}_p)/se(\hat{\theta}_p)\), is called the profile t plot. As curvature measures, profile t plots provide valuable information concerning the nonlinearity of the estimation situation (with respect to each parameter). Additionally, they may be used to determine nominal 100(1 - \(\alpha\))% likelihood intervals,

\[-t(n-p; \alpha/2) \leq \tau(\theta_p) \leq t(n-p; \alpha/2),\]

for individual parameters.

**III. EMPIRICAL RESULTS**

The data used to illustrate the techniques outlined previously were from an experiment to test the effects of reduced water potential on the germination of onion seed. The treatments consisted of 4 water potential levels of 0, -0.662, -1.14, and -1.57 mPa labeled 1 through 4, respectively, which were applied to seeds of the onion cultivar Challenger. The treated seeds were then incubated at 25 °C in light for 20 days. Germination was scored daily as the number of seeds out of 100 showing radicle protrusion greater than 1 mm. The experiment was replicated 8 times.

Summary statistics on the cumulative germination of each of the four treatments are presented in table (1). Mean cumulative germination decreased from 72 to 7% as water potential decreased from 0 to -1.57 mPa. Skewness became more positive as water potential dropped and variability remained relatively constant except...
at the lowest water potential. This treatment exhibited positive skewness, and decreased variability compared to other treatments. The data implied that this difference was due to truncation and more time (> 20 days) would be required to observe final germination at this water potential.

The Weibull model was fitted to the individual treatments using nonlinear estimation as described earlier, and results are presented in table 2. In each treatment the default convergence criteria of the algorithm was satisfied. All parameter estimates were significantly different from zero based on asymptotic t tests, suggesting the model was reasonable and all parameters were required. Again the lowest water potential treatment was a possible exception with the estimate for the parameter C being marginally significant (P = .10).

The differences in parameter estimates between treatments paralleled differences seen in the summary statistics and had biological significance. The maximum attainable germination, estimated by M, decreased as water potential decreased indicating that restricting the availability of water increases the proportion of seeds which are unable to develop sufficient turgor pressure to effect sprouting. The estimates of K, which is related to the rate of germination, also decreased with lowered water potential. Reduced water potential in the solution surrounding the seed decreases the rate at which water is taken up by the seed and may also reduce the rate of various metabolic processes required to accomplish germination. The same biological explanation can be given to changes in the estimates of lag time to initial germination (L), which increased as water potential dropped. Thus the parameter estimates from the Weibull model were consistent with the observed data and had relevant biological interpretations.

The correlational structure of the estimates was consistent in relative magnitude and sign between treatments with no correlations greater than .99 in magnitude. The models did not appear to be overparameterized.

LOF tests found no significant lack of fit in any treatment (lowest p value was .17) indicating that estimated functions intersected the data well. In addition, plots of replication standard deviations vs. replication averages were examined for heteroscedasticity within each treatment. There were no unexpected patterns, and thus, transformations or other variance stabilizing procedures were not required.

Residual analyses on each model were also included and showed no problems. Plots of studentized residuals vs. predicted values and time showed the residuals to behave in a random fashion with no unexpected patterns. They were evenly distributed about zero with acceptable magnitudes. Probability plots verified the correctness of the normality assumption.

The 95% pairwise inference regions for the parameter estimates, given in (2) and (3), as well as approximate 95% marginal confidence intervals, are shown in Figure 1. Only the first treatment is shown since other treatments produced similar results. The least square estimates are marked as a (+) at the center of each ellipse and the marginal intervals (dashed lines) can be read directly from the plots. The direction of the ellipses also indicates the sign of the correlation between parameter estimates. Although the shape of the ellipses is related to the magnitude of the respective correlations, it is not appropriate to compare ellipses in this case given the differences in scales for each respective plot.
Figure 2 represents the data from the specified water potential treatments plotted along with their respective Weibull functions and the 95% inference bands (4). The curves follow the data patterns consistently and individual points do not stray far from their expectation functions. The inference bands follow the curves well with little deviation. This figure clearly demonstrates the effect of decreased water potential on germination and shows the marked difference in germination at the lowest water potential level.

Profile t plots, as described earlier, were prepared for each treatment. However only those for the first treatment are shown here (Figure 3). A straight dashed line at 45° represents the perfect linear estimation condition. The degree of deviation of the profile sum of squares function (presented as a curved solid line) from this reference is an indicator of the amount of nonlinearity of the actual estimation situation. Although slight curvature was evident in parameter estimates M and L, the overall situation was determined to be satisfactory. The profile t plots for other treatments also showed slight but not severe curvature for some parameters. Profile t plots may also be used for determining exact likelihood intervals for individual parameters. The axes for δ and τ can be rescaled to units of the parameter and significance levels, respectively. By tracing a line from a given significance level through the profile sum of squares function, the likelihood interval of the parameter can be found. These values can then be compared to the linear approximation of the interval to give an idea of the nonlinearity of the parameter estimation. For example in the first treatment the estimate of the parameter K was 1.19. The linear approximate 95% confidence interval was .93 to 1.45. Using the profile t plot for K, the exact likelihood interval on K was .94 to 1.49, which is well approximated by the linear approximate interval. Nonlinearity for all parameters was determined to be slight and reparameterization of the expectation function was unnecessary.

An important aspect of using growth curves to describe germination is the ability to perform single and joint tests of hypotheses (5). Such testing procedures allow for detailed analysis of the germination process. As a case in point, comparisons between the control in this study (treatment 1) and the other treatments were made (Figure 4). A single degree of freedom contrast on the maximum attainable germination was made by comparing the estimate of M for the control with the average estimate of M for the other treatments. This test found the treatments to be significantly different than the control in this aspect of germination (P < .0001). Similarly a multiple degree of freedom contrast of the average estimates of K and L, gave a 2 degree of freedom test that had marginal significance (P = .07). Curve comparisons may be done as well. A hypothesis of identical parameters for the control and the average of the other treatments was tested with 4 degrees of freedom and was rejected at p < .0001. From these tests it can be inferred that reduced water potential influenced the whole germination process by affecting the number of seeds and, to a lesser degree, the rate at which they germinated. In sum, reduced water potential increased the germination lag, decreased the rate of germination and significantly reduced the final germination.

The other growth models were also tried on this data. Although the Logistic is commonly used in germination studies, it was found to have a significant lack of
fit. It would seem that the zero skewness characteristic of this model would preclude its use for most germination modelling. The Gompertz fit to the data was marginal. The LOF tests were nonsignificant but the relatively small size of the p values and subsequent residual analysis suggested that this function did not model the process well. The Richards function allows for an adjustable skewness by adding a fourth parameter. However, when fitted to the onion data, the parameter estimates for this model exhibited very large standard errors and high correlations as well. Sensitivity to starting values and extremely long convergence times limited the usefulness of the Richards function. All of the above models had RMS and PRESS values larger than those of the Weibull model. The Weibull model consistently out performed the other models in convergence time, relative magnitudes of standard errors and correlations, and LOF.

Germination data from several crop and weed species were used to assess the Weibull function as a germination model. Seeds of onion, rapeseed, sugarbeet, kochia, matgrass, and medusa head were germinated under a variety of conditions including simulated seed aging, temperature treatments, source of seed, and water potential treatments. In all cases no significant lack of fit was found. Convergence was usually fast and correlations of parameter estimates were reasonable. In a few cases where the convergence was slow or the correlations high, the problem was in part due to insufficient observations between initial and maximum germination. A revised sampling schedule should help remedy this problem. The Weibull model was found to be robust for the description of seed germination over a wide range of species and germination conditions.

IV. CONCLUDING REMARKS

Traditional methods used in seed germination data analysis such as germination indices, moments, coefficient of velocity, and probit analysis fail to describe the time course of germination and they are, for the most part, ambiguous and incomplete. An alternative to utilizing moments and indices is the use of growth models and empirically derived curves which allow germination to be described in terms of three or four coefficients, and hence provide information on location, dispersion in time, rates, and extent of germination. The statistical results from nonlinear estimation of the specified growth model should include, aside from the standard regression statistics, information concerning lack of fit, parameter correlation and inference regions, bands for expectation function, residual structure, and nonlinearity of the estimation situation with respect to individual parameters. The modified four-parameter Weibull model provided a good fit across a relatively wide range of seed species and germination conditions, and the resulting parameter estimates reflected identifiable aspects of germination.

ACKNOWLEDGEMENTS

Contribution from College of Agriculture, University of Idaho, Idaho Agricultural Experiment Station paper number 9102.
REFERENCES


Table 1. Summary statistics for cumulative germination of onion seeds under various water potential treatments.

<table>
<thead>
<tr>
<th>Treatment 1</th>
<th>(0.0 mPa)</th>
<th>Treatment 2</th>
<th>(-.662 mPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean: 72.00</td>
<td>Std: 18.45</td>
<td>Mean: 21.68</td>
<td>Std: 21.68</td>
</tr>
<tr>
<td>Min: 7.00</td>
<td>Skewness: -2.40</td>
<td>Min: 2.00</td>
<td>Skewness: -1.94</td>
</tr>
<tr>
<td>Max: 88.00</td>
<td>Range: 81.00</td>
<td>Max: 89.00</td>
<td>Range: 87.00</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Treatment 3</th>
<th>(-1.14 mPa)</th>
<th>Treatment 4</th>
<th>(-1.57 mPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean: 42.72</td>
<td>Std: 21.29</td>
<td>Mean: 7.07</td>
<td>Std: 5.31</td>
</tr>
<tr>
<td>Min: 1.00</td>
<td>Skewness: -.63</td>
<td>Min: 1.00</td>
<td>Skewness: .76</td>
</tr>
<tr>
<td>Max: 71.00</td>
<td>Range: 70.00</td>
<td>Max: 21.00</td>
<td>Range: 20.00</td>
</tr>
</tbody>
</table>
Table 2. Parameter summaries for the Weibull function fitted to the onion data.

<table>
<thead>
<tr>
<th>Treatment 1</th>
<th>Treatment 2</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Par.</strong></td>
<td><strong>Est.</strong></td>
</tr>
<tr>
<td>M</td>
<td>85.25</td>
</tr>
<tr>
<td>K</td>
<td>1.19</td>
</tr>
<tr>
<td>L</td>
<td>1.95</td>
</tr>
<tr>
<td>C</td>
<td>.45</td>
</tr>
<tr>
<td>RMS: 21.3</td>
<td>PRESS: 2719.0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Treatment 3</th>
<th>Treatment 4</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Par.</strong></td>
<td><strong>Est.</strong></td>
</tr>
<tr>
<td>M</td>
<td>63.28</td>
</tr>
<tr>
<td>K</td>
<td>.27</td>
</tr>
<tr>
<td>L</td>
<td>4.39</td>
</tr>
<tr>
<td>C</td>
<td>1.24</td>
</tr>
<tr>
<td>RMS: 62.5</td>
<td>PRESS: 6944.9</td>
</tr>
</tbody>
</table>
Figure 1. Pairwise plots of the parameter approximate 95% joint inference regions (solid lines), approximate 95% marginal confidence intervals (dashed lines) and least squares estimates (+) for the onion data.
Figure 2. Observed and predicted (solid lines) values of % cumulative germination for the onion data along with the approximate 95 % confidence bands (dashed lines).
Figure 3. Profile t plots for the parameters of the Weibull model fitted to the onion data (treatment 1). The solid line represents the profile t, the dotted line is the linear approximation and the dashed line is the 95% marginal likelihood interval.
Figure 4. Predicted % cumulative germination from the Weibull model for the specified onion seed treatments.
APPENDIX

1. SAS/IML Codes for
Gauss-Newton Algorithm

/* GAUSS-NEWTON ALGORITHM (HARTLEY, 1961) ADAPTED FROM
BATES AND WATTS (1988) TO READ EXTERNAL DATA, USE
WELBULL FUNCTION, AND CALCULATE S.E.'S 
AND STUDENTIZED RESIDUALS.
*/
DATA ONION (KEEP=TRT X GERM);
INFILE C:\ONION.DAT FIRSTOBS=6;
INPUT OBS VAR $ TRT X GERM;
IF TRT=0 AND VAR='C';
IF GERM=0 THEN DELETE;
PROC IML;

/* *********** DEFINE MODULE FOR MODEL *********** */
START MODEL(,THETA, X, Y, RES, GRAD, DERIVS);
IF THETA(1,1) > 100 THEN THETA(1,1) = 100;
IF THETA(2,1) < 0 THEN THETA(2,1) = 0.00001;
IF THETA(3,1) < 0 THEN THETA(3,1) = 0.00001;
IF THETA(4,1) < 0 THEN THETA(4,1) = 0.00001;
TEMP = EXP(-(THETA(3,1)*(X-THETA(2,1))))
#THETA(4,1));
YHAT = THETA(1,1)* (1 - TEMP);
RES = Y - YHAT;
IF DERIVS = 1 THEN DO;
DER_M = 1 - TEMP;
DER_L = -THETA(1,1)*THETA(3,1)*
THETA(4,1)*TEMP #THETA(3,1)
#THETA(4,1)-1);
DER_K = THETA(1,1)*THETA(4,1)*
(TEMP*X-THETA(2,1))
#THETA(3,1)*(X-THETA(2,1))
#THETA(4,1)-1));
DER_C = THETA(1,1)*TEMP*LOG(THETA(3,1))
*X-THETA(2,1))#THETA(3,1)
*X-THETA(2,1))#THETA(4,1);;
GRAD = DER_M||DER_L||DER_K||DER_C;
END;
FINISH;

/* ********** COMPUTATIONAL MODULE ************ */
START NLSFIT(THETA, DAYS, Y, YHAT, CRITERION, 
MAXITER, MINSTEP, TOL, VERBOSE);
RUN MODEL(THETA, DAYS, Y, RESID, GRAD, 1);
P = NROW(THETA);
N = NROW(RESID);
NDOF = N-P;
MULT = SQRT(NDOF/P);
STEP_SIZE = 1;
DO ITER = 1 TO MAXITER;
OLDSSQ = SSQ(RESID);
CALL GSORTH(THETA,RHAT,RANK,GRAD);
IF RANK = 1 THEN DO;
PRINT "SINGULAR DERIVATIVE MATRIX";
STOP;
END;
TAN = QHAT * RESID;
SS_TAN = SSQ(TAN);
INCR = SOLVRHAT,TAN);
CRITERION = MULT * SQRT(SS_TAN/(OLDSQ- 
SS_TAN));
IF VERBOSE THEN PRINT ITER(IFORMAT = 
2.(1), PRINT INFO);, CRITERION (INCR);,
IF CRITERION < TOL THEN UNK DOMORE;
END;
ELSE IF STEPSIZE < MINSTEP THEN DO;
PRINT "STEP FACTOR REDUCED 
BELOW MINIMUM";
END;
END;
THETA = TRIAL;
END;
*/

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STEPSIZE = MIN(1.4 * STEPSIZE);  
RUN MODEL(THETA, DAYS, Y, RESID, GRAD, 1);  
END;  
PRINT "MAXIMUM ITERATIONS REACHED: PROGRAM TERMINATED";  
STOP;  
DOMORE:  
RI = INV(RHAT);  
S = SQRT(N/EWSSQ/NDOF);  
SE = J(1, 10);  
DO I = 1 TO P;  
SE(I, 1) = SQRT(RHAT(I, I)*RI(I, I))*S;  
END;  
THETA = THETA | SE;  
TEMP = EXP(-THETA(1, 1)*(DAYS-THETA(1, 1)));  
YHAT = THETA(1, 1)*(1-TEMP);  
YHAT = YHAT| RESID| SQRT| VECDIAG(QHAT|QHAT);  
FINISH;  
USE ONION VAR| X| GERM;  
READ ALL VAR| X INTO DAYS;  
READ ALL VAR| GERM INTO Y;  
THETA = {100, 99, 5, 5};  
RUN NL5IT|THETA, DAYS, Y, YHAT, CRITERION, 50, 0.01, 0.01, 1);  
RESET NAME;  
PRINT THETA, YHAT;  
RUN;  

2. SAS Codes for the Functional Forms

PROC NL5IT METHOD = GAUSS BEST = 1 DATA = ONION;  
PARMS M = 88 K = .5 L = .99 C = .5;  
BOUNDS 85 < M < 88, K > 0, L > 0, 0 < C < .5;  
TEMP1 = X - 1;  
TEMP = EXP((K*(TEMP1)**C));  
MODEL GERM = M*(1-TEMP);  
DER.M = (1-TEMP);  
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*
3. SAS/IML Codes for Parameter Inference Region

```sas
OPTIONS DEV='PS2EGA';
LIBNAME FERM 'D:';
%LET P1 = C;
%LET P2 = 1;
%LET HAT = hat;

DATA VONY (KEEP=X);
  INFILE 'C:INUNIONIONREP1.DAT' FIRSTOBS=5;
  INPUT REP VAR $ TRT X GERM;
  IF TRT='0' AND VAR='C';
  IF GERM='0' THEN DELETE;

PROC IML;
START VIVVX(HV, THETA);
  TEMP = EXP(-((X-T(I1,II)*T(12,II)))**2);  /* MODULE CALC'S THE 
  DER_M = -T(I1,II)*T(12,II);  /* DERIVATIVE CROSS 
  DER_L = -T(I2,II)*T(12,II);  /* PRODUCTS MATRIX V'V 
  DER_K = T(12,II)*TEMP;  /* = INV(GRAD'GRAD). 
  DER_C = T(13,II)*TEMP;  /* MATRIX IS SETUP AS:
  GRAD = DER_M || DER_L || DER_K || DER_C;  /* V'V
  INVGRAD = INV(GRAD'GRAD);  /* AND C=4. 
  POINT = #2;  /* WHERE M=1, L=2, K=3, C=4. 
  HV = INVGRAD(2,II) || INVGRAD(4,II);  /* BEGIN MODULE. 
  FREE DER_M, DER_L, DER_K, DER_C;  /* END MODULE.
END;

USE VONY;
READ ALL VAR[X] INTO TIME;  /* GET DATA AND PUT X
GET TIME INTO VECTOR.

THETA = (85.25, 98.19, 1.19, .464);  /* DEFINE THETA AS:
M, L, K, AND C.

RUN VIVVX (TIME, HV, THETA);  /* CALL CROSS PROD'S.
```

/* PUT THE ELEMENTS OF 
HV INTO VECTOR 
FOR OUTPUT. 
*/

CREATE CROSS FROM VIVV;
APPEND FROM VIVV;

/* OUTPUT TO DATASET. 
*/

DATA UPPER(KEEP=&P1 &P2) LOWER(KEEP=&P1 &P2);  /* DATA STEP CALC'S THE 
SET CROSS;
Vi = COL1;
Vj = COL2;
Vij = COL3;
J = THE VERT. AXIS.
SEE (2).
SET PARAM EST'S AT 
FINAL VALUES. ALSO 
MSE AND F(2,N-P,.05) 
*/

Mhat = 55.25;
Lhat = 1.98;
Rhat = 1.19;
Chat = .464;
MSE = 21.27;
F = 3.05;

LOLM = &P2&HAT - SQRT((Vij**2*MSE)**F)/ 
( (Vij**2 - Vij*Vij*(Vij**2 - 1)));  /* FIGURE RANGE OF CALC 
UPLM = &P2&HAT + SQRT((Vij**2*MSE)**F)/ 
( (Vij**2 - Vij*Vij*(Vij**2 - 1)));  /* BASED ON THE DERIV. 
INTER = (UPLM - LOLM)/200;  /* OF THE ELLIPSE = 0. 

DO &P2 = (LOLM-INTER) TO (UPLM+INTER) / 
BY INTER;  /* START ELLIPSE CALC'S.
A = Vij;
B = 2*Vij*(&P2 - &P2&HAT);
CC = Vij*(&P2 - &P2&HAT)**2 - (2*MSE)*F;
&PI = (-B + SQRT(B**2 - 4*A*CC))/(2*A) + &PI&HAT;  /* SET ELLIPSE PARAM'S 
OUTPUT UPPER;
&PI = (-B - SQRT(B**2 - 4*A*CC))/(2*A) + &PI&HAT;  /* BASED ON THE ELLIPSE 
OUTPUT LOWER;

END;
PROC SORT DATA=UPPER;  /* SORT TWO HALVES AND 
BY &P2;  /* REASSEMBLE FOR
PROC SORT DATA=LOWER;  /* BETTER PLOTTING.
BY DESCENDING &P2;
```

New Prairie Press
http://newprairiepress.org/agstatconference/1991/proceedings/3
4. SAS Codes for Confidence band on Expectation Function, RMS, PRESS, and Residual Plots

```
DATA OGERM;
  INFILE 'D:\ONREPI.DAT' FIRSTOBS=5;
  INPUT REP VAR $ TRT X GERM;
END;
IF VAR='C' AND TRT=1;
PROC NLIN METHOD=GAUSS BEST=1 DATA=OGERM;
PARMS M=15 K=1.5 L=7 C = .5;
RUN;
```

5. SAS Codes for Nonlinear Parameter Testing

```
DATA ONION;
  INFILE 'C:\ONION.DAT' FIRSTOBS=6;
  INPUT OBS VAR $ TRT X GERM;
RUN;
```

New Prairie Press
http://newprairiepress.org/agstatconference/1991/proceedings/3
TEMPI = (X-L);
TEMP = EXP(-K*(TEMPI)**C);
MODEL GERM = M*(1-TEMP);
DER.M1 = X1*(1-TEMP);
DER.M2 = X2*(1-TEMP);
DER.K1 = (M*C)*TEMPI*EXP(K*(TEMPI))**C;
DER.K2 = (M*C)*TEMPI*EXP(K*(TEMPI))**C;
DER.M1 = X1*(1-TEMP);
DER.M2 = X2*(1-TEMP);
DER.K1 = (M*C)*TEMPI*EXP(K*(TEMPI))**C;
DER.K2 = (M*C)*TEMPI*EXP(K*(TEMPI))**C;

DATA EST;
SET FROM NUN;
IF _TYPE_ = 'ITER' THEN DELETE;
PROC IML;
USE FROM;
READ ALL INTO EST;
N=28;
MSE = ESTIM(1,2)/(N-8);"GET THE"
DPD = ESTIM(12,NROW(ESTIM),3:NCOI.)/SQRT(MSE);"PIECES"
THETA = ESTIM(1,3)/NCOI.;"WRITE"
H1 = [1 0 0 0 0 1 0 0 0];"CONVERGE ON"
H2 = [0 0 0 1 0 0 0 0 -1];"PARAMETERS"
H3 = [0 1 0 0 0 1 0 0 0];"ONE CONSTANT"
H4 = [1 0 0 0 1 0 0 0 0];"(eq M)."
0 1 0 0 0 1 0 0 0;"(eq M)"
0 0 1 0 0 1 0 0 0;"USED TO CALC
0 0 0 1 0 0 1 0 0;"TAU."
0 0 0 0 1 0 0 1 0;"
/* ORDER IS M1, L1, K1, C1, M2, L2, K2, C2 */
MSRI = (H1*THETA)**NIV(H1*DPD*H1)*(H1*THETA)/NROW(H1);"CALCULATE THE"
MSR2 = (H2*THETA)**NIV(H1*DPD*H2)*(H2*THETA)/NROW(H2);"REDUCED MSREG S"
MSR3 = (H3*THETA)**NIV(H1*DPD*H3)*(H1*THETA)/NROW(H3);"USING THE"
MSR4 = (H4*THETA)**NIV(H1*DPD*H4)*(H4*THETA)/NROW(H4);"GEN LNHYP"
F1 = MSR1/MSE;
F2 = MSR2/MSE;
F3 = MSR3/MSE;
F4 = MSR4/MSE;
P1 = 1 - PROBF(F1,NROW(H1),20);
P2 = 1 - PROBF(F2,NROW(H2),20);
P3 = 1 - PROBF(F3,NROW(H3),20);
P4 = 1 - PROBF(F4,NROW(H4),20);
PRINT F1, F2, F3, F4;
/* CALCULATE F"
/* STATISTICS"
/* AND"
/* P VALUES."
/* PRINT RESULTS."

6. SAS Codes for Profile t Plots

/* DEFINE MACRO"
/* VARIABLES FOR"
/* USE IN LATER"
/* CALCULATIONS."
/* THESE ARE"
/* EST.'S FROM"
/* PRIOR NLIN RUN."
/* F IS A CUT OFF"
/* VALUE FOR TAU ="
/* SQRT(F(P,N-P))"/
*/

/* MACRO TAU(DELT);"
DATA _NULL_;"
CALL SYMPUT('MTRY',VAL);"PARAM SCALE.
PROC NLIN METHOD=GAUSS BEST=1 DATA=OGERM OUTEST=EST;
PARMS M = &MTRY K = &KHAT L = &LHAT C = &CHAT;
BOUNDS &MTRY < = M < &MTRY, K > 0, 0 < L < 3, 0 < C < 3;
*/

F2 = MSR2/MSE;
F3 = MSR3/MSE;
F4 = MSR4/MSE;
P1 = 1 - PROBF(F1,NROW(H1),20);
P2 = 1 - PROBF(F2,NROW(H2),20);
P3 = 1 - PROBF(F3,NROW(H3),20);
P4 = 1 - PROBF(F4,NROW(H4),20);
PRINT F1, F2, F3, F4;
/* CALCULATE F"
/* STATISTICS"
/* AND"
/* P VALUES."
/* PRINT RESULTS."

/* SELECT OUT THE"
/* ESTIMATES."
/* SET EST;"
/* IF _TYPE_ = 'ITER' THEN DELETE;"
PROC IML;
USE FROM;
READ ALL INTO EST;
N=28;
MSE = ESTIM(1,2)/(N-8);"GET THE"
DPD = ESTIM(12,NROW(ESTIM),3:NCOI.)/SQRT(MSE);"PIECES"
THETA = ESTIM(1,3)/NCOI.;"WRITE"
H1 = [1 0 0 0 0 1 0 0 0];"CONVERGE ON"
H2 = [0 0 0 1 0 0 0 0 -1];"PARAMETERS"
H3 = [0 1 0 0 0 1 0 0 0];"ONE CONSTANT"
H4 = [1 0 0 0 1 0 0 0 0];"(eq M)."
0 1 0 0 0 1 0 0 0;"(eq M)"
0 0 1 0 0 1 0 0 0;"USED TO CALC
0 0 0 1 0 0 1 0 0;"TAU."
0 0 0 0 1 0 0 1 0;"
/* ORDER IS M1, L1, K1, C1, M2, L2, K2, C2 */
MSRI = (H1*THETA)**NIV(H1*DPD*H1)*(H1*THETA)/NROW(H1);"CALCULATE THE"
MSR2 = (H2*THETA)**NIV(H1*DPD*H2)*(H2*THETA)/NROW(H2);"REDUCED MSREG S"
MSR3 = (H3*THETA)**NIV(H1*DPD*H3)*(H1*THETA)/NROW(H3);"USING THE"
MSR4 = (H4*THETA)**NIV(H1*DPD*H4)*(H4*THETA)/NROW(H4);"GEN LNHYP"
F1 = MSR1/MSE;
/* CALCULATE F"
/* STATISTICS"
/* AND"
/* P VALUES."
/* PRINT RESULTS."

/* SELECT OUT THE"
/* ESTIMATES."
/* SET EST;"
/* IF _TYPE_ = 'ITER' THEN DELETE;"
PROC IML;
USE FROM;
READ ALL INTO EST;
N=28;
MSE = ESTIM(1,2)/(N-8);"GET THE"
DPD = ESTIM(12,NROW(ESTIM),3:NCOI.)/SQRT(MSE);"PIECES"
THETA = ESTIM(1,3)/NCOI.;"WRITE"
H1 = [1 0 0 0 0 1 0 0 0];"CONVERGE ON"
H2 = [0 0 0 1 0 0 0 0 -1];"PARAMETERS"
H3 = [0 1 0 0 0 1 0 0 0];"ONE CONSTANT"
H4 = [1 0 0 0 1 0 0 0 0];"(eq M)."
0 1 0 0 0 1 0 0 0;"(eq M)"
0 0 1 0 0 1 0 0 0;"USED TO CALC
0 0 0 1 0 0 1 0 0;"TAU."
0 0 0 0 1 0 0 1 0;"
/* ORDER IS M1, L1, K1, C1, M2, L2, K2, C2 */
MSRI = (H1*THETA)**NIV(H1*DPD*H1)*(H1*THETA)/NROW(H1);"CALCULATE THE"
MSR2 = (H2*THETA)**NIV(H1*DPD*H2)*(H2*THETA)/NROW(H2);"REDUCED MSREG S"
MSR3 = (H3*THETA)**NIV(H1*DPD*H3)*(H1*THETA)/NROW(H3);"USING THE"
MSR4 = (H4*THETA)**NIV(H1*DPD*H4)*(H4*THETA)/NROW(H4);"GEN LNHYP"
F1 = MSR1/MSE;
DEL = &DEL;

TAU = SIGN(&MTRY - &MHA1) *
    SORT(( SSE - &SS)/(&SS/&DF));
    TABS = ABS(TAU);

FILE 'A:M.DAT' MOD;
PUT @I DEL @IO TAU;

* CALL SYMPUT('MHA1',M);
CALL SYMPUT('KHA1',K);
CALL SYMPUT('CHA1',C);
CALL SYMPUT('T',TABS);

%MEND;

%MACRO LOOP;
%DO HALF=-1 %TO 2;
%DO J=0 %TO 9;
%IF &HALF=-1 %THEN %LET DELT=-&I..&J;
%ELSE
%IF &HALF=1 %THEN %LET DELT=+&I..&J;
%ELSE %LET DELT=+&I..&J;
%TAU(&DEL1);
%IF &T> &F THEN %GOTO LABEL1;
%END;
%LABEL1: %END; %END;
%MEND;

DATA OGERM;
INFILE 'A:ONION.DAT' FIRSTOBS=5;
INPUT REP VAR $ TRT X GERM;
IF VAR='C' AND TRT=0;

%LOOP;