Nonlinear Model of Wheat Starch Granule Distribution at Several Stages of Development[1]

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ABSTRACT

Wheat starch granule size distributions can be satisfactorily described by sets of intersecting hyperbolas on a volume-cumulative number plane. Distinct peaks arise from the intersections when the sets are transformed to other formats. The intersections can be determined by formula after the hyperbolas are transformed to straight lines graphically or by regression. The total mass within a given volume range or the percentage of mass contributed by a given volume can be calculated by formulas derived from integration or differentiation. The intersections could demarcate more than two sizes of granules. This suggestion is illustrated by data for growth of wheat starch granules in two cultivars during three seasons. The possible physiological significance of these findings is discussed, and alternative interpretations of the A-type granule peak are outlined.

We previously described the changes in average starch granule size during the growth of the wheat kernel (Baruch et al 1979). Our conclusion mentioned that intersections of the different equations representing a complete sample granule distribution might indicate boundaries between distinct classes of granules. We describe a technique for identifying such equations and their intersections from granule volume-cumulative number data.

This article presents several formulas for transformation to other data formats discussed in a previous publication (Meredith et al 1977). The formulas are functions of granule volume and of the intercept and slope of the logarithmic plot of the volume-cumulative standardized number curve.

This new technique suggests the physiological growth of more than two classes of granules. We include four examples. Three of the examples show three distinct size classes at maturity.

MATERIALS AND METHODS

Symbols and Definitions

The symbol (V, n) represents paired observations obtained from the Coulter Counter.™ On a graph, each of these ordered pairs is a point. The elements V and n are the first and second coordinates, respectively. The symbol n is the observed number of granules larger than a selected volume, V. To compare values of n between samples, n is standardized by dividing each value by the largest value of n observed in the sample. The standardized value is a decimal fraction and has the symbol N. The symbols a and b are parameters determined by linear regression. The parameter a is the vertical axis intercept, and b is the slope of the graph of the regression line. The mass of granules within a given volume range has the symbol w, and the standardized decimal fraction value found by dividing w by the total mass of the sample has the symbol W. Both N and W for any complete sample must equal one.

Calculation of Best Fit Equations and Intersections

To find equations that describe the volume-cumulative standardized number curve, replicate Coulter Counter granule number counts were averaged at each of several threshold volume settings. The resulting (V, n) pairs of threshold volume and average granule count were treated in the following manner. We corrected the raw cumulative number counts for background and coincidence according to the instructions in the Coulter Manual reduced the corrected raw count to the standardized count, N; transformed the ordered (V, N) pairs to ordered ($\ln V$, $\ln N$) pairs; regressed linearly $\ln N$ on $\ln V$ to select those sets of ($\ln V$, $\ln N$) pairs with a coefficient of determination greater than 0.980; and selected from these highly correlated sets successive equations that intersected close to the end points of their respective generating sets and that recreated V within ±10% of the experimental value of V for each pair of the set.

The values of V for the intersections were found from the equation:

$$V = (a_1/a_2)^{1/(b_1-b_2)}$$

where a_1 and a_2 and b_1 and b_2 were the values of the constants a and b in the nonlinear model $N = aV^b$ on either side of the intersection. Hence, the intersection formed boundaries between two sets of granules described by the same cumulative distribution model with different parameters.

In subsequent investigations, we found that plotting the ($\ln V$, $\ln N$) pairs makes the set groupings visually apparent (Fig. 1). To

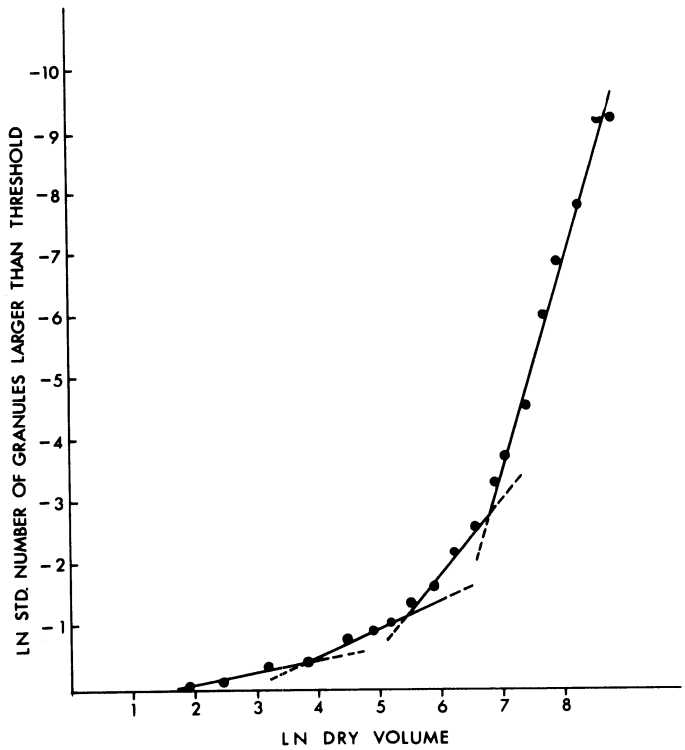

Fig. 1. Logarithm of cumulative standardized number of granules as function of logarithm of threshold volume of counter corrected to anhydrous starch basis. The data are approximated by four straight lines. The example is the distribution of granules of cultivar Cappelle Desprez of the 1971 season, harvested 28 days after ear emergence.

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determine the parameters a and b, one can either regress the sets of points appearing on each line or measure each slope to find b and substitute in the equation:

$$a = N_g / V_g^b$$

to determine a. N_g and V_g are the geometric means of N and V for each set. The intersections are then calculated by the formula already stated.

Samples and Equipment

Starch samples were prepared from experimentally grown wheats that had been sequentially harvested during their development in outdoor plots. The method of preparation was previously described (Jenkins et al 1974; Meredith et al 1970, 1973) along with growth conditions and dating. The 73 samples considered include those for which the raw Coulter Counter data had been used in the previous paper (Baruch et al 1979).

A simple Coulter Counter, model D, was used with a 140-μm orifice and with Isoton™ isotonic saline as the diluent. Counts were made of numbers of granules with volumes exceeding a set threshold volume. The threshold volume equivalent diameters were successively advanced by 2 μm between each observation.

RESULTS

Identification of the Regression Model: The Time Dependence of Development

The least square fit procedure suggests that intersecting hyperbolas of the form $N = aV^b$ (Fig. 2a) fit the sets of cumulative (V, N) pairs observed in naturally occurring wheat starch samples. The intersections of these hyperbolas form boundaries between the sets. The ordinate points in Fig. 3 represent the first coordinates of the boundaries during kernel growth. The movement of these boundaries upward and to the right on the graph indicates how the granule volume is a function of time.

The sliding regression program we have used contains options for linear, logarithmic, and exponential model regressions as well as for the hyperbolic model, $N = aV^b$. We have tried these options and achieved the most plausible intersections and the highest correlations with the hyperbolic model. A few exceptions occurred amongst mature samples where a linear model produced better intersections for the first set of three pairs. Even in these cases, the hyperbolic model produced acceptable results.

When we upset the natural granule distribution by selectively sedimenting part of the sample or by adding a selected small

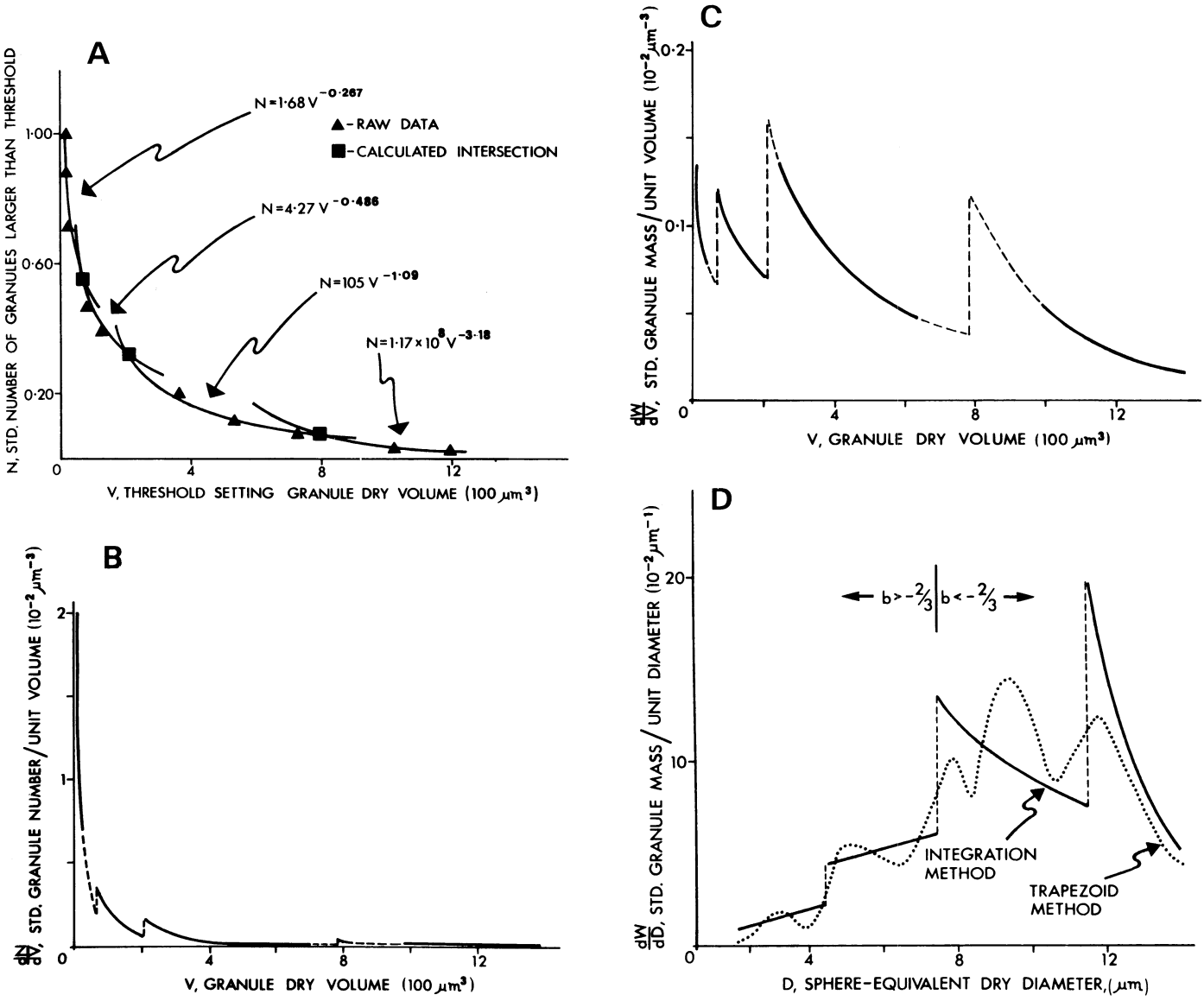

Fig. 2. Relative distribution curves derived by differentiation of the original cumulative (V,N) pairs. Full lines contain experimental points; dashed lines are extrapolated to intersections. Points beyond 1,200 μm are not plotted. **A,** Intersecting nonlinear equations of the form $N = aV^b$; **B,** volume-relative standardized number curve; **C,** volume-relative standardized mass curve; **D,** diameter-relative standardized mass curve. The dotted curve was calculated by trapezoidal quadrature.

volume range, it was necessary to use logarithmic, linear, and exponential models as well as the hyperbolic model to achieve similar precision. But in the 73 natural samples, only the nonlinear model consisting of hyperbolic sets and their resulting intersections uniquely described the volume-cumulative number pattern. The two parameters and the boundary positions that specify this model offer measurable differences between samples. We have yet to decide if these differences correspond to physiological differences of genetic or environmental origin.

DISCUSSION

Transformations to Other Data Formats

The sets of pairs in the equation $N = aV^b$ (Fig. 2) transform to the (V, dN/dV) pairs that form the volume-relative standardized number sets (Fig. 2.), to the (V, dW/dV) pairs that form the volume-relative standardized mass sets (Fig. 2c), and to the (D, dW/dD) pairs that form the diameter-relative standardized mass sets (Fig. 2d).

When we begin with

$$N = aV^b,$$

then

$$dN/dV = abV^{(b-1)}.$$

Because $dW = \rho V dN/(\rho V_T)$, where ρ, the density, cancels, and where V_T represents the total standardized number volume of the sample, then $dW/dV = abV^b/V_T$.

Because the volume of a sphere is $V = \pi D^3/6$, and because $dW/dD = (dW/dV)(dV/dD)$, then

$$dW/dD = \frac{3ab(\pi/6)^{b+1} D^{3b+2}}{V_T}.$$

The volume of starch included between the upper and lower boundaries of (V, N) pairs in a set can be calculated from the equation

$$\int_{V_1}^{V_2} VdN = \frac{ab}{b+1}(V_2^{b+1} - V_1^{b+1}).$$

Summation of the starch volumes of the individual sets gives the total starch standardized number volume V_T. Because the total value of N is one, V_T is also the average volume of a starch granule.

The fraction of the total mass of starch granules in the size range between any volume V_1 and V_3 is given by:

$$W = ab \frac{(V_3^{b+1} - V_1^{b+1})}{V_T(b+1)}.$$

If an intersection occurs at V_2 between V_1 and V_3, then the equation should be applied between V_1 and V_2 and then between V_2 and V_3. This equation changes to a diameter form for use with sieves etc if $\pi D_1^3/6$ replaces V_1 and $\pi D_3^3/6$ replaces V_3.

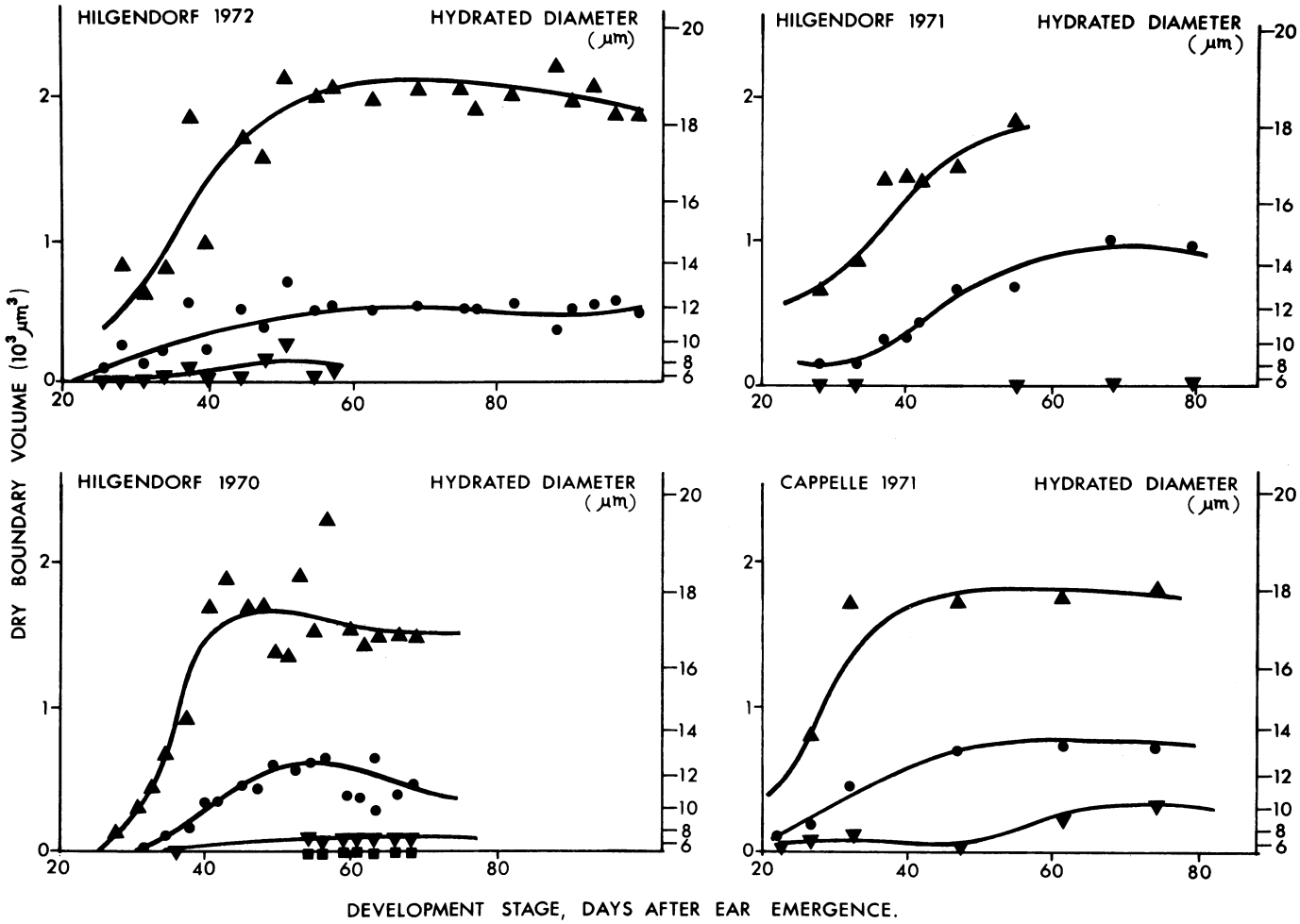

Fig. 3. The increase of boundary volumes as a function of time in sequentially harvested samples of two wheat cultivars and three seasons. Different symbols represent first largest, second largest, etc., boundary volume in each day's sample. The right-hand scale is hydrated diameter.

Comparison with Trapezoidal Quadrature Method

The method of computation outlined in the Coulter Manual uses trapezoidal quadrature of the segments between successive observations. To compute V_T, both this method and the integral method sum areas that are flanked by the N axis and the curves between successive (V, N) pairs and enclosed between successive N coordinate lines. We have used the two methods in parallel to check our calculations. By either method, the total volumes determined agreed within 3% over the 73 samples examined.

The same trapezoidal quadrature computation normally has been used to generate volume (or diameter)-relative standardized mass pairs. The first coordinate of these relative (V, dW/dV) pairs is found according to the formula $V=(V_2+V_1)/2$, where V_2 and V_1 are the first coordinates of successive (V, N) pairs. The second coordinate is found according to the assumption that

$$\frac{(V_2+V_1)(N_2-N_1)}{2(V_2-V_1)} \cong \lim_{\Delta V \to 0} \frac{\Delta W}{\Delta V} = \frac{dW}{dV}$$

In this case the method is subject to all the effects that follow from reducing a finite value to an infinitesimal value. In particular, ΔV should be made small to accurately locate the second coordinate. But the coefficient of error of both ΔW and ΔV will increase in response. Hence, precision of location is achieved at the expense of precision of value.

The use of formal differentiation, as set out in the section on transformation to other data formats, avoids these effects. Our method depends on the validity of the best fit equations through more than two ordered pairs. The increased number of pairs included in the adjustment process leads to better precision, and the V specifies the exact position of dW/dV.

These two uses of trapezoidal quadrature should not be confused. The efficiency of trapezoidal quadrature and formal integration to calculate cumulative quantities between two bounds (eg, total mass of a sample) is nearly equal when sufficient and adequately spaced pairs are chosen. However, the efficiency of trapezoidal quadrature to construct relative distributions is significantly less than formal differentiation. In Fig. 2d, we show relative distributions calculated by both methods.

Discontinuities at Boundaries

The sawtooth appearance of the transformed curves in Fig. 2 may seem alien to the general expectation for the shape of granule distribution curves. However, only the solid portions of the curves are within experimentally bounded equations. The dashed portions arise from extrapolating these equations to either side of their boundary and connecting them along the first coordinate of the boundary. The existence of intersections in the original (V, N) pair distribution inevitably leads to discontinuities in the derived distributions. The precision available and the progression of the intersections in time (Fig. 3) imply that the discontinuities actually exist.

The distribution on the diameter-relative standardized mass plane (Fig. 2d) is divided into two sections. Where b is greater than $-2/3$ the graph slope is positive, and where b is less than $-2/3$ the slope is negative. The fact is deduced by setting the next highest derivative of dW/dD to zero. The two sections produce a peak near this division on this plane because of the passage of b from a value larger than $-2/3$ to one which is less than $-2/3$ when other discontinuity effects have been smoothed. The position of this peak is approximately where the A-granule peak is often observed.

Interpretations of Model

Three possible interpretations arise from the application of the model to our data for developing grains.

The discontinuities may be demarcation points between distinct sets of granules; the distribution within most of these sets is skewed. When we consider each set to have a common period of genesis during grain development, then we imply that some granules grow faster than the majority in their set, but that few granules grow more slowly.

In the second alternative view of growth, we can consider that granule volume is some function of time, as suggested by Fig. 3. That is, genesis of granules continues, but some mechanism may have triggered a new, slower growth rate at each of the discontinuities.

A third possibility is that the sets of hyperbolas are really only approximations to a continuous curve on the volume-cumulative standardized number plane, and both a and b are continuous functions of V. Although such a proposition would exclude the notion of separate sets identified by discontinuities, it would explain the appearance of the A-granule peak on the diameter-relative standardized mass plane as an artifact due to the use of these particular coordinates. While not demonstrating the existence of a separate class of A-type granules, the position of this artifact peak could prove a valuable parameter to distinguish between starch granule distributions.

All the hypotheses are reasonable and should influence our thinking about the physiology of starch granule growth.

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