A Water Relations Analysis of Seed Germination Rates

Kent J. Bradford
Department of Vegetable Crops, University of California, Davis, California 95616

ABSTRACT
Seed germination culminates in the initiation of embryo growth and the resumption of water uptake after imbibition. Previous applications of cell growth models to describe seed germination have focused on the inhibition of radicle growth rates at reduced water potential ($\psi$). An alternative approach is presented, based upon the timing of radicle emergence, to characterize the relationship of seed germination rates to $\psi$. Using only three parameters, a 'hydrot ime constant' and the mean and standard deviation in minimum or base $\psi$ among seeds in the population, germination time courses can be predicted at any $\psi$, or normalized to a common time scale equal to that of seeds germinating in water. The rate of germination of lettuce (Lactuca sativa L. cv Empire) seeds, either intact or with the endosperm envelope cut, increased linearly with embryo turgor. The endosperm presented little physical resistance to radicle growth at the time of radicle emergence, but its presence markedly delayed germination. The length of the lag period after imbibition before radicle emergence is related to the time required for weakening of the endosperm, and not to the generation of additional turgor in the embryo. The rate of endosperm weakening is sensitive to $\psi$ or turgor.

Seed germination is the process of initiating growth of a previously quiescent or dormant embryo. For most seeds, it begins with imbibition of water. Imbibition is generally a triphasic process, with rapid initial water uptake (phase I), followed by a plateau phase with little change in water content (phase II), and a subsequent increase in water content coincident with radicle growth (phase III) (3). In terms of the regulation of germination, phase II is of primary interest, since germination in the physiological sense can be considered to be completed when embryo growth is initiated. It is the length of phase II that is generally extended by dormancy, low or high temperatures, water deficit, or abscisic acid, while factors which promote germination do so by shortening this lag phase. Once the radicle has penetrated any enclosing tissues and is growing, germination is complete and seedling growth has begun.

A number of attempts have been made to apply the Lockhart (19) model for plant cell growth to the initiation of radicle growth during seed germination (e.g., 5, 21, 23, 29). The Lockhart model describes cell growth by the empirical equation

$$dV/Vdt = m(\psi_r - Y), \quad (1)$$

where $dV/Vdt$ (Table I) is the rate of volume increase relative to the total volume, $m$ is an extensibility coefficient relating growth rate to $\psi_r$, $\psi_r$ is the turgor pressure, and $Y$ is the minimum or threshold turgor that must be exceeded for growth to occur. Since water uptake is required for the volume increase during growth, growth rate is also described by

$$dV/Vdt = L(\psi - \psi_r), \quad (2)$$

where $L$ is the hydraulic conductance of the tissue (incorporating pathway geometry, usually unknown), $\psi$ is the water potential of the external medium, and $\psi_r$ is the water potential of the growing cell. These two equations can be combined to give

$$dV/Vdt = \frac{L \cdot m}{L + m(\Delta\psi + \psi_r - Y)}, \quad (3)$$

where $\Delta\psi = \psi - \psi_r$. When $L \gg m$, this equation reduces to Equation 1, as $\Delta\psi$ is small when $L$ is large. If $m \gg L$, then the equation reduces to Equation 2, as $L$ and $\Delta\psi$ will control the rate of growth by determining the rate of water transport (2, 4, 6). In the case of seed germination, where growth is initiated at the end of a period of near equilibrium between the seed $\psi$ and its surroundings, $\Delta\psi$ will be small (3, 15, 23, 29, 30). As the distances for water transport are also small, and the water uptake rates during phase I of imbibition usually far exceed those required to sustain growth, it is unlikely that growth is limited by $L$. Thus, Equation 1 has been used to describe and interpret the water relations of seed germination (4, 5, 21, 22, 29).

The application of Equation 1 is simple only under near-steady-state conditions (constant rate of growth) when $m$, $Y$, and cellular osmotic potential ($\psi_r$) are assumed to be constant (4). Carpita et al. (5) utilized growing excised embryonic axes of lettuce (Lactuca sativa L.) to estimate the parameters of Equation 1. A difference in external $\psi$ of 0.34 MPa was required to maintain equal constant growth rates of red- and far-red-irradiated embryonic axes. The difference was attributed equally to a lower $\psi_r$ (which would increase $\psi_r$) and to a larger $m$ (more extensible cell walls) in red-irradiated seeds. However, the data fit the model of Equation 1 rather poorly, apparently indicating that some of the parameters of the equation were changing during the incubation of axes at a range of $\psi$ values. Schopfer and Plachy (23) used a similar approach to study the water relations of rape (Brassica napus L.) seed germination. They imbibed seeds for varying periods on water, then determined the linear rates of water uptake or loss over 12 h after transfer to solutions of different $\psi$. From these data, they concluded that $m$ increased markedly and $Y$ fell to zero when radicle growth was initiated. However, these
results are difficult to interpret because in some cases the changes in seed volume apparently represented irreversible growth, while in others reversible shrinking and swelling were being measured. Thus, it is likely that the cell wall properties being evaluated (i.e., the elastic versus plastic extensibility) differed depending upon the \( \psi \) of the external solution, as was evidenced by discontinuities in the growth rate versus \( \psi \) curves as growth rate neared zero (23). A more fundamental problem with applying these approaches to the initiation of embryo growth is that during phase II, growth is not occurring, so \( m \) and \( Y \) cannot be evaluated. Once growth is initiated, \( m \) and \( Y \) can be evaluated, but the seeds are then in phase III, and the parameters will refer to cell growth rather than to germination per se. The objective in applying growth models to seed germination is generally to investigate how various factors, such as \( \psi \), temperature, light, or growth regulators, influence the initiation of radicle growth, not how they influence subsequent root growth rates.

An alternative approach is to use radicle emergence as the criterion that growth has been initiated and germination completed, and then quantitate the \( \psi \) required to inhibit germination, or reduce growth rate to zero. At this \( \psi \), \( \psi_{r} = Y \), or \( Y = \psi - \psi_{r} \), assuming \( \psi \) and \( \psi_{r} \) are essentially equal and \( \psi_{r} = \psi - \psi_{r} \). The \( \psi_{r} \) at this \( \psi \), rather than at full imbibition, must be determined, either directly, or by accounting for the changes in embryo volume as \( \psi \) is reduced (3). Thus, \( Y \) (or the \( \psi_{r} \) remaining when germination is just prevented), but not \( m \), can be quantified by this approach (17, 23, 26, 29). However, caution must be exercised in applying this method, as it is possible that \( Y \) itself may change during the lengthy incubations necessary to determine the \( \psi \) that inhibits final germination (8), since reductions in \( \psi \) progressively delay germination, even though the final percentage of germination may be unaffected (8, 16, 23).

In many seeds, the embryo is surrounded by endosperm, perisperm, or other tissues that may restrict radicle growth (8, 12, 28–30). A satisfactory model of seed germination rates should be able to account for, and quantitate, the influence of such enveloping tissues. This has been done only by the germination inhibition approach, comparing intact seeds to embryos from which the enclosing tissues have been removed (15, 17, 21, 26, 29, 30). Some estimates of the contribution of enclosing tissues to \( Y \) (i.e., \( Y_{e} \)) have been obtained by this method, but the cautions cited above apply to these data as well. \( Y_{e} \) has also been estimated from the force required to puncture through the endosperm (8, 13, 21, 28), and while the values obtained correlate well with final germination responses, it is difficult to relate them quantitatively to \( \psi \) or to germination rates within the seed population.

A final limitation of previous applications of growth models to seed germination rates lies in their inability to account for the discontinuous nature of seed germination. The Lockhart model was originally developed to describe single cells, and has been adapted to the growth of tissues (2, 4, 6, 27). Growth in these systems is a continuous variable, whereas seed germination is a discrete variable, since at a given time, an individual seed has either germinated or it has not. However, the times to germination of seeds within a population can be described in statistical terms, as in the mean and variance of germination times (25), and these parameters can then be considered as continuous variables reflecting the response of the seed population to environmental influences. If the Lockhart model is to be applied to germination, it must be modified to account for the special characteristics of seed populations.

I describe here an alternative approach to the application of growth models to seed germination. Statistical methods appropriate to the study of population distributions of quantal responses are employed to derive quantitative parameters that characterize germination responses to \( \psi \). These parameters can then be used to predict germination rates and final germination percentages at any \( \psi \). By estimating the embryo \( \psi_{r} \) at different \( \psi \)s, a model relating seed germination rates to \( \psi \) can be developed that is formally analogous to the cellular growth models, but is based on the rates of initiation of radicle growth within the seed population rather than on water uptake rates.

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**Table I. Following is a list of the symbols and abbreviations used:**

- \( \frac{dV}{dt} \): relative rate of volume increase (h\(^{-1}\))
- \( g \): specific germination percentage of the seed population (%)
- \( GR \): germination rate, or \( 1/t \) (h\(^{-1}\))
- \( GR_{g} \): germination rate of percentage \( g \) of the seed population (h\(^{-1}\))
- \( GR_{g0} \): mean germination rate, or \( 1/\bar{g}_{0} \) (h\(^{-1}\))
- \( L \): cell or tissue conductance to water (MPa\(^{-1}\).h\(^{-1}\))
- \( m \): extensibility coefficient (MPa\(^{-1}\).h\(^{-1}\))
- \( \psi_{o} \): turgor or pressure potential (MPa)
- \( \psi_{t}(g) \): osmotic potential of germination percentage \( g \) (MPa)
- \( \psi_{p} \): turgor or pressure potential (MPa)
- \( \psi_{p}(g) \): base, or minimum, turgor permitting germination (MPa)
- \( \psi_{p0}(g) \): base, or minimum, turgor permitting germination of percentage \( g \) (MPa)
- \( \psi_{b0} \): mean base turgor, or \( \psi_{p0}(50) \) (MPa)
- \( \psi_{w} \): water potential of the water source or imbibition medium (MPa)
- \( \psi_{s} \): water potential of the growing cell or tissue (MPa)
- \( \psi_{b0} \): base, or minimum, water potential permitting germination (MPa)
- \( \psi_{b}(g) \): base, or minimum, water potential permitting germination of percentage \( g \) (MPa)
- \( \psi_{p} \): mean base water potential, or \( \psi_{b}(50) \) (MPa)
- \( \Delta \psi \): water potential difference, \( \psi - \psi_{w} \) (MPa)
- \( RWC \): relative water content, based upon water content after full imbibition on water (%)
- \( \sigma_{b0} \): standard deviation in base water potential within the seed population (MPa)
- \( \sigma_{p00} \): standard deviation in base turgor within the seed population (MPa)
- \( t \): time to radicle emergence (h)
- \( t_{p} \): time to radicle emergence of germination percentage \( g \) (h)
- \( t_{p0} \): mean time to radicle emergence (h)
- \( t_{p0}(\psi) \): time to radicle emergence of germination percentage \( g \) at a given \( \psi \) (h)
- \( \theta_{w} \): hydrotropic constant, or \( (\psi - \psi_{b}(g))_{o} \) (MPa.h)
- \( \theta_{p} \): turgor-time constant, or \( (\psi_{b} - \psi_{b}(g))_{o} \) (MPa.h)
- \( Y \): yield threshold, or minimum turgor for growth (MPa)
- \( Y_{o} \): yield threshold, or minimum turgor for growth, of the embryonic axis (MPa)
- \( Y_{s} \): yield threshold, or minimum embryo turgor required for penetration, of the endosperm envelope or other tissues covering the embryonic axis (MPa)
THEORY

The rate of seed germination is the inverse of the time to radicle emergence or the initiation of embryo growth. Since all seeds in a population do not germinate simultaneously, GR must be defined with reference to a particular g. For example, GR₉₀ would be the inverse of the time required for radicle emergence of 50% of the seeds in the population (1/₉₀). Linear relationships between GR and ψ have been reported (8, 14, 16). Gummerson (14) developed a model to describe seed germination responses to ψ, and included the effects of temperature in the model by using thermal time (i.e. a heat-sums or degree-days approach [1]) instead of actual time. However, since I will deal here with a single constant temperature, I will address only the ψ aspects of the model.

Gummerson (14) proposed that a θₜᵣ could be defined as

\[ θₜᵣ = (ψ - ψₐ(g))tᵣ, \]

where ψ is the seed water potential, ψₐ(g) is the base, or minimum, ψ allowing germination of percentage g, and tᵣ is the time to germination of percentage g. If θₜᵣ is constant for a seed lot, variation in tᵣ among germination percentages is in inverse proportion to the difference between ψ and ψₐ(g) of individual seeds. The increasing tᵣ values of successive fractions of the seed population are therefore a consequence of their increasing ψₐ(g) values. Rearranging Equation 4 gives

\[ GRₜᵣ = 1/tᵣ = (ψ - ψₐ(g))/θₜᵣ, \]

indicating that a plot of 1/tᵣ versus ψ should be linear, with an intercept at 1/t₀ = 0 (or complete inhibition of germination at percentage g) equal to ψₐ(g) and a slope of 1/θₜᵣ. The slope should be identical for all values of g, since θₜᵣ is a constant, while ψₐ(g) will vary with g. Gummerson (14) determined the distribution of ψₐ(g) by plotting 1/tᵣ versus ψ for a range of values of g, finding ψₐ(g) from the intercepts on the ψ axis for each g, then plotting probit (g) as a function of those ψₐ(g) values. The probit transformation linearizes a cumulative normal (Gaussian) distribution, which approximates cumulative germination time courses (7-11, 25). An added feature of a probit plot is that the inverse of the slope of the probit regression line is equal to the standard deviation of the population (11). Thus, θₜᵣ, ψₐₜᵣ(ψₐₜᵣ(50), when g = 50% or probit (g) = 0), and σₚᵣ can be estimated. θₜᵣ, ψₐₜᵣ, and σₚᵣ are assumed to be constant, intrinsic characteristics of the seed population.

A more convenient method of estimating θₜᵣ, ψₐₜᵣ, and σₚᵣ is to adapt a probit procedure described by Ellis et al. (10) for determining minimum temperatures for germination. Rearranging Equation 4,

\[ ψₐ(g) = ψ - (θₜᵣtᵣ). \]

Using Equation 6 to estimate the ψₐ(g) of seeds germinating at each tᵣ, one can plot probit (g) for all observed germination percentages at a range of constant ψs versus ψ - (θₜᵣtᵣ), using different values of θₜᵣ until the best fit, or minimum residual, is obtained in probit analyses. The resulting linear equation will be

\[ \text{probit}(g) = [ψ - (θₜᵣtᵣ) - ψₐₜᵣ]/σₚᵣ, \]

which relates g to tᵣ at any constant ψ. This approach has the advantage that data from all germination percentages at all ψs are utilized in a single regression to identify the optimal value of θₜᵣ. ψₐₜᵣ is determined from the midpoint of the probit line (i.e. when probit (g) = 0), and σₚᵣ is the inverse of the slope.

While Equation 7 can be used to predict g as a function of t at any ψ, it would be useful to be able to express germination times at different ψs on a common time scale and reduce the time courses at any ψ to a single curve, just as the transformation to thermal time allows data at all suboptimal temperatures to be expressed as a single curve (1, 7, 9, 10). Since θₜᵣ is a constant, the time to germination varies in direct proportion to ψ - ψₐ(g) (Equation 4). The maximum possible ψ is 0 MPA, so 0 - ψₐ(g) represents the maximum range of ψ values allowing germination of percentage g. Normalizing Equation 4 by dividing both sides by 0 - ψₐ(g),

\[ θₜᵣ(0 - ψₐ(g)) = [ψ - ψₐ(g)]tᵣ/(0 - ψₐ(g)). \]

The left side of this equation is simply tᵣ(0), the times to germination of seeds in water, while the right side can be reduced to \[ 1 - (ψ/ψₐ(g))]tᵣ(ψ), \] where \( tᵣ(ψ) \) is the time to germination of percentage g at any constant ψ. Thus,

\[ tᵣ(0) = [1 - (ψ/ψₐ(g))]tᵣ(ψ), \]

which states that the times to germination of seeds at any ψ can be normalized to equal the time to germination in water by multiplying \( tᵣ(ψ) \) by the factor \( [1 - (ψ/ψₐ(g))] \). When ψ = 0, the factor equals 1, and when ψ = ψₐ(g), the factor reduces to zero and germination does not occur. At any ψ between 0 MPA and ψₐ(g), the relationship between the actual time to germination for any percentage g and the time to germination in water is given by the same factor.

This model provides a quantitative description of the relationship between seed germination rates and ψ. We can compare the parameters derived from this approach to the terms in Equation 1. Germination rate (GRₜᵣ = 1/tᵣ) replaces dV/V/dt as the rate term. To convert the driving force (ψ - ψₐ(g)) to turgor units, the ψₜ at ψ is equal to ψ - ψₜᵣ, where ψₜᵣ is the osmotic potential of the embryo at that ψ. The base turgor (ψₜᵣ) is the ψₜᵣ remaining when ψ = ψₜᵣ. The variation in GRₜᵣ for different fractions of the population embodied in ψₐ(g) could result from variation in ψₐ(g) among seeds, or from variation in ψₐ(g). The extensibility coefficient (m) is formally analogous to 1/θₜᵣ, as both act as proportionality constants or slopes relating the rate to the driving force. To convert to turgor units, θₜᵣ is replaced by θₜᵣ, which has the same meaning as θₜᵣ and is estimated by the same procedure, but differs numerically from it as the value of ψₜᵣ differs from that of ψₜᵣ. Thus, the Lockhart model can be rewritten in terms appropriate to the description of seed germination rates as

\[ GRₜᵣ = (ψₐ(g) - ψₐₜᵣ(g))/θₜᵣ = (ψ - ψₜᵣ - ψₚᵣ(g))/θₜᵣ. \]

The relative contributions of variation in ψₐ(g) (leading to variation in ψₚᵣ(g)) and in ψₚᵣ(g) to the total variation in GRₜᵣ within the population must be experimentally determined. If actual seed germination time courses can be described by this modified Lockhart model, then for a given g, a plot of GRₜᵣ as a function of ψₚᵣ(g) should be linear, with a slope of 1/θₜᵣ, and an intercept on the turgor axis of ψₚᵣ(g).
The three terms in Equation 10 that could influence the germination rate are \( \psi_p(g) = \psi - \psi_o(g) \), \( \psi_{p,h}(g) \), and \( \theta_p \) analogous to \( \psi_p \), \( Y \), and \( m \) of Equation 1. Factors that alter \( GR \) would presumably act by affecting the values of one or more of these parameters. Plots of \( GR_p \) versus \( \psi_p(g) \) would be affected in different ways depending upon variation in each parameter (Fig. 1). If only \( \psi_p(g) \) varied among seeds in the population, or was affected by environmental or physiological conditions, the slope and intercept of the plot would be unchanged, and \( GR_p \) would vary directly with variation in \( \psi_p(g) \) (Fig. 1A). If only \( \psi_{p,h}(g) \) was affected, the curve would shift to different intercept values, but with the same slope (Fig. 1B). Variation in the inherent rate of progress toward germination for a given \( \psi_{p,h}(g) \) and \( \psi_{p,b}(g) \) (i.e. per unit of ‘turgor-time’) would appear as a change in slope (Fig. 1C). Equivalent observable effects on \( GR_p \) could arise from variation in any of the three parameters. It is also possible that more than one parameter could be influenced by a particular condition. Comparison of \( GR_p \) versus \( \psi_p(g) \) curves of seed lots with and without exposure to conditions that alter \( GR_p \) should reveal which of the parameters has been modified.

**MATERIALS AND METHODS**

Lettuce seeds (Lactuca sativa L. cv Empire) were incubated on blotters saturated with PEG 8000 solutions from 0 to \(-1.2\) MPa at 0.2 MPa intervals. The PEG solutions were prepared according to Michel (20), and the \( \psi_p \) were verified using a vapor pressure osmometer (Wescor model 5200) calibrated against NaCl standards. Four replications of 25 seeds each were placed on two 4.5 cm blotters in Petri dishes and moistened with 3.2 mL of water or PEG solution. The dishes were covered with tightly fitting lids to prevent evaporation and were incubated in a germination chamber at 20°C. Incubation was in the dark except for brief intervals when germination was counted under laboratory (fluorescent) light. Counts were made at 2 h intervals during the periods of most rapid germination, and at longer intervals subsequently, until no additional germination was observed for 48 h. Cut seeds were prepared by cutting the seed transversely at approximately one-third the distance from the radicle end to the cotyledon end of the seed. The sections containing the embryonic axes were incubated as described for intact seeds. Germination was scored at emergence and initial gravitropic bending of the radicle for intact seeds, and at the same stage of radicle growth for cut seeds, since the radicle pushed the embryo back from the endosperm cap rather than protruding through it.

Seed moisture contents at various stages of imbibition were determined by weight loss on oven drying at 130°C for 1 h. The moisture content during the plateau phase of imbibition on water was considered to be 100% \( RWC \) and moisture contents at \( \psi < 0 \) MPa were expressed as percent \( RWC \) (3). Embryo osmotic potentials (\( \psi_p \)) were determined by thermo-couple psychrometry on frozen and thawed tissue using either Merrill-type Peltier-cooled sample chambers or the vapor pressure osmometer. Both types of psychrometers were calibrated with NaCl solutions of known osmotic potential. Seeds were imbibed to the plateau phase of moisture content and were removed from the endosperm envelope by gentle pressure on the cotyledon end. In some cases, the embryos were separated into cotyledons and axes before freezing in sealed microfuge tubes, thawing, and transferring to the psychrometer chambers. All manipulations of embryos were performed in a humidified box to reduce evaporative losses.

Probit analyses were conducted using the PROC PROBIT routine of the SAS statistical package which employs a maximum-likelihood weighted regression method (SAS Institute Inc., Cary, North Carolina) (11). Preliminary analyses were also conducted using standard least-squares linear regression of probit-transformed percentages. It was found that if data greater than 95% of the final germination percentage were excluded (which carry little weight in probit analyses), both methods gave essentially identical estimates of the optimal regression lines.

**RESULTS**

**Germination Responses to \( \psi \)**

Cumulative germination time courses at a range of \( \psi_p \) were determined for intact (Fig. 2A) and cut (Fig. 2B) lettuce seeds. The data are shown on a logarithmic time scale to separate the time courses at high \( \psi \) for clarity, and because the log transformation results in a more normal distribution of ger-
observations, and the population means the logarithmic prediction of termination times (24). Cut seeds, in which the endosperm envelope no longer completely surrounds the embryo, germinated more rapidly at each ψ compared to the intact seeds.

To quantify the water relations parameters of these seeds, the germination percentages were transformed to probits and plotted versus ψ[g] = ψ - (θ/h)[g], using different values of θ/h in repeated probit analyses until the best fit to all data was obtained (Fig. 3). The optimal values of θ/h (19.3 MPa-h for intact seeds and 9.9 MPa-h for cut seeds) were able to reduce the data at all ψs to single distributions of ψ[g]. The ψ[g] values for intact and cut seeds were essentially identical (Fig. 3). The ψ[g] values estimated from this analysis (−1.10 ± 0.02 MPa for intact and −1.18 ± 0.02 MPa for cut seeds) agreed well with the ψ required to inhibit germination percentage by 50%, interpolated from plots of final germination percentage versus ψ (−1.03 and −1.25 MPa for intact and cut seeds, respectively; data not shown). Using the values for θ/h, ψb, and σb, from Figure 3, the original germination time courses could be predicted by Equation 7, transforming probit (g) back to percentages (Fig. 2). That is, the predicted curves in Figure 2 represent the cumulative normal distributions of the function \( \psi - (\theta/h) - \psi_b/\sigma_b \), with time at each \( \psi \). The agreement between the predicted and actual values is quite good overall, particularly at the higher ψs. Some discrepancies between predicted and actual percentages occur at the lowest ψs. By systematically varying each of the terms of the function, it was found that increasing \( \theta/h \) to 25 to 30 MPa·h and allowing \( \psi \) to deviate within ±0.1 MPa from the expected values could account for the differences between the actual and predicted curves at \( \psi \leq -0.6 \) MPa without altering \( \psi_b \) or \( \sigma_b \). An increase in \( \theta/h \) could be due to slower imbibition at low \( \psi \) (3), and the \( \psi \) of PEG solutions can easily vary by 0.1 MPa from the predicted values during prolonged incubations. The predicted curves also become very sensitive to small changes in \( \psi \) as \( \psi \) approaches \( \psi_b(g) \), since GR is proportional to \( \psi - \psi_b(g) \). A difference in \( \psi \) of 0.1 MPa from the expected value changes GR by less than 10% when \( \psi \) is near 0 MPa, but the same...
variation when $\psi - \psi_b(g) = 0.1$ MPa would either double $GR$ or prevent germination completely. The probit regression technique utilizes data from all germination percentages at all $\psi$s tested to determine the values of $\theta_I$, $\overline{\psi}_p$, and $s_\psi$, that best describe the overall population response of germination to $\psi$.

This point can be further illustrated by plotting the data of Figure 2 on a normalized time scale, or $[1 - \psi/\psi_b(g)]t_d(\psi)$, where $\psi_b(g)$ is the $\psi$ predicted for each observed germination percentage, calculated from $\psi - (\theta_I/t_d)$ (see Fig. 3). On this time scale, the germination time courses at all $\psi$s (Fig. 2) are normalized to single curves approximating the time course predicted at 0 MPa (Fig. 4; Eq. 9). Alternatively, the predicted curves of Figure 2 can be generated by dividing the $t_d$ values at $\psi = 0$ MPa by the factor $[1 - \psi/\psi_b(g)]$. This remarkably simple result indicates that both the timing and the final extent of germination are directly proportional to the ratio of $\psi$ to $\psi_b(g)$. The process illustrated in Figure 3 is essentially one of identifying the correct $\theta_I$ and distribution of $\psi_b(g)$ that makes this so.

As noted previously, linear relationships have been reported for mean germination rate ($GR_{50}$) as a function of $\psi$ (8, 14, 16), as would be predicted from Equation 5. When the $GR_{50}$ values from Figure 2 (determined from separate probit analyses of germination percentages at each $\psi$ versus log $t_d$) are plotted versus $\psi$, the data agree well with the theoretical line, $GR_{50} = (\psi - \overline{\psi}_p)/\theta_I$, using the values of $\overline{\psi}_p$ and $\theta_I$ from Figure 3 (Fig. 5A). Parallel curves representing the rates to achieve any $g$ could be similarly constructed using the appropriate value of $\psi_b(g)$ and the same $\theta_I$. Figure 5 illustrates graphically the conclusion noted earlier, that the difference in germination rates of intact and cut seeds was due primarily to the lower $\theta_I$ of the latter, rather than to a significantly lower $\overline{\psi}_p$ for cut seeds.

**Germination Responses to $\psi_p$**

To put the model of Figure 5A in terms of $\psi_p$ rather than $\psi$, $\psi - \psi_b(g)$ must be evaluated at each $\psi$. For the seed lot used here, embryo $\psi$, was $-1.04 \pm 0.03$ MPa ($n = 10$), and no significant difference in $\psi$ was found between the cotyledons and the embryonic axes. To evaluate whether $\psi_g(g)$ varied within the population, embryos were sampled immediately upon radicle protrusion, with successive samples being taken of newly germinated seeds at various times. No significant variation in $\psi_g(g)$ was detected among different germination fractions at the time of radicle emergence, and the $\psi_g(g)$ values obtained were identical to those from random samples of ungerminated seeds during phase II of imbibition (data not shown). Subsequent calculations therefore assume that single values of $\psi$, and $\psi_p$, can be used for all seeds in the

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**Figure 4.** Cumulative germination time courses for intact and cut seeds at a range of $\psi$s (symbols as in Fig. 2) plotted on a normalized time scale. The solid dots represent the predicted time courses in water based upon the parameters derived in Figure 3. When normalized on the basis of $[1 - (\psi/\psi_b(g))]t_d(\psi)$, the families of curves representing germination at different $\psi$s (Fig. 2) collapse to equal the time course in water. Thus, germination at a range of $\psi$s can be expressed on a common normalized scale, as shown from, or the actual time course at any $\psi$ (Fig. 2) can be predicted from that in water according to $t_d(0)/[1 - (\psi/\psi_b(g))]$.

**Figure 5.** Mean germination rates of intact and cut lettuce seeds as functions of $\psi$ (A) or $\psi_p$ (B). The symbols are the mean germination rates (1/t50) at each $\psi$ or $\psi_p$. The lines are those predicted from the parameters derived as in Figure 3 on the basis of either $\psi$ or $\psi_p$, i.e. $GR_{50} = (\psi - \overline{\psi}_p)/\theta_I$, or $GR_{50} = (\psi - \overline{\psi}_{2,0})/\theta_I$. The $\psi_p$ values at each $\psi$ were estimated according to Figure 6.
lot during phase II of imbibition. This result also rules out the possibility that variation in \( \psi_p(g) \) is responsible for variation in \( GR_p \) among seeds in the lot (Fig. 1A).

To estimate \( \psi_p \) as a function of \( \psi \), the effect of the change in embryo volume as \( \psi \) is reduced must be taken into account. In contrast to most vacuolated plant tissues, relatively small reductions in \( \psi \) result in considerable water loss from lettuce seeds (Fig. 6). Assuming that the nonosmotic (apoplastic) volume of the seeds remains constant at 15% over the \( \psi \) range under consideration (3), the \( \psi_p \) at \( \psi < 0 \) MPa could be calculated from the change in RWC (Fig. 6) by dividing the \( \psi_p \) measured in fully imbibed seeds (RWC = 100) by the factor \([ 1 - ((100 \text{- RWC})/85)]\). \( \psi_p \) at each \( \psi \) was then calculated as \( \psi - \psi_p \) (Fig. 6, inset). Tests where \( \psi_p \) was measured directly after equilibration of seeds at low \( \psi \) confirmed that correction for changes in osmotic volume estimated the variation in \( \psi_p \), with RWC to within 0.1 MPa (Table II).

After calculating the embryo \( \psi_p \) at each \( \psi \), the procedure illustrated in Figure 3 was repeated, plotting probit (\( g \)) versus \( \psi_p - (\theta_p/\psi) \), optimizing \( \theta_p \), and determining \( \psi_{p,b} \) and \( \sigma_{\psi_{p,b}} \). These yielded values for intact seeds of 10.2 MPa-h, 0.44 ± 0.01 MPa, and 0.081 MPa for \( \theta_p \), \( \psi_{p,b} \) and \( \sigma_{\psi_{p,b}} \) respectively, and 5.3 MPa-h, 0.40 ± 0.01 MPa and 0.081 MPa for cut seeds. Plotting \( GR_p \) versus \( \psi_p \), the experimental data agree well with the predicted lines based upon these parameters (Fig. 5B). It is also apparent that the experimental data agrees most closely with the theoretical curves where variation in \( GR \) is due to variation in \( \theta_p \) (Fig. 1C), rather than to variation in \( \psi_{p,b} \) (Fig. 1B).

**DISCUSSION**

Since seed germination is evidenced by growth of the embryonic axis, the application of growth models to seed germination should provide information about the factors controlling the rate and extent of germination under various conditions. However, for the reasons discussed in the introduction, the application of cell or tissue growth models to seed germination has been problematic. An individual seed has either germinated (initiated growth) or it has not, i.e. germination is a discontinuous or quantal response. The germination responses of a population of seeds can be analyzed in statistical terms, recognizing the variation in the values of specific parameters among seeds that gives rise to different lag times before growth is initiated. This is analogous to the adaptation of cellular water relations models to tissues by defining bulk tissue parameters on a volume-averaged or weight-averaged basis by summing across individual cell types possessing different characteristics (27). As the times to germination of seeds in a population approximate a normal distribution (particularly on a log time scale), statistical approaches based upon the normal distribution, such as the probit transformation, can be applied to analyze the distributions of germination times (7,10, 24). The parameters derived from such analyses (e.g. the mean and standard deviation of the population for a particular characteristic) can then be used to estimate the response time of any particular fraction of the population.

The method proposed here extends the model proposed by Gummerson (14) for analyzing seed germination responses to \( \psi \). Gummerson’s (14) basic assumption was that the rate of germination is proportional to the difference between \( \psi \) and the minimum \( \psi \) allowing germination of a particular percentage, \( \psi_p(g) \). The variation in times to germination among seeds is a reflection of the differences in their \( \psi_p(g) \) values. It is clear that \( \psi_p(g) \) does vary within seed populations, as final germination percentages are progressively reduced as \( \psi \) is lowered.

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**Table II. RWC and Osmotic Potentials (\( \psi_p \)) of Lettuce Seeds Imbibed either on Water or at \(-1.0\) MPa**

<table>
<thead>
<tr>
<th>Imbibed</th>
<th>Seed Water Content</th>
<th>RWC</th>
<th>Measured ( \psi_p )</th>
<th>Predicted ( \psi_p )</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>76.2 ± 1.5</td>
<td>100</td>
<td>-1.28 ± 0.05</td>
<td>-1.28</td>
</tr>
<tr>
<td>-1.0</td>
<td>60.1 ± 3.1</td>
<td>79</td>
<td>-1.76 ± 0.06</td>
<td>-1.70</td>
</tr>
<tr>
<td>-1.0 to -0</td>
<td>72.2 ± 1.0</td>
<td>95</td>
<td>-1.40 ± 0.06</td>
<td>-1.36</td>
</tr>
</tbody>
</table>

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The relative values were obtained by multiplying the corresponding values by 100.
(Fig. 2) (8, 16, 26, 29, 30). If $\psi(g)$ remains constant when the seed population is incubated at different $\psi$, the time to germination of a particular percentage $g$ at any $\psi > \psi(g)$ should be inversely proportional to $\psi - \psi(g)$ (cf. Eq. 5). The proportionality constant, $1/\theta_{m}$, represents the rate of progress toward germination per unit of hydrotome (MPa·h). The method of repeated probit analyses using different values of $\theta_{m}$, adapted from that proposed by Ellis et al. (10) for determining base temperatures for germination, provides a convenient method of analyzing data from a range of $\psi$s, using the complete germination time courses (Fig. 3). $\theta_{m}$ can also be determined for specific percentage fractions by plotting $G_{R\psi}$ versus $\psi$; the slope of the curve is $1/\theta_{m}$ (Fig. 5A). This approach is useful to get an initial estimate of $\theta_{m}$, but it does not provide information about the variability of $\psi(g)$ unless the analysis is repeated for the entire range of $g$ (14). The probit analysis approach estimates both $\bar{\psi}$ and $\sigma_{\psi}$, which can be used to predict actual germination time courses at any $\psi$ (Fig. 2).

The ability of a single constant value of $\theta_{m}$ to reduce data from a range of $\psi$s to a single distribution of $\psi(g)$ (Fig. 3), also evident in the linear relationship between $GR_{\psi}$ and $\psi$ (Fig. 5A), indicates that $\psi(g)$ remained relatively constant during imbibition and incubation at different $\psi$s. This allows normalization of the germination time courses at any $\psi$ to equal that in water using the time scale of $[1 - \psi/\psi(g)]/\bar{\psi}$ (Fig. 4). In addition, the $\bar{\psi}_{u}$ values estimated by the current approach were in good agreement with the $\psi$s required to inhibit final germination by 50% in both intact and cut seeds. Thus, similar values were obtained using either probit analyses of time courses or final germination inhibition to estimate $\bar{\psi}_{u}$. However, this is not always the case; in tomato (Lycopersicon esculentum Mill.) seeds, for example, $\bar{\psi}_{u}$ of cut seeds is dependent upon the $\psi$ at which the seeds are incubated (8). In this case, the germination inhibition approach estimates $\bar{\psi}_{u}$ values considerably lower than those predicted by probit analysis of germination time courses for seeds imbibed at higher $\psi$s. An advantage of the method presented here is that $\theta_{m}$, $\bar{\psi}$, and $\sigma_{\psi}$ can be estimated from germination time courses at only two or three relatively high $\psi$s where germination still goes to completion and where adaptive changes during incubation are minimized. The parameters obtained from the probit approach at relatively high $\psi$ are more likely to reflect the situation in a seed imbibed in water than are those estimated from germination inhibition experiments conducted at low $\psi$.

Although $\theta_{m}$, $\bar{\psi}$, and $\sigma_{\psi}$ empirically quantify the germination responses of a seed lot to $\psi$, it is the physiological basis of these parameters that is of interest. Germination of lettuce seed has been described as being dependent upon a balance of forces between the capacity of the embryonic axis to expand and the ability of the endosperm envelope to resist axis expansion (18, 21, 26, 29). At the end of phase II of imbibition, the driving force for axis expansion is the extent by which $\psi_{c}$ exceeds $Y_{a}$, the yield threshold of the axis cells. The minimum $\psi_{c}$ allowing axis growth in seeds with the endosperm removed or cut will be an estimate of $Y_{a}$. If the endosperm presents a physical constraint to axis expansion, once the axis cells expand sufficiently to become appressed to the endosperm, it will be the yield threshold of the endosperm ($Y_{e}$) which limits further expansion, rather than $Y_{a}$. During phase II of imbibition, the lettuce endosperm envelope does not limit embryo water uptake, as the water contents of embryos imbibed intact did not increase when removed from the endosperm and transferred to water for short periods prior to the initiation of growth (data not shown). This is in contrast to some other seeds, where the endosperm or testa restrict water uptake by physically limiting the volume of the embryo (8, 15, 30). The minimum $\psi_{c}$ permitting radicle emergence from intact seeds will be an estimate of $Y_{a} + Y_{e}$ or the $\psi_{c}$ required to expand the radicle cells plus the additional pressure needed to exceed the break strength of the endosperm. For the seed lot used here, the difference in $\psi_{c}$ values between intact and cut seeds ($Y_{c}$) was <0.1 MPa (Fig. 5B), indicating that at the time of radicle emergence, the endosperm did not present a significant mechanical resistance to penetration by the embryo. Thus, endosperm weakening, rather than an increase in $\psi_{c}$ of the embryo cells as was previously proposed (3, 26), appears to be the factor regulating the timing of radicle emergence. Cytological and morphological changes in the endosperm cells adjacent to the radicle tip occur prior to or coincident with radicle emergence in lettuce and could be related to endosperm weakening (12, 22). The value of $Y_{c}$ has been shown by the germination inhibition approach to vary among cultivars and seed lots, and with the imbibition temperature and duration (26, 29). For the cultivar and seed lot used here imbibed under optimal conditions, the more rapid germination of cut seeds could be attributed almost entirely to a smaller $\theta_{m}$, rather than to a reduction in $\bar{\psi}_{u}$ (compare Fig. 1, B and C, with Fig. 5B). Of the approximately 9 h reduction in mean time to germination due to cutting (Fig. 4), as much as 3 h could be due to a more rapid initial imbibition rate (shorter phase I) (data not shown). The remaining 6 h reduction in $t_{50}$ may represent the time normally required during phase II for the initiation and completion of the endosperm weakening process. In addition, processes in the axis required for the initiation of growth (such as a reduction in $Y_{a}$) might occur more rapidly in cut than in intact seeds due to, e.g., greater oxygen availability.

Having shown that seed germination rates can be directly related to the turgor of the embryo (Fig. 5B), it remains to be asked why this should be so. The reason for a relationship between $\psi_{c}$ and the final extent of germination is clear, since $\psi_{c}$ must exceed the total yield threshold ($Y_{a} + Y_{e}$) in order for germination to occur. When seeds are imbibed at low $\psi$s, however, the rate of germination is slowed even at $\psi$s where final germination is unaffected (Fig. 2). The $GR$ is slowed more than can be accounted for by reduced imbibition rates at low $\psi$. Lettuce seed imbibition was complete within 24 h even at very low $\psi$ (3), yet germination did not begin until after 60 h at $-1$ MPa (Fig. 2A). Current evidence indicates that $\psi_{c}$ is not changing significantly during phase II, even in seeds incubated for extended periods at $\psi < 0$ MPa, if differences in $RWC$ are taken into account (Table I) (8, 15, 23, 29, 30). Since the lettuce axis has to grow only slightly to protrude through the endosperm, it seems unlikely that the delay is...
due to a long period of slow growth prior to emergence. The length of phase II would seem to be primarily determined by the time required for $Y_a + Y_b$ to fall below $\psi$. Since cut seeds germinate much more rapidly at any $\psi$ than do intact seeds (Figs. 2 and 4), the time required for a change in $Y_a$, rather than in $Y_0$, appears to be the rate-limiting factor in intact seeds. Cracks and breaks between endosperm cells have been observed just prior to visible radicle growth, with the radicle tip apparently forcing the endosperm tissue apart along these cracks (22). This leads to the conclusion that $\psi$ of the embryo, or perhaps of the endosperm cells themselves, influences the rate at which processes related to a reduction in $Y_a$ occur. This would be in agreement with the anatomical observations of Georghiou et al. (12), who noted that characteristic cellular changes (breakdown of storage materials, vacuole formation) in the endosperm opposite the radicle tip that accompany radicle emergence did not occur if the seeds were incubated for up to 48 h in osmotic solutions that prevented germination.

In conclusion, a method has been presented for analyzing seed germination responses to reduced $\psi$ and for quantifying and predicting seed germination time courses at any $\psi$ on the basis of only three fundamental parameters of the seed lot, $\theta_{11}, \psi_0$, and $\sigma_{\psi}$. Germination time courses at a range of $\psi$ values can be presented on a normalized time scale based upon the ratio of the test $\psi$ to the $\psi(g)$ of seeds in the lot. By estimating embryo $\psi$, at various $\psi$s, the same analysis can be used to quantitate the relationship between embryo $\psi$, and $\psi$. Comparison of intact seeds with seeds where the endosperm envelope has been cut indicates that at the time of radicle emergence, the physical restraint by the endosperm is small. Thus, it does not appear that the embryo forces its way through a considerable barrier, but rather that the endosperm presents only a weak resistance when radicle growth commences. The timing of radicle emergence under optimal conditions is controlled primarily by the rate at which weakening of the endosperm (reduction in $Y_a$) occurs. The rate of endosperm weakening is apparently sensitive to $\psi$ or $\psi_0$.

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LITERATURE CITED