Seed Technology

An International Journal
Serving Seed Scientists and Technologists

Volume 26, Number 1 • 2004

Published jointly by:

Association of Official Seed Analysts

Society of Commercial Seed Technologists

ISSN: 1096-0724 Printed in the USA by Allen Press, Lawrence, KS

Applications of Hydrotime Analysis in Seed Testing

Kent J. Bradford* and David W. Still

ABSTRACT

Hydrotime is a way to describe the relationship between water potential (ψ) and seed germination rates and percentages. The minimum ψ that allows germination to be completed is called the base water potential (ψ_b). The germination rate (inverse of time to radicle emergence) increases linearly as the seed ψ increases above ψ_b to its maximum rate in water (ψ = 0 MPa). As ψ_b values vary among individual seeds, germination rates also vary, resulting in lack of uniformity that can be quantified by the standard deviation in ψ_b values (σ_{ψ_b}). The hydrotime constant (θ_H) indicates the inherent speed of germination in a seed lot. Thus, hydrotime analysis quantifies the speed of germination (θ_H), the stress tolerance of germination (ψ_b) and the uniformity of germination for a seed lot (σ_{ψ_b}), which are all useful indicators of seed vigor. Hydrotime analysis of seed lots under diverse conditions allows them to be ranked according to their potential for successful emergence. It is also a valuable tool for developing and assessing seed enhancement treatments such as pelleting and priming. Hydrotime analysis can be simplified into an endpoint test that could be useful for ranking seed lots according to vigor and for diagnosing seed lot potential under stressful conditions.

The Hydrotime Concept in Seed Germination

The hydrotime concept is a unifying model to describe the patterns of germination that occur in response to the water potential (ψ) of the seed's environment. It is similar to thermal time, or degree-days, in which the degrees in excess of a base or threshold temperature (T_b), multiplied by the time to a developmental event (for example, radicle emergence) is a constant. Gummerson (1986) proposed that in analogy with thermal time or degree-days responses in relation to temperature, the time to germination is related to the magnitude of the difference between the ψ of the seed or environment and the physiological ψ threshold for radicle emergence (ψ_b). Interestingly, Gummerson (1986) showed that in the case of germination responses to reduced ψ, the total hydrotime (MPa-hours or MPa-days) to radicle emergence was the same for all seeds in the population, but that individual seeds varied in their threshold ψ at which radicle emergence would be prevented. The following equation describes the basis of the hydrotime model (Bradford, 1990; 1995):

$$\theta_H = [\psi - \psi_b(g)]\, t_g \qquad (1)$$

where θ_H is the hydrotime constant (MPa h), ψ is the actual seed water potential

Kent J. Bradford,* Department of Vegetable Crops, University of California, Davis, CA 95616-8631; David W. Still, Department of Plant Science, California State Polytechnic University, Pomona, CA 97168. Original results of D.W.S. supported in part by California Agriculture Research Initiative. *Corresponding author. Received 19 September 2003.

(MPa), $\psi_b(g)$ is the base or threshold water potential (MPa) defined for a specific germination fraction g, and t_g is the time (h) to radicle emergence of fraction g of the seed population. If θ_H is a constant, then t_g must increase proportionately as ψ is reduced and approaches $\psi_b(g)$.

Equation 1 can be rearranged to illustrate the relationship between ψ and the germination rate ($GR_g = 1/t_g$, the inverse of time to radicle emergence) of fraction g of the seed population.

$$GR_g = 1/t_g = [\psi - \psi_b(g)] / \theta_H \qquad (2)$$

Thus, a plot of GR_g versus ψ gives a straight line with a slope of $1/\theta_H$ and an intercept on the ψ axis equal to $\psi_b(g)$ (Fig. 1A). If this relationship is plotted for different germination percentages (for example, Gummerson, 1986), a series of parallel lines result with a common slope ($1/\theta_H$) but different intercepts [$\psi_b(g)$]. Experimentally, it has been found that in most cases the $\psi_b(g)$ values vary among seeds in the population in a normal or Gaussian distribution (for example, Gummerson, 1986). Thus, the relative frequency of a given ψ_b value in the seed population is a normal bell curve, which can be defined by its mean [$\psi_b(50)$] and standard deviation (σ_{ψ_b}) (Fig. 1B; Bradford, 1997). The term $\psi_b(g)$ represents this distribution of threshold values within the seed population.

The $\psi_b(g)$ values of the seeds in a population, along with the hydrotime constant (θ_H), determine the times to radicle emergence at a given ψ. The difference between the seed ψ and the ψ_b of that seed [referred to as $\Delta\psi(g)$] is

Figure 1. A. Germination rates ($GR_g = 1/t_g$) as a function of ψ for different germination fractions (g). Germination rates for a given fraction increase linearly with ψ above the threshold value [$\psi_b(g)$], which varies among seed fractions. The slope, however, is constant for all fractions and is equal to $1/\theta_H$. B. A normal distribution of ψ_b values, characterized by the mean [$\psi_b(50)$] and standard deviation (σ_{ψ_b}). The relative frequency of occurrence of a particular ψ_b value is indicated by the bell-shaped curve, which is symbolized by $\psi_b(g)$.

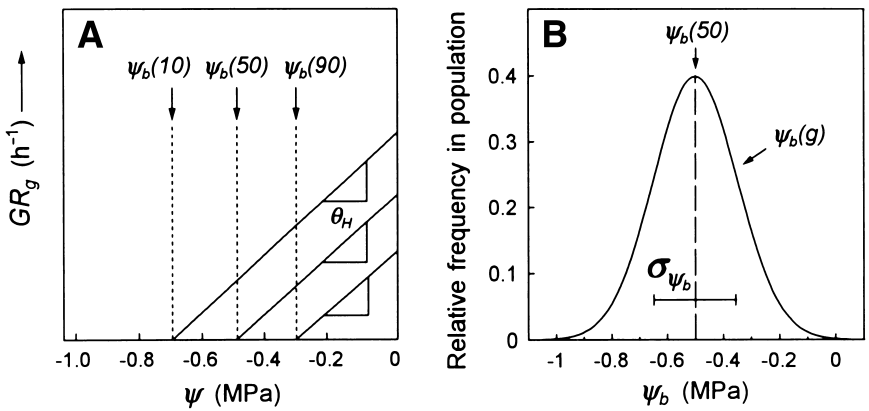
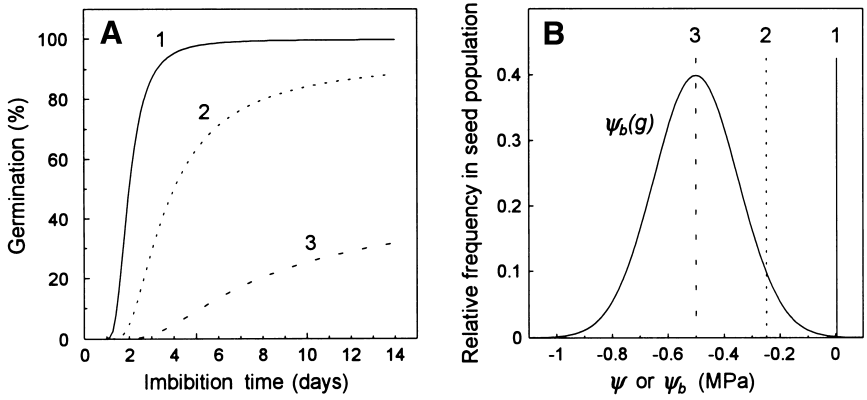


FIGURE 2. A. Germination time courses for a seed population at 0 MPa (curve 1), at –0.25 MPa (curve 2), or at –0.5 MPa (curve 3). B. Relationship of three water potentials to the threshold distribution for germination of a seed population. Imbibition of seeds having the $\psi_b(g)$ distribution indicated [with $\psi_b(50)$ of –0.5 MPa and σ_{ψ_b} of 0.15 MPa] at water potentials 1, 2 and 3 would result in the corresponding germination time courses shown in Panel A.

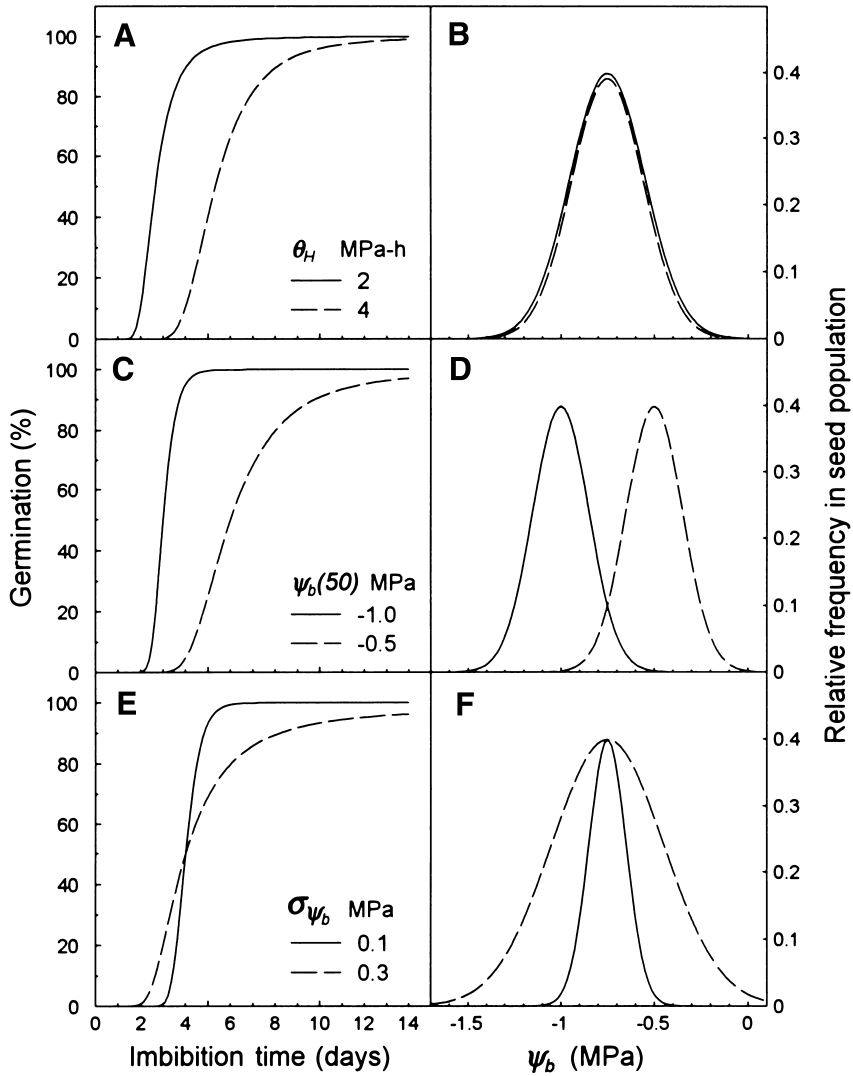


inversely related to t_g; that is, the larger $\Delta\psi(g)$, the smaller is t_g, and vice versa, since θ_H is a constant (Eqn. 1). Since the ψ of pure water is 0 MPa, this represents the highest value of ψ, and the difference between this and the $\psi_b(g)$ values of the seeds is the greatest, so germination is most rapid in water (Fig. 2A, curve 1). If the ψ of the seed is reduced (for example, by imbibing it on a medium containing an osmoticum) (Fig. 2B, line 2), then $\Delta\psi(g)$ will be smaller, and t_g will be increased for all seeds in the population (Fig. 2A, curve 2). Further reduction in ψ results in greater delay in germination, and also in the prevention of germination in an increasing fraction of the seed lot (Fig. 2A, curve 3; Fig. 2B, line 3). As shown in Fig. 2B, when the actual ψ (indicated by the vertical lines) is lower (more negative) than the $\psi_b(g)$ values, those seeds whose values are higher than ψ (to the right of the vertical lines in Fig. 2B) will be prevented from completing germination. Thus, reducing ψ has a predictable effect on the timing and on the final percentage of germination.

The actual magnitude of the effect on germination of a given reduction in ψ is determined by the position and width of the $\psi_b(g)$ distribution, i.e., by $\psi_b(50)$ and σ_{ψ_b}. If the $\psi_b(50)$ distribution is shifted to more negative values, e.g., to –1.0 MPa rather than –0.5 MPa as depicted in Figs. 1B and 2B, then the germination rates of all seeds will be more rapid and final percentages will be higher. Similarly, if the $\psi_b(g)$ distribution in the population shifts to higher (less negative) values, then germination will be slower, and some seeds may not be able to germinate even in water if their ψ_b thresholds are greater than 0 MPa. There is considerable evidence now that this is essentially what happens in dormant seeds (Bradford, 1997; 2002; Meyer et al., 2000; Alvarado and Bradford, 2002). That is, the value of $\psi_b(g)$ for a seed population can be

shifted to higher or lower values by the induction and release of dormancy. The variation among seeds in their ψ_b values, measured by σ_{ψ_b}, results in the distribution of germination times, so greater variation means a wider spread between the first and last seeds to germinate.

Figure 3. Effects of changes in hydrotime parameters on germination time courses. Germination time courses (A) for seed lots having identical $\psi_b(50)$ and σ_{ψ_b} values (B) but different θ_H values. Germination time courses (C) for seed lots having different $\psi_b(50)$ values (D) but identical θ_H and σ_{ψ_b} values. Germination time courses (E) for seed lots having different σ_{ψ_b} values (F) but identical θ_H and $\psi_b(g)$ values.



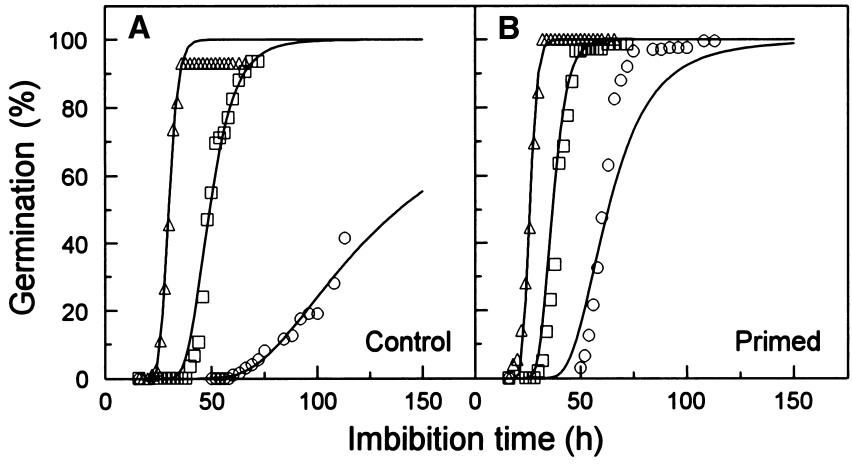
The three parameters that define the hydrotime characteristics of a seed population, θ_H, $\psi_b(50)$, and σ_{ψ_b} are useful indicators of seed quality and vigor. The hydrotime constant, θ_H, indicates the overall speed of germination (Figs. 3A, B). For example, the θ_H values of lettuce (*Latuca sativa* L.) seeds, which generally germinate in a day or two, will be much lower than those for carrot (*Daucus carota* L.) seeds, which may take 7 to 10 days to germinate. An increase in the θ_H value can also be an indicator of seed aging. The $\psi_b(50)$ value of a seed population indicates its inherent stress tolerance. The lower (more negative) that $\psi_b(50)$ is, the lower the ψ that is required to prevent germination and the more rapid germination will be at ψ values higher than the seeds' $\psi_b(g)$ values (Figs. 3C, D). Thus, $\psi_b(50)$ can be considered as a general vigor index and as an index of stress tolerance. Seed quality is also reflected in the uniformity of germination, which is indicated by σ_{ψ_b} (Figs. 3E, F). Thus, the speed, stress tolerance and uniformity of a seed lot can all be quantified by hydrotime analysis.

Application of Hydrotime Analysis to Seed Testing

The application of the hydrotime model to evaluate seed quality is straightforward, although presently somewhat labor-intensive. The model is based upon data from germination time courses, so it requires data for several time points during germination at several water potentials. Thus, replicate samples are imbibed in water and in PEG 8000 solutions of –0.25 and –0.5 MPa, for example. At 20°C, these solutions would contain 0.131 and 0.192 g PEG/g water; as the ψ of PEG solutions changes with temperature, specific solutions should be prepared for the intended temperature (Michel, 1983). It should also be noted that the Michel equations should serve as an approximation only. All solutions should be adjusted to the desired ψ and verified using a vapor pressure osmometer. The percentage of germination (radicle emergence) is then recorded at several times, with the observation times being chosen to provide several values between 16 and 84% germination (i.e., within one standard deviation around the median) for best results. Germinated seeds are removed at each observation time to prevent them from taking up water and altering the ψ of the medium. Once germination is complete (all seeds may not germinate at all ψ), probit regression analysis is used to fit the time courses to the hydrotime model (Bradford, 1990). This can be done easily in a spreadsheet, or a SAS protocol is available from one of the authors (D.W. Still). The values of θ_H, $\psi_b(50)$, and σ_{ψ_b} are then determined from the regression equation. In many cases, differences in seed lots are evident simply from inspection of the resulting time courses.

As an example of the application of hydrotime analysis, control and primed lettuce seeds, both of which were pelleted, were germinated at different ψ as described above (Fig. 4). It is evident, particularly at the lowest ψ, that the primed seeds (Fig. 4B) are germinating more rapidly than the control seeds (Fig. 4A). In this case, this is due to a lower $\psi_b(50)$ value for the primed seeds, resulting in better germination at the lower ψ. In other cases, the effect of seed priming in speeding germination rates was primarily by reducing θ_H, with less effect on $\psi_b(50)$ (Dahal and Bradford, 1990; Bradford and Somasco, 1994).

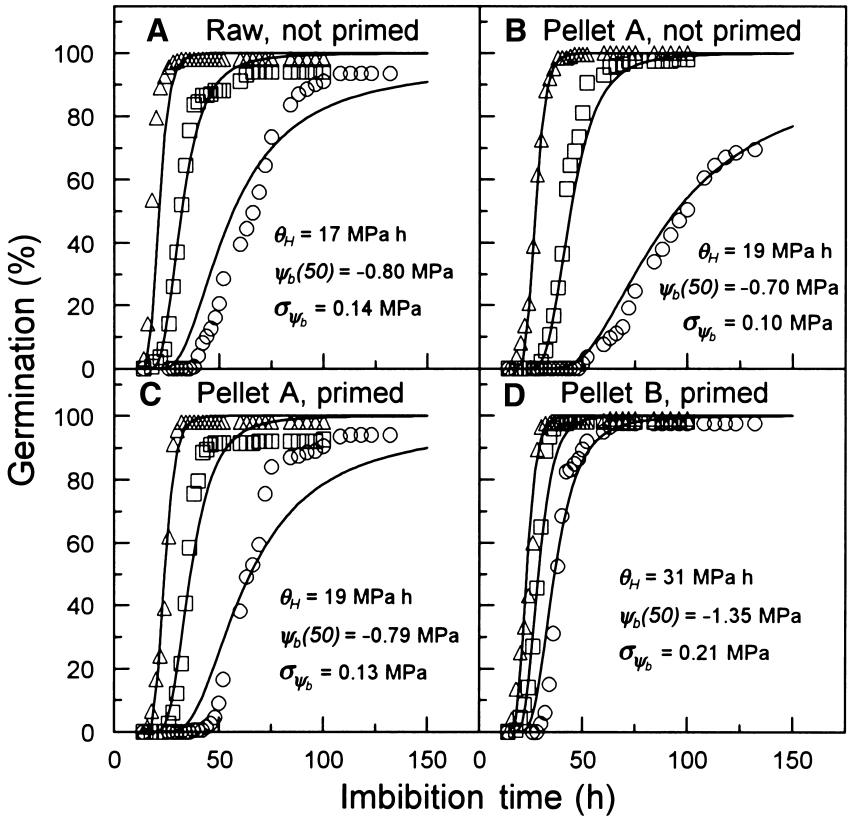
Figure 4. Hydrotime germination time courses of seeds of a butterhead type lettuce cultivar that were pelleted (control) or primed and pelleted (primed) by a commercial seed technology company. Seeds were germinated at 0 (△), −0.25 (□) and −0.5 MPa (○). The hydrotime model was fit to the data, and the resulting parameters were: Control, θ_H = 19 MPa h, $\psi_b(50)$ = −0.64 MPa, and σ_{ψ_b} = 0.08 MPa; Primed, θ_H = 22 MPa h, $\psi_b(50)$ = −0.85 MPa and σ_{ψ_b} = 0.09 MPa. In this case, the major effect of priming was to lower the $\psi_b(50)$.



Experience with a wide array of priming and pelleting methods indicates that, depending upon the cultivar, seed lot, and specific treatments applied, effects on all three parameters of the hydrotime model can be observed. For example, Fig. 5A shows germination time courses of raw (not pelleted) control (not primed) lettuce seeds. Pelleting of these seeds without priming caused some delay in germination that was particularly evident at the lowest ψ (Fig. 5B). Priming the seeds before pelleting overcame this delay and restored performance essentially to that of the raw control seeds (Fig. 5C). However, another type of pellet was even better when combined with the primed seeds, resulting in very rapid germination even at the lowest ψ (Fig. 5D). The different values of the hydrotime parameters in each case indicate how these can be indices of specific types of seed performance in response to different treatments. Our data with lettuce indicate that the hydrotime model is a sensitive indicator of the effects that priming and pelleting treatments have on the seed lot and can be used to monitor production runs or develop new protocols.

Virtually any seed enhancement can have effects on a seed lot that are detectable by comparing the hydrotime parameters obtained before and after the treatment. In particular, any treatment that decreases the goodness of "fit" of the model relative to the raw control seed has potentially altered the physiology of the seed. In most situations, such seed lots have had poorer performance in the field than those treatments in which the fit of the model has been

Figure 5. Hydrotime analysis can be used within a seed technology company to analyze the effects of priming treatments and different pellet formulations. In this example, seeds of a single lot of an iceberg-type lettuce cultivar were primed and pelleted in different ways by a single commercial seed company. The germination of untreated control seed (not primed, not pelleted) is shown in Panel A. Panel B shows the germination of control seeds after pelleting by formulation A. Panel C shows the effect of priming followed by pelleting by formulation A. Priming offset some of the delay due to pelleting. In Panel D, seeds were primed and pelleted by formulation B, which gave better performance than pellet A after priming.



conserved or improved (D.W. Still, data not shown). For example, three of four lots of broccoli (*Brassica oleracea* L. var. *italica* Plenck) seeds tested had very similar hydrotime parameter values at both 20 and 25°C (Table 1). The fourth lot, however, had divergent values and slightly lower final germination percentages (Table 1). This lot performed poorly at higher temperature (Table 1) and in the field when planted under stressful conditions (data not shown). The reduced vigor of seed lot 4 was attributed to a non-optimized phytosanitary seed treatment. Aberrant or uncharacteristic performance in a hydrotime test

Table 1. Hydrotime analysis can be used to evaluate the physiological status of a seed lot. In this example with broccoli, lots 1, 2, and 3 have similar hydrotime parameters and final germination percentages. The physiological status (i.e., hydrotime parameters) of seed lot 4, however, is distinctly different, and when planted under stressful conditions, this lot exhibited poor stand establishment and a relatively high frequency of abnormal seedlings.

	20°C				25°C				30°C
Seed lot No.	θ_H MPa-h	$\psi_b(50)$ MPa	σ_{ψ_b} MPa	Final %	θ_H MPa-h	$\psi_b(50)$ MPa	σ_{ψ_b} MPa	Final %	Final %
1	11	−0.68	0.21	99	9	−0.65	0.16	100	100
2	11	−0.66	0.23	99	8	−0.61	0.15	99	99
3	11	−0.65	0.16	100	9	−0.65	0.17	100	99
4	25	−0.87	0.20	95	20	−0.90	0.24	95	82

can be an indicator of potential problems for seed lots planted under stressful conditions. Similarly, hydrotime analysis has identified lettuce seed lots of comparatively lower hydrotime vigor that have performed poorly when planted in the field. In these cases, conclusions based on hydrotime data were corroborated with suitability tests routinely performed by seed enhancement companies. Although more subtle vigor differences are only detectable by developing an extensive database and making relative comparisons, significant problems are far more obvious. In general, any seed lot in which the germination response predicted from the hydrotime analysis model does not closely match the observed values should be treated with caution. Our experience with lettuce indicates these seed lots do not respond well to pelleting and priming treatments and also have poor stand establishment under environmentally stressful conditions.

A practical limitation to the application of hydrotime analysis to seed testing is the need for repeated observations to obtain a germination time course. If image analysis systems can be used for automated scoring of germination, this limitation would be largely overcome. However, there are also ways to minimize the data required or to convert the test into an end-point threshold type of assay. For example, once familiarity has been gained through experience with a particular species or group of cultivars, germination time courses can be predicted for lots considered to be of acceptable quality. Table 2 shows the predicted times to specific germination fractions or percentages for control and primed lettuce seeds at three water potentials. Based upon these data, observation times can be selected that would be expected to give germination percentages in the 16 to 84% range that is most informative for hydrotime analysis. Seed lots that have exceeded expected percentages by that predicted time would be considered to be of high quality, while those whose germination was less than predicted at a specific time and ψ combination would be suspect and might warrant a retest for more complete data or additional vigor testing.

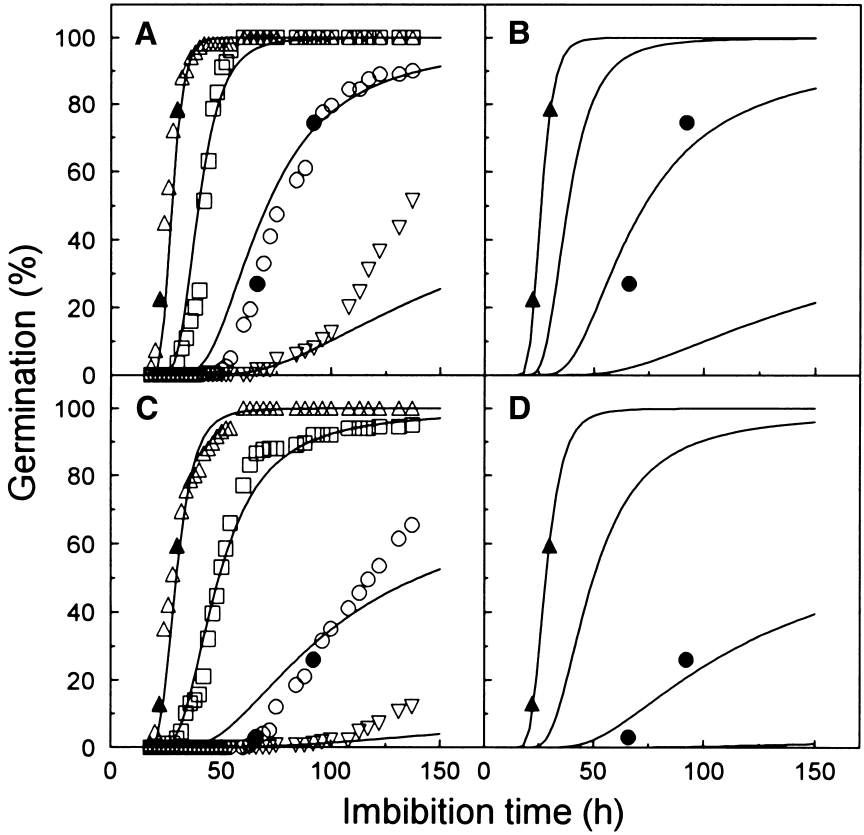
The type of information in Table 2 can also be used to develop the minimum data set required to fit the hydrotime model. Complete germination time courses are shown for two lettuce seed cultivars in Fig. 6 (Panels A, C). However, the minimum number of observations required to fit the regression model is two points at each of two ψ levels. When two observation times selected at only 0 and –0.4 MPa were used to fit the model (closed markers in

Table 2. Through experience with a particular species, a standard time course can be developed to reduce the number of observations required to ascertain the hydrotime parameters of a seed lot. In this example, multiple replications were run on five lettuce cultivars to derive a common time course and estimate the times to specific germination percentages. For statistical purposes relating to probit analysis, observations for the hydrotime model are ideally made between 16 and 84% of the final germination percentage. To reduce the number of observations required, germination percentages can be determined from two time points within this percentage range and at two water potentials with minimal deviation from determinations derived from a complete hydrotime analysis. Average germination times are shown (± standard error of the mean) for each seed fraction of pelleted lettuce seed (iceberg type). The data are for primed and not primed seed (n = 5 cultivars for each treatment).

		Time to germination at each ψ		
Treatment	**Fraction**	**0 MPa**	**–0.25 MPa**	**–0.5 MPa**
	%	---------- (h) ----------		
Primed	10	12.6 (2.2)	20.1 (1.4)	31.8 (1.2)
	20	15.0 (2.0)	25.4 (1.5)	37.9 (1.8)
	30	16.6 (1.9)	29.2 (2.0)	42.5 (2.5)
	40	18.1 (1.8)	32.5 (2.5)	46.1 (3.0)
	50	19.4 (1.8)	35.5 (3.1)	49.7 (3.6)
	60	20.8 (1.8)	38.6 (3.6)	53.2 (4.3)
	70	22.2 (1.9)	41.8 (4.2)	57.0 (4.9)
	80	23.9 (1.9)	45.6 (4.9)	61.4 (5.7)
	90	26.2 (2.1)	51.0 (6.0)	67.5 (6.8)
Not primed	10	16.9 (1.9)	23.2 (2.0)	39.8 (4.1)
	20	19.3 (1.9)	28.9 (2.3)	48.2 (5.0)
	30	21.0 (1.9)	33.0 (2.7)	54.3 (5.6)
	40	22.5 (1.9)	36.5 (3.1)	59.4 (6.2)
	50	23.9 (2.0)	39.7 (3.5)	64.3 (6.8)
	60	25.3 (2.1)	43.0 (4.0)	69.1 (7.3)
	70	26.7 (2.1)	46.5 (4.5)	74.3 (7.9)
	80	28.4 (2.3)	50.6 (5.0)	80.4 (8.6)
	90	30.9 (2.5)	56.2 (5.8)	88.8 (9.6)

Panels A and C), the values of the parameters derived did not differ greatly from those resulting from using the complete data set (Figs. 6B, D). Thus, once relatively complete background information is available for a given seed type, and the overall application of the model has been confirmed, the data requirements can be reduced to the minimal set required for the specific testing application. In some cases, only an endpoint assay after specific times at a reduced ψ would be sufficient to screen whether lots might potentially be

FIGURE 6. To reduce the number of observations, time points can be selected based upon experience with a given species that will be informative for specific water potentials. Once comprehensive data sets are available, as in Panels A and C, they can serve as a guide to select specific observation time points that will be informative at each ψ. As examples of the minimum data required, only two observations at 0 and –0.4 MPa each from Panels A and C (filled markers) are plotted in Panels B and D. When only these data were used to fit the hydrotime model, the predicted curves (lines in B and D) were very similar to those resulting from using the complete data set (A, C). Thus, once experience has been gained with a given seed type, the data requirements for hydrotime analysis can be considerably reduced.



problematic. More complete data could then be collected for suspect lots to be able to diagnose the specific problem that might be present.

CONCLUSIONS

Hydrotime analysis can provide several indices of seed quality relating to stress tolerance, speed and uniformity of germination. Considerable experience with vegetable seed species, backed by parallel field performance tests, indicates that hydrotime analysis can be diagnostic of how seed lots will perform under stressful conditions, including both temperature and salt stress. Although relatively labor-intensive, with experience minimal data sets or quality thresholds can be utilized, greatly reducing the labor requirements to implement hydrotime analysis. The development of automated imaging for scoring germination could also facilitate the application of hydrotime analysis. Extensive experience with the hydrotime model indicates that it reveals seed characteristics that are closely associated with the underlying physiological processes, such as dormancy, light and temperature regulation of germination. Thus, application of the hydrotime model can also feed back into breeding and selection programs to identify lines having improved seed performance and stress tolerance.

ACKNOWLEDGMENTS

The authors wish to acknowledge the technical assistance of Maria Campos in collecting data presented in this paper.

REFERENCES

Alvarado, V., and Bradford, K.J. 2002. A hydrothermal time model explains the cardinal temperatures for seed germination. Plant Cell Env. 25:1061–1069.

Bradford, K.J. 1990. A water relations analysis of seed germination rates. Plant Physiology 94:840–849.

Bradford, K.J. 1995. Water relations in seed germination. Pages 351–396 *in* J. Kigel and G. Galili, eds. Seed Dev. Germ. New York, Marcel Dekker, Inc.

Bradford, K.J. 1997. The hydrotime concept in seed germination and dormancy. Pages 349–360 *in* R. H. Ellis, M. Black, A. J. Murdoch and T. D. Hong, eds. Basic and Applied Aspects of Seed Biology. Boston, Kluwer Academic Publishers.

Bradford, K.J. 2002. Applications of hydrothermal time to quantifying and modeling seed germination and dormancy. Weed Sci. 50:248–260.

Bradford, K.J., and O.A. Somasco. 1994. Water relations of lettuce seed thermoinhibition. I. Priming and endosperm effects on base water potential. Seed Sci. Research 4:1–10.

Dahal, P. and K.J. Bradford. 1990. Effects of priming and endosperm integrity on seed germination rates of tomato genotypes. II. Germination at reduced water potential. J. Exp. Botany 41:1441–1453.

Gummerson, R.J. 1986. The effect of constant temperatures and osmotic potential on the germination of sugar beet. J. Exp. Botany 37:729–741.

Meyer, S.E., S.B. Debaene-Gill, and P.S. Allen. 2000. Using hydrothermal time concepts to model seed germination response to temperature, dormancy loss, and priming effects in *Elymus elymoides*. Seed Sci. Research 10:213–223.

Michel, B.E. 1983. Evaluation of water potential of solutions of polyethylene glycol 8000 both in the absence and presence of other solutes. Plant Physiology 72:66–70.