



Importance of distribution function selection for hydrothermal time models of seed germination

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Summary

The germination of a population of seeds was modelled using the concept of hydrotime or hydrothermal time. Typically, a Normal distribution for base water potential ($\Psi_{b(g)}$) was used within these models to relate variation in $\Psi_{b(g)}$ to the variation in time to germination of a given fraction of seeds. We sought to examine empirically the validity of this assumption, to compare the fit of alternative distributions and make recommendations for improved germination modelling procedures. Eight statistical distributions (Gumbel, Weibull, Normal, Log-Normal, Logistic, Loglogistic, Inverse Normal and Gamma) were fitted to data for four weed species *Hordeum spontaneum*, *Phalaris minor*, *Heliotropium europaeum* and *Raphanus raphanistrum*. Methods for incorporating each of these distributions into hydrotime are presented. For three species (*H. spontaneum*, *P. minor* and *H. europaeum*), the Normal distribution gave the worst fit (with AIC values: –124.2, –296.9 and

–264.5, respectively) and would lead to biased predictions, whereas the Loglogistic distribution consistently provided the best explanation of $\Psi_{b(g)}$ variation in these species (with AIC values: –188.6, –326.2 and –272.1 respectively). All distributions failed to provide an unbiased description of the observed distribution of $\Psi_{b(g)}$ in *R. raphanistrum*. The Normal distribution is not necessarily the best function for base water potential in hydrothermal models and, indeed, may give much more biased predictions than alternative functions. The ‘best’ distribution may vary with species. The distribution of $\Psi_{b(g)}$ within a seed sample should therefore be examined and an appropriate equation selected, before using a model to make predictions.

Keywords: base water potential, germination rate, hydrotime, Loglogistic, normal distribution, *Hordeum spontaneum*, *Phalaris minor*, *Heliotropium europaeum*, *Raphanus raphanistrum*.

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Introduction

Seed germination is a pivotal stage in the life cycle of plants (particularly annuals) that can ultimately affect plant fitness (Harper, 1977). Germination is an irreversible process; once it has started, the embryo will experience either growth or death. Therefore, the successful

recruitment of many plant species is highly dependent on the proper timing of germination (Fenner & Thompson, 2005). Determining what controls the phenology of germination in nature has been an ongoing research objective of germination biologists (Baskin & Baskin, 1998). For most seeds, the process of initiating growth of the embryo begins with imbibition (Bradford, 1990).

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Thus, the temporal pattern of seed germination is highly regulated by the soil water potential, Ψ , of the germination medium, as this determines the equilibrium water content of the seed (Daws *et al.*, 2008). In many studies, it has been found that germination rate, GR_g (the reciprocal of time to a given germination fraction, $1/t_g$), is linearly related to Ψ (Gummerson, 1986; Bradford, 1990; Dahal & Bradford, 1994). In a similar way to the use of thermal time (heat unit) models (Covell *et al.* 1986; Ellis *et al.*, 1986), 'hydrotime' has been developed to model the effect of reduced water potential on progress towards germination (Gummerson, 1986; Bradford, 1990; Welbaum & Bradford, 1991). Specifically,

$$GR_g = 1/t_g = (\psi - \psi_{b(g)})/\theta_H \quad (1)$$

which, after rearranging, gives

$$\theta_H = (\psi - \psi_{b(g)})t_g, \quad (2)$$

where $\psi_{b(g)}$ is the base (or threshold) water potential at or below which (i.e. more negative) the germination of a given seed fraction does not occur and θ_H is the hydrotime constant (with units MPa day). For hydrothermal time, time in the equation is replaced by thermal time. Implicit in this model is the assumption that θ_H is constant for all fractions of the seed population. Thus, a graph of GR against Ψ will show parallel lines for the different fractions, intercepting the Ψ axis at different ψ_b values. The variation in time to germination among seeds within a population is therefore assumed to be the result of variation in their $\psi_{b(g)}$, which has been assumed in most previous studies to follow a Normal distribution (Gummerson, 1986; Bradford, 1990) with a mean ($\psi_{b(50)}$) and standard deviation (σ_{ψ_b}). Thus,

$$\psi_{b(g)} = \psi_{b(50)} + \text{probit}(g)\sigma_{\psi_b} \quad (3)$$

From Eqn (2),

$$\psi_{b(g)} = \psi - \left(\frac{\theta_H}{t_g}\right), \quad (4)$$

which can be substituted into Eqn (3) and rearranged to give

$$\text{probit}(g) = \frac{\Psi - (\theta_H/t_g) - \Psi_{b(50)}}{\sigma_{\psi_b}} \quad (5)$$

Although it is empirical, rather than mechanistic, the above model (Eqn 5) provides parameters that are biologically meaningful. The intrinsic rate of germination, reaction to water stress and uniformity of germination correspond to θ_H , $\psi_{b(50)}$ and σ_{ψ_b} respectively (Bradford, 2002; Bradford & Still, 2004).

As this model has been applied under an increasing variety of contexts, examples have been reported

in which the fit to observations is less than acceptable (Finch-Savage *et al.*, 1998; Kebreab & Murdoch, 1999; Grundy *et al.*, 2000). This naturally leads us to re-examine the validity of the model assumptions. While almost all previous applications of hydrotime and hydrothermal time have assumed a Normal distribution for $\psi_{b(g)}$ (by using probit analysis; Bradford & Still, 2004; Daws *et al.*, 2008; Meyer & Allen, 2009), the assumption has not been tested critically (but see Watta *et al.*, 2010). The Normal distribution was selected for the original model on the basis of adequacy of fit to the data in the original studies and for its mathematical convenience, rather than for any theoretical reasons. The Normal distribution is symmetrical about the mean (so that mean = median = mode) and serves as a reasonable approximation to the central regions of frequency distributions. However, $\psi_{b(g)}$ is in the tail of the distribution (it is defined explicitly as an extreme value, below or at which no germination occurs) and may thus be expected to follow (right) skewed frequency patterns (Brown & Mayer, 1988; Watta *et al.*, 2010).

Recently, Watta *et al.* (2010) reported that using the Weibull distribution in their hydrothermal model, more accurate and unbiased estimates of $\psi_{b(g)}$ and germination dynamics were obtained than when using the Normal distribution. The move from hydrotime to hydrothermal time brings additional assumptions. In its most common form, the hydrothermal model assumes that $\psi_{b(g)}$ and base temperature, T_b (the temperature at and below which germination is prevented), are constant (i.e. $\psi_{b(g)}$ is independent of temperature and T_b is independent of water potential) and the relationship between temperature and GR_g is linear. Even in the suboptimal range of temperature for germination, there are several reports indicating an interaction between $\psi_{b(g)}$ and T_b (Covell *et al.*, 1986; Dumur *et al.*, 1990; Kebreab & Murdoch, 1999; Bradford, 2002; Timmermans *et al.*, 2007) and non-linearity in GR_g responses to temperature (Ellis *et al.*, 1986; Marshall & Squire, 1996; Timmermans *et al.*, 2007). Thus, one can argue that it is better to determine the distribution of $\psi_{b(g)}$ using just a hydrotime model first (avoiding the noise due to the additional hydrothermal assumptions) and then insert this distribution of $\psi_{b(g)}$ into the hydrothermal time model.

In this article, we describe the results of a systematic evaluation of the ability of eight different distributions to describe variation in $\psi_{b(g)}$. These were selected on the basis of previous use in germination studies, theoretical justification and shape. We used data for four species that we have found not to fit the conventional hydrothermal time model.

Materials and methods

Distributions

In addition to the Normal distribution for describing variation in $\Psi_{b(g)}$, we selected seven other distributions for comparison (see Appendix S1 for more details on these distributions).

Gumbel distribution

The inverse cumulative distribution (percentage point function, ppf) to predict $\Psi_{b(g)}$ and the cumulative distribution function (cdf) to predict germination percentage (g) for the Gumbel distribution can be formulated into a hydrotime model as below:

$$\Psi_{b(g)} = \mu - \sigma \cdot \left[\ln \left(\ln \left(\frac{1}{g} \right) \right) \right] \quad (6)$$

$$g = \exp \left[- \exp \left(- \left(\frac{(\Psi - (\theta_H/t_g) - \mu)}{\sigma} \right) \right) \right] \quad (7)$$

where μ and σ are location and scale parameters respectively. If $\Psi_{b(g)}$ follows a Gumbel distribution, then at $\Psi_{b(g)} = \mu$, the fraction of germinated seeds is ≈ 0.366 (in a Normal distribution, it is equal to the median, that is, 0.5).

Weibull distribution

The Weibull distribution can be incorporated in a hydrotime model as:

$$\Psi_{b(g)} = \mu + \sigma \cdot [-\ln(1 - g)]^{\frac{1}{\lambda}} \quad (8)$$

$$g = 1 - \left[\exp \left(- \left(\frac{(\Psi - (\theta_H/t_g) - \mu)}{\sigma} \right)^{\lambda} \right) \right] \quad (9)$$

where λ is the shape parameter that determines the skewness and kurtosis of the distribution. Regardless of the shape value, if $\Psi_{b(g)} - \mu = \sigma$, then the proportion of germinated seeds is ≈ 0.632 . The probability density for $\Psi_{b(g)} < \mu$ is zero and then follows a Weibull distribution with the origin at μ , that is, $\Psi_{b(0)}$.

Logistic distribution

The applicability of the distribution in hydrotime model was evaluated as:

$$\Psi_{b(g)} = \mu + \sigma \cdot \ln \left(\frac{g}{1 - g} \right) \quad (10)$$

$$g = \frac{1}{1 + \exp \left(- \left(\frac{(\Psi - (\theta_H/t_g) - \mu)}{\sigma} \right) \right)} \quad (11)$$

Log-normal distribution

To be applicable in hydrotime analysis, a three-parametric Log-Normal distribution with a threshold parameter, δ , below which the frequency of $\Psi_{b(g)}$ is zero (i.e. $\Psi_{b(0)}$), should be used:

$$\psi_{b(g)} = \delta + \exp(\sigma + \lambda \cdot \text{probit}(g)) \quad (12)$$

$$g = \Phi \left(\frac{(\ln(\Psi - (\theta_H/t_g) - \delta) - \sigma)}{\lambda} \right), \quad (13)$$

where Φ is the Laplace integral or the standard Normal cdf.

Loglogistic distribution

For this distribution, the hydrotime model becomes:

$$\Psi_{b(g)} = \mu + \sigma \cdot \left(\frac{g}{1 - g} \right)^{\frac{1}{\lambda}} \quad (14)$$

$$g = \frac{1}{1 + \left(\frac{(\Psi - (\theta_H/t_g) - \mu)}{\sigma} \right)^{-\lambda}} \quad (15)$$

Inverse normal distribution

The percentage point function for the Inverse Normal distribution does not exist in a simple, closed form. It is computed by numerically inverting the Inverse Normal cumulative distribution function using a bisection method. However, the hydrotime-based cdf can be expressed as follows:

$$g = \Phi \left(\sqrt{\frac{\lambda}{(\Psi - (\theta_H/t_g) - \delta)}} \right) \times \left(\frac{(\Psi - (\theta_H/t_g) - \delta - \sigma)}{\sigma} \right) + \exp \left(\frac{2\lambda}{\sigma} \right) \times \Phi \left(- \sqrt{\frac{\lambda}{(\Psi - (\theta_H/t_g) - \delta)}} \right) \times \left(\frac{(\Psi - (\theta_H/t_g) - \delta - \sigma)}{\sigma} \right) \quad (16)$$

Gamma distribution

The cdf and ppf of a gamma variable cannot be expressed in closed forms and are calculated numerically:

$$g = \frac{1}{\Gamma(\lambda)} \int_0^z (z)^{\lambda-1} \exp(-z) dz, \quad (17)$$

where

$$Z = \frac{(\Psi - (\theta_H/t_g) - \delta)}{\sigma}$$

and Γ is the *gamma function*, defined as

$$\Gamma(\lambda) = \int_0^{\infty} \chi^{\lambda-1} \exp(-\chi) d\chi \quad (18)$$

Seed sources

In this article, we analyse data for four species with contrasting ecologies, providing a strong test of the various model structures. The data were collected by three different researchers acting independently and thus methodology differed slightly between species. Seeds (caryopses) of *Hordeum spontaneum* (C. Koch) Thell. (wild barley) and *Phalaris minor* L. (little canary grass) were collected at maturity from infested wheat fields in June 2009 at Karaj (35°31'N, 50°11'E) and Varamin (35°11'N, 50°20'E), Iran respectively. Seeds were removed from the panicles, cleaned and then kept at room temperature (23–26°C) for about 2 months. *Heliotropium europaeum* L. (Common heliotrope) seeds were harvested at Normanville (35°49'S, 143°43'E), Australia, in April 2001 and were placed into dry storage at 5°C until the time of the experiment (85 days). Mature *Raphanus raphanistrum* L. (wild radish) fruits were collected from Yuna (28°31'S, 115°03'E), Australia, in 1998 and stored at room temperature until March 1999. Seeds were then extracted by hand from their fruit segments.

Experimental designs

For *P. minor*, four replicates of 25 seeds were spread evenly within 9-cm-diameter Petri dishes, each containing a Whatman No 1 filter paper. Each filter paper was moistened with 6 mL of polyethylene glycol (PEG, molecular weight 6000) solutions equivalent to water potentials of –0.2, –0.4, –0.6, –0.8, –1 MPa or distilled water (0 MPa). For *H. spontaneum*, four replicates of 15 seeds were placed in each Petri dish with 7 mL of PEG solutions of 0, –0.3, –0.6, –0.9, –1.2 and –1.5 MPa. The small number of seeds in each dish was necessary to prevent osmotic potential changing significantly as the seeds and their surrounding caryopses imbibed rapidly. The PEG solutions were prepared according to the Michel and Kaufmann (1973) equation for a given temperature. All dishes were covered by plastic film to reduce evaporation. Although the range of temperatures included as treatments in this experiment varied from 4 to 30°C, we will only deal here with constant temperatures of 16°C (photoperiod 12 h), as we are addressing the hydrotime aspect of seed germination. These were approximately the optimal temperatures for the germination of the species (data not shown). Germinated seeds (radicle protrusion of >3 mm) were counted daily for 20 days. The experiment for the two species was repeated once, and data from the two experiments

were pooled to double the number of observations for a robust statistical analysis (there was no interaction between experiment run and treatments).

For *H. europaeum*, five replicates of 100 seeds were placed in 10-cm-diameter Petri dishes containing two discs of Whatman No. 1 filter paper. Five millilitres of a given solution was added to each replicate, and the dishes were sealed with Parafilm. Solutions of PEG (molecular weight 8000) were made according to the equation from Michel (1983), to give osmotic potentials of 0, –0.1, –0.2, –0.3, –0.4 and –0.5 MPa at temperatures (ranging from 20 to 40°C with a 14-h photoperiod setting). However, we used data from 35°C condition, the optimum temperature. Germination (radicle visible) was recorded daily for 21 days.

For *R. raphanistrum*, three replicates of 10-cm-diameter Petri dishes contained 50 seeds on two Whatman 1001 filter papers. Twelve millilitres of PEG (8000) concentrations was added to establish osmotic potentials of –0.1, –0.2, –0.4 or –0.8 MPa or distilled water (0 MPa). Each osmotic potential was put into three incubators set at diurnal temperature fluctuations of 0–10, 5–15 and 15–25°C (night per day, 14-/10-h cycle). As the highest germination percentage and rate were observed at 5–15°C (data not shown), data from this treatment are analysed in this article. Germination (radicle protrusion of >3 mm) counts were made daily for the first 10 days, then every 2–3 days for 31 days and then at 46, 52 and 57 days.

In all experiments, where required, the osmotic solution was replaced with fresh solution over the course of experiment to counter any increase in PEG concentration as a result of imbibition or evaporation. In addition, all data were adjusted for the number of viable seeds remaining ungerminated at the end; that is, seed germination percentages were calculated based on the number of viable seeds at the conclusion of experiment. Seeds were considered viable if they contained a fresh, white endosperm after being cut open (Baskin & Baskin, 1998).

Statistical analysis

All distributions, having been formulated into the hydrotime model, were fitted to data using the PROC NLMIXED procedure of SAS, with the default optimisation technique of dual quasi-Newton algorithm (SAS, 2009). This procedure implements maximum likelihood (ML) to estimate the model parameters and is preferred to the least squares method (PROC NLIN, for example, Watta *et al.* (2010)). The use of the unweighted least squares method for fitting models to germination data has been rejected by O'Neill *et al.* (2004), because it assumes that the variance of each

point on a curve is constant across treatments (time or osmotic potentials), and every point is independent of its predecessors; both assumptions are invalid. In addition, using the PROC NLMIXED with its ML method, more appropriate criteria than r^2 can be obtained, such as the Akaike information criterion (AIC) or the Bayesian information criterion (BIC; Burnham & Anderson, 2002) for the purpose of model selection. Distribution properties (Table 1) were estimated using the Estimate statement, and their corresponding standard errors were approximated with the default delta method (Billingsley, 1986). More discussion about the curve fitting procedure is provided in Appendix S3 (SAS codes can be provided upon request).

In a hydrotime (or hydrothermal) model, there are two types of residual: (i) the residual calculated from the difference between virtual $\Psi_{b(g)}$ (Eqn 2) and predicted $\Psi_{b(g)}$ (e.g. Eqn 1), denoted as RW, and (ii) the residual estimated from the difference between the observed percentage germination and the germination predicted from the cdf of a particular distribution (e.g. Eqn 6), denoted as RG. Both types of residual were plotted against their respective fitted values to evaluate each distribution visually for any systematic bias.

Results

The parameter estimates for eight distributions are summarised in Tables 1–4 for the four species. For *H. spontaneum*, there were marked differences among distributions in terms of precision, as shown by very different AIC values (Table 1). The Loglogistic distribution (AIC = −188.6) gave the best fit to $\Psi_{b(g)}$ data, followed by the Log-Normal and Inverse Normal distributions. The Normal distribution provided the worst fit (AIC value −124.2). The fitted time courses of germination for the models with the highest (Loglogistic) and lowest (Normal) adequacy are shown in Fig. 1. As can be seen, the germination of *H. spontaneum* was overestimated by the Normal distribution for all water potentials higher than −0.9 MPa, and there was generally a poorer agreement between the model and observed values compared with the Loglogistic model. Not only were estimates of the mean (−1.35 MPa), median (−1.61 MPa) and mode (−1.75 MPa) of $\Psi_{b(g)}$ of the Loglogistic model unequal as a result of the asymmetric distribution, but they also differed from the Normal distribution (mean, −1.54 MPa). Overall, all three-parameter models fitted the distribution of $\Psi_{b(g)}$ better than those with two parameters. The threshold parameter, δ (i.e. $\Psi_{b(0)}$), was also fairly consistent for three-parameter models, c. −1.85 MPa. The estimates of the hydrotime constant (θ_H) of all

Table 1 Parameter estimates, distribution properties and measures of goodness of fit for eight statistical distributions used in hydrotime modelling of *Hordeum spontaneum* seed germination

Distribution	Hydrotime constant (θ_H)	Distribution parameters			Distribution properties				AIC
		Location (μ or δ)	Scale (σ)	Shape (λ)	Mean	Median	Mode	SD	
Normal	82.1240 (3.2468)*	−1.5413 (0.0250)	0.2716 (0.0295)	–	−1.5413 (0.0250)	−1.5413 (0.0250)	−1.5413 (0.0250)	0.2716 (0.0295)	−124.20
Gumbel	82.4621 (2.4657)	−1.6447 (0.0199)	0.2365 (0.0172)	–	−1.5082 (0.0205)	−1.6447 (0.0199)	−1.5580 (0.0197)	0.3033 (0.0220)	−150.90
Weibull	83.6895 (1.2540)	−1.8010 (0.0124)	0.2990 (0.0201)	0.7730 (0.0589)	−1.4534 (0.0188)	−1.6149 (0.0153)	−1.8010 (0.0124)	0.4547 (0.0477)	−180.00
Log-Normal	83.5457 (1.3058)	−1.8463 (0.0223)	−1.4461 (0.1199)	1.0763 (0.1042)	−1.4260 (0.0251)	−1.6108 (0.0147)	−1.7723 (0.0142)	0.6212 (0.1073)	−185.90
Logistic	82.0464 (3.0710)	−1.5431 (0.0239)	0.1644 (0.0170)	–	−1.5431 (0.0239)	−1.5431 (0.0239)	−1.5431 (0.0239)	0.2982 (0.0309)	−129.80
Loglogistic	83.3760 (1.3481)	−1.8530 (0.0260)	0.2470 (0.0317)	1.6383 (0.1746)	−1.3493 (0.0562)	−1.6060 (0.0146)	−1.7491 (0.0186)	†	−188.60
Inverse Normal	83.601 (1.2863)	−1.8645 (0.0225)	0.2940 (0.0738)	0.4233 (0.0208)	−1.4413 (0.02125)	−1.6133 (0.02379)	−1.7713 (0.01261)	0.5079 (0.0606)	−185.00
Gamma	83.7265 (1.2888)	−1.7985 (0.0122)	0.5169 (0.0749)	0.6503 (0.0899)	−1.4624 (0.0170)	–	−1.7985 (0.0122)	0.4168 (0.0344)	−177.50

*Values in the parentheses indicate standard error.

†SD could not be estimated for the Loglogistic model.

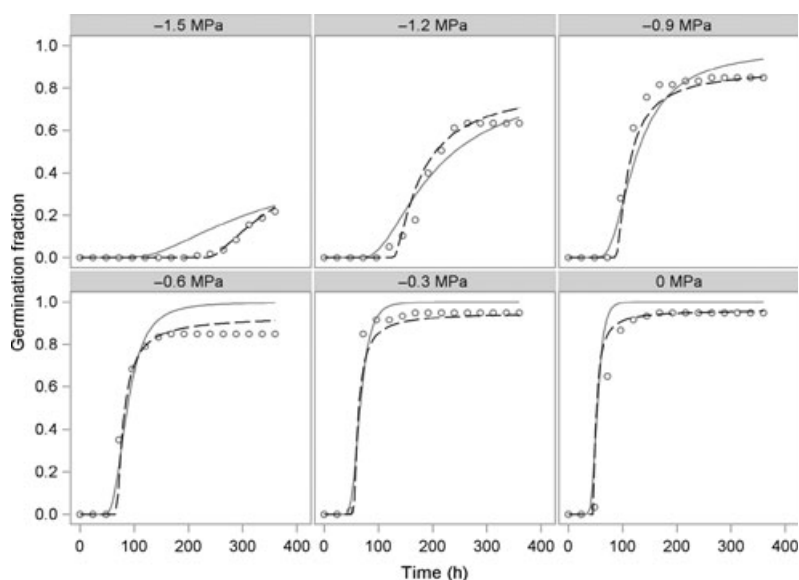


Fig. 1 Cumulative germination of *Hordeum spontaneum* seeds at various water potentials. Fitted lines are the hydrotime model based on the Normal (as originally formulated: solid line) and the Loglogistic (the model with the best fit: dashed line) distributions. Circles show the observed mean cumulative percentage germination. Parameters of each distribution are shown in Table 1.

distributions were similar (about 83 MPa h). Although the standard deviation (SD) was not estimable for the Loglogistic distribution in *H. spontaneum*, values obtained from the two next best distributions (Log-Normal and Inverse Normal) indicated that the spread of $\Psi_{b(g)}$ is about double that estimated by the Normal or other two-parameter distributions (Table 1). The accuracy of distributions was further evaluated by inspecting residual plots (Figs 2 and 3). The use of Normal, Logistic and Gumbel distributions led to highly biased predictions in terms of both germination (RG) and $\Psi_{b(g)}$ (RW) residuals. Such a distinct curvature in residuals was not observed with any of three-parameter distributions. The Loglogistic distribution showed the least bias based on RG (Fig. 2), but the Inverse Normal and Log-Normal were more accurate in terms of RW (Fig. 3).

For *P. minor*, the Loglogistic distribution again resulted in the best fit, with the smallest AIC (−326.2; Table 2). The Normal distribution gave the poorest fit (AIC = −296.9), and the other symmetrical distribution (Logistic) was only slightly better (AIC = −299.4). The Inverse Normal and Log-Normal distributions were again the next most precise distributions (Table 2). The normal distribution marginally overestimated the germination extent at the higher water potentials (less negative) and was also unsatisfactory in predicting the initiation of germination (lag phase), while the Loglogistic model had a better match with the real data in this respect (Fig. 4). Inspection of residuals also indicated that all asymmetrical models resulted in less biased predictions of germination and $\Psi_{b(g)}$ (Appendix S2, Fig. B1 and Fig. B2, respectively) compared with Normal and Logistic distributions (i.e. symmetrical models).

All three-parameter distributions shared a common threshold, δ (or location, μ), parameter of −2.61 MPa (Table 2). The mean $\Psi_{b(g)}$ estimated from the Loglogistic distribution (−1.02 MPa) was considerably different from that of the Normal distribution (−1.54 MPa). The Loglogistic distribution also predicted a median and a mode of −1.44 and −1.81 MPa, respectively, again in contrast to the Normal and Logistic distributions. All other three-parameter distributions also predicted larger (less negative) mean, median and θ_H , but smaller (more negative) mode compared with the symmetrical distributions.

For *H. europaeum*, all distributions provided good fit to germination data, with the Loglogistic distribution being again marginally the most suitable distribution (Table 3). In this species, however, the worst fit to $\Psi_{b(g)}$ data, was given by the Gumbel and Weibull distributions and not by the Normal (Table 3). As for the two previous species, the germination of *H. europaeum* was overpredicted by the Normal distribution at the highest water potentials (i.e. >−0.3). However, it performed equally to the Loglogistic model at lower osmotic potentials (Fig. 5). The location parameter (μ or δ) ranged from −5.11 MPa for the Log-Normal distribution to −1.86 MPa for the Weibull distribution, with a location estimate of −4.25 MPa for the Loglogistic distribution. However, the distributions did not vary markedly in parameters related to the rapidity (i.e. θ_H) or evenness (i.e. SD) of germination, with average values of 115.9 MPa h and 0.42 MPa respectively. Despite the good fits to germination data (Fig. 5 and Appendix S2, Fig. B3), none of the distributions were able to predict $\Psi_{b(g)}$ accurately for values smaller (more negative) than about −1.25 MPa (Appendix S2, Fig. B4). They all continuously underpredicted $\Psi_{b(g)}$

Fig. 2 Scatter plots of fitted cumulative germination proportion against residuals (RG) for eight statistical distributions used in hydrotime modelling of *Hordeum spontaneum* seed germination. Dashed lines are a cubic polynomial fitted to the residuals for better visualisation of trends.

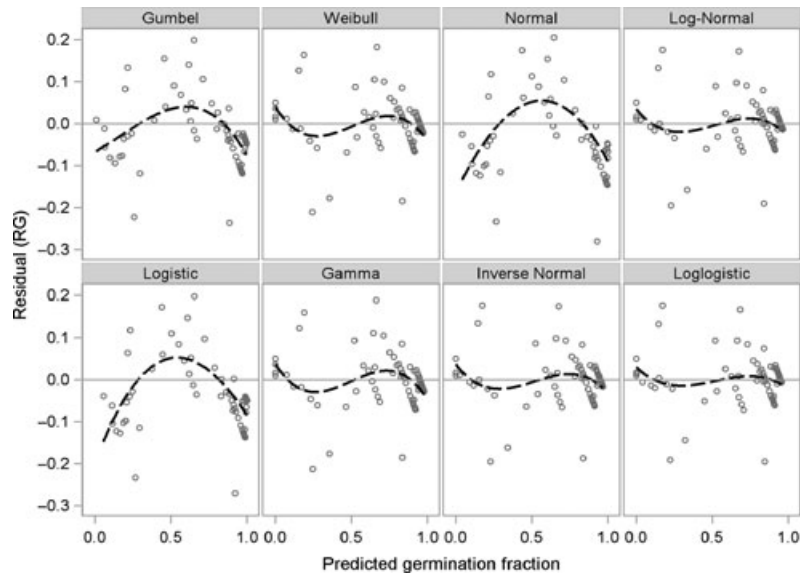
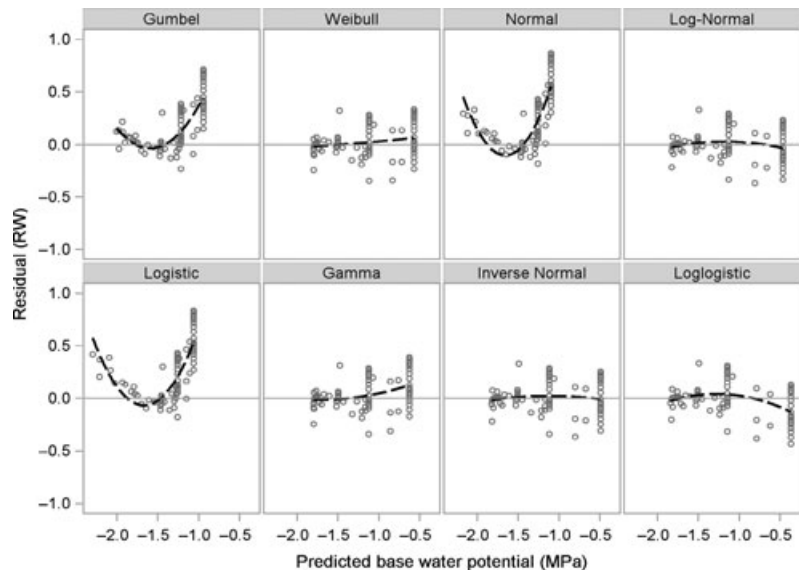


Fig. 3 Scatter plot of fitted base water potential against residuals (RW) for eight statistical distributions used in hydrotime modelling of *Hordeum spontaneum* seed germination. Dashed lines are a quadratic polynomial fitted to residuals for better visualisation of trends.



beyond that value, except for the Logistic (and to lesser extent for the Loglogistic), where the systematic departure of residuals from the zero reference line was less evident.

In *R. raphanistrum*, the fitted distributions appeared to be symmetrical, as the mean, median, mode and SD were reasonably consistent within a distribution (Table 4). The shape parameter (λ) in the Weibull distribution, however, was larger than 3.6 (and thus the mode, -0.38 MPa, was greater than the mean), suggesting a left-skewed distribution. The estimated means, medians, modes and θ_H were also similar among the different distributions. The results of the hydrotime model for *R. raphanistrum*, whatever the distribution, should be applied with caution as the residual plots indicated that the outputs of all models

were highly biased (Appendix S2, Fig. B5 and B6). The goodness of fits of the Normal and Weibull distributions were slightly better than, or similar to, the other distributions (Table 4). However, even for these two models, the fitted curves of cumulative germination were poor (Fig. 6). Figure 6 shows clearly that germination probability for all seeds subjected to an osmotic potential of 0, -0.1 and -0.4 MPa has been underestimated, while at -0.2 MPa, it has been overestimated. This systematic bias was also seen in their corresponding residual plots (RG; Appendix S2, Fig. B5). Similarly, for RW, there was an apparent relationship between estimated $\Psi_{b(g)}$ and RW (Appendix S2, Fig. B6). The Weibull distribution gave the best estimates of $\Psi_{b(g)}$, followed by the Normal distribution.

Table 2 Parameter estimates, distribution properties and measures of goodness of fit for eight statistical distributions used in hydrotime modelling of *Phalaris minor* seed germination

Distribution	Hydrotime constant (θ_H)	Distribution parameters			Distribution properties			
		Location (μ or δ)	Scale (σ)	Shape (λ)	Mean	Median	Mode	AIC
Normal	218.3000 (12.0046)*	-1.5407 (0.0549)	0.9561 (0.0477)	–	-1.5407 (0.0549)	-1.5407 (0.0549)	-1.5407 (0.0549)	-296.90
Gumbel	196.6500 (9.1507)	-1.7560 (0.0518)	0.7553 (0.0333)	–	-1.3200 (0.0385)	-1.7560 (0.0518)	-1.4792 (0.0428)	-319.50
Weibull	181.0500 (9.5057)	-2.3976 (0.1443)	1.2841 (0.1204)	1.2916 (0.1327)	-1.2101 (0.0604)	-1.4307 (0.0405)	-1.9919 (0.0865)	-318.30
Log-Normal	180.0300 (10.0843)	-2.7163 (0.2937)	0.2498 (0.2092)	0.6441 (0.1194)	-1.1367 (0.0854)	-1.4326 (0.0411)	-1.8685 (0.0547)	-323.40
Logistic	218.9700 (12.0767)	-1.5442 (0.0547)	0.5818 (0.0295)	–	-1.5442 (0.0547)	-1.5442 (0.0547)	-1.5442 (0.0547)	-299.40
Loglogistic	181.3700 (9.5603)	-2.6775 (0.2548)	1.2388 (0.2318)	2.4508 (0.4099)	-1.0208 (0.1152)	-1.4387 (0.0397)	-1.8076 (0.0543)	-326.20
Inverse Normal	180.1100 (9.3341)	-2.8030 (0.2580)	1.6434 (0.1960)	4.0288 (1.8172)	-1.1595 (0.0674)	-1.1990 (0.0697)	-1.8819 (0.0548)	-322.70
Gamma	182.4900 (9.6831)	-2.4958 (0.1779)	0.7141 (0.0944)	1.8036 (0.3885)	-1.2078 (0.0550)	–	-1.9219 (0.0701)	-320.10

*Values in the parentheses indicate standard error.

Table 3 Parameter estimates, distribution properties and measures of goodness of fit for eight statistical distributions used in hydrotime modelling of *Heliotropium europaeum* seed germination

Distribution	Hydrotime constant (θ_H)	Distribution parameters			Distribution properties			
		Location (μ or δ)	Scale (σ)	Shape (λ)	Mean	Median	Mode	AIC
Normal	117.8300 (4.6649)*	-0.8244 (0.0229)	0.4142 (0.0162)	–	-0.8244 (0.0229)	-0.8244 (0.0229)	-0.8244 (0.0229)	-284.50
Gumbel	111.7700 (4.4371)	-0.9417 (0.0259)	0.3450 (0.0140)	–	-0.7425 (0.0205)	-0.8152 (0.0259)	-0.9417 (0.0223)	-258.80
Weibull	116.4800 (4.9157)	-1.8652 (0.2173)	1.1854 (0.2111)	2.8792 (0.5503)	-0.8085 (0.0286)	-0.8215 (0.0233)	-0.8430 (0.0412)	-259.50
Log-Normal	115.9800 (4.4821)	-5.1185 (1.7701)	1.4580 (0.4117)	0.0952 (0.0389)	-0.8017 (0.0228)	-0.8212 (0.0220)	-0.8600 (0.0282)	-264.60
Logistic	118.2700 (4.4170)	-0.8261 (0.0215)	0.2498 (0.0095)	–	-0.8261 (0.0215)	-0.8261 (0.0215)	-0.8261 (0.0215)	-271.20
Loglogistic	115.9600 (4.3990)	-4.2535 (0.2038)	3.4317 (0.2035)	13.9203 (1.8144)	-0.7925 (0.0279)	-0.8218 (0.0208)	-0.8571 (0.0281)	-272.10
Inverse Normal	115.1100 (4.4900)	-3.7540 (1.0535)	2.9344 (1.0561)	152.5600 (16.2140)	-0.8196 (0.0219)	-0.8475 (0.0227)	-0.9030 (0.0379)	-264.20
Gamma	115.9500 (7.2993)	-3.7294 (0.1236)	0.0577 (0.0056)	50.7599 (4.7743)	-0.8018 (0.0339)	–	-0.8595 (0.0389)	-264.40

*Values in the parentheses indicate standard error.

Fig. 4 Cumulative germination of *Phalaris minor* seeds at various water potentials and the comparison of germination time courses predicted by the hydrotime model based on the Normal (as originally formulated: solid line) or the Loglogistic (the best fit distribution: dashed line) distributions. Parameters of each distribution are shown in Table 2.

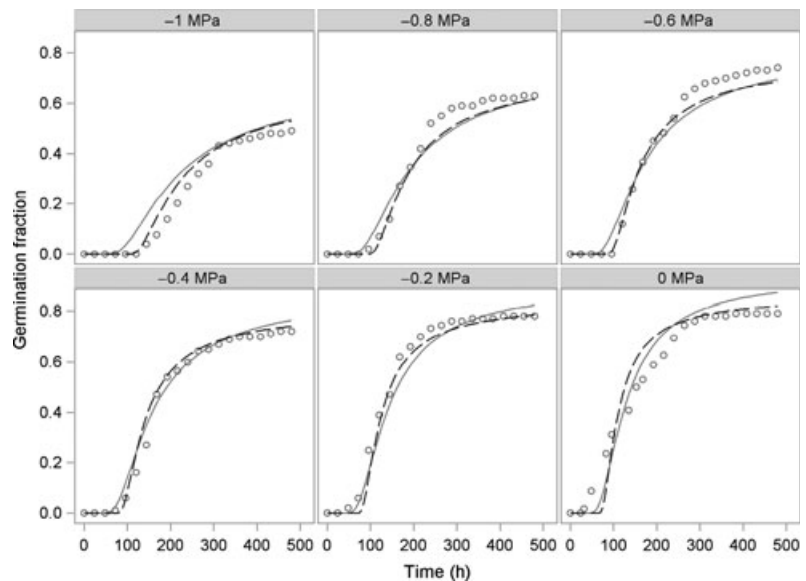
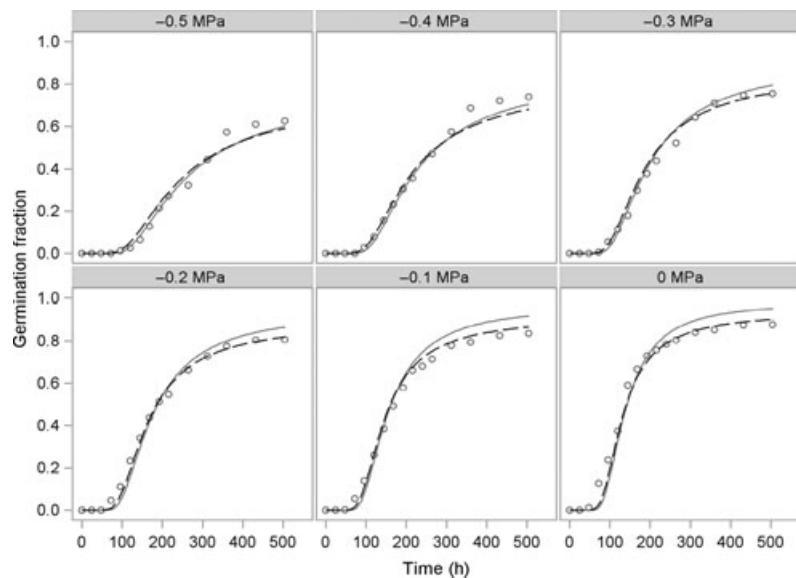


Fig. 5 Cumulative germination of *Heliotropium europaeum* seeds at various water potentials and the comparison of germination time courses predicted by the hydrotime model based on the Normal (solid line) or the Loglogistic (the best fit distribution: dashed line) distributions. Parameters of each distribution are shown in Table 3.



Discussion

Hydrotime analysis, although an empirical method, is considered by many researchers to have physiologically and ecologically relevant parameters (Allen *et al.*, 2000; Alvarado & Bradford, 2002; Allen, 2003) and, in its standard form, provides several useful indices of seed quality relating to stress tolerance ($\Psi_{b(50)}$), speed (θ_H) and uniformity (σ_{Ψ_b}) of germination (Bradford, 2002; Bradford & Still, 2004). Despite its popularity, the generality of its assumptions has not been examined systematically. If these assumptions do not hold, at least approximately, in a particular situation, misleading interpretations can easily arise. A central assumption is the Normal distribution of $\Psi_{b(g)}$ within the seed sample, which in turn determines the esti-

imated variation in time to germination. Almost exclusively, the cumulative Normal distribution (probit transformation) has been taken for granted in hydrotime and hydrothermal time models (Alvarado & Bradford, 2002; Batlla & Benech-Arnold, 2004; Kochy & Tielborger, 2007). We found that in comparison with seven other distributions, it provided the least precise and least accurate fit to data sets of three of four species. Our results support a conclusion that $\Psi_{b(g)}$ may often be right-skewed, and a more appropriate distribution is needed for prediction of germination.

The Loglogistic distribution was consistently the most flexible distribution in describing the observed $\Psi_{b(g)}$ and the time to germination of *H. spontaneum*, *P. minor* and *H. europaeum*. But, just as with the Normal, this does not necessarily mean that the

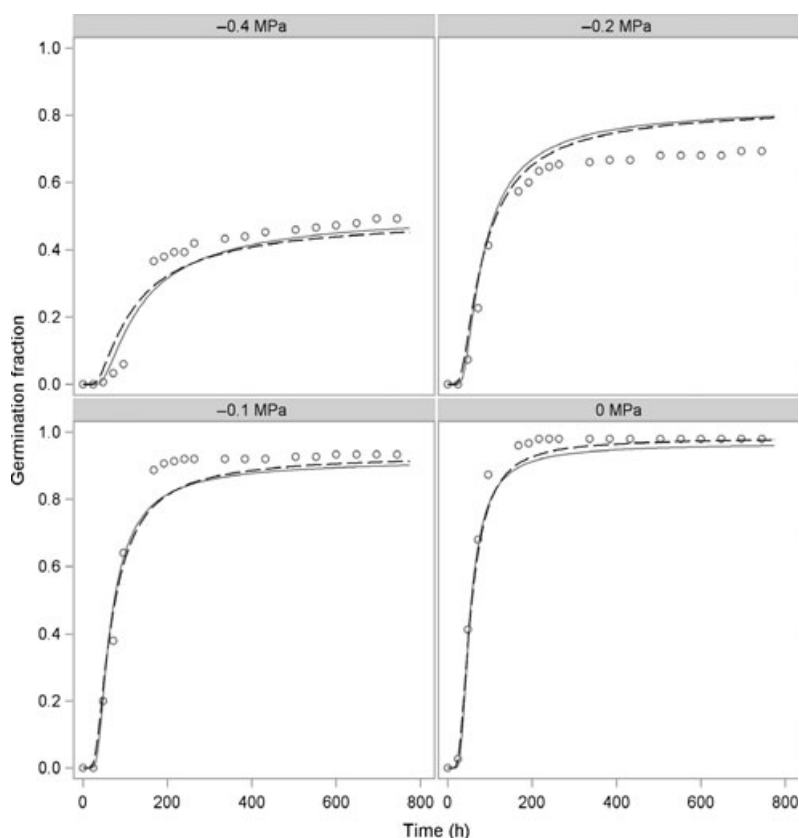


Fig. 6 Cumulative germination of *Raphanus raphanistrum* seeds at various water potentials and the comparison of germination time courses predicted by the hydrotime model based on the Normal (as originally formulated: solid line) or the Weibull (dashed line) distributions. Parameters of each distribution are shown in Table 4.

distribution has universal applicability. The Inverse Normal and Log-Normal distributions were also found to give reasonable fits to data from *H. spontaneum* and *P. minor*, while in the case of *H. europaeum*, the Logistic distribution was superior. The predictions of the Loglogistic distribution were less prone to systematic errors compared with Normal distribution. Contrary to our preliminary expectations, based on the interpretation of $\Psi_{b(g)}$ as an extreme value, both generalised extreme value distributions (i.e. the Gumbel and the Weibull, see Appendix S1) tested in this article were inferior in describing the pattern of $\Psi_{b(g)}$ variation. The only exception was for *R. raphanistrum*, the least skewed data set, where the Weibull was as efficient as the Normal distribution. Watta *et al.* (2010), in comparing just two distributions, also found that the Weibull was more precise and accurate than the Normal in hydrothermal time modelling of *Buddleja davidii* and *Pinus radiata* germination. However, the hydrotime model for *R. raphanistrum* should be applied with caution, as the fits of all models were highly biased.

Our data for *R. raphanistrum* showed a symmetrical, or even a left-skewed, distribution for $\Psi_{b(g)}$. Thus, most right-skewed distributions (especially the Loglogistic) provided poorer fits to this data set. However, residual plots (Appendix S2, Fig. B5 and B6) indicated that the hydrotime model in general fails to give an unbiased fit to the data. As no distribution had any

profound advantage over others, the lack of fit in this species may be due to the inadequacy of the hydrotime method to describe germination response of *R. raphanistrum* to water potentials. The hydrotime model assumes that the slope of GR_g vs. osmotic potential is constant for all percentiles, but that was not the case for *R. raphanistrum* (data not shown). In addition, Bloomberg *et al.* (2009) hypothesised that $\Psi_{b(g)}$ may vary as a function of germination time course, most specifically at low temperatures. In their study, seeds of *Pinus radiata* were incubated for 50 days at various constant temperatures and water potentials; it was found that there was an apparent change in $\Psi_{b(g)}$ for seeds taking more than 25 days to germinate. In our study, the germination test was conducted for 57 days, so it might be plausible that the $\Psi_{b(g)}$ of *R. raphanistrum* seeds changed over time. However, it may reflect either a real process that needs inclusion in our models or it may be an artefact of conducting germination tests under artificial conditions. For this species, we also used fluctuating temperatures, which has been found to reduce the $\Psi_{b(g)}$ of several species (Huarte & Benech-Arnold, 2005). The same increased tolerance to water stress might have happened in *R. raphanistrum* seeds leading to poor fits of the hydrotime model.

A further important feature in population germination studies is the possibility of distinct subpopulations (e.g. flushes of late germination resulting from loss of

Table 4 Parameter estimates, distribution properties and measures of goodness of fit for eight statistical distributions used in hydrotime modelling of *Raphanus raphanistrum* seed germination

Distribution	Hydrotime		Distribution parameters				Distribution properties			
	constant	(θ_H)	Location (μ or δ)	Scale (σ)	Shape (λ)	Mean	Median	Mode	SD	AIC
Normal	23.1497	(1.3864)*	-0.4081 (0.0111)	0.2326 (0.0126)	–	-0.4081 (0.0111)	-0.4081 (0.0111)	-0.4081 (0.0111)	0.2326 (0.0126)	-130.30
Gumbel	23.1561	(1.3321)	-0.4908 (0.0129)	0.1923 (0.0112)	–	-0.3799 (0.0103)	-0.4908 (0.0129)	-0.4204 (0.0109)	0.2466 (0.0144)	-125.30
Weibull	23.0556	(1.4039)	-1.2787 (0.7724)	0.9527 (0.7804)	4.2590 (3.6798)	-0.4121 (0.0221)	-0.4046 (0.0143)	-0.3840 (0.0659)	0.2297 (0.0228)	-129.40
Log-Normal	23.1629	(1.3888)	-8.2783 (3.5028)	2.0629 (0.4451)	0.0294 (0.0131)	-0.4058 (0.0111)	-0.4092 (0.0111)	-0.4160 (0.0117)	0.2317 (0.0125)	-128.10
Logistic	23.3289	(1.4380)	-0.4098 (0.0115)	0.1426 (0.0082)	–	-0.4098 (0.0115)	-0.4098 (0.0115)	-0.4098 (0.0115)	0.2587 (0.0148)	-127.20
Loglogistic	23.3297	(1.4374)	-1.2439 (0.0543)	0.8250 (0.0494)	6.0000 (–)†	-0.3800 (0.0106)	-0.4190 (0.0112)	-0.4639 (0.0124)	0.2768 (0.0165)	-125.20
Inverse Normal	23.1881	(1.3603)	-1.0468 (0.0294)	0.6269 (0.0236)	5.0000 (–)†	-0.4198 (0.0108)	-0.4341 (0.0112)	-0.4626 (0.0119)	0.2220 (0.0125)	-127.40
Gamma	23.1778	(1.6760)	-2.7243 (0.1625)	0.0229 (0.0014)	101.0900 (7.1668)	-0.4029 (0.0119)	–	-0.4259 (0.0127)	0.2309 (0.0127)	-127.70

*Values in the parentheses indicate standard error.

†SE could not be estimated.

innate dormancy), which can result in a step form of germination progress curve (e.g. Yoshioka *et al.* 1998). In such cases, the common unimodal distributions will not be expected to give a reasonable fit; however, O'Neill *et al.* (2004) showed that the Inverse Normal distribution has the potential to account for this situation. A new class of inverse Gaussian type distribution was recently introduced by Sanhueza *et al.* (2008) that can also model bimodality, but this has not yet been incorporated into most statistical software packages. Nonetheless, we need to be cautious that some distributions are difficult to fit and parameter estimate algorithm may fail to converge. This failure is more likely to happen with complicated models like the Inverse Normal distribution, which we found very sensitive to the initial values used for parameter estimates. However, the use of starting values sufficiently close to solution estimates can usually overcome this problem.

In conclusion, it would appear that in some data sets at least, the assumption of a Normal distribution is clearly inappropriate and will lead to both poor description of data and poor predictions if used in models based on hydrotime or hydrothermal time. This is a considerable cause for concern, as many less statistically able researchers will not find it easy to formulate models themselves. Experience with ANOVA and other basic analyses (e.g. Cousens, 1988) is that many researchers apply existing methods 'off the shelf', without checking assumptions, and thus frequently make errors. There is no justification, at this stage, for the universal replacement of the Normal distribution with a single alternative. The assumptions of the model need to be tested before being applied. However, the Loglogistic distribution is a more flexible and appropriate candidate than the Normal, its parameters remain physiologically informative, and it should be considered strongly when using a hydrotime or hydrothermal time approach for modelling of seed germination. For example, the threshold (δ) or location (μ) parameter in three-parameter models delineate the osmotic potential at which the probability of germination is zero (or in other words, the osmotic potential from which germination begins). This parameter provides a realistic estimation of the $\Psi_{b(0)}$, which is not inferable from a Normal distribution. A better appreciation of the lag phase is the pragmatic advantage of the inclusion of this parameter into hydrotime models of germination.

Further work is needed to both confirm our findings and to develop seed germination models capable of incorporating the various departures from model assumptions discussed above. The data sets in our study were used as illustrative examples, from contrasting species and under different laboratory conditions,

to test the specific question of whether the distribution assumption is important. It was not intended that this should be a definitive analysis of germination under all possible conditions for these species. For clarity of presentation to readers, we chose only to examine germination at near optimum temperatures, even though a range of temperatures were available. Having established the important principles and compared candidate alternative models, we are now in a position to conduct more comprehensive, broad-ranging analyses of these and other existing data sets. The so-called cure model (Onofri *et al.*, 2011), which is an extension of parametric survival analysis, seems to be a very promising candidate for modelling seed germination, as it accounts for both the biological interpretations and statistical interferences.

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Supporting information

Additional supporting information may be found in the online version of this article:

Appendix S1 A description of statistical distributions used for hydrotime analysis of seed germination.

Appendix S2 Scatter plots of fitted cumulative germination proportion against residuals and fitted base water potential against residuals for three plant species, *Phalaris minor*, *Heliotropium europaeum* and *Raphanus raphanistrum*.

Appendix S3 Statistical compromises when fitting equations to seed germination frequency data.