

A computational procedure for a hydrotime concept of seed germination

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SUMMARY

The paper presents a computational approach for modeling the germination process in relation to water potential according to Gummerson and Bradford's hydrotime concept. The procedure is based on a generalized linear model with a probit link function. In order to present the model process in a population, the data were simulated. Successive computational steps led to informative results and a graphical presentation.

Key words: seed germination, hydrotime concept, generalized linear model, cumulative normal distribution, link function, probit

1. Introduction

The germination of seeds is a complex physiological process differentiated among species and dependent on many environmental factors, like temperature, available water, light and nutrients. One of the factors of primary importance commonly used for the modeling of this process is water availability in the soil environment. This factor is usually expressed in terms of water potential as a result of differences in water potentials. The potential of pure water is 0, whereas the presence of solutes reduces the chemical potential of water, denoted by a negative value. In the soil, seeds imbibe the water up to the equilibrium with the water potential of the medium. The duration of the imbibition phase determines the timing of seed germination, and is mathematically described by the so-called hydrotime model.

The hydrotime model was initially suggested by Gummerson (1986) and then developed in many papers by Bradford and Bradford et al. (i.e. 1990, 1994, 1995, 1996, 2002). It is in essence a population model that relates water availability and germination timing. The hydrotime model describes the relation between the germination rate GR_g of a given percentage g and the magnitude of the difference between seed water potential ψ and a physiological base (threshold) water potential for radicle emergence ψ_b . Germination rate GR_g is the inverse of time to germination t_g for a given percentage g , $GR_g = 1/t_g$, and the threshold ψ_b is the lowest (most negative) water potential ψ at which a given seed can complete germination, or the highest (least negative) ψ that prevents germination. The values of germination rate GR_g as a function of ψ are linear and parallel, having a common slope and intercepting the X axis at different ψ_b values. Since the slopes are the same, the total hydrotime (MPa-hours or MPa-days) to radicle emergence ratio is the same for all the seeds in the population, but individual seeds vary in terms of the threshold $\psi_b(g)$ at which radicle emergence would be prevented.

The hydrotime model defined by Gummerson (1986) and Bradford (1990) is of the form:

$$GR_g = 1/t_g = (\psi - \psi_b(g))/\theta_H, \quad (1)$$

where GR_g is the germination rate of a given percentage g , t_g is the time to germination, ψ is the seed water potential, $\psi_b(g)$ is the base or threshold water potential for a specific germination fraction g , and θ_H is the hydrotime constant. It is worth pointing out that GR_g is related explicitly to germination rate, while Gummerson's model is an attempt to relate linearly the rate and water potential ψ as expressed in the second algebraic identity of eq. (1).

Using the parameters from the hydrotime model the germination time courses at different ψ can be normalized on a common time scale (h) to the time course that would occur in water for that seed population. Taking $t_g(0)$ as the time to germination in water and $t_g(\psi)$ as the time to germination at any other ψ , the relationship between $t_g(0)$ and $t_g(\psi)$ can be written in the form:

$$t_g(0) = [1 - \psi/\psi_b(g)] \cdot t_g(\psi). \quad (2)$$

In analysis of the experimental assumptions and statistical interpretation of the results, the following should be taken into account. Firstly, the term g refers to a specific fraction or percentage of a total seed population, so recalculating the fraction or percentage on the basis of only the number of germinated seeds under given conditions is inappropriate for this model. And secondly, the $\psi_b(g)$ values vary among seeds in the population in a normal distribution, with median $\psi_b(50)$ and variance $\sigma_{\psi_b}^2$.

In an interpretation of the model from the physiological point of view, it should be noted that the three parameters θ_H , $\psi_b(50)$, and σ_{ψ_b} enable the prediction of complete germination time courses at any ψ given the assumption of uniformity of other environmental factors, and the median $\psi_b(50)$ of $\psi_b(g)$ values reflects the average water stress tolerance of the population: the higher (less negative) the $\psi_b(50)$ value, the slower the germination rate of the population; and the higher the medium ψ values (less stress) at which germination is inhibited.

The objective of the study was to present a procedure for statistical analysis of data from a simulated seed germination experiment according to the hydrotime concept formulated by Gummerson (1986) and Bradford (1990).

2. Method of analysis

The initial data processing step was probit¹ analysis. Seed germination is described by a binary response model, where the response Y of an individual seed can take one of two possible outcomes, for convenience denoted 0 and 1. For example, $Y = 1$ when the seed germinates or 0 when the seed does not germinate. Suppose that \mathbf{x} is a vector of explanatory variables and $\Pr(Y = 1|\mathbf{x})$ is the response probability to be modeled, so the probit model takes the form:

$$\Pr(Y = 1|\mathbf{x}) = \Phi(\mathbf{x}^T \mathbf{b}),$$

¹ The term “probit” was invented by Chester Bliss (1934). It stands for probability unit.

where Φ is the standard cumulative normal probability distribution and \mathbf{xb} is the probit score. Thus the predicted values in a probit model are like Z-scores. For example, if a predicted score is 0 the chance of germination is 50%, and analogously if the predicted score is 1 the chance of germination is 84.1%, etc.

In the study a generalized linear model with a probit link function was used to calculate probit values, according to the equation:

$$Y' = \Phi^{-1}(p) = b_0 + \sum b_i X_i,$$

where Y' is the probit transformed value – a continuous response variable that reflects the scale of germination from 0 to 1; p is the proportion (p =germinated seeds/total number of seeds), inverse $\Phi(p)$ is the $g=100p\%$ quintile from the standard normal distribution, b_0 is a constant, the b_i 's are regression coefficients, and the X_i 's are predictor variables – water potentials ψ and germination rate (GR_g).

The above model, regardless of the regression coefficients or values X_i , always produces predicted values of Y' in the range of 0 to 1, i.e. if $Y' \geq 0$ then $Y = 1$ and if $Y' < 0$ then $Y = 0$.

The estimates of the model were used to calculate three parameters: θ_H , $\psi_b(50)$, and σ_{ψ_b} , to describe the germination process in a population. The whole computational procedure, including a numerical example, and a presentation of the final results appear below.

3. Numerical example

Theoretical behavior patterns of seeds in a population were presented using simulated data. It was assumed that the number of germinated seeds was determined at five water potentials: 0, -0.2, -0.4, -0.6, -0.8 MPa. For simplification and clear presentation of the procedure, data were simulated from 30 time intervals of 10 h (Table1). Statistical analyses were performed using the statistical package STATISTICA[®], and all complementary calculations were performed in an EXCEL[®] spreadsheet.

Table 1. Percentage of germinated seeds – simulated data.

Water potential (ψ)	Time (h)														
	10	20	30	40	50	60	70	80	90	100	110	120	130	140	150
0.0	1	49	76	86	91	93	94	95	96	96	96	97	97	97	97
-0.2	0	26	55	69	76	81	83	85	87	88	89	89	90	90	91
-0.4	0	11	32	46	55	61	65	68	70	72	73	74	75	76	76
-0.6	0	3	14	24	32	37	41	44	47	49	51	52	53	54	55
-0.8	0	1	5	10	14	18	21	23	25	27	28	29	30	31	32
	160	170	180	190	200	210	220	230	240	250	260	270	280	290	300
0.0	97	97	98	98	98	98	98	98	98	98	98	98	98	98	98
-0.2	91	91	91	92	92	92	92	92	92	92	93	93	93	93	93
-0.4	77	77	78	78	79	79	79	79	80	80	80	80	80	81	81
-0.6	56	56	57	57	58	58	59	59	59	60	60	60	60	61	61
-0.8	32	33	34	34	35	35	35	36	36	36	36	37	37	37	37

1. The general linear model:

$$E(Y) = p = b_0 + b_1\psi + b_2GR_g .$$

The link of function $b_0 + b_1\psi + b_2GR_g$ to p in a linear form is through the $\Phi^{-1}(p)$.

2. Data were generated by the statistical package based on germination percentage simulated data (Table 1). The simulated data were obtained from real data of an experiment with *Matricaria maritima* subsp. *inodora* to present model results of the analysis for population. The real data and resulting analyses according to the presented procedure may be found in the paper by Bochenek et al. (2007).

Y	X_0	ψ	GR_g
1	1	0	0.1000
...
1	1	0	0.0033
1	1	-0.2	0.1000
...
1	1	-0.2	0.0033
1	1	-0.4	0.1000
...

1	1	-0.4	0.0033
1	1	-0.6	0.1000
...
1	1	-0.6	0.0033
1	1	-0.8	0.1000
...
1	1	-0.8	0.0033
0	1	0	0.1000
...
0	1	0	0.0033
0	1	-0.2	0.1000
...
0	1	-0.2	0.0033
0	1	-0.4	0.1000
...
0	1	-0.4	0.0033
0	1	-0.6	0.1000
...
0	1	-0.6	0.0033
0	1	-0.8	0.1000
...
0	1	-0.8	0.0033

3. Estimation of parameters:

	b_0	b_1	b_2
Estimates	-2.2176	-2.9878	45.1053
Standard error	0.0330	0.0510	1.2407

4. Calculation of parameters of the distributions $GR_g(\psi)$ at different water potentials.

ψ (MPa)	0	-0.2	-0.4	-0.6	-0.8	Standard error
$GR_{50}(\psi)$	0.0492	0.0359	0.0227	0.0094	-0.0038	0.0222

5. Calculation of $GR_g(\psi)$ values at different probabilities from the normal distribution with $GR_{50}(\psi)$ and the common standard error for the following water potentials. For example at the water potential of 0, $GR_g(0) \sim N(0.0492, 0.0222^2)$, thus probability = 0.9 = $F(0.0776)$ and analogically at the water potential of -0.4, $GR_g(-0.4) \sim N(0.0227, 0.0222^2)$, thus probability = 0.8 = $F(0.0413)$.

Table 2. Germination rate (h^{-1}) for different percentages of germinated seeds at different water potentials.

Probability	Water potential, ψ (MPa)					Percentage of germinated seeds
	0	-0.2	-0.4	-0.6	-0.8	
0.9	0.0776	0.0643	0.0511	0.0378	0.0246	10
0.8	0.0679	0.0545	0.0413	0.0280	0.0149	20
0.7	0.0609	0.0475	0.0343	0.0210	0.0078	30
0.6	0.0549	0.0415	0.0283	0.0150	0.0018	40
0.5 $GR_{50}(\psi)$	0.0492	0.0359	0.0227	0.0094	-0.0038	50
0.4	0.0436	0.0303	0.0170	0.0038	-0.0094	60
0.3	0.0376	0.0242	0.0110	-0.0022	-0.0154	70
0.2	0.0306	0.0172	0.0040	-0.0093	-0.0224	80
0.1	0.0208	0.0075	-0.0057	-0.0190	-0.0322	90

The above data are presented graphically in Figure 1, which shows the effect of water potential on seed germination rate for different cumulative fractions of seeds. As expected, the lines representing germination fractions are parallel to different intercepts and have the same slopes.

The simple regression between water potential and germination rate gives slopes equal to 0.06624, and the inverse of this value is the hydrotime constant

$$\theta_H = \frac{1}{0.06624} = 15.1.$$

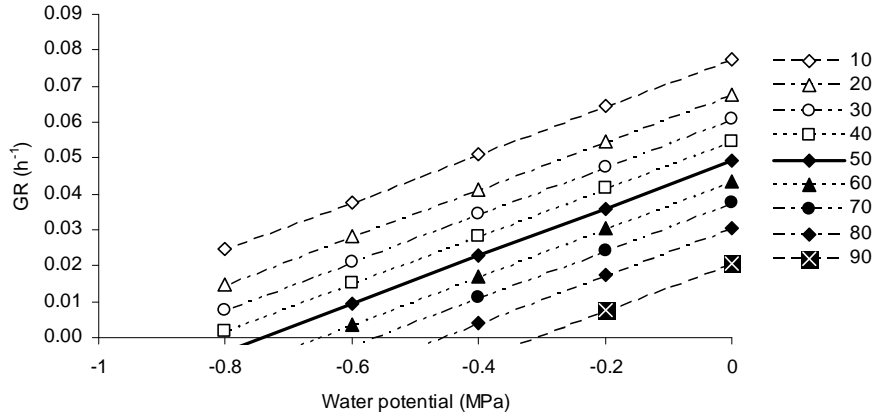


Figure 1. Effect of water potential on seed germination rate (GR) for different cumulative germination fractions.

The hydrotime constant θ_H was then used to calculate the thresholds preventing radicle emergence at different percentages g , $\psi_b(g)$ according to equation (1)

$$\psi_b(g) = \left(\psi - \frac{\theta_H}{t_g} \right).$$

The probits regressed on the values of $\psi_b(g)$ give a straight line (Fig.2) with estimates that permit the calculation of the parameters of normal distribution, $\psi_b(50)$ and σ_{ψ_b} . It is noteworthy that the empirical points lay exactly on the line.

The threshold of medium $\psi_b(50)$ is the middle point of the line intersecting the X axis at probit = 0:

$$\psi_b(50) = -\frac{2.2191}{2.9900} = -0.7422$$

and σ_{ψ_b} is the inverse of the slope: $\sigma_{\psi_b} = \frac{1}{2.9900} = 0.3345$.

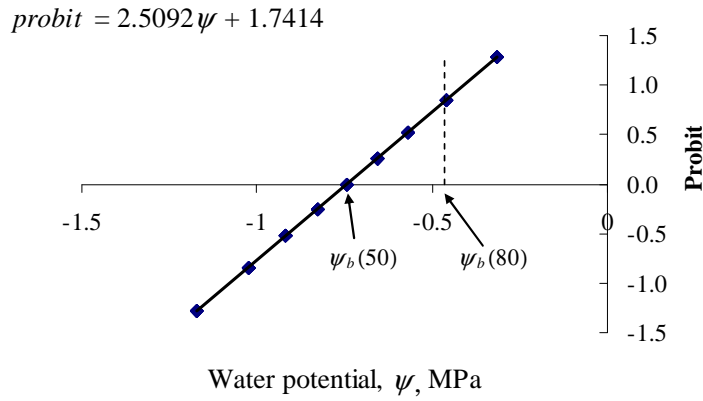


Figure 2. Simple regression of probits and water potentials.

With the known parameters $\theta_H = 15.1$, $\psi_b(50) = -0.7422$, $\sigma_{\psi_b} = 0.3345$ the final presentation of the results may take different forms, depending on the context of process interpretation, i.e. a comparison at different environmental factors.

1. Prediction of germination time courses of seeds in a population within a range of water potentials (ψ) as influenced by imbibition time (h) (in reality the empirical points vary about the lines) (Fig.3).

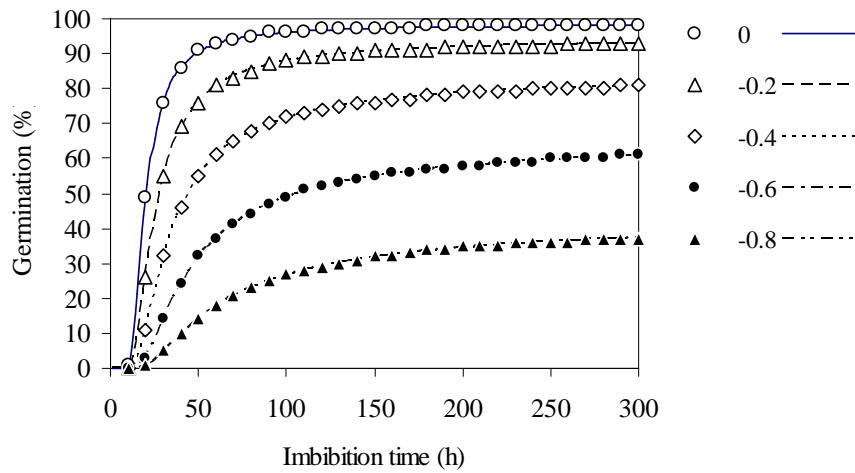


Figure 3. Germination time courses of seeds at ranges of water potentials (ψ , in MPa) as influenced by imbibition time (h)

2. Prediction of the germination process in a population of a given species depending on water potential in the form of normal distribution (Fig.4).

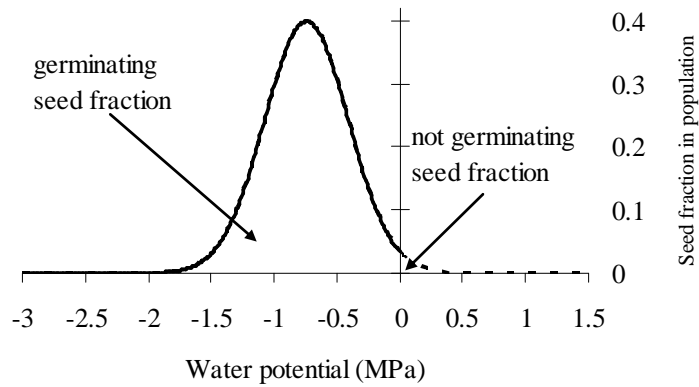


Figure 4. Probability distribution of $\psi_b(g)$.

3. Presentation of normalized germination time courses on the basis of accumulated hydrotime according to equation (4) (Fig.5).

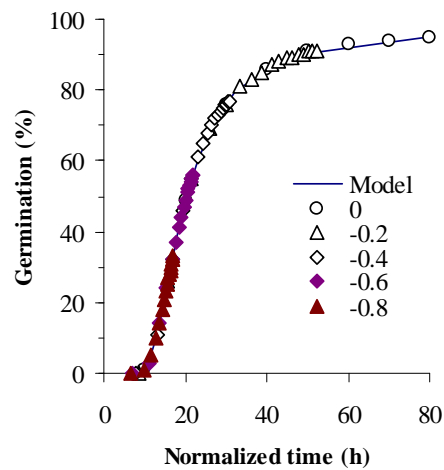


Figure 5. Normalization of germination time courses on the basis of accumulated hydrotime.

4. Interpretation of the results and discussion

The hydrotime model is an attempt to capture, in a simple mathematical form, a very common, but at the same time very complex, natural phenomenon connected with seed germination. The advantage of the hydrotime model expressed in eq. 1 is that it requires only three parameters to quantify and fully predict the germination behavior of a given plant. Once θ_H , $\psi_b(50)$ and σ_{ψ_b} are known for a given seed population, germination time courses at any ψ can be generated simply (Fig.3). Seed dormancy is a reflection of high (more positive) values of the $\psi_b(g)$ threshold, and that means that break dormancy (chilling, after-ripening, hormones, etc.) shifts the $\psi_b(g)$ distribution to lower (more negative) values (Alvarado, Bradford 2002, 2005, Bochenek, Gołaszewski 2007, Żuk-Gołaszewska et al. 2007). This provides a direct linkage between the depth of dormancy, germination rate and the likelihood of successful seedling establishment (Bradford, 1996). The germination time courses at any ψ can be normalized to a common time course equivalent to that in water (Fig.5). The fact that such normalization is possible is an indication of the degree to which the population-based threshold model accounts for germination behavior (Bradford 2002). A restraint imposed by tissues surrounding the embryo radicle can be a major determinant of the threshold water potential. Enzymatic weakening of these tissues probably is a key event regulating the timing of radicle emergence (Welbaum et al. 1998). The three parameters of the model have obvious biological significance, defining the sensitivity and variation in sensitivity of the seed population to changes in ψ and the total hydrotime required for germination. The ability of the same model to account for germination in water as well as in lower ψ suggests that the germination behavior of seeds even under optimal conditions is a reflection of their underlying water relations characteristics (Bradford 1995).

It should be noted that the statistical analysis presented in our paper is one of the methods that may be adopted to calculate the parameters of the hydrotime empirical model. The analysis can be performed by probit or logit analysis or

with the use of the Weibull function (see Bradford 2002, Brown 1987, Ellis et al. 1987, Dumur et al. 1990, Schimpf et al. 1977, Willenborga et al. 2005). Generally, a developed hydrotime model is a mathematical idealization for seed germination in a population, and the results of the model do not imply a successful prediction in natural conditions, where many environmental factors can modify the germination process.

As for the probit analysis applied in our study, various computational approaches are possible depending on the considered hypotheses. It may be done for water potentials (and other environmental stresses) treated as a qualitative variable(s) and for water potentials as a quantitative variable treated as a sample of possible water potentials. The estimates of the parameters of the hydrotime model according to the first and the second approach are usually convergent, while if there are only two water potentials the two approaches give the same results. In our study we conducted the procedure according to the second approach. Application of a generalized linear model with probit link function seems reasonable in our case, because it guarantees estimation of the statistically valid hydrotime model parameters; the procedure is relatively simple because it is based on well-known rules of the general regression approach and because the generalized linear models module with different link functions is a standard easy-to-use statistical tool in most modern statistical packages.

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