The Richards function and quantitative analysis of germination and dormancy in meadowfoam (*Limnanthes alba*)

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Abstract

While investigating germinability in the new-crop meadowfoam (*Limnanthes alba* Benth.), it was desired to gain maximum information despite minimal seed material and prior knowledge. Extended use of the Richards function in a factorial germination experiment proved very powerful. The functions yielded estimates of four correlated coefficients, requiring multivariate analysis of variance (MANOVA) to provide valid *F*-tests among germination profiles. These germination functions provided more rigorous discrimination among treatments than the univariate final germination level. The functions for the imbibants KNO_3 and GA_{4+7}, in darkness at 10/5°C, were best, being characterized by high mean absolute germination rate, relatively short duration, and high final germination (upper asymptote). The results provided insights into possible dormancy mechanisms.

Keywords: dormancy, germinative maturity, *Limnanthes alba*, meadowfoam, non-linear regression, Richards function

Introduction

Meadowfoam (*Limnanthes alba* Benth.) is a winter herbaceous annual native to the Pacific Northwest United States. It is valuable as a potential new crop because its seed oil is a source of highly saturated long chain (C_{20} and C_{22}) fatty acids (Chang and Rothfus, 1977). High-quality waxes, lubricants, detergents and plasticizers are among the potential uses of meadowfoam oil (Higgins *et al.*, 1971). Meadowfoam, however, has some traits which are a challenge to its domestication, such as seed dormancy. Early research on meadowfoam germination (Toy and Willingham, 1966; Cole, 1974) has indicated that normal germination occurs at about 5–15°C, with little germination as temperature approaches 20°C. Indications of secondary dormancy (Cole, 1974; Nyunt and Grabe, 1987) and primary dormancy (Nyunt and Grabe, 1987; Jolliff *et al.*, 1994) have been found.

Most germination studies have analysed the effects of environmental or genetic factors on the final germination percentage. However, studies on seed dormancy have emphasized a need for the measurement of suitable parameters describing germination in respect to time and germination rate. A regression model for evaluating germinative development would be helpful in understanding the biology of seed germination and the biological significance of environmental responses.

The advantages of fitting functions to growth or germination data are manifold and these, together with the rationale of curve fitting, are given by Hunt (1979). These "functional" approaches to growth (or germination) analysis fit cumulative growth data to either polynomial approximations of exponential functions or to explicitly defined sigmoid models (Venus and Causton, 1979). With determinate data, statistically comparable descriptions can be achieved by either model, but practical observations favour the use of asymptotic sigmoid models. Notable among these is the Richards function. This is a comprehensive collection of sigmoids, with special cases including the monomolecular, von Bertalanffy, Mitzerlich, logistic and Gompertz functions (Richards, 1959). Although the Richards function provides a very realistic description of plant growth (Causton and Venus, 1981), it has not been used widely in seed germination studies.

The objective of the present study was to utilize and evaluate biological applications of quantitative

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analysis of seed germination to identify optimum germination test procedures for dormancy-breaking in this new crop and to obtain maximum information from limited seed material. As no information was available on meadowfoam after-ripening, the analysis method had to be able to detect low as well as high dormancy; that is, it had to be both precise and robust. This quantitative analysis consisted of multiple discriminant (canonical variate) analysis and multivariate analysis of variance (MANOVA) of sets of Richards function statistics. The paper presents derivatives needed to fit these functions with a non-linear regression routine (in SAS) and demonstrates the efficacy of the subsequent multivariate analysis in examining germinability and dormancy.

Material and methods

Experiment design

Dormancy-breaking germination methods were investigated using a range of conditions and media. The germination conditions were light (continuous white light) and darkness (Factor A), together with two regimes of alternating temperatures (Factor B), 10/5°C and 15/10°C (12 h for each temperature). Combined factorially, these conditions defined four separate experiments performed in germination cabinets, which were pooled into a combined ANOVA analysis.

The experimental material was drawn from 4-month-old bulk seed of 250 plants from the open-pollinated composite "Moginie" with an unknown "residual" dormancy level, this being the only seed stock available at the time. The experimental unit consisted of fifty randomly selected seeds[1] in a 9 cm Petri dish on Whatman No. 1 filter paper with 5 ml germination medium. Within each germination cabinet, four imbibants provided dormancy-breaking treatments (Factor C) as follows: distilled water, 0.2% KNO_3, GA_3 (100 mg l^{-1}) and GA_{4+7} (100 mg l^{-1}) (ISTA, 1999). Seed samples were divided into putative "maturity" groups, brown (presumed "mature") and green seeds (presumed "immature") (Factor D). A further dormancy-breaking treatment was imposed – pre-chilling (7 d at 5°C in darkness) and non-chilling (Factor E). The design of each cabinet experiment, therefore, was a 4 × 2 × 2 factorial, replicated into three blocks (shelves in a cabinet). These cabinet experiments were combined through an A × B factorial pooling (cabinets) of the individual C × D × E factorials (within cabinets). Richards functions were fitted to each experimental unit (EU) separately, and the model was applied to the MANOVA subsequently using the four Richards statistics as data vectors.

Seeds were considered germinated when the embryo had just ruptured the testa. The numbers of germinated seeds were counted and removed every day during the first 10 d of incubation and at 2 d intervals thereafter. The test was terminated when there was no further germination for two consecutive periods. Ungerminated seeds were tested in 1% tetrazolium solution for viability (Perry, 1987). Germination was expressed as the percentage of germinated seeds based on total viable seeds in the experimental unit.

Curve fitting

The Richards function was fitted to establish germination "profiles" for each experimental unit separately, using procedure NLIN (NonLINear regression) of the SAS statistical package. The Marquardt iterative option was used, as this behaved the most robustly and consistently with the data set. This method regresses the residuals onto the model partial derivatives with respect to the Richards parameters until the estimates converge (SAS Institute Inc., 1990). NLIN also supplies the standard errors and correlations among the Richards function coefficients.

The Richards function is defined as

$$L = A(1 \pm e^{(\beta - \kappa t)})^{-1/\upsilon}, \quad (1)$$

where L is the germination percentage at time t (d), A is the upper asymptote of germination, ν estimates the inflexion point of the curve, β is the x-axis placement parameter, and κ is the rate of change parameter. The negative alternative is used when ν is negative, and vice versa (Causton and Venus, 1981).

The derivatives required for the NLIN program are different from those given by Causton and Venus (1981), who used Newton–Raphson iterations and differentials based on MINRES (minimum-residual). The present SAS package requires differentials with respect to the Richards parameters that do not appear to be available in the literature. Therefore, their solution was an important prerequisite for this fitting, and they are provided here for future reference. The first partial derivatives for L (germination or growth)

[1] As a composite approaches a panmictic population in structure, the results will represent the mean equilibrium values as estimated from random plant samples (of 50 seeds) replicated into the factorial treatment structures described.

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with respect to each parameter of the Richards function are as follows:

$$\frac{\partial L}{\partial A} = (1+e^{(\beta-\kappa t)})^{-1/\nu}$$

$$\frac{\partial L}{\partial \beta} = -\frac{A}{\nu} e^{(\beta-\kappa t)}(1+e^{(\beta-\kappa t)})^{-\frac{1+\nu}{\nu}}$$

$$\frac{\partial L}{\partial \kappa} = \frac{At}{\nu} e^{(\beta-\kappa t)}(1+e^{(\beta-\kappa t)})^{-\frac{1+\nu}{\nu}}$$

$$\frac{\partial L}{\partial \nu} = A\nu^{-2}(1+e^{(\beta-\kappa t)})^{-\frac{1}{\nu}} \cdot \ln(1+e^{(\beta-\kappa t)}).$$

It transpired that the shapes of many of the germination profiles were similar to the Gompertz function (a special case of the Richards function, when $\nu = 0$). In that case, the estimated functions would not converge in NLIN using the Richards function model and its derivatives. Such data were re-fitted using a direct Gompertz function, together with its derivatives. These alternative fits always converged and resulted in higher coefficients of determination (η^2).

The Gompertz function may be written:

$$L = A\exp(-e^{(\beta-\kappa t)}), \qquad (2)$$

where the parameters have the same general meaning as in the Richards function (notice ν is absent).

Next, the first partial derivatives of L with respect to parameters of the Gompertz function are as follows:

$$\frac{\partial L}{\partial A} = \exp(-e^{(\beta-\kappa t)})$$

$$\frac{\partial L}{\partial \beta} = -A \cdot \exp(-e^{(\beta-\kappa t)}) \cdot e^{(\beta-\kappa t)}$$

$$\frac{\partial L}{\partial \kappa} = At \cdot \exp(-e^{(\beta-\kappa t)}) \cdot e^{(\beta-\kappa t)}.$$

Having obtained derivatives for both the Richards and Gompertz functions, we completed the trilogy with the logistic (a special case of the Richards function, when $\nu = 1$) (see Appendix). (We had no direct need for these, however; but they may be useful for reference when using NLIN for growth analysis.)

Two biologically useful variables were calculated from the fitted statistics, both defined by Richards (1959). A weighted mean absolute germination rate (R) can be obtained as $A\kappa/2(\nu+2)$, using the statistics in lieu of the parameters. Another useful statistic is $2(\nu+2)/\kappa$, which represents the time required for the major portion of germination to occur and can be described as the duration of germination (D).

Analysis of variance

The univariate analysis of variance (ANOVA) of the experiment design was conducted for observed (not Richards-fitted) final germination percentages (after 21 d imbibition). The data were transformed to arcsin (percentage/100)$^{1/2}$ values prior to analysis as, by their nature, they were expected to follow a binomial distribution (Steel and Torrie, 1981). The appropriate complex F-tests (Crump, 1946; Satterthwaite, 1946) for some effects, e.g. pooling effects, were obtained by the THWAITE program (I.L. Gordon, unpublished). MANOVA was conducted on the four sample statistics (A, b, k and n) describing each fitted Richards function. For the Gompertz function, ν was set to 0.0001 to facilitate a single MANOVA for all experimental units, as suggested by Richards (1959). The four statistics describing the germination profiles were not transformed for MANOVA, as they were expected to approximate normal. This follows from the central-limit theorem, namely that variates that are sums of several independent and similarly distributed effects tend to be normally distributed (Morrison, 1990). The NLIN fittings amount to a series of linear functions each involving large numbers of data and so satisfy this requirement. The MANOVA model had a similar structure to the univariate one, replacing effects with vectors of Richards statistics.

Results

Final germination

The final germination mean-square for imbibants was significant ($F_{4,20}$ = 7.584, $P < 0.01$), and the mean germination levels are shown in Table 1. GA_{4+7} and KNO_3 resulted in high germination, whereas water gave the lowest value. The final germination was higher in darkness than in light ($F_{2,4}$ = 6.698, $P < 0.10$); green seeds germinated slightly higher than brown ($F_{4,5}$ = 4.071, $P < 0.10$). Temperature effects were not significant ($F_{3,3}$=1.699). Pre-chilling was also not significantly different from lack of chilling ($F_{3,7}$ = 2.260). This univariate attribute was expected to be somewhat imprecise when compared with the Richards function germinative profiles.

Germination profiles

Most germination profiles (141/192 experimental units) followed the Gompertz function ($\nu \cong 0$) and were re-fitted as such. Two germination profiles were considered to be logistic functions, with ν close to 1, but

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Table 1. The main effects on final germination (univariate) of meadowfoam

	F-test	Item	Germination
Temperature	ns	10/5°C	1.3718 (96.1)
		15/10°C	1.3346 (94.5)
Light	(*)	Darkness	1.3981 (97.1) a
		Light	1.3084 (93.3) b
Chilling	ns	Pre-chilling	1.3560 (95.5)
		Non-chilling	1.3504 (95.2)
Imbibant	**	GA_{4+7}	1.4273 (98.0) a
		KNO_3	1.4226 (97.8) a
		GA_3	1.3458 (95.0) b
		Water	1.2172 (88.0) c
Seed colour	(*)	Green	1.3705 (96.0) a
		Brown	1.3360 (94.6) b

Values in parentheses are the untransformed values (%).
**: significant at the 1% probability level; (*): significant at the 10% probability level; ns: not significant.
Means within each column which have a different letter are significantly different by the *t*-test at the 5% level for imbibant and at the 10% level for light and seed colour.

the Richards parameters accommodated these. Others (39 experimental units) followed unspecified Richards functions ($0 < \nu < 1$). The coefficients of determination (η^2) were very high for all these curves, from 0.9313 (the lowest) to 0.9999 (Fig. 1). The NLIN procedure was, therefore, highly successful in describing the germination profiles, either as a Richards function or as a Gompertz function when $\nu \rightarrow 0$ caused non-convergence. One of the strengths of this approach was flexibility in allowing the optimum function to be used. They all contain four parameter-estimates, which can be subsequently submitted to MANOVA to enable exploration of the effects of light, imbibant, etc.

Main effects

The MANOVA significance tests [Rao's *F*-approximation of Wilks' Λ (Cooley and Lohnes, 1971)] showed that all main effects on germination profiles were highly significant ($P < 0.01$), except for chilling treatments (Table 2; Fig. 2). Canonical (multiple) discriminant scores were used subsequently to discriminate between treatments. The discriminant scores indicated that each of the germination profiles for the four imbibants were significantly different ($P < 0.01$) (Table 2; Fig. 2a). While the duration of germination (D) was similar for all four imbibants, the mean absolute germination rate (R) was higher in KNO_3 and GA_{4+7} than in GA_3 and water. The KNO_3 and GA_{4+7} imbibants also yielded a higher asymptote. Temperature markedly ($P < 0.01$) influenced the germination profiles (Table 2; Fig. 2b). The warm regime (15/10°C) prolonged the duration of germination (D) and decreased the mean absolute germination rate (R). Light significantly ($P < 0.01$) affected the germination profiles (Table 2; Fig. 2c), such that the mean absolute germination rate (R) was considerably less in light than in darkness. However, the duration of germination (D) was similar for the

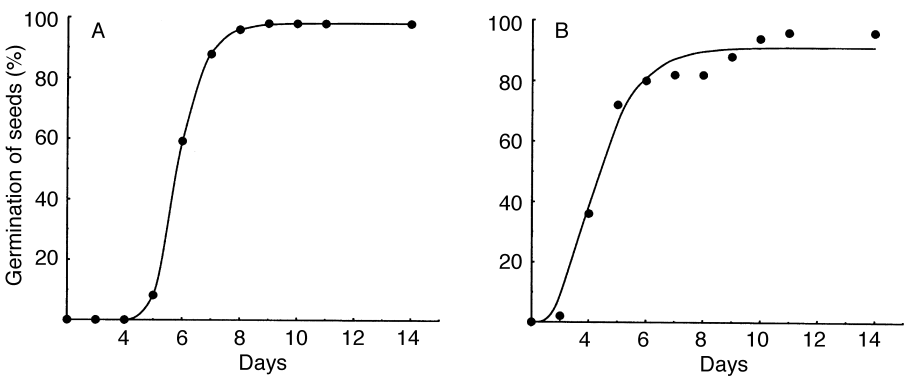

Figure 1. Primary data for two experimental units fitting the Richards function. The coefficients of determination were 0.9999 for (A), the best fit, and 0.9313 for (B), the worst fit.

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Table 2. The statistics of mean Richards functions and their discriminant scores, estimates of germination rate (R) and duration (D) for main effects and significant interactions

		Statistics of function				Discriminant	R	D
Source	Item	A	b	k	n	scores	(% d^{-1})	(d)
Temperature	10/5°C	91.8732	6.2257	1.1103	0.1361		23.90	3.8
**	15/10°C	90.5349	3.4188	0.7937	0.1340		16.82	5.4
Light	Dark	93.5969	5.1815	1.0011	0.2099		21.20	4.4
**	Light	88.8112	4.4631	0.9030	0.0603		19.46	4.6
Seed colour	Green	92.2975	5.1767	1.0296	0.1353		22.26	4.1
**	Brown	90.1105	4.4678	0.8745	0.1349		18.45	4.9
Chilling	Non-chilling	91.3271	4.9321	0.9862	0.1116		21.33	4.3
ns	Pre-chilling	91.0809	4.7125	0.9179	0.1586		19.36	4.7
Imbibant	KNO_3	95.2590	5.1984	1.0132	0.2388	10.7835 a	20.09	4.5
**	GA_{4+7}	92.9230	4.8898	0.9594	0.1084	4.5772 b	21.14	4.4
	GA_3	91.2449	4.6228	0.9248	0.0996	0.0094 c	21.56	4.4
	Water	85.3893	4.5781	0.9107	0.0936	–15.3686 d	18.57	4.6
Temp × Light	10/5 × Dark	94.6124	6.7394	1.1702	0.1759	–12.7404 d	25.44	3.7
**	10/5 × Light	89.1340	5.7121	1.0504	0.0922	–3.5852 c	22.38	4.0
	15/10 × Dark	92.5814	3.6236	0.8319	0.2439	5.6468 b	17.16	5.4
	15/10 × Light	88.4883	3.2140	0.7555	0.0284	10.6775 a	16.48	5.4
Temp × Col	10/5 × Green	92.9006	6.8214	1.2074	0.1648	–22.9588 d	25.91	3.6
**	10/5 × Brown	90.8458	5.6300	1.0132	0.1033	–12.7618 c	21.88	4.2
	15/10 × Green	91.6944	3.5320	0.8517	0.1049	19.6701 a	18.55	4.9
	15/10 × Brown	89.3753	3.3056	0.7358	0.1673	16.0608 b	15.17	5.9
Temp × Imbib	10/5 × GA_3	92.9506	6.1207	1.1231	0.1300	7.1985 a	26.91	3.4
*	10/5 × GA_{4+7}	92.3941	7.1067	1.2624	0.1668	4.4912 ab	24.50	3.8
	10/5 × KNO_3	95.0999	5.7851	1.0187	0.1493	3.2298 b	22.54	4.2
	10/5 × Water	87.0482	5.8904	1.0372	0.0900	–1.0523 c	21.60	4.0
	15/10 × GA_3	92.8954	3.0355	0.7265	0.0571	–4.6717 d	16.93	5.3
	15/10 × GA_{4+7}	90.0957	3.2901	0.7641	0.0324	–3.8271 cd	16.40	5.7
	15/10 × KNO_3	95.4180	3.9944	0.9002	0.3283	2.1662 b	18.45	5.2
	15/10 × Water	83.7304	3.3552	0.7842	0.1267	–7.5289 e	15.44	5.4
Temp × Chill	10/5 × N-ch × G	92.4465	7.2786	1.3314	0.1980	0.9445 bc	28.00	3.3
× Col	10/5 × N-ch × B	91.6956	5.5014	0.9782	0.0900	–4.0565 de	21.46	4.3
**	10/5 × P-ch × G	93.3548	6.3643	1.0835	0.1316	–5.5840 e	23.73	3.9
	10/5 × P-ch × B	89.9960	5.7587	1.0482	0.1165	–1.7185 cd	22.29	4.0
	15/10 × N-ch × G	91.6485	3.4740	0.8429	0.0696	4.3230 a	18.66	4.9
	15/10 × N-ch × B	89.5179	3.4740	0.7923	0.0889	2.3606 ab	16.98	5.3
	15/10 × P-ch × G	91.7403	3.5898	0.8605	0.1403	4.4427 a	18.44	5.0
	15/10 × P-ch × B	89.2326	3.1372	0.6793	0.2458	–0.7059 c	13.49	6.6
Light × Chill	Dark × N-ch × G	94.8903	5.5088	1.0997	0.1534	6.5926 a	24.23	3.9
× Col	Dark × N-ch × B	92.5819	4.8683	0.9473	0.1416	2.7381 bc	20.48	4.5
*	Dark × P-ch × G	93.7166	5.2694	1.0219	0.2160	5.0334 ab	21.61	4.3
	Dark × P-ch × B	93.1988	5.0793	0.9354	0.3287	5.0073 ab	18.72	5.0
	Light × N-ch × G	89.2048	5.2440	1.0746	0.1142	–3.8118 d	22.67	3.9
	Light × N-ch × B	88.6316	4.1071	0.8232	0.0374	–5.2740 d	17.91	4.9
	Light × P-ch × G	91.3784	4.6846	0.9220	0.0559	0.4954 c	20.49	4.5
	Light × P-ch × B	86.0298	3.8166	0.7921	0.0336	–10.7891 e	16.75	5.1

Continued

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Table 2. *Continued*

Source	Item	Statistics of function				Discriminant scores	*R* (% d^{-1})	*D* (d)
		A	*b*	*k*	*n*			
Temp × Light × Imbib × Col **	10/5 × Dk ×G × GA_3	90.2946	9.1348	1.5636	0.3618	–14.1667 o	29.89	3.0
	10/5 × Dk × B × GA_3	95.9309	6.6499	1.1667	0.2064	–5.2759 kl	25.36	3.8
	10/5 × Dk × G × GA_{4+7}	95.4930	7.3031	1.2778	0.1916	–7.2344 lm	27.83	3.4
	10/5 × Dk × B × GA_{4+7}	94.2823	6.0752	1.1194	0.0392	–3.6295 jk	25.88	3.6
	10/5 × Dk × G × KNO_3	97.7419	5.1956	1.0031	0.2446	1.5235 i	21.84	4.5
	10/5 × Dk × B × KNO_3	96.0952	6.1283	0.9659	0.1677	–6.7973 l	21.41	4.5
	10/5 × Dk × G × Water	90.9831	6.8435	1.1406	0.0666	–9.6142 mn	25.11	3.6
	10/5 × Dk × B × Water	90.1080	6.5848	1.0125	0.1296	–11.1928 n	21.42	4.2
	10/5 × L × G × GA_3	90.9524	7.0643	1.2793	0.0572	–7.6719 lm	28.28	3.2
	10/5 × L × B ×GA_3	86.3984	5.5780	1.0400	0.0419	–5.1575 kl	22.00	3.9
	10/5 × L × G × GA_{4+7}	90.4982	7.0154	1.2750	0.2190	–7.0944 lm	26.00	3.5
	10/5 × L × B × GA_{4+7}	91.5590	4.0892	0.8201	0.0705	1.6924 i	18.13	5.0
	10/5 × L × G ×KNO_3	94.3077	6.7745	1.1789	0.1572	–6.6065 l	25.77	3.7
	10/5 × L × B × KNO_3	92.2551	5.0423	0.9268	0.0276	–2.1218 j	21.08	4.4
	10/5 × L × G × Water	86.9643	5.2405	0.9412	0.0203	–5.1666 kl	20.26	4.3
	10/5 × L × B ×Water	80.1372	4.8929	0.9422	0.1436	–4.9111 jkl	17.61	4.6
	15/10 × Dk × G × GA_3	93.2618	3.6062	0.9012	0.1295	7.9556 abcd	19.73	4.7
	15/10 × Dk × B × GA_3	91.5693	3.1440	0.7076	0	5.2069 defgh	16.20	5.7
	15/10 × Dk × G × GA_{4+7}	94.4825	3.4671	0.8667	0	8.0989 abc	20.47	4.6
	15/10 × Dk × B × GA_{4+7}	95.3273	2.7411	0.6827	0.1807	9.3876 ab	14.92	6.4
	15/10 × Dk × G × KNO_3	96.4699	4.2424	0.9045	0.4833	5.9527 cdefg	17.57	5.5
	15/10 × Dk × B ×KNO_3	95.9583	4.5645	0.9426	0.6510	5.0161 efgh	17.06	5.6
	15/10 × Dk × G × Water	89.7309	3.3204	0.8288	0	6.3572 cdef	18.59	4.8
	15/10 × Dk × B × Water	83.8513	3.9027	0.8211	0.5066	1.4927 i	13.73	6.1
	15/10 × L × G × GA_3	89.1419	3.4256	0.7735	0	3.9955 fghi	17.24	5.2
	15/10 × L × B × GA_3	86.4097	2.9847	0.6739	0	3.5038 ghi	14.56	5.9
	15/10 × L × G × GA_{4+7}	93.5009	3.1452	0.7497	0.0474	7.1496 bcde	17.12	5.5
	15/10 × L × B × GA_{4+7}	88.2707	2.7886	0.6070	0	3.8792 fghi	13.39	6.6
	15/10 × L × G × KNO_3	96.0877	3.5370	0.9426	0.1789	10.7204 a	20.78	4.6
	15/10 × L × B × KNO_3	93.1560	3.6335	0.8110	0	5.0177 efgh	18.89	4.9
	15/10 × L × G × Water	80.8797	3.5121	0.8463	0	2.1069 i	17.11	4.7
	15/10 × L × B × Water	80.4597	2.6855	0.6404	0	2.4846 hi	12.88	6.2

**: significant at the 1% probability level; *: significant at the 5% probability level.
Temp: temperature; L: light; Dk: dark; Imbib: imbibant; Col: seed colour; G: green; B: brown; *R*: mean absolute rate of germination; *D*: duration of germination.
Discriminant scores within each column which have a different letter are significantly different by the *t*-test at the 5% level.

two light conditions. Thus, darkness resulted in a higher upper asymptote. Response of germination to seed colour was also detected ($P < 0.01$) (Table 2; Fig. 2d). The mean absolute germination rate (*R*) was higher in green seeds, while the duration of germination (*D*) in green seeds was 0.8 d shorter than in brown seeds. The upper asymptote of germination was considerably higher in green seeds than in brown seeds. However, the main functions between pre-chilling and non-chilling were not significantly different (Table 2; Fig. 2e).

First-order interaction

While the main effects summarize the overall trends, more information about germinability and dormancy may be revealed in the interactions. A significant interaction ($P < 0.01$) between temperature and light was found for the germination profiles (Table 2; Fig. 3a), with greater light reduction in the cooler temperature regime. A similar differential effect ($P < 0.01$) was found for seed colour × temperature (Table 2; Fig. 3b). The rate of brown seed germination was slower at the cooler temperature, as shown by both *R* and *D*. Another interaction affecting germination profiles involved combinations of temperature and imbibant ($P < 0.05$) (Table 2; Fig. 3c). Germination profiles for KNO_3 at both temperatures were similar; but with GA_{4+7}, GA_3 and water, the cooler regime (10/5°C) resulted in higher mean absolute germination rates (*R*) and shorter germination durations (*D*). In the cooler regime, germination profiles of GA_{4+7}, GA_3 and KNO_3 formed an overlapping series and were all different compared with the profile of water. In the warmer (15/10°C)

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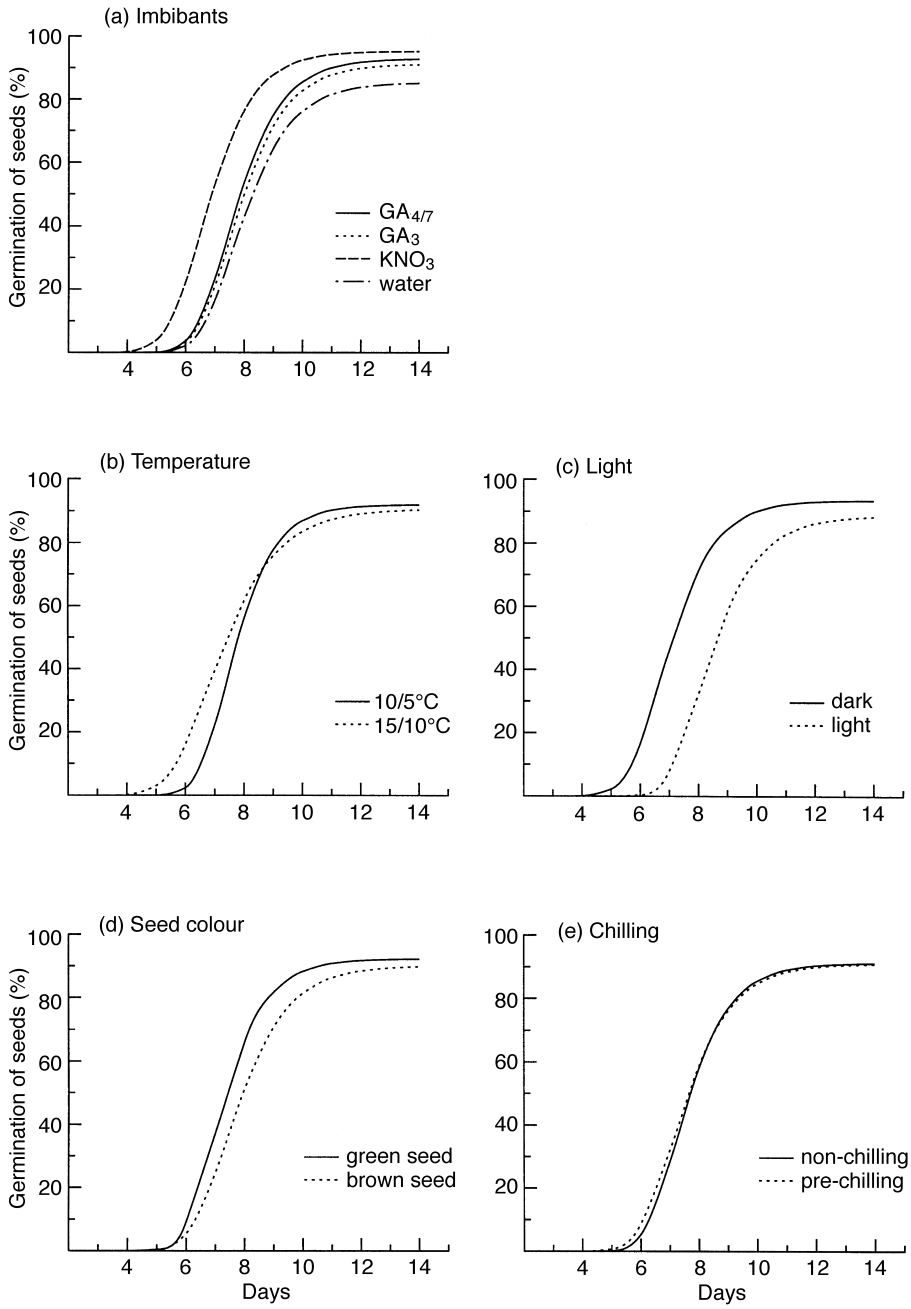

Figure 2. The mean Richards functions of seed germination for main effects.

regime, however, the germination profiles of the four imbibants fell into three different groups, with the two gibberellins being together in one group.

Second-order interaction

The second-order interactions involving temperature, chilling and seed colour were strongly significant ($P <$ 0.01) (Table 2; Fig. 4a). Differences between chilling treatments were found only in green seeds at 10/5°C and in brown seeds at 15/10°C. The germination profiles differed between seed colours at 10/5°C, regardless of chilling treatments. Similar colour separation was found at 15/10°C, but only after pre-chilling. Non-chilled green seeds germinating at 10/5°C had a high mean absolute germination rate (R) and short duration of germination (D).

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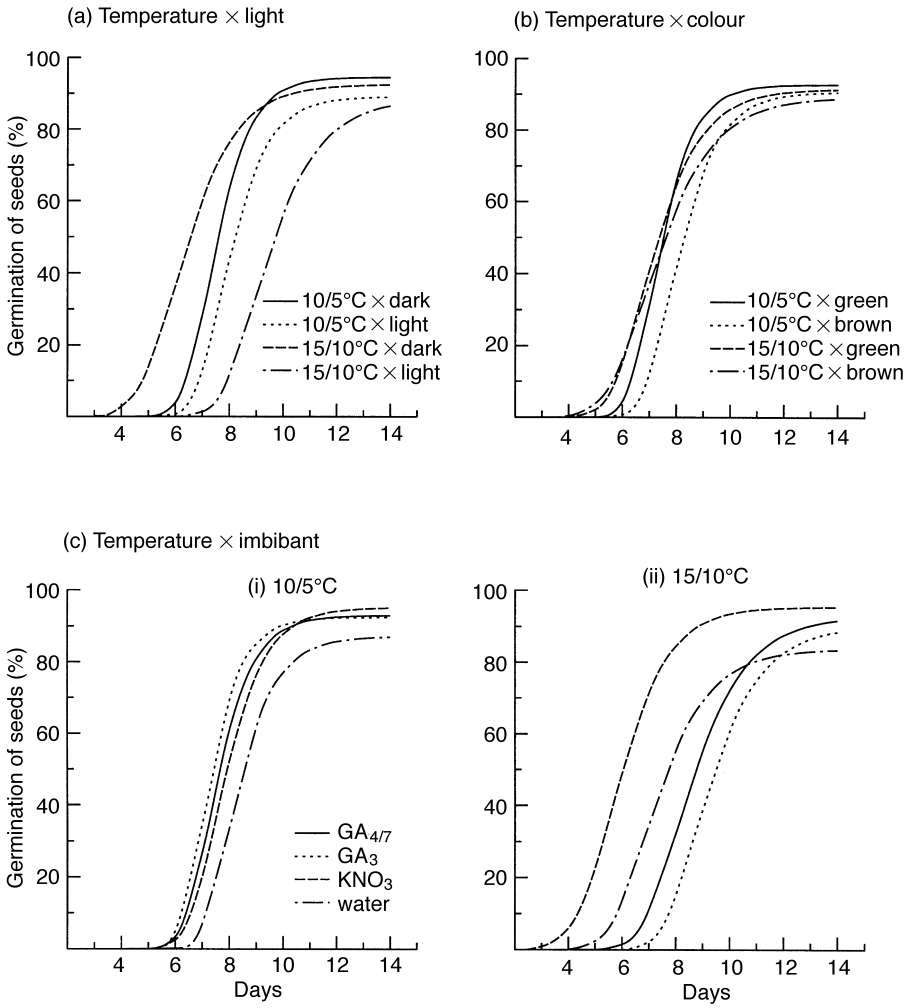

Figure 3. The mean Richards functions of seed germination for first-order interactions.

There was also an interaction ($P < 0.05$) between light, chilling and seed colour (Table 2; Fig. 4b). The effects of chilling on germination profiles were found only in the light for both seed colours. Non-chilled green seeds in darkness had a high mean absolute germination rate, short duration of germination (D), and a high upper asymptote of germination.

Third-order interaction

There was also a significant ($P < 0.01$) third-order interaction among temperature, light, imbibant and seed colour (Table 2; Fig. 5). GA_{4+7} and KNO_3 treatments resulted in high mean absolute germination rates (R) and short durations of germination (D) in darkness at 10/5°C for both seed colours, which resulted in a high upper asymptote of germination (A). A similar result was found in the GA_3 treatment, but only for brown seeds. With green seeds, GA_3 in darkness at 10/5°C yielded the highest mean absolute germination rate (29.89) compared with the other combinations, but it had a relatively short duration (3.0 d). Thus, it resulted in a low asymptote. However, water gave a low mean absolute germination rate (R) and a low asymptote (A), regardless of temperature, darkness and seed colour.

Discussion

Curve fitting

The germination profiles of meadowfoam seeds clearly were sigmoidal (Figs 1–5), which is not unexpected (Bewley and Black, 1994). In this study, non-linear functions (Richards or Gompertz)

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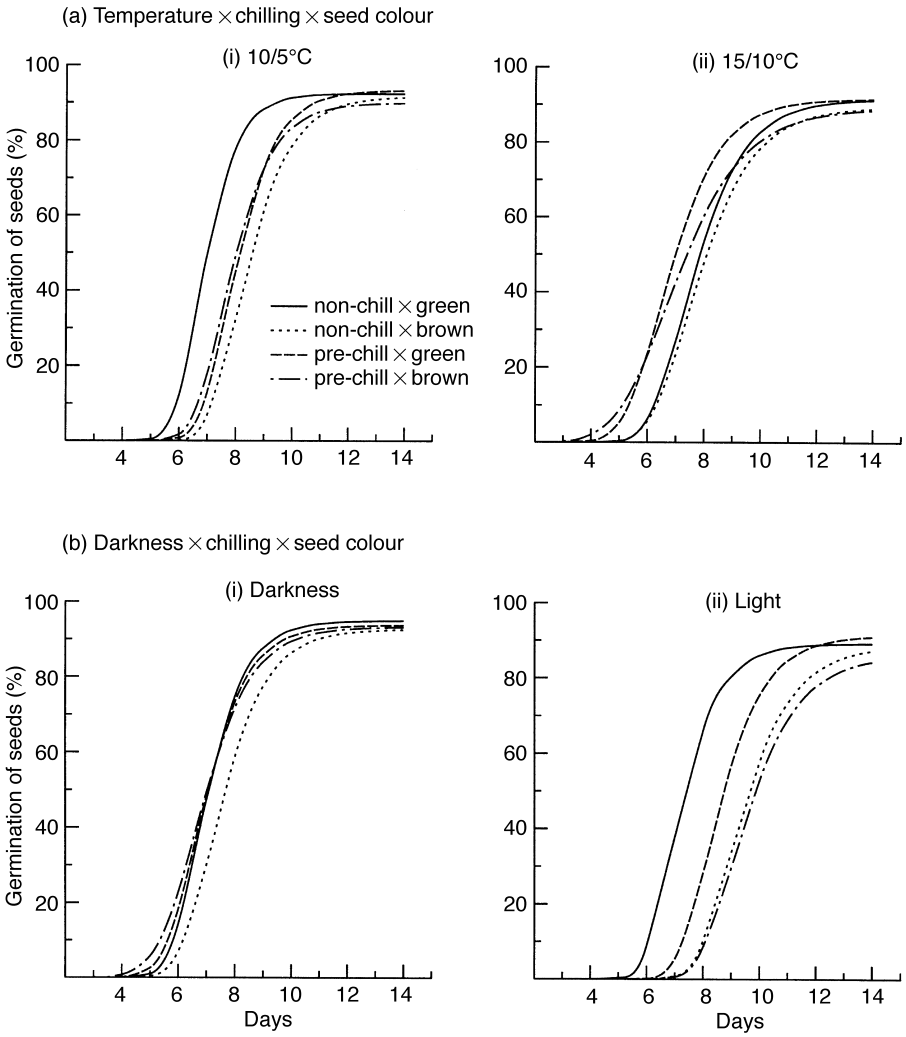

Figure 4. The mean Richards functions of seed germination for second-order interactions.

provided convenient and credible descriptions of the progress of seed germination. (Since the Gompertz function is a special case of the Richards function, we can use the term "Richards function" as a generalization.) The Richards function has also proved superior to other curve-fitting approaches for seed germination of cress (Lehle and Putnam, 1982). Moreover, the multivariate analysis of variance (MANOVA), which provided a valid simultaneous analysis of all four (correlated) statistics which define any Richards function, made it possible to test significant differences among the functions (germination profiles) themselves. Subsequently, the functions allowed estimation of other aspects, such as germination percentages at key times and germination rates. Results may be presented as "mean functions" following MANOVA, as has been done here. A similar result has been reported by Schimpf *et al*. (1977), who used only the logistic function to fit the germination curve (only two of our Richards functions approached the logistic). Most of the germination profiles were Gompertz functions (141/192 functions), and a further 39 functions were between the Gompertz and logistic ($0 < \nu < 1$) ("unspecified Richards functions"). This result agreed with Nichols and Heydecker (1968), who noted that most germination curves were positively skewed, a fact which the (symmetrical) logistic cannot accommodate. A major advantage of the four-parameter Richards function is the flexibility of curve type: it is possible to include mixed types (logistic, Gompertz, unspecified Richards) in the one analysis, as the parameter ν accounts for this. This permits the use of the optimum function rather than forcing all curves into one type. We have used this to maximum advantage by fitting each experimental unit

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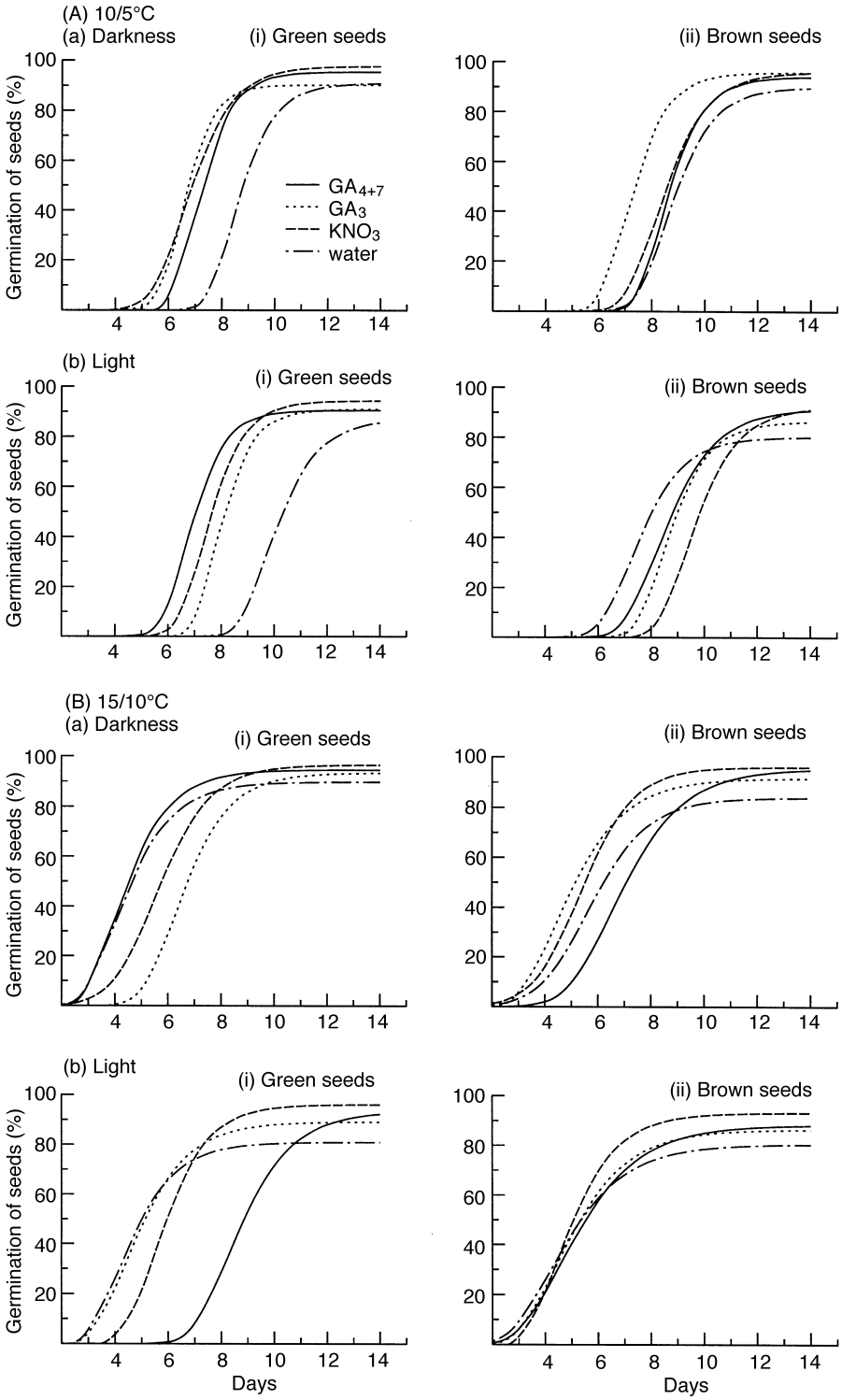

Figure 5. The mean Richards functions of seed germination for third-order interactions.

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separately, followed by MANOVA. Compared with the univariate final germination level (ANOVA) (Table 1), multivariate analysis of variance (MANOVA) was much more sensitive in discriminating among germination profiles (Table 2). Whether this degree of sensitivity will always be useful biologically is a point of caution: it may sometimes be over-sensitive with respect to *a priori* knowledge from physiology. However, in this case, it has revealed much information, despite the unknown effects of after-ripening.

The Richards function yields estimates of A, β, κ and ν, of which only A and ν appear to provide clear stand-alone biological information. Parameter A gives the asymptotic maximum germination of seeds, and ν describes the type (or shape) of the germination curve. The point of inflexion is a function of ν (in fact $L_I/A = (\nu + 1)^{-1/\nu}$, Causton and Venus, 1981). The parameter ν has a profound effect on the Richards function, as it changes x-axis placement and slope as well as inflexion. Slope is complex in any case, being influenced not only by κ and ν, but also by A [in fact, slope $(\rho) = (\kappa L)/(\nu A^{\nu})(A^{\nu} - L^{\nu})$; Causton and Venus, 1981]. Furthermore, as all four statistics are correlated, only MANOVA can supply valid F-tests for profile testing. However, as this work has shown, these complexities are all surmountable, and highly satisfactory results can emerge (Table 2). The established complexities may even extend beyond our *a priori* knowledge to interpret them, thereby signalling a need to reappraise our hypotheses.

Further useful comparisons of curves were made from the two biologically useful derived statistics, namely, the mean absolute germination rate (R) and the germination duration (D) (Table 2). The mean absolute rate (R) should be considered together with the asymptote (A) if a useful picture of profile shape is to be obtained (Causton and Venus, 1981). The duration of germination loosely measured the time required for the major portion of germination. However, it does not correspond to the time of 50% germination, nor to the time of inflexion, nor to the time between $0.05A$ and $0.95A$: it is another measure of time in such functions.

These secondary statistics led to a better understanding of the seed germination profiles, since rate and duration were affected differently by the different factors. For example, in this study, they showed that GA_3 treatment of green seeds at 10/5°C in darkness yielded a higher mean absolute rate with a shorter duration of germination, which resulted in a low upper asymptote compared with GA_{4+7} and KNO_3 (Table 2; Fig. 5). The derived parameters (R and D) have also been employed by Dennett *et al.* (1978, 1979) in the description of leaf growth in *Vicia faba*.

Germination and dormancy

The Richards function results indicated that KNO_3 and GA_{4+7} treatments in darkness at 10/5°C were good procedures for obtaining maximum seed germination of meadowfoam, since they gave high mean absolute rates (R) and high germination with short durations (Table 2; Fig. 5). The results agreed with those of Toy and Willingham (1966) and Cole (1974) that meadowfoam seeds do not require light for germination, and germination may even be inhibited by exposure to light. Mmolawa (1987) also suggested that KNO_3 may be effective in alleviating dormancy in meadowfoam seeds (as in other species).

Other workers found that accessions of *Limnanthes* showed optimum germination at constant temperatures of about 5–10°C, but that germination dropped sharply at temperatures above 15°C and was near zero at 20°C (Toy and Willingham, 1966; Cole, 1974). Jolliff *et al*. (1994) reported similar results. The present study has shown that the warm regime (15/10°C) increased heterogeneity of individual germination times, as reflected by changes in both mean absolute germination rate (R) and duration of germination (D) (Table 2), while there was no effect on the final germination level (Table 1; Fig. 2b).

Germination testing and dormancy

One of our practical objectives was to define a procedure for breaking dormancy in meadowfoam. Based on our results (Table 2; Fig. 5), we could define the dormancy-breaking test as: imbibant 0.2% KNO_3 solution (for economic reasons) in darkness, temperature 10/5°C (12/12 h) without pre-chilling, and assessment at 14 d from imbibition (test M). The test results with KNO_3, therefore, measure the basic germinative ability of the natural whole-seed system: that is "germinative maturity". Poor germination in this test would represent "immaturity". A "standard" germination test usually employs water as the imbibant (ISTA, 1999) at constant temperature. From this study, darkness, a cool temperature (10°C), and no pre-chilling would be recommended, with assessment at 14 d (test S). The difference between the two tests would represent dormancy (D) plus non-viability (I), the latter being separated following a tetrazolium test (Perry, 1987).

Thus:

maturity (%) = M/V × 100,	where V (viable seeds) = total – I;
dormancy (%) = (M−S)/V × 100,	(on a live-seed basis); or
dormancy (%) = (M−S)/M × 100,	(on a maturity basis).

From the present results, the asymptote of KNO_3 at 10/5°C of this seed-lot was 95.1% ± 0.3 (Fig. 3c). We

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do not have a simple "standard" test in these treatments: the nearest was water at 10/5°C. The asymptote of water at 10/5°C was 87.0% ± 0.3. Using that estimate, this seed-lot had 8.1% dormancy. The standard error of the KNO_3 asymptote (from pooled errors of the NLIN fits) provides a standard error for germinative maturity, and was, in this case, 0.3% (as noted already). A standard error for dormancy is also obtainable, as that of the difference between the two asymptotes ($A_{KNO_3} - A_{H_2O}$). As these are independent estimates, the standard error is:

$$\hat{\sigma}_{\text{Dorm}} = (\hat{\sigma}^2_{A_{KNO_3}} + \hat{\sigma}^2_{A_{H_2O}})^{1/2}.$$

For this case, the standard error for dormancy was 0.5%. Thus, not only do we have an estimate of dormancy, but the methodology has permitted an acceptable level of precision.

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Appendix

The first partial derivatives of L with respect to parameters of the logistic function are as follows:

$$\frac{\partial L}{\partial A} = (1 + e^{(\beta - \kappa t)})^{-1}$$
$$\frac{\partial L}{\partial \beta} = -A \cdot e^{(\beta - \kappa t)}(1 + e^{(\beta - \kappa t)})^{-2}$$
$$\frac{\partial L}{\partial \kappa} = At \cdot e^{(\beta - \kappa t)}(1 + e^{(\beta - \kappa t)})^{-2}.$$

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