Prediction of seed longevity: a modification of the shape of the Ellis and Roberts seed survival curves

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Abstract

The prediction of the viability of stored seeds is important both for the management of germplasm collections and for the management of commercial seed production and storage. The Ellis and Roberts model for seed viability during storage is examined, and an inadequacy of the model highlighted. A modification is proposed, based on the 'control mortality' probit model developed for insecticide bioassays, to take proper account of variation in initial viability. This new 'control viability' model, relating seed viability to storage time, is fitted to data from a carrot seed storage experiment and found to fit well for a range of storage environments. A relationship, similar to that proposed by Ellis and Roberts for the effects of storage conditions on the rate of loss of viability, is fitted to the estimated rates from this new model. Data from a second carrot seed storage experiment are used to validate this relationship.

Keywords: carrot, model, moisture, seed viability, storage, temperature

Introduction

Reliable estimates of seed longevity are important both for the management of germplasm collections and for the management of commercial seed production and storage. For example, accurate predictions of the rate of loss of viability of seed lots stored under differing conditions would provide valuable information for their management, to preserve maximum viability following harvest and prior to sale. It would also be valuable in forming decisions on whether it is worthwhile storing unsold stocks of seed to a successive year by giving estimates of viability losses under known storage conditions.

Working with cereal grains, Roberts and Ellis (Roberts, 1960, 1972, 1973; Ellis and Roberts, 1980a,b) developed equations to predict seed longevity, which are now widely used. The 'improved' model (Ellis and Roberts, 1980a) relates probit percentage viability, v, to storage period, t (in days), as

$$v = k_i - \left(\frac{1}{\sigma}\right)t \qquad (1)$$

where k_i is the initial probit percentage viability and σ is the standard deviation of the distribution of deaths in time. In addition to the effects of temperature and moisture, the main environmental factors influencing longevity, the 'improved' equations include allowances for genotype and pre-storage environment. In the development of this model, Ellis and Roberts assume that the frequency of individual deaths in time in a population stored at constant conditions can be described by the Normal distribution. They then use probit analysis to quantify both the 'initial' viability of the seed lot, which depends only on genotype and seed quality, and the rate of loss of viability under given sets of conditions.

While the assumption of a Normal distribution of deaths in time is convenient, there are sufficient variations from this pattern in the literature to indicate that this may not be fully justified (Ellis and Roberts, 1977; Priestley *et al.*, 1985; Kraak and Vos, 1987; Roos and Davidson, 1992). The model suggests that viability is 100% at some time prior to the start of storage, and that differences between seed lots are accounted for simply by shifting the viability curve by a fixed amount along the time axis. The adoption of this model means that those seeds that are non-viable at the start of storage are assumed to be part of the Normal distribution describing deaths in time. Since little is known about the events or conditions that contributed to this non-viability it seems more logical to ignore these seeds in the construction of a model. Figure 1a shows an example set of viability curves constructed following the Ellis and Roberts (1980a) model, where the initial viability ranges between 60 and 100%, causing the time to 50% viability to vary between 50 and 450 d. Notice how this form of model produces parallel viability curves, with a given change

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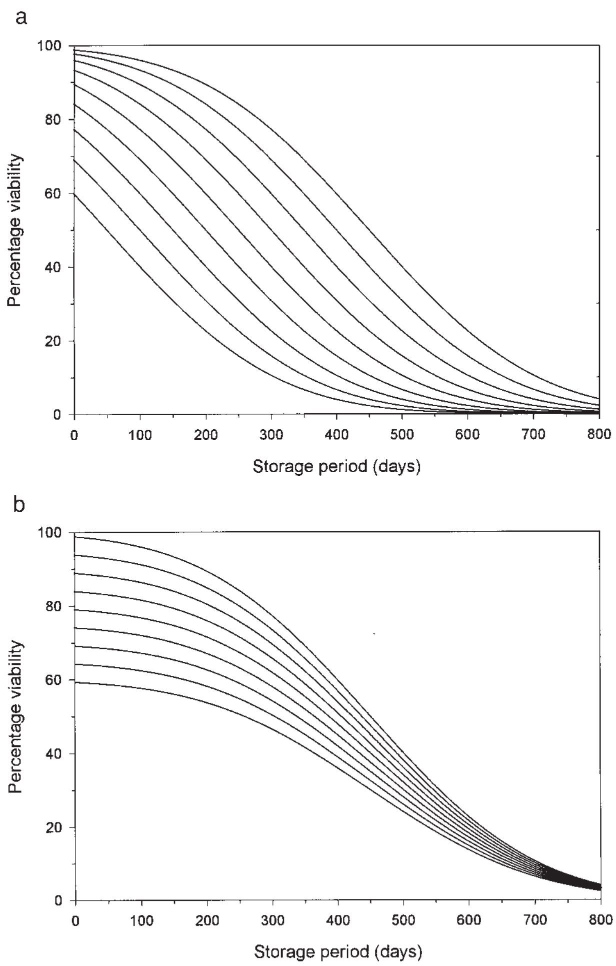

Figure 1. Sets of simulated viability curves constructed following (a) the original Ellis and Roberts model with initial viability being altered by shifting the curve along the time axis, and (b) the modified 'control viability' model with initial viability altered by including an additional 'control viability' parameter in the model.

in initial viability indicating a fixed reduction or increase in the time to any particular percentage viability.

Wilson, McDonald and St. Martin (1989) suggest a correction for the initial proportion of non-germinated seeds, an approach also discussed by Ellis and Roberts (1980a), achieved by scaling the observed data so that the initial percentage of viable seeds is 100%. This approach, however, must assume a fixed, known value of the initial viability, whereas in practice only an estimate, with associated error, is available. Problems can also occur in the statistical fitting of the model to data that have been corrected in this way, with replicate observations possibly exceeding this fixed initial viability.

Using data from two carrot seed longevity experiments we have attempted to estimate the Ellis and Roberts viability constants for carrot, and propose an extension to their model to take proper account of the variability in initial non-viability. Our alternative model is based on the extended probit model proposed by Finney (1971) to take account of 'control mortality'. This model was originally developed for analysing the effect of the concentration of pesticide on insect deaths, allowing proper account to be taken of the deaths that would occur in the absence of any pesticide. Using this model, differences in the level of natural mortality do not affect the values of the slope and intercept parameters, just the value of the additional 'control mortality' parameter. For the seed longevity problem we adopt a similar approach that allows a proper account to be taken of the variability in the initial level of non-viability of seed lots. Figure 1b shows simulated viability curves following our alternative 'control viability' model, with the initial percentage viability varying from 60–100%, but the time to reach 50% of this initial viability remaining unchanged. Rather than shifting the viability curve along the time axis, this alternative model compresses the curve so that it starts at time zero from an upper asymptote at the estimated initial percentage viability. The values of the slope and intercept parameters do not vary between these curves, just the value of the 'control viability' parameter, representing the estimated initial viability of the seed lot.

In previous publications concerned with modelling the loss of viability of stored seeds, model validation appears to have related only the accuracy of the prediction of the times to particular percentage viability levels. We feel that any model validation should apply to the whole viability curve, and adopt this approach for our modified model.

Materials and methods

Seed lot 1

Batches of carrot seed cv. Chantenay red-cored Supreme (Elsoms, lot 1933) were raised to 10, 15, 20 and 30% moisture content (wet weight basis). These moisture contents were achieved by adding an appropriate amount of water to seeds in a bottle, which was then shaken every hour for 4 h, and left to equilibrate at 5°C for 3 d. The quantity of water to be added was determined by oven drying a sample of seed at 130°C for 1 h. Batches of seed from each moisture content treatment were heat sealed in laminate foil packets and then stored at 5, 10 and 20°C (± 0.25°C) for periods ranging from 2 weeks to 2 years depending upon treatment (Table 1). Storage temperatures were checked on a daily basis with a thermocouple mounted within the storage chamber. Three replicate batches, each of 100 seeds, were stored for each of the combinations of temperature, moisture content and time. After treatment, seeds were

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Table 1. Sampling schedule and treatments for the first carrot seed experiment

Seed storage treatment		Actual moisture content	Sampling intervals	Number of samples
Temperature	Moisture content			
5°C	10%	10%	3 monthly for 2 years	8
5°C	15%	14%	1 monthly for 2 years	24
5°C	20%	18%	1 monthly for 2 years	24
5°C	30%	25%	1 monthly for 1 year	12
10°C	10%	10%	2 monthly for 2 years	12
10°C	15%	14%	1 monthly for 1 year	12
10°C	20%	18%	1 monthly for 1 year	12
10°C	30%	25%	1 monthly for 1 year	12
20°C	10%	10%	2 monthly for 2 years	12
20°C	15%	14%	1 monthly for 1 year	12
20°C	20%	18%	1 monthly for 1 year	12
20°C	30%	25%	weeks 2, 4, 6, 8, 10, 12, 16 and 20	8

Table 2. Sampling schedule and treatments for the second carrot seed experiment

Seed storage treatment		Sampling intervals	Number of samples
Temperature	Moisture content		
5°C	10%	2 monthly for 14 months, then 3 monthly for 21 months	14
5°C	15%	2 monthly for 14 months, then 3 monthly for 21 months	14
5°C	20%	2 monthly for 14 months, then 3 monthly for 21 months	14
5°C	30%	1 monthly for 7 months	7
10°C	15%	2 monthly for 14 months, then 3 monthly for 21 months	14
10°C	20%	1 monthly for 15 months	15
10°C	30%	2 weekly for 20 weeks	10
20°C	15%	1 monthly for 13 months	13
20°C	20%	2 weekly for 18 weeks	9
20°C	30%	twice weekly for 45 days	13
40°C	15%	daily for 14 days	14
40°C	20%	daily for 9 days	9
1°C	40%	1 monthly for 10 months	10
5°C	40%	2 weekly for 14 weeks	7
10°C	40%	weekly for 12 weeks	12
40°C	10%	weekly for 7 weeks	7
60°C	10%	daily for 4 days	4

germinated in the light on filter paper (Whatman No. 1) at 20°C in plastic boxes (125 mm × 80 mm × 20 mm). Germinated seedlings were counted after 7 and 14 d (ISTA, 1985). The initial viability of the seeds was estimated using 3 batches of 100 untreated seeds that were germinated at the start of storage.

Seed lot 2

An independent experiment was performed a year later to provide data to validate the modified model. Batches of carrot seed cv. Chantenay red-cored Supreme (produced at HRI, lot 4/78) were similarly raised to various moisture contents and then stored under various temperature regimes for periods of between 2 weeks and 2 years depending upon treatment (Table 2). In this experiment, 4 replicate batches of 100 seeds of each treatment combination were stored and after treatment seeds were germinated as described above. Untreated, control batches were again included to estimate the initial viability of the seeds.

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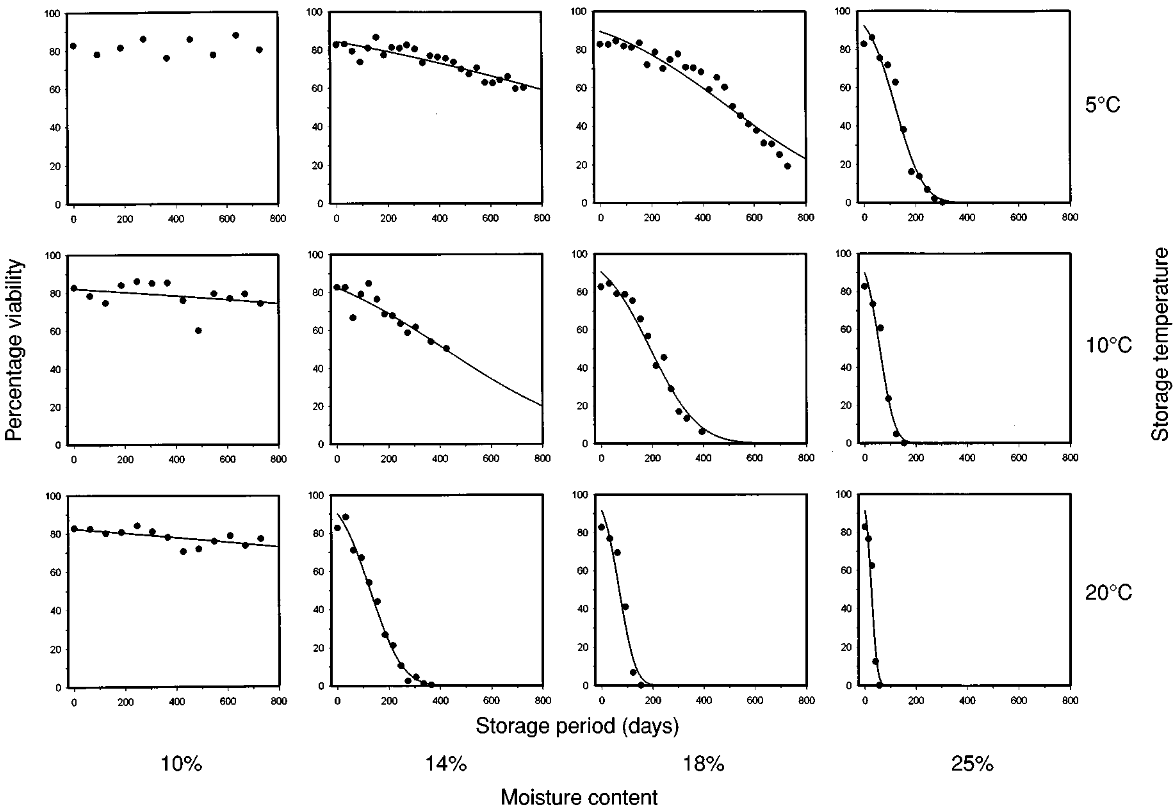

Figure 2. Fitted curves under the Ellis and Roberts (standard probit) model (equation 1) for 11 of the 12 storage environments in the first carrot seed experiment. Plotted points are the means of 3 replicates, calculated on the probit scale.

Model development – shape of response curve

The original model proposed by Roberts (1973) assumes that the frequency of individual deaths in time in a population stored in constant conditions is described by the Normal distribution with parameters $\bar{p}$, the mean viability period, and σ, the standard deviation of the distribution of deaths in time. Thus seed survival curves, plotting percentage viability against time, are 100 times the complement of cumulative Normal distributions. The model further assumes that the standard deviation for the distribution, σ, is directly proportional to the mean viability period, $\bar{p}$, and that the log of the mean viability period is linearly related to storage temperature and moisture content. The 'improved' viability equations proposed by Ellis and Roberts (1980a) separate the constants involved in this system of equations into those that are affected solely by storage conditions, and those that are only affected by genotype, seed lot and pre-storage conditions. They further suggest a modified relationship for the log of the mean viability period as a function of storage temperature and moisture content.

In the improved model (equation 1) the initial probit percentage viability, k_i, is not affected by storage conditions, only by genotype, seed lot and pre-storage conditions. It can thus be determined by germination tests for the particular seed lot at the time of storage, or by fitting probit viability curves to data from rapid-deterioration storage treatments. The standard deviation of the distribution of deaths in time, and hence the slope of the seed survival curve, is not affected by genotype, seed lot or pre-storage conditions but can be related to the storage temperature, T, and moisture content, M.

Following Ellis and Roberts, an estimate of the initial percentage viability was obtained from the results of the germination test on the untreated seeds, yielding a value of 83% with a 95% confidence interval for this estimate ranging from 78–87%. The fitted standard probit curves for 11 of the 12 storage temperature by moisture content treatment combinations are shown in Figure 2, with the fitted parameters shown in Table 3. The fitted curve for the 5°C, 10% moisture content treatment is not included as the estimated slope was fractionally, though not significantly, greater than zero. These fitted curves,

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Table 3. Parameters for the Ellis & Roberts (1980a) probit viability model fitted to results from the first carrot seed experiment

Seed storage treatment		Estimated slope of probit curve $(1/\sigma)$	Estimated initial probit percentage viability (k_i)	Estimated initial percentage viability
Temperature	Actual moisture content			
5°C	14%	−0.00097	1.010	84
5°C	18%	−0.00249	1.251	90
5°C	25%	−0.01177	1.430	92
10°C	10%	−0.00033	0.920	82
10°C	14%	−0.00223	0.939	83
10°C	18%	−0.00681	1.311	91
10°C	25%	−0.02180	1.276	90
20°C	10%	−0.00039	0.927	82
20°C	14%	−0.01021	1.288	90
20°C	18%	−0.02017	1.375	92
20°C	25%	−0.05483	1.359	92

particularly those for the two higher moisture content treatments, clearly show the major problem with the Ellis and Roberts model. The data show systematic deviations from the fitted line, resulting in the overestimation of percentage viability after short storage periods, and the underestimation of the time to, say, 50% viability. For example, for the 5°C, 18% moisture content treatment, the Ellis and Roberts model underestimates the percentage viability between 300 and 500 d storage, and overestimates it for storage periods up to 150 d and beyond 600 d. Note also the variation in the estimates of the initial probit percentage viability obtained from each of the fitted curves (Table 3). Ellis and Roberts suggest the use of just one such fit to provide an alternative and 'better' estimate of the initial viability level than can be obtained from a simple germination test, but given the observed variability in these estimates, the reliability of a value so obtained is questionable.

This systematic deviation of the fitted curve from the observed data is primarily due to the constraints imposed by the probit curve, which must reach horizontal asymptotes at both 100% and 0% viability, with the shape of the curve being symmetrical about 50%. The choice of this model is based on the assumption that the times to death of individual seeds within a population stored under constant conditions follow a Normal distribution. However, the conditions prior to the start of storage are rarely constant, if known at all. It is therefore incorrect to assume that those seeds that are non-viable at the start of storage form part of the same distribution of times to death as those that are viable at this time. Given the lack of information about the viability state of these seeds at times prior to the start of storage, it seems more sensible to assume no information about the distribution of their times of loss of viability. One approach, as mentioned earlier, is to restrict the estimation of viability only to those that were viable at the start of storage (Wilson, McDonald and St. Martin, 1989). This approach, however, can cause problems, since the initial percentage viability is not known but only estimated. It is thus possible for observed levels of viability during storage to be greater than this estimated initial level, leading to re-scaled percentages greater than 100%, and the consequent problems of including these data values in the probit curve fitting.

An alternative approach can be developed by adapting the 'control mortality' probit model used in insecticide assays (Collett, 1991; Morgan, 1992; Fenlon, 1995), originally proposed by Finney (1971). This model allows for the possibility that even when a zero dose of insecticide is applied to a population of insects, some proportion of the population, c_M (referred to as the 'control mortality' level), will die. Thus the overall proportion of insects that die at a particular dose is the sum of the proportion, c_M, that die naturally, and a proportion p (given by the probit function) of the remaining proportion $(1 - c_M)$, a result sometimes known as Abbott's formula (Abbott, 1925). The parameter c_M is estimated from the data, and observed values less than the 'control mortality' can still contribute to the curve fitting.

For the problem being considered here we similarly introduce an extra parameter. The parallel between the insecticide model and the seed viability problem is more obvious if we consider the percentage of non-viable seeds rather than the percentage of viable seeds. Then we have a percentage of the seed population that is non-viable prior to storage with the percentage non-viability increasing with increasing storage period. Algebraically this model can be written as

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$$\%non\text{-}viability = 100\left(c_{NV} + \left(1 - c_{NV}\right)p\right) \quad (2)$$

where c_{NV} is the control non-viability, i.e. the non-viability of the seed lot at the start of storage, and

$$p = \Phi\left(a + bt\right) \quad (3)$$

where Φ( ) is the cumulative Normal function (i.e. the inverse of the probit function), t is the storage period, and a and b are, respectively, the intercept and slope parameters.

The direct comparison of this new 'control viability' probit model with the original Ellis and Roberts viability model requires it to be expressed in terms of the percentage of viable seeds rather than the percentage of non-viable seeds. The percentage viability is simply 100 minus the percentage non-viability, so

$$\%viability = 100 - 100\left(c_{NV} + \left(1 - c_{NV}\right)p\right) \quad (4)$$

can be re-written as

$$\%viability = 100\left(1 - c_{NV}\right)\left(1 - p\right) \quad (5)$$

Now

$$1 - p = 1 - \Phi\left(a + bt\right) \quad (6)$$

which can be re-expressed as

$$1 - p = \Phi\left(-a - bt\right) \quad (7)$$

since one minus the cumulative Normal function of a value is equal to the cumulative Normal function of minus that value. Then, writing $(1 - c_{NV})$, the control or initial proportion viability, as c_V, we have

$$\%viability = 100 \times c_V \times \Phi\left(A + Bt\right) \quad (8)$$

with $A = -a$ and $B = -b$.

The standard probit model used by Ellis and Roberts is a special case of this model, and is appropriate where 100% of seeds are viable prior to storage. To obtain it, first set $c_V = 1$ and divide both sides of the equation by 100

$$\frac{\%viability}{100} = 1 \times \Phi\left(A + Bt\right) \quad (9)$$

and then apply the probit transformation to the left-hand side of the equation rather than the cumulative Normal function to the right-hand side

$$\Phi^{-1}\left(\frac{\%viability}{100}\right) = A + Bt \quad (10)$$

where $A = k_i$ and $B = 1/\sigma$, and the left-hand side of the equation is the probit percentage viability (denoted 'v' in the notation used by Ellis and Roberts).

For the 'control viability' model, the initial percentage viability is obtained by setting $t = 0$ in equation 8, and is therefore calculated as $100 \times c_v \times \Phi(A)$. Where 100% of seeds are initially viable (i.e. $c_v = 1$), parameter A is equivalent to k_i, but where c_v takes a value less than one, the approximate relationship is

$$\Phi\left(k_i\right) = c_v \times \Phi\left(A\right) \quad (11)$$

The fitted 'control viability' probit curves for the data from the first carrot experiment are shown in Figure 3, with the fitted parameter values given in Table 4. Unlike the curves fitted using the original Ellis and Roberts probit model there is no indication of systematic deviation from the fitted curve for the 'control viability' probit model. In addition, the mean residual deviances for the 'control viability' probit model are mostly smaller, and never substantially larger, than the corresponding values for the Ellis and Roberts model (Table 4).

Model development – modelling of slope parameter

Having used our modified viability model to obtain good descriptions of the data for each treatment combination separately, the next step is to unify these descriptions by modelling the fitted parameters in terms of the storage temperature and moisture content values. In comparison with the original Ellis and Roberts (1980a) model, the 'control viability' model has an extra parameter to estimate. However, as with the original model, the only parameter that should be dependent on the storage conditions is the probit slope, B, the other two parameters being affected only by genotype, seed lot and pre-storage conditions.

In order for the initial percentage viability to be constant across all storage conditions, the values of both the control viability parameter, c_v, and the probit intercept parameter, A, must be kept constant across the range of storage conditions. Forcing the control viability parameter to be constant is relatively simple. For each of the probit curve fits shown in Table 4, the data from all assessment times was used in the estimation of the control viability parameter. As can be seen from the results in Table 4 a different parameter estimate is produced for each treatment combination. However, where sufficient information about the pre-storage viability is available from pre-storage germination tests, a single estimate of the control viability parameter can be obtained using only these data, hence removing this potential source of variability in the estimated initial percentage viability. A value for the control viability parameter can thus be obtained in an identical way to the initial percentage viability parameter of the original Ellis and Roberts model. For the first carrot experiment the estimated value of the control viability parameter was 0.83 (with a 95% confidence limit from 0.78 to 0.87).

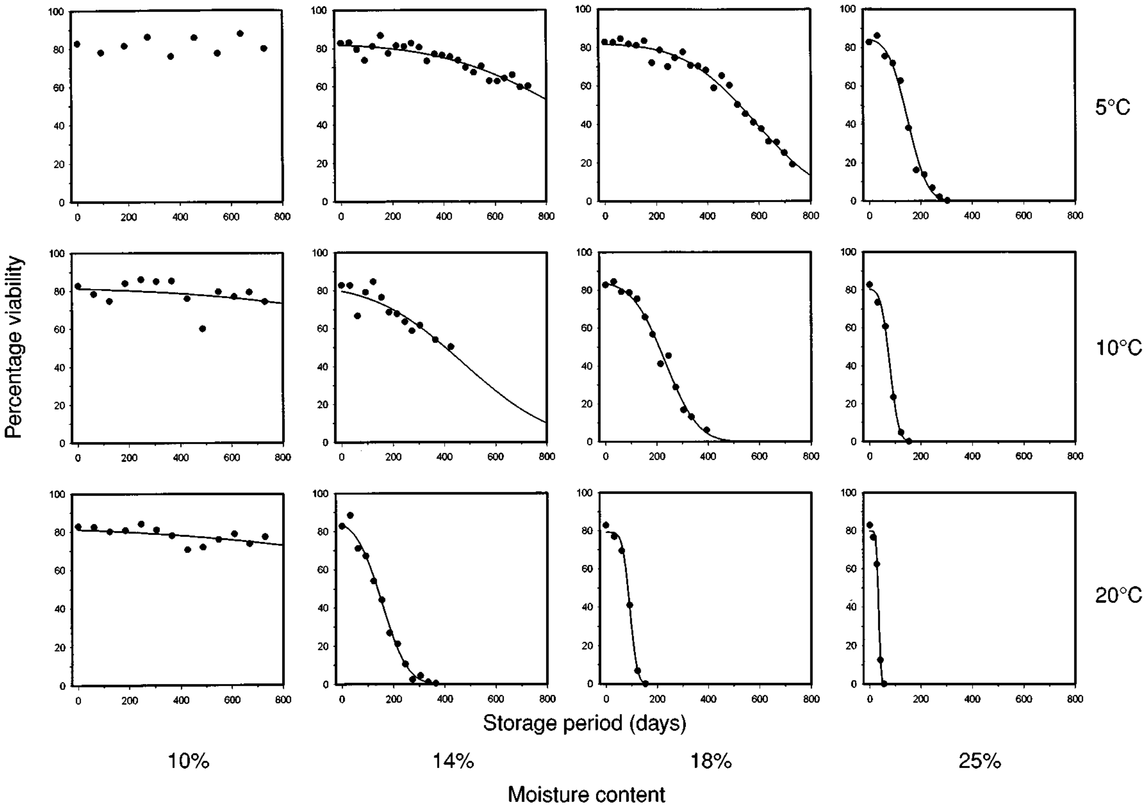

Figure 3. Fitted curves under the modified 'control viability' probit model (equation 8) for 11 of the 12 storage environments in the first carrot seed experiment. Plotted points are the means of 3 replicates, calculated on the probit scale.

Table 4. Parameters for the 'control viability' probit model (equation 8) fitted to results from the first carrot seed experiment (each treatment combination fitted separately), and comparison of mean residual deviances for the Ellis and Roberts (1980a) probit and 'control viability' probit models

Seed storage treatment		Parameters			Initial percentage viability	Mean residual deviance	
Temperature	Actual moisture content	c_v	B	A	$(100 \times c_v \times \Phi(A))$	Control viability	Ellis & Roberts
5°C	14%	0.8262	−0.00244	2.31729	81.8	1.367	1.507
5°C	18%	0.8186	−0.00464	2.72225	81.6	0.988	2.050
5°C	25%	0.8465	−0.01681	2.52205	84.1	1.586	3.343
10°C	10%	0.8263	−0.00115	2.13828	81.3	4.350	4.250
10°C	14%	0.8335	−0.00361	1.71292	79.8	2.475	2.414
10°C	18%	0.8443	−0.00985	2.29102	83.5	1.100	2.113
10°C	25%	0.8057	−0.03637	2.85355	80.4	0.677	3.692
20°C	10%	0.8346	−0.00094	1.89846	81.0	1.900	1.808
20°C	14%	0.8560	−0.01277	1.96029	83.5	1.635	1.935
20°C	18%	0.7915	−0.04428	4.08929	79.1	1.066	7.065
20°C	25%	0.7971	−0.13048	4.43815	79.7	0.881	8.348

Having fixed the control viability parameter to be constant across all storage treatments, we similarly need to fix the probit intercept parameter, A. A single parameter estimate for A can be obtained by

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Table 5. Observed and predicted probit slopes for the 'control viability' probit model for the first carrot seed experiment

Seed storage treatment		Probit slope parameter		
Temperature	Actual moisture content	Observed[1]	Predicted[2]	Residual mean deviance[3]
5°C	10%	−0.00001	−0.00147	3.155
5°C	14%	−0.00264	−0.00277	1.399
5°C	18%	−0.00417	−0.00524	9.573
5°C	25%	−0.01622	−0.01597	1.614
10°C	10%	−0.00164	−0.00259	6.214
10°C	14%	−0.00572	−0.00490	3.983
10°C	18%	−0.01028	−0.00925	2.789
10°C	25%	−0.03229	−0.02818	3.556
20°C	10%	−0.00171	−0.00807	307.800
20°C	14%	−0.01484	−0.01525	2.022
20°C	18%	−0.02889	−0.02883	2.431
20°C	25%	−0.07888	−0.08780	4.852

[1] Observed slopes, *B*, obtained by fitting equation 8 with the intercept parameter, *A*, constrained to be the same for all treatments
[2] Predicted slopes calculated using equation 14
[3] Residual mean deviances comparing observed response with that predicted by 'control viability' probit model (equation 8) with slopes calculated using equation 14

simultaneously fitting 'control viability' probit curves to all 12 treatments. Constraining the intercept parameter to be the same for all treatments and fixing the control viability parameter at the value estimated above, the slope parameters are then allowed to vary with treatment. For the first carrot experiment the estimated common value of the probit intercept parameter is 2.43 (SE 0.043). Combining the estimates of the probit intercept parameter and the control viability parameter gave an estimated initial percentage viability of 82%.

The estimated probit slopes obtained from the simultaneous fitting of the 'control viability' probit model to all 12 treatment combinations (Table 5) were then used to estimate the relationship between the probit slope parameter and the storage conditions. Ellis and Roberts (1980a) proposed two possible relationships between the standard deviation, σ (minus the reciprocal of the slope parameter), and the moisture content, M, and storage temperature, T.

$$\log(\sigma) = K_L - C_1 M - C_2 T \qquad (12)$$

$$\log(\sigma) = K_E - C_W \log(M) - C_H T - C_Q T^2 \qquad (13)$$

The choice of which equation to use is discussed in detail in Ellis and Roberts (1980a), the model with linear effects being recommended over small ranges of moisture content and storage temperature, and that with curvilinear effects for wider ranges. Two further equations can immediately be developed from the above with combinations of linear and curvilinear effects for the situations where one of the storage conditions varies over a wide range whilst the other varies over a narrow one.

Estimates of the parameters in these equations (either K_L, C_1 and C_2 or K_E, C_W, C_H and C_Q) are obtained by regressing the estimated slopes from the individual fits against the appropriate moisture content and storage temperature values. The arguments given by Ellis and Roberts (1980a) for the forms of these relationships are still valid for the modified 'control viability' model proposed here, and so the same approach can be used. As stated above, Ellis and Roberts' models were expressed in terms of the logarithm of the standard deviation of the distribution of deaths in time, but they can be equivalently expressed in terms of the logarithm of minus the reciprocal of the probit slope parameter, B.

None of the four models for storage temperature and moisture content fitted the slopes from all 12 treatment combinations particularly well, with particularly poor prediction of the slopes for treatments stored at 10% moisture content. However, since the final assessment of viability for these treatments was greater than 70%, the slope parameter estimates were unreliable. Omitting the data from these treatments resulted in all four models giving much better fits to the observed probit slopes. The fitted equation for the linear / linear form is given below, with the predicted probit slopes from this model given in Table 5.

$$\log\left(\frac{-1}{B}\right) = 8.683 - 0.1591M - 0.1136T \qquad (14)$$

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Models including a quadratic temperature term fitted slightly better than those with only the linear term, although one immediately apparent problem is that the coefficient of the quadratic temperature term is positive, whilst the arguments put forward by Ellis and Roberts (1980a) suggest that it should be negative. A possible explanation, also discussed by Ellis and Roberts, is that the range of temperatures tested in this experiment was not wide enough to show the true shape of the curvilinear response to temperature. Similarly, the models including a linear moisture content term fitted slightly better than those with a log-linear moisture content term, though these latter models gave better predictions of the probit slopes for the 10% moisture content treatments. A measure of the goodness-of-fit of the overall 'control viability' probit model to the data from this first experiment, for the linear / linear form of the probit slope model, is given by the mean residual deviances shown in Table 5. Of the 12 treatment combinations, only the slope for the 20°C, 10% moisture content treatment was not particularly well predicted, a pattern seen for all four of the model forms.

Model validation

Data from an independent carrot storage experiment were used to further demonstrate the appropriateness of the 'control viability' probit model, and to validate the models detailed above to describe the relationship between the probit slope and the storage conditions. As for the first set of data, both the original Ellis and Roberts model (equation 1) and the modified 'control viability' model (equation 8) were fitted separately to the data from each storage treatment in this second experiment. Again, systematic deviations from the Ellis and Roberts model were apparent, with the 'control viability' model generally fitting better (data not shown). As can be seen from Table 2, a wider range of storage conditions were tested in this experiment, some within the range tested in the first experiment, but many requiring extrapolation of the models to obtain predicted probit slopes.

Before assessing the adequacy of the four models for the probit slopes as functions of the storage conditions, estimates of the two seed-lot parameters, A and c_V are required. An estimate of the 'control viability' parameter, c_V, can easily be obtained from the results of a germination test prior to storage. A large test of 6800 seeds provided an estimate of 0.888 (95% confidence limit from 0.881 to 0.896) for the seed lot used in this second experiment. Obtaining an estimate of the probit intercept parameter, A, was less easy. One approach, suggested by Ellis and Roberts (1980a), is to fit a probit curve to the results from a rapid-deterioration test. Using the data from the most extreme of the storage treatments in this second experiment (60°C, 10% moisture content, zero viability reached after 3 d) we obtained a value of 3.71 (SE 0.359). A possible problem with this approach, as seen here, is that such an estimate may be fairly imprecise. If this approach is taken then the imprecision of this estimate should be allowed for in predicting the time to, say, 50% viability using the complete 'control viability' probit model. In this validation exercise we can obtain a more precise estimate by adopting the same approach as for the first experiment, simultaneously fitting 'control viability' probit curves to all treatment combinations, with the 'control viability' parameter fixed at the value calculated above, and the probit intercept parameter constrained to be the same for all treatments. For the second experiment the value of this combined intercept estimate is 3.52 (SE 0.038), obtained from a fit excluding the data for the 5°C, 10% moisture content treatment, since no 'control viability' probit fit could be obtained for this treatment. Combining this value with that obtained for the 'control viability' parameter gives an initial percentage viability for the seed lot used in this second experiment of 89%.

The estimated slopes obtained from the combined fit of the 'control viability' probit model (equation 8) to all treatments in the second experiment, together with the predicted parameter values from the linear / linear form for the slope model (equation 14), are given in Table 6. A measure of the goodness-of-fit of the 'control viability' probit model to the data from the second experiment, using the above estimates of the seed-lot parameters and equation 14 to calculate the slopes of the probit curves, is given by the residual mean deviances shown in Table 6.

Of the storage conditions tested in the second experiment, only six fell within the range of conditions in the first experiment (see Table 6). Of these interpolated storage conditions, for only one (10°C, 15% moisture content) was the probit slope poorly predicted. For this treatment, all four models gave predictions that seriously overestimated the observed slope, resulting in large mean residual deviances for the fits based on these predictions. In contrast, of the 11 extrapolated storage conditions, for only one (5°C, 10% moisture content) was the observed slope predicted well by more than two of the models. Slopes for the more extreme storage conditions, notably the high temperature treatments but also the high moisture content treatments, were particularly poorly predicted.

Discussion

The 'control viability' probit model developed in this paper has been shown to fit the data from both carrot

Table 6. Observed and predicted probit slopes for the 'control viability' probit model for the second carrot seed experiment

Seed storage treatment		Probit slope parameter		Residual mean deviance[3]
Temperature	Moisture content	Observed[1]	Predicted[2]	
5°C	10%	–	*−0.00147*	*1.519*
5°C	15%	−0.00257	**−0.00325**	**5.632**
5°C	20%	−0.00747	**−0.00721**	**1.838**
5°C	30%	−0.03486	*−0.03537*	*0.996*
10°C	15%	−0.00431	**−0.00574**	**25.61**
10°C	20%	−0.01254	**−0.01271**	**1.493**
10°C	30%	−0.04948	*−0.06241*	*13.89*
20°C	15%	−0.01505	**−0.01787**	**9.888**
20°C	20%	−0.04951	**−0.03960**	**13.10**
20°C	30%	−0.12932	*−0.1944*	*56.28*
40°C	15%	−0.47992	*−0.1733*	*139.4*
40°C	20%	−0.68033	*−0.3840*	*64.17*
1°C	40%	−0.02864	*−0.1102*	*644.6*
5°C	40%	−0.07086	*−0.1736*	*304.8*
10°C	40%	−0.07893	*−0.3064*	*827.3*
40°C	10%	−0.16735	*−0.07824*	*90.78*
60°C	10%	−2.8246	*−0.7588*	*168.1*

[1] Observed slopes, *B*, obtained by fitting equation 8 with the intercept parameter, *A*, constrained to be the same for all treatments
[2] Predicted slopes calculated using equation 14 (interpolated values shown in bold, extrapolated values shown in italics)
[3] Residual mean deviances comparing observed response with that predicted by 'control viability' probit model (equation 8) with slopes calculated using equation 14

experiments much better than the standard probit model on which the Ellis and Roberts equations are based. Systematic deviations, similar to those observed with the carrot data, are also apparent in the results published in Ellis and Roberts (1977) and Ellis and Roberts (1980a, Fig. 4), though there the response was plotted against a probit transformed axis. Where the initial percentage viability is close to 100% there will be little to choose between the two models. However, where this initial level is much lower than 100%, as with the two data sets analysed in this paper, then the 'control viability' model must be used in order to provide an accurate description of the loss of viability of stored seeds.

The lack of precision of the probit slope estimates obtained for treatments which had slow rates of deterioration indicates how important it is to monitor the viability of seeds under each set of storage conditions for sufficiently long to enable the accurate description of the viability response. The unreliable estimates produced when monitoring is completed too early, as with the 10% moisture content treatments in the first experiment, introduce problems in obtaining a satisfactory fit for the relationship between probit slope and storage conditions. Only with responses measured accurately over a wide range of storage conditions can a reliable relationship between the rate of loss of viability, as described by the probit slope, and the storage conditions be obtained.

One problem still to be resolved satisfactorily is the estimation of the probit intercept parameter, *A*. Obtaining a value, as suggested by Ellis and Roberts, by fitting a 'control viability' probit curve to data from a single rapid-deterioration treatment appears to give a fairly imprecise estimate. Incorporating this level of imprecision into predictions based on the model will lead to imprecise predictions of potential storage times. One alternative might be to use a series of rapid-deterioration treatments, estimating a common intercept value for the set of curves.

In conclusion, the 'control viability' probit model provides a better description of the loss of viability in storage for any individual storage environment than the Ellis and Roberts (1980a) improved model. With the provision of fitted curves for a wide enough range of storage conditions the relationship between the rate of loss of viability and storage conditions could be reliably estimated. Combining this relationship with the 'control viability' probit model will provide a useful tool for predicting the longevity of stored seeds.

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