Seed ageing, survival, and the improved seed viability equation; forty years on

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Invited Review

Seed ageing, survival and the improved seed viability equation; forty years on

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Abstract

Seed Science and Technology published 'The quantification of ageing and survival in orthodox seeds' four decades ago. That paper explained the improved seed viability equation, which quantifies seed longevity in response to storage environment, and the implications for seed ageing and seed quality, including likely seedling emergence. Developments in seed science, and anhydrous biology more widely, associated with that well-cited paper are summarised here.

Keywords: ageing, longevity, moisture content, seed, survival, temperature, viability

Introduction

The first volume of *Seed Science and Technology* included the paper that introduced the terms orthodox and recalcitrant to distinguish between species with contrasting seed storage behaviour (Roberts, 1973). The former category describes the common situation in plants where the longevity of mature seeds is improved in a predictable manner by reduction in the moisture content and/or temperature of seed storage. That 'predictable manner' was the subject of a further paper in *Seed Science and Technology* eight years later entitled 'The quantification of ageing and survival in orthodox seeds' (Ellis and Roberts, 1981a), hereafter the 1981 paper.

The 1981 paper was based upon an invited presentation to the Triennial Congress of the International Seed Testing Association (ISTA) held in Vienna, Austria, in 1980. It had two strands. First, it outlined the then new, improved seed viability equation developed to

estimate seed survival period in response to hermetic storage in (constant) temperature and moisture content regimes (Ellis and Roberts, 1980a, b). Second, it positioned that equation in the wider context of seed ageing and seed quality. Seed producers seek to maximise, and seed suppliers safeguard, the latter, and the 1981 paper developed earlier ideas on this topic concerning the several different facets of seed quality (Ellis and Roberts, 1980c), or seed vigour which ISTA had then recently defined (Perry, 1978).

Above all, the 1981 paper strove to link (variation in) individual seed lifespans with the longevity of the (homogeneous) seed population (seed lot) from which individual groups of seeds are sampled. More specifically, the paper presented wide-ranging evidence that the proportion of seeds within a homogeneous seed population that had aged so considerably that they had lost viability, when sampled and tested in a standard germination test, provided an indicator of the ageing accumulated within those seeds within that population that remained viable (and so their range of potential performance). I summarise subsequent developments from the 1981 paper here.

Seed longevity and storage environment

The improved seed viability equation was a development from the approach of Roberts (1960) in which the seed survival curve (loss in viability over the period stored in a constant, hermetic environment) was described by a negative cumulative normal distribution; and semi-logarithmic relations between the mean viability period (for example, the time taken for viability to be reduced to 50% if the original viability was 100%) and each of seed storage moisture content and temperature. That worked well for the seed lot from which it was derived and over limited ranges of seed storage temperature and moisture content; for example, 25-45°C and 12-18% moisture content in single seed lots of barley (*Hordeum vulgare* L.), broad bean (*Vicia faba* L.), and pea (*Pisum sativum* L.) (Roberts and Abdalla, 1968). Roberts (1972) pointed out three problems with his equation, however. First, it was only applicable to the seed lot from which the parameters of the responses had been derived. Second, it was unclear if it could be applied over wide ranges of moisture content. Third, it was unlikely that extrapolation to more extreme temperatures (whether high or low) was accurate.

The improved seed viability equation (Ellis and Roberts, 1980a) comprises two equations. The first

$$v = K_i - p / \sigma \tag{1}$$

estimates probit percentage viability (v) after p days in storage, where K_i is a constant for the seed lot (equivalent to initial probit viability) and σ is the standard deviation of the frequency distribution of seed deaths in time (days). The second equation

$$\log_{10} \sigma = K_E - C_W \log_{10} m - C_H t - C_Q t^2 \tag{2}$$

quantifies the effect of seed storage moisture content (m, % fresh weight) and temperature (t, °C) in constant, hermetic environments on seed longevity (σ) where K_E, C_W, C_H and C_Q are species constants (Ellis and Roberts, 1980a). The value σ is a particular period of

longevity (for example, the period from 97.7 to 84.1% or from 84.1 to 50% viability), common to both equations. These two equations can be combined by replacing σ in (1) with the antilogarithm of $(K_E - C_W \log_{10} m - C_H t - C_Q t^2)$, viz.

$$v = K_i - p / 10^{K_E - C_W \log_{10} m - C_H t - C_Q t^2} \tag{3}$$

The improved seed viability equation demonstrated unequivocally that there are continuous relations across wide ranges of each of temperature and moisture content with seed storage longevity; and provided a tool to provide quantitative advice on seed storage. Its publication predated the advent of personal, desktop computers (and, for example, spreadsheet software). To make the improved seed viability equation easier to apply with the appropriate viability constant values to derive estimates of longevity, the seed viability nomograph was constructed, for barley in the first instance. This is reproduced here (figure 1). There are two features of the seed viability nomograph, beyond the ease of estimating longevity using only a ruler, worth highlighting. First, it is possible to solve for any one of the five variables (longevity, initial viability, final viability, storage temperature, moisture content), not just longevity, if the remaining four variables are fixed; or, for example, to identify the many different combinations of seed storage temperature and moisture content that would provide a similar loss in viability over a defined storage period. Second, a slight "shake" of the ruler on the temperature and moisture content scales (A and B, respectively, in figure 1) provides a dramatic change on the scale for σ (C, longevity). This is because of the logarithmic scale for σ. This exemplifies the high sensitivity of seed longevity to comparatively small changes in the storage environment.

Figure 1 represents old technology. An online resource is available which enables the improved seed viability equation to be applied to estimate longevity, final viability, storage temperature, or moisture content using either user-provided estimates of the seed viability constants or those published for many species: the Seed Information Database (Royal Botanic Gardens Kew, 2022).

Temperature and longevity
Seeds can be exposed to a wide range of temperatures after harvest, from sub-zero temperatures close to -20°C in genebanks, through a wide range of "natural" storage regimes (typically 0-30°C, but sometimes wider) to those in heated-air seed dryers operating at temperatures as high as 70°C (or even 90°C for very brief periods). Analyses over limited temperature ranges provided a constant Q_{10}, the temperature coefficient for change in rate of loss in seed viability per 10°C rise in temperature. Comparisons of values calculated at warm temperatures, however, were always lower than those calculated at hot temperatures (Ellis and Roberts, 1980a; 1981a). The improved seed viability equation modified the negative semi-logarithmic relation between longevity and temperature of Roberts (1960), which provides a constant Q_{10}, by adding a negative quadratic term (Ellis and Roberts 1980 a, b). Hence, the improved seed viability equation accepted that Q_{10} was not constant but increased in value the warmer the temperature. This approach has not only been shown to work well across a wide range of temperature (from -13 to +90°C), but it has also identified that the relative effect of temperature on air-dry seed longevity is invariant amongst many contrasting species (Ellis *et al*., 1982; Dickie *et al*., 1990; Ellis and Hong, 2007a).
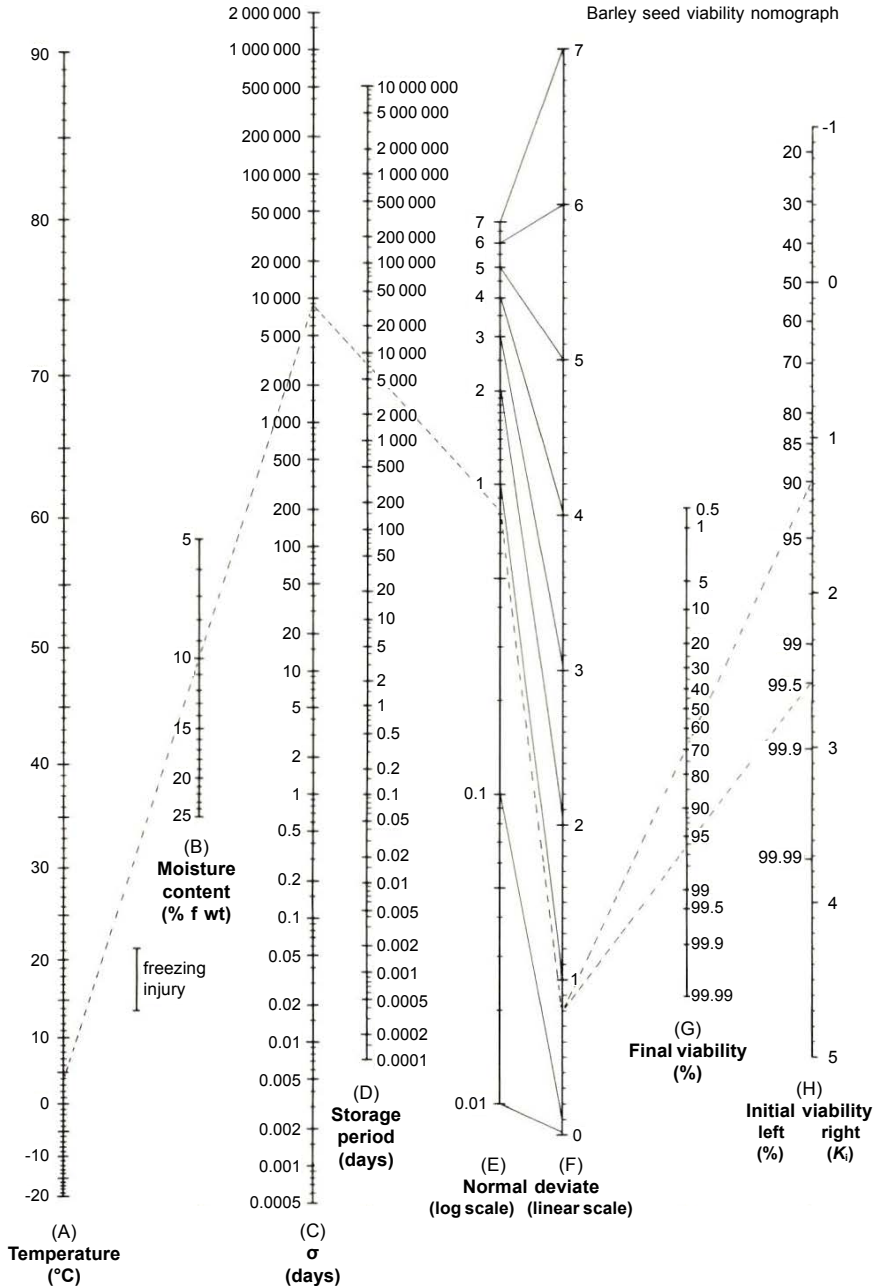
Figure 1. Seed viability nomograph for barley (*Hordeum vulgare* L.). This solves the improved seed viability equation (3) where $K_E = 9.983$, $C_W = 5.896$, $C_H = 0.040$, and $C_Q = 0.000428$. Calculations are made by straight lines (e.g., using a ruler) across groups of three neighbouring variables (scales, **A**, **B**, **C**; or **C**, **D**, **E**; or **F**, **G**, **H**). The broken lines show an example calculation to predict viability (scale **G**) after hermetic storage for 20 years (scale **D**) at a constant temperature of 4°C (scale **A**) with 10% moisture content (scale **B**) for two different seed lots, one with 90% and one with 99.5% initial viability (scale **H**). From Ellis and Roberts (1980a; 1981a).

Whilst the temperature term $(- C_H t - C_Q t^2)$ in the improved seed viability equation demonstrated a continuous relation between longevity and temperature across a wide range, the inclusion of the quadratic term implies that an optimum value of seed storage temperature (at which longevity is calculated to be maximal) can be provided by extrapolation. This extrapolated value is estimated to be -67°C from the estimates of C_H and C_Q derived for lettuce (*Lactuca sativa* L.) by Kraak and Vos (1987) or -47°C for those shown in figure 1 for barley. There is no evidence to support such extrapolation, however. The coolest temperatures to which the improved viability equation has been applied successfully, provided moisture contents are dry enough to avoid freezing damage, are -10°C (Kraak and Vos, 1987) or -13°C (Dickie *et al*., 1990).

Oxygen, moisture content and longevity
All models are simplifications of the real world. The improved seed viability equation was developed from observations in hermetic storage. It does not account for the effect of oxygen on seed deterioration. Despite this, the equation together with the species-specific viability constant values has been applied successfully to estimate the survival of different seed lots in hermetic storage (e.g. Ellis and Roberts, 1981b) and also in open storage (e.g. Fabrizius *et al*., 1999).

There is substantial interaction between the effects of moisture and oxygen on seed longevity, however. Moreover, the negative logarithmic relation between seed moisture content and longevity is subject to limits at high (Ibrahim and Roberts, 1983; Ibrahim *et al*., 1983) and low moisture contents (Ellis *et al*., 1988). In essence, the negative logarithmic relation between moisture content and longevity in air-dry storage applies across seed water potentials between about -350 and -14 MPa (Roberts and Ellis, 1989).

The upper limit coincides with the threshold for respiration and equates to seed moisture contents between 15 and 28%, varying with seed oil content. Providing sufficient oxygen is available for respiration, seed longevity increases with increase in water potential above this value providing considerable longevity in fully-imbibed seeds if germination is prevented by dormancy, whereas in the absence of oxygen there may be a slight further decline in longevity with increase in water potential until longevity reaches a minimum value (Ibrahim and Roberts, 1983; Ibrahim *et al*., 1983; Roberts and Ellis, 1989). Hence the benefit of oxygen to seed survival at very high moisture contents is considerable and there is good evidence that a certain degree of damage to seeds during air-dry storage can be reversed if the seeds are exposed to oxygen at very high moisture contents (Villiers and Edgecumbe, 1975; Ward and Powell, 1983; Butler *et al*., 2009a).

In contrast, the damaging effect of oxygen on seed longevity at low moisture contents in air-dry storage can be considerable (in some circumstances). The magnitude of the damage from oxygen depends upon the moisture content of seed in air-dry storage (Ellis and Hong, 2007b). Seed longevity in both open and hermetic storage is similar at high moisture contents within the air-dry range; reducing seed storage moisture content improved longevity in both cases, with a negative logarithmic relation between these variables; but the gradient (C_W in equation (2)) was far shallower in open storage with longevity at low moisture contents far less in open than in hermetic storage (Ellis and Hong, 2007b).

The low-moisture-content limit to the negative logarithmic relation between seed moisture content and longevity varies between about 2 to 6% moisture content with little or no effect on longevity from drying below this value in hermetic storage (Ellis *et al.*, 1988, 1989, 1990a). The value of this limit varies amongst species depending on seed oil content and coincides with the shoulder of the seed moisture content – relative humidity isotherm, where almost all of the water bound at weak sites has been removed by desiccation and the water remaining within the seeds is bound at strong sites (Roberts and Ellis, 1989). The value of the low-moisture-content limit to negative logarithmic relationships between seed longevity is slightly greater the cooler the storage temperature, increasing by about 1.5% moisture content with a 35°C reduction in seed storage temperature in one study (Ellis and Hong, 2006).

Estimates of C_W vary considerably amongst species, with higher values detected for those with starchy than those with oily seeds (Ellis and Roberts, 1981a; Dickie *et al.*, 1990) although C_W may show some variation amongst species with similar seed oil contents (Demir *et al.*, 2011). The variation in C_W reflects differences in moisture sorption isotherms amongst the species: if the logarithmic relation between longevity and moisture content in equation (2) is replaced by a semi-logarithmic relation between longevity and equilibrium relative humidity then different species provide similar slopes (Roberts and Ellis, 1989).

Orthodox, intermediate, and recalcitrant seed storage behaviour
Defining the lower and upper moisture content limits to the improved seed viability equation has been helpful in distinguishing amongst contrasting patterns of seed storage behaviour. Roberts (1973) introduced the two terms orthodox and recalcitrant to distinguish between species with contrasting seed storage behaviour. Orthodox seeds showed storage behaviour which obeyed the rules of the improved seed viability equation (longevity improved in a predictable manner by reduction in seed storage moisture content and temperature). Recalcitrant seeds, on the other hand, were so defined because their longevity in different environments failed to conform to the seed viability equation. The latter category typically comprised species with seeds that were damaged by even limited desiccation, behaving very much like the parent plants.

However, seeds of certain species were known to survive some desiccation but nevertheless deteriorate quickly in some (typically drier and/or cooler), but not all, air-dry storage environments. Once the lower and upper moisture content limits to the improved seed viability equation had been defined in orthodox seeds, essentially the wide range of moisture contents encompassed by variation in the water bound at weak sites (Roberts and Ellis, 1989), it became clear that there was a group of species with seeds that did survive desiccation to higher moisture contents within the air-dry range and could show considerable longevity under such conditions. For example, seeds of *Coffea arabica* L. survived both considerable desiccation to around 9% moisture content, a water potential of c. -90 MPa, and 12 months' subsequent hermetic storage at 15°C, but lower moisture contents and/or cooler temperatures increased the rate of deterioration during seed storage (Ellis *et al.*, 1990b). Clearly this group of species did not meet the definition of orthodox seed storage behaviour; they did not show the continuous relations between seed storage

longevity and wide ranges of moisture content and temperature. Nonetheless, the seeds could survive in certain well-defined air-dry storage environments. They were therefore classified as showing intermediate seed storage behaviour, i.e. intermediate between the orthodox and recalcitrant categories (Ellis *et al*., 1990b; 1991a, b, c; Hong and Ellis, 1996; Lima *et al*., 2014).

The Seed Information Database (Royal Botanic Gardens Kew, 2022) provides a list of seed storage behaviour for over 9,500 species. This online list was developed from an earlier account for around 7,000 species by Hong *et al.* (1996). That publication provides further information on approaches to and difficulties in assessing seed storage behaviour.

A continuum in desiccation tolerance and seed storage behaviour across species has also been suggested (Pammenter and Berjak, 1999; Walters, 2015) with, for example, marked differences amongst the many species with recalcitrant seeds with three subdivisions of highly-, moderately- and minimally-recalcitrant behaviour (Farrant *et al*., 1988); and similarly amongst species with orthodox seeds (e.g. Ellis *et al*., 2018; 2019; Hay *et al*., 2022); as well as amongst the orthodox, intermediate and recalcitrant categories. Moreover, the developmental status of and method of drying also affects seed desiccation tolerance and longevity in storage (Pammenter and Berjak, 1999). Certainly, not only do both desiccation tolerance and longevity in air-dry storage change during seed development and maturation in orthodox (e.g., Demir and Ellis, 1992a) and recalcitrant species (e.g. Hong and Ellis, 1990), but desiccation tolerance to very low moisture contents can be delayed or even lost during the development and maturation of orthodox seeds in stressful environments (Ellis and Hong, 1994) or afterwards during the early imbibition of dried seeds (Hong and Ellis, 1992). These considerations do not affect the utility of the three categories of seed storage behaviour, however. In suitable environments, orthodox seeds can survive long-term storage, intermediate seeds medium-term storage, and recalcitrant seeds short-term storage even though within each category species may differ greatly in seed longevity. Knowledge of the predictable survival of mature orthodox seeds in the air-dry state is applied widely by humankind; not only throughout agriculture, horticulture and forestry in crop species, but also in the *ex situ* conservation of wild plant species (e.g., Chapman *et al*., 2019) with over 90% of the world's seed plants producing desiccation-tolerant seeds (Wyse and Dickie, 2017).

Seed survival curves
Equation (1) quantifies the seed survival curve as a negative cumulative normal distribution in a constant storage environment where the distribution of seed deaths in time is normal with standard deviation σ (days). This is the same assumption as the original viability equation of Roberts (1960). It can be fitted by probit analysis. It is important to note this seed-to-seed variability within an homogenous seed population (or seed lot): if individual seeds had identical longevity (no variation amongst the individual seeds) then they would all die at the same time in the same storage environment and tests of seed lots would provide either 0% or 100% viability only, with no intermediate values, and the shape of the seed survival curve would be rectangular. Several improvements to equation (1) or the methods used to fit the viability equations have been suggested.

Germination tests are typically used to estimate seed viability after different periods of experimental storage to provide the data that can then be subjected to probit analysis, but germination tests to assess viability can be confounded by seed dormancy. This problem can be reduced by using dormancy-breaking treatments immediately before or during the germination test procedure, but since dormancy is reduced during air-dry storage it can be the case that attempts to define the seed survival curve end up defining a combination of loss in dormancy as well as loss in viability. To account for loss in both dormancy and viability occurring simultaneously during storage, Kebreab and Murdoch (1999) introduced the following multiplicative probability model to quantify loss in dormancy:

$$g = 100 \times [\Phi^{-1} (K_d + \beta_l p)] \times [\Phi^{-1} (K_i - (p/\sigma)] \tag{4}$$

where g is ability to germinate normally (%), K_d is initial ability to germination (i.e. non-dormant seed) in normal equivalent deviates (NED), β_l is loss in dormancy (NED day^{-1}), Φ^{-1} is the cumulative normal function (i.e. the inverse of the probit function), with K_i, p, and σ as equation (1). This approach has been shown to work well with seeds of weeds (Kebreab and Murdoch, 1999) and crops (Whitehouse *et al*., 2018).

Mead and Gray (1999) modified equation (1) to account for the proportions of dead or empty seeds in the original seed lot as follows:

$$\% \textit{Viability} = 100 \times c_V \times \Phi^{-1} [K_i - (p/\sigma)] \tag{5}$$

where c_V is 'control viability'. This type of approach in which all observations are converted by a common factor so that the value at zero time is 100% had been used before (e.g. Roberts and Abdalla, 1968), but rather than relying on a single original germination test to provide the estimate of c_V, Mead and Gray (1999) incorporated estimation of c_V into probit analysis (thereby including all sample test results into its estimation). This modification worked well with seed storage data of carrot (*Daucus carota* L.) with no systematic deviation from fitted survival curves and smaller error overall.

The statistical software available to researchers has improved greatly since the improved viability equation was first developed. In the 1970s, it was necessary to use a stepwise approach to fitting the improved seed viability equation: estimates of longevity were first obtained by probit analysis for each separate storage environment in accordance with equation (1); these estimates of longevity were then subjected to multiple regression analysis in accordance with equation (2) (Ellis and Roberts, 1980b). Hay *et al*. (2003) applied the FITNONLINEAR directive in GenStat to fit the improved viability equation, together with the inclusion of the control viability term, in a single analytical step to seed survival data for *Arabidopsis thaliana* (L.) Heynh. They showed that their approach accounted for the full variability of the data set and provided smaller standard errors for the estimates of the viability constants – and hence the prospect of less error in predicting seed longevity.

Fluctuating storage environments
Many seed storage environments are not constant, although of course hermetic storage can maintain seed moisture content. The improved seed viability equation can accommodate a change from one constant environment to another by calculating a new estimate of

K_i at the start of the period of storage in the new environment. In other words, the estimate of σ is a function of the current storage environment only, with no effect of change in environment *per se* (Ellis and Roberts, 1981a; Hung *et al*., 2001), provided the environment doesn't transgress the moisture content limits.

This approach is unhelpful where the environment fluctuates many times, however. The relations between environment and longevity are not linear, see equation (2). Hence, calculating mean moisture content and/or mean temperature under-estimates longevity in a fluctuating storage environment (Hung *et al*., 2001). Instead, the effective value of, for example, temperature should be calculated to account for the precise relation between storage environment and longevity. Hung *et al*. (2001) provided an example table of effective temperatures where 12 hours day^{-1} was spent in each extreme of a variable temperature storage environment; for example, for 12 hours at 5°C and 12 hours at 35°C each day the effective temperature is 30.3°C (in other words, greatly above the mean of 20°C). The practical importance of this to commercial seed storage is clear: minimise storage temperature, of course, but especially the maximum temperature in fluctuating temperature conditions.

Predicting seed longevity
Models serve to summarise and quantify our understanding (to date) and to educate – but may also be used to predict and so identify where gaps in knowledge remain. A good example of the latter for the seed viability equation is the omission of terms to quantify the effect of oxygen and interaction with that of moisture (see above). As Hay *et al*. (2003) have noted, estimates of the viability constants with a large standard error may well lead to inaccurate model predictions. Moreover, equation (3) comprises five constants (K_E, C_W, C_H, C_Q and the seed lot constant, K_i) and there is also the potential for error in defining the temperature and moisture content of storage and maintaining these at constant values.

When the improved viability equation was developed it was shown that the constants for barley were able to predict (retrospectively) mean viability periods for independent data for one seed lot in nine different environments and for two further seed lots (of different varieties) in five different environments (Ellis and Roberts, 1980a). A further comparison of predictions for 36 complete seed survival curves in barley (six seed lots, each in six different environments) showed good or excellent agreement for 17 curves, and reasonable agreement but with some under-prediction of actual seed survival in the remaining 19 (Ellis and Roberts, 1981b). Similarly, estimates of the viability constants for onion (*Allium cepa* L.) were able to quantify well independent observations for the survival curves of five of six seed lots stored in one environment and of a further seed lot stored in five different environments (Ellis and Roberts, 1981a); and independent studies in pepper (*Capsicum annuum* L.) showed good agreement between predicted and actual loss in seed viability during storage (Demir *et al*., 2009).

Hence, there was a strong case for the viability constant values K_E, C_W, C_H and C_Q to be invariant within a species (Ellis and Roberts, 1980a, 1981a), and for C_H and C_Q to be invariant across orthodox species (Ellis and Roberts, 1981a; Dickie *et al*., 1990; Ellis and Hong, 2007a), and so for σ to be the same for different seed lots of one species stored in an identical environment. However, different estimates of K_E, or of σ in a common

environment, have been reported amongst different seed lots (e.g., Zewdie and Ellis, 1991; Ellis *et al*., 1992; Hay *et al*., 1997, 2003; Lyall *et al*., 2003). One of the largest comparisons of predictions from the improved viability equation against independent observations has been provided in wheat (*Triticum aestivum* L.). The longevity of 285 seed lots of wheat (produced in three different years in different regimes and harvested at different stages of development) in hermetic storage at 40°C with 14.1-15.7% moisture content was compared with independent estimates from the seed viability equation by Yadav and Ellis (2018). Half of the seed lots provided good agreement with the estimates of longevity (σ) from the viability equation whereas some (most of those stored at 14.1-14.8% moisture content) survived longer than predicted.

Caution should always be applied therefore in placing excessive reliance on the estimates of longevity from the improved viability equation and on the assumption that σ has the same value for different seed lots stored in an identical environment. Ellis *et al*. (2018; 2019) provide good examples of very many seed lots where σ did have the same value within each of 32 genera in one environment during long-term storage but where in a further 29 genera this was not the case (in some genera no loss in viability was detected, but in other genera loss in viability was probably confounded with loss in dormancy [Poaceae] or loss in hardseededness [Fabaceae] during storage).

Seed quality and crop establishment

The 1981 paper provided the following successive foundations to link the survival of the seed lot with wider aspects of seed quality:
    a.   individual seeds age during post-harvest storage, declining in individual per-formance (if sown), before they eventually die;

    b.   the time course of decline in one constant environment is similar for all individual seeds within a homogenous seed lot;

    c.   but all seeds die after different periods of ageing in one storage environment, this being normally distributed [equation (1)];

    d.   hence at any one point in time all the individual seeds undergoing ageing within a homogenous seed lot are at different stages on the same pathway of decline and so can differ in performance if sown at that time;

    e.   and the cumulative normal distribution can be applied to quantify these aspects of variation in the performance (if sown) of individual seeds within a seed lot, and between seed lots.

A detailed demonstration of the above was provided for the defective phase, the phase preceding seed death during which the live seeds are no longer capable of germinating normally and instead produce abnormal seedlings – see figures 10-12 in Ellis and Roberts (1981a). The wider implications for eight different aspects of seed decline within different seeds' lifespans within one seed lot was also presented – see figure 18 in Ellis and Roberts (1981a). There are three consequences of the above that I emphasise here.

Seed quality development

Although different aspects of seed quality are associated, the relationships are often curvilinear and thus estimates of one aspect of seed quality may be more sensitive to differences in seed quality than another (Ellis and Roberts, 1981a). For example, the onset and development of ability to germinate and tolerate desiccation during early seed development shows that germination test results are well able to distinguish differences in seed quality then. However, once 100% ability to germinate is approached the germination test cannot detect the subsequent improvement in seed quality that occurs – whereas field emergence ability and seed storage longevity do (figure 2). Moreover, as the seeds mature yet more and maximum quality is approached longevity is better able to detect further improvement than field emergence. In this example there was a narrow peak of maximum quality before it declined but this was a much wider period, a plateau, in a warmer year (Pieta Filho and Ellis, 1991a) and, for example, in a fleshy-fruited crop (Demir and Ellis, 1992b).
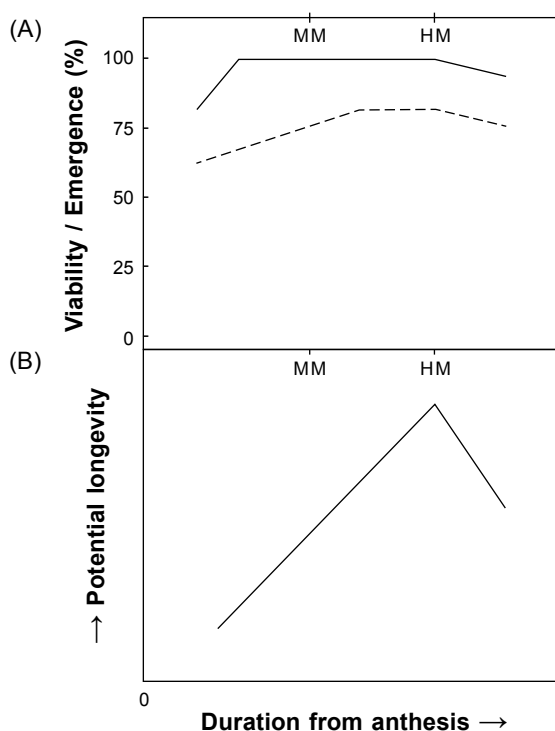

Figure 2. Comparison of outline temporal patterns of seed quality development and decline during seed development and maturation (duration from anthesis) amongst three different methods of assessing seed quality (abridged from results for barley seeds produced in 1988 (Pieta Filho and Ellis, 1991a, b)): ability to germinate in standard laboratory tests (panel **A**, continuous line); emergence of seedlings from the field seedbed (panel **A**, dashed line); longevity in air-dry hermetic storage (panel **B**). MM = mass maturity; HM = harvest maturity. From Ellis (2019).

The use of longevity as a more sensitive test of seed quality than ability to germinate amongst good quality seed lots in investigations of seed development and maturity has shown that: there are different temporal patterns of seed quality improvement amongst environments as well as amongst species (Ellis, 2019); that it may be possible for seed quality improvement to be resumed after harvest (Butler *et al.*, 2009a, b); and that warmer seed drying temperatures can benefit the longevity of seed harvested moist (Whitehouse *et al.*, 2018).

Seed versus seedling vigour

I have limited use of the term seed vigour until now, mainly because its definition (Perry, 1978) includes the consequences of factors beyond seed ageing, particularly seed health and physical damage to seeds. For example, the phenomenon of imbibition injury in grain legumes such as pea (*Pisum sativum* L.) which reduces seedling emergence in the field, particularly in cold and wet seedbeds, is a consequence of physical damage (Powell and Matthews, 1978).

Seedling emergence and crop establishment

The 1981 paper outlined how ageing of individual seeds within a seed lot would be expected to affect seedling emergence under stress in the field (where the word stress means an environment poorer than the optimum conditions of a laboratory germination test). It showed that a given stress in the seedbed which resulted in different field emergence (percentage of seeds sown that established seedlings) and also in different relative field emergence (percentage of viable seeds sown that established seedlings) was a constant difference between the probit values of each of laboratory germination and field emergence (figure 16 in Ellis and Roberts, 1981a). This approach has subsequently been confirmed in further investigations (Khah *et al.*, 1986; Ellis and Dolman, 1988; Wheeler and Ellis 1992a).

To determine an appropriate seed sowing rate to establish a crop with the desired plant population density also requires farmers to forecast conditions in the seed bed in advance. Whilst it is not possible to predict soil temperature and moisture accurately in advance, it is often possible to anticipate if seedbed conditions will be comparatively good or poor. Table 1 provides examples of such an approach to indicate how seed sowing rates in three crops might need to be adjusted to cope with various combinations of different quality seed lots and different seedbed conditions using the approach outlined in the 1981 paper. This approach was also outlined in diagrammatic form (figure 3), based on results for onion (*Allium cepa* L.) in the UK, for use by growers to input their own data for seed lot viability and emergence (Wheeler and Ellis, 1992b). Table 1 and figure 3 illustrate why it is advisable to sow better-quality seed lots for sowing in poorer seedbeds and restrict poor-quality seed lots to sowing when good seedbed conditions are expected. For example, the combination A3 with B5 in figure 3 will provide a superior outcome for growers than A1 with B4.

Crop yield is affected by plant population density and so reduced emergence due to poor seed quality can affect yield unless seed sowing rates are adjusted accordingly. The curvilinear relations between yield and plant population density are unlikely to be affected

by differences in seed quality above the minimum germination level for sale, however, at least in UK broadacre crops (Khah *et al*., 1989). The exception might be if sowing is delayed in short-duration crops sown late because seedling emergence is slower from lower viability seed lots (Khah *et al*., 1986). Hence where seed lot viability is reduced by ageing the surviving seeds show reduced performance in that they are slower to germinate and the seedlings slower to emerge, but subsequent surviving seedling growth rates appear to be unaffected by ageing (Khah *et al*., 1989; Wheeler and Ellis, 1991). In other words, ageing reduces seed vigour but not seedling vigour (though seedling vigour may be damaged by other causes of poor seed quality).

Table 1. Guide to likely field emergence (%) of spring wheat (*Triticum aestivum* L.), winter oilseed rape (*Brassica napus* L.) and onion (*Allium cepa* L.) seed lots differing in viability (laboratory test result) if sown at Reading into seedbeds when poor or good conditions are expected. (From Khah *et al*., 1986; Ellis and Dolman, 1988; Wheeler and Ellis, 1992b).

Seed lot viability (%)	Field emergence (%)					
	Spring wheat		Winter oilseed rape		Onion	
	Poor[1]	Good[1]	Poor[2]	Good[2]	Poor[3]	Good[3]
70[4]	–	–	–	–	23	54
80[4]	–	–	–	–	33	66
85	29	67	51	70	40	73
90	37	75	61	78	50	80
95	52	85	74	87	64	89
98	67	93	85	94	84	95

[1] The difference between probit percentage emergence and probit percentage viability was 0.6 in good or 1.6 in poor spring seedbeds at the University's experimental site.

[2] The difference between probit percentage emergence and probit percentage viability was 0.5 in good or 1.0 in poor late summer seedbeds at the University's experimental site.

[3] The difference between probit percentage emergence and probit percentage viability was 0.4 in good or 1.3 in poor spring seedbeds at the University's experimental site.

[4] The minimum germination level for sale in the UK is 70% for onion, but 85% for the other two crops. Hence, estimates of seedling emergence provided for onion only at 70 and 80% viability.

Seed testing implications

The main consequence of the 1981 paper for seed testing in the seed supply chain will hopefully have been to support the education and training of seed analysts, seedsmen and seedswomen. For my part, I used the 1981 paper to provide a framework for my teaching of seed science to undergraduates and postgraduates across subsequent decades. Beyond the somewhat vague and optimistic phrase of "improved understanding", I suggest three principal implications for seed testing arose from the 1981 paper. They relate to the seed testing year; minimum germination levels for sale; and seed lot integrity.
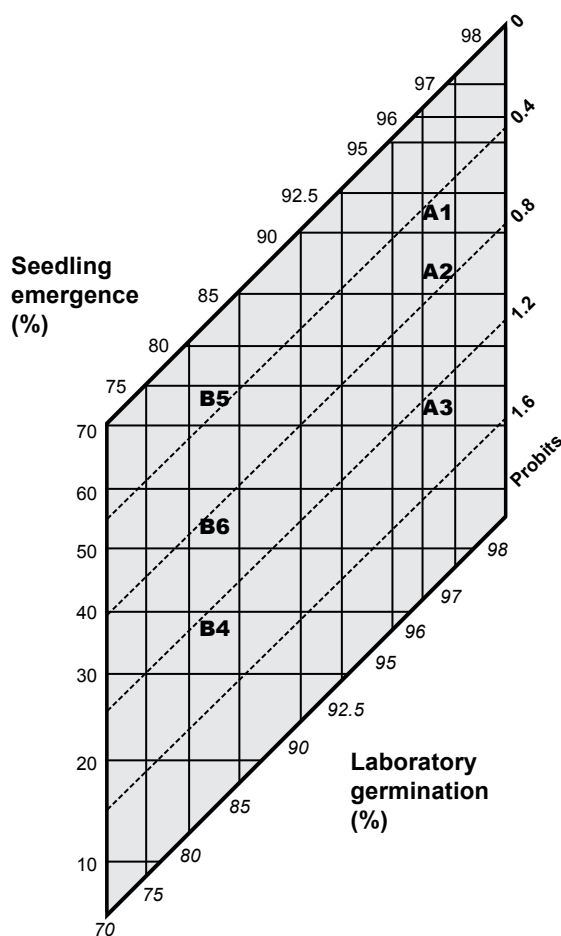
Figure 3. Rough guide to calculating likely seedling emergence (%, left-hand and top axes) of seed lots differing in ability to germinate in standard laboratory tests (%, bottom axis) in contrasting seedbed conditions. The diagonal lines represent the difference (right-hand axis) between probit percentage laboratory germination and probit percentage seedling emergence. A1, A2, A3, B4, B5, B6 represent (hypothetical) results for two seed lots (A and B) sown in six different seedbed environments (from 1 or 5 [best], through 2 or 6, to 3 or 4 [poorest]). In the best environments (A1, B5) seedling emergence was about 0.4 probits lower than laboratory germination and in the poorest 1.3 probits lower (A3, B4). These two values were those determined from investigations with seed lots of onion (*Allium cepa* L.) sown at Reading into extreme (from very good to the poorest at which sowings would still be made) seedbeds. To use the guide for other crops in different seedbeds: (i) plot historical results for as many sets of results for laboratory germination and seedling emergence at the site as are available on the graph; (ii) identify the extreme observations on the diagonal axis (labelled probits); (iii) locate where the laboratory germination test result of the seed lot to be sown (vertical lines) intersects the two probit value diagonals identified in (ii); (iv) read off the two values at these intersections for likely seedling emergence when poor or good conditions are expected; (v) use these values to adjust seed sowing rates appropriately in the light of the conditions expected for the seedbed at sowing. From Wheeler and Ellis (1992b).

A seed lot test certificate has a limited period of validity: within a seed testing year (in the UK). Hence, a seed lot tested at the beginning of that year might be sold up to 12 months later, but retesting is necessary for sale in each subsequent seed testing year. The seed viability nomographs for each of barley and onion in the 1981 paper clearly showed why it is essential to retest seed lots for sale regularly and provided a tool to estimate expected loss in viability in these two contrasting crops in a wide range of environments: onion requires much cooler and/or drier storage environments (in practice a controlled environment for periods beyond short-term storage) than barley (where drying and ambient temperatures can suffice in the UK, especially if there is little carry over of seed between years). This approach has been extended to compare the suitability of contrasting ambient sites for short-term storage by a seed company (Ellis, 1988).

Implicit in the 1981 paper is strong support of the importance of an explicit minimum germination level for sale. Many farmers and growers involved with seed but downstream of seed producers and seed analysts overlook that seeds are a living input to crop production (e.g., equating seed with inanimate fertilizers); they might assume that a poor quality seed lot of only 50% viability could be compensated for by sowing in the field at twice the rate of a high quality seed lot of 100% viability in order to achieve the same plant population density. Such an opinion is equivalent to assuming the same relative field emergence for contrasting seed lots sown in the same seedbed. Both are possible in the special case where the seedbed environment provides optimum conditions for germination and emergence (i.e. similar to the laboratory germination test), but this is unlikely. In the poor early spring seedbed conditions outlined in table 1 where a spring wheat (*Triticum aestivum* L.) seed lot with 98% normal germination would be expected to show 67% emergence, a seed lot showing only 50% normal germination would be expected to show under 10% emergence (and in practice the emerging cereal seedlings would be likely to be outcompeted by weeds). Hence, minimum germination levels for sale are vital to growers.

By definition, a seed lot is a homogenous, limited quantity of seed harvested from one field at the same time and treated identically (e.g. dried, transported, packaged) through-out its subsequent history. Given that the ageing accumulated by, and the subsequent performance of, the surviving seeds in a seed lot is linked to the proportion of seeds that has already died in that population (Ellis and Roberts, 1981a), it follows that the consequence of mixing (sometimes euphemistically described as 'blending') of two or more seed lots would provide a new seed bulk which is not merely heterogeneous (requiring enhanced sampling approaches), but for which the result of a germination test has the potential to mislead the grower in terms of likely seedling emergence (particularly in poor seedbed conditions). Hence, it is important to maintain the integrity of the seed lot.

Anhydrous biology

The seed industry is well used to regulation. The oversight of trade in other aspects of anhydrous biology is also necessary. The use of entomopathogenic fungi to control locusts and grasshoppers is one method of biological control that relies upon the production of conidia and their storage, distribution, and application. Many aspects of this commercial

supply chain mimic seed production and supply. Estimates of conidia survival periods are required by regulators to ensure the high quality of these biological control agents throughout the delivery chain; biological control agents generally have a shorter shelf life that chemical biocides. The improved seed viability equation has been shown to describe well the survival of conidia of entomopathogenic fungi in response to storage environment (Hong *et al*., 1997). Moreover, as with seeds, there is a low moisture content limit to the negative logarithmic relation between conidia longevity and moisture content (Hong *et al*., 1998). The improved seed viability equation has also been applied to pollen storage (Hong *et al*., 1999). These applications emphasise the economy of nature in anhydrous biology.

Such economy in the quantitative response of storage longevity to the air-dry environment across seed, conidia and pollen (where tolerant to desiccation; the phenomenon of contrasting storage behaviour is not unique to seeds) points to common mechanisms for the ability of plant cells and tissues to not just survive considerable desiccation but to survive for substantial periods once dried. One such substantial period is provided by the discovery of viable seeds of several species after 123 years in the foundation stone of the Nuremburg City Theatre (Aufhammer and Simon, 1957). Reviews of the basic mechanisms thought to underly desiccation tolerance, air-dry seed survival and their acquisition during seed development include Oliver and Bewley (1997), Pammenter and Berjak (1999), Walters (2015), Leprince *et al*., (2017) and Ballesteros *et al*. (2020). What is clear from these reviews and from applied research on seed science with orthodox seeds is that more than one mechanism in protecting seeds from desiccation and in enabling considerable longevity is involved. These are not necessarily invoked simultaneously with, for example, improvement in K_i associated with oligosaccharide accumulation early in seed development and maturation, but associated with the accumulation of low-molecular-weight, heat-stable proteins later on as seeds mature (Sinniah *et al*., 1998). Further, some damage to protective mechanisms can be repaired (Oliver and Bewley, 1997), not only for seed survival during or after a period of storage (Villiers and Edgecumbe, 1975; Ibrahim *et al*., 1983), but also much earlier during seed development and maturation for subsequent seed longevity (Ellis and Yadav, 2016; Yadav and Ellis, 2016). Moreover, improved understanding of the plant cell properties and mechanisms underlying desiccation tolerance raises the potential for new approaches to be developed to improve seed storage life (Ballesteros *et al*., 2020).

Discussion

I well remember seeing the first issue of *Seed Science and Technology* in 1973; and how modern it (hard copy of course then) looked. Progress is hard to measure in real time, but reflection shows considerable progress in seed science and technology in the journal across 50 volumes.

Orthodox seeds in the dry state remain an enigma; whether they are quiescent or simply dead remains unknown unless or until they are withdrawn from storage and then tested. There is also the paradox that to determine how to maintain seed viability in suitable environments for long enough, one must first discover the combinations of

conditions that kill seeds. No model is perfect, but the real test of a model is whether it is useful. I trust that the improved seed viability equation has provided a useful scaffold for greater understanding of seed science and technology over the four decades since its original development.

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