

Temperature and Seed Storage Longevity

J. B. DICKIE*, R. H. ELLIS†, H. L. KRAAK‡, K. RYDER§
and P. B. TOMPSETT*

*Jodrell Laboratory, Royal Botanic Gardens, Kew, Wakehurst Place, Ardingly, Haywards Heath, West Sussex RH17 6TN, UK, †Department of Agriculture, University of Reading, Earley Gate, P.O. Box 236, Reading RG6 2AT, UK, ‡Government Seed Testing Station, Binnenhaven 1, 6709 PD Wageningen, The Netherlands and §Statistics Department, AFRC Institute of Arable Crops Research, Rothamsted Experimental Station, Harpenden, Herts AL5 2JQ, UK

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ABSTRACT

Seed survival data for eight diverse species, namely the cereal barley (*Hordeum vulgare* L.), the grain legumes chickpea (*Cicer arietinum* L.), cowpea [*Vigna unguiculata* (L.) Walp.] and soya bean [*Glycine max* (L.) Merr.], the timber trees elm (*Ulmus carpiniifolia* Gleditsch.), mahogany (*Swietenia humilis* Zucc.), and terb (*Terminalia brassii* Exell.), and the leaf vegetable lettuce (*Lactuca sativa* L.) were compared over a wide range of storage environments (temperatures from -13 °C to 90 °C, seed moisture contents from 1.8 to 25% f.wt) using a viability equation developed previously. In accordance with that equation, the effect of temperature on seed longevity was dependent upon the temperature range. The temperature coefficients of the viability equation did not differ significantly ($P > 0.05$) among the eight species despite their contrasting taxonomy. Thus the quantitative relation between seed longevity and temperature does not vary among diverse species. The same conclusion was obtained for the coefficients of a proposed alternative model of the relation between seed longevity and temperature. The implications of the two temperature models in the viability equation for extrapolations to low and very low temperatures are discussed.

Key words: Seed storage, seed longevity, seed moisture, temperature, viability equation, genetic resources conservation, *Cicer arietinum* L., *Glycine max* (L.) Merr., *Hordeum vulgare* L., *Lactuca sativa* L., *Swietenia humilis* Zucc., *Terminalia brassii* Exell., *Ulmus carpiniifolia* Gleditsch., *Vigna unguiculata* (L.) Walp.

INTRODUCTION

Seeds of the majority of crops and many non-cultivated species show orthodox storage characteristics, i.e. they can be dried to low moisture contents without damage and, over a wide range of conditions, their longevity increases with decrease in seed storage moisture content and temperature in a quantifiable and predictable way (Roberts, 1973). Despite this similarity in seed storage behaviour among diverse taxa, it has not been clear whether the response of longevity to these variables of the storage environment differs quantitatively among diverse species with orthodox seeds.

Relations between seed survival and storage duration, temperature and moisture content have been quantified by the following seed viability equation proposed by Ellis and Roberts (1980a),

$$v = K_i - p / 10^{K_E - C_W \log_{10} m - C_H t - C_Q t^2}, \quad (1)$$

where v is probit percentage viability after p days in storage at $m\%$ moisture content (f.wt) and t

°C, K_i is a constant specific to the seed lot, and K_E , C_W , C_H and C_Q are species viability constants. Equation (1) was developed by analyzing data for barley (*Hordeum vulgare* L.) seed survival, obtained over wide ranges of seed storage moisture content (5–25%) and temperature (3–90 °C) (Ellis and Roberts, 1980b). The equation has since been successfully fitted to similarly comprehensive data sets for a further six species (Ellis, Osei-Bonsu and Roberts, 1982; Tompsett, 1986; Kraak and Vos, 1987). In addition, the basic tenets of this equation have been shown to apply in a wide range of otherwise contrasting species (Ellis and Roberts, 1981; Ellis, Hong and Roberts, 1986, 1988, 1989).

The power term of eqn (1) is the logarithm of the standard deviation, σ , of lifespans of individual seeds in a population under the storage conditions indicated (Ellis and Roberts, 1980a), so that

$$\log_{10} \sigma = K_E - C_W \log_{10} m - C_H t - C_Q t^2, \quad (2)$$

where σ is a measure of the longevity of seeds, since it is the time in days during which percentage viability is reduced by one on the probit scale (e.g.

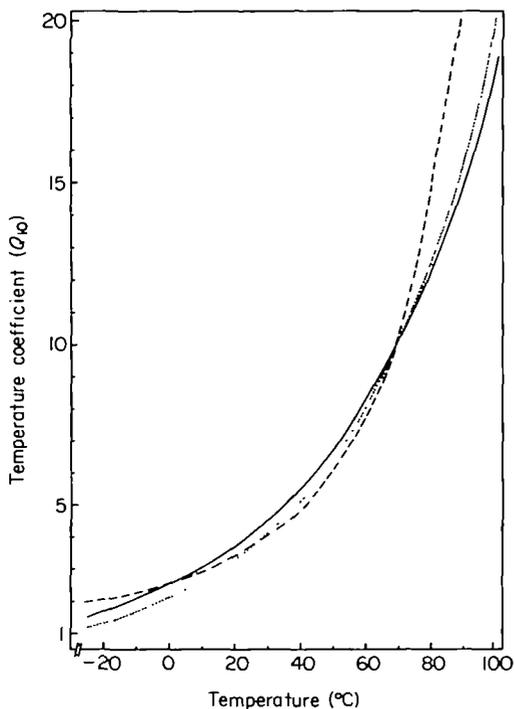


FIG. 1. Relations between estimates of the temperature coefficient for increase in the rate of loss of seed viability per 10 °C rise in temperature (Q_{10}) and temperature. The continuous curve shows the relation determined by Ellis and Roberts (1980*b*, 1981) for barley, where relations between seed longevity and temperature from 3 °C to 90 °C were quantified by eqn (2). The dotted and broken lines show the relations between Q_{10} and temperature determined here for eight species (Table 1) when relations between seed longevity and temperatures between -13 °C and 90 °C were quantified by eqns (2) and (4), respectively.

from 97.7% to 84.1% or from 84.1% to 50.0%). Thus, the coefficient C_w indicates the response of seed longevity to moisture content, while C_H and C_Q together indicate the response to temperature.

The effect of temperature on the rate of biochemical or physiological processes is often described by the temperature coefficient, Q_{10} ; the ratio of the rate of a process at one temperature, t °C, to the rate at another 10 °C lower, i.e. $t-10$ °C. The expression $-C_H t - C_Q t^2$ describes an effect of temperature on seed longevity, whereby the Q_{10} increases as temperature increases (Ellis and Roberts, 1980*a*). The continuous line in Fig. 1 illustrates the relation determined between Q_{10} and temperature for seed longevity in barley (Ellis and Roberts, 1980*a*). Ellis and Roberts (1981) showed that previously published data on the longevity of

seeds of twenty crop and flower species conformed to this relation. These and other similarities in relations between temperature and seed longevity among species have tempted speculation that the effect of temperature may not differ among species (Ellis *et al.*, 1982; Dickie, McGrath and Linington, 1985; Ellis, 1988).

In contrast, differences in the estimates of the coefficient C_w (the effect of moisture content on longevity) have appeared substantial (e.g. Ellis, 1988). These differences have been shown to be significant where direct comparisons of the value of C_w have been made between species (Ellis *et al.*, 1982; Tompsett, 1986; Ellis, Hong and Roberts, 1989).

In this paper we have combined the data from our separate studies with diverse taxa in order to test two hypotheses: first, that the effect of storage temperature on seed longevity does not vary among species, but second, that interspecific differences do occur in the effect of seed storage moisture content on longevity. We also consider an alternative temperature term in the viability equation with the aim of providing alternative estimates of longevity when extrapolating to very low temperatures.

DISSERTATION

A total of 359 estimates of σ , obtained from 359 seed survival curves under a very wide range of different storage environments (temperatures between -13 °C and 90 °C, and moisture contents between 1.8% and 25%) in our three laboratories, were pooled for the following eight contrasting (Table 1) species: *Cicer arietinum* L. (chickpea), *Glycine max* (L.) Merr. (soya bean), *Hordeum vulgare* L. (barley), *Lactuca sativa* L. (lettuce), *Swietenia humilis* Zucc. (mahogany), *Terminalia brassii* Exell. (terb), *Ulmus carpiniifolia* Gleditsch. (elm), and *Vigna unguiculata* (L.) Walp. (cowpea). The analyses were retrospective in that data for seven of the eight species (all but mahogany), treated separately or in small groups, have already provided estimates of the species' viability constants (Ellis and Roberts, 1980*b*; Ellis *et al.*, 1982; Tompsett, 1986; Kraak and Vos, 1987). In the present study statistical analysis was first carried out using GENSTAT 5 (GENSTAT Committee, 1987) to fit eqn (2).

The analysis supports the hypotheses, that for the eight species: (i) there are significant differences between the estimates of the two coefficients K_E and C_w (Table 1) ($P < 0.001$ for each), and (ii) the temperature coefficients C_H and C_Q could be regarded as having the common values $C_H = 0.0329$ (s.e. 0.00171) and $C_Q = 0.000478$ (s.e.

TABLE 1. Values of the seed viability constants K_E and C_W and K_B and C_W for eight species when temperature terms are constrained to common values with information illustrating taxonomic and physical diversity among the eight

Species (common name) Family	Number of seed survival curves	Viability constant values				Mean seed wt (mg)	Typical oil content (% d.wt)	Seed type and life-form	Source of data
		eqn (2)		eqn (4)					
		K_E (s.e.)	C_W (s.e.)	K_B (s.e.)	C_W (s.e.)				
<i>Cicer arietinum</i> L. (chickpea) Leguminosae	65	8.901 (0.158)	4.847 (0.124)	12.039 (0.315)	4.789 (0.127)	300–500*	5*	Non-endospermic Tropical annual grain	Ellis <i>et al.</i> (1982)
<i>Glycine max</i> (L.) Merr. (soya bean) Leguminosae	58	7.525 (0.155)	4.086 (0.130)	10.676 (0.309)	4.035 (0.133)	100–670*	18*	Non-endospermic Annual grain	Ellis <i>et al.</i> (1982)
<i>Hordeum vulgare</i> L. (barley) Gramineae	50	9.144 (0.271)	5.342 (0.221)	12.425 (0.348)	5.390 (0.230)	25–58*	2*	Endospermic Temperate annual grain	Ellis and Roberts (1980 b)
<i>Lactuca sativa</i> L. (lettuce) Compositae	39	6.985 (0.169)	4.200 (0.170)	10.173 (0.323)	4.177 (0.175)	0.6–1.3*	35*	Endospermic Temperate annual herb	Kraak and Vos (1987)
<i>Swietenia humilis</i> Zucc. (mahogany) Meliaceae	27	5.393 (0.123)	2.391 (0.123)	8.568 (0.302)	2.346 (0.127)	125–400*	67† (embryo)	Non-endospermic Tropical tree	Tompsett (unpubl. res.)
<i>Terminalia brassii</i> Exell. (terb) Combretaceae	46	5.016 (0.196)	2.161 (0.205)	8.281 (0.340)	2.209 (0.211)	< 10†	71† (embryo)	Non-endospermic Tropical tree	Tompsett (1986)
<i>Ulmus carpiniifolia</i> Gleditsch. (elm) Ulmaceae	24	5.830 (0.166)	3.035 (0.152)	9.046 (0.320)	3.061 (0.156)	< 10†	14†	Non-endospermic Temperate tree	Tompsett (1986)
<i>Vigna unguiculata</i> (L.) Walp. (cowpea) Leguminosae	50	9.401 (0.177)	5.118 (0.142)	12.524 (0.329)	5.042 (0.146)	100–120*	2§	Non-endospermic Tropical annual grain	Ellis <i>et al.</i> (1982)

Sources: * Cromarty, Ellis and Roberts, 1982; † P. B. Tompsett, unpubl. res.; § Ellis, 1988.

0.0000204) ($P > 0.05$). Comparison of the continuous and dotted lines in Fig. 1 shows that these common values for C_H and C_Q result in a very similar relation between Q_{10} and temperature to that determined previously for barley alone, save that the curves cross at around 70 °C.

It is difficult to determine seed longevity at sub-zero temperatures in combination with low seed moisture contents, because under these conditions longevity is protracted. Consequently investigations lasting several years show little or no difference in germination (e.g. Harrison and Carpenter, 1977; Ellis, 1984). Nevertheless, there is a need to provide some estimate of longevity at sub-zero temperatures. In particular, the management of seed accessions for genetic resources conservation in long-term seed stores requires estimates of longevity at around -20 °C. Despite the dangers involved, it is often necessary to estimate seed longevity at sub-zero temperatures by extrapolation from observations at higher temperatures. Roberts and Ellis (1977) showed that extrapolation of linear relations between the logarithm of longevity and higher temperatures overestimated longevity at -20 °C. Subsequently, Ellis and Roberts (1980a, 1981) proposed that the decline in the Q_{10} for rate of loss of viability with decreasing temperature down to -20 °C could be described by the inclusion of the quadratic temperature term ($-C_Q t^2$) in the viability equation. The empirical adoption of that term in the viability equation has enabled the formulation of a model [eqn (2)] which takes into account the fact that Q_{10} for rate of loss of viability is not stable but shows continuous variation with temperature (Fig. 1).

The difficulty, however, with the inclusion of the quadratic temperature term in the viability equation is that it imposes a reversal in the relation between longevity and temperature at the point t_L , defined by

$$t_L = -C_H / (2C_Q). \quad (3)$$

This implies that seed longevity will decline as temperature declines below t_L . Using the estimates of the common values of C_H and C_Q determined here in this equation gives an estimate of -34.4 °C for t_L . Earlier estimates of C_H and C_Q for barley (Ellis and Roberts, 1980b) or the three grain legumes (Ellis, 1988) give broadly similar values of -46.7 °C and -30 °C, respectively. These values approximately coincide with the temperature of about -40 °C at which homogeneous nucleation of ice microcrystals within supercooled intracellular water occurs (Merryman and Williams, 1981). However, there is little evidence to support a reversal in the trend of increasing longevity with a decrease in temperature, and in any case there

are unlikely to be significant amounts of free water present in seeds at moisture contents suitable for long-term storage. Moreover, further extrapolation of the model to around -196 °C (i.e. to the temperature of liquid nitrogen) predicts extremely short seed life spans, while there is evidence that this is not so (e.g. Harrison and Carpenter, 1977; Stanwood and Roos, 1979).

Inspection of the curve for Q_{10} against temperature (Fig. 1) suggests an exponential function as an alternative to the quadratic temperature component of the viability equation, such that

$$\log_{10} \sigma = K_B - C_W \log_{10} m - a10^x, \quad (4)$$

where the two species viability coefficients K_B and C_W have modified values after fitting the new temperature term and two new coefficients a and b are associated with this term.

Equation (4) was fitted to the data using the latest version (3.08) of MLP (Ross, 1987). This was done indirectly, however. To improve numerical accuracy in the estimation of the coefficients, it was necessary to fit the equation in a modified but equivalent form, where the term $a10^x$ was replaced by $aR^{x/10}$. Mirroring the results of fitting eqn (2), the estimates of a and R could be considered to be common for all eight species, since they did not differ significantly ($P > 0.05$), where $a = 3.154$ (s.e. 0.2342) and $R = 1.139$ (s.e. 0.0068) and the value of b derived from the estimate of R is 0.00565. As in eqn (2) the estimates of both K_B and C_W (Table 1) differed significantly among species ($P < 0.001$). Thus the application of eqn (4) to this data-set endorses the conclusions drawn from that of eqn (2): as indicated by the broken line in Fig. 1, the effect of temperature on seed longevity increases with increase in temperature, and the quantitative relation between temperature and seed longevity does not vary among these eight species.

Clearly, some caution is necessary in extrapolating these conclusions from eight species to the rest of those members of the flowering plant kingdom with orthodox seeds. Nevertheless, these eight represent four of the ten super-orders of flowering plants (Heywood, 1978). Moreover, the variation in life form, habitat and seed characteristics among the eight is substantial (Table 1). Although three of the species are grain legumes and contribute almost half of the observations, testing failed to reveal any significant effect ($P > 0.05$) of these three on the overall estimates of C_H and C_Q in eqn (2). Thus, the possibility of their having undue influence on these estimates can be ignored. In view of this wide coverage from the flowering plant kingdom we believe that these results give solid support to the hypothesis that the quantitative influence of

temperature on the longevity of all orthodox seeds is the same.

The principal reason for the development of an alternative temperature term in the viability equation concerned extrapolation to low and very low temperatures. However, even without extrapolation comparison of the two discontinuous lines in Fig. 1 shows differences in the relations between Q_{10} and temperature for loss in seed viability resulting from the application of eqns (2) and (4). Between about 30 °C and 70 °C the exponential temperature model in viability eqn (4) provides slightly lower estimates of Q_{10} than the quadratic temperature model of eqn (2), while outside this range the situation is reversed (Fig. 1). Both models are the result of empirical fitting and at present there is no objective way of choosing which to use; from a total sum of squares of 570.5 the residual sums of squares for the two models were 11.95 for the quadratic and 12.51 for the exponential, both based on 341 degrees of freedom, showing that the models are indistinguishable.

While there are no serious discrepancies between -13 °C and 90 °C, there is a tendency for the exponential temperature model to overestimate comparative longevity (longevity adjusted for species differences in response to different seed moisture contents) at the lower extreme of this temperature range (Fig. 3), which is not evident for the quadratic temperature model (Fig. 2). It should be noted, however, that because of the calculations required to obtain comparative longevities in Figs 2 and 3 some of the error shown must arise from the moisture content terms. Note also that corresponding values of comparative longevity differ between the two figures because of the difference (about 3.2) between the estimates of K_E and K_B for all the species (Table 1).

Also shown in Figs 2 and 3 are estimates of elm seed longevity at -75 °C from Tompsett (1986). These four observations are far removed from the main body of the data (-13 °C to 90 °C) and are therefore likely to be highly influential in fitting either of the models. In addition, the survival

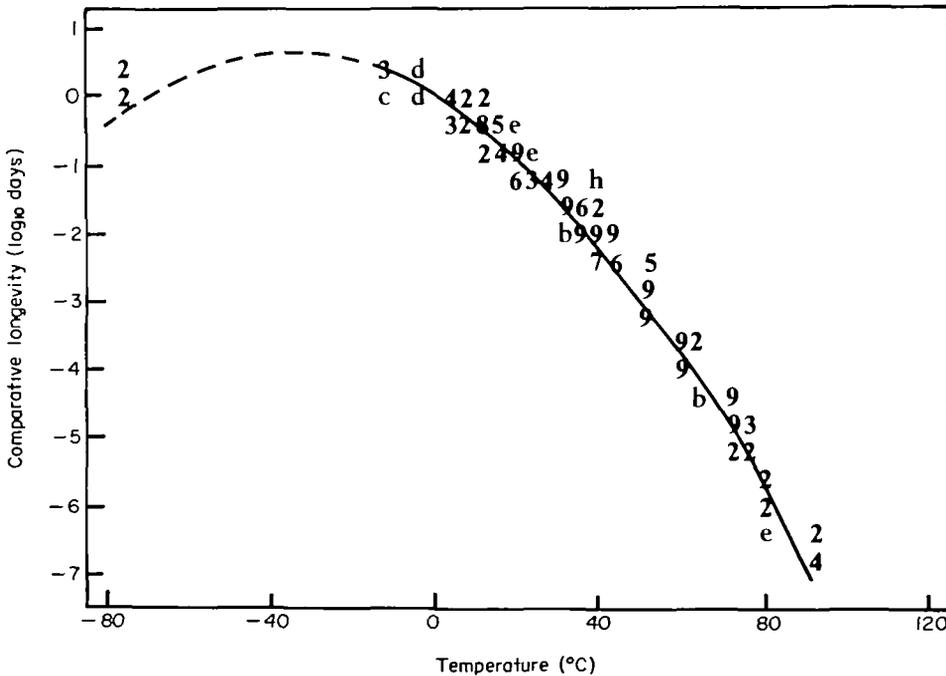


FIG. 2. Relations between comparative seed longevity and temperature for lettuce (a), mahogany (b), elm (c), terb (d), barley (e), cowpea (f), soya bean (g) and chickpea (h), when described by eqn (2), where comparative longevity = $\log_{10}\sigma - K_E + C_W \log_{10}m$. The continuous curve shows the relation fitted between -13 °C and 90 °C. The broken curve shows extrapolation to -75 °C. For reasons discussed in the text the observations at -75 °C were not included in the analyses. The numbers 2-8 indicate two to eight coincident observations on several species, respectively, while 9 indicates nine or more (frequently many more) coincident observations. The letters indicate single observations for the species corresponding to those letters in the list above. There was no systematic deviation from the fitted line by any of the eight species.

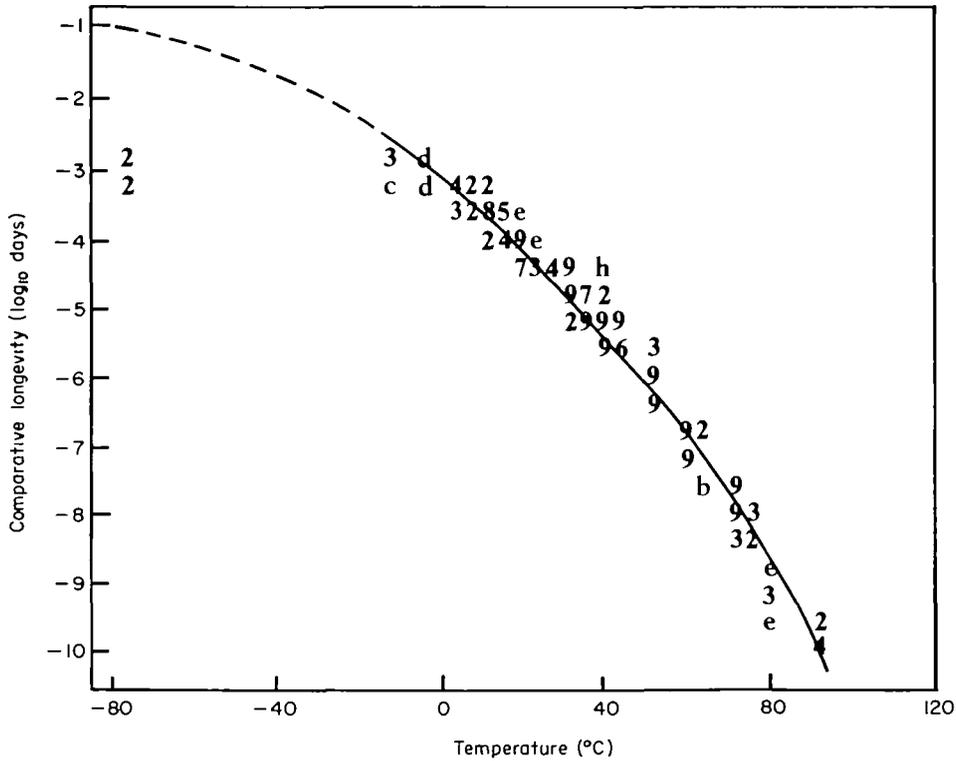


FIG. 3. Relations between comparative seed longevity and temperature described by eqn (4), where comparative seed longevity = $\log_{10}\sigma - K_b + C_{\psi} \log_{10}m$. Remaining details as Fig. 2.

curves from which they were obtained showed comparatively little loss in viability during the period of investigation and were also ill-fitting as shown in Figs 2 and 3. Consequently these points were omitted from the analyses. Nevertheless, comparisons with the extrapolations of the two temperature models of longevity at this low temperature provide some preliminary indication of the merits of each equation. Figure 2 clearly shows that the quadratic equation underestimates the four points at -75°C , while Fig. 3 shows that the exponential equation overestimates them. In contrast to our earlier arguments for the development of an alternative temperature model, the estimates given by the quadratic equation are closer to the four points than those given by the exponential (while the standard errors of these estimates have not been calculated, those for the quadratic equation would be expected to increase as temperature decreases below -13°C , whereas those for the exponential equation would remain almost constant). In fact it can be seen that reduction in storage temperature from -13°C to -75°C had little effect on the recorded estimates

of elm seed longevity. This may indicate the difficulty in determining the rate of loss of viability accurately under very good seed storage conditions when the period of investigation is limited to only a few years. Nevertheless, such observations illustrate current uncertainties concerning the supposed advantages to longevity of reducing temperatures below the range of -10°C to -20°C currently used for long-term seed storage in gene banks.

Genebank managers need estimates of seed longevity at about -20°C . The estimates provided by the quadratic equation are markedly more conservative; for example, barley seeds stored at -18°C and 6% moisture content would be expected to lose one probit of viability in 728 and 1488 years by extrapolation of the quadratic and exponential equations respectively. The balance of arguments for the present, then, supports the continued use of eqn (2) to describe the relation between seed longevity and temperature between -20°C and 90°C .

The effect of moisture content on seed longevity, described by C_{ψ} in eqn (2) and by C_{ψ} in eqn (4),

varies significantly among the eight species examined. This observation confirms earlier separate studies on parts of the data (Ellis *et al.*, 1982; Tompsett, 1986) and challenges assumptions by Harrington (1963) and Priestley, Cullinan and Wolfe (1985) that a given change in seed storage moisture content would affect the longevity of all species equally. Inspection of Table 1 indicates a weak negative association between the values of C_w and oil content among the species, in the manner noted by Ellis (1988). Oil content influences seed water relations and is a major determinant of equilibrium relative humidity (Pixton, 1967), which is, in turn, related to water potential. Roberts and Ellis (1989) and Ellis *et al.* (1989) have produced evidence for a common response of seed longevity to moisture in oily and non-oily seeds, provided seed moisture status is measured by water potential (from equilibrium relative humidity), rather than by gravimetric water content. While the result of a subsidiary analysis (not reported here) on the present five annual species lends indirect support to such a hypothesis, the pattern of variation in C_w for the three tree species is not so clearly resolvable. Consequently these and other similar species might be included in subsequent tests of that hypothesis.

CONCLUSIONS

We conclude that the quantitative relation between seed longevity and temperature does not vary among diverse species, whereas the slope of the logarithmic relation between longevity and storage moisture content does. We confirm that the quadratic temperature term in the viability equation developed by Ellis and Roberts (1980*a*), eqn (2), provides an effective method for quantifying relations between seed longevity and temperature from around -20°C to 90°C . A strategy for future research into the seed storage longevity of orthodox species might be to accept the values of the viability constants C_H and C_Q determined here for a wide temperature range; $C_H = 0.0329$ (s.e. 0.00171), $C_Q = 0.000478$ (s.e. 0.0000204). Attention could then be concentrated on quantifying relations between longevity and moisture at single, convenient and accurately-controlled constant temperatures, in the manner adopted by Ellis *et al.* (1986, 1988, 1989), using these values of C_H and C_Q to estimate values for the constant K_E .

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