

A Model of the Effect of Temperature and Moisture on Pollen Longevity in Air-dry Storage Environments

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Data on the survival of pollen of *Typha latifolia* L. stored for up to 261 d over seven different saturated salt solutions (providing 0.5 to 66% relative humidity) and six different constant temperatures (from -5 to $+45$ °C) were analysed to quantify the effect of air-dry storage environment on pollen longevity. Pollen survival curves conformed much more closely to negative cumulative normal distributions than to negative exponential relations. Estimates of p_{50} (storage period required to reduce pollen viability to 50%), provided by negative cumulative normal distributions, were available from 37 different storage environments in which pollen viability was reduced below 50%. Once observations at 0.5% and 5.5% relative humidity were excluded from analysis, there was a negative logarithmic relation between these estimates of longevity and pollen moisture content (% wet basis) and a curvilinear semi-logarithmic relation between longevity and temperature. When the negative logarithmic relation between longevity and moisture content was replaced by a negative semi-logarithmic relation between longevity and the relative humidity of the storage environment the resultant model was less satisfactory, principally because pollen longevity over saturated solutions of calcium nitrate (43–62% relative humidity) and sodium nitrite (60–66% relative humidity) were consistently greater and smaller, respectively, than fitted values. Notwithstanding these errors, comparison between the fitted relations and observations at the two lowest relative humidities provided estimates of the lower-relative-humidity limits to these relations. These provisional estimates varied with storage temperature being lowest at 25 °C (< 5.5 % relative humidity). However, there was no linear trend to that variation ($P > 0.25$): the mean estimate was 11.9 (s.e. = 1.4)%. The considerable similarities among models of pollen longevity in air-dry storage, and their estimated lower limits, and those developed previously for orthodox seeds and spores are discussed.

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Key words: *Typha latifolia* L., pollen, storage, survival, longevity, relative humidity, moisture content, temperature.

INTRODUCTION

Although there are several exceptions [e.g. *Zea mays* L. (Buitink *et al.*, 1996)], the pollen of many species of flowering plants survives considerable desiccation and can be stored in air-dry environments successfully for *ex situ* plant genetic resources conservation (Harrington, 1970; Roberts, 1975; Hoekstra, 1995; Barnabás and Kovács, 1997). Reducing temperature, moisture content or oxygen partial pressure increases the longevity of desiccation-tolerant pollen in air-dry storage (Roberts, 1975). There have been several investigations of the effects of duration, temperature and moisture content of storage on pollen survival (e.g. Henny, 1978; Kobayashi, Ikeda and Nakatani, 1978; Ganeshan, 1985). Nevertheless, until recently no single investigation had systematically combined wide ranges of each of temperature and moisture content. Thus insufficient data were available to enable a model of pollen longevity in response to both variables to be developed. However, Buitink *et al.* (1998) have recently reported the survival of pollen of *Typha latifolia* L. during storage in a

wide range of different environments. This paper reports analyses of that data which show that models developed previously to quantify the effect of different environments on the longevity of other propagules, namely seeds and fungal spores, stored air-dry (Ellis and Roberts, 1980; Hong, Ellis and Moore, 1997) can also be applied successfully to pollen. We also consider the limit to the application of the model in low relative humidity environments.

MATERIALS AND METHODS

Observations

Full details of pollen collection, storage and germination procedures were provided by Buitink *et al.* (1998). Briefly, about 0.2 g of pollen of *T. latifolia* L. was stored in the dark within 500 ml desiccators over phosphorus pentoxide or saturated solutions of six different salts in factorial combination with six different constant (± 1 °C) temperatures (Table 1). Sub-samples were removed after different durations of storage, in order to estimate pollen viability, for up to 261 d. Pollen moisture content (wet basis, w.b.) was estimated by drying nine 10 mg samples of pollen at 95 °C for 24 h and determining the loss in weight on drying;

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TABLE 1. The relative humidity (RH, %) provided by phosphorus pentoxide and saturated salt solutions at different temperatures (°C) and pollen moisture content (m.c., %, wet basis) in these environments

Salt	−5 °C		5 °C		15 °C		25 °C		35 °C		45 °C	
	RH	m.c.	RH	m.c.	RH	m.c.	RH	m.c.	RH	m.c.	RH	m.c.
P ₂ O ₅ *	0.5	2.4	0.5	0.8	0.5	0.1	0.5	0.1	0.5	−0.01†	0.5	0.4
ZnCl ₂	5.5	4.7	5.5	3.4	5.5	2.7	5.5	2.4	5.5	1.7	5.5	1.5
LiCl	15	5.1	15	4.4	14	4.1	13	3.4	11.5	2.9	11	2.4
KC ₂ H ₃ O ₂	26	6.3	26	5.5	25	4.8	25	4.2	22	4.0	19	3.4
MgCl ₂	33.5	6.6	33.5	6.1	33	5.7	32.5	5.4	32.5	4.9	31.5	4.1
Ca(NO ₃) ₂	62	12.5	61	10.8	56	9.5	50.5	7.2	43	6.0	43	5.4
NaNO ₂	66	13.6	66	12.8	66	12.4	64	11.0	62	8.5	60	7.8

* Relative humidity over phosphorus pentoxide expected to be no greater than the value shown (Buitink *et al.*, 1998).

† i.e. sample increased in weight during oven drying and re-weighing.

all weighings were accurate to 0.001 mg. Estimates of the variation in the relative humidity provided by phosphorus pentoxide or each saturated salt solution with temperature (Table 1) are from Vertucci and Roos (1993).

Model development

Roberts (1975) suggested that pollen survival probably declines exponentially during storage, but noted that in some circumstances it may conform to negative cumulative normal distributions. Accordingly, the observations for pollen survival during storage in each environment were subjected to regression analysis in accordance with the equation

$$\log_{10} G = a - bp \quad (1)$$

where G is the germination percentage after p days in storage, b is the gradient of the regression line, and a is the intercept (but germination values of 0% were altered to 0.01% before transformation), and to probit analysis in accordance with the seed viability equation

$$\nu = K_i - p/\sigma \quad (2)$$

where ν is probit percentage viability after p days in storage, K_i is the intercept and σ is the standard deviation of the frequency distribution of pollen deaths in time (Ellis and Roberts, 1980), using GLIM (Baker and Nelder, 1978). Relations between longevity (σ , d), temperature (t , °C), and moisture content (m , % w.b.) are quantified by

$$\log_{10} \sigma = K_E - C_W \log_{10} m - C_H t - C_Q t^2 \quad (3)$$

in the seed viability equation where K_E , C_W , C_H and C_Q are constants (Ellis and Roberts, 1980). Equation (3) incorporates a negative logarithmic relation between seed longevity and moisture content, but a negative semi-logarithmic relation between seed longevity and equilibrium relative humidity (r , %) provides an alternative in which

$$\log_{10} \sigma = K_E - C_R r - C_H t - C_Q t^2 \quad (4)$$

where C_R is a constant (Roberts and Ellis, 1989). Equations (3) and (4) and modifications thereof, were applied to the estimates of pollen longevity by multiple regression using GLIM.

RESULTS

Pollen survival curves

The number of observations for each survival curve (pollen viability plotted against duration of storage) varied considerably. For example, only two observations (before storage commenced and 0.1% germination after storage) were available for pollen stored at 60% RH and 45 °C, while 21 observations were available for pollen stored at 5.5% RH and 25 °C.

The 42 survival curves were fitted as negative exponentials [eqn. (1)] and as negative cumulative normal distributions [eqn. (2)] to a total of 489 observations of pollen viability.

Constraining all 42 pollen survival curves to a common origin when described by negative exponentials provided no significant increase in residual deviance ($P > 0.25$), the coefficient of determination being reduced from 0.73 to 0.70. The value of this common intercept was 2.129 (s.e. = 0.030), but this is equivalent to 134.6% whereas initial germination varied only from 89.9 to 90.7% among the 42 environments (mean 90.3%).

In contrast, constraining all 42 pollen survival curves to a common origin by negative cumulative normal distributions provided a significant increase in residual deviance ($P < 0.005$), the coefficient of determination being reduced from 0.93 to 0.92. The value of this common intercept was 1.323 (s.e. = 0.055). This is equivalent to 90.7% viability, and so almost identical to the initial mean estimate of pollen viability. The separate estimates of K_i were equivalent to initial viability values between extremes of 78.8% (storage over phosphorus pentoxide at 35 °C) and 95.9% (storage over a saturated solution of calcium nitrate at 25 °C), i.e. a somewhat wider range than that observed. Analysis of variance showed that the estimates of K_i did not vary with storage temperature ($P > 0.25$), but varied considerably with relative humidity ($P < 0.025$), the mean values of K_i being minimum (1.163; equivalent to 87.5% initial viability) when stored over phosphorus pentoxide and maximum when stored over a saturated solution of calcium nitrate (1.502; equivalent to 93.3% initial viability).

In addition to the much greater coefficient of determination, and the fact that initial viability was substantially

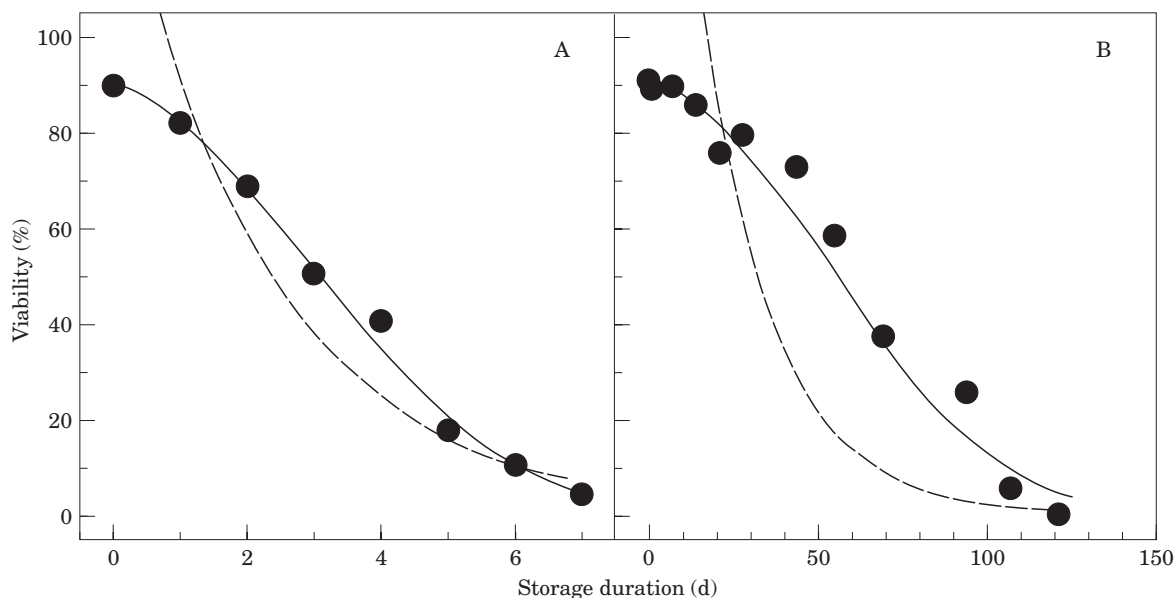


FIG. 1. Survival of pollen of *Typha latifolia* (●) stored in desiccators over phosphorus pentoxide at 45 °C (about 0.5% RH) (A) or over a saturated solution of calcium nitrate at 15 °C (56% RH) (B). The curves shown are best-fit negative exponentials (---) or negative cumulative normal distributions (—).

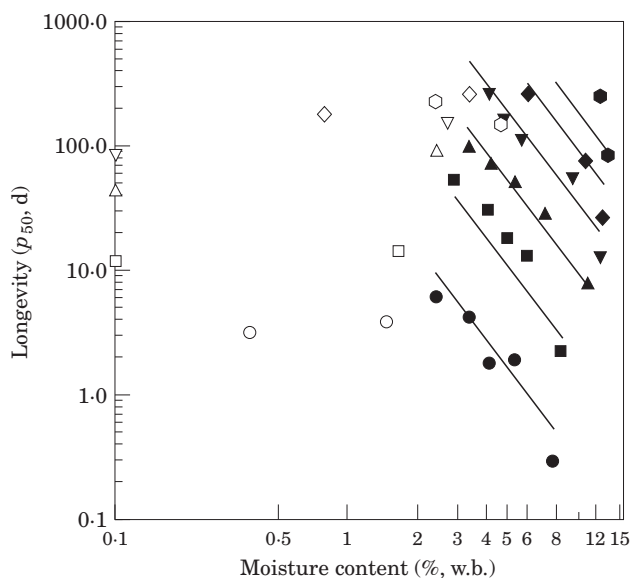


FIG. 2. Negative logarithmic relationship between the longevity (p_{50} , d) and moisture content (% w.b.) of pollen of *Typha latifolia* stored at 45 °C (○, ●), 35 °C (□, ■), 25 °C (△, ▲), 15 °C (▽, ▼), 5 °C (◇, ◆), or -5 °C (○, ●). Solid lines shown were fitted to the observations denoted by solid symbols. The model fitted is quantified in Table 2.

overestimated by negative exponentials, the pollen survival curves conformed much more closely to negative cumulative normal distributions than to negative exponentials. Figure 1 shows a comparison of the two approaches to describe pollen survival curves for storage at 0.5% RH with 45 °C (Fig. 1A) and 56% RH with 15 °C (Fig. 1B). The estimates of initial germination for pollen stored over phosphorus pentoxide at 45 °C and a saturated solution of calcium nitrate at 15 °C resulting from the negative exponentials were 140 and 222%, respectively. In contrast, the estimates

resulting from the negative cumulative normal distribution were 91.2 and 92.7%, respectively (the actual values being 89.9 and 90.3%, respectively).

Relations between pollen longevity, temperature and moisture content

Given that the estimates of K_1 for the individual survival curves described by negative cumulative normal distributions varied among storage environments significantly and substantially (from values equivalent to 78.8% initial viability at 35 °C with 0.5% RH, to 95.9% at 25 °C with 50.5% RH), eqns (3) and (4) were applied using estimates of p_{50} (duration of storage which reduced pollen viability to 50%) or p_{70} (duration of storage which reduced pollen viability to 70%) rather than σ . The former duration is the more accurately estimated, since it represents the mean of the distribution of pollen deaths in time, and 37 storage environments (a total of 438 observations of pollen viability) provided results in which viability had been reduced either close to or below 50%. In addition, a further five storage environments (42 in total) provided estimates of p_{70} .

These estimates of p_{50} are plotted against moisture content with both axes plotted on logarithmic scales in Fig. 2. The estimates of pollen moisture content at different temperatures over phosphorus pentoxide were somewhat variable (Table 1). In addition, longevity at the second lowest moisture content at each temperature of pollen stored over saturated solutions of zinc chloride (Table 1), was less than that at the next higher moisture content (Fig. 2). Consequently the observations for storage over phosphorus pentoxide and saturated solutions of zinc chloride were excluded from analysis (open symbols in Fig. 2). In accordance with eqn. (3), among the remaining 25 observations there was a negative logarithmic relation between pollen longevity and moisture content at each storage

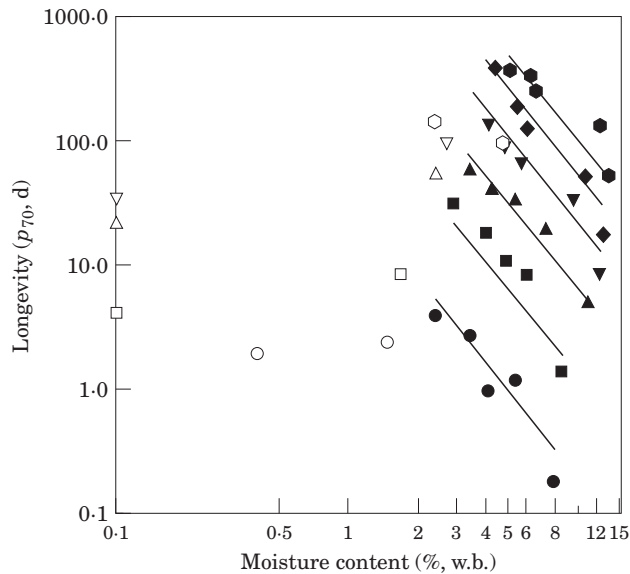


FIG. 3. Negative logarithmic relationship between the longevity (p_{70} , d) and moisture content (% wet basis) of pollen of *Typha latifolia* stored at 45 °C (○, ●), 35 °C (□, ■), 25 °C (△, ▲), 15 °C (▽, ▼), 5 °C (◇, ◆), or -5 °C (○, ●). Solid lines shown were fitted to the observations denoted by solid symbols. The model fitted is quantified in Table 2.

temperature with longevity being greater the cooler the temperature (Fig. 2). At all six storage temperatures (-5 to +45 °C), extrapolation of the fitted relations overestimated the longevity of pollen stored over phosphorus pentoxide or saturated solutions of zinc chloride (open symbols in Fig. 2) considerably. Note that the longevity of pollen stored at -5 °C over saturated solutions of zinc chloride (5.5% RH, 4.7% moisture content) was much less than that for pollen stored over phosphorus pentoxide (0.5% RH, 2.4% moisture content), whereas at warmer temperatures longevity was either similar or greater over saturated solutions of zinc chloride (Fig. 2). The lower-moisture-content limit to the application of eqn. (3) cannot be estimated precisely from these results, however.

The relations between p_{70} , moisture content and temperature in 42 storage environments were broadly similar to those for p_{50} , but of course p_{70} was less than p_{50} (Fig. 3, Table 2).

TABLE 2. Estimates of the coefficients which quantify relations between the longevity (p_{50} or p_{70}), moisture content (m), relative humidity (r) and temperature (t) of pollen of *Typha latifolia* stored air-dry

Equation fitted	R^2 (d.f.)	K_E (s.e.)	C_W (s.e.)	C_R (s.e.)	C_H (s.e.)	C_Q (s.e.)
$\log_{10} p_{50} = K_E - C_W \log_{10} m - C_H t - C_Q t^2$	0.952 (21)	4.607 (0.244)	2.464 (0.223)	—	0.0304 (0.0080)	0.00065 (0.00016)
$\log_{10} p_{70} = K_E - C_W \log_{10} m - C_H t - C_Q t^2$	0.965 (26)	4.193 (0.166)	2.298 (0.178)	—	0.0261 (0.0053)	0.00070 (0.00012)
$\log_{10} p_{50} = K_E - C_R r - C_H t - C_Q t^2$	0.953 (21)	3.406 (0.143)	—	0.0236 (0.0021)	0.0246 (0.0076)	0.00057 (0.00016)
$\log_{10} p_{70} = K_E - C_R r - C_H t - C_Q t^2$	0.963 (26)	3.006 (0.084)	—	0.0212 (0.0017)	0.0179 (0.0054)	0.00067 (0.00013)

These relations are shown by solid lines in Figs 2–5 and were fitted to observations denoted by solid symbols therein.

Relations between pollen longevity, temperature and relative humidity

Over most of the range of relative humidities investigated, there was a negative semi-logarithmic relation between pollen longevity (p_{50}) and relative humidity at each temperature and an increase in longevity the cooler the temperature in accordance with eqn. (4) (solid symbols and solid lines in Fig. 4). Deviations from the model quantified by eqn. (4) were observed, however, at the two highest relative humidities investigated: over saturated solutions of calcium nitrate and sodium nitrite actual longevity were consistently greater and smaller, respectively, than the fitted values (Fig. 4). All observations at the two lowest relative humidities (open symbols between 0 and 5.5% RH in Fig. 4) showed considerably lower longevity than provided by extrapolation of the relations fitted at higher relative humidities. Indeed, relations between longevity and relative

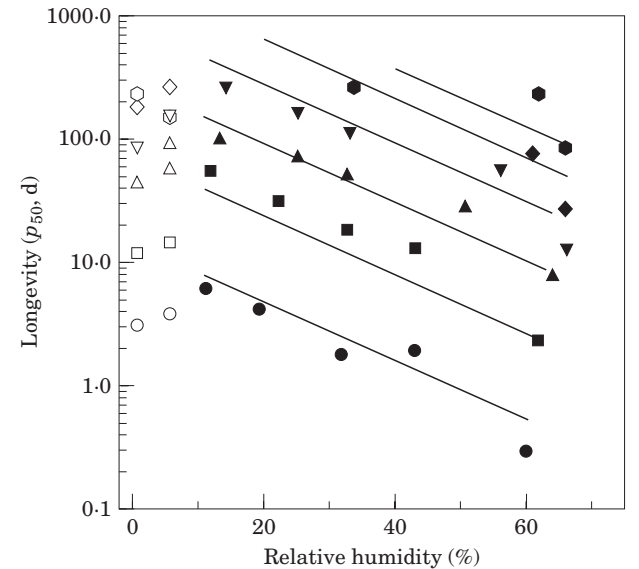


FIG. 4. Relationship between the longevity (p_{50} , d) and equilibrium relative humidity (%) of pollen of *Typha latifolia* stored at 45 °C (○, ●), 35 °C (□, ■), 25 °C (△, ▲), 15 °C (▽, ▼), 5 °C (◇, ◆), and -5 °C (○, ●). Solid lines shown were fitted to the observations denoted by solid symbols. The model fitted is quantified in Table 2.

TABLE 3. Relations between the longevity (p_{50} or p_{70}) of pollen of *Typha latifolia* and higher ranges of relative humidity (r) stored at six different temperatures

Temperature (°C)	Range of RH (%)	Equation fitted	P	R^2	LRHL (%)
45	11–60	$\log p_{50} = 1.103 - 0.0250r$ (s.e. = 0.166) (s.e. = 0.0045)	< 0.025	0.912	14.5
	11–60	$\log p_{70} = 0.8989 - 0.0252r$ (s.e. = 0.1763) (s.e. = 0.0047)	< 0.025	0.904	13.8
35	22–62	$\log p_{50} = 2.169 - 0.0278r$ (s.e. = 0.186) (s.e. = 0.0044)	< 0.025	0.953	13.5
	11.5–62	$\log p_{70} = 1.860 - 0.0260r$ (s.e. = 0.124) (s.e. = 0.0032)	< 0.005	0.956	14.1
25	5.5–64	$\log p_{50} = 2.203 - 0.0179r$ (s.e. = 0.114) (s.e. = 0.0030)	< 0.005	0.897	< 5.5
	5.5–64	$\log p_{70} = 1.973 - 0.0179r$ (s.e. = 0.122) (s.e. = 0.0032)	< 0.01	0.873	< 5.5
15	14–66	$\log p_{50} = 2.796 - 0.0225r$ (s.e. = 0.174) (s.e. = 0.0040)	< 0.025	0.913	11.8
	14–66	$\log p_{70} = 2.494 - 0.0206r$ (s.e. = 0.166) (s.e. = 0.0038)	< 0.025	0.907	8.8
5	33.5–66	$\log p_{50} = 3.337 - 0.0267r$ (s.e. = 0.523) (s.e. = 0.0094)	< 0.025	0.889	18.5
	15–66	$\log p_{70} = 2.908 - 0.0228r$ (s.e. = 0.152) (s.e. = 0.0034)	< 0.025	0.938	14.8
–5	15–66	$\log p_{70} = 2.855 - 0.0144r$ (s.e. = 0.149) (s.e. = 0.0033)	< 0.025	0.864	< 15.0

The estimates of the lower-relative-humidity to each relation (LRHL) are provided by the intersect between each of these negative relations with the positive relation provided by observations at lower relative humidities. See text for more details.

humidity were generally (i.e. except at -5°C) positive among this cohort of observations, in contrast to the negative semi-logarithmic relation detected at higher relative humidities.

Since positive relations between longevity and relative humidity were (generally) detected at the lowest relative humidities and negative semi-logarithmic relations detected at higher relative humidities, it is possible to estimate the intersections between these two relations at each temperature. This was done at each temperature in turn for the seven relative humidity treatments. At each temperature, all observations were initially analysed as a single (negative) relation between pollen longevity and relative humidity. The data were subsequently analysed iteratively in two separate sets with progressively more observations being apportioned to the lower relative humidity set, the model which provided the lowest total residual deviance providing the best-fit model. The resultant models at higher relative humidities are quantified in Table 3. In most cases, the positive relations at low relative humidities were provided by the straight line between observations at 0.5 and 5.5% relative humidity. The advantage of this approach is that it is objective. The drawbacks are that the positive relations at low relative humidities tend to be constructed from only two or three observations at each temperature, and the overall models sometimes underestimate longevity for observations near the point of intersection. For example, at 15 and 35 °C the approach provided sensible models, with the fitted lines intersecting at 11.8 and 13.5% RH, respectively (Table 3).

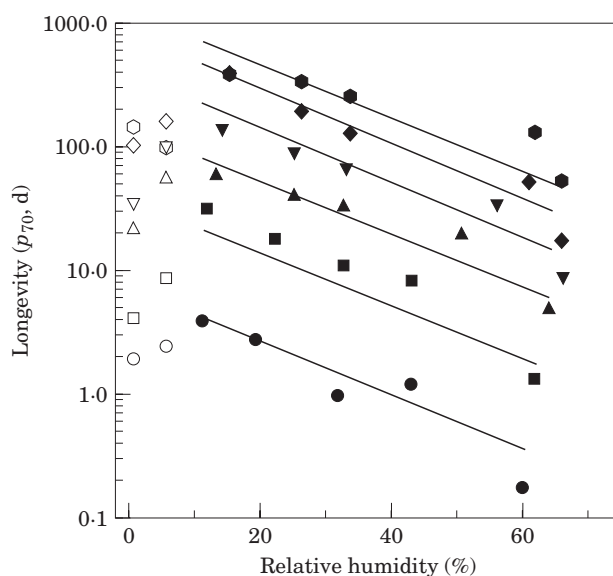


FIG. 5. Relationship between the longevity (p_{70} , d) and equilibrium relative humidity (%) of pollen of *Typha latifolia* stored at 45 °C (○, ●), 35 °C (□, ■), 25 °C (△, ▲), 15 °C (▽, ▼), 5 °C (◇, ◆) and -5°C (○, ●). Solid lines shown were fitted to the observations denoted by solid symbols. The model fitted is quantified in Table 2.

However, at 45 °C this objective procedure provided estimates of the intersect greater than the lowest values in the upper relative humidity range and so longevity at these

relative humidities (e.g. at 11% RH and 45 °C) was underestimated. Similar analyses were carried out for p_{70} (Fig. 5, Table 3). Whereas both periods of longevity resulted in similar estimates of the low-relative-humidity limit at 25, 35 and 45 °C, differences were larger at 5 and 15 °C. Nevertheless, both sets of analyses showed that the lowest estimates were obtained for storage at 25 °C.

DISCUSSION

The original paper by Buitink *et al.* (1998) considered loss in pollen viability in the context of intracellular glasses. In this paper, we have shown that the effect of air-dry storage environment on pollen longevity can be quantified by the seed viability equations developed almost two decades ago (Ellis and Roberts, 1980; Roberts and Ellis, 1989), and which have recently been shown to apply also to fungal spores (Hong *et al.*, 1997). These models demonstrate that there are considerable similarities in the storage behaviour of contrasting propagules in anhydrous biology.

The combination of the constants C_H and C_Q quantify the response of propagule longevity to temperature. These constants quantify a response in which the temperature coefficient (Q_{10}) for change in rate of loss in viability per 10 °C difference in temperature changes with temperature (Ellis and Roberts, 1981; Dickie *et al.*, 1990). The results of the current study demonstrate the considerable similarities between the relative effect of temperature on pollen longevity (for p_{50} , $C_H = 0.0304$, $C_Q = 0.00065$, Table 2) with that on seed longevity ($C_H = 0.0329$, $C_Q = 0.00048$) determined previously (Dickie *et al.*, 1990).

As with seeds (Roberts and Ellis, 1989) and fungal spores (Hong *et al.*, 1997), negative logarithmic relations between pollen longevity and moisture content (Figs 2 and 3) provided better fits to observations than negative semi-logarithmic relations between pollen longevity and equilibrium relative humidity (Figs 4 and 5).

This is particularly pertinent given the somewhat anomalous results for storage over saturated solutions of calcium nitrate (43% RH at 45 °C to 62% RH at -5 °C) and sodium nitrite (60% RH at 45 °C to 66% RH at -5 °C) where all observations of longevity were substantially greater and smaller, respectively, than the fitted trend (Figs 4 and 5). In particular, at a storage temperature of 45 °C the treatment at 43% RH provided greater longevity than that at 31.5% RH (Figs 4 and 5). It is possible, therefore, that the method of controlling relative humidity affected pollen survival. This is not unknown. For example, Kobayashi *et al.* (1978) reported better survival of pollen of three *Citrus* species when stored over a saturated solution of magnesium chloride rather than over sulphuric acid at the same relative humidity.

On the other hand, the accuracy of gravimetric determinations of pollen moisture content is constrained by the limited amount of pollen available for such destructive tests. Moreover, one advantage of the semi-logarithmic relation between longevity and equilibrium relative humidity is that it provides a constant (i.e. C_R) which can be used to compare species (Roberts and Ellis, 1989) and also propagules.

The estimate of C_R for pollen of *T. latifolia* (0.0236) is close to that for conidia of the entomopathogenic fungus *Metarhizium flavoviride* (0.0269) (Hong *et al.*, 1998), but somewhat less than that for orthodox seeds (0.0346) (Ellis *et al.*, 1990b). These estimates of C_R imply that each reduction in relative humidity by 8.7, 11.2 and 12.7% doubles the longevity of orthodox seeds, conidia of *Metarhizium flavoviride* and pollen of *T. latifolia*, respectively. The estimate for C_R of about 0.0236 may well be applicable to pollen of other species. For example, from limited results of the longevity of pollen of *Papaver rhoeas* L. stored at about 75 or 40% RH (Hoekstra, 1995) we estimate $C_R = 0.029$.

There are several reports in the literature of the prolonged longevity of very dry pollen. For example, storage at 0% RH provided the greatest survival of pollen of *Permistera typhoideum* (Harrington, 1970) and 1% RH resulted in good survival of pollen of *Ginkgo* sp. and *Prunus domestica* L. (Snyder and Clausen, 1974). However, almost a quarter of a century ago Roberts (1975) recognized that there is a low-moisture-content limit below which further reduction in moisture does not increase pollen longevity. He suggested then that this limit lies somewhere between moisture contents in equilibrium with 8 to 25% RH.

The current results (Figs 2–5) confirm that there are low-moisture-content limits to the negative logarithmic relation between pollen longevity and moisture content, and low-relative-humidity limits to the negative semi-logarithmic relation between pollen longevity and relative humidity, as has been shown for seeds (Ellis, Hong and Roberts, 1988, 1989, 1990a; Ellis *et al.*, 1990b, 1992; Vertucci and Roos, 1990, 1991, 1993; Vertucci, Roos and Crane, 1994) and fungal spores (Hong *et al.*, 1997, 1998). The procedure used here to estimate the low-relative-humidity limit was problematic: the resultant models tended to underestimate pollen longevity at these values, and so tend to overestimate the low-relative-humidity limit. Ideally, more observations would be required to estimate these limits more accurately. Nevertheless, the approach is objective and provided estimates between extremes of < 5.5 and 18.5% RH (Table 3); i.e. a very similar range to that suggested by Roberts (1975) from much more limited data. The variation in these estimates showed no continuous linear trend with temperature ($P > 0.25$). The mean estimate was 11.9 (s.e. = 1.4)% RH. However, a curvi-linear trend was almost apparent ($0.1 > P > 0.05$) whereby the estimates of the low-relative-humidity limit were driest (5.5% RH) at the intermediate temperature of 25 °C. We cannot explain why such a curvi-linear trend should occur. Given the considerable difficulties in estimating the low-relative-humidity limit accurately, we would wish to see this trend confirmed in subsequent research before accepting it as real.

The mean estimate for the low-relative-humidity limit of 11.9% RH is close to the estimate of 10–12% RH at 20 °C determined in orthodox seeds stored hermetically at 65 °C (Ellis *et al.*, 1988, 1989, 1990a, b, 1992, 1996), that of 11% RH for conidia of *Metarhizium flavoviride* stored hermetically at 50 °C (Hong *et al.*, 1998), and that of 14% RH for seeds of *Taxus brevifolia* Nutt. stored at 5 to 35 °C (Walters-Vertucci, Crane and Vance, 1996), but lower than the range 19–30% RH for orthodox seeds stored at -5 to

65 °C (Vertucci and Roos, 1990, 1991, 1993; Vertucci *et al.*, 1994). Given the 'glass ceiling' apparent in Figs 2–5, corresponding to the maximum storage period of 261 d (i.e. the maximum estimates of longevity may have been artificially constrained by the duration of the investigation), observations from longer durations of storage and more environments are required to provide more accurate estimates of the low-moisture-content and low-relative-humidity limits to negative relations between pollen longevity and moisture in air-dry storage. Nevertheless, the present results indicate considerable similarities among pollen, orthodox seeds and fungal spores in these limits and in the quantitative response of longevity to storage environment.

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