

## **Low-moisture-content Limits to *Nothofagus* Seed Longevity**



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### Summary

Analysis of the relationship between seed moisture content and longevity in hermetic storage at 65°C provided evidence that the increase in longevity with reduction in seed moisture content was subject to a low-moisture-content limit ( $m_c$ ) in both *Nothofagus alpina* and *N. obliqua*. Initial broken-stick regression models gave provisional estimates for  $m_c$  of 6.1% (20% RH at 20°C) and 4.6% (15% RH at 20°C) for *N. alpina* and *N. obliqua*, respectively. However, longevity initially increased and then declined in a curvilinear pattern with further reduction in seed storage moisture content below these values. Cubic and quadratic models also described these patterns well, with few differences in residual error between the three models, although the curvilinear models provided much lower estimates of the optimum moisture content for longevity (2.7%). At selected low moisture contents, there was no significant difference ( $P > 0.25$ ) between the seed survival curves of both species. Thus, alternative broken-stick models, with no effect of moisture content within this range (broadly 2–4%), were fitted, which provided estimates for  $m_c$  of 4.8% (15% RH at 20°C) in both species.

### Introduction

The seed viability equation's negative logarithmic relationship between seed longevity and moisture content applies across a wide range of moisture contents (Ellis and Roberts, 1980), but is nevertheless subject to certain limits at each extreme (Roberts and Ellis, 1989). Below a low-moisture-content limit ( $m_c$ ) to this relation, longevity generally neither increases nor decreases with further reduction in moisture content (Ellis *et al.*, 1988, 1989, 1990a, 1992; Ellis *et al.*, 1990b). The value of  $m_c$  varies considerably among species, but if expressed in terms of seed equilibrium relative humidity (RH) then the variation narrows to 10–15% RH at 20°C (Roberts and Ellis, 1989). However, estimates of  $m_c$  have been limited principally to crop seeds. Little information is available for seeds of wild species, and particularly trees.

The objective of this study was to determine the response of seed longevity to a wide range of seed storage moisture contents, especially at low moisture contents, in *Nothofagus obliqua* (Mirb.) Oerst. and *N. alpina* (Poepp. & Endl.) Oerst. (*Fagaceae*), two timber trees from Chile.

## Materials and Methods

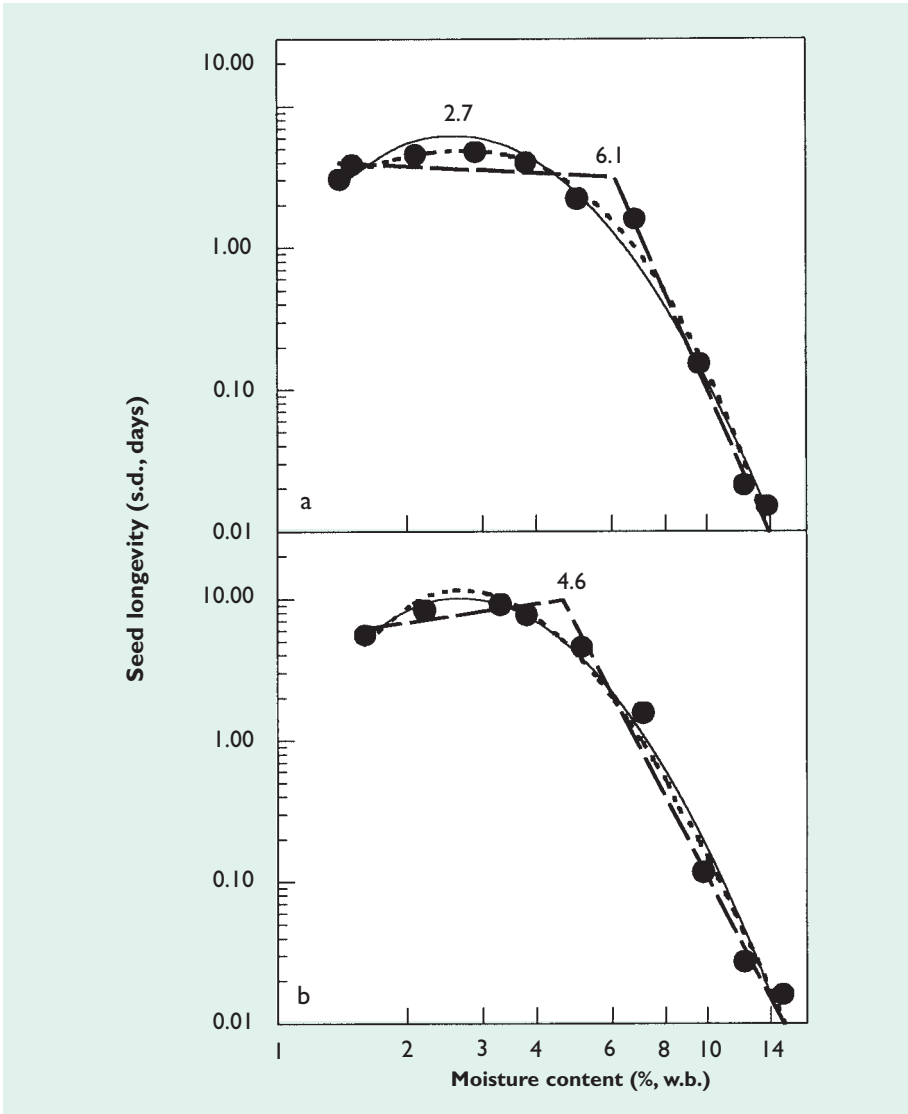
Seed of *Nothofagus obliqua* and *N. alpina*, obtained from Chile, were adjusted to different moisture contents within the air-dry range. For each species, samples of seeds at different moisture contents were stored hermetically at 65°C for different periods up to 30 d. After experimental storage, seeds were humidified for 1–2 days at 20°C, to avoid imbibition damage (Hong & Ellis, 1996), then imbibed in a 50 ppm solution of GA<sub>3</sub> for 24 h, to break dormancy. They were then tested for ability to germinate between moist rolled paper towels within a polythene bag for 30 d at 25°C. Seed viability was estimated by ability to germinate normally (ISTA, 1999).

Seed survival curves at moisture contents between 1.4 and 15% were fitted by probit analysis in accordance with the improved viability equation (Ellis and Roberts, 1980). The negative logarithmic relationship between seed longevity ( $\sigma$ , the standard deviation of frequency distribution of seed deaths in time) and moisture content was analysed using broken-stick, quadratic and cubic regression models (Genstat, 1997). The broken-stick model estimated  $m_c$  directly as the intersect between two linear relations. The estimate of  $m_c$  provided by quadratic and cubic regression models was that value where the slope was zero.

## Results

The negative logarithmic relationship between *Nothofagus* seed longevity and moisture content was subject to a low-moisture-content limit ( $m_c$ ) in both species (Figure 40.1). Initial broken-stick regression models provided estimates for  $m_c$  of 6.1% (20% RH at 20°C) for *N. alpina* and 4.6% (15% RH at 20°C) for *N. obliqua*. However, longevity initially increased and then slightly decreased in a curvilinear pattern with reduction in seed storage moisture content below these estimates of  $m_c$ . Quadratic and cubic regression models (Figure 40.1) gave maximal longevity at around 2.7% moisture content (<10% RH at 20°C) in both species. Comparison of the residual errors of the three models applied showed few differences (Table 40.1), despite considerable differences in the resultant estimates of  $m_c$ .

In *Nothofagus alpina*, comparison (Figure 40.2a) by probit analysis of seed survival curves at the five lowest seed storage moisture contents (from 1.4% to 3.8%) showed no significant differences ( $P > 0.25$ ). However, a similar analysis for the four lowest moisture contents (1.6% to 3.8%) in *N. obliqua*



**Figure 40.1** Effect of moisture content ( $m$ ) on seed storage longevity (standard deviation of the frequency distribution of seed deaths in time) at 65°C for *Nothofagus alpina* (a) and *N. obliqua* (b). Both axes are logarithmic. The relationships are quantified by broken-stick (— —), quadratic (—) and cubic (· · ·) models. Models for *N. alpina*: **Broken-stick:**  $\log_{10}\sigma = 0.62 - 0.16 * \log_{10}m$ , to  $m \leq$  break point (BP);  $\log_{10}\sigma = 0.62 - 6.65 * \log_{10}m + (-6.65 + 0.16)(\log_{10}m - BP)$ , for  $m \geq$  BP. **Quadratic:**  $\log_{10}\sigma = -0.06 + 4.14 * \log_{10}m - 5.03 * \log_{10}m^2$ . **Cubic:**  $\log_{10}\sigma = 0.38 + 0.81 * \log_{10}m + 1.19 * \log_{10}m^2 - 3.25 * \log_{10}m^3$ . *N. obliqua*: **Broken-stick:**  $\log_{10}\sigma = 0.70 + 0.43 * \log_{10}m$ , to  $m \leq$  BP;  $\log_{10}\sigma = 0.70 - 6.12 * \log_{10}m + (-6.12 - 0.43)(\log_{10}m - BP)$ , for  $m \geq$  BP. **Quadratic:**  $\log_{10}\sigma = -0.02 + 4.60 * \log_{10}m - 5.4 * \log_{10}m^2$ . **Cubic:**  $\log_{10}\sigma = -0.39 + 7.13 * \log_{10}m - 9.62 * \log_{10}m^2 + 2.05 * \log_{10}m^3$ .

**Table 40.1 Errors from fitting four different regression models to describe the relationship between the logarithm of seed storage longevity ( $\sigma$ ) at 65°C and the logarithm of moisture content ( $m$ ) for *Nothofagus alpina* and *N. obliqua***

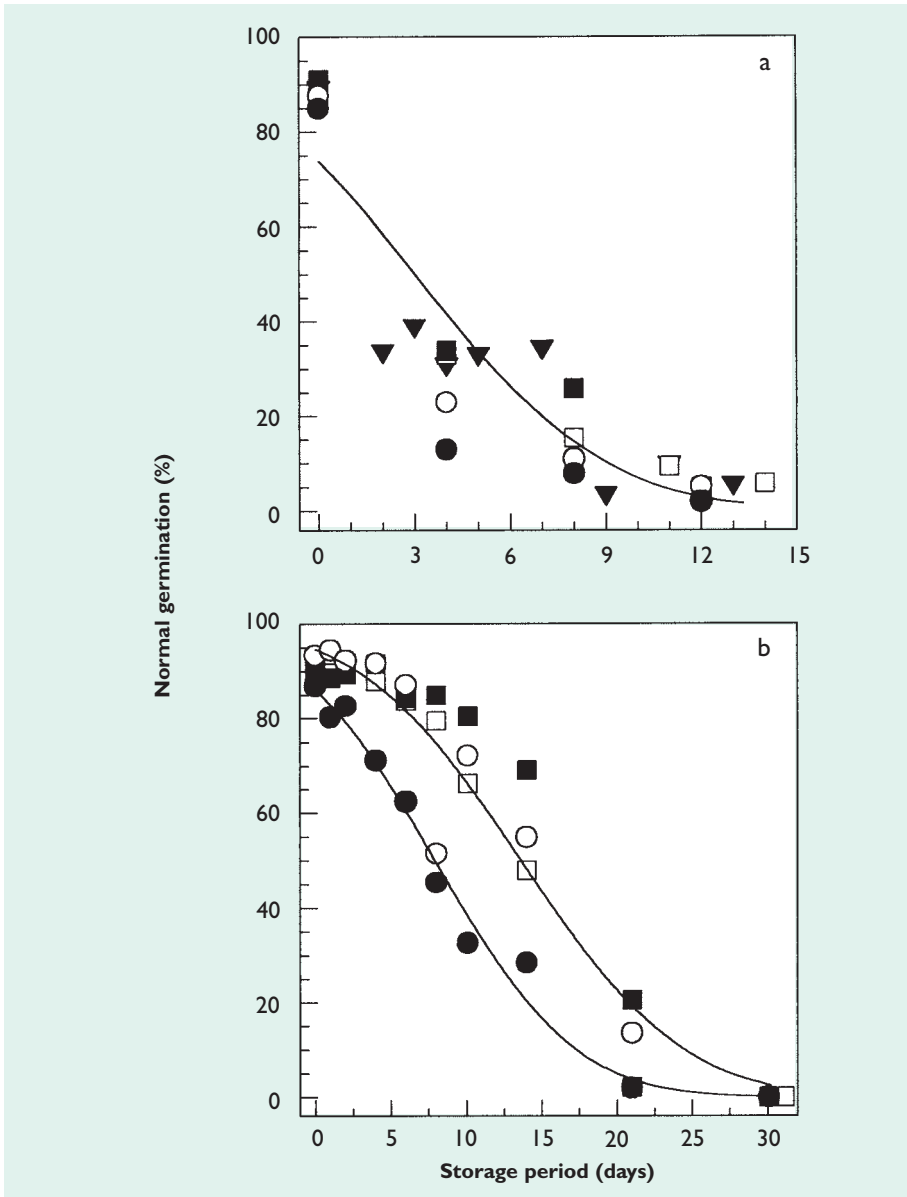
Species/ Model	d.f.	RSS	MSS	R <sup>2</sup>	No. of parameters
<b><i>Nothofagus alpina</i></b>					
Broken-stick (negative slope where $m < m_c$ )	6	0.097	0.016	0.99	4
Quadratic	7	0.155	0.022	0.98	3
Cubic	6	0.102	0.017	0.98	4
Broken-stick (no slope where $m < m_c$ )	7	0.241	0.034	0.93	3
<b><i>Nothofagus obliqua</i></b>					
Broken-stick (positive slope $m < m_c$ )	5	0.134	0.027	0.96	4
Quadratic	6	0.185	0.031	0.98	3
Cubic	5	0.167	0.033	0.97	4
Broken-stick (no slope where $m < m_c$ )	5*	0.124	0.021	0.96	3

\*d.f. is 5 instead of 6 because the lowest moisture content data point was excluded from this analysis. See text for further explanation.

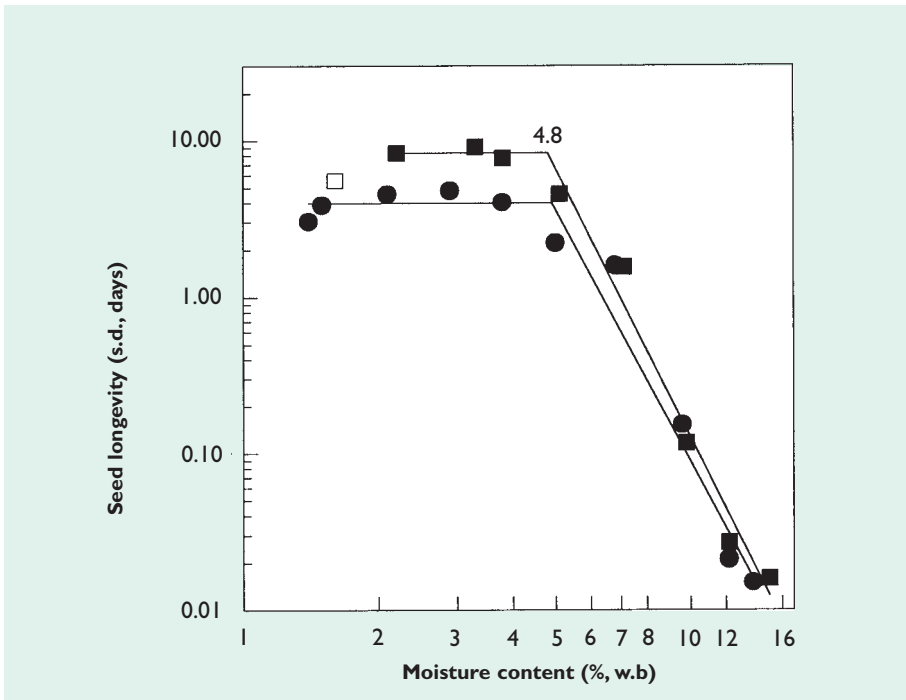
(Total sums of squares were 8.736 in *N. alpina* and 10.143 in *N. obliqua*, respectively).

(Figure 40.2b) did show significant differences ( $P < 0.01$ ) among the four survival curves. Once the lowest moisture content seed survival curve (1.6%) was excluded from the data set, however, no significant reduction in error was detected by constraining the remaining data to a common curve ( $P > 0.25$ ) (Figure 40.2b). Thus, at 65°C, survival at 1.6% moisture content for *N. obliqua* was poorer than at 2.2–3.8% moisture content. This was a consequence of differences in intercept (i.e., loss in viability before storage, presumably during desiccation) rather than slope: no significant increase in error ( $P > 0.5$ ) was detected when all four of these survival curves were constrained to a common slope.

An alternative broken-stick model was fitted with no effect of moisture content between 1.4 and 3.8% (*N. alpina*) or 2.2. and 3.8% (*N. obliqua*) on seed storage longevity (i.e., the slope in Figure 40.3 is zero over these ranges). This model gave estimates for  $m_c$  of 4.8% moisture content (15% RH at 20°C) in both species. The residual error of this model was larger ( $P < 0.05$ ) for *N. alpina* than the original broken-stick model (Table 40.1), but nevertheless provided an estimate of  $m_c$  (Figure 40.3) below which longevity did not change (compare with Figure 40.1a).



**Figure 40.2** (a) Seed survival data for *Nothofagus alpina* stored hermetically at 65°C with 1.4 (●), 1.5 (○), 2.1 (■), 2.9 (□) and 3.8% (▼) moisture content. Survival curves were fitted by probit analysis with a common  $K$ , ( $0.64 \pm 0.046$  probits) and common slope ( $-0.21 \pm 0.008$ ; i.e., 4.7 d to lose 1 probit viability). (b) Survival of *N. obliqua* seeds stored hermetically at 65°C with 1.6 (●), 2.2 (○), 3.3 (■) and 3.8% (□) moisture content. The survival curve at 1.6% moisture content has the same slope ( $-0.12 \pm 0.04$ ; i.e., 8.5 d to lose 1 probit viability) as the single curve for 2.2, 3.3 and 3.8% moisture content. However, the intercept of the latter is  $1.61 \pm 0.047$  probits (95% initial germination) compared with  $0.99 \pm 0.037$  (equivalent to 84% initial germination) for the curve at 1.6% moisture content.



**Figure 00.3** Relationship between seed moisture content and longevity (standard deviation of the frequency distribution of seed deaths in time) at 65°C for *Nothofagus alpina* (●) and *N. obliqua* (■, □). Both axes are logarithmic. A broken-stick model with a horizontal line at moisture contents below  $m_c$  was fitted to both species. The lowest data point (□) for *N. obliqua* was omitted from the analysis. Model for *N. alpina*:  $\log_{10}\sigma = 0.60$ , to  $m \leq$  break point (BP);  $\log_{10}\sigma = 4.38 - 5.42 * \log_{10}m$ , for  $m \geq$  BP. *N. obliqua*:  $\log_{10}\sigma = 0.91$ , to  $m \leq$  BP;  $\log_{10}\sigma = 4.8 - 5.71 * \log_{10}m$ , for  $m \geq$  BP.

## Discussion

In general, the relationship between the logarithms of each of seed longevity and moisture content at 65°C observed in these two *Nothofagus* species followed a similar broken-stick pattern to that described previously in various crop species (Ellis *et al.*, 1988; 1989; 1990a, b). But it was also sufficiently variable to be described by a continuous curvilinear function.

Vertucci and Roos (1990) and Vertucci *et al.* (1994) used a quadratic function to describe the relation between seed vigour and storage moisture content in several crop species. They concluded that optimum moisture contents for seed storage were in equilibrium with 19–27% RH; i.e., appreciably greater than the value of 10–15% RH at 20°C suggested by Roberts and Ellis (1989). In the present study, the curvilinear function, albeit to log-transformed data, provided an optimum moisture content as low as 2.7%, in equilibrium with <10% RH at 20°C.

Clearly, in the present study, desiccation to the lowest moisture content studied for *N. obliqua* of 1.6% was damaging to viability prior to storage (Figure 40.2b) and, although not significant, this may have been the case for *N. alpina* also at 1.4% moisture content (Figure 40.2a). This tallies with previous research showing damage to seeds from extreme desiccation (Nutile 1964; Vertucci and Leopold 1987; Roberts and Ellis, 1989; Vertucci and Roos, 1990; Vertucci *et al.*, 1994).

The identification of damage from desiccation to the lowest moisture content studied (1.4%) helps to illustrate that at slightly greater values (i.e., about 2 to 4%) there was no effect of moisture content on longevity. Once moisture content was increased above 4.8%, however, longevity was reduced progressively in accordance with the viability equation of Ellis and Roberts (1980).

In both *Nothofagus* species, the relative humidities in equilibrium at 20°C with  $m_c$  (15% RH) provided by the final broken-stick model were broadly similar to those estimated previously for the longevity of crop seeds (Ellis *et al.*, 1988; 1989; 1990a,b), orchid seeds (Pritchard *et al.*, 1999), seeds of a tree – *Taxus brevifolia* (Walters-Vertucci *et al.*, 1996), pollen (Buitink *et al.*, 1998; Hong *et al.*, 1999) and even fungal spores (Hong *et al.*, 1998).

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## References

- Buitink, J., Walters, C.W., Hoekstra, F. and Crane, J. (1998). Storage behaviour of *Typha latifolia* pollen at low water contents: Interpretation on the basis of water activity and glass concepts. *Physiologia Plantarum* **103**: 145–153.
- Ellis, R.H., Hong, T.D. and Roberts, E.H. (1988). A low-moisture-content limit to logarithmic relations between seed moisture content and longevity. *Annals of Botany* **61**: 405–408.
- Ellis, R.H., Hong, T.D. and Roberts, E.H. (1989). A comparison of the low-moisture-content limit to the logarithmic relation between seed moisture and longevity in twelve species. *Annals of Botany* **63**: 601–611.
- Ellis, R.H., Hong, T.D. and Roberts, E.H. (1990a). Moisture content and the longevity of seeds of *Phaseolus vulgaris*. *Annals of Botany* **66**: 341–348.
- Ellis, R.H., Hong, T.D., Roberts, E.H. and Tao, K.L. (1990b). Low moisture content limits to relations between seed longevity and moisture. *Annals of Botany* **65**: 493–504.
- Ellis, R.H., Hong, T.D. and Roberts, E.H. (1992). The low-moisture-content limit to the negative logarithmic relation between seed longevity and moisture content in three subspecies of rice. *Annals of Botany* **69**: 53–58.
- Ellis, R.H. and Roberts, E.H. (1980). Improved equations for the prediction of seed longevity. *Annals of Botany* **45**: 13–30.
- Genstat 5 Committee (1997). *Genstat 5 Release 3 reference manual*. Clarendon Press, Oxford, UK.
- Hong, T.D. and Ellis, R.H. (1996). *A protocol to determine seed storage behaviour*. In: J.M.M. Engels and J. Toll (eds). IPGRI Technical Bulletin No. 1. International Plant Genetic Resources Institute, Rome, Italy.
- Hong, T.D., Jenkins, N.E., Ellis, R.H. and Moore, D. (1998). Limits to the negative logarithmic relationship between moisture content and longevity in conidia of *Metarhizium flavoviride*. *Annals of Botany* **81**: 625–630.
- Hong T.D., Ellis, R.H., Buitink, J., Walters, C., Hoekstra, F.A. and Crane, J. (1999). A model of the effect of temperature and moisture content on pollen longevity in air-dry storage environments. *Annals of Botany* **83**: 167–173.
- ISTA (1999). International Rules for Seed Testing, Rules 1999. *Seed Science and Technology* **27**: Supplement, 1–333.

- Nutile, G.E. (1964). Effect of desiccation on viability of seeds. *Crop Science* **4**: 325–328.
- Pritchard, H., Poynter, A. and Seaton, P. (1999). Interspecific variation in orchid seed longevity in relation to ultra-dry storage and cryopreservation. *Lindleyana* **14**: 92–101.
- Roberts, E.H. and Ellis, R.H. (1989). Water and seed survival. *Annals of Botany* **63**: 39–52.
- Vertucci, C.W. and Leopold, A.C. (1987). The relationship between water binding and desiccation tolerance in tissues. *Plant Physiology* **85**: 232–238.
- Vertucci, C.W. and Roos, E.E. (1990). Theoretical basis of protocols for seed storage. *Plant Physiology* **94**: 1019–1023.
- Vertucci, C.W., Roos, E.E. and Crane, J. (1994). Theoretical basis of protocols for seed storage III. Optimum moisture content for pea seed stored at different temperatures. *Annals of Botany* **74**: 531–540.
- Walters-Vertucci, C., Crane, J. and Vance, N. (1996). Physiological aspects of *Taxus brevifolia* seeds in relation to seed storage characteristics. *Physiologia Plantarum* **98**: 1–12.

