Seed Science Research (2002) **12**, 31–37 DOI: 10.1079/SSR200195

Seed storage behaviour of *Fagus sylvatica* and *Fagus crenata*

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Abstract

The seed storage behaviour of *Fagus sylvatica* and *F. crenata* was investigated. A large fraction of seeds of both species survived desiccation to about 3% moisture content (MC) (in equilibrium with 10% relative humidity at 20°C). Nevertheless, viability was reduced significantly and progressively by desiccation from 14% to 3% MC. In addition, during subsequent hermetic storage at constant temperatures of 20 to –20°C in *F. sylvatica* and 10 to –20°C in *F. crenata* seeds, viability was lost more rapidly with reduction in MC below about 7.6–11.5% (40–71% relative humidity at 20°C). Thus, *Fagus sylvatica* and *F. crenata* exhibited intermediate seed storage behaviour. Survival at –20°C with 7.8–11.5% (*F. sylvatica*) and 7.6% MC (*F. crenata*) was comparatively good, with 64–84% of seeds remaining able to germinate normally after 2 years of hermetic storage, although this was neither appreciably better nor worse than at 0–10°C. Optimum seed storage environments, within the range investigated, were provided by combining temperatures of –10 to –20°C with 7.8–11.5% (*F. sylvatica*) or 7.6–9.5% (*F. crenata*) MC.

Keywords: *Fagus sylvatica*, *Fagus crenata*, desiccation, seed storage behaviour, orthodox, intermediate

Introduction

Seeds with orthodox storage behaviour tolerate desiccation to moisture contents (MCs) below 5%, and reduced storage temperature and MC increase their longevity in air-dry storage in a predictable way (Roberts, 1973). Seeds showing intermediate storage behaviour tolerate some desiccation, but longevity in dry storage is decreased by reduction in temperature below about 10°C and/or MC values below those in equilibrium with about 40–50% relative humidity (Ellis *et al*., 1990).

The seed storage behaviour of *Fagus sylvatica* L. is uncertain. Bonner (1990) defined *F. sylvatica* as sub-orthodox, due to its poor longevity under typical storage conditions for orthodox tree seeds. Gosling (1991) proposed that *F. sylvatica* exhibits intermediate seed storage behaviour due to its poor longevity under the best-available tree seed storage conditions (10% MC, –10°C). In contrast, Poulsen (1993) classified *F. sylvatica* as orthodox, because viability was maintained – within certain limits – during 2 years of storage at 5.4% MC and –8°C. Poulsen and Knudsen (1999) confirmed this classification recently after 6 years of further storage.

Fagus crenata B., a closely related species, has also been recently classified as showing orthodox seed storage behaviour (Koyama *et al*., 1997) because viability was not reduced greatly after 2 years of storage at 2°C and 8.7% MC.

Although some recent literature suggests that *F. sylvatica* and *F. crenata* exhibit orthodox seed storage behaviour, further investigation is warranted because substantial seed viability reduction has been reported when these seeds are dried to and/or stored at MC between 5 and 9% (Suszka, 1974, 1975; Schubert, 1990; Poulsen, 1993; Šindelar, 1995; Suszka *et al*., 1996; Koyama *et al*., 1997). To resolve this issue, we investigated the response of seed survival to (1) progressively greater desiccation to low and very low (<5%) MC; and (2) subsequent hermetic storage in a wide range of comparatively cool and dry environments.

Materials and methods

Seeds of *F. sylvatica* (European beech) were collected from the ground 1 week after the beginning of seed

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shedding in October 1997 at Whiteknights, The University of Reading. *F. crenata* (Japanese beech) seeds were purchased from the Ito-Nouen Nursery, Shimosugi, Japan, in December 1997 and delivered to Reading by courier post. Empty nuts of *F. sylvatica* and *F. crenata* were discarded by passing the nuts throughout a ventilated air column. After further cleaning using sieves and a ventilated air column, 150 seeds were sampled to determine initial viability. Initial MCs (fresh-weight basis), determined using the low-constant-temperature-oven method (ISTA, 1999), for *F. sylvatica* and the *F. crenata* were 21 and 17.6%, respectively. Seeds were stored sealed in laminated aluminium foil bags at these MCs and 2–5°C for 5 d after receipt while they were cleaned and initial MCs determined.

The samples of *F. sylvatica* and *F. crenata* were then divided into six sub-lots and dried in a forced-air drying cabinet (15–17% relative humidity at 15–17°C) to different MCs between 2.6 and 14.7%. Desiccation to the lowest MC (<5%) was achieved by further drying over regularly regenerated silica gel at 20°C. Desiccation to approximately 3% MC took 6 d (*F. crenata*) to 20 d (*F. sylvatica*). After drying, each sub-sample of *ca*. 2500 seeds was stored hermetically in a laminated aluminium foil bag at 2–5°C for 5–8 d to allow moisture equilibration within and among the seeds. Seed equilibrium relative humidity was determined at 20°C using a Humiditat IC1 (Novasina, Zurich).

Seed viability was estimated by long-duration germination tests, which broke dormancy. Before each germination test, seeds of both species were humidified to 15% MC above water at 20°C to avoid imbibition damage (Hong and Ellis, 1996). Seeds were then placed between moist rolled paper towels within polythene bags in an incubator at 3–5°C until germination was completed. Each sample of 80–100 seeds was divided into four (*F. sylvatica*) or two (*F. crenata*) replicates. Normal germination (ISTA, 1999) was assessed.

Seeds that remained ungerminated after long test durations were evaluated regularly to determine whether they remained fresh or were obviously dead (ISTA, 1999); if fresh seeds remained, then germination tests were continued. Selected initial samples of *F. sylvatica* seeds, which did not germinate after 180 d at 3–5°C, were treated with 2,3,5-triphenyl-tetrazolium chloride solution (1%, buffered at pH 6.5–7.5) (ISTA, 1999) to check viability. All seeds that had not germinated after 180 d failed to stain as viable, i.e. reliance on the fresh/obviously dead classification was appropriate. Therefore, in later samples it was assumed that seeds that did not germinate within 180 d at 3–5°C were dead. Ungerminated seeds of *F. crenata* were not subjected to a tetrazolium test. For all viable seeds to germinate, 150–180 d (*F. sylvatica*) or 120 d (*F. crenata*) at 3–5°C were required; this is compatible with the results of Thomsen (1997).

A factorial design was used to study the response of *F. sylvatica* seed survival to storage MC and temperature. Seeds were stored at five temperatures (20, 10, 0, –10 or –20°C) combined with six MCs from 2.6 to 13.5%, and with storage periods from 0 to 730 d. Seed numbers were limited for *F. crenata*, and so only two storage temperatures (10 and –20°C) were used, but with a similar range of MCs (2.9–14.5%) and storage periods. Each treatment combination was represented by a sample of 80–100 seeds, sealed in a laminated aluminium-foil packet (12 × 12 cm).

The relationship between seed viability (percent normal germination, angular-transformed) and seed MC following desiccation was analysed by general linear regression models (Genstat, 1997). A similar approach was applied initially to determine the effect of MC, temperature and period of storage on seed viability in *F. crenata* and *F. sylvatica* (Genstat, 1997). In those analyses, storage temperature and MC were declared as factors, with storage period as a continuous variable.

Results

Both seed lots showed high initial viability, 97 and 90% normal germination for *F. sylvatica* and *F. crenata*, respectively. The majority of seeds of both species survived desiccation to the lowest MCs studied, 2.6–2.9% (10–12% RH at 20°C). Nevertheless, progressive reductions in viability with desiccation were detected ($P < 0.005$) (Fig. 1). The gradient of this desiccation damage did not differ ($P > 0.5$) between *F. sylvatica* and *F. crenata*. Within the MC range investigated, desiccation did not affect the subsequent time taken to germinate (data not shown). Relations between equilibrium relative humidity at 20°C and seed MC for *F. sylvatica* and *F. crenata* were similar (Fig. 2).

Seeds of *F. sylvatica* lost viability during 730 d in most storage environments, but especially at higher MCs combined with warmer temperatures (Fig. 3). Significant effects of storage period ($P < 0.001$), temperature ($P < 0.001$), MC ($P < 0.05$) and the interaction between temperature and MC ($P < 0.001$) on *F. sylvatica* seed viability were detected. Viability was lost completely after 150 d at 20°C and 13.5 or 11.5% MC, and almost completely after 400 d at 10°C and 13.5 or 11.5% MC. A substantial reduction in viability also occurred in seeds stored at sub-zero temperatures with 13.5% MC. Loss in viability was least in seeds stored at –10 and –20°C with 11.5% MC. Within the first 180 d of storage, seeds at 7.8% and lower MCs lost viability at all temperatures investigated, but thereafter little further loss in

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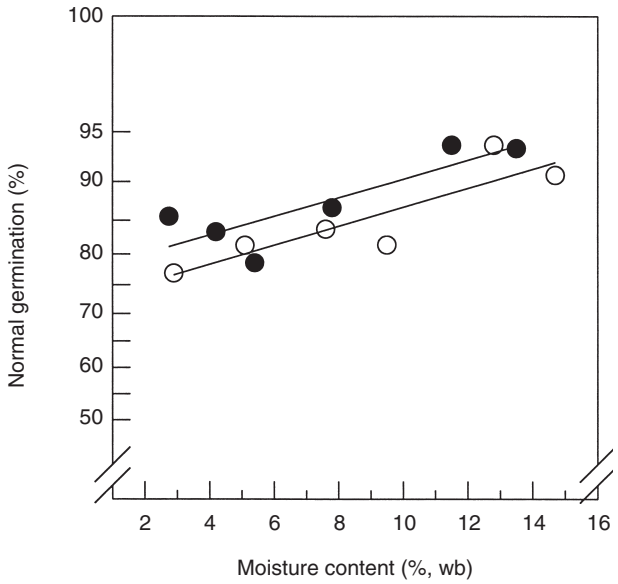

Figure 1. Effect of desiccation to different seed moisture contents on the seed viability (normal germination percentage, angular scale) of *F. sylvatica* (●) and *F. crenata* (○). The fitted lines have intercepts of 61.4° (i.e. 77%) and 58.0° (i.e. 72%) for *F. sylvatica* and *F. crenata*, respectively, with a common slope of 1.045° $\%^{-1}$ (SE 0.31).

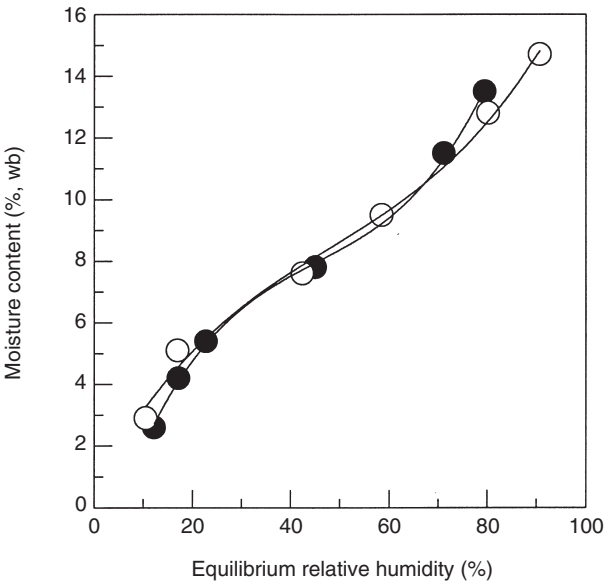

Figure 2. Relationship between equilibrium relative humidity at 20°C and seed moisture content for *Fagus sylvatica* (●) and *F. crenata* seeds (○).

viability was observed. This pattern is consistent with the absence of significant interactions ($P > 0.1$) between seed MC and storage period, and between storage period and temperature.

In order to better understand the response of seed survival to different combinations of seed storage temperature and MC, a contour plot of seed survival after 730 d storage against seed storage MC and temperature was prepared. Maximum survival of *F. sylvatica* seeds occurred at around 11.5% MC combined with –10 and –20°C (Fig. 4a). To determine whether or not the contour patterns shown in Fig. 4a were significant, a further regression analysis of survival in response to temperature, MC and storage period (all as continuous variables) was undertaken. This showed a significant effect ($P < 0.001$) of including linear and quadratic components to describe the effect of both storage temperature and MC, and linear, quadratic and cubic components for the storage period. In addition, several interactions were significant (Fig. 4). The model obtained by this empirical regression analysis accounted for only 52% of total variance (whole data set from 0 to 730 d). Nevertheless, it was sufficient to confirm that there was a range of optimal combinations of seed storage MC and temperature at which seed survival of *F. sylvatica* after 730 d was greatest (Fig. 4b). This range (Fig. 4b) coincided reasonably well with that at which ≥ 60% viability was recorded after 730 d storage (Fig. 4a).

F. crenata seeds lost viability more rapidly during storage at the two higher (12.8 and 14.7%) and the two lower (2.9 and 5.1%) MCs studied, at both temperatures, than at the two intermediate MCs (7.6 and 9.5%) (Fig. 5). Significant effects of MC and storage period ($P < 0.001$), but not temperature ($P > 0.05$), on viability were detected. Indeed, the pattern of viability loss was similar at both 10°C and –20°C at most MCs. However, all the first-order interactions among MC, temperature and storage period were significant ($P < 0.05$), presumably as a result of the differences apparent at 9.5–12.8% MC. Similar to the results in *F. sylvatica* at 2.6% MC, viability was reduced substantially during the first 200 d of storage at 2.9% MC, but with little subsequent change over the subsequent 600 d of storage.

Discussion

Figures 3–5 provide clear evidence that both *F. sylvatica* and *F. crenata* show intermediate seed storage behaviour. This conclusion is at variance with those of Poulsen (1993) for *F. sylvatica* and Koyama *et al*. (1997) for *F. crenata*. Nevertheless, the actual results for comparable storage environments are rather more compatible. At 4°C, Poulsen (1993) showed slightly poorer survival following storage at 5.4% than at 9.0% MC, while at –8°C survival at 5.4% MC was poorer that at 12.1% MC; no results were provided for intermediate moisture contents. Comparison with Fig. 4 shows that these environments provided results that were all close to the 60% contour line. Similar

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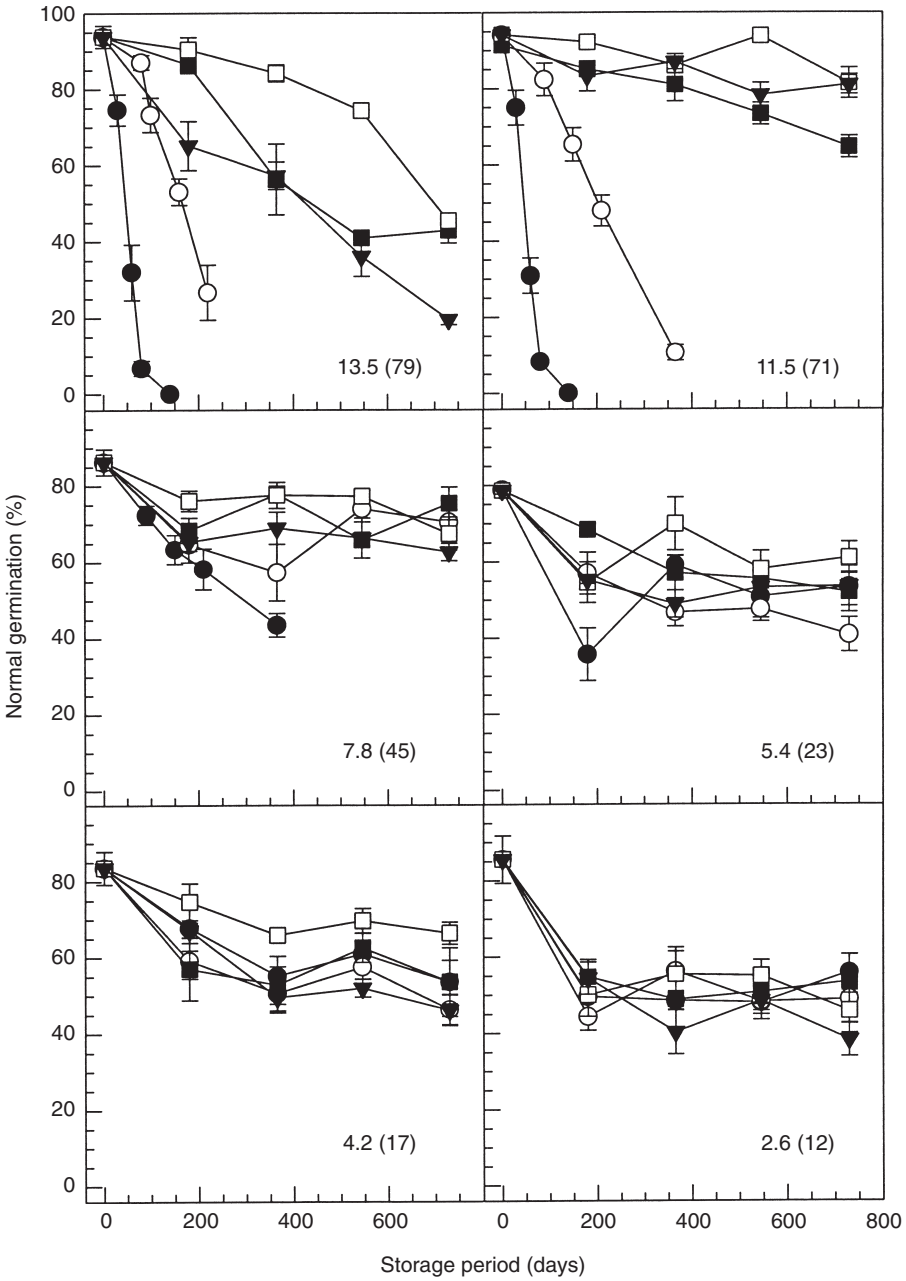

Figure 3. Effect of seed storage moisture content (MC), temperature and period on seed viability (ability to germinate normally) in *F. sylvatica*. Storage temperatures were 20°C (●), 10°C (○), 0°C (■), –10°C (□) and –20°C (▼). Seed MC (%) and equilibrium RH at 20°C (%, in parentheses) are given, bottom right. Vertical bars represent ± SE for each germination test result.

results to those of Poulsen (1993) were obtained after 10 years of storage, in which seeds at 6.2% MC showed 18% less germination compared with those at 9.0% MC (Suszka *et al*., 1996). Koyama *et al*. (1997) concluded that *F. crenata* is orthodox because seeds stored for 2 years at 2°C with 8.7% MC showed good survival. That observation is also broadly compatible with our results at 10°C and –20°C with 7.6% MC (Fig. 5), but the wider range of storage environments in our study showed that both further desiccation (Fig. 1) and subsequent storage at lower MCs (Fig. 5) result in greater damage.

In both species, seed survival curves at the highest MCs investigated were unimodal. For example, in *F.*

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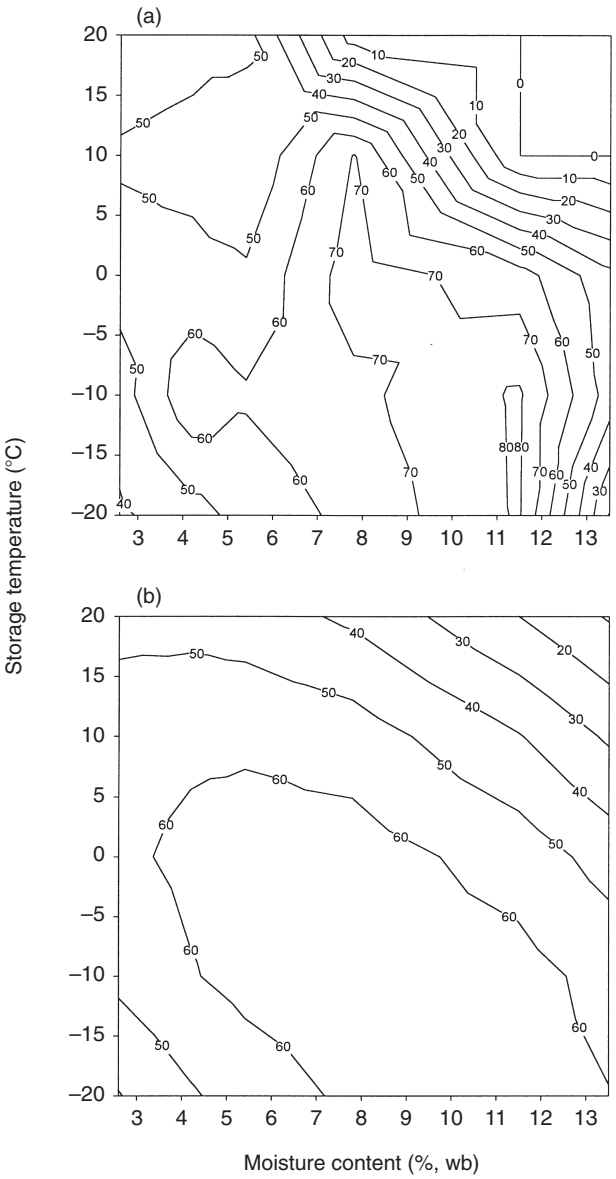

Figure 4. Contour plot of (a) observed viability (% normal germination) after 730 d hermetic storage at different combinations of seed moisture content (MC) and temperature of *F. sylvatica*. All observations from 0 to 730 d storage (Fig. 3) were analysed to provide the following regression model: viability (angular % normal germination) = 55.43 (± 4.96) – [0.1504 (± 0.025) × storage period] + [0.328 (± 0.112) × temperature] + [4.7 (± 1.24) × MC] + [0.0004 (± 0.000085) × (storage period)^2] – [0.0213 (± 0.0047) × (temperature)^2] – [0.244 (± 0.072) × (MC)^2] – [0.3 × 10^{-6} (± 0.8 × 10^{-7}) × (storage period)^3] – [0.0019 (± 0.00086) × (storage period × MC)] – [0.086 (± 0.015) × (temperature × MC)]. The effect of all the above terms in the model was significant ($P < 0.05$). The contour plot (b) shows the fitted values of this model for a storage period of 730 d.

sylvatica at 20°C with 13.5% MC, the curve is clearly a negative cumulative normal distribution (Fig. 3). In contrast, at the lowest MCs (2.6–2.9%) studied, the pattern was bimodal with the majority of the loss in viability occurring within the first 200 d of storage (Figs 3, 5). The contradiction between homogeneity in survival at higher MCs, but heterogeneity in survival at low MCs warrants further investigation; it is a potential tool to better understand seed desiccation tolerance.

The contour plots for *F. sylvatica* (Fig. 4) show that, among comparatively cool and dry environments, it is possible to identify a discontinuity in the general trend for seed storage survival to improve with reduction in each of seed storage MC and temperature. Indeed, Fig. 4 illustrates that results from wide combinations of different seed storage temperature and MCs are helpful in determining seed storage behaviour. The raw data suggest that the discontinuity is stronger for MC than for temperature, with the storage environments providing the least loss in viability clustering around 11.5% MC at –10°C to –20°C (Fig. 4a). The fitted model suffers from being arbitrary with little physiological justification, but provides a broader range of least-damaging environments. Despite this limitation, we suggest that this approach represents a sensible methodology to apply when attempting to define appropriate environments for short- and medium-term seed storage of species with intermediate seed-storage behaviour. Somewhat in contrast to the raw data (Fig. 4a), the fitted model suggests that the 'optimal' MC increases with reduction in storage temperature from 0 to –20°C (Fig. 4b). This particular model output is consistent with the theory that the optimum MC for seed storage increases with reduction in temperature (Vertucci *et al.*, 1994). There is some possibility, therefore, that this theory, developed for orthodox seeds, might explain intermediate seed storage behaviour.

In *F. crenata*, a similar storage MC of 7.6% was least damaging, but a longer period of storage would have been required to distinguish between the two storage temperatures of 10°C and –20°C (Fig. 5). Nevertheless, given the results for seed storage survival after 2 years at 7.6% and 9.5% MC, the cooler of these two temperatures might be preferable.

One difference between the current results for these two *Fagus* species and those species in which intermediate seed storage behaviour was first investigated, e.g. *Coffea arabica* (Ellis *et al.*, 1990) or *Elaeis guineensis* (Ellis *et al.*, 1991), is that the discontinuity in the trend of seed storage survival with temperature was detected at a much warmer temperature in the latter two species. One explanation

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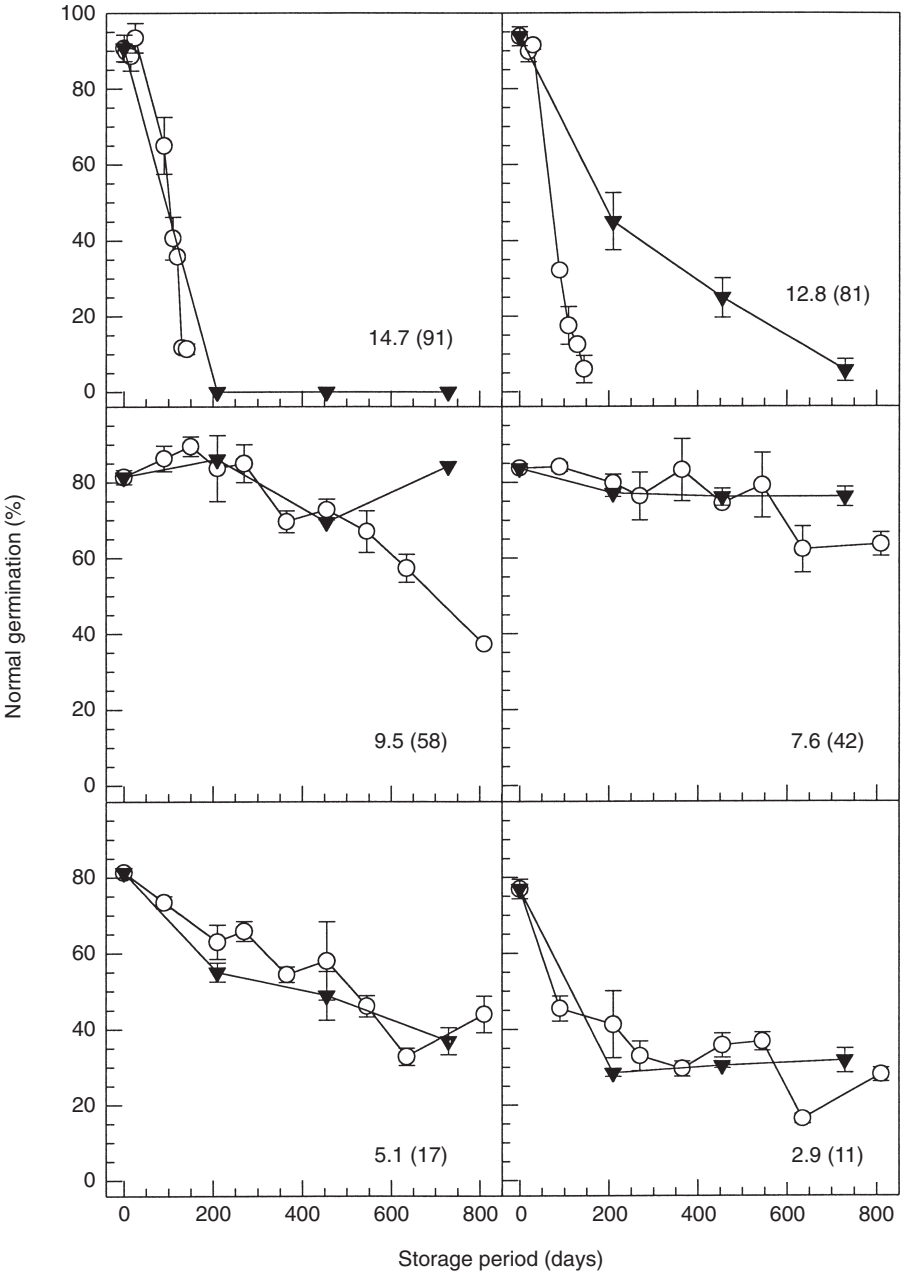

Figure 5. Effect of seed storage moisture content, period and temperature, 10°C (○) and –20°C (▼), on the viability of *F. crenata* seeds. Further details as Fig. 3.

for the difference is that the latter species originated from warmer climates than *Fagus* species (Hong and Ellis, 1996).

Acknowledgements

We thank the Instituto de Investigaciones Agropecuarias, Chile, for a research scholarship to Pedro León-Lobos, to Dr Shigeru Suzuki for help with the purchase of *F. crenata* seeds and supporting information, and Drs Tran Dang Hong, Alistair Murdoch and Peter Gosling for advice.

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Received 3 September 2001
accepted after revision 9 November 2001

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