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Assessment of germination and vigour status of 24-year-old seeds of a *Sophora* hybrid

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Abstract Germination and vigour of 24-year-old seeds collected from a single individual of a hybrid *Sophora* and stored at room temperature are compared with seeds from the 2001 season from the same tree. Germination of machine-cleaned seed showed the hard seed character was still evident after 24 yr. Hand-chipped seeds of the 2001 harvest gave 93% germination; 1977 seeds were 8%. Of the 2001 harvest there was little difference in germination between machine-cleaned seeds and seeds removed by hand from pods. The majority of 1977 embryos were dead within their hard seed coat. Hand-chipped and machine-scarified seeds of both harvests were subjected to two accelerated ageing treatments designed to impose physiological stress. Germination of 1977 seeds was suppressed to zero but 2001 seeds were minimally affected. Tetrazolium tests for viability showed that viability was lost from most 1977 seeds. The loss in germinability and viability of the 1977 seeds was accompanied by a loss in vigour of remaining viable seeds. Leakage of solutes from 1977 seeds was greater than from 2001 seeds, suggesting cell membrane and cytoplasmic damage.

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INTRODUCTION

Physical or hard seed coat dormancy is well known for many legume species (Crocker & Barton 1957; Baskin & Baskin 1998). The high mechanical resistance of such seeds imposes dormancy by restricting water and/or oxygen uptake and may also protect the embryo from seed predators (Mayer & Poljakoff-Mayber 1989). Germination of individual seeds from a season's seed production is dependent on degradation of the testa and, thus, is spread over time and space optimising dispersal.

The hard-seed-coat dormancy of the New Zealand endemic species of *Sophora* (kowhai) is well known (e.g., Sykes & Godley 1968). Kowhai seeds possess a tough, dense seed coat which, for *S. microphylla*, accounts for about 40% of the seed mass (Murray 1979). Natural loss of testa impermeability in kowhai may occur by biotic (e.g., Greenfield 2000) or abiotic means such as abrasion by wind, sand, and water. Persistence in seed banks even in the absence of contributing trees (Partridge 1989) is a consequence of this seed trait. Commonly used scarification methods include mechanical or hand abrasion or cutting, acid treatment, and heat treatment (Metcalf 1995).

The hard seed coat with its associated buoyancy in water may be a significant factor in the widespread distribution of *Sophora* sect. *Edwardsia* (Sykes & Godley 1968) and its possible migration from South America (Pena et al. 2000) or from the north-western Pacific Ocean (Hurr et al. 1999). Seeds of *Sophora chrysophylla* of the Hawaiian Islands have similar properties (Akamine 1951). Dispersal of the genus *Sophora* across the South Pacific probably occurred within the last 2–5 million years (Hurr et al. 1999).

Seed ageing results in loss of integrity of a number of structural and functional components of seed tissues, including chromosomal and DNA damage which can reduce transcription fidelity and membrane deterioration accelerated by free radical production (Hendry 1993). Aged seeds exhibit symptoms ranging from reduced viability or germinability to full viability but with abnormal development of the seedling reflecting reduced seed vigour (Bewley & Black 1994). Intermediate levels of impairment between these extremes would also be expected.

In the experiments reported here we tested the germination and vigour properties of 24-year-old kowhai seeds against the performance of seeds of the current harvest from the same individual tree.

MATERIALS AND METHODS

Collections of kowhai seeds were made in 1977 and 2001 from a mature specimen tree at the Turitea Campus, Massey University, locally identified as *Sophora microphylla*. The New Zealand representatives of *Sophora* have recently been the subject of taxonomic revision (Heenan et al. 2001). Consequently, the tree has been identified as a hybrid, most likely between *S. microphylla* and *S. godleyi* (P. B. Heenan pers. comm.). This tree fruits heavily each year, a variable property of *S. microphylla* in natural or ornamental plantings (Rattenbury 1979).

The 1977 harvest was machine cleaned (Westrup LA-H Laboratory polisher, 10 × 10 mm cylindrical screen followed by Clipper Office and Tester air screen cleaner, model no. 400/C; upper screen, round 5.95 mm diameter; lower screen, round 3.18 mm diameter; airflow 0.0138 $m^3 s^{-1}$) following collection to remove pod material but to preserve seed integrity. Seeds were stored at room temperature in a closed container that was opened at least once annually for removal of seeds. The same machine cleaning process was used to prepare seeds of the 2001 harvest but a control "hand-cleaned" sample of 4 replicates of 50 seeds was also taken to test for potential seed damage by the machine cleaning. Bulk seeds from both harvests were prepared in 4 replicate lots using a seed divider and kept at room temperature until used. Germination testing was performed on 4 replicates of 50 seeds using the between-paper-roll method according to International Seed Testing Association (ISTA 1999) Rules.

Table 1 Percent germination of fresh and aged kowhai seeds. "AAtime" is hours of accelerated ageing. Data from 4 replicates were arcsin transformed for statistical analysis. Two means (on the same variable) with the same suffix are not significantly different (based on the 5% LSD). Hand-cleaned seeds had pods removed by hand; machine-cleaned had pods removed by machine; chipped seeds were scarified with a scalpel; forsberg seeds were scarified by machine. Germination categories follow ISTA (1999).

			Germination category				
Year	AAtime	Treatment	Normal	Abnormal	Hard	Ungerm.	Dead
1977	0	chipped	8.0 b	1.0 a	1.0 a	0.0 a	90.0 f
		forsberg	1.0 a	2.0 ab	39.0 c d	7.0 c	50.9 d
		machine-cleaned	0.0 a	0.0 a	88.9 f	1.0 ab	10.0 bc
	72	chipped	1.0 a	0.0 a	0.0 a	0.5 a	98.5 g
		forsberg	0.5 a	1.5 ab	25.6 bc	3.6 bc	68.8 de
		machine-cleaned	0.0 a	0.0 a	89.5 f	0.0 a	10.5 bc
	120	chipped	0.0 a	0.5 a	2.5 a	0.0 a	97.50 g
		forsberg	1.0 a	0.0 a	17.5 b	2.5 ab	79.0 ef
		machine-cleaned	0.0 a	0.0 a	87.9 f	0.5 a	11.6 c
2001	0	chipped	93.0 f	5.0 bc	0.5 a	0.0 a	1.5 a
		forsberg	17.5 c	5.0 bc	72.5 e	4.0 bc	1.0 a
		hand-cleaned	0.0 a	0.0 a	99.5 g	0.0 a	0.5 a
		machine-cleaned	0.0 a	0.0 a	100.0 g	0.0 a	0.0 a
	72	chipped	88.0 f	9.0 c	0.0 a	0.0 a	3.0 ab
		forsberg	34.4 d	10.2 c	47.8 d	3.6 bc	4.0 ab
		machine-cleaned	0.5 a	1.0 a	97.5 g	0.0 a	1.0 a
	120	chipped	78.7 e	6.6 bc	1.5 a	0.5 a	12.6 bc
		forsberg	29.1 cd	11.8 c	43.1 d	4.2 bc	11.8 bc
		machine-cleaned	0.0 a	0.0 a	98.5 g	0.0 a	1.5 a

Seed germination was assessed as normal or abnormal seedlings, hard seeds, fresh ungerminated seeds, and dead seeds as defined by ISTA (1999) after 7 days and 17 days. Reported results refer to the 17-day counts. Seeds were tested either directly (testa presumed intact), or following scarification by hand chipping with a scalpel or by use of a machine scarifier (Forsberg Line scarifier; rpm 1425; medium grit sandpaper for 10 s). This yielded some testa disruption with minimal embryo damage (Table 1).

Accelerated ageing (AA) was used to test stress tolerance of germination and vigour in both seed lots. Two periods of stress were imposed by keeping seeds at 41°C and approx. 100% relative humidity in a closed chamber for 72 or 120 hr. Seeds were counted for normal germination, abnormal germination, hard seed, fresh ungerminated seed, and dead seed.

Seed vigour was also tested by conductivity determination of seed sample leachates. Four replicates of 15 seeds (chipped or intact) were soaked in 28 ml glass-distilled water at 20°C and conductivity (µSiemens) of the solution surrounding the seeds was measured periodically.

Seed viability of 1977 and 2001 samples was tested using the topograhical tetrazolium test based on the ISTA Rules (ISTA 1999) for *Sophora* spp. The test is a chromogenic assay for detection of cellular respiratory activity. Seeds were hand-chipped and then soaked for 24 hr at 25°C in a 1% (w/v) tetrazolium chloride solution. Four replicates of each seed population were assessed. Embryos were examined and classified as either viable or non-viable according to the staining pattern. The criteria used for attributing viability were staining of the radicle with continuity of staining from the axis to the cotyledons and at least 50% staining of the cotyledons.

Table 2 Viability (% ± SEM) of fresh and aged kowhai seeds assessed by tetrazolium staining of embryo tissues as continuous staining in all embryo parts or substantial but not necessarily continuous staining. Difference in means between 1977 and 2001 seeds were highly significant ($P < 0.001$).

Year	Continuous staining	Substantial staining
1977	18.0 (± 1.2)	26.0 (± 3.5)
2001	75.8 (± 0.3)	99.0 (± 1.0)

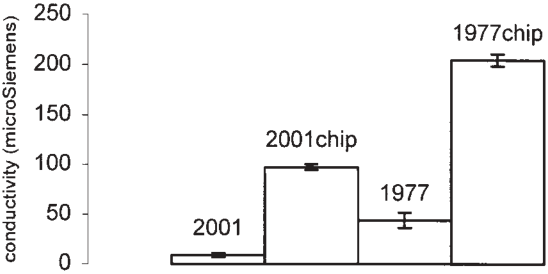

Fig. 1 Leakage of electrolytes from scarified and intact kowhai seeds. Mean conductivity (in µSiemens) from 3 replicate leaching solutions was measured after 19 hr at 20°C . Bars are standard errors of the means ($P < 0.001$).

RESULTS

Both 1977 and 2001 seeds showed the hard-seeded trait (Table 1). The machine cleaning of pods to release seeds had no effect on the testa integrity of 2001 seeds; thus, the machine-cleaned 1977 seeds are similar to the machine-cleaned 2001 seeds. Hand-scarified 1977 seeds exhibited reduced germination (8%) compared with the 2001 seeds (93%) (Table 1). Some limited seed coat permeability was induced for 2001 seeds (18% germination), but most seeds (73%) remained water impermeable. Thus, the Forsberg scarifier was relatively ineffective for the length of time used. It also removed the residual germination of the poorly germinating 1977 seeds.

The results suggest that the majority of 1977 embryos were dead within their hard seed coats by 2001. Embryo viability in the fresh and aged seeds was 76% and 18%, respectively, using tetrazolium (Table 2). With a relaxed criterion of substantial but not necessarily continuous staining, viability was estimated at 99% and 26%, respectively. A *t*-test to compare the two years using arcsin transformation of the data indicated significant differences ($P < 0.001$) whether or not variability about the 95% level was assumed equal to that at the 20–30% level.

Residual germination of 1977 seeds was reduced to zero by the accelerated ageing conditions of high temperature and humidity (Table 1). Germination of 2001 seeds was reduced (to 88%). Germination of 2001 Forsberg-scarified seeds was significantly improved by accelerated ageing. Germination promotion of unscarified 2001 seeds, by accelerated ageing treatment, was not seen, however. The 120-hr AA treatment stress also suppressed 1977 germination to zero but reduced 2001 germination only a little (Table 1).

Vigour of the 1977 seeds was also shown to be impaired by increased leakiness of seed tissues to ionic solutes contained within them (Fig. 1). Conductivity of unscarified 1977 seed leachates was 44.0 ± 7.9 (µSiemens) by 19 hr, and for 2001 seeds was 8.9 ± 1.9 (µSiemens). For scarified seeds, conductivity was 204.4 ± 10.2 (µSiemens) for 1977 seeds and 97.4 ± 3.1 for 2001 seeds.

DISCUSSION

The seeds of this *Sophora* hybrid retained the hard-seeded character typical of the genus, after 24 years of storage. The seeds of both harvests were indistinguishable in terms of colour (dull yellow) and size. A relationship has been sugested between testa colour and seed coat impermeability, as coloured seed coats are often thicker (Morris et al. 1968; Khan et al. 1996). Generally, it would be expected that permeability may be reduced by a physical barrier of greater cell numbers, differences in cell density, phenolic oxidation, lignin content, or cutinisation. Although the seeds of kowhai contain phenolics (Markham & Godley 1972), such differences are unlikely to be factors in distinguishing these seed populations, at least in terms of their possible contribution to seed coat integrity. Loss of kowhai seeds in soil seed banks may be partly, at least, due to soil microbes (Greenfield 2000), although physical scarification remains the most likely mechanism.

Germinability following scarification deteriorated after 24 years' storage, to produce a population containing only some 8% of seeds capable of producing normal seedlings under standardised germination conditions. The tetrazolium assay used here to indicate seed viability status showed that a somewhat greater fraction of the 1977 seeds possessed respiratory activity enough to germinate: 18 to 26% depending on the stringency of the criterion used. The subjectivity involved in estimating viability of native seeds using this assay has been questioned by Burrows (1995). In the present study the tetrazolium estimate tended to exceed germination data, suggesting that some of the aged seeds contained living tissues but of insufficient quantity to support germination. By contrast, the viability of 2001 seeds was estimated at 76 to 99% depending on the criteria adopted, and actual germination was 93%.

Accelerated ageing was used to apply physiological stress to chipped seeds in order to reveal any differences in vigour of germinating seeds. It was expected that the germinating seeds from the 1977 population might be more sensitive to the stress imposed and that this would be shown by reduced germination in the standardised germination test following the treatment. Accelerated ageing reduced germination to zero in the 1977 seeds, indicating that seeds that were capable of germination were of low vigour. By contrast, seeds of the fully germinable 2001 population were only slightly sensitive to the stress periods. Seeds of this population are thus shown to possess high vigour. This is further supported by the higher levels of leakage of solutes from 1977 seeds. Leakage of solutes from 1977 seeds was greater than from 2001 seeds, suggesting cell membrane and cytoplasmic damage. The conductivity test allows the two populations to be further distinguished, and raises questions about the relationship of germinability and viability to cell and tissue function in seeds. There is a possibility that the hybrid nature of the single tree sampled here may be a factor in the loss of seed germinability over time recorded here when our data are compared with recent seed ageing studies of other *Sophora* species (Norton et al. 2002).

There was no indication that the stress imposed by accelerated ageing affected seed coat permeability to improve germination of unscarified seeds in either population, a possible consequence of incubating hard seeds in high humidity and temperature. Accelerated ageing for both time periods, however, did increase seed coat permeability in the subsequent germination testing (34 and 29%) compared with the untreated scarified seeds (18%).

Vigour of the 1977 seeds was also shown to be impaired by increased leakiness of seed tissues to ionic solutes contained within them. The 1977 seeds were about twice as leaky as 2001 seeds when scarified, indicating loss of tissue integrity, and suggestive of membrane damage of embryo cells. Unscarified 1977 seeds were also some 5 times leakier than 2001 seeds, suggesting some loss of testa integrity. Although the hard seed coat characteristic was retained by the older seeds in terms of water permeability, this is clear evidence of some degree of testa damage. Scarified seeds were 5 times leakier than unscarified 1997 seeds, and 11 times leakier for 2001 seeds. The reduced ratio for the old seeds also suggests testa damage. In addition, the possibility of some seeds contributing more variation than others to the population means is suggested by the greater standard errors for the 1977 seeds. A range of damage states is thus likely.

We conclude that 1977 seeds have retained their hard seed property but have low viability compared with highly germinable fresh seed. Accelerated ageing treatment and conductivity measurement of seed-leached solutes further differentiates the 24-year-old seeds from the 2001 seed population.

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