# Seed fill, viability and germination of NSW species in the family Rutaceae

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***Abstract*:** The New South Wales Seedbank (at Mount Annan Botanic Garden) stores seeds of both common and threatened species for conservation, research and restoration or revegetation projects. The value of the collections depends on our ability to germinate seeds once they have been retrieved from storage. The collection includes 129 collections representing 93 taxa in the family Rutaceae, but seed viability in Rutaceae is variable, germination cues are poorly-understood and problems are likely to arise in trying to grow plants from seed.

In this study we quantified seed fill and/or viability and germination for 112 species in the Rutaceae family. For many of the species, this is the first time that these seed characteristics have been recorded. We found that seed fill (0–100%) and seed viability (0–97%), were highly variable, with 80% of collections having low viability (<75%). There was also a trend for threatened species to have lower seed fill than common species, while viability and germination were similar. This review reaffirms the need for further study of seed characteristics in Rutaceae.

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## Introduction

Plant species in the family Rutaceae make up a significant component of the understorey in many temperate Australian plant communities, particularly in low-nutrient habitats, as well as a high proportion of regionally endemic species (Auld 2001). In New South Wales 26% of 252 native Rutaceae species are threatened (Botanic Gardens Trust 2008).

A representative collection of seeds is an important component of both common and threatened species conservation. Long-term seed storage is a requirement of many threatened species Recovery Plans and a recommended action in many Threatened Species Priorities Action Statements (Department of Environment and Climate Change 2007). Seeds of common species such as Geijera parviflora are also needed for growing from seeds by groups such as Greening Australia (D. Carr, pers.comm.).

However problems are likely to arise in trying to grow Rutaceae family plants from seeds as seed viability is variable and germination cues are poorly understood (Roche et al. 1997, Auld 2001, Floyd 2008). While many ornamental genera including *Boronia*, *Correa* and *Crowea* can be propagated from cuttings this does not allow much genetic variability to be retained. Seed research in Rutaceae has been hampered by low seed numbers and poor viability, making it difficult to collect sufficient seeds to study germination and dormancy. However, with an increase in conservation initiatives such as *ex situ* seed banking, it is imperative that effective methods for successful germination are identified (Smith et al. 2003).

The first step in determining whether seeds can be used to produce healthy plants is to determine whether seeds are filled and viable. Seed fill is a measure of the proportion of outwardly undamaged seeds that have all the tissues essential for germination (that is, an intact endosperm and embryo). **Seed fill** has not often been documented separately to seed viability, as seeds must be filled to be viable (although the converse is not true, that is, not all filled seeds are viable). **Seed viability** – the number of seeds that germinate – is more easily assessed, although it is not suitable for species that have a high level of dormancy. Seed viability can also be measured using a cut test, with the additional step of determining whether the endosperm and embryo are healthy (usually firm and white). Seed viability (including seed fill) appears to be a critical issue in Australian Rutaceae with its extreme variability in seed lot viability (Roche et al. 1997, Auld 2001).

Little progress has been made in understanding germination of Australian Rutaceae since the study by Roche et al. (1997) and the review of Sydney species by Auld (2001). Germination of five threatened species of Rutaceae from the NSW Seedbank was very low (0–12%) when treated with Instant Smoke Plus Seed primer, with viability of 12–38% (Offord et al. 2004).

Seeds of Sydney region Rutaceae have a high level of dormancy on release from the parent (Auld 2001). Physiological dormancy is the most common type of dormancy in temperate species of Rutaceae (Baskin & Baskin 1998) indicating that the embryo has low growth potential (Baskin & Baskin 2004). A review of seed dormancy classification for shrub species in south eastern Australia assumed all Rutaceae had physiological dormancy (Ooi 2007). In some cases of physiological dormancy, germination is stimulated by gibberellic acid ($GA_3$) (Baskin & Baskin 2004) and $GA_3$ treatment is often required for laboratory germination of Rutaceae from around the world, including *Dictamnus albus*, *Diplolaena grandiflora*, *Melicope ternata* and *Ruta chalepensis* (Liu et al. 2008). Bell et al. (1993) cite unpublished studies of $GA_3$ enhancing germination in *Boronia fastigiata* and *Boronia megastigma*. Natural germination cues for Australian Rutaceae species include fire, heat and smoke (Paynter & Dixon 1991; Dixon et al. 1995; Roche et al. 1997; Auld 2001). A summary of treatments used to stimulate germination in previous studies on Australian Rutaceae is presented in Appendix 1.

The aim of this study was to assess seed fill and viability and improve our understanding of factors influencing laboratory-based germination of seeds in the NSW Rutaceae species. Measurements of imbibition (water uptake), embryo size and morphology, and germination responses to stimulants such as smoke water and $GA_3$were recorded for some species, as a step towards classification of seed dormancy (Baskin & Baskin 2004).

## Methods

The New South Wales Seedbank (located at Mount Annan Botanic Garden) currently (September 2008) stores 129 Rutaceae collections representing 93 taxa. Collections prior to 2004 comprise 46 collections (36% of all Rutaceae collections), while more recent collections since the start of the NSW Seedbank – Millennium Seed Bank partnership (2004–2008) comprise 83 collections (64%). The partnership, known as SeedQuest NSW, is an international collaborative project that has enhanced seedbanking and associated research in NSW (www.rbgsyd.nsw.gov.au/seedbank) and contributes to the global effort to conserve 10% of the world's flora as seed collections by 2010 (www.kew.org/msbp/index.htm). Seed quantities are recorded for 113 collections, with 66% comprising fewer than 1000 seeds (74 collections). Thirty-two collections (28%) have 1000–5000 seeds and only 7 collections (6%) have more than 5000 seeds. Seed fill was studied in collections made prior up to 2006; viability and germination studied in collections made between 2004 and 2006 and seed weight studied in collections made after 2005.

Data on seed weight, fill, viability and germination were collected during routine seedbanking operations. Seed weight for three replicates of 50 seeds was measured and results presented as the mean ± standard error for individual seeds. Seed fill was determined by x-ray or a cut test. The cut test was either performed on a separate seed sample to the germination test, or on seeds remaining at the conclusion of a germination test if a separate sample was not set aside due to low seed numbers. In the latter cases care must be taken in interpretation of the cut test results as a component of seed viability as seed viability may have been lost during the course of the germination test. Seed viability was determined after the germination test as the sum of the seeds that had germinated in addition to those with a firm white endosperm and embryo when assessed by a cut test. Seed fill and viability were compared using a paired one-sample *t*-test in Genstat (Lawes Agricultural Trust 2007).

Germination studies were generally conducted on dried seeds (equilibrated at 15%RH and 15°C in a dry room) although several species were studied fresh (within one month of collection and prior to drying or storage) or following freezer storage at -18°C (Table 2). The age of seeds at testing ranged from 5 days to 3.2 years (see Table 2). Germination tests were conducted on water agar (control), on water agar following 18 hrs soaking in Kings Park smoke solution at 1:100 dilution (smoke), on water agar incorporating $GA_3$ (250ppm), or on water agar incorporating $GA_3$ following 18 hrs soaking in Kings Park smoke solution at 1:100 dilution (smoke + $GA_3$). Due to limited seed numbers, not all seed collections received all treatments (see Table 2). All germination tests were conducted with 12 hrs light/12 hrs dark in incubators at temperatures shown in Table 2. Sample sizes for seed fill, viability and germination were dependant on the size of collections. Seeds were divided into replicates (separate Petri dishes) wherever possible.

Viability and germination data were analysed using the Generalised Linear Mixed Model analysis in Genstat (Lawes Agricultural Trust 2007), with a binomial distribution and a logit link function. Wald tests were used to determine which factors (genera, species and/or germination treatments) were significant. Wald tests are analogous to *F*-tests in ANOVA, but are used to test the significance of fixed model terms that have an asymptotic $\chi^2$ (chi-squared) distribution (Payne 2003). A Least Significant Difference (LSD) test was used to determine which predicted means were significantly different between germination treatments. A critical *t* value ($t^{0.025}_{\text{deviance d.f.}}$) of 2 was used, as the $\chi^2$distribution approaches 2 for increasing degrees of freedom.

Embryo type was characterised for 17 species: *Asterolasia buckinghamii*, *A. elegans*, *Boronia anemonifolia*, *B. anethifolia*, *B. ledifolia*, *B. occidentalis*, *B. serrulata*, *Eriostemon*

*australasius*, *Geijera salicifolia*, *G. parviflora*, *Leionema dentatum*, *Melicope hayesii*, *Phebalium squamulosum* subsp. *gracile*, *Philotheca trachyphylla*, *Zieria granulata*, *Z. laxiflora* and *Z. prostrata*. Embryo morphology was determined by making a transverse section of seeds and assigning an embryo type according to the classification of Martin (1946).

Imbibition experiments to detect the presence of physical dormancy were conducted on *Zieria granulata*, *Z. laxiflora* and *Z. prostrata* as well as on two species of *Geijera* from other seedbanks (three collections of *Geijera parviflora* and one collection of *Geijera linearifolia*). For imbibition experiments, seeds were weighed, then placed on moist filter paper in Petri dishes for five minutes, removed from dishes, blotted dry and re-weighed for a measurement at time 0. Measurements were then made using the same method after 72 hours for *Geijera* species. and 122 hours for *Zieria* species. with seeds kept at room temperature during imbibition. Three replicates of 100 seeds each were used for *Zieria granulata*, *Z. laxiflora* and *Z. prostrata,* while six replicates of five seeds each were used for three collections of *Geijera parviflora* and one collection of *G. linearifolia*. The percentage increase in seed mass was determined using the calculation:

$$\% \text{ increase in mass} = [(W_1-W_d)/W_d] \times 100,$$

Where $W_1$ and $W_d$= mass of imbibed and dry seeds, respectively (Turner et al. 2006).

**Table 1: Seed weight, seed fill and threat status for Rutaceae collections from NSW Seedbank. Seed weight**: average single seed weight (mg) ± SE under dry room conditions. **Seed fill**: percentage, sample size and method with G=germination, C=cut test and X=x-ray. **Threat status**: *TSC, EPBC* codes E=endangered, V=vulnerable; ROTAP codes 2=species with very restricted distribution in Australia and maximum geographic range<100km, 3=species with range >100km in Australia but only occurring in small populations, E=endangered, V=vulnerable, R=rare, C=species represented in a national park or other reserve, a=adequately reserved with total population >1000 plants, i=inadequately reserved with total populations <1000 plants, -=recorded in reserves but population size unknown (Briggs & Leigh 1988).

| | | Seed weight | Seed fill | | | Threat listing | | |
|---|---|---|---|---|---|---|---|---|
| **Accession** | **Species** | **Average seed weight (mg) (±SE)** | **% filled** | **Sample size** | **Method** | **TSC** | **EPBC** | **ROTAP** |
| 20040115 | *Asterolasia buckinghamii* | | 100 | 10 | G | | | |
| 20020784 | *Asterolasia buxifolia* | | 65 | 20 | X | E | | |
| 20051377 | *Asterolasia correifolia* | | 75 | 20 | X | | | |
| 20071235 | *Asterolasia elegans* | 4.35 (0.52) | | | | E | E | 2ECa |
| 20051418 | *Asterolasia hexapetala* | 3.30 (0.58) | 75 | 20 | X | | | 2RC- |
| 20071301 | *Boronia algida* | 1.43 (0.58) | | | | | | |
| 20051564 | *Boronia anemonifolia* | | 100 | 25 | C | | | |
| 20061157 | *Boronia anemonifolia* | | | | | | | |
| 20051507 | *Boronia anethifolia* | 1.61 (0.64) | 96 | 25 | C | | | |
| 20051506 | *Boronia boliviensis* | 6.98 (1.45) | | | | E | | |
| 873596 | *Boronia falcifolia* | | 40 | 20 | X | | | |
| 20071238 | *Boronia floribunda* | 1.43 (0.41) | 80 | 20 | C | | | |
| 20071309 | *Boronia fraseri* | 5.88 (1.20) | | | | | | 2RCa |
| 20051411 | *Boronia glabra* | 9.04 (1.40) | 100 | 20 | X | | | |
| 842476 | *Boronia glabra* | | 50 | 20 | X | | | |
| 20051624 | *Boronia ledifolia* | | 72 | 25 | C | | | |
| 20061206 | *Boronia ledifolia* | 6.51 (1.37) | 60 | 10 | C | | | |
| 913622 | *Boronia ledifolia* | | 36 | 11 | X | | | |
| 20051501 | *Boronia microphylla* | 1.22 (0.52) | | | | | | |
| 20051526 | *Boronia microphylla* | 1.16 (0.69) | | | | | | |
| 866149 | *Boronia mollis* | | 45 | 20 | X | | | |
| 20051415 | *Boronia occidentalis* | 1.33 (0.47) | 100 | 25 | C | | | |
| 873598 | *Boronia pinnata* | | 50 | 20 | X | | | |
| 20061197 | *Boronia repanda* | 3.47 (1.43) | 30 | 20 | C | E | E | 2E |
| 20061178 | *Boronia rigens* | | 0 | 20 | C | | | |
| 20071287 | *Boronia rigens* | 1.14 (0.32) | | | | | | |
| 20071166 | *Boronia rosmarinifolia* | 10.68 (1.80) | | | | | | |
| 20051623 | *Boronia serrulata* | | 100 | 25 | C | | | 2RC- |
| 20051625 | *Boronia serrulata* | | 92 | 25 | C | | | 2RC- |
| 20061117 | *Boronia thujona* | 0.96 (0.32) | 100 | 10 | C | | | |
| 20061238 | *Boronia thujona* | 1.19 (0.26) | | | | | | |
| 20020795 | *Boronia umbellata* | | 5 | 20 | X | V | | 2VC- |
| 20061144 | *Crowea exalata* | 4.84 (1.49) | 50 | 20 | C | | | |
| 842663 | *Crowea exalata* | | 80 | 20 | X | | | |
| 873436 | *Crowea exalata* | | 25 | 20 | X | | | |
| 20051611 | *Crowea saligna* | 7.25 (0.93) | 20 | 20 | C | | | |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 20051612 | *Eriostemon australasius* | 6.06 (0.61) | 20 | 20 | C | | | |
| 873599 | *Eriostemon australasius* | | 37.5 | 8 | X | | | |
| 843027 | *Eriostemon australasius* subsp. *australasius* | | 65 | 20 | X | | | |
| 913464 | *Flindersia schottiana* | | 100 | 10 | X | | | |
| 20060007 | *Geijera parviflora* | 23.70 (1.87) | 76 | 99 | C | | | |
| 872916 | *Geijera parviflora* | | 95 | 20 | X | | | |
| 872921 | *Geijera parviflora* | | 100 | 20 | X | | | |
| 890212 | *Geijera parviflora* | | 100 | 20 | X | | | |
| 923903 | *Geijera parviflora* | | 100 | 20 | X | | | |
| 923862 | *Geijera salicifolia* | | 100 | 20 | X | | | |
| 863021 | *Halfordia kendack* | | 100 | 20 | X | | | |
| 20061168 | *Leionema carruthersii* | 2.48 (1.29) | 10 | 20 | C | | | 3RC- |
| 20051626 | *Leionema dentatum* | | 100 | 35 | C | | | |
| 877288 | *Leionema dentatum* | | 90 | 20 | X | | | |
| 20061119 | *Leionema diosmeum* | 1.81 (0.36) | 90 | 20 | C | | | |
| 20051190 | *Leionema elatius* subsp. *beckleri* | 1.12 (0.73) | 20 | 20 | C | | | |
| 20051491 | *Leionema lamprophyllum* | 1.03 (0.54) | | | | | | |
| 20071259 | *Leionema ralstonii* | 4.44 (1.12) | | | | V | V | 2VCi |
| 20061203 | *Leionema rotundifolium* | 4.94 (1.26) | 40 | 10 | C | | | 3RC- |
| 20020916 | *Leionema* sp. Colo River (P.H. Weston 2423) | | 50 | 20 | X | | | |
| 850823 | *Melicope elleryana* | | 100 | 20 | X | | | |
| 873898 | *Melicope hayesii* | | 30 | 20 | X | | | |
| 861402 | *Melicope micrococca* | | 60 | 20 | X | | | |
| 20051369 | *Nematolepis squamea* | | 55 | 20 | X | | | |
| 20071271 | *Phebalium bifidum* | 3.52 (0.82) | | | | | | |
| 20051447 | *Phebalium glandulosum* subsp. *glandulosum* | 1.71 (0.22) | 100 | 10 | G | | | |
| 20051470 | *Phebalium nottii* | 3.02 (1.06) | 85 | 20 | X | | | |
| 20051481 | *Phebalium obcordatum* | 2.18 (0.83) | 100 | 10 | G | | | 3RCa |
| 20051407 | *Phebalium squamulosum* subsp. *gracile* | 2.63 (0.86) | 75 | 20 | G | | | |
| 933424 | *Phebalium squamulosum* subsp. *gracile* | | 85 | 20 | C | | | |
| 20051474 | *Phebalium stenophyllum* | 2.90 (0.89) | 100 | 10 | G | | | |
| 20051198 | *Philotheca buxifolia* | | 16 | 25 | C | | | |
| 20051426 | *Philotheca ciliata* | 3.90 (1.38) | 79 | 19 | X | | | |
| 20051448 | *Philotheca difformis* | 3.45 (0.91) | 5 | 20 | X | | | |
| 864893 | *Philotheca difformis* subsp. *difformis* | | 0 | 20 | X | | | |
| 873451 | *Philotheca difformis* subsp. *difformis* | | 15 | 20 | X | | | |
| 20051490 | *Philotheca ericifolia* | 1.94 (0.65) | 100 | 10 | G | V | V | 3RC- |
| 20051197 | *Philotheca hispidula* | 10.51 (2.70) | 100 | 20 | G | | | |
| 20051195 | *Philotheca myoporoides* | 7.43 (1.43) | 70 | 20 | X | | | |
| 20051422 | *Philotheca salsolifolia* | 7.81 (1.15) | 90 | 20 | X | | | |
| 20071043 | *Philotheca scabra* | 9.98 (1.47) | | | | | | |
| 20061167 | *Philotheca trachyphylla* | 4.44 (1.15) | 64 | 14 | C | | | |
| 842876 | *Philotheca trachyphylla* | | 75 | 20 | X | | | |
| 20061122 | *Zieria arborescens* | 1.35 (1.05) | 84 | 150 | C | | | |
| 20061172 | *Zieria buxijugum* | 0.89 (0.39) | 10 | 20 | C | E | E | 2E |
| 20051419 | *Zieria cytisoides* | 3.11 (0.51) | 90 | 20 | X | | | |
| 20061171 | *Zieria formosa* | 0.99 (0.40) | 15 | 20 | C | E | E | 2E |
| 20041359 | *Zieria granulata* | | 100 | 30 | C | E | E | 2VCi |
| 20061125 | *Zieria ingramii* | | 0 | 20 | C | E | E | 2V |
| 20071243 | *Zieria ingramii* | 3.80 (1.22) | | | | E | E | 2V |
| 20071233 | *Zieria involucrata* | 1.67 (0.64) | | | | E | V | 2VCa |
| 20020783 | *Zieria involucrata* | | 55 | 20 | X | E | V | 2VCa |
| 20051605 | *Zieria laevigata* | 3.19 (1.01) | 68 | 25 | C | | | |
| 20041352 | *Zieria laxiflora* | | 100 | 28 | C | | | |
| 20061175 | *Zieria littoralis* | 1.85 (0.67) | 90 | 20 | C | | | |
| 20061173 | *Zieria parrisiae* | 1.21 (0.77) | 30 | 20 | C | E | E | 2E |
| 20061174 | *Zieria parrisiae* | | 5 | 20 | C | E | E | 2E |
| 20071312 | *Zieria pilosa* | 3.31 (0.83) | | | | | | |
| 913530 | *Zieria pilosa* | | 65 | 20 | X | | | |
| 20071159 | *Zieria prostrata* | 1.29 (0.73) | | | | E | E | 2E |
| 20020791 | *Zieria prostrata* | | 67 | 30 | C | E | E | 2E |
| 20061152 | *Zieria smithii* | 1.19 (0.62) | 90 | 10 | C | | | |
| 20061164 | *Zieria smithii* | 1.69 (0.19) | 80 | 20 | C | | | |
| 20071158 | *Zieria smithii* | 1.28 (0.65) | | | | | | |
| 923860 | *Zieria smithii* | | 80 | 20 | X | | | |
| 20061189 | *Zieria southwellii* | 1.36 (0.55) | 90 | 20 | C | | | |

## Results

We quantified seed fill and/or viability and germination for 112 Rutaceae species. Seed weights (recorded for 56 species) were highly variable (Table 1), e.g. in the Tribe Boronieae there was an order of magnitude difference between the smallest (*Zieria buxijugum*) and largest (*Boronia rosmarinifolia*). *Geijera parviflora*, the only representative of the tribe Zanthoxyleae in this study, was the largest at 23.7 mg. There is considerable variation in seed weight even within a genus, e.g. *Boronia thujona* (0.96 mg) compared to *Boronia rosmarinifolia* (10.68 mg) or *Philotheca ericifolia* (1.94 mg) compared to *Philotheca hispidula* (10.51 mg).

At the generic level *Philotheca* had the largest seeds (mean seed weight of 8 species was 6.18 mg se=1.12), then *Boronia* (15 species) 3.6 mg se=0.85, *Phebalium* (6 species) 2.66 mg se=0.26, *Leionema* (6 species) 2.63 mg se=0.68 and *Zieria* (15 species) 1.87 mg se=0.24.

Seed fill was highly variable ranging from none to 100%, even between different collections of the same species (Table 1). Collections with no filled seed were made from *Boronia rigens*, *Philotheca difformis* subsp. *difformis* and *Zieria ingramii*#. Low seed fill (≤30%) was recorded for *Boronia repanda*#, *B. umbellata*#, *Crowea exalata* (1 of 3 collections studied), *C. saligna*, *Eriostemon australasius* (1 of 2 collections), *Leionema carruthersii*#, *L. elatius* subsp. *beckleri*, *Melicope hayesii*, *Philotheca buxifolia* subsp. *buxifolia*, *P. difformis* (3 of 3 collections), *Zieria buxijugum*, *Z. formosa*# and *Z. parrisiae*# (2 of 2 collections). Seed fill was generally significantly higher than seed viability ($P<0.001$). Low seed fill was more prevalent (44%) in threatened species# than in common species (15%). (# indicates species listed under the NSW *Threatened Species Conservation* Act, Federal *Environmental Protection Biodiversity Conservation* Act or as *Rare or Threatened Australian Plants*.)

Viability data was pooled and averaged across germination treatments, as there was no significant difference in viability between treatments at the end of germination tests. Viability of filled seeds was highly variable (0–97%), with 80% of collections having low viability (<75%) (Table 2). Low viability was found in all 11 threatened species tested, with only one (*Zieria granulata*) having viability >60%.

Similar germination results were recorded for both threatened and common species, with about half the collections germinating well (>80%) when given one or more treatments. Percent germination across the treatments was significantly different ($P<0.001$), with $GA_3$ in combination with smoke water (average 62% germination) > $GA_3$ (40%) > no treatment (13%) or smoke treatment (9%) (Table 2). These results indicate that a proportion of seeds are non-dormant at the time of testing as germination of untreated seed can occur, albeit at a low level. A greater proportion has non-deep physiological dormancy, with germination stimulated by $GA_3$.

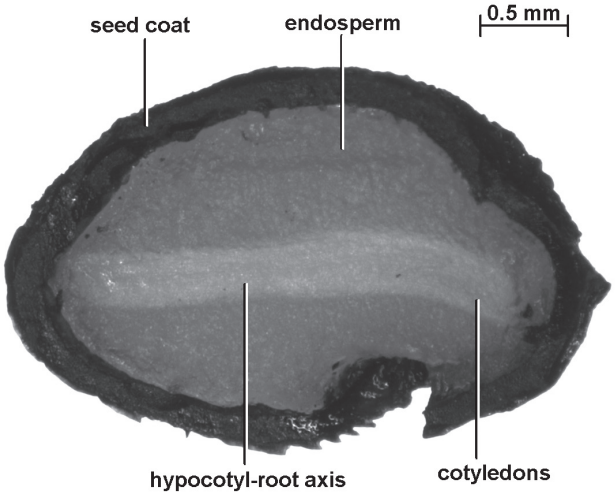


**Fig. 1.** *Zieria laxiflora* seed showing linear embryo (root axis and cotyledons).

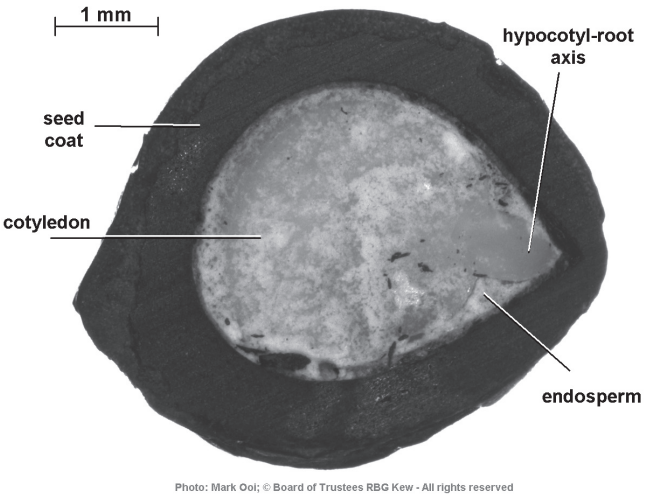


**Fig. 2.** *Geijera salicifolia* var. *latifolia* seed showing spathulate embryo (hypocotyl-root axis and cotyledon).

Water was imbibed by all five species examined, with increases of 30–51% for *Zieria* species and 45–58% for *Geijer*a species (Table 3). In a separate study, seeds of five *Boronia* species (*B. anemonifolia*, *B. anethifolia*, *B. ledifolia*, *B. serrulata* and *B. occidentalis*) were found to imbibe water with an increase in seed weight of 16–34% over 72 hrs (A. Martyn, unpublished data). Imbibition is a prerequisite for germination and germination occurred without scarification in 34/38 species tested (Table 2) indicating that physical dormancy was not present. Embryos were fully developed in all 17 species, with linear embryos for all species (for example, *Zieria laxiflora*, Figure 1) except *Geijera parviflora* and *G. salicifolia* var. *latifolia* (Figure 2) which have spathulate embryos.

**Table 2: Seed age, germination conditions, viability, final germination and time to germination for Rutaceae collections from NSW Seedbank.** Germination conditions: storage conditions prior to germination test, seed age, day and night temperature during germination test (all with 12 hrs light/12 hrs dark), seeds per replicate and number of replicates. Viability: percentage ± SE averaged at end of all germination treatments. Germination data: percentage of viability adjusted germination (VAG) ± SE for untreated (control), smoke, gibberellic acid (GA3) and smoke+GA3 treated seeds. Bold indicates significantly difference to untreated seeds. Time to germination: time to 1st (T 1st) and last (T last) germination in days for all successful treatments.

| | | | | | | | | | Viability of germination % ± se | | | | | Time (days) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Accession | Species | Conditions prior to germ | Seed Age (days) | Day Temp (°C) | Night Temp (°C) | Seeds per replicate | No of replicates | Av viable seed (%) (±SE) | no treatment | Smoke | GA3 | Smoke +GA3 | no treatment | Smoke | GA3 | Smoke +GA3 |
| 20040115 | *Asterolasia buckinghamii* | dry room | 1160 | 27 | 12 | 10 | 1 | 50 | | | | 0 | | | | |
| 20051377 | *Asterolasia correifolia* | dry room | 505 | 27 | 12 | 15 | 2 | 39 (0.8) | 0 (0) | | | **81** (2.1) | | | | 28–98 |
| 20051418 | *Asterolasia hexapetala* | dry room | 499 | 27 | 12 | 10 | 2 | 55 (2.7) | 52 (3.2) | | | **100** (0) | 158–338 | | | 42–294 |
| 20051564 | *Boronia anemonifolia* | dry room | 245 | 25 | 15 | 25 | 3 | 97 (0.8) | 4 (1.6) | **26** (1.3) | 19 (1.2) | **38** (1.7) | 72–135 | 53–200 | 42–188 | 43–125 |
| 20061157 | *Boronia anemonifolia* | fresh | 43 | 20 | 10 | 25 | 3 | 69 (1.3) | 9 (0.9) | | | **27** (1.7) | 89–96 | | | 38–80 |
| 20051507 | *Boronia anethifolia* | dry room | 465 | 27 | 12 | 20 | 3 | 87 (1.6) | 6 (1.4) | | | **64** (1.8) | 17 | | | 14–66 |
| 20051506 | *Boronia boliviensis* | dry room | 472 | 27 | 12 | 9 | 1 | 56 | | | | 80 | | | | 34–83 |
| 20051411 | *Boronia glabra* | dry room | 499 | 27 | 12 | 10 | 2 | 68 (1.6) | 0 (0) | | | **100** (0) | | | | 22–42 |
| 20051526 | *Boronia microphylla* | dry room | 463 | 27 | 12 | 10 | 2 | 20 (1.9) | 0 (0) | | | **100** (0) | | | | 133–200 |
| 20051501 | *Boronia microphylla* | dry room | 474 | 27 | 12 | 10 | 1 | 30 | | | | 100 | | | | 34–63 |
| 20051415 | *Boronia occidentalis* | dry room | 492 | 33 | 18 | 20 | 3 | 73 (1.6) | 7 (0.6) | 2 (1.2) | | **74** (1.4) | 96 | 206 | | 35400 |
| 20061117 | *Boronia thujona* | dry room | 5 | 27 | 12 | 10 | 1 | 40 | 25 | 75 | | 100 | 217 | 105–217 | | 26–61 |
| 20061144 | *Crowea exalata* | dry room | 244 | 27 | 12 | 5 | 5 | 8 (1.6) | 67 | | | **100** | 184 | | | 265 |
| 20051611 | *Crowea saligna* | dry room | 538 | 27 | 12 | 10 | 2 | 0 (0) | | | | | | | | |
| 20051612 | *Eriostemon australasius* | dry room | 523 | 27 | 12 | 15 | 2 | 2 (0.8) | | | | | | | | |
| 20060007 | *Geijera parviflora* | dry room | 239 | 27 | 12 | 25 | 3 | 80 | 0 | | | | | | | |
| 20051626 | *Leionema dentatum* | dry room | 509 | 27 | 12 | 15 | 2 | 5 (0.8) | 50 (5.9) | | | **100** | 155 | | | |
| 20061119 | *Leionema diosmeum* | dry room | 252 | 27 | 12 | 14 | 1 | 39 | 17 | | | 80 | 75 | | | 115–164 |
| 20051190 | *Leionema elatius* subsp. *beckleri* | dry room | 526 | 27 | 12 | 12 | 2 | 6 (1.0) | | | | | 121–184 | | | |
| 20061203 | *Leionema rotundifolium* | dry room | 229 | 27 | 12 | 15 | 1 | 30 | 60 | | | 25 | 164 | | | 70 |
| 20051369 | *Nematolepis squamea* | dry room | 506 | 27 | 12 | 15 | 2 | 50 (1.1) | 65 (1.2) | | | **88** (3.0) | 57–121 | | | 42–124 |
| 20051447 | *Phebalium glandulosum* subsp. *glandulosum* | dry room | 490 | 27 | 12 | 10 | 1 | 30 | | | | 33 | | | | 133 |
| 20051470 | *Phebalium nottii* | dry room | 482 | 27 | 12 | 10 | 2 | 75 (1.3) | 0 (0) | | | **28** (2.0) | | | | 36–63 |
| 20051481 | *Phebalium obcordatum* | dry room | 481 | 27 | 12 | 10 | 1 | 50 | | | | 0 | | | | |
| 20051407 | *Phebalium squamulosum* subsp. *gracile* | dry room | 499 | 27 | 12 | 10 | 2 | 35 (0.0) | 0 (0) | | | **63** (3.0) | | | | 27–59 |
| 20051474 | *Phebalium stenophyllum* | dry room | 482 | 27 | 12 | 10 | 1 | 10 | | | | 100 | | | | 207 |
| 20051426 | *Philotheca ciliata* | dry room | 497 | 27 | 12 | 15 | 2 | 63 (2.4) | 0 (0) | **6** (2.1) | **58** (3.5) | **67** (3.5) | | 154 | 23–110 | 28–144 |
| 20051448 | *Philotheca difformis* | dry room | 488 | 27 | 12 | 10 | 2 | 0 (0) | | | | | | | | |
| 20051490 | *Philotheca ericifolia* | dry room | 475 | 27 | 12 | 10 | 1 | 20 | | | | 100 | | | | 49–133 |

| | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 20051197 | *Philotheca hispidula* | dry room | 514 | 27 | 12 | 10 | 2 | 5 (1.9) | 0 | | 0 | | | | |
| 20051195 | *Philotheca myoporoides* | dry room | 514 | 27 | 12 | 10 | 2 | 20 (1.9) | 50 | | **0** (0) | 64–121 | | | |
| 20051422 | *Philotheca salsolifolia* | dry room | 497 | 27 | 12 | 10 | 2 | 43 (0.9) | 47 (3.1) | 29 | **75** (4.2) | 201–204 | 120–183 | | 120 |
| 20061167 | *Philotheca trachyphylla* | dry room | 239 | 27 | 12 | 10 | 1 | 10 | | | 100 | | | | 83 |
| 20061122 | *Zieria arborescens* | fresh | 54 | 27 | 12 | 25 | 3 | 82 (2.3) | 5 (1.3) | 4 (1.3) | **54** (3.3) | **63** (3.1) | 166–176 | 191–262 | 47–273 | 41–166 |
| 20061172 | *Zieria buxijugum* | dry room | 238 | 27 | 12 | 20 | 1 | 0 | | | | | | | | |
| 20051419 | *Zieria cytisoides* | dry room | 498 | 27 | 12 | 10 | 2 | 85 (1.9) | 0 (0) | | | **73** (1.1) | | | | 34–73 |
| 20061171 | *Zieria formosa* | dry room | 238 | 27 | 12 | 20 | 1 | 25 | 25 | | | 100 | 265 | | | 26–61 |
| 20041359 | *Zieria granulata* | dry room | 854 | 27 | 12 | 10 | 2 | 42 (2.6) | 8 (2.4) | | | **63** (1.5) | 23 | | | 28–49 |
| 20041359 | *Zieria granulata* | freezer | 267+366 | 20 | 20 | 20 | 3 | 70 (1.5) | 2 (1.2) | | **44** (0.9) | | 29 | 21–369 | | |
| 20041352 | *Zieria laxiflora* | dry room | 867 | 27 | 12 | 10 | 2 | 80 (2.7) | 33 (2.7) | | | **82** (1.7) | 23–182 | | | 28–120 |
| 20041352 | *Zieria laxiflora* | freezer | 280+379 | 20 | 20 | 20 | 3 | 68 (1.5) | 0 (0) | | **29** (1.3) | | | 21–210 | | |
| 20061175 | *Zieria littoralis* | dry room | 238 | 27 | 12 | 10 | 5 | 76 (1.6) | 75 (2.2) | | | **100** (0) | 44–291 | | | 26–87 |
| 20061173 | *Zieria parrisiae* | dry room | 238 | 27 | 12 | 10 | 5 | 27 (1.7) | 5 | | | **100** (0) | 40 | | | 71–124 |
| 20061152 | *Zieria smithii* | dry room | 242 | 27 | 12 | 5 | 5 | 62 (0.9) | 23 (1.7) | | | **93** (1.7) | 212 | | | 47–112 |
| 20061164 | *Zieria smithii* | dry room | 239 | 27 | 12 | 10 | 5 | 86 (1.0) | 0 (0) | | | | | | | |

**Table 3: Percentage imbibition (average % increase in seed weight ± SE) for seven seed collections (five Rutaceae species).**

| | Imbibition (%) | |
|---|---|---|
| **Species** | **Average** | **Std error** |
| *Zieria granulata* | 30.3 | 2.0 |
| *Zieria prostrata* | 51.3 | 3.0 |
| *Zieria laxiflora* | 33.7 | 1.7 |
| *Geijera parviflora* 1 | 58.5 | 10.8 |
| *Geijera parviflora* 2 | 45.4 | 8.0 |
| *Geijera parviflora* 3 | 53.1 | 12.7 |
| *Geijera linearifolia* | 45.4 | 19.1 |

## Discussion

### *Implications for seedbanking*

This study confirms that seed fill, viability and germination are highly variable in NSW Rutaceae, as observed in previous studies from Western Australia (Roche et al. 1997) and the Sydney region (Auld 2001). To ensure optimal regeneration of plants from both conservation seedbanks that aim for long-term seed storage, and restoration seedbanks that have more rapid turnover of seed collections, it is necessary to take these issues into account. For example to plan collecting trips over several seasons to enable the collection of sufficient seeds for germination, or use sufficient seeds in viability and germination tests to account for the presence of empty seeds in a collection.

Distinguishing between seed fill and viability may assist in determining whether problems are occurring in the seed banking or regeneration stage, as seed fill is a fixed characteristic for a collection, while viability will be maximal at collection and decline during storage. Problems with seed fill occur before natural dispersal or seed collection and can be an inherent species characteristic, the result of inbreeding depression in small populations, or a result of seed predation or environmental conditions such as prolonged drought impacting on pollination or seed development (reviewed by Fenner & Thompson 2005). Predation had a significant impact on seed fill for *Zieria laevigata* and *Zieria prostrata* with up to 50% of seeds lost to predators for both species in some locations (Armstrong 2002, NSW NPWS 1998). Further study of seed production and pre-dispersal seed losses over several years (as suggested by Auld 2001), is needed especially for threatened species such as *Zieria parrisiae*.

Our results indicate that threatened species were more likely to have low seed fill than common species, though viability and germination were similar. This suggests that poor seed fill is a contributing factor to threat status. Observations of seed or fruit presence (e.g. Shapcott et al. 2005) may

significantly overestimate reproductive activity if seed fill is not taken into account.

Problems with seed viability can be minimised by ensuring seeds are collected as close to maturity as possible (including bagging seeding branches if practical), followed by thorough cleaning, appropriate storage (cool dry conditions) and monitoring of viability during storage (Smith et al. 2003). Studies of seed viability during conservation storage are also required for the Rutaceae family, as little information is available (studies on seed persistence in soil do not necessarily relate to seed viability in storage). A study on the Western Australian Rutaceae species *Geleznowia verrucosa* measured a decline of 10–11% viability in only 175 days at room temperature (Paynter & Dixon 1990). Determining and maintaining seed viability is a key factor not only in seedbanking but also in utilization of seeds for restoration (Thompson et al. 2001).

The Rutaceae seeds examined have physiological dormancy (according to the definition of Baskin & Baskin 2004) as they are capable of imbibing water (ruling out physical dormancy), have fully developed embryos (ruling out morphological dormancy) and respond to germination stimulants such as $GA_3$. Results of this study and others (e.g. Ooi 2007) refute the suggestion that the seed coat may act as a physical barrier to imbibition in Rutaceae (Auld 2001). Imbibition experiments should be conducted for a wider range of Rutaceae species, to consolidate these results. It is important to note that imbibition studies should be conducted over a period of at least 72 hrs, as the time course of some previous studies has been too short (e.g. 5 hrs, Mildenhall 2002).

Germination of Rutaceae seeds can be significantly improved using a combination of smoke and $GA_3$. Two important processes conducted in *ex situ* seed banks, namely assessing seed viability using germination, and growing plants from seed for recovery of threatened species, could potentially be enhanced by the use of this treatment. The additive effect of smoke and $GA_3$ increases germination for some crop species (van Staden et al., 2000; Kępczyński et al., 2006) and several Australian native species (Cochrane et al. 2002). Little is known about the role, timing and location of gibberellin activity in relation to environmental cues for germination, particularly for seeds from wild-sourced species, however evidence suggests that smoke may increase the sensitivity of seeds to gibberellins and other hormones (van Staden et al., 2000; Schwachtje & Baldwin 2004)

*Implications for seed ecology*

Classification of seed dormancy offers a structured approach to collecting basic information on seed characteristics and can help identify likely factors required for dormancy break. For example, the physiological dormancy identified in this study may be broken by seasonal temperature changes, dry afterripening, stratification and wetting and drying cycles (Merritt et al. 2007). These natural cues may have been acting when regeneration of the following species was noted without passage of a fire: *Asterolasia elegans* (Benson & McDougall 2001), *Boronia coerulescens* subsp. *coerulescens* and *B. filifolia* (Bonney 2003), *Leionema lachnaeoides* (NSW NPWS 2001), *Zieria lasiocaulis* (NSW NPWS 2002) and *Zieria granulata* (Department of Environment & Conservation 2005).

The extrapolation of laboratory germination outcomes to germination in nature has some limitations. Storage conditions and seed age can affect subsequent germination (Baskin, Thompson & Baskin 2006). For example, seeds may experience afterripening in dry room storage prior to germination. However, for many species there has been little opportunity to study fresh seeds and laboratory based studies on stored seeds, along with dormancy classification, provide a starting point for further investigations.

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## Appendix 1

| Treatment | Species with positive germination response | Reference | Species with negative or no germination response | Reference |
|---|---|---|---|---|
| Scarification or seed coat removal | *Geleznowia verrucosa* | Paynter & Dixon 1991 | *Boronia, Eriostemon, Zieria*, *Phebalium* | Whitehorne & McIntyre 1975 |
| | *Geijera linearifolia*, *Eriostemon angustifolius* subsp. *angustifolius* | Bonney 2003 | *Leionema lachnaeoides* | Mildenhall 2002 |
| Leaching | *Boronia ledifolia* | Benson & McDougall 2001 | *Geleznowia verrucosa* | Paynter & Dixon 1991 |
| | *Geijera linearifolia*, *Eriostemon angustifolius* subsp. *angustifolius* | Bonney 2003 | | |
| Scarification or seed coat removal + leaching | *Boronia ledifolia, B. denticulata* | Whitehorne & McIntyre 1975 | | |
| | *Crowea saligna, C. exalata* | Whitehorne & McIntyre 1975 | | |
| | *Eriostemon australasius* | Langkamp 1987 | | |
| | *Geijera parvifolia* | Whitehorne & McIntyre 1975 | | |
| | *Zieria smithii* | Whitehorne & McIntyre 1975 | | |
| Gibberellins | *Phebalium daviesii* | Lynch & Appleby 1996 | | |
| | *Boronia fastigiata*, *B. megastigma* | Bell et al. 1993 | | |
| Smoke or smoke products | *Boronia fastigiata, B. megastigma, B. tenuis, B. viminea* | Roche et al. 1997 | *Boronia fastigiata* | Dixon et al. 1995 |
| | *Crowea saligna* | Langkamp 1987 | *Boronia spathulata* | Roche et al. 1997 |
| | *Diplolaena dampieri and Geleznowia verrucosa* | Roche et al. 1997 | *Correa reflexa* var. *cardinalis* | Roche et al. 1997 |
| | *Geleznowia verrucosa* | Dixon et al. 1995 | *Leionema lachnaeoides* | Mildenhall 2002 |
| | *Philotheca spicata* | Dixon et al. 1995 | *Phebalium anceps* | Dixon et al. 1995 |
| Heat | *Asterolasia elegans* | Auld 2001 | *Leionema lachnaeoides* | Mildenhall 2002 |
| | *Boronia ledifolia* | Auld 2001 | | |
| | *Eriostemon australasius* | Auld 2001 | | |
| | *Leionema* | Auld 2001 | | |
| | *Zieria arborescens, Z. involucrata, Z. laevigata* | Auld 2001 | | |