Chapter 4

GERMINATION REQUIREMENTS FOR AUSTRALIAN SPECIES

OF *FRANKENIA* L. (FRANKENIACEAE).

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ABSTRACT


Understanding optimum germination requirements for *Frankenia* L. will inform arid zone ecology as well as *Frankenia* revegetation studies. Here, we examine germination rates and germination success for 20 Australian species of *Frankenia* at three temperatures (17°C, 23°C, and 29°C), the light requirements for germination, and seedling emergence success from soil. The results were as follows: (1) Overall, in most species germination rates were highest at 17°C and decreased as temperature increased. (2) Germination success ($T_{50}$ – time to 50% germination) varied between species with temperature. At 17°C, all species reached $T_{50}$ within 28 days. At 23°C, $T_{50}$ varied significantly between species, ranging from $T_{50}$ by Day 2 (*F. serpyllifolia*, *F. setosa*) to none by Day 28 (*F. confusa*, *F. pauciflora* var. *pauciflora*). At 29°C, eight species did not reach $T_{50}$ by Day 28. (3) There was a positive association between seed exposure to light and germination, with smaller-seeded species requiring light to germinate. (4) Seedling emergence from soil was low and only three of the 19 species reached $T_{50}$ by Week 8. These results are discussed in the context of identifying *Frankenia* species for remediation and revegetation projects, and for suitability as garden species.

*Key words*: Australian arid zone plants, germination, halophytes, revegetation, seedling emergence
Chapter 4 Germination requirements for Frankenia

INTRODUCTION

Successful germination strategies are crucial to the survival of an individual plant and therefore also the population or species. Seeds have evolved a series of adaptations, including temperature preferences and light requirements, to ensure that germination is triggered when environmental conditions are favorable for seedling establishment. While germination strategies have been described for a wide diversity of the world’s flora, relatively few detailed studies have been undertaken for the 15000 plant species endemic to Australia. Furthermore, relatively little information exists linking the available laboratory data to field conditions (Mott & Groves 1981).

Laboratory research into the germination requirements of Australian native plants species has shown that, in general, seeds germinate over a range of temperatures (see Bell 1999). Species specific studies identified optimum temperatures for germination across species, whereby germination is delayed but not prevented below and above the temperature optima. The effect of temperature on seed germination is important as it determines the time of year (season) for successful seedling establishment (Fenner & Thompson 2005). Data on the light requirements for germination in Australian plant species are sparse. The response of seeds to light prevents germination in areas that are unfavourable for seedling establishment (Fenner & Thompson 2005). In most research, the appearance of the hypocotyl at the soil surface – not the emergence of the radical from the seed – is the first sign that germination has occurred. Mortality between germination and hypocotyl emergence is predicted to be high, although it is seldom measured (Fenner & Thompson 2005).

We investigate germination in Australian species of the halophyte plant genus Frankenia. Frankenia is a cosmopolitan genus occurring in Mediterranean, semi-arid, and arid regions. The largest fraction of the world’s diversity of Frankenia occurs in Australia. Currently, 47 species are recognized in Australia with only one, F. pulverulenta L., not being endemic. Comprehensive published data on Frankenia germination are limited to Brightmore’s (1979) study of F. laevis L., a European species. Limited data on germination in Australian Frankenia species are included in some botanical tomes (e.g. Elliot & Jones 1986; Wrigley & Fagg 2003). The aim of this study was to obtain baseline data on optimum light and temperature requirements for germination in Australian species of Frankenia. We also examine hypocotyl emergence success from a soil medium to examine potential mortality rates between germination and seedling emergence. This information is important from a horticultural perspective. Only a few Australian Frankenia species are currently cultivated.
(notably *F. pauciflora* DC) despite their recognized tolerance to soil salinity, and to drought conditions. There is also potential for including *Frankenia* in remediation and revegetation projects in areas affected by salinity (Elliot & Jones 1986; Easton & Kleindorfer 2008a, 2008b). Currently, the propagation of *Frankenia* is generally by cuttings. Propagation by seed has met with limited success (Elliott & Jones 1986). This may be due to non-optimal germination conditions (as opposed to poor seed viability), which we endeavour to elucidate here.

**MATERIALS AND METHODS**

**Test species**

*Frankenia* are commonly small shrubs, sub-shrubs or cushion-bushes with pink, pale purple or white (generally) five-petalled flowers, and recurved, salt encrusted leaves. They occur in habitats such as coastal cliffs, margins of salt lakes, saltmarshes and salt-pans, at the base of sand-hills, and in depressions (gilgai) on gibber plains (Summerhayes 1930; Barnsley 1982; Whalen 1986; Badman 1999). This study uses the taxonomic relationships introduced by Summerhayes (1930) and revised by Barnsley (1982). Table 1 lists the 20 *Frankenia* species (with authorities) included in this study, the location of the populations, and the mean seed mass of each species. (Mean seed mass was calculated after individually weighing 150 seeds per population on a Mettler Toledo MX/UMX microbalance.) We include two varieties of *F. pauciflora*. A recent revision of the *F. pauciflora* group demonstrated that these two varieties, *F. pauciflora* var. *fruticulosa* from South Australia and *F. pauciflora* var. *pauciflora* from Western Australia, are sufficiently molecularly unrelated to warrant their inclusion as separate species (Craigie 2007).

Seeds were collected by one of the authors (L. Easton) between 2001 and 2005\(^1\), and stored in airtight containers at optimal storage conditions following the protocol of Wrigley and Fagg (2003) (see Chapter 3). Seeds from all species were periodically germinated over time to check for loss of viability. No degradation in seed germinability, or indication of seed dormancy was found during this time period. Seed age at germination was between one and two years from collection. *Frankenia* seeds have been demonstrated to remain highly viable

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\(^1\) Herbarium voucher specimens for each population, collection details including month and year of seed collection, and number of plants sampled per population, are available from the authors at Flinders University, School of Biological Sciences.
for at least seven years (Easton & Kleindorfer 2008a). No indication of seed dormancy has been observed (see Chapter 3).

**Light Requirements**

Germination experiments followed the protocol of the Integrated Screening Programme (Hendry & Grime 1993). Eighteen species were tested for light requirements (see Table 1). Treatments were prepared using two 500 ml clear plastic screw-top containers each containing 20 seeds, per species. Seeds were sown onto Whatman Number 1 filter papers, which sat on perforated PVC disks supported by 10 mm legs. Distilled water was added to the level of the disks, thus constantly base-watering the seeds (adapted from Zubrinich 1990). Because we tested what enhances seed germination and not what inhibits seed germination, base-watering allowed seeds to be kept at maximum water availability. Containers were sealed to prevent evaporation.

One container for each species was subjected to one treatment (‘dark’ or ‘light’). The ‘dark’ treatment was attained by covering the relevant containers with two layers of aluminium foil. All containers were placed into a 23°C controlled temperature growth cabinet for 28 days, and illuminated with Silvanian Gro-lights (25 μmol m$^{-2}$ s$^{-1}$, 400–700 nm) on a 14-hour day/10-hour night regime. Previous studies have demonstrated that using a constant temperature adequately emulates the naturally occurring fluctuating (‘alternating’) diurnal temperatures for germination of arid zone species (Mott & Groves 1981). On Day 28, the aluminium foil was removed from the ‘dark’ treatments, and germinated seeds from the ‘light’ and ‘dark’ treatments were counted. Seeds were considered germinated if the radicle had emerged. All containers were left uncovered for an additional seven days to test for further germination.

The light requirement for germination was calculated using the Relative Light Germination (RLG) index (Milberg et al. 2000);

$$RLG = 1 - \frac{Gd}{(Gd + Gl)}$$

where $Gd$ is the number of seeds that germinated in the ‘dark’ treatment, and $Gl$ is the number of seeds that germinated in the ‘light’ treatment. The RLG indices are a range of values from 1 (light essential for germination) to 0 (no light required for germination). Seeds were considered germinated with the emergence of the radicle. Any seeds infected by fungi
were scored as non-viable. The ungerminated seeds were dissected to establish their possible viability (see Verger et al. 2003).

**Germination response to temperature**

Seventeen species were subjected to three temperatures to investigate germination response to temperature (see Table 1). Germination containers were prepared as per light requirement experiments. Experimental design followed a completely randomized block design as per Khan and Rivzi (1994). Each treatment (temperature) had four replicates of 15 seeds per population per species. The following arrangements of effects were tested: population effect (four replicates per population), species effect (between one and three populations per species, depending on seed availability), and treatment effect (17°C, 23°C, or 29°C). Thus four replicates of each population of each species were subjected to 17°C, 23°C, or 29°C. This experimental design produced a total of 504 units (containers).

Containers were placed in growth cabinets at 17°C, 23°C, or 29°C, and illuminated with silvanian gro-lights (25 μmol m⁻² s⁻¹, 400–700 nm) on a 14-hour day/10-hour night regime. Seeds were checked every second day, at which time germinated seeds were counted and removed. Seeds were considered germinated with the emergence of the radicle. Any seeds infected by fungi were scored as non-viable and removed. The experiment was terminated after 28 days and the ungerminated seeds were dissected (i.e. cut test) to establish their possible viability (see Verger et al. 2003).

Germination rates (defined as the percentage of seeds that germinated at each 2-day interval) by Day 8 were estimated using a modified Timson Index:

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\text{Germination rate} = \sum [(G^1/t)+ (G^2/t)+ \ldots + (G^t/t)]
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where \(G\) is the mean percentage of seed germination at 2-day intervals and \(t\) is the total number of days of the germination period. The greater the value the more rapid is the germination. A Nested (Hierarchical) Design ANOVA was used to calculate the differences in germination rates between species and between temperatures at Day 8.

Germination success (defined as the percentage of the total number of seeds per species that germinated) was calculated using the ‘Time (in days) to 50% germination (T₅₀)’
index (Trudgill et al. 2000). A Nested (Hierarchical) Design ANOVA was used to calculate the differences in germination success between species per temperature at Days 8 and 28.

**Seedling emergence in sand**

Concurrent to ‘seed response to germination’ experiments, we tested for seedling emergence from sand. Seeds from the same seed lot were used for these experiments. Nineteen species were tested for germination success from sand, identified by the emergence of the hypocotyl. Small plastic seedling pots (5 cm x 5 cm) were filled with washed sand. Water saturated sand was achieved by standing the sand-filled pots in trays of water, and this method of base-watering was maintained throughout the experiment (see Ahmad & Ismail 1995; Wrigley & Fagg 2003). Each pot was planted with 10 seeds (seeds placed on the soil surface to satisfy the light requirement – see RESULTS). Four replicates per population of between one and three populations (depending on availability of seed) per species were included (see Table 1). Pots were illuminated with Silvanian Gro-lights (as previous) on a 14-hour day/10-hour night regime. Ambient temperatures varied between 23°C and 29°C. These temperatures were estimated to represent the daily ambient average temperatures in horticultural glasshouse environments (Ransom Seed Laboratory 2003). Plants were fertilized monthly with a \( \frac{1}{3} \) strength Hoagland’s solution (Hoagland & Snider 1933).

Pots were checked weekly for the first two months and number of seedlings counted. The pots were then checked monthly for a further four months to monitor for any additional germination, and for seedling mortality. Germination success over the initial 2-month period was calculated using the ‘**Time to 50% germination (T_{50})**’ index (Trudgill et al. 2000). Mortality over the six month period (i.e. seedling survival to six months) was calculated as the percentage of seedlings remaining alive at six months.

**RESULTS**

**Light requirements**

Table 2 lists the light requirements, as RLG indices, for *Frankenia* species included in this study. The two largest-seeded species (*F. eremophila*, *F. setosa*) had the lowest RLG indices (0.21 and 0.23 respectively). Two smaller-seeded species (*F. confusa*, *F. irregularis*) had a light prerequisite for germination (i.e. an RLG index of 1.00). However, while the RLG
indices generally increased with decreasing seed mass, there was no significant difference in the RLG indices between the smaller-seeded species (RLG 0.81 ±0.08 se) and the larger-seeded species (RLG 0.67 ±0.06 se) (ANOVA: F = 1.99, df = 40, P = 0.10).

Seven populations (LE01043, LE03013, LE03032, LE03038, LE03061, LE05024, LE05025) had less than 50% germination success (T50) over the 28-day period indicating that factors other than light, water, and an ambient temperature of 23°C were required for optimum germination in these populations.

**Germination and temperature**

Figure 1 shows germination rates to Day 8 for each species for each temperature treatment (17°C, 23°C, and 29°C) and overall, calculated using a modified Timson Index. There were significant differences in germination rates between species and/or between temperatures. At 17°C, *F. eremophila, F. foliosa, F. planifolia* and *F. setosa* had high germination rates, while *F. confusa, F. interioris, F. pauciflora var. pauciflora* and *F. sessilis* had low germination rates. At 23°C, *F. cinerea, F. eremophila* and *F. serpyllifolia* had high germination rates while *F. confusa* and *F. pauciflora var. pauciflora* had low germination rates. At 29°C, germination rates were lower for all species. *Frankenia planifolia* and *F. serpyllifolia* had the highest germination rates. Conversely, *F. confusa* and *F. pauciflora var. pauciflora* had negligible germination rates. All species had higher germination rates at 17°C than 23°C except *F. cinerea, F. interioris* and *F. sessilis*. Notably, the germination rate for *F. pauciflora var. pauciflora* was two-fold lower at 23°C than at 17°C.

Figure 2 shows germination percentages and T50 per species per temperature to Day 28. Overall, germination by Day 28 was significantly different between species (F = 4.73, df = 44, P<0.001), ranging from 95.5% (*F. planifolia*) to 28% (*F. pauciflora var. pauciflora*).

At 17°C, all species reached T50 by Day 28. *Frankenia pauciflora var. pauciflora* had the lowest germination success (T50 on Day 8). At Day 28, germination percentages were significantly different between species (F = 11.5, df = 22, P<0.001) ranging from *F. setosa* (100%) to *F. pauciflora var. pauciflora* (53%). Twelve of the 17 species had greater than 80% germination by Day 28. *Frankenia setosa* had the highest germination, and 97% of seeds had germinated by Day 4. A further four species (*F. cordata, F. fecunda, F. foliosa, F. gracilis*) reached T50 by Day 4.

At 23°C, ten of the 17 species had greater than 80% germination by Day 28. However, germination percentages were significantly different between species (F = 5.43, df = 22, P<0.001), ranging from *F. planifolia* (98%) to *F. pauciflora var. pauciflora* (27.5%). Two
species (*F. serpyllifolia*, *F. setosa*) had reached $T_{50}$ by Day 2, and 11 species had reached $T_{50}$ by Day 4. Conversely, two species (*F. confusa*, *F. pauciflora* var. *pauciflora*) had not reached $T_{50}$ by Day 28.

At 29°C, germination percentages at Day 28 were significantly different between species ($F = 9.12$, df = 22, $P<0.001$), ranging from *F. planifolia* (89%) to *F. pauciflora* var. *pauciflora* (3%). Only *F. planifolia* achieved greater than 80% germination. *Frankenia serpyllifolia* and *F. planifolia* reached $T_{50}$ by Day 2 and Day 4 respectively. Conversely, eight species had not reached $T_{50}$ by Day 28 (*F. confusa*, *F. fecunda*, *F. foliosa*, *F. laxiflora*, *F. magnifica*, *F. pauciflora* var. *pauciflora*, *F. setosa*, *F. tetrapetala*).

**Seedling emergence in sand**

Table 3 lists the percentage of seedlings that had emerged from soil by Week 8, and the number of seedlings still alive at six months. It also lists the number of seedlings that emerged after Week 8. Seedling emergence success ($T_{50}$) was significantly different between all species ($F = 2.38$, df = 151, $P<0.05$) with only three of the 19 taxa (*F. cinerea*, *F. eremophila*, *F. sessilis*) reaching $T_{50}$. Four additional species reached 40% germination success ($T_{40}$) by Week 8 – *F. foliosa* (Week 5), *F. tetrapetala* (Week 6), and *F. interioris* and *F. laxiflora* (Week 8).

**DISCUSSION**

This study demonstrates that temperature and light requirements for germination vary between species of *Frankenia*. Light did not inhibit germination for any of the species tested; in fact, light appears to be necessary for promoting seed germination in some species, notably in the smaller-seeded species. For example, *F. irregularis* and *F. confusa* (seed mass 67 μg) had an RLG index of 1.00 indicating that light was essential for germination. Many studies (e.g. Milberg *et al.* 2000; El-Kablawy & Al-Rawai 2005; Ramírez-Padilla & Valverde 2005) have shown a light requirement for germination of small seeds. Small seeds germinating in darkness below the soil surface may produce seedlings that are unable to reach the soil surface. In arid regions this would occur when seeds fall into cracks, especially in clay-based soils, or are buried by wind-borne sand (Wood 1937; Maun & Lapierre 1986; Facelli & Ladd 1996). The results of our study suggest that germination becomes less reliant on a requirement for light with increasing seed mass within *Frankenia*. However, the overall pattern was a non-
significant trend. The relatively larger and faster growing seedlings from the larger-seeded species may be more successful in emerging from burial.

The effects of the interaction between temperature and seed mass on germination in *Frankenia* are discussed in Chapter 3. Here we examined the germination rates (particularly during the first week after sowing) and $T_{50}$ of individual species. This is valuable information for identifying species for horticultural and revegetation applications.

The effect of temperature on seed germination is significant as it determines the time of year (season) for successful seedling establishment (Bell 1999; Fenner & Thompson 2005). Published data on seedlings of European and American species of *Frankenia* documents that seedlings are only observed after higher than average rainfall (Brightmore 1979; Allison 1992, 1995; Escudero et al. 1999). For *Frankenia*, fruit remains attached indefinitely to the parent plant. The seeds remain within the fruit until the fruit fully hydrates (e.g. after high rainfall). Hydration splits the fruit thus releasing and dispersing the seeds. Further persistent rainfall (or flooding) is required for seed imbibition and for germination to proceed (Brightmore 1979). Water must also remain available for seedlings to become established.

In Australia, *Frankenia* occur in regions where persistent rain generally falls in the cooler winter months. If higher than average rainfall is necessary for seedling establishment in *Frankenia*, average winter temperatures should be correlated with the highest germination rates and the greatest germination success. Daily mean temperatures for winter in the regions of *Frankenia* distribution in Australia averaged between 15°C to 18°C (1961–1990, Australian Bureau of Meteorology 2008). The results of this study demonstrated that overall, germination rates and germination success were highest at temperatures correlating to winter in regions of *Frankenia* distribution. An increase of only 6°C (17°C to 23°C) changed germination rates and $T_{50}$ in all the *Frankenia* species tested in this study. Generally, seed germination decreased with increasing temperature, although the magnitude of the decrease was species specific. A further increase of 6°C in temperature to 29°C significantly further reduced germination rates and $T_{50}$ in all species. Changes in germination rates and $T_{50}$ due to relatively small temperature increases suggest a narrow temperature window for optimum germination in *Frankenia*, despite the fact that naturally occurring *Frankenia* experience extreme temperature fluctuations.

However, some species were less temperature specific. *Frankenia cordata*, *F. eremophila*, *F. planifolia*, and *F. serpyllifolia* had high germination at all temperatures including 29°C, suggesting that temperatures between 17°C and 29°C are suitable for good germination. These are all larger-seeded species and often associated with clay-pans (see
Chapter 4 Germination requirements for Frankenia

Chapter 5) although their distribution is diverse. *Frankenia cordata* occurs around Alice Springs, NT. *Frankenia eremophila* is an isolated coastal species occurring only along the Great Australian Bight. *Frankenia planifolia* and *F. serpyllifolia* have a wide distribution across central Australia and are part of a complex of species that may in fact be con-specific (Whalen 1986).

Conversely, germination was extremely low for the *F. pauciflora* complex, including the closely related *F. confusa* (Craigie 2007), even at 17°C. This coastal species complex may require temperatures lower than 17°C for optimum germination, or may require alternating diurnal temperatures. In general, using constant temperatures for germination studies adequately represents the mean diurnal temperature range (Mott & Groves 1981). However in some cases, alternating diurnal temperatures are required to promote germination (Harty & McDonald 1979). For example, *Spinifex hirsutus* occurs in regionally close proximity to *F. pauciflora* along the west coast of Western Australia. These coastal *Frankenia* may also require specific alternating diurnal temperatures to promote germination. *Frankenia pauciflora* is the most widely commercial cultivated Australian species of *Frankenia* and its low germination success may underpin the concept that *Frankenia* has limited propagation success from seeds.

On the contrary, we have demonstrated that germination across all species, except *F. pauciflora* var. *pauciflora* is high, given optimum temperatures. However, there was poor seedling emergence from sand across all *Frankenia* species. Only three species reached T₃₀ over the initial 8-week period. Two of these species, *F. eremophila* and *F. sessilis*, also had greater than 70% seedling survival at six months. These two species commonly occur in close geographical proximity in the ‘far west coast’ of South Australia. Note that *F. eremophila* also had high germination at all temperatures. Unfortunately, *F. eremophila* is not a good candidate for horticultural promotion, as we have yet to observe any flowering in this species under glasshouse conditions over a 7-year period².

Failure of seedling emergence from sand may have been attributable to non-optimum temperatures (ambient temperature ranged between 23°C and 29°C), or alternately, to seedling interactions related to the properties of the sand. Suppression of seedling growth by mechanical resistance of soil mediums has been reported for other species (see Barley & Greacen 1967; Collis-George & Yoganathan 1985; Tobe & Gao 2007).

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² Flowering information on the species included in this study are available from the author and are lodged at Flinders University, School of Biological Sciences.
In this study, seedling survival was poor. This is evidenced by our finding that only nine of the 19 species tested had >60% seedling survival after six months. As all treatments were base-watered (as per Ahmad & Ismail 1995), fatalities were not due to drought stress. Interestingly, five of these nine species had low initial seedling emergence (less than T₀). Four are smaller-seeded species that are molecularly closely related within the \textit{F. pauciflora} complex (Craigie 2007). Of the three remaining larger-seeded species, \textit{F. latior} and \textit{F. serpyllifolia} are also closely related and may be conspecific (Whalen 1986). \textit{Frankenia crispa} is a rare, isolated and little known species that occurs in north-western Victoria and the ‘Mid-north’ of South Australia. The high seedling survival despite the low seedling emergence may be related to \textit{F. crispa} persistence despite the paucity of populations.

Seed germination has a direct impact on species distribution and abundance, especially in arid and semi-arid environments (Bell 1999; Ramírez-Padilla & Valverde 2005). Knowledge of seed germination is essential for designing studies on reproductive ecology and particularly on seedling ecology. It is also crucial for the development of conservation management plans, which could include the rare \textit{Frankenia} species (e.g. \textit{F. plicata} Melville, \textit{F. drummondii} Benth.) or the small, isolated, little-known species (e.g. \textit{F. bracteata} Turcz., \textit{F. confusa}, \textit{F. crispa}, \textit{F. glomerata}, \textit{F. subteres} Summerh.). These species are only known from roadside verges or on private land (Briggs & Leigh 1996). The uncertain future of these species is of concern and efforts should be made to bring them into cultivation, where at least their continued existence will be secure (Wrigley & Fagg 2003). The success of propagating \textit{Frankenia} from seeds should be enhanced as more information on optimum requirements for seed germination and seedling survival becomes available.

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Chapter 4 Germination requirements for Frankenia

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Chapter 4 Germination requirements for Frankenia


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Chapter 4 Germination requirements for Frankenia


### Table 1. Species included in experiments, noting location of population samples, identification numbers, and average weights of seeds. Superscript (a) indicates populations included in germination in vials experiments; superscript (b) indicates populations included in germination in soil experiments; and superscript (c) indicates populations included in germination in dark experiments.
Table 2. Relative light requirement (RLG) indices for *Frankenia* species. Species are categorized as ‘smaller-seeded species’ and ‘larger-seeded species’ to highlight the relative differences in light requirements between these categories. RLG range from 1 (a light requirement for germination) to 0 (no light required for germination).
Table 3. Percentage of seeds that germinated in soil to Week 8 (W8), percentage of plants that were alive after 6 months (M6), and the number of seeds that germinated after Week 8. *Frankenia pauciflora* var. *pauciflora* (a) indicates coastal varieties of this species. *Frankenia pauciflora* var. *pauciflora* (b) indicates inland varieties of this species.
Chapter 4 Germination requirements for Frankenia

Figure 2. Percentage of seeds that germinated over 28 days at 17°C, 23°C, and 29°C, and time (in days) to reach 50% germination. Species are numbered as follows: (1) *F. cinerea*, (2) *F. confusa*, (3) *F. cordata*, (4) *F. eremophila*, (5) *F. fecunda*, (6) *F. foliosa*, (7) *F. gracilis*, (8) *F. interioris*, (9) *F. laxiflora*, (10) *F. magnifica*, (11) *F. pauciflora var. fruticulosa*, (12) *F. pauciflora var. pauciflora*, (13) *F. planifolia*, (14) *F. serpyllifolia*, (15) *F. sessilis*, (16) *F. setosa*, and (17) *F. tetrapetala*. Dashed lines represent the smaller-seeded species, while solid lines represent the larger-seeded species.