Chapter 6

EFFECTS OF SALINITY LEVELS AND SEED MASS ON GERMINATION IN AUSTRALIAN SPECIES OF *FRANKENIA* L. (FRANKENIACEAE).

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ABSTRACT

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Halophyte species demonstrate differing levels of salt tolerance. Understanding interspecific variation to salinity levels is of value from both the scientific perspective, which includes the identification of traits associated with salinity tolerance, as well as from an applied perspective, which includes identifying plant species for specific salinity restoration and remediation projects. This paper investigates the effects of salinity on germination of 12 Australian species of the plant genus *Frankenia* L. (Frankeniaceae). We use saline solutions that corresponded to the average soil-water salinity concentrations in the arid zones of inland Australia. These solutions consisted of 10mM calcium chloride, 30mM magnesium sulphate, and 450mM sodium chloride. The aims of our study were: (1) to investigate germination (germination rates, germination success) of *Frankenia* seeds to four salinity levels (0%, 10%, 20%, 30%), (2) to test for possible interaction effects between seed mass, germination, and salinity, and (3) to examine the effect of salinity levels on the inhibition of germination and/or seed damage. Species varied in their salt tolerance for germination rates and success. Species with larger seeds had higher germination rates and germination success for high salinity levels. Several species did not germinate well at any salinity level. Finally, no seeds were adversely affected by exposure to high salinity levels pre-germination. There is potential for including some *Frankenia* species in remediation and revegetation projects in areas affected by salinity, and also as garden plants in saline regions.

Key words: Australian arid zone plants, germination rates, germination success, salinity

INTRODUCTION

In 1995, global estimates indicated that at least 1.5 billion hectares of land were affected by salinity (Yenson 1995). The National Land and Water Resources audit found that in Australia, approximately 5.7 million hectares were at risk or affected by salinity. This report predicted that within 50 years, the land area in Australia affected by salinity would increase to 17 million hectares. Soil salinization results from brine contamination associated with oil and gas production, a build up of ions in irrigated regions, excessive land clearance, and coastal incursions (Keiffer & Ungar 2001). Plant growth is detrimentally impacted by salinity. Regions affected by salinity may become unvegetated if not remediated, due to the unfavourable properties of saline soil and the consequent soil erosion (Keiffer & Ungar 2001). However, salinity is not detrimental to all plants, and some plant species known as halophytes, grow naturally on saline soils. Boyko (1966) was the first to suggest that halophytic plants could be used to rehabilitate saline soils (i.e. phytoremediation). Ravindran *et al.* (2007) demonstrated that soil salinity levels, as measured by electrical conductivity (EC), can be reduced by the cultivation of halophytes on soils affected by salinity by accumulating salts in their plant tissues. Halophytes can also lower water tables, and their roots bind soil thus preventing soil erosion (see www.crcsalinity.com).

Halophytes are exposed to enormous variations in temperature and salinity. Surface soils have salinity levels ranging from 2 – 100 times that of the subsoil, and present an even more extreme environment to seeds than to the established plant (Ungar 1978). Thus, the critical stages in the life histories of halophytes are germination and seedling establishment. The success of a plant species depends on its seeds remaining viable during times of high salinity levels, and its ability to germinate readily when salinity stress is reduced (Khan *et al*. 2006). However, seed germination, even for most halophytes, is extremely sensitive to soil salinity, and maximum germination occurs under non-saline conditions (Vicente *et al*. 2004). An increase in salinity levels causes both a reduction in percentage of seeds germinating and a delay in the initiation of the germination process, which can cause complete inhibition of the germination process (Pujol *et al*. 2000).

Timing of germination is crucial in the life cycle of halophytes. Germination in a salt-stressed environment exposes seedlings to high risks of mortality. Excess salt reduces a seedling's ability to extract water from the soil, causing wilting and eventual death (Woodell 1985; Ungar 1991; Gutterman 1994; Khan & Ungar 1996, 1998; Keiffer & Ungar 1997). Some salts (e.g. sodium and magnesium) are also toxic to plants (Ungar 1991). In Australia,

seeds of halophytic species typically germinate early in the spring when soil salinity levels are reduced, which promotes seedling establishment prior to the high salt-stressed conditions caused by hot, dry summers. However, many halophytes have the potential to germinate at any time of the year under favourable conditions (rainfall, optimum temperatures, and reduced salinity).

Germination has also been linked to seed mass. Large seeds tend to have faster germination rates when compared to small seeds (e.g. Fenner 1983, 1992; Milberg *et al*. 1996; Easton & Kleindorfer 2008a, 2008b). As a consequence, seedlings from larger-seeded species should be able to establish under a range of environmental conditions that cannot be exploited by smaller-seeded species. Large-seededness in plants may be an adaptive strategy in drought prone regions (see Baker 1972; Salisbury 1974; Wulff 1986; Mazer 1990; Geritz *et al*. 1999; Susko & Lovett Doust 2000; Westoby *et al.* 2002). Increased salinity, which results in a physiologically induced drought, may also induce larger seed masses (Fenner 1992). Rapid seed germination, often characteristic of halophytes, may be an adaptive strategy to utilize any soil water with reduced salinity levels – even of short duration (Khan & Ungar 1996). However, the correlation between germination rates in halophytes and seed mass has received limited investigation (Leishman & Westoby 1994).

Halophyte species exhibit different levels of salt tolerance (Vicente *et al.* 2004). A better understanding of interspecific variation to salinity stress is constructive from both the scientific perspective, which includes the identification of traits associated with salinity tolerance, as well as from an applied perspective, which includes identifying plant species for specific restoration and remediation projects (Hester *et al*. 2001). This paper investigates the effects of salinity on germination in Australian species of *Frankenia* L. (Frankeniaceae). *Frankenia* is a cosmopolitan halophytic plant genus that occurs in Mediterranean, semi-arid, and arid regions. Ecological data on *Frankenia* as a taxon, and specifically the 47 species of Australian *Frankenia*, are limited. Published data on *Frankenia* germination are limited to Brightmore's (1979) study of *F. laevis* L. (a European species), and botanical tomes (e.g. Elliot & Jones 1986; Wrigley & Fagg 2003). Only a few Australian species are currently cultivated (e.g. *F. pauciflora, F. connata*) despite their recognized tolerance to soil salinity and drought conditions. There is also potential for including *Frankenia* in remediation and revegetation projects in areas affected by increasing salinity.

We compare seed germination in 12 Australian *Frankenia* species that have been subjected to various levels of salinity. We test for interspecific variation in germination rates and germination success, also in relation to large- and small-seededness. Six of these 12

species have low numbers of ovules per flower and thus produce low numbers of larger seeds per fruit ('larger-seeded species' *sensu* Easton & Kleindorfer 2008a) and six species have high numbers of ovules per flower and produce many smaller seeds per fruit ('smaller-seeded species' *sensu* Easton & Kleindorfer 2008a). Larger- and smaller-seeded *Frankenia* species often occur in close geographical proximity, even within the same community. This provides a good opportunity to examine selective pressures that have shaped these geographically co-occurring reproductive strategies.

The aims of our study were: (1) to investigate germination (germination rates, germination success) of *Frankenia* seeds at four salinity levels (0%, 10%, 20% 30%), (2) to test for possible interactions between seed mass, salinity, and germination, and (3) to examine the effects of salinity levels on inhibition of germination and/or seed damage. We predicted higher germination rates and germination success in larger-seeded species overall and notably at higher salinities. We also predicted high recovery rates in *Frankenia* species after exposure to high salinities.

MATERIALS AND METHODS

Test species

Table 1 lists the population locations, and the mean seed mass of the species included in this study. We follow the taxonomic descriptions proposed by Summerhayes (1930), and revised by Barnsley (1982). Mean seed mass was calculated by individually weighing 150 seeds per population on a Mettler Toledo MX/UMX microbalance. Twelve *Frankenia* species were included in this study, six species with a low number of ovules per flower and larger seeds (*F. cordata* J.Black*, F. interioris* Ostenf.*, F. serpyllifolia* Lindl.*, F. sessilis* Summerh.*, F. setosa* W.Fitzg., F. *tetrapetala* Labill.), and six species with a high number of ovules per flower and smaller seeds (*F. cinerea* A. DC.*, F. fecunda* Summerh.*, F. foliosa* J.Black*, F. laxiflora* Summerh., and two varieties of *F. pauciflora* DC). A recent revision of *F. pauciflora* demonstrated that two varieties of *F. pauciflora*, one from South Australia and the other from Western Australia, are sufficiently unrelated to warrant their inclusion as two separate species in this study (Craigie 2007).

Seeds were collected from naturally occurring populations in early autumn from 2001 to 2005,[1] and stored in air tight containers at optimal seed storage conditions (see Wrigley & Fagg 2003). 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. *Frankenia* seeds have been demonstrated to retain viability for at least seven years (Easton & Kleindorfer 2008a).

Pilot study

A preliminary trial by T. Playford and L. Easton (2003, Flinders University, unpublished data) tested the effects of four levels of salinity (0%, 25%, 50% and 75% seawater) at three temperatures (16°C, 24°C, 30°C) on germination in two species of Australian *Frankenia,* one larger-seeded species (*F. serpyllifolia*), and one smaller-seeded species (*F. foliosa*). These two species co-occur along the Oodnadatta Track in the 'Far North' of South Australia. There were statistically significant differences in the germination rates between these two species, (Students t-test: $t = 4.42$, $df = 47$, $P<0.001$), with higher germination rates at all temperatures per salinity levels in the larger-seeded species.

Salinity experiments

Germination experiments followed the protocols of the Integrated Screening Programme (Hendry & Grime 1993). To examine the effects of salinity on germination rates and germination success, replicates of 15 seeds were sown on Whatman Number 1 filter paper in 500 ml plastic containers. The filter papers were placed on round perforated PVC disks supported by 10 mm legs, thus suspending the filter paper and seeds over a 100 ml reservoir of solution (adapted from Zubrinich 1990). The filter paper and the seeds were thus constantly base-watered (see Ahmad & Ismail 1995). Containers were sealed to prevent evaporation and maintained at 20°C (as per Ransom Seed Laboratory 2003, and see Chapter 4) in a growth cabinet 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 affected with fungal infection were scored as non-viable and removed to prevent the fungal spores from spreading to other seeds.

[1] Herbarium specimens and 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.[1]

The main salt components in saline soils are Na^{+}, Mg^{2+}, Ca^{2+}, Cl^{-} and SO_4^{-} (Shanberg 1975). However, many studies examining the effects of salinity on plants have been carried out using only sodium chloride (NaCl) or dilutions of seawater (Tobe *et al*. 2000). We used saline solutions that corresponded to the average soil-water salinity concentrations in the arid zones of inland Australia (see Kroker 1996). These solutions consisted of 10mM calcium chloride, 30mM magnesium sulphate, and 450mM sodium chloride. Four salinity concentrations (0%, 10%, 20%, 30%) were tested, based on preliminary studies for salt tolerance limits of several *Frankenia* species (Kroker 1996; T. Playford & L. Easton, unpublished data), and demonstrated by Brightmore (1979) with *F. laevis*. Electrical conductivities of the solutions are 0.4 dS/cm, 15.5 dS/cm, 26.2 dS/cm, and 38.9 dS/cm respectively.

Germination rates

Experimental design followed a completely randomized block design (see Khan & Rivzi 1994). We tested for the following effects: population and species (three populations with four replications each for each of the 12 species), seed mass (assigned into two seed categories of 'larger-seeded' and 'smaller-seeded' species), and treatment (0%, 10%, 20%, 30% saline solutions). This experimental design produced a total of 576 units (containers).

Statistical analyses utilized SPSS Version 14. To satisfy the requirements of normality, data were arc-sine transformed. The germination rate for each species was estimated using a modified Timson Index;

$$\textbf{Germination rate} = \sum[(G^1/t) + (G^2/t) \ldots\ldots + (G^t/t)]$$

where ***G*** is the percentage of seed germination at 2–day intervals, and ***t*** is the total number of days of the germination period. A greater value of ***G*** indicates a faster germination rate. We used a Nested (Hierarchical) Design ANOVA to calculate differences in germination rates between species at each of the four salinity levels on Day 2, Day 4, Day 6 and Day 8.

Germination success

Germination success for each species was calculated using the **'Time** (in days) **to 50% germination (T_{50})'** index (Trudgill *et al*. 2000). A Nested Design ANOVA calculated differences in T_{50} between species at the four salinity levels.

Recovery experiments

After 28 days, solutions were discarded and ungerminated seeds were placed over a reservoir of distilled water for a further 14 days to test whether the saline solutions had inhibited germination (Khan & Ungar 1996; Pujol *et al*. 2000; Rubio-Cas *et al.* 2003). We used 14 days as a cut-off because little additional germination occurred after 10 days from transfer to distilled water (also see Khan & Ungar 1996; Ramírez-Padilla & Valverde 2005). It was not necessary to do viability tests (e.g. using tetrazolium) on ungerminated seeds at the end of experiment since most of the seeds that remained ungerminated showed clear signs of rotting or fungal infection.

Percentage recovery germination was calculated using the following formula;

$$\textbf{Recovery \% = [a/(c-b)].100}$$

where **a** is the total number of seeds germinated after being transferred to distilled water, **b** is the total number of seeds germinating in the saline solution, and **c** is the total number of seeds (modified from Khan & Gulzar 2003).

Effect of seed mass

Analyses testing for the effect of seed mass on germination rates followed the protocol outlined in Chapter 3. We used a Nested (Hierarchical) Design ANOVA to investigate differences in germination rates between larger-seeded and smaller-seeded species every two days over a 28–day period, using temperature and seed mass as fixed factors, and species as a random factor. Type III Sum of Squares were calculated for each test to adjust for the effect of different species that were nested within each seed mass category.

Partial Eta Squares (η_p^2) – a measure of effect size – were calculated. Measures of effect size quantify the degrees of association or correlation between an effect (e.g. a main effect or an interaction) and the dependent variable (Becker 2000). If the value of the measure of association is squared, as it is for η_p^2 it can be interpreted as the proportion of variance in the dependent variable (i.e. germination) that is attributed to each effect, and is calculated as;

$$\eta_p^2 = SS_{effect}/(SS_{effect} + SS_{error}).$$

Statistically, η_p^2 is the proportion of the Sum of Squares effects plus Sum of Squares error variances that are attributed to the effects.

Finally, standard linear regression analyses assessed the relationship between mean seed mass per species and germination rates (using the modified Timson Index) at each

salinity level, to test the null hypothesis of no relationship between seed mass and germination rate.

RESULTS

Germination rates

Figure 1 shows germination rates per species as calculated by a modified Timson Index at Day 2, Day 4, Day 6, and Day 8 per salinity level (0%, 10%, 20%, and 30%). Germination rates were significantly different (at $P<0.001$ – see Table 2) between species per salinity level at $P<0.001$ at each 2-day interval except Day 2 at 10%, 20%, and 30%, Day 4 at 20%, and Day 8 at 30%, where germination rates were not significantly different (see Table 2). Species varied in their germination rates per salinity level. For example, *F. cordata* had the highest germination rate on Day 4 at 0% salinity, on Day 6 at 10% salinity, and on Day 8 at 20% and 30% salinity, whereas *F. foliosa* had the highest germination rates on Day 6 at 0%, on Day 8 at 10%, and had nominal germination at 20% and 30%. Only a few *F. laxiflora* seeds germinated for any salinity levels until Day 4 or later. *Frankenia cordata, F. interioris, F. serpyllifolia* and *F. setosa* had the highest germination rates for all salinity levels. *Frankenia setosa* had the highest germination rates at 20% and 30% of all the *Frankenia* species being tested. *Frankenia pauciflora* (WA), *F. laxiflora* and *F. fecunda* had consistently low germination rates. Interestingly, *F. foliosa* had amongst the highest germination rates at Day 2 for 0% and 10% salinity, but amongst the lowest germination rates for 20% and 30% salinity levels. Also, *F. pauciflora* (SA) had high germination rates at Day 2 for 0% salinity but among the lowest germination rates for all other salinity levels.

A further notable trend was that species with high germination rates were larger-seeded species. Larger-seeded species had high germination rates for up to 30% salinity levels. Smaller-seeded species had relatively lower germination rates. *Frankenia sessilis* and *F. tetrapetala* had slower germination rates than the other larger-seeded species. The latter two species have a coastal distribution, and appeared to respond to salinity levels similarly to *F. pauciflora* (SA) and *F. pauciflora* (WA), which are also coastal species (see below).

Germination success

Figure 2 shows the germination success of the 12 species calculated over 28 days at the four salinity levels. There were significant differences in germination success between

species (measured as time in days to reach 50% germination; T_{50}) for all salinity levels. At 0% salinity, time to T_{50} ranged from Day 4 (*F. cordata, F. interioris, F. serpyllifolia, F. setosa*) to Day 10 (*F. pauciflora* (WA)) (ANOVA: F = 2.27, df = 11, P<0.01). In addition, T_{75} (time to 75% germination) was reached by nine of the 12 species by Day 28. At 10% salinity, time to T_{50} ranged from Day 4 (*F. serpyllifolia*) to Day 12 (*F. sessilis*), with the exception of *F. pauciflora* (WA) which had not reached T_{50} by Day 28 (only 48.7%) (F = 3.94, df = 11, P<0.001). Only five of the 12 species reached T_{75} by Day 28.

At 20% salinity, only *F. setosa* reached T_{50} (by Day 10) (F = 20.69, df = 11, P<0.001), and also reached T_{75} within the 28 day period. However *F. serpyllifolia* had 48% germination success. Five further species (*F. cinerea, F. interioris, F. laxiflora, F. pauciflora* (SA), *F. tetrapetala*) reached T_{25} by Day 28. At 30% salinity only *F. setosa* reached T_{50} (by Day 22) (F = 24.4, df = 11, P<0.001), and a further two species (*F. interioris, F. serpyllifolia*) reached T_{25} by Day 28. *Frankenia foliosa* and *F. pauciflora* (WA) had very low germination (3.33% and 2.78% by Day 28 respectively).

Recovery

Table 3 lists the percentage of seeds that germinated per species per salinity level by Day 28, and the percentage of ungerminated seeds that germinated in distilled water over 14 days after being transferred from the saline solutions. Overall, germination to Day 28 (i.e. the combination of all the salinity levels) ranged from 32.9% *(F. pauciflora* (WA)) to 75.4% (*F. setosa*). Percentage recovery after transfer of ungerminated seeds to distilled water ranged from 36% (*F. pauciflora* (WA)) to 87.8% (*F. interioris*) with an average recovery of 68%. This suggests that seed viability is not adversely affected by exposure to highly saline water, and germination rates may be enhanced by exposure to high salinity for some species.

In 0% salinity, all species had greater than 69% germination by Day 28, and six species had greater than 90% germination. With the exception of *F. pauciflora* (SA), there was generally no recovery after transferring ungerminated seeds to distilled water as expected. At 10% salinity, all species had between 60% and 92% germination, with the exception of *F. pauciflora* (WA) (47.8%). Five species had greater than 80% germination, notably *F. interioris* (91.7%). Recovery after transfer to distilled water ranged from 7.7% for *F. setosa* (but note the original high germination of 85.6%) to 65% for *F. pauciflora* (SA), *F. tetrapetala* and *F. sessilis*. At 20% salinity, all but three species had greater than 20% germination by Day 28, notably *F. setosa* (76.1%) and *F. serpyllifolia* (48.3%). Recovery after transfer to distilled water was greater than 48% for all species (i.e. higher than recovery

after exposure to 10% salinity). At 30% salinity, germination ranged between 2.8% (*F. pauciflora* (WA)) and 53.3% (*F. setosa*). All but four species had less than 10% germination. All species with high germination were larger-seeded species. Recovery was greater than 48% for all species, and three species had greater than 70% recovery, notably *F. interioris* (92.6%).

Effect of seed mass

Table 4 lists the effect of the interaction between salinity*species(seed mass category) on germination rates for Day 2, Day 4, Day 6, and Day 8, and on germination success for Day 8 and Day 28. Notable are the significant differences in germination rates at Day 2 and Day 4, and in germination success at Day 8 and Day 28. Table 5 lists the differences in germination rates between species(seed mass category) (henceforth called the SSM) at Day 2, Day 4, Day 6 and Day 8, and in germination success at Day 28 for each salinity level. Germination rates were significantly different between SSM on Day 2 at 10% salinity, Day 2 at 20% salinity, and Day 8 at 30% salinity.

We examined the Partial Eta Squared (η_p^2) values to identify the degree of association between germination and SSM. First we examined differences in germination between larger-seeded and smaller-seeded species by combining the results of all four salinity categories (see Table 5). The largest interaction occurred on Day 2, accounting for 67% of the total variance in the performance scores (i.e. germination).

Next we examined differences in germination rates that could be attributed to the SSM effect within each of the four salinity levels (0%, 10%, 20%, 30%). The days for which germination was influenced by SSM varied between salinity levels. At 0% salinity, the highest SSM effect occurred on Day 6 (44%). At 10% salinity, the highest SSM effect occurred on Day 2 (60%). At 20% salinity, the highest SSM effect occurred on Day 2 (84%). At 30% salinity, the highest SSM effect occurred on Day 8 (66%). The importance of seed mass for germination success increased with salinity levels. Germination success at Day 28 attributable to SSM effect was most pronounced for 20% salinity (61%) and 30% salinity (78%).

Finally, standard linear regression analyses calculated the relationship between mean seed mass per species and germination rates (Timson Index at Day 28) for each salinity level, to test the null hypothesis of no relationship between seed mass and germination rate. The null hypothesis was supported for 0% salinity ($r^2 = 0.13$, $F = 1.42$, $P = 0.26$), and 10% salinity ($r^2 = 0.30$, $F = 4.33$, $P = 0.06$). This finding suggests that only 13% and 30% respectively of the increase in germination rates could be explained by an increase in seed mass during the 28-

day period. Examination of residual plots revealed that within the smaller-seeded species, an increase in seed mass did not necessarily correlate with an increase in germination rates. However, there was a significant relationship between seed mass and germination rates for 20% salinity ($r^2 = 0.77$, $F = 32.43$, $P<0.001$), and 30% salinity ($r^2 = 0.85$, $F = 55.89$, $P<0.001$), indicating that 77% and 85% respectively of the increase in germination rates could be explained by an increase in seed mass at these higher salinity levels during the 28–day period.

DISCUSSION

This study provides evidence that germination in *Frankenia* species varies in relation to salt tolerance and seed mass. Some species did not germinate well at any salinity level (e.g. *F. pauciflora* (WA)). In contrast, several species had high germination rates and success for salinity levels ranging from 10% – 30% (EC: 15.45 dS/m – 38.87 dS/m) (e.g. *F. setosa, F. interioris*), and others showed delayed germination until seeds were in contact with the saline water for up to eight days (e.g. *F. cinerea, F. laxiflora*). This study also found an effect of seed mass on germination rate in relation to salinity levels: larger-seeded species had higher germination rates and germination success in general, but notably at higher salinity levels. No seeds of *Frankenia* species were adversely affected by exposure to high salinity levels pre-germination.

Germination rates and germination success decreased as salinity levels increased (as expected), although there was statistically little difference in germination success between 0% and 10% salinity for most species. The notable exception was *F. pauciflora* (WA). We have previously documented this species as having poor germination success (see Chapter 4), and suggested that temperatures less than 17°C may be necessary for optimal germination activity. The constant temperature of 20°C applied in this study may not be conducive for successful germination in *F. pauciflora* (WA). Temperature has been reported to modulate seed germination under saline conditions (Khan & Gulzar 2003).

Frankenia seeds remained viable after exposure to high salinities. In this study, the overall germination percentage (the sum of all germination in all salinities) was greater than 40% for all species except *F. pauciflora* (WA), and there was a high percentage of germination recovery when ungerminated seeds were transferred to distilled water (>50% per species, and up to 88% in *F. interioris*). Specifically, seeds germinated quickly when salinity

stress was removed. Exposure to high salinity is documented to prime seeds in many salt tolerant species before germination (Ungar 1996) and may be occurring here.

Halophytes have developed strategies to delay germination until conditions are suitable for seedling establishment. Some species restrict germination at salinities less favourable for seedling establishment, but do not prevent germination. Alternately, halophytes can inhibit all germination at salinities beyond the tolerance of the species (Ungar 1978). It appears that in most Australian *Frankenia* species, germination is restricted by salinity although the germination rates are species specific and vary with salinity levels. Only *F. pauciflora* (WA) appears to have some mechanism to inhibit germination when subjected to salinity. A study by Brightmore (1979) on the European species *F. laevis* (the only other published account on the effects of varying salinity levels on germination in *Frankenia*) demonstrated 75% germination at 0% salinity, 30% germination at 20% salinity, and 15% germination at 25% salinity, but only 1% at greater than 33% salinity. We also noted 75% germination or greater at 0% salinity (i.e. no salinity stress) in all species except *F. pauciflora* (WA) (69%), and peaked at 97% for *F. interioris*. At 30% salinity, most species had greater than 5% germination (except *F. pauciflora* (WA) 2.8%, *F. foliosa* 3.3%, *F. sessilis* 4.4%), and three species had greater than 25% germination (*F. interioris* 25%, *F. serpyllifolia* 27%, *F. setosa* 53%).

Also noteworthy are the slow germination rates of *F. foliosa, F. pauciflora* (SA), *F. pauciflora* (WA), *F. sessilis* and *F. tetrapetala* at the higher salinity levels. These are all coastal species (often found in saltmarshes) except for *F. foliosa* (found around the edges of inland salt lakes) and are thus all subjected to highly saline conditions (note that seawater has an EC of approximately 55 dS/m). Slow germination rates may be linked to seed dispersal. It is suggested that the main mode of seed dispersal in *Frankenia* is by water, and in the case of the above mentioned species, seawater (Brightmore 1979). Seeds of these species would need to remain dormant while dispersal occurs. Presumably, the plesiomorphic *Frankenia* were smaller-seeded coastal species, which were globally dispersed by water – specifically, across oceans (see Summerhayes 1930; Brightmore 1979). Their seeds would have needed mechanisms to prevent them from germinating in saline water and to remain viable when subjected to high salinity over long periods of time. *Frankenia pauciflora* (WA) is considered the ancestral Australian *Frankenia* species (Summerhayes 1930). The poor germination in this species and the implied narrower window for germination success may be an ancestral characteristic.

Effect of seed mass

Our study supports the hypothesis that germination is correlated with seed mass under saline (or physiologically induced drought) conditions. The largest seeded *Frankenia* species *F. setosa*, *F. serpyllifolia*, *F. cordata*, and *F. interioris* had the highest germination rates and the highest germination success, which was most pronounced at the higher salinity levels (greater than 25% germination at 30% salinity). This would be beneficial if rainfall events were small or of short duration, and dilution of salinity levels of the soil-water would be minimal. However, this bet-hedging strategy assumes that seedlings will be able to establish quickly. Rapid soil-water loss through evaporation and infiltration, and the equally rapid return to high salinity levels would follow minor rainfall events (in maybe just hours). Any germinating seeds could soon dehydrate and die.

However, the smaller-seeded *Frankenia* species (e.g. *F. fecunda, F. foliosa, F. laxiflora*) are successful in inland arid regions and have not been outcompeted by the larger-seeded species. They have slow germination rates and low germination success at 20% and 30% salinity. *Frankenia foliosa* has greatly reduced germination even at 10% salinity. A prerequisite for low soil-water salinity to induce germination would ensure that minor rainfall events would not trigger germination, and the problems encountered by rapidly establishing seedlings in rapidly drying environments would be moderated.

Seedling survival may depend on the concentration of salinity levels across the soil profile (Gul *et al*. 2001). Salt concentrations in the soil surface layers around clay pans, gilgais, and dry water courses are often considerably higher than the concentration in the sub-surface soil layers. Thus, while soil surface layers may be encrusted with salt, the rooting zones are less saline (Badman 1999; Gul *et al.* 2001). Larger-seeded *Frankenia* species are commonly found near clay pans, gilgais and dry water channels (see Chapter 5). The rapid germination rates associated with these larger-seeded species would enable developing seedlings to establish quickly into the less saline sub-surface layers. Higher salinity in the crustal layers at the germination stage would not necessarily signify adverse conditions for seedling establishment. *Frankenia* species found around salt lakes, saltmarshes and salt-pans are generally smaller-seeded species (see Chapter 5). Evaporation and soil-water infiltration results in a return to concentrated salt crusts or depressions of the extremely highly saline water. The sub-surface layers would continue to be highly saline. Slow germination rates and low tolerance to salinity at the germination stage would favour these species. Only significant rainfall events would dilute soil-water salinity levels for the period of time needed for plants to establish beyond the early seedling stage to a more salt tolerant stage.

Conclusion

Arid, drought prone areas are often claimed to favour large-seededness. Here we show that even within arid zones, micro-habitats may be important in determining optimal seed mass. Variation in salt tolerance for germination may be important in explaining small-scale variation in vegetation patterns among *Frankenia* species within close geographical proximity. The results of this study provide indirect evidence that selection in some Australian *Frankenia* species to develop larger seeds (a result of the reduction in ovule number) has facilitated their establishment and reproduction under conditions that were less favourable for smaller-seeded *Frankenia* species.

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Species	Reference number	Location	GPS co-ordinates	Mean seed mass (±se) µgms
F. cinerea	LE03038	Shark Bay, WA	S26°10'10" E113°40'55"	NR
	LE03037a	Shark Bay, WA	S26°01'18" E113°35'07"	61 (±2)
	LE03057	Lake King, WA	S35°05'24" E119°36'37"	95 (±3)
F. cordata	LE05006	Ormiston Gorge, NT	S23°40'45" E132°42'42"	391 (±18)
	LE05007	Rainbow Valley, NT	S24°19'57" E133°37'53"	295 (±16)
	LE05011	Curtin Springs, NT	S25°21'01" E131°50'47"	375 (±9)
F. fecunda	LE03013	Leonora, WA	S29°01'59" E121°29'13"	52 (±25)
	LE03018	Lake Miranda, WA	S27°41'58" E120°32'32"	48 (±2)
	LE03023	Lake Austin, WA	S27°36'07" E117°53'3 1"	82 (±2)
F. foliosa	LE01004	West Finnis Springs, SA	S29°30'05' E137°24'29"	115 (±4)
	LE01037	Birdsville Track, SA	S29°20'13" E139°19'32"	81 (±4)
	LE04001	Strzelecki Track	S29°33'27" E139°25'08"	43 (±1)
F. interioris	LE03007	Coolgardie, WA	S30°58'20" E121°03'11"	343 (±9)
	LE03012	Lake Goongarrie, WA	S30°00'56" E121°09'46"	320 (±85)
	LE03065	Mundrabilla, WA	S31°54'37" E127°21'26"	286 (±62)
F. laxiflora	LE03014	Leonora, WA	S28°16'52" E 121°07'26"	86 (±4)
	LE03031	Mt Narryer, WA	S28°42'13" E 115°53'24"	105 (±4)
	LE03047	Hines Hill, WA	S31°34'55" E117°58'37"	72 (±1)
F. pauciflora (SA varieties)	LE04021	Coorong, SA	S36°03'20" E139°35'21"	106 (±7)
	LE05025	Pt Rickaby, SA	S34°40'50" E 137°29'37"	145 (±7)
	LE05026	Pt Gawler, SA	S34°38'35" E138°26'22"	128 (±8)
F. pauciflora (WA varieties)	LE03005	Kambalda, WA	S31°13'20" E 121°30'01"	57 (±2)
	LE03037b	Shark Bay, WA	S35°05'24" E 119°36'37"	59 (±5)
	LE03039	Leeman, WA	S29°41'23" E114°32'36"	59 (±2)
F. serpyllifolia	LE01038	Birdsville Track, SA	S29°12'38" E138°23'58"	452 (±9)
	LE02007	Bungadillina Creek, SA	S28°16'33" E 135°50'16"	724 (±21)
	LE02013	Mt Barry Station, SA	S28°13'40" E 134°48'12"	537 (±16)
F. sessilis	LE03001	Cactus Beach,	S32°03'56" E132°59'37"	239 (±9)
	LE03066	SA Head of Bight, SA	S31°28'28" E131°05'41"	194 (±25)
	LE01007	Eucla, WA	S31°42'49" E 128°53'06"	138 (±3)
F. setosa	LE01041	Carnarvon Range, WA	S25°08'44" E 120°14'20"	688 (±22)
	LE03035	Gascoyne Junction, WA	S24°51'18" E 115°18'20"	1214 (±34)
	LE03040	Kalbarri, WA	S27°45'23" E 114°08'21"	832 (±28)
F. tetrapetala	LE03056	Lake Newton, WA	S32°57'39" E119°36'33"	135 (±3)
	LE03058	Newdegate, WA	S33°11'29" E 119°12'49"	135 (±3)
	LE03061	Grasspatch, WA	S33°25'09" E 121°42"32"	185 (±11)

Table 1. Species included in experiments, site reference numbers, location, and GPS co-ordinates for each population, and mean weights (with standard errors) of seeds per population, in micrograms. 'NR' denotes data not recorded. 'SA' denotes South Australia. 'WA' denotes Western Australia. 'NT denotes Northern Territory.

Salinity level	Day 2	Day 4	Day 6	Day 8
0%	5.667 ***	10.88 ***	6.38 ***	3.48 ***
10%	1.32 (NS)	7.33 ***	4.95 ***	4.49 ***
20%	0.96 (NS)	1.45 (NS)	3.71 ***	3.03 ***
30%	0.00	2.84 ***	4.46 ***	1.57 (NS)

Table 2. Results of ANOVAs calculated for differences in germination rates between *Frankenia* species at 0%, 10%, 20%, and 30% salinity levels, for Day 2, Day 4, Day 6, and Day 8. 'NS' denotes no significant difference. '***' denotes $P<0.001$. For all calculations d.f. = 108.

	0% salinity		10% salinity		20% salinity		30% salinity		All salinities	
	% germinate	% recover	% germinate	% recover	% germinate	% recover	% germinate	% recover	% germinate	% recover
F. cinerea	91.7	0.0	83.3	26.7	28.9	89.1	8.3	80.0	53.1	75.1
F. cordata	90.0	0.0	83.3	20.0	23.9	74.5	14.4	74.7	52.9	65.8
F. fecunda	78.3	10.3	61.7	46.4	14.4	59.7	7.2	76.1	40.4	59.4
F. foliosa	92.2	7.1	73.3	47.9	20.6	87.4	3.3	82.8	47.4	77.3
F. interioris	96.7	0.0	91.7	60.0	36.7	90.4	25.0	92.6	62.5	87.8
F. laxiflora	75.0	0.0	73.9	14.9	28.3	62.8	7.8	66.9	46.3	51.4
***F. pauciflora* (SA)**	93.9	45.5	69.4	65.5	27.8	83.8	7.8	85.5	49.7	80.7
***F. pauciflora* (WA)**	68.9	0.0	47.8	13.8	12.2	48.7	2.8	48.0	32.9	36.0
F. serpyllifolia	94.4	0.0	88.9	35.0	48.3	83.9	26.7	84.1	64.6	76.9
F. sessilis	92.2	14.3	63.9	64.6	16.1	94.7	4.4	88.4	44.2	84.3
F. setosa	86.7	0.0	85.6	7.7	76.1	53.5	53.3	63.1	75.4	44.1
F. tetrapetala	86.1	8.0	67.8	65.5	25.0	88.9	10.0	82.1	47.2	77.1

Table 3. Germination percentages at solution salinities of 0%, 10%, 20% and 30%, and percentage germination after transferring ungerminated seeds into distilled water for a further 14 days.

	df	df denominator	Mean Squares	F-value	Significance level	Partial Eta Squared
GERMINATION RATES						
Day 2						
Seed category	1	10	94.71	0.365		0.322
Species(seed category)	10	30	257.67	6.093		0.035
Salinity	3	30	272.25	6.438	***	0.670
Seed category*salinity	3	30	10.74	0.254	**	0.392
Salinity*species(seed category)	10	30	257.67	6.093	***	0.670
Day 4						
Seed category	1	10	3800.41	8.521	*	0.460
Species(seed category)	10	30	446.00	5.352	***	0.641
Salinity	3	30	8206.16	98.48	***	0.908
Seed category*salinity	3	30	138.79	1.665		0.143
Salinity*species(seed category)	10	30	446.00	5.352	***	0.641
Day 6						
Seed category	1	10	718.29	4.60	***	0.973
Species(seed category)	10	30	156.02	1.002		0.315
Salinity	3	30	6236.92	40.065		0.250
Seed category*salinity	3	30	402.47	2.585	***	0.800
Salinity*species(seed category)	10	30	156.02	1.002		0.250
Day 8						
Seed category	1	10	36.80	0.362		0.035
Species(seed category)	10	30	101.69	0.866		0.224
Salinity	3	30	2023.26	17.229	***	0.633
Seed category*salinity	3	30	557.45	4.747	**	0.322
Salinity*species(seed category)	10	30	101.70	0.866		0.224
GERMINATION SUCCESS						
Day 8						
Seed category	1	10	4842.19	5.844	*	0.369
Species(seed category)	10	30	828.60	3.052	**	0.504
Salinity	3	30	47516.23	175.00	***	0.946
Seed category*salinity	3	30	157.134	0.579		0.055
Salinity*species(seed category)	10	30	271.56	3.052	**	0.504
Day 28						
Seed category	1	10	5941.84	5.150	*	0.340
Species(seed category)	10	30	1153.66	4.696	***	0.610
Salinity	3	30	43724.77	177.987	***	0.947
Seed category*salinity	3	30	137.90	0.561		0.053
Salinity*species(seed category)	10	30	1153.66	4.700	***	0.610

Table 4. Summary of the interaction effects of salinity, seed mass category and species on germination rates, calculated using Nested Design ANOVAs, at Day 2, Day 4, Day 6 and Day 8, and for germination success at Day 8 and Day 28. (*) indicates a significance level of $P<0.05$, (**) indicates a significance level of $P<0.01$, and (***) indicates a significance level of $P<0.001$.

Salinity	Day	Effect	df	df denominator	Mean squares	F-value	Significance level	Partial Eta Squared
0%								
Rates	2	Seed category	1	10	34.20	0.182	*	0.454
		Species(seed category)	10	24	187.51	1.808		0.018
	4	Seed category	1	10	948.33	3.599		0.265
		Species(seed category)	10	24	263.52	1.204		0.334
	6	Seed category	1	10	125.89	0.512		0.049
		Species(seed category)	10	24	245.82	1.893		0.441
	8	Seed category	1	10	498.33	6.669	*	0.400
		Species(seed category)	10	24	74.72	0.691		0.224
Success	28	Seed category	1	10	531.53	2.717		0.214
		Species(seed category)	10	24	195.60	0.868		0.266
10%								
Rates	2	Seed category	1	10	44.44	0.452		0.043
		Species(seed category)	10	24	98.27	3.58	**	0.599
	4	Seed category	1	10	1378.64	4.663		0.318
		Species(seed category)	10	24	295.65	1.848		0.435
	6	Seed category	1	10	47.518	0.399		0.038
		Species(seed category)	10	24	119.024	1.272		0.346
	8	Seed category	1	10	43.25	0.508		0.048
		Species(seed category)	10	24	85.10	1.334		0.357
Success	28	Seed category	1	10	1283.91	3.024		0.232
		Species(seed category)	10	24	424.51	0.861		0.264
20%								
Rates	2	Seed category	1	10	48.28	0.489		0.047
		Species(seed category)	10	24	98.74	12.425	***	0.838
	4	Seed category	1	10	1684.56	15.221	**	0.604
		Species(seed category)	10	24	110.67	1.931		0.446
	6	Seed category	1	10	1229.55	7.765	*	0.437
		Species(seed category)	10	24	158.34	1.953		0.449
	8	Seed category	1	10	60.114	0.465		0.511
		Species(seed category)	10	24	129.33	1.300		0.351
Success	28	Seed category	1	10	2203.83	2.738		0.215
		Species(seed category)	10	24	805.02	3.689	**	0.606
30%								
Rates	2	Seed category	1	10	0.00			
		Species(seed category)	10	24	0.00			
	4	Seed category	1	10	205.25	7.851	*	0.440
		Species(seed category)	10	24	26.15	0.743		0.236
	6	Seed category	1	10	522.73	5.235	*	0.344
		Species(seed category)	10	24	99.86	1.680		0.412
	8	Seed category	1	10	1107.48	6.718	*	0.402
		Species(seed category)	10	24	164.85	4.659	**	0.660
Success	28	Seed category	1	10	2336.27	5.019	*	0.334
		Species(seed category)	10	24	465.53	8.670	***	0.783

Table 5. Summary of the interaction effect of seed mass and species on germination rates, calculated using Nested Design ANOVAs, at Day 2, Day 4, Day 6 and Day 8, and for germination success at Day 28, at 0%, 10%, 20%, and 30% salinity. (*) indicates a significance level of $P<0.05$, (**) indicates a significance level of $P<0.01$, and (***) indicates a significance level of $P<0.001$

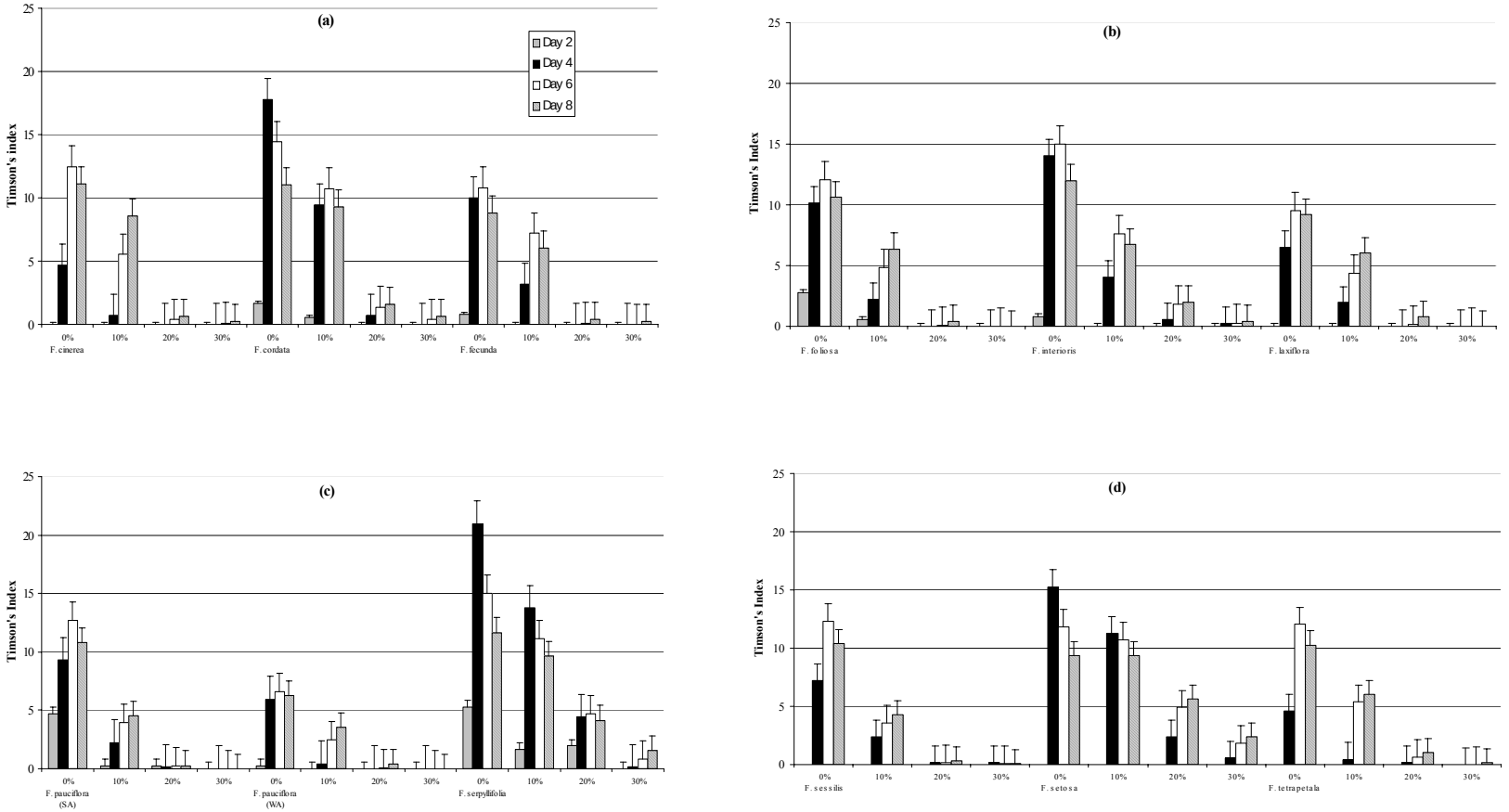

Figure 1. Germination rates, calculated using a modified Timson Index at Days 2, Day 4, Day 6, and Day 8, at salinity levels of 0%, 10%, 20%, and 30%. (a) comprises *F. cinerea, F. cordata* and *F. fecunda*. (b) comprises *F. foliosa, F. interioris* and *F. laxiflora.* (c) comprises *F. pauciflora* (SA), *F. pauciflora* (WA) and *F. serpyllifolia.* (d) comprises *F. sessilis, F. setosa* and *F. tetrapetala.*

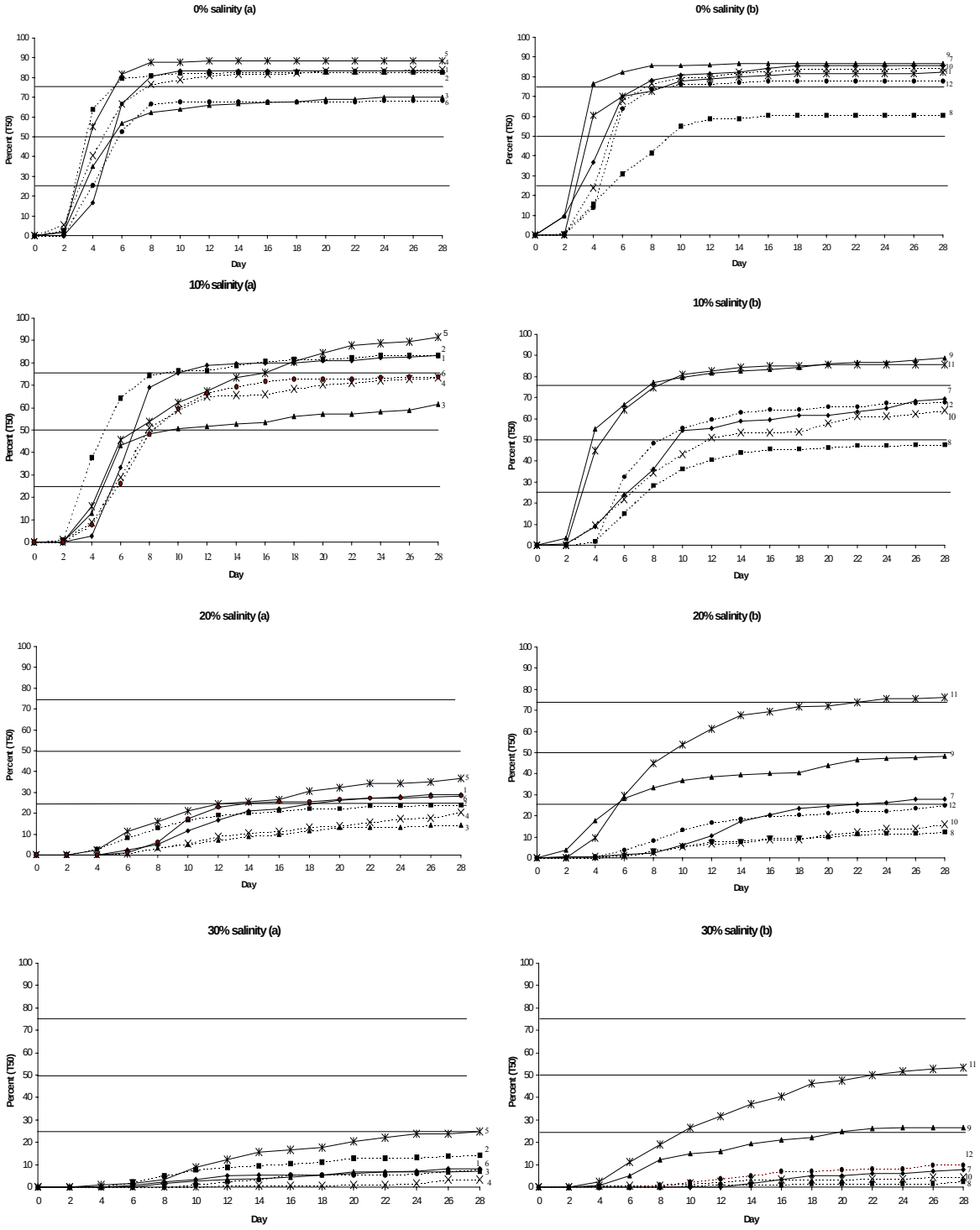

Figure 2. Germination success of each species for each salinity level (0%, 10%, 20%, 30%) over 28 days. Species are designated numbers as follows: (1) *F. cinerea*, (2) *F. cordata*, (3) *F. fecunda*, (4) *F. foliosa*, (5) *F. interioris*, (6) *F. laxiflora*, (7) *F. pauciflora* (SA), (8) *F. pauciflora* (WA), (9) *F. serpyllifolia*, (10) *F. sessilis*, (11) *F. setosa*, and (12) *F. tetrapetala.*