

Determination of Response of Rare and Poorly  
Known Western Australian Native Species to  
Salinity and Waterlogging  
Project 023191



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### Executive summary

Six threatened understorey species endemic to southern Western Australia (*Acacia trulliformis*, *Austrostipa geoffreyi*, *Banksia oligantha*, *Dryandra mucronulata* subsp. *retrorsa*, *Hakea tuberculata* and *Orthrosanthus muelleri*) were screened for their tolerance to sodium chloride (NaCl) during seed germination and their tolerance to the single and combined effects of salt and waterlogging as seedlings.

Water uptake under elevated saline conditions was significantly slower for *Acacia*, *Banksia*, *Dryandra* and *Orthrosanthus* but was not restrictive to germination. Seeds of all species except *Banksia* and *Austrostipa* recovered well from saline pre-treatment (200 and 400 mM NaCl for 5, 15 or 30 days) when transferred to non-saline conditions, suggesting seed resilience to temporary hyper-saline conditions. Germination of *Banksia*, *Dryandra* and *Hakea* seeds under constant saline conditions (100, 150 and 200 mM NaCl) was not significantly different from a control, although under highly saline conditions (400 mM NaCl) germination was significantly reduced. No germination occurred at the highest salinity in *Austrostipa*, *Banksia* or *Orthrosanthus*. Temperature, provenance, salinity and their interaction significantly affected germination in two seedlots of *Acacia* that were tested under different temperature regimes. In all species increasing the concentration of NaCl increased the rate of germination.

Treatment with hyper-saline water had a significant effect on seedling survival for most species, although mortality varied greatly between treatments and between species. Although plants of some species survived under saline and / or waterlogged conditions for up to 12 weeks, their growth was generally impeded compared to the controls. All species except *Banksia* and *Dryandra* had 100% survival under fresh waterlogged conditions after 12 weeks. *Austrostipa* exhibited a low salinity tolerance during germination but exceptional tolerance to growth under both saline and/or waterlogged conditions.

This investigation has identified a number of threatened understorey species that exhibit salt tolerance during germination and / or tolerance to salt and waterlogging stress as seedlings. Field experiments would need to test the validity of these results before their use in revegetation of secondary salinised landscapes. Seeds of these species have been conserved *ex situ*.

## **Introduction**

In Western Australia an abundance of salt in the soil profile and clearing of native vegetation has given rise to a fragmented landscape that has resulted in extensive salinisation and hydrological change. Dryland salinity is considered a major environmental problem (Lambers 2003) with loss of biodiversity one of the key problems caused by salinity and associated waterlogging. In the agricultural wheat growing zone of Western Australia alone 1500 of the 4000 recorded plant species occur in a landscape position where the risk of salinity is high. Some 450 of these are endemic and at risk of extinction (Keighery *et al.* 2000).

Seed germination is a major limiting factor for the establishment of plants under saline conditions (Bewley and Black 1994). Both halophyte and non-halophyte germination is generally highest under fresh water conditions and an increase in salinity can lead to a reduction in final germination percentage as well as germination rate (Rozema 1975; Ungar 1978; Ungar 1995; Houle *et al.* 2001; Baskin and Baskin 2001; Khan and Gulzar 2003). Salt can effect the germination of seed by decreasing the ability of seed to imbibe water (osmotic) and/or by facilitating the uptake of ions in amounts that may be toxic (ionic) (Ayers 1952; Marcar 1994). Germination may also be influenced by the types of salts involved (Ryan *et al.* 1975) and dormancy may be retained until rainfall dilutes osmotic potentials to levels that permit imbibition (Bell *et al.* 1993).

Increased salinity can also reduce plant growth and survival through osmotic effects, water deficit, toxic effects of ions and imbalance of uptake of essential nutrients (Ashraf and Harris 2004). Increases in salinity can reduce root elongation and production, as well as reducing shoot production and growth.

Tolerance to salinity varies with the developmental stage of plants (Ayers 1952; Ladiges *et al.* 1981; Mayer and Poljakoff-Mayber 1989; van der Moezel and Bell 1987b; Myers and Couper 1989; Pearce-Pinto *et al.* 1990; Zedler *et al.* 1990; Bewley and Black 1994; Niknam and McComb 2000) and may effect seed, seedlings, juvenile and adult plants to varying degrees.

To make matters worse, secondary salinisation in Western Australia is associated with rising groundwater levels and discharge of saline groundwater, therefore most saline land is also waterlogged (McFarlane and Williamson 2002). Waterlogging is defined as the presence of excess water in the root zone of plants (Moore and McFarlane 1998) which leads to anaerobic conditions and poor gas exchange. The effect of waterlogging on plant growth depends on the rate of oxygen depletion, accumulation of toxins, the proportion of the root zone affected, limitations on root elongation and the availability and uptake of nutrients (McFarlane and Williamson 2002). Plants vary in their ability to survive periods of anaerobic soil conditions. Oxygen deficiency in the soil has a direct effect on plant functions such as respiration and root growth.

The combined effect of waterlogging and salinity may have a greater effect than the effect of each factor alone (Barrett-Lennard 1986; van der Moezel *et al.* 1988; van der Moezel *et al.* 1991; Craig *et al.* 1990; Barrett-Lennard 2002; Marcar 2002; Munns 2002). It is important to determine response to both salinity and waterlogging as

seasonal and periodic waterlogging is often a major limitation to plant survival and growth. A high degree of tolerance to both waterlogging and salinity is therefore required for the successful rehabilitation of secondary salinised land in Australia and an understanding of tolerance at the different life cycle stages of a plant is important for successful revegetation (Bell 1999).

Currently there is little information available on the response of native understorey or threatened species to elevated salt or waterlogged conditions. Previous laboratory and glasshouse screening trials have concentrated on identifying Australian native tree or shrub species such as *Eucalyptus* (van der Moezel and Bell 1987a and 1987b; van der Moezel *et al.* 1988; Pearce-Pinto *et al.* 1990; van der Moezel *et al.* 1991), *Melaleuca* (van der Moezel and Bell 1987a and 1987b; van der Moezel *et al.* 1991), *Acacia* (Craig *et al.* 1990; Rehman *et al.* 1996) and *Casuarina* (El-Lakany and Luard 1982; van der Moezel *et al.* 1988) that exhibit tolerance to salinity and/or waterlogging either at the seed or seedling stage. A range of potential native and non-native salt-tolerant forage crops have also been identified (reviewed by Rogers *et al.* 2005).

This project investigated the impact of salinity and waterlogging on the germination and seedling growth of a number of rare and poorly known understorey plant species from southern Western Australia. The capacity of seeds to maintain viability for extended periods of time during exposure to hyper-saline conditions then initiate germination when salinity stress is reduced was also investigated. The main objective of the project was to provide a better basis for evaluating species at risk from hydrologic change and to assist in the identification of salt and inundation tolerant species prior to field evaluation for revegetation. Excess seed material has been stored for long-term conservation as an insurance policy against extinction in the wild.

## **Materials and Methods**

### *Species selection and seed collection*

Species for screening were selected from a list of threatened endemic vascular plants at risk of extinction in southern Western Australia (G. Keighery pers. comm. 2000). These included *Acacia trulliformis* R. S. Cowan & Maslin, *Austrostipa geoffreyi* S. W. L. Jacobs & J. Everett, *Banksia oligantha* A. S. George, *Dryandra mucronulata* A. S. George, *Hakea tuberculata* R. Br., *Orthrosanthus Muelleri* Benth. and *Myoporum turbinatum* Chinnock (used in seedling trials only) (Table 1). Screening for salt tolerance was carried out by adding NaCl to increase salt levels. NaCl dominates over other salts in the root zone of salt affected plants in Australia and was considered valid to use on its own in this project (Noble and Rogers 1994).

Mature fruits were collected between November 2003 and January 2004 from natural populations to ensure genetic representation of each species. *Acacia* legumes were collected from two sites (CO and SM) to investigate provenance variation during germination. Insufficient follicles were collected from a second provenance of *Dryandra* for adequate provenance screening. Seed of *Astartea* sp. Jerrdaccuttup was also collected for screening but subsequently proved too difficult to germinate for screening for the

current salinity trials. Vegetatively propagated material of *Myoporum* was obtained for pot trials to assess salinity and waterlogging in seedlings. Seeds were separated from their fruits and cleaned before storing dry at room temperature for between one and two months prior to germination. Excess seed material from each species was stored for long-term conservation at the Department of Conservation and Land Management's Threatened Flora Seed Centre, to be used in future restoration projects, if appropriate. None of these species with the possible exception of *Austrostipa geoffreyi* and *Myoporum turbinatum* have been previously described as halophytic.

**Table 1. Western Australian endemic species selected for salt and waterlogging tolerance investigation, including family, geographic distribution, habitat, rainfall (mm), fruit type and seed mass (gm).**

Species	Family	Geographic Distribution (South West Land Division)	Habitat	Rainfall (mm) based on long term average	Fruit type	Seed mass (gm) n=30
<i>Acacia trulliformis</i> R. S. Cowan & Maslin	Mimosaceae	Ongerup	Depressions	<400 mm	Legume	0.0080
<i>Austrostipa geoffreyi</i> S. W. L. Jacobs & J. Everett	Poaceae	Lake King	Salt lake margins	<400 mm	Caryopsis	0.0024
<i>Banksia oligantha</i> A. S. George	Proteaceae	Wagin to Narrogin	Sandy flats	<600 mm	Follicle	0.0132
<i>Dryandra mucronulata</i> A. S. George	Proteaceae	Cranbrook to Broomehill	Rocky ridges	<600 mm	Follicle	0.0112
<i>Hakea tuberculata</i> R. Br.	Proteaceae	Augusta to Cape Arid	Low winter-wet	<1000 mm	Follicle	0.0088
<i>Myoporum turbinatum</i> Chinnock	Myoporaceae	East of Esperance	Salt lake margins	<600 mm	Nut	0.0071
<i>Orthrosanthus Muelleri</i> Benth.	Iridaceae	Tenterden to Ongerup	Low winter-wet	<400 mm	Capsule	0.0008

## Experimental Design

### *Experiment 1. Seed germination and salt tolerance*

This experiment investigated the ability of seeds to tolerate constant saline conditions during germination.

Seeds were incubated in 9cm plastic Petri dishes on filter paper (Whatman #2) over synthetic sponge moistened with 15 ml of sodium chloride (NaCl) in increasingly saline environments (0, 100, 150, 200 and 400 mM NaCl) and incubated in a germination cabinet under a 12 h/12 h photoperiod at a constant 15°C. A further 100 seeds of *Acacia* from each seedlot were incubated at 25°C at 0, 100, 200 and 400 mM NaCl to investigate temperature response to germination under constant saline conditions.

### *Experiment 2: Imbibition and recovery investigation*

This experiment investigated the ability of seeds to imbibe under hyper-saline conditions and the capacity of seeds for recovery and maintenance of viability for extended periods

of time during exposure to hyper-saline conditions (pre-incubation phase) then initiate germination when salinity stress is reduced (incubation phase).

Seeds were pre-incubated in 9cm plastic Petri dishes on filter paper (Whatman #2) over synthetic sponge moistened with 15 ml of sodium chloride (NaCl) at increasingly concentrated solutions (0, 200 and 400 mM) for 5, 15 and 30 days prior to rinsing with distilled water and placing on filter paper over sponge watered with 15 ml distilled water. Seeds were incubated in a germination cabinet in the laboratory under a 12 h/12 h photoperiod at a constant 15°C. For the first 12 hours of pre-incubation, seeds were weighed at 2 hourly intervals, then at 24, 36, 48, 72 and 120 hours. The fresh weight of the seeds was recorded before the experiment.

*Acacia* seeds have a typically hard water-impermeable seed coat which requires pre-treatment to stimulate germination. To facilitate germination a small portion of the seed coat was mechanically scarified by removing 2 mm of the testa with a scalpel. The enclosing glumes are an important mechanism causing dormancy in many grasses so that prior to incubation the glumes and awns of *Austrostipa* were removed to enable the caryopsis to germinate. No other species was given pre-treatment to stimulate germination.

In both Experiment 1 and Experiment 2 four replicates of 25 seed were used in each treatment in a randomised complete block design. Petri dishes were placed in sealed plastic containers to prevent evaporation of water and thus increases in concentration of salts in the dishes. Germination was scored twice weekly until all seedlings had germinated or died, the latter assessed by a squash test. Germination was determined as radicle emergence. Germinated seedlings were removed from the dishes at each scoring period and grown on under nursery conditions to be used in Experiment 3 (see below). Seedlings were potted into sterilised commercial potting mix (1 peat: 1 loam: 1 coarse river sand) in 5 cm x 5 cm x 15 cm black plastic pots, and later transferred to square 9 cm x 9 cm x 19 cm or 7 cm x 7 cm x 16 cm (control treatment) pots for inclusion in Experiment 3. Round 17 cm x 17 cm pots were used for the saline drained treatment delivering saline solution through drippers for ease of handling. In this treatment four plants were placed in each pot. A low phosphate mix was used for the three proteaceous species (*Banksia*, *Hakea* and *Dryandra*). Slow release fertiliser (Osmocote for native plants) was added to all pots after one month.

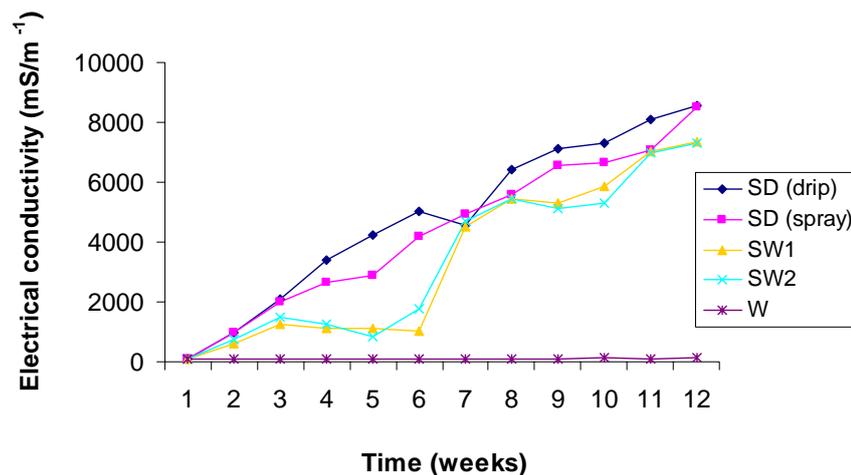
### *Experiment 3. Seedling growth and survival*

This pot experiment investigated the single and combined effects of salt and waterlogging on seedling survival and growth.

Six treatments (non-saline drained control [C], saline drained [SD-spray] and [SD-drip], non-saline waterlogged [W] and saline waterlogged – full [SW1] and half [SW2]) were applied to 6-month old seedlings of each species for 12 weeks under glasshouse conditions. The seedlings were sourced from seedlings germinated during Experiments 1 and 2 of this study. Twenty seedlings per species were used for each experimental treatment, except for *Orthrosanthus*. Insufficient plants of this species were produced

due to low germination. Six plants were available for the control and five in all other treatments. Each plant was treated as a replicate in a randomised block design with a single plant in each pot. Pots were placed in plastic tanks (1 m x 1 m x 0.29 m) (Photo1). Each tank held 125 pots (126 pots in control) containing an individual plant, except for treatment SD-spray. This tank held five pots containing four plants each (20 plants of five species). Insufficient plants were available of *Myoporum* and *Orthrosanthus* for inclusion in this treatment. Each pot in waterlogging experiments W and SW1 were subjected to fully waterlogged conditions in which water levels were held at the soil surface in pots. 80 litres of solution were added to fully waterlogged treatment (fresh and saline). Pots in SW2 treatment were waterlogged where levels of solution were more than half way up the pot (60 litres of solution). The salt used to obtain the required salinity in this experiment (SD, SW1 and SW2) was NaCl. Tanks were drained weekly and refilled with new solution. NaCl concentrations were increased weekly by 100 mM NaCl from an initial < 10 mM NaCl (scheme water) for 12 weeks in treatments S, SW1 and SW2. Non-saline drained pots (C) were irrigated with scheme water three times daily for 15 minutes. SD-spray treatment received saline solutions via spray irrigation to the base of the plant whereas SD-drip received saline solutions via drippers to the root zone of each plant three times daily. The salinity of each solution was measured weekly (Fig. 1). The glasshouse had natural lighting and daily minimum and maximum temperatures ranged from 10<sup>0</sup>C to 28<sup>0</sup>C in January to 9<sup>0</sup>C and 26<sup>0</sup>C in May.

Glasshouse trials commenced on January 18, 2005 and continued for 12 weeks until April 21, 2005 for all treatments except SD-drip. This treatment commenced 3 March and ran until 30 May 2005.



**Fig. 1.** Electrical conductivity (mS/m<sup>-1</sup>) of treatment solutions measured mid-week. Salt (NaCl) was increased by 100 mM each week to a maximum of 1100 mM.

### *Statistical analysis*

Individual seed weights (mg) were recorded on commencement of the imbibition study and at every weighing period until 120 hours. Total percent germination (TPG) was recorded for all treatments for each species. Mean time to germinate (MTG) was calculated for each treatment for each species, using the equation:

$$\text{MTG} = \sum (n \times d) / N$$

where: n = number of seeds germinated between scoring intervals; d = the incubation period in days at that time point and N = total number of seeds germinated.

Percent germination data from experiments 1 and 2 were arcsin square root transformed prior to Analysis of Variance (ANOVA).

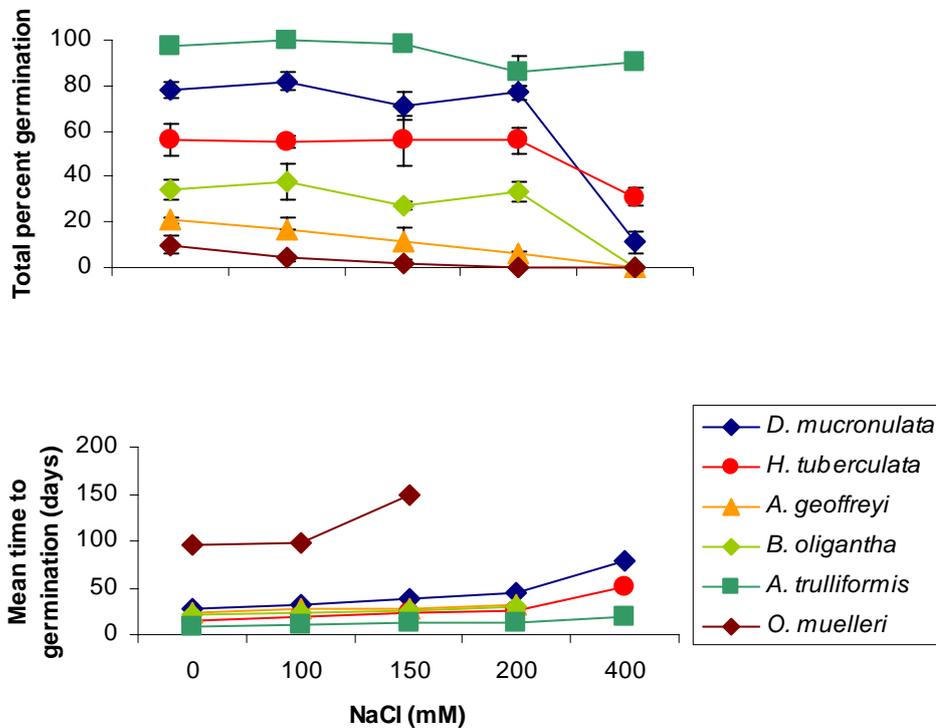
Individual survival data of each plant in experiment 3 were analysed using logistic regression. Data were analysed using the SAS statistical software and procedures GLM and LOGISTIC respectively (SAS Institute Inc, 2003). Plant growth parameters (height and width in two directions) and foliar symptoms of salt and/or waterlogging injury were recorded weekly for 12 weeks from commencement of the experiment (Photo 2). Foliar symptoms included wilting and drying of apical shoot, leaf reddening, necrosis, chlorosis and leaf loss. Height was measured from the base of each seedling to the tip of the apical shoot. To compare growth rates between treatments, an index of above-ground volume was calculated for each plant (height x width x width) at weeks 1 and 12. Average growth rate between weeks 1 and 12 was then determined, and analysed by ANOVA after logarithmic transformation, to render the residuals approximately normal and homoscedastic using standard diagnostic tests (SAS procedure UNIVARIATE; SAS Institute Inc, 2003). Growth rates were also scaled as a percentage relative to the control treatment to facilitate interpretation.

## **Results**

### *Experiment 1. Seed germination and salt tolerance*

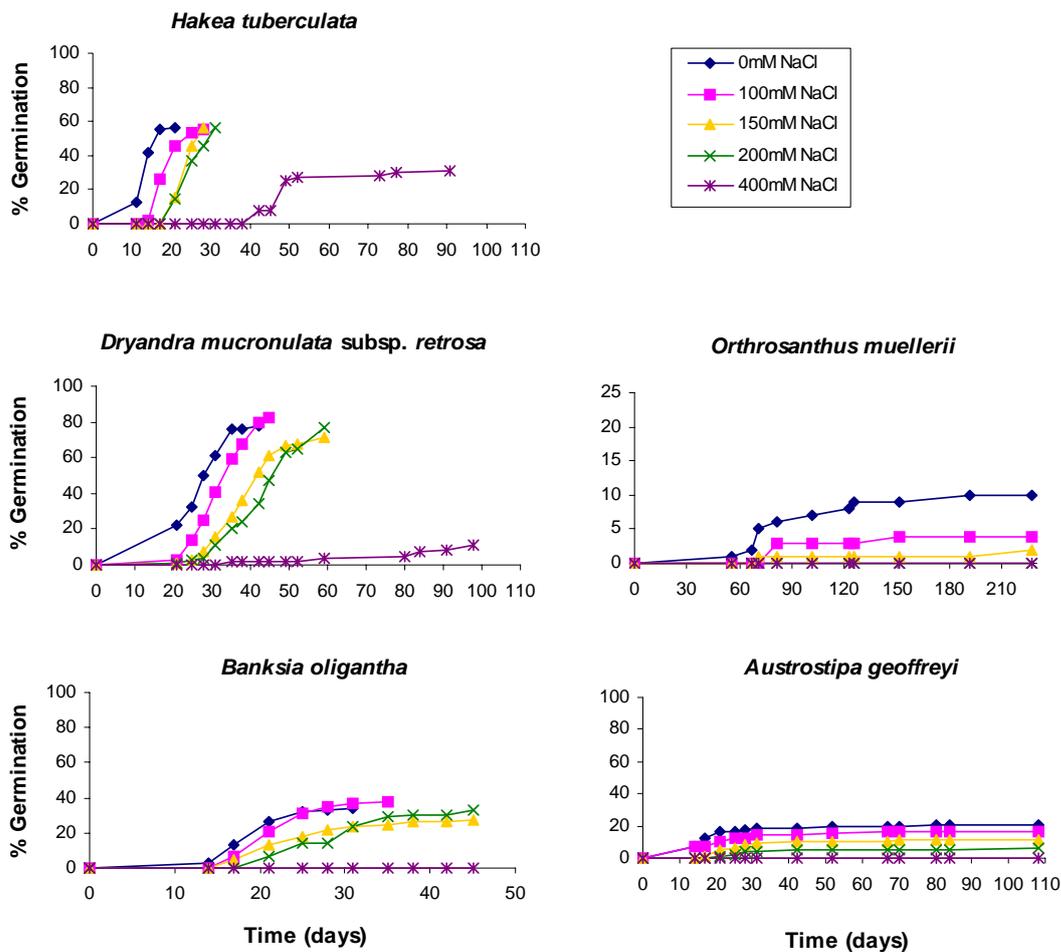
For three species (*Banksia*, *Dryandra* and *Hakea*) germination at 100, 150 and 200 mM NaCl was not significantly different from germination under non-saline conditions, although under highly saline conditions (400 mM NaCl) germination was significantly reduced (Fig. 1). No germination occurred at 400 mM NaCl for *Austrostipa*, *Banksia* and *Orthrosanthus* seeds. *Hakea* germination at 400 mM NaCl was reduced by 24-25% relative to all other treatments and at 400 mM NaCl *Dryandra* experienced a 60% or more reduction in percent germination relative to all other treatments. Seeds of *Orthrosanthus muelleri* had low and sporadic germination under all conditions but the effect of increasing concentrations of NaCl generally reduced percent germination. There was no difference in percent germination in *Austrostipa* between non-saline and low saline (100 mM NaCl) treatment but these results were significantly different to those at 200 or 400 mM NaCl. In all species increasing the concentration of NaCl

increased the rate of germination for seeds incubated under constant saline conditions (Fig. 2 and 3).



**Fig. 2. Total percent germination and mean time to germination in days for seed of six species of native plants from southern Western Australia under increasingly saline conditions (0-400 mM NaCl).**

At 25<sup>0</sup>C increasing salinity progressively inhibited germination of *Acacia* seeds, with increasingly significant differences between treatments and the control. At 15<sup>0</sup>C there were no significant differences in percent germination between 100 or 150 mM NaCl and the control for seedlot SM or between 100 mM NaCl and the control for seedlot CO. At 400 mM NaCl seedlot CO exhibiting a 50% reduction in percent germination over seedlot SM. An increase in incubation temperature substantially increased the mean time to germination for all treatments for both seedlots, by a factor of 2 – 4 times (Figure 3). At 15<sup>0</sup>C mean time to germination was similar for both seedlots for each treatment but overall mean time to germination progressively increased as salinity increased.

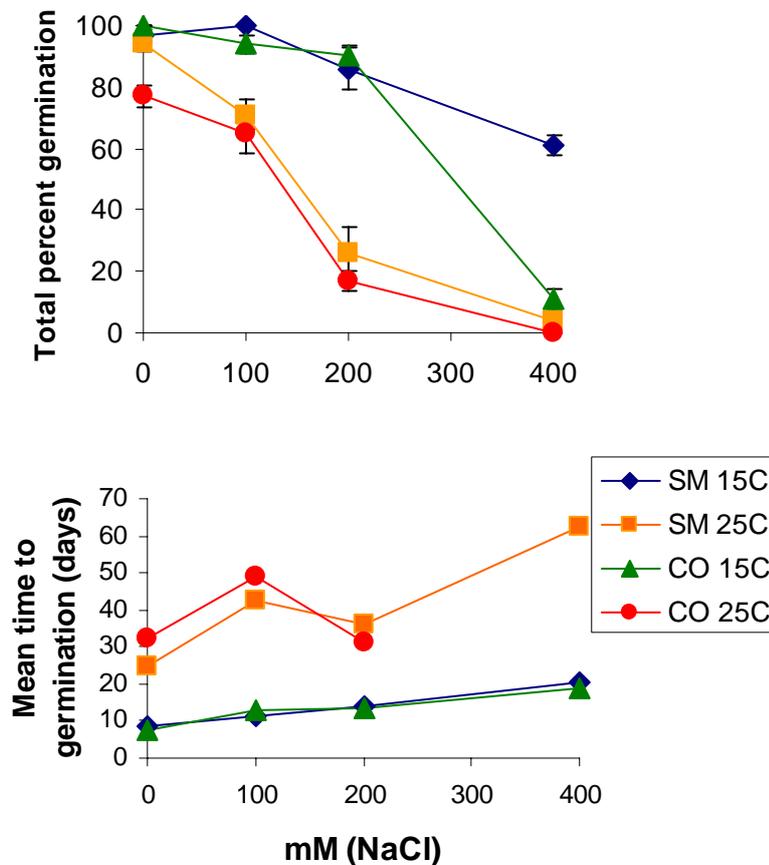


**Fig. 3.** Cumulative percent germination over time in days for five native understorey species from south Western Australia in a range of salinities (0-400 mM NaCl).

Temperature, provenance, salinity and their interaction significantly affected percent germination in *Acacia* (Table 2), but the size of the main effects for each factor is so large that the interactions are of no practical significance and can be discounted (Mead 1988).

**Table 2.** Three way ANOVA testing the effect of temperature, salinity and provenance and the interaction between these variables on percent germination in *Acacia trulliformis*.

Source of variation	Sum of squares	df	Mean square	F	Significance
Temperature	5.27725977	1	5.27725977	234.42	<.0001
Provenance	0.64965595	1	0.64965595	28.86	0.0002
Salinity	8.14921746	3	2.71640582	169.01	<.0001
Temperature x provenance	0.03297297	1	0.03297297	1.46	0.2495
Temperature x salinity	0.63322730	3	0.21107577	13.13	<.0001
Provenance x salinity	0.66317704	3	0.22105901	13.75	<.0001
Temperature x provenance x salinity	0.73776983	3	0.24592328	15.30	<.0001

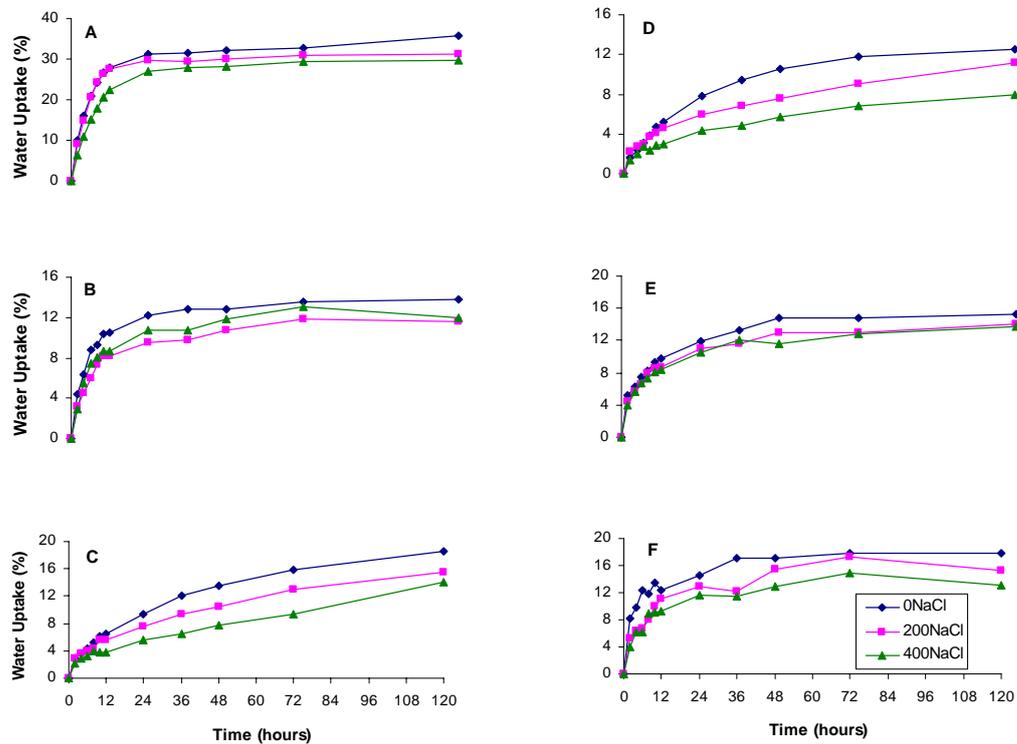


**Fig. 4.** Total percent germination and mean time to germination in days for seeds of two seedlots *Acacia trulliformis* (SM and CO) at two constant temperatures (15°C and 25°C) under increasingly saline conditions (0-400 mM NaCl).

#### *Experiment 2: Imbibition and recovery investigation*

There was a significant ( $p < 0.05$ ) reduction in water uptake during the imbibition phase for *Banksia* and *Dryandra* as the osmotic potential decreased with increasing salinities (Fig. 5).

In *Acacia* and *Orthrosanthus* water uptake at 200 mM NaCl was not significantly different to uptake at 400 mM NaCl, but imbibition for each species under both saline conditions was significantly different relative to the controls. In *Hakea* there was no difference in imbibition between non-saline and saline conditions even at the highest salinity. There was a significant difference in water uptake at moderate salinity (200 mM NaCl) and under non-saline conditions for *Austrostipa* seeds, although no difference in imbibition at 400 and 0 mM NaCl. Ability to imbibe under moderate and high saline conditions was similar.



**Figure 5.** Percentage water uptake over 120 hours at 0, 200 and 400 mM NaCl for A *Acacia trulliformis*; B *Austrostipa geoffreyi*; C *Banksia oligantha*; D *Dryandra mucronulata*; E *Hakea tuberculata*; and F *Orthrosanthus muelleri*.

Overall, seeds of all species recovered well from saline pre-treatment when transferred to non-saline conditions, suggesting seed resilience to temporary hyper-saline conditions (Table 3). *Banksia* and *Austrostipa* were the only two species that exhibited large declines in percent germination under hyper-saline conditions despite rinsing. Mean time to germination (MTG) increased for each increase in salt solution but did not increase greatly between rinsing times. MTG ranged from 8 days in *Acacia* under non-saline conditions to over 100 days for *Orthrosanthus* under hyper-saline conditions. A comparison of percent germination under constant saline conditions (Experiment 1) and after rinsing (Experiment 2) indicates that rinsing had a highly significant effect on all species except *Acacia*. For three species (*Austrostipa*, *Dryandra* and *Orthrosanthus*) the timing of the rinsing (5, 15 or 30 days from saline pre-incubation) was not important. Only at the highest salinity did *Austrostipa* exhibit a decline in germination when the time to rinsing increased to 15 or more days. Saline treatment had a significant effect on all species except *Orthrosanthus* with declines in percent germination evident at 400 mM NaCl compared to the controls. Pre-incubation under hyper-saline conditions for 15 or 30 days prior to rinsing effectively doubled percent germination of *Orthrosanthus* seed over the control indicating a possible priming effect.

**Table 3. Total percent germination ( $\pm$  SE) and mean time to germination in days after saline pre-incubation phase (0, 200 and 400 mM NaCl for 0, 5, 15 and 30 days) and recovery on distilled water for seed of six species of native plants from southern Western Australia**

	Days to rinsing	0 mM NaCl		200 mM NaCl		400 mM NaCl	
		final germ (%)	MTG (days)	final germ (%)	MTG (days)	final germ (%)	MTG (days)
<i>Acacia trulliformis</i> (SM only at 15°C)	0	97 $\pm$ 1.9	8.6	86 $\pm$ 6.6	13.8	61 $\pm$ 3.4	20.3
	5	97 $\pm$ 1.9	8.1	96 $\pm$ 1.6	9.6	87 $\pm$ 2.5	9.7
	15	96 $\pm$ 1.0	8.1	94 $\pm$ 3.8	12.6	61 $\pm$ 1.0	21.2
	30	94 $\pm$ 1.2	7.9	90 $\pm$ 4.2	14.1	70 $\pm$ 7.4	21.6
<i>Austrostipa geoffreyi</i>	0	21 $\pm$ 1.0	23	6 $\pm$ 1.0	31	0	0
	5	31 $\pm$ 5.3	24.3	23 $\pm$ 1.9	21.5	20 $\pm$ 2.8	22.2
	15	30 $\pm$ 5.3	30.4	35 $\pm$ 5.7	27.8	9 $\pm$ 4.4	39.3
	30	29 $\pm$ 5.4	23.5	33 $\pm$ 5.5	33.4	9 $\pm$ 1.9	40.4
<i>Banksia oligantha</i>	0	34 $\pm$ 4.2	20.4	33 $\pm$ 4.4	29.7	0	0
	5	40 $\pm$ 4.3	21.3	31 $\pm$ 1.0	20.4	18 $\pm$ 3.5	17.7
	15	46 $\pm$ 8.1	21.8	41 $\pm$ 5.5	26.9	21 $\pm$ 3.8	34.2
	30	39 $\pm$ 6.8	21.2	28 $\pm$ 4.4	32.1	10 $\pm$ 3.5	41.8
<i>Dryandra mucronulata</i>	0	78 $\pm$ 3.8	27.8	77 $\pm$ 3.0	43.7	11 $\pm$ 5.0	77.7
	5	98 $\pm$ 1.2	26.7	79 $\pm$ 1.0	31.1	78 $\pm$ 3.5	31.2
	15	86 $\pm$ 2.6	33.6	86 $\pm$ 3.5	37.6	73 $\pm$ 4.7	43.5
	30	81 $\pm$ 3.4	33.3	86 $\pm$ 3.8	40.3	74 $\pm$ 8.1	53.9
<i>Hakea tuberculata</i>	0	56 $\pm$ 6.7	14.1	56 $\pm$ 5.7	25.5	31 $\pm$ 3.8	51.3
	5	72 $\pm$ 8.5	15.7	80 $\pm$ 1.6	15.8	66 $\pm$ 3.8	16.1
	15	75 $\pm$ 6.0	16.3	68 $\pm$ 5.7	20	59 $\pm$ 4.2	23.3
	30	61 $\pm$ 7.5	16.4	58 $\pm$ 11.5	29.1	66 $\pm$ 4.2	35.1
<i>Orthrosanthus muelleri</i>	0	10 $\pm$ 4.2	96	0	0	0	0
	5	11 $\pm$ 2.6	67.6	11 $\pm$ 3.4	68.1	8 $\pm$ 1.6	70
	15	8 $\pm$ 1.6	70.6	9 $\pm$ 1.0	80.8	19 $\pm$ 2.5	103.9
	30	8 $\pm$ 3.7	91	9 $\pm$ 4.4	100.9	22 $\pm$ 4.2	126

### Experiment 3. Seedling growth and survival

Treatment with hyper-saline water had a statistically significant effect on plant survival for most species, although mortality varied greatly between treatments and between species (Table 4).

*Banksia* was the least tolerant of salt and water stress, applied singly or in combination. *Austrostipa* was the most tolerant with 100% survival in all treatments. Many species grown in free draining treatments with added salt suffered higher mortality than that of waterlogging and salt combined. Young plants of *Myoporum* and *Austrostipa* were highly tolerant under all conditions (95%-100% survival) although 50% of plants of the former survived in treatment SD-spray; plants of *Banksia* and *Dryandra* suffered complete mortality in all treatments except the control and waterlogging without salt after 12 weeks; plants of *Hakea* were highly tolerant to waterlogging without salt but were sensitive to the combined effects of salt and waterlogging and to salt alone applied

**Table 4. Percentage survival of seven native Western Australian species subjected to glasshouse salinity and waterlogging pot trials under five treatments and a control (no treatment) over a 12 week period.** [Significance ( $P<0.05$ ) of each treatment relative to other treatments is indicated by the different letters as determined by pairwise comparisons using Wald's test at  $\alpha=0.05$ ].

Treatment	% Survival						
	<i>Acacia trulliformis</i>	<i>Hakea tuberculata</i>	<i>Orthrosanthus muellerii</i>	<i>Banksia oligantha</i>	<i>Austrostipa geoffreyi</i>	<i>Dryandra mucronulata</i>	<i>Myoporum turbinatum</i>
C	100 <sup>a</sup>	100 <sup>a</sup>	100 <sup>a</sup>	100 <sup>a</sup>	100 <sup>a</sup>	100 <sup>a</sup>	100 <sup>a</sup>
SD-spray	0 <sup>b</sup>	0 <sup>b</sup>	0 <sup>c</sup>	0 <sup>b</sup>	100 <sup>a</sup>	0 <sup>b</sup>	45 <sup>b</sup>
SD-drip	100 <sup>a</sup>	65 <sup>c</sup>	-	0 <sup>b</sup>	100 <sup>a</sup>	0 <sup>b</sup>	-
W	100 <sup>a</sup>	100 <sup>a</sup>	100 <sup>a</sup>	35 <sup>c</sup>	100 <sup>a</sup>	95 <sup>c</sup>	100 <sup>a</sup>
SW1	0 <sup>b</sup>	15 <sup>d</sup>	20 <sup>b</sup>	0 <sup>b</sup>	100 <sup>a</sup>	0 <sup>b</sup>	95 <sup>c</sup>
SW2	30 <sup>c</sup>	25 <sup>d</sup>	100 <sup>a</sup>	0 <sup>b</sup>	100 <sup>a</sup>	0 <sup>b</sup>	95 <sup>c</sup>

via spray; and *Orthrosanthus* plants were highly tolerant to waterlogging with and without salt but not tolerant to salt alone. Seedlings of *Acacia* and *Orthrosanthus* survived 12 weeks of partially waterlogged conditions but not fully waterlogged conditions, although the survival of the *Acacia* in the former was significantly reduced compared to the control.

Over the trial period visible signs of stress leading to plant death in the saline drained and waterlogged treatments began around week 5 and included browning and wilting of growing tips and progressive leaf death.

Although plants of some species may have survived under saline and / or waterlogged conditions for up to 12 weeks, their growth was generally impeded compared to the controls (Table 5). There were significant decreases ( $p<0.05$ ) in plant growth under treatment SD-drip for *Acacia* and *Hakea* but not for *Austrostipa*.

**Table 5. Relative growth rate of seven native Western Australian species subjected to glasshouse salinity and waterlogging pot trials over a 12 week period. Growth rates are expressed as a percentage of the untreated control.** [Significance ( $P<0.05$ ) of each treatment relative to other treatments is indicated by the different letters as determined by Dunnett's test]

Treatment	Relative Growth Rate %						
	<i>Acacia trulliformis</i>	<i>Hakea tuberculata</i>	<i>Orthrosanthus muellerii</i>	<i>Banksia oligantha</i>	<i>Austrostipa geoffreyi</i>	<i>Dryandra mucronulata</i>	<i>Myoporum turbinatum</i>
C	100 <sup>a</sup>	100 <sup>a</sup>	100 <sup>a</sup>	100 <sup>a</sup>	100 <sup>a</sup>	100 <sup>a</sup>	100 <sup>a</sup>
SD-spray	-	-	-	-	72 <sup>bd</sup>	-	83 <sup>a</sup>
SD-drip	57 <sup>b</sup>	47 <sup>b</sup>	-	-	48 <sup>c</sup>	-	-
W	67 <sup>ab</sup>	88 <sup>ab</sup>	141 <sup>a</sup>	64 <sup>b</sup>	143 <sup>c</sup>	96 <sup>a</sup>	113 <sup>a</sup>
SW1	-	53 <sup>b</sup>	-22 <sup>a</sup>	-	66 <sup>d</sup>	-	104 <sup>a</sup>
SW2	47 <sup>b</sup>	73 <sup>b</sup>	151 <sup>a</sup>	-	95 <sup>ab</sup>	-	99 <sup>a</sup>

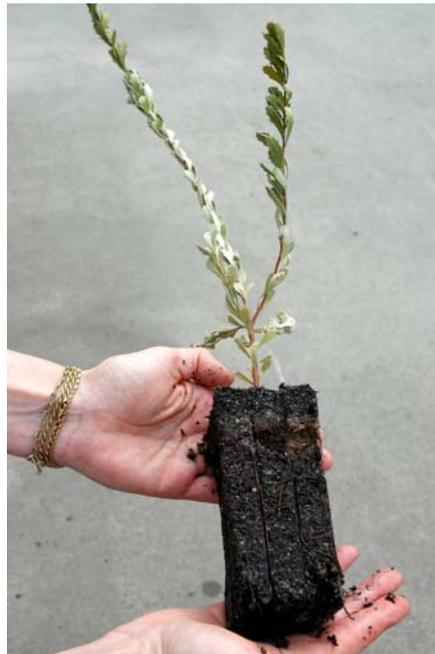


**Photo 1. Glasshouse pot trial to investigate effects of salt and waterlogging on seedlings of six species of native plant from southern Western Australia.**



**Photo 2. Measurement of seedling growth parameters in glasshouse pot trials to investigate effects of salt and waterlogging on six species of native plant from southern Western Australia.**

Waterlogging caused no significant reduction in percentage plant growth relative to controls except for *Banksia* which suffered a 36% decline in relative growth. *Acacia* but not *Hakea* showed a significant decline ( $P < 0.05$ ) in relative growth in treatment SW2. There was no significant reduction in relative plant growth of *Myoporum* under all treatments. Although *Orthrosanthus* showed an increase in relative plant growth in treatment W and SW2 compared to the control, and a decrease in growth in SW1, these changes were not significant due to the small number of plants tested.



**Photo 3. Blackened roots of *Acacia trulliformis* after saline waterlogging.**

## Discussion

This investigation has demonstrated that for some species salinity delayed germination at salinities that did not prevent germination (demonstrated by an increase in germination time) or inhibited germination by either preventing germination without loss of viability (demonstrated by the seeds ability to recover). The proportion showing this trait varied with the species and the duration of exposure to high salinity which is a relationship that has been reported elsewhere (Keiffer and Ungar 1997; Baskin *et al.* 1988).

The species differed greatly in their response to constant saline conditions during germination. The threshold salinity for a significant reduction in germination for *Austrostipa* and *Orthrosanthus* was 100 mM and 0 mM NaCl respectively. This threshold was 400 mM NaCl for *Dryandra*, *Hakea*, *Banksia* and *Acacia* suggesting considerable tolerance to salt in these non-halophytic species during the germination phase. The mean time to germination increased exponentially with increases in salinity

for all species. Increases in salinity are known to cause a decrease in level and rate of germination in both halophytic (Khan and Ungar 1999; Rubio-Casal *et al.* 2003) and non-halophytic species (Van der Moezel and Bell 1987a; Pearce-Pinto *et al.* 1989) as well as an initial delay in germination (Ungar 1978). Factors affecting speed of germination and growth can influence the composition and abundance of species within a community. Many species exhibit narrow regeneration niches and may undergo replacement by other more salt or waterlogging tolerant species under increasingly saline conditions, thus allowing the establishment and expansion of non-native or non-desirable species due to differential tolerance to salinity between species and differential germination and growth between species. Species that germinate and grow rapidly will develop root systems that can utilise ground water thereby reducing competition for topsoil moisture. Delays in germination due to osmotic pressure may push species to germinate under less favourable environmental conditions thereby compromising their establishment and survival.

A seeds ability to tolerate saline conditions for germination is partially dependent on the incubation temperature (Ungar 1978; Baskin and Baskin 2001; Khan *et al.* 2001) with percent germination decreasing at higher temperatures under conditions of low osmotic potential. The interaction between salinity and temperature on germination plays a major role in determining timing of germination and will influence whether plants will successfully establish in salinising environments. Recruitment of the majority of the study species occurs in autumn and winter after the onset of rains when temperatures decrease and soil salinity would be lowered.

The generally significant decrease in percent germination for *Acacia* with increased temperature suggests that germination in this species would be inhibited during the warmer months of the year when soil salinities may be higher. Projected increases in temperatures associated with climate change may also have the potential to severely affect the natural regeneration of this rare species in the future. The significant difference in germination percent noted between the two seedlots under the highest salt treatment may indicate an adaptive strategy at the morphologic, physiologic and/or ecological level to success within a stressful environment (van der Moezel and Bell 1987a). Further research needs to more fully investigate the combined effects of temperature, salt stress and provenance for seedling growth and survival. Unfortunately many rare species are only known from a single location and provenance testing is not possible.

Our data suggest that water uptake under elevated saline conditions was significantly slower for at least four of the species under investigation but was not restrictive to germination. That a large percentage of non-germinating seed recovered after being transferred from saline conditions to distilled water suggests the inhibitory effect is mostly osmotic and not toxic (Ungar 1995; Houle *et al.* 2001). After summer dispersal, small seeds are often located near the soil surface where salt loads may be high and vary due to evaporation of ground water. Rainfall may quickly leach surface salt and stimulate seed imbibition during the winter months. Thus for successful establishment, seeds need to remain viable in high salinities and germinate when salinity decreases. The ability of seeds of these species to remain dormant under high salt loads suggests the

potential to survive temporary saline habitats and could be adaptive in preventing germination during summer rains when saline solutions of surface soil haven't been diluted sufficiently (Bell *et al.* 1993). Under hyper-saline conditions seed survival rather than germination may be more important for persistence in the landscape.

The effect of salt and the combined effects of salt and waterlogging on seedlings manifested in slow growth and seedling mortality for most species over the 12-week trial period. The time taken to develop salt- and/or waterlogged-induced foliar symptoms was approximately five weeks. At low to moderate salinities Epstein (1980) suggested that salt exclusion is the main adaptive strategy in plants and therefore reflected in growth and yield. At high salinities ion toxicity is the main cause of plant death so survival is the primary strategy (Levitt 1980). The majority of species tested showed lower relative growth and survival under the combined saline and waterlogged conditions than to waterlogging alone. Ladiges *et al.* (1981) reported increases in shoot and root growth after waterlogging, but a decline after salt waterlogging for *Melaleuca ericifolia*. In contrast, Marcar *et al.* (2002) reported less effect of salt than of waterlogging with respect to growth reduction in *Eucalyptus grandis* and *E. globulus*. Partial saline waterlogging showed improved survival over fully waterlogged and saline conditions for the majority of species although *Banksia* was highly sensitive to both waterlogging and saline conditions. Less oxygen is available to roots in fully waterlogged as compared to half waterlogged treatments and plant tolerance to flooding is variable. Oxygen deficiency has a direct effect on plant function and reductions in relative growth rate in saline and non-saline waterlogged treatments may be due to the anaerobic conditions experienced by plants over the 12 weeks (Ladiges and Kelso 1977). Non-saline waterlogging of plants for 12 weeks was not detrimental to survival in five of the seven species tested. In *Banksia* and *Dryandra* the effect of waterlogging appeared to be a blackening of fibrous roots due to anaerobic conditions (Photo 3).

Of the two drained saline treatments survival of plants of all species but *Dryandra* and *Banksia* was higher when salt was applied via a dripper system. There was a relationship between sensitivity to SW1 treatment and salt applied via spray with all species but *Austrostipa* and *Myoporum* suffering 100% mortality after 12 weeks treatment. Direct application of saline water to lower leaves was more detrimental at the same concentrations than direct application to the plants root zone.

*Austrostipa* exhibited a low salinity tolerance during germination but exceptional tolerance to growth under both saline and/or waterlogged conditions similar to that reported by Myers and Couper (1989) for the halophytic grasses *Puccinellia ciliata* and *P. pereme*. The inhibiting effect of salt on seed germination was reversible except at the highest salinity, as seeds from the saline treatments germinated after being rinsed in distilled water. Similar recovery responses have been noted for halophytic species but not so for non-halophytes (Khan and Ungar 1997; Khan and Ungar 1999, Khan *et al.* 2001). Post-emergence, seedlings of *Austrostipa* displayed little to no reduction in plant growth despite highly saline and/or waterlogged conditions. In fact, waterlogged *Austrostipa* seedlings exhibited a more than 40% increase in growth when inundated and a high proportion of waterlogged seedlings of all species survived the 12 week trial period. The interaction between substrate salinity and plant development is evident in

*Austrostipa* where the mechanisms that allow tolerance to salinity develop over time appearing only in established seedlings. It is possible that salt tolerance in this species may be more critical in later developmental phases when the roots encounter saline subsoils (van der Moezel and Bell 1987a). Many halophytes also have special adaptations that prevent germination in saline soils (Ungar 1978) and can only germinate when salts have been leached after heavy rains. This perennial grass inhabits the margins of salt lakes on raised dunes and has the potential to be inundated by saline water. Although highly salt and waterlogging tolerant during growth, seed germination may represent a controlling factor in the distribution and abundance of this species in the wild.

In the case of *Orthrosanthus*, preconditioning of seed in sodium chloride may have acted as a primer for increasing germination as seeds were stimulated to germinate at higher percentages than controls (almost double) after exposure to hyper-saline conditions (400 mM NaCl) for 15 or 30 days. Continuous incubation of seeds of this species in any concentration of NaCl inhibited germination suggesting that constant saline conditions impose secondary seed dormancy in this species. Both survival and growth under waterlogged and saline waterlogged conditions was good in this species, although further investigations into dormancy mechanisms are required as germination was low under control conditions.

## **Conclusions**

This study simplified environmental variation by testing constant conditions over time (eg temperature and moisture) rather than variations in conditions which are more akin to natural fluxes (Niknam and McComb 2000). Nevertheless, the benefits of using laboratory and glasshouse facilities mean that screening of plants can occur at any time of year on a range of species without the variability of salt and waterlogging in the field (Rogers *et al.* 2005). Studies such as this one can provide some degree of insight into the potential loss of biodiversity under changed hydrology, and/or the resilience of components of the vegetation understory. These studies can point to the probability of persistence in a changing landscape. They are only indicative and can alert land managers to the level of threat to individual species already threatened by other processes such as habitat fragmentation, small population size, disease and weed invasion. Seeds of many species in southern Western Australia may undergo periods of inundation under hyper-saline conditions prior to the onset of autumn-winter rains that would dilute saline waters, therefore understanding the behaviour of seeds and seedlings under salt and water stress is necessary for the management of native ecosystems. It is encouraging to consider that a number of the study species are non-halophytes yet have the potential to retain viability and recover after exposure to saline conditions. The majority of species are also highly waterlogging tolerant with a number being able to survive short periods of saline inundation. Field experiments would need to test the validity of these results. Ultimately though, the loss of some vegetation components to rising water tables is inevitable but understanding which vegetation is tolerant to changed hydrological conditions will allow restoration efforts to be more successful.

This project has contributed to the conservation of Western Australian terrestrial biodiversity by helping to consolidate species recovery plans and assisting in the reversal of species decline through *ex situ* conservation actions. Seed in store secures the long-term future of seed-bearing plants. This project has provided knowledge and information that will promote biodiversity conservation through the identification of species at risk of extinction in salinising landscapes, and will provide information to assist in the management of these species. The project has clearly identified genetic resources that are sustainable within a salinising landscape. There is a clear public benefit in identifying native species at risk of extinction, and/or the provision of information on species tolerance to hydrologic change that can be utilised for revegetation. There will be high levels of public benefit if species are held *ex situ* and made available for restoration works based on their tolerance to adverse conditions. Breakthroughs in developing technologies in seed germination can reduce the cost of growing target species for restoration. Further investigations into the ecology and biology of threatened species and the current and future impacts of salinity on terrestrial plant biodiversity are warranted.

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