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# Seedling growth responses of *Banksia littoralis* and *Melaleuca preissiana* to soil salinity

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## Abstract

*Banksia littoralis* (Swamp Banksia) and *Melaleuca preissiana* (Moonah) are two tree species that are commonly associated with wetland fringes and inter-dunal depressions of the Swan Coastal Plain. These low-lying areas are prone to hydrological disturbances and salinisation. The growth and tolerance of *B. littoralis* and *M. preissiana* seedlings to increased soil salinity was examined via a pot trial that lasted for 70 days with weekly watering of 0, 50, 100 and 150 mM of NaCl solution. No mortality was observed for either species within any of the salt treatments. *M. preissiana* seedlings showed no difference in their growth response or above-ground biomass allocation. *B. littoralis* seedlings showed a substantial reduction in relative growth rate and net assimilation rate for solutions greater than 100 mM NaCl. This resulted from an overall reduction in shoot dry mass, with less leaf area being produced per unit leaf mass, and caused recently produced leaves to become dehydrated. These results suggest that *M. preissiana* seedlings are capable tolerating soils with an electrical conductivity of at least 1650 $\mu S\ cm^{-1}$, whereas seedling growth of *B. littoralis* is significantly reduced for soil conductivity > 980 $\mu S\ cm^{-1}$. From this study it can be concluded that soil salinisation will have a more detrimental impact on *B. littoralis* seedling recruitment than co-occurring *M. preissiana* seedlings.

**Keywords:** Moonah, relative growth rate, salt tolerance, Swamp Banksia, Swan Coastal Plain

## Introduction

*Banksia littoralis* R. Br. (Proteaceae) and *Melaleuca preissiana* Schauer (Myrtaceae) are two tree species from the Swan Coastal Plain which are entirely dependant on accessing groundwater for their survival, and as such are restricted to the fringes of wetlands and low-lying inter-dunal depressions that dominate the area. These low-lying areas are more prone to becoming salinised, and approximately 70% of wetlands have been lost due to increased draining, filling or land clearing since European settlement (Halse 1989). While many of the Swan Coastal Plain wetlands are not highly saline (i.e. conductivity >5 000 $\mu S\ cm^{-1}$), areas derived from marine, estuarine, or littoral deposits (especially some of the loamy soils surrounding wetlands) have been found to have high soluble and exchangeable sodium contents (Cochrane *et al*. 1994), dominated by sodium chloride (Halse 1989). Water quality data collected in December 1987 from 16 Perth wetlands ranged from 505 to 10 270 $\mu S\ cm^{-1}$ (Davis *et al.* 1991), whereas more recent data collected from 19 different wetlands ranged from 0.11 to 4.51 ppt (171 to 7203 $\mu S\ cm^{-1}$) (John & Kemp 2006).



Hydrological alteration, nutrient enrichment and salinisation resulting from human activity are the main factors placing fringing wetland vegetation at risk (Davis & Froend 1999). For *M. preissiana,* seedling recruitment events are episodic, and over time may not compensate for the loss of older plants within populations (Froend *et al.* 1993). *M. preissiana* survives changes in the underlying hydrology by its extensive root system and moderate flood tolerance of seedlings (Froend *et al.* 1993), with any change in population structure noticeable only after many decades (Groom *et al.* 2001). The seedlings of *B. littoralis* are also flood tolerant (Groom 2004a), but are more susceptible to declining groundwater levels (Groom *et al.* 2001).

This paper compares the growth response of *B. littoralis* and *M. preissiana* seedlings to increased soil salinity. The response of these two wetland tree species to the effects of increased soil salinisation is unknown, and will have significant impacts on the species' ability to recruit in an environment already being impacted by declining groundwater levels and episodic flooding. If Swan Coastal Plain wetlands do become more saline, there are implications for the long-term survival of the fringing vegetation, with populations being unable to offset adult plant mortality by seedling recruitment.

## Methods

### Experimental setup

The salinity trials were conducted in a naturally lit plant house at the Bentley Campus of Curtin University of Technology. Six- to nine-month old seedlings of *B. littoralis* and *M. preissiana* were sourced from a local Perth nursery. All seedlings were grown from seeds collected from unverified natural populations inhabiting Perth's northern Swan Coastal Plain. Seedlings were transplanted into cylindrical pots (8.2 cm diameter, 14.9 cm height) about two weeks prior to the commencement of the experiment, using a potting medium composed of six parts coarse river sand, three parts composted pine bark, and one part slow release, low phosphate fertiliser (Osmocote® Native Gardens, Scotts Australia). Salinity solutions were applied weekly as analytical grade NaCl dissolved in deionised water at 50 mM, 100 mM, and 150 mM concentrations while a control received deionised water only. Watering was conducted so as to allow a small degree of flushing to occur. Five seedlings per species were randomly selected and assigned to each salinity treatment.

### Harvest and growth parameters

Four seedlings per species were harvested the day before the salinity trial commenced. Shoots were excised from roots and divided into leaf and stem components for each seedling. After 70 days of watering with NaCl solutions all surviving seedlings were similarly harvested and partitioned into their stem, leaf and root components. In addition, the fresh weight of the youngest fully expanded leaf (YFEL) from each seedling was measured. Fresh leaves of each plant were digitally scanned and then analysed for projected area using the Image J software (http://rsb.info.hih.gov/ij). All plant material was then oven dried at 70°C for 72 hours, after which dry weights of all components were taken.

Relative growth rate (RGR) and net assimilation rate (NAR) was calculated according to McGraw & Garbutt (1990). In addition, the leaf area ratio (LAR), measured as the ratio of projected leaf area (photosynthetic surface) to aboveground biomass; leaf weight ratio (LWR), calculated as a percentage biomass allocation to leaves; specific leaf area (SLA), measured as total leaf area divided by total leaf mass were calculated. For the youngest fully expanded leaf of each seedling, the degree of succulence (measured as leaf water content per unit leaf area) and SLA were determined.

### Soil analysis

Soil samples were collected for each species-treatment combination after 70 days watering with their respective salt solution, and allowed to air dry for two weeks. Sub-samples were then mixed at a rate of 1:5 with deionised water, shaken for one minute, and allowed to stand for approximately five minutes. Electrical conductivity was then recorded using a portable meter (WP-81, TPS, Brisbane, Australia) from the supernatant.

### Statistical analyses

Interaction between species and treatment blocs was analysed by factorial ANOVA and statistical difference between treatments within species groups was analysed by one-way ANOVA using SPSS version 15.0 (SPSS Inc. Chicago, USA). Homogeneity of variances was assessed using Levene's test and log or root transformed as required, with data presented as untransformed means. Scheffé's test was used for multiple comparisons of means at a significance level of 0.05.

## Results

There was no mortality recorded for either species in any of the salt treatments. *Banksia littoralis* showed a general reduction in shoot biomass with increasing salinity. Seedlings growing in 150 mM NaCl had a 37% reduction in biomass compared to the controls (Table 1), resulting in the lowest relative growth rate and net assimilation rates (a measure of overall efficiency of a seedling in generating biomass) recorded. The addition of different levels of salt solutions had no significant impact on leaf area ratio (a measure of leafiness) or leaf weight ratio (a measure of biomass allocation to leaves) but did result in a significant decrease in specific leaf area (a measure of relative leaf density) at 150 mM NaCl. Salinity levels greater than 100 mM NaCl resulted in less leaf area being produced per unit leaf weight, and less water occurring in the youngest fully expanded leaves per gram dry weight (Table 1).

In contrast to the *Banksia*, *M. preissiana* displayed no appreciable change in shoot biomass across treatments (Table 2), with no change in relative growth rate or net assimilation rate. There was no significant difference in seedling leaf area ratio, specific leaf area or degree of succulence. Overall *M. preissiana* seedlings grew faster, with a higher leaf area ratio and specific leaf area than *B. littoralis*.

After 70 days of watering with salt solutions the soil electrical conductivity increased as the concentration of salt solution increased (Table 3), with the conductivity data for *M. preissiana* soil being 244 to 560 μS $cm^{-1}$ higher than for *B. littoralis* soil at the two highest NaCl concentrations.

## Discussion

It can be expected that species restricted to winter-wet depressions, wetlands and boarding water courses in southwestern Australia should display some degree of salt tolerance. On the Swan Coastal Plain, vegetation occupying low-lying inter-dunal areas or fringing wetlands are the most prone to environmental change. Our data suggests that *M. preissiana* seedlings are capable of growing in soils with an electrical conductivity of 1650 μS $cm^{-1}$ for at least 70 days without any adverse effect on seedling growth or biomass allocation. This is higher than the known salt tolerance limits for *M. preissiana* trees (500–1000 μS $cm^{-1}$; Bennett & George 2002). In contrast, it is expected that *B. littoralis* seedlings would not tolerate prolonged exposure to soil electrical conductivities greater than 1090 μS $cm^{-1}$ due to a decline in relative growth and net assimilation rates. At soil conductivity greater than 980 μS $cm^{-1}$ *B. littoralis* seedlings respond to the increased salt by a reduction in shoot weight and total leaf area produced, resulting in a significant

**Table 1**

Growth parameters of *Banksia litoralis* seedlings after 70 days of watering with different concentrations of NaCl solution. Data presented as means (se) for 5 seedlings. Superscripts indicate subsets between treatments based on Scheffé's test after differences were found by one-way ANOVA. % change is in relation to control (0 mM) data. SLA = specific leaf area; YFEL = youngest fully expanded leaf; RGR = relative growth rate; NAR = net assimilation rate. ns = not significant ($P$>0.05)

| | | | | | ***P* value** | **% change** | | |
|---|---|---|---|---|---|---|---|---|
| | **0 mM** | **50 mM** | **100 mM** | **150 mM** | | **50 mM** | **100 mM** | **150 mM** |
| Shoot weight (g) | $1.8^{a}$ (0.1) | $1.8^{a}$ (0.2) | $1.4^{ab}$(0.1) | $1.2^{b}$ (0.04) | 0.001 | –1 | –23 | –37 |
| Leaf area ratio (cm$^2$ g$^{-1}$ shoot) | 51.7 (3.8) | 52.1 (2.7) | 49.2 (1.9) | 49.8 (1.6) | ns | 1 | –5 | –4 |
| Leaf weight ratio (% leaf shoot$^{-1}$) | 67.4 (3.3) | 66.5 (2.9) | 68.6 (2.8) | 72.9 (1.4) | ns | –1 | 2 | 8 |
| SLA (cm$^2$ g$^{-1}$) | $76.6^{a}$ (3.3) | $78.3^{a}$ (1.6) | $71.8^{ab}$ (1.3) | $67.8^{b}$ (1.5) | 0.006 | 2 | –6 | –11 |
| SLA-YFEL (cm$^2$ g$^{-1}$) | $82.5^{a}$ (7.1) | $87.7^{a}$ (7.2) | $68.4^{b}$ (1.8) | $68.6^{b}$ (3.0) | 0.030 | 6 | –17 | –17 |
| Succulence-YFEL (mg g$^{-1}$) | $20.8^{a}$ (2.7) | $19.4^{a}$ (0.6) | $14.0^{b}$ (2.0) | $14.3^{b}$ (1.7) | 0.040 | –6 | –32 | –31 |
| Shoot RGR (mg g$^{-1}$ d$^{-1}$) | 6.7 | 6.6 | 3.1 | 0.1 | – | –2 | –55 | –98 |
| Shoot NAR (mg g$^{-1}$ d$^{-1}$) | 0.130 | 0.127 | 0.062 | 0.002 | – | –3 | –52 | –98 |

**Table 2**

Growth parameters of *Melaleuca preissiana* seedlings after 70 days of watering with different concentrations of NaCl solution. Data presented as means (se) for 5 seedlings. See Table 1 for description of abbreviations.

| | | | | | ***P* value** | **% change** | | |
|---|---|---|---|---|---|---|---|---|
| | **0 mM** | **50 mM** | **100 mM** | **150 mM** | | **50 mM** | **100 mM** | **150 mM** |
| Shoot weight (g) | 2.2 (0.1) | 1.9 (0.2) | 2.3 (0.3) | 2.2 (0.2) | ns | –12 | 6 | –7 |
| Leaf area ratio (cm$^2$ g$^{-1}$ shoot) | 90.1 (3.5) | 101.8 (9.8) | 87.9 (8.7) | 93.5 (1.8) | ns | 13 | –2 | 4 |
| Leaf weight ratio (% leaf shoot$^{-1}$) | 73.2 (5.8) | 74.5 (1.6) | 74.4 (2.3) | 77.2 (0.1) | ns | 2 | 2 | 5 |
| SLA (cm$^2$ g$^{-1}$) | 126 (12) | 136 (12) | 117 (8.4) | 121 (2) | ns | 9 | –7 | –4 |
| SLA-YFEL (cm$^2$ g$^{-1}$) | 114 (7) | 132 (7) | 123 (20) | 126 (4) | ns | 16 | 8 | 10 |
| Succulence-YFEL (mg $H_2O$ g$^{-1}$) | 11.0 (0.4) | 13.6 (1.3) | 11.3 (1.5) | 12.7 (0.8) | ns | 24 | 3 | 16 |
| Shoot RGR (mg g$^{-1}$ d$^{-1}$) | 21.3 | 19.4 | 22.1 | 20.3 | – | –9 | 4 | –5 |
| Shoot NAR (mg g$^{-1}$ d$^{-1}$) | 0.236 | 0.191 | 0.251 | 0.217 | – | –19 | 6 | –8 |

**Table 3**

Soil electrical conductivity readings (μS cm$^{-1}$) after 70 days of watering with salt solutions. Data are means of 2 replicates.

| | **0 mM** | **50 mM** | **100 mM** | **150 mM** |
|---|---|---|---|---|
| *B. littoralis* | 210 | 933 | 984 | 1090 |
| *M. preissiana* | 114 | 986 | 1228 | 1650 |

decrease in specific leaf area (a measure of relative leafiness), as well as significant dehydration of the youngest fully expanded leaf.

Soil salinity levels have important implications on the recruitment, growth and survival of the native vegetation fringing wetlands, particularly when salinity levels rise beyond naturally occurring levels due to human interference. Seedling establishment is crucial for the long-term viability of *B. littoralis* and *M. preissiana* populations, with recruitment events only occurring episodically when conditions are suitable. For *M. preissiana*, recruitment tends to occur on mass during the winter months following a fire, when there is sufficient soil moisture to ensure adequate root growth (Froend *et al.* 1993). Seedling recruitment within the fringing embankments is influenced by wetland water levels (Froend *et al.* 1993) with seedling long-term survival ultimately dependent on underlying groundwater levels. For both species, an increase in soil salinity would have a detrimental impact on seedling recruitment levels, although clearly *M. preissiana* is more salt tolerant than *B. littoralis*.

The decline in *B. littoralis* and *M. preissiana* population vigour on Perth's Swan Coastal Plain over the past 40 years has been attributed to a regional decline in groundwater levels, resulting from the combination of declining annual rainfall and localised groundwater abstraction (Groom *et al.* 2001). Deep-rooted species fringing wetlands are more prone to water stressors because they are obligately dependent on subsurface groundwater (Groom 2004b; Canham *et al.* 2009). *B. littoralis* and *M. preissiana* seedlings can survive flooding conditions for at least one month (Froend *et al.* 1993; Groom 2004a) but are unable to survive periods of submergence. Studies on species salt tolerance typically investigate interaction with waterlogging, as together these stressors can cause severe damage to plants (Barrett-Lennard 2003). Although the combined effect of salinity and waterlogging on the growth and physiology of our two target species is unknown, it has been reported that waterlogging with freshwater has little effect on the growth and survival within the genus (van der Moezel *et al.* 1991). For *Melaleuca* species inhabiting areas prone to inundation, experimental pot trials have shown a tolerance of at least 42000 $\mu S\ cm^{-1}$ soil conductivity with 22 days of waterlogging (*M. cuticularis*; Carter *et al.* 2006) and 62000 $\mu S\ cm^{-1}$ after waterlogging for 10 weeks (*M. ericifolia*; Salter *et al.* 2007). Soil salinisation is well known to inhibit growth, alter plant morphology and leaf physiology in species not adapted to tolerate salinity (Kozlowski 1997). If Swan Coastal Plain wetlands become more saline the survival and population vigour of species restricted to wetland fringes and low-lying depressions will be negatively impacted.

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