The impact of overhead irrigation with saline-sodic and alkaline water on plant health and productivity

Revised 24 Feb 2016

Final Report

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Extended summary

- The irrigation of unamended coal seam water (CS water) as part of the land amendment irrigation (LAI) program offers several advantages compared to other approaches. However, it is unknown whether the overhead irrigation of this saline and alkaline unamended water (for example, through centre-pivots) will cause direct damage to the foliage of *Leucaena leucocephala* cv Tarramba (leucaena), *Medicago sativa* cv L91 (lucerne), *Chloris gayana* cv Topcut (Topcut Rhodes grass) and cv Reclaimer (Reclaimer Rhodes grass) or affect seed germination. Three studies were conducted to address these questions.
- The first study aimed to provide a rapid preliminary assessment of the potential deleterious effects of saline and alkaline water on the foliage. Unamended CS water was obtained from Pleasant Hills (Queensland) with an electrical conductivity (EC) of 6.5 dS/m. Three treatments were prepared for overhead irrigation, consisting of 0% CS water (100% deionised water, control), 50% CS water (50% deionised water, EC 3.2 dS/m), and 75% CS water (25% deionised water, EC 4.6 dS/m). These waters were overhead-irrigated a total of 15 times across a five week period. This preliminary experiment demonstrated that:
    - Leucaena was comparatively sensitive to CS water, with some visual damage evident for 50% CS water (EC of 3.2 dS/m, this also causing a slight reduction in chlorophyll fluorescence (CF)). As expected, this damage was more pronounced for 75% CS water.
    - Lucerne was less sensitive to overhead irrigation with CS water than leucaena. Shoot biomass was not significantly reduced in any treatment up to 4.6 dS/m, but CF was reduced slightly (but not significantly) and the 75% CS water resulted in visual damage to the leaves.
    - Topcut Rhodes grass was the most tolerant species examined. Upon completion of the experiment, the plants were generally healthy and even 75% CS water did not appear to stress the foliage when assessed using visual symptoms, CF, or shoot biomass.
- A second more detailed study examined the potential deleterious effects of saline-sodic (EC ≤15 dS/m) and alkaline (≤2000 mg/L, $CaCO_3$ equivalent) water on foliage of Reclaimer Rhodes grass and leucaena in a range of growing-conditions:
    - Foliage of leucaena was sensitive, with necrosis and chlorosis evident for saline water at an EC ≥3 dS/m, but alkalinity had no significant effect. This damage to the foliage reduced shoot fresh mass for saline-treatments (an EC of 6 dS/m corresponding to a 50% reduction in fresh mass) but not for alkaline-treatments. Chlorophyll fluorescence in leucaena was also reduced, indicating that plants suffered stress.
    - Shoot fresh mass of Reclaimer Rhodes grass was not reduced in any treatment (up to 15 dS/m or 2000 mg/L $CaCO_3$ equivalent) nor was CF reduced. Thus, Rhodes grass was more tolerant than leucaena to overhead irrigation with saline water.
    - Growing conditions influenced the magnitude of the deleterious effects, with salinization of the soil increasing tolerance to foliar-applied saline water. In contrast,

plants grown in ambient conditions (i.e. outside the glasshouse) were less tolerant to foliar-applied salt.
    - This study has demonstrated that whilst saline-sodic and alkaline water can potentially be used for overhead irrigation, leucaena and Topcut Rhodes grass cannot be grown in a mixed farming system when overhead irrigated with CS water with an EC >3 dS/m since the species differ substantially in salt tolerance.
- The third study examined the effect of salinity (0-20 dS/m), sodium adsorption ratio (SAR, 30-∞) and alkalinity (0-2000 mg/L as $CaCO_3$) on seed germination of leucaena, Topcut Rhodes grass and Reclaimer Rhodes grass in a laboratory study.
    - For leucaena, germination decreased when EC increased above 15 dS/m, but SAR had no effect on germination. Root length was longest at EC 4 dS/m and SAR 5, and root growth declined when EC >10 dS/m.
    - Leucaena germination and root length was not affected by alkalinity (up to 2000 mg/L $CaCO_3$ equivalent), confirming results with overhead irrigation.
    - For Topcut Rhodes grass, germination was not significantly affected by the salinity, SAR or alkalinity tested. This Rhodes grass cultivar is highly tolerant towards the salinity and alkalinity found in CS water during germination and shoot growth when soils are amended with gypsum as part of the LAI.
    - For Reclaimer Rhodes grass, germination decreased when salinity increased above 15 dS/m, suggesting that the germination of this cultivar is less salt tolerant than Topcut Rhodes grass.
    - SAR had no significant effect on germination of Reclaimer, but there was a trend showing least germination at SAR ∞ (i.e. in Ca-free solutions), but this finding is of little practical importance since soils will be amended with gypsum during LAI.
    - Alkalinity had no significant effect on germination of Reclaimer.
- The studies showed little differences in plant responses to CS water or saline solutions, therefore, the detrimental effect is due to Na and Cl in CS water.
- Under the current experimental conditions, overhead irrigation with water or 3-6 dS/m is safe for both the germination and growth of Rhodes grass, but the limit for leucaena is ≤ 3 dS/m. However, growth conditions (such as temperature, humidity, pests and diseases) will influence the nature and extent of the deleterious effects caused by the overhead irrigation of CS water – these factors need to be taken into account when developing an appropriate irrigation regime in the field.

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Introduction

This Santos-UQ project investigated the effect of saline, sodic and alkaline water on plant foliage and seed germination. The project consisted of three subprojects. The first project ("A preliminary assessment of the potential impacts of the overhead irrigation of saline and alkaline water on plant foliage") investigated the effect of coal seam (CS) water from Pleasant Hills (EC 6.5 dS/m) at two dilutions on the foliage of *Leucaena leucocephala* cv Tarramba (leucaena), Medicago sativa cv L91 (lucerne), and *Chloris gayana* cv Topcut (Topcut Rhodes grass). In that study, leaf age was not monitored and leucaena plants had mature foliage when irrigations began. In the second project ("Seed germination under saline and alkaline conditions"), the effect of saline and alkaline solutions on germination of seeds of two Rhodes grass cultivars (Topcut and Reclaimer) and leucaena was investigated. In the third and final project ("Overhead irrigation of saline-sodic and alkaline water: Examination of the potential deleterious effects on foliage of Rhodes grass and leucaena"), solutions spanning a range of salinities and alkalinities were applied to Reclaimer Rhodes grass and leucaena with carefully controlled foliage age. In addition, the study investigated the effect of environmental conditions (soil salinity, glasshouse and external) on resistance of plants to saline overhead irrigation.

For sake of continuity and clarity, project one (first overhead irrigation study) and project three (second overhead irrigation study) are discussed together since they deal with overhead irrigation, and project two (seed germination study) is discussed last.

In low-rainfall environments, the extraction of saline-sodic and alkaline ground waters (such as for coal seam gas (CSG) production) is potentially valuable for agricultural production. For example, the Great Artesian Basin (Australia) which is the largest artesian basin in the world, contains an estimated 65,000 million ML of groundwater (Nevill et al., 2010). This groundwater (including the CS water) can be beneficially used to increase agricultural production. However, much of the water in the Great Artesian Basin is saline and alkaline, with the electrical conductivity (EC) values typically ranging from 1 to >10 dS/m (Great Artesian Basin Consultative Council, 1998). Therefore, it is important that the irrigation of these waters does not result in degradation of the soil resource and that it does not reduce plant growth.

The potentially adverse effects of salts within the rooting environment (soil) are well-known, causing plant osmotic stress, ion toxicity, and decreased photosynthesis and growth (Munns, 2002; Paz et al., 2012; Tester & Davenport, 2003). However, little information is available regarding the direct effect of the overhead irrigation of saline and alkaline waters on plant foliage. A report by FAO (1985) indicated that for equal water quality, plant physiological responses vary between overhead and direct irrigation of soil. For example, whilst *Citrus sp.* displayed foliar symptoms when sprinkler-irrigated with water containing 3 mM Na and Cl, no effects were observed when the same water was applied through flood and furrow irrigation. Similarly, a comparative study on bell pepper (*Capsicum frutescens*) by Bernstein and Francois (1973) examined yield response from furrow, drip, and sprinkler irrigation with water at an EC of 4 dS/m, with these authors finding a reduction in yield of 18% for furrow irrigation, 2% for drip irrigation, and 59% for sprinkler irrigation. However, the large

yield decrease for sprinkler irrigated pepper plants was caused by cumulative salt absorption by rooting-system and foliage. Therefore, an estimate of the foliar damage caused by Na and Cl directly absorbed by leaves was not possible. The effects of the overhead irrigation of CS water on cotton (*Gossypium hirsutum*), barley (*Hordeum vulgare*) and Italian ryegrass (*Lolium multiform*) was investigate by Beletse et al (2008). The irrigation water with a total alkalinity of 4712 mg/L (as $CaCO_3$ equivalent) and at an EC of 7.5 dS/m caused leaf scorching only in cotton. However, in the experiment of Beletse et al. (2008) (as seen in Bernstein and Francois (1973)) the irrigation water was able to move through the soil profile, thereby plant symptoms were caused by both root and foliar exposure to salinity and alkalinity. Interestingly, CS water produced only minor symptoms in leaves of cotton, thus suggesting great variability to salt and alkali tolerance between plant species. Maas (1985) reported that sprinkling irrigation with saline water can produce foliar injury such as chlorosis and necrosis due to increased foliar absorption of Na and Cl, however, the magnitude of these symptoms varies substantially across plant species. Foliar toxicity symptoms tend to appear in older leaves and under hot and dry conditions (Maas, 1985; Maas et al., 1982; McCune, 1991). This is possibly due to the high evapotranspiration rate and enhanced accumulation of Na and Cl on leaf surfaces.

Although evidence from published research suggests that the overhead irrigation of saline-sodic and alkaline water can potentially produce adverse effects on plant foliage, in almost all previous studies, irrigation water was applied to both plant foliage and to the soil (resulting in additive impacts).

The two studies reported here aimed to establish a threshold for the safe overhead irrigation of CS water by examining the potential deleterious effects of saline-sodic and alkaline water when applied to the foliage of Reclaimer Rhodes grass and leucaena. The effect of growth conditions was also examined, with plants grown either inside the glasshouse or in ambient conditions (i.e. external to the glasshouse). Furthermore, whilst most plants were grown in a non-saline soil from which the foliar-applied saline water was excluded, some plants were grown in a salinised soil. Plant performance was assessed using a range of parameters, including visual symptoms, chlorophyll fluorescence (CF), and fresh mass production. The results of these experiments will assist in the development of management guidelines for the beneficial use of saline-sodic and alkaline water in overhead irrigation programs.

Germination and early growth of plants can also be affected by salinity. Germination is considered to have taken place when the seed swells due to water uptake and the pre-formed radicle in the seed expands and breaks through the seed coat. Thus, initial phases of germination require water uptake and may be inhibited by high salinities due to osmotic stress. By contrast, continued root and shoot growth requires cell division and elongation and the cell division process may be affected by either osmotic effects or ion toxicities (Lambers et al. 2008). In some plants, germination is less affected than root growth [e.g. *Bouteloua gracilis* (Zhang et al. 2012); *Zea mays* (Zhang and Zhao 2011); *Triticum aestivum* (Lin et al. 2012)] whereas in other plants, root growth may be less affected than germination [e.g. *Buchloe dactyloides* (Zhang et al. 2012)].

The chemical properties of saline topsoils, or soils irrigated with CS water, may impair seed germination and establishment of plant species. While the effect of saline conditions on seed germination is reasonably well understood, large species differences exist (Ashkan and Jalal 2013;

Mahmood et al. 1996; Tobe et al. 2003). Calcium ions are considered to alleviate the effects of Na toxicity due to membrane effects, but few studies have investigated the effect of Na/Ca ratio, expressed in this study as sodium adsorption ratio (SAR) on germination (Tobe et al. 2002; 2004; Tobe et al. 2003; Zehra et al. 2012). By contrast, the effect of alkalinity on germination of pasture species is poorly researched, and alkalinity is considered to be more detrimental than salinity to both seed germination and root growth (Li et al. 2010; Lin et al. 2012; Zhang et al. 2015; Zhang and Zhao 2011). The aim of the third study was to investigate the effect of increasing salinity and alkalinity on the germination of leucaena, Topcut and Reclaimer Rhodes grass. These salt-tolerant species are used for mixed pasture systems in Queensland and may be overhead-irrigated with CS water. It was hypothesised that seed germination would be inhibited at high salinities and that there would be differences in salt tolerance between the pasture species.

Materials and methods

Briefly, in the first overhead irrigation study, *Leucaena leucocephala* cv Tarramba (leucaena), *Medicago sativa* cv L91 (lucerne), *Chloris gayana* cv Topcut (Topcut Rhodes grass) were studied. Plants were grown in non-saline soil (commercial potting mix) and overhead irrigated with deionised water or CS water from Pleasant Hills (EC 6.5 dS/m) diluted to 50% (EC 3.2 dS/m, pH 9.5, 790 mg/L alkalinity as $CaCO_3$) or 75% strength (EC 4.6 dS/m, pH 9.5, 1140 mg/L alkalinity as $CaCO_3$). No individual leaves were tagged in this study and plants were irrigated up to 15 times. The second overhead irrigation study utilised only two species (leucaena and Reclaimer Rhodes grass). In this study solutions with a range of salinities were prepared (0-15 dS/m) and the effect of increasing alkalinity (0-2000 mg/L as $CaCO_3$) at a basal salinity of 4 dS/m were investigated over 30 irrigations. Furthermore, some of the plants were grown in saline soil (ECse 10 dS/m) or outside the glasshouse to test the effect of environmental conditions on plant growth. In the germination study, the effect of salinity, SAR and alkalinity on seed germination of leucaena, and Topcut and Reclaimer Rhodes grass species was determined in the laboratory.

For detailed explanation of the experimental variables and set-up please refer to the Appendices describing the respective studies.

Results and Discussion

Foliar damage from overhead irrigation

Foliar damage was consistent in the two overhead irrigation studies, despite the first study using dilute CS water and the second study using defined salt solutions (Figure 1). Results from both studies showed that damage to leaves differed between species and increased with concentration and frequency of irrigation.

For leucaena, some leaflets irrigated with the 50% (EC = 3.2 dS/m) or 75% (EC = 4.6 dS/m) CS water showed chlorotic and necrotic lesions, corresponding to areas where the water droplets had accumulated and dried (Figure 1). As expected, the magnitude of these symptoms was substantially greater at 75% CS water than at 50% CS water. For plants in both the 50% and 75% CS water treatments, the extent of damage to the foliage was variable, with some leaflets showing no symptoms whilst others appeared to have substantial damage. In addition, for plants irrigated with 75% CS water, it was also noted that the formation of chlorotic lesions was followed by a general chlorosis of the entire leaflet prior to their eventual abscission – this becoming particularly noticeable after ca. 7-8 irrigations.

Figure 1. Foliar symptoms of salt stress on leucaena cv Tarramba leaves after 15 irrigations. Top row: plants irrigated with DI water (control) (top left), 50% strength CS water (EC 3.2 dS/m) (top centre), or 75% strength CS water (EC 4.6 dS/m) (top right). Bottom row: plants irrigated with DI water (control) (bottom left), or saline solutions with 3 dS/m (bottom centre) or 5 dS/m (bottom right).

Leucaena irrigated with saline water (not diluted CS water) also showed chlorotic and necrotic lesions, appearing after only three irrigation events, with the magnitude of this damage increasing with increasing salinity (being observed in all treatments with an EC ≥3 dS/m) and with an increasing number of irrigations (Figure 1). Foliar abscission occurred in severely damaged leaves, with this abscission becoming particularly pronounced after ca. 10 irrigations for the 8 dS/m treatment.

Symptoms appeared more severe in the second irrigation study than in the first study and this can be attributed to the age of the leaves: in the first study, leaves were fully formed and mature, whereas in the second study, leaves were young and just fully expanded.

The effects of CS water on Rhodes grass were similar to those described for leucaena, although the symptoms generally did not appear to be any worse at 75% CS water than at 50% CS water (Figure 2). Application of CS water resulted in the formation of chlorotic and necrotic lesions, these often being confined to the leaf margins. Interestingly, visual assessment indicated that whilst the leaf tissues were initially damaged by the foliar-application of CS water, the severity of symptoms appeared to decrease over time. It is possible that shedding of affected leaves and replacement with leaves better adapted to saline irrigation resulted in improved appearance of plants. For this reason, individual leaves/tillers were marked in the second irrigation study to ensure leaves received the intended number of irrigations.

Figure 2. Foliar symptoms of salt stress on Rhodes grass after 15 irrigations. Top row: plants of cultivar Topcut irrigated with DI water (control) (top left), 50% strength CS water (EC 3.2 dS/m) (top centre), or 75% strength CS water (EC 4.6 dS/m) (top right). Bottom row: plants of cultivar Reclaimer irrigated with DI water (control) (bottom left), or saline solutions with 3 dS/m (bottom centre) or 5 dS/m (bottom right).

In the second irrigation study, Rhodes grass irrigated with salt solution responded similarly to grass irrigated with CS water (Figure 2). Symptoms were not were observed until ca. 10 irrigations, when necrotic and curly leaf tips developed in most plants (particularly those growing in saline soil). However, there was little indication that leaves were shed due to saline overhead irrigation. It needs to be cautioned that the cultivars used in differed between the first and second study (Topcut vs Reclaimer), and this may affect results slightly. Overall, these results confirm that Rhodes grass is salt tolerant. Rhodes grass is a halophyte and is able to tolerate relatively high salinity concentrations in soil due to its ability to accumulate excess salt within its leaves (Kopittke et al., 2009), and to secrete salt in excess through bicellular glands (Kobayashi et al, 2007). Salt excretion may occur for salt taken up through roots or through leaves.

Figure 3. Foliar symptoms of salt stress on lucerne cv L91 after 15 irrigations. Left: DI water (control); centre: 50% strength CS water (EC 3.2 dS/m); right: 75% strength CS water (EC 4.6 dS/m).

The shoots of lucerne (only used in the first irrigation study) also showed chlorotic and necrotic lesions resulting from the application of 50% or 75% CS water (the severity of the symptoms being higher at 75% CS water) (Figure 3). The severity of these symptoms did not appear to decrease over time. Lucerne was not part of the second irrigation study, therefore was not irrigated with salt solution.

Effect of alkalinity

The effects of increasing alkalinity (at constant salinity of 4 dS/m) in the overhead irrigation water were examined in the second overhead irrigation study. For both leucaena and Reclaimer Rhodes grass, alkalinity values of up to 2000 mg/L ($CaCO_3$ equivalent) had no adverse impact on foliage other than that attributable to the basal salinity (4 dS/m) (Figure 4), i.e. the observed damage was caused by the salinity, not the alkalinity of the irrigation water.

Figure 4. Effect of combined salinity and alkalinity on leucaena cv Tarramba (left side) and Reclaimer Rhodes grass (right side) after 15 irrigations. Plants were irrigated with solutions containing 4 dS/m and: 0 alkalinity (control) (top row): 250 mg/L alkalinity ($CaCO_3$) (centre row): and 2000 mg/L alkalinity ($CaCO_3$) (bottom row).

Only one study was identified in the literature where the effect of alkaline water application on plant foliage was investigated. Beletse et al. (2008) examined the effect of sprinkler irrigation on cotton, Italian ryegrass, and barley (salt tolerant crops) with sodium bicarbonate ($NaHCO_3$) rich water from CSG operations in South Africa. Despite the water used by Beletse et al. (2008) having a total alkalinity of 4712 mg/L (as $CaCO_3$ equivalent) and at an EC of 7.5 dS/m (this being substantially higher than the water used in the present study) these authors found that there was no significant foliar damage, with only cotton having scorched leaves. Thus, based upon the evidence in the present study and that reported previously, it seems that overhead irrigation of alkaline water up to 2000 mg/L ($CaCO_3$ equivalent) is not deleterious to plant foliage of Reclaimer Rhodes grass, whereas in leucaena, the salinity was more detrimental than the alkalinity.

Effect of salt stress on chlorophyll fluorescence

Chlorophyll fluorescence (CF) is considered to indicate functioning of the photosystem. For leucaena irrigated with CS water, a significant interaction (P<0.001) was found between the irrigation number and the water quality, thereby indicating that the water quality influenced the CF. In leucaena, CF decreased (indicating stress) with increasing irrigation and CS water concentration (Figure 5). Therefore, leucaena photosynthesis appears to be sensitive to overhead irrigation with saline and alkaline water.

Leucaena overhead-irrigated with saline or alkaline water up to six times, showed no effect on CF (Figure 6). The only exception to this was at the highest salinity treatment (8 dS/m) in ambient conditions, with the slight decrease in fluorescence in this treatment indicating stress. However, the decrease in CF became more marked with increasing irrigations, with salinity causing a decrease in fluorescence in treatments with EC values of ≥ 3 or 4 dS/m (Figure 6), whilst alkalinity decreased fluorescence in treatments with a total alkalinity of ≥ 250 mg/L ($CaCO_3$ equivalent) (Figure 6d). These results confirm the observation made in the first overhead irrigation study, i.e. leucaena experiences stress with increasing salinity and number of irrigations.

For Rhodes grass, the irrigation of either 50% or 75% CS water did not significantly decrease CF at any of the three measurement periods (P=0.208) (Figure 5). However, it was found that CF decreased significantly between irrigation events (P<0.001), with CF significantly lower after 11 or 15 irrigations than following only five irrigations. It is not clear what caused this gradual reduction in CF in Rhodes grass (which was consistent regardless water quality), although it was possibly due to general aging of the leaves.

Similarly, Rhodes grass irrigated with saline or alkaline water showed no impact on CF in any treatment, regardless of the number of irrigations (Figure 7). It should be noted, however, that fluorescence tended to decrease with increasing irrigation, regardless of treatment, again this could be due to aging of the leaves.

For lucerne, the interaction between the irrigation number and water quality was not significant (P=0.475), but the water quality had a significant effect on CF (P<0.001) (Figure 5).

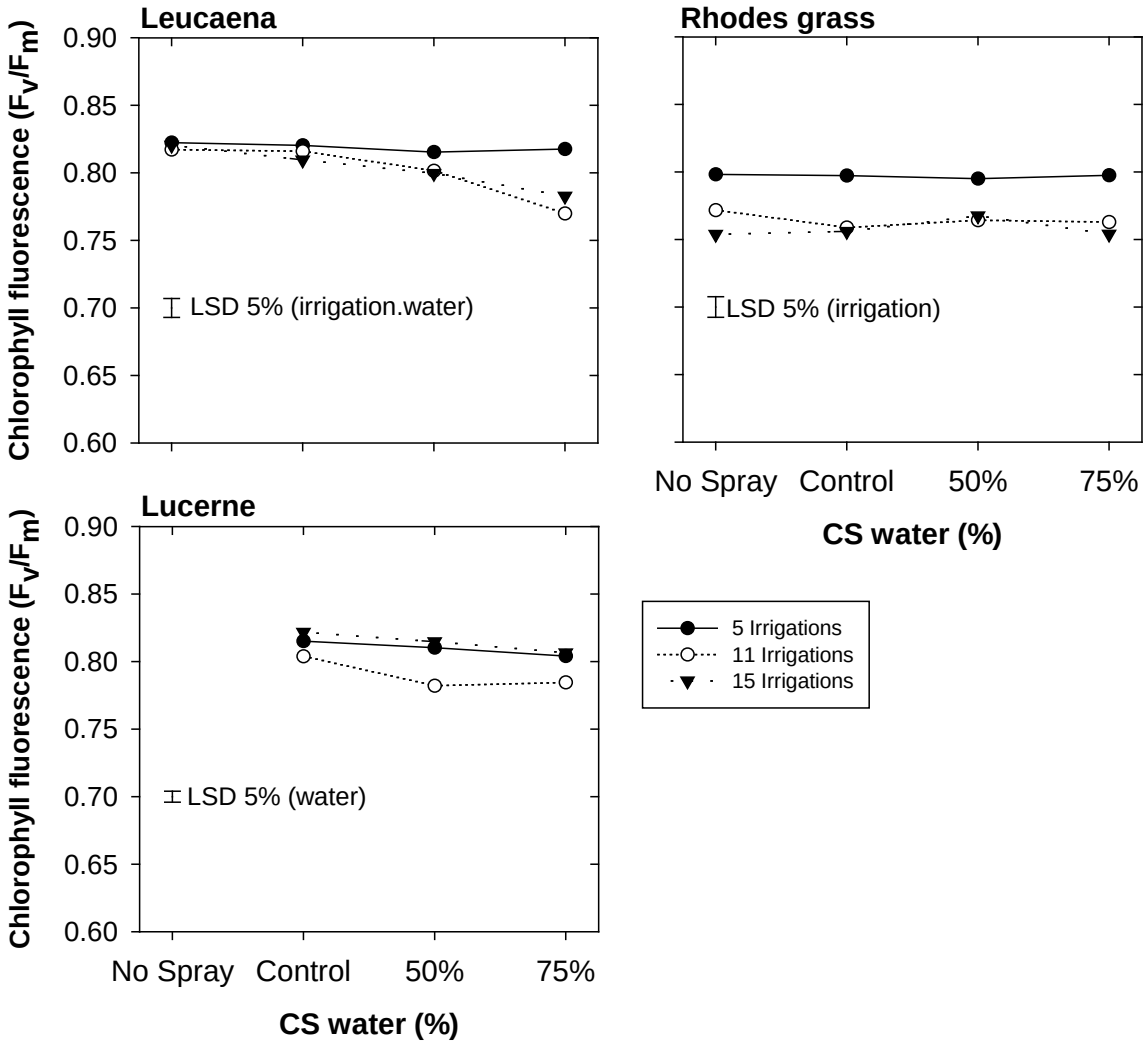

Figure 5. Effect of overhead irrigation with CS water on CF of leucaena, Rhodes grass, and lucerne leaves after 1 hour dark adaptation. Leaves were measured in plants that had received no overhead irrigation ("No spray") or in plants that had been overhead-irrigated a total of five, 11, or 15 times. Each point is the arithmetic mean of 18 measurements. The vertical bars represent the least significant difference. Where there was a significant interaction between the irrigation number and water quality ("irrigation.water"), the LSD value can be used to compare any two points on the plot. Where the CF was influenced significantly by the number of irrigations ("irrigation"), the LSD value can only be used to compare the effect of the number of irrigations on CF at a single water quality. Where the CF was influenced significantly by the water quality ("water"), the LSD value can only be used to compare the effect of water quality on CF within a single irrigation-event. There were no spare lucerne plants available which were not being overhead-irrigated during the experimental period, and hence a 'No Spray' value could not be determined.

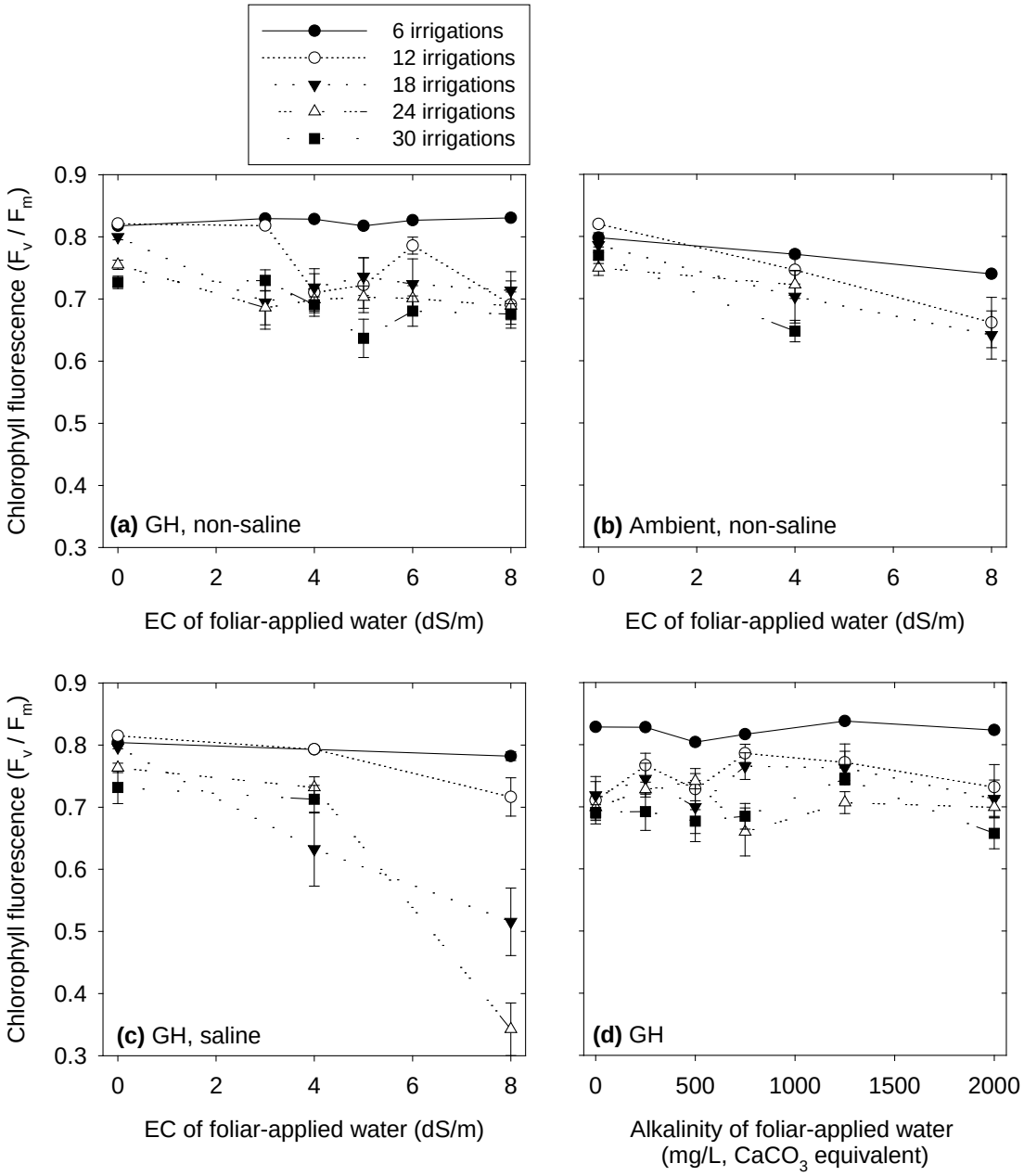

Figure 6. Chlorophyll fluorescence of leucaena overhead irrigated with saline water (a, b, and c) and EC 4 dS/m alkaline water (d). Measurements were taken on five leaves of equal age (previously tagged) that were randomly selected from plants that received a total of 6, 12, 18, 24 and 30 irrigations. Each point represents the arithmetic mean of 15 measurements. Plants overhead irrigated with saline water at an EC of 8 dS/m and growing in: (i) saline soil (b); and (ii) ambient (c) had shed their fully mature leaves before a total of 30 and 24 irrigations, respectively. As a consequence, CF values could not be determined.

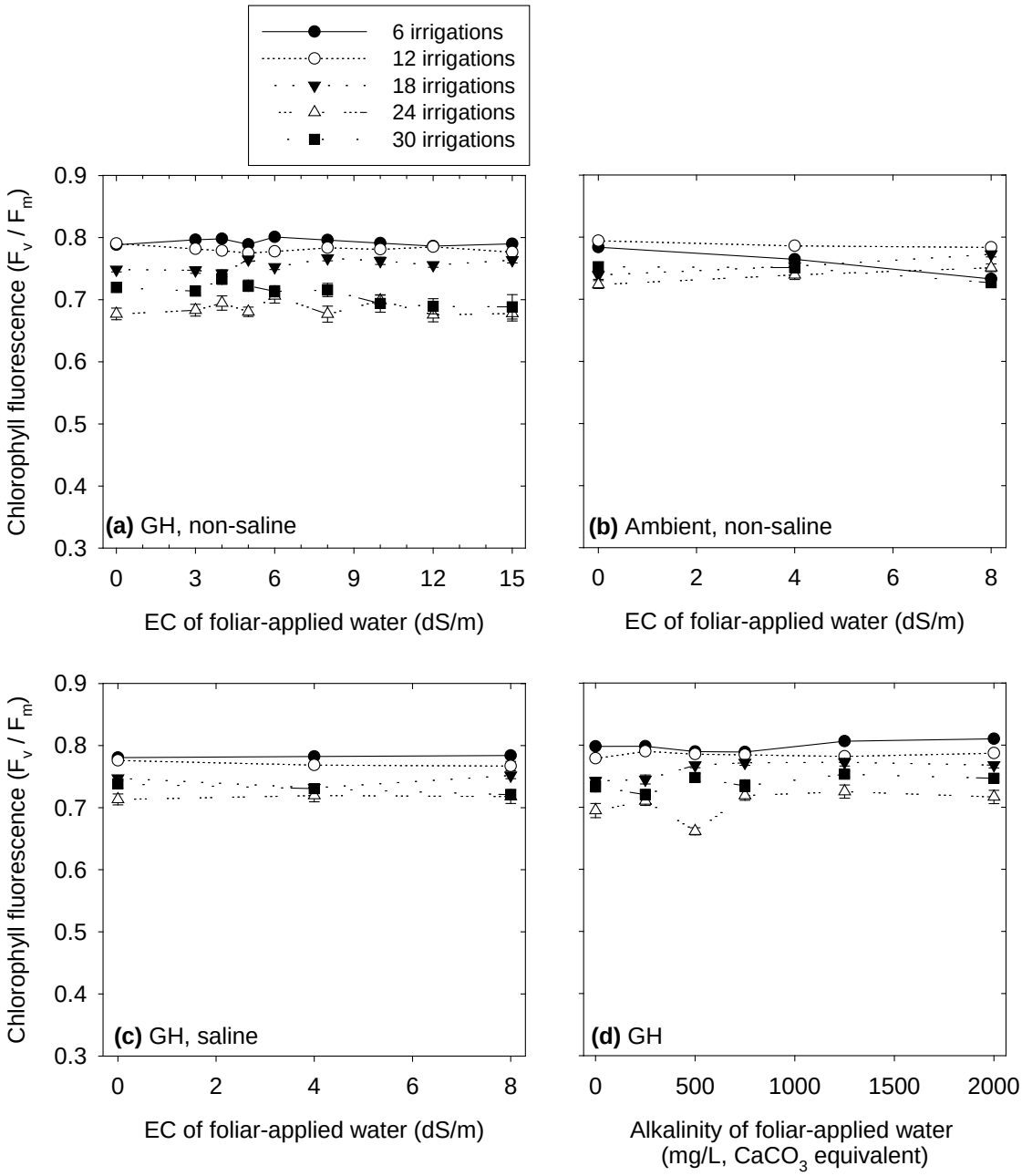

Figure 7. Chlorophyll fluorescence of Rhodes grass overhead irrigated with saline water (a, b, and c) and EC 4 dS/m alkaline water (d). Measurements were taken on five leaves of equal age (previously tagged) that were randomly selected from plants that received a total of 6, 12, 18, 24 and 30 irrigations. Each point represents the arithmetic mean of 15 measurements.

For all three plant species, the relationship between CF and tissue Na concentration was poor, indicating that increasing tissue Na concentrations could not explain changes in fluorescence (data not shown). Similarly, no consistent relationship was found between CF and tissue concentrations of

K, Ca, P, S, or Mg, again indicating that changes in mineral nutrition could not explain changes in CF (data not shown – see Appendices A1 & A2).

Overall, the result show that CF is a sensitive measure of plant stress and that Rhodes does not suffer stress due to overhead irrigation with up to 15 dS/m water, whereas leucaena and lucerne is sensitive to saline water when the EC is >3 dS/m. Notably, the response of the Rhodes grass and leucaena to saline water or CS water was very similar, indicating that it is mainly the Na and Cl in CS water that is detrimental to plants.

Effect of salt stress on biomass

In the first overhead irrigation study, biomass increased over the five week period of irrigation with CS water, but the quality of the irrigation water did not have a significant effect on the fresh mass of the shoots for either Rhodes grass or lucerne (leucaena biomass was not measured). While there were no significant differences, however trend lines suggested that Rhodes grass was unaffected by CS water, whereas lucerne showed a slight decrease in biomass (Figure 8). Not unexpectedly, a significant difference (P<0.001) was found between the mass of Rhodes grass (90 g/pot) and lucerne (42 g/pot) shoots averaged across all three treatments (Figure 8).

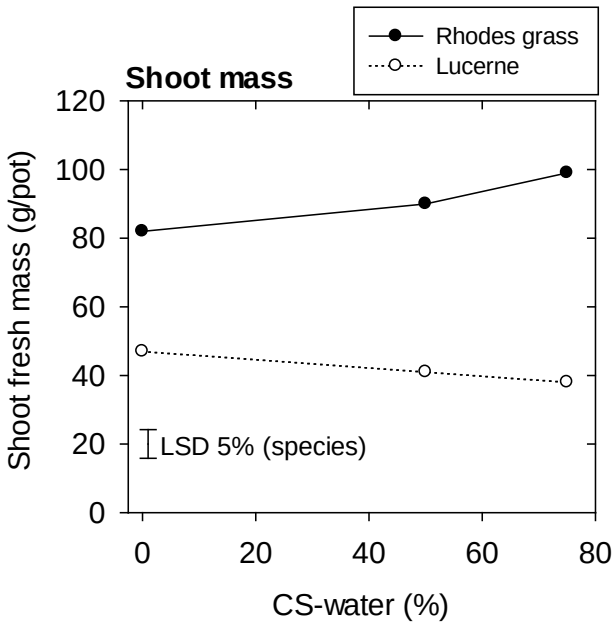

Figure 8. The fresh mass of shoots for Rhodes grass or lucerne overhead-irrigated 15 times with solutions which contained 0, 50, or 75% CS water (the remainder being deionised water). Both plant species were grown for four weeks prior to imposing the treatments for a further five weeks. The LSD value presented allows comparison between plant species only (there was no significant interaction between species and water composition, nor did the water composition cause significant differences in shoot fresh mass within species).

In the second overhead irrigation study, saline water clearly decreased leucaena biomass with increasing salinity (Figure 9a). Interestingly, when leucaena was grown in a saline soil (ECse 10 dS/m), the tolerance to overhead irrigation with saline water appeared to increase. In contrast, the data suggest that leucaena grown outside the glasshouse was more sensitive to overhead irrigation with saline water. Indeed, for leucaena grown in ambient conditions, overhead irrigation with water at an EC of 4 dS/m resulted in a ca. 60% reduction in fresh mass (c.f. the 34% reduction when grown in the glasshouse) (Figure 9a). Note that overall biomass production outside the glasshouse was less than inside the glasshouse, presumably due to lower air temperatures.

Although salinity had an adverse impact on the shoot fresh mass of leucaena (Figure 9a), alkalinity (up to 2000 mg/L as $CaCO_3$ equivalent) had no additional adverse impact on plant shoot mass above that caused by the basal salinity (4 dS/m) of the alkalinity treatments (Figure 9b).

Salinity and alkalinity caused no reduction in fresh mass for any treatment with Rhodes grass – fresh mass values at an EC of 15 dS/m and at an alkalinity of 2000 mg/L $CaCO_3$ equivalent at EC 4 dS/m being similar to that in the corresponding controls (Figure 10).

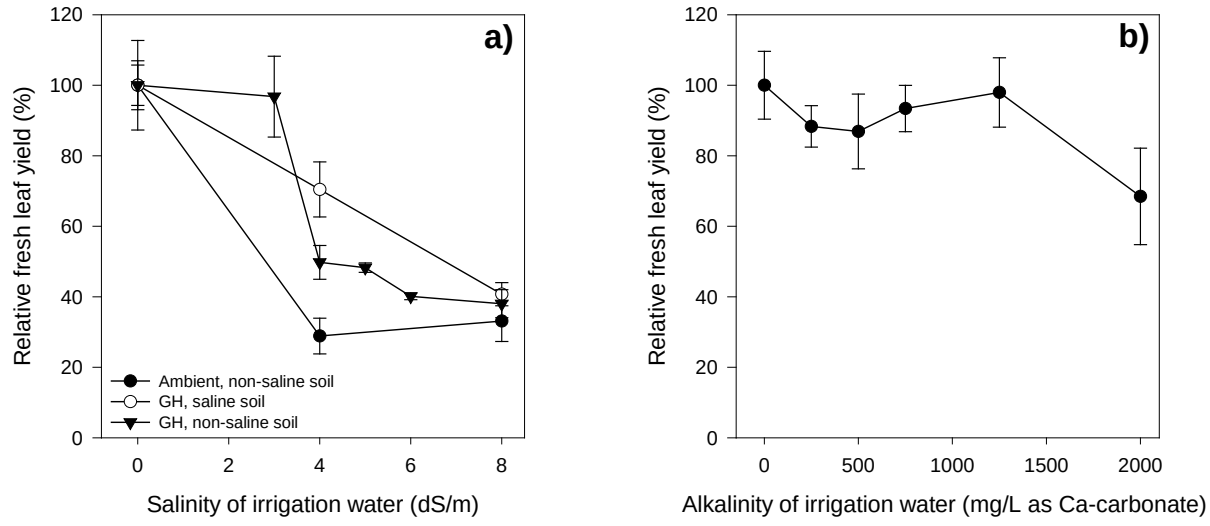

Figure 9. Fresh mass of leucaena leaves overhead irrigated 30 times with either saline (a) or alkaline water (b). Plants were grown in the glasshouse (GH) or in ambient conditions (i.e. outside the glasshouse), and were either grown in a non-saline or saline soil (ECse 10 dS/m). Alkaline water contained increasing amount of alkalinity in a background salinity of 4 dS/m.

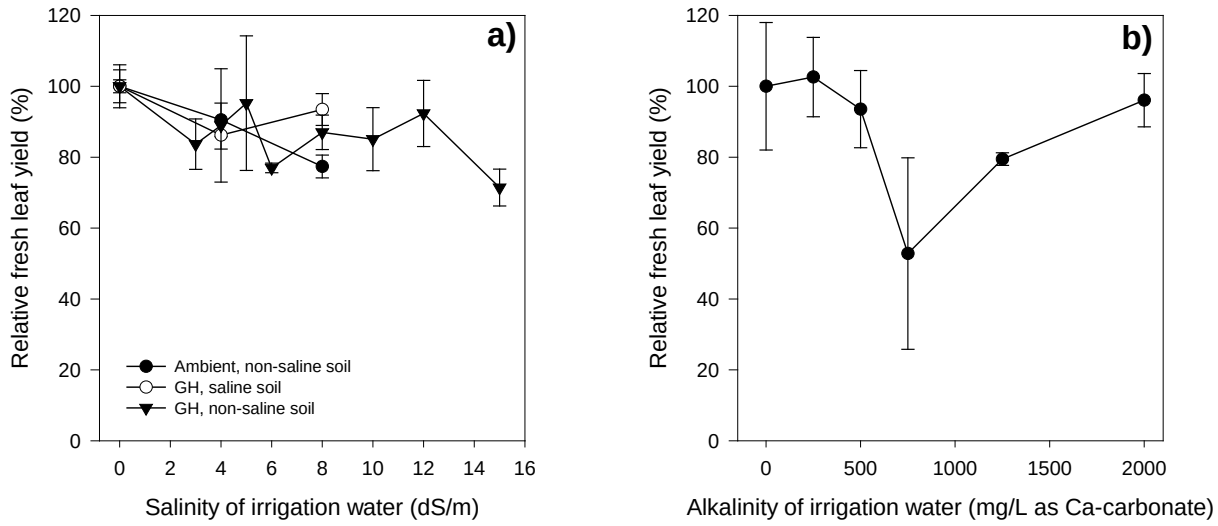

Figure 10. Fresh mass of Rhodes grass overhead irrigated 30 times with either saline (a) or alkaline water (b). Plants were grown in the glasshouse (GH) or in ambient conditions (i.e. outside the glasshouse), and were either grown in a non-saline or saline soil. Alkaline water contained increasing amount of alkalinity in a background salinity of 4 dS/m.

Influence of growth conditions

In leucaena, a non-halophyte species, the magnitude of foliar damage increased for all growing conditions, with increasing water salinity (EC ≥3 dS/m) and number of irrigations. However, plants grown in saline soils without overhead irrigation showed little damage, indicating that leucaena is capable of withstanding high soil salinity (ECse 10 dS/m) but not foliar irrigation with mildly saline water (EC 4 dS/m) (Figure 11). For Rhodes grass (halophyte species) only plants grown in either ambient (non-glasshouse conditions) or in saline soil, showed an increase in the severity of symptoms with increasing salinity of the irrigation water (Figure 11).

The cumulative effect of root and foliar exposure to salinity, therefore, increased foliar necrosis and chlorosis in leucaena and Rhodes grass. Similar results were observed in a study conducted by Benes et al (1996), in which barley leaves from plants grown in saline soil at an EC of 9.6 dS/m and overhead irrigated with saline water of equal EC, displayed increased leaf scorching than plants growing in non-saline soil but only sprayed with saline water at an EC of 9.6 dS/m. Transpiring leaves of plants exposed to saline soil, undergo rapid modifications in cell water content caused by osmotic stress (against which the plant can initially adjust), however, when Na and Cl accumulate in the cytoplasm (and the vacuole can no longer compartmentalise these ions) enzyme activity is compromised (Munns, 2002). In addition, osmotic adjustment, ion compartmentalisation and salt excretion (in Rhodes grass), are processes that require a substantial amount of energy, that is taken away from resources otherwise used for plant growth (Raven, 1985). Therefore, plant osmotic adjustment combined with foliar injury were possible causes for the reduced overall growth in saline soil conditions.

Foliar injury was also evident in both plant species grown in ambient (non-glasshouse) conditions (Figures 12 and 13). Plants water loss though stomata tend to increase with hot and dry weather and under these conditions, several authors (Bernstein, 1975; Maas, 1985; McCune & Silberman, 1991) reported an increase in foliar injury following overhead irrigation with saline water. In the second overhead irrigation study, glasshouse and external ambient average maximum temperatures were similar. However higher levels of humidity were maintained under glasshouse conditions through a water-wall-air circulating system. Higher humidity levels, therefore, could have decreased the severity of symptoms when compared to plants grown in ambient conditions because of the reduced transpiration rate in glasshouse conditions.

Figure 11. Comparison between plants grown in non-saline or saline soil. Left hand column: non-saline soil, irrigated 15 times with DI water (0 dS/m, control). Centre column: grown in saline soil (ECse 10 dS/m) and irrigated 15 times with DI water (EC 0 dS/m). Right hand column: grown in saline soil (ECse 10 dS/m) and irrigated 15 times with saline water (EC 4 dS/m). Top row: Topcut Rhodes grass. Bottom row: leucaena.

Figure 12. Leucaena grown inside the glasshouse (left side) or outside (right side) and irrigated 15 times with saline solution of 0 dS/m (control) (top row) or 4 dS/m (bottom row).

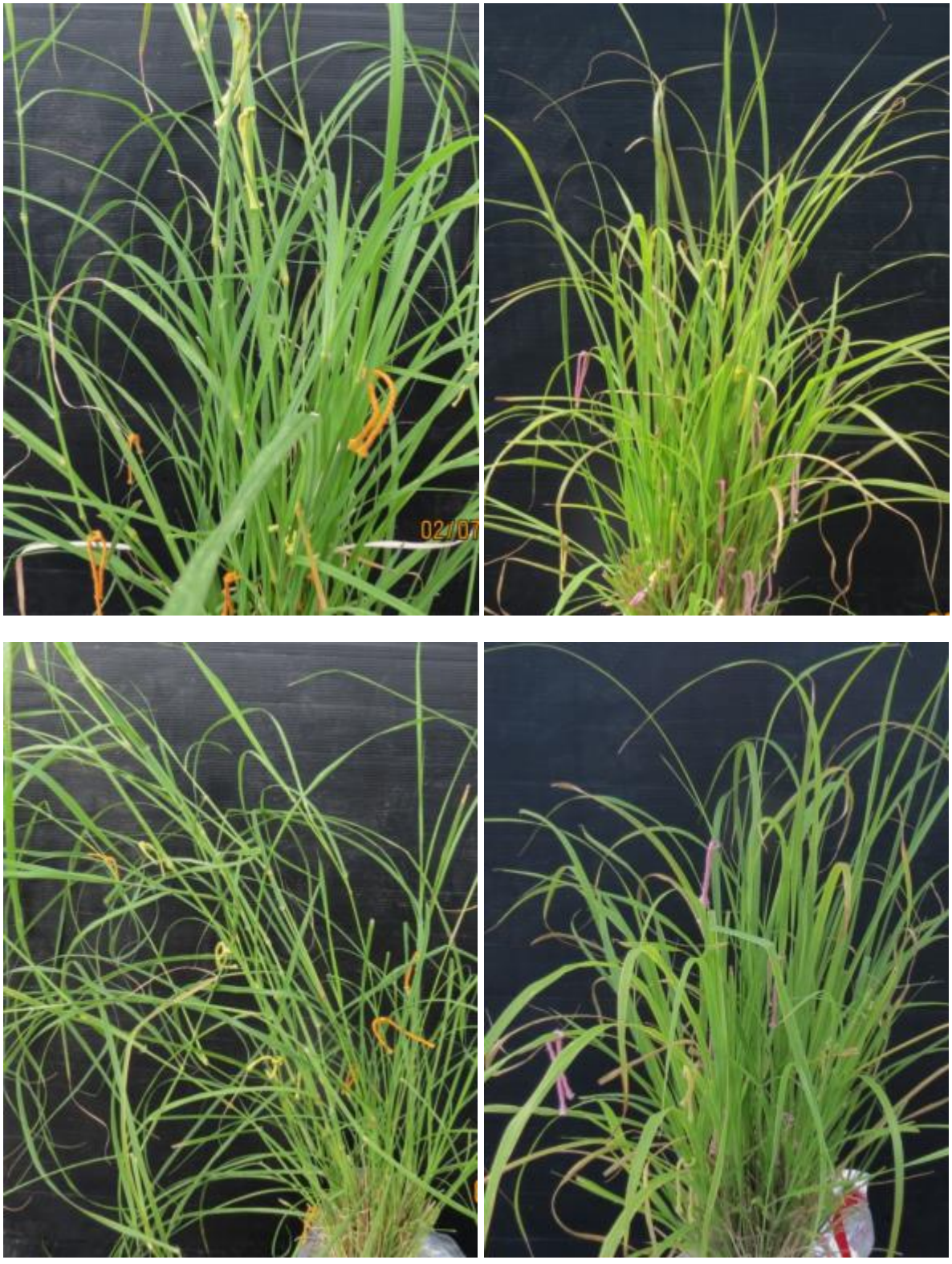

Figure 13. Reclaimer Rhodes grass grown inside the glasshouse (left side) or outside (right side) and irrigated 15 times with saline solution of 0 dS/m (control) (top row) or 4 dS/m (bottom row).

Effect of overhead irrigation on tissue Na and Cl concentrations

In plants irrigated with CS water, tissue concentrations of Na increased with irrigation water salinity and number of irrigation events. Tissue Na concentrations in leucaena were lower (6 mg/kg after 15 irrigations with 75% CS water) than in the other two species (12 mg/kg for lucerne and 8-10 mg/kg for Rhodes grass) (Figure 14). Similar results were obtained for leucaena and Rhodes grass overhead irrigated with saline or alkaline water (Figure 15).

Given that Rhodes grass was also the species which appeared to be the most tolerant of overhead irrigation with CS water (see elsewhere), the formation of damage in leaf tissues of different species does not appear to be necessarily related to the concentration of Na taken up into the tissues *per se*. Interestingly, unlike some other species, Rhodes grass is known to accumulate (c.f. exclude) Na in shoot tissues during growth in saline rooting media (Smith 1981; Kopittke et al. 2009). It may be that the hairiness (and greater water droplet retention) of lucerne and Rhodes grass resulted in more Na accumulation than in leucaena. Despite leucaena having lower Na accumulation, it was more damaged by Na and it may be that the damage caused is related to a Na detoxification mechanisms (i.e. shedding of leaves high in Na).

Although Na-dominated salinity can sometimes induce deficiencies of other cations (particularly Ca), leaf tissue concentrations of elements other than Na were not consistently effected by the overhead irrigation of CS water (Appendices A1 & A2).

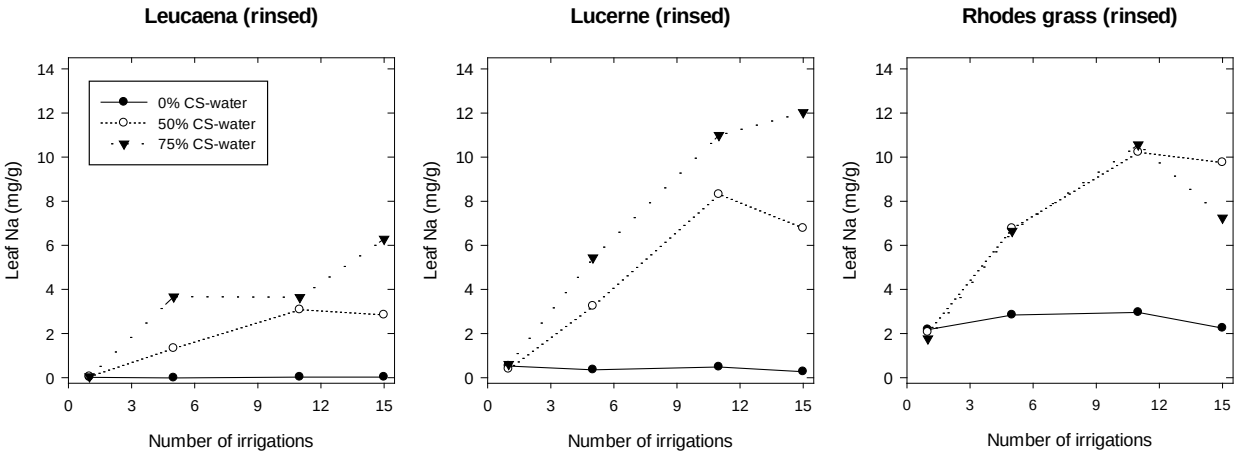

Figure 14. Effect of number of overhead irrigations on the Na concentrations in leaf tissues of leucaena, lucerne, and Rhodes grass when using 0% CS water, 50% CS water, or 75% CS water. Tissue concentrations were determined after 1, 5, 11 and 15 irrigations. Between irrigations with the relevant treatment (CS water), care was taken to ensure that no other water was sprayed onto the leaves (for example, rainfall), and hence any salts were allowed to accumulate. Immediately following harvest, leaves were rinsed in deionised water to remove any free salts from the leaf surface. Tissues from all replicates were combined prior to analysis and hence no error bars can be presented.

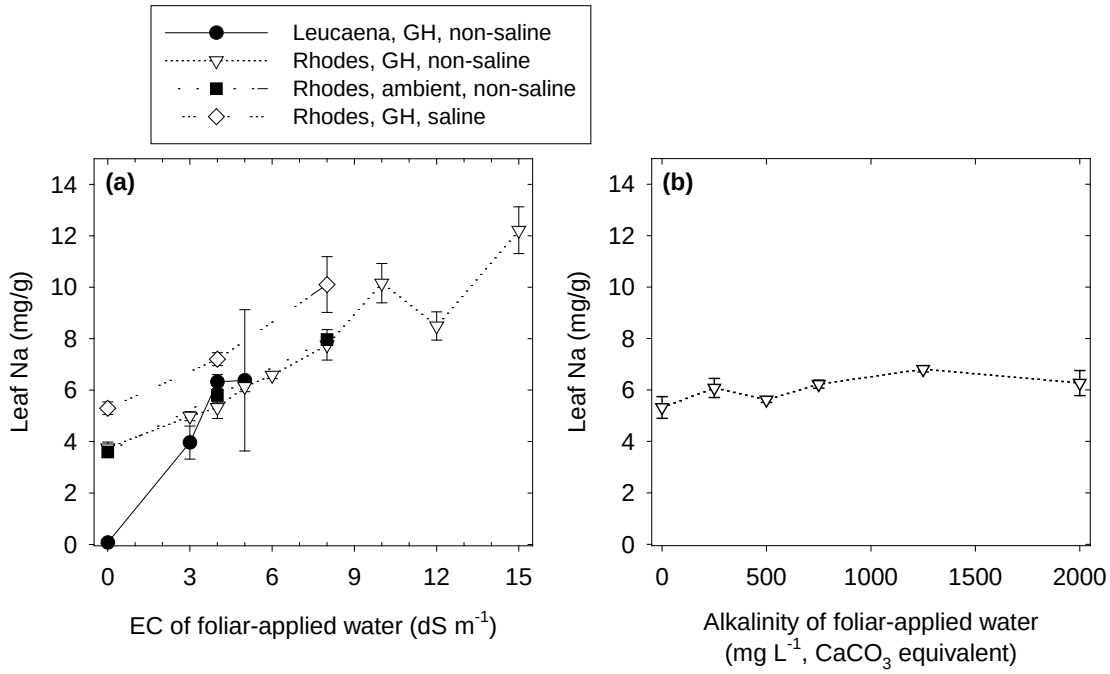

Figure 15. Tissue concentrations of Na (a,c) for leucaena and Rhodes grass overhead irrigated 30 times with either (a) saline or (b) alkaline water. Plants were grown in the glasshouse (GH) or in ambient conditions (i.e. outside the glasshouse), and were either grown in a non-saline or saline soil. The vertical bars represent the standard deviation of three replicate measurements.

Seed germination

Germination of leucaena seed was inhibited by 10% when the EC reached 11 dS/m, and 50% inhibited when the EC reached 20 dS/m (Figure 16) and SAR had no significant effect on germination. This indicates that germination of leucaena cv Tarramba seed is highly salt tolerant.

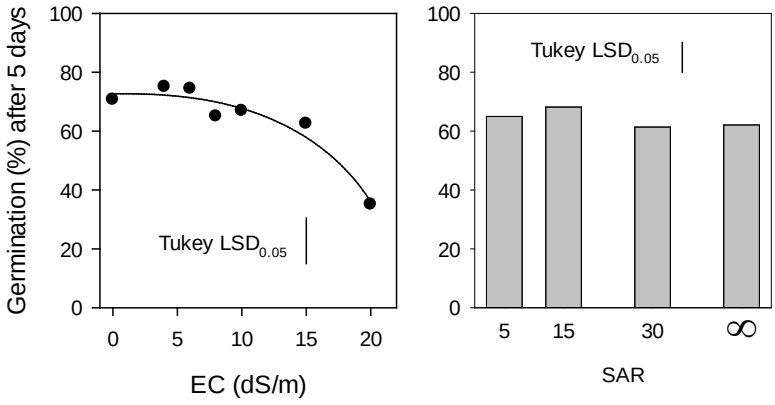

Figure 16. Effect of increasing salinity and SAR on germination percentage of leucaena cv Tarramba seed after 5 days. The vertical line represents Tukey's LSD at 5% significance level.

Alkalinity had no significant effect on germination percentage or average root length (Figure 17) after 5 days. This is remarkable since germination in many species is generally inhibited by increasing alkalinity (Li et al. 2010; Lin et al. 2011; Tewari et al. 1999; Zhang and Zhao 2011). The root growth inhibition observed in this study is insignificant and may not affect root growth in soil since soils are buffered with regards to pH changes. Furthermore, the application of elemental sulfur will limit soil solution pH fluctuations during CS water irrigation. Even raw CS water with pH 9 is unlikely to affect leucaena germination and growth since the highest alkalinity (2000 mg/L) had pH 9 (Appendix A2), similar to the pH in CS water.

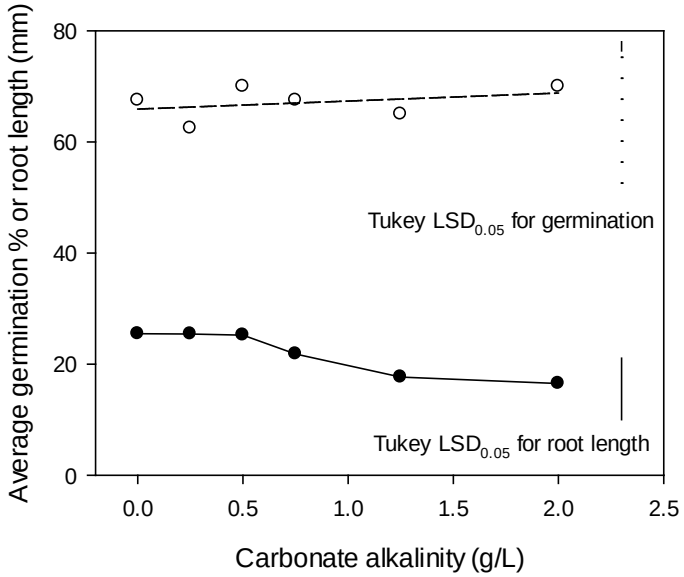

Figure 17. Effect of alkalinity on leucaena seed germination percentage (open circles with dashed line) and root length (solid circles with solid line) after 5 days. Symbols are the mean values of five replicates. The vertical line represents Tukey's LSD at 5% significance level.

Germination of Topcut Rhodes grass seed after 9 days was not affected by salinity or SAR (Figure 18), with germination percentages ranging from 58% (EC 20 dS/m) to 48% (0 dS/m), and from 55% (SAR ∞) to 49% (SAR 30). Alkalinity had no significant effect on seed germination rates, ranging from 47% (control) to 57% (750 mg/L as carbonate) and 47% (2000 mg/L as carbonate) (Figure 18). However, the germination rate of the species was low and variable, possibly due to some dormancy or seed quality factors.

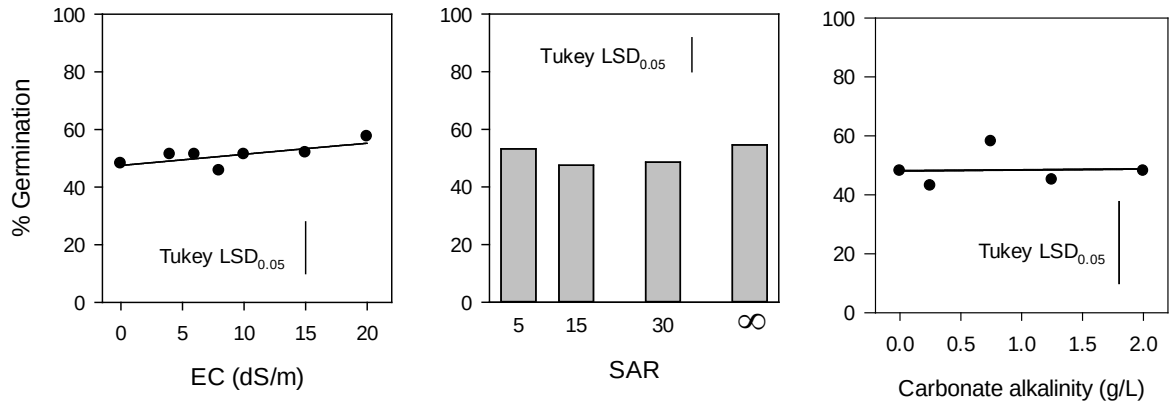

Figure 18. Germination percentage of Topcut seed after 9 days as affected by increasing salinity, SAR and alkalinity. The vertical lines represent Tukey's LSD at 5% significance level.

Germination of Reclaimer Rhodes grass seed ranged from 28% in the control, to 22% at 15 dS/m and decreased to 15% at 20 dS/m (Figure 19). Reclaimer Rhodes grass was claimed to have higher salt tolerance than Topcut Rhodes grass, but our germination results appear to indicate Topcut to be more salt tolerant. However, it must be cautioned that the germination percentage in the control was very low and results should be treated with caution. It is interesting to note that the response to salinity appears to differ between these closely related cultivars.

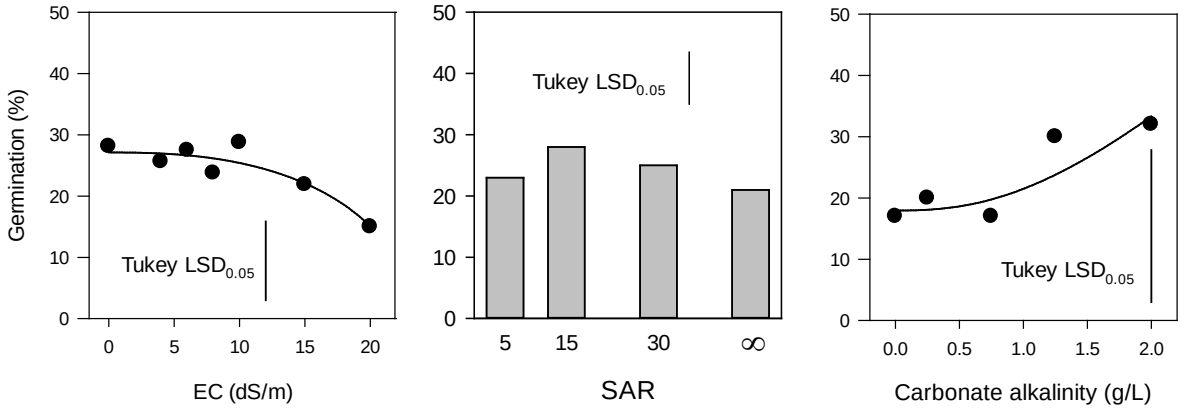

Figure 19. Germination percentage of Reclaimer Rhodes grass seed after 8 days as affected by increasing salinity, SAR and alkalinity. The vertical lines represent Tukey's LSD at 5% significance level.

The SAR had no significant effect on germination, but a trend suggested that germination is lower in the control (SAR ∞, no Ca added) than in SAR 5-30 treatments (Figures 19). This points to a requirement for Ca during germination and it would be expected that the Ca requirement would also persist during root growth (which was not measured in this study). The protective effect of low Ca

concentrations (<1 mM) on seed germination is known [e.g. for *Phragmites* (Zehra et al. 2012)], but Tobe et al. (2002); Tobe et al. 2003) found that Ca only alleviates Na toxicity on root growth but does not overcome Na inhibition to germination.

Conclusion

Leucaena was salt tolerant during seed germination, and can grow in saline soil (ECse 10 dS/m), but was sensitive when overhead irrigated with saline water (EC >4 dS/m), which resulted in foliar necrosis, chlorosis and leaf abscission. The shedding of newly developing leaves under overhead irrigation with water of salinity >4dS/m is of concern since this would prevent fodder production. Therefore caution should be exercised when overhead irrigating leucaena with water >3 dS/m.

Topcut and Reclaimer Rhodes grass were salt tolerant during seed germination and growth. Neither saline soil with ECse 10 dS/m nor overhead irrigation with water up to 15 dS/m affected plant growth and biomass production. Therefore, these species are most suitable for land amendment irrigation with CS water. Considering that Rhodes grass is also tolerant of poor soil conditions (e.g. waterlogging), Rhodes grass may be the most suitable species for land amendment irrigation.

Lucerne was only subjected to limited testing, but was salt tolerant during overhead irrigation with CS water with 3.2 dS/m with limited damage at 4.6 dS/m (seed germination was not tested). No alkalinity tolerance testing was conducted with lucerne. Lucerne appeared slightly more salt tolerant than leucaena but less tolerant than Rhodes grass.

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Appendix A1 – Overhead irrigation study 1

A preliminary assessment of the potential impacts of the overhead irrigation of saline and alkaline water on plant foliage

Final Report

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11 July 2014

(Revised 23 August 2014)

A1.1 Executive summary

- The irrigation of unamended coal seam water (CS water) as part of the land amendment irrigation (LAI) program offers several advantages compared to other approaches. However, it is unknown whether the overhead irrigation of this saline and alkaline unamended water (for example, through centre-pivots) will cause damage to the foliage of the plants. Therefore, this present preliminary study examined whether the overhead irrigation of CS water caused damage to the foliage of three plant species (leucaena, Rhodes grass, and lucerne) when grown in a glasshouse at The University of Queensland.
- Unamended CS water was obtained from Pleasant Hills (Queensland) with an electrical conductivity of 6.5 dS/m. Three treatments were prepared for overhead irrigation, consisting of 0% CS water (100% deionised water, control), 50% CS water (50% deionised water, EC 3.2 dS/m), and 75% CS water (25% deionised water, EC 4.6 dS/m). These waters were overhead-irrigated a total of 15 times across a five week period.
- It was found that overhead irrigation with either 50% (EC of 3.2 dS/m) or 75% (EC of 4.6 dS/m) CS water did indeed cause damage to the foliage of some plants, although the magnitude of the damage varied substantially between species. However, overall, the damage caused to the foliage by overhead irrigation of 50% CS water was considered to be modest and did not impact upon plant biomass.
- Rhodes grass was the most tolerant species examined. Upon completion of the experiment, the plants were generally healthy and even 75% CS water did not appear to stress the foliage when assessed using visual symptoms, chlorophyll fluorescence(CF), or shoot biomass.
- Lucerne was more sensitive to overhead irrigation with CS water, and even though shoot biomass wasn't significantly reduced in any treatment, CF was reduced slightly (but significantly) and the CS water resulted in visual damage to the leaves (particularly for 75% CS water).
- Leucaena was also more sensitive to CS water than was Rhodes grass, with some visual damage evident for 50% CS water (this also causing a slight reduction in CF). As expected, this damage was more pronounced for 75% CS water.
- The results of this preliminary study suggest that with careful monitoring and adaptive management, it is likely that the overhead irrigation of 50% CS water (3.2 dS/m) can be successful without damage to the foliage of some species (Rhodes grass) or with only comparatively minor damage (leucaena and lucerne).
- This preliminary study has not taken into account a number of factors which would influence plant performance in the field. For some factors, the experiment conducted here is likely to have resulted in increased damage relative to that expected in the field. In particular, this study utilized a fine mist for irrigation, there was no 'rainfall' to allow washing of the leaf surfaces, and for leucaena only old leaves were monitored (in the field new leaves would grow to replace the old leaves). Other management and environmental factors will also influence the effects of CS water and need to be considered, for example relative humidity in the glasshouse would be higher than field conditions.
- Finally, here we have utilized only two treatments containing CS water (and we have not separated the effects of alkalinity and salinity), but the composition of CS water is highly

variable – care needs to be taken when extrapolating this data to situations where the water composition differs.

A1.2 Introduction

A1.2.1 Overview

Evidence from past and ongoing research indicates that land amendment irrigation (LAI) systems of coal seam water (CS water) offer a robust alternative to engineering approaches such as the use of reverse osmosis or associated water amendment facilities (AWAFs). A potential disadvantage of this approach, however, is that CS water contains elevated levels of Na^+ and Cl^- ions, and has high alkalinity. The potential detrimental effects of unamended CS water on soils may be overcome by the application of gypsum ($CaSO_4.2H_2O$) to reduce the sodium absorption ratio (SAR) to a safe level and by the application of elemental sulphur to neutralize the alkalinity. Information is lacking, however, on whether the overhead irrigation of unamended CS water, as occurs with LAI, has direct detrimental effects on the foliage of plants.

A1.2.2 Review of the Literature: Salt spray effects on plant foliage

Previously, a review of the scientific and technical literature was carried out by The University of Queensland to assess the likelihood of damage to the foliage of leucaena, Rhodes grass, oat, lucerne, and forage sorghum (*A Literature Review of the Impacts of Overhead Irrigation with Saline Water on Plant Foliage*, July 2014: Blamey, Menzies, Kopittke). It was identified that very little is known regarding the direct foliar effects of CS water, and even less on the crops of interest. This review of literature concluded that:

- Little information is available in the scientific and technical literature on the direct effects of saline and alkaline water on plant foliage, and that there is even less information on the effects of saline and alkaline water on the foliage of leucaena, Rhodes grass, oat, lucerne, and forage sorghum;
- No scientific information was found on the direct effects of CS water on the foliage of these crops;
- It is impossible to have any degree of certainty predicting plant responses to overhead irrigation with CS water given differences among plant species in water retention on leaves and the lack of a relationship between the effects of Na and Cl uptake from the soil and those resulting from foliar-applied Na and Cl.

Elevated soil salinity (high concentrations of salts) and sodicity (high Na concentration) are worldwide problems that have reduced plant growth for millennia (Bernstein 1975). These problems commonly occur in, but are not limited to, arid, semi-arid, and sub-humid environments. Plants growing in littoral environments, near the ocean and brackish or salt lakes, also face salinity problems through wind-blown salt that contaminates the soil, adds salt directly to the foliage, or both. Soil salinity and sodicity are not limited to natural environments. Human actions have increased these problems through clearing of native vegetation that has caused dryland salinity and through irrigation that has greatly increased soil salinity and sodicity. In both instances, problems have occurred through changes to on-site and/or off-site hydrology.

In contrast to soil salinity effects, only a few reports have addressed the direct impacts of saline water on plant foliage. Indeed, the response of 71 crops was detailed by Maas (1985), but only 19 crops were listed regarding their response to saline foliar sprays. McCune (1991) also presented an overview of information available at that time. The effects of saline water on plant foliage include leaf chlorosis, necrosis, and distortion but these are not specific for "saline particles and many cannot be distinguished from those induced by drought." McCune (1991) noted that various factors impact on the extent of foliar damage. These include: (1) the amount, duration, and frequency of exposure, (2) size and chemical composition of the droplets, (3) environmental factors such as light, temperature, relative humidity, and precipitation, and (4) plant species and stage of development of individual plants.

Many of the individual studies addressed the effects of highly saline waters (e.g. sea water, water from de-iced roads). The remaining few studies evaluated a diverse range of saline waters; commonly a single study evaluated the effects of only one saline water (e.g. based on an industrial emission) on a limited number of plant species. The composition of the saline water and plant species investigated are often not those of interest to Santos. Importantly, however, there is a poor relationship between the effects on plants of salts absorbed via the roots and those applied to the foliage. These limitations, along with differences among plants in sensitivity to specific ions, make it difficult to assess the possible effects of CS water application to plant foliage.

The FAO (1985) initially published summary information on the relative tolerance of selected crops to foliar injury from saline water applied in sprinkler irrigation (Table A1.1).

Table A1.1. Relative tolerance of some crops to foliar injury from Na and Cl in saline water applied by sprinklers after Maas (1985).

Concentrations of Na^+ or Cl^- (mM)			
<5	**5 - 10**	**10 – 20**	**>20**
almond	grape	lucerne	cauliflower
apricot	pepper	sorghum	cotton
citrus	potato	maize	sugarbeet
plum	tomato	barley	sunflower
		safflower	
		sesame	
		cucumber	

Salt spray has many direct effects on plant foliage in natural environments and in those influenced by human activities. The sea shore is probably the most important natural environment in which foliage may be affected by ocean spray. Situations in which humans increase salt spray effects include sprinkler irrigation with saline water, with possible attendant effects on the soil, and through drift of saline water from industrial sites.

Plants differ greatly in their sensitivity to salts applied as foliar sprays. Bernstein (1975) claimed that "symptoms of leaf injury by foliarly absorbed salts are the same as those caused by salts absorbed by the roots", a conclusion supported by Maas et al. (1982). Absorption of salts by the roots is continuous provided there is sufficient soil moisture while foliar absorption occurs only during "the 10% or less of the time that leaves are wetted by the sprinklers" (Bernstein 1975). It is uncertain if this conclusion is correct, however. For example, Burkhardt (2010) investigated the fate of saline aerosols, concluding that most fine (<2.5 µm) aerosols are hygroscopic and often deliquescent on transpiring leaves. These concentrated solutions may be taken up through both the cuticle and stomata. Burkhardt (2010) concluded that hygroscopic particles may work as desiccants, and are therefore deleterious to foliage.

A search of the literature relating to the application of alkaline waters to the soil or to the foliage revealed little information on plant effects and no results on direct foliar effects. Working with lucerne seedlings (5-weeks-old) in sand culture, Peng et al. (2008) reported the effects of irrigation with saline (1403 to 7014 mg/L NaCl) (24 to 120 mM NaCl) and alkaline (pH 7.03 to 10.32) waters. Salinity and alkalinity reduced lucerne biomass, with saline and alkaline water being more severe than either stress alone.

A solution culture experiment by Javid et al. (2012) showed that salinity 2900 mg/L (50 mM) NaCl and alkalinity induced by 420 mg/L (5 mM) $NaHCO_3$ (pH 8.5), alone or in combination, decreased the growth of *Brassica juncea* after 4 weeks of stress. Alkalinity alone was more detrimental than salinity alone, with the greatest decrease with the combined treatments. A similar effect was observed by Paz et al. (2012) with *Lotus tenuis* exposed to a root-zone salinity of 5840 mg/L (100 mM) NaCl and alkalinity of 840 mg/l (10 mM) $NaHCO_3$. Research by Li et al. (2010) with lucerne evaluated the effects of equimolar NaCl plus Na_2SO_4 (30 to 150 mM) and of $NaHCO_3$ plus Na_2CO_3 (10 to 50 mM) in the rooting zone. Solution pH ranged from 7.01 to 7.05 and from 9.80 to 10.1, respectively. As found previously, alkali salts had a much greater detrimental effect than neutral salts on plant growth. The alkali salts also had a greater effect than the neutral salts on Na^+ in leaves, accompanied by greater decreases in K^+, Ca^{2+}, and Mg^{2+}.

While alkalinity appears to be more damaging to plants than salinity alone, caution is needed in interpreting these results which relate to ions absorbed by the roots. Direct foliar effects may be different from those in which alkaline waters applied as foliar sprays as found for salinity effects.

A1.2.3 Aims

Given the paucity of knowledge regarding the effects of saline and alkaline waters on the foliage of plants, the present study aimed to address these uncertainties by providing a rapid initial assessment as to whether the overhead irrigation of saline and alkaline CS water damages plant foliage.

In particular, this research program aimed to ascertain whether or not CS water applied via sprinkler irrigation damages the foliage of the crops of interest. The research outlined here aims to provide a rapid initial assessment of whether it is likely that saline and alkaline water is likely to damage plant foliage as a result of overhead irrigation. Study 2 of this research program aims to provide a more comprehensive analysis of the potential deleterious effects of CS water, and in particular, aims to separate the effects of salinity and alkalinity – this potentially allowing more rigorous management of the CS water.

A1.3 Materials and methods

A1.3.1 Experimental design

The research was conducted in a glasshouse at The University of Queensland, St Lucia, from April to July 2014. During this time, the maximum temperature in the glasshouse was 35°C and the minimum was 15°C. The experiment consisted of a total of nine treatments (three plant species, with three water qualities), each with six replicates, thereby yielding a total of 54 experimental units.

A1.3.1.1 Plants and growth conditions

The three plant species investigated were leucaena (*Leucaena leucocephala* ssp. *glabrata* cv. Tarramba), Rhodes grass (*Chloris gayana* cv. Topcut), and lucerne (*Medicago sativa* L. cv. L91). For the leucaena, two plants were grown in each 5 L pot to a height of ca. 2 m before being trimmed to ca. 0.4 m to encourage new growth. For both Rhodes grass and lucerne, a total of ca. six seedlings were established per 2 L pot. Plants were grown in pots filled with commercial potting mix to which a basal application of slow-release Osmocote fertiliser had been applied. All plants were grown for four weeks (either from planting [for Rhodes grass and lucerne] or from coppicing [for leucaena]) prior to imposing treatments. During this initial growth period, plants were watered daily with deionised water. Additional Osmocote (*Osmocote Plus Trace Elements - Total All Purpose*) fertiliser was applied every four weeks throughout the experimental period. For leucaena, Mallet (Imidacloprid) was applied to control psyllids two weeks after commencing the experiment.

A1.3.1.2 Water quality

The potential deleterious effects of CS water were investigated by using three treatments, with an appropriate quantity of CS water obtained from Pleasant Hills (Queensland). This water was highly saline (electrical conductivity [EC] of 6.5 dS/m) and alkaline (pH of 9.5 and total alkalinity of 1520 mg/L of $CaCO_3$ equivalent) (Table A1.2). The three treatments consisted of: 0% CS water (100% deionized water, control), 50% CS water (50% DI water, ca. 3.2 dS/m), and 75% CS water (25% DI water, ca. 4.6 dS/m) (Table A1.2).

Table A1.2. Chemical composition of the three treatments investigated: 0% CS water (control, 100% deionised water), 50% CS water (50% CS water and 50% deionised water), and 75% CS water (75% CS water and 25% deionised water). For reference, data are also presented for undiluted CS water with an EC of 6 dS/m (100% CS water) even though this was not utilized as a treatment in the current experiment.

	Treatment 1 (0% CS water)	Treatment 2 (50% CS water)	Treatment 3 (75% CS water)	100% CS water
pH	**5.6**	**9.5**	**9.5**	**9.5**
EC (dS/m)	**-**	**3.2**	**4.6**	**6.5**
Ca (mg/L)	**-**	**1**	**2**	**3**
Mg (mg/L)	**-**	**<1**	**<1**	**1**
Na (mg/L)	**-**	**801**	**1220**	**1510**
K (mg/L)	**-**	**7**	**12**	**15**
Cl (mg/L)	**-**	**648**	**907**	**1100**
S (mg/L)	**-**	**5**	**9**	**11**
Total alkalinity (mg/L as $CaCO_3$)	**-**	**787**	**1140**	**1520**
SAR	**-**	**221**	**238**	**193**

A1.3.2 Water irrigation and sample collection

Plants were irrigated three times per week using the appropriate water. The water was applied as a fine mist (Pope, microjet sprinklers) using irrigation chambers that were connected to a pump and a 120 L container with the relevant water. The volume of water used per irrigation event was 60 L per chamber. The duration of each irrigation event was 15 min (this being similar to the duration a plant might be irrigated for when using centre-pivots), with a total of 15 irrigation events applied over a

five week period. No attempt was made to prevent the overhead irrigation water from entering the soil. However, the daily application of deionized water (applied directly to the soil whilst avoiding the foliage) prevented the accumulation of salts in the growth medium.

To allow for analyses of major plant nutrients (plus Na), plant foliage was harvested following 0, 5, 10, and 15 irrigations. For leucaena, two leaves were harvested from each plant, whilst two tillers were collected from each pot for lucerne and Rhodes grass. For each treatment, one leaf or tiller was rinsed with deionised water to remove salt from the foliar surface. For the remaining harvested leaf tissues, the salt was not removed prior to analysis. The length of time required to allow adequate removal of salts from the leaf surface (whilst minimising the leaching of salts from the internal leaf tissues) was determined in a preliminary experiment in the laboratory, being 1.5 to 2 min. For leucaena, new shoots were removed during the course of the experiment to ensure that all leaves sampled for tissue analysis had received an equal number of irrigations. However, this was not feasible for lucerne and Rhodes grass due to their growth form.

At the same time as collecting tissues samples for analysis (i.e. after 0, 5, 10, and 15 irrigations), chlorophyll fluorescence (CF) was measured using an Optiscience OS30p+ hand-held CF meter. Chlorophyll fluorescence was measured at night following >1 h dark-adaptation. Data are presented as the ratio F_v/F_m which is a widely used measure of the maximum efficiency of Photosystem II. Measurements were taken on six leaves per replicate.

Upon conclusion of the experiment, the fresh mass of the shoots was measured for each experimental unit for Rhodes grass and lucerne (Table A1.3). It was not useful to obtain measurements of biomass for leucaena given that the majority of the biomass had been formed prior to commencement of the overhead irrigation of the CS water. A visual assessment was used to observe growth and identify any symptoms of toxicity. A detailed photographic record was kept, with photos collected after every irrigation event.

For both biomass and CF, a two-way analysis of variance (GenStat v7.1) was used to determine if there were any significant differences between treatments.

Table A1.3. Summary of activities

Date	Activity
Commencement	Prepare leucaena, and seed lucerne and Rhodes grass
Week 1	Build irrigation chambers with pump, reservoirs, prepare glasshouse space
Week 4	Irrigations 1-3, photograph plants
Week 5	Irrigations 4-6, CF, photograph plants
Week 6	Irrigations 7-9, photograph plants
Week 7	Irrigations 10-12, CF, photograph plants
Week 8	Irrigations 13-15, CF, photograph plants
Week 9	Harvest plants, determine biomass, dry in oven for tissue analysis
Week 10	Conduct tissue analyses
Week 11	Report preparation
Week 12	Report preparation and delivery

A1.4 Results and discussion

A1.4.1 Visible effects of overhead irrigation with CS water on plant foliage

As expected, growth during the experimental period was good and plants in the control treatment appeared to be healthy. However, the overhead irrigation of CS water resulted in the formation of visual damage to the foliage, with the magnitude of this damage being greater at 75% CS water than at 50% CS water.

For leucaena, some leaflets irrigated with the 50% or 75% CS water showed chlorotic and necrotic lesions – these apparently corresponded to areas where the water droplets had accumulated and dried (Figure A1.1). As expected, the magnitude of these symptoms was substantially greater at 75% CS water than at 50% CS water. For plants in both the 50% and 75% CS water treatments, the extent of damage to the foliage was variable, with some leaflets showing no symptoms whilst others appeared to have substantial damage. In addition, for plants irrigated with 75% CS water, it was also noted that the formation of chlorotic lesions was followed by a general chlorosis of the entire leaflet prior to their eventual abscission – this becoming particularly noticeable after ca. 7-8 irrigations.

The effects of CS water on Rhodes grass were similar to those described for leucaena, although the symptoms generally did not appear to be any worse at 75% CS water than at 50% CS water (Figure A1.2). Again, the overhead application of CS water resulted in the formation of chlorotic and necrotic lesions, these often being confined to the leaf margins. Interestingly, visual assessment indicated that whilst the leaf tissues were initially damaged by the foliar-application of CS water, the severity of symptoms actually decreased over time (Figure A1.3). Indeed, following ca. six irrigations, the symptoms were generally not apparent and the plants were typically healthy in appearance (Figure A1.3). It is possible that adaptation in leaf morphology/physiology resulted in this decreased damage, although further studies are needed to confirm this hypothesis.

The shoots of lucerne also showed chlorotic and necrotic lesions resulting from the application of 50% or 75% CS water (the severity of the symptoms being higher at 75% CS water) (Figure A1.4). However, in contrast to Rhodes grass, the severity of these symptoms did not appear to decrease over time.

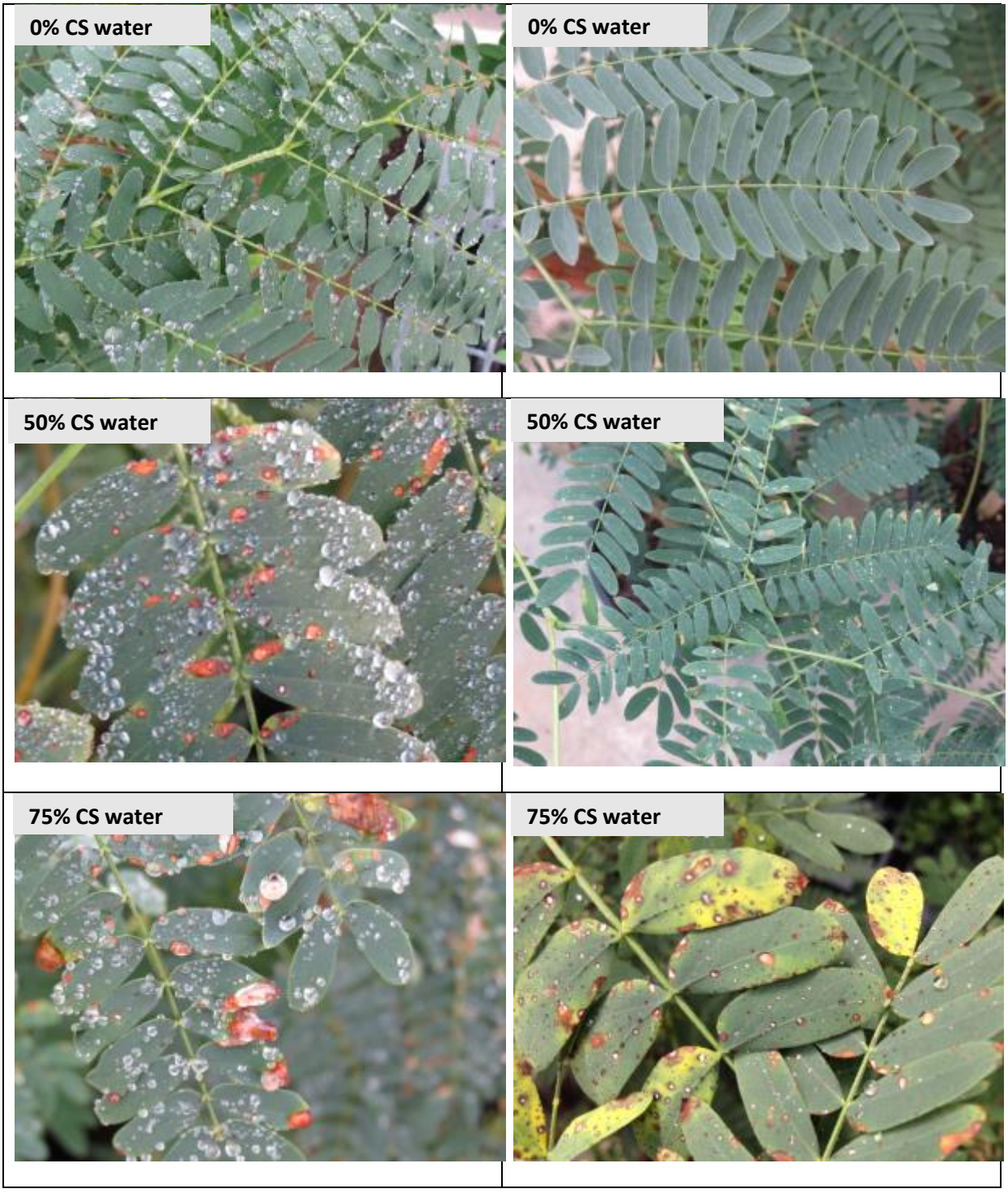

Figure A1.1. Leucaena leaves overhead irrigated with 0% CS water (top), 50% CS water (middle), or 75% CS water (bottom). Images are presented immediately following irrigation (left) to show the accumulation of water on the foliage, or following drying of the retained water (right). For the foliage exposed to either 50% or 75% CS water, note that (i) the severity of symptoms varied between leaflets (with not all leaflets damaged as badly as shown below), and (ii) some leaflets have been shed (particularly at 75% CS water). For all images, plants had been irrigated 15 times.

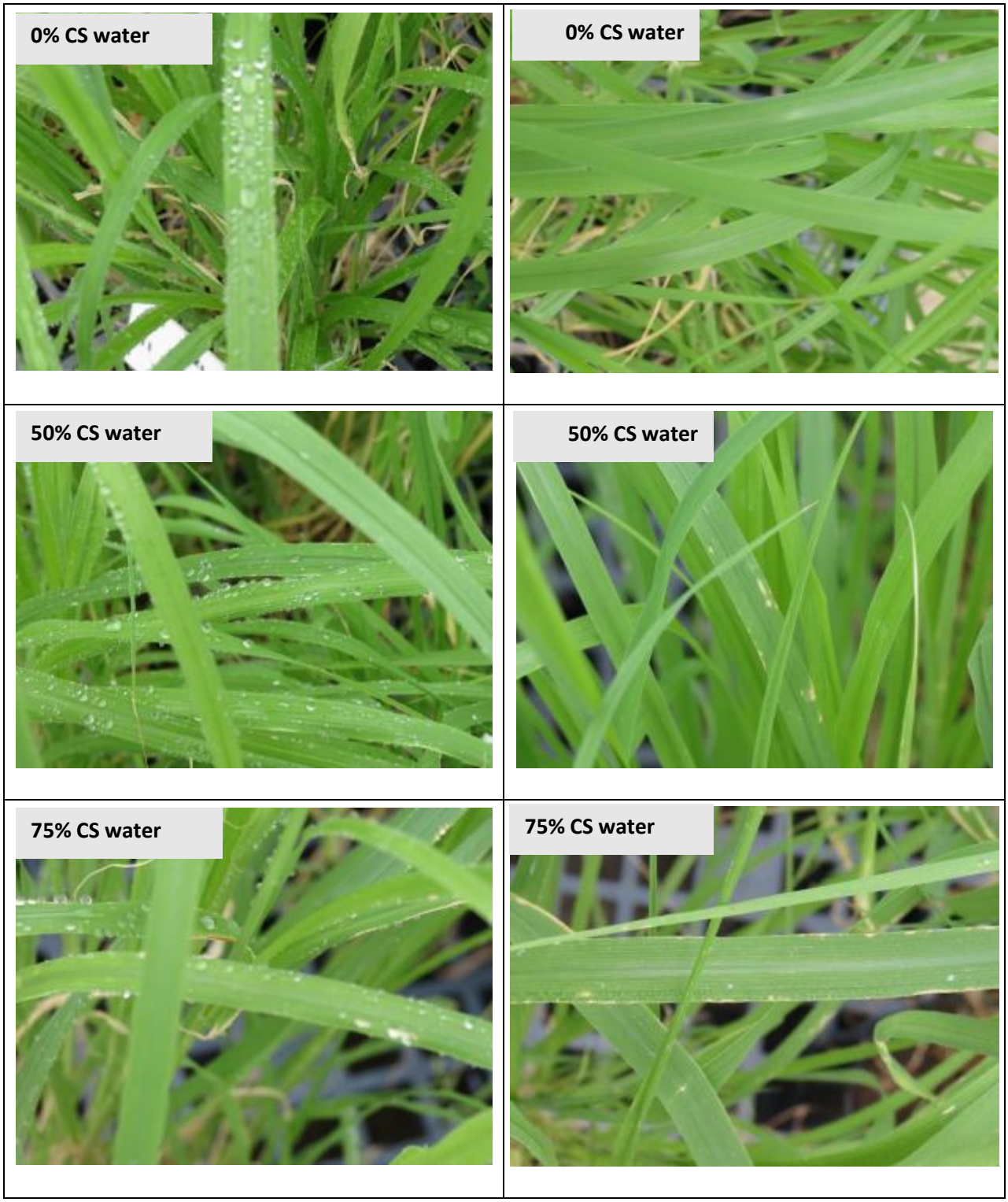

Figure A1.2. Shoot of Rhodes grass overhead irrigated with 0% CS water (top), 50% CS water (middle), or 75% CS water (bottom). Images are presented immediately following irrigation (left) to show the accumulation of water on the foliage, or following drying of the retained water (right). For all images, plants had been irrigated 15 times.

Figure A1.3. Shoots of Rhodes grass overhead irrigated either four times (top) or 10 times (bottom) with 50% CS water, showing the apparent decrease in severity of symptoms.

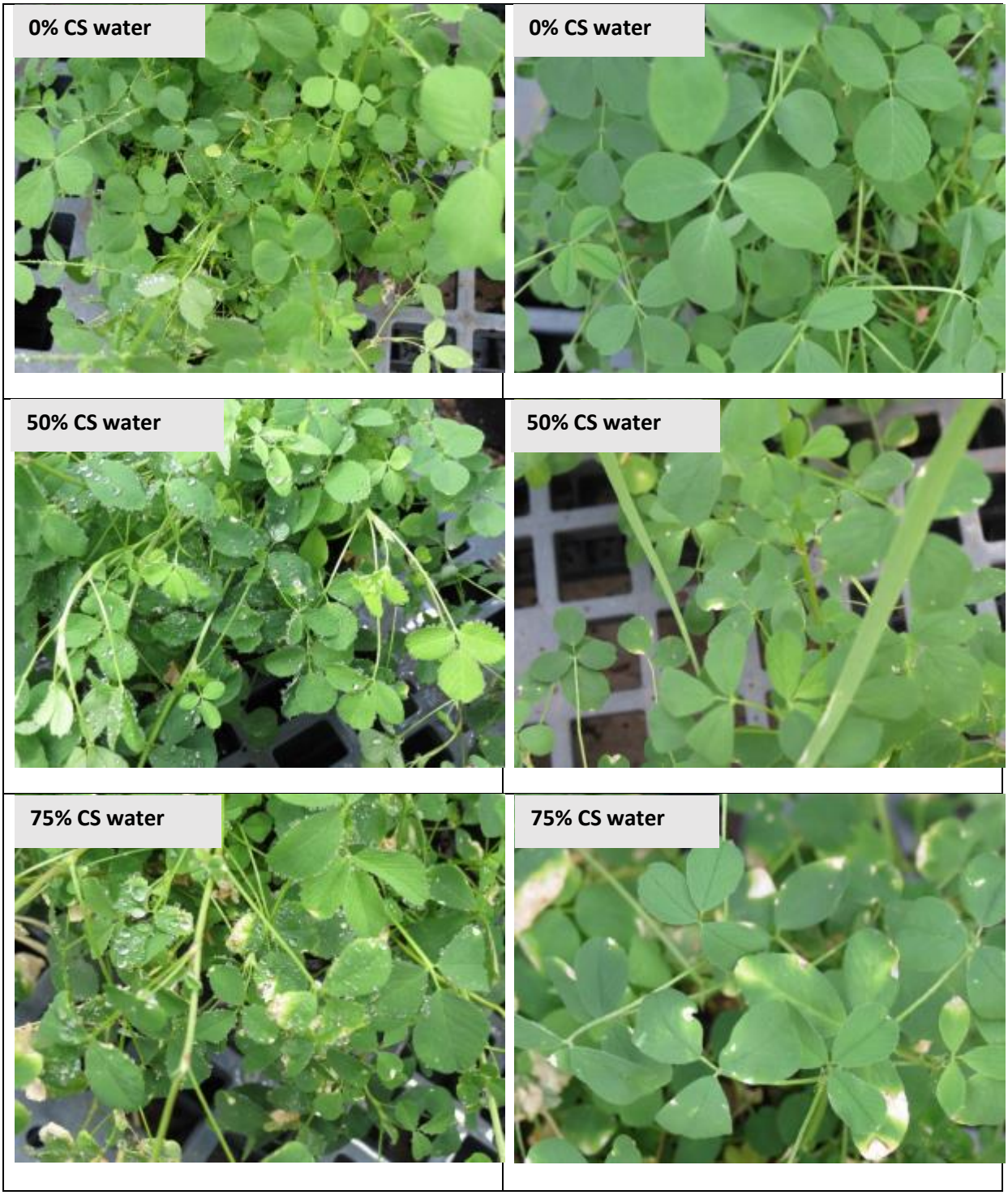

Figure A1.4. Shoot of lucerne overhead irrigated with water with 0% CS water (top), 50% CS water (middle), or 75% CS water (bottom). Images are presented immediately following irrigation (left) to show the accumulation of water on the foliage, or following drying of the retained water (right). For all images, plants had been irrigated 15 times.

A1.4.2 Biomass

Although biomass increased substantially during the five-week irrigation period, the quality of the irrigation water did not have a significant effect on the fresh mass of the shoots for either Rhodes grass or lucerne (Figure A1.5 and Figure A1.6). Indeed, there was no significant interaction between shoot fresh mass and the treatment EC (P=0.061), nor did the EC of the treatment have a significant effect on biomass (P=0.738, Figure A1.5). Not unexpectedly, a significant difference (P<0.001) was found between the mass of Rhodes grass (90 g/pot) and lucerne (42 g/pot) shoots averaged across all three treatments (Figure A1.5).

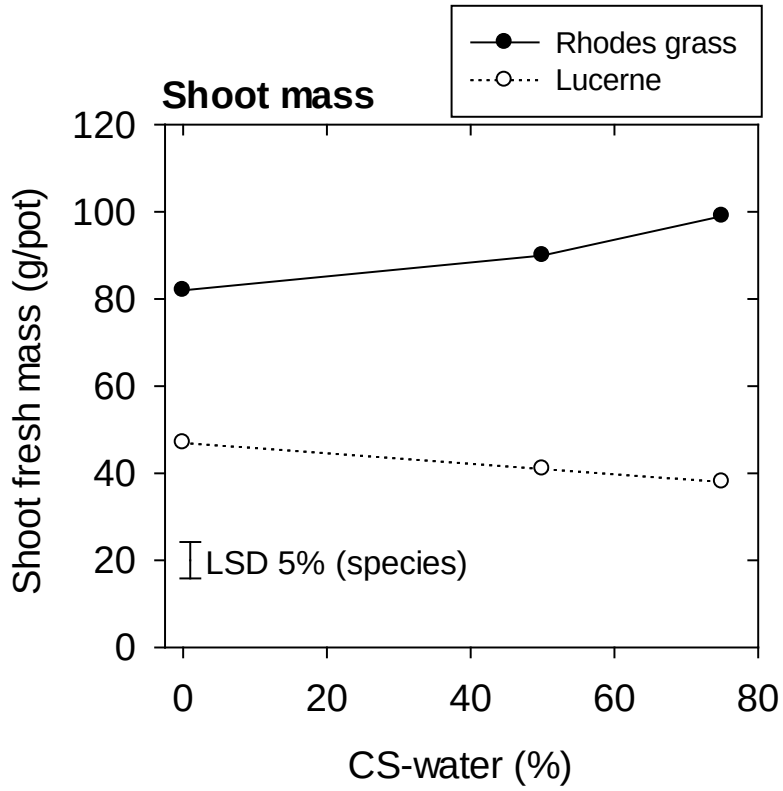

Figure A1.5. The fresh mass of shoots for Rhodes grass or lucerne overhead irrigated with solutions which contained 0, 50, or 75% CS water (the remainder being deionised water). Both plant species were grown for four weeks prior to imposing the treatments for a further five weeks. The LSD value presented allows comparison between plant species only (there was no significant interaction between species and water composition, nor did the water composition cause significant differences in shoot fresh mass within species).

Figure A1.6. Comparison of leucaena (top), Rhodes grass (middle) and lucerne (bottom) overhead irrigated using 0% CS water (left), 50% CS water (middle), or 75% CS water (right) a total of 15 times over five weeks.

A1.4.3 Chlorophyll fluorescence

Chlorophyll fluorescence was assessed using the F_v/F_m ratio, where F_m is maximum fluorescence and F_v is variable fluorescence (i.e. the difference between maximum and minimum fluorescence) – this providing a measure of maximum quantum yield in Photosystem II as an indication of environmental stresses (Maxwell and Johnson 2000). The average value of F_v/F_m for vascular plants growing under non-limiting conditions is generally ca. 0.79 to 0.84 (Maxwell and Johnson 2000). In the present study, values for F_v/F_m ranged between 0.75 to 0.82, and hence were largely within the range expected and did not appear to indicate substantial stress (Figure A1.7). Regardless, for both leucaena and lucerne, the irrigation of 75% CS water was observed to result in a significant (albeit comparatively small) decrease in CF, but overhead irrigation with CS water had no significant effect for Rhodes grass.

For leucaena (P<0.001), a significant interaction was found between the irrigation number and the water quality, thereby indicating that the water quality influenced the CF (but that the pattern of this response varied depending upon the number of irrigation events) (Figure A1.7). Specifically, for leucaena, it was observed that the quality of the water had no significant effect on CF when measured after five irrigations, but when it was measured after 11 (or 15) irrigations, CF had decreased significantly in the 75% CS water treatment (although not the 50% CS water treatment) (Figure A1.7). Therefore, there appears to be a cumulative impact of overhead irrigation with saline and alkaline water on photosynthesis in leucaena.

For Rhodes grass, the irrigation of either 50% or 75% CS water did not significantly decrease CF at any of the three measurement periods (P=0.208). However, it was found that CF decreased significantly between irrigation events (P<0.001), with CF significantly lower after 11 or 15 irrigations than following only five irrigations. It is not clear what caused this gradual reduction in CF in Rhodes grass (which was consistent regardless water quality), although it was possibly due to general aging of the plants.

For lucerne, the interaction between the irrigation number and the water quality was not significant (P=0.475), but the water quality had a significant effect on CF (P<0.001). For example, after 15 irrigations, CF was 0.822 in the control but had decreased significantly to 0.807 in the 75% CS water.

For all three plant species, the relationship between CF and tissue Na concentration was poor, indicating that increasing tissue Na concentrations could not explain changes in fluorescence (Figure A1.8 - A1.10). Similarly, no consistent relationship was found between CF and tissue concentrations of K, Ca, P, S or Mg, again indicating that changes in mineral nutrition could not explain changes in CF (Figure A1.8 - A1.10).

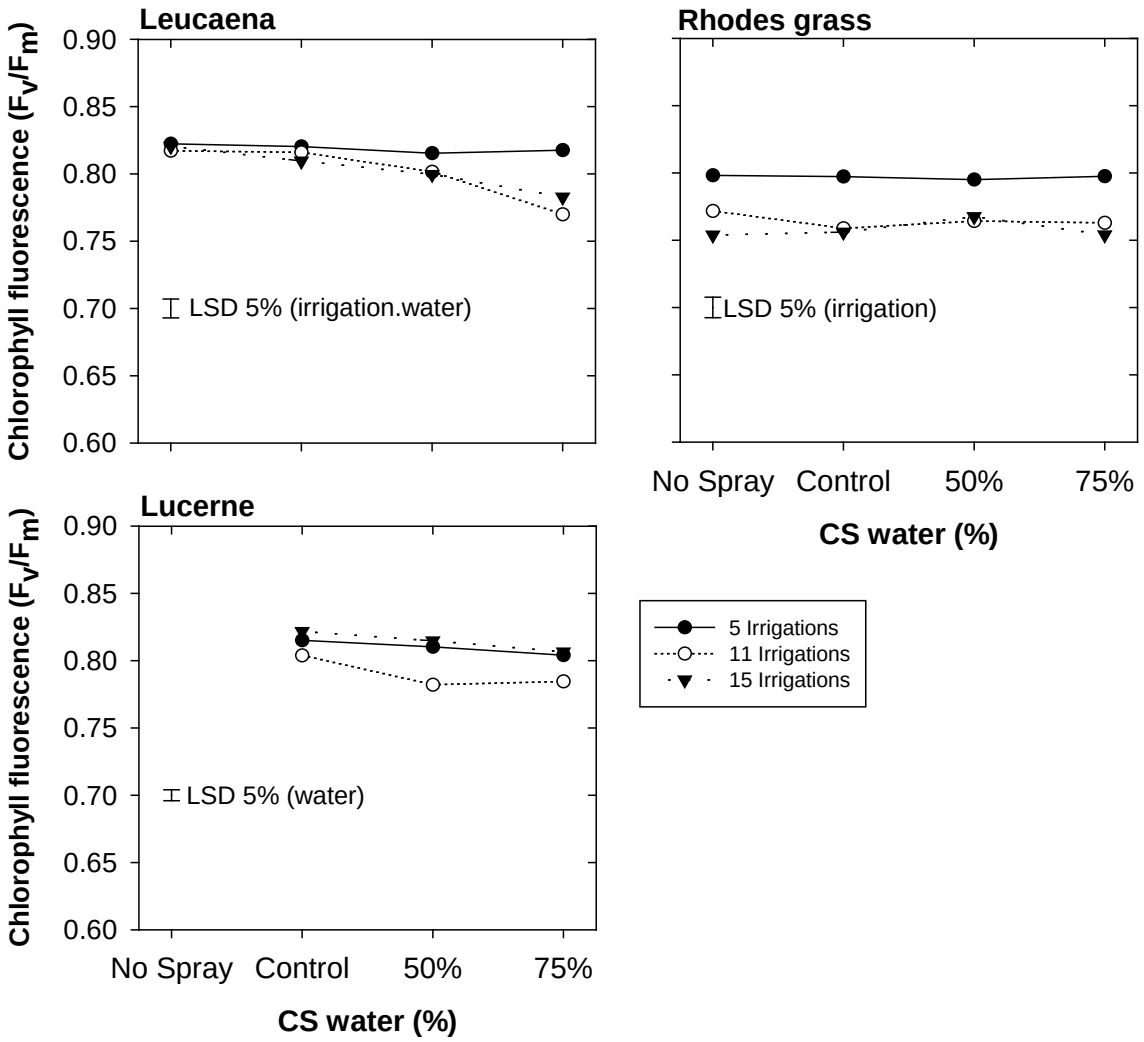

Figure A1.7. Effect of overhead irrigation with CS water on CF of leucaena, Rhodes grass, and lucerne leaves after 1 hour dark adaptation. Leaves were measured in plants that had received no overhead irrigation ("No spray") or in plants that had been overhead-irrigated a total of five, 11, or 15 times. Each point is the arithmetic mean of 18 measurements. The vertical bars represent the least significant difference. Where there was a significant interaction between the irrigation number and the water quality ("irrigation.water"), the LSD value can be used to compare any two points on the plot. Where the CF was influenced significantly by the number of irrigations ("irrigation"), the LSD value can only be used to compare the effect of the number of irrigations on CF at a single water quality. Where the CF was influenced significantly by the water quality ("water"), the LSD value can only be used to compare the effect of water quality on CF within a single irrigation-event. There were no spare lucerne plants available which were not being overhead-irrigated during the experimental period, and hence a 'No Spray' value could not be determined.

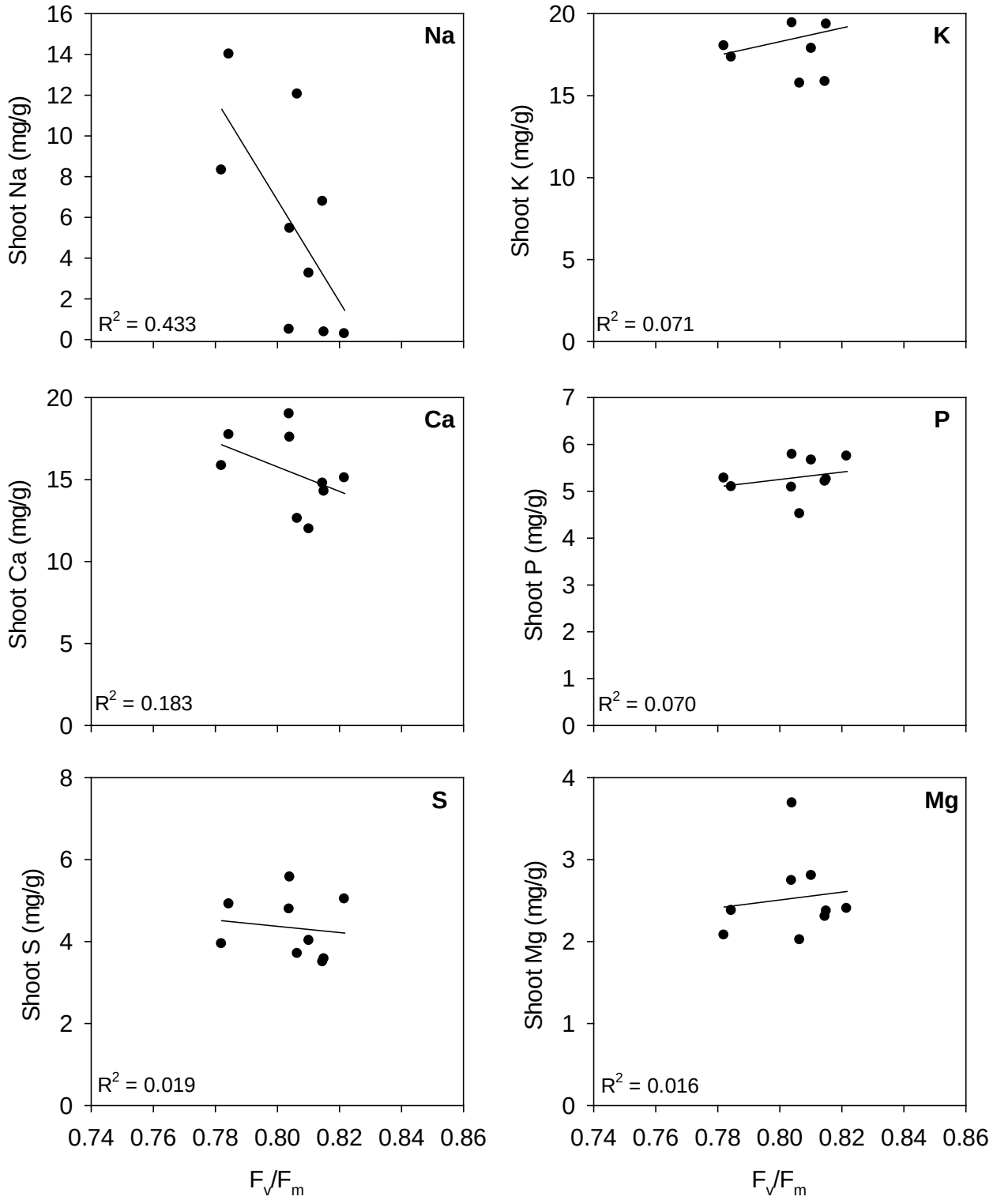

Figure A1.8. Relationships between CF and shoot tissue concentrations of Na, K, C, P, S or Mg for leucaena. Data are pooled across all three water qualities (0%, 50%, and 75% CS water) following overhead irrigation 5, 10, or 15 times.

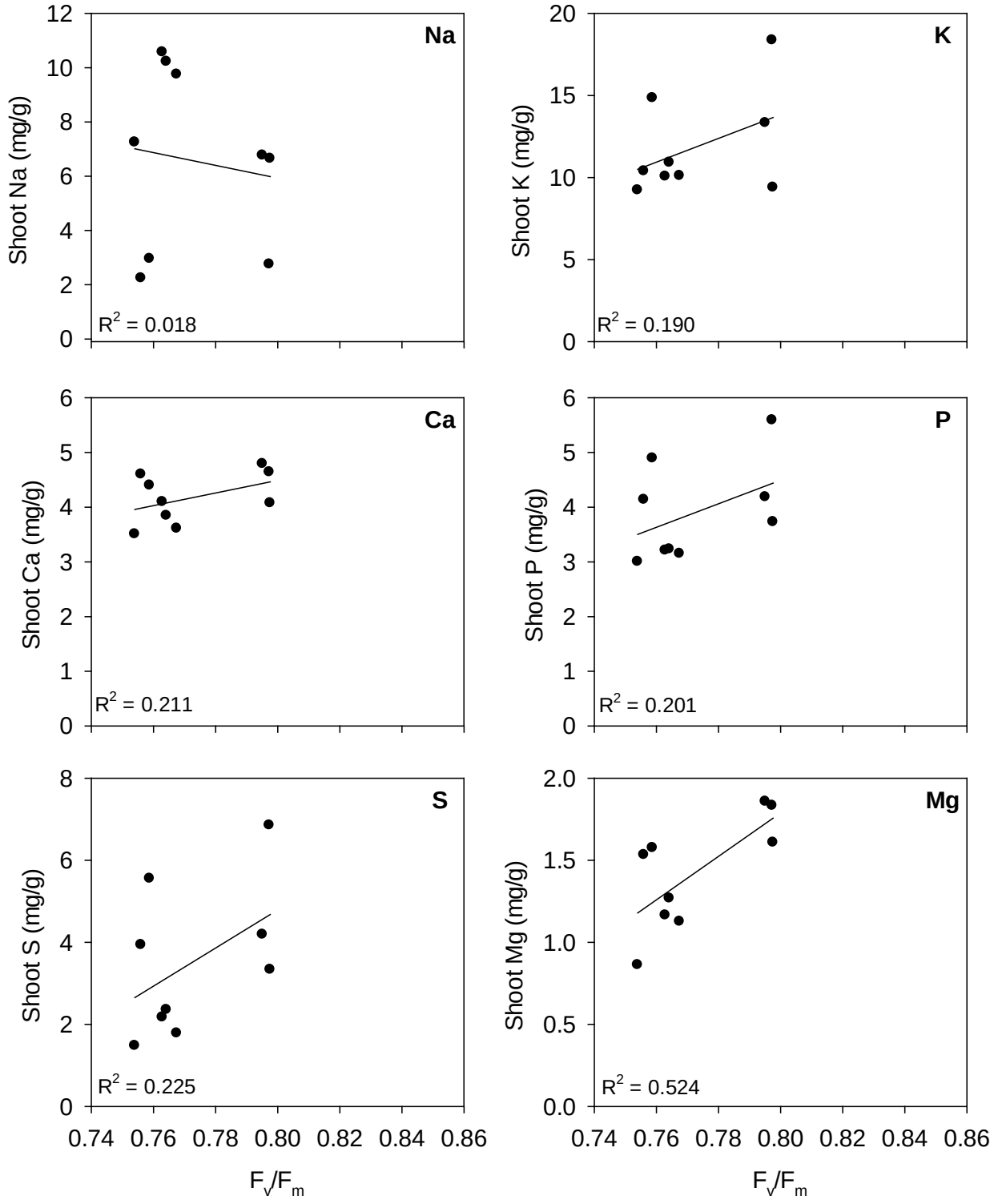

Figure A1.9. Relationships between CF and shoot tissue concentrations of Na, K, C, P, S or Mg for Rhodes grass. Data are pooled across all three water qualities (0%, 50%, and 75% CS water) following overhead irrigation 5, 10, or 15 times.

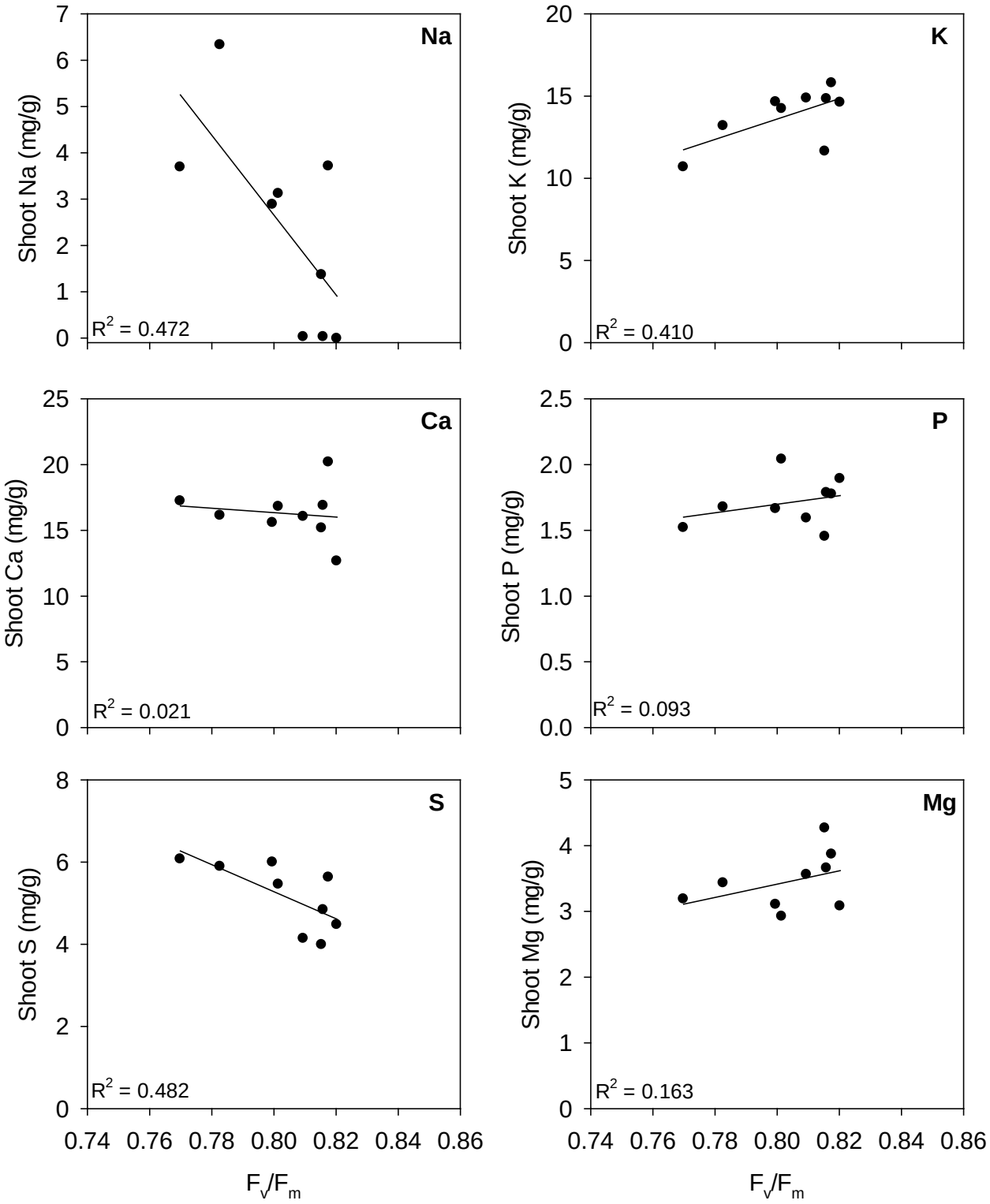

Figure A1.10. Relationships between CF and shoot tissue concentrations of Na, K, C, P, S or Mg for lucerne. Data are pooled across all three water qualities (0%, 50%, and 75% CS water) following overhead irrigation 5, 10, or 15 times.

A1.3.4 Analyses of leaf tissues

As expected, leaf tissue concentrations of Na tended to increase both as the percentage of CS water increased and as the number of irrigations increased, although differences were observed between the three plant species (Figure A1.11).

Tissue concentrations tended to be higher in Rhodes grass and lucerne than in leucaena (Figure A1.11). Therefore, given that Rhodes grass was also the species which appeared to be the most tolerant of overhead irrigation with CS water (see elsewhere), the formation of damage in leaf tissues of different species does not appear to be necessarily related to the concentration of Na taken up into the tissues *per se*. Interestingly, unlike some other species, Rhodes grass is known to accumulate (c.f. exclude) Na in shoot tissues during growth in saline rooting media (Smith 1981; Kopittke et al. 2009).

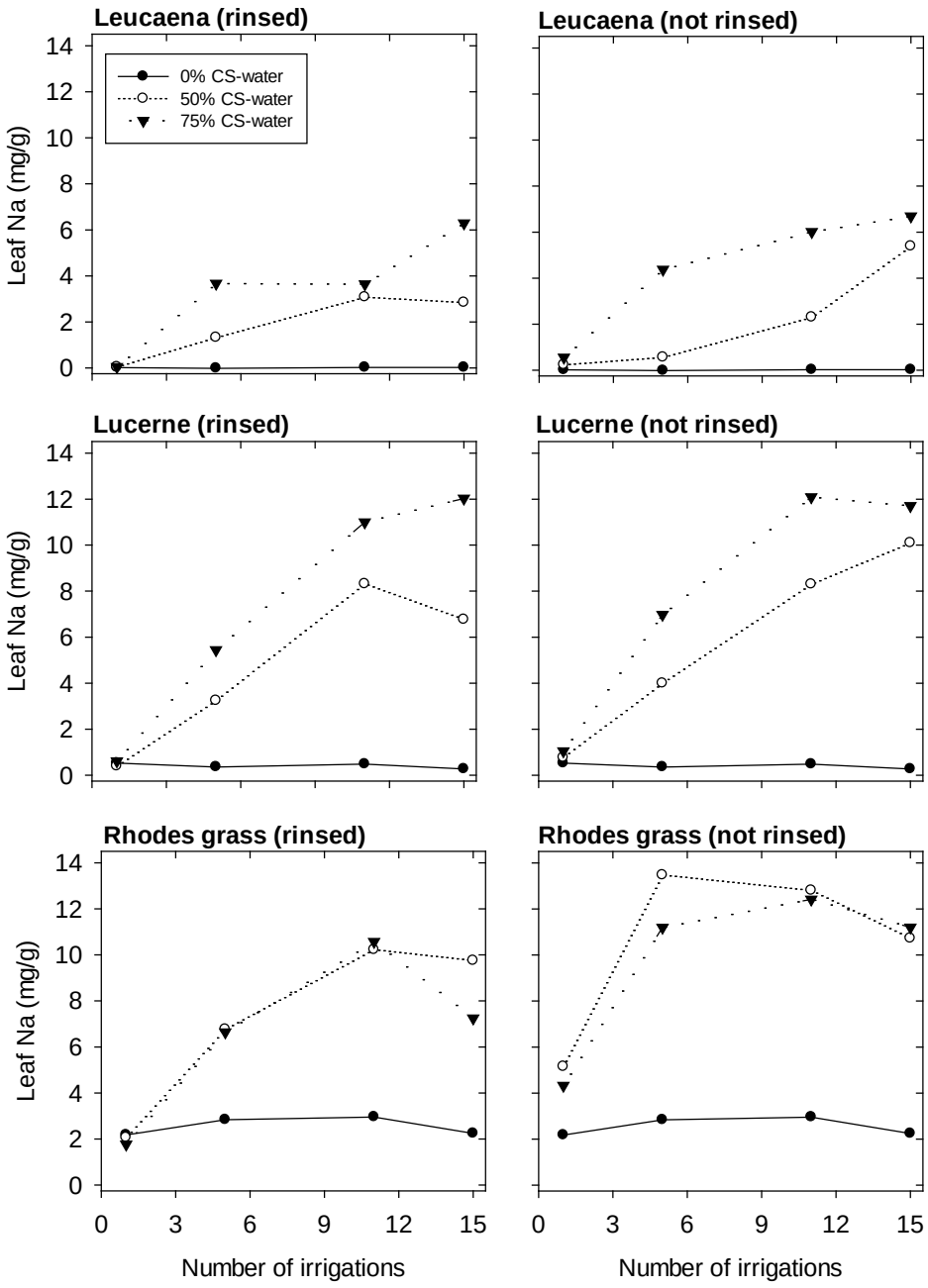

Figure A1.11. Effect of overhead irrigation on the Na concentrations in leaf tissues of leucaena, Rhodes grass and lucerne when using 0% CS water, 50% CS water, or 75% CS water. Tissue concentrations were determined after 1, 5, 11 and 15 irrigations. Between irrigations with the relevant treatment (CS water), care was taken to ensure that no other water was sprayed onto the leaves (for example, rainfall), and hence any salts were allowed to accumulate. Immediately following harvest, leaves were rinsed in deionised water to remove any free salts on the leaf surface. Tissues from all replicates were combined prior to analysis and hence no error bars can be presented.

For Rhodes grass, concentrations of Na in leaves that were not rinsed prior to analysis tended to be ca. 2-4 mg/g higher than in rinsed leaves, with this difference representing the amount of Na retained on the outside leaf surfaces (Table A1.4). On average, this difference between rinsed and unrinsed leaves for Rhodes grass (3.4 mg/g) was higher than for both lucerne (1.0 mg/g) and leucaena (0.85 mg/g) (Table A1.4). It is possible that these observed differences result from variations in leaf morphology (such as hairiness of the leaf surface) although further studies are required to confirm this hypothesis.

Despite internal Na concentrations increasing 3.0 mg/g (averaged across all plant species, from 4.5 to 7.5 mg/g) between 5 and 15 irrigations, the external Na concentration appeared to remain relatively constant (being 2.4 mg/g after 5 irrigations but 1.8 mg/g after 15 irrigations) (Table A1.4, Figure A1.11). It is also noteworthy, that tissue concentrations of Na may have perhaps reached a 'plateau' towards the end of the present experiment (Figure A1.11), although further investigation is required. However, if this hypothesis is confirmed, it would suggest that periodic rinsing of the leaf surface would have a reduced impact on foliar Na levels following prolonged exposure to saline irrigation water.

Table A1.4. Leaf tissue concentrations of Na following overhead irrigation with 50% CS water or 75% CS water 5, 10 or 15 times. Values are presented for the difference in Na concentration between rinsed and unrinsed leaves.

		5 irrigations			10 irrigations			15 irrigations		
	CS water (%)	**Rinsed Na (mg/g)**	**Not rinsed Na (mg/g)**	***Diff. (mg/g)***	**Rinsed Na (mg/g)**	**Not rinsed Na (mg/g)**	***Diff. (mg/g)***	**Rinsed Na (mg/g)**	**Not rinsed Na (mg/g)**	***Diff. (mg/g)***
Leucaena	*50*	1.36	1.38	*0.02*	3.12	2.30	*-0.82*	2.88	5.39	*2.51*
	75	3.71	4.38	*0.67*	3.69	6.02	*2.33*	6.33	6.69	*0.36*
Rhodes	*50*	6.76	13.5	*6.74*	10.2	12.8	*2.60*	9.75	10.7	*0.95*
	75	6.64	11.2	*4.56*	10.6	12.4	*1.80*	7.25	11.2	*3.95*
Lucerne	*50*	3.24	3.99	*0.57*	8.31	8.30	*-0.01*	6.77	10.1	*3.33*
	75	5.44	6.98	*1.54*	10.9	12.1	*1.20*	12.0	11.7	*-0.30*

Although Na-dominated salinity can sometimes induce deficiencies of other cations (particularly Ca), leaf tissue concentrations of elements other than Na were not consistently effected by the overhead irrigation of CS water (Table A1.5).

Table A1.5. Leaf tissue concentrations of selected elements following 15 irrigations with 0% CS water, 50% CS water or 75% CS water. Data are presented only for leaf tissues that were rinsed in deionised water following harvest. Tissues from all replicates were combined prior to analysis and hence no indication of error can be presented.

	CS water (%)	Na (mg/g)	K (mg/g)	Ca (mg/g)	P (mg/g)	S (mg/g)	Mg (mg/g)
Leucaena	*0*	0.02	14.9	16.0	1.59	4.13	3.56
	50	2.88	14.6	15.6	1.66	5.99	3.10
	75	6.33	13.2	16.1	1.68	5.89	3.43
Rhodes grass	*0*	2.24	10.4	4.60	4.14	3.94	1.53
	50	9.75	10.1	3.61	3.15	1.78	1.13
	75	7.25	9.23	3.51	3.00	2.48	0.86
Lucerne	*0*	0.27	21.8	15.1	5.75	5.03	2.40
	50	6.77	15.8	14.8	5.21	3.50	2.30
	75	12.0	15.7	12.6	4.51	3.70	2.02

A1.3.5 Other environmental and management factors

The present experiment has been conducted in a glasshouse at The University of Queensland. However, it is known that a range of management and environmental factors are likely to influence the extent to which irrigation waters damage the foliage of plants. Some of these factors are mentioned below, but were outside the scope of the current experiment. Nevertheless, it would be crucial to consider these factors when developing an irrigation program.

In general, it can be assumed that high evaporative demand increases the severity of foliar damage resulting from saline sprays. Bernstein (1975), for example, concluded that the foliage of plants is less affected by salinity when relative humidity was high than when it was low. An FAO (1985) report concluded that greater leaf absorption of ions occurs "mostly during periods of high temperature and low humidity". Toxicity occurs through the absorption of Na^+, Cl^- or both. A high frequency of irrigation and alternate drying and wetting of leaves during sprinkler irrigation (as imposed in the current study) was considered the most damaging.

Maas (1985) recommended night time overhead irrigation; if day time overhead irrigation is necessary, hot, dry and windy days should be avoided. Maas et al. (1982) found that increasing the frequency of sprinkling pepper (*Capsicum annuum*) foliage with saline solution increased salt uptake and leaf injury evident as epinasty, chlorosis, and necrosis.

A1.5 Conclusions

In the present study, we have examined the effects of the overhead irrigation of CS water on leucaena, Rhodes grass and lucerne. It was found that overhead irrigation with either 50% (EC of 3.2 dS/m) or 75% (EC of 4.6 dS/m) CS water did indeed cause visual symptoms of damage to the foliage, although the magnitude of the damage varied substantially between species.

The results for Rhodes grass showed that overhead irrigation of CS water did not decrease biomass, nor did it decrease CF. Interestingly, although examination of foliar symptoms suggested that CS water initially had deleterious effects on the foliage, the magnitude of these effects decreased over time (upon completion of the experiment, the foliage appeared to be generally healthy).

In leucaena, leaflets showed the formation of chlorotic and necrotic lesions when irrigated with either 50% or 75% CS water, although the symptoms were much less pronounced when irrigated with 50% CS water. It was noticed that a number of leaflets were shed by plants when irrigated with 75% CS water – the severity of this increasing with time (although shoot biomass could not be assessed for this plant species). These results for leucaena were supported by measurements of CF (as an indicator of stress) which showed that 50% CS water caused in a slight (but not significant) decrease, whilst 75% CS water resulted in a significant decrease.

For lucerne, the results were similar to those described for leucaena above, although the severity of symptoms caused by the application of CS water was reduced. Specifically, the application of CS water caused the formation of chlorotic and necrotic lesions on the foliage (particularly for the 75% CS water). Interestingly, as observed for Rhodes grass, overhead irrigation of the CS water did not cause a significant decrease in shoot fresh mass (although there was a slight decline). Again, CF was observed to decrease significantly (although still within the general range considered to the normal) following irrigation with CS water.

The data presented here demonstrate that species selection is important given that CS water can potentially have deleterious effects on the foliage of plants when applied as overhead irrigation. As expected, the magnitude of the deleterious effects was higher for 75% CS water than for 50% CS water due to its higher salinity and alkalinity. Differences were observed between the three species examined, with Rhodes grass appearing to be the most tolerant, with no apparent damage caused by either 50% or 75% CS water. For both lucerne and leucaena, the severity of foliar-damage was greater for 75% CS water than for 50% CS water. However, based upon a number of parameters, foliar damage at 50% CS water was rather modest.

This preliminary study has not taken into account a number of factors which would influence plant performance in the field. Here, we have utilized only two treatments containing CS water, but the

composition of CS water is highly variable – care needs to be taken when extrapolating this data to situations where the composition of CS water differs. Importantly, it was not an aim of this preliminary study to separate the deleterious effects alkalinity and salinity.

Finally, management practices and environmental factors will also influence the effects of overhead irrigation with CS water on plants and need to be considered. For example, periodic rainfall (or use of good quality irrigation waters) will likely wash excess salts from the outer leaf surfaces, thereby reducing the potential accumulation of salt. Also, the frequency (and duration) of irrigation will influence the extent to which irrigation waters damage the foliage, as will a range of environmental factors such as temperature and humidity. Regardless, the present preliminary study has indicated that, with careful monitoring and adaptive management, it is likely that the overhead irrigation of CS water of 3.2 or 4.6 dS/m EC can be successful without damage to the foliage of Rhodes grass. Greater care is required with overhead irrigation of leucaena or lucerne where CS water of EC 3.2 dS/m may cause minor damage to foliage.

A1.6 Potential Future Research

This research has provided a rapid initial assessment of whether it is likely that saline and alkaline water will damage plant foliage as a result of overhead irrigation. Study 2 of this research program aims to provide a more comprehensive analysis of the potential deleterious effects of CS water. In particular, the separation of the effects of salinity and alkalinity could allow more rigorous adaptive management of CS water irrigation projects. Any future research should consider the following:

- It would be useful to measure pan evaporation for the duration of the experiment, given its importance in influencing other experimental factors.
- In addition to recording fresh mass of plant tissues, it might be useful to also measure dry mass.
- For tissue analyses, foliar Cl concentrations could be measured (this being the dominant anion).
- Analysis of replicated tissue samples would allow statistical analyses of this data.
- By tagging leaves, it would be possible to track their age and hence ensure that there was uniformity between analyses of the various plant species regarding the duration of exposure to overhead irrigation.
- The confounding effects of salinity and alkalinity could be separated in order to ascertain their importance.

A1.7 References

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A1.8 Appendix 1. Shoot fresh mass of Rhodes grass and lucerne following 15 irrigations

Treatment	Plant Species	Shoot mass (g/pot)
Control	Rhodes grass	97.2
Control	Rhodes grass	80.3
Control	Rhodes grass	69.1
Control	Rhodes grass	73.0
Control	Rhodes grass	98.6
Control	Rhodes grass	76.1
Control	Lucerne	55.1
Control	Lucerne	42.3
Control	Lucerne	45.9
Control	Lucerne	43.5
50% CS water	Rhodes grass	91.5
50% CS water	Rhodes grass	102.8
50% CS water	Rhodes grass	60.8
50% CS water	Rhodes grass	90.7
50% CS water	Rhodes grass	110.6
50% CS water	Rhodes grass	86.0
50% CS water	Lucerne	29.4
50% CS water	Lucerne	35.6
50% CS water	Lucerne	42.6
50% CS water	Lucerne	47.2
50% CS water	Lucerne	40.7
50% CS water	Lucerne	51.7
75% CS water	Rhodes grass	82.0
75% CS water	Rhodes grass	124.3
75% CS water	Rhodes grass	87.7
75% CS water	Rhodes grass	90.2
75% CS water	Rhodes grass	118.6
75% CS water	Rhodes grass	88.8
75% CS water	Lucerne	48.0
75% CS water	Lucerne	37.8
75% CS water	Lucerne	43.4
75% CS water	Lucerne	32.6
75% CS water	Lucerne	31.5
75% CS water	Lucerne	36.2

A1.8 Appendix 2. Output from ANOVA of shoot fresh mass

```
***** Analysis of variance *****

Variate: Mass

Source of variation     d.f.       s.s.       m.s.    v.r.  F pr.
Species                    1    21057.9    21057.9  140.34  <.001
Treatment                  2       91.9       46.0    0.31  0.738
Species.Treatment          2      925.2      462.6    3.08  0.061
Residual                  30     4501.6      150.1
Total                     35    26576.6


* MESSAGE: the following units have large residuals.

*units* 15          -29.6   s.e. 11.2
*units* 26           25.7   s.e. 11.2

***** Tables of means *****

Variate: Mass

Grand mean  66.3

  Species      lucerne Rhodes_grass
                 42.1         90.4

 Treatment     3_dS   4-5_dS  control
               65.8     68.4     64.6

      Species Treatment     3_dS   4-5_dS  control
      lucerne               41.2     38.2     46.8
 Rhodes_grass               90.4     98.6     82.4

*** Standard errors of means ***

Table              Species   Treatment     Species
                                          Treatment
rep.                    18          12           6
d.f.                    30          30          30
e.s.e.                2.89        3.54        5.00

*** Standard errors of differences of means ***

Table              Species   Treatment     Species
                                          Treatment
rep.                    18          12           6
d.f.                    30          30          30
s.e.d.                4.08        5.00        7.07

*** Least significant differences of means (5% level) ***

Table              Species   Treatment     Species
                                          Treatment
rep.                    18          12           6
d.f.                    30          30          30
```

```
l.s.d.                8.34       10.21       14.44

 159  APLOT [RMETHOD=simple] fitted,normal,halfnormal,histogram
 160  AGRAPH [METHOD=means]

LSD Species.Treatment

 161  LSD Species.Treatment

 WARNING: F-test is not significant at the 5% level
 Prob. of F = 0.06058  and so LSDs are probably inappropriate!


 ***** Table of Ranked Means *****

 Interaction for factors:     Species   Treatment

     Species   Treatment  rep.        Mean

     lucerne      4-5_dS     6       38.23
     lucerne        3_dS     6       41.20
     lucerne     control     6       46.80
 Rhodes_grass     control     6       82.35
 Rhodes_grass        3_dS     6       90.41
 Rhodes_grass      4-5_dS     6       98.58

   ++  LSD = 14.44


LSD Species
 162  LSD Species

 F-test is significant at the 5% level

  ***** Table of Ranked Means *****

     Species  rep.        Mean

     lucerne    18       42.08 a
 Rhodes_grass    18       90.45 b


NB: Means with same subscript are not significantly different at the 5%
level

    ++  LSD = 8.339
```

A1.8 Appendix 3. Chlorophyll fluorescence for leucaena, Rhodes grass, and lucerne

Species	Number of irrigations	Treatment	F_v/F_m
Leucaena	5	No Spray	0.8224
Leucaena	5	Control	0.8203
Leucaena	5	50%	0.8154
Leucaena	5	75%	0.8176
Leucaena	11	No Spray	0.8172
Leucaena	11	Control	0.8159
Leucaena	11	50%	0.8015
Leucaena	11	75%	0.7699
Leucaena	15	No Spray	0.8205
Leucaena	15	Control	0.8095
Leucaena	15	50%	0.7996
Leucaena	15	75%	0.7827
Rhodes grass	5	No Spray	0.7982
Rhodes grass	5	Control	0.7973
Rhodes grass	5	50%	0.7951
Rhodes grass	5	75%	0.7976
Rhodes grass	11	No Spray	0.7717
Rhodes grass	11	Control	0.7588
Rhodes grass	11	50%	0.7642
Rhodes grass	11	75%	0.7629
Rhodes grass	15	No Spray	0.7538
Rhodes grass	15	Control	0.7560
Rhodes grass	15	50%	0.7675
Rhodes grass	15	75%	0.7540

Lucerne	5	No Spray	
Lucerne	5	Control	0.8151
Lucerne	5	50%	0.8103
Lucerne	5	75%	0.8041
Lucerne	11	No Spray	
Lucerne	11	Control	0.8039
Lucerne	11	50%	0.7821
Lucerne	11	75%	0.7845
Lucerne	15	No Spray	
Lucerne	15	Control	0.8217
Lucerne	15	50%	0.8147
Lucerne	15	75%	0.8065

A1.8 Appendix 4. Output from ANOVA of chlorophyll fluorescence, leucaena

```
***** Analysis of variance *****

Variate: CF

Source of variation     d.f.(m.v.)      s.s.       m.s.    v.r.  F pr.
irrigationsteps            2       0.0228175  0.0114087   15.17  <.001
solutions                  3       0.0472610  0.0157537   20.95  <.001
irrigationsteps.solutions
                           6       0.0202963  0.0033827    4.50  <.001
Residual                303(45)    0.2278231  0.0007519
Total                   314(45)    0.3096963


***** Tables of means *****

Variate: CF

Grand mean  0.8077

 irrigationsteps   11irri   15irri    5irri
                   0.8011   0.8031   0.8189

 solutions    50%CS    75%CS  DIwater  nospray
             0.8055   0.7901   0.8153   0.8200

 irrigationsteps solutions    50%CS    75%CS  DIwater  nospray
          11irri             0.8015   0.7699   0.8159   0.8172
          15irri             0.7996   0.7827   0.8095   0.8205
           5irri             0.8154   0.8176   0.8203   0.8224


*** Standard errors of means ***

Table          irrigationsteps   solutions irrigationsteps
                                           solutions
rep.                   120          90          30
d.f.                   303         303         303
e.s.e.             0.00250     0.00289     0.00501

(Not adjusted for missing values)

*** Standard errors of differences of means ***

Table          irrigationsteps   solutions irrigationsteps
                                           solutions
rep.                   120          90          30
d.f.                   303         303         303
s.e.d.             0.00354     0.00409     0.00708

(Not adjusted for missing values)

*** Least significant differences of means (5% level) ***

Table          irrigationsteps   solutions irrigationsteps
```

```
                                        solutions
rep.                    120          90          30
d.f.                    303         303         303
l.s.d.              0.00697     0.00804     0.01393

(Not adjusted for missing values)

 382  APLOT [RMETHOD=simple] fitted,normal,halfnormal,histogram
 383  AGRAPH [METHOD=means]

LSD irrigationsteps.solutions
 384  LSD irrigationsteps.solutions

 F-test is significant at the 5% level


 ***** Table of Ranked Means *****

 Interaction for factors:irrigationsteps      solutions

irrigationsteps      solutions  rep.        Mean

      11irri       75%CS    30      0.7699 a
      15irri       75%CS    30      0.7827 a
      15irri       50%CS    30      0.7996 b
      11irri       50%CS    30      0.8015 bc
      15irri     DIwater    30      0.8095 bcd
       5irri       50%CS    30      0.8154 cd
      11irri     DIwater    30      0.8159 d
      11irri     nospray    30      0.8172 d
       5irri       75%CS    30      0.8176 d
       5irri     DIwater    30      0.8203 d
      15irri     nospray    30      0.8205 d
       5irri     nospray    30      0.8224 d


NB: Means with same subscript are not significantly different at the 5%
level


   ++  LSD = 0.01393
```

A1.8 Appendix 5. Output from ANOVA of chlorophyll fluorescence, Rhodes grass

```
***** Analysis of variance *****

Variate: CF

Source of variation     d.f.(m.v.)      s.s.        m.s.     v.r.  F pr.
irrigationsteps            2        0.1057490  0.0528745  159.40  <.001
solutions                  3        0.0015175  0.0005058    1.52  0.208
irrigationsteps.solutions
                           6        0.0050827  0.0008471    2.55  0.020
Residual                 292(56)    0.0968578  0.0003317
Total                    303(56)    0.1713531


***** Tables of means *****

Variate: CF

Grand mean  0.77308

 irrigationsteps   11irri   15irri    5irri
                  0.76442  0.75782  0.79702

 solutions    50%CS    75%CS  DIwater  nospray
            0.77559  0.77148  0.77068  0.77459

 irrigationsteps solutions    50%CS    75%CS  DIwater  nospray
          11irri            0.76423  0.76290  0.75880  0.77173
          15irri            0.76747  0.75400  0.75597  0.75383
           5irri            0.79506  0.79755  0.79728  0.79820


*** Standard errors of means ***

Table          irrigationsteps   solutions irrigationsteps
                                           solutions
rep.                   120          90          30
d.f.                   292         292         292
e.s.e.            0.001663    0.001920    0.003325

(Not adjusted for missing values)

*** Standard errors of differences of means ***

Table          irrigationsteps   solutions irrigationsteps
                                           solutions
rep.                   120          90          30
d.f.                   292         292         292
s.e.d.            0.002351    0.002715    0.004703

(Not adjusted for missing values)

*** Least significant differences of means (5% level) ***

Table          irrigationsteps   solutions irrigationsteps
                                           solutions
```

```
rep.                  120          90          30
d.f.                  292         292         292
l.s.d.           0.004628    0.005343    0.009255

(Not adjusted for missing values)

1095  APLOT [RMETHOD=simple] fitted,normal,halfnormal,histogram
1096  AGRAPH [METHOD=means]

LSD irrigationsteps.solutions
1097  LSD irrigationsteps.solutions

 F-test is significant at the 5% level


 ***** Table of Ranked Means *****

 Interaction for factors:irrigationsteps      solutions

irrigationsteps      solutions  rep.        Mean

      15irri     nospray    30      0.7538 a
      15irri       75%CS    30      0.7540 a
      15irri     DIwater    30      0.7560 ab
      11irri     DIwater    30      0.7588 abc
      11irri       75%CS    30      0.7629 abcd
      11irri       50%CS    30      0.7642 bcd
      15irri       50%CS    30      0.7675 cd
      11irri     nospray    30      0.7717 d
       5irri       50%CS    30      0.7951 e
       5irri     DIwater    30      0.7973 e
       5irri       75%CS    30      0.7975 e
       5irri     nospray    30      0.7982 e


NB: Means with same subscript are not significantly different at the 5%
level


   ++  LSD = 0.009255
```

A1.8 Appendix 6. Output from ANOVA of chlorophyll fluorescence, lucerne

```
***** Analysis of variance *****

Variate: CF

Source of variation     d.f.(m.v.)      s.s.        m.s.     v.r.  F pr.
irrigationsteps            2        0.0296583  0.0148292   19.54  <.001
solutions                  2        0.0111490  0.0055745    7.34  <.001
irrigationsteps.solutions
                           4        0.0026794  0.0006699    0.88  0.475
Residual                231(30)     0.1753313  0.0007590
Total                   239(30)     0.2172904



***** Tables of means *****

Variate: CF

Grand mean  0.8048

 irrigationsteps   11irri   15irri    5irri
                   0.7902   0.8143   0.8098

 solutions    50%CS    75%CS  DIwater
             0.8023   0.7984   0.8136

 irrigationsteps solutions    50%CS    75%CS  DIwater
          11irri             0.7821   0.7845   0.8039
          15irri             0.8147   0.8065   0.8217
           5irri             0.8103   0.8041   0.8151


*** Standard errors of means ***

Table          irrigationsteps   solutions irrigationsteps
                                           solutions
rep.                     90          90          30
d.f.                    231         231         231
e.s.e.              0.00290     0.00290     0.00503

(Not adjusted for missing values)

*** Standard errors of differences of means ***

Table          irrigationsteps   solutions irrigationsteps
                                           solutions
rep.                     90          90          30
d.f.                    231         231         231
s.e.d.              0.00411     0.00411     0.00711

(Not adjusted for missing values)

*** Least significant differences of means (5% level) ***

Table          irrigationsteps   solutions irrigationsteps
```

```
                                        solutions
rep.                    90          90          30
d.f.                   231         231         231
l.s.d.             0.00809     0.00809     0.01402

(Not adjusted for missing values)

 895  APLOT [RMETHOD=simple] fitted,normal,halfnormal,histogram
 896  AGRAPH [METHOD=means]

LSD irrigationsteps.solutions
 897  LSD irrigationsteps.solutions

 WARNING: F-test is not significant at the 5% level
 Prob. of F = 0.47506  and so LSDs are probably inappropriate!


 ***** Table of Ranked Means *****

 Interaction for factors:irrigationsteps      solutions

irrigationsteps      solutions  rep.        Mean

      11irri       50%CS    30      0.7821
      11irri       75%CS    30      0.7845
      11irri     DIwater    30      0.8039
       5irri       75%CS    30      0.8041
      15irri       75%CS    30      0.8065
       5irri       50%CS    30      0.8103
      15irri       50%CS    30      0.8147
       5irri     DIwater    30      0.8151
      15irri     DIwater    30      0.8217


   ++  LSD = 0.01402
```

A1.8 Appendix 7. Concentrations of elements measured in plant shoot tissues

Species	Irrigation number	CS-Water (%)	Rinsed?	Na (mg/g)	K (mg/g)	Ca (mg/g)	P (mg/g)	S (mg/g)	Mg (mg/g)
Leucaena	0	n.a.	n.a.	0.15	16.39	11.54	1.95	3.79	2.49
Rhodes grass	0	n.a.	n.a.	5.53	22.58	5.50	5.77	7.77	2.10
Lucerne	0	n.a.	n.a.	0.40	17.27	17.83	4.13	3.81	2.68
Rhodes grass	1	50	yes	2.06	12.69	3.49	3.47	4.22	1.43
Leucaena	1	50	yes	0.08	13.71	12.67	1.77	5.15	3.71
Lucerne	1	50	yes	0.39	21.07	14.07	5.95	4.56	2.67
Lucerne	1	75	yes	0.61	16.42	15.62	6.01	4.15	2.90
Rhodes grass	1	75	yes	1.77	10.47	3.79	3.79	3.53	1.55
Leucaena	1	75	yes	0.09	15.27	18.24	1.83	5.06	3.69
Leucaena	1	0	n.a.	0.02	16.87	12.76	1.93	4.28	3.18
Rhodes grass	1	0	n.a.	2.18	19.23	5.35	5.97	7.13	2.06
Lucerne	1	0	n.a.	0.53	20.76	18.11	4.24	2.90	2.47
Lucerne	1	75	no	1.05	19.86	16.76	4.46	3.84	2.50
Rhodes grass	1	75	no	4.33	19.86	5.43	6.00	7.29	1.93
Leucaena	1	75	no	0.56	14.74	13.50	1.91	5.00	3.58
Rhodes grass	1	50	no	5.15	27.48	5.84	6.30	7.11	2.07
Lucerne	1	50	no	0.77	18.15	13.75	4.64	2.79	1.97
Leucaena	1	50	no	0.24	13.88	11.17	1.97	4.75	2.89
Lucerne	5	50	yes	3.24	17.86	11.97	5.66	4.02	2.80
Rhodes grass	5	50	yes	6.76	13.33	4.79	4.19	4.19	1.86
Leucaena	5	50	yes	1.36	11.64	15.17	1.45	3.99	4.26
Rhodes grass	5	75	yes	6.64	9.39	4.08	3.73	3.34	1.61
Lucerne	5	75	yes	5.44	19.42	17.57	5.78	5.57	3.69
Leucaena	5	75	yes	3.71	15.79	20.18	1.77	5.62	3.87
Lucerne	5	0	n.a.	0.36	19.34	14.26	5.25	3.57	2.37
Rhodes grass	5	0	n.a.	2.75	18.37	4.64	5.59	6.85	1.83
Leucaena	5	0	n.a.	-0.01	14.61	12.65	1.89	4.47	3.08
Leucaena	5	75	no	1.38	14.81	11.26	2.10	5.50	3.24
Rhodes grass	5	75	no	11.20	14.89	4.34	4.59	4.02	1.57
Lucerne	5	75	no	6.98	18.77	12.89	4.66	4.38	2.13
Leucaena	5	50	no	0.56	13.34	13.33	1.91	5.32	3.18
Lucerne	5	50	no	3.99	17.34	18.12	4.22	4.32	2.66
Rhodes grass	5	50	no	13.47	17.50	4.65	5.11	5.14	1.87
Rhodes grass	11	50	yes	10.22	10.91	3.84	3.23	2.36	1.27
Lucerne	11	50	yes	8.31	18.02	15.84	5.28	3.94	2.08
Leucaena	11	50	yes	3.12	14.23	16.80	2.04	5.45	2.92
Rhodes grass	11	75	yes	10.57	10.07	4.10	3.21	2.17	1.17
Leucaena	11	75	yes	3.69	10.68	17.22	1.52	6.07	3.19
Lucerne	11	75	yes	13.99	17.33	17.72	5.09	4.91	2.38
Leucaena	10	75	no	6.02	13.33	13.42	1.93	5.96	3.17
Lucerne	10	75	no	12.09	18.14	16.03	5.10	4.20	2.42

Rhodes grass	10	75	no	12.42	12.75	3.75	3.46	2.65	1.19
Leucaena	10	50	no	2.30	14.26	14.61	1.82	5.66	3.07
Lucerne	10	50	no	8.30	16.93	18.06	4.33	3.70	2.27
Rhodes grass	10	50	no	12.80	12.68	3.97	4.06	2.89	1.41
Leucaena	10	0	n.a.	0.02	14.83	16.87	1.79	4.83	3.65
Lucerne	10	0	n.a.	0.49	21.10	18.99	5.08	4.79	2.74
Rhodes grass	10	0	n.a.	2.96	14.85	4.40	4.90	5.55	1.58
Leucaena	15	0	n.a.	0.02	14.86	16.04	1.59	4.13	3.56
Lucerne	15	0	n.a.	0.27	21.76	15.09	5.75	5.03	2.40
Rhodes grass	15	0	n.a.	2.24	10.39	4.60	4.14	3.94	1.53
Rhodes grass	15	75	no	11.20	10.64	3.92	3.11	2.06	1.13
Lucerne	15	75	no	11.71	17.16	12.83	4.29	3.95	2.12
Leucaena	15	75	no	6.69	13.53	15.23	1.72	5.97	3.01
Lucerne	15	50	no	10.09	12.37	15.44	5.14	4.17	2.19
Leucaena	15	50	no	5.39	12.12	17.61	1.80	6.21	3.25
Rhodes grass	15	50	no	10.72	9.56	3.96	3.46	1.98	1.15
Leucaena	15	50	yes	2.88	14.64	15.58	1.66	5.99	3.10
Rhodes grass	15	50	yes	9.75	10.11	3.61	3.15	1.78	1.13
Lucerne	15	50	yes	6.77	15.84	14.76	5.21	3.50	2.30
Leucaena	15	75	yes	6.33	13.18	16.12	1.68	5.89	3.43
Rhodes grass	15	75	yes	7.25	9.23	3.51	3.00	1.48	0.86
Lucerne	15	75	yes	12.03	15.75	12.61	4.51	3.70	2.02

Appendix A2 – Overhead irrigation study 2

Overhead irrigation of saline-sodic and alkaline water: Examination of the potential deleterious effects on foliage of Rhodes grass and leucaena

Honours Thesis

Submitted for the degree of Hons B EnvSci,

School of Agriculture and Food Sciences, The University of Queensland, St. Lucia

14 October, 2015

Federico Cicchelli

Project Supervisors: Dr P. Kopittke & Dr B. Wehr

Note: graphs and data have been reanalysed and as such information presented here has been superseded

THE UNIVERSITY OF QUEENSLAND
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A2.1 Abstract

Saline-sodic and alkaline groundwater provides a potentially valuable resource provided its irrigation does not decrease plant growth. Although the adverse effects of salts within the rooting environment are well-studied, surprisingly little is known regarding the direct effects of overhead irrigation of saline-sodic and alkaline water on plant foliage. The present study examined the potential deleterious effects of saline-sodic (electrical conductivity, EC, ≤ 15 dS/m) and alkaline (≤ 2000 mg/L, $CaCO_3$ equivalent) water on foliage of Rhodes grass (*Chloris gayana* cv. *Reclaimer*) and leucaena (*Leucaena leucocephala* ssp. *glabrata* cv. Tarramba) in a range of growing-conditions. Foliage of leucaena was sensitive, with necrosis and chlorosis evident for saline water at an EC ≥ 3 dS/m and alkaline water containing ≥ 500 mg/L ($CaCO_3$ equivalent). This damage to the foliage reduced shoot fresh mass for saline-treatments (an EC of 6 dS/m corresponding to a 50% reduction in fresh mass) but not for alkaline-treatments, with CF also reduced. In contrast to leucaena, shoot fresh mass of Rhodes grass was not reduced in any treatment nor was CF reduced. It was noted that growing conditions influenced the magnitude of the deleterious effects, with salinization of the soil increasing tolerance to foliar-applied saline water. In contrast, plants grown in ambient conditions (i.e. outside the glasshouse) were less tolerant to foliar-applied salt. This study has demonstrated that whilst saline-sodic and alkaline water can potentially be used for overhead irrigation, differences exist between plant species and growing conditions also influence the observed tolerance.

A2.2 Introduction

In low-rainfall environments, the extraction of saline-sodic and alkaline groundwaters (such as for coal seam gas [CSG] production) is potentially valuable for agricultural production. For example, the Great Artesian Basin (Australia) which is the largest artesian basin in the world, contains an estimated 65,000 million ML of groundwater (Nevill et al., 2010). This groundwater (including the coal seam water) can be beneficially used to increase

agricultural production. However, much of the water in the Great Artesian Basin is saline and alkaline, with the electrical conductivity values typically ranging from 1 to >10 dS/m (Great Artesian Basin Consultative Council, 1998). Therefore, it is important that the irrigation of these waters does not result in degradation of the soil resource and that it does not reduce plant growth.

The potential adverse effects of salts within the rooting environment (soil) are well-known, causing plant osmotic stress, ion toxicity, and decreased photosynthesis and growth (Munns, 2002; Paz et al., 2012; Tester & Davenport, 2003). However, little information is available regarding the direct effect of the overhead irrigation of saline-sodic and alkaline waters on plant foliage. A report by FAO (1985) indicated that for equal water quality, plant physiological responses vary between overhead and direct irrigation of soil. For example, whilst *Citrus sp.* displayed foliar symptoms when sprinkler-irrigated with water containing 3 mM Na and Cl, no effects were observed when the same water was applied through flood and furrow irrigation. Similarly, a comparative study on bell pepper (*Capsicum frutescens*) by Bernstein and Francois (1973) examined yield response form furrow, drip, and sprinkler irrigation with water at an EC of 4 dS/m, with these authors finding a reduction in yield of 18% for furrow irrigation, 2% for drip irrigation, and 59% for sprinkler irrigation. However, the large yield decrease for sprinkler irrigated pepper plants, was caused by cumulative salt absorption by rooting-system and foliage. Therefore, an estimate of the foliar damage caused by Na and Cl directly absorbed by leaves is not possible. The effects of the overhead irrigation of CS water on cotton (*Gossypium hirsutum*), barley (*Hordeum vulgare*) and Italian ryegrass (*Lolium multiform*) was investigate by Beletse, Annandale, Steyn, Hall, and Aken (2008). The irrigation water with a total alkalinity of 4712 mg/L (as $CaCO_3$ equivalent) and at an EC of 7.5 dS/m caused leaf scorching only in cotton. However, in the experiment of Beletse et al. (2008) (as seen in Bernstein and Francois (1973)) the irrigation water was able to move through the soil profile, thereby plant symptoms were caused by both root and foliar exposure to salinity and alkalinity. Interestingly, CS water produced only minor symptoms in leaves of cotton, thus suggesting great variability to salt and alkali tolerance between plant species. Maas (1985) reported that sprinkling irrigation with saline water can produce foliar injury such as chlorosis and necrosis due to increased foliar absorption of Na

and Cl, however, the magnitude of these symptoms substantially change across plant species. Foliar toxicity symptoms tend to appear in older leaves and under hot and dry conditions (Maas, 1985; Maas, Clark, & Francois, 1982; McCune, 1991). This, possibly due to the high evaporation rate and enhanced accumulation of Na and Cl on leaf surface.

Although evidence from past research suggests that the overhead irrigation of saline-sodic and alkaline water can potentially produce adverse effects on plant foliage, in almost all previous studies, irrigation water was applied to both plant foliage and to the soil (resulting in additive impacts). The present study aimed to establish the threshold for the safe overhead irrigation of CS water by examining the potential deleterious effects of saline-sodic and alkaline water when applied to the foliage of Rhodes grass (*Chloris gayana* Kunth) and leucaena (*Leucaena leucocephala*). The effect of growth conditions was also examined, with plants grown either inside the glasshouse or in ambient conditions (i.e. external to the glasshouse). Furthermore, whilst most plants were grown in a non-saline soil from which the saline foliar-applied water was excluded, some plants were grown in a salinised soil. Plant performance was assessed using a range of parameters, including visual symptoms, CF, and fresh mass production. The results of this experiment will assist in the development of regulatory guidelines for the beneficial use of saline-sodic and alkaline water in overhead irrigation programs.

A2.3 Materials and methods

A2.3.1 Soil preparation and experimental design

This experiment aimed to investigate whether saline and alkaline water has deleterious effects when it is overhead-irrigated and exposed directly to plant foliage. The surface 0-25 cm of a non-saline Red Kandosol (Ultisol) (Isbell, 2002; Soil Survey Staff, 2003) was collected from a field irrigation site near Roma (Queensland, Australia) (25.713° S; 148.992° E). The soil was air-dried, sieved to 10 mm, and placed in 2.8 L pots. Given the low pH of the soil (pH of 3.9, 1:5 water), $Ca(OH)_2$ was concomitantly mixed through the soil at a rate of 1.5 g/kg – this being determined from a preliminary experiment as being sufficient to increase to ca.

pH 5.5. A basal application of gypsum was applied at a rate equivalent of 3 t/ha (2 g/kg) as is common agricultural practice for these soils in the field. Furthermore, a basal application of slow release fertiliser (Osmocote Exact Standard, 5 g/pot) was mixed through the soil to ensure a minimum rate equivalent of ca. 50 kg/ha of P and ca. 150 kg/ha of N. Additional liquid fertiliser (Grow Force Flow Feed EX7) was applied after seedlings were established (see later) and every three weeks until plants were harvested.

Overhead irrigation with two types of water was investigated for potential adverse effects on plant foliage, with treatments either increasing in salinity or increasing in alkalinity (Table A2.1). For each of these two water types (i.e. saline or alkaline), the experiment investigated two plant species (Rhodes grass and leucaena), two types of irrigation water applied to the soil (non-saline or saline), and two growth conditions (glasshouse or ambient). Thus, the experiment consisted of a total of 37 treatments (Table A2.1) with three replicates, yielding a total of 111 experimental units arranged in a randomised block design. The salinity (i.e. electrical conductivity, EC) and alkalinity values chosen (Table A2.1) exceed that to be used for the irrigation of CS water, but were selected because they cover the range of values likely to influence plant growth (FAO, 1985; Maas et al., 1982) and hence would enable limits to be defined for the safe overhead irrigation of waters.

Table A2.1. Treatments used to evaluate the effects of overhead irrigation of saline-sodic and alkaline water on growth of Rhodes grass and leucaena.

Treatment	Species	Foliar-irrigating EC (dS/m)	Soil-irrigating EC (dS/m)	Alkalinity (mg/L $CaCO_3$ equivalent)	Growth conditions
1	Rhodes grass	0	0	0	Glasshouse
2	Rhodes grass	3	0	0	Glasshouse
3	Rhodes grass	4	0	0	Glasshouse
4	Rhodes grass	5	0	0	Glasshouse
5	Rhodes grass	6	0	0	Glasshouse
6	Rhodes grass	8	0	0	Glasshouse
7	Rhodes grass	10	0	0	Glasshouse
8	Rhodes grass	12	0	0	Glasshouse
9	Rhodes grass	15	0	0	Glasshouse
10	Leucaena	0	0	0	Glasshouse
11	Leucaena	3	0	0	Glasshouse
12	Leucaena	4	0	0	Glasshouse
13	Leucaena	5	0	0	Glasshouse
14	Leucaena	6	0	0	Glasshouse
15	Leucaena	8	0	0	Glasshouse
16	Leucaena	4	0	250	Glasshouse
17	Rhodes grass	4	0	500	Glasshouse
18	Rhodes grass	4	0	750	Glasshouse
19	Rhodes grass	4	0	1250	Glasshouse
20	Rhodes grass	4	0	2000	Glasshouse
21	Rhodes grass	4	0	250	Glasshouse
22	Leucaena	4	0	500	Glasshouse
23	Leucaena	4	0	750	Glasshouse
24	Leucaena	4	0	1250	Glasshouse
25	Leucaena	4	0	2000	Glasshouse
26	Rhodes grass	0	10	0	Glasshouse
27	Rhodes grass	4	10	0	Glasshouse
28	Rhodes grass	8	10	0	Glasshouse
29	Leucaena	0	10	0	Glasshouse
30	Leucaena	4	10	0	Glasshouse
31	Leucaena	8	10	0	Glasshouse
32	Rhodes grass	0	0	0	Ambient
33	Rhodes grass	4	0	0	Ambient
34	Rhodes grass	8	0	0	Ambient
35	Leucaena	0	0	0	Ambient
36	Leucaena	4	0	0	Ambient
37	Leucaena	8	0	0	Ambient

A2.3.2 Plant growth and soil-applied irrigation

All plant-growth experiments were conducted at The University of Queensland (St Lucia, Australia) from February to August 2015. During this period, the average maximum temperature in the glasshouse was 30 °C and the minimum was 17 °C (outside the glasshouse, corresponding temperatures were 29.5 °C and 10 °C).

The two plant species, Rhodes Grass (*Chloris gayana* cv. *Reclaimer*) and leucaena (*Leucaena leucocephala* ssp. *glabrata* cv. Tarramba), were selected as being economically important species for ruminant production in Australia. Seeds of Rhodes grass and leucaena were placed in the appropriate 2.8 L pots and allowed to grow for seven weeks before being thinned to either 10 plants (Rhodes grass) or two plants (leucaena) per pot. Plants were then grown for a further four weeks prior to commencement of overhead irrigation and then a further three months during overhead irrigation (see below). During the experimental period, Rhodes grass tillers and leucaena leaves were tagged with cotton strings (Figure A2.1) to identify those which were irrigated for the entire duration of the experiment from newly formed ones (i.e. older leaves received all treatment applications).

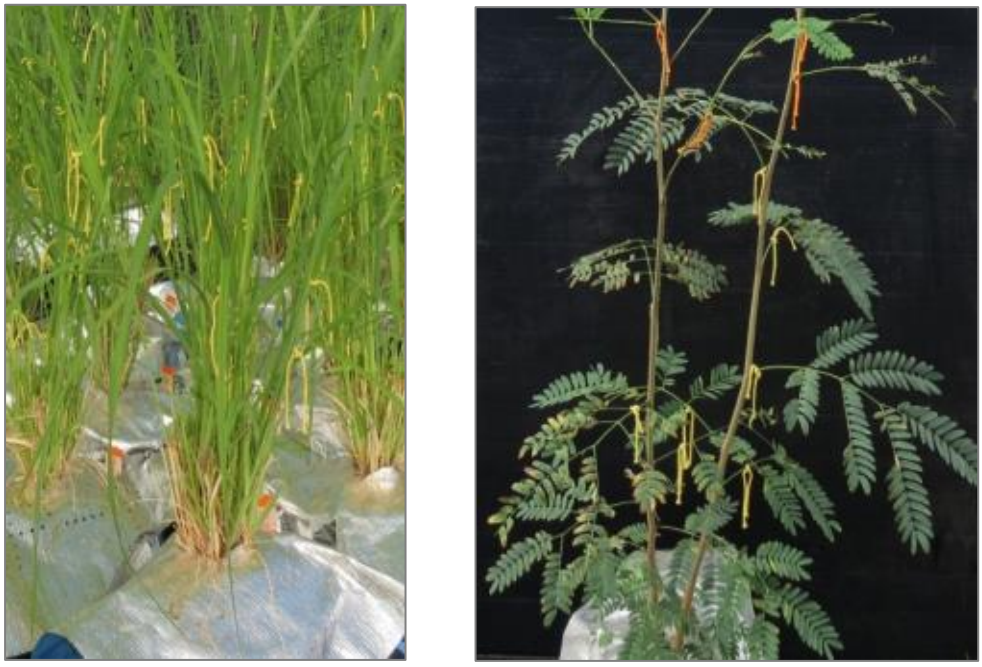

Figure A2.1. Images showing Rhodes grass tillers (on the left) and leucaena leaves (on the right) tagged with cotton strings. This is to identify those leaves that were exposed to all irrigation events. Also note

that the soil was covered (in all treatment pots) to minimise water absorption during overhead irrigation, thereby limiting the treatment availability for plant root uptake.

As required for growth, water was applied to the soil throughout the entire duration of the experiment. For all pots, water was applied directly to the soil, taking care to avoid contact with the foliage. This soil-applied water was either non-saline (deionised water) or had a salinity of 10 dS/m (Table A2.1 and Table A2.2) and was applied twice per week at a rate sufficiently high (i.e. 300 to 800 mL per pot) to ensure thorough leaching of the soil. For the saline soil-applied water (10 dS/m), the leachate was collected (Figure A2.2) and analyzed. The EC values measured for the leachate ranged between 8.6 and 18 dS/m, with an average value of 11.4 dS/m.

Figure A2.2. Image showing the double-pot system used for treatments in which plants were growing into saline soil. Saline water was used to flush the soil contained in the black pot. The leachate collected into the second pot (white bucket) was used to monitor the soil liquid phase electrical conductivity (e.g. salinity).

A2.3.3 Overhead irrigation

The saline and alkaline solutions required for overhead irrigation were prepared in 200 L containers using NaCl and $NaHCO_3$ (Table A2.2). Plants were overhead-irrigated using irrigation chambers connected to water pumps (Flowjet water system, Figure A2.3) with solutions applied as a fine mist using mist sprayers (Netafim microjet). During each 30-min irrigation event, the water was cycled on for 1 min and off for 9 min to reduce consumption of water whilst keeping the leaves continuously wet (Maas et al., 1982). A total of 30 irrigation events were conducted over a three month period.

Table A2.2. Treatment solutions used for the investigation of the potential adverse effects of salinity and alkalinity on plant foliage following overhead irrigation (Table A2.1).

Electrical conductivity (dS/m)	Alkalinity (mg/L as $CaCO_3$ equivalent)	NaCl (g/L)	$NaHCO_3$ (g/L)
0	0	0	0
3	0	1.6	0
4	0	2.1	0
5	0	2.7	0
6	0	3.2	0
8	0	4.3	0
10	0	5.5	0
12	0	6.6	0
15	0	8.3	0
4	250	1.8	0.42
4	500	1.5	0.84
4	750	1.3	1.3
4	1250	0.86	2.1
4	2000	0.26	3.4

The soil was covered with a protective foil in all treatment pots (Figure A2.1) to limit the movement of the saline and alkaline water applied by overhead-irrigation into the soil. Furthermore, to ensure that the saline-sodic and alkaline overhead irrigation water had not infiltrated into the rooting environment, at completion of the irrigation trial, soil samples (0 to 3 cm depth) were collected from several pots to examine whether any infiltration of overhead-irrigated water had influenced soil pH and EC. However, no increase in pH or EC was evident in any treatment (Table A2.4 in Appendix).

Figure A2.3. Images of the overhead irrigation system. On the left, the system set-up; plants were irrigated in chambers (right) connected to water pumps (middle) and 200L drums (left) containing relevant treatments. On the right, details of an irrigation chamber; water piping equipped with Netafim microjet nozzles was fitted above the structure to enhance plant foliage wetting.

A2.3.4 Measurements

Leaves were regularly examined visually to assess for symptoms of toxicity. Chlorophyll fluorescence (Optiscience OS30p+) was measured after 6, 12, 18, 24 and 30 irrigations with measurements taken at night following leaves dark adaptation (>2 h) on five leaves of equal age (i.e. leaves exposed to all irrigation events) that were randomly selected from each experimental unit. Data are presented as the ratio between variable fluorescence (F_v) and

maximum fluorescence (F_m) which indicates the efficiency of the Photosystem II in plant leaves (Maxwell & Johnson, 2000).

A2.3.5 Plant tissue harvest and analysis

Upon completion of the irrigation trial (e.g. 30 irrigation events), all plants were harvested and fresh mass measured. Plant foliage was immediately rinsed with deionised water to remove accumulated salt from the surface of the leaves. The rinsing time required for the removal of soluble salts from the leaf surface was determined in a preliminary experiment using five randomly selected leaves from both species. Briefly, an EC electrode was placed in a glass beaker with 250 mL of deionised water and a magnetic stirrer. A leaf was placed in the water, with any salt on the leaf surface dissolving – measurements of solution EC were taken every 3 s for a total of 180 s. By measuring the increase in the solution EC, it was determined that a rinsing time of ca. 18 s was appropriate. Following rinsing, plant material was dried (65 °C) for 4 d and dry mass recorded. For tissue analysis, samples of leaves exposed to all 30 irrigation events were ground and open-vessel digested using a 5:1 mixture of nitric (HNO_3) and perchloric ($HClO_4$) acids, prior to analysis by ICP-OES. Tissue Cl concentrations were determined colorimetrically on samples digested with 0.1 M HNO_3.

A2.3.6 Statistical analyses

Changes in the fresh mass of the shoot tissues were analysed using regression analyses (SYSTAT 13, Cranes Software, India), fitting curves of the general form

$$\text{FreshMass} = b/\exp[(cT)^h]$$

where b is the maximum fresh mass in toxicant-free conditions, c is a strength coefficient that increases with the strength of the toxicant, T is the toxicant intensity (i.e. the level of salinity or alkalinity), and h is a shape coefficient (Kinraide, 1999). Curves were plotted where R^2 was ≥0.5, an arbitrary decision to ensure meaningful determination of relationships between fresh mass and toxicant variables.

A2.4 Results

A2.4.1 Visual observations

Plant growth during the experimental period was good, with all plants in the control treatment appearing healthy (Figures A2.4 – A2.5). For plants growing in the saline soil irrigated with water at 10 dS/m (without additional saline overhead irrigation), as expected, some symptoms were observed for both Rhodes grass and leucaena; these symptoms being brown and necrotic leaf tips in Rhodes grass (Figure A2.9) and chlorosis in leucaena (Figure A2.7).

Despite good growth in these control treatments, the overhead irrigation of saline and alkaline water was found to have deleterious effects and caused foliar damage in both plant species (Figures A2.4 – A2.11). However, the magnitude of this foliar damage depended upon the plant species, composition of the irrigation water, the salinity of the soil, and the growing conditions (glasshouse or ambient) – these factors are considered in detail below.

Firstly, giving consideration to the saline overhead irrigation water, chlorosis and necrosis was observed for leaves of both plant species in areas where the water droplets had accumulated and successively dried (Figure A2.4). For leucaena, these chlorotic and necrotic lesions were observed after only three irrigation events, with the magnitude of this damage increasing with increasing salinity (being observed in all treatments with an EC ≥3 dS/m) and with an increasing number of irrigations. Foliar abscission occurred in severely damaged leaves, with this abscission becoming particularly pronounced after ca. 10 irrigations for the 8 dS/m treatment in all growing conditions. Although the same symptoms were observed in Rhodes grass, they were less pronounced. Indeed, symptoms were not were observed until ca. 10 irrigations, when necrotic and curly leaf tips developed in most plants (particularly those growing in saline soil). Interestingly, for Rhodes grass grown in the glasshouse, the severity of these symptoms did not increase markedly with increasing EC, with the symptoms observed at 8 dS/m being similar to those observed at 15 dS/m. Furthermore, Rhodes grass grown in either ambient (non-glasshouse) conditions or in saline soil showed an increase in the severity of symptoms (Figure A2.10). For example, whilst symptoms were

observed for glasshouse-grown plants at an EC of 8 dS/m (see above, Figure A2.9), similar symptoms were observed in the corresponding plants grown in ambient conditions at an EC of 4 dS/m (Figure A2.10).

Secondly, the effects of increasing alkalinity (at constant salinity, 4 dS/m) in the overhead irrigation water were examined. As described above, a solution with an EC of 4 dS/m (and no added alkalinity) resulted in chlorosis and necrosis of the leaf tissue – this being more severe in leucaena. However, increases in alkalinity (at constant EC) did not result in a marked increase in the severity of the symptoms (Figure A2.8 and Figure A2.11) on leaves in areas where water droplets accumulated and dried. However, although the magnitude of the symptoms did not increase, visual observation indicated that necrosis was more severe (and chlorosis less severe) compared to the saline-sodic treatments.

Figure A2.4. Photos displaying Rhodes grass (top-middle) and leucaena plants (middle and bottom) following an irrigation event to show the water accumulation pattern on leaves, and after drying of the retained water (middle and bottom-right).

Figure A2.5. Series of photos showing leucaena leaflets from plants grown in glasshouse conditions and overhead-irrigated with control water (top-left), besides, with increasing water salinity and at an EC of: (i) 4 dS/m (top middle-left); (ii) 6 dS/m (middle); and (iii) 8 dS/m (bottom-right). In the image showing the foliage irrigated with saline water at an EC of 8 dS/m, leaves were exposed to 15 irrigation events, this because in all treatment replicates, the fully mature leaflets were shed before completion of the irrigation trial. For all other photos, plants were irrigated till completion of the experiment (e.g. 30 irrigation events).

Figure A2.6. Images showing leucaena leaflets from plants grown in non-glasshouse conditions (e.g. external ambient) and overhead-irrigated with control water (top-left), besides, with increasing water salinity and at an EC of: (i) 4 dS/m (top-right); and (ii) 8 dS/m (bottom). Leaflets exposed to either 4 dS/m or 8 dS/m saline water, had been irrigated 15 times and in all treatment replicates, the fully mature leaflets were shed before completion of the experiment. The foliage irrigated with control water (top-left) was irrigated 30 times.

Figure A2.7. Series of photos displaying leucaena leaflets from plants were grown in saline soil (under glasshouse conditions) and overhead-irrigated with control water (top and bottom-left), besides, with increasing water salinity and at an EC of: (i) 4 dS/m (top-middle-right); and (ii) 8 dS/m (bottom-right). In the image showing the foliage irrigated with saline water at an EC of 8 dS/m, leaves were exposed to 15 irrigation events and in all treatment replicates, the fully mature leaflets were shed before completion of the irrigation trial. For all other photos, plants were irrigated till completion of the experiment.

Figure A2.8. Series of images showing the damage on leucaena leaflets, in plants grown in glasshouse conditions and overhead-irrigated with increasingly alkaline (constant salinity) water and with mg/L $CaCO_3$ equivalent of : (i) 250 (top); (ii) 500 (middle-left); (iii) 1250 (middle-right); and (iv) 2000 mg/L (bottom-right). In the images showing leaflets irrigated with alkaline water, 250 mg/L of L $CaCO_3$ equivalent (top), leaves were exposed to 30 irrigation events. For all remaining images, the displayed leaflets received 15 irrigations.

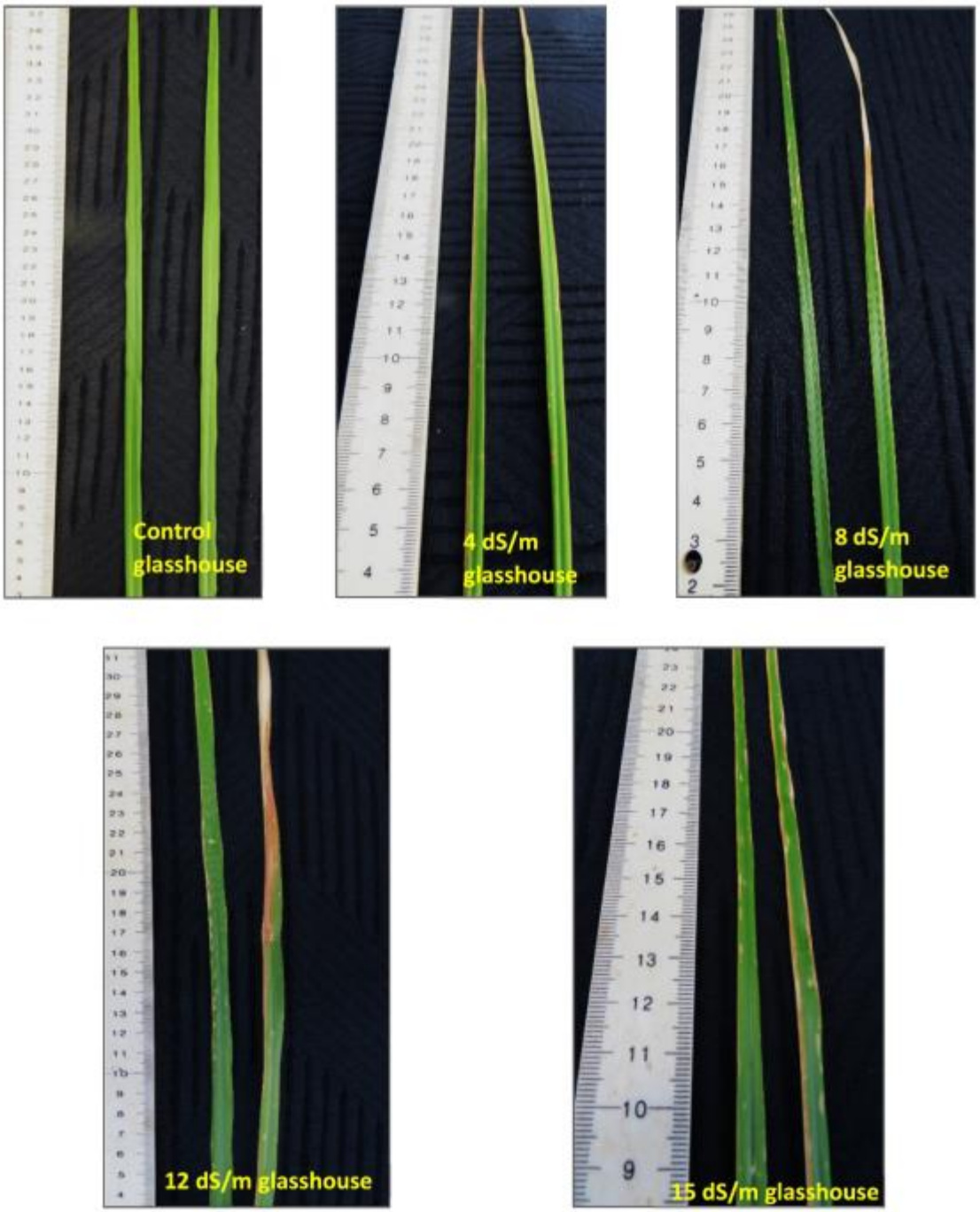

Figure A2.9. Series of photos showing Rhodes grass leaves from plants grown in glasshouse conditions and overhead-irrigated with control water (top-left), besides, irrigated with increasing water salinity and at an EC of: (i) 4 dS/m (top-middle); (ii) 8 dS/m (top-right); (iii) 12 dS/m (bottom-left); and (iv) 15 dS/m (bottom-right). For all images, leaves were irrigated for the entire duration of the experiment.

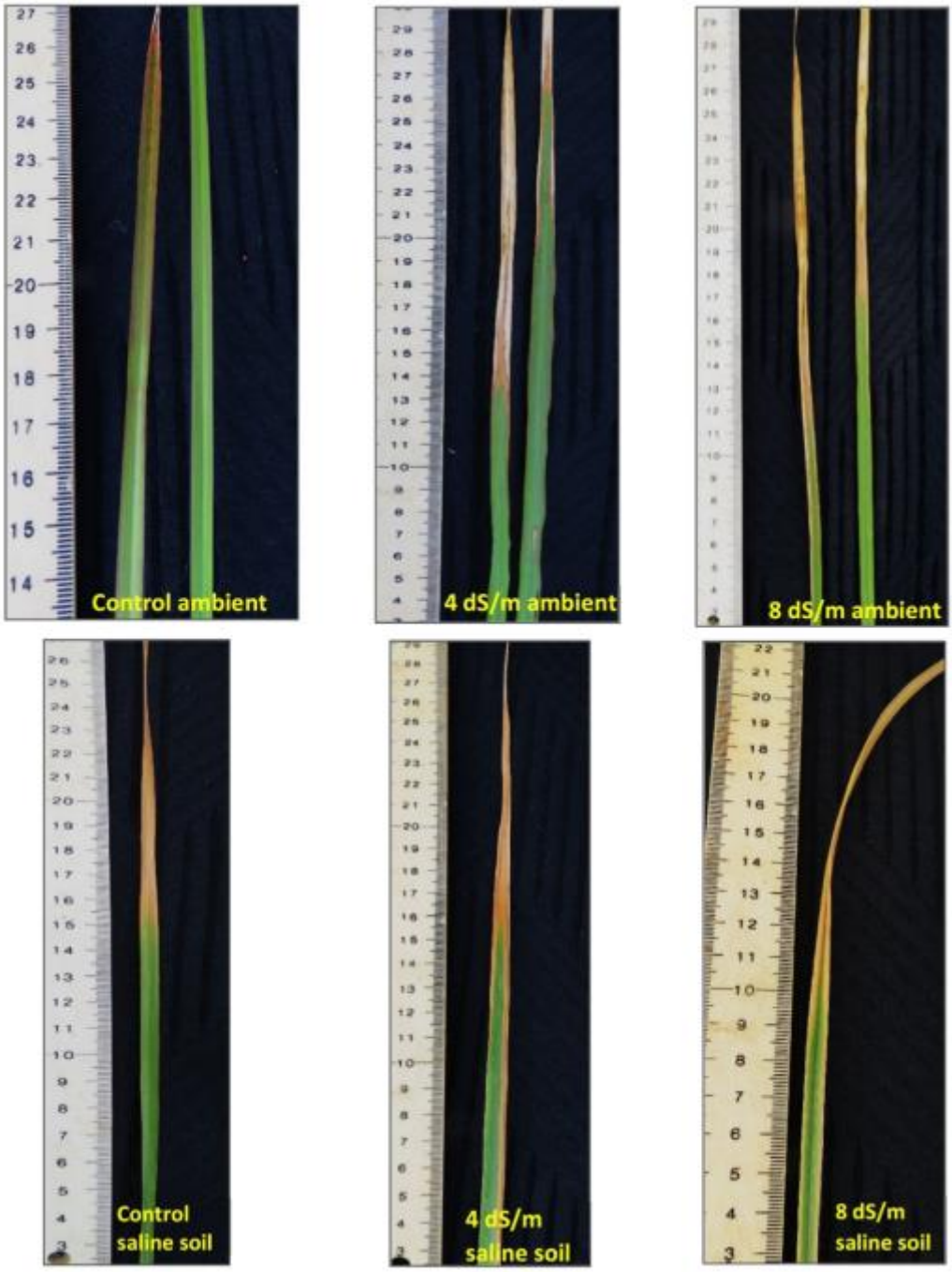

Figure A2.10. Series of images showing Rhodes grass leaves from plants grown in non-glasshouse conditions (top) and plants grown in saline soil (bottom). Plants were overhead-irrigated with control water (top and bottom left), besides, irrigated with increasing water salinity and at an EC of: (i) 4dS/m (top and bottom middle); and (ii) 8dS/m (top and bottom right). For all images, leaves were irrigated for the entire duration of the experiment.

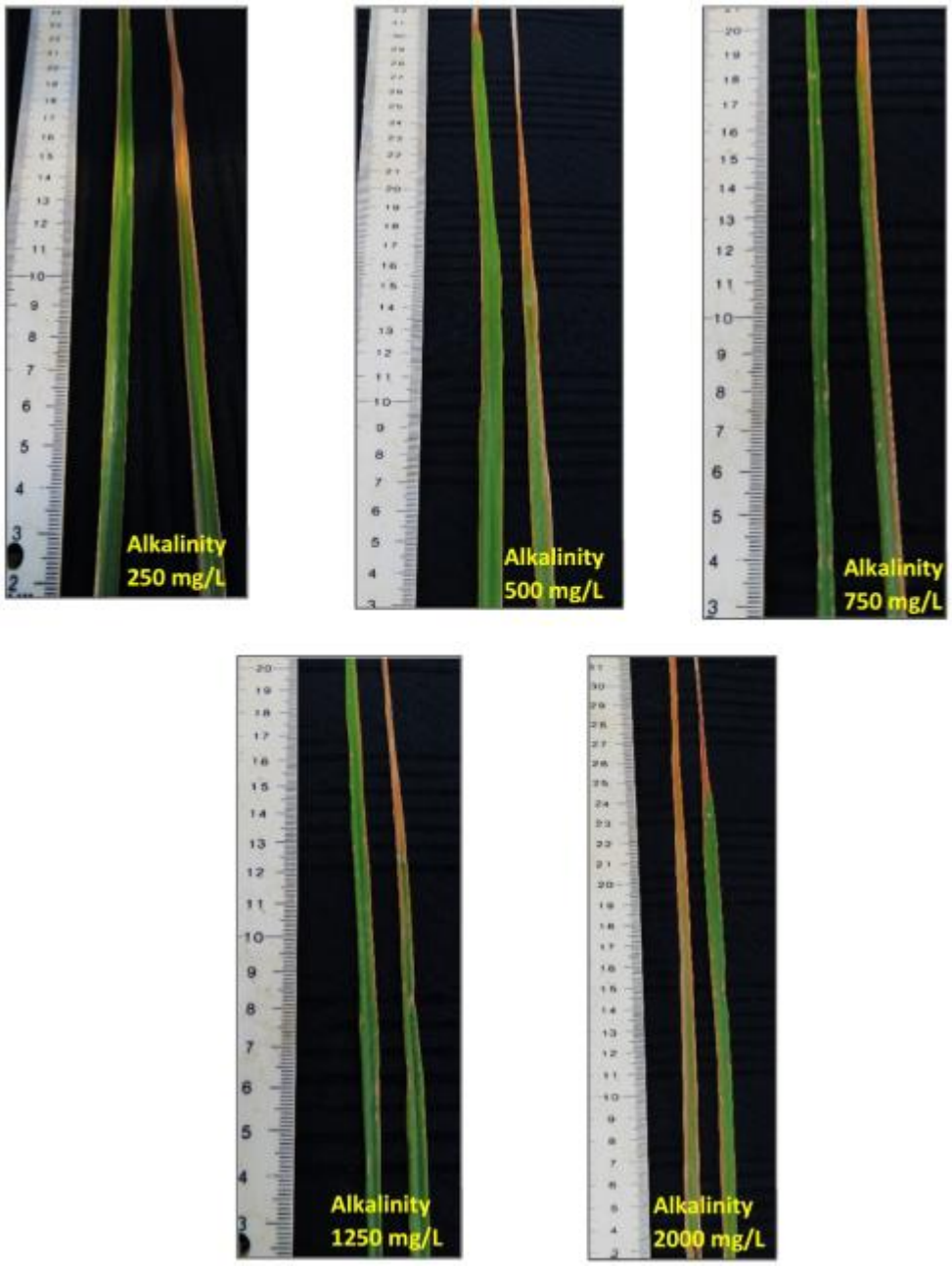

Figure A2.11. Sequence of photos displaying foliar damage in Rhodes grass leaves from plants grown in glasshouse conditions and overhead-irrigated with increasingly alkaline (constant salinity) water and with mg/L $CaCO_3$ equivalent of : (i) 250 (top-left); (ii) 500 (top-middle); (iii) 750 (top-right); (iv) 1250 (bottom-left); and (v) 2000 mg/L (bottom-right). For all images, plants were irrigated till completion of the experiment.

A2.4.2 Plant biomass

For the overhead irrigation water, increasing salinity resulted in a substantial decrease in the fresh mass of glasshouse-grown leucaena, with an EC of 6 dS/m calculated to correspond to a 50% reduction in fresh mass (Figure A2.12a). Interestingly, when the

leucaena was grown in a saline soil, the tolerance to overhead-irrigated saline water appeared to increase. For example, when leucaena was grown in the saline soil (EC of ca. 10 dS/m), shoot fresh mass was reduced by only 12% reduction when overhead-irrigated with water at an EC of 4 dS/m (c.f. 34% reduction in the non-saline soil at an EC of 4 dS/m). In contrast, the data suggest that leucaena grown outside the glasshouse was more sensitive to saline water when overhead-irrigated. Indeed, for leucaena grown in ambient conditions, overhead irrigation with water at an EC of 4 dS/m resulted in a ca. 60% reduction in fresh mass (c.f. the 34% reduction when grown in the glasshouse) (Figure A2.12a). (Note that overall biomass production outside the glasshouse was less than inside the glasshouse, presumably due to lower air temperatures). Although salinity had an adverse impact on the shoot fresh mass of leucaena (Figure A2.12a), alkalinity (up to 2000 mg/L as $CaCO_3$ equivalent) had no additional adverse impact on plant shoot mass above that caused by the basal salinity (4 dS/m) (Figure A2.12b).

Whilst salinity reduced fresh mass of leucaena (above), there was no reduction in fresh mass for any treatment with Rhodes grass – fresh mass values at an EC of 15 dS/m and at an alkalinity of 2000 mg/L ($CaCO_3$ equivalent) being similar to that in the corresponding controls (Figure A2.13). (Again, as observed for leucaena, overall growth in the ambient treatments was lower than in the control, and growth in the saline-soil treatments was lower than in the corresponding non-saline treatments.)

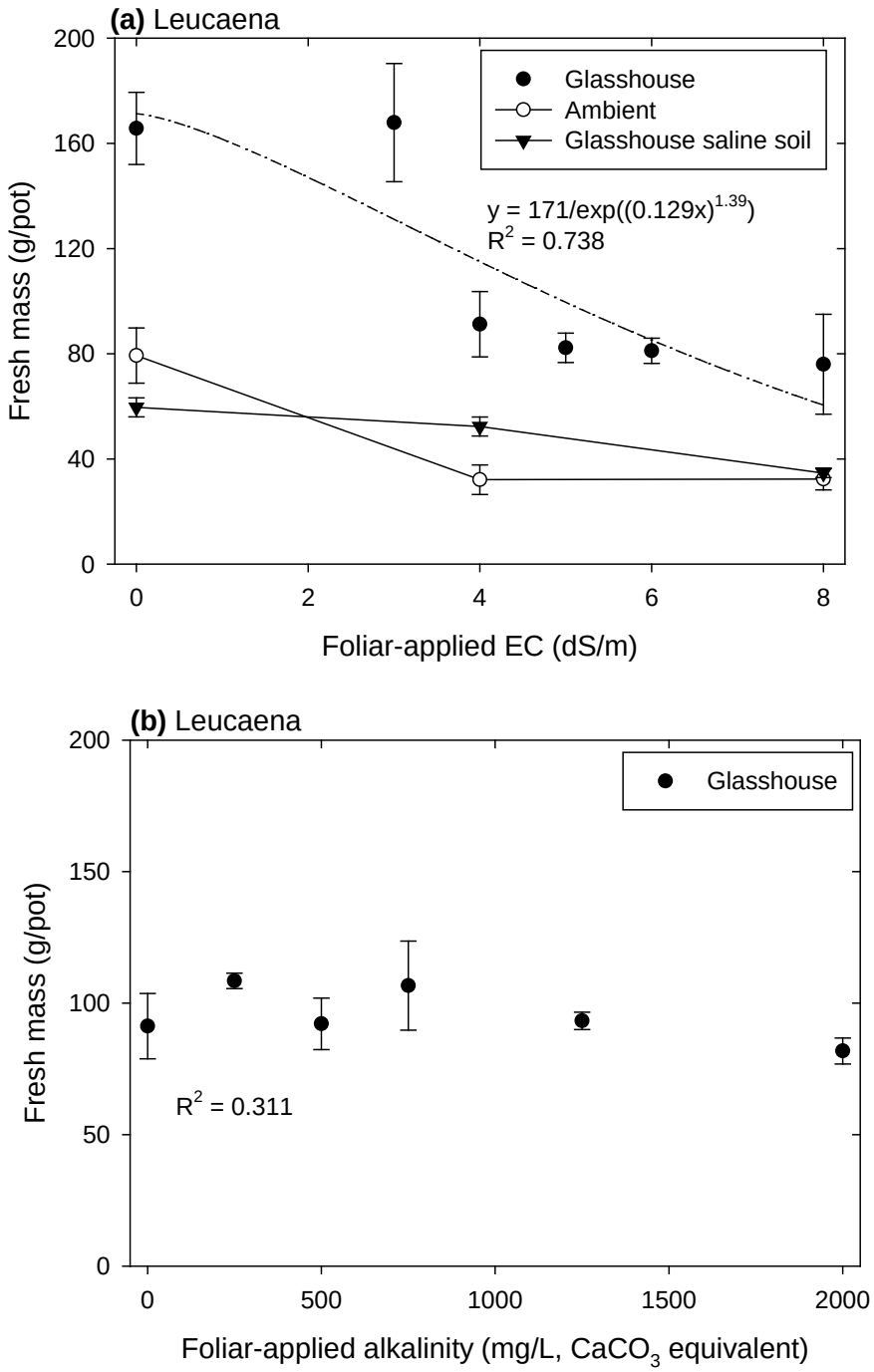

Figure A2.12. The fresh mass for leucaena overhead-irrigated with saline (a) and alkaline water (b). Fresh mass values in the foliar-applied salinity treatments are presented comparing glasshouse, ambient and saline soil growing conditions (a). For the water with increasing alkalinity, all treatments also had an EC of 4 dS/m. For glasshouse-grown plants in non-saline soil, non-linear regressions were used to examine the effect of salinity and alkalinity on plant growth (regressions are shown where the R^2 value is >0.5).

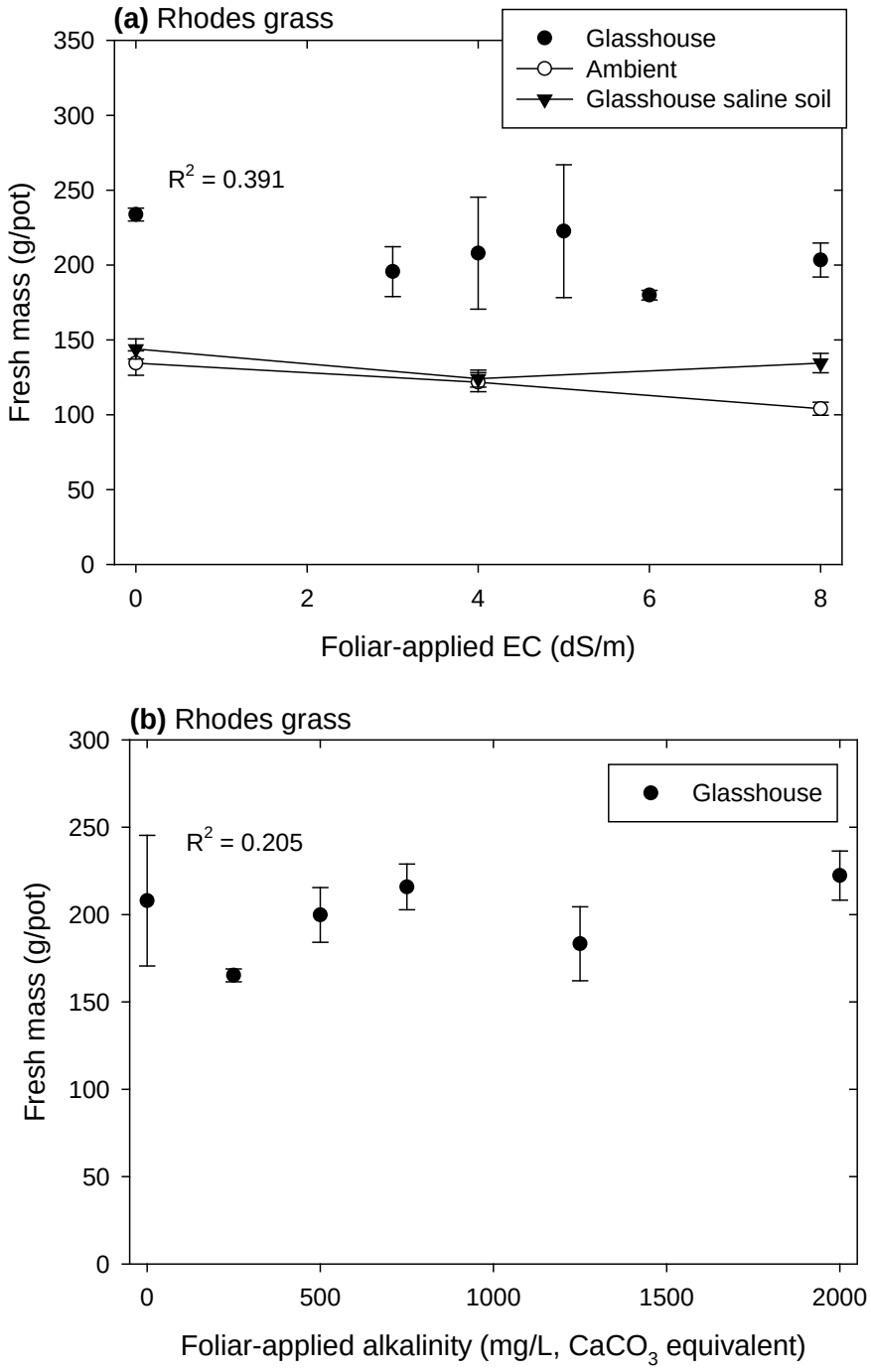

Figure A2.13. The fresh mass for Rhodes grass overhead-irrigated with saline (a) and alkaline water (b). Fresh mass values in the foliar-applied salinity treatments are presented for plants grown in glasshouse, ambient and saline soil growing conditions (a). For the water with increasing alkalinity, all treatments also had an EC of 4 dS/m. For glasshouse-grown plants in non-saline soil, non-linear regressions were used to examine the effect of salinity and alkalinity on plant growth (regressions are shown where the R^2 value is >0.5).

A2.4.3 Chlorophyll fluorescence

Chlorophyll fluorescence was examined using the ratio $F_{v/}F_m$, where F_v is the variable fluorescence and F_m the maximum fluorescence. The ratio $F_{v/}F_{m,}$ provides a measure of the quantum yield in the plant Photosystem II, or the potential quantum efficiency, that is widely recognised as an indicator of the efficiency of plant photosynthetic activity (Maxwell & Johnson, 2000). Vascular plants growing in non-limiting conditions have a $F_{v/}F_m$ ratio of ca. 0.83, with lower values indicating that plants are exposed to environmental stress (Maxwell & Johnson, 2000).

For leucaena overhead-irrigated up to six times, this saline or alkaline water had no impact on CF regardless of the treatment (Figure A2.14). The only exception to this was at the highest salinity treatment (8 dS/m) in ambient conditions, with the slight decrease in fluorescence in this treatment indicating stress. However, the decrease in CF became more marked with increasing irrigations, with salinity causing a decrease in fluorescence in treatments with EC values of ≥3 or 4 dS/m (Figure A2.14), whilst alkalinity decreased fluorescence in treatments with a total alkalinity of ≥250 mg/L ($CaCO_3$ equivalent) (Figure A2.14d).

For Rhodes grass, it was found that salinity and alkalinity had no impact on CF in any treatment, regardless of the number of irrigations (Figure A2.15). It should be noted, however, that fluorescence tended to decrease with increasing irrigation, regardless of treatment.

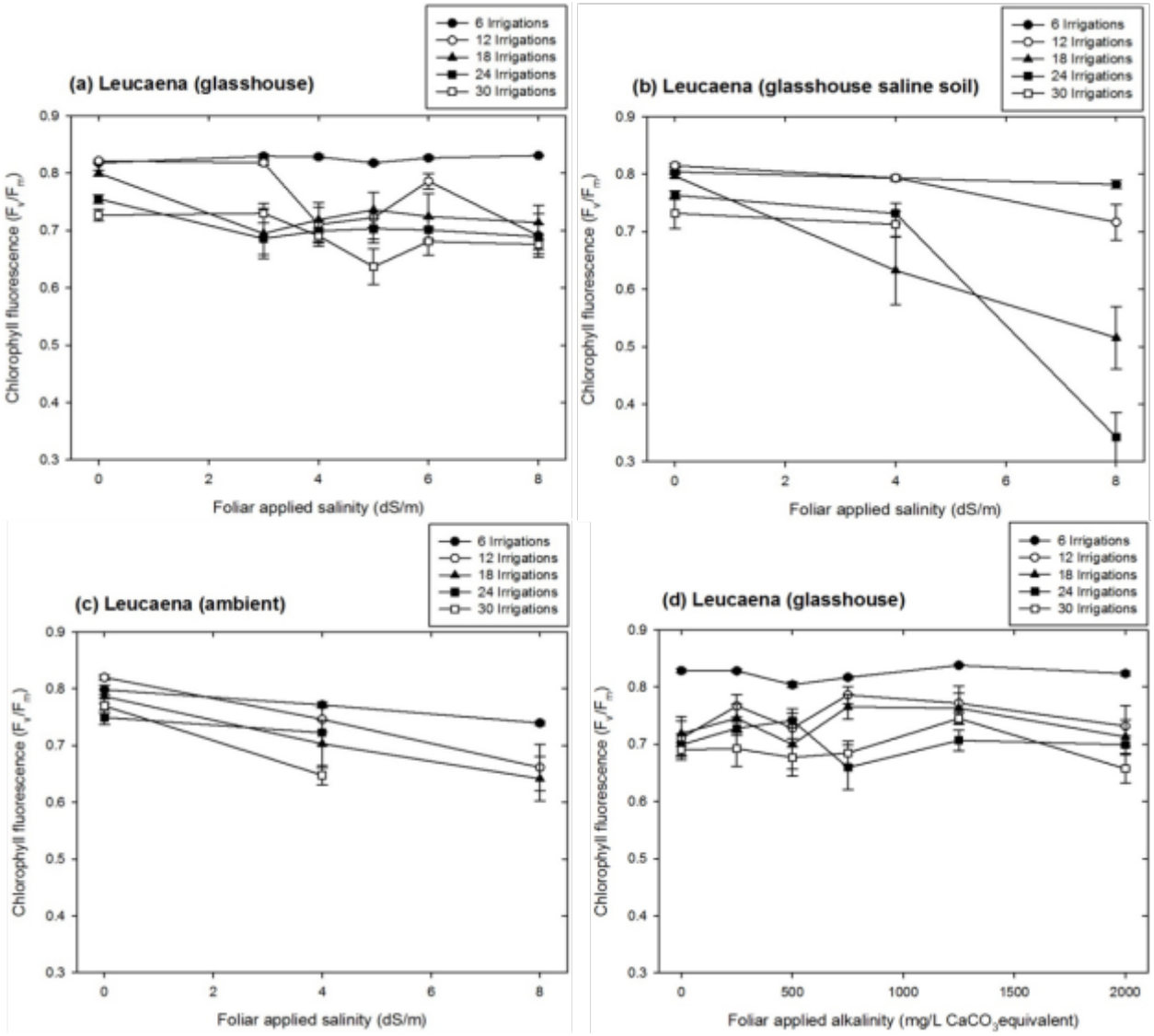

Figure A2.14. Chlorophyll fluorescence of leucaena overhead-irrigated with saline water (a, b, and c) and alkaline water (d). Measurements were taken on five leaves of equal age (previously tagged) that were randomly selected from plants that received a total of 6, 12, 18, 24 and 30 irrigations. Each point represents the arithmetic mean of 15 measurements. Plants overhead irrigated with saline water at an EC of 8 dS/m and growing in: (i) saline soil (b); and (ii) ambient (c) had shed their fully mature leaves before a total of 30 and 24 irrigations, respectively. As a consequence, chlorophyll values could not be determined.

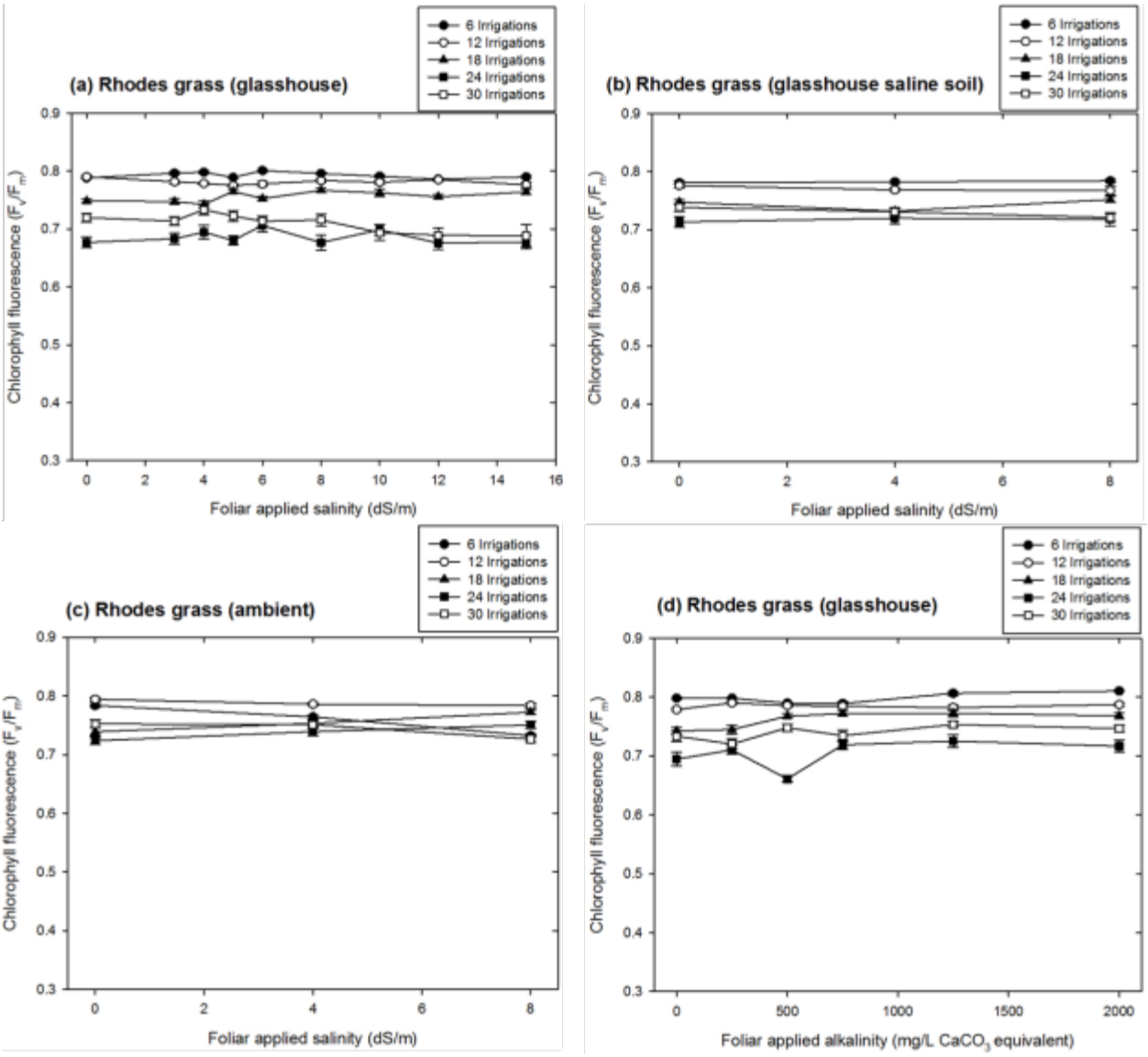

Figure A2.15. Chlorophyll fluorescence of Rhodes grass overhead-irrigated with saline water (a, b, and c) and alkaline water (d). Measurements were taken on five leaves of equal age (previously tagged) that were randomly selected from plants that received a total of 6, 12, 18, 24 and 30 irrigations. Each point represents the arithmetic mean of 15 measurements.

A2.4.4 Effects of the saline-sodic and alkaline water on elemental concentrations in leaf tissue

Elemental analyses were performed for fully mature leaves that received all 30 treatment applications. However, for leucaena, there was insufficient tissue to allow for analysis at many of the higher salinity treatments and for all alkalinity treatments (see Figure A2.16).

As expected, for all treatments in both species, leaf tissue Na and Cl concentrations increased with increasing salinity, with Na increasing to ca. 0.5 to 1.2% and Cl increasing to 1.5 to 2.8% (Figure A2.16a,b and Figure A2.17a,b). At any given EC for the overhead irrigation water, leaf tissue concentrations of Na in Rhodes grass were higher in plants in the saline soil than for plants grown in the non-saline soil (Figure A2.17a). Surprisingly, however, it was observed that tissue Na concentrations were lower for plants grown in ambient conditions than for plants grown in the glasshouse (Figure A2.17a), presumably due to lower growth and decreased transpiration. Regardless, the tissue Na concentrations for Rhodes grass observed here (up to ca. 1.2%) are lower than those reported to be associated with toxicity in this species (ca. 2.5 to 3%) (Kopittke, Kopittke, & Menzies, 2009) – this being consistent with the observation that the fresh mass of Rhodes grass was not observed to be reduced in any treatment (Figure A2.13). Unfortunately, we are unaware of any critical tissue values for leucaena for either Na or Cl.

Although salinity often causes a reduction in tissue Ca concentrations, overhead irrigation of saline or alkaline water did not result in a marked decrease in Ca in any treatment for either plant species (Figure A2.16c, Figure A2.17c, and Figure A2.18b). Indeed, in all treatments, the average tissue Ca concentrations of 2.7% for leucaena and 0.80% for Rhodes grass were higher than the critical value for deficiency, being ca. 0.7% for leucaena and ca. 0.25 - 0.5% for Rhodes grass (Reuter & Robinson, 1997).

Finally, although salinity can also reduce tissue concentrations of K, again, the overhead irrigation of saline and alkaline water had no apparent effect, regardless of species or treatment (Figure A2.16d, Figure A2.17d, and Figure A2.18b). Again, measured tissue concentrations of K (average of 0.9% for leucaena and 0.5% for Rhodes grass) were higher than the corresponding critical values (0.5% for leucaena and 0.2% for Rhodes grass), indicating that growth was not limited by K deficiency in any treatment.

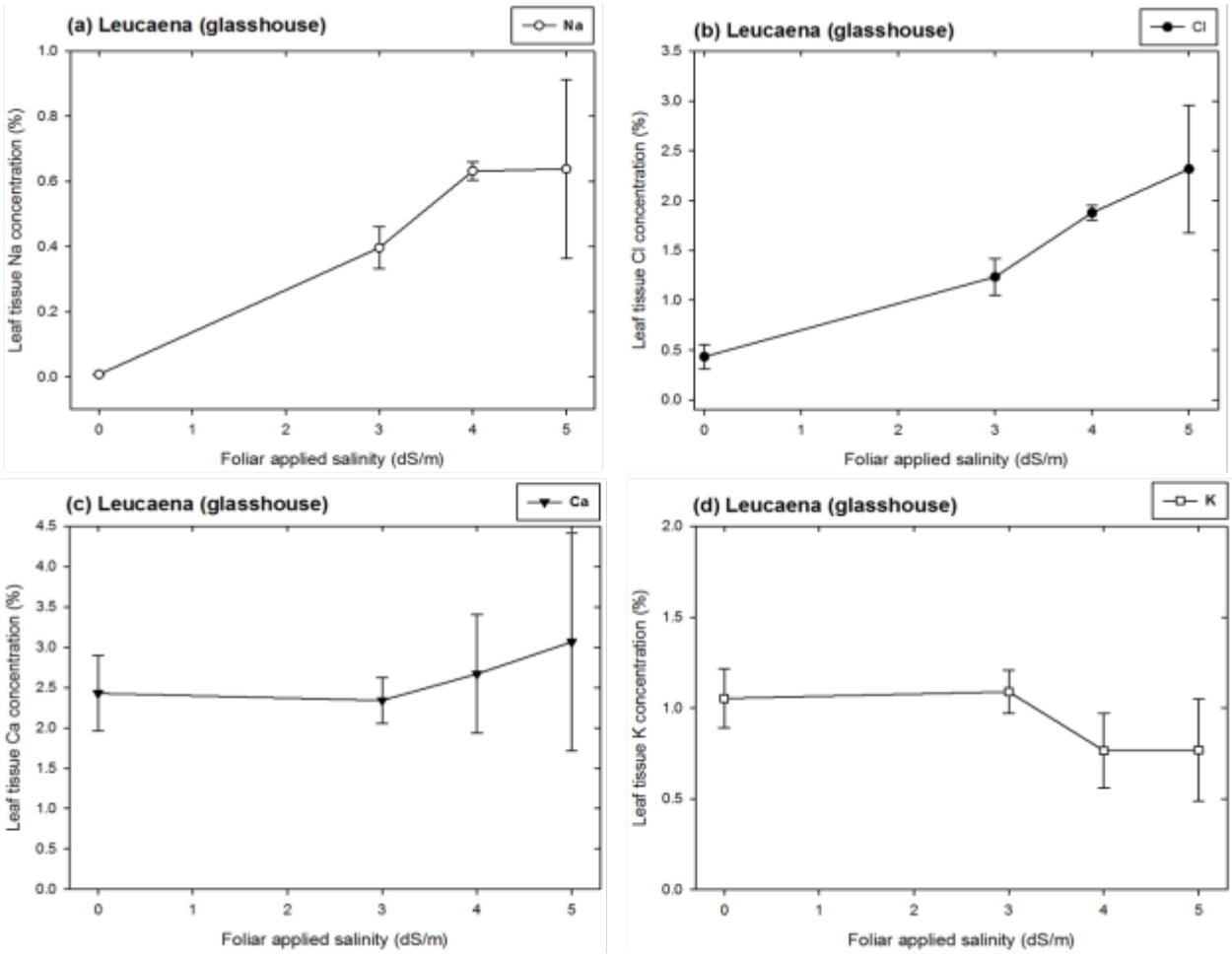

Figure A2.16. Effect of overhead irrigation with saline water on the Na (a), Cl (b), Ca (c) and K (d) concentrations in leaf tissue for leucaena plants grown in glasshouse conditions. Tissue concentrations were determined after a total of 30 irrigation events from fully mature leaves (that were tagged and received all treatment applications). Prior to analysis, leaves were rinsed to remove the salt accumulated on the leaf surface. There was insufficient plant tissues available for collection in experimental units exposed to irrigation water at an EC of 6 and 8 dS/m, thereby concentrations could not be determined and presented.

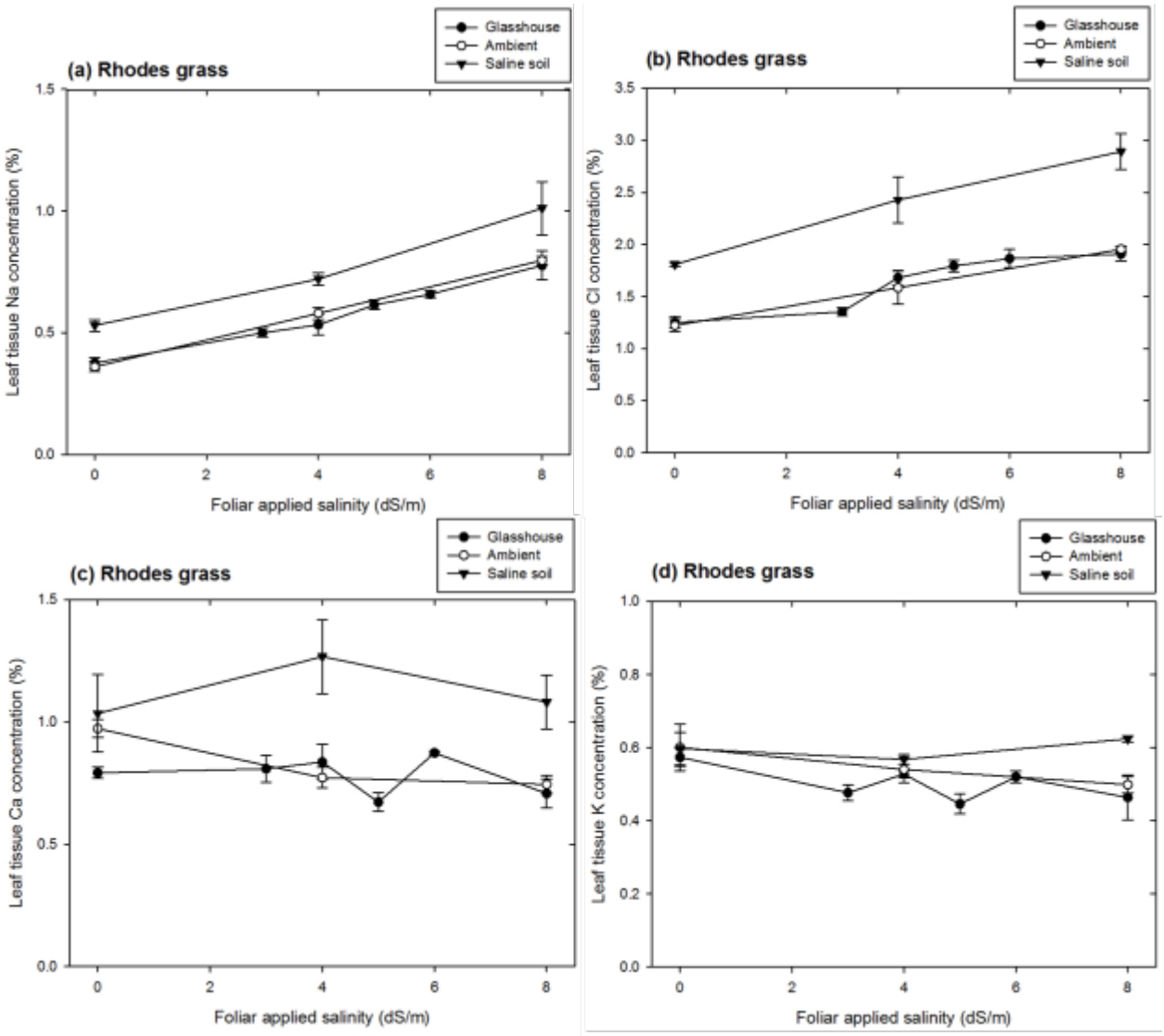

Figure A2.17. Effect of overhead irrigation with saline water on Rhodes grass leaf concentrations of Na (a), Cl (b), Ca (c) and K (d) comparing glasshouse, ambient and saline-soil conditions. Tissue concentrations were determined after a total of 30 irrigation events from fully mature leaves (that were tagged and received all treatment applications). Prior to analysis, leaves were rinsed to remove the salt accumulated on the leaf surface.

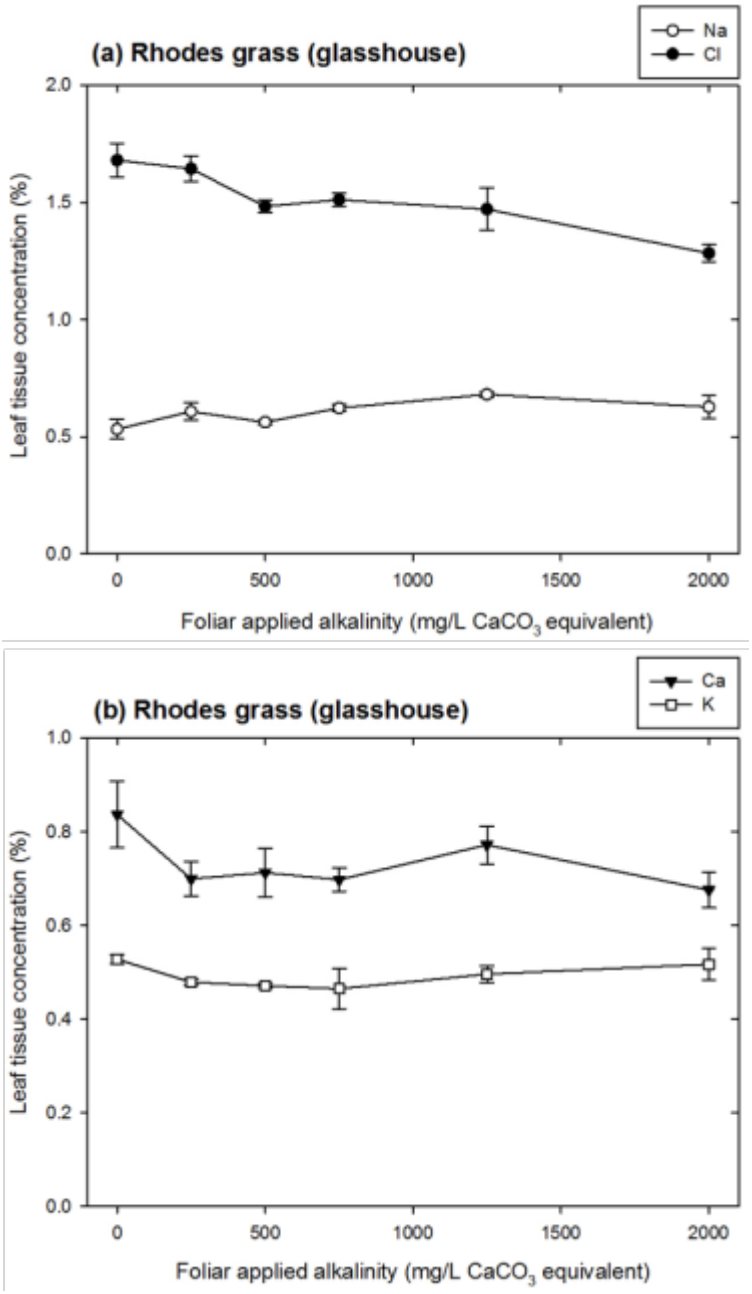

Figure A2.18. Effect of overhead irrigation with alkaline water on the Na, Cl (a) and Ca, K (b) concentrations in leaf tissue for Rhodes grass plants grown in glasshouse conditions. Tissue concentrations were determined after a total of 30 irrigation events from fully mature leaves (that were tagged and received all treatment applications). Prior to analysis, leaves were rinsed to remove the salt accumulated on the leaf surface.

A2.5 Discussion

Elevated soil salinity (high concentrations of salts) and sodicity (high Na concentration) are worldwide problems that have reduced plant growth for millennia. However, in contrast to salinity within the rooting medium (i.e. soil), few reports have addressed the direct impacts of overhead irrigation of saline water on plant foliage. Here, it has been shown that the overhead irrigation of saline (with EC values up to 15 dS/m) and alkaline (up to 2000 mg/L, as $CaCO_3$ equivalent) irrigation waters exerted deleterious effects on the foliage of leucaena and Rhodes grass, resulting in the development of chlorotic and necrotic lesions (and in severe cases, foliar abscission). Specifically, it was observed that the overhead irrigation with saline-sodic and alkaline water resulted in: (i) foliar morphological damage, (ii) reduction in plant fresh mass, (iii) decrease in CF (F_v/F_m), and (iv) an increase of leaf Na and Cl tissue concentrations (please note that in Rhodes grass, tissue Cl concentrations decreased following irrigation with alkaline water (Figure A2.18a)). However, differences were observed between the two plant species examined here; whilst water with an EC >3 dS/m reduced growth of leucaena, growth of Rhodes grass not reduced in any treatment investigated. Interestingly, for leucaena, the salinisation of the rooting medium appeared to increase tolerance to the overhead irrigation of saline water.

Adverse effects of the overhead irrigation of saline water

The overhead irrigation of saline (NaCl) water was found to have deleterious effects, causing foliar damage in both plant species (Figures A2.5, A2.6, A2.7, A2.9, and A2.10). The magnitude of the effects was different between the two plant species (see later discussion) and foliar damage was evidenced in leucaena by the formation of chlorotic and necrotic lesions (in severe cases, foliar abscission) (Figures A2.5 – A2.7), decreased fresh mass (Figure A2.12a), and decreased CF (Figure A2.14a, b and c). It is likely that foliar damage observed in both plant species is caused by increased localized Na and Cl absorption in the tissues immediately underlying the tissues where water droplets accumulated and dried. Indeed,

bulk concentrations of Na in the foliar tissue increased to ca. 0.65% in leucaena (FigureA2.16a) and ca. 1.2% in Rhodes grass (Figure A.17a). For Rhodes grass, although these bulk tissue Na concentrations were lower than that reported to be toxic when grown in saline soils (Kopittke et al., 2009) the concentrations of Na in tissues underling the area where the droplets dried are almost certainly higher – this likely resulting in the observed chlorosis and necrosis. This is consistent with that observed by Maas (1985), who reported a linear increase of Cl absorption (and foliar injury) in bush beans with the increase of salt concentration (EC) in water droplets accumulated on leaves during sprinkling-irrigation.

Bernstein (1975) stated that "symptoms of leaf injury by foliar absorbed salts are the same as those caused by salts absorbed by the roots", a conclusion supported by Maas et al. (1982). However, in the present study, other than a general reduction in growth, the most apparent visual symptom of the overhead irrigation of saline water was the formation of chlorotic and necrotic lesions on the leaf surface where the water accumulated and dried – this not being a typical symptom of plants exposed to excess salts within the rooting medium. Indeed, the observations of the present study are in agreement with Gorham, Papa, and Aloy-Lleonart (1994) who stated "that tolerance to salt applied as salt spray or in the soil are different mechanisms". The formation of chlorotic and necrotic lesions observed in the present study have also been reported by Mantel, Mead, Hoffman, and Francois (1989) in plum trees (*Prunus saliciva*) sprinkled-irrigated weekly with saline water at an EC >3.3 dS/m. In the present study with Rhodes grass and leucaena, these foliar symptoms (chlorosis and necrosis) occurred in areas where water had droplets accumulated during overhead irrigation and successively dried (Figure A2.4). With prolonged irrigation for leucaena (but not Rhodes grass), these symptoms extended to the whole leaf surface with increasing number of irrigations and water salinity. Similarly, Mantel et al. (1989) reported that foliar necrosis extended from the margins, to the entire leaf surface in plum trees when sprayed with highly saline water and following 14 irrigation events. The chlorosis and necrosis of foliage exposed to saline water has also been reported by Vollenweider and Günthardt-Goerg (2005), Armbruster and Mulchi (1984) and McCune (1991). Other impacts of foliar-applied saline water have also been reported by Burkhardt (2010) who found that the overhead irrigation of water containing 584 mg/L NaCl to leaves of apple (*Malus*

domestica) leaves of tomato (*Lycopersicon esculentum*) resulted in decreased photosynthesis and decreased transpiration.

Adverse effects of the overhead irrigation of alkaline water

For both leucaena and Rhodes grass, alkalinity values of up to 2000 mg/L ($CaCO_3$ equivalent) had no adverse impact on foliage other than that attributable to the basal salinity (with the basal EC in all alkaline solutions being 4 dS/m) (Figure A2.8 and Figure A2.11). Only one study was identified in the literature where the effect of alkaline water application on plant foliage was investigated. Beletse et al. (2008) examined the effect of sprinkler irrigation on cotton, Italian ryegrass, and barley (salt tolerant crops) with sodium bicarbonate ($NaHCO_3$) rich water form CSG-operations in South Africa. Despite the water used by Beletse et al. (2008) having a total alkalinity of 4712 mg/L (as $CaCO_3$ equivalent) and at an EC of 7.5 dS/m (this being substantially higher than the water used in the present study) these authors found that there was no significant foliar damage, with only cotton having scorched leaves. Thus, based upon the evidence in the present study and that reported previously, it seems that overhead irrigation of alkaline water up to 2000 mg/L ($CaCO_3$ equivalent) is not deleterious to plant foliage.

Differences between plant species

Substantial differences were observed between leucaena and Rhodes grass. For leucaena exposed to 30 irrigations, the adverse effects were particularly prevalent for water with an EC ≥3 dS/m, this being evidenced by the formation of chlorotic and necrotic lesions (in severe cases, foliar abscission) (Figure A2.5 – A2.8), decreased fresh mass (Figure A2.12a), and decreased CF (Figure A2.14). Although leaves of Rhodes grass were observed to develop chlorotic and necrotic lesions (Figure A2.9 – A2.11), the fresh mass of Rhodes grass was not reduced in any treatment (Figure A2.13), nor was CF reduced relative to the control (Figure A2.15).

The high tolerance of Rhodes grass to salt within the rooting environment is well documented in the scientific literature (Kopittke, Blamey, Sheldon, & Menzies, 2009; Russell, 1976; Shaw, 1999). Rhodes grass is a halophyte and is able to tolerate relatively high

salinity concentrations in soil due to its ability to accumulate excess salt within its leaves (Kopittke et al., 2009), and to secrete salt in excess through bicellular glands (Kobayashi, Masaoka, Takahashi, Ide, & Sato, 2007). Indeed, in the present study Na and Cl uptake occurred following foliar application, thereby absorption and translocation from the leaf surface to internal tissue. However, salt glands in Rhodes grass may have been still able to accumulate the excess of salt, therefore increasing plant tolerance to foliar applied salinity.

Influence of growth conditions

In leucaena, a non-halophyte species, the magnitude of foliar damage increased for all growing conditions, with increasing water salinity (EC ≥3 dS/m) and number of irrigations. For Rhodes grass (halophyte species) only plants grown in either ambient (non-glasshouse conditions) or in saline soil, showed an increase in the symptoms severity with increasing water salinity (Figure A2.10). The cumulative effect of root and foliar exposure to salinity, therefore, increased foliar necrosis and chlorosis in either, leucaena and Rhodes grass. Similar results were observed in a study conducted by Benes, Aragüés, Austin, and Grattan (1996), in which, barley leaves from plants grown in saline soil at an EC of 9.6 dS/m and overhead irrigated with saline water at equal EC, displayed increased leaf scorching than plants growing in non-saline soil but only sprayed with saline water at an EC of 9.6 dS/m. Transpiring leaves of plants exposed to saline soil, undergo rapid modifications in cells water content caused by osmotic stress (against which plant can initially adjust), however, when Na and Cl accumulate in the cytoplasm (and vacuole cannot compartmentalise these ions anymore) enzymes activity result compromised (Munns, 2002). In addition, despite being vital for the survival of the plant, the osmotic adjustments, ions compartmentalisation and salt excretion (in Rhodes grass), are processes that require a substantial amount of energy, that is taken away from resources otherwise used for plant growth (Raven, 1985). Therefore, plant osmotic adjustments (energy demanding) in conjunction with foliar injury were possible causes for the reduced overall growth showed by Rhodes grass and leucaena in saline soil conditions.

Foliar injury was also evident in both plant species grown in ambient (non-glasshouse) conditions. Plants water loss though stomata tend to increase with hot and dry weather and under these conditions, several authors (Bernstein, 1975; Maas, 1985; McCune & Silberman, 1991) reported an increase in foliar injury following overhead irrigation with saline water. In the present study, average maximum temperature in the glasshouse and external ambient was similar, however, in glasshouse conditions the humidity was kept high through a water-wall-air circulating system. Higher humidity level, therefore, could have decreased the symptoms severity when compared to plants grown in non-glasshouse conditions because of the reduced transpiration rate in glasshouse conditions.

A2.6 Conclusion and future directions

The foliar-application of saline-sodic water caused toxic effects that were independent of salts within the rooting environment. However, the severity of these toxic effects varied depending upon the plant species, the presence of excess salts in the rooting environment, and the growth conditions. In leucaena, foliar necrosis and chlorosis was evident following irrigation with saline water at an EC >3 dS/m, with foliar abscission particularly pronounced after 10 irrigations at high EC values. This foliar-applied saline-sodic water also caused a reduction in shoot fresh mass (with a 50% reduction at an EC of 6 dS/m) and CF, but an increase in tissue concentrations of Na and Cl. In contrast to these observations, although some foliar damage was observed for Rhodes grass (including chlorosis and necrosis), there was no significant reduction in shoot fresh mass in any treatment. It was also observed that growing conditions influenced the tolerance to foliar-applied salt – although a salinised soil increased foliar necrosis and chlorosis, the magnitude of the growth reduction due to the foliar-application of salt decreased. Furthermore, it was observed that growth in glasshouse conditions decreased the deleterious effects of the foliar-applied salt, presumably due to differences in temperature and humidity. Finally, at an EC of 4 dS/m, irrigation of waters containing an alkalinity of up to 2000 mg/L (as $CaCO_3$ equivalent) had no adverse impact on shoot fresh mass for either species. Thus, whilst it has been shown that saline-sodic and

alkaline water can potentially be used for overhead irrigation, care must be taken to ensure that growth of sensitive species is not reduced, with the magnitude of the growth reduction depending upon growth conditions.

It must be noted that the present study has taken into account several important factors in the evaluation of the effects of saline and alkaline water on plant foliage. However, the data extrapolation is not possible in either, different water chemical composition or other plant species. In addition, given that growing condition substantially affect tolerance to foliar applied salinity and alkalinity, further studies are needed to evaluate plant responses in field conditions. Soil chemical and physical characteristics, changing climatic conditions and possibly specific amendment programs are likely to affect plant tolerance to foliar applied salt and alkali. Indeed, the findings from this research can contribute to partially fulfill the uncertainties to date present in the scientific and technical information. Focus of the future research might include the examination of plant physiological responses (ionic balance and photosynthesis ratio) and adaptive mechanisms, following the overhead irrigation with saline and alkaline water under different growing conditions (e.g. different levels of soil salinity and temperature/humidity).

A2.7 Acknowledgements

I would like to extend my deepest gratitude to my supervisors Dr Peter M. Kopittke and Dr Bernhard J. Wehr for their guidance and support throughout the year. Their patience and willingness to assist with uncountable questions made the completion of the project possible. Most important, their academic knowledge and work ethic was exceptionally valuable for my learning experience as a student and will serve as a model for my future career. A sincere gratitude also to the soil group and all my former lecturers for their precious contribution towards academic formation. I would like to acknowledge Cui Li for the SEM analysis of plant material (not presented); UQ analytical chemistry laboratory for the leaf tissue nutrient concentrations analysis; Christiane De Camargo for assistance in leaves tagging; Gaius Leong for assistance with biomass data collection and Prapa Taranet, without whom CF data collection would have resulted not feasible.

I would like to acknowledge Santos Ltd. for funding this project and associated scholarship, which allowed the completion of the project with the best possible technical support and personal dedication, in particular, I acknowledge Dr Scott Dalzell for his suggestions and knowledge on this field of study.

A sincere and warm thank you to my friends Gaius and Prapa for their personal support; my parents Gaetano and Vincenzina for their love; my loved cousin Donato Cicchelli and its wife Briana, without whom I would have not begun a new life in Australia and finally, Christiane De Camargo whose patience and daily encouragement was fundamental to overcome the many challenges I encountered during my studies.

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A2.9 Appendix 1

Table A2.4. Soil salinity (measured through electrical conductivity (EC)) and pH (1:5 water) measurements (mean and standard errors (S.E.)), following a total of 30 irrigation events with saline-sodic and alkaline water on Rhodes grass and leucaena. For each treatment, a soil sample was collected from the top-soil (ca. 0 – 3 cm depth) of each replicate pot (e.g. three samples) in the glasshouse growing conditions.

		Overhead Irrigation Solution		Soil			
				EC (dS/m)		pH	
Treatment	Plant Species	EC (dS/m)	Alkalinity (mg/L $CaCO_3$ equivalent)	Mean	S.E.	Mean	S.E.
29	Leucaena	0	0	0.47	0.01	6.6	0.09
26	Rh. grass	0	0	1.35	0.08	5.9	0.11
10	Leucaena	0	0	2.60	0.08	5.6	0.22
1	Rh. grass	0	0	2.09	0.05	5.5	0.07
12	Leucaena	4	0	1.67	0.34	5.3	0.07
3	Rh. grass	4	0	2.26	0.04	5.5	0.27
15	Leucaena	8	0	1.65	0.31	5.6	0.02
6	Rh grass	8	0	2.70	0.04	5.4	0.16
23	Leucaena	4	750	2.30	0.17	5.4	0.11
18	Rh grass	4	750	2.06	0.01	5.6	0.18
25	Leucaena	4	2000	1.73	0.11	5.8	0.09
20	Rh grass	4	2000	2.08	0.46	6.0	0.34

Appendix A3 –Seed germination study

Seed germination under saline and alkaline conditions

Revised Report to Santos Ltd

23 April 2015

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A3.1 Executive summary

- The effect of salinity, sodium adsorption ratio (SAR) and alkalinity on seed germination of *Leucaena leucocephala* cv Tarramba, *Chloris gayana* cv Topcut (Topcut Rhodes grass) and cv Reclaimer (Reclaimer Rhodes grass) was tested in a laboratory study. Root growth in response to the treatments was measured in leucaena.
- Land amendment irrigation (LAI) may utilize CS water with EC 4 dS/m, SAR 150 and pH 9, with soils concomitantly amended with sulfur and gypsum to lower the SAR and pH of the CS water in the soil. This present research investigated if germination of the plant species was affected by the application of saline, sodic, and alkaline water.
- Seeds were exposed to salinities ranging from 0-20 dS/m (equivalent to up to 210 mM NaCl) and SAR ranging from 0-30. Alkalinity effects were tested with sodium bicarbonate (0-2000 mg/L $CaCO_3$ equivalent) at a constant salinity of 4 dS/m.
- Seeds were germinated in darkness for 2 days at 25°C, and then transferred to low light (laboratory bench) and germination was evaluated 5 days after sowing (leucaena) or 8-9 days after sowing (Rhodes grass). No further increases in germination with increasing time were observed.
- For leucaena:
    - Germination decreased when EC increased above 15 dS/m, but SAR had no effect on germination. Thus, leucaena is tolerant of saline soils and would not likely be impaired by salt accumulation in the topsoil due to overhead irrigation with saline water up to EC 4 dS/m.
    - The influence of solution salinity and SAR on root length was variable. Best growth was observed with EC 4 dS/m and SAR 5, and root growth declined when EC >10 dS/m. Again, these results indicate that leucaena root growth is unlikely to be affected by overhead irrigation with CS water of EC 4 dS/m if there is gypsum applied to soil to lower the SAR.
    - Germination and root length was not affected by alkalinity (up to 2000 mg/L $CaCO_3$ equivalent), making it unlikely that leucaena will be affected in the field with overhead irrigation with CS water at pH 9.
- For Topcut Rhodes grass seed, across the range of water quality investigated in the present study, germination was not significantly affected by salinity, SAR or alkalinity, but there was a slight trend of increasing germination with increasing salinity. It is expected that this Rhodes grass cultivar would be highly tolerant of the salinity and alkalinity found in CS water when soils are amended with gypsum as part of the LAI.
- For Reclaimer Rhodes grass seed, germination decreased when salinity increased above 15 dS/m, suggesting that seed germination of this cultivar is less salt tolerant than Topcut Rhodes grass. Increasing SAR had no significant effect on seed germination, but there was a trend showing less germination at SAR 0 (i.e. in Ca-free solutions), but this finding is of little practical importance since soils will be amended with gypsum during LAI. Alkalinity had no significant effect on seed germination, but the trend showed increasing germination with increasing alkalinity. Seed germination of this cultivar appears slightly less salt tolerant than Topcut

Rhodes grass, but under CS water irrigation it is likely Reclaimer Rhodes grass will still perform satisfactorily.

- In summary, overhead irrigation with CS water and LAI with gypsum application is unlikely to affect seed germination and early growth of seedlings of leucaena cv Tarramba, and Rhodes grass cv Topcut and Reclaimer.

A3.2 Introduction

Plant species differ in the ability of their seed to germinate and grow in saline environments. Germination is considered to have taken place when the seed swells due to water uptake and the pre-formed radicle in the seed expands and breaks through the seed coat. Thus, initial phases of germination require water uptake and may be inhibited by high salinities due to osmotic stress. By contrast, continued root and shoot growth requires cell division and elongation and the cell division process may be affected by either osmotic effects or ion toxicities (Lambers et al. 2008). In some plants, seed germination is less affected than root growth [e.g. *Bouteloua gracilis* (Zhang et al. 2012); *Zea mays* (Zhang and Zhao 2011); *Triticum aestivum* (Lin et al. 2012)] whereas in other plants, root growth may be less affected than seed germination [e.g. *Buchloe dactyloides* (Zhang et al. 2012)].

Saline soils, or irrigation of soil with saline water may increase salinity and affect seed germination and early establishment of seedlings. The accumulation of salts on the soil surface due to water evaporation can exacerbate this effect and increase the soil solution salinity to very high values.

Land amendment irrigation (LAI) with coal seam water (CS water), which is saline, sodic and alkaline, may change soil properties in the topsoil and impair the establishment of plant species. While the effect of saline conditions on seed germination is reasonably well understood, large species differences exist (Ashkan and Jalal 2013; Mahmood et al. 1996; Tobe et al. 2003). Calcium ions are considered to alleviate the effects of Na toxicity due to membrane effects, but few studies have investigated the effect of Na/Ca ratio, expressed in this study as sodium adsorption ratio (SAR) on seed germination (Tobe et al. 2002; 2004; Tobe et al. 2003; Zehra et al. 2012). By contrast, the effect of alkalinity on seed germination of pasture species is poorly researched, and alkalinity is considered to be more detrimental than salinity to both germination and root growth (Li et al. 2010; Lin et al. 2012; Zhang et al. 2015; Zhang and Zhao 2011). Santos GLNG intends to irrigate soils with CS water containing up to 900 mg/L sodium, pH 9 and EC 4.0 dS/m. The aim of this study was to investigate the effect of increasing salinity and alkalinity on the seed germination of three pasture species, *Leucaena leucocephala* cv Tarramba, a shrub legume, and *Chloris gayana* cv "Topcut" and cv "Reclaimer", both salt tolerant cultivars of Rhodes grass. These salt-tolerant species are used for mixed pasture systems in Queensland under overhead irrigation with CS water. It was hypothesised that seed germination would be inhibited at high salinities and that there would be differences in salt tolerance between the pasture species.

A3.3 Materials and methods

Seeds from *Leucaena leucocephala* cv Tarramba were scarified by making a small incision in the testa at the cotyledon end of the seed using a nail clipper. Seed from Rhodes grass (*Chloris gayana*) cultivars Reclaimer and Topcut were obtained from Selected Seeds Pty Ltd (Pittsworth QLD).

Eight seeds of the varieties were placed in sterile Petri dishes (50 mm diameter with absorbent cellulose disks, model PD-47B, Advantec Japan) containing 2.2 mL of treatment solutions. Each Petri dish with eight seeds constituted a replicate. Dishes were germinated in darkness for 2 days and then transferred to a laboratory bench with ambient light at 23-26°C. Each treatment was replicated five times. Dishes were completely randomised after each evaluation. The number of germinated

seeds in each treatment was counted. Root length was measured for leucaena seedlings only, and seeds with roots longer than 4 mm were considered to have germinated.

Treatment solutions tested the effect of salinity, expressed as electrical conductivity (EC) ranging from 0-20 dS/m, sodium adsorption ratio (SAR), ranging from 0, 5, 15 and 30, and alkalinity (0-2000 mg/L as $CaCO_3$ equivalent, ranging from pH 6.6 to 9.4) on seed germination. Solutions were prepared using stock solutions (0.25 M) of NaCl, $CaCl_2$ or $NaHCO_3$ (Appendix 1). The electrical conductivity was measured with an EC meter and the SAR calculated from the concentration of Na and Ca in the solutions using the formula:

$$SAR = \frac{[Na]}{\sqrt{[Ca]}}$$

with the [Na] and [Ca] concentration on millimolar basis.

The solute potential (Ψs) of the solutions was estimated using the formula:

$$\Psi s = iCRT$$

with C the molar concentration of the salt, R the gas constant, T the temperature in Kelvin, and i number of molecules in the dissociated salt.

Results were analysed with Proc GLM in SAS to determine significance levels (Tukey) and possible interactions between main effects (salinity, EC and alkalinity).

A3.4 Results and Discussion

A3.4.1 Leucaena

Germination percentage of leucaena seed after 5 days was significantly affected by salinity (as expressed by the EC) but not by the SAR (Table A3.1), and the average root length after 5 days was affected by both EC and SAR (Table A3.2).

Table A3.1. ANOVA of leucaena germination percentage after 5 days in response to EC and SAR.

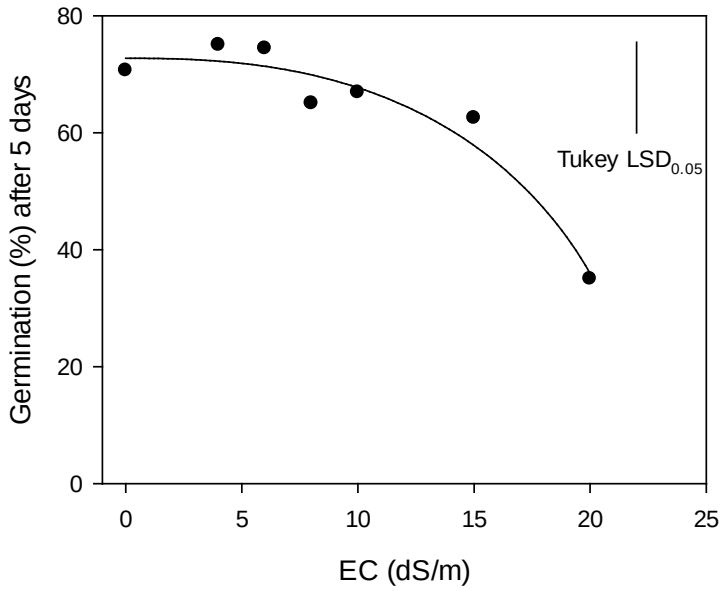
Source	DF	Type I SS	Mean Square	F Value	Pr>F
EC	6	22495.5	3749.3	14.14	<0.0001
SAR	3	1003.3	334.5	1.26	0.291
EC*SAR	18	4379.5	243.3	0.92	0.5588

Table A3.2. ANOVA of average root length of leucaena after 5 days in response to EC and SAR.

Source	DF	Type I SS	Mean Square	F Value	Pr>F
EC	6	4867.1	811.2	19.85	<0.0001
SAR	3	1157.3	385.8	9.44	<0.0001
EC*SAR	18	1022.0	56.8	1.39	0.1508

Since germination percentage was not affected by SAR, the data were pooled for EC. Seed germination was not significantly affected by solution EC up to 15 dS/m (P>0.05), however germination declined (P<0.05) from 71% (control) to 35% for the 20 dS/m treatment. This suggests that salinity in excess of 15 dS/m inhibited germination of leucaena cv Tarramba. This points to a remarkable salinity tolerance of this leucaena cultivar given that Miah (2013) suggested that leucaena can only tolerate 20 mg/L NaCl (corresponding to an EC of ca. 0.042 dS/m), but we suspected this threshold may be typographic error in the manuscript. Evaporation of solution during the five days was minimal (no significant difference in weight of the dish was recorded), but water uptake by germinating seeds may have concentrated salts further. The minimal water loss due to evaporation was due to the design of the Petri dishes which had close fitting lids. Germination percentages after 5 days were not different from those recorded after 21 days (data not shown), and for simplicity, only germination after 5 days data are presented.

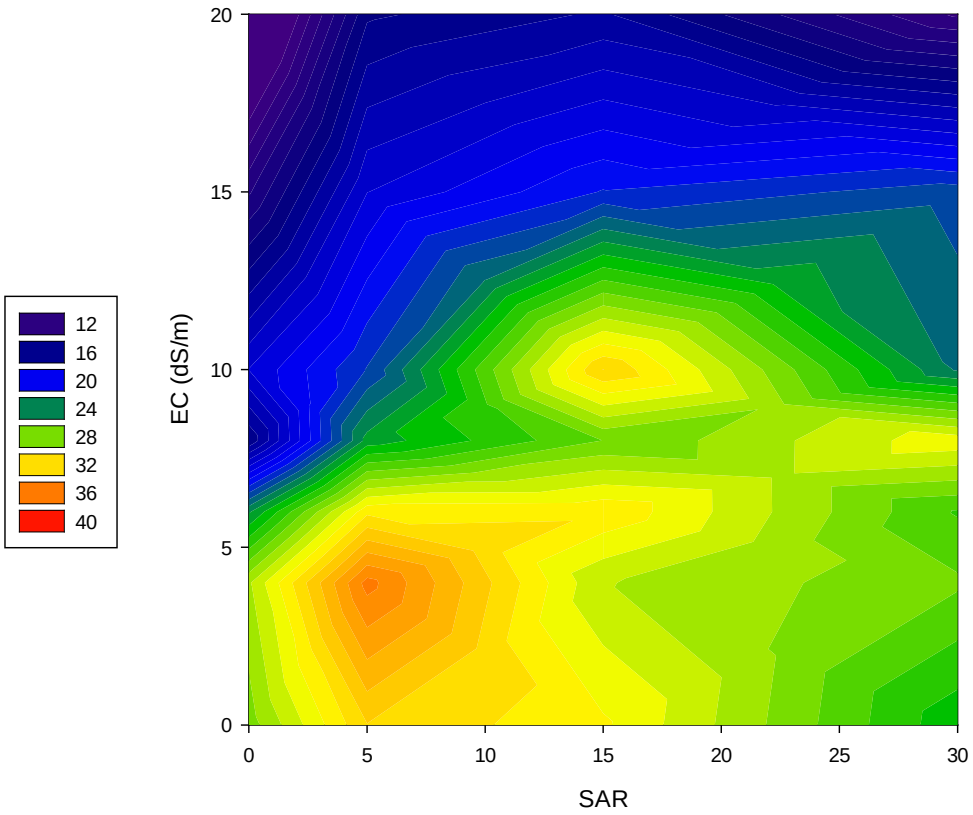
The EC for 10% and 50% inhibition of germination was determined from Figure A3.1 using the regression curve $y^2=5288.7-2.23x^{2.5}$. The EC for 10% and 50% reduction were calculated as 11.6 dS/m and 20.0 dS/m, respectively. This indicates that leucaena cv Tarramba is highly salt tolerant.



FigureA3.1. Germination percentage of leucaena cv Tarramba after 5 days. Treatments with different SAR and same EC were combined since SAR had no significant effect on germination. The solid points are the mean of 20 replicates. The curve represents the best-fit for $y^2=5288.7-2.23x^{2.5}$, $r^2=0.939$. The vertical line represents Tukey's LSD at 5% significance level.

Average root length of leucaena seedlings after 5 days was significantly affected by both EC and SAR, but there was no statistical interaction between these parameters on root length (Table A3.2). The root length decreased from 28 mm in the control, to 24 mm at 8 dS/m, and reached a minimum of 12 mm at 20 dS/m (Figure A3.2).

Root length in the control was significantly lower (19 mm) at SAR 0 than in the SAR 5-30 treatments (24-27 mm) (Figure A3.2). The lower root length in the SAR 0 treatment, which consisted of deionised water, indicated that Ca ions are required for root growth. Indeed, cell division and elongation is known to require Ca ions (Lambers et al. 2008), and the Ca ions supplied by the cotyledons may not have been sufficient to meet plant demand for the five day duration due to immobility of Ca within the phloem. As root growth is rarely constant for longer than five days in deionised water due to the depletion of nutrients from seed reserves, we did not record root length after more than five days. Such nutrient deficiencies would confound the interpretation of the impacts of salt and alkalinity on root length.



FigureA3.2. Root length (in mm) of leucaena cv Tarramba seedlings after 5 days in response to EC and SAR.

Root growth is often more sensitive to salinity than germination, e.g. in maize (Zhang and Zhao 2011) or lucerne (Li et al. 2010). Our results confirm this statement, with NaCl salinities greater than 10 dS/m decreasing root growth (compared to >15 dS/m affecting germination). The observed root growth inhibition may have been caused by osmotic effects or ion imbalances and further research is required to identify the physiological cause. Increasing solute potential appeared to decrease germination and root growth (Figure A3.3) in a linear manner, but the correlation between germination percentage or root length, and solute (osmotic) potential was weak. Although plants can tolerate osmotic potentials of -15 bars (permanent wilting point), growth of leucaena was impaired with salinities >25 mM (Anthraper and DuBois 2003; Hansen and Munns 1988), corresponding to a solute potential of -1.3 bar. The decrease in seed germination and root length

with increasing salinity can thus be ascribed to osmotic stress. However, the possibility of ionic stress cannot be excluded. A cursory analysis of the extent of germination inhibition per unit concentration of different ions showed very similar rates (slopes) for Na and Cl (Figure A3.3), but germination inhibition was greater for Ca, suggesting that Ca is more inhibitory to seed germination in leucaena than either Na or Cl. On the other hand, neither ion had a markedly different effect on root length (Figure A3.3). To obtain more definite results, further research would be required into the effect of ions on seed germination and root growth.

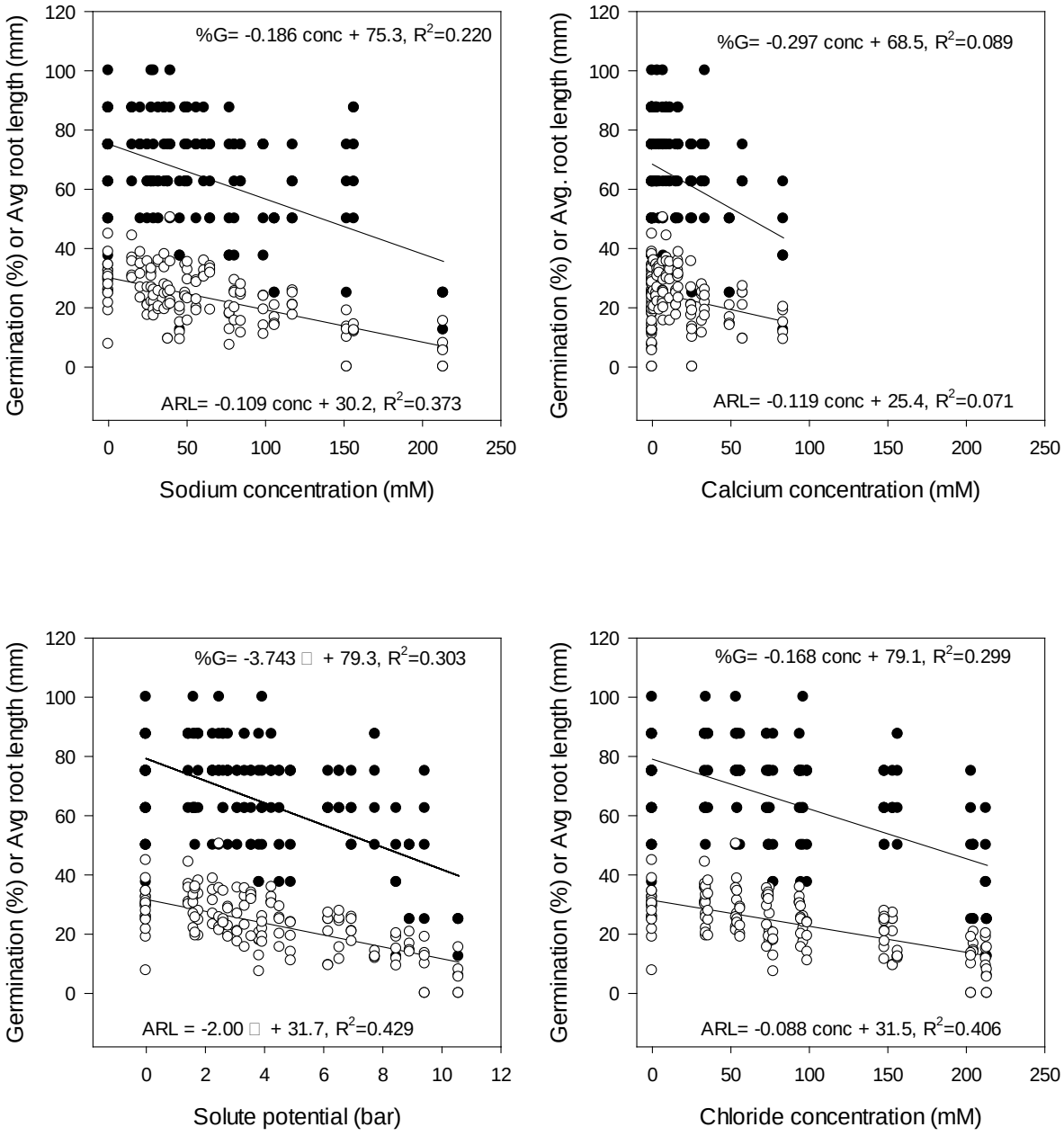


Figure A3.3. Effect of Na concentration (top left), Ca concentration (top right), Cl concentration (bottom right) and calculated solute potential (bottom left) on seed germination (filled symbols) and root length (open symbols) of leucaena cv Tarramba 5 days after sowing.

Alkalinity had no significant effect on seed germination percentage (Table A3.3) or average root length (Table A3.4) after 5 days.

Table A3.3. ANOVA of seed leucaena germination percentage 5 days after sowing in response to alkalinity.

Source	DF	Type I SS	Mean Square	F Value	Pr>F
Alkal	5	213.542	42.708	0.21	0.9560

Table A3.4. ANOVA of average root length of leucaena 5 days after sowing in response to alkalinity.

Source	DF	Type I SS	Mean Square	F Value	Pr>F
Alkal	5	415.819	83.164	2.60	0.0511

Although root length after 5 days was not significantly affected (P<0.0511), there was a trend with root length decreasing from 25 mm in the control, to 16 mm with 2000 mg/L carbonate alkalinity (Figure A3.4). On the other hand, increasing alkalinity showed no effect on seed germination (Figure A3.4). This is remarkable since seed germination is generally inhibited by increasing alkalinity (Li et al. 2010; Lin et al. 2011; Tewari et al. 1999; Zhang and Zhao 2011). The root growth inhibition observed in this study is insignificant and may not affect root growth in soil since soils are buffered with regards to pH changes in addition to application of elemental sulfur to limit pH fluctuations during CS water irrigation. Even raw CS water with pH 9 is unlikely to affect leucaena cv Tarramba seed germination and growth since the highest alkalinity (2000 mg/L) had pH 9 (Appendix 1), similar to the pH in CS water.

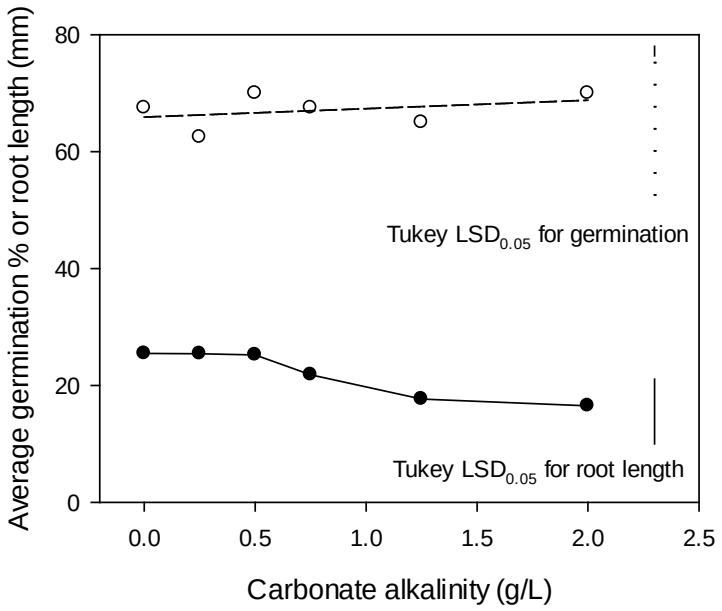


Figure A3.4. Effect of alkalinity on leucaena seed germination percentage (open circles with dashed line) and root length (solid circles with solid line) after 5 days. Symbols are the mean values of five replicates. The vertical line represents Tukey's LSD at 5% significance level.

A3.4.2 Topcut Rhodes grass

Germination of *Chloris gayana* cv Topcut (Topcut Rhodes grass) after 9 days was not affected by salinity or SAR (Table A3.5), with germination percentages ranging from 58% (EC 20 dS/m) to 48% (0 dS/m), and from 55% (SAR 0) to 49% (SAR 30) (Figure A3.5). Although this cultivar of Rhodes grass is very salt tolerant, we have no comparative values for other cultivars, but it is remarkable that this cultivar was not affected by EC 20 dS/m in the germination assay. On the other hand, germination was inhibited at >100 mM NaCl (corresponding to an EC of 10 dS/m) in *Panicum turgidum* (El-Keblawy 2004), >10 dS/m in *Cynodon dactylon* (Mahmood et al. 1996) and >2 dS/m in *Sporobolus arabicus* (Sheikh and Mahmood 1986). Thus, Topcut is highly salt tolerant among grasses and this may be related to the fact that Rhodes grass is able to accumulate salt in its foliage and excrete salt through salt glands on the leaves (Oi et al. 2012). Yet, the seedlings in the current trial had not yet developed foliage and could not have excreted salt. They must have another mechanism to tolerate salt during the germination process.

Table A3.5. ANOVA of seed germination of Topcut Rhodes grass after 9 days in response to EC and SAR.

Source	DF	Type I SS	Mean Square	F Value	Pr>F
EC	6	1607.1	267.9	0.74	0.6197
SAR	3	1271.2	423.7	1.17	0.3252
EC*SAR	18	4955.3	275.3	0.76	0.7427

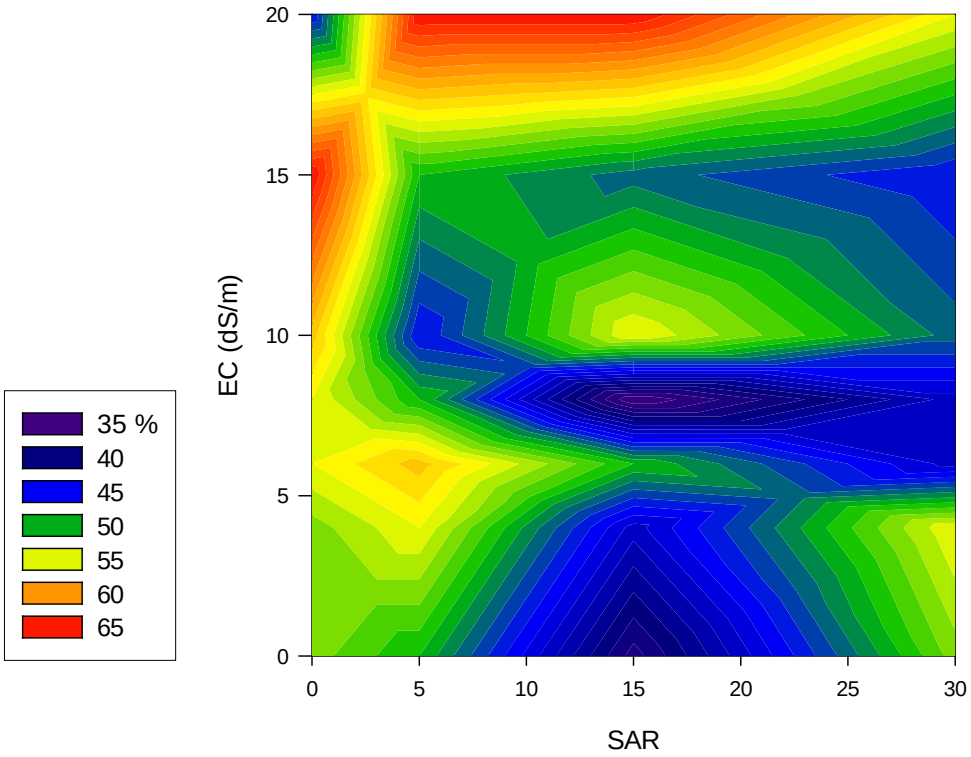


Figure A3.5. Germination percentage of Topcut Rhodes grass seed after 9 days in response to EC and SAR.

Alkalinity had no significant effect on germination rates, ranging from 47% (control) to 57% (750 mg/L as carbonate) and 47% (2000 mg/L as carbonate) (Figure A3.6). Zhang et al. (2011) found that maize germination was inhibited by increasing alkalinity, but in other grass species, alkalinity had little effect [e.g. *Leymus chinensis*, (Lin et al. 2011)]. Since Rhodes grass seeds have long awns which were easy to confuse with roots, we have not measured root length in Rhodes grass. Germination after 9 days was not significantly lower than at 14 days, therefore, we are only reporting germination after 9 days.

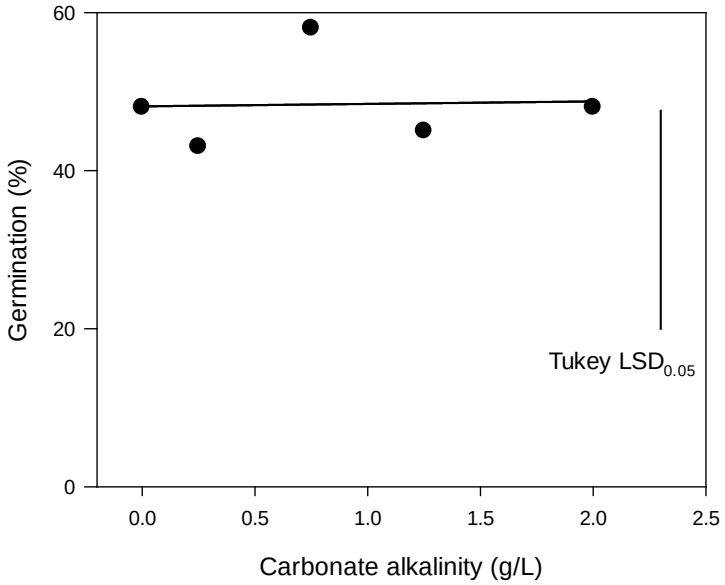


Figure A3.6. Germination percentage of Topcut Rhodes grass seed after 9 days as affected by increasing alkalinity. Solid points are the mean of 5 replicates, the line represents the fit for y=48.1+0.311x. The vertical line represents Tukey's LSD at 5% significance level.

Interestingly, seed germination was not inhibited due to toxic ion effects or osmotic potential, since germination percentages appeared to increase with all ion concentrations and osmotic potential (Figure A3.7), but it must be cautioned that the correlations were weak and need further investigation before drawing firm conclusions. The increase in the extent of germination appeared to be greater with Ca than with Na or Cl, suggesting that Ca is particularly beneficial for germination of this cultivar. It is unlikely that additional Ca overcomes salt stress or ion toxicity (since both Na and Cl increased germination rate) and further work is required to better understand this observation.

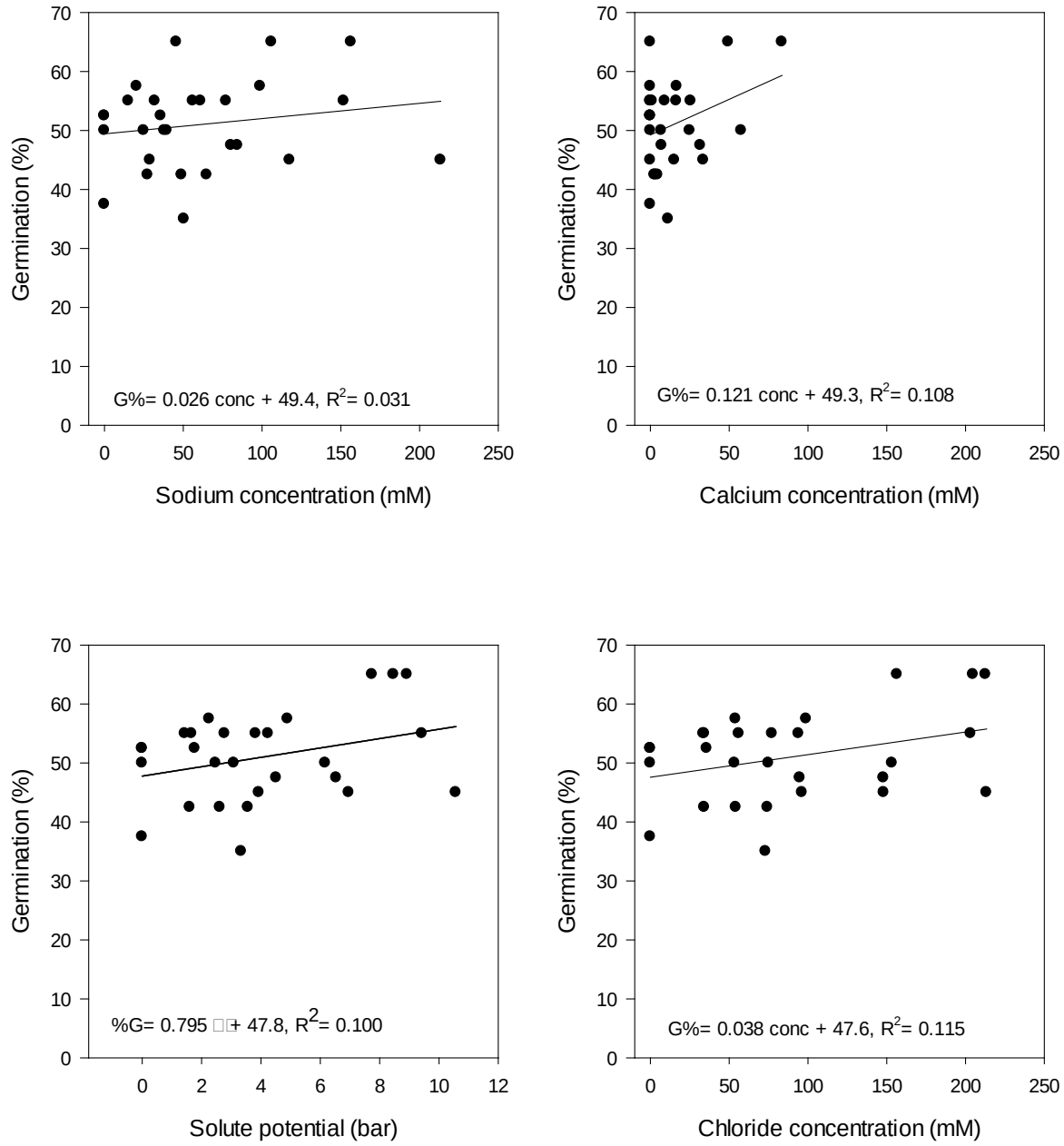


Figure A3.7. Effect of Na concentration (top left), Ca concentration (top right), Cl concentration (bottom right) and solute (osmotic) potential (bottom left) on germination of Topcut Rhodes grass seed after 9 days.

A3.4.3 Reclaimer Rhodes grass

Overall, Reclaimer Rhodes grass had a lower germination percentage than Topcut, irrespective of treatments. Germination after 8 days was significantly affected by salinity but not by the SAR (Table A3.6).

Table A3.6. ANOVA of seed germination percentage of Reclaimer Rhodes grass after 8 days.

Source	DF	Type I SS	Mean Square	F Value	Pr>F
EC	6	2781.2	463.5	2.52	0.0249
SAR	3	869.4	289.8	1.58	0.1986
EC*SAR	18	2763.4	153.5	0.84	0.6546

Germination ranged from 28% in the control, to 22% at 15 dS/m and decreased to 15% at 20 dS/m (Figures A3.8 and A3.9). Reclaimer Rhodes grass was claimed to have higher salt tolerance than Topcut Rhodes grass, but our results show Topcut to be more salt tolerant in the seed germination trial. It is interesting to note that the response to salinity differed between these closely related cultivars.

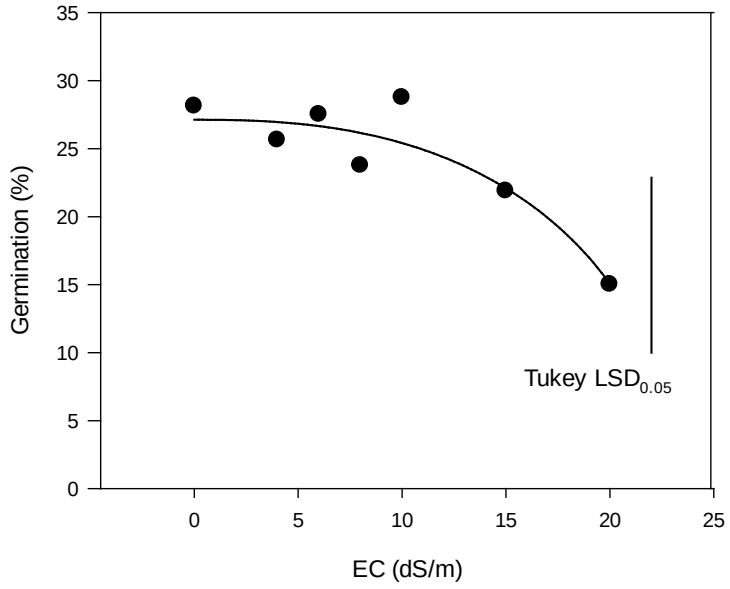


Figure A3.8. Germination percentage of Reclaimer Rhodes grass seed after 8 days as affected by increasing salinity. Solid points are the mean of 20 replicates. The curve represents the best-fit for $y^2=735.9-0.285x^{2.5}$, $r^2=0.853$. The vertical line represents Tukey's LSD at 5% significance level.

The EC required for 10% and 50% inhibition of germination was calculated from the fitted curve ($y^2=735.9-0.285x^{2.5}$) as 11.9 dS/m and 20.6 dS/m, respectively. These values are lower than for Topcut Rhodes grass, but similar to the values determined for leucaena cv Tarramba. Thus, in terms of seed germination, this cultivar is still very salt tolerant, but not as tolerant as Topcut Rhodes grass.

The SAR had no significant effect on germination, but a trend suggested that germination was lower in the control than in SAR 5-30 treatments (Figures A3.9 and A3.10). This points to a requirement for Ca during germination and it would be expected that the Ca requirement would also persist during root growth (which was not measured in this study). The protective effect of low Ca concentrations (<1 mM) on seed germination is known [e.g. for *Phragmites* (Zehra et al. 2012)], but Tobe et al. (2002; Tobe et al. 2003) found that Ca only alleviates effects of Na toxicity on root growth but does not overcome Na inhibition to germination.

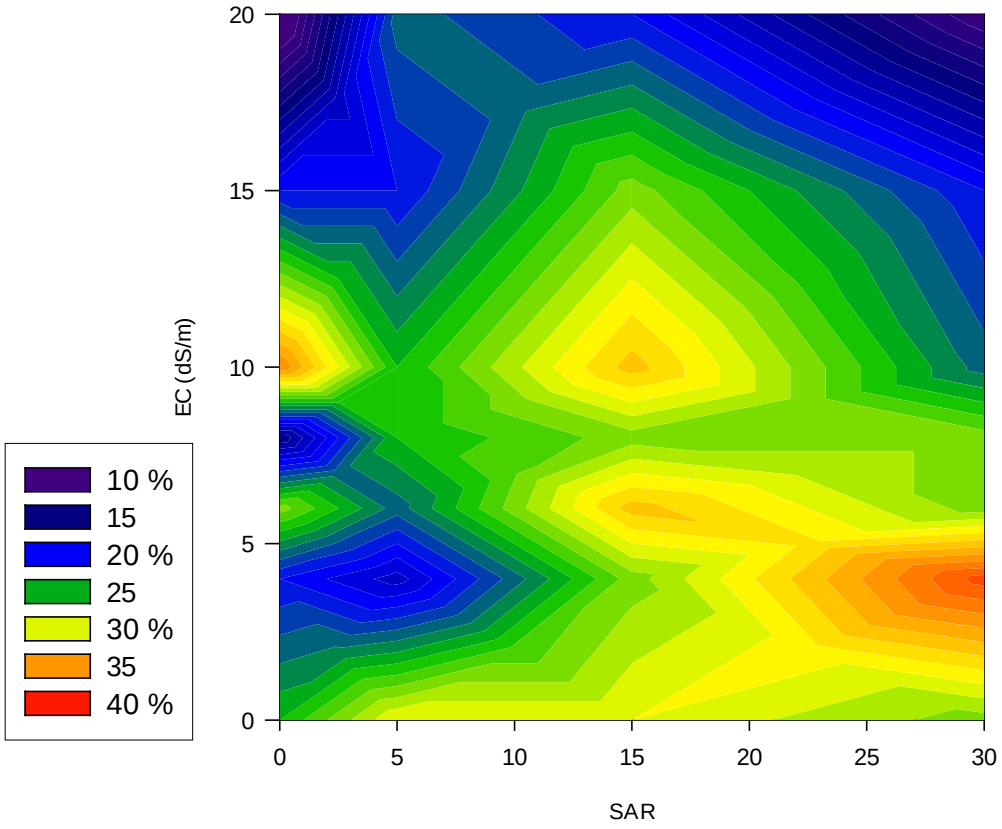


Figure A3.9. Reclaimer Rhodes grass seed germination percentage as affected by EC and SAR after 8 days.

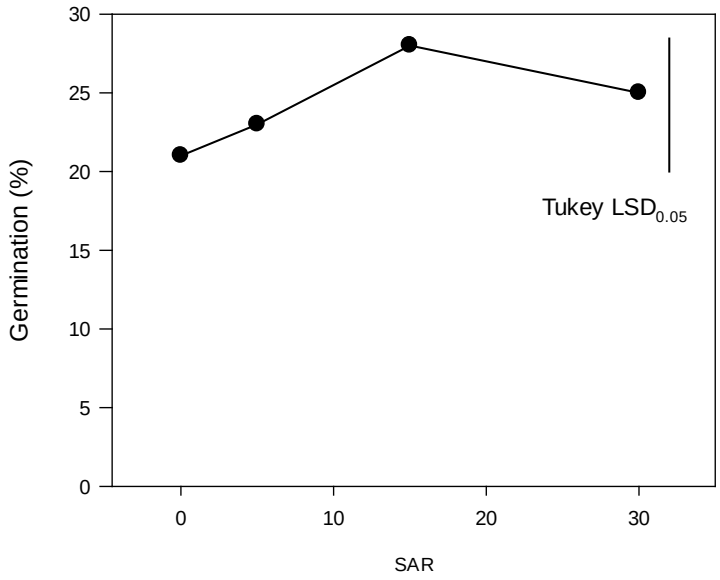


Figure A3.10. Germination percentage of Reclaimer Rhodes grass seed after 8 days as affected by different SAR. Symbols represent the mean of 35 replicates. The vertical line represents Tukey's LSD at 5% significance level

Alkalinity had no statistically significant effect on seed germination, but the trend suggests that germination decreased from 32% at 2000 mg/L carbonate to 17% in the control (Figure A3.11). This is an unexpected result and would require detailed physiological investigation to better understand the underlying mechanisms. Interestingly, in *Vigna aconitifolia*, only concentrations greater than 100 mM $NaHCO_3$ inhibited germination (Patil et al. 2012). Zhang and Zhao (2011) observed little inhibition to germination by alkalinity in maize. On the other hand, alkalinity inhibition of germination and root growth was observed in both wheat (Lin et al. 2012) and lucerne (Li et al. 2010).

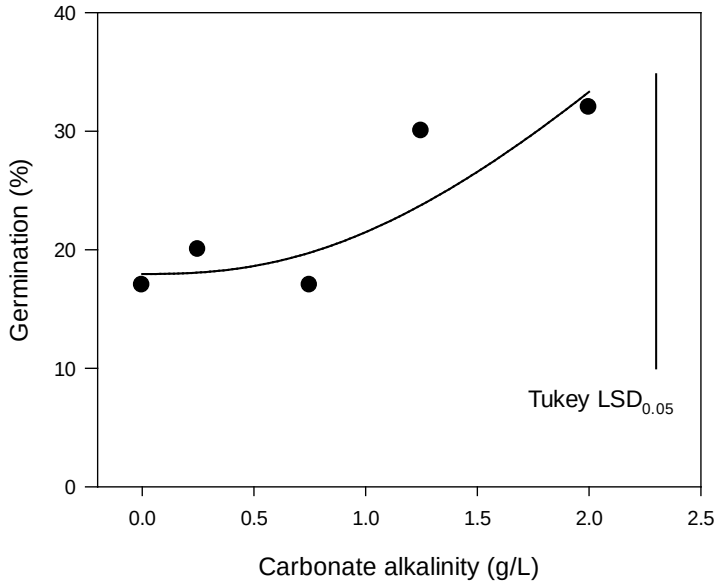


Figure A3.11. Germination percentage of Reclaimer Rhodes grass seed after 8 days as affected by increasing alkalinity. Solid points are the mean of 5 replicates. The curve is the best-fit of y^2= 322.7+139.4$x^{2.5}$, r^2 = 0.751. The vertical line represents Tukey's LSD at 5% significance level.

Expressing the salinity-SAR interaction as concentrations of ions showed that the rate of inhibition of germination appeared to differ little between the Na, Ca and Cl ions, and the solute (osmotic) potential (Figure A3.12), but correlations were weak and no firm conclusions can be drawn. This suggests that inhibition of germination is not due to toxicity of a particular ion, but due to osmotic effects. In soil grown Rhodes grass, exposure of individual roots to 200 mM NaCl (with a solute potential of 9.9 bar) was shown to stimulate root growth of exposed roots (Waisel 1985) but this concentration was strongly inhibitory to shoot growth of Rhodes grass when whole plants were grown in saline solution (Guggenheim and Waisel 1977; Taleisnik et al. 1997). Therefore, single roots may overcome salinity stress by being supplied with water from parts of the root system that is not salinized. However, if the entire roots system is exposed to salinity, growth will be affected.

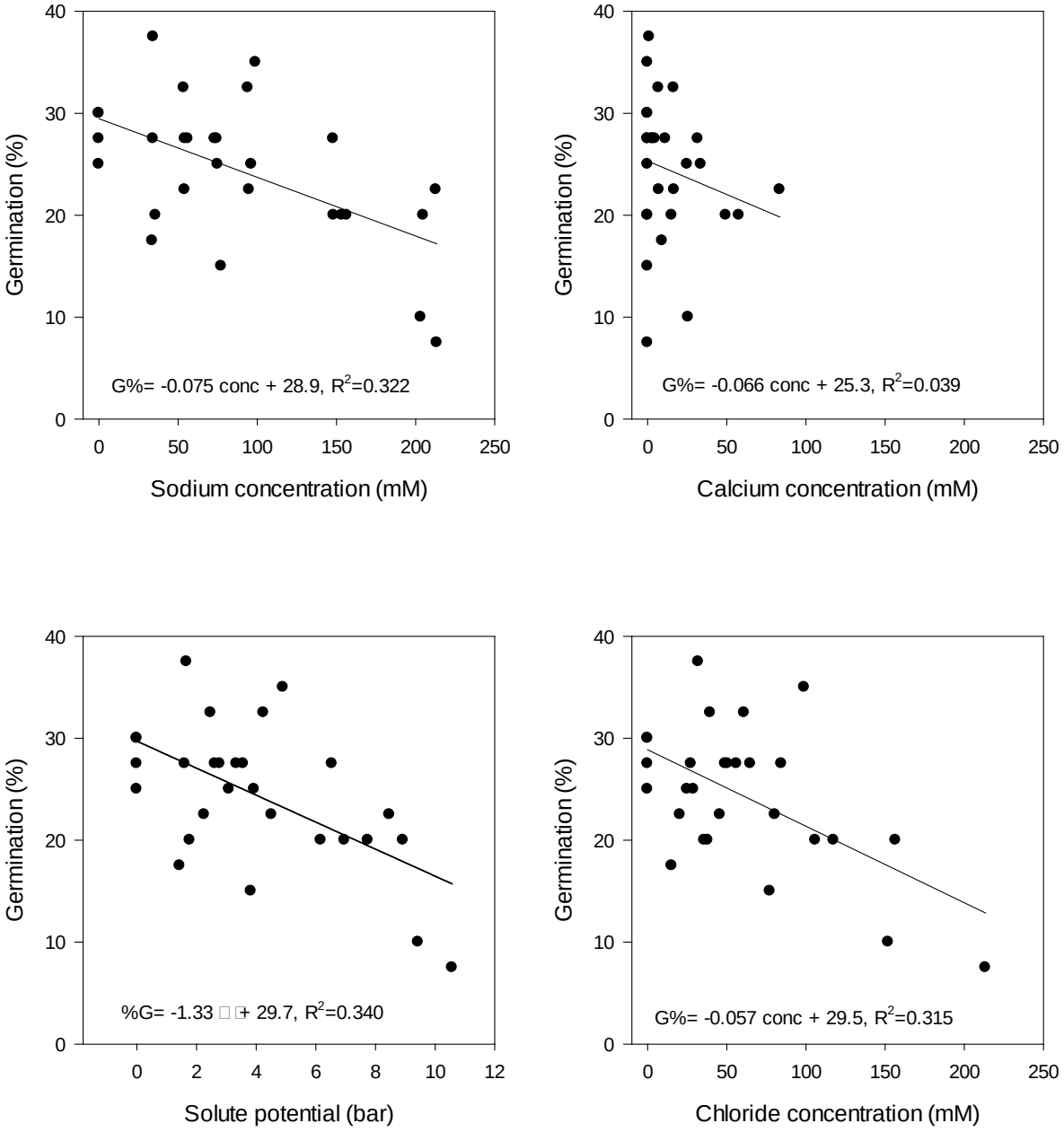


Figure A3.12. Effect of Na concentration (top left), Ca concentration (top right), Cl concentration (bottom right) and solute (osmotic) potential (bottom left) on germination of Reclaimer Rhodes grass seed after 8 days.

A3.5 Conclusion

This study showed that the three plant species are remarkably salt tolerant during the germination phase and only salinities of 20 dS/m in the solution are detrimental to germination, resulting in 50% decrease in seed germination in leucaena and Reclaimer Rhodes grass, whereas Topcut Rhodes grass seed germination was not affected by salinity up to 20 dS/m. The SAR affected germination of Reclaimer Rhodes grass seed and root growth in leucaena, but had no effect on Topcut Rhodes grass. It is likely that the seedlings require a small amount of Ca to maintain cell wall and plasma membrane function since only the SAR 0 treatment (which contained no Ca) decreased germination and growth. This suggested that the Ca activity in the cell wall and plasma membrane plays a role and even in the presence of excess Na ions, the Ca concentration is maintained in the apoplast. This study investigated germination of seeds on germination paper; we chose the germination paper method since it is rapid to perform and avoids confounding factors such as adsorption/desorption of ions and effects of water availability.

It has been observed that different germination media may influence the outcome of salt tolerance screening (Zhang et al. 2011), with agar-based or hydroponic media showing more toxicity than the paper based test, but the responses are species specific. It is possible that high salt concentrations applied to agar and soil may displace other cations from the exchange phase and the displaced cations (mainly divalent cations such as Ca or Mg) are inhibitory to germination. Likewise, different soils may have different effects on germination, but no research has been found to confirm this and it is possible that sensitivity to salinity in soil may be greater than in the germination paper tests. The salinity threshold determined in this study cannot be directly extrapolated to field germination results. Evaporation of water from the soil surface may increase the salinity in the surface layer to values found in saturated salt solutions (e.g. 220 dS/m for NaCl). Thus, surface salinity is not directly related to the salinity of the irrigation water but to the build-up of salt due to evaporation. Long-term irrigation with low salinity water can still result in salinisation of the surface soil, but irrigation with highly saline water will exceed the seed threshold sooner than low-salinity water. If accumulation of salt in the surface layer can be avoided (for instance by frequent irrigation that prevents drying-off of the surface layer), irrigation with water having salinities of up to 15 dS/m will permit germination of Rhodes grass and leucaena seeds. Leaching irrigation of soil during LAI may flush salt from the surface layer in sandy soils, but not in low-conductivity soils (clay soils). If salts cannot be leached from the surface 5-10 cm due to low hydraulic conductivity, it needs to be established if the salinity of the soil solution in the surface is below 15 dS/m, otherwise germination inhibition is likely. Soil solution salinities for soils from IR7, IR8 and IR6(3) ranged from 4.1-6.0 dS/m prior to CS water application and increased up to 10.3 dS/m after 9 ML CS water. Thus, it is unlikely that salinities in field soil will increase to values inhibitory to seed germination, especially, if frequent irrigations are applied that avoid drying-out of the surface soil.

In the Moonie column study (Bianca Das M.Sc. thesis), germination of Topcut Rhodes grass seed in unamended Moonie Vertosol was poor and it was suggested that this may be due to the high salinity of the soil (17 dS/m in soil solution), with higher values likely in the surface crust. Also, the alkalinity

was high (pH 9.6), corresponding to a carbonate alkalinity of 2000 mg/L. The results reported here reveal that Topcut Rhodes grass would not be affected by these salinities and alkalinities, and the results from the Moonie soil germination study indicate that soil germination results may be different to the germination paper results. This is likely due to evaporative water loss from the surface and concentration of salts in the surface crust in soil studies.

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A3.7 Appendix 1. Preparation of treatment solutions

Solutions for EC and SAR tests were prepared using 0.25 M stocks of NaCl and $CaCl_2$, mixed, and made up to 50 mL. Solutions for the alkalinity tests were prepared using 0.25 M stocks of $NaHCO_3$ and NaCl, mixed, and made up to 50 mL.

SAR ∞

EC (dS/m)	NaCl (mL)	$CaCl_2$ (mL)	Na (mM)	Ca (mM)	Cl (mM)	Ψs (bar)
0	0	0	0	0	0	0
4.0	7.17	0	35.9	0.0	35.9	1.9
6.0	11.24	0	56.2	0.0	56.2	2.8
8.1	15.46	0	77.3	0.0	77.3	3.8
10.2	19.80	0	99.0	0.0	99.0	4.9
15.0	31.30	0	156.5	0.0	156.5	7.7
19.6	42.69	0	213.5	0.0	213.5	10.6
	Make up to 50 mL					

SAR 5

EC (dS/m)	NaCl (mL)	$CaCl_2$ (mL)	Na (mM)	Ca (mM)	Cl (mM)	Ψs (bar)
0	0	0	0	0	0	0
3.6	3.04	1.85	15.2	9.2	33.7	1.4
5.8	4.10	3.36	20.5	16.8	54.1	2.3
7.7	5.00	5.00	25.0	25.0	75.0	3.1
9.4	5.80	6.73	29.0	33.6	96.3	3.9
14.0	7.60	11.55	38.0	57.7	153.5	6.2
18.3	9.14	16.71	45.7	83.5	212.8	8.5
	Make up to 50 mL					

SAR 15

EC (dS/m)	NaCl (mL)	$CaCl_2$ (mL)	Na (mM)	Ca (mM)	Cl (mM)	Ψs (bar)
0	0.00	0.00	0	0	0	0
3.9	5.50	0.67	27.5	3.3	34.2	1.6
5.8	7.92	1.39	39.6	6.9	53.5	2.5
7.6	10.10	2.27	50.5	11.3	73.2	3.3
9.5	12.20	3.31	61.0	16.5	94.1	4.3
13.8	16.90	6.35	84.5	31.7	148.0	6.5
17.8	21.20	9.89	106.0	49.4	204.9	8.9
	Make up to 50 mL					

SAR 30

EC (dS/m)	NaCl (mL)	$CaCl_2$ (mL)	Na (mM)	Ca (mM)	Cl (mM)	Ψs (bar)
0	0	0	0	0	0	0
3.9	6.40	0.23	32.0	1.1	34.3	1.7
6.0	9.80	0.53	49.0	2.6	54.3	2.6
8.0	13.00	0.94	65.0	4.7	74.4	3.6
9.6	16.10	1.44	80.5	7.2	94.9	4.5
14.2	23.50	3.07	117.5	15.3	148.2	7.0
18.2	30.40	5.13	152.0	25.6	203.3	9.4
	Make up to 50 mL					

Alkalinity

Carbonate (mg/L)	$NaHCO_3$ (mL)	NaCl (mL)	EC (dS/m)	Na (mM)	Cl (mM)	HCO_3 (mM)	Ψs (bar)	pH
0	0	0	0	0	0	0	0	6.65
250	1.00	6.70	4.1	38.5	33.5	5.0	1.9	8.79
500	2.00	5.80	4.1	39.0	29.0	10.0	1.9	8.78
750	3.00	4.90	4.1	39.7	24.7	15.0	2.0	8.95
1250	5.00	3.20	3.8	41.2	16.2	25.0	2.0	8.96
2000	8.00	1.00	3.8	44.8	4.8	40.0	2.2	9.43
	Make up to 50 mL							

A3.7 Appendix 2. Detailed germination results

Germination of leucaena cv. Tarramba after 5 days.

EC (dS/m)	SAR	Rep	Germin. %	Avg Root length	Root lengths of individual seedlings						
0	∞	1	50	19.0	20	14	21	21			
0	∞	2	75	21.7	35	15	33	12	10	25	
0	∞	3	62.5	25.8	26	40	41	8	14		
0	∞	4	87.5	32.6	18	44	44	46	30	32	14
0	∞	5	87.5	38.9	65	40	35	36	40	17	39
4	∞	1	87.5	24.7	35	41	21	19	15	22	20
4	∞	2	62.5	27.8	45	15	17	46	16		
4	∞	3	75	33.5	22	14	70	33	32	30	
4	∞	4	87.5	38.0	41	45	30	38	33	40	39
4	∞	5	87.5	19.4	22	30	10	5	23	20	26
6	∞	1	75	20.0	32	5	24	6	19	34	
6	∞	2	87.5	29.1	34	40	36	37	39	8	10
6	∞	3	50	22.8	22	22	38	9			
6	∞	4	75	28.5	32	29	31	41	22	16	
6	∞	5	75	19.0	20	24	15	21	22	12	
8	∞	1	37.5	12.7	16	6		16			
8	∞	2	50	20.5	19	20	25	18			
8	∞	3	87.5	17.9	26	24	8	5	28	25	9
8	∞	4	75	18.2		10	14	19	26	22	18
8	∞	5	37.5	7.3		5	10	7			
10	∞	1	37.5	14.0		12	24	6			
10	∞	2	75	24.0	27	27	38	21	18	13	
10	∞	3	75	23.8	14	20	32	14	25	38	
10	∞	4	75	19.3	25	22	17	15	25	12	
10	∞	5	50	11.0	20	6	10	8			
15	∞	1	50	12.5	20	12	12	6			
15	∞	2	87.5	11.9	12	10	13	10	15	12	11
15	∞	3	87.5	12.3	15	10	11	20	7	7	16
15	∞	4	75	14.3	15	19	16	8	18	10	
15	∞	5	62.5	12.4	7	12	17	15	11		
20	∞	1	12.5	6.0		6					
20	∞	2	25	8.0	6	10					
20	∞	3	25	15.5	15	16					
20	∞	4	25	5.5	6	5					
20	∞	5	0	0.0							

EC (dS/m)	SAR	Rep	Germin. %	Avg root length	Root lengths of individual seedlings							
0	5	1	62.5	26.0	22	13	34	35	26			
0	5	2	50	25.0	16	16	22	46				
0	5	3	87.5	34.4	55	53	20	26	25	47	15	
0	5	4	100	44.9	69	56	44	56	50	17	40	27
0	5	5	50	29.5	15	63	22	18				
4	5	1	87.5	36.9	41	50	41	34	50	31	11	
4	5	2	62.5	30.8	39	44	19	35	17			
4	5	3	75	44.3	40	50	36	65	39	36		
4	5	4	87.5	35.6	65	55	36	50	25	9	9	
4	5	5	87.5	30.0	20	14	30	25	42	34	45	
6	5	1	50	23.3	21	22	25	25				
6	5	2	75	31.3	25	11	33	45	44	30		
6	5	3	75	34.8	35	35	32	52	11	44		
6	5	4	87.5	38.7	34	54	46	32	49	30	26	
6	5	5	75	26.8	41	22	5	22	22	49		
8	5	1	75	21.0	20	23	20	44	10	9		
8	5	2	62.5	35.6	33	45	48	46		6		
8	5	3	50	20.8	14	30	34	5				
8	5	4	62.5	17.4	20	16	24	12	15			
8	5	5	75	26.8	14	38	35	30	38	6		
10	5	1	62.5	21.8	38	18	21	17	15			
10	5	2	62.5	19.2	21	20	5	21	29			
10	5	3	50	17.3	15	20	27	7				
10	5	4	75	26.0	26	24	30	25	31	20		
10	5	5	100	24.0	31	21	31	10	8	40	39	12
15	5	1	25	9.5	11	8						
15	5	2	62.5	9.4	16	8	12	6	5			
15	5	3	75	20.8	34	22	16	12	25	16		
15	5	4	62.5	25.0	20	35	20	30	20			
15	5	5	62.5	27.2	21	31	36	27	21			
20	5	1	12.5	15.0	15							
20	5	2	37.5	11.7	20	10	5					
20	5	3	50	19.0	11	12	31	22				
20	5	4	37.5	9.3	13	9	6					
20	5	5	62.5	20.2	18	21	22	15	25			

EC (dS/m)	SAR	Rep	Germin. %	Avg root length	Root lengths of individual seedlings							
0	15	1	75	32.3	52	42	25	25	15	35		
0	15	2	75	30.3	34	20	45	22	20	41		
0	15	3	62.5	31.2	45	35	25	16	35			
0	15	4	75	29.8	35	45	26	25	35	13		
0	15	5	87.5	29.1	57	21	50	39	16	11	10	
4	15	1	62.5	31.8	35	29	26	42	27			
4	15	2	62.5	22.0	29	22	18	20	21			
4	15	3	87.5	30.6	35	25	35	15	41	22	41	
4	15	4	100	33.1	52	32	25	52	22	24	17	41
4	15	5	62.5	26.8	41	20	30	25	18			
6	15	1	50	22.3	28	31	20	10				
6	15	2	100	50.5	54	53	53	50	60	48	55	31
6	15	3	75	35.5	36	46	31	4	61	35		
6	15	4	75	25.7	23	38	25	25	35	8		
6	15	5	87.5	21.3	30	5	27	6	41	25	15	
8	15	1	87.5	32.7	52	52	42	30	30	10	13	
8	15	2	62.5	29.4	30	28	29	28	32			
8	15	3	62.5	35.4	59	39	45	29	5			
8	15	4	87.5	15.6	25	30	11	18	5	5	15	
8	15	5	75	23.0	34	24	20	14	25	21		
10	15	1	75	30.8	22	41	40	35	12	35		
10	15	2	62.5	30.6	25	25	33	45	25			
10	15	3	75	32.5	15	37	27	50	32	34		
10	15	4	87.5	35.9	45	42	29	40	58	30	7	
10	15	5	75	30.3	40	25	31	30	25	31		
15	15	1	25	15.5	16	15						
15	15	2	75	11.5	21	11	9	16	6	6		
15	15	3	75	24.3	9	26	36	30	25	20		
15	15	4	62.5	25.2	31	16	25	18	36			
15	15	5	62.5	27.8	21	35	35	27	21			
20	15	1	25	14.5	14	15						
20	15	2	50	20.8	16	12	24	31				
20	15	3	50	16.8	21	21	19	6				
20	15	4	50	14.5	10	20	16	12				
20	15	5	25	14.0	20	8						

EC (dS/m)	SAR	Rep	Germin. %	Avg root length	Root lengths of individual seedlings						
0	30	1	62.5	24.8	29	10	26	39	20		
0	30	2	75	30.3	29	29	12	38	39	35	
0	30	3	75	34.5	40	33	41	26	45	22	
0	30	4	37.5	7.7	8	10	5				
0	30	5	75	27.8	20	32	30	36	12	37	
4	30	1	50	35.3	40	28	32	41			
4	30	2	87.5	19.4	37	6	35	27	5	21	5
4	30	3	62.5	25.6	41	27	40	10	10		
4	30	4	62.5	36.0	28	51	31	54	16		
4	30	5	62.5	20.4	26	6	20	30	20		
6	30	1	75	24.5	31	25	26	7	20	38	
6	30	2	87.5	23.7	30	27	19	16	27	30	17
6	30	3	87.5	34.6	30	44	35	37	30	26	40
6	30	4	62.5	25.0	21	23	29	31	21		
6	30	5	62.5	23.8	30	30	21	31	7		
8	30	1	75	34.0	35	41	15	19	49	45	
8	30	2	50	32.5	31	34	27	38			
8	30	3	75	33.2	35	24	45	20	40	35	
8	30	4	50	31.8	32	32	29	34			
8	30	5	62.5	19.2	15	51	20	5	5		
10	30	1	75	29.3	35	40	36	21	15	29	
10	30	2	50	25.5	30	30	22	20			
10	30	3	37.5	20.0	21	29	10				
10	30	4	62.5	15.6	22	10	12	24	10		
10	30	5	75	25.0	29	20	30	15	26	30	
15	30	1	75	17.5	21	25	15	18	9	17	
15	30	2	50	21.0	11	25	23	25			
15	30	3	50	25.8	23	30	35		15		
15	30	4	62.5	25.0	22	29	37	12	25		
15	30	5	62.5	20.8	21	28	18	22	15		
20	30	1	25	10.0	15	5					
20	30	2	0								
20	30	3	50	19.0	26	16	7	27			
20	30	4	75	13.5	11	16	8	15	21	10	
20	30	5	62.5	12.6	9	12	14	19	9		

EC (dS/m)	Alkal (mL)	Rep	Germin. %	Avg root length	Root lengths of individual seedlings						
4	0	1	75	21.3	17	21	30	28	22	10	
4	0	2	62.5	32.8	32	37	42	17	36		
4	0	3	62.5	27.2	45	25	20	36	10		
4	0	4	62.5	21.4	26	19	18	20	24		
4	0	5	75	24.7	42	21	29	19	31	6	
4	1	1	62.5	16.0	26	6	10	18	20		
4	1	2	62.5	32.8	32	37	42	17	36		
4	1	3	62.5	27.2	45	25	20	36	10		
4	1	4	87.5	34.1	26	29	30	49	36	40	29
4	1	5	37.5	17.0	20	11	20				
4	2	1	75	36.3	20	56	27	28	21	66	
4	2	2	62.5	22.6	22	26	26	19	20		
4	2	3	62.5	23.8	22	26	24	26	21		
4	2	4	87.5	23.1	28	26	16	31	22	23	16
4	2	5	62.5	20.2	20	30	31	13	7		
4	3	1	87.5	22.1	25	29	30	25	21	5	20
4	3	2	62.5	20.6	16	21	31	19	16		
4	3	3	87.5	19.1	20	15	20	29	15	15	20
4	3	4	50	26.8	25	20	31	31			
4	3	5	50	20.5	24	10	26	22			
4	5	1	75	17.0	21	5	26	14	21	15	
4	5	2	75	24.0	32	27	30	20	20	15	
4	5	3	62.5	23.0	36	20	25	16	18		
4	5	4	37.5	7.3	5		6	11			
4	5	5	75	17.0	27	18	21	5	26	5	
4	8	1	62.5	17.0	15	22	16	17	15		
4	8	2	62.5	17.0	18	18	24	13	12		
4	8	3	75	16.5	25	15	18	22	7	12	
4	8	4	87.5	14.0	19	12	19	21	5	12	10
4	8	5	62.5	18.0	14	15	22	25	14		

Germination of Topcut Rhodes grass after 9 days.

EC (dS/m)	SAR	Number of germinated seeds (out of 8)					Avg (%)
		Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	
0	∞	1	4	6	5	5	52.5
4	∞	5	3	4	4	5	52.5
6	∞	3	5	5	5	4	55
8	∞	5	2	5	4	6	55
10	∞	4	8	2	4	5	57.5
15	∞	4	5	4	6	7	65
20	∞	0	4	4	4	6	45
0	5	2	6	5	3	4	50
4	5	2	5	4	5	6	55
6	5	6	5	6	3	3	57.5
8	5	3	4	5	3	5	50
10	5	1	5	4	5	3	45
15	5	5	7	1	2	5	50
20	5	6	5	3	6	6	65
0	15	2	5	2	4	2	37.5
4	15	3	4	4	4	2	42.5
6	15	6	5	4	4	1	50
8	15	4	2	3	3	2	35
10	15	4	6	3	8	1	55
15	15	5	5	3	4	2	47.5
20	15	4	6	7	4	5	65
0	30	6	4	3	3	5	52.5
4	30	5	3	5	4	5	55
6	30	3	4	2	4	4	42.5
8	30	5	3	3	3	3	42.5
10	30	3	3	4	5	4	47.5
15	30	3	5	3	5	2	45
20	30	4	7	5	2	4	55

EC (dS/m)	Alkal (mL)	Number of germinated seeds (out of 8)					Avg (%)
		Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	
4	0	4	2	6	3	4	47.5
4	1	2	4	3	4	4	42.5
4	3	4	4	4	5	6	57.5
4	5	2	5	4	4	3	45
4	8	5	2	4	5	3	47.5

Germination of Reclaimer Rhodes grass after 8 days.

EC (dS/m)	SAR	Number of germinated seeds (out of 8)					Avg (%)
		Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	
0	∞	2	1	3	2	2	25
4	∞	1	2	1	2	2	20
6	∞	2	4	2	2	1	27.5
8	∞	0	2	1	3	0	15
10	∞	3	3	3	2	3	35
15	∞	2	2	2	1	1	20
20	∞	1	0	1	0	1	7.5
0	5	3	1	3	2	3	30
4	5	1	3	1	1	1	17.5
6	5	2	2	1	1	3	22.5
8	5	5	1	2	1	1	25
10	5	3	4	2	0	1	25
15	5	1	1	1	3	2	20
20	5	3	1	0	3	2	22.5
0	15	3	2	3	2	2	30
4	15	3	2	1	4	1	27.5
6	15	4	3	1	4	1	32.5
8	15	2	2	1	2	4	27.5
10	15	3	3	3	2	2	32.5
15	15	2	1	2	2	4	27.5
20	15	2	0	0	3	3	20
0	30	2	4	2	2	1	27.5
4	30	4	1	4	4	2	37.5
6	30	3	4	2	2	0	27.5
8	30	2	2	3	3	1	27.5
10	30	2	1	1	3	2	22.5
15	30	2	0	2	2	2	20
20	30	0	0	0	2	2	10

EC (dS/m)	Alkal (mL)	Number of germinated seeds (out of 8)					Avg (%)
		Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	
4	0	2	2	1	0	2	17.5
4	1	0	1	3	2	2	20
4	3	1	1	3	2	0	17.5
4	5	3	2	3	3	1	30
4	8	3	4	3	2	1	32.5

Appendix A4 - Revised paper accepted by Agricultural Water Management

Overhead-irrigation with saline and alkaline water: Deleterious effects on foliage of Rhodes grass and leucaena

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A4.1 Abstract

Saline and alkaline water represents a potentially valuable resource provided its irrigation does not decrease plant growth. Although the adverse effects of salts within the rooting environment are well-studied, comparatively little is known regarding the direct effects of overhead-irrigation of saline and alkaline water on plant foliage. The present study examined the potential deleterious effects of saline (electrical conductivity, EC, ≤ 15 dS m^{-1}) and alkaline (≤ 2000 mg L^{-1}, $CaCO_3$ equivalent) water on foliage of Rhodes grass (*Chloris gayana* cv. Reclaimer) and leucaena (*Leucaena leucocephala* ssp. *glabrata* cv. Tarramba) under a range of growing-conditions. Foliage of leucaena was sensitive, with necrosis and chlorosis evident for saline water at an EC ≥ 3 dS m^{-1} and alkaline water containing ≥ 500 mg L^{-1} ($CaCO_3$ equivalent). For leucaena, this damage to the foliage reduced relative shoot fresh mass and chlorophyll fluorescence for saline-treatments, but alkalinity did not reduce relative shoot fresh mass or chlorophyll fluorescence in any treatment. In contrast to leucaena, relative shoot fresh mass of Rhodes grass was not reduced by foliar-applied salinity in any treatment (nor did alkalinity reduce growth of Rhodes grass). It was noted that growing conditions influenced the magnitude of the deleterious effects, with salinization of the soil slightly increasing tolerance to foliar-applied saline water for leucaena. This study has demonstrated that whilst saline and alkaline water can potentially be used for overhead irrigation, differences in observed tolerance exist between plant species, and are influenced by growing conditions.

Keywords: Chlorophyll fluorescence; Leaf morphology; Plant growth; Toxicity.

A4.2 Introduction

In low-rainfall environments, the extraction of saline and alkaline groundwaters provides a potentially valuable resource for agricultural production. For example, the Great Artesian Basin (Australia), the largest artesian basin in the world, contains an estimated 65,000 million ML of groundwater (Nevill et al., 2010). This groundwater (including the water extracted from coal seams during natural gas production) can be beneficially used to increase agricultural production. However, much of the water in the Great Artesian Basin is saline and alkaline, with the electrical conductivity (EC) values typically ranging from 1 to > 10 dS m^{-1} (Great Artesian Basin Consultative Council, 1998). Therefore, it is important that the irrigation of these waters does not result in degradation of the soil resource and that it does not reduce agricultural production.

The potential adverse effects of salts within the rooting environment (soil) are well-known, causing plant osmotic stress, ion toxicity, and decreased photosynthesis and growth (Munns, 2002; Paz et al., 2012; Tester and Davenport, 2003). However, comparatively little information is available regarding the direct effect of the overhead irrigation of saline and alkaline waters on plant foliage. A report by FAO (1985) indicated that for equal water quality, plant physiological responses vary between overhead and direct irrigation of soil. For example, whilst *Citrus* sp. displayed foliar symptoms when sprinkler-irrigated with water containing 3 mM Na and Cl (corresponding to an EC of ca. 0.4 dS m^{-1}), no effects were observed when the same water was applied through flood and furrow irrigation. Similarly, studying the yield of bell pepper (*Capsicum frutescens*) irrigated using furrow, drip, and sprinkler at an EC of 4 dS m^{-1}, Bernstein and Francois (1973) found a reduction in yield of 18 % for furrow irrigation, 2 % for drip irrigation, and 59 % for sprinkler irrigation. However, in this study of Bernstein and Francois (1973), the reduction in yield for the sprinkler-irrigated plants was due to the combined absorption of salt by both the roots and the foliage, and hence an estimate of the foliar damage caused by Na and Cl absorbed directly by leaves is not possible (also see Sevostianova et al. (2011), for example). The effects of the overhead-irrigation of coal seam water (CS-water) on cotton (*Gossypium hirsutum*), barley (*Hordeum vulgare*) and Italian ryegrass (*Lolium multiform*) were evaluated by Beletse et al. (2008) using water with a total alkalinity of 4712 mg L^{-1} ($CaCO_3$ equivalent) and an EC of 7.5 dS m^{-1}. These authors found that overhead-irrigation caused leaf scorching in cotton but not in the other species, but again, the potential movement of this saline and alkaline water into the rooting media prevents separation of the deleterious effects caused by exposure to the shoots from those due to exposure to the roots. Maas (1985) reported that overhead-irrigation with saline water can produce foliar injury (chlorosis and necrosis) due to increased foliar absorption of Na and Cl, however, the magnitude of these symptoms differs substantially depending upon the plant species.

Although previous studies have demonstrated that the overhead-irrigation of saline and alkaline water can potentially produce adverse effects on plant foliage, these studies have generally applied the saline irrigation water to both plant foliage and to the soil, thereby making it difficult to separate the effects of salts within the soil from those applied to the foliage. The present study aimed to

establish the threshold for the safe overhead irrigation of saline and alkaline water (including CS-water) by examining the potential deleterious effects of synthetic irrigation waters when applied to the foliage of Rhodes grass (*Chloris gayana* Kunth) and leucaena (*Leucaena leucocephala* (Lam.) de Wit ssp. *Glabrata* (Rose) Zárate) – these two species being widely used for fodder within the Great Artesian Basin region. The effect of growth conditions was also examined, with plants grown either inside the glasshouse or in ambient conditions (i.e. external to the glasshouse) and either in a non-saline soil or in a saline soil. Plant performance was assessed using a range of parameters, including visual symptoms, chlorophyll fluorescence, and fresh mass production. The results of this experiment will assist in the development of regulatory guidelines for the beneficial use of saline and alkaline water in overhead-irrigation programs.

A4.3 Materials and methods

A4.3.1 Soil preparation and experimental design

This experiment aimed to investigate whether saline and alkaline water has deleterious effects when it is overhead-irrigated and exposed directly to plant foliage. The surface 0-25 cm of a non-saline Red Kandosol (Ultisol) was collected from a field irrigation site northeast of Injune (Queensland, Australia) (25.713° S; 148.992° E). The soil was air-dried and sieved to 10 mm. Given the low pH of the soil (pH 3.9, 1:5 water), $Ca(OH)_2$ was added at a rate of 1.5 g kg^{-1} – this being determined from a preliminary experiment as being sufficient to increase pH to ca. 5.5. A basal application of gypsum was added at a rate equivalent of 3 t ha^{-1} (2 g kg^{-1}) as is common agricultural practice for these soils in the field. Furthermore, a basal application of slow release fertiliser (Osmocote Exact Standard, 5 g pot^{-1}) was mixed through the soil, providing on a surface area basis the equivalent of ca. 150 kg ha^{-1} of N and ca. 50 kg ha^{-1} of P. After mixing the soil with amendments, 2.8 L was placed in pots and wetted up on a capillary mat. Additional liquid fertiliser (Grow Force, Flow Feed EX7) was applied after seedlings were established (see later) and every three weeks until plants were harvested.

Two types of overhead-irrigation water were investigated for their potential adverse effects on plant foliage, with treatments either increasing in salinity or increasing in alkalinity (Table A4.1 and Supplementary Table A4.S1). For each of these two water types (i.e. saline or alkaline), the experiment investigated two plant species (Rhodes grass and leucaena), two soil salinities (non-saline or saline), and two environmental conditions (glasshouse or ambient, although only a limited number of treatments were grown in ambient conditions). For the saline overhead-irrigation water, NaCl was added at rates sufficient to increase EC to 0, 3, 4, 5, 6, 8, 10, 12, or 15 dS m^{-1} for Rhodes grass and 0, 3, 4, 5, 6, or 8 dS m^{-1} for leucaena (Table A4.1 and Supplementary Table A4.S1). For the alkaline overhead-irrigation water, NaCl and $NaHCO_3$ were added at rates sufficient to increase alkalinity to 0, 250, 500, 750, 1250, or 2000 mg L^{-1} ($CaCO_3$ equivalent), with all alkalinity treatments having a basal EC of 4 dS m^{-1} (Table A4.1 and Supplementary Table A4.S1). The salinity (i.e. EC) and alkalinity values chosen exceeded values found in CS water, but were selected because they cover the range of values

likely to influence plant growth (FAO, 1985; Maas et al., 1982b) and hence would enable limits to be defined for the safe overhead-irrigation of these two crops.

The experiment consisted of a total of 37 treatments (Supplementary Table A4.S1) with three replicates, yielding a total of 111 experimental units arranged in a randomised design. Specifically, the treatments consisted of (i) Rhodes grass and leucaena grown in the glasshouse and overhead-irrigated with waters with increasing EC values (Treatments 1-15), (ii) Rhodes grass and leucaena grown in the glasshouse and overhead-irrigated with waters with an increasing alkalinity but a constant EC of 4 dS/m (Treatments 16-25), (iii) Rhodes grass and leucaena grown in the glasshouse in a saline soil and overhead-irrigated with waters with increasing EC values (Treatments 26-31), and (iv) Rhodes grass and leucaena grown in ambient conditions and overhead-irrigated with waters with increasing EC values (Treatments 32-37).

A4.3.2 Plant growth and soil-applied irrigation

All plant-growth experiments were conducted at The University of Queensland (St Lucia, Australia). During the experimental period, the average maximum temperature in the glasshouse was 30 °C (± 2.3 °C, standard deviation) and the minimum was 17 °C (± 2.2 °C), whilst outside the glasshouse, corresponding temperatures were 30 °C (± 3.6 °C) and 10 °C (± 3.2 °C). Inside the glasshouse, average humidity was 60 % (± 15 %) and average dewpoint was 13 °C (± 3.8 °C), whilst outside the glasshouse, average humidity was 74 % (± 24 %) and average dewpoint was 11 °C (± 4.5 °C).

The two plant species, Rhodes Grass (*Chloris gayana* cv. Reclaimer) and leucaena (*Leucaena leucocephala* ssp. *glabrata* cv. Tarramba), were selected as being economically important fodder species in Australia. Plants were grown for seven weeks before being thinned to either 10 plants (Rhodes grass) or two plants (leucaena) per pot. Plants were then grown for a further four weeks prior to commencement of overhead irrigation and then a further three months during overhead-irrigation (see below). During the experimental period, Rhodes grass tillers and leucaena leaves were tagged to identify those that were irrigated for the entire duration of the experiment from newly formed ones. For brevity, hereafter, 'old leaves' describes leaves that were exposed to all 30 irrigations whilst 'new leaves' describes leaves that were exposed to < 30 irrigations.

As required for growth, water was applied to the soil throughout the entire duration of the experiment. For all pots, water was applied directly to the soil, taking care to avoid contact with the foliage. This soil-applied water was either non-saline (deionized water) or had a salinity (EC) of 10 dS m^{-1} (Table A4.1 and Supplementary Table A4.S1) and was applied twice per week at a rate sufficiently high (i.e. 300 to 800 mL per pot) to ensure thorough leaching of the soil. For the water (10 dS m^{-1}) applied to salinize the soil, the leachate was collected and analysed, with EC values ranging from 8.6

to 18 dS m^{-1} (average of 11.4 dS m^{-1}). The EC of the leachate varied depending upon the amount of water taken up from the soil by the plant between irrigations.

A4.3.3 Overhead irrigation

The saline and alkaline solutions required for overhead-irrigation were prepared in 200 L containers using NaCl (99.5 %, Pacific Salts, Australia) and $NaHCO_3$ (Technical Grade, ST172, ChemSupply, Australia) prepared with deionised water (Table A4.1, Table A4.2, and Supplementary Table A4.S1). Plants were overhead-irrigated using irrigation chambers connected to water pumps with solutions applied as a fine mist using mist sprayers. During each 30-min irrigation event, the water was cycled on for 1 min and off for 9 min to reduce consumption of water whilst keeping the leaves continuously wet (Maas et al., 1982b). A total of 30 irrigation events were conducted every 3-4 d over a three month period.

The soil was covered with a protective foil in all treatment pots to limit the movement of the saline and alkaline overhead-irrigation water into the soil and to minimise evaporation from the soil surface. At completion of the irrigation trial, soil samples (0 to 3 cm depth) were collected from several pots to examine whether any infiltration of overhead-irrigated water had influenced soil pH and EC – no increase in pH or EC was evident in any treatment (data not presented).

A4.3.4 Observations, measurements, harvest, and analyses

Leaves were regularly examined visually for symptoms of toxicity. Chlorophyll fluorescence (OS30p+, Opti-Sciences, New Hampshire, USA) was measured after 6, 12, 18, 24 and 30 irrigations with measurements taken at night following at least 2 h dark adaptation (Lichtenthaler and Babani, 2004) on five leaves of equal age (i.e. leaves exposed to the same number of irrigation events) that were randomly selected from each experimental unit. A preliminary test had identified that 2 h dark adaptation was sufficient in the present study. Chlorophyll fluorescence was examined using the ratio between variable fluorescence (F_v) and maximum fluorescence (F_m), F_v/F_m. The ratio F_v/F_m, provides a measure of the quantum yield in the plant Photosystem II, or the potential quantum efficiency, that is widely recognised as an indicator of the efficiency of plant photosynthetic activity (Maxwell and Johnson, 2000). Vascular plants growing in non-limiting conditions have a F_v/F_m ratio of ca. 0.83, with lower values indicating that plants are exposed to environmental stress (Maxwell and Johnson, 2000).

After 30 irrigations, five leaves (that had received all 30 treatment irrigations) were randomly collected from leucaena and Rhodes grass treatments. The fresh leaf material was preserved by immediately placing in 3 % glutaraldehyde in 0.1 M sodium cacodylate at 3.5 °C. Microwave processing was performed using a Pelco BioWave (Ted Pella Inc., California, USA) with a ColdSpot

water recirculating device. Following fixation with glutaraldehyde, samples were post-fixed with 1 % osmium tetroxide, subjected to a dehydration series using ethanol (30, 50, 70, 90, 100, and 100 %) before critical point drying (Wendt et al., 2004). Samples were coated in Au and examined using scanning electron microscopy (SEM) (JSM-6460LA, JEOL, Tokyo, Japan) at 15 kV using energy-dispersive X-ray spectroscopy (EDS) for elemental analyses.

Upon completion of the irrigation trial (i.e. after 30 irrigation events), all plants were harvested and fresh mass measured. Plant foliage was immediately rinsed with deionised water to remove accumulated salt from the surface of the leaves. The rinsing time required for the removal of soluble salts from the leaf surface was determined in a preliminary experiment. Briefly, an EC electrode was placed in a glass beaker with 250 mL of deionised water and a magnetic stirrer. The EC increased rapidly when a leaf was placed in the beaker due to dissolution of salt adhering to the leaf surface, but the EC stabilized due to slow diffusion of salt from the inside of the leaf. The discontinuity in slope was taken as the time required to dissolve salt while avoiding diffusion. Measurements of solution EC were taken every 3 s for a total of 180 s, and it was determined that a rinsing time of ca. 18 s was appropriate to dissolve surface salt. Following rinsing, plant material was dried (65 °C) for 4 d and dry mass recorded. Elemental analyses were performed for fully mature leaves that received all 30 treatment applications. However, for the tissues of leucaena that received all 30 treatment applications, there was insufficient tissue to allow for analysis at many of the higher salinity treatments and for all alkalinity treatments. Samples were ground and open-vessel digested using a 5:1 mixture of nitric and perchloric acids, prior to analysis by ICP-OES (Martinie and Schilt, 1976). Tissue Cl concentrations were determined colorimetrically (Rayment and Higginson, 1992) using samples digested with 0.1 M nitric acid.

Data were analysed using a two-way analysis of variance using GenStat v17 (VSN International). Comparisons between means were made using Fisher's protected least significant difference (LSD) test.

A4.4 Results

A4.4.1 Visual observations and SEM analyses

All plants in the control treatments appeared healthy with no signs of nutrient deficiencies. For plants growing in the saline soil irrigated with water at 10 dS m^{-1} (without additional saline overhead-irrigation), symptoms were observed for foliage of both Rhodes grass and leucaena, with necrotic leaf tips in Rhodes grass and chlorotic leaves in leucaena. Overhead-irrigation of saline and alkaline water was found to have deleterious effects and caused foliar damage in both plant species. The magnitude of this damage was dependent upon plant species, composition of the irrigation water, the salinity of the soil, and the growing conditions (glasshouse or ambient).

Firstly, giving consideration to the saline overhead-irrigation water, chlorosis and necrosis was observed for leaves of both plant species (Supplementary Fig. A4.S1) in areas where the water droplets had accumulated and dried. For leucaena, these chlorotic and necrotic lesions were observed after only three irrigation events, with the magnitude of this damage increasing with increasing salinity (being observed in all treatments with an EC ≥ 3 dS m^{-1}) and with an increasing number of irrigations. Foliar abscission occurred in severely damaged leaves, with this abscission becoming particularly pronounced after ca. 10 irrigations at 8 dS m^{-1}. Although the same chlorotic and necrotic symptoms were observed in Rhodes grass, they were less pronounced. Indeed, symptoms were not were observed until ca. 10 irrigations, when necrotic and curly leaf tips developed in most plants (particularly those growing in saline soil).

Secondly, the effects of increasing alkalinity in the overhead irrigation water were examined at constant salinity (EC of 4 dS m^{-1}). As described above, a solution with an EC of 4 dS m^{-1} (and no added alkalinity) resulted in chlorosis and necrosis of the leaf tissue – this being more severe in leucaena. However, increases in alkalinity did not result in a marked increase in the severity of the symptoms for either species, although leaves were perhaps more necrotic than chlorotic.

Examination of leaves using SEM confirmed the damage to the leaf surface, presumably corresponding to the chlorotic and necrotic areas. At high alkalinity (2000 mg L^{-1}, $CaCO_3$ equivalent), salts were observed on the leaf surface in the damaged areas (Fig. A4.1). Analyses using SEM-based EDS revealed that the salts contained Ca, C, and O, presumably as $CaCO_3$ (Supplementary Fig. A4.S2), with the Ca in the crystals presumably derived from the necrotic plant tissue and from trace amounts present as impurities in the overhead irrigation water from the salts (Table A4.2). This effect was particularly evident when examining the distribution of salt crystals at the boundary between chlorotic and necrotic tissues (Fig. A4.1c), with the crystal structure observed corresponding to that of Ca-carbonate (Goffredo et al., 2011).

A4.4.2 Shoot fresh mass

Growth in the control treatments was good, with a final shoot fresh mass of ca. 160 g pot^{-1} for glasshouse-grown leucaena and 240 g pot^{-1} for Rhodes grass. However, plant growth in these control treatments was influenced by the growing conditions (i.e. glasshouse versus ambient, non-saline soil versus saline soil). Presumably due to lower temperatures, shoot fresh mass was reduced when grown in ambient conditions, being 80 g pot^{-1} for leucaena and 140 g pot^{-1} for Rhodes grass for the control treatments. Similarly, shoot fresh mass was reduced for glasshouse-grown plants in saline soil, being 60 g pot^{-1} for leucaena and 150 g pot^{-1} for Rhodes grass.

For leucaena, when compared to the corresponding control in the same growing conditions, the foliar-application of saline water resulted in a significant decrease in relative fresh leaf mass (Fig. A4.2a), with a significant interaction found between the EC of the foliar-applied water and the growing conditions (P = 0.002). Thus, whilst an increase in EC resulted in a decrease in relative fresh mass, the magnitude of this decrease depended upon the growth conditions (i.e. whether the plants were grown in the glasshouse or in ambient conditions, and whether the soil was non-saline or saline) and whether it was the old leaves or new leaves being examined. Indeed, the deleterious impact of foliar-applied saline water was significantly less for the new leaves than for the old leaves (Fig. A4.2a), presumably because, at least in part, the new leaves had been exposed to fewer irrigations than the old leaves. For example, for leucaena irrigated with water at an EC of 8 dS m^{-1}, the average relative fresh mass of new leaves was 46 %, compared to 0 % for old leaves (averaged across all treatments). It was also noted that effect of the foliar-applied saline water was significantly lower when plants were grown in a saline soil – this being most pronounced for the old leaves at an EC of 4 dS m^{-1} (Fig. A4.2a). Finally, for foliar-applied saline water, although relative fresh mass tended to be lower in ambient-grown plants than in glasshouse-grown plants, this difference was not significant (Fig. A4.2a). Although salinity had an adverse impact on the shoot relative fresh mass of leucaena (Fig. A4.2a), alkalinity (up to 2000 mg L^{-1} as $CaCO_3$ equivalent) had no additional adverse impact on plant relative shoot mass above that caused by the basal salinity (4 dS m^{-1}) (Fig. A4.2b). However, it must be noted that the old leaves for leucaena were not included in the analyses for alkalinity, given that their growth at an alkalinity of 0 mg L^{-1} (basal EC of 4 dS m^{-1}) was so poor (Fig. A4.2a).

Rhodes grass relative biomass was not affected by foliar application of either saline (Fig. A4.3a) or alkaline (Fig. A4.3b) water. For the foliar-application of saline water, no significant interaction was found (P = 0.345), nor did the EC of the overhead-irrigation water have a significant effect on relative fresh mass (P = 0.467) (Fig. A4.3a). However, significant differences were found between leaf ages (P < 0.001), specifically, relative fresh mass tended to be higher in old leaves than in new leaves (Fig. A4.3a). Similarly, although the alkalinity of the foliar-applied water had no significant effect on relative fresh mass of Rhodes grass (P = 0.171), significant differences were found between old and new leaves (P = 0.040) (Fig. A4.3b).

A4.4.3 Chlorophyll fluorescence

For leucaena overhead-irrigated up to six times, saline or alkaline water generally had no impact on chlorophyll fluorescence regardless of the treatment (Fig. A4.4a-d). However, the decrease in chlorophyll fluorescence became more marked with increasing irrigations, with salinity causing a decrease in fluorescence in treatments with EC ≥ 3 or 4 dS m^{-1} (Fig. A4.4a-c), whilst alkalinity had no impact on fluorescence in any treatment (Fig. A4.4d).

For Rhodes grass, it was found that salinity and alkalinity had no impact on chlorophyll fluorescence in any treatment, regardless of the number of irrigations (Fig. A4.5). It should be noted, however, that fluorescence tended to decrease with increasing irrigations, regardless of treatment.

A4.4.4 Elemental concentrations in leaf tissues

As expected, for all treatments in both species, leaf tissue Na and Cl concentrations increased nearly linearly with increasing salinity, with Na increasing to ca. 0.5 to 1.2 % (dry mass basis) and Cl increasing to 1.5 to 3 % (Fig. A4.6). For both Na and Cl, at any given EC, tissue concentrations were similar for both Rhodes grass and leucaena (Fig. A4.6) even though growth was reduced at high EC in leucaena but not Rhodes grass (Fig. A4.2 and Fig. A4.3). Leaf salt concentrations were higher in Rhodes grass grown in saline soil (Fig. A4.6a,b). Tissue concentrations of Na in Rhodes grass were similar for glasshouse-grown plants and for those grown in ambient conditions (Fig. A4.6a,b). Regardless, the bulk tissue Na concentrations for Rhodes grass observed here (up to ca. 1.2 %) are lower than those reported to be associated with toxicity in this species (ca. 2.5 to 3 %) (Kopittke et al., 2009) – this being consistent with the observation that the relative fresh mass of Rhodes grass was not reduced in any treatment (Fig. A4.3). Unfortunately, we are unaware of any critical tissue values for leucaena for either Na or Cl, nor are we aware of any critical tissue values for Rhodes grass for Cl.

Although salinity often causes a reduction in tissue Ca concentrations, overhead irrigation of saline or alkaline water did not result in a decrease in Ca in any treatment for either plant species (data not presented). Indeed, in all treatments, the average tissue Ca concentrations of 2.7 % for leucaena and 0.80 % for Rhodes grass were higher than the critical value for deficiency, being ca. 0.4-0.5 % for leucaena (Pinkerton et al., 1997) and ca. 0.25-0.5 % for Rhodes grass (Jones et al., 1991). Irrigation with alkaline irrigation water did not change tissue Na concentrations, but resulted in a slight decrease in tissue Cl concentrations (Fig. A4.6c,d).

A4.5 Discussion

Elevated soil salinity and sodicity are worldwide problems that impact plant growth. However, in contrast to salinity within the rooting medium (i.e. soil), few reports have addressed the direct impacts of overhead-irrigation of saline or alkaline water on plant foliage. Here, it has been shown that the overhead-irrigation of saline water (EC values up to 15 dS m^{-1}) exerts deleterious effects on the foliage of leucaena and Rhodes grass. Specifically, it was observed that the overhead-irrigation with saline water resulted in: (i) foliar morphological damage, (ii) reduction in plant relative fresh

mass, (iii) decrease in chlorophyll fluorescence, and (iv) an increase of leaf Na and Cl tissue concentrations. Overhead irrigation with alkaline water (up to 2000 mg L^{-1}, as $CaCO_3$ equivalent) did not exacerbate foliar damage above that caused by the background salinity of 4 dS m^{-1}. However, the magnitude of the damage varied depending upon the composition of the water, the plant species, and the growth-conditions.

A4.5.1 Adverse effects of the overhead-irrigation of saline water

The overhead-irrigation of saline (NaCl) water caused visual foliar damage in both plant species. Although the magnitude of the effects differed between the two plant species (see later), this foliar damage occurred as chlorotic and necrotic lesions (in severe cases, foliar abscission), decreased relative fresh mass (Fig. A4.2 and Fig. A4.3), and decreased chlorophyll fluorescence (Fig. A4.4 and Fig. A4.5). It is likely that this foliar damage observed in both plant species is caused by increased localized Na and Cl absorption in the tissues underneath the foliar surfaces where water droplets accumulated and dried. Salts, presumably $CaCO_3$ (Supplementary Fig. A4.S2) that precipitated in the highly alkaline solution from impurities in the salt (Table A4.2), were observed in the chlorotic and necrotic tissues in the high alkalinity treatment, but few were observed on the surface of healthy tissues (Fig. A4.1b-d). Indeed, with each subsequent irrigation, it was observed that the droplets frequently dried in the same locations where damage had previously occurred – this suggesting that water droplets were more likely to be retained on damaged leaf surfaces than healthy leaf surfaces (perhaps due to reduced surface tension). Regardless, it was observed that average bulk concentrations of Na in foliar tissue increased to ca. 0.5 % in leucaena and ca. 1.2 % in Rhodes grass (Fig. A4.6a). For Rhodes grass, although these bulk tissue Na concentrations were lower than the 3 % reported to be toxic when grown in saline soils (Kopittke et al., 2009), the localized concentrations of Na in tissues underlying the area where the droplets dried (Fig. A4.1) would have been substantially higher than the bulk value of 1.2 % – this likely resulting in the observed chlorosis and necrosis. These observations are consistent with those of Maas (1985), who reported a linear increase of Cl absorption (and foliar injury) in bush beans, with the increase of salt concentration (EC) in water applied by sprinkling-irrigation.

Bernstein (1975) stated that “symptoms of leaf injury by foliarly absorbed salts are the same as those caused by salts absorbed through the roots”, with the findings of the present study in agreement – both foliar-applied and root-absorbed salts resulted in the chlorosis and necrosis of the leaf tissue. The chlorotic and necrotic lesions observed in the present study have also been reported by Mantel et al. (1989) in plum (*Prunus saliciva*) sprinkle-irrigated weekly with saline water at an EC ≥ 3.3 dS m^{-1}. In a manner similar to the present study, Mantel et al. (1989) reported that the severity of symptoms increased with the number of irrigation events, with foliar necrosis extending from the margins to the entire leaf surface when sprayed with highly saline water and following 14 irrigation events (Mantel et al., 1989). The chlorosis and necrosis of foliage exposed to saline water has also been reported by Vollenweider and Günthardt-Goerg (2005), Armbruster and Mulchi (1984), and McCune (1991).

A4.5.2 Adverse effects of the overhead-irrigation of alkaline water

For both leucaena and Rhodes grass, alkalinity values of up to 2000 mg L^{-1} ($CaCO_3$ equivalent) had no adverse impact on either foliage relative fresh mass or chlorophyll fluorescence other than that attributable to the basal salinity (the basal EC in all alkaline solutions being 4 dS m^{-1}) (Fig. A4.2 to Fig. A4.5). The only toxic effect of alkalinity appeared to be an increase in necrosis (coupled with a decrease in chlorosis) in leucaena, but the severity of this was not sufficient to influence overall growth. We are aware of only one previous study where the effect of alkaline water application on plant foliage was investigated. Beletse et al. (2008) examined the effect of sprinkler-irrigation on cotton, Italian ryegrass, and barley (salt tolerant crops), and despite having an alkalinity of 4712 mg L^{-1} ($CaCO_3$ equivalent) and an EC of 7.5 dS m^{-1}, these authors found that there was no notable foliar damage other than for cotton which had scorched leaves. Thus, based upon the evidence in the present study and that reported previously, it seems that overhead-irrigation of alkaline water up to 2000 mg L^{-1} ($CaCO_3$ equivalent) is not deleterious to plant foliage.

A4.5.3 Differences between plant species

Substantial differences were observed between leucaena and Rhodes grass, particularly regarding the saline water. For leucaena exposed to 30 irrigations, adverse effects were particularly prevalent for water with an EC ≥ 3 dS m^{-1}, with the formation of chlorotic and necrotic lesions, partial or complete leaf abscission, decreased relative fresh mass (Fig. A4.2), and decreased chlorophyll fluorescence (Fig. A4.4). Although leaves of Rhodes grass were observed to develop chlorotic and necrotic lesions, the relative fresh mass of Rhodes grass was not reduced in any treatment (Fig. A4.3), nor was chlorophyll fluorescence reduced relative to the control (Fig. A4.5). Indeed, relative fresh mass of Rhodes grass was not reduced in any treatment despite having similar tissue concentrations of Na and Cl as leucaena (Fig. A4.6).

The high tolerance of Rhodes grass to salt within the rooting environment is well documented in the scientific literature (Russell, 1976). Rhodes grass is a halophyte and is able to tolerate relatively high salinity concentrations in soil due to its ability to accumulate excess salt within its leaves (Kopittke et al., 2009) and to secrete excess salt through bicellular glands (Kobayashi et al., 2007). For the present study examining only two plant species, tolerance to foliar-applied salt was in correspondence with their tolerance to salt in the rooting environment. It has also been reported that the tolerance of crops to saline sprinkling waters may differ to their tolerance to soil salinity (Maas et al., 1982b) – "susceptibility to damage by foliarly absorbed salts depends more on leaf characteristics and rate of foliar absorption than on tolerance to soil salinity" (Bernstein, 1975).

A4.5.4 Influence of growth conditions

Leucaena grown in saline soil was less susceptible to stress from foliar applied salt (Fig. A4.2a) thereby suggesting that the mechanisms used to tolerate a saline rooting environment can potentially reduce the severity of damage caused by overhead-irrigation of saline water. As a result, for leucaena, it seems that the salt within the soil and the salt applied to the foliage share some commonalities in their mechanisms of toxicity. However, this is somewhat in contrast to that reported by Gorham et al. (1994) who stated "that tolerance to salt applied as salt spray or in the soil are different mechanisms". Also, Benes et al. (1996) found that leaves of barley grown in a saline soil (EC of 9.6 dS m^{-1}) and overhead-irrigated with saline water (9.6 dS m^{-1}) had increased scorching relative to those growing in a non-saline soil. In the present study, however, it must be noted that salinization of the rooting environment only reduced damage from overhead-irrigation in the old leaves of leucaena and not the new leaves (Fig. A4.2a).

In the present study, despite differences in their absolute fresh mass (see *Results*), comparison of relative fresh mass showed that the magnitude of the growth reduction caused by the saline water was similar for both the glasshouse- and ambient-grown plants (Fig. A4.2 to Fig. A4.6) despite slight differences in temperature and humidity. This is in contrast to that reported previously, with foliar toxicity symptoms tending to appear in older leaves and under hot and dry ambient conditions (Maas, 1985; Maas et al., 1982a; Maas et al., 1982b; McCune, 1991) due to the high evaporation rate and enhanced accumulation of Na and Cl on leaf surfaces.

A4.6 Conclusions

For both leucaena and Rhodes grass, overhead-irrigation of saline water was found to damage the foliage by inducing chlorosis and necrosis. For leucaena, saline water with an EC ≥ 3 dS m^{-1} caused a reduction in the relative mass of the leaves and also caused a reduction in chlorophyll fluorescence. However, alkalinity of up to 2000 mg L^{-1} ($CaCO_3$ equivalent) had no additional deleterious effect on leucaena above that caused by the basal salinity in these treatments. In contrast to leucaena, Rhodes grass was more tolerant to saline overhead irrigation water, with relative mass of leaves not reduced by water with EC values ≤ 15 dS m^{-1}. The growing conditions were found to influence the magnitude of the deleterious effects, with salinization of the soil slightly increasing tolerance to foliar-applied saline water for leucaena. This study has demonstrated that whilst saline and alkaline water can potentially be used for overhead irrigation, differences in tolerance exist between plant species, and this is influenced by growing conditions.

Acknowledgements

Financial support was provided by Santos GLNG. We acknowledge funding from the Australian Research Council (ARC) for an ARC Future Fellowship for Peter Kopittke (FT120100277). The assistance of two anonymous reviewers is also acknowledged.

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Table A4.1. Rates at which NaCl and $NaHCO_3$ were added to prepare the solutions listed in Supplementary Table A4.S1.

Electrical conductivity (dS m^{-1})	**Alkalinity (mg L^{-1}, $CaCO_3$ equivalent)**	**NaCl (mM)**	**$NaHCO_3$ (mM)**
0	0	0	0
3	0	27	0
4	0	36	0
5	0	46	0
6	0	55	0
8	0	74	0
10	0	94	0
12	0	110	0
15	0	140	0
4	250	31	5.0
4	500	26	10
4	750	22	16
4	1250	15	25
4	2000	4.4	41

Table A4.2. Measured concentrations of major cations and anions measured in the solutions used for overhead irrigation.

Electrical conductivity (dS m^{-1})	Alkalinity (mg L^{-1}, $CaCO_3$ equivalent)	Ca (mM)	Cl (mM)	K (mM)	Mg (mM)	Na (mM)
0	0	0.001	0.16	0.001	0.000	0.001
3	0	0.036	29	0.009	0.026	32
4	0	0.045	36	0.011	0.032	38
5	0	0.055	47	0.014	0.042	50
6	0	0.065	55	0.016	0.050	60
8	0	0.070	73	0.022	0.061	85
10	0	0.091	90	0.030	0.089	96
12	0	0.12	110	0.038	0.12	120
15	0	0.16	140	0.052	0.17	150
4	250	0.034	33	0.012	0.028	41
4	500	0.028	29	0.012	0.022	43
4	750	0.029	25	0.009	0.020	42
4	1250	0.026	17	0.007	0.014	47
4	2000	0.019	7.9	0.004	0.007	51

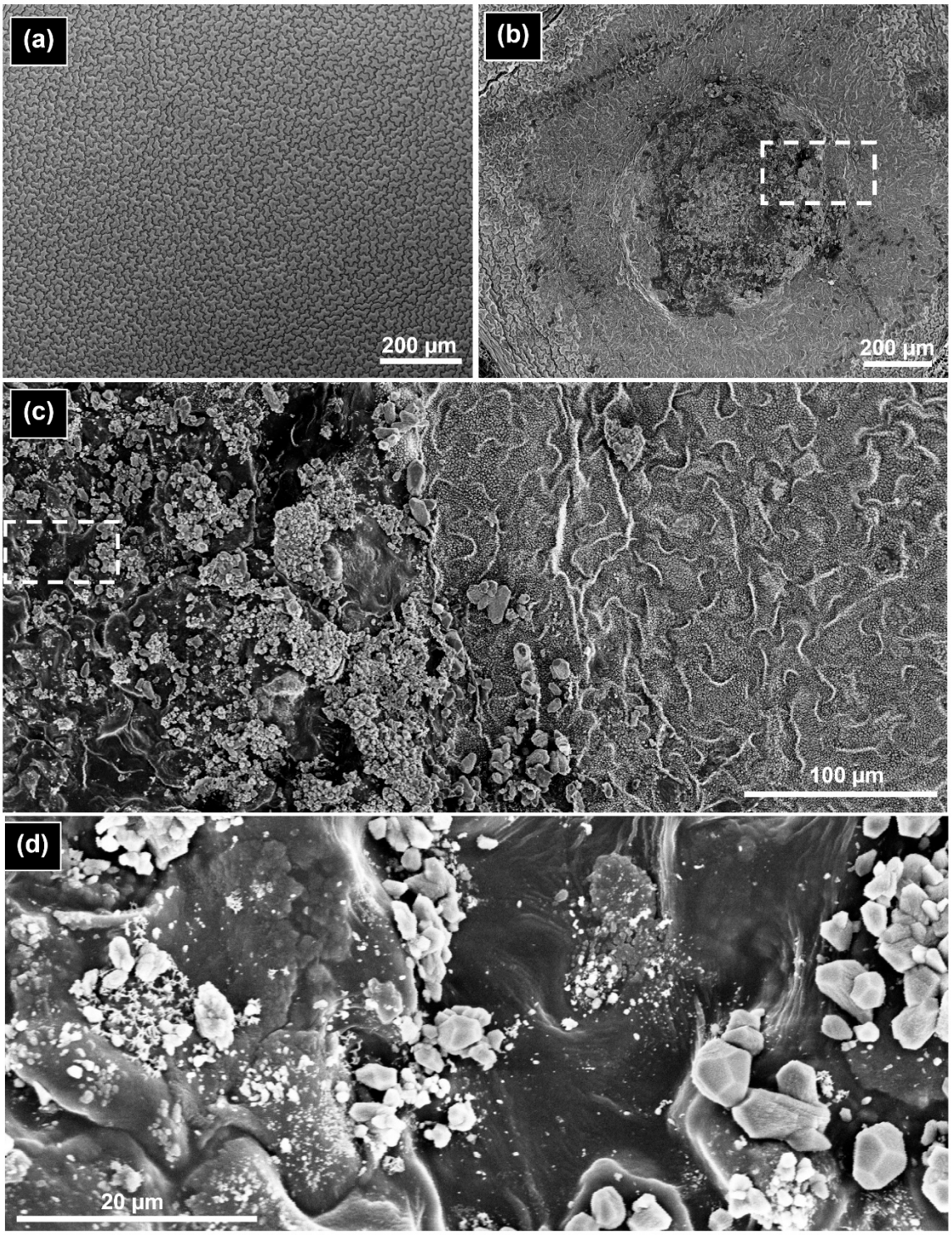

Fig. A4.1. Scanning electron micrographs of the adaxial surface of a leucaena leaf overhead-irrigated with water (a) from the control, and (b-d) at an alkalinity of 2000 mg L^{-1} ($CaCO_3$ equivalent) with a basal electrical conductivity of 4 dS m^{-1}. The image in (b) shows a circular chlorotic and necrotic lesion surrounded by healthy tissue, with the image in (c) is a close-up of the rectangle in (b), and the area in (d) is a close-up of the rectangle in (c). Leaves had received all 30 treatment irrigations.

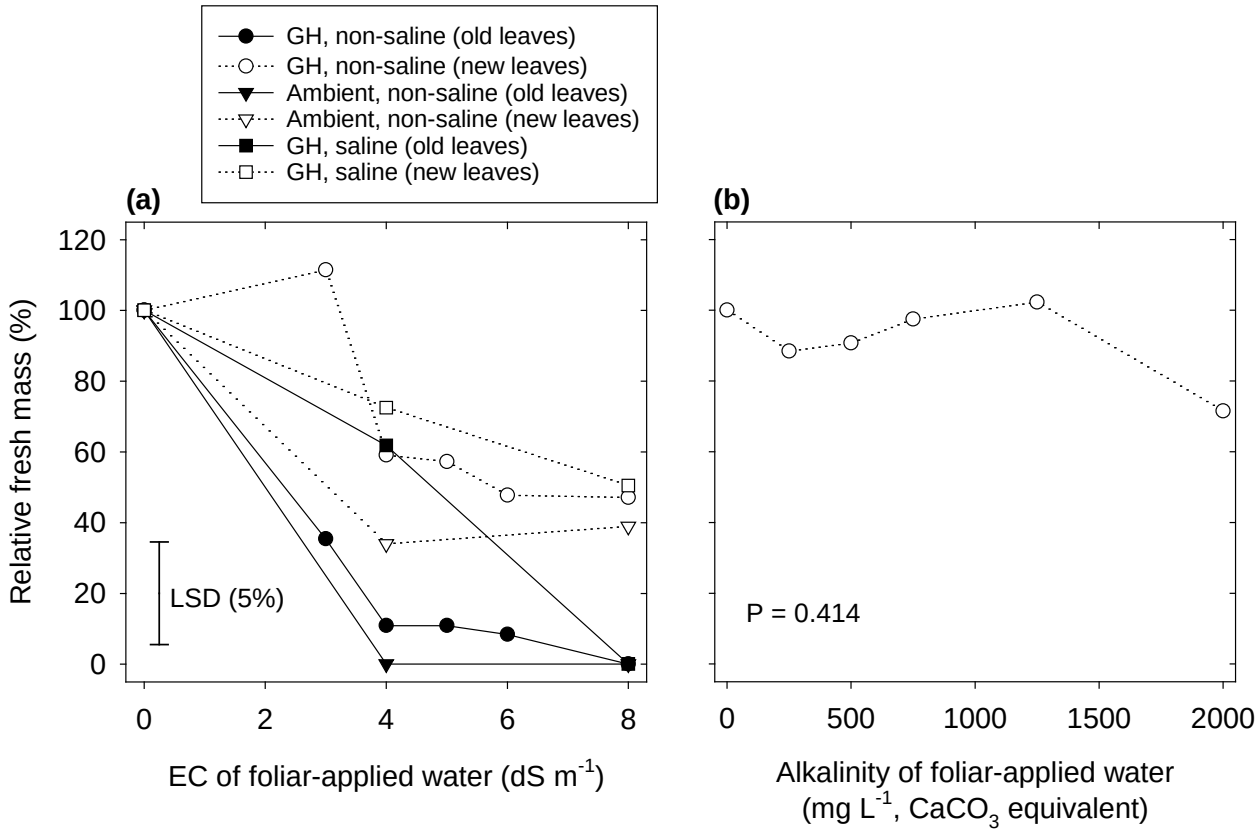

Fig. A4.2. Relative fresh mass (calculated from the corresponding control, Supplementary Table A4.S2) of leucaena leaves overhead-irrigated with either (a) saline or (b) saline (4 dS m^{-1}) and alkaline water. Plants were grown in the glasshouse (GH) or in ambient conditions (i.e. outside the glasshouse), and were either grown in a non-saline or saline soil. 'Old leaves' were overhead-irrigated 30 times, whilst 'new leaves' were exposed to less than 30 irrigations. Data are not presented for 'old leaves' in the alkalinity treatments (all with an EC of 4 dS m^{-1}), with almost all of these leaves abscised irrespective of treatment.

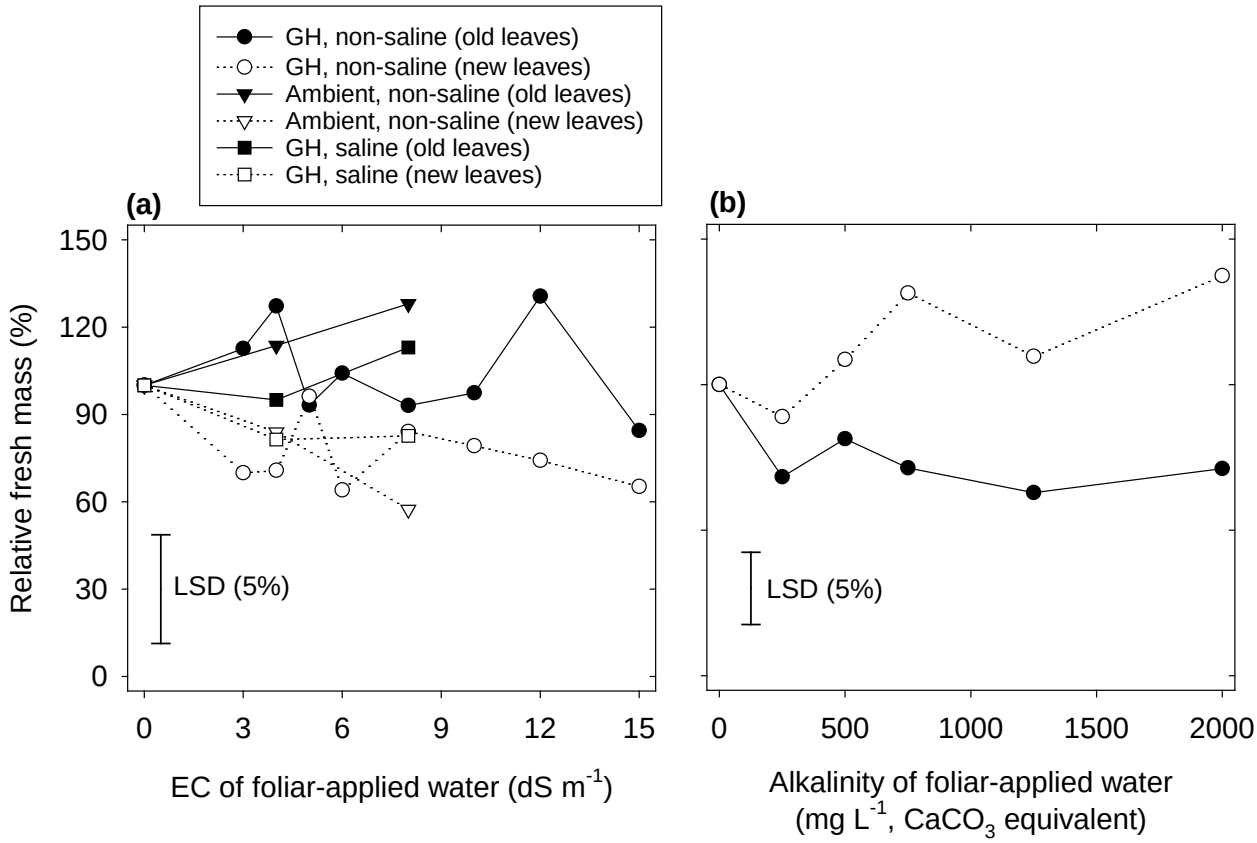

Fig. A4.3. Relative fresh mass (calculated from the corresponding control, Supplementary Table A4.S2) of Rhodes grass overhead-irrigated with either (a) saline or (b) saline (4 dS m^{-1}) and alkaline water. Plants were grown in the glasshouse (GH) or in ambient conditions (i.e. outside the glasshouse), and were either grown in a non-saline or saline soil. 'Old leaves' were overhead-irrigated 30 times, whilst 'new leaves' were exposed to less than 30 irrigations.

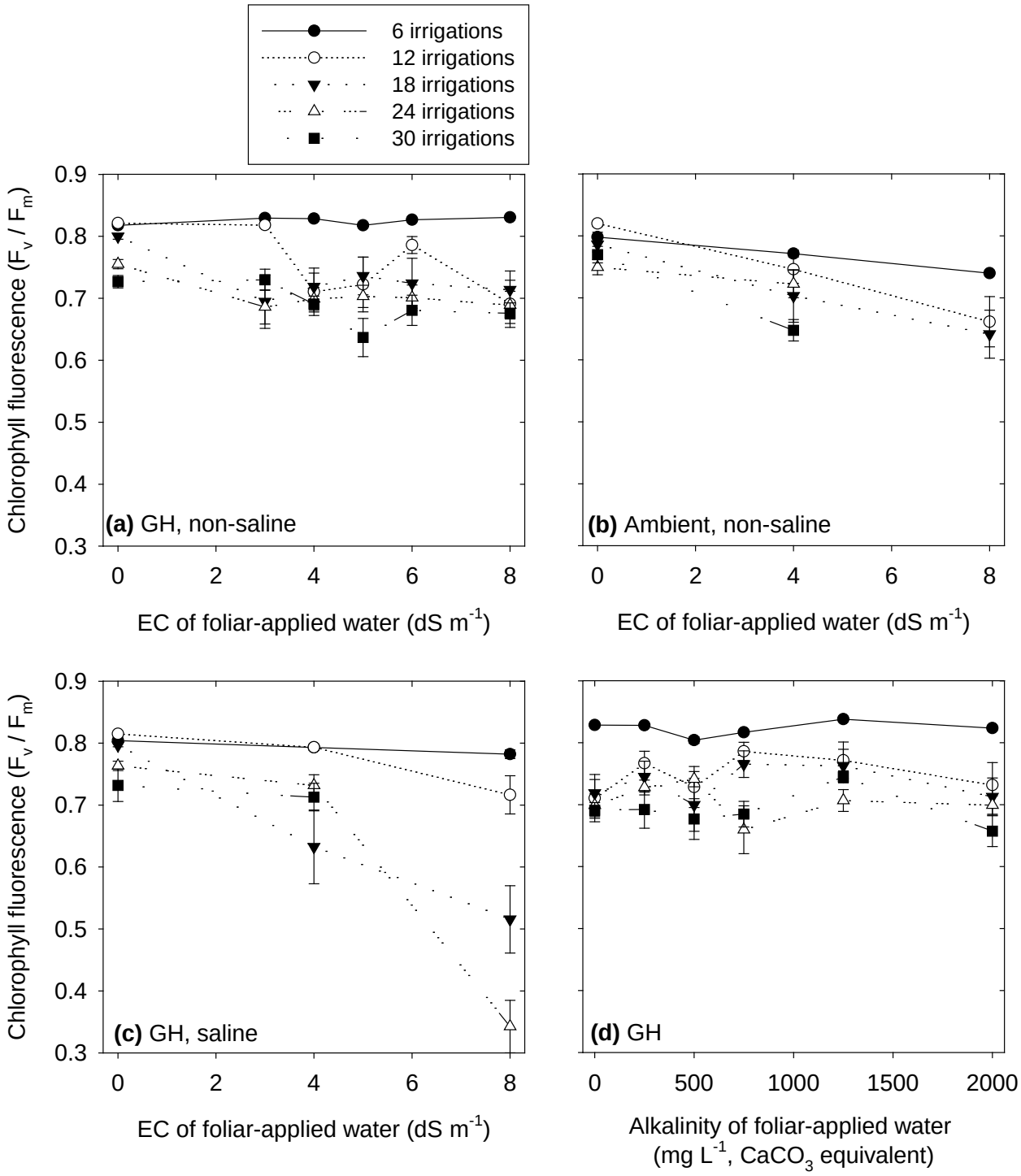

Fig. A4.4. Chlorophyll fluorescence for leucaena overhead-irrigated with either (a-c) saline or (d) saline (4 dS m^{-1}) and alkaline water. Plants were grown in the glasshouse (GH) or in ambient conditions (i.e. outside the glasshouse), and were either grown in a non-saline or saline soil. The vertical bars represent the standard deviation of replicate measurements.

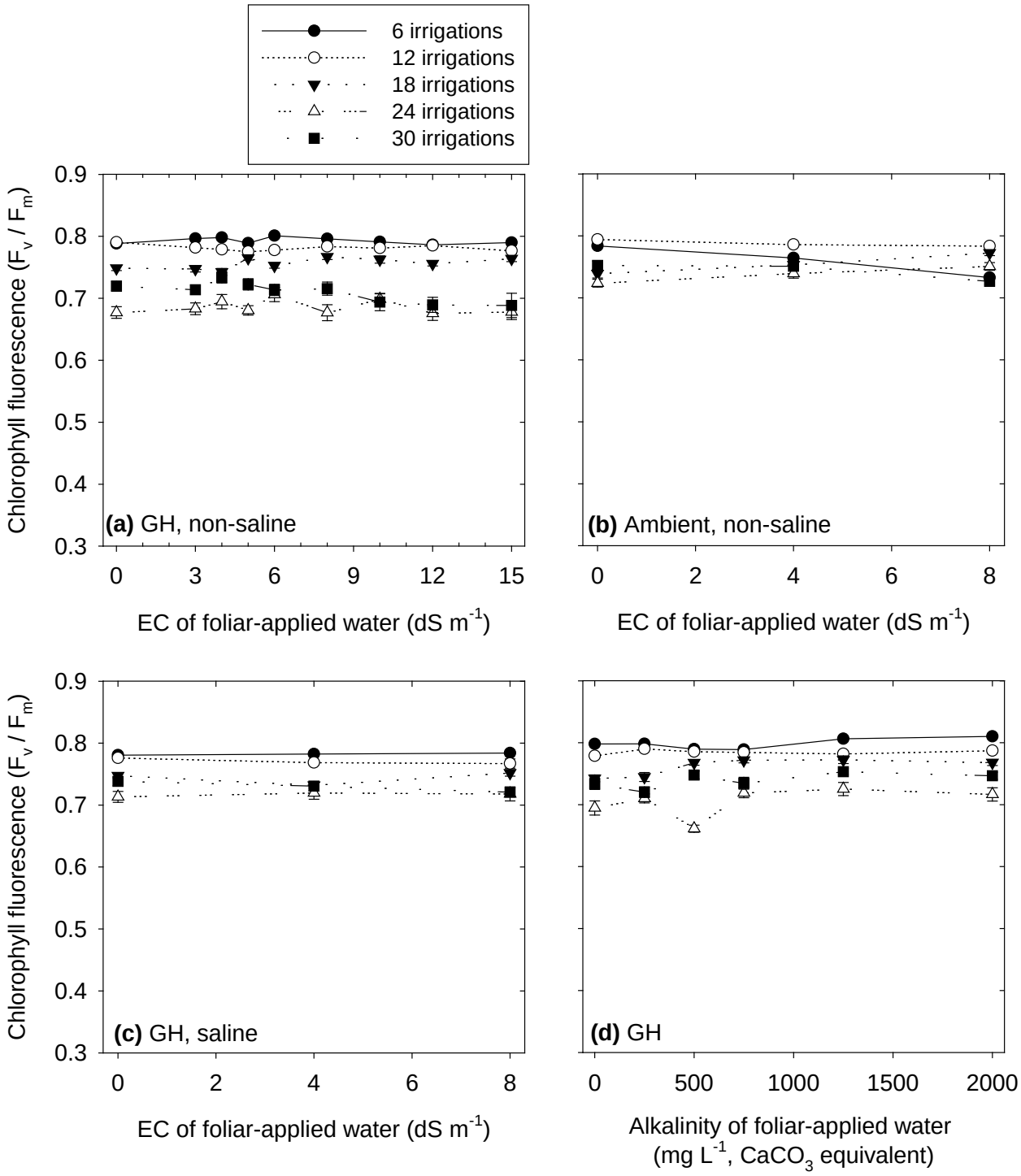

Fig. A4.5. Chlorophyll fluorescence for Rhodes grass overhead-irrigated with either (a-c) saline or (d) saline (4 dS m^{-1}) and alkaline water. Plants were grown in the glasshouse (GH) or in ambient conditions (i.e. outside the glasshouse), and were either grown in a non-saline or saline soil. The vertical bars represent the standard deviation of replicate measurements.

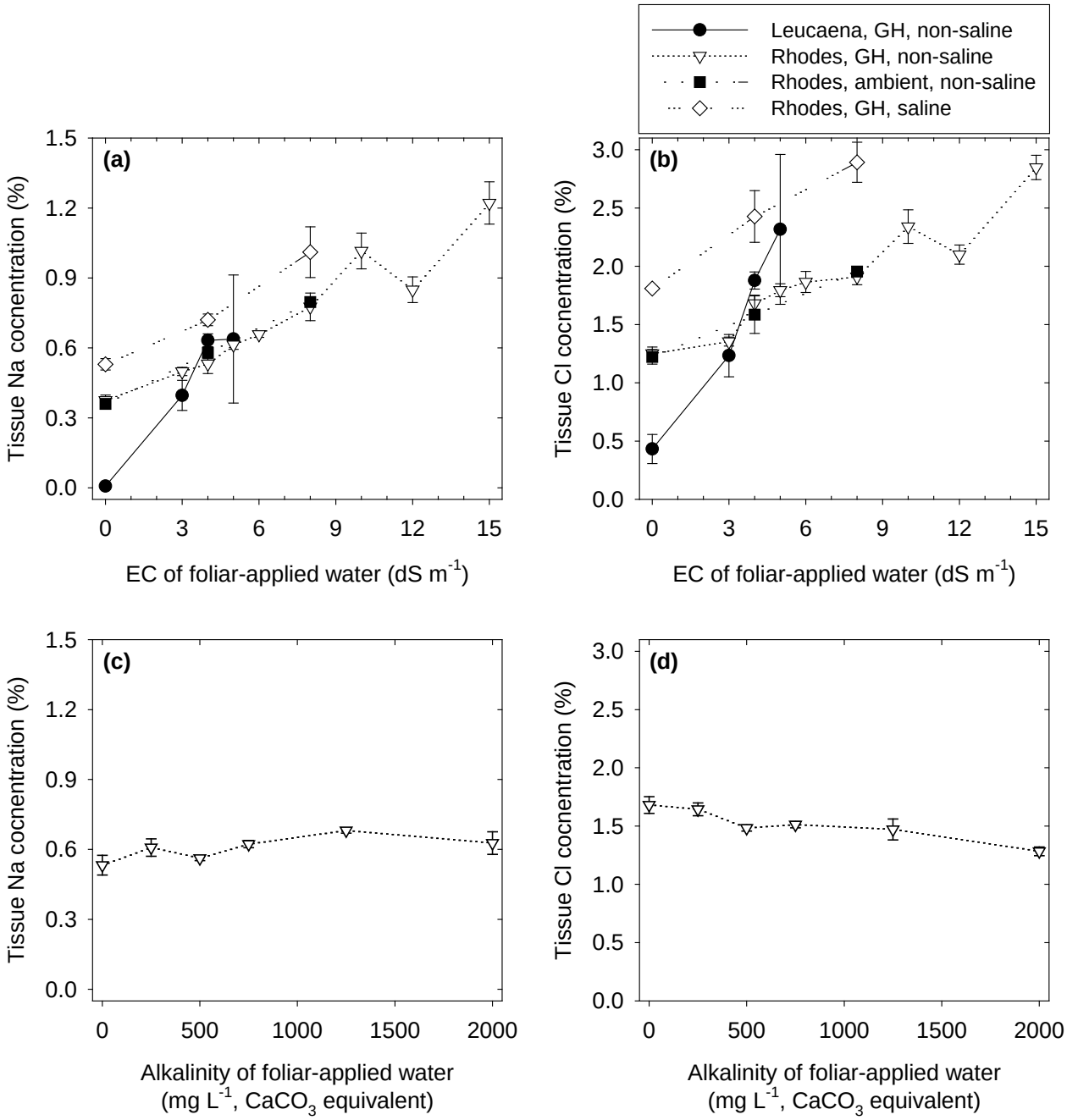

Fig. A4.6. Tissue concentrations of Na (a,c) and Cl (b,d) for leucaena and Rhodes grass overhead-irrigated with either (a,b) saline or (c,d) saline (4 dS m^{-1}) and alkaline water. Plants were grown in the glasshouse (GH) or in ambient conditions (i.e. outside the glasshouse), and were either grown in a non-saline or saline soil. The vertical bars represent the standard deviation of three replicate measurements.

A4.8 Supplementary information

Overhead-irrigation with saline and alkaline water: Deleterious effects on foliage of Rhodes grass and leucaena

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Table A4.S2. The 37 treatments used for the overhead-irrigation of leucaena and Rhodes grass. Salts were added as either NaCl or $NaHCO_3$ to increase the electrical conductivity (EC) and alkalinity. Plants were grown either in the glasshouse (GH) or in ambient conditions outside the glasshouse, and were either grown in a non-saline soil or in a saline soil (due to leaching with water at 10 dS m^{-1}).

Treatment	Species	Foliar-irrigating EC (dS m^{-1})	Soil-irrigating EC (dS m^{-1})	Alkalinity (mg L^{-1}, $CaCO_3$ equivalent)	Growth conditions
1	Rhodes grass	0	0	0	GH, non-saline
2	Rhodes grass	3	0	0	GH, non-saline
3	Rhodes grass	4	0	0	GH, non-saline
4	Rhodes grass	5	0	0	GH, non-saline
5	Rhodes grass	6	0	0	GH, non-saline
6	Rhodes grass	8	0	0	GH, non-saline
7	Rhodes grass	10	0	0	GH, non-saline
8	Rhodes grass	12	0	0	GH, non-saline
9	Rhodes grass	15	0	0	GH, non-saline
10	Leucaena	0	0	0	GH, non-saline
11	Leucaena	3	0	0	GH, non-saline
12	Leucaena	4	0	0	GH, non-saline
13	Leucaena	5	0	0	GH, non-saline
14	Leucaena	6	0	0	GH, non-saline
15	Leucaena	8	0	0	GH, non-saline
16	Rhodes grass	4	0	250	GH, non-saline
17	Rhodes grass	4	0	500	GH, non-saline
18	Rhodes grass	4	0	750	GH, non-saline
19	Rhodes grass	4	0	1250	GH, non-saline
20	Rhodes grass	4	0	2000	GH, non-saline
21	Leucaena	4	0	250	GH, non-saline
22	Leucaena	4	0	500	GH, non-saline
23	Leucaena	4	0	750	GH, non-saline
24	Leucaena	4	0	1250	GH, non-saline
25	Leucaena	4	0	2000	GH, non-saline
26	Rhodes grass	0	10	0	GH, saline
27	Rhodes grass	4	10	0	GH, saline
28	Rhodes grass	8	10	0	GH, saline
29	Leucaena	0	10	0	GH, saline
30	Leucaena	4	10	0	GH, saline
31	Leucaena	8	10	0	GH, saline
32	Rhodes grass	0	0	0	Ambient, non-saline

33	Rhodes grass	4	0	0	Ambient, non-saline
34	Rhodes grass	8	0	0	Ambient, non-saline
35	Leucaena	0	0	0	Ambient, non-saline
36	Leucaena	4	0	0	Ambient, non-saline
37	Leucaena	8	0	0	Ambient, non-saline

Table A4.S3. Shoot fresh mass of the six control treatments. Plants were grown either in the glasshouse (GH) or in ambient conditions outside the glasshouse, and were either grown in a non-saline soil or in a saline soil (due to leaching with water at 10 dS m^{-1}).

Trt	Species	Foliar-irrigating EC (dS m^{-1})	Soil-irrigating EC (dS m^{-1})	Alkalinity (mg L^{-1}, $CaCO_3$ equivalent)	Growth conditions	Shoot fresh mass (g/pot)
1	Rhodes grass	0	0	0	GH, non-saline	240
10	Leucaena	0	0	0	GH, non-saline	160
26	Rhodes grass	0	10	0	GH, saline	150
29	Leucaena	0	10	0	GH, saline	60
32	Rhodes grass	0	0	0	Ambient, non-saline	140
35	Leucaena	0	0	0	Ambient, non-saline	80

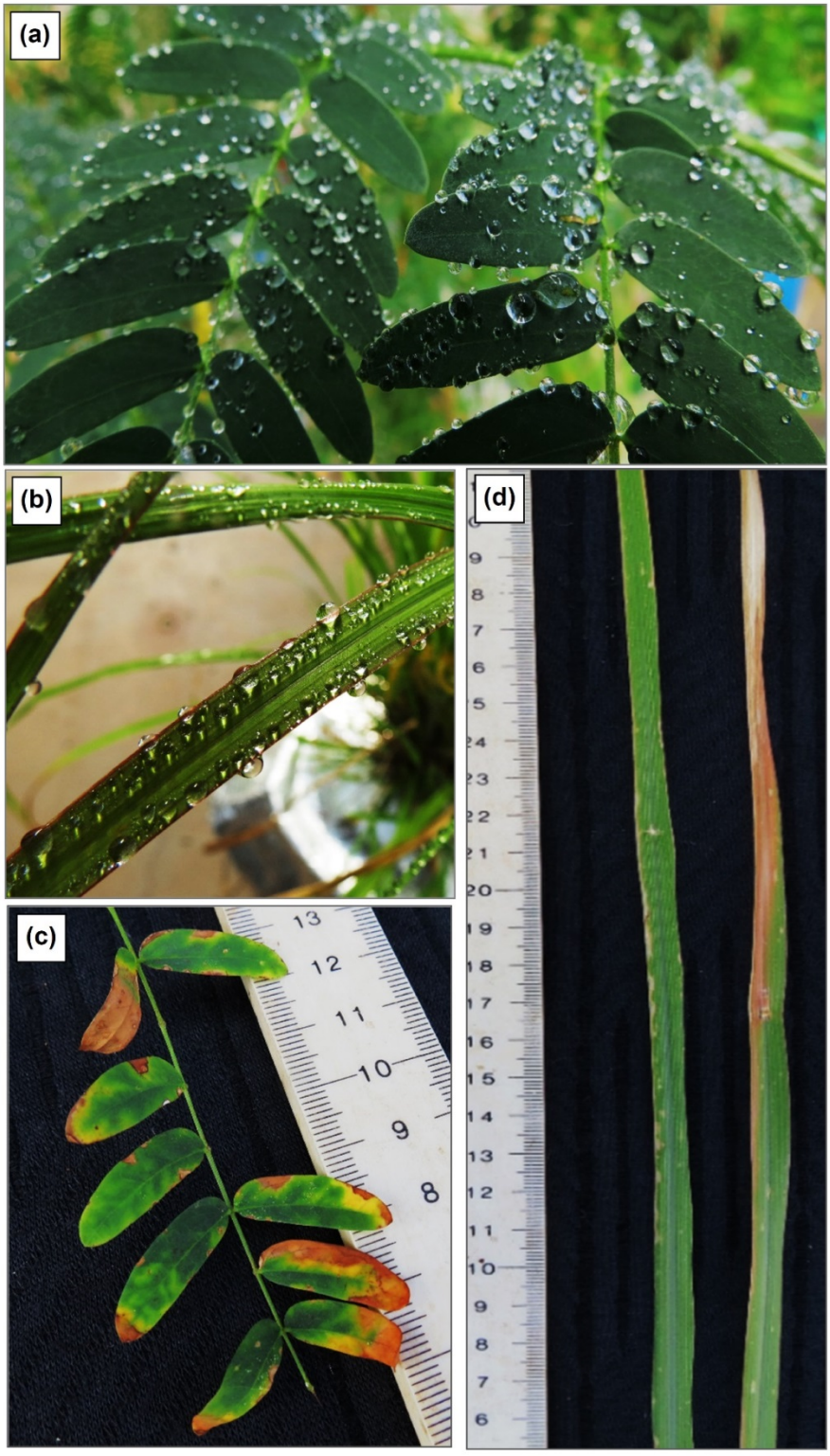

Fig. A4.S20. Images of leaves of glasshouse-grown (a,c) leucaena and (b,d) Rhodes grass overhead-irrigated with water (a,b) containing no added salinity and alkalinity, (c) at an electrical conductivity (EC) of 4 dS m^{-1}, or (d) at an EC of 12 dS m^{-1}. In (a) and (b), the images were taken immediately after irrigation. In (c) and (d), the leaves were first allowed to dry. Note the chlorosis and necrosis in (c) and (d).

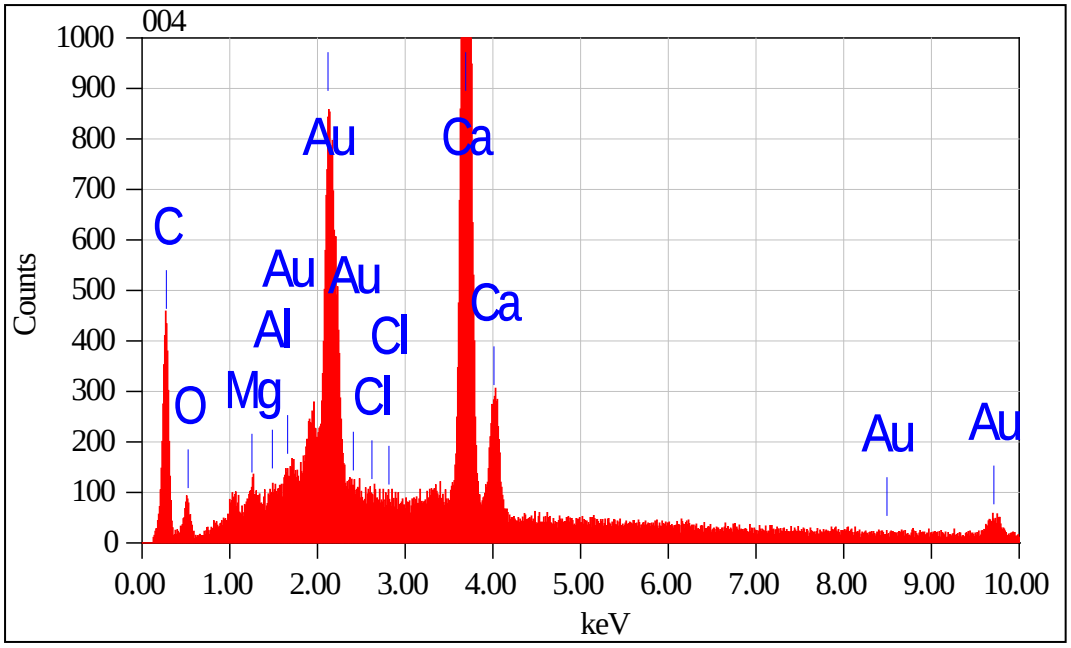

Fig. A4.S21. Energy-dispersive X-ray spectroscopy analyses of salts on the surface of leucaena leaves overhead-irrigated with water containing an alkalinity of 2000 mg L^{-1} ($CaCO_3$ equivalent) with a basal electrical conductivity of 4 dS m^{-1}. The samples were coated with Au prior to examination using scanning electron microscopy.