Use of nitrogen fertiliser to support salinity tolerance and promote edible biomass production in saltbush (*Atriplex nummularia*) forage systems.

Sarah M Rich [1], Ed G. Barrett-Lennard[2], Joshua K. Hendry[1], Matt G Wilmot[1] and Hayley C. Norman[1]

[1] CSIRO, Floreat, WA.
[2] DPIRD, South Perth WA.

Abstract (250 words)
Across southern Australia, there has been uptake in planting perennial plants such as oldman saltbush (*Atriplex nummularia*) to fill summer/autumn feedgaps in livestock production systems. Oldman saltbush is a halophytic, woody, native shrub that is adapted to saline and arid conditions. There is a need to better understand the agronomic practices that will optimise edible biomass production and nutritional value of these plantings. The objective of this project was to assess the influence of nitrogen (N) and salt (as NaCl) concentrations on the growth of Anameka™ oldman saltbush, in a glasshouse and in the field.

A glasshouse experiment was conducted on 2-year-old shrubs to ascertain growth responses to five levels of nitrogen (N) and three salinity levels (NaCl). Under these controlled conditions, addition of N significantly increased edible biomass, even under high salinities, where the N presumably acted to bolster salinity tolerance mechanisms. A threshold level of 5 mM of N was found beyond which edible biomass did not continue to increase, although this interacted with salinity, being 2.5 mM of N at high salinity. Two field experiments were run to investigate the use of N to boost biomass, with inconclusive results. These experiments highlight the benefits of nitrogen management for saltbush planting, especially when growing them on marginal saline country but wide, deep root systems may mean older shrubs can access nitrogen from depth.

Keywords
Saltbush, perennial forage, nitrogen fertiliser, salinity tolerance

Introduction

Atriplex nummularia Lindl (oldman saltbush) is a woody perennial shrub that is planted by farmers in saline and semi-arid farming systems as forage for livestock and to improve environmental health. It evolved within the arid and semi-arid regions of southern Australia and occurs as a dominant species in communities over a 4000 km range (Parr-Smith, 1982).
The drought and salt tolerance of oldman saltbush allows farmers to plant these shrubs onto saline and arid land that is marginal for crop and pasture legume production (Barrett-Lennard et al., 2005, Monjardino et al., 2014). They can improve profitability of mixed farming systems by complementing poor quality forage; in Mediterranean and semi-arid areas they are generally utilised for ruminant feeding in autumn when the feedbase is dominated by poor quality crop residues (Malcolm and Pol, 1986). Oldman saltbush is a rich source of crude protein (CP), vitamin E and minerals that are essential to the diet of ruminants (Norman et al., 2013, Revell et al., 2013, Malcolm and Pol, 1986). Oldman saltbush is not recommended as a sole diet due low to moderate digestibility of the organic matter (OM), excessive salt and sulphur accumulation and production of toxic secondary compounds such as oxalate (Al Daini et al., 2013, Norman et al., 2010, Masters et al., 2007).
Anameka™ cultivar was commercialised in 2015 and more than 6.5 million shrubs have been planted across southern Australia. There is a need to develop a better understanding of agronomic practices that will optimise growth of edible dry matter (EDM; leaves and stems < 3mm) and the nutritional value of the EDM. The objective of this project was to assess the influence of nitrogen (N) and salt (as NaCl) concentrations on the growth of Anameka™ oldman saltbush, in a glasshouse and in the field.

Methods

Controlled environment experiment

A glasshouse experiment was conducted to ascertain plant growth responses to five levels of nitrogen (N) and three salinity levels (NaCl). The experiment was conducted over summer in a glasshouse with daytime maximum ranging from 26.1 - 35.0 °C and night time minimum from 16.6 -23.2 °C. Daylight hours over the nine week experiment reduced from 13:30 to 12:10 h.

Anameka™ cuttings were vegetatively propagated through tip cuttings by a commercial nursery (Chatfields Tree Nursery, Tammin, WA). Cuttings, all originating from a single genotype, were grown in seedling trays for six months and then planted into 15 cm round pots. Six months later, the shrubs were replanted into 30 L rectangular pots. All pots were filled with a low nutrient sand-based potting mix (CSIRO Special Mix; RichGro, Jandakot, WA, Australia). Plants were provided with non-limiting tap water three times a week until they were two years old when experimental treatments were applied.

Over the two years the shrubs had been pruned occasionally to maintain them below 0.5 m in height, and they were supplied 15 g of a complete slow-release fertiliser (Scotts Osmocote plus trace elements potted plants, Evergreen Garden Care Australia Pty Ltd, Bella Vista, NSW, Australia) on three occasions. At the start of the experiment, the soil medium and shrubs were very nutrient poor. This management was imposed to represent mature shrubs growing on the nutrient poor sandy soils of Western Australia (a significant niche for oldman saltbush planting).

Prior to starting the experiment 400 ml of leachate was collected from four pots which had been watered with distilled water. The leachate was tested for a range of nutrients (CSBP Plant and Soil Laboratories, Bibra Lake, Western Australia) and found to contain such minimal nutrition it would offer no background interference to the experiment.

The experiment was a fully randomised design with replicates blocked on glasshouse benches. Pots were rotated on the benches once a fortnight to reduce the potential of edge effects. Plants were watered with a nutrient solution which was made up weekly using distilled water. The factorial experiment had 15 nutrient solution treatments with 5 levels of nitrogen (0, 2.5 mM, 5 mM, 10mM and 20mM) and 3 levels of salinity as NaCl (10 mM, 100 mM and 500 mM). Nitrogen was supplied as a 1:1 ratio of NO_3 and NH_4. All 15 treatments had a nutrient solution base designed for Australian native shrubs (mM): 3.0 $CaCl_2$, 2.0 $MgSO_4$, 0.2 KH_2PO_4, 4.0 KCl, 0.1 Ethylenediamine-N, N'-bis (2-hydroxyphenylacetic acid) ferric sodium complex (EDDHA-FeNa) and (μM): 4.6 H_3BO_3, 0.5 $MnSO_4$, 0.2 $ZnSO_4$, 0.2 $CuSO_4$, 0.1 Na_2MoO_4. The nutrient solution was adjusted to pH 6.1, using KOH.

Each potted shrub received 2 L of nutrient solution 3 times weekly. This amount was determined to allow pots to be fully flushed with fresh solution avoiding any accumulation of salts in the soil. To avoid nutrient or salinity shock to the plants at high treatments levels all plants were stepped up to treatment-strength nutrient solution. Pre-treatment was one week at 25 % of treatment solution and the following week at 50 % solution before 100 % solution at the start of the third week.

Harvest and biomass measurements

All plants were destructively harvested, seven weeks after the start of full-strength nutrient treatments. To harvest, the main stem was severed at the soil surface. The aboveground biomass was sorted into edible dry matter (EDM; leaves and stem material < 3 mm diameter) and woody stems (remaining stem material < 3 mm diameter). The plant material was weighed then oven dried (60 °C) for 48 h prior to determining dry weight of plant components.

Field experiment

Two sites were established near Dongara with Anameka™ rooted cuttings in July of 2019. The nursery raised from cuttings were propagated 6 months prior to planting at Chatfield's Tree Nursery in Tammin, Western Australia. The shrubs were hand planted with a pottiputki planting tool in rows across the paddocks, with 5 m between rows and 2 m between shrubs within a row. At the time of planting, sites were ripped and mounded with a Chatfield's Tree Planter to enhance root penetration through the soil profile.

Within each row, replicates of 15 shrubs were subject to the same nutrient treatments in August of 2019. The sites were blocked (n = 3 replicates) and nutrient treatments were allocated to groups of 15 adjacent plants randomly within blocks. Nitrogen fertiliser was applied as calcium nitrate at the rate of 60 g per shrub (100 kg/ha). Each shrub was fertilised by hand with the fertiliser sprinkled over the soil under the shrub canopy. Biomass production was assessed in March 2020 using the 'Adelaide' technique (Andrew et al., 1979).

Results

The addition of N and NaCl significantly increased total EDM after 9 weeks of treatment ($p = <0.001$ Figure 1). Addition of N resulted in an increase in biomass of all tissue types (thick and thin stems, and leaves), however, leaf biomass accumulation was the major contributor to increasing plant size. Salinity level impacted the amount of biomass accumulation, with 500 mM NaCl significantly reducing plant accumulation EDM at and above 5mM of N, compared to lower salinity levels. Within the lower salinity levels, N availability significantly increased biomass accumulation until 5 mM N from which there was no significant difference in edible biomass accumulation with additional N ($p > 0.05$).

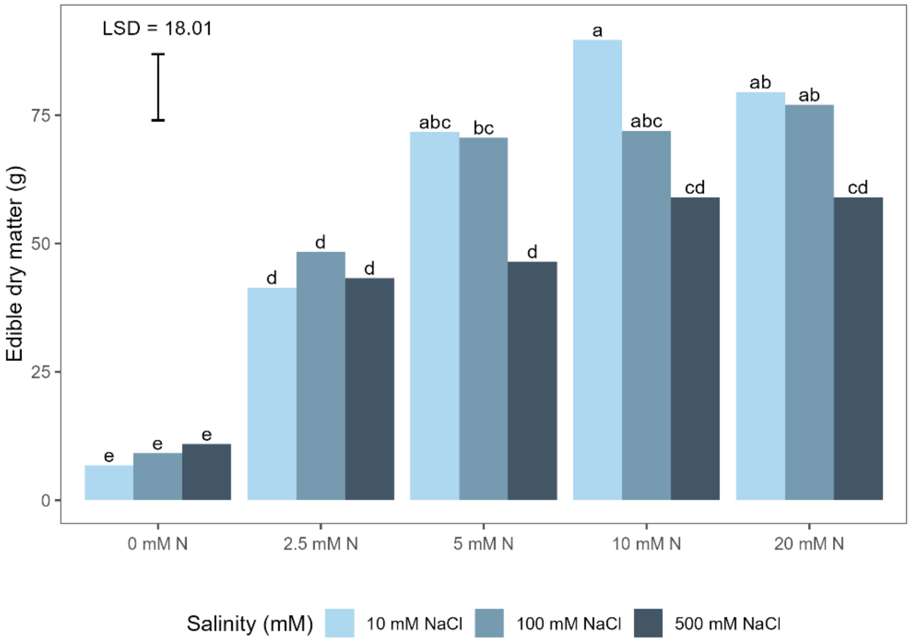

Figure 1. Total dry edible matter from final destructive harvest of two-year old oldman saltbush grown in the glasshouse under varying salinity and nitrogen nutrient solution treatments for 9 weeks. Letters indicate significant difference p > 0.05 (n=4).

The field data yielded some inconsistent results (Figure 2). The Forsyth site established faster than the Gilliam site, producing significantly more EDM in the first 8 months, and showing no significant difference ($p > 0.05$) in EDM production, regardless of nitrogen application. At the Gillam site, the fertilised plants produced significantly more EDM than the unfertilised plots. The addition of 100 kg/ha calcium nitrate quadrupled biomass production from the saltbushes at this site.

The early establishment conditions at the sites may have influenced root architecture, with rainfall in the first two years from planting (2019, 319 mm, and 2020, 330 mm) being well below the long-term mean (441 mm) so that seedlings may have prioritised root growth to seek deep moisture and therefore, these roots may have been able to exploit nutrient stores from depth. Producers should consider test strips of fertiliser or encourage nitrogen fixing understory legumes.

Conclusion

From this work we can conclude that agricultural plantings of oldman saltbush may respond to nitrogen fertiliser, especially while young with smaller root systems, however due to the deep-rooted nature of this species, standard soil testing to 0.2 m (commonly used to inform crop and pasture fertiliser application) may not be indicative of fertiliser requirements in older plantings. Further work with more detailed soil testing to depth, across different soil types, is needed. There appears to be a case for encouraging adapted annual legumes in the understorey to fix nitrogen.

These experiments add to the body of work showing the high saline tolerance of this species and highlight the benefits of nitrogen management if they are being used as a forage, especially when growing them on marginal saline country. These data show that once established, these plants will survive at very high salinities, even when on nutrient deprived soils and if also provided with some basic nutrition will flourish and grow well, producing extensive new growth even under salinities of up to 500 mM of NaCl.

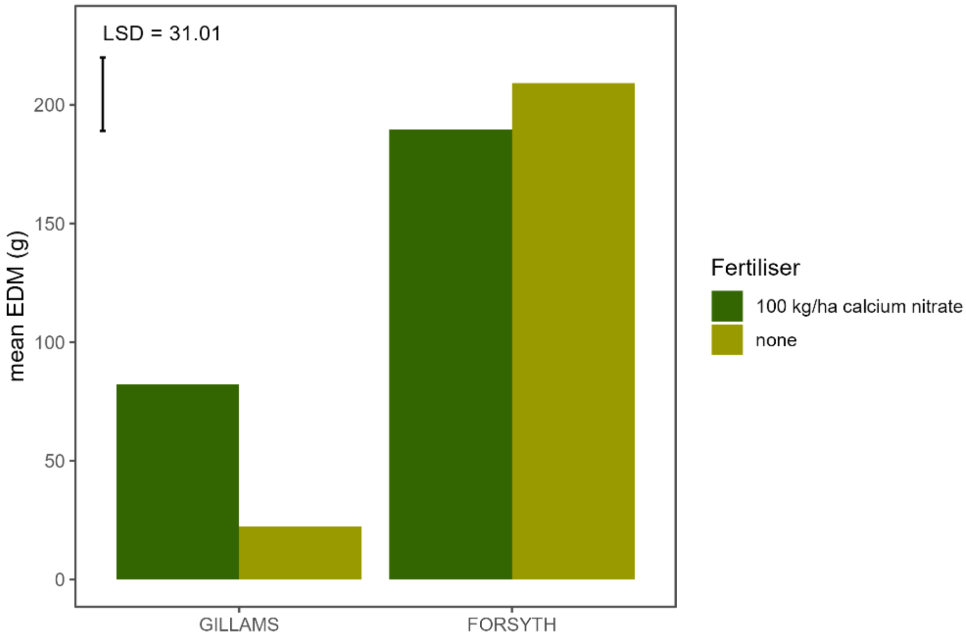

Figure 2. Mean edible dry matter (EDM) produced by Oldman saltbush shrubs under two different N-fertiliser regimes at two sites near Dongara, Western Australia. EDM is presented mean/plant from Adelaide scoring seven months after fertiliser application. (n= 3).

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