





# **Final report**

# No more gaps with superior shrub systems

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### Abstract

This project aimed to conduct collaborative research with producers to (1) collect paddock scale productivity and economic data demonstrating that sheep can utilise saltbush systems to improve productivity, (2) quantify benefits arising from using fertilisers and/or adapted annual legumes within systems, and (3) investigate opportunities to halve establishment costs through elite seed lines that can be planted in nurseries or seeded directly in the field. We generated data and developed extension messages and products to support adoption of shrub systems on marginal soils. Adoption of these systems allow producers to increase whole-farm stocking rates, a key driver of profitability. It also improves the ability to manage seasonal risk.

At three locations, we measured substantial productivity of Anameka<sup>™</sup> shrub systems that were planted on soils that have constraints that impact productivity of traditional crops and pastures. The shrub paddocks were grazed during late summer or autumn, the time of year in Mediterranean-type climates when a tonne of green feed has up to ten times greater economic value than a tonne of green feed in spring. The shrubs, planted at commercial densities of 830-950 shrubs/ha, yielded from 0.5 to 1.4 t/ha of edible dry matter, and this complemented 1.2 to 4.8 t/ha of low-quality dead understorey. The saline, low rainfall paddock in Baandee (300mm mean annual rainfall), supported up to 1026 autumn grazing days/ha with 230 g/day of liveweight gain and a condition score gain of 0.5 units (with low levels of supplementation for the pregnant ewes). The deep white sand at Dongara (400mm mean annual rainfall), supported up to 545 summer/autumn grazing days/ha with 135 g/day of sheep growth and a condition score gain of 0.4 units. The site provided eight times more grazing days/ha than the adjacent crop stubble. One Mallee saltbush shrub genotype at produced the equivalent of 4.3 tonnes/ha of edible biomass. The deep white sand site in Cranbrook (500mm mean annual rainfall), supported up to 430 sheep grazing days/ha in autumn with 40 g/day of liveweight gain and an increase in condition score of 0.7 units. The methodology for the research site experiments only allowed one grazing event each year the project was focussed on filling the autumn feed gap. It is probable that the sites could be grazed opportunistically at other times of the year to extract more value from the annual biomass when it is green. These sites could generate additional value if they can be used to improve twin lamb survival through the provision of shelter during lambing in winter.

A new generation of saltbush and Mallee saltbush shrubs, to complement Anameka<sup>™</sup> saltbush, have been identified. These have capacity to double existing biomass growth rates and allow the future potential for less expensive nursery-raised seedlings and in-paddock direct seeding. The research team generated critical data regarding commercial cleaning of seed, seed coatings to improve handling and temperature requirements for optimal germination. Information has been created and extended regarding shrub grazing management and fertilisers to optimise shrub system productivity.

The project exceeded its communicational goals. Over four years we delivered 2068 face to face contacts (1040 producers and 1028 industry representatives) over 48 events in three states. We also delivered a Landline TV story and TV news stories, 9 radio interviews, three You-tube-style communication products (https://vimeo.com/733868413), 6 magazine articles (in Feedback and Beyond the Bale), 43 online or newspaper media articles and more than 30,700 social media views. The team worked with the Gillamii Centre and DPIRD to update and innovate 'Saltland Genie' (a salinity web app) and deliver three, two-day masterclasses in regional locations, attended by 72 producers and 29 industry representatives. We worked with producer groups to establish two MLA-funded PDS projects, with activities on 10 additional farms. At the conclusion of the project, we obtained Future Drought Fund support to establish 6 producer-scale Anameka<sup>TM</sup> saltbush systems in

NSW and WA. Saltbush is a leading option for drought mitigation in low to medium rainfall zones as it is adapted to semi-arid environments. We have developed a proposal to progress direct seeding through the WA Agricultural Research Collaboration. The work resulted in eight scientific publications, with three more in the later stages of drafting. The work was the subject of invited keynote presentations at national and international conferences.

From 2019, Anameka<sup>™</sup> sales have doubled and 6 million shrubs (with a lifespan of 15+ years) have now been planted by 328 producers over 8,500ha. We have enabled new nursery capacity in NSW and WA to meet increasing demand. Adoption of native shrub systems on soils that are marginal for cropping, provides critical nutrients for sheep or cattle at a time when they are scarce to complement poor quality crop and pasture residues. Producers can reduce both the cost and inconvenience of supplementation and the shrub paddocks provide a good place for confinement feeding while winter pastures establish, thus increasing biomass production in other parts of the farm. Targeting perennial use on land that is marginal for cropping removes one of the major barriers to adoption in mixed farming systems. Other research has demonstrated additional benefits such as reduced dryland salinity, enhanced ecosystem function, reduced methane emissions intensity (Mallee saltbush), higher animal welfare through improved antioxidant status. These combine to improve eco-credentials of meat and wool production systems.

### **Executive summary**

#### Background

Nutritional gaps present the largest production cost to grass-fed meat and wool producers across Mediterranean and dry areas of Australia. Gaps lead to suboptimal ruminant production and conservative whole-farm stocking rates. A recent modelling study found that average stocking rates in the WA wheatbelt may be about 40% lower than the calculated optimum, although the top 25% most profitable producers are close to optimally stocked (Young *et al.* 2022). Sheep producers often stock at rates that align to expectations of decile 2-4 (low rainfall) seasons. This is driven by the high perceived and actual downside risk associated with sustaining livestock through poor seasons. Filling feed gaps has been one of the top WALRC priorities for the last four years. Strategies to address key nutrient gaps are likely to give confidence to mixed farmers to increase whole-farm stocking rates while potentially lowering the risk profile of their enterprises. Drought-tolerant native shrubs, grown on soils that are marginal for crop production, provide nutrients to complement, and thereby improve the feed conversion ratio of crop and pasture residues during summer/autumn. These shrubs lift farm profitability by reducing supplementation requirements, allow deferred grazing of regenerating pastures, and buffering between-season variation in forage supply. The provide vitamins and minerals that are limited in summer and assist animals to manage oxidative stress.

Previous research has shown that sheep offered Anameka<sup>™</sup> saltbush or Mallee saltbush, while grazing cereal residues, maintained weight, and had 20% greater wool growth than sheep offered cereals alone (Li *et al.* 2018). In respiration chambers, inclusion of Mallee saltbush led to 26% less methane (Li *et al.* 2018). Barriers to adoption of shrubs include the high opportunity cost of using soils that are suited to cropping, the up-front cost of establishment, uncertainty about agronomic and grazing management and a lack of on-farm data quantifying benefits.

This project conducted research and extension to address these barriers. The target audience was producers, producer groups and the wider livestock industries. The information that has been

gathered in the project has been used to inform mixed producers of the opportunity to utilise shrubs to overcome nutrient gaps and give them confidence to increase stocking rates. We will identify elite seed lines for potential commercialisation and develop guides to optimise establishment, productivity, and grazing management.

#### Objectives

This project aimed to conduct collaborative research with producers to; (1) collect paddock scale productivity and economic data demonstrating that sheep and/or cattle can utilise shrubs to improve productivity and utilisation of crop residues, (2) quantify benefits arising from using fertilisers and/or adapted annual legumes within shrub systems, and (3) investigate opportunities to halve establishment costs through seed lines that can be planted in nurseries or direct seeded. There was a significant industry engagement activity. Increased adoption of shrub systems by producers will congruently improve landscape function and reduce the impact of dryland salinity.

#### Methodology

A series of four on-farm paddock-scale field experiments across the agro-ecological zones of WA were used to obtain grazing data and compare shrub genotypes. Glass house and field experiments were used to investigate salt tolerance and fertiliser responses. Components of seed ecology that inform direct seeding were investigated in laboratories, glasshouses and in on-farm experiments.

#### **Benefits to industry**

Benefits to industry arising from this research include

- Data, increasing awareness and extension products to support adoption of shrub systems on marginal soils to improve whole-farm stocking rates and manage seasonal risk.
- New genotypes of shrubs that have capacity to double existing productivity and allow the potential for direct seeding. Direct seeding could halve establishment costs and allow reintroduction of higher feeding value native shrubs to rangeland areas.
- Information regarding shrub grazing management and fertilisers to assist producers to optimise system productivity.
- Adoption of these systems will lead to additional benefits such as reduced dryland salinity, enhanced ecosystem function, possibly reduced methane emissions (Mallee saltbush), higher animal welfare and improved eco credentials of meat and wool.
- We raised awareness and provided information to support adoption of shrub systems. Over four years we delivered 2068 face to face contacts (1040 producers and 1028 industry representatives) over 48 events in three states. We also delivered a Landline TV story and TV news stories, 9 radio interviews, three You-tube-style communication products (https://vimeo.com/733868413), 6 magazine articles (in Feedback and Beyond the Bale), 43 online or newspaper media articles and more than 30,700 social media views.
- The team worked with the Gillamii Centre and DPIRD to update and innovate 'Saltland Genie' (a salinity web app) and deliver three, two-day masterclasses in regional locations, attended by 72 producers and 29 industry representatives. We worked with producer groups to establish two MLA PDS projects, with activities on 10 farms.

- Anameka<sup>™</sup> adoption continues to be strong. From 2019, sales have doubled and 6 million shrubs (with a lifespan of 15+ years) have now been planted by 328 producers over 8500ha across southern Australia. We now have a nursery based in NSW who are producing up to half a million shrubs this season.
- For the research industry, eight scientific publications have resulted from the research and three more are in the later stages of drafting. The work was the subject of invited keynote presentations at national and international conferences.
- The shrubs are being utilised in the new MLA 'Shade and Shelter' projects as they could have significant impact on twin lamb survival. The team successfully gained Future Drought Fund support to establish 6 producer-scale Anameka<sup>™</sup> saltbush systems demonstrations in areas of NSW and WA where adoption is low, but the opportunity is high.

#### Future research and recommendations

As a result of this project and associated industry consultation, we recommend that

- The commercialisation of an elite saltbush seed line or a combination of several lines. Discussions with industry are underway to scope the appetite for the product and seed production and sales logistics.
- While we have identified elite Mallee saltbush genotypes that have high biomass production
  and nutritional value and tolerate deep infertile sands, they remain relatively unpalatable to
  sheep. We have identified a cohort that have higher palatability than the original population,
  however, these are grazed after the majority of saltbush has been consumed. We suggest
  waiting on the outcomes of the 'shade and shelter' MLA-funded projects before deciding on
  a commercialisation strategy. More work is required to understand why sheep do not eat
  the shrubs and their role in methane mitigation. There is an opportunity to select a genotype
  that is very productive on marginal sandy soils and reduces methane emissions.
- The key findings regarding shrub management and agronomy are already being presented to industry. The variability in fertiliser responses across sites is likely due to the deep-rooted shrubs accessing fertiliser at depth. Farmers are unlikely to sample soils to these depths, so messages need refinement.
- Use of adapted annual legumes remains a key opportunity but weed management has emerged as an issue. *Matricaria* (Globe Chamomile) is a threat to shrub systems in the eastern wheatbelt of WA. We recommend the development of herbicide and weed management recommendations for shrub systems.
- We believe that we have the information, technology, and genotypes to progress direct seeding (for agricultural and possibly rangeland zones). We hope to continue this work in partnership with industry, possibly through a WA Agricultural Research Collaboration Application (submitted June 2023) and CSIRO Drought Mission support. We will maintain active conversations with MLA and AWI to discuss opportunities for co-investment.

# Table of contents

Abst	ract		2
Exec	utive	summary	3
1.	Backg	ground	9
	History	y of development of oldman saltbush and Mallee saltbush 1	0
2.	Objec	ctives1	2
3.	Meth	odology1	6
	3.1 (	Objective 11	6
	3.1.1	Site locations and characterisation1	.6
	3.1.2	Grazing studies 2	1
	3.1.3	Plant measurements 2	2
	3.1.4	Assessing nutritional value	2
	3.1.5	Statistical analyses	3
	3.2 (	Objective 2 2	3
	3.2.4	Application of nitrogen and/or phosphate fertilisers – field experiment 1 2	9
	3.2.5	Application of nitrogen fertilisers at different rates – field experiment 2 3	0
	3.3 (	Objective 3	0
	3.3.5	Seed coating for glasshouse and field experiments	7
	3.3.6	Impact of sowing depth on seedling emergence	8
	3.3.7	Comparing coated and 'naked' seeds in a commercial nursery	9
	3.3.8	Seed coating and field emergence	9
	3.3.9	Seed and bract characteristics of the elite seed lines	.0
	3.4 (	Objective 44	1
4	Resul	ts and Discussion4	1
	4.3 (	Objective 14	1
	4.3.1	Grazing studies	.1
	Plant e	establishment and survival4	.7
	4.3.2	Biomass production 4	.8
	4.3.4	Management of grazing intensity6	4

4.3.5	Relative palatability64
4.3.6	Nutritional value of shrubs67
4.3.7	Feeding value70
4.4	Objective 2
4.4.1	Adapted legumes
4.4.2	Salt tolerance of Mallee saltbush and saltbush; glasshouse experiment 181
4.4.3	Impact of salinity and nitrogen fertilisers on growth and nutritional value of saltbush - glasshouse experiment 2
4.4.4	Application of nitrogen and/or phosphate fertilisers – field experiment 1 93
4.4.5	Application of nitrogen fertilisers at different rates – field experiment 2 94
4.5	Objective 3
4.5.5	Seed coating for glasshouse and field experiments 108
4.5.6	Impact of depth of burial on seedling emergence 108
4.5.7	Comparing coated and 'naked' seeds in a commercial nursery
4.5.8	Seed coating and field emergence 110
4.5.9	Seed and Bract characteristics of the elite seed lines
4.4	Objective 4115
	<b>Objective 4. 115</b> walks and communication events       115
Field	•
Field TV an	walks and communication events
Field TV an Social	walks and communication events
Field TV an Social Field	walks and communication events
Field TV an Social Field	<ul> <li>walks and communication events</li></ul>
Field TV an Social Field Other Engag	walks and communication events
Field TV an Social Field Other Engag	<ul> <li>walks and communication events</li></ul>
Field TV an Social Field Other Engag Print Anam	<ul> <li>walks and communication events</li></ul>
Field TV an Social Field Other Engag Print Anam Stude	walks and communication events
Field TV an Social Field Other Engag Print Anam Stude Scien	<ul> <li>walks and communication events</li></ul>
Field TV an Social Field Other Engag Print Anam Stude Scien	walks and communication events

5

6	Future research and recommendations	
7	References	140
8	Appendices	145
	8.1 Acknowledgements	145
	8.2 Field walks	145
	8.3 Selection of media articles	148
	8.2 Shrub guideline – summary of key extension messages	152

## 1. Background

Nutritional gaps present the largest production cost to grass-fed meat and wool producers across Mediterranean and dry areas of Australia. Gaps lead to suboptimal ruminant production and conservative whole-farm stocking rates. The mixed farming zone od southern Australia (300-650 mm annual rainfall) covers approximately 50 million ha of farmland and approximately 19,000 producers. Previous economic modelling found that the gross margin of a sheep enterprise in the mixed farming region increased by 15% for every 20% increase in farm stocking rate from a conservative base level (Friend et al. 2018). Despite the importance of stocking rate, sheep-meat and wool producers manage the risk associated with the cost of feeding supplements by running very conservative stocking rates. A recent modelling study found that average stocking rates in the WA wheatbelt may be about 40% lower than the calculated optimum, although the top 25% most profitable producers are close to optimally stocked (Young et al. 2022). Sheep-meat and wool producers often stock at rates that align to expectations of decile 2-4 (low rainfall) seasons. This is driven by the high perceived and actual downside risk associated with sustaining livestock through poor seasons. Filling feed gaps has been one of the top WALRC priorities for the last four years. In NSW, the majority of the 170 producers interviewed by Hackney (2017) ranked feed gaps as a significant limitation to livestock production. Strategies to address key nutrient gaps are likely to give confidence to mixed farmers to increase whole-farm stocking rates and while potentially lowering the risk profile of their enterprises.

Perennial forages provide an opportunity to fill the summer/autumn feed gap, however, very few producers in mixed farming zones are willing to put cropping land under perennials due to reduced flexibility in crop rotations and the high opportunity cost associated with forgoing high-value crops in good seasons. Recent extensive planting of canola to take advantage of high global prices is an example of why perennials are not adopted on 'good' cropping soils. Drought-tolerant native shrubs, grown on soils that are marginal for crop production, provide nutrients to complement, and thereby improve the feed conversion ratio of crop and pasture residues during summer/autumn. These shrubs lift farm profitability by reducing supplementation requirements, allowing deferred grazing of regenerating pastures, and buffering between-season variation in forage supply. They provide vitamins and minerals that are limited in summer and assist animals to manage oxidative stress. Previous research has shown that sheep offered Anameka<sup>™</sup> saltbush or Mallee saltbush, while offered cereal residues, maintained weight, and had 20% greater wool growth than sheep offered cereals alone (Li et al. 2018; CSIRO 2016 - AWI Project 84099). In respiration chambers, Li et al. (2018) also found the inclusion of Mallee saltbush in the diet led to 26% less methane. Barriers to adoption of shrubs include the high opportunity cost of using soils that are suited to cropping, the up-front cost of establishment, uncertainty about agronomic and grazing management and a lack of on-farm data quantifying benefits.

This project conducted research and extension to address these barriers. The target audience was producers, producer groups and the wider livestock industries. This project aimed to conduct collaborative research with producers to; (1) collect paddock scale productivity and economic data demonstrating that sheep and/or cattle can utilise shrubs to improve productivity, (2) quantify benefits arising from using fertilisers and/or adapted annual legumes within shrub systems, and (3) investigate opportunities to halve establishment costs through seed lines that can be planted in nurseries or direct seeded. Increased adoption of shrub systems by producers will congruently improve landscape function and reduce the impact of dryland salinity.

The information that has been gathered in the project has been used to inform mixed producers of the opportunity to utilise shrubs to overcome nutrient gaps and give them confidence to increase stocking rates. We identified elite seed lines for potential commercialisation and develop guides to optimise establishment, productivity, and grazing management. Throughout the project we have sought to highlight the investment by MLA and AWI in a project that addresses producer needs.

#### History of development of oldman saltbush and Mallee saltbush

#### **Oldman saltbush**

Oldman saltbush (*Atriplex nummularia* Lindl.) is a woody perennial shrub that is planted by farmers in southern Australia as forage for livestock and to improve environmental health. It is native to Australia and occurs as a dominant species in widespread communities over a 4000 km range in arid and semi-arid zones (Parr-Smith 1982). The drought tolerance mechanisms of oldman saltbush include deep roots (> 4 m), osmotic control and slow growth when water is scarce (Barrett-Lennard 2003). These mechanisms are associated with salinity tolerance; oldman saltbush grows on soils with root-zone salinities as high as 600 mM sodium chloride (NaCl), (Ashby and Beadle 1957) and growth can be enhanced at lower salinities (25–200 mM NaCl). It does not exclude salts at the roots (Araújo *et al.* 2006) and therefore its main salinity tolerance mechanisms are salt exclusion from the leaves and osmotic adjustment (Flowers and Colmer 2008). This involves transport, accumulation, and compartmentalisation of inorganic ions in the vacuoles while synthesising and accumulating organic solutes in the cytoplasm to maintain the osmotic equilibrium in cells (Munns and Tester 2008). Biomass can contain up to 25% salt and this has implications for the livestock consuming the biomass.

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 and are generally grazed by sheep and cattle 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), sulphur (S), vitamin E and minerals that are essential to the diet of ruminants (Revell *et al.* 2013, Norman *et al.* 2019, Masters *et al.* 2019). The S and salt content supports more than 20% greater wool growth (Li *et al.* 2018; CSIRO 2016). The feeding value of oldman saltbush as a sole diet is generally poor, due to a combination of slow biomass production, low to moderate digestibility of the organic matter (OM), excessive salt accumulation and production of toxic secondary compounds such as oxalate (Norman *et al.* 2004; Masters *et al.* 2007; Norman *et al.* 2010a; Al Daini *et al.* 2013). However, it offers significant potential as a component of the diet to complement poor quality crop residues in summer and autumn.

A collaborative research project, involving farmers, agronomists, livestock scientists and systems modellers identified low feeding value, particularly metabolizable energy (ME) content, as the key constraint that limited profitability of saltbush systems (Sustainable Grazing on Saline Lands Project, 2007). Pre-experimental modelling indicated that improving shrub organic matter digestibility (OMD) by 10% would increase profits by three times the increment associated with increasing biomass production by 10%, or reducing the cost of establishment by 10% (O'Connell *et al.* 2006; Thomas *et al.* 2009; Monjardino *et al.* 2010).

A collection of oldman saltbush germplasm was initiated in 2006, with 60,000 plants from two subspecies collected from 27 sites (provenances) across Australia (Hobbs and Bennell 2008). The collection was grown across 3 nursery sites in Condobolin (NSW), Tammin (WA) and Monarto (SA). Survival and growth were assessed prior to grazing with flocks of 1-year-old sheep at stocking densities of 25 and 50 sheep/ha. Sheep grazing pressure allowed for subsequent measurement of recovery from grazing (associated with long-term biomass production) and relative palatability. Across the three experimental sites, sheep demonstrated a consistent and statistically significant preference for specific provenances (Norman and Masters 2023). Metabolism crate feeding experiments enabled the development of laboratory and NIRS methods that accurately and inexpensively predicted nutritional value of Australian shrubs (Norman and Masters 2010; Norman *et al.* 2010). There was significant variation between provenances in OMD, salt, crude protein (CP), minerals and biomass growth. Nutritional analyses indicated that sheep preferred shrubs with higher digestibility and lower salt content, this allowed rapid identification of elite material (Norman and Masters 2023).

After consideration of palatability, biomass and NV, 90 of the 60,000 shrubs (the best 30 from each nursery site) were selected and vegetatively cloned as cuttings. When this cohort was compared to the average of the original collection, they had 20% higher OMD, were consistently preferred by sheep and produced 8 times more biomass than the mean of the original collection. These cuttings were planted into replicated blocks at 3 experimental sites across Australia and assessed for agronomic and nutritional traits over 3 years. Again, sheep were used to rank the genotypes for relative palatability, and this was included with agronomic traits as a selection criterion. In the final phase of the work, the best 12 genotypes were identified, vegetatively cloned and planted in replicated block experiments at 13 diverse sites across southern Australia. Genotypes were again assessed for survival, productivity and nutritional value. The most promising 4 genotypes and an industry standard were harvested and fed to sheep in a final energy and nitrogen metabolism experiment (Norman *et al.* 2016). This led to commercialisation of Anameka<sup>™</sup> (a clonal cultivar) in 2015, just 9 years after the establishment of the collection (Norman and Masters 2023). Whole-farm economic analysis suggests that Anameka<sup>™</sup> can double the profitability of saltbush plantations on farms. More than 6 million Anameka have now been planted by 328 farmers across southern Australia.

#### Mallee saltbush

Mallee saltbush (*Rhagodia preissii* Sub spp. Preissii Moq.), is another *Chenopodiaceae* species that is also native to WA. It is a woody shrub that grows 0.6 - 2 m tall, with leaves being linear to narrowly obovate. It is found naturally on clay and sandy soils, on coastal sand dunes and saline soils. *Rhagodia preissii* is the first species of the rhagodia genus to be commercially cultivated, and it is planted by farmers because it performs well on nutrient-poor sandy soils, is drought tolerant and produces substantial amounts of dry matter (Kotze *et al.* 2011). It has also shown to be tolerant to frost events, with only negligible frost damage after frost events (Revell *et al.* 2008).

Through the screening of potential Australian shrub species for agriculture and revegetation, Mallee saltbush was found to be one of the shrubs with the highest biomass production and digestibility (Revell *et al.* 2013, Norman *et al.* 2017; CSIRO 2016). Other beneficial aspects of Mallee saltbush are the lower salt content of biomass (as compared to saltbush), with total ash contents averaging 12 %, low oxalate content and high crude protein and S content (Norman *et al.* 2017). Grazing experiments have shown low palatability of Mallee saltbush for inexperienced sheep, however, with increasing

exposure the consumption increased indicating an adaptive response and it was also found that there was a large variation in the palatability between individual plants.

In 2015, 100 'families' of Mallee saltbush 200 were collected from ten sites across the natural range in WA. Using a similar methodology to the Anameka<sup>™</sup> project, the collection was planted in replicated blocks at 3 research sites and assessed for agronomic and nutritional traits, as well as sheep preferences. The most promising genotypes were included in this project.

#### Summary of economic and systems benefits generated by shrubs

Economic studies demonstrate that these shrubs, without any genetic improvement, can lift wholefarm profitability, reduce cost of production (by reducing supplementary feed requirements) and reduce whole-farm risk by buffering feed supply (O'Connell *et al.* 2006, Thomas *et al.* 2009; Monjardino *et al.* 2010). The supplementary grain bill for several farms that were part of one study was halved (CSIRO 2016 - AWI Project 84099). Saltbush and Mallee saltbush have the advantage of being productive on marginal soils, so adoption comes with a low opportunity cost. The models indicate that shrubs allow producers to sustainably increase whole-farm stocking rates without increasing risk, therefore a 5% increase in sheep meat and beef production is feasible. Based on modelling using producer data and on-farm measurement, the wool income derived from the utilisation of unimproved marginal land ranged from \$24/ha in a low rainfall zone to \$197/ha in a medium rainfall zone. Improved shrubs (such as Anameka<sup>TM</sup>) increased the annual wool income from \$39 to \$381/ha respectively, roughly doubling the profitability of each hectare of marginal land (CSIRO 2016 - AWI Project 84099). The cost of establishment is a one-off expense and there is little ongoing cost.

Other benefits that these shrubs have been shown to provide include, shade and shelter for livestock (Revell *et al.* 2013, Masters *et al.* 2023), vitamin E (to maintain health, manage heat stress and improve shelf-life of meat, Pearce *et al.* 2010, Fancote *et al.* 2013) and plant secondary compounds which possibly reduce methane and inhibit the larval development of worms (Kotze *et al.* 2011). Perennial shrubs been shown to utilise out-of-season rainfall, lower water tables (reducing soil surface salinity) and provide a range of ecosystem services (Norman *et al.* 2008).

# 2. Objectives

Our objectives were to conduct collaborative research to deliver high-value shrub systems to improve utilisation of the summer/autumn/early winter feed base in Mediterranean and low rainfall mixed farming systems. We assumed that better and less expensive shrubs, with matched agronomy packages, will stimulate adoption by producers. We anticipate that the agronomy and grazing management guides that have been developed in this project will reduce risk, increase shrub biomass production/ha and provide further confidence for producers to adopt.

**Objective 1.** Collect data on sheep and/or cattle diet selection, productivity and economic data quantifying the opportunity to use saltbush and/or Mallee saltbush to provide nutrients that complement crop/pasture residues in summer and autumn. By identifying and working with 3 producer groups in areas where adoption of shrubs such as Anameka<sup>TM</sup> has been lower than

# expected, the studies will capture data describing the existing production system and barriers to shrub adoption.

#### This objective was successfully achieved.

Research sites to address the objective were established in 2019 on soils that were marginal for cropping on four commercial farms in Baandee (305 mm mean annual rainfall), Dongara (400 mm), Cranbrook (500 mm) and Cunderdin (458 mm). Two sites were saline valley floors (Baandee and Cunderdin), while the others were deep infertile white sands. Baandee, Cranbrook and Dongara were grazed with Merino sheep during summer/autumn 2021 and again in summer/autumn 2022. These sites include measurement of preferences amongst shrub genotypes, recovery from grazing and changes in liveweight and condition score of sheep. Data have been collected at 4 sites to describe and compare biomass availability, botanical composition (understorey and shrubs) and nutritional value of species.

We collected data comparing productivity, nutritional value, relative palatability, and recovery from grazing. We have compared the shrub genotypes across sites for all aspects of feeding value to identify priority genotypes for commercialisation. The shrub paddocks (predominantly Anameka<sup>™</sup> with nested experimental blocks of ~ 800 other saltbushes and Mallee saltbush) supported from 181 to 1025 sheep grazing days/ha in autumn, the time of year in Mediterranean climates when a tonne of green feed has up to 10 times the value of a tonne of green feed in spring. The design for the research site experiments only allowed one grazing event per year in autumn as the project was focussed on filling the autumn feed gap. It is probable that the sites could be grazed opportunistically at other times of the year to extract more value from the annual biomass when it is green. These sites could generate additional value if they provide an opportunity to improve twin lamb survival (the subject of another MLA investment).

**Objective 2** Complete on-farm shrub agronomy studies to identify and evaluate management practices that optimise profitability of shrub systems. These practices will include the use of adapted annual legumes in the understorey, application of nitrogen fertilisers and management of grazing intensity.

#### This objective was successfully achieved.

Adapted legumes (French serradella) were utilised on the deep sandy sites at Dongara and Cranbrook, while subterranean clover, burr medic, bladder clover and woolly clover were sown at Baandee. The project team conducted in-paddock fertiliser experiments at 3 locations, two in Dongara and one in Baandee. Due to complexity with variability in soil nitrogen across and within the soil profile, combined with deep rooted shrubs (>4m), we also conducted two controlled environment experiments in a glasshouse.

The impact of heavy grazing intensity on biomass production was tested at Baandee, where blocks of shrubs were excluded from grazing and the growth rates were compared to heavily grazed neighbouring shrubs. There was no difference in biomass a year after grazing. The three grazed research sites also provided data regarding recovery from grazing.

**Objective 3** Complete on-farm testing of 3 high-energy saltbush and 3 high-palatability Mallee saltbush seed lines that readily establish from seed, with a view to identifying and rapidly

commercialising the release of material that optimises profitability and has lower up-front establishment costs. These experiments will be conducted alongside experimental sites associated with outputs 1 and 2, so data can be used for sensitivity analysis in the economic models.

# The aims were successfully achieved as described but there is more work to be done to enable reliable direct seeding of elite shrubs on farms.

This objective focussed on barriers to on-farm direct seeding. Mature oldman saltbush and Mallee saltbush happily persist in harsh saline, infertile and/or semi-arid areas for many decades. Both however have very small seed that are very challenging to establish outside of a very well controlled nursery environment. This inability to readily establish is likely to be an ecological strategy to minimise competition at the site of the mother plant. To deliver this objective, we worked with a leading tree nursery and a group of farmers who specialise in shrub establishment from seed (Gillamii Centre). Prior to this project, success has been associated with establishment on sandy soils, with very specialised niche seeding machines, in areas with reliable summer rainfall.

A series of laboratory, nursery and field experiments were conducted to gain a better understanding of saltbush seed ecology, methods to determine seed fill in woody bracts, commercially viable methods to remove seed from bracts, responses to temperature and salinity during establishment (to identify the best time to sow) and impact of depth of burial on establishment. Using commercially sourced seed, we developed methods to coat the seed (to make it a suitable size for commercial sowing machines) and tested direct seeding with various growth promotors and insecticides. There was an issue with seed quality that constrained the work – however it has highlighted the importance of using a microscope to check for damage to seed after cleaning. With a view to selecting elite seed lines for commercialisation, we investigated bract fill and seed quality of the elite lines.

We have identified and tested several methods to remove seed from bracts and have a protocol for seed coating. The methodology for cleaning was refined to optimise flow and minimise damage to the exposed radicle. We have compared establishment of coated and naked seed in a commercial nursery. We sowed coated seed with added insecticides and growth promoters in Kellerberrin (2021), Cranbrook (2021) and on both a clay and sandy soil in York (2022 - at the break of season and again in winter). While we have made significant progress, there is more work to be done if we wish to establish oldman saltbush from seed in heavy soils or in areas with a dry and hot summer. It is our belief that there is a market for both nursery-raised material (much less risky but more expensive) and direct sown material (less expensive, less constrained by nursery capacity and can be taken to areas where cultivation is an issue but much riskier in many environments so a higher opportunity cost on the land unit).

#### **Objective 4** Completed two field walks at each of the 6 research sites, 3 print media stories, 'howto' guides for establishment and management of shrubs and 3 peer-reviewed publications. Data packaged for MLA training programs as required.

#### We surpassed our goals for this extension focussed objective.

This shrub work continues to generate significant producer, industry and media attention. Over four years we delivered 2068 face to face contacts (1040 producers and 1028 industry representatives) over 48 events in three states. We also delivered a Landline TV story and TV news stories, 9 radio interviews, three You-tube-style communication products (https://vimeo.com/733868413), 6

magazine articles (in Feedback and Beyond the Bale), 43 online or newspaper media articles and more than 30,700 social media views. The team worked with the Gillamii Centre and DPIRD to update and innovate 'Saltland Genie' (a salinity web app) and deliver three, two-day masterclasses in regional locations, attended by 72 producers and 29 industry representatives. Anameka<sup>™</sup> adoption continues to be strong with more than 6 million shrubs planted by 325 producers across southern Australia. We now have a nursery based in NSW who are producing up to half a million shrubs this season and we are increasing the communication and media activity in eastern Australia. Eight scientific publications have resulted from the research and three more are in the later stages of drafting. We worked with Gillamii Centre, Fitzgerald Biosphere group and Merredin and Districts Farm Improvement group to establish two MLA PDS projects, with activities on 10 farms. The shrubs are being utilised in the new MLA 'Shade and Shelter' projects as they could have significant impact on twin lamb survival. The team successfully gained Future Drought Fund support to establish 6 producer-scale Anameka<sup>™</sup> saltbush systems demonstrations in areas of NSW and WA where adoption is low, but the opportunity is high.

## 3. Methodology

### 3.1 Objective 1

This objective had three aims. The first was to demonstrate the potential of shrub systems on marginal soils and capture information regarding productivity, feeding value, grazing management and recovery from grazing to producers. The sites were a focus for field walks and communication events. Two of the three sites feature in case studies that were published in MLA's Feedback magazine (see appendix). The second aim was to compare Anameka<sup>™</sup> saltbush with other elite clones and saltbush seed lines that were derived from the clones. The final aim was to investigate the productivity and relative palatability of the Mallee saltbush lines that were selected for higher agronomic potential.

A limited number of forage species can tolerate the combined stress of salinity and arid conditions, but species from the Chenopodiaceae family, especially species of the *Atriplex* genera have gained a lot of interest due to their persistence in the dry climates, saline, and infertile soils, and their value as feed for livestock within these areas (Le Houerou 1992, Ben Salem *et al.* 2010; Norman *et al.* 2013).

#### 3.1.1 Site locations and characterisation

Three primary research sites were in Baandee (near Kellerberrin in the eastern wheatbelt), Dongara (northern wheatbelt) and Cranbrook (great southern zone) of Western Australia. An additional site was planted at the Cunderdin Agricultural College, some data on shrub growth and nutritional value were captured but it was not grazed due to departure of the staff member who championed the work, combined with COVID restrictions. All were planted on soils that were considered 'marginal' for cropping – the Baandee and Cunderdin sites were relatively saline, and the Dongara and Cranbrook sites were infertile sands. Other research sites were planted in Esperance, Spencers Brook and Yealering, however, COVID severely restricted ability to conduct timely measurement at Esperance, and the Spencers Brook and Yealering sites were accidently overgrazed during establishment.

The soils at the sites were sampled for analysis (CSBP, Bibra Lake, Western Australia). The Baandee site was on a historically over-grazed and compacted saline sandy loam (see Table 3.1). The Cranbrook and Dongara sites were situated on infertile deep white sands that are unprofitable to crop and have a tendency towards wind erosion in summer. The size of the sites was determined by the host producers (3.5 to 12.4 ha) and represented a 'marginal' land unit that was a likely target for shrub systems. The sites comprised an experimental area with a randomised replicated block planting (block number = 8 to 9) and each genotype was represented by four adjacent shrubs within the block. The sites were planted to the shrubs at commercial densities of between 833-952 plants/ha (Table 3.2). At the time of planting, they were ripped and mounded with a Chatfield's Tree Planter and shrubs were hand planted in single rows, with spacings of 2m between shrubs. The experimental shrubs were planted mechanically with the tree planter. Shrubs were nursery raised from seed or cuttings, with propagation 6 months prior to planting. The sites were planted to

shrubs on 16 July 2019 at Baandee, 28 August 2019 (Cranbrook), 27 July 2019 at Cunderdin and 7 July 2019 at Dongara.

Long-term average annual rainfall at the experimental sites ranged from 305mm (Baandee) to 499mm (Cranbrook, Fig. 3.1). Rainfall in the establishment year (2019) and the year following (2020) was well below the long-term mean at all sites, demonstrating the adaptation and resilience of the shrubs. This did however lead to some higher than anticipated shrub deaths at the two sandy sites as they had very poor water holding capacity. The Dongara site was impacted by an erosion event caused by a deteriorating tropical cyclone after planting. While there were some losses, the remaining shrubs, many with exposed roots, did remarkably well to survive. Fig. 3.2 shows a picture of a shrub with a root ball that dried out with the wind erosion event, and then regrew from the stem when it rained.

Trait	Unit		Site	
		Baandee	Cranbrook	Dongara
Gravel	%	5-10	0	0
Texture		1.5	1.5	1.5
Ammonium Nitrogen	mg/kg	4	2	5
Nitrate Nitrogen	mg/kg	8	4	24
Phosphorus Colwell	mg/kg	26	30	15
Potassium Colwell	mg/kg	22	< 15	36
Sulphur	mg/kg	30.4	5.0	6.7
Organic Carbon	%	0.87	0.52	0.77
Conductivity	dS/m	0.673	0.250	0.070
pH (CaCl2)		6.0	6.3	5.4
рН (Н2О)		6.7	6.9	6.2

#### Table 3.1 Soil characterisation data from the 3 primary shrub research sites.

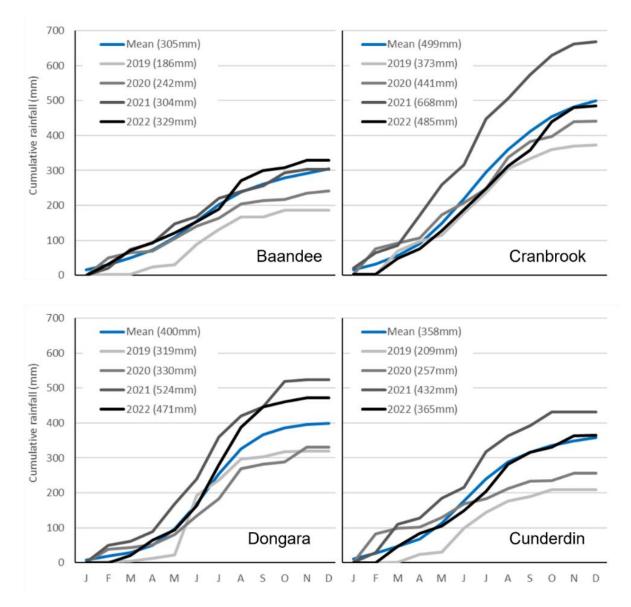


Fig. 3.1. Cumulative long-term mean and annual rainfall at the primary research sites. Note the establishment year (2019) and the next (2020) was well below the long-term mean at all sites.



Fig. 3.2. A saltbush plant from Dongara in 2020. Note the root growth from nodes above the root ball and the exposed shrubs in the background.



Fig. 3.3. The Baandee site at the time of planting in 2019 (top) and in 2022 (bottom).



Fig. 3.4. The Cranbrook site at the time of planting in 2019 (left) and in 2022 (right).



Fig. 3.5. The Dongara site at the time of planting in 2019 (top) and in 2021 (bottom).



Fig. 3.6. The Cunderdin site 3 months after planting in 2019 (left) and in 2021 (right).

#### 3.1.2 Grazing studies

Grazing studies were conducted with approval from the CSIRO Wildlife, Livestock and Laboratory Animal Ethics Committee (AEC# 2020-34), in compliance with the Australian Code of Practice for care and use of animals for scientific purposes (8th edition 2013). We utilised the host farmers commercial Merino flocks and all animals that were used in the experiment were healthy, vaccinated and given an oral drench before grazing. They were weighed and condition scored (using the method of Suiter 1994) before, after and during grazing.



Fig. 3.7. Selecting sheep for the autumn grazing experiment at Dongara in 2021.

#### 3.1.3 Plant measurements

Understory herbage mass and shrub biomass was measured at regular intervals over 4 years. Individual shrub biomass was assessed by the 'Adelaide technique' (Andrews *et al.* 1979). Herbage mass was estimated using visual estimation within quadrats, assessed every 5m along three transects within each paddock. The visual estimation was calibrated with 25 herbage mass cuts. Sward composition was estimated in each quadrat drop using the BOTANAL technique (t'Mannetje and Haydock 1963). Plant herbage samples from each legume species within each plot were collected through grab samples along three transects within plots at the same time herbage mass was measured.

Relative preference during grazing was determined through individual shrub defoliation scores where (0 = no evidence of defoliation to 5 = defoliated to sticks). Each defoliation unit corresponded to approximately 20% of the edible dry matter (EDM).



Fig. 3.8. Differences in relative palatability in the early stages of grazing at Baandee. Note the four heavily defoliated shrubs are the same genotype. CSIRO's Matt Wilmot pictured.

#### 3.1.4 Assessing nutritional value

Shrubs and understorey were sampled for *in vitro* nutritive value in spring and in summer/autumn each year, with the summer/autumn measurements corresponding to animal measurements. Three replicate blocks were sampled at each site, with each sample comprising 5 x 10cm tip cuttings from each of the four plants of the same genotypes within the replicate. (three samples per genotype per sampling date). The samples were dried at 65°C for 48 hours and ground to pass through a 1mm screen using a Tecator Cyclone© mill.

The plant nutritional value (NV) analyses; *in vitro* (pepsin-cellulase) dry matter digestibility (DMD), and organic matter digestibility (OMD), neutral detergent fibre (NDF), acid detergent fibre (ADF), crude protein (CP) and organic matter (OM) were estimated by near infrared spectroscopy (NIRS)

according to the methods described in Norman *et al.* (2020). Briefly, the methods used to build and test the NIRS calibrations included pepsin-cellulase in vitro DMD and OMD (in duplicate and calibrated with in vivo standards in each run). NDF and ADF were measured sequentially on the same samples using an Ankom 200/220 Fibre Analyser in accordance with the operating instructions for this equipment (Ankom ® Tech. Co. Fairport, NY, USA) and AFIA methods 1.8A and 1.9A (AFIA 2014). Total nitrogen (N) was determined by combustion using a Leco FP-428 N Analyser (Sweeney and Rexroad 1987) and CP was estimated by multiplying N by 6.25. Anions (nitrate, phosphate, oxalate, sulphate and chloride) were extracted with slight modifications of Cataldi *et al.* (2003) and analysed by high performance liquid chromatography (HPLC) using suppressed conductivity.

#### 3.1.5 Statistical analyses

Statistical analyses, including analysis of variance (NV data), REML (cross site comparison data), principal components analysis and regression were undertaken using GenStat (Version 21, Lawes Agricultural Trust, Rothamsted Experimental Station, UK). A significance level of 5 % (P< 0.05) was applied to all the statistical analyses.

#### 3.2 Objective 2

Objective 2 was addressed through a series of field and glasshouse experiments.

#### 3.2.1 Use of adapted annual legumes in the understorey

Each of the research sites was under sown with an appropriate annual legume. At the two sandy sites, this was French Serradella (cv. Margurita). At Baandee a greater number of legumes were potentially adapted to the site, so subterranean clover (cv. Mawson), bladder clover (cv. Bartolo) and burr medic (cv. Cavalier) were sown. All legumes were sown at the break of season using the best-practice agronomic recommendations.



Fig. 3.9. Baandee site after sowing annual legumes in the understorey in 2021 (top) and the French serradella at Cranbrook.

# **3.2.2** Difference in salt tolerance between Mallee saltbush and saltbush - glasshouse experiment 1.

#### With MSc student Aslak Christensen (University of Copenhagen)

While oldman saltbush and Mallee saltbush both belong to the *Chenopodiaceae* family, little was known about the salt tolerance of Mallee saltbush prior to this project. Given the need for perennials to be adapted to soils that are marginal for cropping, there was some urgency to get a greater understanding of the salt tolerance of Mallee saltbush to support extension messages.

The effect of salinity on plants is primarily due to water stress/osmotic stress causing stunted growth because of a decrease in the osmotic potential of the soil solution. The negative growth effects of salinity can also be caused by toxic concentrations of specific ions such as Na+ and Cl-, and these might also result in nutritional imbalances of other minerals. This has significant implications for grazing animals. Reduced growth due to salinity stress occurs in two phases with osmotic stress

being rapidly induced and followed by the accumulation of ions at toxic levels. Shoot growth is generally more severely affected than root growth.

The aim of this study was first to determine the salinity tolerance of the clonal cuttings of Anameka<sup>TM</sup> and a genotype of Mallee saltbush, and the resultant biomass growth at different salinity levels. The second aim was to investigate the impact of increasing salinity on the nutritive value of the biomass. It was hypothesized that the clonal cuttings of both species will survive and maintain growth under salinities ranging from 0 - 500 mM (~seawater salinity), but that the reduction in biomass associated with increased salinity level will vary between species. An additional hypothesis was that the nutritive value of the biomass will be reduced as salinity increases due to a higher ash and oxalate content in the shoot DM.

#### Plants, potting media and pre-experimental management

This glasshouse salinity experiment was undertaken with 50 nursery raised seedlings propagated from vegetative cuttings of oldman saltbush cv. Anameka<sup>™</sup> and cuttings taken from a selected genotype of Mallee saltbush acc. Cln 40. Seedlings where approximately 15 cm tall and four months old at initiation of the experiment.

Seedlings were grown for 30 days in a glasshouse under controlled conditions and organized in a complete randomized block design. Blocks were moved around during the experiment to avoid any edge effect and pots were spaced further apart as the seedling grew larger to avoid shading. The soil mixture was an infertile coarse sand with low clay/silt content, a neutral pH (CaCl<sub>2</sub>) of 6.6 and a low salinity (conductivity 0.14 dS/m), 15 mg kg- of nitrogen (urea/ammonium), 111 mg kg- potassium, 65 mg kg- S and 17.5 mg kg- of P. Clonal cuttings were planted in free-draining pots of 3.4 L (height: 23.5 cm and width: 13.65 cm) containing 2.8  $\pm$  0.02 kg of soil, and the bottom of the pots were fitted with a thin sheet of cloth to cover the drainage holes, still allowing water to drain but keeping soil in the pots. The water holding capacity of the pots was 1.09  $\pm$ 0.01 kg.

The daily temperatures ranged on average from 13.1 - 24.8 °C over the experiment and the humidity ranged from 40 - 85 % (Fig. 3.10), with a photoperiod of roughly 12 hours of light and dark. Clonal cuttings were transferred to pots in the greenhouse 10 days before initiation of the experiment, to acclimatize the plants.

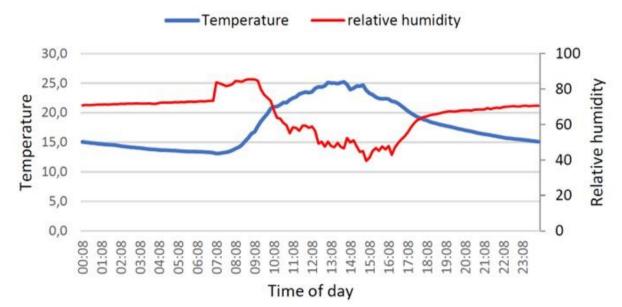


Fig. 3.10. Mean daily glasshouse temperature and humidity during the experiment.

#### **Glasshouse treatments**

Plants were exposed to five 5 NaCl salinity treatments (0, 50, 100, 200 and 500 mM NaCl), with 10 seedlings at each treatment for both species. This corresponded to EC values of 0.8, 7.15, 13.5, 22.1 and 53.9). Plants were irrigated every day, with 300 mL of salt solution per pot, to provide sufficient leaching and counter accumulation of salts above the desired level throughout the experiment. A nutrient mixture similar to 1/4 Hoagland solution was added to the salt solution to avoid acidification of the soils due to leaching of base cations by the high volume of leaching water. Plants were harvested 30 days after initiation of the salt treatments. Seedlings were cut at the root base and stem and leaf fresh biomass was immediately sorted and weighed. Stems and leaves were dried in an oven at 70 °C for 48 hours to determine dry weights. Laboratory analyses were performed for factors impacting on nutritional value of biomass (as per 3.1.4 methodology).

ANOVA was used to determine the significance of difference between treatments in the shoot dry matter yield, feed analysis parameters, content of anions and the effect on the mineral profile. A significance level of 5 % (P< 0.05) was applied to all of the statistical analyses. Correlation analysis was used to assess the interaction among the different parameters with increasing salinity level in the watering solution and in the analysis of feed value parameters.



Fig. 3.11. Pots in the glasshouse at the start of the experiment

# **3.2.3** Impact of salinity and nitrogen fertilisers on growth and nutritional value of saltbush - glasshouse experiment 2.

A second glasshouse experiment was conducted testing the impact of nitrogen fertiliser on saltbush growth and how this interacts with salinity. The objective of this experiment was to assess the influence of combinations of N and salt (as NaCl) concentrations on growth and nutritional value, in a glasshouse. We focussed on saltbush in this experiment as the Mallee saltbush is less widely planted. The addition of N has been shown to lead to a significant growth response (Al Daini *et al.* 2013, Eissa and Ahmed 2016), however no studies show the optimal level of N for growth. Our first

hypothesis was that increasing NaCl in the soil solution will decrease biomass growth while increasing N will increase shoot growth rates.

In response to increasing salt, halophytes like saltbush typically osmotically adjust the cytoplasm of leaf cells through the synthesis of N containing 'compatible solutes' (Flowers and Colmer 2008). For example, *Atriplex spongiosa* F. Muell. had a 2.3-fold increase in the concentration of glycine betaine (containing 12.0% N) in leaves as the salinity increased from 50 to 750 mM NaCl (Storey and WynJones 1979). Our final hypothesis is that saltbush plants subject to higher levels of salinity will have a greater response to N than those with less saline conditions.

#### Plants, potting media and pre-experimental management

Anameka<sup>™</sup> saltbush was cloned from cuttings by a commercial nursery (Chatfield's Tree Nursery, Tammin, WA). Cuttings were grown in decomposed pine-bark medium in 4x4x8 cm seedling trays for 6 months and then planted up into 15 cm round pots. Six months later, seedlings were moved into 30 L rectangular pots. Pots were filled with a low nutrient sandy potting mix. Plants were provided with non-limiting fresh water three times a week until they were two years old when experimental treatments were applied. All shrubs were pruned occasionally to maintain them below 50cm in height.

The plants were supplied 15g of a complete slow-release fertiliser (Scotts Osmocote plus trace elements potted plants, Evergreen Garden Care Australia Pty Ltd, Bella Vista, NSW, Australia) at three points over the two years, however the soil medium and shrubs were very nutrient poor at the time of treatment implementation. This management was imposed to represent mature shrubs growing on the nutrient poor sandy soils of WA (a significant niche for shrub planting). Prior to starting the experiment 400 ml of leachate was collected from 4 pots which had been watered with distilled water. The leachate was tested for a range of nutrients and found to contain such minimal nutrition it would offer no background interference to the experiment.

At the start of the experiment all plants were sorted by size, the largest and smallest plants were discarded, and remaining plants were stratified so each treatment was assigned to four replicates that had a similar mean size. 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.

#### **Glasshouse treatments**

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 ration of NO3 and NH4. All 15 treatments had a nutrient solution base designed for Australian native shrubs (mM): 3.0 CaCl2, 2.0 MgSO4, 0.2 KH2PO4, 4.0 KCl, 0.1 Ethylenediamine-N,N'-bis(2-hydroxyphenylacetic acid) ferric sodium complex (EDDHA-FeNa) and ( $\mu$ M): 4.6 H3BO3, 0.5 MnSO4, 0.2 ZnSO4, 0.2 CuSO4, 0.1 Na2MoO4. The nutrient solution was adjusted to pH 6.1, using KOH.

Each potted shrub received 2 L of nutrient solution, three times weekly. This amount was determined to allow pots to be fully flushed with fresh solution avoiding any accumulation of salts in the soil. This was verified by measuring the EC (Hanna Combo HI 98129) in the nutrient solutions and comparing it to leachate collected between 20 and 50 minutes after watering. Leachate values were similar to nutrient solutions. To avoid nutrient or salinity shock to the plants at high treatment levels

all plants were stepped up to treatment-strength nutrient solution. Pre-treatment was one week at 25 % of treatment solution and one week at 50 % solution.

#### Plant growth measures

On the day experimental treatments started and every 14 days afterwards, plant growth was measured non-destructively. Each pot was photographed from each side using a photobooth in the glasshouse which allowed for the photos to be taken under identical conditions each time. A green area index was determined by analysed the photos using the Canopeo green canopy image analysis tool (Patrignani and Ochsner 2015) within Matlab (Mathworks, Inc.Natick, MA). Green area index from images taken on the day of harvest were correlated to measured leaf area and dry mass. There was a high positive linear correlation (r2 =0.94 and r2 =0.96 for leaf dry mass and leaf area respectively) and so this relationship was used to calculate individual plant increases in estimated leaf area and biomass accumulation over the period of the experiment.

Leaf thickness and succulence were determined on fully expanded leaves after seven weeks of treatment. Ten representative fully-expanded leaves were collected from each plant and transported directly to the laboratory for processing. Leaves were cut in small batches and processed immediately precluding the need for rehydration in the lab (Garnier *et al.* 2001). Fresh weights and leaf area (LA; leaf area meter Li-Cor LI-3100, Lincoln, NE, USA) were determined and then leaves dried at 60 C for 48 h so dry weight could be measured. From these measurements the specific leaf area (SLA, the ratio of leaf area to leaf dry mass) and leaf dry matter content (LDMC, the ratio of leaf dry mass). Leaf thickness (LT) was then estimated flowing the methods of Vile et. al. (2005; LT = (SLA x LDMC)-1). Leaf succulence (LS) was estimated by dividing leaf water content by leaf surface area LS = (FW-DW)/(LA) (Mantovani 1999).

#### Harvest and biomass measurements

All plants were destructively harvested, seven weeks after the start of full-strength nutrient treatments. Immediately prior to harvesting, plants were photographed so all harvested biomass measures could be correlated with green area index determined by image analysis (see above). To harvest, the main stem was severed at the soil surface. Any lower leaves and stems which were sandy or may have had nutrient solution poured onto them during watering were rinsed free of salts dried to the plant surface or adhered Na+. The wash solution contained 5 mM CaSO4 and was osmotically balanced to the three salinity treatments using sorbitol (Negrão *et al.* 2017).

The soil was gently shaken from the roots and the roots subsampled for root tissue > 2 mm. Root samples were then washed in the same osmotically balanced solutions, patted dry with paper towel and immediately frozen prior to freeze drying for chemical analysis.

The above ground biomass was sorted into EDM (leaves and small, soft green stems < 3mm diameter) and woody stems (remaining stem material < 3 mm diameter). Green leaf area was determined using a leaf area meter, (Li-Cor LI-3100, Lincoln, NE, USA). A subsample of each type of plant material was immediately frozen prior to freeze drying and the remainder oven dried for determining total dry weight. Samples were processed and analysed according to the methods described in section 3.1.4.



Fig. 3.12. Saltbushes in the glasshouse and the 15 stock solutions.

#### 3.2.4 Application of nitrogen and/or phosphate fertilisers – field experiment 1

Fertiliser treatments were applied to a subset of Anameka<sup>™</sup> saltbushes at two sites in Dongara in spring 2019 and spring 2020. Each site consisted of 4 replicated blocks with 10 adjacent Anameka<sup>™</sup> shrubs applied the same treatment within the block. Treatments included calcium nitrate, superphosphate, both fertilisers together and an unfertilised control. Calcium nitrate was applied at the rate of 60g/shrub and superphosphate at the rate of 70g/shrub. This equated to approximately 100-120 kg/ha for each fertiliser. Each shrub was fertilised by hand with the fertiliser sprinkled over the soil under the shrub canopy. Biomass was sampled for nutritional value and analysed according to the methods described in the 3.1.4 methodology section. Data were analysed using ANOVA in GenStat 19.



Fig. 3.13. The nitrogen response experimental site in Dongara (property of Craig Forsyth).

#### 3.2.5 Application of nitrogen fertilisers at different rates – field experiment 2

To better understand the N rate responses, fertiliser treatments were applied to Anameka<sup>™</sup> shrubs at Baandee in June 2021. The treatments included five rates of calcium nitrate application (equivalent to 0, 20, 40, 60, 80 kg/ha), across six replicated blocks with 10 replicate shrubs for each treatment per block. Biomass production was assessed in October 2021 and again in February 2022. Biomass was sampled for nutritional value in October 2021. Shrubs within blocks were sampled (10 cm tip cuttings) and bulked so that there were six samples for each treatment. The samples were analysed for nutritional traits and anions as described in the 3.1.4 methodology section.

### 3.3 Objective 3

The low establishment rate of saltbush when direct-seeded is attributed to inhibitory effects of the bracteoles (often called bracts, pericarps or fruits) surrounding the seeds. The bracts can range in form and characteristics from being thin and membranous to heavy lignified structures, and they are thought to regulate the timing of germination and assist in dispersal of the seeds (Garvin and Meyer, 2003). We hypothesise that the light, woody bracts allow for dispersal by water during unusually wet seasons, depositing the seeds in a moist, favourable location for establishment. The exact mechanism for the inhibitory effect has shown to vary between species, from controlling the timing of water imbibition, mechanical inhibitory effects of salts in the bracts and possibly chemical compounds in the bracts delaying germination (Ungar and Khan, 2001)

Several studies have shown that bract removal increases germination rate and total germinated seeds under laboratory conditions (Stevens *et al.* 2006). Additionally, many of the bracts don't contain fully developed seeds, which further reduce the establishment rate when using seeds within bracts. It is incredibly difficult for farmers to assess the number of seeds that are in a bract sample, as they require careful dissection. It is not uncommon for farmers to attempt to plant bracts with poor seed fill.

Unfortunately, simply removing the bract does not improve field establishment. The lack of success when sowing cleaned seeds of oldman saltbush is, at least partly, due to a lack of knowledge on the requirements for optimal germination. It could also be due to the small seed size and the consequent need for superficial sowing depths, making the seed prone to drought if there is a lack of follow up rain after germination (Barret-Lennard *et al.* 1991). The seeds are very laborious and time-consuming to collect and clean, and therefore very costly to obtain. There is a need for knowledge on optimal germination conditions, to make good use of the seed available.

Objective 3 was addressed over a series of sequential experiments.

#### 3.3.1 Seed morphometric analysis and viability tests

In the first study we performed a morphometric analysis of bracts and seeds to assess if current seed processing techniques can be optimised, or if new approaches need to be developed to improve the quality of oldman saltbush seed batches. A preliminary quality assessment was performed using an x-ray scan, to determine average seed fill of a batch of intact bracts from a population (Fig. 3.14 and

3.15). These were divided in two subsamples based on size: <3.15 mm and >3.15mm. Five replica of 25 bracts of each subsample were tested in the x-ray. Where the x-ray image was not clear enough to determine the presence of a seed, the bracts were subjected to a cut test. In-depth seed morphometric analysis was performed to answer the following questions: How effective is x-ray to assess seed fill? Does bract size/weight have effect on seed fill? Does the bract or seed size/weight have effect on seed fill? Does the bract or seed size/weight have effect on seed fill? Does the bract or seed size/weight have effect on seed fill? Does the bract or seed size/weight have effect on seed fill? Does the bract or seed size/weight have effect on seed fill? Does the bract or seed size/weight have effect on seed fill? Does the bract or seed size/weight have effect on seed fill? Does the bract or seed size/weight have effect on seed fill? Does the bract or seed size/weight have effect on seed fill? Does the bract or seed size/weight have effect on seed fill? Does the bract or seed size/weight have effect on seed fill? Does the bract or seed size/weight have effect on seed fill? Does the bract or seed size/weight have effect on seed fill?

The experiment was performed according to the following procedure. Weights were determined for 100 bracts randomly selected from the batch. Each bract was placed on 10x10 mm grid and area was recorded. The bracts were x-rayed to assess seed fill and recorded as filled, empty or unknown. Each was then dissected to determine the presence or absence of a seed and the seed was weighed. Seeds were placed on 10x10 grid on corresponding place to record size. They were then transferred to agar at a corresponding position (according to grid) for germination testing. A time lapse camera recording a frame every 10 minutes was used to obtain a germination time for each seed.

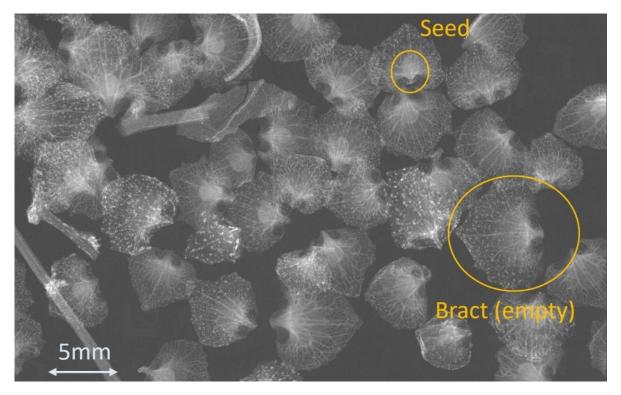


Fig. 3.14. An x-ray of woody saltbush bracts showing some with seed while others are empty.

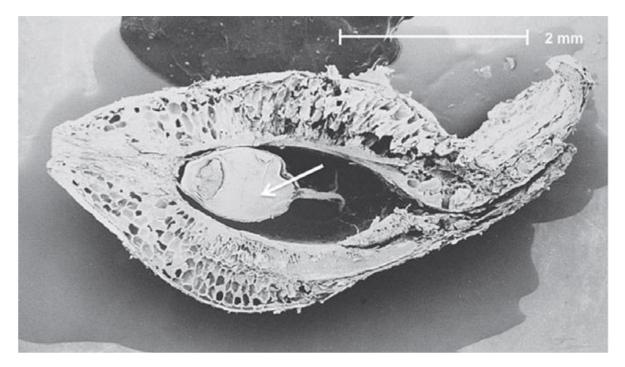


Fig. 3.15. Cross-section of an Atriplex fruit (scanning electron micrograph image). The seed (arrow) is enclosed between two large bracts. There is also a substantial air-filled pore enclosed by the bracts and surrounding the seed. *Reproduced from Barrett-Lennard et al. (2016), © 2016 Society for Ecological Restoration, Wiley.* 



Fig. 3.16. Images showing the fruits and seeds of Mallee saltbush (top) and oldman saltbush (bottom).

# **3.3.2** Removing seeds from fruits/bracts and bract pre-treatments to improve processing efficiency while not inhibiting germination

Two experiments were performed to test novel seed processing techniques that could improve the efficiency of debracting and determine if such treatments had any effect on seed viability. Methods that were tested included hand processing (rubbing bracts between two corrugated rubber sheets), a Venables pasture seed cleaner and a Kimseed serradella dehulling machine.

Two research questions were addressed. Do pre-treatments improve efficiency of mechanical extraction of oldman saltbush seed from the bract? Do pre-treatments improve or inhibit germination of cleaned seed compare to untreated cleaned control? Two sets of different pre-treatments were evaluated, in the first water is used in different settings (still water or flowing water), and in the second acid and enzymes were used.

#### Water pre-treatment

Does imbibition associated with the high salt content (up to 42%) of bracts affect germination? Are compounds leaching from the bract in the water affecting germination? This experiment was performed with two controls (bract without treatment and seeds without treatment) and three treatments (bract in still water, bract in flowing water and seeds in still water, Fig. 3.17). Each treatment was performed on 4 different imbibition times: 6 min, 1 h, 6 h and 12 h.



Fig. 3.17. Bracts in still (left) and flowing (right) water in the water pretreatment experiment.

#### Acid and enzymes pre-treatment

In this experiment, untreated seeds were used as control and four treatments were performed: bracts in cellulose solution (CL), bracts in Pectinase (PN), bracts in Hydrochloric acid 36% (HA) and bracts in Sulphuric acid 20% (SA). Each treatment was performed on four different imbibition times: 6 min, 1 h, 6 h and 12 h. After each treatment the bracts were rinsed in water. For acid treatments, bracts were also immersed in a neutralising basic solution for 5 min, following previously described methodologies (Stevens *et al.* 2006; Pedrini *et al.* 2018). For both experiments, after the treatment, seeds were dried in a food dehydrator at 35°C for 3 hours.

#### Seed extraction efficiency testing

To evaluate how a treatment could improve seed extraction, treated and dried bracts where manually processed with rubber mat and customised (Venable) hand sander lined with rubber to simulate the action of a seed thresher and a commercially available Kimseed dehuller. One gram of pre-treated and dried bracts was rubbed between the mat and hand sander for 30 seconds (Fig. 3.18). Using sieves and airflow the cleaned seed are removed and counted. The uncleaned fraction is then threshed again for one minute and the resulting cleaned seeds separated and counted. For the larger machines, 100 g of dried bract was passed through the machine at a wide setting, using sieves to separate out cleaned seed from remaining bracts which were then subsequentially passed through smaller settings on the machinery (Fig. 3.18). All cleaned seed was counted, assessed via microscopy for damage and germination tested.

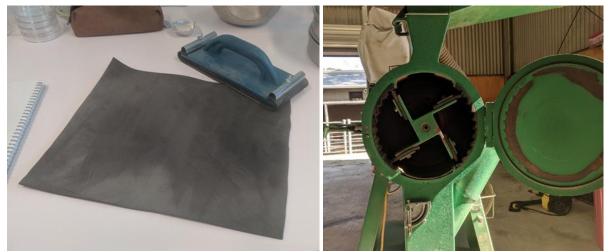


Fig. 3.18. Rubber mat and Venable seed cleaner. The proportion between the seed cleaned in the first 30 second and seed cleaned in the subsequent minute is used as indication of extraction efficiency.

#### **Germination tests**

Standard germination test was performed on filter paper, imbibed with 14 ml of water in 90mm petri dishes, sealed in glad wrap, with five replicates of 30 seeds per treatment. Seeds were incubated at 15°C, 12 hours light/dark cycle. Germination was scored twice a day for the first three days and then daily for eight days.

#### 3.3.3 Does seed size/weight affect germination?

There are considerable differences in seed mass between the elite seed lines. In addition, seeds can be screened to remove the smallest seeds from a batch if small seeds are problematic. This experiment investigated the optimal seed size for germination and seedling development. Based on plant ecology literature (Black 1958), seed mass is expected to be positively correlated with total germination percentage and rate of healthy seedling development. This is because bigger seeds tend to have greater energy resources.

Seeds of oldman saltbush and Mallee saltbush were collected and cleaned. Seeds from a naturalised population of oldman saltbush were collected in January 2019 from a farmer's field in Cranbrook

WA, dried (40 °C for three days) and stored for later use. Pure seed was obtained by removing the bracts enclosing the seed by gently rubbing the intact, dried fruits between corrugated rubber matting to obtain clean and undamaged seed. Seeds from a population of Mallee saltbush were collected on the 18 April 2019 from an experimental plot at CSIRO in Floreat WA. The fleshy fruits containing the seed (Fig. 3.16) were macerated and washed through sieves of different sizes to obtain only the clean seed, which was then dried (40 °C for three days), cleaned and vacuum packed.

All germination experiments were performed according to the ISTA germination test (ISTA, 2011). Four replicates of 100 randomly selected seeds were set to germinate in germination trays called the "Jacobsen apparatus" (Fig. 3.19). The apparatus consists of a transparent box with a germination plate inside upon which filter paper substrates with seeds are placed. The substrate is kept continuously moist by means of a wick, which extends down into the underlying water bath. Seeds were spaced uniformly and adequately apart on the filter paper substrate. This setup ensures appropriate oxygen and water availability during the course of the experiment (ISTA, 2011).

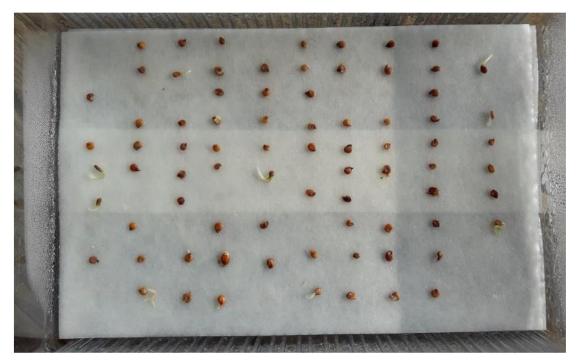


Fig. 3.19. Germinating seeds of oldman saltbush in a Jacobsen apparatus. In the middle can be seen the wick that extends down into the water bath below the germination plate.

All the germination tests were conducted in plant growth chambers (Conviron, controlled environment systems) with 12 hours of light (6 am to 6 pm), and 12 hours of darkness. The light source provided white light imitating natural light conditions with a photon flux density of 342  $\mu$ mol/m<sup>2</sup>/s of PAR (photosynthetic active radiation, 400 to 700 nm). Germinated seeds were counted and removed three times a day during the light period for all replicates, and all experiments lasted for roughly a month.

Seeds were sorted into size classes using metal hand sieves with round holes ranging from 0.75 mm, 1 mm, 1.25 mm, 1.5 mm, 1.75 mm, and 2 mm. Sieves were placed on top of each other and then shaken with the seed on top for five min, to divide them into different size fractions. Each individual sieve was thoroughly cleaned to obtain all seeds, which were then counted, and the 100-seed weight recorded. The correlation between individual seed mass/weight and hours to germination

and the subsequent development of seedlings was examined by weighing 300 individual seeds of oldman saltbush and Mallee saltbush on an analytical scale where the weight was recorded in mg with three decimals. The weighed seeds were then placed in germination trays and their position on the filter paper noted, and germination trays were placed in plant growth chambers at 10 °C under a light regime of 12 hours of light and 12 hours of dark. When seeds germinated, they were left in the germination tray and were not removed before a healthy seedling had developed (Fig. 3.20).

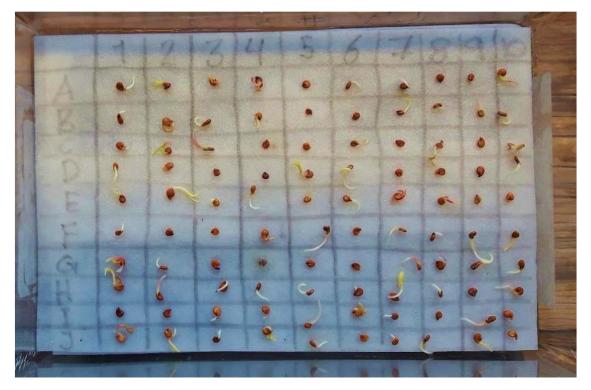


Fig. 3.20. Individually weighed seeds of oldman saltbush placed on a filter paper for germination, that was divided into cells to keep track of each individual seed.

Free draining pots were filled with sand (particle size: 0.4 - 0.9 mm), and 25 holes of five mm depth were made, and seeds gently placed within and covered with sand. The pots were then placed in a plant growth chamber at 10 °C with 12 hours of light and 12 hours of dark. The pots were checked once a day for emerged seedlings during the light period and pots were irrigated from below to ensure sufficient water availability continuously during the experiment. A seedling was characterised as fully developed when the cotyledons had fully emerged.

Statistical analyses were performed using R version 3.5.3 (http://www.R-project.org). The seed germination data was modelled using a cumulative distribution model function of the standard log-logistic distribution.

#### 3.3.4 Impact of temperature and salinity on seed germination rates

The aim of these experiments was to examine the germination responses of oldman saltbush and Mallee saltbush under a range of temperatures and salinities to ascertain the optimal conditions for successful establishment by direct seeding. It is hypothesized that seeds of both species will have optimal germination temperatures at around 10-15 °C in non-saline conditions, similar to the conditions in the wet winter months in Australia, where these species are naturalised.

All germination experiments were performed according to the ISTA germination test (ISTA, 2011). 4 replicates of 100 randomly selected seeds were set to germinate in Jacobsen apparatus. Seeds were spaced uniformly and adequately apart on the filter paper substrate. All the germination tests were conducted in plant growth chambers (Conviron, controlled environment systems) with 12 hours of light (6 am – 6 pm), and 12 hours of darkness. The light source provided white light imitating natural light conditions with a photon flux density of 342  $\mu$ mol/m2/s of PAR (photosynthetic active radiation, 400 to 700 nm).

Four replicates of 100 seed of both species were set to germinate in plant growth chambers under four constant temperature regimes of 5 °C, 10 °C, 20 °C, and 30 °C, at 12 hours of light and 12 hours of dark. The selected temperatures were intended to illustrate the range of temperatures the seeds would experience over the course of the year in WA.

Seeds were placed under five different salinities of sodium chloride solution: 0 mM, 50 mM, 100, mM, 200 mM, and 500 mM, to access the salinity tolerance of the seeds upon germination and the effect of increasing salinity on germination percentage and rate. For each salinity level and species four replicates of 100 seeds were used and placed into plant growth chambers for germination at 10 °C with 12 hours of light and 12 hours of darkness. Seeds were counted three times a day during the light period and germinated seeds removed. A total of 8 replicates of 100 seeds of each species was germinated under each of the five salinity levels.

Statistical analyses were performed using R version 3.5.3 (http://www.R-project.org). Analysis of dose-response curves were described with a three-parameter asymmetric sigmoid Weibull curve, by use of the add-on package drc (Ritz and Streibig, 2005). The seed germination data was modelled using a cumulative distribution model function of the standard log-logistic distribution.

#### 3.3.5 Seed coating for glasshouse and field experiments

Clean saltbush seed (250g) was purchased from a commercial supplier for the direct seeding experiments as the team were unable to collect from the field due to COVID travel restrictions at the time in WA. Coating was performed by Dr Simon Pedrini, a seed ecologist and coating expert from Curtin University (Fig. 3.21). The seed coating treatments included,

- clay coating only,
- clay coating + a health promoting compound (salicylic acid),
- clay coating + a residual pesticide,
- clay coating + a health promoting compound + a residual pesticide,
- control naked seed, and
- control unprocessed seed in bract.



Fig. 3.21. Coated and uncoated seeds, ready for the experiments.

#### 3.3.6 Impact of sowing depth on seedling emergence

In preparation for field seeding, a sowing depth experiment was conducted in the glasshouse. Saltbush seeds are incredibly small so sowing depth is critical as they do not have sufficient energy reserves to emerge from depth. If they are sown too shallow, the seedling is at greater risk of desiccation. If sown too deep, the plant cannot emerge. In the experiment, we compared emergence rate and success from the following treatments,

- coated seed on the soil surface,
- coated seed sown at 1mm depth,
- coated seed sown at 5mm depth,
- coated seed sown at 10mm depth,
- coated seed sown at 20mm depth, and
- naked seed sown at 1mm depth.

The coated and naked seeds (n=100) were the same as the ones used in the previous experiment. They were carefully planted at the prescribed depths in three replicate trays per treatment. The trays were watered with fine misters twice per day for a month and emerged seedlings were counted daily (Fig. 3.22).



Fig. 3.22. Depth of sowing experiment designed to identify an optimal sowing depth for the field experiment. The photos on the right show the 20 mm sowing depth (before covering) and the soil surface sowing treatment. CSIRO's Josh Hendry pictured.

#### 3.3.7 Comparing coated and 'naked' seeds in a commercial nursery

Coated and naked seed were planted in a commercial nursery (Chatfield Tree Nursery, WA) in summer 2021. The seeds (n=3456) were planted with a robotic planter with a setting of one seed per cell. Trays contained 72 cells and there were 24 trays planed for both naked and coated seed (seed originated from the same batch). Seeds were planted at 1mm depth into a commercial seed raising mix. They were watered immediately and placed onto a bench in the nursery in a randomised design. After three months of growth under nursery conditions, seedlings were graded and counted.

### 3.3.8 Seed coating and field emergence

Previous work suggests that naked seeds do not establish under 'real world' conditions. The coating has been designed to offer the protection of the bract and allow for shallow burial as they can be placed on the surface in a furrow. Saltbush seeds are very small and so there will be a trade-off between depth, emergence and plant establishment. Understanding the influence of various management (depth, coatings, sowing method) and abiotic (soil moisture and temperature) factors on seed germination, emergence and establishment is important if we are to achieve successful field sown shrubs.

In 2021, we used precision cereal sowing equipment to sow saltbush seeds (naked, with various coatings and in bract) in the wheatbelt (Kellerberrin). The 2ha site comprised a non-saline sandy

loam soil and each treatment was replicated in 6 blocks with 20m sowing runs. Seeds were placed at a depth of 5mm. Seedlings (from the same seed line) were planted as a control.

In 2001, we also tested the seed coating treatments using the niche seeding methodology used in Cranbrook. At this site we dropped the seed on the surface of the niche created by the seeder and covered the seed with either vermiculite (industry practice and expensive) or just white sand (to simulate burial at 5mm). The site was incredibly wet due to excessive rainfall, so seeding was delayed until late September. This delay could have impacted survival as seeds had less time to establish roots prior to the hot and dry summer conditions.



Fig. 3.23. A bract emerging from the saltbush establishment experiment at Cranbrook in 2021.

A second generation of field trials were hand sown in May and June of 2022 to test the emergence and establishment of seedlings from the various seed coating treatments, plus naked and seed in bract. Trials were sown into two paddocks near York, one a heavy clay loam and the second a grey sand. The experimental layout was a split plot design with paired 1 m rows of 10 mm and 1 mm sowing depths (surface sown seed lightly covered with sand to prevent predation/blowing away). Fifty seeds were hand sown, and all treatments replicated six times. Emergence was monitored at 4 and 8-10 weeks. Soil and air temperature and local rainfall were logged at each site.

#### 3.3.9 Seed and bract characteristics of the elite seed lines

While commercial seed was purchased for the sowing experiments, we maintained a focus on the characteristics of seed and bract collected from the selected saltbush genotypes. This was to ensure genetic gains in nutritional value (the trait that has the most influence on profitability) were not lost. The female clones were established with alternate rows of the high nutritional value male (clone H) in a 5ha block in Tammin, Western Australia. In January 2023, bracts from 50 female plants per clonal line were collected, bulked and subject to a range of tests. This included measurement of

mean bract dry weight, seed fill, seed mass and seed germinability. All tests utilised four, randomly selected 100-unit replicate samples. This seed is currently undergoing laboratory screening for ability to tolerate moisture deficits during germination (June 2023). It is also being coated for further field establishment research.

# 3.4 Objective 4

Objective 4 concerned the packaging and dissemination of information about the project and the use of shrubs to fill feed gaps. It was severely interrupted by COVID travel restrictions however the team found creative ways to meet milestones and had a significant push in the final year of the project.

# 4 Results and Discussion

# 4.3 Objective 1

#### 4.3.1 Grazing studies

The three research sites were all grazed during the summer/autumn of 2021 and 2022. This is the time of year when feed is scarce and has its highest value to the farming system. At Dongara, an adjacent crop stubble was opportunistically grazed at the same time with the same class of sheep. Data regarding timing of grazing, stock numbers and sheep productivity are presented in Table 4.1. Data are also presented for mean biomass availability. Understorey sward composition of the sites is presented in Tables 4.2 A-C.

#### Autumn 2021

2020 was an incredibly dry season in Western Australia, so saltbush and understorey biomass for grazing in Autumn 2021 was unlikely to have been optimised. The sites supported 30 to 50 days of grazing during late summer and autumn at 8.5 to 13.5 sheep/ha.

The site at Cranbrook was grazed with weaner ewes  $(31.5 \pm 0.66 \text{ kg})$  liveweight and condition score, CS,  $2.1 \pm 0.03$ ) from 1 Mar until the 20 April (50 days) at a density of 8.6 sheep/ha (430 grazing days/ha). The ewes gained 40 g/head.day of liveweight and a CS increase of 0.77. This was facilitated by 4068 kg/ha of dry understorey feed on offer (FOO) and 499 kg/ha of shrub edible dry matter (EDM) (Fig. 4.1).



Fig. 4.1. Merino ewes grazing the research site at Cranbrook in April 2021.

The site at Baandee was grazed with two-year-old ewes ( $67.6 \pm 0.79$  kg liveweight and CS  $2.7 \pm 0.05$ ) from 23 Mar until the 22 April (30 days) at 13.5 sheep/ha (405 grazing days/ha). The ewes gained 20 g/head.day of liveweight and a CS increase of 0.5. This was facilitated by 1227 kg/ha of dry understorey FOO and 454 kg/ha of shrub EDM. Given the incredibly poor quality of the understorey (with DMD values of <35% and CP <7.5%), modest growth was a good achievement. The host producers were happy with the result, especially given the marginal value of the soils (Fig. 4.2).



Fig. 4.2. Merino ewes grazing the research site at Baandee in March 2021.

Dongara was grazed with two-year-old ewes ( $63.4 \pm 0.7$  kg liveweight and CS  $2.6 \pm 0.02$ ) from 24 Mar until the 28 April (35 days) at 8.5 sheep/ha (298 grazing days/ha). Unfortunately, the ewes were shorn during grazing at short notice and the team were unable to get to the site to capture wool weights. The measured mean liveweight loss (98 g/head.day) is likely to be associated with wool removal. The host producer averaged 4.5 kg/head of wool production across the flock. Given this, the ewes probably gained liveweight and this assumption is supported by a modest increase in mean condition score (0.3 units). Using 4.5 kg as the benchmark of wool removal, is possible that the sheep gained 31 g/head.day of liveweight. These production data are largely consistent with our expectations. At Dongara here was 2351 kg/ha of understory FOO at the start of grazing. This

comprised senesced annual ryegrass (59%), serradella (14%), barley grass (8%) and the remaining 19% was capeweed, brome grass and couch grass. The nutritional value of the understorey was incredibly poor with a mean DMD of 45%, ADF of 51%, NDF of 68%, and CP of 3.7% (Fig. 4.3).

At all sites, addition of a small amount of grain supplementation or management of the understorey to increase the legume content would enable higher rates of growth as energy would be the primary limiting factor.

#### Autumn 2022

The site at Cranbrook was grazed with maiden ewes ( $50.8 \pm 0.67$  kg liveweight and CS  $3.29 \pm 0.06$ ) from 7 April until the 28 April (21 days) at a density of 8.6 sheep/ha (181 grazing days/ha). The ewes gained 165 g/head.day of liveweight and a condition score decrease of 0.17. It is possible that the sheep had not eaten before being weighed at the start of the experiment. In autumn 2022, there was 3946 kg/ha of dry understorey FOO and 661 kg/ha of shrub EDM.

In 2022 at Baandee, pregnant ewes with mean liveweight of  $81.0 \pm 0.45$  kg and mean condition scores of  $3.1 \pm 0.03$  units grazed the plot from 5 May until the 9 June. We more than doubled the previous year's stocking density to 29.3 ewes/ha and they were on the plots for 35 days (1025 grazing days/ha). The pregnant ewes gained 231 g/head.day and 0.5 condition score units. Given the lack of understorey (1225 kg/ha) and poor nutritional value of the understorey, the ewes were offered a supplement of 1100kg of export oaten hay (up to 232 g/head.day) and approximately 200kg of barley grain (42 g/head.day).

In Dongara in 2022, the shrub plot and an adjacent wheat stubble paddock were each grazed by 150 Merino weaners ( $35.6 \pm 0.32$  kg liveweight and CS  $2.6 \pm 0.03$ ). The sheep in the saltbush plot (12.1 sheep/ha) gained 135 g/day of weight and 0.44 condition score units (544 grazing days/ha). In contrast, the sheep in the stubble pot (1.5 sheep/ha) gained 95 g/day of weight and 0.36 condition score units (67.5 grazing days/ha).



Fig. 4.3. Merino wethers grazing the research site at Dongara in March 2021.

#### Conclusions

The shrub paddocks (predominantly Anameka<sup>™</sup> with an experimental block of other saltbushes and Mallee saltbush) supported from 181 to 1025 sheep grazing days/ha in autumn, the time of year in Mediterranean-type climates when a kilogram of green feed has up to 10 times the value of a kilogram of green feed in spring. Baandee, a low rainfall environment supported up to 1025 grazing days/ha with 230 g/head.day of sheep growth (and moderate supplementation for the pregnant ewes). This equated to 2.8 sheep grazing days/ha.year. The deep white sandy site in higher rainfall Cranbrook supported up to 430 sheep grazing days in autumn with 40 g/head.day of sheep growth. This equated to 1.2 grazing days/ha.year. The deep white sand at Dongara supported up to 544 grazing days/ha with 135 g/head.day of sheep growth. This equated to 1.5 grazing days/ha.year. The adjacent stubble at Dongara yielded just 67 grazing days/ha.year at 95 g/head.day of sheep growth (or 0.18 grazing days/ha.year).

The design for the research site experiments only allowed one grazing event per year in autumn as the project was focussed on filling the autumn feed gap. It is probable that the sites could be grazed opportunistically at other times of the year to extract more value from the annual biomass when it is green. Previous research demonstrated that sheep grazing saltbush and annual legume understorey pastures chose to select about 50:50 shrub and understorey in autumn and 10:90 in spring (Norman *et al.* 2010c). These sites could generate additional value if they offer an opportunity to improve twin lamb survival by 17% through the provision of shelter in winter (Masters *et al.* 2023).

		Ваа	ndee			Cranb	rook				Dongara	1		
	202	1	202	2	2022	1	202	2	2021	(shrub)	2022 (sh	rub)	2022 (stu	bble)
	Mean	sem	Mean	sem	Mean	sem	Mean	sem	Mean	sem	Mean	sem	Mean	sem
Date onto plots	23 Mar		5 May		1 Mar		7 Apr		24 Mar		14 Feb		14 Feb	
Date mid grazing	n/a		25 May		n/a		n/a		n/a		8 Mar		8 Mar	
Data removed	22 Apr		9 Jun		20 Apr		28 Apr		28 Apr		31 Mar		31 Mar	
Days on plots	30		35		50		21		35		45		45	
Paddock size (ha)	4.6		4.6		3.5		3.5		12.4		12.4		99.8	
Sheep/plot	62		135		30		30		105		150		150	
Sheep/ha	13.5		29.3		8.6		8.6		8.5		12.1		1.5	
Grazing days/ha	405		1026		430		181		298		545		68	
Liveweight on (kg)	67.6	0.79	81.0	0.45	31.5	0.66	50.85	0.67	63.4	0.66	35.7	0.31	35.6	0.22
Liveweight mid (kg)	n/a		84.3	0.61	n/a		n/a		n/a		42.4	0.33	41.5	0.33
Liveweight off (kg)	68.2	0.67	89.1	0.47	33.5	0.53	54.32	0.68	*60.0	0.48	41.8	0.32	39.9	0.32
Condition score on	2.7	0.05	3.13	0.03	2.08	0.03	3.29	0.06	2.6	0.02	2.2	0.02	2.2	0.02
Condition score mid	n/a		3.44	0.04	n/a		n/a		n/a		2.6	0.03	2.6	0.03
Condition score off	3.2	0.04	3.64	0.03	2.85	0.06	3.12	0.05	2.9	0.03	2.6	0.03	2.6	0.02
Condition score change	0.5		0.51		0.77		-0.17		0.3		0.44		0.36	
Mean daily change (g/day)	20.0		231.1		40.0		165.2		*-98.1	*(or 31g)	135.3		94.7	
Shrub EDM (g/shrub)	477		937		599		794		367		1695.0		n/a	
Shrub density (shrubs/ha)	952		952		833		833		833		833		n/a	
Shrub EDM (kg/ha)	454		892		499		661		306		1412			
Understorey FOO on	1227		**1255		4068		3946		2351		4820		2417.0	
(kgDM/ha)														
Understorey FOO off	50		50				2952				3568		~1000	
(kgDM/ha)														

Table 4.1 Grazing data from Cranbrook, Baandee and Dongara in 2021 and 2022.

\*The sheep at Dongara were shorn unexpectedly during grazing and the team were unable to get to the site to capture wool weights. The host producer estimated they were cutting 4.5 kg wool/head. If this were accurate, the sheep would have gained 31 g/head.day of liveweight during grazing.

\*\*Over the 35 day grazing period the pregnant ewes were offered 1100kg of export oaten hay (232 g/head.day) and approximately 200kg of barley grain (42 g/head.day).

	Species	1/10/2020	16/03/2021	22/04/2022	15/09/2023	9/03/2023
Grass	Ryegrass	52.1	73.6	43.6	39.2	58.8
	Barley grass	1.0	0.3	10.3	10.6	19.4
Legume	Bladder clover	9.2	0.0	0.0	1.7	0.0
	Burr medic	3.5	6.2	22.4	18.5	13.3
	Woolly clover	0.0	0.0	1.1	7.1	0.0
	Rose clover	0.0	0.0	0.0	2.6	0.0
	Subclover					
	(Mawson)	0.0	0.0	0.0	5.7	0.0
Forb	Wild radish	30.2	7.2	16.3	7.1	0.0
	Rolypoly	0.3	10.1	0.0	0.0	0.0
	Wild geranium	3.7	2.6	6.3	1.3	0.0
	Matricaria	0.0	0.0	0.0	6.3	8.5

#### Table 4.2b. Botanical composition (%DM) of the understorey food on offer at Cranbrook.

	Species	8/04/2022	28/04/2022	27/03/2023
Grass	Annual ryegrass	0.9	0.0	0.0
	Oats	10.0	13.5	0.0
	Couch grass	5.6	9.1	18.8
	Subtropical (lovegrass)	4.3	26.2	0.0
	Brome grass	21.5	14.4	71.1
	Winter grass	0.0	7.2	0.0
Legume	French serradella	41.5	12.7	0.0
Forb	Erodium	10.6	5.6	0.0
	Wireweed	5.5	6.2	4.0
	Dock	0.0	5.1	0.0
	Flatweed	0.0	0.0	6.1

# Table 4.2c. Botanical composition (%DM) of the understorey food on offer at Dongara.

		24/03/2021	14/02,	/2022	30/03/2022
	Species	Shrub plot	Shrub plot	Stubble plot	Shrub plot
Cereal	Wheat stubble	0.0	0.0	89.6	0.0
Grass	Annual ryegrass	56.9	28.3	7.6	5.2
	Brome grass	3.8	0.7	0.0	0.0
	Cooch grass	6.9	2.1	0.0	0.0
	Subtropical grass	0.3	0.0	0.0	1.6
	Windmill grass	0.0	0.0	0.0	0.0
Legume	French serradella	1.0	1.2	0.0	0.5
	Blue Lupin	0.0	0.0	0.0	0.0
Forb	Mulla-mulla	20.4	34.2	0.0	7.9
	Wild Radish	5.8	25.0	0.0	19.1
	Paddy melon	2.9	3.9	2.8	0.0
	Erodium	0.0	0.4	0.0	36.2
	Capeweed	2.1	4.2	0.0	29.6

#### Plant establishment and survival

Seedling death rates at Baandee were generally low (Table 4.3). On average more than 99.5 % of saltbushes survived establishment while Mallee saltbush establishment success was 94%. This was despite only 186 mm of rainfall (60% of long-term average) in 2019 and 80% of average in 2020. Over the next 3 years, several mature saltbushes died (resulting in a 98.8% success rate over the four years). There were marginally fewer deaths in the seed lines compared to the clonal lines, but the differences were not significant. Death rate of the Mallee saltbush was higher than the death rate of saltbushes, with an overall death rate of 23.9% (76.1 % success). Of the Mallee saltbush, the RPCIn 40 genotype had 64% failure and RPL9 had 31% failure. RPB3 was the most successful with only 5.6% failure (the same rate as the worst saltbush genotype).

Failure rates of shrubs at Cunderdin were also generally low with 4% failure to establish for saltbush clones, 8% for saltbush seed lines and 14% for Mallee saltbush (Table 4.3). Anameka<sup>™</sup> suffered 8% death rates, the seed DH line had a 25% failure rate. Of the three Mallee saltbush genotypes at the site, the clonal line Cln 40 all established successfully, while the seed line RP S7 had 25% mortality during establishment.

The two sandy sites had much higher shrub death rates than the saline loam sites, this is likely associated with poor water holding capacity of coarse sandy soils and the very dry seasons in 2019 and 2020 (see Fig. 3.1). Dongara had just 80% of the long-term average rainfall in 2019 and 2020. A tropical cyclone also created havoc at Dongara with a significant wind erosion event several months after planting. At Dongara, 15% of saltbush clones failed to establish as well as 7% of the seed lines and 8% of the Mallee saltbush. Clone B was a notable failure with 36% mortality.

At Cranbrook, 15% of the saltbush clones failed to establish, compared to 7% of the saltbush seed lines and Mallee saltbush. In 2019, Cranbrook only had 75% of the long-term average rainfall (373 mm). There were further casualties after the heavy grazing events (shrubs were very heavily grazed in an attempt to get sheep to eat the Mallee saltbush so we could determine recovery growth rate after defoliation). This was especially noticeable on clone C, consistently the most palatable genotype. At the end of the experiment clone C had suffered 47% mortality. Clones G and H, Anameka seed, Seed GH and Seed EH were the most persistent saltbush varieties.

At all sites, the West Australian oldman healthy, nursery raised saltbush seedlings from subspecies, *spathulata*, failed to establish and persist.

Establishment from seedlings is generally reliable, incredibly dry seasons and a cyclone did not have as large an impact on survival as we anticipated. The loss of mature shrubs after heavy grazing was more of a concern. The key message is that if farmers want to utilise all the shrub biomass in a paddock, they should avoid diverse plantations as it puts significant pressure of the relatively palatable genotypes.

Genotype		Baande	e	С	ranbroo	k		Dongar	а	(	Cunderc	lin
	Est.	>1 yr	Total	Est.	>1 yr	Total	Est.	>1 yr	Total	Est.	>1 yr	Total
Anameka	3	3	6	17	3	19	6	6	11	8	0	8
Clone A	0	0	0	19	3	22	11	0	11	8	0	8
Clone B	0	0	0	17	8	25	36	0	36	4	0	4
Clone C	0	0	0	31	17	47	22	0	22	4	0	4
Clone E	0	0	0	8	25	33	17	0	17	0	0	0
Clone G	0	0	0	6	6	11	6	3	8	4	0	4
Clone H	0	3	3	6	8	14	11	0	11	0	0	0
Anameka seed	0	0	0	6	0	6	6	0	6	8	0	8
Seed AH	0	0	0	3	8	11				4	0	4
Seed BH	0	0	0	11	14	25	0	0	0	4	0	4
Seed CH	0	3	3	8	11	19				4	0	4
Seed DH	0	0	0	14	0	14	14	0	14	25	0	25
Seed EH	0	0	0	0	6	6	8	0	8	8	0	8
Seed GH	0	0	0	6	3	8	8	0	8	4	0	4
Spathulata	0	6	6	86	8	94	89	0	89	71	0	71
RP A12	0	8	8	22	0	22						
RP B3	0	6	6				6	0	6			
RP B15				11	3	14						
RP B2				19	3	22						
RP B8				17	0	17						
RP C1				31	11	42						
RP C17				6	8	14						
RP Cln 40	8	56	64	8	11	19	11	0	11	0	4	4
RP G17				6	6	11						
RP L14				22	0	22						
RP L9	14	17	31	25	3	28						
RP M3	8	3	11	8	0	8						
RP S7				28	3	31				25	0	25
RP T5										17	0	17
Mean saltbush	0	1	1	15	10	25	15	1	17	4	0	4
clones	Ŭ	1	1	15	10	25	15	-	17	-	Ū	-
Mean saltbush	0	0	0	7	6	13	7	0	7	8	0	8
seed	5	5	5	-	-		-	5	-	-	5	-
Mean Mallee saltbush	6	18	24	17	4	21	8	0	8	14	1	15

Table 4.3. Mortality (%) of shrub genotypes during and after establishment at the research sites.

Deaths were recorded 12 months after establishment (Est.) and at the end of the experiment (> 1 year).

#### 4.3.2 Biomass production

Mean EDM (leaves and stems < 3mm) of shrubs (± SEM) are presented in Figures 4.5, 4.6, 4.7 and 4.8 for the four research sites. These rates have been converted from g/shrub to kg/ha based on the shrub density at the sites. Figures are presented in preference to tables as it allows the reader to see differences in defoliation associated with grazing (sufficient grazing pressure to defoliate the Mallee saltbush was difficult to achieve) and it also allows a visualisation of leaf drop, recovery, growth rate after grazing and variability within a genotype (through the standard error of the mean). Each graph has Anameka<sup>™</sup> at the site as a reference (black bold).

#### Baandee

Data are presented in Figs 4.5 A to D. The very dry seasons in 2019 (with just 186 mm of rainfall or 60% of long-term mean) and 2020 (80% of long-term mean) are apparent in the slow shrub growth rates. All saltbushes follow a similar pattern of biomass production. At the time of the first grazing in March 2021, saltbush EDM ranged from 250 to 700 kg DM/ha. The graphs demonstrate that some shrubs were defoliated more than others during grazing, this may impact on growth rates as some shrubs will have a greater photosynthetic area. The shrubs would have had well established roots and they responded well to an average rainfall season in 2021. In January, the best saltbush genotype (seed CH) averaged 2.02 t/ha of EDM, while the worst (clone C) averaged 1.0 t/ha. Clone C and Anameka had been very heavily grazed in comparison to the other shrubs. In 2022, we intensified the stocking rate to put greater pressure on the shrubs. The saltbushes were defoliated to bare sticks. Unfortunately, the majority of Mallee saltbushes still had 0.5 t EDM/ha, we were unable to push the animals hard enough to achieve full defoliation of these shrubs. Recovery rates until April 2023 indicate that the most productive saltbushes were the seed lines rather than their clonal parents, Anameka seed had 1.5 t/ha of EDM.

The saltbushes were generally more productive than the Mallee saltbush at this site. RP M3 and RP B3 were significantly more productive than the other genotypes before grazing in 2022, however, RP A12 recovered from grazing faster. RPCB 40 was preferred to the other Mallee saltbush genotypes and was completely defoliated in 2022 – this had a massive impact on recovery rate and survival. The WA saltbush subspecies, spathulata, did not achieve more than 0.5 t/ha of EDM.

There was clearly some EDM lost between January 2022 and grazing in May. It is unclear if this was due to the plants getting drought stressed and dropping leaves or an accidental grazing event. If it was a leaf drop event, we should consider grazing the shrubs earlier in summer.



Fig. 4.4. Shrubs at Baandee in autumn 2022. Saltbushes have been defoliated to sticks (with some material above sheep height) but the sheep refused to eat some of the Mallee saltbush shrubs.

#### Cranbrook

Data from the sandy site in Cranbrook are presented in 4.6A to D. It is immediately apparent that the saltbushes were much less productive than those at Baandee, with a maximum of 0.6 t/ha of EDM (seed GH). The saltbush seed lines were all more productive than the parent clones. Again, oldman saltbush subspecies spathulata failed to perform.

Note the scale change when presenting the Mallee saltbush data (4.6D and E) – this is apparent when comparing the Mallee saltbush to Anameka<sup>™</sup>. Mallee saltbush were much more productive than saltbush with 1.2 t/ha growth by autumn 2021 and 2 t/ha in Autumn 2023. These shrubs had an advantage of not being completely defoliated during grazing (compared to the saltbushes), so had higher photosynthetic areas to power regrowth. Mallee saltbush RP B8, RP B2 and RP M3 are clearly the most productive shrub genotypes at the site. The large standard errors of the lines indicate the large variability in growth at this site

#### Dongara

The biomass data demonstrate that the shrubs struggled to establish at Dongara. 2019 and 2020 had about 80% of long-term average rainfall and the wind erosion event removed a lot of topsoil from the shrubs. By the first grazing in 2021, there was less than 250 kg/ha of saltbush EDM. While growth was slow compared to the other sites, by October 2022, the most productive saltbush (seed EH) had 1.8 t EDM/ha. Anameka<sup>™</sup> was one of the least productive genotypes at the site. As for the sandy Cranbrook site, Mallee saltbush clearly flourished at Dongara. RP B3 was the standout, with 4.3t EDM/ha of biomass in June 2022 (Fig. 4.7).

#### Cunderdin

The shrubs established rapidly at this site with several genotypes averaging 0.8t/ha after 320 days of growth (Fig. 4.8). Some seed lines were significantly more productive than the clonal parents. Anameka<sup>™</sup> and Mallee saltbush RP T5 produced the most biomass during the experiment (Fig. 4.8).

#### Summary

As a rule, the seed lines were more productive than the clonal parents and these differences range from ~10% (Cunderdin and Dongara) to ~30 % (Baandee and Cranbrook). At the deep white sandy sites in Dongara and Cranbrook, the Mallee saltbush genotypes have produced twice as much EDM as the saltbushes. At the sandy loam / loamy clay sites in Baandee and Cunderdin, there were few differences in EDM between saltbush and Mallee saltbush. The saltbush was disadvantaged by preferential grazing.

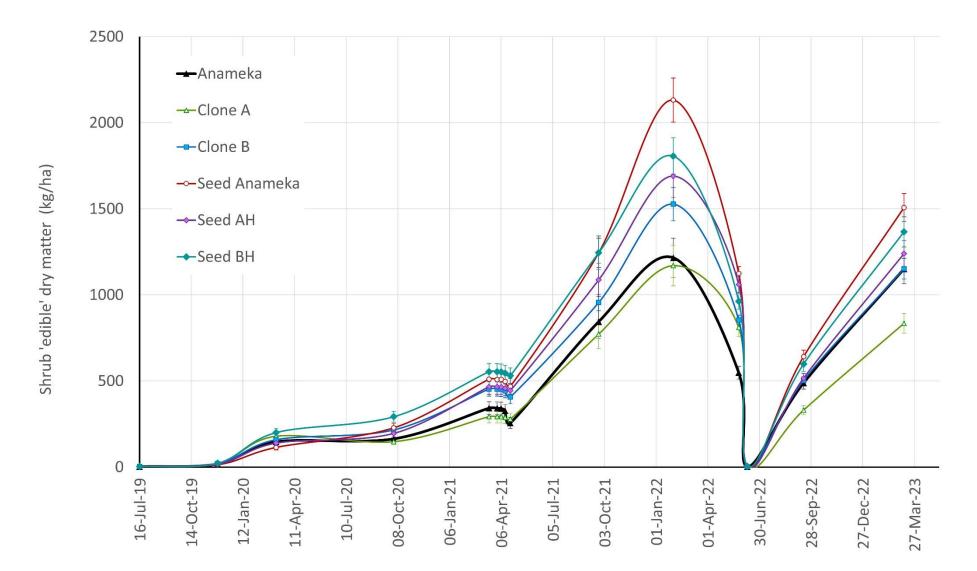


Fig. 4.5A. 'Edible' dry matter production (kg DM/ha) of shrub (saltbush) genotypes at Baandee. Gazing was initiated on 24 March 2021 and 5 May 2022.

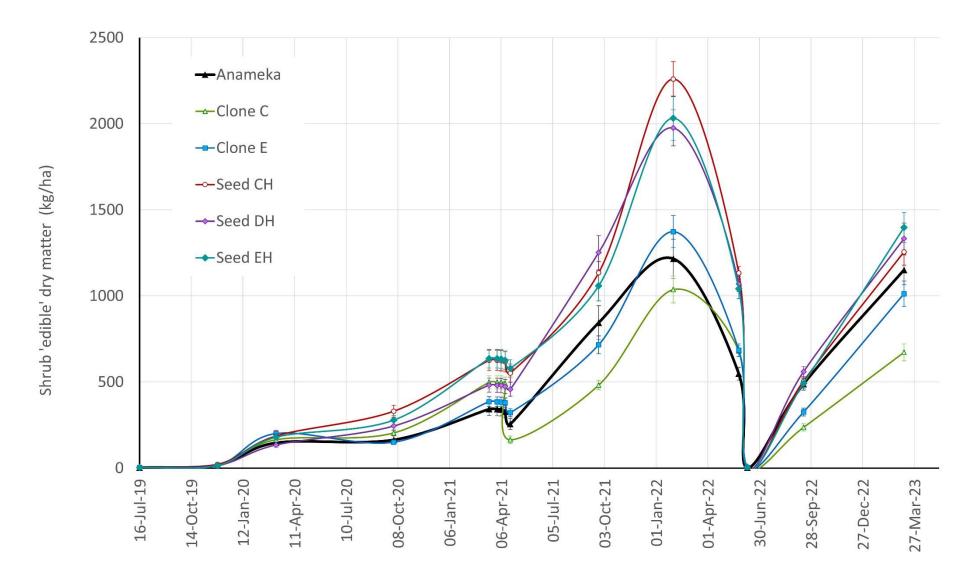


Fig. 4.5B. 'Edible' dry matter production (kg DM/ha) of shrub genotypes (saltbush) at Baandee. Gazing was initiated on 24 March 2021 and 5 May 2022

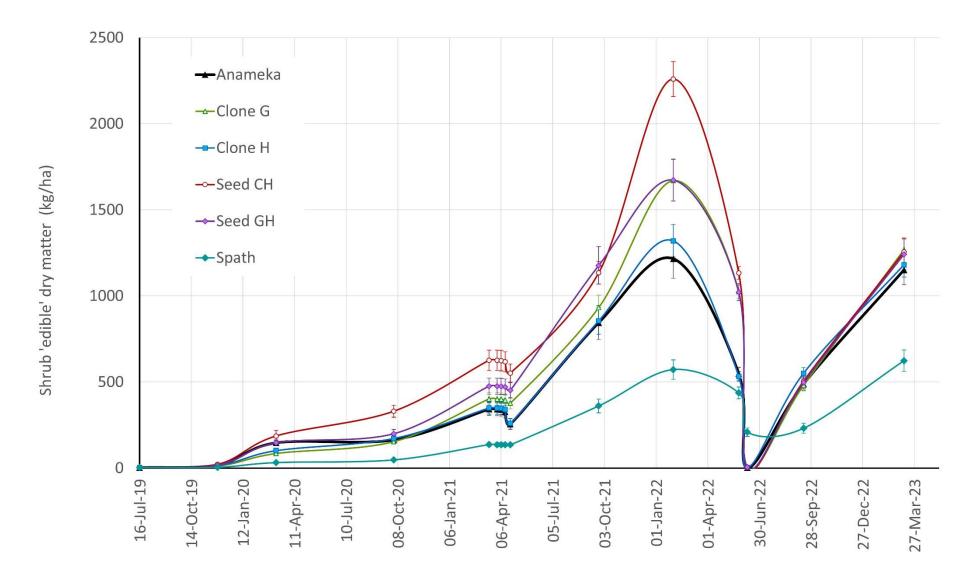


Fig. 4.5C. 'Edible' dry matter production (kg DM/ha) of shrub genotypes (saltbush) at Baandee. Gazing was initiated on 24 March 2021 and 5 May 2022

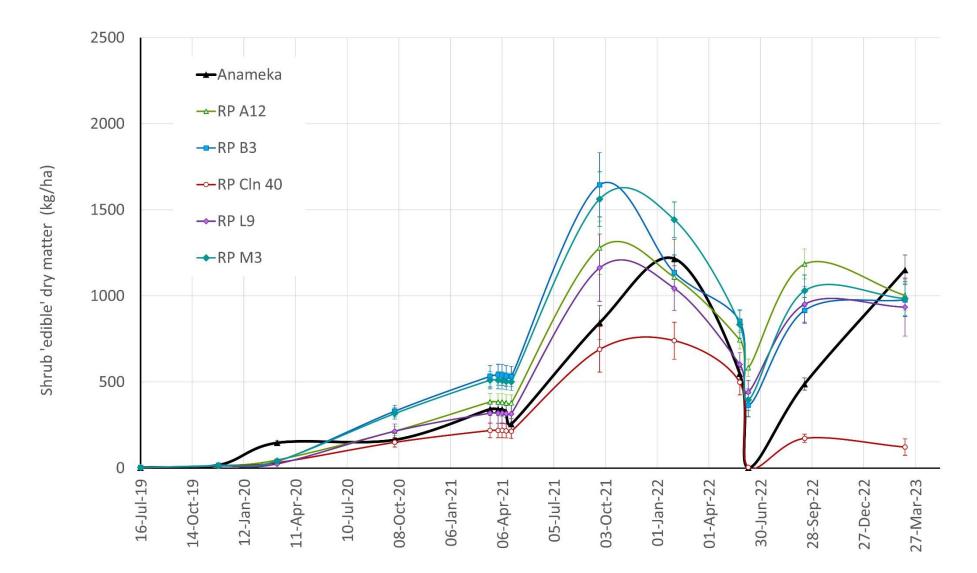


Fig. 4.5D. 'Edible' dry matter production (kg DM/ha) of shrub genotypes (Mallee saltbush) at Baandee. Gazing was initiated on 24 March 2021 and 5 May 2022

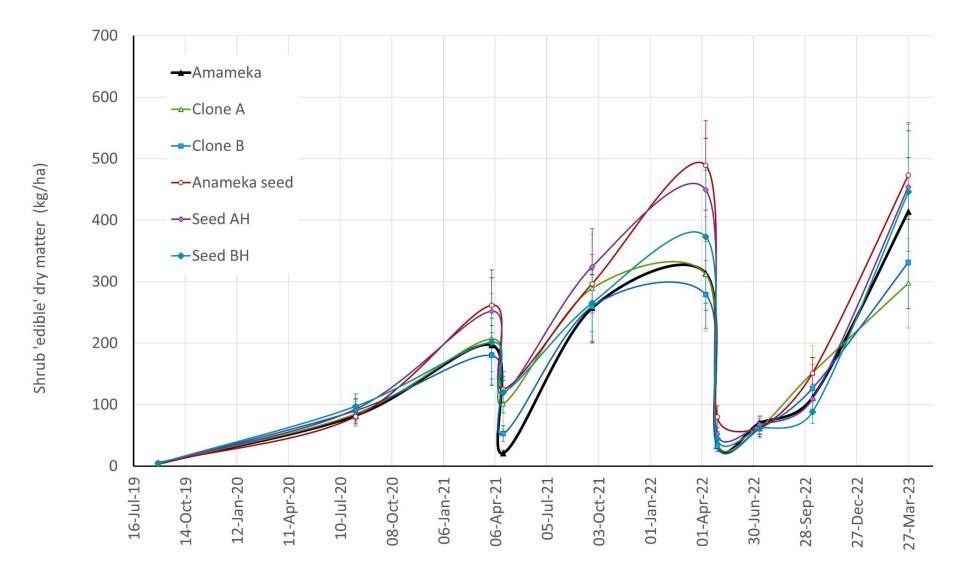


Fig. 4.6A. 'Edible' dry matter production (kg DM/ha) of shrub (saltbush) genotypes at Cranbrook. Gazing was initiated on 31 March 2021 and 8 April 2022

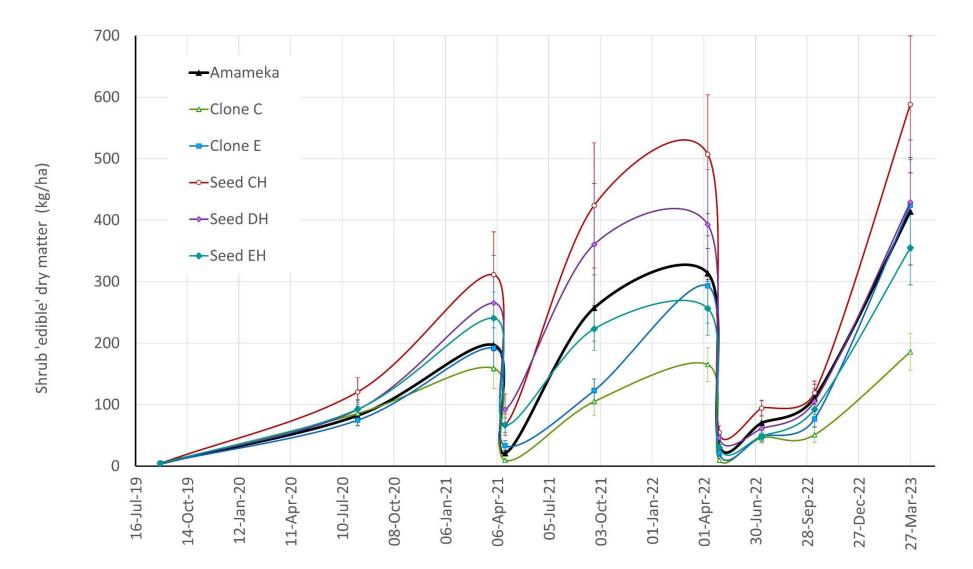


Fig. 4.6B. 'Edible' dry matter production (kg DM/ha) shrub (saltbush) genotypes at Cranbrook. Gazing was initiated on 31 March 2021 and 8 April 2022.

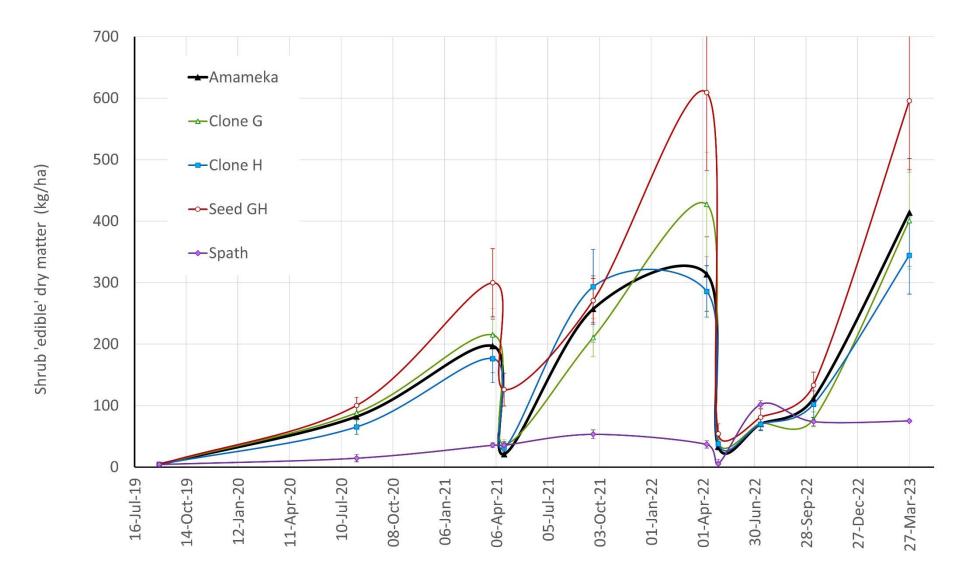


Fig. 4.6C. 'Edible' dry matter production (kg DM/ha) shrub (saltbush) genotypes at Cranbrook. Gazing was initiated on 31 March 2021 and 8 April 2022

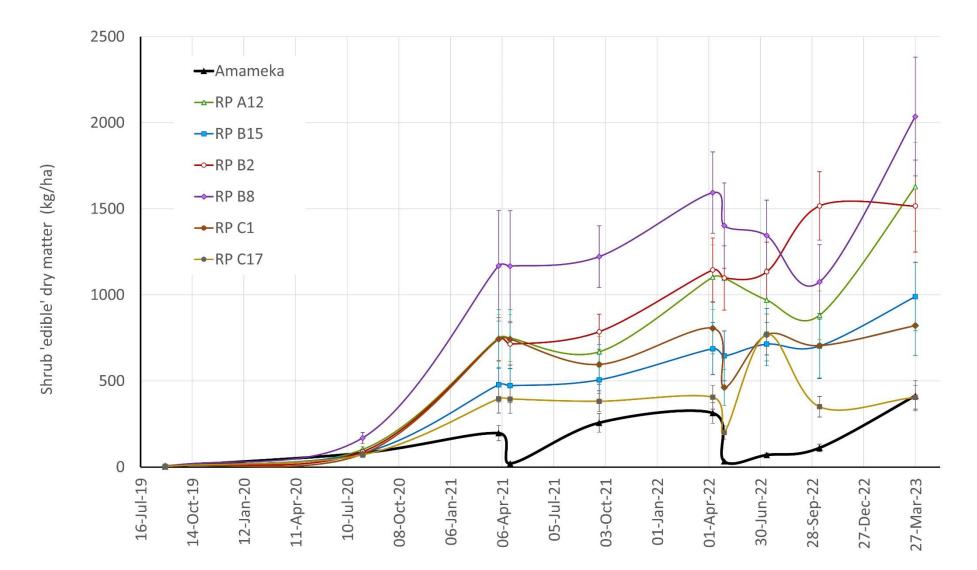


Fig. 4.6D. 'Edible' dry matter production (kg DM/ha) shrub (Mallee saltbush) genotypes at Cranbrook. Gazing was initiated on 31 March 2021 and 8 April 2022. Note change in scale on y axis from previous graphs.

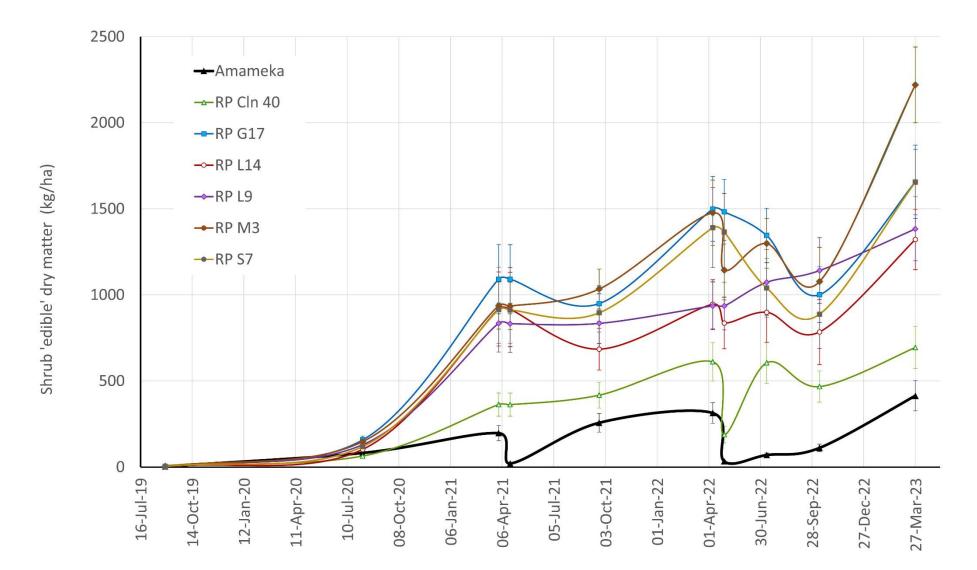


Fig. 4.6E. 'Edible' dry matter production (kg DM/ha) of the Mallee saltbush genotypes at Cranbrook. Gazing was initiated on 31 March 2021 and 8 April 2022. Note change in sale from previous graphs.

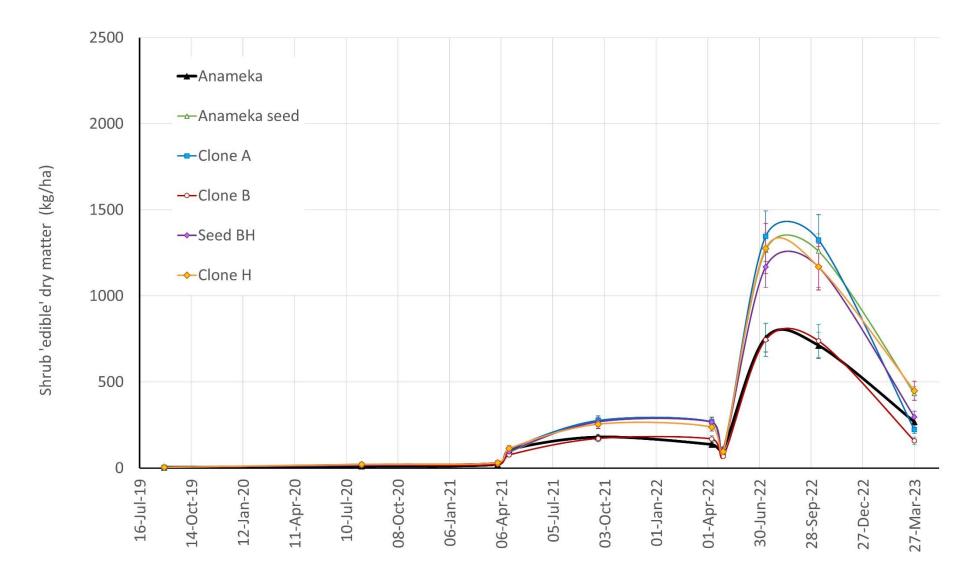


Fig. 4.7A. 'Edible' dry matter production (kg DM/ha) of the saltbush genotypes at Dongara. Gazing occurred on 14 February 2021 and 31 March 2022

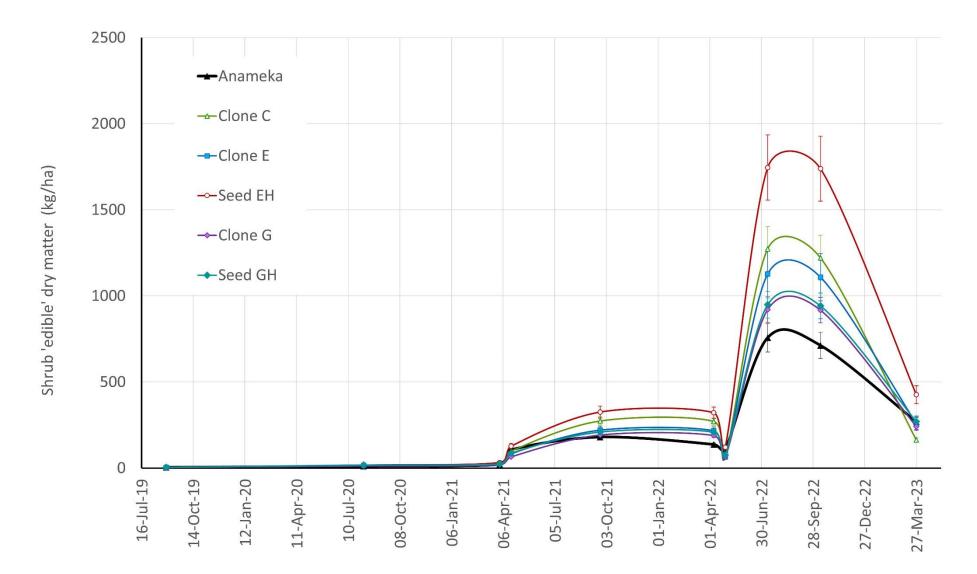


Fig. 4.7B. 'Edible' dry matter production (kg DM/ha) of the saltbush genotypes at Dongara. Gazing occurred 14 February 2021 and 31 March 2022

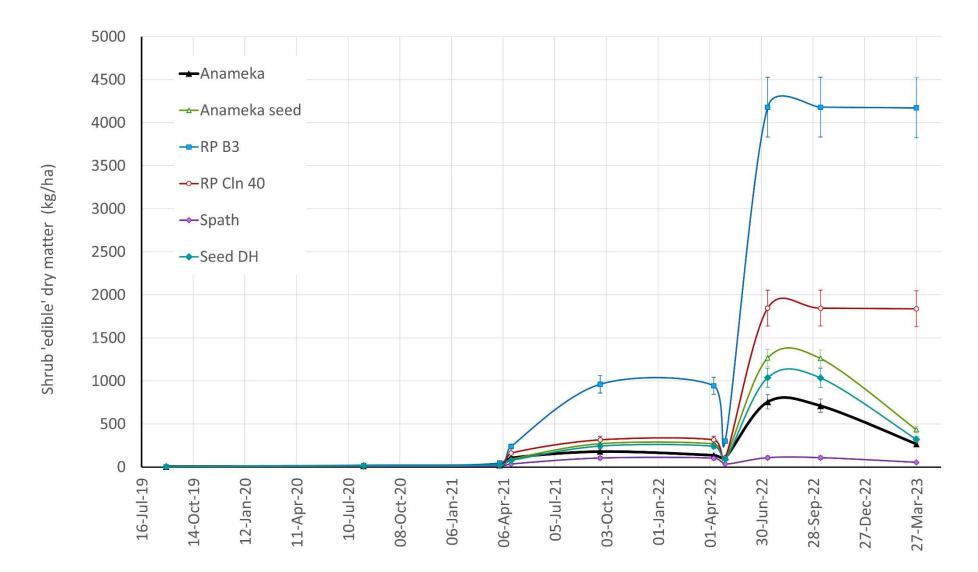


Fig. 4.7C. 'Edible' dry matter production (kg DM/ha) of the Mallee saltbush genotypes at Dongara. Gazing occurred 14 February 2021 and 31 March 2022. Note the change in scale, the other graphs for the site peaked at 2500 kg/ha DM.

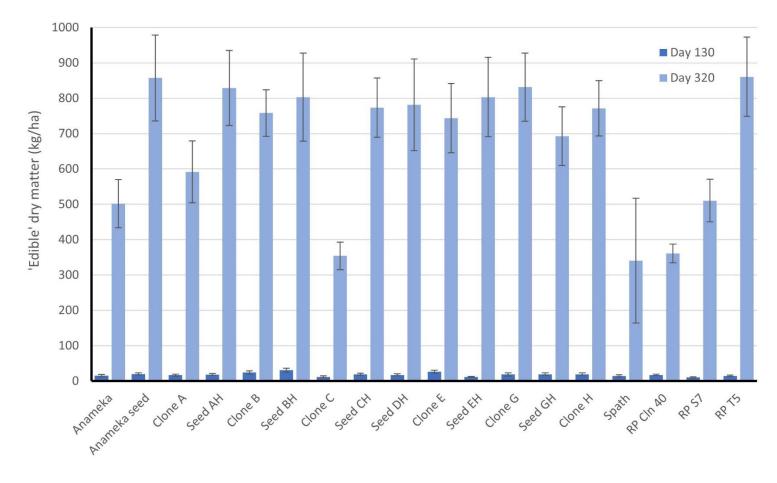


Figure 4.8. 'Edible' dry matter production (kg EDM/ha) during the first year of growth at the Cunderdin research site.

#### 4.3.4 Management of grazing intensity.

Blocks of 5 shrubs at Baandee (n=4) were fenced off to prevent grazing in autumn 2022 (Fig. 4.9). The aim is to demonstrate that excluding shrubs from grazing may not lead to more biomass availability the following season. Regardless of scientific merit, these shrubs have become a talking point for local producers. They are also a useful tool to demonstrate productivity of the shrubs when the bulk of the shrubs in the paddock had been grazed to sticks.

It was clear from Fig. 4.10 that the shrubs that were heavily grazed grew at a much greater rate than those in the exclusion cages. The "use it or lose it" message is still relevant.



Fig. 4.9 Shrubs at Baandee in exclusion cages as part of a grazing management experiment.

#### 4.3.5 Relative palatability

Palatability of particular shrub is a function of the nutritional value, secondary plant compounds that impact flavour or position of the shrub in the landscape (i.e. close to water troughs). Sheep can only select for or against nutrients and compounds that firstly induce positive or negative feedback (in the gut and brain) and secondly can be detected by the animal. It is always relative to the other options that an individual animal must meet its dietary needs. For this reason, relative palatability is expressed for each grazing event at each site separately. Sheep are incredibly sensitive, recent work demonstrated that sheep would change dietary preferences to alleviate vitamin E deficiency (Amanoel *et al.* 2016).

The relative palatability data from the research sites in 2021 and 2022 are presented in Table 4.4 There were significant differences in sheep preferences between species and between genotypes at each measurement time during grazing. For each season, palatability was rated approximately midway through grazing and again at the end of grazing.

Saltbush was always significantly preferred to Mallee saltbush at each site and measurement period. There was also significant variation between genotypes for both species. Saltbush clones were often preferred over the seed lines generated from the same clones. The most palatable genotypes were Anameka (Anameka), clone C and clone H. When pushed, sheep would eat Mallee saltbush RP Cln 40, this has had implications for persistence of these genotypes if planted in mixtures and overgrazed.

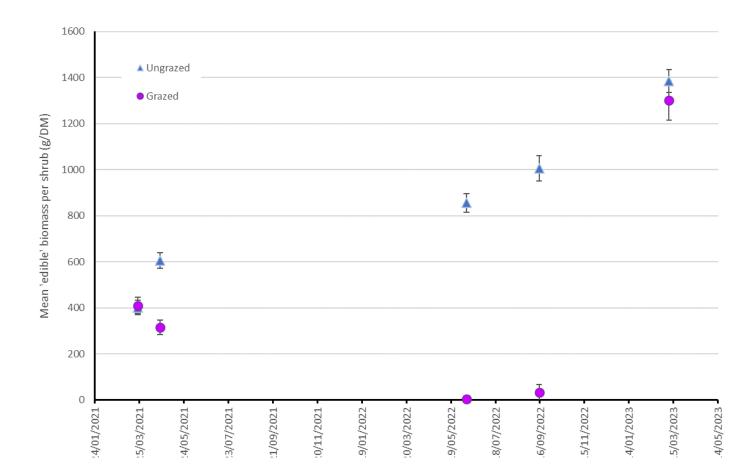


Fig. 4.10. 'Edible' dry matter on Anameka<sup>™</sup> shrubs at Baandee that are open to annual grazing or protected in exclusion fence.

	Baandee											Cran	brook			Dongara				
	Early (21)		Mid (21)		Late (21	)	Early (22	2)	Late (22	)	Late (21	)	Late (22)	)	Early (21	)	Early (22	2)	Late (22	.)
Genotype	m	se	m	se	m	se	m	se	m	se	m	se	m	se	m	se	m	se	m	se
Anameka	4.7	0.9	25.5	3.2	65.6	2.9	33.8	3.5	99.8	0.1	85.5	2.8	89.3	0.5	19.4	3.3	4.8	1.2	63.8	1.7
Clone A	1.4	0.2	4.1	0.7	32.5	2.6	1.9	0.5	99.7	0.1	56.6	3.6	91.2	0.6	2.0	0.7	2.0	0.7	82.2	1.4
Clone B	2.3	0.2	11.5	1.9	47.5	2.7	3.6	0.6	99.8	0.1	68.5	3.8	88.6	0.8	1.9	1.0	0.9	0.6	78.7	1.1
Clone C	1.3	0.2	66.4	3.6	94.2	0.4	14.7	3.1	99.8	0.1	93.4	1.0	95.5	1.1	0.8	0.7	4.3	1.1	85.7	1.0
Clone E	1.6	0.2	14.6	2.5	64.2	2.5	2.0	0.4	99.8	0.1	78.5	2.7	91.3	0.6	1.2	0.3	1.3	0.6	75.7	1.2
Clone G	1.9	0.4	6.1	0.7	33.6	3.3	3.2	0.7	99.7	0.1	81.5	1.1	89.7	0.7	1.4	0.5	0.3	0.3	75.2	1.1
Clone H	2.3	0.3	22.1	3.0	62.6	1.5	41.1	1.3	99.7	0.1	78.8	2.7	88.2	1.0	5.3	1.3	7.5	1.6	65.6	1.3
Anameka seed	2.2	0.3	7.4	1.3	40.0	3.0	2.1	0.4	99.7	0.1	48.8	3.1	84.6	1.0	1.4	0.4	0.6	0.4	66.5	1.2
Seed AH	2.0	0.4	5.4	0.7	24.9	2.7	3.3	0.6	99.8	0.1	40.1	3.8	89.4	0.6						
Seed BH	1.6	0.1	3.9	0.6	12.9	1.6	3.8	1.4	99.7	0.1	34.7	2.9	88.1	0.7	0.3	0.1	0.0	0.0	74.7	1.0
Seed CH	1.3	0.2	10.9	1.8	43.5	3.6	0.8	0.2	99.7	0.1	70.2	4.4	90.3	0.9						
Seed DH	1.8	0.3	6.6	1.5	28.2	3.3	3.4	0.6	99.7	0.1	60.3	3.1	89.4	0.6	0.9	0.4	0.3	0.3	69.7	1.2
Seed EH	1.3	0.2	8.1	1.4	34.4	4.6	5.9	2.4	99.6	0.1	66.1	3.7	88.3	0.8	0.6	0.3	0.3	0.3	75.5	1.3
Seed GH	0.8	0.2	4.4	0.9	14.7	1.8	2.1	0.5	99.6	0.1	57.7	3.2	91.2	0.9	0.8	0.4	0.3	0.3	72.4	1.5
Spathulata	0.8	0.2	1.1	0.3	1.5	0.2	1.3	0.6	50.6	4.2	0.1	0.2	30.0	0.5	1.5	0.5	0.0	0.0	43.8	8.8
RP A12	1.5	0.2	1.5	0.2	5.2	1.2	0.3	0.3	19.1	5.1	0.2	0.1	0.5	0.4						
RP B3	1.2	0.2	2.1	0.4	3.4	0.6	0.0	0.0	53.5	6.7					1.3	0.4	0.0	0.0	0.3	0.3
RP B15											1.1	0.4	12.0	3.6						
RP B2											0.5	0.2	7.9	3.4						
RP B8											0.4	0.2	10.8	5.0						
RP C1											0.3	0.2	43.5	5.4						
RP C17											0.5	0.2	51.2	2.9						
RP Cln 40	1.0	0.2	1.7	0.4	4.1	0.7	0.0	0.0	98.7	0.6	0.1	0.1	71.6	3.2	0.2	0.2	0.0	0.0	0.6	0.4
RP G17											0.1	0.1	1.2	0.7						
RP L14											0.8	0.4	15.2	3.6						
RP L9	1.0	0.3	1.2	0.2	5.4	1.5	0.0	0.0	30.4	6.4	0.4	0.2	0.6	0.4						
RP M3	1.5	0.2	2.2	0.4	3.4	1.0	0.1	0.1	52.9	5.5	0.7	0.3	21.1	4.9						
RP S7											0.5	0.3	1.2	0.8						
All genotypes	1.8		13.2		40.0		8.2		96.4		34.3		56.4		2.6		1.5		62.0	
Saltbush (num)	1.9		14.1		42.8		8.7		99.7		65.8		89.7		3.0		1.9		73.8	
Mallee saltbush	1.2		1.7		4.3		0.1		50.9		0.5		19.7		0.8		0.0		0.5	
Between species	< 0.001	0.9	<0.001	7.8	< 0.001	7.7	< 0.001	4.4	< 0.001	6.3	< 0.001	5.3	< 0.001	4.9	0.0	1.8	<0.001	1.1	<0.001	1.2
Between genotypes	<0.001	0.8	<0.001	4.5	<0.001	6.1	<0.001	3.6	<0.001	6.6	<0.001	2.3	<0.001	2.4	<0.001	2.6	<0.001	1.8	<0.001	3.1

Table 4.4. Relative palatability (percentage defoliation) of shrubs at each site during grazing events in 2021 and 2022. In some seasons, rankings occurred during the grazing event (ie early, mid or late). A heat map for each measurement time highlights the preferred (green) or avoided (red) shrubs.

At Baandee, when the data were restricted to just include the female clonal lines and seed derived from the female clones (all with a common male parent – clone H), the clonal parents had statistically higher relative palatability (P<0.001 for all time periods except for day 30 in 2022 when P=0.004). This is demonstrated in Fig. 4.11.

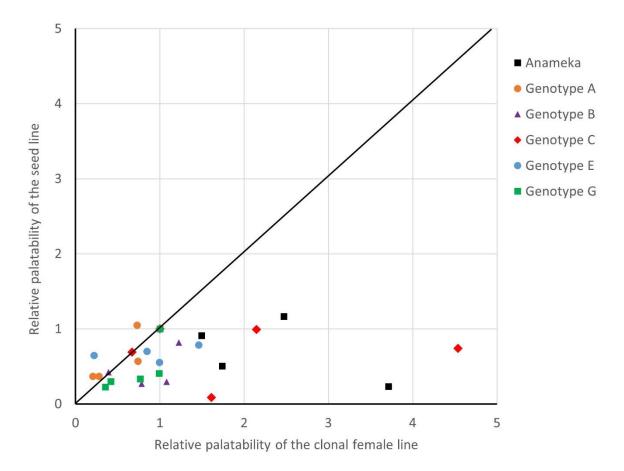


Fig. 4.11. Relative palatability of the clonal female and seed (generated from the female and crossed to a common male clone H) at each of the times that defoliation was scored at Baandee (for each time period, percentage defoliation was normalised so the mean for all shrubs a time period was 1). The chart indicates that the clonal line was preferred to its seed line for the majority of comparisons – genotype A was the notable exception.

#### 4.3.6 Nutritional value of shrubs

Three replicated blocks of shrubs were sampled at the four experimental sites every spring and early autumn for four years (Baandee, Cranbrook and Dongara) or two years (Cunderdin). In total 1212 samples were analysed in the laboratory, developing a strong understanding of variation associated with site of growth, season of sampling, species, subspecies, plant family and genotype. Data were analysed by REML to allow for a comparison of genotypes across sites despite differences in genotypes at some of the locations. For all traits there are significant differences associated with site of growth, species, plant family and genotype. There were no differences associated with season, the nutritional value of shrubs remained constant between the spring and autumn

#### (Table 4.5).

On average the elite saltbushes had 20.1% ash (salt) content, while the local subspecies spathulata had 26.4% ash. The mean of Mallee saltbush was lower with 16.3% ash. The data for saltbush are consistent with our expectations. The salt accumulation by Mallee saltbush was higher than has been previously reported. The mean ash content at Baandee (20.8%) was higher than that at Cranbrook (17.5%).

The organic matter digestibility (OMD) of the selected saltbushes was not very different to the Mallee saltbush genotypes (67.6% compared to 70.7%). The spathulata was poor, this also corresponded to higher ADF and NDF for the species. Of the saltbushes, clone A had the highest OMD (69%), and this was statistically similar to seed AH, Anameka seed, clone B, seed BH, clone G and seed GH. The high OMD of the saltbushes was not surprising given the cohort of material that was been tested had been heavily selected for energy values.

The digestible organic matter in the dry matter (DOMD% - an estimate of energy value) of saltbushes (53.9%) was lower than Mallee saltbush (59%), this is partially associated with the higher ash content of the saltbush (ie they have less organic matter that could be digested to begin with). This is not always a problem for livestock if they are only eating shrubs as a part of a diet and are able to excrete the salt with access to fresh water. Spathulata is more representative of a 'wild' saltbush and had a mean DOMD of 44%.

Site of growth impacted DOMD, shrubs at Baandee had significantly higher values than shrubs at Dongara. Seed BH had the highest DOMD (54.8%), and this value was statistically similar to the values for Anameka seed, clone A, seed AH, clone B, seed BH, clone G, seed GH, clone H and seed DH. The selected saltbushes with the poorest (and statistically similar) DOMD were clone C, clone C seed, clone 38 and clone E seed. The best Mallee saltbush (RP A12) had a mean DOMD of 61.6%, the poorest (RP17) was only 52.6%.

The selected saltbushes had consistently higher crude protein (CP) content with a mean across sites of 17%, compared to 13.5% for Mallee saltbush and 11% for the spathulata. These levels would meet the maintenance CP requirement for most classes of stock. However, it is likely that some of this nitrogen is associated with small, non-protein compounds. This N would only be converted to protein in the rumen if there was sufficient energy in the diet. The genotypes with the highest CP content were clone A, clone C and clone E. The worst saltbushes were the clone B and Anameka seed genotypes.

Table 4.6 presents the correlations between nutritional traits, calculated on all 1212 observations. All of the relationships were statistically significant and as expected. DOMD (energy value) was positively correlated with CP and OMD, and negatively correlated with fibre (ADF and NDF) and ash.

<u> </u>		DOMD	OMD	CP	ADF	NDF	Ash
Species	Genotype	(%)	(%)	(%DM)	(%OM)	(%OM)	(%DM)
Oldman saltbush	Anameka	53.6	67.1	16.7	16.7	27.5	20.0
subsp.	Anameka seed	54.2	67.3	16.2	16.9	28.6	19.4
nummularia	Clone A	54.4	69.0	18.8	15.2	25.4	21.1
	Seed AH	54.1	67.7	16.5	16.7	28.1	19.9
	Clone B	54.5	67.3	14.9	16.6	28.6	18.9
	Seed BH	54.8	68.2	15.5	16.2	27.9	19.7
	Clone C	52.3	66.3	18.5	17.0	27.6	21.0
	Seed CH	52.8	66.5	16.7	17.0	28.4	20.6
	Clone E	52.2	66.7	18.3	16.3	26.3	21.6
	Seed EH	52.5	66.4	17.7	17.0	28.1	20.9
	Clone G	54.7	67.9	16.2	16.2	27.2	19.4
	Seed GH	54.2	67.7	15.4	16.4	27.9	19.9
	Clone H	53.6	67.2	16.7	16.5	27.3	20.1
	Seed DH	53.8	66.9	15.8	17.2	29.3	19.5
subsp. spathulata	Spath	43.6	58.0	10.2	19.1	29.3	26.5
Mallee saltbush	RP A12	61.6	72.4	12.8	14.2	23.0	15.0
	RP B15	59.2	70.1	16.6	16.7	26.9	15.5
	RP B2	57.8	68.2	16.0	17.9	28.6	15.1
	RP B3	60.0	72.7	13.4	13.3	22.2	17.4
	RP B8	58.6	69.9	14.7	16.7	26.5	16.2
	RP C1	54.7	67.0	15.2	18.2	29.3	18.2
	RP C17	52.6	65.8	14.9	18.6	29.4	20.0
	RP Cln 40	55.7	68.1	11.3	16.8	27.1	18.1
	RP G17	59.5	70.2	11.6	16.2	26.0	15.1
	RP L14	57.9	68.6	14.5	17.6	28.4	15.4
	RP L9	60.6	70.6	12.0	15.6	25.7	14.2
	RP M3	60.5	70.0	13.7	16.4	26.2	13.6
	RP S7	59.3	70.4	14.0	16.0	25.6	15.9
	RP T5	61.2	72.5	15.9	15.1	23.7	15.7
Mean of subspecies	OMSB	53.9	67.6	17.0	16.3	27.3	20.1
	RP	59.1	70.7	13.5	15.4	25.0	16.3
	Spath	44.4	60.5	11.0	18.2	28.3	26.4
Mean of sites	Baandee	56.5	71.3	18.8	14.1	23.2	20.8
	Cranbrook	55.0	66.7	14.7	17.4	29.1	17.5
	Cunderdin	54.9	67.6	14.1	17.3	29.1	18.8
	Dongara	53.0	66.6	14.8	16.4	26.4	20.3
LSD Genotypes*	LSD (5%) max rep	1.31	1.77	1.63	1.09	1.80	0.92
	LSD (%) min rep	2.17	2.93	2.71	1.81	2.98	1.52
Significance of	Species	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Main effects	Site	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
(p value)	Genotype	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
	Season	n.s	n.s	n.s	n.s	n.s	n.s

# Table 4.5. Mean nutritional value of genotypes across sites, predicted by REML (to account forgenotypes not being present at each site).

\*Max reps is appropriate for saltbushes as they were at all sites, min rep more appropriate for Mallee saltbush comparisons.

		1	2	3	4	5
OMD (%)	6	-0.897	0.105	0.620	0.848	-0.827
NDF (%OM)	5	0.949	-0.429	-0.674	-0.525	-
DOMD (%)	4	-0.613	-0.437	0.342	-	
CP (%DM)	3	-0.640	0.400	-		
Ash (%DM)	2	-0.379	-			
ADF (%OM)	1	-				

# 4.3.7 Feeding value

Feeding value (FV) is the worth of a forage in terms of livestock production, it is a function of both how much of the forage is eaten and the utilisation of that forage. Nutritional value (NV) is defined by the nutrient content or animal response per unit of feed intake (Ulyatt 1973). These characteristics, along with biomass availability and voluntary feed intake are critical for livestock production.

Given variability between the sites (some favouring different species) and inconsistent genotypes at the sites (associated with lack of seed resources at the start of the project), it is difficult to consistently compare feeding value across sites. A REML approach was explored but the data were incredibly difficult to interpret. The simplest way to compare feeding value of the shrubs is to compare them to Anameka<sup>™</sup>, a consistent and genetically uniform control at each site and the industry best practice. Using the data for Anameka<sup>™</sup> at each site, a percentage variation compared to Anameka<sup>™</sup> was calculated (ie if the number was 50, the plant had 50% lower value than Anameka<sup>™</sup> for the measurement, and if it was 150, it had a 50% higher value than Anameka<sup>™</sup>. These data are presented in Tables 4.7 to 4.10. A heat map has been imposed on each to give a rapid visual comparison – the more green, the higher the component of feeding value. Conversely, the more red, the less positive value for the trait.

#### Baandee

As a general rule, the saltbush clonal lines were less productive than the seed lines but had higher nutritional value and relative palatability (Table 4.7). The Mallee saltbush had higher DOMD (energy), lower fibre and lower ash but poorer crude protein and relative palatability. Given difficulty in getting sheep to eat them, they had low feeding value despite higher energy content. Individual genotypes had advantages and disadvantages, many of the saltbushes did not have higher nutritional value or relative palatability than Anameka<sup>™</sup>, but some of the seed lines (such as Anameka seed, seed CH and seed BH) were much more productive.

#### Dongara

At Dongara, Mallee saltbush RP B3 was a standout for biomass production, DOMD, OMD, Ash and ADF but CP and palatability were poor. Anameka seed, clone H and seed EH were the most productive shrubs, each producing more than 50% greater biomass than Anameka<sup>™</sup> (Table 4.8).

#### Cranbrook

Cranbrook was characterised by highly palatable but relatively unproductive saltbushes and very productive and unpalatable Mallee saltbush (Table 4.9). The Mallee saltbushes had higher DOMD and lower fibre but also lower CP than the saltbushes. Seed CH and seed GH were standout

saltbushes at the site. Seed EH had poor biomass production. Of the Mallee saltbushes, RP B8 and RP M3 appear to have the highest feeding value, assuming animals will eat them.

#### Cunderdin

At Cunderdin, Anameka seed, clone G, seed AH, seed BH and seed EH had greater than 50% productivity, compared to Anameka<sup>™</sup> (Table 4.10).

#### Across sites - focussing on the saltbushes

Figures 4.10 to 4.14 compare the saltbush genotypes across the four experimental sites. Most of the seed lines are consistently more productive than Anameka<sup>™</sup>. There is variation among the clones with some more productive than Anameka<sup>™</sup> at some sites and less productive at others (Fig. 4.12). Anameka seed and seed CH are standouts across sites. Seed EH was good at all sites but poor at Cunderdin.

Fig. 4.13 demonstrates that few saltbush genotypes are more palatable relative to Anameka<sup>™</sup>. Of the nine observations across sites, clone C was preferred on six occasions and clone H of four occasions. The seed lines were all less preferred to Anameka<sup>™</sup>.

DOMD is presented in Fig. 4.14. This is the trait where there is the least variability, which is unsurprising given the very heavy selection pressure that was applied to the cohort during the early stages of domestication. Anameka seed, seed BH and seed GH have similar or higher DOMD to Anameka<sup>™</sup>.

In terms of crude protein, clone B and seed BH clearly have lower CP content than clone A, clone C, clone E and seed EH (Fig. 4.15). Ash content was higher in clone A, clone C, clone E and seed EH (Fig. 4.16).

#### Where to from here?

Plant breeders tend to create a selection index and use it to select the best-bet genotypes. It is more complex for these shrubs as there are clear trade-offs between the key elements of feeding value - biomass production, DOMD, CP, ash and relative palatability. Palatability may be a good predictor of voluntary feed intake (especially for the highly palatable and highly unpalatable shrubs) but palatability is always a function of what else is on offer. Given no choices, sheep or cattle may happily consume one of the less preferred genotypes. For Mallee saltbush it is unclear if relative unpalatability is linked to compounds or mineral profiles that are potentially toxic. Although we have seen no evidence of toxicity, and we have not measured any compounds of concern, we have not managed the sheep to eat only Mallee saltbush and cannot rule out this possibility.

Our clonal Mallee saltbush selection (Cln B40) did have higher relative palatability but struggled to persist after heavy grazing. It is relatively prostrate so may be more useful as a shelter plant than the other genotypes (less chance of an ewe losing sight of a lamb), although long term persistence is a concern. There were several standouts in terms of biomass production and DOMD, including RP B3, RP B8, RP M3 and RP A12. These lines should be prioritised for further investigation.

There is no clear standout in terms of clonal lines, Anameka<sup>™</sup> remains a sound choice for commercialisation. There is clearly an opportunity to commercialise a saltbush seed line to complement Anameka<sup>™</sup> in the market. Anameka seed and seed CH are perhaps the most promising, seed BH is productive but has lower CP content. Seed EH was promising at three sites but performed poorly at another. Any are likely to be suited to nursery raised seedlings – seed production traits and ability to establish from seed should be considered.

		Nutritio	onal va	lue			Relative palatability							
Genotype	2020	2021	2022	2023	DOMD	OMD	СР	Ash	ADF	Early 21	Mid 21	Late 21	Early 22	Late 22
Anameka	100	100	100	100	100	100	100	100	100	100	100	100	100	100
Clone A	122	86	148	73	100	101	101	103	95	30	16	50	6	100
Clone B	108	132	156	100	102	100	87	91	103	49	45	72	11	100
Clone C	112	146	124	58	97	98	104	104	108	28	260	144	43	100
Clone E	136	113	125	88	96	97	102	106	104	34	57	98	6	100
Clone G	57	117	187	110	101	99	91	94	104	40	24	51	9	100
Clone H	69	102	97	103	100	99	99	99	103	49	87	95	122	100
Anameka seed	78	149	205	131	101	100	97	96	104	47	29	61	6	100
Seed AH	95	136	193	108	101	100	99	97	102	43	21	38	10	100
Seed BH	136	162	176	119	103	100	92	92	104	34	15	20	11	100
Seed CH	127	183	207	109	97	98	100	102	105	28	43	66	2	100
Seed DH	92	141	195	116	100	99	93	96	110	38	26	43	10	100
Seed EH	122	186	190	121	96	97	104	103	111	28	32	52	17	100
Seed GH	100	139	186	108	99	99	90	97	106	17	17	22	6	100
Spathulata	22	40	80	54	89	81	73	125	105	17	4	2	4	51
RP A12	32	112	136	87	116	108	72	74	79	32	6	8	1	19
RP B3	26	156	156	85	109	105	77	87	80	26	8	5	0	54
RP Cln 40	21	64	91	11	102	98	63	85	112	21	7	6	0	99
RP L9	16	93	110	81	113	104	68	73	91	21	5	8	0	30
RP M3	28	149	152	86	114	104	81	67	96	32	9	5	0	53
Mean	79	127	153	92	102	99	89	94	101	32	37	45	14	84

Table 4.7. Using Anameka as a standard (100%), percentage differences in components of feeding value at Baandee (green is positive, red is negative).

	'Edil	ble' DM in aut	umn		Nutrit	ional va	lue		Rel	ative palatak	oility
Genotype	2021	2022	2023	DOMD	OMD	СР	Ash	ADF	Early 21	Early 22	Late 22
Anameka	100	100	100	100	100	100	100	100	100	100	100
Clone A	130	196	83	101	104	116	111	88	10	42	129
Clone B	99	123	59	99	98	89	95	104	10	18	123
Clone C	125	198	61	99	101	113	108	97	4	88	134
Clone E	122	158	97	98	101	108	112	93	6	28	119
Clone G	85	138	90	103	102	103	99	94	7	6	118
Clone H	138	174	167	101	102	100	101	97	27	155	103
Anameka seed	136	196	162	100	100	98	99	102	7	12	104
Seed BH	144	195	111	102	103	98	104	93	2	0	117
Seed DH	110	176	119	97	97	89	101	105	4	7	109
Seed EH	147	235	159	97	100	106	108	96	3	6	118
Seed GH	110	152	101	100	100	93	101	98	4	6	114
Spathulata	53	75	21	87	94	75	130	99	8	0	69
RP B3	212	690	1556	115	111	84	88	78	7	0	0
RP Cln 40	114	231	686	108	106	64	91	93	1	0	1
Mean	122	202	238	100	101	96	103	96	13	31	97

Table 4.8. Using Anameka as a standard (100%), percentage differences in components of feeding value at Dongara (green is positive, red is negative).

		ible' DN autumn			Nutritio	onal va	lue		Relative p	alatability
	2021	2022	2023	DOMD	OMD	СР	Ash	ADF	2021	2022
Anameka	100	100	100	100	100	100	100	100	100	100
Clone A	105	99	72	102	102	124	101	94	66	102
Clone B	91	89	80	101	101	97	100	96	80	99
Clone C	81	53	45	96	97	117	106	107	109	107
Clone E	98	93	102	98	100	127	109	97	92	102
Clone G	109	136	97	102	102	99	98	97	95	100
Clone H	90	91	83	98	98	96	102	101	92	99
Anameka seed	133	156	114	102	101	98	96	101	57	95
Seed AH	128	143	110	102	102	103	100	99	47	100
Seed BH	102	119	108	101	101	94	99	98	41	99
Seed CH	158	162	142	99	99	104	102	103	82	101
Seed DH	135	125	104	103	101	97	93	101	71	100
Seed EH	122	82	86	98	99	106	102	105	77	99
Seed GH	152	194	144	102	102	97	98	98	67	102
Spathulata	18	12	18	73	79	62	135	131	0	34
RP A12	381	351	393	111	105	86	74	96	0	1
RP B15	243	219	239	109	104	104	76	103	1	13
RP B2	378	365	366	107	101	100	73	111	1	9
RP B8	594	508	492	108	103	91	79	103	0	12
RP C1	377	257	198	101	99	95	90	112	0	49
RP C17	201	129	98	97	97	92	100	115	1	57
RP Cln 40	184	195	168	98	97	72	94	109	0	80
RP G17	554	478	400	110	104	71	73	100	0	1
RP L14	466	301	319	107	101	90	75	109	1	17
RP L9	424	298	334	111	104	82	67	102	0	1
RP M3	476	471	536	109	102	84	68	108	1	24
RP S7	464	443	400	108	102	80	75	104	1	1
Mean	236	210	198	102	100	95	92	104	40	63

# Table 4.9. Using Anameka as a standard (100%), percentage differences in components of feedingvalue at Cranbrook (green is positive, red is negative).

	'Edibl	e' DM		Nutriti	onal va	lue	
	First spring	First autumn	DOMD	OMD	СР	Ash	ADF
Anameka	100	100	100	100	100	100	100
Clone A	110	118	104	105	114	104	88
Clone B	158	151	105	103	81	92	92
Clone C	78	71	101	101	111	99	97
Clone E	175	148	99	100	101	103	98
Clone G	122	166	104	103	99	98	92
Clone H	127	154	102	102	110	100	95
Anameka seed	130	171	103	101	94	96	99
Seed AH	121	165	100	100	88	98	103
Seed BH	202	160	104	103	81	98	93
Seed CH	122	154	101	100	89	100	100
Seed DH	114	156	105	104	108	98	92
Seed EH	76	160	102	103	107	105	93
Seed GH	127	138	105	106	86	102	89
Spathulata	94	68	86	98	72	149	90
RP Cln 40	114	72	110	109	83	96	86
RP S7	67	102	115	110	94	83	82
RP T5	96	171	117	111	95	78	86
Mean	119	135	104	103	95	100	93

 Table 4.10. Using Anameka as a standard (100%), percentage differences in components of feeding value at Cunderdin (green is positive, red is negative).

At Cunderdin, Anameka seed, clone G, seed AH, seed BH and seed EH had greater than 50% productivity, compared to Anameka<sup>™</sup>.

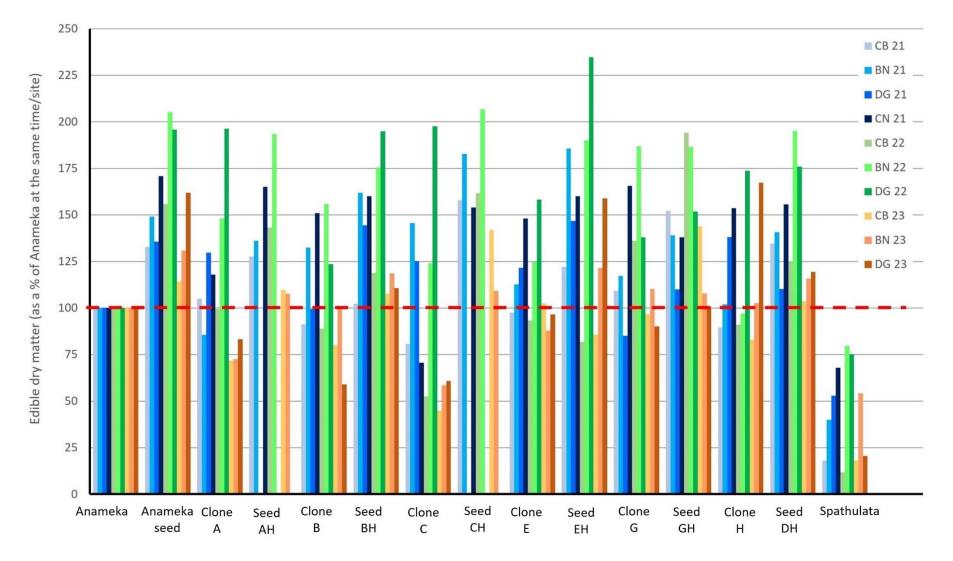


Fig. 4.12. Edible dry matter of saltbushes at each site in autumn, expressed as a percentage of the mean of Anameka at the site. Numbers above 100 indicate that the genotype was more productive than Anameka<sup>™</sup>. CB = Cranbrook, BN = Baandee, CN = Cunderdin and DG = Dongara.

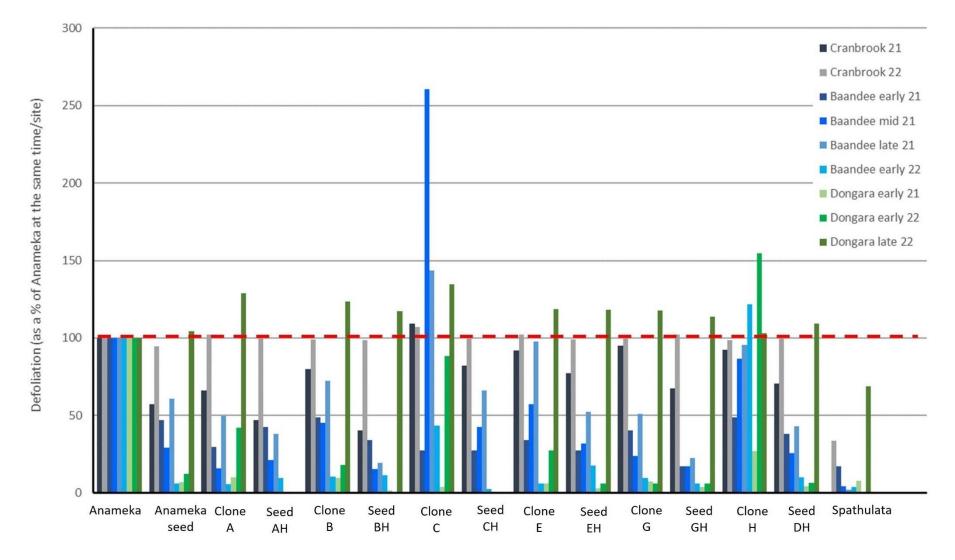


Fig. 4.13. Defoliation of saltbushes at each site in autumn, expressed as a percentage of the mean of Anameka<sup>™</sup> at the site. Numbers above 100 indicate that the genotype was more preferred to Anameka<sup>™</sup>.

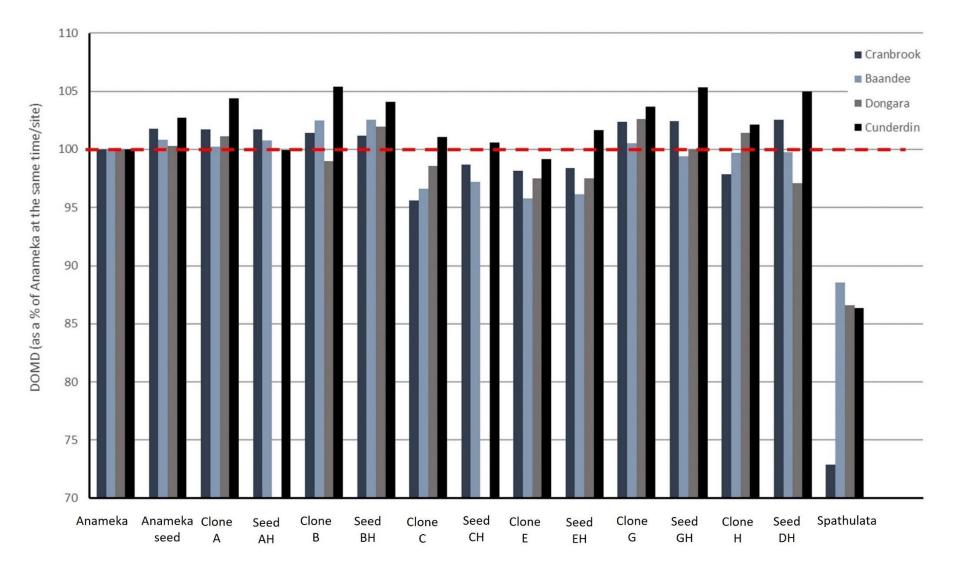


Fig. 4.14. Digestible organic matter in the dry matter of saltbushes at each site, expressed as a percentage of the mean of Anameka<sup>™</sup> at the site. Numbers above 100 indicate that the genotype had higher DOMD than Anameka<sup>™</sup>.

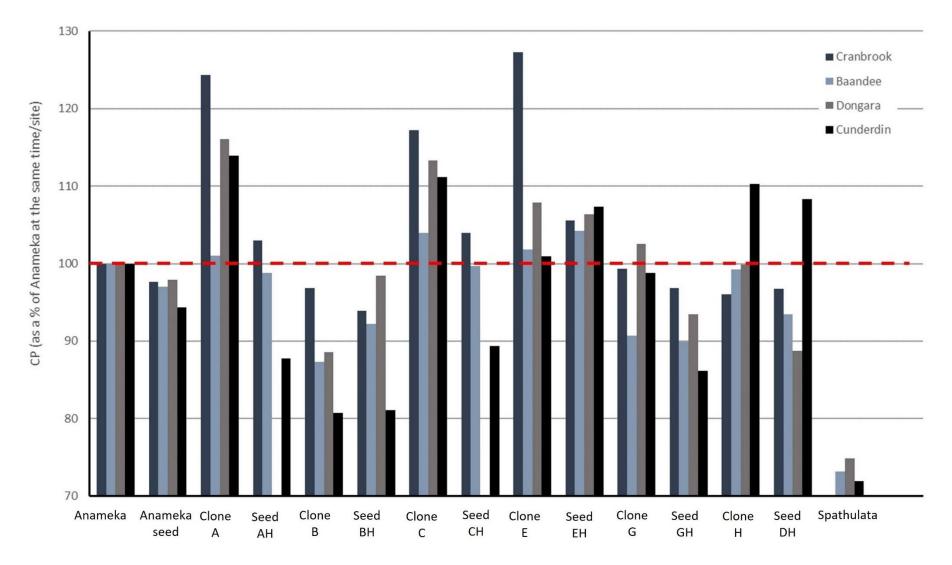


Fig. 4.15. Crude protein content of saltbushes at each site, expressed as a percentage of the mean of Anameka<sup>™</sup> at the site. Numbers above 100 indicate that the genotype had higher crude protein than Anameka<sup>™</sup>.

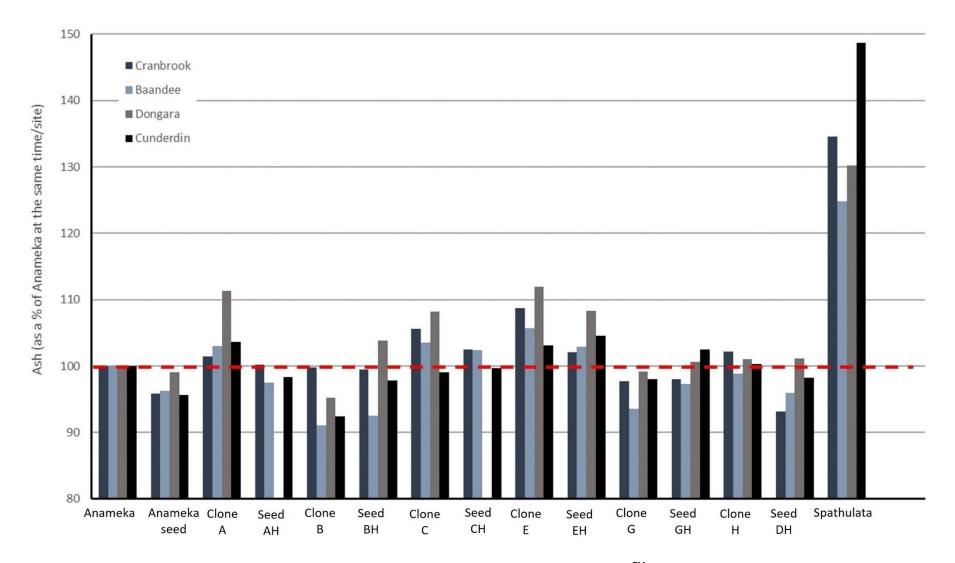


Fig. 4.16. Ash content of saltbushes at each site, expressed as a percentage of the mean of Anameka<sup>™</sup> at the site. Numbers above 100 indicate that the genotype had higher ash content than Anameka<sup>™</sup>.

# 4.4 Objective 2

### 4.4.1 Adapted legumes

Legume content of Banndee ranged from 6 to 36% (Table 4.2A). Burr medic was he most successful of the legumes (up to 22% of feed on offer), followed by woolly clover, Mawson subclover and bladder clover. The French serradella content of the pasture at Cranbrook was initially 42% but this did not persist (dropped to 7%, Table 4.2B; Fig. 4.17). It is probable the nitrogen that was fixed, and lack of weed control and grazing during the growing season, enabled bromegrass to dominate in the following years. The legume content of Dongara was never more than 2% (Table 4.2B). This site was not sown to legumes due to COVID restrictions at the critical times. It became apparent that it is difficult to manage weeds when they take advantage of the fixed nitrogen and lack of spring grazing. *Matricaria* (Globe Chamomile) and brome grass were the biggest concerns. It is probable that grass selectives can be used to control brome grass (if the producer has firebreak scale spraying equipment). Matricaria is a bigger concern as it is a forb so more difficult to selectively manage. This weed is colonising the eastern wheatbelt of WA and may become a disincentive to adoption of shrub systems. Research is required to get a better understanding of herbicide options for saltbush and Mallee saltbush.



Fig. 4.17. Cranbrook, July 2022. Growth of the serradella understorey demonstrating the system potential on deep sands (left). While saltbush provided better autumn grazing value, the Mallee saltbush has much greater shelter potential during lambing (right).

### 4.4.2 Salt tolerance of Mallee saltbush and saltbush; glasshouse experiment 1

### With MSc student Aslak Christensen (University of Copenhagen)

All plants survived the five salinity treatments, but with clear effects on growth. From the harvest of plants at the five salinity levels (Fig. 4.18) difference in plant and leaf size could be observed, with decreases in leaf size at 500 mM NaCl for oldman saltbush clonal cuttings and with chlorosis starting to appear on leaves of Mallee saltbush at the 500 mM salinity level. There was a significant effect of

increasing salinity level on the shoot growth of both species (Fig. .4.19). A curvilinear growth response was exhibited by saltbush at increasing salinity levels. The increased growth at 50 mM and same productivity at 100 mM, compared to the no-salt control, for oldman saltbush is expected as it is a halophyte (a plant that gives a growth response to salt). With 500mM (roughly seawater salinity), the saltbush yielded 59% of the no salt control.

Mallee saltbush is not a halophyte, and it demonstrated a continuous linear growth reduction at increasing salinity levels. Regardless, all plants survived the relatively short experiment and Mallee saltbush was still able to maintain growth at 500 mM, with a reduction in shoot growth to 40% when compared with the 0 mM treatment. It is unclear if the Mallee saltbush would have maintained this given persistence salinity, however it does have capacity to manage moderate levels of salinity or transient salinity. Long-term persistence of Mallee saltbush will be investigated using the saline sites developed in (CSIRO 2016 - AWI Project 84099).



Fig. 4.18. Clonal cuttings of oldman saltbush (top) and Mallee saltbush (bottom) showing the difference in growth response under the five salinity treatments (increasing from left to right), at the end of the glasshouse experiment before harvesting.

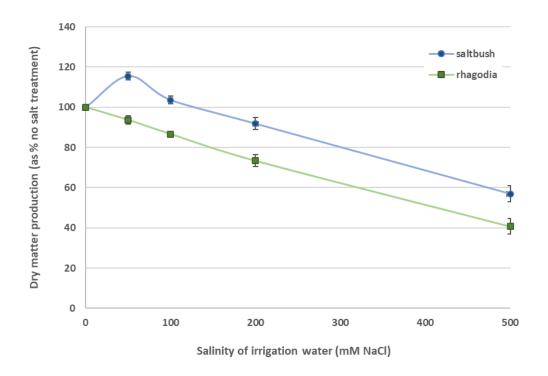


Fig. 4.19. The biomass growth at five salinity levels of clonal cuttings of Oldman saltbush and Mallee saltbush depicted as % growth relative to the 0 mM treatment

At the onset of salinity, the immediate plant response is a transient reduction of stomatal aperture triggered by the osmotic stress caused by the increasing electrical conductivity in the soil solution, and a reduction in leaf growth (Munns and Tester, 2008). Within hours a new reduced level of transpiration stabilizes. However, many dicotyledonous halophytes, but not all, have optimal growth conditions in concentrations of 50 - 360 mM NaCl (Flowers *et al.* 2010). Clonal cuttings of oldman saltbush of the genotype Anameka<sup>TM</sup> had optimal growth at 0 - 200 mM with the highest growth at 50 mM, illustrating the fact that Na+ can be considered an essential nutrient for oldman saltbush and hence stimulate growth at lower salinities.

Due to the high ash content of 23% at 0 mM it appears that Mallee saltbush actively accumulates salts, probably as an adaptive feature to dry and saline environments. Despite its high ash content at non-saline conditions, it had a negative growth response to even low salinities. It maintained growth at 500 mM NaCl in the soil solution, although with some signs of old leaves dying due to excess salt accumulation. This suggests that ionic stress sets in before 500 mM (~seawater salinity), indicating that this species is reaching its salinity threshold, the level at which it is able to survive. The physiological adaption of salt bladders in oldman saltbush increase its salinity tolerance, in contrast to Mallee saltbush, where such adaptive features have not been documented.

#### **Nutritional value**

For oldman saltbush, increasing salinity of the irrigation water was correlated to a significant increase in mean OMD, ash, S and nitrate. Salinity was negatively correlated with ADF and NDF (both on an OM basis), DOMD, Cu and phosphate. For Mallee saltbush, increasing salinity of the irrigation water was correlated to a significant increase in ash and chloride. Salinity was negatively correlated with ADF and NDF (both on an OM basis), oxalate and nitrate (Tables 4.11 and 4.12). Both species

had an increase in ash content associated with increasing salinity; 28.1% to 38.4% of shoot DM for saltbush.

		Oldr	nan salt	tbush			Mallee	e saltbus	h	
	0	50	100	200	500	0	50	100	200	500
	mМ	mМ	mМ	mМ	mМ	mM	mМ	mМ	mМ	mМ
Ash (%DM)	28.1	29.8	31.1	32.9	38.4	23.0	24.6	26.3	27.9	31.0
OM (%DM)	71.9	70.2	68.9	67.1	61.6	77.0	75.4	73.7	72.1	69.0
OMD %	65.8	65.1	66.0	66.0	68.0	70.5	69.1	71.8	70.3	67.9
DOMD %	48.8	47.6	47.6	46.8	45.6	54.7	52.9	54.0	52.1	48.9
NDF (%OM)	16.4	15.4	13.9	13.9	11.0	16.3	16.3	15.9	14.9	13.2
ADF (%OM)	9.9	9.1	8.5	8.4	6.4	11.2	10.9	10.2	9.7	9.1
CP (%DM)	24.4	26.3	28.1	29.4	27.5	22.5	22.5	23.8	23.1	20.6

Table 4.11. Nutritive value parameters examined for dry shoot biomass of clonal cuttings of Anameka<sup>™</sup> saltbush and Mallee saltbush grown at five different salinity levels.

# Table 4.12. Anion and mineral analysis quantifying the content of oxalate, nitrate, sulphate,phosphate, chloride and minerals in the shoot dry matter.

		Old	man salt	bush			Ma	allee salt	bush	
	0	50	100	200	500	0	50	100	200	500
	mМ	mМ	mМ	mМ	mМ	mМ	mМ	mM	mМ	mМ
Chloride (mg/gDM)	50.7	73.6	89.5	114.1	124.3	29.1	54.9	73.5	99.0	127.7
Nitrate (mg/gDM)	96.9	102.6	103.5	46.8	14.8	104.2	110.8	97.6	57.4	14.1
Phosphate										
(mg/gDM)	25.1	22.9	24.5	22.3	14.9	13.2	13.7	19.3	22.0	16.7
Sulphate (mg/gDM)	7.7	7.8	8.1	10.8	15.7					
Oxalate (mg/gDM)	51.5	57.8	41.4	15.5	12.4	53.4	50.1	54.6	39.8	27.1
Na (%DM)	3.7	7.6	9.4	9.1	11.2	2.7	4.9	6.9	8.7	8.7
K (%DM)	4.7	2.6	2.4	2.0	2.0	4.7	3.4	3.0	1.3	1.2
Ca (%DM)	1.4	0.8	0.8	0.7	0.7	1.3	0.8	0.7	0.6	0.8
Mg (%DM)	1.1	0.7	0.4	0.4	0.4	1.1	0.9	0.8	0.6	0.6
S (%DM)	0.5	0.5	0.6	0.6	0.7	0.2	0.3	0.3	0.3	0.2
P (%DM)	0.7	0.7	0.8	0.7	0.5	0.4	0.5	0.6	0.6	0.5
Fe (mg/kgDM)	114.5	96.1	120.1	94.7	87.5	104.4	80.4	117.9	85.7	86.3
Cu (ug/kgDM)	13.7	12.2	12.7	11.5	10.4	23.3	29.8	37.2	29.3	17.8
Mn (mg/kgDM)	85.3	105.3	118.5	89.3	104.3	170.9	187.2	258.4	202.6	166.3
Zn (mg/kgDM)	105	69.9	74.9	58.4	73.2	275	274	321	298	211
B (mg/kgDM)	60.6	60.1	77.3	73.9	74	59.3	53	57.6	59.8	47.4

Analysis of inorganic anions and oxalate showed that there was a significant interaction between salinity level and their concentration in the shoot DM. There was a significant increase in chloride content when increasing the salinity for both species, increasing the content from 5.1% to 12.4% of DM in saltbush and increasing the content from 2.9 to 12.7% in Mallee saltbush (Table 4.12). Both

species showed similar high contents of nitrates and oxalates in the shoot DM, and experienced significant reductions at increasing salinity. Nitrate was reduced from approximately 10% to 1.4% of shoot DM when increasing the salinity from 0 – 500 mM, while the oxalate level decreased from 5.2% to 1.2% of shoot DM in saltbush and 2.7% from 5.3% of shoot DM in Mallee saltbush. The oxalate content is highest at the optimum salinity level in relation to growth, 50 and 0 mM NaCl for oldman saltbush and Mallee saltbush, respectively. Oldman saltbush experienced a significant increase from 0.8 - 1.6% sulphate in the shoot DM. The analysis of the mineral profile revealed a significant interaction between increasing salinity level and the Na and Mg concentration in the shoot DM for both species.

The ash content in the shoot biomass of Mallee saltbush when grown at non-saline conditions were much higher than the 12 % previously reported (Norman *et al.* 2017). Due to the higher ash content in the shoot DM, the DOMD was only 55 % compared with previous reports of 66 % (Revell *et al.* 2013). Despite the higher ash content, the shoot DM of Mallee saltbush had a high CP content of 22.4 % at 0 mM, this is in excess of the maintenance and growth requirements for sheep and cattle. The nutritive value of Mallee saltbush is significantly reduced from 200 to 500 mM, correlating with its growth that was also significantly reduced from 200 to 500 mM NaCl. Hence, plants growing on saline land would produce less energy per hectare both due to reduction in growth and ME, compared with non-saline environments.

Oldman saltbush had a OMD of 66 to 68 % at 0 to 500 mM NaCl. The metabolizable energy content in the shoot DM of oldman saltbush was reduced from 6.9 MJ kg at 0 mM to 6.3 MJ kg at 500 mM NaCl, and from 8.1 to 6.9 MJ kg for Mallee saltbush. This reduction is primarily caused by a decrease in OM due to a higher ash content in the DM. Oldman saltbush plants had a smaller decrease in ME with increasing salinity, despite having a higher increase in ash content than Mallee saltbush. The cause of this difference can be found in increasing OMD values with increasing salinity levels for oldman saltbush plants, which to some extent counter the negative effects of reduced OM on the ME.

When grown under saline conditions, halophytes have a high requirement for N due to the synthesis of nitrogen containing compatible solutes used to osmotically adjust the symplasm of leaf cells (Flowers and Colmer, 2008). The higher growth at elevated salinity levels of oldman saltbush at elevated salinity levels correlates with a higher accumulation of N, which is likely destined for the synthesis of compatible solutes containing N, such as glycine betaine. Therefore, the actual CP contents might be significantly lower due to the presence of these non-protein compounds. This N will only be available for conversion into microbial protein in the rumen, provided there is sufficient ME available in the diet or if it's included in a protein-deficient diet (Masters *et al.* 2001). This is also the case for the CP in the shoot DM of Mallee saltbush, that despite not having an increase in growth or N content with increasing salinity, still had a high ash content that would require a high concentration of compatible solutes in the symplasm for osmotic regulation.

When examining the mineral profile at different salinity levels, significant effects were observed. Both species experienced a similar increase in Na+ and Cl- with increasing salinity. All halophytes rely on the controlled uptake and compartmentalisation of Na+, K+, and Cl- into vacuoles and the synthesis of organic compatible solutes, with Na/K ratios being 2.5 to 21 times higher in the vacuole compared with the cytosol (Flowers and Colmer, 2008). It seems that oldman saltbush is better able to compartmentalise salts than Mallee saltbush, reflected in the 7 % higher ash content at 500 mM

Salinity	1	-																		
ADF	2	-0.97	-																	
Ash	3	0.99	-0.99	-																
OMD	4	0.93	-0.89	0.91	-															
DOMD	5	-0.95	0.96	-0.97	-0.77	-														
СР	6	0.46	-0.54	0.54	0.25	-0.65	-													
NDF	7	-0.96	0.99	-0.98	-0.89	0.95	-0.59	-												
Ca	8	-0.59	0.70	-0.66	-0.31	0.81	-0.85	0.71	-											
Cl	9	0.87	-0.90	0.91	0.70	-0.95	0.83	-0.91	-0.84	-										
К	10	-0.62	0.73	-0.70	-0.36	0.83	-0.88	0.74	1.00	-0.88	-									
Mg	11	-0.63	0.75	-0.71	-0.44	0.80	-0.93	0.78	0.94	-0.89	0.96	-								
Na	12	0.79	-0.89	0.85	0.61	-0.91	0.80	-0.90	-0.93	0.92	-0.95	-0.96	-							
Oxalate	13	-0.85	0.80	-0.86	-0.78	0.84	-0.70	0.83	0.56	-0.92	0.62	0.69	-0.71							
S	14	0.93	-0.95	0.95	0.91	-0.89	0.63	-0.98	-0.63	0.90	-0.68	-0.78	0.86	-0.88	-					
Cu	15	-0.92	0.92	-0.94	-0.71	0.99	-0.62	0.90	0.81	-0.93	0.83	0.75	-0.87	0.80	-0.82	-				
Fe	16	-0.69	0.62	-0.69	-0.44	0.77	-0.31	0.57	0.57	-0.65	0.56	0.37	-0.51	0.57	-0.44	0.85	-			
Mn	17	0.16	-0.37	0.22	0.11	-0.27	0.31	-0.38	-0.53	0.23	-0.52	-0.56	0.58	0.10	0.32	-0.19	0.18	-		
NO3	18	-0.93	0.84	-0.92	-0.85	0.88	-0.46	0.84	0.48	-0.85	0.52	0.52	-0.63	0.93	-0.83	0.87	0.76	0.19	-	
PO4	19	-0.97	0.93	-0.95	-0.88	0.91	-0.28	0.89	0.53	-0.76	0.55	0.49	-0.70	0.71	-0.81	0.91	0.78	-0.13	0.88	-
Zn	20	-0.40	0.50	-0.48	-0.08	0.67	-0.88	0.50	0.95	-0.75	0.94	0.85	-0.78	0.51	-0.44	0.70	0.57	-0.33	0.40	0.34
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19

Table 4.13A Correlations between glasshouse salinity treatments and nutritional traits for oldman saltbush

Correlations in bold black are significant (P < 0.05)

Salinity	1	-																		
ADF	2	-0.93	-																	
Ash	3	0.96	-0.99	-																
OMD	4	-0.67	0.41	-0.51	-															
DOMD	5	-0.96	0.86	-0.92	0.81	-														
CP	6	-0.74	0.45	-0.54	0.92	0.79	-													
NDF	7	-0.99	0.94	-0.96	0.64	0.95	0.72													
Ca	8	-0.39	0.64	-0.61	0.02	0.42	-0.20	0.37	-											
Cl	9	0.93	-0.99	0.99	-0.49	-0.90	-0.48	-0.93	-0.68	-										
К	10	-0.83	0.95	-0.93	0.42	0.83	0.33	0.85	0.78	-0.97	-									
Mg	11	-0.80	0.95	-0.92	0.33	0.78	0.25	0.81	0.83	-0.96	0.99	-								
Na	12	0.78	-0.95	0.92	-0.24	-0.74	-0.19	-0.78	-0.84	0.95	-0.98	-0.99	-							
Oxalate	13	-0.95	0.87	-0.90	0.77	0.97	0.78	0.97	0.34	-0.89	0.84	0.78	-0.73	-						
S	14	-0.37	0.08	-0.13	0.44	0.29	0.73	0.39	-0.71	-0.04	-0.14	-0.22	0.24	0.37	-					
Cu	15	-0.58	0.29	-0.36	0.79	0.61	0.92	0.61	-0.43	-0.30	0.19	0.09	-0.02	0.69	0.86	-				
Fe	16	-0.37	0.26	-0.32	0.80	0.59	0.53	0.38	0.25	-0.36	0.45	0.37	-0.26	0.59	-0.02	0.47	-			
Mn	17	-0.29	-0.01	-0.07	0.78	0.41	0.79	0.32	-0.51	-0.03	-0.01	-0.12	0.22	0.49	0.70	0.91	0.65	-		
NO3	18	-0.97	0.93	-0.94	0.61	0.92	0.69	0.99	0.35	-0.92	0.85	0.81	-0.78	0.97	0.40	0.62	0.38	0.34	-	
PO4	19	0.31	-0.64	0.54	0.33	-0.21	0.38	-0.36	-0.78	0.60	-0.71	-0.76	0.80	-0.27	0.50	0.39	0.09	0.56	-0.41	
Zn	20	-0.72	0.42	-0.52	0.93	0.78	1.00	0.70	-0.21	-0.46	0.32	0.23	-0.17	0.77	0.73	0.92	0.55	0.81	0.67	0.40
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19

Table 4.13B Correlations between glasshouse salinity treatments and nutritional traits for Mallee saltbush

. Correlations in bold black are significant (P < 0.05)

Oldman saltbush and Mallee saltbush are some of the Australian shrub species with the highest S content, with averages in this study ranging from 2 to 3 g/kg for Mallee saltbush and 5 to 7 g/kg for oldman saltbush, similar to what has previously been recorded for these species (Revell *et al.* 2013). Sulphur is primarily utilized by ruminants together with N in the production of microbial CP and therefore it's the ratio of N:S that's important, with the recommended N:S ratio being 12.5:1 (SCA, 2007). Sulphur is especially important for wool growth. The N:S ratio was 7.8:1 at 0 mM NaCl and 6.2:1 at 500 mM for oldman saltbush and 18:1 and 16.5:1 at 0 and 500 mM NaCl respectively for Mallee saltbush. Based on the N:S ratio there is an excess of sulphur in oldman saltbush, which could be advantageous for wool producing sheep as they have a high sulphur requirement (Norman *et al.* 2004). The high N to S ratio in Mallee saltbush could potentially contribute to its lower palatability.

Considering that the digestibility of the DM is one of the most critical profit drivers in the use of these perennial halophytic shrubs, it seems that the shoot DM of Mallee saltbush had a better nutritive value at non-saline conditions compared with oldman saltbush shoot DM. Mallee saltbush had a lower ash content, better digestibility and higher ME at all salinity levels. The nutritive value of oldman saltbush became similar to Mallee saltbush as salinity increased.

The increasing ash content reduces the feeding value, not only due to its effect on NV by decreasing ME but also the impact on voluntary feed intake (VFI). Low concentration of salts can stimulate feed intake by increasing water use and thereby the rate of passage of feed in the rumen, but high NaCl concentrations have shown to reduce the feed intake significantly (Masters *et al.* 2005b). The high ash content of oldman saltbush has also shown to cause mineral imbalances of Mg, Ca, and K when feed to sheep as the sole source of feed (Mayberry *et al.* 2010). Besides salt, saponins and high oxalate levels have been reported to reduce the palatability of Mallee saltbush for grazing livestock when grown on non-saline sandy soils, whereas oxalate and nitrate can reduce the VFI of Oldman saltbush (Norman *et al.* 2017). Based on the results from this study it seems that nitrate and oxalate are present at levels that could be toxic and could result in a decreased VFI. The high salt content will likely reduce the risk of animals consuming toxic amounts of these compounds. There was also a negative correlation between increasing salinity level and oxalate concentration in the shoot DM of both species.

# 4.4.3 Impact of salinity and nitrogen fertilisers on growth and nutritional value of saltbush - glasshouse experiment 2

The plants responded in an astounding manner to the addition of nitrogen, even under very high (500 mM or sea water equivalent) salinity (Fig. 4.21). Plant biomass increase was almost entirely driven by an increase in leaf mass. Leaf dry weight increased steadily with the addition of nitrogen (Fig. 4.22), differing significantly between nitrogen levels (p < 0.001), and interacting significantly with salinity level (p < 0.001). Generally, the three highest rates of nitrogen (5, 10 and 20 mM of ammonium nitrate) produced the most leaf growth, with little difference between them. The no nitrogen treatment produced a very minimal increase in leaf area by the end of the experiment, likely due to the addition of other nutrients. While the 2.5 mM nitrogen treatment produced a growth response, this was significantly smaller than the higher nitrogen levels at low and medium salinity. High salinity (500 mM) produced a significantly (p < 0.001) smaller growth response to the lower salinities which did not differ significantly (p = 0.47) from each other in their growth response.

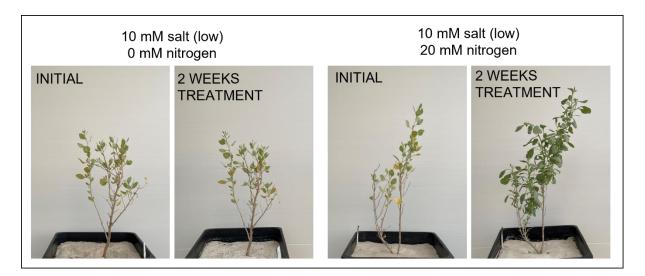
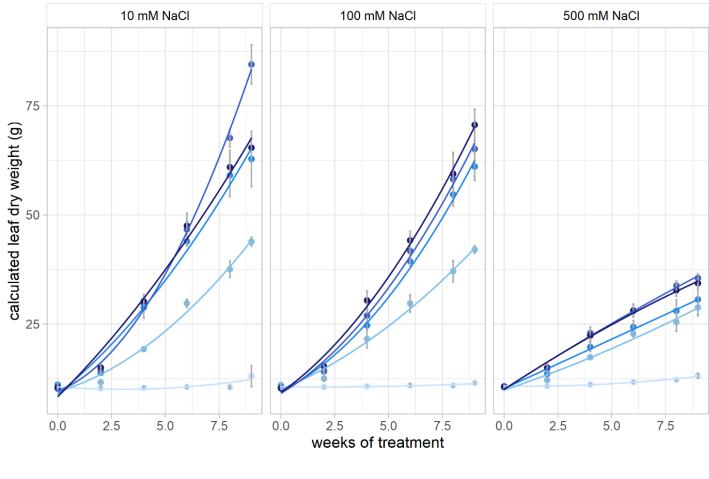


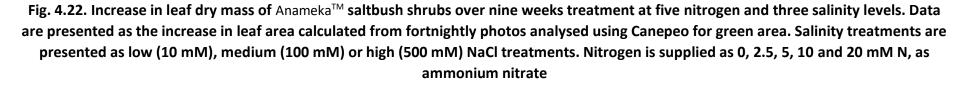
Fig. 4.20: Example of saltbush response to nitrogen treatments after 2 weeks of stepping up treatments with reduced strength nutrient solution (25 % solution concentrations in week one and 50 % in week two).



Fig. 4.21. Comparative growth of Anameka<sup>™</sup> saltbush shrubs after 9 weeks treatment at differing nitrogen and salinity levels.



Nitrogen (mM) → 0 mM N → 2.5 mM N → 5 mM N → 10 mM N → 20 mM N

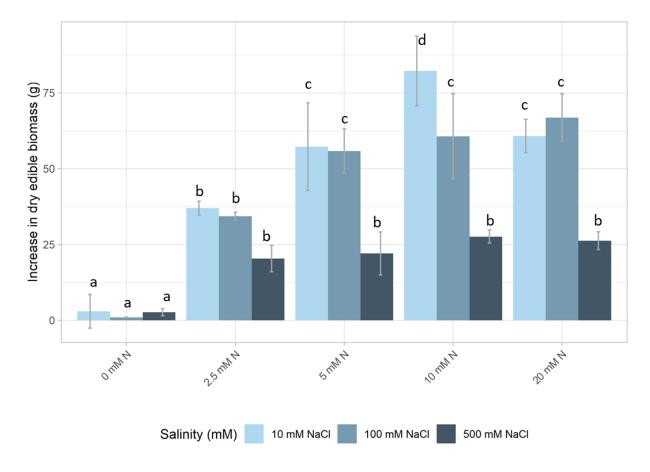


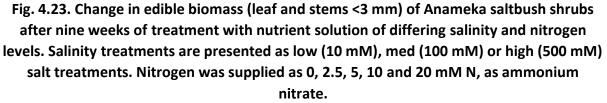
Changes in leaf biomass were driven by changes in leaf area and thickness. The addition of nitrogen resulted in edible matter per plant (dry mass of leaves and thin stems) increasing significantly over the nine weeks of treatment (Fig. 4.22, Table 4.14). High salt levels produced the smallest amount of growth, however the plants coped well with 100 mM of salinity, in many cases producing as much growth under 100 mM as under very low levels of salinity (10 mM). The largest increase in edible biomass was produced by the addition of 10 mM nitrogen under low salt, however even at higher salinities (100 mM) large amounts of biomass can be produced with the addition of nitrogen.

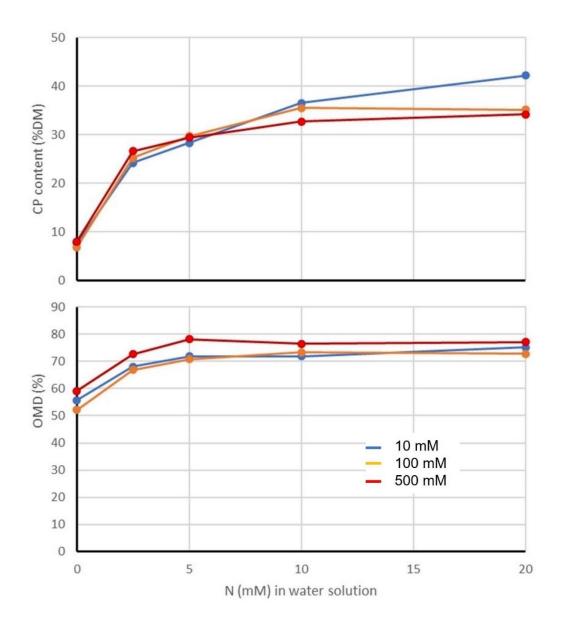
Nutritional value of biomass changed rapidly with nitrogen application from the low nitrogen control but these plateaued from 5 to 10 mM in the solution where the plants appeared to reach sufficient nitrgen. Data for CP and OMD are presented in Fig. 4.24.

# Table 4.14. Dry mass and leaf area from final destructive harvest of A. nummularia grown undervarying salinity and nitrogen treatment for nine weeks. Edible biomass is the sum of leaves and<br/>thin stems (< 3 mm) which would be consumed by sheep and cattle.</td>

Salinity (mM NaCl)	Nitrogen (mM)	Leaf (g)	Woody Stems >3mm (g)	Stems <3 mm (g)	Edible biomass (g)	Leaf area (m2)
	0	3.98	7.23	3.51	7.48	0.04
	2.5	33.94	21.94	7.43	41.37	0.79
10	5	60.87	38.65	10.83	71.70	1.64
	10	74.37	43.33	14.56	88.93	2.23
	20	67.27	35.42	12.53	79.80	1.94
	0	5.12	8.08	4.05	9.17	0.04
	2.5	39.74	18.12	8.59	48.33	1.09
100	5	59.06	26.27	11.54	70.59	1.38
	10	61.54	28.05	10.42	71.96	1.86
	20	66.03	31.99	10.99	77.02	1.81
	0	6.86	8.94	3.60	10.46	0.06
	2.5	35.30	9.49	7.94	43.24	0.54
500	5	38.10	10.22	8.37	46.47	0.64
	10	50.18	12.30	10.19	60.37	0.88
	20	51.22	12.25	8.72	59.93	1.13
	LSD	14.95	7.35	4.00	18.01	0.39
Effect	Nitrogen	<0.001	<0.001	<0.001	<0.001	<0.001
(P value)	Salinity	0.001	<0.001	n.s.	0.002	<0.001
	Nitrogen x Salinity	0.059	<0.001	n.s.	0.088	<0.001







# Fig. 4.24. Crude protein and organic matter digestibility of biomass from the second glasshouse experiment.

In summary, saltbush responds well to nitrogen application in terms of biomass growth and nutritional value of the biomass, until a critical limit is reached. This limit is to some degree associated with the salinity at the site as saltbush needs non-protein N compounds for osmoregulation. It is possible that field responses could be determined but the nitrogen availability and salinity of the site would drive the response.

### 4.4.4 Application of nitrogen and/or phosphate fertilisers – field experiment 1

Our objective was to assess the influence of nitrogen and phosphate levels on the growth and nutritional value of oldman saltbush in the field. The addition of nitrogen was shown to lead to a significant increase in growth in a glasshouse, however, no studies show a growth response to nitrogen application in the field. There was little information regarding phosphate at the start of this project. As a general rule, Australian natives are well adapted to low phosphate environments. Fertiliser treatments were applied to a subset of saltbushes at two sites Dongara in spring 2019 and again in spring 2020. Calcium nitrate was applied at the rate of 60g per shrub and superphosphate at the rate of 70g/shrub. This equated to approximately 100 to 120 kg/ha for each fertiliser.

The field data yielded some mixed messages (Table 4.15). At Forsyth's (sand over clay), the only significant difference was that the calcium nitrate and superphosphate treatment yielded significantly less biomass (~30%) than the unfertilised control. At Gillam's (deep white sand), the unfertilised control produced significantly less biomass than the fertilised plots. There was a significant response to superphosphate and to calcium nitrate, but the combination of fertilisers did not improve biomass production. In the first year at Gilliam's, addition of calcium nitrate quadrupled biomass production from the saltbushes.

	Forsyth Ma	r 2020	Gillam Mar	2020	Gillam Mar	2021	Gillam June	2021
Treatment	Mean	sem	Mean	sem	Mean	sem	Mean	sem
No fertiliser	107	8.6	41	4.3	82	3.8	71	2.5
Superphosphate	111	7.8	83	12.9	112	7.1	117	8.1
Calcium nitrate	101	5.5	167	17.8	113	4.9	119	6.9
Calcium nitrate +								
superphosphate	80	6.1	107	8.7	95	3.4	95	4.3
P value	0.002		<0.001		<0.001		<0.001	
LSD (5%)	17		31		11		12	

# Table 4.15. Biomass growth responses from fertiliser treatments applied at the two sandy researchsites at Dongara (Forsyth and Gillam). Data are expressed as a percentage of the mean.

# 4.4.5 Application of nitrogen fertilisers at different rates – field experiment 2

To better understand the N rate responses, fertiliser treatments were applied to Anameka<sup>™</sup> shrubs at Baandee in June 2021. The treatments included five rates of calcium nitrate application (from none to 80 kg/ha), across six replicated blocks with ten replicate shrubs for each treatment per block. Biomass production was assessed in October 2021 and again in February 2022. Biomass was sampled for nutritional value in October 2021. Shrubs within blocks were sampled and bulked so that there were six samples for each treatment.

There was no significant biomass production response to the fertiliser applications at Baandee (Fig. 4.25). It is probable that the mature shrubs were mining historical fertiliser applications at depth. The soil was less sandy than the Dongara sites and would have had greater nutrient holding capacity. There were no significant differences in nutritional value traits that were associated with fertiliser treatment (Table 4.16). This is not surprising as the lack of nitrogen was not inhibiting growth.

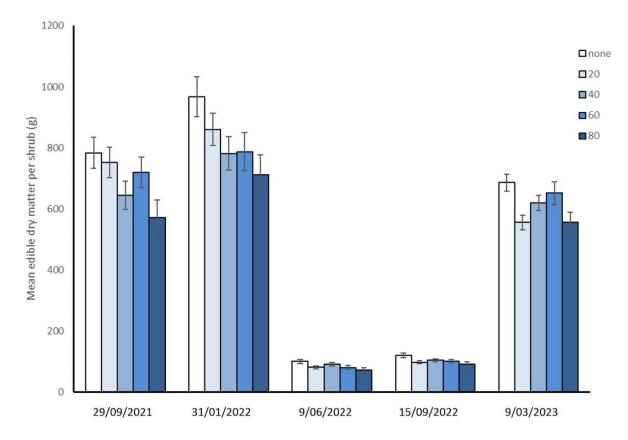


Fig. 4.25. 'Edible' dry matter of Anameka<sup>™</sup> at the Baandee research site. Six replicate blocks of ten shrubs were subject to five levels of nitrogen fertiliser application over two seasons.

		levels of	calcium nitrate	fertiliser.			
Calcium nitrate	NDF	ADF	Hemicellulose	OMD	DOMD	СР	ash
(g/shrub)	(%DM)	(%DM)	(%DM)	(%)	(%)	(%DM)	(%DM)

Table 4.16. Nutritional value of Anameka<sup>™</sup> oldman saltbush plants at Baandee, subject to five

Calcium nitrate	NDF	ADF	Hemicellulose	OMD	DOMD	СР	ash
(g/shrub)	(%DM)	(%DM)	(%DM)	(%)	(%)	(%DM)	(%DM)
0	33.2	17.7	15.6	70.3	51.7	19.3	26.6
20	34.9	18.4	16.5	68.8	50.4	17.5	26.8
40	34.7	18.9	15.8	69.0	51.8	18.1	25.0
60	35.1	18.8	16.3	68.3	50.1	17.1	26.7
80	34.5	18.4	16.1	69.2	50.8	17.6	26.7
Mean	34.5	18.4	16.0	69.1	50.9	17.9	26.4
P value	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

In summary, both saltbush and Mallee saltbush respond to nitrogen fertiliser, if the nutrient is limiting plant growth. There is no point applying nitrogen if the plants have sufficient (as appeared to be the case in Baandee). Shrubs on saline land have higher nitrogen requirements to optimise growth, however salinity has a negative impact on growth, so shrub biomass returns on a nitrogen investment may be lower. Although we have too few sites to make firm recommendations, it is probable that sandy sites and areas without a history of fertiliser application are more likely to provide a yield response to nitrogen. There are no clear recommendations for industry as the

decision to apply nitrogen is going to be very site specific and may not be guided by soil nitrogen in the top 30 cm (the trait that is measured to inform crop and pasture fertiliser application) as the shrubs are very deep rooted. We now have a better understanding of the salt tolerance of Mallee saltbush. While it is not a true halophyte, it has capacity to grow on saline soils where the salinity of the soil solution is less than seawater salinity.

# 4.5 Objective 3

Elite seed lines were compared in the first objective. They have two pathways to market, firstly as seeds that are grown in nurseries (at a potential lower cost than Anameka<sup>™</sup> as they are mechanically planted compared to manual propagation by cuttings), or they are direct seeded *in situ*. For saltbushes that are grown in harsh environments or where rainfall over summer is rare, risk during the establishment phase is acute. For a species where individual shrubs happily persist in harsh saline or semi-arid areas for many decades, oldman saltbush is very challenging to establish from seed in the field. During project development it became clear that there were a number of issues that needed to be addressed if we were to establish elite saltbush and Mallee saltbush genotypes directly on farms. Of the two species, oldman saltbush is much more challenging to establish from seed due to the woody bract.

Germination and emergence are the most precarious stage in a plant's lifecycle and seedlings are particularly vulnerable to environmental stress. Despite these constraints, plants colonise extreme environments over the planet. For long-lived perennial species such as oldman saltbush or Mallee saltbush, competition with the mother plant can be acute (to the detriment of the mother plant and the offspring); they likely utilise a range of methods to 'discourage' seedling establishment in the immediate vicinity of the parent plants and 'encourage' dispersal to new areas. Members of the research team co-authored a journal paper that argues that for many species, establishment in extreme situations is not because seed is adapted to germinate in extreme environments but because it falls into, and is spatially and temporally nurtured within, more benign "recruitment niches" (Barrett-Lennard *et al.* 2016. This principle has importance for revegetation in extreme environments such as the world's drylands and builds upon the legacy of research by CV Malcolm and colleagues between 1976 and 1982 on saltland revegetation.

Recruitment niches can be constructed based on an understanding of the key requirements that seeds need in their immediate environment for establishment. In the 1980's Malcolm's team developed a "niche seeder" capable of distributing and precisely placing fruits of saltbushes (bracts) in an elevated "V"-shaped mound (to decrease risk of waterlogging and allow some salt leaching from sandy soils), covering the bracts with vermiculite (to decrease capillarity and therefore salinity at the soil surface), and spraying the placements with black paint (to increase soil temperatures) (Barrett-Lennard *et al.* 2016). Figure 4.26 shows a niche seeder that is still operated by Ian Walsh and Sam Lehmann in Cranbrook, WA. Other studies in arid environments have demonstrated that establishment can be improved using stones on the soil surface to develop appropriate recruitment niches. While niche seeding is successful in some areas (most likely where there is sandy soils and relatively reliable summer rainfall), it still requires specialised equipment, a 'good' season, heavy tillage to create a good soil bed and vermiculite. Black paint is no longer used.

Common methods for establishing oldman saltbush is through planting nursery raised seedlings. Nursery production of plug plants for translocation rely on automated air seeder. To compensate for potential emergence shortcomings, multiple seeds per plug (3 to 5) are usually sown. This approach guarantees that all plugs are filled, but if more than one seedling emerges in a single plug, the excess seedlings need to be manually removed, considerably adding to the labour costs.

In 2016, we calculated that a saltbush seedling established from seed was approximately half the cost of a seedling from a nursery (CSIRO 2016 - AWI Project 84099). The denser the plantation that is desired, the more significant the cost. Vermiculite was a significant expense. The other major cost to direct seeding is the time that seedlings take to reach a size where the paddock can be grazed (higher opportunity cost for the land) and the risk of failure is higher. A paddock that is planted to seedlings can be grazed (albeit carefully), the autumn after establishment. Directly seeded paddocks are likely to be out of production for at least twelve months, two years if there is a need to fill gaps with seedlings. A final complication is that saltbush bract are woody and it is incredibly difficult to pull them apart to see if there are a seed within. It is possible for farmers to plant bract with exceptionally low seed fill, and the native seed industry does not always sell seed or bract with seed fill and germination percentages.



Fig. 4.26. Niche seeding machine from Cranbrook (Walsh and Lehmann families).

This inability of long-lived perennial shrubs such as saltbush to readily establish is likely to be an ecological strategy to minimise competition at the site of the mother plant and improve long-term survival. A series of laboratory, nursery and field experiments were conducted to gain a better understanding of saltbush seed ecology, methods to determine seed fill in woody bracts, methods to remove seed from bracts, response to temperature during establishment, impact of depth of burial on establishment, direct seeding with coated seed (with various growth promotors and insecticides), and bract fill/seed quality of the elite seed lines.

Seed processing and enhancing technologies can provide solutions for both the nursery production industry, and the farmers that intend to achieve successful saltbush field establishment by direct seeding. Seed processing techniques aim to reduce or remove the external structures enveloping the

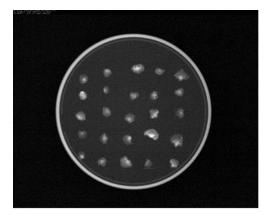
seed (bract) that limit seed handling and mechanical delivery. The bract could also be responsible for reduced germination, staggered germination and complicate the application of seed enhancement.

The objective to progress towards lower cost seedlings and in-field seeding was addressed across a range of experiments.

### 4.5.1 Seed morphometric analysis and seed viability tests

The seed of oldman saltbush is encapsulated in two hard-woody bracts. The edges of the bract are flat, of soft paper-like consistency, while the centre of the bract, where the seed is located, is bulged, thicker and of hard-wooden texture. The dispersal units (bract and seed) are very heterogeneous in shape and size. It is not uncommon for dispersal units to contain underdeveloped seed, or no seed at all. The current seed sorting equipment that relies on differential density between dispersal unit with seed and without seed (eg air flow separator), different size (sieves) or different shapes (gravity table) have so far been ineffective in correctly separating the seed-filled units from the empty ones.

A preliminary quality assessment was performed using the x-ray scan, to determine average seed fill of the batch of intact bracts (bracts). Bracts were divided in two subsamples based on size: <3.15 mm and >3.15mm. Five replicates of 25 fruits of each subsample were tested in the x-ray (Fig. 4.27).



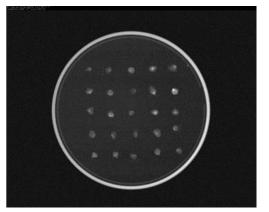


Fig. 4.27. X-ray imaging of oldman saltbush bracts. The image on the left is the "big" fraction <3.15mm, on the left is the small fraction >3.15 mm

#### How effective is x-ray to assess seed fill?

Across the mixed saltbush population, 51.6% of the bracts contained seeds, with bigger bracts having a greater proportion of seed fill. The "big" fraction contained  $66 \pm 5.7\%$  seeds, while the "small" bracts contained significantly lower  $38 \pm 5.8\%$  seeds (Table 4.17).

With x-ray it was not possible to clearly identify all the seeds presents in the fruits. However, when germination testing was performed on seed extracted from the bracts, seed not identified originally with x-ray (either unknown or empty), did not germinate (Table 4.17). This means that x-ray might not be accurate to determine seed fill, however, it is a good predictor of seed viability and germinability. The x-ray technology is not commonly available to tree nurseries and is more likely to remain a research tool.

X ray		Cleaned	Germir	nation
			Yes	No
With seed	52	50 (2 empty)	35	15
Empty	32	6 (26 empty)	0	6
Unknown	16	15 (1 empty)	0	15

Table 4.17. Result of x-ray and germination analysis

### Does fruit size/weight and density have an effect on seed fill?

The average weight of a bract is  $10.1 \pm 0.63$  mg with an area of  $26.0 \pm 1.14$  mm<sup>2</sup>. For cleaned seed the average weight is  $0.9 \pm 0.06$  mg and the volume:  $2.3 \pm 0.07$  mm<sup>2</sup>. Seed and fruit volume required to calculate density was determined with the equitation described in Ganhão & Dias (2019). For all morphometric parameters there was a statistically significant difference among filled and empty fruit. The most valuable parameter is density, because it is the property required to allow effective separation of filled and empty fruit with most seed cleaning equipment. However, there was not enough difference, and the distribution overlap is too large, for seed processing methods based on density separation such as air flow separator, to be effective. Therefore, if 100% seed fill has to be achieved, we suggest a complete removal of the bract is performed. This will also facilitate seed viability testing for commercial sale.

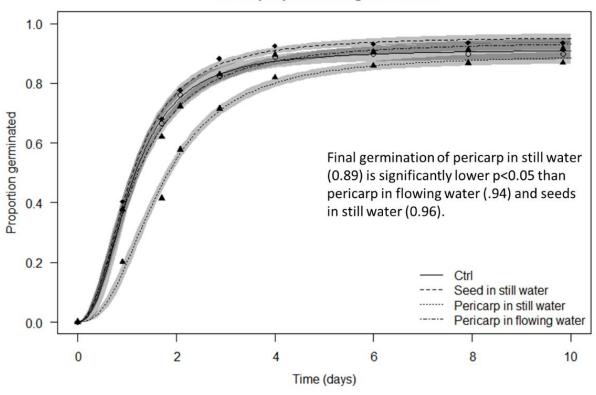
# 4.5.2 Removing seeds from fruits/bracts and bract pre-treatments to improve processing efficiency while not inhibiting germination

It was clear from the previous experiment that oldman saltbush seeds needed to be debracted to progress the industry goal of lower cost establishment. Removal of the woody bract is difficult and requires mechanical or chemical intervention. These series of experiments explore the options.

To evaluate how a treatment could improve seed extraction, treated and dried bracts where manually processed with rubber mat and customised (Venable) hand sander lined with rubber to simulate the action of a seed thresher. A serradella dehuller was also tested in the later part of the project using similar methodologies.

### Water pre-treatment

No difference was detected in cleaning efficiency between the control and any of the water pretreatment. Difference in germination were detected among treatments (Fig. 4.28). Bracts soaked in still water had a delay in germination and lower final germination, compared to bracts soaked in flowing water and cleaned seed in still water. A potential explanation is the leaching of salt and germination inhibiting compounds (ABA) from the bract that is then imbibed by the seed. A delay in germination in similar conditions has been described for sugar beet, due to ABA (Ignatz *et al.* 2019).



#### Water preprocessing treatment

Figure 4.28. Differences in germination were instead detected among water treatments.

#### Acid and enzymes pre-treatment

Improvement in cleaning efficiency was detected when using acids, however, no significant improvement was found with enzymes (Fig. 4.29). Hydrochloric acid 36% (Ha) and sulphuric acid 20% (Sa), showed improved cleaning efficiency after 1 h of soaking, with improved efficacy at longer exposure. However, germination was significantly reduced for all HA treatment longer that 1 hour and for SA treatment longer than 6 h. The recommendation when using acid debracting is to use hydrochloric acid for 30 min or less or sulphuric acid from 3 to 6 hours.

While effective this has implications for use in seed nurseries where staff will require training and PPE to use acids safely. Mechanical debracting is potentially a more attractive option for commercial scale seed cleaning.

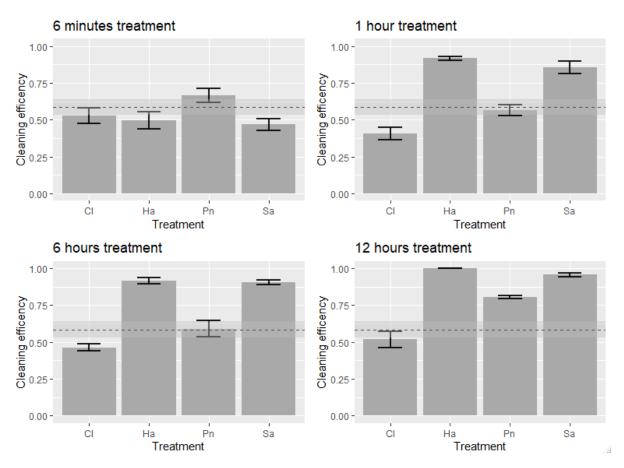


Fig. 4.29. Impact of acid pre-treatments for different time periods on seed cleaning efficiency.

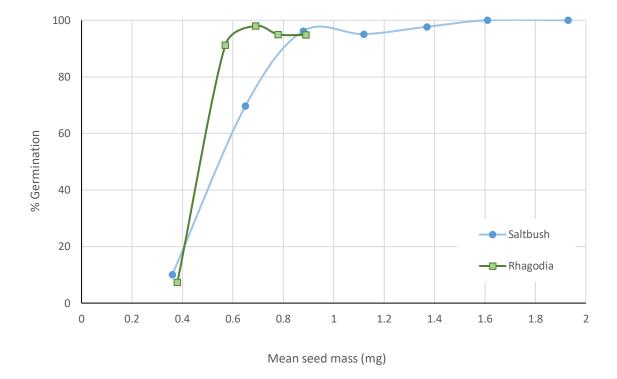
#### **Mechanical threshing**

We evaluated cleaning efficiency and effect on germination using two machines, a seed thresher (drum with rubber beaters) and dehuller (grinding disks). There was no difference in germination performance, but the processes appear to be more efficient (in terms of bract cleaning rate) with the dehuller.

#### 4.5.3 Does seed size/weight affect germination?

The average 1000 seed weight was  $600 \pm 53$  mg for Mallee saltbush and  $980 \pm 41$  mg for saltbush based on the 100 seed weights recorded for the two temperature and salinity experiments, in total 72 replicates of 100 seed for each species. Based on weighing of 300 individual seeds of each species and size sorting of 3000 seeds of each species, the variance in seed weight and size was found to be large for seeds of oldman saltbush, while the seeds of Mallee saltbush was more uniform in seed size and weight.

The correlation between seed size and germination and the development of healthy seedlings is extremely relevant when selecting the seeds for sowing operations. Both species have a high germination percentage and it is clear that there is an increasing germination percentage when the seed size increases, although maximum germination seems to occur for all seeds above 0.75 mg for Oldman saltbush and above 0.5 mg for Mallee saltbush (Fig. 4.30). It is also clear that there is no significant differences or trend in hours to germination with increasing seed size, for both species



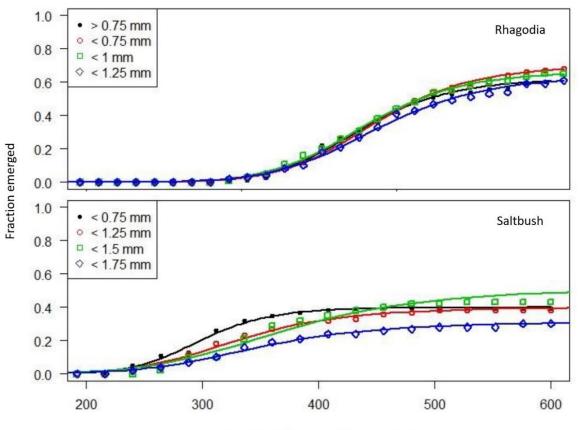
(data not presented). There is a lag period of around six days before the Mallee saltbush seeds start to germinate, while the seeds of oldman saltbush readily germinate.

Fig. 4.30. The relationship between seed size and germination percentage for oldman saltbush and Mallee saltbush.

Seeds of oldman saltbush emerged faster than the Mallee saltbush seeds, correlating with the faster germination rate also found for this species. There was a significant increase in time taken to achieve 50% emergence (t50 values) with increasing seed size up to 1.5 mm for oldman saltbush. There was no correlation between increasing size and maximum (total) emergence, with only the <1.5 mm and <1.75 mm fractions being significantly different. The seeds of Mallee saltbush had a slower emergence rate and smaller seedlings, but a more uniform one with no significant differences in time taken to achieve 50% emergence or the total emergence percentage (Fig. 4.32). The total emergence percentage was on average for all four size-actions 65.8 % for Mallee saltbush and only 40.5 % for oldman saltbush for all four size fractions.



Fig. 4.31. On the left is shown emerged seedlings of Mallee saltbush and on the right emerged seedlings of oldman saltbush.



Hours from the start of the experiment

Fig. 4.32 Emergence curves for four different seed size fractions of oldman saltbush and Mallee saltbush sown in 5 mm depth in sand.

Both species have a good development of seedlings from germinated seeds, and when looking at the percentage of healthy developed seedlings from the germinated seeds, it was clear that a higher percentage of Mallee saltbush seeds develops into healthy seedlings. On average of all the germinated seeds  $98 \pm 3.5$ % of the Mallee saltbush seeds developed into a healthy seedling while

only 88 ± 7.1% of oldman saltbush seeds developed successfully. The few germinated seeds that didn't develop properly either died after germination or had deformed development. In this experiment there was no correlation between seed size and the number of hours to germination for both species. This could be due to the large variation in hours to germination within each weight group. There was a positive relationship between increasing germination percentage and seedling development with increasing seed mass. The large variation found in seed germination might be due to the fact the at least oldman saltbush is an outcrossing species, resulting in the seeds collected all being different genetic combinations, yielding the observed variation in time to germination. The reproductive physiology of Mallee saltbush remains largely unexplored.

The data indicate that as long as the seed weight is above 0.75 mg for oldman saltbush and above 0.5 mg for Mallee saltbush the seeds should be viable and able to develop into healthy seedlings, given that optimal conditions are present. This is important especially if more efforts were to be put into each individual seed by methods such as coating or priming to improve the germination of each individual seed. It also makes the cleaning and sorting of the seed easier, by simply avoiding the smallest.

#### 4.5.4 Impact of temperature and salinity on seed germination rates

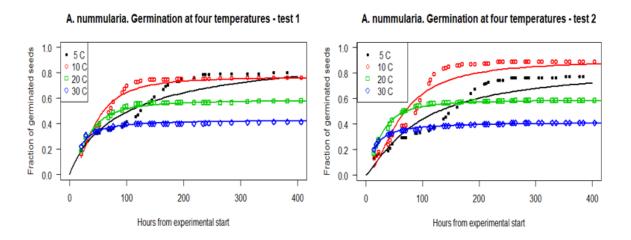
Optimum planting time will be a trade-off between temperature and the likelihood that there will be sufficient ongoing rainfall for establishment. Germination has been shown to progress rapidly at a temperature regime of 8 °C at night to 20 °C during the day for four saltbush species and oldman saltbush was most tolerant of lower temperature regimes (Malcolm *et al.* 1982). Another study found that the optimal temperature for oldman saltbush to be 15 °C in relation to total germination and rate of germination (Uchiyama, 1981). The same study also found temperatures from 25°C and above reduced germination 50 % or more, and that seeds below 0.5 mg had below 10 % germination, whereas seeds above 0.5 mg had 83 % or higher germination percentage. These results should only be considered indicative of the optimal germination temperature and seed size for successful germination, since there can be large variability between genotypes, and that the results have not verified by others.

The current consensus on why the clean seeds don't establish when they are planted in the field is that they are so small that they start to germinate upon rain, but then dry out before the next rainfall event. This is a combination of the small seed size (and therefore low energy storage) and the consequent requirement for a low seeding depth, that makes the seed prone to water stress in the early developmental stages. It is probable that providing optimal temperature and water availability by sowing at the best time of the year will improve establishment from seed.

The temperatures that were tested were 5, 10, 20 and 30°C. The highest maximum germination percentage was obtained at the 10 °C treatment for both species, with 10 °C being significantly higher than the three other temperatures for Mallee saltbush (Fig. 4.33). The 10 °C was only significantly higher than the 5°C treatment for oldman saltbush in one of the temperature tests, but significantly different from the 20 and 30°C treatment. There was a significant decrease in *t50* values for both species at increasing temperatures.

Generally, oldman saltbush germinated faster than Mallee saltbush, with an average t50 of 52 hours (2.1 days) at 10°C compared with a t50 value of 202 hours (8.4 days) for Mallee saltbush at 10 °C. There was a significant decrease in t50 values with increasing temperatures, indicating that the seeds germinate faster at higher temperatures. To ensure a good establishment by direct seeding, a

fast germination and seedling development is required to utilize the available moisture in the soil after a rainfall event, ensuring root development and persistence through potential dry periods after germination. Although rapid germination is desired, higher temperatures come at a cost of reduced maximum germination percentage above 10 °C. Oldman saltbush had on average similar maximum germination at the 5 and 10 °C treatment of 82.5 and 83 %, 63 % at 20 °C and 41.5 % at 30 °C. Mallee saltbush was found to be more sensitive to temperatures upon germination, with no or few seeds germinating at 30 °C and only roughly 40 % of seeds germinating at the 5 and 20 °C treatments, compared with on average 78.5 % germination at the 10 °C treatment. These results were obtained using constant temperatures; however, temperatures fluctuate during day and night, and hence using different thermo periods could have given a more accurate measure of optimal sowing time.





R.preissii. Germination at four temperatures - test 1

R. preissii. Germination at four temperatures - test 2

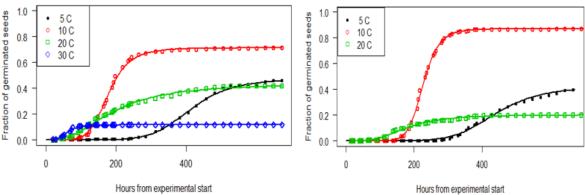
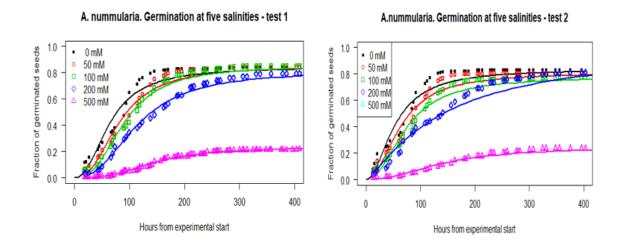


Fig. 4.33. Germination curves shown for oldman saltbush and Mallee saltbush under 4 constant temperatures. The 30 °C treatment in temperature test 2 for Mallee saltbush is not shown since there were no seeds that germinated.

#### Impact of salinity on germination

The salinity levels of the germination solution were 0, 50, 100, 200 and 500 mM NaCl. The water level in the germination trays were observed before the experiment started and after termination and no considerable water loss was recorded, indicating that there was no accumulation of salts in the germination media due to evaporation of water.

Similar t50 values and maximum germination percentage were found between the two salinity tests performed for both species at the five designated salinity levels. The highest maximum germination percentage was for oldman saltbush reached at 0-200 mM, with no significant differences in test 1 but some significant differences in test 2 for 0-200 mM. 500 mM had a significant effect on maximum germination in both tests, reducing germination dramatically. Mallee saltbush had maximum germination at 0-50 mM, with a significant effect on maximum germination starting to occur at 100 mM, and at 200 mM a significant reduction is recorded (Fig. 4.34). There were no Mallee saltbush seeds that germinated at 500 mM. There was a significant effect on t50 values with increasing salinity at all salinity levels.



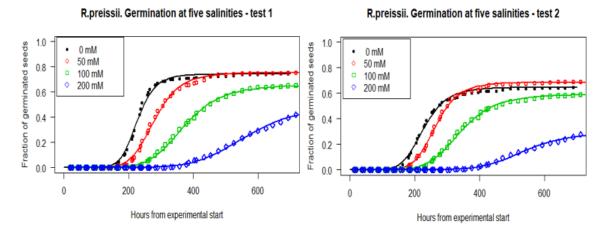


Fig. 4.34 Germination curves shown for oldman saltbush and Mallee saltbush when germinated under 5 different salinity levels. No curve is generated for Mallee saltbush at the 500 mM treatment since no germination was recorded.

It is clear from the two salinity tests performed that oldman saltbush has a very high salinity threshold at the germination stage with no reductions in maximum germination at 200 mM. But there is still a significant increase in the time to 50% germination values, illustrating the delay in seed germination which is also observed for many other species (Gul *et al.* 2013). At 500 mM on average, 27 % of oldman saltbush seeds still germinated, which is a significantly higher percentage than the 10 % previously reported (Uchiyama, 1987). This difference can be explained by the fact that the

seeds used in this study were collected from a saline field. It has been documented for oldman saltbush that seeds collected from plants growing in a high salinity medium had a higher salt tolerance at the germination stage compared with seeds originating from plants grown in a low salinity medium (Uchiyama, 1987). This knowledge of the environment interaction on seed germination could be utilized to produce seeds that are better suited for establishment on saline soils by collecting seeds from plants growing in saline areas.

Seeds of Mallee saltbush had a lower salinity threshold compared with oldman saltbush, with significant reductions in maximum germination starting to occur at 100 mM, and significant for both salinity tests at 200 mM. The delay in germination was also higher for Mallee saltbush, increasing the t50 by 324 hours (14 days) on average when the salinity increased from 0 – 200 mM. This contrasted with only 69 hours (3 days) for oldman saltbush. However, taken in relative terms the t50 increases with 242 % for Mallee saltbush and 221 % oldman saltbush. So, the effect on t50 is similar but in practice, the longer delay and reduction in maximum germination can make it more difficult to achieve the successful establishment of Mallee saltbush on saline soil. Especially in soils with higher salinity levels, since there was complete inhibition of germination at 500 mM. It's important to note that seeds of Mallee saltbush were collected from a non-saline area, and therefore it should be verified whether more salt – tolerant seeds could be obtained from plants grown on saline land, to improve establishment on saline land for this species.

It has previously been found that germinating seeds typically have a lower stress tolerance than that of seedlings or mature plants, which seems to be the case for Oldman saltbush and Mallee saltbush, that both exhibit a lower salinity tolerance at germination than at the early growth stages (Dodd and Donovan, 1999).

For many halophyte species, it has been shown that seeds inhibited by a salinity level above the species threshold, will resume germination again when the salinity level is lowered (Khan and Gul, 2006). This mechanism ensures that the seeds will persist in the seed bank despite high salinity stress under high evaporative conditions, and it might be that Mallee saltbush utilises this mechanism. It has been shown that Oldman saltbush seeds exhibit this mechanism, resuming germination when the saline conditions are removed (Uchiyama, 1987). Hence, if seeds are sowed too early in the winter in relation to leaching of salts, they would eventually germinate when the salinity level falls below the threshold for the seed. Although it remains unknown how large a fraction of seeds that remain viable after enduring extreme salinities in the soil solution.

In determining the time of sowing this trade-off between maximum germination and rate of germination (t50) should be considered in relation to the risk of water stress and drying out of the topsoil at the time of germination. The trade-off is less important for oldman saltbush than it is for Mallee saltbush since it is more sensitive to temperatures above or below 10 °C. Taking the annual weather pattern of WA into consideration, the water availability is higher and the temperature lower during winter, creating ideal conditions for germination. The 10-degree temperature optimum found in this study correlates best with the months of June-August in WA, which are also the months with the most rainfall. This indicates that these species have naturally adapted for germination during the winter months when the temperature is lower and the water availability high. Compared with the spring where there is a higher temperature and the time span between rainfall events is greater, increasing the risk of drying out of germinating seeds before proper seedling development.

One factor that could impair the sowing of seeds in winter at the onset of the rains could be a high soil salinity level, inhibiting/delaying germination and thereby might make it more favourable to delay sowing until more leaching has occurred. Currently, direct seeding is only recommended for

sandy soils, due to the faster leaching of salts from these soils after the onset of rains in May. The low salinity threshold and sensitivity to warmer temperatures above 10 °C makes the window for the sowing of Mallee saltbush quite narrow compared to Oldman saltbush.

#### 4.5.5 Seed coating for glasshouse and field experiments

The commercial seeds were successfully coated for the experiments and germination tests revealed that the coating did not inhibit germination (data not presented, Fig.4.35).



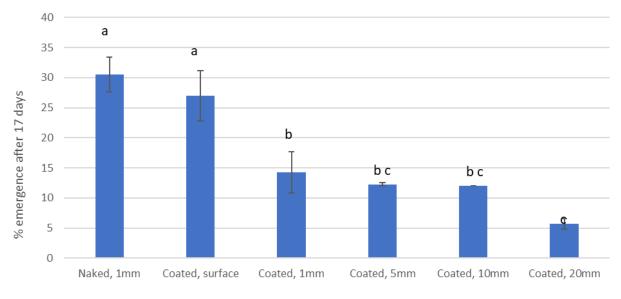
Fig. 4.35. Simone Pedrini (Curtin University) and Ed Barrett-Lennard (DPIRD) testing different seed coating formulations and subsequent germination of coated seeds.

### 4.5.6 Impact of depth of burial on seedling emergence

Very small seeds have poor energy reserves so emergence from depth is less likely. In preparation for field seeding, we ran a depth of sowing experiment in the glasshouse. Saltbush seeds are incredibly small so sowing depth is critical. If they are sown too shallow, the seedling is at greater risk of desiccation. If sown too deep, the plant cannot emerge. This experiment compared emergence of coated seed on the soil surface with being sown to 1 mm, 5 mm, 10 mm, 20 mm and naked seed at 1 mm sowing.

We found the seed performed far better with either surface or 1 mm sowing, than at deeper depths (Fig. 4.36). Moving from 1 mm to 5 mm burial halved the emergence rate. These data were in contrast for those of a closely related saltbush species with a similar seed size, *A. amnicola,* where emergence was similar at 5 or 10 mm depths (Stevens *et al.* 2006).

These data indicate that mechanical sowing on farms will be challenging as the seeds must be placed close to the soil surface. It is possible however as this is similar to seeding small-seeded pasture legumes such as biserrula or gland clover.



Seed treatment and sowing depth

Fig. 4.36. Emergence data, 17 days after sowing from the glasshouse depth of emergence experiment.

#### 4.5.7 Comparing coated and 'naked' seeds in a commercial nursery

Coated and naked seed were planted in a commercial nursery (Chatfield Tree Nursery, WA) in summer 2021. The seeds (n=3456) were planted with a robotic planter with a setting of one seed per cell. Trays contained 72 cells and there were 24 trays planed for both naked and coated seed (seed originated from the same batch). Seeds were planted at 1mm depth into a commercial seed raising mix (Fig. 4.37). They were watered immediately and placed onto a bench in the nursery in a randomised design. After 3 months of growth under nursery conditions, seedlings were graded and counted.

Naked seeds had a statistically higher success rate compared to the coated seed (p < 0.001). The naked trays averaged 18.0 ± 0.99 seedlings per tray (24% success) while the coated seeds averaged 10.0 ± 1.31 seedlings per tray (14% success). There was some evidence that the coated seed resulted in less seedlings that were considered 'poor quality' by the nursery. Under ideal conditions in the commercial nursery, the seed coating does not offer an advantage, in fact it appears to penalise establishment. This is not entirely unexpected given the nursery system has been optimised for naked seeds.



Fig. 4.37. Using a mechanised planter to sow naked and coated seeds at Chatfields Tree Nursery in Tammin (Dustin McCreery and Matt Wilmot pictured).

#### 4.5.8 Seed coating and field emergence

#### 2021 - Controlled environment and hand sowing field trials

The results from the field experiments at Kellerberrin (Fig. 4.38) and Cranbrook were disappointing with very few seedlings established. It is impossible to determine the failures in 2021 were due to seed coating, season, soil type, planting depth or predation by insects. We decided to move back towards more controlled field studies in 2022.



Fig. 4.38 Seeding the saltbush establishment experiment at Kellerberrin in 2021.

#### 2022 - Controlled environment and hand sowing field trials

Trials were sown into two paddocks near York, one a heavy clay loam and the second a grey sand. Experimental layout was a split plot design with paired 1 m rows of 10 mm and 1 mm sowing depths (surface sown seed lightly covered with sand to prevent predation/blowing away, Fig. 4.39).

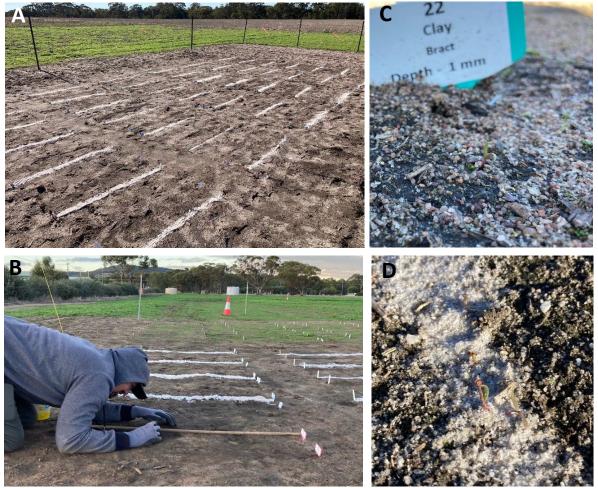


Fig. 4.39. Oldman saltbush emergence and establishment hand sown field trials. Paired rows were hand sown to two depths (A & B) and some seedlings established from all treatments (C), however by far the most successful treatment was that which tested undamaged seed (D; see 4 seeds emerging in close proximity). CSIRO's Josh Hendry pictured.

Initial counts of the May trial were somewhat disappointing with only low numbers of seedlings establishing and these results added motivation to work out what was constraining seedling establishment. While germination rates (using a standard ranking of the seed imbibing and radicle emerging from the seed coat) on petri dishes were good, we struggled to get high rates of seedling establishment. We conducted some petri dish germination experiments where we incubated the seed for 10 days post germination and found that much of the seed failed to elongate beyond initial germination and some of those seeds which did start growing behaved in an odd fashion with the roots growing upwards.

Microscopy studies (Fig. 4.40A) of the seed revealed a high rate of seed damage, which in these tiny seeds, was not evident to the naked eye. The seed we are using in our field experiments (both coated and naked) has been commercially cleaned and the process of removing the bract appears to have broken the tip of the radicle (first root) off many seeds (Fig. 4.40B) or damaged the seed in other ways (Fig. 4.40C). This results in seed either failing to germinate, germinating but producing a low vigour seedling or in the case of the missing radicle tip producing a seedling where the root has lost its gravitropism and therefore does not bend downwards into the soil and grows upwards, desiccating in the air.

Glasshouse experiments in soil trays comparing the emergence of undamaged seed with that of seed that was either damaged or missing radicle tips showed that although some initial germination occurred in all treatments the final establishment of both damaged seed treatments was zero while the undamaged seed had a  $30.4 \pm 5.2$  % establishment rate, which is quite good for such a small seeded native species.

Using a combination of microscopy counts and petri dish experiments we have calculated that the seed we have been using is at most only 20 % viable. While this is a disappointing finding, it does add some context to our lack of success with saltbush seedling establishment so far.

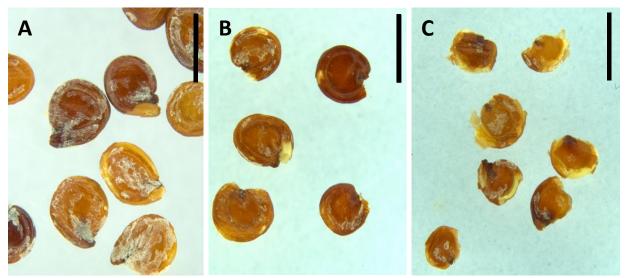


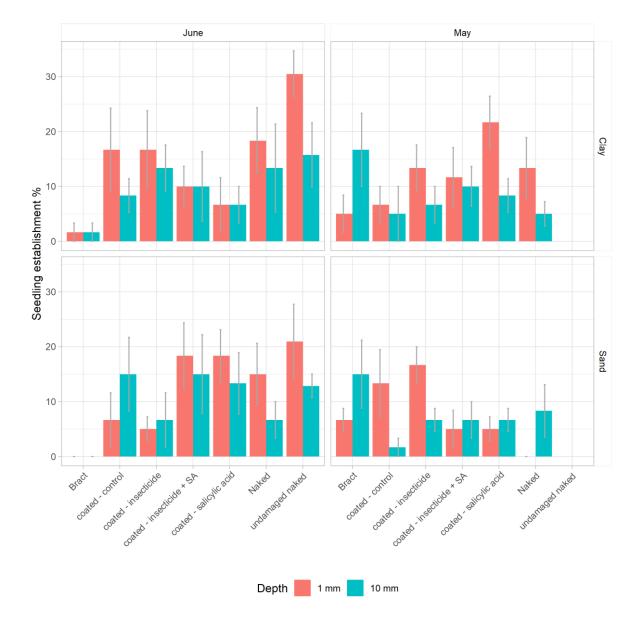
Fig. 4.40 Atriplex seed from commercial cleaning. Around 20 % of the seed is undamaged (A). The remaining seed either has the radicle tip (first root) snapped off (B) or is damaged in some other way (C; chips out of the cotyledons, seed broken in half, seed coats really damaged). Scale bar is 2 mm.

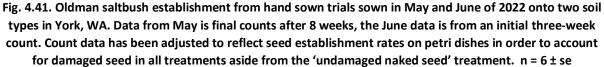
We have experimented with a range of bract removal and seed cleaning options. We have tested both a thresher and a dehuller and have prototyped a method which allows us to quickly clean large amounts of seed using the dehuller and separating it from all the bract chaff using a air column separator. We plan to coat seed of several varieties and move forward with our controlled environment studies using this undamaged seed.

We continued with our June sowing time even though we now realised the coated seed was somewhat comprised, however, we also added an extra treatment of undamaged seed (hand sorted under the microscope from the seed used in the naked treatment to ensure it was the same variety/age etc). Adjusted seedling establishment is presented in Fig. 4.41, this data is presented as a

germination percentage, calculated on only 20 % of the seeds being viable; also note that the June sowing data is from an initial three week count and final data (especially for the bract treatments) will likely be slightly higher.

There was no treatment effect of the seed coatings (p = 0.252), however even when analysed against adjusted seed emergence the undamaged seed had a higher establishment rate (p = 0.0007). It is difficult to know if the coating treatments produced no impact or if the data is confounded due to seed damage. Regardless, the fact there is no negative impact, and coated seed can be machine sown is an encouraging finding. Our emergence rate was also not significantly influenced by site (p=0.289) or sowing depth (p=0.1719).





Now we have a protocol to produce clean naked seed in a method where we can be more confident of the seed viability after germination, we are planning to use a few key varieties in controlled environment cabinets to understand the key impact of temperature and moisture availability during germination in April 2023, we started an experiment to investigate impact of water availability with a large range of water potentials (produced osmotically in a laboratory). From this experiment we will determine several key water potentials and move to understanding how the seeds respond in a soil environment. Using a new soil-based germination and emergence protocol (developed at CSIRO for studying canola seed) we will test germination and emergence from varying depths of soils which differ in soil moisture.

#### 4.5.9 Seed and Bract characteristics of the elite seed lines.

Bracts and seeds were collected from the elite clonal lines in January 2023 as a source of seeds for coating for future field testing. There was significant variability I individual seed mass, with seeds from clone B and clone G being bigger than those of Anameka<sup>™</sup> or clone E (Table 4.18). The clone E seeds are below the critical size thresholds identified in previous experiments. The germinability in these seeds however was not related to seed size and germinability ranged from 75 to 100%. Bract size and weight also varied significantly. The largest bracts were three times the mass of the smallest bracts.

	Mean bract dry wt		Bracts with	Bracts with seeds		Individual seed mass			Germination (%)	
	(1	mg)	(%	)		(m	g)			
Line	mean	sem	mean	sem	mean		sem	mean	sem	
Anameka seed	7.8	0.31	35.0	1.47	(	).99	0.048	82.4	3.87	
Seed AH	11.1	0.40	55.3	2.87	1	1.21	0.025	100.0	0.00	
Seed FH	11.0	0.51	66.5	4.09	-	1.15	0.024	90.2	2.68	
Seed BH	5.5	0.06	19.0	2.12	-	1.38	0.056	87.1	4.35	
Seed CH	8.6	0.15	66.8	1.89	(	0.86	0.022	85.8	2.63	
Seed DH	18.3	0.44	66.8	2.96	-	1.08	0.025	95.1	2.89	
Seed EH	4.9	0.18	50.0	2.48	(	0.71	0.033	95.1	2.89	
Seed GH	14.8	0.48	31.5	2.90	-	1.40	0.053	75.3	3.17	
Grand mean	10.2		48.8		1	1.10		88.9		

Table 4.18. Bract weight, fill, seed size and germinability of the elite seed lines (data from the seed
production site in 2023).

#### 4.5.10 Where to next for direct seeding?

We have made significant progress in understanding the seed ecology of the two species and have made some novel contributions to the literature. We can now successfully clean seeds and remove the bracts in a way that is commercially viable. We have a greater understanding of the vulnerability of the hypocotyl of oldman saltbush seeds and have protocols to minimise damage. We know the optimal temperatures for germination and impact of salinity on germination. We can also successfully coat seeds to make them bigger (to flow through commercial seeders) and to allow for use of insecticides in the seed coating (so there is less need for blanket insecticide application and improved opportunities for rangeland revegetation). Damaged seed compromised the field experiments and both species offer challenges for paddock scale establishment.

We believe that we have the information, technology and genotypes to progress direct seeding. We hope to continue this work in partnership with industry through a WA Agricultural Research Collaboration Application. We will contact MLA and AWI to discuss the opportunity.

### 4.4 Objective 4.

#### Field walks and communication events

Completed two field walks at each of the 6 research sites, 3 print media stories, 'how-to' guides for establishment and management of shrubs and 3 peer-reviewed publications. Data packaged for MLA training programs as required.

We have delivered 2068 industry face to face contacts where the project experiments and extension messages have been discussed (1040 to producers and 1028 to extension agents, scientists and other industry members) over 48 events during the project. Sixteen of these events occurred at the project research sites (Table 4.19).

The team have worked with Gillamii Centre to deliver two-day saltland masterclass workshops in regional locations (Cranbrook, Ravensthorpe and Jerramungup). These workshops involve seminars and field-based activities as farmers plan the rehabilitation of a saline site with saltbush. The workshops were attended by 72 producers and 29 industry representatives.



Fig. 4.42. Farmers participating in a two day saltland training workshop in Jerramungup in 2020.



Fig. 4.43. Farmers participating in a two day saltland training workshop in Cranbrook in 2020.

Media	Date	Agency	Title
Radio	22/06/2022	ABC South West WA (Kit Mochan)	Interview with DPIRD's Richard George discussing Anameka saltbush, AM Radio, 6:24 am
	22/06/2022	ABC South West WA (Belinda Varischetti)	Interview with Hayley Norman, Saltbush Improvement, AM Radio, , 12:43 pm
	28/11/2022	ABC South West WA (Sophie Johnson)	Pre-recorded interview with Hayley Norman, AM Radio, 6:20 am
	6/07/2023	2GB (Sophie Clarke)	The drought resilient shrub supporting farmers during dry times
	6/07/2023	<u>4BC</u>	The drought-resistant shrub supporting farmers during dry times
	6/07/2023	MMM Gold Coast	News at 11am
	6/07/2023	ABC North and West SA	Regional Drive, live interview
	6/07/2023	ABC Bega	Live interview
	6/07/2023	ABC Bathurst	Live interview
TV	16/04/2023	ABC National Landline	The Silent Flood: Draining the silent flood of salinity in the wheatbelt of WA.
	5/07/2023	7 Regional WA	Interview

### TV and radio – 9 radio interviews, a Landline story and regional news.

Date	Who	Theme	Reads	Likes	Reposts
10/07/2023	Norman	Saltbush media release	1991	39	7
10/07/2023	Norman	Saltbush TV story	28		
10/07/2023	Colgrave	Saltbush media release		29	
10/07/2023	Purtell	Saltbush media release	527	16	
10/07/2023	Taylor	Saltbush media release			
6/06/2023	GGA SW drought hub	Anameka and saltbush research	1498	11	3
5/07/2023	Farmer Dean Wyatt	Anameka saltbush delivery	1044	13	
10/07/2023	Norman	Saltbush media release	1954	4	
10/07/2023	Facey Group	FDF project	802	8	4
10/07/2023	The Rural	Saltbush media release	152		
10/07/2023	CSIRO #1	Saltbush media release	3974	39	13
10/07/2023	CSIRO #2	Saltbush media release	4047	16	5
10/07/2023	COSMOS Magazine	Saltbush media release	49		
10/07/2023	Pulse Actions Plus	Saltbush media release	309		
	Chatfield Tree				
10/07/2023	Nursery	Saltbush video - old views	2023		
	Chatfield Tree				
10/07/2023	Nursery	Saltbush video - new views	533		
10/07/2023	WALRC	Silage Transformational Feedbase	1261	14	1
11/07/2023	Di Myberry	Stobbs paper - featuring TF and saltbush	407	5	1
17/07/2023	Facey Group	Field walk at research site	1646	15	5
11/08/2023	CSIRO	Saltbush video	7261	39	13
		Promoted saltbush and forage			
22/08/2023	CSIRO Publishing	improvement publication	535	5	4
11/09/2023	FBG	Spring field day at saltbush site with CSIRO	129		
		TOTAL	30170	253	56

### Social Media –.30,170 views, 253 'likes' and 56 reposts.



Fig. 4.44. The Landline story featured the saltbush germplasm development site at Tammin and discussed the evolution of the research and key messages. Unfortunately, references to funding bodies were edited out.

Field walks and communication events with producers and industry - 2068 face to face contacts (1040 producers and 1028 industry representatives) over 48 events in three states

Date	Location	Project site	Producer group	Producers	Industry	Purpose
5/02/2019	Cranbrook, WA	Yes (Lehmann)	Gillamii	4	4	Look at site and discuss research design
7/02/2019	Tammin, WA	Yes (York,	WA Farmers	120	20	Talked about the value of shrubs within systems and
		germplasm site)	Federation			introduced the project
12/03/2019	Kojonup, WA	No	WALRC &	30	10	Presented a talk about sheep nutrition and briefly discussed
			Southern Dirt			the project.
13/03/2019	Donnybrook, WA	No	WALRC & Local	55	10	Presented a talk about the opportunities to provide
			farmers			antioxidant supplements to cattle through saltbush.
						Discussed the project
22/03/2019	Swan Hill, Vic	No	Western Murray	130	20	Dustin McCreery was guest speaker at an Anameka saltbush
			Land			workshop. He discussed the project on behalf of H. Norman
			Improvement			
22/05/2019	Yealering, WA	No	CFIG	4	5	Discussed the project and selected the research site.
23/05/2019	Cranbrook, WA	Yes (Lehmann)	Gillamii	4	4	Discuss interactions with the state-government funded
						'Regenerating Saline and Marginal Landscapes in south west
						WA' project (Genie App). Filming of saltbush extension
						messages
19/08/2019	Balkuling, WA	No		15	5	Discussed opportunities for saltbush on deep sandy soils
23/08/2019	Perth, WA	CSIRO		4	3	Hosted a national delegation of producers and extension
		glasshouse				agents at CSIRO – Looked at the shrub development work in
						the glasshouse and CSIRO shrubs
28/08/2019	Muresk, WA	Yes (Muresk)			3	Informal field walk at the Muresk site with 3 interstate
						researchers
4/09/2019	Lameroo, SA	No	Murray	30	20	Field walk (for the DLPS legume project). Discussed saltbush
			Sustainable			and the project with the group on several occasions
			Farming Systems			
8/10/2019	Baandee, WA	Yes (Luers)	MADFIG	4	2	Informal field walk at the Baandee site, looking at
						establishment
10/10/2019	Cranbrook, WA	Yes (Lehmann)	Gillamii	15	10	Field walk at the research sites and discussion of project
						outcomes.

11/10/2019	Cranbrook, WA	Yes (Lehmann)	Gillamii	15	10	Salinity Masterclass with a 45-minute saltbush and sheep nutrition lecture
23/03/2020	WA into COVID loo	ckdown, inter and in	ntra state travel restri	ctions		
3/04/2020	Manypeaks, WA	No	WALRC	22		Virtual field walk. Novel forages, beef production, mineral deficiencies and filling feed gaps. 22 live online (downloaded >200 times)
5/05/2020	Augusta, WA	No	WALRC	18		Virtual field walk. Sheep nutrition, forage production and soil constraints
13/05/2020	Pinjarra, WA	No	WALRC	19		Virtual field walk Pinjarra. Forage production, feed gaps, novel beef supply chains and ryegrass toxicity
1/08/2020	CSIRO allows over	night travel again				
5/08/2020	Ravensthorpe, WA	No	RAIN & Gillamii	25	10	Saltland & shrub masterclass and field walks
7/09/2020	Kalannie, WA	No	SLCC	8	12	Soil and Land Conservation Council tour to Kalannie. Visited an Anameka plantation & discussed the project
20/09/2020	Jerramungup, WA	No	Stirlings to Coast & Gillamii	32	9	2-day saltbush and saltland masterclass (41 producers and consultants)
22/09/2020	Jerdacuttup, WA	No	Stirlings to Coast	24	6	Field walk discussing shrub establishment and management.
31/01/2021	WA into COVID loo	kdown, inter and i	ntra state travel restri	ctions		
3/02/2021	Fremantle, WA	No	WALRC & AAAS	40	260	Invited keynote, Stobbs Memorial Lecture (Hayley Norman) at the Australian Association of Animal Scientists Conference
15/03/2021	Toodyay, WA	No	Regen Ag WA & Perth NRM	10	20	Saltbush as a feature in regenerative ag systems
23/04/2021	Perth, WA	No	University of WA		29	The project discussed at a lecture to postgraduate students
14/05/2021	Manypeaks, WA	No	WALRC & Stirling's to Coast	85	10	Discussed the opportunity to use native shrubs to address mineral deficiencies o the south coast
24/04/2021	WA into COVID loo	kdown, inter and ir	ntra state travel restri	ctions		
19/05/2021	Perth, WA	No		6	28	The seed coating and establishment work presented at an industry forum in Perth
16/06/2021	Dongara, WA	Yes (Gillam)	NACC	6		Visited the site and then a Chapman Valley Anameka plantation. Talked about saltbush within farming systems
17/06/2021	Binnu	No	WALRC & NACC	80	20	Managing marginal land and shrubs for lambing protection.
12/08/2021	Williams, WA	No	Sheep's Back	100	50	Saltbush to fill feed gaps in farming systems (2 x 20 minute talks)

13/06/2021	Baandee, WA	Yes (Luers)	MADFIG			Soil and Land Conservation Council visit to the Baandee
12/00/2021	<b>T</b>	) ( (   )	CL CC		C	Research site
13/09/2021	Tammin and	Yes (Luers)	SLCC	4	6	WA Soil and Land Conservation Council will visited research
	Baandee, WA					sites and the nursery at Tammin to discuss the environmental
23/03/2022	Perth, WA	No	ACCC	4	16	benefits arising from shrub systems. Shrub research presented to a small group of scientists and
25/05/2022	Pertil, WA	NO	ALLL	4	10	industry representatives
29/04/2022	Dorth 14/A	No	Lipivorsity of M/A	6	15	Lecture to students at UWA including some discussion of the
29/04/2022	Perth, WA	NO	University of WA	0	12	shrub research
1/06/2022	Orange to	No	Central West	25	14	Discussion of the shrub project over a series of farm visits
1/00/2022	Condobolin, NSW	NO	Central West	23	14	and field walks in NSW as part of the DLPS project tour
27/06/2022	Pingelly, WA	No	WALRC	10	70	WALRC winter forum
29/06/2022	Baandee, WA	Yes (Luers)	MADFIG	10	3	Toured the nursery, a commercial site and the Baandee
25/00/2022	baandee, wA	Tes (Lucis)	MADIN		5	research site with 3 MLA research managers
30/06/2022	Adelaide, SA	No			50	Project presented at a CSIRO Framing Systems workshop
22/08/2022	Perth, WA	No	WALRC	3	170	WALRC and AAAS Livestock Matters Forum (keynote)
29/10/2022	Perth, WA	No		2	40	'10 Big Things' climate change forum and workshop
4/10/2022	Nannup and	No		3	3	WALRC farmer/industry field information gathering tour
., _0, _0	Boyanup, WA			•	•	(with Matt Camarri, Rob Bell, Sam Taylor)
11/10/2022	Cranbrook, WA	Yes (Lehmann)	WALRC, Gillamii	35	3	Field walk at Cranbrook site
24/10/2022	Tammin &	Yes (Luers)	MADFIG	2	8	Saltbush and understory establishment workshop (with
, -, -	Baandee, WA	()				Chatfield's, Curtin, DPIRD)
19/01/2023	Pingaring, WA	No	WALRC	1	3	Farm tour and feedbase discussion and collection of
						background info (for WALRC field walk), Dean Wyatt's farm.
2/02/2023	Albany, WA	No	Gillamii	4	7	Gillamii Centre Workshop
2/03/2023	Cranbrook, WA	Yes (Sam	Gillamii, SLCC	6	8	Soil and Land Conservational Council visit to research sites
		Lehmann)				
23/03/2023	Pingaring, WA	No	WALRC	22	20	WALRC Autumn Field Walk - filling feed gaps
4/04/2023	Baandee, WA	Yes (Luers)	MADFIG	8	12	Baandee Field Walk
			TOTAL	1040	1028	



Fig. 4.45. Field walks at Binnu (top), Tammin (middle) and Cranbrook (bottom).

#### Other extension outputs

The project has delivered ten extension products including 6 magazine articles, "How to" factsheet (used as the basis for a You-Tube clip), two videos for the Future Drought Fund (promoting saltbush adoption), two extension videos (as part of the Saltland Genie package) and the team actively contributed to the development of the Saltland Genie App.

Date	Extension	Title
March 2018	Beyond the Bale	Closing the Autumn Feedgap
October 2018	ECOS Magazine	Oldman saltbush helps farm profits and ecosystem health
May 2020	MLA Feedback Magazine	Superfood for sheep
May 2020	MLA Feedback Magazine	Just add saltbush (interview of host farmer Sam Lehmann)
January 2022	MLA Feedback Magazine	Super saltbush plugs the feed gap
January 2022	MLA Feedback Magazine	Indestructible saltbush earns its keep (interview with host producer Rex Luers
September 2023	Beyond the Bale	Filling the feed gap with shrub systems
Launched	Saltland Genie	As a member of the technical steering committee for Gillamii
March22		Centre, contributed to the revision and development of the Saltland Genie App and associated extension outputs
2022	Extension You-Tube clip (with DPIRD)	Optimising Anameka fodder shrubs for dryland sheep production
2022	Extension You-Tube clip (with DPIRD)	Saltbush seedling system
June 2022	Future Drought Fund Conference	Anameka saltbush featured in a keynote video address to the Future Drought Fund conference. Online.
April 2023	Future Drought Fund Conference	Anameka saltbush featured in a video clip filmed for the 2023 Future Drought Fund conference. Online in June 2023
Updated	Handout for field walks	Guidelines for optimising saltland pastures (Hayley Norman, Ed
continuously	and used as the basis for	Barrett-Lennard, Matt Wilmot, Dustin & Lisa McCreery from
,	talks and the You-Tube clips	Chatfield's Tree Nursery). See appendix



Fig. 4.46. Filming at the Baandee site in May 2022.



Sam farms with his wife

Site selection and

"As the plots get older, the

Enterprise:



Four years after the release of a new and easily digestible saltbush variety, woolgrowers are making otherwise unproductive agricultural land profitable, particularly in mixed cropping and livestock businesses.

Filling the late summer/early autumn feed gap is a constant challenge for woolgrowers in dryland farming regions, particularly Western Australia's central wheatbelt.

With limited nutritional value available in a dry stubble paddock, purchasing and planning for alternative feed sources approximately five kilojoules of energy to each sheep daily.

'Each sheep requires a daily intake of around 12-13 kilojoules, or up to 28 kilojoules during lambing, to stay in good condition, meaning we still need to supplement the feed throughout autumn, usually with hay and grain," he says. a significant percentage of this autumn feed gap," he says.

"But that aside, this area of land is unproductive for anything other than bluebush or saltbush, and so it didn't take much convincing for us to plant the area out to the new Anameka<sup>™</sup> variety."

Typically, 800-Looo plants are required per



### Is saltbush right for you?

- Do you have country which is less productive and has a nutrient gap during summer and autumn? Saltbush can lift productivity of poorer areas.
- Do you want to reduce reliance on supplementary grain? Saltbush can provide nutrients to complement crop residue and help fill feed gaps.
- Do your stock have vitamin E deficiency? Saltbush contains high levels of vitamin E and minerals associated with antioxidant pathways.
- Are you developing

#### Saltbush superpowers

### III

According to research by CSIRO, sheep grazing Anameka saltbush as well as cereal stubbles maintained weight and grew 20% more wool than sheep just grazing cereal residues.

ome producers have observed an increase in win survival when ewes are ambed down in saltbush.

#### N

According to research by CSIRO, increasing the adoption of shrub systems will improve landscape health and reduce the impact of salinity.

### This MLA/AW

This MLA/AWI project investigates the on-farm benefits of the next generation of shrubs

### Tips to fit saltbush into your system

#### Seedlings

Chatfield's Tree Nursery in WA is the commercialisation partner for Anameka saltbush. In response to producers' success with Anameka, Chatfield's has increased distribution capacity with an additional propagation nursery in WA and a new distributor in NSW to service growing demand in eastern states.

#### Site selection

Old man saltbush, including Anameka, is very tolerant to salinity but not to waterlogging.

"It appears to be going really well in sandy areas, particularly where there's some gravel underneath, and also in rocky country," Dr Norman said,

Anameka will also happily grow in good country, but you need to look at why you are putting them in and make a decision based on your farming system and what works for you."

#### Establishment

Dr Norman said the general rule is to plant no more than 700 plants/ha. In higher rainfail areas or to manage a shallow water table, about 1,000 plants/ha is a good option.

\*Consider putting in an understorey before the shrubs, choosing species which suit the country.

"If possible, get the shrubs into moist soil or plant before a rainfall event. Plant the seedlings deep so less than 4cm of the shoot is above the soil surface," she said. LIVESTOCK NUTRITION

## Superfood for sheep

A recently released variety of old man saltbush has hit a sweet spot with producers and their sheep, paving the way for new research into getting more value out of forage shrubs.

Anameka<sup>th</sup> saltbush was commercialised in 2015 and, according to project leader Dr Hayley Norman at CSIRO, the uptake is faster than anticipated, with one million stems expected to be sold this year.

"More than half the sales of Anameka so far have been repeat sales, which is exciting because it means people had success with it," Dr Norman said.

The old man saltbush research began under the auspices of the Future Farm Industries Cooperative Research Centre (2007–2014) and continues with funding from MLA and Australian Wool Innovation (AWI).

Anameka saltbush was selected for its higher nutritive values and relative palatability. It's a valuable addition to livestock enterprises, providing options for deferred grazing, filling the feed gap, and providing antioxidant vitamin and mineral supplements.

Anameka seedlings are grown from

The research team is working with producers across WA to collect productivity and economic data to demonstrate how sheep and cattle can utilise saltbush shrubs to improve weight gain when grazing adjacent crop stubbles.

The project will also investigate management strategies to improve saltbush growth and utilisation.

#### Super supplement

Dr Norman said drought-tolerant shrubs such as saltbush provide nutrients to complement other summer-autumn forage options, including pastures or crop stubbles.

- Saltbush can lift farm profitability by: • reducing supplementation
- requirements
- allowing deferred grazing of regenerating pastures
- buffering the impact of low forage supply in poor seasons
- providing antioxidant, protein, vitamin and mineral supplements.

In particular, saltbush is rich in vitamins E and A, zinc, copper, sulphur, manganese and selenium, which may contribute to pathways that reduce

### RESEARCH IN

#### PROJECT NAME

No more gaps with superior shrub systems

#### RESEARCH ORGANISATIONS CSIRO

#### FUNDING ORGANISATIONS MLA and Australian Wool

Innovation GOAL

To find economical drought-tolerant shrub solutions to bridge seasonal nutritional gaps.

DURATION 31 March 2019 - 31 March 2022

#### KEY FINDINGS TO DATE

- Saltbush is not just for saline and arid areas; it performs well in other environments
- There is significant variability in feeding value between shrub varieties.

#### IMPORTANT NOTE:

As saltbush contains oxalates

### Super saltbush plugs feed gap

S altbush not only enhances livestock nutrition to bridge feed gaps – new research is showing how nitrogen can super-size growth of this reliable performer in non-saline and sandy soils.

Producers will see gains from either strategic nitrogen application or by incorporating adapted annual legumes to fix nitrogen in soil.

CSIRO's saltbush research, funded by MLA and Australian Wool Innovation (AWI), continues to deliver valuable insights into how this drought-tolerant super shrub can help carry producers in Mediterranean and low-rainfalt mixed farming systems through the autumn feed gap.

#### Long-term research

The old man saltbush research began in 2007, under the auspices of the Future Farm Industries Cooperative Research Centre (2007–14).

Researchers honed in on the saltbush variety Anameka<sup>TM</sup> for its ability to be productive in non-saline and sandy soils, and its responsiveness to nitrogen fertilisers.

The CSIRO-led team are progressing research to develop seed lines from the nutritious and palatable cohort of plants that delivered the Anameka variety to market.

#### Sheep taste-tes

CSIRO project leader Dr Hayley Norman said livestock played a key role in selecting Anameka.

"Anameka was identified as the best plant from 60,000 individuals – the national

Figure 1: Ohanges in dry edible biomass of Anameka saltbash from differing nitrogen and saline treatment levels (presented as see water salinity equivalents).

research team relied on the sheep themselves, who consistently chose the shrubs with the highest nutritional value," Hayley said.

"There was no way we could put 60,000 plants through a laboratory, and the innate ability of the sheep to select saltbush varieties for optimum energy and nutrients that best met their needs was a unique facet of our study."

#### On-farm bena

After commercialisation in 2015, Anameka saltbush has been a hero, offering a solution to the balancing act that producers in Mediterranean and low-rainfall mixed farming systems face during the autumn feed gap, when livestock rely on crop stubble and supplementation to carry them through.

With saltbush providing key nutrients including protein, sulphux minerals and Vitamin E during this challenging period, costly and time-intensive supplementation can be delayed, and grazing of new pastures following the late autumn rains can be deferred.

Saltbush can remain productive for decades on a range of soil types that are marginal for crops and its evolution in semi-and parts of Australia makes it incredibly tokerant of *low-rainfall* seasons once established.

#### RESEARCH UPDATE

WHAT'S IT ABOUT? To find economical, droughttolerant shrub solutions to bridge seasonal nutritional page.

WHY IT MATTERS Anameko satibuel is a polalable, nutriticus addition to liversock enterprises and is heipful in filling line liensi gap, providing anti-oxidant vitamin and immerski supplemente, and supporting befores direction.

WHERE'S IT UP TO?

WHO'S INVOLVED? MLA, CSIRO, Australia Wool Innovation

#### Nitrogen key to saltbush vigou

Satibush likes nitrogen, and knowing how much, under what conditions is an aim of CSIRO Research Scientist Dr Sarah Rich.

After seeing a growth response to nitrogen in field research sites in Dongara and Baandee, WA, she moved to the glasshouse, growing 100 plants under 15 different treatments of nitrogen and salt. Figure 1 fleft) shows an up to 40 times

ncrease in biomass production when

### ON FARM

all and the second

### 'Indestructible' saltbush earns its place

A sheep producer Rex Luers' involvement with saltbush research has given him a first-hand experience with the reliable and nutritious Anameka<sup>™</sup> variety, which is now a stalwart in his grazing strategies.

Rex has grown salzbush at his Kellerbernin property as part of an MLA/Austrelian Wool Innovation-funded, CSIRO-led research project since 2019 (see previous page). Despite being in a low-rainfall region

(L3 zone), Rex grazed 2,000 dry sheep equivalent (DSE) on one Anameka stand planted as part of the study.

#### Plugging the feed ga

Anameka lines have proven to be high-value feed gap fillers, particularly in September and October, prior to grazing harvest stubbles and from April to June, before opening rains.

The highly productive variety has produced from 1–3.5kg of edible dry metter (EDM) per shrub each year, almost It EDM ha. This complements the annual legume and ryograss understorey.

#### Making ga

Saltbush has been a profitable addition to the Lucrs' family grazing system and Rox is vory happy with the outcome of the trials.

"We expect our plantations will give us 8,000 grazing days of good feed," Rex said. As part of the study. Rex onzed the saltbush

As part of the study, Rex grazed the satibush over summer and autumn 2022 and was able to double the stocking density to 29.3 eves/ha, with eves on the plots for 35 days (1.025 grazing days/ha).

Despite intending to use saltbush primarily for maintenance, he's seen pleasing weight While salibush cannot be 100% of a diet, Rex was able to reduce supplementation – an accied borus "We'l use it for a confinement feeding area after the season break and also for a quick graze

during summer, especially with young sheep, for vitamin E supplementation," Nex said. Given the lack of understorey (1,225kg/ha by autum) and high-said dick, the owes were offered a supplement of 1300kg of oxport outen hay (22) phead/digy and approximately 200kg of barley grain (42) thead/digy).

#### Testing the Bmits

Confining sheep in saltbush stands while waiting for winter pastures to establish after a late seasonal break is a common factic but it results in shrubs being completely defolated to sticks.

Current experiements at the property are working out whether there's any benefit in excluding shrubs from grazing in order to generate more biomass availability the following season.

Researchers are testing the 'use it or lose it' principal, with some of the shrubs isolated from grazing in exclusion cages. Research at other locations has shown that satbush copes well with severe defoliation if given time to recover and there's lifel benefit in defening grazing for more than a year.

Rex has earmarked two more areas on the property for salitbush plantings and has been surprised at how hardy the plants are. REX LUERS, Kellerberrin, WA

ENTERPRISE Merino sheep

LIVESTOCK 900 ewes, 200 hoggets, 1,050 lambs

PASTURES Medics, natural pestures and sowr pastures of oats and barley

SOIL Loam/days and loams

RAINFALL

343000

#### LESSON LEARNT

Once established, don't be



#### Engaging with PDS and other producer led projects.

The team assisted two producer groups to design and get MLA support for PDS projects.

We worked with the Gillamii Centre Group to develop a successful PDS for the 2021 MLA round. Team members have assisted with site selection and planting best-practice saltbush-based pastures. CSIRO provides training in animal and plant measurement techniques, animal ethics applications and analytical capability for nutritional analysis as an in-kind contribution.

We worked with MADFIG. (eastern wheatbelt of WA) to develop a successful PDS for the 2022 MLA round. We have provided training in plant and animal measurement and assisted in the selection and design of 5 paddock-scale (10-20 ha) demonstration sites. Site will be ready for grazing in 2023.

We worked with a team of producers from the Northern Agriculture Catchment Council to develop shrub demonstration sites that deliver autumn feed and reduce salt and fertiliser runoff into the Chapman River.



Fig. 4.47. The research team assisting MADFIG. to design their PDS sites on the Giles and Gethin farms near Merredin in WA.



Fig. 4.48. The Gillamii PDS project near Cranbrook in WA.

Date	Agency	Title
5/09/2018	Farm Weekly	Cunderdin college sets up summer grazing
14/02/2019	Countryman	Saltbush shrubs combat salinity
14/02/2019	Countryman	Bumper turnout for field days and forum
26/02/2019	Farm Weekly	Saltbush demand doubles production
2/11/2020	South West	Establishing and grazing saltbush
	Catchments Council	
	Website and	
	Newsletter.	
22/06/2022	ABC	Growing salt-tolerant plants will fix land damaged due to
		European farming methods, WA farmer says.
30/03/2023	Farm Weekly	Livestock Matters Sheep Industry Insights
16/04/2023	Farm Weekly	Livestock Matters Forum Pingaring
24/04/2023	Sheep Central.	Saltbush helps sheep feed gaps and salinity in Stirling
		Ranges
24/04/2023	Sheep Central.	Saltbush helps sheep feed gaps and salinity in Stirling
		Ranges.

5/07/2023       CSIRO Metal release times       Drought-resident shrub to go east to support famers in dry times         5/07/2023       The Canberra Times       Pass the saltbush: Farmers go native ahead of El Nino         5/07/2023       Perth Now       Pass the saltbush: Farmers go native ahead of El Nino         5/07/2023       Perth Now       Pass the saltbush: Farmers go native ahead of El Nino         5/07/2023       Perth Now       Pass the saltbush: Farmers go native ahead of El Nino         5/07/2023       FarmOnline National       Anameka saltbush CSIRO survives drought and makes sheep more productive         5/07/2023       Stock Journal       Anameka saltbush CSIRO survives drought, makes sheep more productive         5/07/2023       Tasmanian Examiner       Pass the saltbush: Farmers go native ahead of El Nino         5/07/2023       Newcastle Herald       Pass the saltbush: Farmers go native ahead of El Nino         5/07/2023       The Land       Anameka saltbush CSIRO survives drought, makes sheep more productive         5/07/2023       Farm Weekly       Anameka saltbush CSIRO survives drought, makes sheep more productive         5/07/2023       Stock Journal       Anameka saltbush CSIRO survives drought, makes sheep more productive         5/07/2023       Farm Weekly       Anameka saltbush CSIRO survives drought, makes sheep more productive         5/07/2023       Stock Journal       A	5/07/2023	CSIRO Media release	Drought resistant shrub to go east to support farmers in dry
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#### Anameka<sup>™</sup> growers in eastern Australia.

We worked with Chatfield's Tree Nursery (Anameka<sup>™</sup> licensee) to expand capacity of prob production, with a focus on eastern Australia. Tulla Natives in Wakool NSW, and Katanning Tree Nursery in WA both now grow the shrubs. Tulla Nursery has propagated 150 000 plants for NSW/Vic in the 2022 season after expanding the nursery to increase production. They have again doubled capacity in 2023. All four nurseries that grow Anameka sell-out and have a waitlist.

The team at Tulla Nursery have now got two Chatfield's Tree Planters and have had great success with planting (see picture below). This is going to be an important component of Future Drought Fund demonstration activities in eastern Australia in 2023, where three ~10ha sites will be established with producer groups and Select Carbon.



Fig. 4.49. On-farm stand of Anameka<sup>™</sup> saltbush, established with a tree planter, in Swan Hill, Vic.

#### **Students trained**

Aslak H. C. Christiansen. University of Copenhagen. Master thesis in Agricultural development. "Effects of increasing salinity level on growth and feeding value of the two perennial halophytic shrubs Oldman saltbush and Mallee saltbush, and the effect of temperature, salinity, seed weight and seed size on germination".

#### Scientific publications associated with the project

We exceeded the publication goals for the project with 8 scientific papers published and 3 more in advanced draft format.

- Norman HC, Duncan EG and Masters DG (2019). Halophytic shrubs accumulate minerals associated with antioxidant pathways. **Grass and Forage Science**. 74, 345-355.
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Fig. 4.50. The Baandee sign was erected in 2021 and the site is generating a lot of interest from producers using the main east/west highway.

### 5 Conclusion

Oldman saltbush shrub systems offer a number of benefits to the red meat and wool industries, through the provision of out-of-season feed that is high in nutrients that are lacking in summer and autumn. It grows on land that is marginal for cropping so has a much lower opportunity cost in mixed farming systems (a major barrier to adoption of perennial pastures in these zones). The systems offer a significant number of grazing days, especially when considering they are provided in autumn when nutrients are scarce, and they are obtained from soils where there are few production options. The shrubs are adapted to semi-arid areas so maintain productivity in poor seasons, assisting producers to manage risk associated with climate variability and drought. Both saltbush and Mallee saltbush offer shade and shelter opportunities and are likely to provide nutrients that assist animals to deal with heat wave events associated with climate change. Both offer environmental benefits including a reduction in salinity through deep roots and water use in summer, carbon, methane mitigation (Mallee saltbush) and a wide range of ecosystem services.

The broad aims of the project were to (1) raise awareness, (2) identify elite saltbush and Mallee saltbush genotypes that can be established by seed, and (3) conduct research to improve agronomic and grazing management and move towards in-paddock establishment.

This project generated data and conducted extension activities to raise awareness of these shrubs as a promising farming system option. Despite COVID restrictions, the team delivered 2068 industry face to face contacts where the project experiments and extension messages have been discussed (1040 to producers and 1028 to extension agents, scientists and other industry members) over 48 events during the project. Sixteen of these events occurred at the project research sites. The team have worked with Gillamii Centre to deliver two-day saltland masterclass workshops in regional locations (Cranbrook, Ravensthorpe and Jerramungup). These workshops involve seminars and fieldbased activities as farmers plan the rehabilitation of a saline site with saltbush. The workshops were attended by 72 producers and 29 industry representatives.

We have identified elite oldman saltbush seed lines that have similar nutritional value to Anameka (industry best-practice) and reliably produce at least 50% more biomass. These are ready for commercialisation although we are keen to explore some aspects of seed ecology before making a final decision. We have identified adapted Mallee saltbush lines that are incredibly productive on deep infertile sandy soils and high digestibility (energy) and crude protein. There are still some issues with palatability that require further consideration and they could be screeded for methane mitigation potential.

We have made significant progress in understanding the seed ecology of oldman saltbush and Mallee saltbush. We can advise industry how to clean seeds and remove the bracts with commercially viable methods. We have a greater understanding of the vulnerability of the hypocotyl of oldman saltbush seeds and have protocols to minimise damage during de-bracting. We know the optimal temperatures for germination and impact of salinity on germination. This is not being expanded to include water potential. We can also successfully coat seeds to make them bigger, so that they flow through commercial seeders. We believe that we have the information, technology and genotypes to progress direct seeding in agricultural zones and southern rangelands. There is a little more work to be done in this area.

### 5.5 Key findings

#### Shrubs within farming systems

- Nutritional gaps present the largest production cost to grass-fed meat and wool producers across Mediterranean and dry areas of Australia.
- The mixed farming zone (300-650 mm annual rainfall) covers approximately 50 million ha of farmland and approximately 19,000 producers. Previous economic modelling found that (1) the gross margin of a sheep enterprise in these regions increased by 15% for every 20% increase in farm stocking rate from a conservative base level, and (2) that average stocking rates in the WA wheatbelt may be about 40% lower than the calculated optimum (Friend *et al.* 2018; Young *et al.* 2022).
- Strategies to address key nutrient gaps are likely to give confidence to mixed farmers to increase whole-farm stocking rates and while potentially lowering the risk profile of their enterprises.
- Perennial forages provide an opportunity to fill the summer/autumn feed gap, however, very few producers in mixed farming zones are willing to put cropping land under perennials due to the high opportunity cost.
- Drought-tolerant native shrubs, grown on soils that are marginal for crop production, provide nutrients to complement, and thereby improve the feed conversion ratio of crop

and pasture residues during summer/autumn. These shrubs lift farm profitability by reducing supplementation requirements, allow deferred grazing of regenerating pastures, and buffer between-season variation in forage supply. The provide vitamins and minerals that are limited in summer and assist animals to manage oxidative stress.

- Barriers to adoption of shrubs include the high opportunity cost of using soils that are suited to cropping, the up-front cost of establishment, uncertainty about agronomic and grazing management and a lack of on-farm data quantifying benefits.
- Increased adoption of shrub systems by producers will congruently improve landscape function and reduce the impact of dryland salinity.

#### Productivity of shrubs and sheep grazing value

- The shrub paddocks supported up to 1025 sheep grazing days/ha in autumn with moderate sheep growth. This is a time of year when sheep typically lose weight withour grain supplementation. The low rainfall Baandee site (with a saline sandy loam) supported pregnant ewes for 1025 grazing days/ha with 230 g/day of liveweight change (with low levels of supplementation). This equates to 2.8 grazing days/ha across the year. The deep white sandy sites supported 430 sheep grazing days/ha in autumn with 40 g/head.day of sheep growth (Cranbrook), and up to 544 grazing days/ha with 135 g/head.day of sheep growth (Dongara). The adjacent stubble at Dongara yielded just 67 grazing days/ha.year with 95 g/head.day of sheep growth. The experimental design only allowed one grazing event per year in autumn, it is probable that the sites could be grazed opportunistically at other times of the year to extract more value from the annual biomass when it is green. These sites could generate additional value if they provide an opportunity to improve twin lamb survival (the subject of another MLA investment).
- As a general rule, the saltbush seed lines produced more biomass than the clonal parents. In the low rainfall Baandee site, saltbush 'edible' biomass production of mature shrubs, planted at commercial densities, ranged from 1.0 to 2.02 t EDM/ha, and the Mallee saltbush species were generally less productive than the saltbushes. At Cranbrook, the saltbushes were less productive on the deep sands (0.6 t EDM/ha, compared to 2.0 t EDM/ha from the Mallee saltbush genotypes. Mallee saltbush clearly flourished at the sandy site in Dongara with one genotype producing 4.3 t EDM/ha. At Cunderdin, the saltbushes had produced 0.8 t EDM/ha after just 320 days of growth. The saltbushes were disadvantaged by preferential grazing.
- In terms of relative palatability to sheep, saltbush was always significantly preferred to Mallee saltbush at each site and every measurement period. There was significant variation between genotypes for both species. Saltbush clones arising from the CRC Future Farm Industries project were generally preferred over the F1 seed lines generated from crossing the same clones. The most palatable genotypes were Anameka<sup>™</sup>, clone C and clone H. When pushed, sheep would eat the Mallee saltbush RP Cln 40 clone in preference to other Mallee saltbush genotypes, this has had implications for the productivity and persistence of the genotype.
- There were significant differences between species and among genotypes within species for all the nutritional traits that were measured. The OMD of the saltbushes was not very different to the Mallee saltbush genotypes (67.6% compared to 70.7%). The high OMD of the saltbushes was not surprising given the cohort of material had been heavily selected for energy values. The digestible organic matter in the dry matter (DOMD% an estimate of energy value per kg of EDM) of saltbushes (53.9%) was lower than Mallee saltbush (mean of 59% with a range of 53 to 62%), this was associated with the higher salt content of the saltbush. This is not always a problem for livestock if they are only eating shrubs as a part of their diet and have access to fresh water. DOMD of the oldman saltbush WA subspecies spathulata (more representative of a 'wild' or unimproved saltbush) was 44%. The

saltbushes had consistently higher CP content with a mean across sites of 17 %DM, compared to 13.5% DM for Mallee saltbush and 11% DM for the spathulata. These levels would meet the maintenance CP requirement for most classes of stock. Some of this nitrogen is associated with small, non-protein compounds. This would only be converted to protein in the rumen if there was sufficient energy in the diet.

- Feeding value is the worth of a forage in terms of livestock production, it is a function of both how much of the forage is eaten and the utilisation of that forage. Our focus to date has been on energy (digestibility) and relative palatability to select the best-bet genotypes. There are clear trade-offs between the key elements of feeding value biomass production, DOMD, CP, ash and relative palatability. There is no new standout in terms of clonal saltbush lines, Anameka<sup>™</sup> remains a sound choice for the market. There is clearly an opportunity to commercialise a saltbush seed line to complement Anameka<sup>™</sup>. Seed line 10 and seed line 35 are perhaps the most promising, seed line 29 is productive but has consistently lower CP content. Seed line 48 was promising at three sites but performed poorly at another. Any are likely to be suited to nursery raised seedlings seed production traits and ability to establish from seed should be considered before making a final decision.
- Our clonal Mallee saltbush selection (Cln B40) did have higher relative palatability but struggled to persist after heavy grazing. There were several Mallee saltbush standouts in terms of biomass production and DOMD, including RP B3, RP B8, RP M3 and RP A12. These lines should be prioritised for further investigation.

#### Agronomic management of shrub systems.

- Blocks of shrubs at Baandee were excluded from grazing in autumn 2022. The adjacent heavily grazed shrubs grew at a much greater rate so there were similar amounts of biomass the following season. The "use it or lose it" message is still relevant.
- Two sites had a reasonable legume content in the understory at the start of the experiments. It became apparent that this is difficult to manage as weeds take advantage of the fixed nitrogen and absence of spring grazing and herbicide manipulation. Matricaria (Globe Chamomile) and brome grass were the biggest weed concerns. It is probable that grass selective herbicides can be used to control brome grass (if the producer has firebreak-scale spraying equipment or can lift over the shrubs). Matricaria is a bigger concern as it is a forb so more difficult to selectively manage with herbicides that will not impact the shrubs. This weed is rapidly colonising the eastern wheatbelt of WA and may become a disincentive to adoption of shrub systems. We recommend research activities to obtain a better understanding of herbicide options for saltbush and Mallee saltbush.
- A glasshouse experiment examined the relative salt tolerance of oldman saltbush and Mallee saltbush. Treatments ranged from fresh to seawater level salinity. All plants survived the five salinity treatments, but with clear effects on growth. A curvilinear growth response was exhibited by saltbush at increasing salinity levels (some salt improves growth), while growth of Mallee saltbush was inhibited by any addition of salt. This suggests that Mallee saltbush is not a halophyte but can tolerate salinity to some degree. At seawater salinity, Anameka<sup>™</sup> yielded 59% of the fresh water control and Mallee saltbush yielded 40% of the fresh water control with evidence of damage to leaves. While Mallee saltbush is less salt tolerant, it has significant capacity to manage moderate levels of salinity or transient salinity.
- The salinity of the soil solution impacts on the nutritional value of shrubs. For oldman saltbush, increasing salinity of the irrigation water was correlated to a significant increase in mean OMD, ash (or salt), sulphur and nitrate. Salinity was negatively correlated with fibre, DOMD, copper and phosphate. For Mallee saltbush, increasing salinity of the irrigation water was correlated to a significant increase in ash and chloride. Salinity was negatively

correlated with fibre, oxalate and nitrate Both species had an increase in ash content associated with increasing salinity.

- A second glasshouse experiment explored the impact of N fertilisers on saltbush growth and nutritional value. Edible dry matter production increased steadily with the addition of nitrogen. Saltbush responds to nitrogen application in terms of greater biomass production and higher CP and OMD of the biomass until a critical limit is reached. This limit is to some degree associated with the salinity at the site as saltbush needs non-protein nitrogen compounds to osmoregulation.
- Fertiliser treatments were applied to a subset of saltbushes at two sandy sites Dongara at rates of 100 -120 kg/ha. The data yielded some mixed messages. At one site the only significant difference was that the calcium nitrate + superphosphate treatment yielded significantly less biomass (~30%) than the unfertilised control. At the other site, the unfertilised control produced significantly less biomass than the fertilised plots. In the first year at this site, addition of calcium nitrate quadrupled biomass production from the saltbushes. There were significant responses to superphosphate and to calcium nitrate, but the combination of fertilisers did not improve biomass production.
- Five rates of calcium nitrate application (from none to 80 kg/ha) were applied to saltbushes at Baandee. There was no significant biomass production response to the fertiliser applications at this site. It is probable that the mature shrubs were mining historical fertiliser applications at depth.

#### Direct seeding of saltbush

- X-ray methods were investigated to allow for measurement of seed fill within bracts. It was not possible to clearly identify all the seeds presents in the bracts. However, when germination test was performed on seed extracted from the bracts, none of the seed that were not identified with the x-ray germinated. X-ray might not be accurate to determine seed fill, however, it is a very good predictor of seed viability. This is arguably the more important trait.
- Saltbush bracts are problematic for direct seeding due to inhibitory impact on germination, uncertainty regarding seed fill, and issues with flow in commercial seeding equipment. Several experiments were conducted to develop commercially viable seed cleaning. Soaking bracts in still or running water did not improve cleaning efficiency however hydrochloric acid for 30 min or less or sulphuric acid from 3 to 6 hours did improve bract removal without compromising seed viability.
- Two mechanical bract removal systems were identified, a Venable drum thresher and a serradella dehuller (grinding disks). The dehuller was to most efficient equipment but care must be taken to not damage the delicate seeds.
- Both species had high germination percentage of clean seeds. We found an increasing germination percentage when the seed size increases, although maximum germination seems to occur for all seeds above 0.75 mg for oldman saltbush and above 0.5 mg for Mallee saltbush. There is a lag period of around 6 days before the Mallee saltbush seeds start to germinate, while the seeds of oldman saltbush readily germinate.
- It is important to investigate the health of germinating oldman saltbush seeds. Damage to the radicle from threshing is not apparent until the seed has started growing. Standard seed testing protocols can miss this as the seeds are generally removed as soon as the radicle emerges.
- Optimum planting time will be a trade-off between temperature, salinity, soil mositure and the likelihood that there will be sufficient ongoing rainfall for establishment. The highest maximum germination percentage occurs at 10°C treatment for both species, although saltbush germinated as well at 5°C.

- The highest maximum germination percentage was for oldman saltbush reached at salinity levels <200 mM NaCl for saltbush or > 50 mM for Mallee saltbush. Saltbush is a better option for direct seeding saline areas and the seeding must occur in winter or early spring to optimise germination.
- Saltbush seeds were successfully coated with some batches having a growth promoting, compound, insecticide or both. The coat did not impact germination. This is an important step towards in-field establishment as they seeds are tiny and not suited to commercial crop seeding equipment. The coating did not improve success in a commercial nursery where it is standard practice to plant 2-3 seeds per cell.
- Saltbush seedlings emerged far better at the surface or 1 mm sowing depts in a glasshouse. Moving from 1 mm to 5 mm burial halved the emergence rates.
- Field sowing was tested in four locations and microscopy studies of the commercially purchased seed revealed a high rate of seed damage, which was not evident to the naked eye at the time of coating and the first experiments. In 2021, we failed to achieve establishment using a commercial seeder at Kellerberrin or a niche seeder at Cranbrook. In 2022, two field sites were hand sown at 1 and 10 mm depths. Naked seed that had been hand sorted under a microscope to ensure no damage was added to the experiments and it was found that only 20% of the coated seeds would have been viable. Using this to adjust the data, we achieved 30% seed emergence, a promising result. There was no impact of coating, depth or time of sowing.
- The elite seed lines that were tested in the first activity have significant variation in seed size, bract size and they all produce seed that is highly germinable. We will use this information, with the outcomes of the first activity to select a cohort for future direct seeding experiments.

### 5.6 Benefits to industry

The benefits to industry from this research include,

- Data, awareness raising and extension products to support adoption of shrub systems on marginal soils to improve whole-farm stocking rates and manage seasonal risk.
- New genotypes of shrubs that have capacity to double existing productivity and allow the potential for direct seeding.
- Information regarding shrub grazing management and fertilisers to assist producers to optimise system productivity.
- Adoption of these systems will lead to additional benefits such as reduced dryland salinity, enhanced ecosystem function, possibly reduced methane emissions (Mallee saltbush), higher animal welfare and improved eco credentials of meat and wool.

### 6 Future research and recommendations

The major challenges that impacted this project were a very dray establishment season in 2019, COVID travel restrictions in Western Australia (restricted ability for timely measurements and extension activities) and a damaged batch of commercially sourced saltbush seed that was used in the coating and field establishment experiments. Despite these challenges, we believe that we made great progress on several fronts.

- We recommend the commercialisation of an elite saltbush seed line or a combination of several lines. Discussions with industry are needed to scope the appetite for the product and seed production logistics.
- While we have identified elite Mallee saltbush genotypes that have high biomass production and nutritional value and tolerate deep infertile sands, they remain highly unpalatable. We suggest waiting on the outcomes of the 'shade and shelter' MLA projects before deciding on a commercialisation strategy. More work is required to understand why sheep do not eat the shrubs and their role in methane mitigation.
- The key findings regarding shrub management and agronomy are already being presented to industry. The variability in fertiliser responses across sites is likely due to shrubs accessing nutrients at depth. Farmers are unlikely to sample soils to these depths, so extension messages are uncertain. Use of adapted annual legumes remains a key opportunity but weed management is an ongoing issue. Matricaria is a threat to shrub systems in eastern WA. We recommend the development of broadleaf herbicide recommendations for shrub systems.
- We believe that we have the information, technology and genotypes to progress direct seeding (for agricultural and possibly rangeland zones). We hope to continue this work in partnership with industry, possibly through a WA Agricultural Research Collaboration Application in May 2023. We will contact MLA and AWI to discuss the opportunity to co-invest.

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### 8 Appendices

### 8.1 Acknowledgements

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### 8.2 Field walks



WESTERN AUSTRALIA



## Come have a look at what edible shelter has done for Sam

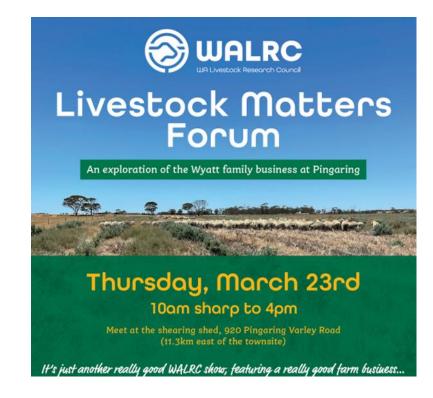
Tuesday, October 11, 2-5pm On site Brickhouse Road, Cranbrook More details at www.walrc.com.au/events | RSVP to @walrc1 or Esther 0418 931 938







CSIRO





## **Livestock Matters**

### Where research meets farm in the city

Monday, August 22 at the Esplanade, Fremantle with dinner to follow

In this exciting re-vamp of the former Great Livestock Industry Day Out, we call on producers to come to town to get to know the people responsible for the next big leaps in productivity. But this is not any ordinary gathering. Central to the day will be the opportunity to meet our next wave of emerging livestock industry researchers. And, in a new WALRC initiative, we launch the Farm-a-Friend student program.

#### Session 1

- 8.30am Registration
- 9.00am Welcome with WALRC Chair Dr Bronwyn Clarke

9.10am Keynote address: Michael Craig runs a mixed broadacre livestock and cropping enterprise at Harrow in the western districts of Victoria that until recently included 18,000 sheep and 500 breeders. A succession plan that came into fruition last year resulted in a 50 per cent reduction in land-holding and some system changes that came with that, but throughout this process, a culture of innovation and adoption of quality research has kept the business strong. Considered an 'early adopter' of all things high-tech, Michael will tell the story of science-based changes made to his business over the last five years. Michael discusses ceasing mulesing; DNA and EID tracking, blood testing work for abortion causing disease issues in the beef herd and



## You are invited to be part of the WALRC Study Tour to Ridgefield

### Monday, June 27

#### 10am-5pm with dinner to follo

This strictly by-invitation event is d x researcher information exchange the full WALRC team and a select g projects, to gain a thorough unders at Ridgefield and for researchers to leading producers. All researchers to Ridgefield are invited to participate to thank former WALRC chair Dr Ti

- All researchers with a project at Ridgefie
- All recipients of the WALRC scholarship I
   To sheep researchers receiving this invitulike to be on it please contact us to disexchange opportunities the day provides

Logistics

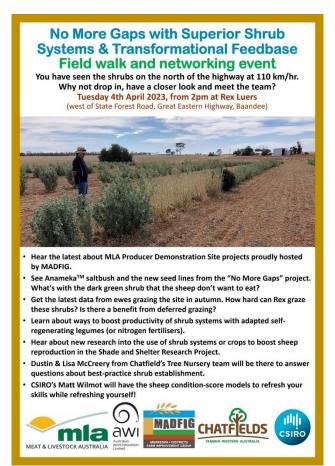






You are invited to join Sam, a couple of fellow farmers who are learning new ways to provide shelter for ewes, and the research team from CSIRO, Murdoch and UWA - to see just how much has changed in five years.

This is an all-weather event – we walk the paddocks irrespective – talking as we go - with wrap-up discussions and hospitality to follow at the Cranbrook Sporting Cub. This field tour is part of the collaborative shade and shelter project and is proudly supported by WAERC and Gillamit.



#### 8.3 Selection of media articles

16 Farm Weekly Thursday, March 30, 2023

By BROOKE LITTLEWOOD PINGARING farmer Dean

Wyatt's main aim is simple: build a robust agricultural business, which remains flexible and sustainable into the future. His father Ian's line of dividing using the flexible of the flexible

the future. His father lan's line of thinking was similar in the Jate 1980s, when saltbush was planted to tackle rising salimity in cropping country. Many years of trial and error followed, when some of the initial varieties – mainly qualibrush and wavy leaf – proved unproductive. While salimity still has a detrimental impact on some parts of the Wyatt family's farm, saltbush now has multi-purpose for Mr Wyatt and wife Danica. The perennial shrub has boosted lamb survival rates on marginal land by up to 12 per cent and provided shade, shelter and fodder. About 50 producers were last Thurday given insight into the Wyats' enterprise, at the WA Livestock Research Council's Livestock Matters forum.

forum



Into four paddocks Grains: wheat, barley and Chrosols: 3100 matod evess and 1200 eve weaners Mating: 75-25 Morino/ White Sutfolk Non-mulesed since 2020 All eve lambs tagged with EIDs

As well as saltbush, Mr Wyatt and keynote speakers including – CSIRO agricultural scientist Hayley Norman, The University of WA's School of Agriculture and Environment and Institute of Agriculture associate professor Dominique Blache and Murdoch University sheep research scientist Serina Hancock, AgPro Management

Continued on page 18



Dean Wyatt (left) and his father Ian Wyatt hosted the WA Livestock Research Council's Livestock Matters forum last week which focused on shade and shelter.

WEEKLY NEWS



### Farm Weekly Thursday, March 30, 2023 19

 $\square$  About 50 producers were given insight into the Wyatts' enterprise, at the WA Livestock Research Council's Livestock Matters forum.

#### 20 Fern Weekly Thursday, March 30, 2023

Q Continued from page 319

When interviewed by AgPro Management contailute Ed Regardt at Livenick, Mattery, My Wyst aud the biggert challenger with mon-molening was Rystrika in 12-18-monthoid, full-flement beggins, which contind a longer longth of word than a matted even. Sheep were samilly shown in Match, however this year all non-nulesed sheep will be

"I think be proactive, not reactive," We Wyart and. He said he may change his treatment at the craffs for blowflies and foce and then us CLiK at weeming. In response, Bullevier Raral Entreprises' David Gring provided an update on immericide reastance on the

Eastern States. He ceased mulesing in 20 and has been Responsible Wool Standard (RWS)

accredited since last year. Mr Grieg said 75pc of the

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WA Liverback Respect Council electrics officer Eather curses succession with Las Icentrel and Dean Wyatt,

HILLS CONCRETE PRODUCTS

#### Laura Garland, Anderson Rams principal Lpring Anderson Rams principal Lpring Anderson Rams Samup Seef producer Matt Camary attended the field day.

+ 30. 2023 21

in the

Page 149 of 153





02 Aug 2018 Farm Weekly, Perth

Author: Courtney Walsh • Section: General News • Article Type: News Item Audience : 12,167 • Page: 64 • Printed size: 646.00cm² • Market: WA Country: Australia • ASR: AUD 1,726 • words: 1336 • Item ID: 989305577

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Page 1 of 3

## Anameka offers feed options

#### By COURTNEY WALSH

INVESTING in Anameka saltbush is providing options for the Richards family who run a mixed farming enterprise at Quairading.

Robyn and Greg Richards, who farm 5000 hectares with their son Haydyn, his wife Jess and two young children, said there were plenty of upsides to putting hectares of their land to Anameka. Robyn first heard about

Anameka at the local CRC about four years ago and immediately realised there were potential benefits there. "Paonla from the Fastern

"People from the Eastern States had come over to promote it," Robyn said, "They were talking about

the amount of protein in the plant and the fact that sheep prefer to eat the Anameka over the Old Man saltbush which eot me interested."

which got me interested." Robyn went home and told "If we're being realistic we'd have to say we have about 1000 hectares of salt country which is too much and the first choice would be reclaiming it to good land which would be a battle.

"I don't know if there is a cure to salt land but by utilising the salt land by putting it to Anameka that will also benefit our sheep, it is the next best option we have."

More than 4000 Merino ewes are running on the property which are mated to Poll Dorsets, Suffolks and a few SAMMs.

For Dorsels, sanous and a few SAMMs. Greg said they haven't done the figures on weight gain by weighing the sheep on and off the country planted to Anameka, but just by eyeballing it, he said the sheep seemed to like it. "The paddocks that they are

in at the moment with the

In terms of looking at which parts of the better country on the property to lock up with the Anameka shrubs, Greg said it would be a matter of thinking about where it would be most useful.

"We've looked at putting it around the shearing shed as a feed and shelter option in a holding paddock," he said, "Creek lines and laneways

are other options for it but a 20ha paddock of the stuff is definitely on the cards for us.<sup>3</sup> Greg said the primary use of that paddock would be for fattening lambs and supplementary feed in the autumn gap or potentially

even for lambing. When asked if they would

recommend trying Anameka to their neighbours, Robyn

"The sheep seem to do really well on the Anameka so

yes, I think so," she said.

#### 30 LIVESTOCK

# Bumper turnout for field day and forum

Farmers and agricultural representatives from across the State ventured to the central Wheatbelt last week for WAFarmers' livestock field day and forum. Last Thurisday, more than 50 farmers attended the event which included a toor of the Kytagh Prediot, near Tammin, and the York milly's Anameka Farm.

In the autornoon, the Kollerker in Recreation Centre hosted delegates for a series of speeches. Murdicch University professor John Howieson and CSIRO's Rick Lewellyn addressed the crowd alongside well-known agricultural Innovator Annie Brox and WA Llyestock Research Council chairman The Work.

University of WA professor Boss Kingwell also discussed a sheep flock's role in farm profitability, while Australian Wool Innovation research general manager Jane Littlejohn held a quick-fire question-and-answer session before the











The team with Minister McTiernan, WA Minister for Agriculture and Regional Development (planting a saltbush) at an on-farm extension event in 2022.

### 8.2 Shrub and saltland guideline – key extension messages

### **Guidelines for optimising saltland pastures**

Hayley Norman, Ed Barrett-Lennard, Matt Wilmot, Dustin & Lisa McCreery from Chatfield's Tree Nursery

#### 1. Understand your motivations and plan accordingly

Saltland systems deliver a range of benefits and vary in complexity and cost of establishment. An understanding your prime motivations, combined with site capability, allows you to choose the optimal system. Key questions.

- Which are most important to you aesthetics, biodiversity, lowering saline water tables, carbon balance or productivity/profitability?
- Do you have livestock and a feed gap in autumn/winter? Are you wanting forage to complement adjacent crop stubbles or a place for confinement feeding?
- Do you have issues with vitamin E and mineral deficiencies?
- Are you seeking shade and shelter for stock?

#### 2. What is the site capability?

Very few plants tolerate waterlogging combined with salinity (samphire and seaweed).

- How far down is the water table? How often is the soil waterlogged or inundated? How saline is the soil solution in the root zone (ie salt concentration x moisture availability)?
- How much rainfall do you receive and what is your soil texture and pH?
- Naturalised plant indicator species, piezometers, soil tests and EM38 surveys can help (see Saltland Genie)
- Lock up the samphire zone start with the bits that will give an economic return!

### 3. Species and cultivar selection

Choose the right species according to your motivations and land capability.

- Shrubs. Anameka<sup>™</sup> oldman saltbush to optimise profitability for your best 'marginal' land. Oldman saltbush does not like waterlogging and subspecies spathulata (the WA local) have low nutritional value.
- River saltbush is better in wetter areas, oldman saltbush for arid areas.
- Bluebush is cheap and cheerful perennial pasture (throw in a few and they self-seed).
- Where possible legumes (& rhizobia) shrubs and grasses love free nitrogen. Select what works in your area and soil type. Can you get it in before the shrubs?
- In saline systems tall wheatgrass and puccinella may have a role.

#### 4. Water-table drawdown

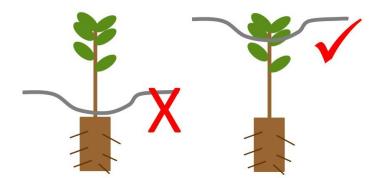
Keep plants leafy in summer if you want them to use out-of-season rainfall or draw down on the watertable. No shrub will utilise water that is saltier than seawater and acidic (we see 1.5 times seawater salinity with a pH of 3). In these cases, the shrubs use the rainfall and reduce recharge over time.

#### 5. Design.

- Don't forget the understorey & spend more on the better 'marginal' soils
- Shrub density 700 1200 plants per ha, depending on rainfall, access to a watertable and salinity and pH of the watertable.
- Layout to optimise stock visibility, movement, and shelter and to avoid waterlogging.
- Do you need to and how will you manage the understorey and weeds?

### 6. Establishment.

• Use a tree planter to scalp the weeds, rip to improve root penetration and press the root ball into the ground (gotta love the Chatfield's planter for this).



- Plant shrub seedlings deep (few leaves visible above ground). •
- Direct seeding of shrubs can be more challenging and better in the areas with summer rainfall. Ensure saltbush bracts contain a viable seed (many don't).
- Can you get an understorey in before the perennials? Don't forget to inoculate legumes.

#### 7. Animal nutrition and wool growth.

- Saltbush and bluebush accumulate up to 25% salt in leaves, salt limits feed intake. These **species should be** ~1/3 of the diet. These plants accumulate salt in non-saline soils.
- Understorey, adjacent crop stubbles or supplements proved the rest of the diet.
- Saltbush is high in crude protein and sulphur access increases wool growth by 25% and is likely to improve wool strength.
- Saltbush and other salt-tolerant shrubs are high in vitamins E and A and the minerals associated with antioxidant pathways (S, Se, Mn, Zn and Cu). These are important when managing heat stress and often limited in autumn.
- Shrubs can have excessive oxalate so be aware of the risk of calcium deficiency, especially if you are supplementing with cereal grain.
- Saltbush and bluebush have marginal energy value (45-55% organic matter digestibility). Energy supplements will be needed for growing and reproducing stock. Anameka<sup>™</sup> was selected for higher digestibility (64% OMD).
- Salt tolerant shrubs are a great complement to crop and pasture residues and reduce the need for supplementary feed.

#### 8. Do they know it's food?

Sheep offered novel forages do not know they are safe to eat, and it will take naive animals time to learn to incorporate them into a balanced diet. Train lambs - with mum or even in utero.

#### 9. Drinking water.

Provide plenty of cool non-saline drinking water, they need to flush the salt (up to 10 litres/day in hot weather). The more salt in the water, the less salt they will eat.







