Direct seeding of chenopod shrubs for saltland and rangeland environments

Future Farm Industries CRC Technical Report 10

Sub-series: Farming Systems


Future Farm Industries CRC


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Direct seeding of chenopod shrubs for saltland and rangeland environments

Future Farm Industries CRC Technical Bulletin

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ABSTRACT

There are currently two ways of establishing chenopod shrubs: sowing from seed using a niche seeder, or planting nursery-raised seedlings with a tree planter. Planting seedlings is the more reliable method, but is relatively expensive (in excess of $450 per hectare). On the other hand, direct seeding using the specialised “niche seeder” is much less expensive ($100-150 per hectare), but is also less reliable. This project aimed to investigate alternative methods of direct seeding chenopod shrubs for saltland and rangeland areas by developing a greater understanding of their seed biology and agronomic requirements. Our aspiration was that shrubs should be established using more conventional farm machinery.

This bulletin reports on a combination of seed biology and agronomic research to develop reliable, low-cost direct seeding options for chenopod shrubs. Experiments into the impact of changing environmental conditions on seeds were studied in the laboratory, and field experiments were conducted to test the applicability of these insights in the field using conventional modified farm seeding machinery.

As a result of this work, a successful direct seeding package using farm seeding equipment (modified for wide row spacings and depth control) was developed for *Atriplex nummularia* (old man saltbush), the most widely planted saltbush species across southern Australia. The nine key elements of the package are:

1. Select suitable paddocks for introduction of new shrubs
2. Prepare a weed-free seedbed using two knockdown herbicide applications (4-6 weeks and 1-2 weeks before seeding) and commence control of rabbits and kangaroos
3. Sow the best seed, by ensuring:
   a. Large fruits, with a high proportion of viable seeds, have been selected
   b. Seed is of subspecies *nummularia* (not subsp. *spathulata*)
   c. Fruits have been harvested within the previous six months and stored in a cool, dry environment
   d. Bracts are retained around the seeds
4. Sow into moisture in late winter - early spring (depending on district)
   a. If the area to be sown is waterlogged, defer sowing until later in spring
   b. If insufficient soil moisture, defer sowing until the following year
5. Use a sowing rate of ~10 fruits/m (if germination rate is 15%) to provide at least one plant for every 2 m of row; use higher rates for seed of lower germination
6. Set the seeder up to sow into furrows with trailing press wheels
7. Sow to a depth of 5-10 mm (very critical)
8. Control weeds and pests (insects, mites, kangaroos and rabbits)
9. Defer grazing until seedlings are well established

This establishment method has also been shown to work for *Rhagodia preissii* (mallee saltbush).
This project was not able to develop reliable direct seeding packages for other *Atriplex* species, including *A. amnicola* and *A. undulata*. Further work is needed to understand the triggers for their germination, before these species can be direct-seeded with conventional machinery. Direct sowing of *M. brevifolia* and *M. pyramidata* appears to be problematic in much of southern Australia, due to their requirement for temperatures >30°C for germination, which do not occur within the normal winter growing season. An exception to this would be areas with more reliable summer rainfall, such as northern New South Wales, where sowing could be deferred until late spring-early summer. An alternative strategy for establishing *M. brevifolia*, is to encourage natural recruitment of seedlings from seed produced on surrounding bushes (if it is already present in the area), or to transplant a low density of nursery-raised seedlings, which could then act as a seed source for natural recruitment (if it is not already present).

*The first successful direct seeding of Atriplex nummularia at Meckering, WA*
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1 **BACKGROUND**

Much of the Australian landscape is salt affected. Western Australia (WA) is the most affected State, with around 1.1 Mha of agricultural land severely affected by dryland salinity and a further 1.7-3.4 Mha at risk of salt-derived degradation (George *et al.* 2008). Saltland pastures, comprised of halophytic chenopod shrubs (family Chenopodiaceae) and other understorey species, have contributed significantly to the productive use of saline land. Three main species of saltbush have been established commercially on saltland: the two Australian natives, *Atriplex nummularia* Lindl. (old man saltbush) and *A. amnicola* Paul G. Wilson (river saltbush), and the Argentinean species, *A. undulata* D. Dietr (wavy leaf saltbush). *Rhagodia preissii* Moq. (Mallee saltbush), another native chenopod shrub, has also been promoted for use on non-saline land, particularly in the northern agricultural region of WA. Other native species, including *A. semibaccata* R.Br. (creeping saltbush), *A. cinerea* Poir. (grey saltbush), *A. bunburyana* F.Meull. (silver saltbush), *Maireana brevifolia* (R.Br.) Paul G.Wilson (small leaf bluebush), *M. pyramidata* (Benth.) Paul G.Wilson (sago bush) and *M. aphylla* R.Br. Paul G.Wilson (cotton bush), are widely naturalised on saltland and methods to either establish them directly, or to encourage a greater rate of seedling recruitment, could enhance the productivity of salt-affected land. The majority of these species are native to the rangelands and simple methods of establishment could also lead to cost-effective rehabilitation of degraded rangeland areas.

1.1 **Establishment Techniques**

Farmers seeking to establish halophytic shrubs on saltland can either: (a) seed directly using niche seeders, such as the “Mallen” niche seeder (manufactured by W.C. Diamond) and the KimSeed seeder (manufactured by Kimberley Seeds); or (b) they can use commercial tree planters to plant nursery-raised seedlings. The “Mallen” niche seeder was developed by C.V. Malcolm and colleagues in the 1970s (Malcolm and Allen, 1981). It deposits *Atriplex* fruits at 1-3 m intervals (placements) along the centre furrow of a raised M-shaped mound (see Figure 1). The banks are raised to reduce waterlogging and the central furrow acts to capture rainfall and to leach salts from the surface. The fruits are mixed with a vermiculite mulch to reduce salinity and black paint is sometimes added to increase soil temperature. Other niche seeders operate in a similar way.

Direct seeding of saltbush species with niche seeders has had mixed success, and often leads to poor establishment. Firstly, less than 5% of fruits commonly result in successful establishment. Therefore, 50 fruits per placement are recommended, with the aim of obtaining at least one successfully established plant per placement (Barrett-Lennard *et al.* 2003; Moore *et al.* 2006). Secondly, many placements fail to establish any bushes. Vlahos (1997) conducted a survey of saltbush establishment on 63 sites over 22 locations in south-western Australia and found that more than half the sites had less than 25% of placements with successful establishment.
The use of nursery-raised seedlings is generally much more reliable than niche seeding (Barrett-Lennard et al. 1991). However, direct seeding (~$100–150/ha) is far cheaper than the planting of nursery-raised seedlings (more than $450/ha). Farmers, therefore, face a trade off between risk and price: the establishment technique that is most reliable is expensive to implement, while the accessibly priced technique is too risky. Furthermore, the cost of sowing these species for rehabilitation of large pastoral zone areas is currently prohibitive and too risky. If prices for demonstrably reliable saltland and rangeland pasture establishment can be brought down to ~$120/ha, there would be substantial farmer and grazer adoption. Furthermore, there is a high chance that the developed techniques would be appropriate to the adoption of direct seeding for applications in the rangeland and arid areas of the wheatbelt.

Figure 1. Direct seeding with the “Mallen” niche seeder.

There are two approaches to developing direct seeding techniques for more reliable chenopod shrub establishment. One is to better engineer the sowing niche. Maximum growth of shrubs is possible with stands of ~1,000 stems per hectare (Malcolm et al. 1988; Barrett-Lennard 1993). Thus, an outlay of $100–$120 per ha for establishment equates to a cost of 10–12 cents per seed placement. This allows for the possibility of relatively expensive micro-engineering options to help overcome a number of stresses affecting establishment, including salinity, waterlogging, inundation, drought, weeds, insects, grazing, and low temperatures or frost (Malcolm, 1972; Jennings et al. 1993; Barrett-Lennard et al. 2003). The alternative viewpoint is to develop direct seeding methods in which farmers can use their own seeding equipment. This project elected to follow the latter approach, with the main target area being soils more favourable to chenopod growth – mildly to moderately saline areas, not subjected to prolonged waterlogging. These areas are also likely to support growth of grass and leguminous understorey species that can markedly increase livestock production (Barrett-Lennard et al. 2005; Norman et al. 2010).
1.2 Seed biology

Relatively little is known about the seed germination requirements of many of the main chenopod shrub species of interest. A greater understanding of their seed biology and the environmental factors that trigger germination is needed before more reliable establishment methods can be developed. Such issues include physical or embryo dormancy, the role of bracteoles enclosing the seed, and optimal temperature and moisture conditions for germination.

As the harsh micro-environment in which Atriplex and other halophytic species grow, is often characterised by high salinity and drought, the development of seed treatments that maximise the potential of the seed to germinate and emerge rapidly could significantly enhance establishment success (Powell 1998). This includes priming (pre-germinating) seeds with water or germination stimulants. This technique has been successfully used for a range of species with plant signalling chemicals including karrikinolide (KAR1, the active ingredient in smoke, formerly known as butenolide) (Flematti et al., 2004), gibberellic acid (GA3), benzoic acid (Senaratna et al., 2003), salicylic acid (Senaratna et al., 2000) and cytokinins.

In a pilot project, Kings Park and Botanical Garden and the Department of Agriculture and Food Western Australia (DAFWA) identified several possible dormancy alleviation and germination stimulation strategies for old man saltbush, river saltbush and wavy leaf saltbush, each of which have different germination requirements (Stevens et al. 2006). For A. amnicola, the presence of light and the substitution of light by 1000 ppm of GA3 improved germination under laboratory conditions and resulted in a four-fold increase in seedling emergence in the field. For A. undulata, removing bracteoles from the fruit increased germination by 15% under laboratory conditions and gave a 1.5-fold improvement in seedling field emergence. On the other hand, bracteole removal and light had small positive effects on germination of A. nummularia under laboratory conditions, but this did not translate into improved emergence of seedlings in the glasshouse or field. The effects of seed priming on seedlings varied with species. Priming with water significantly increased emergence percentage of river saltbush, but had no effect on old man or wavy leaf saltbushes. Salicylic acid and kinetin improved the rate of emergence of all three species at various levels of salinity, while GA3 also improved germination of wavy leaf saltbush.

This bulletin reports on a combination of seed biology and agronomic research to develop reliable, low-cost direct seeding options for chenopods. It examines the potential of seed treatments identified by Stevens et al. (2006) for use in the field and the development of new seeding equipment solutions. In particular, the use of conventional farm machinery for sowing is examined, to encourage farmers to sow their own seed.

2 Project details

A four-year project, titled “Reliable establishment of non-traditional perennial pasture species”, commenced on 1 July 2006 as part of the former Cooperative Research Centre for Plant-based Management of Dryland Salinity (Salinity CRC), with industry funding from Meat & Livestock Australia, Australian Wool Innovation Limited and the former Land and Water Australia. Management of the project was continued by the Future Farm Industries Cooperative Research Centre (FFI CRC), upon its inception in July 2007. The project consisted of four sub-projects: (i) “Establishment of saltland and rangeland species”; (ii) “Establishment of exotic warm-season grasses and legumes”; (iii) “Establishment of native grasses and legumes”; and (iv)
“Recruitment of native grasses in native pastures”. This bulletin focuses on results from the sub-project “Establishment of saltland and rangeland species”.

The saltland pastures component of the project consisted of interlinked laboratory and field components. Kings Park conducted laboratory and glasshouse studies to gain an understanding of the seed biology and germination requirements of the species and also examined a range of potential germination-enhancing seed treatments. DAFWA used this information to develop agronomic and seeding machinery solutions to develop agronomic packages for establishment.

2.1 Species examined

Ten species (see Table 1) were examined during the project and their fruits and seeds are shown in Figure 2. Most work was conducted with the main commercially utilised species, *Atriplex nummularia*, *A. amnicola*, *A. undulata* and *Maireana brevifolia*. However, the greatest success in developing a reliable establishment package was achieved with *A. nummularia* and this will be the main focus in this bulletin.

There are two recognised subspecies of *Atriplex nummularia* Lindl. – *Atriplex nummularia* Lindl. subsp. *nummularia* and *Atriplex nummularia* subsp. *spathulata* Aellen (Anon. 2011). Subspecies *spathulata* can be distinguished from subsp. *nummularia* by having smaller fruits and much shorter petioles. The two species are shown in Figure 3. Subspecies *nummularia* generally has higher nutritive value and palatability characteristics than subspecies *spathulata* (H.C. Norman, pers. comm.). Subspecies *nummularia* was, therefore, used in the laboratory and most of the field studies.

**Table 1. Species examined in this Bulletin.**

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<thead>
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<th>Scientific name</th>
<th>Common name</th>
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<tr>
<td><em>Atriplex nummularia</em> Lindl.</td>
<td>Old man saltbush</td>
</tr>
<tr>
<td><em>A. amnicola</em> Paul G.Wilson</td>
<td>River saltbush</td>
</tr>
<tr>
<td><em>A. undulata</em> D. Dietr</td>
<td>Wavy leaf saltbush</td>
</tr>
<tr>
<td><em>A. semibaccata</em> R.Br.</td>
<td>Creeping saltbush</td>
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<tr>
<td><em>A. bunburyana</em> F.Meull.</td>
<td>Silver saltbush</td>
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<tr>
<td><em>A. cinerea</em> Poir.</td>
<td>Grey saltbush</td>
</tr>
<tr>
<td><em>Maireana brevifolia</em> (R.Br.) Paul G.Wilson</td>
<td>Small leaf bluebush</td>
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<tr>
<td><em>M. pyramidata</em> (Benth.) Paul G.Wilson</td>
<td>Sago bush</td>
</tr>
<tr>
<td><em>M. aphylla</em> (R.Br.) Paul G.Wilson</td>
<td>Cotton bush</td>
</tr>
<tr>
<td><em>Rhagodia preissii</em> Moq.</td>
<td>Mallee saltbush</td>
</tr>
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Figure 2. Fruits and seed of Atriplex amnicola, A. undulata, A. nummularia, A. semibaccata, A. bunburyana, A. cinerea, Maireana brevifolia, M. pyramidata and Rhagodia preissii. Two commonly sown saltland perennial grass species, Puccinellia ciliata and Thinopyrum ponticum, are also shown.

Figure 3. Fruits and leaves of subsp. nummularia (left hand side) and subsp. spathulata (right hand side) of Atriplex nummularia.
3.1 Seed quality

Seed batches can vary widely in quality. Data from Vlahos et al. (1991) showed that seed batches of *A. amnicola* often had less than 25% germinable seed, with less than 10% of samples having more than 50% germination. Seed quality is affected by seed age, purity, dormancy and the proportion of weed seeds. Poor seed quality results in low germinability and is a major limitation to successful establishment from direct seeding. “Seed purity” is defined as the percentage of viable seed by weight in a seed batch. High quality seed batches have high levels of seed purity. However, if there is a high proportion of dormant seed, the seed batch may have high seed purity, but low germinability. Once this dormancy is overcome, the seed is then capable of high germinability.

Three factors affect seed quality: (i) the amount of seed fill in the fruits at harvest; (ii) the amount of inert harvest debris, empty or damaged seeds in the seed batch; and (iii) the duration and conditions of storage. The amount of seed fill at harvest is affected by plant maternal environment, particularly the extent of fertilisation and embryo abortion (Barrett-Lennard et al. 2003). Some of the important chenopods for saltland are dioecious, with male and female flowers occurring on separate bushes, while others are monoecious.

Fertilisation occurs when wind-blown pollen reaches female flowers. If pollination does not occur, fruits develop, but embryos do not. Saltbush fruits ripen over a period of 6-7 months. A proportion of embryos do not develop, with the proportion being higher if plants are under stress (Barrett-Lennard et al. 2003). For example, Strawbridge (1995) found in *A. amnicola* across three different sites that 80-90% of developing fruits contained embryos in early seed development (May-June), but when fruits were ripe in November-December, only 25-45% of fruits contained embryos.

Harvest timing is also critical and is a compromise between maximum seed set and the risk of seed shedding. Seed batches can contain empty florets, immature seed, dormant seed and dead seed in addition to viable seed; these amounts will form a higher proportion of the seed sample if the seed cleaning process is inefficient. Sufficient time is needed post-harvest to overcome any after-ripening seed dormancy issues. This is influenced by seed water content and ambient temperature, while extended lengths of storage under sub-optimal conditions can desiccate and decay seed embryonic tissue.

In general, saltbush seed germination decreases over time (Barrett-Lennard et al. 2003). Therefore, once any seed dormancy issues have been overcome, the freshest seed possible should be used. Storage affects the longevity of seed in varying ways. Beadle (1952) showed that *A. nummularia* seeds were most germinable within four years of age, but eight years after harvest germinability had declined from 92% to 10%, while for *A. vesicaria*, germinability declined to 0% after five years.

In the present study, some experiments were conducted to provide further understanding and quantification of factors limiting seed quality of priority species.

**Materials and methods**

Seed quality was determined in ten seed batches of eight chenopod species acquired from commercial companies and from DAFWA seed increase plots. The commercial seed batches represented samples sold over the counter to farmers.
The process for determining seed quality was as follows. Debris was first separated from fruits by hand. Filled seeds (those with well developed embryos) within the fruits were identified by counting with a Faxitron MX-20 x-ray machine to assess the result of the cleaning process (see Figure 4). Seeds (four replicates of 25) were then incubated in moistened Petri dishes over a 14 day period at 16°C, after which the seed purity of the batch was calculated. Following germination testing, any ungerminated seeds were checked for viability using the tetrazolium staining method described by the International Seed Testing Association (Anon. 1999).

![Figure 4](image1.png)

**Figure 4.** X-ray plates taken with a Faxitron MX-20 showing empty and filled fruits (containing well developed embryos) in the bracts of Atriplex nummularia (left) and A. amnicola (right).

**Results and discussion**

Table 2 summarises the seed quality results. Seed fill percentage of several batches was low, including *A. undulata* (34%), *A. nummularia* (54%) and *A. amnicola* (57% and 66%), while the three *Maireana* and *A. bunburyana* batches all had > 85% seed fill. Germination of the filled fruits was lowest for *M. brevifolia* (36% and 43%) and highest for *M. pyaimidata* (97%). The two *A. amnicola* batches ranged from 55% - 82%, indicating differences within the species. Percentage seed purity was lowest for the *A. undulata* batch (57%), while the *A. amnicola* and *A. nummularia* batches had 75% seed purity.

The x-ray machine was much more successful in identifying fruits filled with well developed embryos than were visual assessments, as empty seeds or seeds encased in bracts or florets often looked the same as filled fruits.

Observations with a range of species suggest that morphological seed properties favouring separation from harvest debris and the threshing ability of seed cleaning equipment play an important role in the viability of cleaned seed. For example, in contrast to chenopods, legume seed batches tend to have a much higher level of purity, as they are easily separated from debris, due to their heavier seed weight and more uniform seed size. The light fruits and encasing of the seed within bracteoles makes chenopod seeds more difficult to separate from debris during threshing.

These factors may contribute to the poor seed purity with many chenopod seed batches.
Table 2. Summary of seed quality attributes of commercial seed batches of 8 chenopod shrub species. Tests were undertaken after a minimum drying period of 14 days at 16°C and 25% relative humidity.

<table>
<thead>
<tr>
<th>Species</th>
<th>% filled fruits(^1)</th>
<th>% germination of filled fruits(^2)</th>
<th>% seed purity by weight of viable seeds(^3)</th>
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<tr>
<td>Atriplex amnicola (a)</td>
<td>57</td>
<td>55</td>
<td>70</td>
</tr>
<tr>
<td>A. amnicola (b)</td>
<td>66</td>
<td>82</td>
<td>77</td>
</tr>
<tr>
<td>A. undulata</td>
<td>34</td>
<td>87</td>
<td>57</td>
</tr>
<tr>
<td>A. nummularia</td>
<td>54</td>
<td>93</td>
<td>71</td>
</tr>
<tr>
<td>A. semibaccata</td>
<td>70</td>
<td>67</td>
<td>79</td>
</tr>
<tr>
<td>A. bunburyana</td>
<td>89</td>
<td>70</td>
<td>90</td>
</tr>
<tr>
<td>A. cinerea</td>
<td>77</td>
<td>89</td>
<td>76</td>
</tr>
<tr>
<td>Maireana brevifolia (a)</td>
<td>86</td>
<td>36</td>
<td>94</td>
</tr>
<tr>
<td>M. brevifolia (b)</td>
<td>91</td>
<td>43</td>
<td>95</td>
</tr>
<tr>
<td>M. pyramidata</td>
<td>86</td>
<td>97</td>
<td>90</td>
</tr>
</tbody>
</table>

\(^1\)Percentage of fruits filled with seed as determined by x-ray analysis

\(^2\)Percent germination after 14 days of fruits screened for >95% filled seed

\(^3\)Seed purity of unscreened sample, denoted by percentage by weight of viable seeds

3.2 Seed dormancy

Following harvest, many species display a period of post-harvest seed dormancy, where seeds are viable but are not able to germinate. High levels of seed dormancy at sowing present a problem for two reasons. Firstly, establishment is likely to be much lower than expected given the seeding rate. Secondly, seeds that later come out of dormancy and germinate on summer-autumn rain are less likely to successfully establish.

Many chenopods exhibit physiological dormancy that is usually relieved over a period of time, whereby seeds undergo biochemical and physiological changes enabling seeds to eventually germinate. Seeds in this state are usually referred to as being in a state of ‘primary innate dormancy’. As seeds fulfil their after-ripening requirement, they then enter conditional dormancy, during which they tend to germinate over a narrow range of environmental conditions. During the progression of dormancy loss, the range of conditions over which the seed will germinate gradually widens, until it is able to germinate over the full range of conditions as dictated by its genotype.

The rate at which dormancy loss occurs has been linked to temperature and moisture conditions experienced by the seed. Non-dormant seeds of some species may re-enter dormancy if environmental conditions remain unfavourable for germination. Such seeds become conditionally dormant then eventually completely dormant. There is little or no information in the literature pertaining to after-ripening and patterns of dormancy for most project species, particularly those of highest priority. An understanding of these characteristics
is important for the seed industry, particularly where storage and germinability of product are concerned.

Research to date indicates that secondary dormancy exists for *M. brevifolia* and *R. preissii*. Seed stocks of these species have been relieved of dormancy through the use of plant signalling agents (see chemical seed enhancement section).

### 3.3 Temperature requirements and optimum sowing time

The interactions of temperature, light and moisture play a critical role in regulating seed germination in the field. A series of experiments was conducted to determine the optimum temperature and light treatments on germination. This information provides a basis to estimate best seasonal “sowing windows” in the field to maximise germination and emergence, particularly for lesser-known species. It also forms the basis for comparing the effects that physical and chemical germination enhancement treatments might have in modifying these “sowing windows”.

**Materials and methods**

Seeds (four replicates of 25) were incubated in Petri dishes with constant temperatures (light/dark) in the range between 5°C and 40°C or with alternating temperatures (12hr/12hr light/dark) of 18/7°C, 26/13°C or 33/18°C. These temperatures represent typical late winter, early to mid spring and late spring to early summer field temperatures, respectively, in southern Australia. Light/dark and dark/dark regimes were only compared at 20°C.

**Results and discussion**

The optimum temperature regimes for germination are presented in Table 3. Also shown are proposed “sowing windows” for each species, based on comparing optimum temperature regimes with typical field temperatures in the central wheatbelt of WA. These “sowing windows” are a guide only and need to be modified for cooler and warmer regions of southern Australia. Sowing times also need to be considered in the context of surviving summer drought and the ability of seedlings to compete with weeds and insects. So, for example, while a species may be suited for germination in spring, growth and biomass production may not be sufficient to enable seedlings to survive summer drought; such species could be sown in autumn to give plants extra time to establish.

*Atriplex nummularia*, *A. undulata* and *A. semibaccata* have germination temperature regimes best suited to late winter to early spring sowing, while *A. amnicola*, *A. bunburyana* and *A. cinerea* appear better suited to spring sowings. *A. cinerea* has equally good germination across alternating and constant temperatures, suggesting this species is well suited to the northern agricultural regions of WA and New South Wales (NSW), with the possibility of expansion into the northern rangelands. Sufficient soil moisture for germination and early growth is also critical in addition to optimum temperatures; late sowing is risky in southern Australia, with establishment success being dependant on good root development prior to onset of the summer drought.

*M. brevifolia* and *M. pyramidata* have higher temperature requirements for germination and appear better suited to germination in late spring and summer. This clearly presents difficulties for direct seeding in much of southern Australia, where summer rainfall is sporadic and unreliable. However, these species could be established more reliably in areas with a higher reliability of summer rainfall, such as northern NSW and Queensland.
Table 3. Constant temperature ranges and alternating temperature gradients for maximum germination percentage (%G) after 14 days for eight chenopod shrub species. The potential sowing window in the central wheatbelt of Western Australia, corresponding to these temperatures, is also shown (in yellow).

<table>
<thead>
<tr>
<th>Species</th>
<th>Optimum temperatures (°C)</th>
<th>Potential sowing window¹</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Constant</td>
<td>Alternating</td>
</tr>
<tr>
<td>Atriplex amnicola</td>
<td>15-25</td>
<td>18/7-26/13</td>
</tr>
<tr>
<td>A. undulata</td>
<td>10-20</td>
<td>26/13-33/18</td>
</tr>
<tr>
<td>A. nummularia</td>
<td>10-20</td>
<td>18/7-26/13</td>
</tr>
<tr>
<td>A. semibaccata</td>
<td>15</td>
<td>18/7-26/13</td>
</tr>
<tr>
<td>A. bunburyana</td>
<td>15-25</td>
<td>18/7</td>
</tr>
<tr>
<td>A. cinerea</td>
<td>20-30</td>
<td>26/13-33/18</td>
</tr>
<tr>
<td>Maireana brevifolia</td>
<td>25-30</td>
<td>26/13-33/18</td>
</tr>
<tr>
<td>M. pyramidata</td>
<td>25-30</td>
<td>26/13-33/18</td>
</tr>
</tbody>
</table>

¹Late sowing risky in southern Australia and dependant on plant available water

3.4 Genotypic differences in A. nummularia for ability to establish from direct seeding

Work conducted by Clive Malcolm in the 1980s demonstrated that some A. amnicola bushes had more seedling recruitment surrounding the mother plant than others (Moore et al. 2006). He also showed that seed derived from such plants had a greater ability to establish from seed and that this was a heritable effect (Malcolm et al. 2003).

Similar differences in the amount of seedling recruitment were observed between bushes of A. nummularia. This led to the hypothesis that genotypic differences occur within A. nummularia for ability to establish from direct seeding and that such plants are derived from bushes that recruit seedlings more readily.

Materials and methods

Seeds of A. nummularia ssp. nummularia were collected from the property of Michael Lloyd at Pingaring, WA. The saltbush stand had been sown 16 years previously. Seeds were collected from three types of bush: (A) bushes from the original sowing with no surrounding seedling recruits (Mature non-recruiter); (B) bushes from the original sowing with a high density of seedling recruits surrounding them (Mature recruiter); and (C) young plants in close proximity to mature recruiters, which were presumably derived from type B bushes (Immature recruiters). This is shown diagrammatically in Figure 5. Seeds were collected on three plants of each type on 28 February 2009.

Two glasshouse experiments were conducted to determine whether seeds from bushes with high recruitment levels established more readily when sown into soil. These were conducted to confirm that emergence differences were seed related and not confounded by preferential predation and grazing or different soil types at the collection site.
In both experiments, fruits were first checked for seed fill using the Faxitron MX-20 x-ray machine and any unfilled seeds were removed from the sample by hand. Following germination testing, any ungerminated seeds were checked for viability using the tetrazolium staining method described by the International Seed Testing Association (Anon. 1999). Only confirmed viable seeds were used for analyses.

Experiment 1 aimed to examine emergence of seedlings from seeds collected from the three different types (A, B and C) of bush. Three bushes of each type were tested (nine treatments in total). Twenty-five bracted seeds from each bush were planted to a depth of 5 mm in free-draining 150 mm diameter pots filled with non-saline sand and patted down firmly. The experiment contained four replicates. Pots were watered daily. Seedling emergence was counted every seven days for 42 days. Counts were adjusted for viability.

Experiment 2 examined the results from the first experiment more closely. Two contrasting types were used, an Emergent type (designated Bush I) and a Semi-emergent type (designated Bush G) (Table 4). For this experiment only confirmed viable seeds were used. Four replicates of 25 bracted seeds were sown into two soil types (sand and loam). Seeds were sown in 150 mm diameter free-draining plastic pots to a depth of 5-7 mm and patted down firmly. Pots were watered daily. Emergence was scored every seven days over a 21 day period.

**Results and discussion**

For Experiment 1, germination percentage after 35 days is shown in Table 4 (also Figure 6). Bushes were categorised as being “Emergent” if their germination of viable seeds was >75%, “Semi-emergent” if their germination was 25-74% of viable seeds and “Non-emergent” if their germination was <25% of viable seeds.

The results indicate that the recruiting pattern of genotypes of *A. nummularia* do not necessarily indicate high germinability when sown at a depth of 5 mm. Some individual genotypes, such as those from Bush I (an Emergent type), had high viability and good germinability, while others, such as those from Bushes A and D (Non-emergent types), had high viability and low germinability. Notably, the ability to recruit seedlings had no influence on their ability to emerge from sowing. For example, Bush B (an Emergent type) was classed as a mature non-recruiter but had 100% germination. Furthermore, when germination percentages were averaged within bush types, the mature non-recruiter, mature recruiter, and immature recruiter types had germination values of 69%, 56% and 67%, respectively (data not shown).
Table 4. Percentage of viable seed and germination percentage with standard errors 35 days after sowing of seedlings derived from different Atriplex nummularia bushes, when sown at 5 mm depth (mean of 4 replicates).

<table>
<thead>
<tr>
<th>Bush No</th>
<th>Bush type</th>
<th>% viability</th>
<th>% germination</th>
<th>Emergence type</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Mature non-recruiter</td>
<td>44%</td>
<td>15 ± 2.5</td>
<td>Non-emergent</td>
</tr>
<tr>
<td>B</td>
<td>Mature non-recruiter</td>
<td>52%</td>
<td>100 ± 1.9</td>
<td>Emergent</td>
</tr>
<tr>
<td>C</td>
<td>Mature non-recruiter</td>
<td>72%</td>
<td>86 ± 1.2</td>
<td>Emergent</td>
</tr>
<tr>
<td>D</td>
<td>Mature recruiter</td>
<td>28%</td>
<td>13 ± 2.0</td>
<td>Non-emergent</td>
</tr>
<tr>
<td>E</td>
<td>Mature recruiter</td>
<td>48%</td>
<td>42 ± 2.0</td>
<td>Semi-emergent</td>
</tr>
<tr>
<td>F</td>
<td>Mature recruiter</td>
<td>56%</td>
<td>87 ± 9.0</td>
<td>Emergent</td>
</tr>
<tr>
<td>G</td>
<td>Immature recruiter</td>
<td>72%</td>
<td>55 ± 4.1</td>
<td>Semi-emergent</td>
</tr>
<tr>
<td>H</td>
<td>Immature recruiter</td>
<td>52%</td>
<td>79 ± 1.9</td>
<td>Emergent</td>
</tr>
<tr>
<td>I</td>
<td>Immature recruiter</td>
<td>72%</td>
<td>100 ± 4.3</td>
<td>Emergent</td>
</tr>
</tbody>
</table>

Figure 6. Glasshouse pot experiment showing differences in emergence of Atriplex nummularia seedlings derived from different bushes from Pingaring, WA
In Experiment 2 the Emergent type (Bush I) had markedly higher emergence 21 days after sowing than the Semi-emergent type (Bush G) in both soils (Figure 7), confirming the results of Experiment 1. There was a soil type effect for the Emergent type, with an emergence rate in the loamy soil of almost 80%, compared to 38% in sandy soil. This difference was not seen in the Non-emergent type.

These results show that the likely germination success of seed lots cannot be predicted based on observations of plant recruitment around mother plants in the field. Rather, actual measurements of establishment from individual seed lots are required. However, multiple clones of individual parent plants can be made from cuttings (Barrett-Lennard et al. 2003). This provides potential opportunities for commercial seed merchants (working with researchers) to develop their own propriety saltbush lines, selected for high establishment by: (a) identifying combinations of male and female plants that produce germinable seed; (b) cloning the two parent plants involved (the female will be known, the male will be from a nearby pollen source); and (c) establishing seed production nurseries using this material.

Figure 7. Percent emergence after 21 days in either a sand or loamy soil of an Emergent (Bush I) and Semi-emergent (Bush G) genotype of A. nummularia grown in the glasshouse.

3.5 Germination response to temperature in types with different abilities to emerge

Differences in emergence responses between seeds from A. nummularia Bush I and Bush G could be explained by differences in their temperature requirements for germination. Therefore, the relationship between temperature and germination was examined in both genotypes.

Materials and methods

Four replicates of 25 naked seeds of each genotype were plated into 90 mm vented Petri dishes containing 0.6% water agar solution, impregnated with 0.1% plant preservation mixture. Petri plates were then sealed with plastic wrap and placed in alternating 12/12 hour temperature cabinets set to 20/10°C, 26/13°C and 35/20°C under a diurnal light/dark regime. Germination was scored every second day over a 14 day incubation period.
Results and discussion

There were no major differences in germination percentage between the Emergent and Semi-emergent *A. nummularia* genotypes in the 20/10°C and 26/13°C temperature regimes (Figure 8). However, a marked difference was found at 35/20°C, with the Semi-emergent type having a much lower germination percentage than the Emergent type. At this temperature regime, germination percentage of the Emergent type was also significantly less that at 20/10°C. This result suggests that emergent saltbushes may be better at germinating under high temperatures that occur on some days in spring.

![Figure 8. Percent germination after 14 days of Emergent and Semi-emergent types of *A. nummularia* in the laboratory under three different alternating temperature regimes.](image)

3.6 The role of bracteoles in germination

Seeds of most Chenopod species are surrounded by lignified bracts, which can vary from being small and light in *Atriplex nummularia* to heavily lignified (woody) in *Maireana pyramidata* (see Figure 1). It is believed these bracteoles play an ecological role by regulating the timing of germination and aiding seed dispersal (Ungar and Khan 2001). Stevens *et al.* (2006) found bracteoles retard germination of *A. nummularia*, *A. undulata* and *A. amnicola* seed in laboratory studies. However, while debracted seed of *A. amnicola* germinated and survived better under field conditions, bracted seed of *A. nummularia* performed better.

Two genotypes of *A. nummularia*, obtained from the property of Michael Lloyd at Pingaring, WA, were examined to better understand the role of bracteoles. One of these (an Emergent type) was obtained from Bush H, that was found to have high emergence rates in the glasshouse when sown at 5 mm depth. The other (a Non-emergent type) was from Bush A, which was found to have low emergence rates in the glasshouse. This provided a unique opportunity to compare seed and bract properties between plants from the same environment to gain an understanding of the factors responsible for high emergence and establishment in bracted and naked seed.

Laboratory experiments were conducted to examine germination following bract removal to repeat the results of Stevens *et al.* (2006) in other chenopod species.
**Materials and methods**

Bracts from most Chenopod species were removed using a Kimseed scarifier set at 1.6 - 2.2 mm plate width. This method was effective at removing bracts without damaging the seed. *Maireana brevifolia* bracts, however, were removed by hand rubbing on a rubber mat, due to fragility of the seeds. De-bracted seeds were separated from debris using sieves and a vacuum aspirator.

Bracted seeds with high viability were selected for experiments using a Faxitron MX-20 x-ray machine to ensure seeds within bracts had fully developed embryos. Treatments 1 and 2 consisted of bracted and de-bracted seeds plated onto Petri dishes lined with glass filter papers. The germination medium for Treatment 3 used bracts from individual species ground to <500 µm, autoclaved and spread evenly to a 0.5 mm depth. This treatment was designed to investigate the presence of germination-inhibiting allelopathic compounds in the bract material. All treatments were conducted under an 18/7°C 12/12 hour regime temperature under dark conditions.

Germination, defined as 1 mm radicle emergence followed by continued growth, was scored regularly over a 14-day incubation duration. Final germination percentage and germination rate index was calculated using Equations 1 and 2.

**Final germination % at \( t \) = \[ \frac{\sum G_t}{r} \times 100 \] (1)**

Where: \( t \) = incubation period (days)

\( G \) = total germinated seeds across treatment Petri dishes

\( r \) = number of replicates

**Germination rate index (GRI) = \[ \sum \frac{(D_i - D_{i-1})}{i} \] \right) (2)**

Where: \( I \) = is the germination count day

\( D_i \) = the percentage of seeds germinated at time “i”

\( D_{i-1} \) is the percentage of seeds adjudged germinated the previous count day (from Maguire, 1962)
Results and discussion

Table 5. Effect of de-bracting chenopod seeds on percentage germination and germination rate index (GRI) after 14 days in Petri dishes. All de-bracted seed treatments were significantly different (P<0.01) to controls.

<table>
<thead>
<tr>
<th>Species</th>
<th>Bracted seed (control)</th>
<th>De-bracted seed (additional % compared to control)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% germination</td>
<td>GRI (% germination/day)</td>
</tr>
<tr>
<td>Atriplex amnicola</td>
<td>24</td>
<td>3</td>
</tr>
<tr>
<td>A. nummularia</td>
<td>62</td>
<td>9</td>
</tr>
<tr>
<td>A. undulata</td>
<td>56</td>
<td>6</td>
</tr>
<tr>
<td>A. semibaccata</td>
<td>49</td>
<td>9</td>
</tr>
<tr>
<td>A. bunburyana</td>
<td>72</td>
<td>14</td>
</tr>
<tr>
<td>A. cinerea</td>
<td>6</td>
<td>0.5</td>
</tr>
<tr>
<td>Maireana brevifolia</td>
<td>17</td>
<td>7</td>
</tr>
<tr>
<td>M. pyramidata</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

For most Chenopod species, de-bracted seed substantially enhanced both germination percentage and germination rate under laboratory conditions (Table 5). De-bracting appeared to be a necessity for reliable germination of Maireana pyramidata and Atriplex cinerea, but had only a small germination enhancing effect on A. bunburyana. The results for A. amnicola, A. undulata and A. nummularia, were similar to the laboratory results of Stevens et al. (2006).

3.7 Emergence of bracted and de-bracted seed of Emergent and Non-emergent A. nummularia types

Field evidence in Section 4.5 suggests naked (debracted) Atriplex seeds tend to establish better than bracted seed in lower areas of the landscape, where there is greater soil moisture. This suggests that bracteoles may regulate seed germination through either: (i) physical properties, such as their ability to hold water, regulate water uptake or buffer moisture loss during drying; or (ii) osmotic adjustment, due to the presence of salts. Underpinning germination is the requirement for seeds to reach a critical seed moisture content, in order for germination to proceed. Factors responsible for a seed within a bracteole having a reduced capacity to reach the critical seed water content could be: (i) bracteole structure impeding the flow of moisture; (ii) seed testa properties presenting a moisture barrier; or (iii) potential osmolytic effects from salts that may be present in the bracteoles.

This section examines different moisture conditions on germination of bracted and debracted A. nummularia types and the role of bracteoles on imbibition of water. A general discussion integrating the results of Section 3.7 follows.
3.7.1 Germination under moist and dry conditions

Materials and methods

Seeds of the Emergent and Non-emergent A. nummularia types were dried at 16°C, 25% relative humidity for seven days and then separated from inert material with a vacuum aspirator. Bracted seed was x-rayed using the Faxitron MX-20 x-ray machine and any unfilled seeds were removed from the sample by hand. Following germination testing, any ungerminated seeds were checked for viability using the tetrazolium staining method described by the International Seed Testing Association (Anon. 1999). Only confirmed viable seeds were used for analyses. For each genotype, one batch of seeds (naked seeds) was de-bracted gently using a serradella dehuller and separated from inert material using a vacuum aspirator. The other batch had intact bracts. The germinability of A. nummularia seed sources was compared with a single source of A. amnicola seed (either naked or bracted).

Two groups of 48 pots (150 mm diameter) were set up in an open area at Kings Park between April 23 and May 4, when average temperatures were 27.6/13.5°C (Bureau of Meteorology 2009). Half the pots were filled with loam and the other half filled with sand. In each pot 30 naked or bracted seeds of the Emergent or Non-emergent A. nummularia types or of naked or bracted A. amnicola seeds were planted to a depth of 5 mm. Four replicates of each species per moisture treatment were used and these were randomly positioned within the treatment area. Pots allocated to the “wet” treatment were watered daily while pots allocated to the “dry” treatment were watered once every five days. A moisture probe (MPM-160-B, ITC international) was used to measure pot soil moisture contents. Five random pots containing sand and eight of loam from wet and dry irrigation treatments were selected weekly and measured at 10:00 am. The number of emerged seedlings was scored twice weekly for 21 days, with a further measurement after 30 days.

A laboratory control was set up using four replicates of Petri dishes for bracted and naked seeds of both genotypes of A. nummularia and for A. amnicola. For each replicate, either 25 bleached naked or bracted seeds were placed on filter paper (Whatmans No. 1) with 8 mm of tissue culture water. The naked and bracted seeds were bleached for 7 and 10 minutes, respectively, before being plated. The plates were incubated at 26/13°C on a diurnal light/dark regime (Contherm 6000 CP), and the number of seedlings which germinated was recorded every two days until germination ceased. Germination was defined as emergence of a 1 mm root radicle, provided that growth continued.

Data were analysed by one-way Analysis of Variance (ANOVA), unpaired t-tests and the Tukey test at a significance level of α = 0.05 using Microsoft Excel 2007 and GenStat version 11. Percentages and averages are presented with standard errors.

Results

On average the water content of the dry pots was 14.0 ± 0.72%, compared to the wet pots with 18.9 ± 0.39%. The wet pots filled with loam had a significantly higher water content (19.7 ± 0.68%) than the pots filled with sand (16.3 ± 0.58%) (t = 0.042).

Figures 9 and 10 show that bracted seeds of A. amnicola had a lower emergence rate than naked seeds in both soil types and moisture treatments. However, the emergence rate was higher in the loam than the sand for both the bracted (t < 0.01) and naked seeds (t < 0.01). Within the loam, there was no significant difference between the dry and moist treatments for either bracted (t = 0.38) or naked seeds (t = 0.07), nor was there a significant difference between the moist and wet treatments for bracted (t = 0.18) and naked seeds (t = 0.754) in the sand.
Overall, bracted seeds of *A. nummularia* had a lower emergence rate than naked seeds (Figures 9 and 10). The exception to this was bracted seeds of the Non-emergent type, which had a 35% greater emergence rate than naked seeds in the moist treatment in loamy soil (Figure 10). Furthermore, bracted seeds of the Emergent type had a higher emergence rate in the sand than the loam (t <0.001). There was no statistical difference between the emergence of naked seeds of the Emergent type and either bracted or naked seeds of the Non-emergent type in the two soils. There were also no significant differences between the emergence of naked or bracted seeds in the dry or moist treatments, apart from: (i) bracted seeds of the Non-emergent type, which had a higher emergence rate in the moist loam than the dry loam (t = 0.039); and (ii) the dry and moist sand treatments for both the bracted (t <0.001) and naked (t =0.02) seeds of the Non-emergent type.

Naked seeds of *A. amnicola* and both *A. nummularia* types also had a greater germination rate than bracted seeds in the laboratory control (Figure 11). The bracted seeds of *A. amnicola* had the highest germination rate of all species.

![Figure 9](image_url)

**Figure 9.** The percentage and standard error of seedlings which emerged in sand in moist and dry conditions over a period of 14 days. Pots were planted with either naked (open symbols) or bracted (closed symbols) seeds of either Emergent *A. nummularia* (squares), Non-emergent *A. nummularia* (triangles), or *A. amnicola* (circles).
Figure 10. The percentage and standard error of seedlings which emerged in moist and dry loamy soil over a period of 14 days. Pots were planted with either naked (open symbols) or bracted (closed symbols) seeds of either Emergent A. nummularia (squares), Non-emergent A. nummularia (triangles) or A. amnicola (circles).

Figure 11. Germination percentage and standard error after 14 days in Petri dishes under laboratory conditions of bracted or de-bracted seeds of A. amnicola and A. nummularia types A (Non-emergent) and H (Emergent). Treatments with the same letter are not significantly different (P=0.05).
3.7.2 Imbibition of Emergent and Non-emergent A. nummularia types

**Materials and methods**

Seeds were prepared as in Section 3.6.1. Six groups of three petri dishes were plated with 10 bracted seeds of the two A. nummularia types, while another two groups were plated with 10 naked seeds per plate. All plates were lined with filter paper (Whatmans No. 1) to which 8 mL of tissue culture water was added. Plates were incubated at 20/10°C on a light/dark regime (Contherm 6000 CP). Bracted seeds were removed from the plates at 24, 48 and 72 hours after being imbibed. The seeds were then separated from the bracteoles and both were weighed. Both the bracteoles and seeds were dried at 107°C for 24 hours and re-weighed to determine the overall water content. Seeds from the naked seed treatment were weighed at 1, 2, 4, 8, 24, 48 and 72 hours after being imbibed; these were returned to Petri dishes after weighing. After 72 hours, the seeds were dried at 107°C for 24 hours and re-weighed to determine water content. Data were analysed as in Section 3.6.1.

**Results**

Overall, bracteoles of Emergent and Non-emergent A. nummularia types had higher water contents than both naked seeds and seeds enclosed within the bracteole, while naked seeds also tended to contain more moisture than those enclosed by bracteoles (Figure 11). Bracteoles of the Emergent type had a significantly higher water content than the Non-emergent type ($t = 0.02$). Seeds of the Emergent type enclosed within bracteoles also had higher water contents than the Non-emergent type after 24 hours of imbibition, but there were no significant differences between their water contents after 48 and 72 hours ($t = 0.26$ and $t = 0.52$, respectively). No significant differences were found between the water content of the two types of naked seeds ($t = 0.202$).

The percentage seed water content needed for germination was approximately 42% (Figure 12). Naked seeds germinated after approximately 20 hours. This was more rapid than seeds enclosed within bracteoles, which began to germinate after approximately 48 hours for the Non-emergent type and after about 72 hours for the Emergent type.

![Figure 12](image-url)  
**Figure 12.** Percent water content and standard error of bracteoles (triangles), naked seeds (squares) and seeds enclosed by bracteoles (circles) over time after soaking in water. The imbibition times for both Emergent (open symbols) and Non-emergent (closed symbols) A. nummularia types are shown, along with the critical seed moisture content for germination (42% water content).
3.7.3 Elutes of A. nummularia bracteoles

Materials and methods

Seeds were prepared as in Section 3.6.1. Five grams of bracteoles of both the Emergent and Non-emergent A. nummularia types were crushed to a particle size of less than 500 μm, then soaked in 100 mL of tissue culture water for 24 hours. The solutes were filtered to remove particulates. The salt concentration of both elutes was measured using an EC meter and the molarity of both elutes calculated, assuming the salt was NaCl. Three replicates of 20 seeds of both A. nummularia types were placed on Petri dishes layered with filter paper (Whatmans No. 1). Each seed type was subjected to 10 mL of the two elutes and the plates incubated at 20/10°C on a diurnal light/dark regime (Contherm 6000 CP). The number of seeds germinated was recorded every two days until no further germination took place. Data were analysed as in 3.6.1.

Results

Salt concentrations in the elutes of the bracts of the Emergent and Non-emergent genotypes were similar, with the Emergent type containing 2.780 ppk of salt (47.57 mM if salt is NaCl) and the Non-emergent type containing 2.783 ppk (47.63 mM if salt is NaCl). The emergence rate of Emergent type seeds was higher than Non-emergent type seeds with both elutes (Figure 13). Furthermore, seeds of the Emergent type had a higher germination percentage in the Emergent type elute than in the Non-emergent type solution (t = 0.02), but there were no differences for the Non-emergent type seeds that germinated in the two elutes (t = 0.374).

![Figure 13. Germination percentage and standard error after 14 days in Petri dishes under laboratory conditions of seeds from A. nummularia Bush A (Non-emergent) and Bush H (Emergent) germinated in elutes made from Bush A and Bush H bracteoles. Treatments with the same letter are not significantly different (P=0.05).](image_url)

General discussion - Emergence of bracted and de-bracted seed of Emergent and Non-emergent types

Bracted seeds of A. amnicola and A. nummularia tend to have a lower emergence rate than de-bracted seeds. This suggests that bracteoles either contain an inhibiting factor that retards germination or they need to absorb additional moisture to allow seeds to germinate. There also
appear to be genotypic differences within *A. nummularia* for ability to emerge from within bracteoles, with bracted seeds of the Non-emergent type having a higher emergence percentage than naked seeds in the moist loam treatments. This may be because these bracteoles did not need to absorb as much water as those of the Emergent type before germination occurred.

Seeds of *A. amnicola* and the Non-emergent *A. nummularia* type had the same emergence rates in moist and dry soil, while the Emergent *A. nummularia* type had a higher emergence percentage in the moist soil. This suggests that Emergent type seeds require a higher moisture content than Non-emergent type seeds before germination can occur.

Bracteoles of *A. nummularia* appear to prolong the time taken for seeds to absorb enough water to germinate. This is suggested by the more rapid germination of naked seeds in the imbibition experiment. It appears that bracteoles need a water content of ~75% before the seed water content is high enough (~42% water content) to enable germination to proceed. The results also suggest a difference between the two types of *A. nummularia*, as the Non-emergent type required less moisture within the bracteole than the Emergent type for seeds to germinate. However, the water content of Non-emergent type bracted seeds declined after 48 hours, indicating this type of *A. nummularia* may be more drought-prone than the Emergent type. These results suggest it is unlikely that differences in the testas of the seeds are responsible for the differences in germination between the two types of *A. nummularia*, as there were no differences in water content of their naked seeds. Instead it appears that a physical property of the bracteole is responsible for reducing the seed imbibition in bracted seed of the Emergent type.

Salt does not appear to be responsible for retarding germination in *A. nummularia*. This is because there was no difference between the salt concentrations in the two types of bracteoles, a result supported by experiments conducted by Stevens *et al.* (2006). Furthermore, the elute germination experiment suggests that chemical properties of *A. nummularia* do not affect the germination of Non-emergent type seeds. The small difference between the percentage of Emergent type seeds that germinated in the Emergent and Non-emergent type elutes may have been due to a structural property of the bracteole that retards germination, as it was observed that Emergent type bracteoles were more uniform in size and shape than Non-emergent type bracteoles.

In conclusion, bracted seeds of *A. amnicola* and *A. nummularia* tend to have a lower germination rate in the glasshouse than naked seeds in both loamy and sandy soils in dry and moist conditions, suggesting that the bracteoles have a role in regulating germination. It appears that bracteoles of *A. nummularia* need to have a moisture content of ~75% before the seeds inside can absorb enough moisture to germinate. As a result, it takes longer for bracted seeds to germinate than naked seeds. However, this may have an advantage under field conditions, as bracteoles of *A. nummularia* will only allow the seeds to germinate under optimal conditions, hence increasing the chance of seedling survival. Future research is needed to identify structural or chemical properties of the bracteole such as size, pH, the presence of hydrophobic chemicals/waxes and the presence of allelopathic substances which may be responsible for germination differences between Emergent and Non-emergent types of *A. nummularia*. 


3.8 Seed priming to enhance germination

The germinability of a seed is regulated by its seed dormancy state and interactions with temperature, moisture and light. Priming (pre-germinating) seeds with water or germination stimulants has been successfully used for a range of species with plant signalling chemicals including karrikinolide (Flematti et al., 2004), gibberellic acid (GA₃), benzoic acid (Senaratna et al., 2003), and kinetin and salicylic acid (Senaratna et al., 2000). Kinetin is a cytokinin, a class of plant hormone that promotes cell division, shoot and root morphogenesis, chloroplast maturation, cell enlargement and auxiliary bud release and senescence. The function of this plant hormone requires auxins to be present. In contrast, salicylic acid is an inducer of systemic acquired resistance (SAR) to diseases in plants (Raskin 1992; Conrath et al. 1995) and is known to interact with plant respiration (Bourbouloux et al. 1998) and protein synthesis (Jin et al. 2000). Work by Dat et al. (1998) and Senaratna et al. (2003) shows that the use of salicylic acid increases thermo tolerance in seedlings. The mode of action of these chemicals during germination is unknown. However, combined with other plant signalling chemicals, both kinetin and salicylic acid may provide additional support to improving germination, emergence and plant establishment.

Stevens et al. (2006) found GA₃ at 1000 ppm improved germination of A. amnicola under laboratory conditions and resulted in a four-fold increase in seedling emergence in the field. Priming with water significantly increased emergence percentage of A. amnicola, but had no effect on A. nummularia or A. undulata. Salicylic acid and kinetin improved the rate of emergence of all three species at various levels of salinity, while GA₃ also improved germination of A. undulata.

A series of experiments was conducted to examine whether plant signalling compounds could improve germination and emergence of the chenoopod species A. semibaccata, A. bunburyana, M. brevifolia and R. preissii, particularly when physiological dormancy was active. Their effectiveness at different moisture levels was also examined.

3.8.1 Efficacy of plant signaling chemicals in the laboratory at critical moisture stress

Materials and methods

Seeds of all species used in these experiments were cleaned of inert material and dried for a minimum of 14 days at 16°C and 25% relative humidity. Seed batches for all experiments had >98% viability, which was confirmed using the tetrazolium staining method described by the International Seed Testing Association (Anon. 1999).

In order to test the efficacy of plant signalling chemicals, the critical moisture stress level for germination was calculated for each species. Critical moisture stress for seed germination was defined as the water potential where germination levels are 75% of maximum germination. The effect of moisture stress on germination was investigated by supplementing germination media with polyethylene glycol (PEG, molecular weight 8000), equating to the solute potentials of 0, -0.25, -0.5, -0.75, -1, -1.25, -1.5 and -2 MPa. The osmotic potentials were calculated by equation 3, where x is the concentration of PEG8000 by %w/v (Michel and Kaufmann 1973).

\[ \Psi_{\text{PEG8000}} (\text{MPa}) = -7.60x^2 - 33.03x + 4.83 \]  \hspace{1cm} (3)

Four replicates of 25 seeds of each species were plated into 90 mm vented Petri dishes containing a filter paper with 8 mL of each solute potential. Petri dishes were subsequently sealed with plastic to retard evaporation and placed into a 20°C 12/12 hour light/dark constant incubator (TLMRIL model, Thermoline, QLD, Australia). This temperature was chosen as the osmolytic regression in equation (1) is correlated with temperature. Germination, defined as 1 mm radicle emergence followed by continued growth, was scored regularly over a 14-day
incubation duration. Final germination percentage and germination rate index was calculated using equations 1 and 2 in Section 3.5. All germination data were arcsine-transformed and an ANOVA was conducted to compare the significance between treatments. Regressions and their equations in relation to the osmolytic gradient were plotted using CurveExpert 1.4. These equations were used to determine the critical moisture stress for each species at 20°C.

Once the critical moisture stress levels had been determined, the effects of seven plant signalling agents and various combinations of them on improving germination percentage and germination rate were investigated for each species (Table 6). Seeds were primed in treatments for 18 hours, extracted, rinsed under water and patted dry with a paper towel. Primed seeds were left to dry for two days at 22°C and 55% relative humidity, then transferred to a 16°C, 25% relative humidity room to dry for a further four days. Four replicates of 25 seeds of each treatment were plated into 90 mm vented Petri dishes containing a filter paper, to which 8 mL of the critical moisture stress solute potential for each species was added. Controls consisted of treated seed plated into Petri dishes with filter paper containing only water. Petri dishes were subsequently sealed with plastic to retard evaporation and placed into a 20°C dark constant incubator (TLMRIL model, Thermoline, QLD, Australia).

Table 6. Germination enhancing chemicals used in experiments and their concentrations.

<table>
<thead>
<tr>
<th>Seed enhancing agent</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gibberellic acid (GA₃)</td>
<td>0.28 mM</td>
</tr>
<tr>
<td>Smoke water (SW)</td>
<td>1%</td>
</tr>
<tr>
<td>Ethylene as ethaphon (Eth)</td>
<td>20 mM</td>
</tr>
<tr>
<td>Karrikinolide (formerly Butenolide) (KAR₁)</td>
<td>0.67 µM</td>
</tr>
<tr>
<td>Potassium nitrate (KNO₃)</td>
<td>0.30 M</td>
</tr>
<tr>
<td>Kinetin (K)</td>
<td>0.05 mM</td>
</tr>
<tr>
<td>Salicylic acid (SA)</td>
<td>0.5 mM</td>
</tr>
</tbody>
</table>

Germination was scored regularly over a 14-day incubation duration. Final germination percentage and germination rate index across treatments were calculated using equations 2 and 3. All germination data was arcsine-transformed and an ANOVA was conducted to compare significance between treatments. This enabled identification of optimum plant signalling chemical combinations (PSCC) for glasshouse and field trials.
Results

Table 7. Optimal plant signalling chemical combinations for improved germination of four chenopod species, using moisture stress to differentiate control (no treatment) and priming treatments. Degrees of significance between control and priming treatments are denoted by asterisks where * = P <0.05, **= P <0.01 and *** = P <0.001. Standard errors are in parenthesis.

<table>
<thead>
<tr>
<th>Species</th>
<th>Best treatmenta</th>
<th>% germination after 14 days at CMSb</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>Atriplex semibaccata</td>
<td>GA₃+SA</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>A. bunburyana</td>
<td>KAR₁+SA</td>
<td>6 (1.3)</td>
</tr>
<tr>
<td>Maireana brevifolia</td>
<td>GA₃+SA+K</td>
<td>2 (0.4)</td>
</tr>
<tr>
<td>Rhagodia preissii</td>
<td>GA₃+SA</td>
<td>4 (1.9)</td>
</tr>
</tbody>
</table>

aGA₃ = gibberellic acid (0.28 mM), SA = salicylic acid (0.5 mM), KAR₁ = karrikinolide (0.67 µM), K = kinetin (0.05 mM)
bCritical moisture stress, defined as the water potential that retards germination by 75% of maximum germination

Seven plant signalling chemical combinations were identified to be significantly effective in improving germination, using moisture stress to differentiate treatments (Table 7). These were GA₃+SA for A. semibaccata, KAR₁+SA for A. bunburyana, GA₃+K+SA for M. brevifolia and GA₃+SA+K for R. preissii. Under critical moisture stress conditions, the respective treatments improved germination in A. semibaccata from 0% to 20%, in A. bunburyana from 6% to 24%, in R. preissii from 4% to 29% and to a lesser extent in M. brevifolia from 2% to 11% (Table 7).

3.8.2 Efficacy of plant signalling chemicals in glasshouse and field conditions

Materials and methods

Three sets of treated seeds of A. semibaccata, A. bunburyana, M. brevifolia and R. preissii were prepared, one each for glasshouse and field trials and one for laboratory controls.

The following treatments were prepared: (i) priming with H₂O; (ii) priming with the optimum chemical signalling chemical combination for each species (shown in Table 7); and (iii) no treatment (control).

Field trials were sown on 18 September 2008 into a free-draining, sandy soil at the University of Western Australia Shenton Park Field Station. Prior to seeding, the site was rotary hoed and received two applications of glyphosate at 2 L/ha (a.i. 680 g/L), three and two weeks prior to sowing. The site also received 150 kg/ha NPK fertiliser four weeks prior to sowing. Seeds were hand-sown into 1 m long rows, 15 cm apart, to a depth of 7-9 mm. The trial had a Latin square design with four replicates. Pyrethrin was applied one week post-sowing for insect control. Plots were scored for emergence weekly for 30 days, then fortnightly over a 120 day period.

The laboratory controls were run on 10 September 2008 using the laboratory germination methodology described in previous sections. An alternating 26/13°C 12/12 hour temperature
regime was used and seeds were plated on 0.6% water agar impregnated with 0.1% plant preservation mixture. Treatments were incubated in the dark.

Glasshouse trials were sown on 21 May 2009 at 5-7 mm depth into 150 mm diameter pots containing free-draining white sand to a 140 mm depth. Four replicates of 50 seeds of each treatment were used. An alternating 27/15°C 12/12 hour temperature regime was used. Pots were irrigated daily and emergence was scored weekly over a 120 day period.

**Results**

Under the laboratory conditions, plant signalling chemical combinations significantly increased germination in *A. bunburyana*, *M. brevifolia* and *R. preissii* (Table 8). Under these conditions, *M. brevifoli* and *R. preissii* displayed very strong physiological dormancy, which was overcome by the plant signalling chemical treatments. Under glasshouse conditions, chemical combinations again improved emergence significantly after 30 days in *M. brevifolia* and *R. preissii*. In this environment *A. semibaccata* also had increased germination, but *A. bunburyana* did not. However, there was a general lack of transfer of the successful laboratory and glasshouse treatments to the field environment, with only *R. preissii* having significantly greater emergence than the untreated control (Table 8). *A. bunburyana* and *M. brevifolia* failed to emerge over the trial duration.

**Table 8.** Percent germination in the laboratory after 14 days and percent emergence in the glasshouse and field after 30 days of seeds primed with water or primed with the optimum plant signalling chemical combination (PSCC) shown in Table 7, compared with untreated controls. Standard errors are shown in parentheses. Within each environment, PSCC treatments within a species that differ significantly (*P* <0.05) from controls are denoted by an asterisk (*).

<table>
<thead>
<tr>
<th>Species</th>
<th>Laboratory</th>
<th>Glasshouse</th>
<th>Field</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>H₂O</td>
<td>PSCC</td>
</tr>
<tr>
<td><em>A. semibaccata</em></td>
<td>32 (4.0)</td>
<td>53 (9.6)</td>
<td>100 (0.0)*</td>
</tr>
<tr>
<td><em>A. bunburyana</em></td>
<td>12 (3.0)</td>
<td>27 (4.9)</td>
<td>39 (5.5)*</td>
</tr>
<tr>
<td><em>M. brevifolia</em></td>
<td>8 (0.0)</td>
<td>5 (2.7)</td>
<td>37 (5.3)*</td>
</tr>
<tr>
<td><em>R. preissii</em></td>
<td>16 (4.6)</td>
<td>24 (8.0)</td>
<td>95 (3.5)*</td>
</tr>
</tbody>
</table>

**Discussion**

Under laboratory and glasshouse conditions plant signalling chemical combinations improved the germination and emergence of Chenopod species. However, there was a general lack of translation of this benefit to field emergence, except in species with significant physiological dormancy issues associated with the seed at the time of treatment. These results suggest that whilst the use of plant signalling chemicals are useful in improving germination percentage, rate and germination tolerance to moisture stress, there needs to be a greater understanding of the biological and environmental factors that reduce this benefit under field conditions.

The efficacy of plant signalling chemicals in glasshouse and field conditions could be dictated by several factors: chemical dependencies, seed dormancy status and stability of the chemicals to different temperatures. The use of kinetin may be an example of a chemical dependency. Here the efficacy of salicylic acid and kinetin to improve germination under
moisture stress in laboratory conditions was a common theme across species. However, the use of kinetin in combination did not always work. Kinetin, a cytokinin, induces cell division but requires auxin to be present in order for it to be effective and the ratio of auxin to cytokinin is crucial during cell division (Mok and Mok, 1994). Auxins in plant tissues promote the production of ethylene, which along with GA$_3$, interacts with light as a cue for germination. It is noteworthy that A. semibacatta and R. preissii did not require the presence of kinetin to benefit from priming and that both species have strong requirements for light in order to germinate. The efficacy of kinetin in species that did not respond significantly to the chemical may be due to a lack of auxins in their seed.

The efficacy of chemicals to enhance germination and emergence can be affected by the dormancy status of seeds at the time of treatment. For example, the efficacy of GA$_3$ as a plant signalling chemical for several Chenopod species appears to depend on the after-ripening status of their seeds (Hilhorst and Karssen, 1992). Gibberellic acids (GA) are not directly responsible for breaking down dormancy, but in combination with after-ripening relief, their inclusion aids the development of a GA-responsive system. GA biosynthesis in seeds occurs during seed imbibition and functions independently from dormancy (Karssen and Lacka, 1986). For example, the R. preissii seed in this experiment was four months-old, and likely to have been in a state of after-ripening. The priming combination of GA$_3$ and salicylic acid produced a higher germination percentage of R. preissii, even under field conditions. Low germination in the laboratory of untreated seed showed it to be highly dormant, but highly responsive to GA$_3$.

The potential to observe chemical hydro-priming benefits on seed germination and emergence is dependent on sampling frequency, experimental duration and optimal temperatures for germination and emergence. For many non-dormant species, the priming benefit is largely due to increases in the rate of germination and emergence. Hence, benefits from these chemicals occur within the first few days after imbibition and differences between priming and control treatments diminish with time.

Both A. bunburyana and M. brevifolia failed to emerge throughout the course of the field trial. Both species performed poorly under glasshouse conditions as well, with low emergence rates compared with germination percentages in laboratory controls. Previous trials of both species in irrigated plots at South Perth also performed poorly. In that trial however, considerable mortality (23%) in M. brevifolia occurred in the first four weeks, while no emergence was recorded in the same species at Shenton Park. This indicates that high moisture contents are required for germination of M. brevifolia in sandy soils.

These experiments suggest field application of chemical hydro-priming appears feasible in R. preissii seed when it is highly dormant. While this appears to benefit emergence in such species, carryover to plant establishment and first year survival remains unresolved. The use of this enabling technology has the potential to improve emergence, but the lack of translation to the field environment suggests that there are other variables related to priming that need to be understood. Future research should focus on developing an understanding of seed and chemical integrity after priming and how this relates to the seedbed environment.
While there are numerous reports on the effects of chemical priming on seed germination in the literature, very little emphasis has been placed on post-treatment handling (i.e. drying and storage) of treated seeds. This is a considerable oversight, as the method of drying and storage of primed seeds affects the degree and longevity of priming benefits and seed viability. Knowledge of these effects is particularly important if priming technology is to be used by the seed industry, as appropriate post-treatment seed handling methods will need to be implemented to optimise treatment benefits.

**Materials and methods**

*A. nummularia* and *A. amnicola* were used in the first experiments, due to their positive response to priming treatments. Seeds were debracted and osmo-primed (-1.0 MPa, PEG 8000), with the addition of GA₃ (0.28 mM), kinetin (0.05 mM) and salicylic acid (0.5 mM), for 24 hrs. Seeds were washed and bench dried for one hour before being placed into drying chambers at 20°C. Three relative humidity (RH) chambers containing solutions of LiCl were used to give equilibrated 30, 50 and 80% RH levels. Seeds were stored for 18 days with the following drying treatments:

(i) 18 days at 30% RH;
(ii) six days at 50% RH, then 12 days at 30% RH; and
(iii) six days at 80% RH, then six days at 50% RH, then six days at 30% RH.

Seeds were sampled and germination tested at 18/7°C dark/dark on days 0, 1, 18 and 36 following treatment.

**Results and discussion**

Slow drying rates retained priming benefits longer for both *A. nummularia* and *A. amnicola*, with the slowest drying rate having greatest effect (Figure 14). These results indicate that at 20 °C storage, the priming benefits of the moderate drying rate treatment (six days at 50% RH, followed by 12 days at 30% RH) tend to last for about 25 days in *A. nummularia* and 30 days in *A. amnicola*. The slow drying treatment (six days at 80%, 6 days at 50% RH and 6 days at 30% RH) increased this to about 36 days in *A. nummularia* and more than 36 days in *A. amnicola*. Priming benefits were quickly lost with rapid drying at 30% RH. In *A. nummularia*, rapid drying also resulting in significantly reduced germination after seven days.

These results have important implications for post-treatment handling of seeds primed with plant signalling chemicals. Rapid drying of fully hydrated seeds after priming may weaken or even rupture intracellular membranes in embryonic tissue, thus lessening seed shelf life.
Figure 14. Germination percentage over time in *Atriplex nummularia* (A) and *A. amnicola* (B), following osmo-priming and drying under different relative humidity regimes.
4 AGRONOMY STUDIES

4.1 Bracted vs unbracted seed – glasshouse and field emergence

Laboratory studies in Section 3.5 (Table 5) and from Stevens et al. (2006) showed that removing bracts from the seed substantially enhanced both germination percentage and germination rate of most Chenopod species. Two experiments were initiated to see if these results could be translated to improved emergence in small field plots and pots in the glasshouse.

4.1.1 Bracted and de-bracted seed of A. amnicola and A. nummularia

The first experiment involved bracted and de-bracted seed of A. amnicola and A. nummularia in the glasshouse.

Results

Glasshouse results showed that A. nummularia and A. amnicola differed in their ability to emerge in the de-bracted state (Figures 15-17). Under such conditions de-bracted A. amnicola seeds had significantly higher emergence than bracted seeds (Figure 15) – a similar result to that obtained under laboratory conditions (Section 3.5). In A. nummularia, however, the emergence of bracted seeds was significantly higher than de-bracted seeds, a result at odds with those from the laboratory. This result was examined further in field plots.

Figure 15. Seedling emergence of bracted (+) and de-bracted (-) Atriplex amnicola and A. nummularia 22 days after sowing into pots in the glasshouse.
Figure 16. Emergence of bracted and de-bracted fruits of Atriplex amnicola

Figure 17. Emergence of bracted and de-bracted fruits of Atriplex nummularia
4.1.2 Bracted and de-bracted seed of A. cinerea, M. brevifolia and M. pyramidata

This experiment involved comparing the emergence of bracted and de-bracted seeds of A. cinerea, M. brevifolia and M. pyramidata in the field with germination in the laboratory.

Materials and methods

Field trials were sown on 23 October 2008 into a free-draining sandy soil at the University of Western Australia Shenton Park Field Station. Prior to seeding, the site was rotary hoed and received two applications of glyphosate at 2 L/ha a.i. 680 g/L, three and two weeks prior to sowing. The site also received 150 kg/ha NPK fertiliser four weeks prior to sowing. Seeds were hand-sown into 1 m long rows, 15 cm apart, to a depth of 7-9 mm. The trial had a Latin square design with four replicates. Pyrethrin was applied one week post-sowing for insect control. Plots were scored for emergence weekly for 30 days.

The laboratory controls were run on 10 September 2008 using the laboratory germination methodology described in previous sections. An alternating 26/13°C 12/12 hour temperature regime was used and seeds were plated on 0.6% water agar impregnated with 0.1% plant preservation mixture. Treatments were incubated in the dark. Germinating seedlings were scored twice weekly for 30 days.

Results and discussion

Under laboratory conditions, de-bracting markedly increased germination percentage of each species and increased the germination rate index of A. cinerea and M. pyramidata (Table 9). Without de-bracting no germination occurred for M. pyramidata, while only 1% of seeds germinated of A. cinerea.

However, the results for seedling emergence in the field trial were quite different to the laboratory germination results (Table 9). Emergence of bracted M. pyramidata was significantly higher than for de-bracted seed, in direct contrast to the laboratory results, where no germination occurred of bracted seeds. There were no differences between emergence of bracted and de-bracted seeds for A. cinerea or M. brevifolia in the field. Clearly other factors operate in the field to regulate seed germination. The role of bracts in these species requires further investigation.

Table 9. Field emergence and laboratory germination over 30 days following sowing. GRI = germination rate index (% germination/day) and ERI = emergence rate index (% seedlings/day). Values in parenthesis represent +/- one standard error.

<table>
<thead>
<tr>
<th>Species</th>
<th>Treatment</th>
<th>Laboratory</th>
<th>Field</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>% germination</td>
<td>GRI</td>
</tr>
<tr>
<td>A. cinerea</td>
<td>Bracted</td>
<td>1.0 (1.0)</td>
<td>0.1 (0.1)</td>
</tr>
<tr>
<td></td>
<td>De-bracted</td>
<td>88.0 (3.6)*</td>
<td>5.7 (0.3)*</td>
</tr>
<tr>
<td>M. brevifolia</td>
<td>Bracted</td>
<td>21.5 (3.0)</td>
<td>0.8 (0.1)</td>
</tr>
<tr>
<td></td>
<td>De-bracted</td>
<td>38.5 (5.4)*</td>
<td>2.0 (0.5)</td>
</tr>
<tr>
<td>M. pyramidata</td>
<td>Bracted</td>
<td>0.0 (0.0)</td>
<td>0.0 (0.0)</td>
</tr>
<tr>
<td></td>
<td>De-bracted</td>
<td>93.0 (1.0)*</td>
<td>14.7 (0.2)*</td>
</tr>
</tbody>
</table>

* denotes significant differences (P<0.05) between treatments within species.
4.2 Seeding depth

A small plot trial was conducted to examine the effect of seeding depth on emergence of *A. nummularia*. Population differences in ability to establish from sowing at different depths were also examined.

**Materials and methods**

The site consisted of a sandy soil at DAFWA headquarters in South Perth, WA. Three seed sources were compared: seed collected from *A. nummularia* bushes on properties at Lake Grace and Tincurrin, WA, and a commercial batch of seed purchased in 2008.

Each plot comprised a row of 400 mm length, with furrows formed by hand to a depth of either 5 mm or 20 mm, into which 50 germinable seeds were sown and filled in by sand. The trial consisted of four replicates arranged in a randomised complete block design, giving a total of 24 plots. The trial was sown on 29 October 2008. Plots were irrigated by overhead sprinklers daily. Emerged seedlings were counted weekly for seven weeks until December 8. Measurements were made on the percentage of viable seeds, which were determined using the tetrazolium staining method described by the International Seed Testing Association (Anon. 1999).

**Results and discussion**

Some seeds of each population emerged from a 5 mm sowing depth, but no seeds established from 20 mm (Figure 18). The other interesting observation was an apparent difference in ability to establish from a 5 mm sowing depth between different *A. nummularia* seed sources (Figure 18). This difference was upheld, even when differences in seed viability were taken into account. These results contrast with a nursery experiment by Vlahos (1997), who found that covering fruits of *A. amnicola* with 2 mm and 5 mm of soil decreased germination by 50% and 95%, respectively.

**Figure 18.** Percentage germination (of viable seeds) of three populations of *A. nummularia*, following sowing at a depth of either 5 mm or 20 mm in the field at South Perth. Populations A and B represent seed collected from bushes at Lake Grace and Tincurrin, respectively, while population C was commercially purchased seed.
4.3 Machinery configuration for sowing A. nummularia and A. amnicola

A trial was sown to determine the most appropriate seeding point configuration for direct sowing A. amnicola and A. nummularia. The use of depth wheels for sowing depth precision was also investigated.

Materials and methods

Field experiments were conducted on a mildly saline, sandyloam site at Meckering, WA, sown on 14 August 2007. The site was sprayed with glyphosate at 2 L/ha a.i. 680 g/L on 18 July 2007 and again one day prior to sowing. The insecticide Dominex® was also applied with the later spraying.

Chemically primed and de-bracted seeds of either A. nummularia or A. amnicola were sown, with sowing rates adjusted to 50 germinable seeds per metre (calculated from prior germination tests). Subsequent examination of the A. nummularia seed showed it to belong to subsp. nummularia.

Three different sowing tyne treatments were compared:

(i) t-boot knife points (TKP), which form a negligible furrow;
(ii) 8"-wide scarifying points cut in half, which enable placement of seeds on the side of furrows; and
(iii) 8"-wide scarifying points, which place seeds in the bottom of 50 -70 mm deep furrows

The wide point tynes in treatment (iii) were similar to what would often be used by a seeding combine. Trailing press wheels were used in each treatment. Depth wheels were also used in treatments (i) and (ii), but not in treatment (iii). Tynes were attached to an experimental cone seeder and changed for different sowing runs.

The trial contained four replicates arranged in a randomised block design. Seed was sown to an approximate depth of 5 mm into two 5 m rows spaced 1.1 m apart, with 2 m bare ground buffers in between. Monthly establishment counts were conducted on emerged seedlings for the first three months, with a final count conducted six months after sowing.

Results and discussion

Rainfall and temperatures at Meckering are shown in Figure 19. Soil moisture conditions were favourable for germination. Seeds were sown into moist soil and 58 mm of rain fell over the following six weeks (until 30 September), with a further 23 mm in October (Figure 19). However, only 47 mm fell over the five months between 1 November and 30 March.

Establishment of A. nummularia was favoured by sowing with knife points rather than with seed placed in furrows (Figure 20). Seedling counts on 6 September (23 days after sowing) showed that the t-boots resulted in 3.7 seedlings/m, whereas the half wide point and wide point (minus depth wheels) configurations had 1.2 and 0.1 seedlings/m, respectively. However, there was a decline in plant survival over summer in the rows sown by t-boots, while more plants emerged in late summer in rows sown by the wide point tynes, so that by the end of summer, establishment differences were much smaller.
Establishment densities of *A. amnicola* were much lower than for *A. nummularia*, indicating that its establishment in the field is more difficult than suggested from glasshouse results. The knife points and half wide point configurations gave the best initial establishment (Figure 21). However, late emergence during summer was again observed in rows sown by the wide point tyne, so that by the end of summer there were no differences in establishment density between tyne treatments.

Overall, the treatments involving a depth wheel had higher seedling emergence for both species. It is likely that the lower emergence from the wide point tynes was at least partly due to insufficient control of sowing depth, as this treatment did not have a depth wheel, with seeds being placed too deep or too shallow.

Even though initial emergence was low, the formation of a furrow appears to have had some benefits for over-summer seedling survival. This is most likely attributable to its better ability to harvest rainfall for use by seedlings.

A possible constraint for *A. amnicola* is a requirement for warmer soil temperatures than those present in mid August at Meckering. *A. amnicola* originates from areas north of the agricultural zone, where winter temperatures are warmer. (The requirement of *A. amnicola* for warmer temperatures at germination was confirmed by the laboratory results in Section 3.1.) Later sowing times may be more successful, if sufficient soil moisture is present. More northerly sites, with warmer soil temperatures, may also lead to greater establishment success.
Figure 20. Established seedlings/m of row from four counts over time with A. nummularia, following sowing with t-boots, half-wide scarifying points and wide scarifying points (with no depth wheel).

Figure 21. Established seedlings/m from four counts over time with A. amnicola, following sowing with t-boots, half-wide scarifying points and wide scarifying points (with no depth wheel).

This trial demonstrated for the first time, the possibility of successful direct seeding of A. nummularia in the field using conventional farm machinery (Figure 22).
4.4 Seed treatments to enhance establishment of *Atriplex nummularia* and *A. amnicola*

The aim of this experiment was to determine whether seed enabling treatments that were successful in enhancing germination of *A. nummularia* and *A. amnicola* in the laboratory and glasshouse could be transferred to a mildly saline field site.

**Materials and methods**

This field experiment was conducted adjacent to the experiment on the mildly saline site at Meckering, WA described in Section 4.2 and site preparation was the same. The trial was sown on 14 August 2007. Rainfall and maximum and minimum temperatures at Meckering are shown in Figure 19.

Seeds of *A. nummularia* ssp. *nummularia* and *A. amnicola* were either bracted or de-bracted (naked). These were subjected to three different priming treatments:

(i) primed with water and a combination of gibberellic acid (GA$_3$), kinetin (K) and salicylic acid (SA) at the rates shown in Table 6

(ii) primed with water alone

(iii) not primed

Seeds were primed in treatments for 18 hours, extracted, rinsed under water and patted dry with a paper towel. Primed seeds were then left to bench dry for two days at 22°C, 55% relative humidity, then transferred to a 16°C, 25% relative humidity room to further dry for another four days.

The trial contained four replicates arranged in a randomised block design and consisted of 48 plots divided into four banks of 12. Seed was sown using half 8”-wide scarifying points (HWP) to an approximate depth of 5 mm into two 5 m rows spaced 1.1 m apart, with 2 m buffers. Sowing rates were adjusted to 50 germinable seeds per metre (calculated from prior
germination tests). Establishment counts were conducted on emerged seedlings 39 days after sowing. Plant heights were measured 70 days after sowing.

**Results and discussion**

Bracted seeds of *A. nummularia* had significantly higher seedling emergence across all treatments than de-bracted seeds (Figure 23). Seedling counts 39 days after sowing showed that of the bracted seed treatments, chemically primed seeds had the best overall emergence, with 16.4 seedlings/m, while un-primed and water primed seeds had 11 and 10 seedlings per metre, respectively. All de-bracted seeds had poor emergence (≤ 2 seedlings/m), although there was a small but non-significant response to water and chemical priming. There was no significant effect of the priming chemicals on plant height 70 days after sowing (Figure 23).

These results show that bract removal is detrimental to *A. nummularia* in the field, supporting the observations of Stevens *et al.* (2006) and the glasshouse results in Section 4.1.1.

**Figure 23.** The effect of chemical priming (gibberellic acid, salicylic acid and kinetin), water priming and un-primed seed on the emergence 39 days after sowing and height (mm) 70 days after sowing bracted (+) and de-bracted (-) Atriplex nummularia seed at Meckering, WA on 14 August 2007.

Seedling emergence of *A. amnicola* was much lower than *A. nummularia* for all treatments (Figure 24), reflecting the results of Section 4.2. Bracted seeds had a higher emergence than de-bracted seeds for each priming treatment, in contrast to the results of Stevens *et al.* (2006) and the glasshouse results in Section 4.1.1. These results suggest bract removal inhibits germination of *A. amnicola* in the field but enhances it in the laboratory or glasshouse. The reason for this discrepancy is not known.

Water-primed *A. amnicola* seeds had the highest seedling emergence per metre (1.5 seedlings/m), but there was no stimulus provided by the chemical priming (Figure 23). Chemical priming had a small positive effect on plant height 70 days after sowing for bracted seeds, but there was no difference for naked seeds (Figure 24).
Figure 24. The effect of chemical priming (gibberellic acid, salicylic acid, and kinetin), water priming and un-primed seed on the emergence 39 days after sowing and height (mm) 70 days after sowing bracted (+) and de-bracted (-) Atriplex amnicola seed at Meckering WA on 14 August 2007.

4.5 Seed treatments to enhance establishment of A. undulata and M. brevifolia

The aim of this experiment was to determine whether seed enabling treatments in the laboratory and glasshouse that were successful in enhancing germination of A. undulata and M. brevifolia could be transferred to a mildly saline field site.

Materials and methods

This field experiment was conducted adjacent to the experiment on the mildly saline site at Meckering, WA described in Section 4.2 and site preparation was the same. The trial was sown on 14 August 2007. Rainfall and maximum and minimum temperatures at Meckering are shown in Figure 19.

Three seed treatments were compared:

(i) un-primed bracted seed;
(ii) un-primed de-bracted (naked) seed; and
(iii) de-bracted seed chemically primed with a combination of gibberellic acid (GA$_3$), kinetin (K) and salicylic acid (SA) at the rates shown in Table 6

The trial contained four replicates arranged in a randomised block design and consisted of 24 plots divided into four banks of six. Seed was sown using half 8"-wide scarifying points (HWP) to an approximate depth of 5 mm into two 5 m rows spaced 1.1 m apart, with 2 m buffers. Sowing rates were adjusted to 50 germinable seeds per metre (calculated from prior germination tests). Establishment counts were conducted on emerged seedlings at monthly intervals for three months.
Results and discussion

Seedling emergence of both species was poor, particularly for *M. brevifolia* (Figures 25 and 26). Initial seedling counts were higher for bracted seed of *A. undulata*, conforming to field results for *A. nummularia* and *A. amnicola*, and conflicting with the laboratory results of Stevens *et al.* (2006).

![Figure 25. Seedling emergence from three counts over time of bracted (+) and de-bracted (-) un-primed seed and chemically primed de-bracted seed of *Atriplex undulata* sown at Meckering, WA on 14 August 2007.](image1)

![Figure 26. Seedling emergence from three counts over time of bracted (+) and de-bracted (-) un-primed seed and chemically primed de-bracted seed of *Maireana brevifolia* sown at Meckering, WA on 14 August 2007.](image2)
The low emergence rates for both species, indicates that a much greater understanding of their environmental requirements for germination is required before direct seeding can be contemplated. A major constraint, particularly for *M. brevifolia*, is likely to have been a requirement for warmer soil temperatures. This trial was sown in mid August, whereas the laboratory studies in Section 3.1 indicate *M. brevifolia* is more likely to germinate and establish after summer rains. Later sowing times and the use of other sowing configurations, such as t-boots, need to be further investigated for these species.

4.6 **The effect of salinity and waterlogging on *A. nummularia* and *A. amnicola* seedling emergence**

A trial was sown along a salinity transect at the Meckering site to determine the effect of salinity and waterlogging on the emergence of *A. nummularia* and *A. amnicola* seedlings. The effect of seeding point configuration on seedling emergence at different locations along the transect was also investigated.

**Materials and methods**

The site was located at Meckering within 100 m of the experiments described in Sections 4.2-4.4, but was located lower in the landscape. The site consisted of a 16 m saline transect, ranging from low salinity levels at the point of highest elevation to moderate levels at the point of lowest elevation. An EM38 survey was conducted on 16 October. ECₖ values were derived from a calibration curve between EM38 readings (horizontal orientation) and are presented in Figure 27. The soil also contained more clay than in the other experiments and soil moisture was higher, particularly at the lower end of the transect. The trial was sown on 14 August 2007 and site preparation was the same as described in Sections 4.2-4.4. Rainfall and maximum and minimum temperatures at Meckering are shown in Figure 19.

![Figure 27](image-url)  

Figure 27. ECₖ values (dS/m), estimated from a calibrated EM38 survey on 16 October 2007 along a salinity gradient at Meckering, WA.
De-bracted seeds of *A. nummularia* subsp. *nummularia* and *A. amnicola* that had been chemically primed with a combination of gibberellic acid (GA₃), kinetin (K) and salicylic acid (SA) at the rates shown in Table 6 were used.

Plots were sown on 14 August 2007 with a cone seeder using two sowing treatments:

(i) half 8"-wide scarifying points (HWP); and
(ii) t-boot knife points (TKP).

**Results and discussion**

The half wide point configuration gave a better establishment than the knife points, in contrast to the results in Section 4.2. One possible reason for this is that the soil in this experiment was more compact and did not have the same degree of furrow collapse (with consequent burial of seed) as in the sandier site. Furthermore, at this site the t-boots could not break into the soil, resulting in a much less favourable seed bed for germination.

Establishment of *A. nummularia* (Figure 28) and *A. amnicola* (Figure 29) did not appear to be affected by salinity, although it is important to note that most of the salt had been leached from the soil surface by the time of sowing. Of interest was the observation that plant numbers in this experiment were higher than in the adjacent experiment, which accumulated less moisture. For instance, *A. amnicola* and *A. nummularia* had seedling emergence of 3.8 and 6.3 plants/m, respectively, in the wetter transect area, compared to only 1 plant/m in the drier area of the adjacent trial. This supports laboratory results in Sections 3.5 and 3.6 that suggest bracts may act as a “moisture sponge” to prevent seeds drying out during the germination process.

![Graph](image-url)  
**Figure 28.** The effect of salinity on the germination of *Atriplex nummularia* seed (de-bracted and chemically primed) sown by either 1/2 wide point or t-boots sowing configurations at Meckering, WA. The trial was sown on 14 August 2007 and measurements were taken on 16 October (70 days after sowing).
Figure 29. The effect of salinity on the germination of Atriplex amnicola seed (de-bracted and chemically primed) sown by either 1/2 wide point or t-boots sowing configurations at Meckering, WA. The trial was sown on 14 August 2007 and measurements were taken on 16 October (70 days after sowing).
5 PROOF OF CONCEPT DIRECT SEEDING TRIALS

The 2007 trial produced some very promising results, suggesting for the first time that direct seeding of *Atriplex nummularia* was feasible with conventional farm machinery, especially on a mildly saline, sandy duplex soil. However, the direct seeding of *A. amnicola*, *A. undulata* and *Maireana brevifolia* resulted in low establishment.

With these results in mind, it was decided to test the direct seeding of these species on other soil types and in different regions to further develop the establishment package. In particular, sites in the warmer regions of the agricultural areas of WA were used to investigate if warmer temperatures might have a bearing on establishment.

5.1 Success at Morawa

A trial was sown at Morawa in the north-eastern agricultural region of WA. This site was chosen to represent the effect of warmer soil temperatures on chenopod establishment than in the 2007-sown Meckering trial. The effect of a different soil type was also examined.

**Materials and methods**

The site was located near Morawa on a red loamy soil. Establishment of *Atriplex nummularia*, *A. amnicola*, *Maireana brevifolia* and *Rhogodia preissii* was examined. The *A. nummularia* seed was subsequently found to be of subsp. *nummularia*.

Chemically primed seeds of *A. nummularia*, *A. amnicola*, *M. brevifolia* and *R. preissii* were sown with their bracts intact, in contrast to de-bracted seeds sown in the 2007 field trial. Sowing rates varied from 100 germinable seeds per metre for *A. nummularia* to 300 for *R. preessii* (calculated from prior germination tests) (see Table 10).

The trial was sown into moist soil on 21 July 2008 using a cone seeder with t-boot knife points, in combination with depth wheels. Trailing press wheels pressed seeds against the soil. The site was sprayed with 2 L/ha glyphosate on 20 June and again on the day of sowing. The insecticide Dominex® was also applied two days after sowing. Temperature probes were placed 1 cm beneath the soil surface to measure soil temperatures.

The trial contained four replicates arranged in a randomised block design. Seeds were sown to a depth of 5 mm into plots consisting of two 5 m rows, spaced 1.1 m apart and separated by 2 m bare ground buffers. Emerged seedlings were counted 8 weeks after sowing.

**Results and discussion**

The site received good rainfall through August (Figure 30), which provided ideal moisture conditions for germination.

Establishment rate of the *A. nummularia* seedlings was 49% of that expected on the basis of the rate of germinable seed sown (Table 10, Figure 32). This confirmed that the 2007 success with direct seeding could be repeated on a loamy soil type.

Establishment of *Rhogodia presseii* was also successful, with 23% of germinable seeds establishing as seedlings (Table 10, Figure 33). This was the first time that *R. presseii* had been successfully direct seeded, and demonstrated the feasibility of sowing this species with conventional farm machinery.
However, the *A. amnicola* and *M. brevifolia* sowings again resulted in poor establishment (Table 10). This was despite mean maximum soil temperatures of the Morawa site at 1 cm depth being 2-5 °C warmer than the other sites (Figure 31). These results demonstrate that further information is needed on the constraints for direct seeding *A. amnicola* and *M. brevifolia*.

Figure 30. Rainfall (mm) received at Dumbleyung, Meckering, Marchegee and Morawa, WA in 2008 between 20 July and 12 September. Arrows show time of sowing at each site.

Figure 31. Maximum soil temperature (°C) measured 1 cm below the surface at Dumbleyung, Meckering, Marchegee and Morawa, WA in 2008 between 20 July and 12 September.
Figure 32. Successful establishment of Atriplex nummularia at Morawa, WA following direct seeding

Figure 33. The first successful establishment by direct seeding of Rhagodia preissii at Morawa, WA
Table 10. Germination percentage, seeding rate, expected germination and observed establishment densities 8 weeks after sowing of A. nummularia, A. amnicola, M. brevifolia and R. presseii sown at Morawa, WA on 21 July 2008. The percentage of seedlings emerged to that expected is shown in parentheses.

<table>
<thead>
<tr>
<th>Species</th>
<th>Germination (%)</th>
<th>Seeding rate (seeds/m)</th>
<th>Expected germination (seeds/m)</th>
<th>Observed seedlings/m</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. nummularia</td>
<td>39%</td>
<td>100</td>
<td>39</td>
<td>19 (49%)</td>
</tr>
<tr>
<td>A. amnicola</td>
<td>22%</td>
<td>130</td>
<td>29</td>
<td>2 (7%)</td>
</tr>
<tr>
<td>M. brevifolia</td>
<td>50%</td>
<td>150</td>
<td>75</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>R. presseii</td>
<td>50%</td>
<td>300</td>
<td>150</td>
<td>34 (23%)</td>
</tr>
</tbody>
</table>

5.2 Effect of different seed sources on establishment of A. nummularia

Three additional field trials were sown in 2008 to test proof of concept of direct seeding A. nummularia using conventional seeding machinery on different soil types and locations.

Materials and methods

Sites were sown at Meckering (adjacent to the 2007 experiments described in Section 4.2), at Dumbleyung (350 km south-west of Perth) and Marchegee (300 km NNE of Perth). The Dumbleyung site consisted of a mildly saline grey loam clay, while the Marchegee site was a brown loam. Daily rainfall at each site between mid-July and mid-September of 2008 is shown in Figure 30, while maximum soil temperatures 1 cm below the surface are shown in Figure 31.

A different seed source of A. nummularia to that sown at Meckering in 2007 and Morawa in 2008 was used. This was subsequently found to belong to subsp. spathulata. The seed also had low germinability. Even after retaining the heaviest 30% of fruits it only had 10% germinability.

Three seed treatments were examined:

(i) Bracted unprimed seed (graded to retain the heaviest 30% of seed)

(ii) Bracted unprimed seed (ungraded for seed weight)

(iii) Bracted seed (graded to retain the heaviest 30% of seed) and primed with 0.05 mM of kinetin

Two different seeding point configurations (t-boot seeding knife points and half 8”-wide scarifying points) were also examined for each seed treatment. These were sown in combination with depth wheels for accurate seed placement, with trailing press wheels.

The trial contained four replicates arranged in a randomised block design. Seed was sown to a depth of 5 mm into two 8 m rows spaced 1.1 m apart, with 2 m bare ground buffers in between. Sowing rates of the graded seeds were adjusted to give a target of approximately 20 germinable seeds per metre.

Trials were sown at Marchegee on 21 July, Dumbleyung on 25 August and Meckering on 26 August. Sites were sprayed six weeks prior to sowing with 2 L/ha glyphosate for weed control and again on the day of sowing. The insecticide Dominex® was also applied with the later spraying. Emerged seedlings were counted eight weeks after sowing.
Results and discussion

No seedlings of *A. nummularia* emerged at Dumbleyung, Meckering or Marchegee for any of the seed treatments or sowing configurations. The sites were moist at the time of sowing and conditions for germination appeared to be reasonable, apart from Dumbleyung, which received little rainfall for the three weeks post-sowing (Figure 30). Rainfall conditions for Marchegee were very similar to those at the successful Morawa site. Thus, it appears that poor moisture conditions for germination and establishment were not the main reasons for the seeding failures at Dumbleyung, Meckering and Marchegee.

The greatest difference between the sites appeared to have been the source of *A. nummularia* seeds. Subsequent examination of the seeds sown at Dumbleyung, Meckering and Marchegee showed them to belong to subsp. *spathulata*. This compared with subsp. *nummularia* for the seeds sown at Morawa in 2008 and at Meckering in 2007. Thus, it appears there are subspecies differences within *A. nummularia* for ability to establish from direct seeding in August-September, with subsp. *nummularia* apparently being much better suited.
Refining the establishment package for *A. nummularia*

Previous experiments at Meckering in 2007 and Morawa in 2008, described in Section 5, showed that *A. nummularia* could be successfully established from seeds using conventional seeding equipment. Related trials also suggested subspecies differences in ability to establish from seeds, with subsp. *spathulata* being more difficult to establish from seed than subsp. *nummularia*. Other glasshouse and laboratory experiments described in Section 3.4 showed genotype differences within ssp. *nummularia* for ability to establish. Laboratory experiments, described in Section 3.7, also showed that germination could be stimulated by priming seeds with chemical signalling compounds, such as kinetin, although this had not previously been translated successfully to the field.

Two trials in different environments and soil types were sown in 2009 to confirm previous findings and to further refine the direct seeding package for *A. nummularia*. The following hypotheses were tested:

1. *A. nummularia* can be successfully established from seed using conventional seeding machinery
2. The use of priming with the chemical signalling compound, kinetin, enhances germination in the field
3. Subspecies *nummularia* can be more readily established from seed than ssp. *spathulata*
4. Genotypes of subsp. *nummularia* differ in their ability to establish from seed

**Materials and methods**

Four *A. nummularia* populations were compared with two seed treatments: (i) primed with kinetin; and (ii) unprimed. Three of the populations were of subsp. *nummularia*, with the fourth being a commercial seed source of subsp. *spathulata*. Two genotypes of subsp. *nummularia* were obtained from the property of Michael Lloyd at Pingaring, WA. One of these (Emergent type) was obtained from a bush (Bush H) that was observed to have a high density of seedling recruits surrounding it, which was subsequently found to have high establishment rates in the glasshouse. The other (Non-emergent type) was from a bush (Bush A) with no seedling recruits, which was found to have low establishment rates in the glasshouse. Further details of the Emergent and Non-emergent types are given in Section 3.4. The third subsp. *nummularia* genotype was a commercial source of cv. De Koch.

Seeds with intact bracts were separated from any debris with a vacuum aspirator and x-rayed using a Faxitron MX-20 x-ray machine. Any seeds lacking embryos were removed from the sample by hand. The remaining seeds were checked for viability using the tetrazolium staining method described by the International Seed Testing Association (Anon. 1999). Only confirmed viable seeds were used for experiments.

Primed seeds were soaked in 0.05 mM kinetin for 18 hours, extracted, rinsed under water and patted dry with a paper towel. Seeds were then left to bench dry for two days at 22°C and 55% relative humidity and were then transferred to a 16°C, 25% relative humidity room to further dry for another four days.

Trials were sown on a well-drained loamy soil 15 km east of Mingenew (29°13’02"S, 115°35’40"E) and on a saline, waterlogged, sandy loam 13 km south of Wagin (33°25’35"S, 114°34’32"E).
117°22'35"E). Weed control consisted of a double knockdown with glyphosate at 2 L/ha (a.i. 680 g/L) on 17 July and 19 August at Mingenew, and 3 August and 31 August at Wagin.

Plots consisted of 8 m single rows, into which 200 seeds of each treatment were sown unto uncultivated soil using an experimental cone seeder. Treatments were replicated four times in a randomised block design. Seeds were sown 5-10 mm below the surface into 3 cm deep furrows. Depth wheels were used to ensure precise seed placement. Seeds were pressed into the soil surface with press wheels. Sowing dates in 2009 were 27 August at Mingenew and 7 September at Wagin. The Mingenew site was sprayed with Talstar and Lorsban on 3 September, while the Wagin site was sprayed with Talstar on 14 September.

Seedling counts were conducted at Mingenew on 29 September (34 days after sowing) and at Wagin on 7 October (30 days after sowing). Analyses of Variance were conducted on treatment means to determine whether establishment densities differed between genotypes and priming treatments.

Results and discussion

Monthly rainfall totals and mean maximum and minimum temperatures from 1 July 2009 to 30 June 2010 are shown for Wagin in Figure 34 and for Mingenew in Figure 35. Germination conditions were very favourable at Wagin. The soil surface was very moist at sowing and remained moist until mid-October, with 51 mm of rain falling in the five weeks after sowing (Figure 34). Conditions for germination were less favourable at Mingenew. At the time of sowing, the soil was dry in the top 2 cm, but seeds were placed into moisture in the bottom of furrows (and pressed into moisture with press wheels). However, only 34 mm of rain fell in the five weeks after sowing and the soil surface was intermittently dry during this period, as temperatures increased rapidly (Figure 35).

Figure 34. Monthly rainfall totals and mean maximum and minimum temperatures from 1 July 2009 to 30 June 2010 at Wagin, WA (Data from the Australian Bureau of Meteorology).
Figure 35. Monthly rainfall totals and mean maximum and minimum temperatures from 1 July 2009 to 30 June 2010 at Mingenew, WA (Data from the Australian Bureau of Meteorology).

Photographs of the Wagin and Mingenew sites are shown in Figures 36 and 37, respectively. Seedling counts per metre of row are shown for Wagin in Table 11 and for Mingenew in Table 12. Establishment was much higher at Wagin. Significant differences occurred between saltbush populations at both sites, with the Emergent type having significantly higher plant numbers than the Non-emergent type. Subspecies *spathulata* also had very low establishment at both sites. There were no significant effects of priming, and no significant genotype x priming interactions at either site.

These results confirm that *A. nummularia* can be established by direct seeding using conventional seeding equipment. A precise sowing depth of 5-10 mm appears to be critical for success. Use of kinetin as a seed priming agent appears to be less important.

These trials confirmed the unsuitability of ssp. *spathulata* for direct sowing. An extremely low proportion of seedlings emerged, in spite of them having high viability. The reason for the large difference between subspecies in ability to establish from direct seeding is unknown and requires further investigation to better understand the mechanisms.

These results confirm previous glasshouse indications of genotypic differences within subsp. *nummularia* for ability to establish from seed, with the Emergent type having markedly higher emergence. Seed of the Emergent type has been forwarded to the Future Farm Industries CRC saltbush breeder, for inclusion of ability to establish from seed as a breeding objective. These results also suggest that a wider examination within and between *A. nummularia* populations is likely to find even larger differences for ability to establish from direct seeding.

Establishment densities of the best treatment (Un-primed, Emergent type) at Wagin resulted in more than 4 plants/m (Table 11). This is clearly denser than the 1-2 plants/m required for long-term stands and indicates that the sowing rate was higher than required. This implies that sowing rates for this seed batch could have been reduced to around 10 seeds/m for a satisfactory establishment.
Table 11. Seedlings per metre of Atriplex nummularia populations with and without seed priming with 0.05 mM kinetin at Wagin, WA

<table>
<thead>
<tr>
<th>Population</th>
<th>Primed</th>
<th>Un-primed</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>de Koch</td>
<td>0.91</td>
<td>1.19</td>
<td>1.05</td>
</tr>
<tr>
<td>Emergent type</td>
<td>2.34</td>
<td>4.19</td>
<td>0.37</td>
</tr>
<tr>
<td>Non-emergent type</td>
<td>2.25</td>
<td>1.66</td>
<td>1.95</td>
</tr>
<tr>
<td>ssp. spathulata</td>
<td>0.00</td>
<td>0.16</td>
<td>0.08</td>
</tr>
<tr>
<td>Mean</td>
<td>1.38</td>
<td>1.80</td>
<td>1.59</td>
</tr>
</tbody>
</table>

Genotype difference $P<0.001$

l.s.d. 1.217

Priming difference Not significant

Genotype x priming differences Not significant

Figure 36. High seedling density of the “Emergent” genotype of old man saltbush following direct seeding at Wagin on 7 September 2009. Photograph taken on 24 November 2009.
Table 12. *Seedlings per metre of Atriplex nummularia populations with and without seed priming with 0.05 mM kinetin at Mingenew, WA*

<table>
<thead>
<tr>
<th>Population</th>
<th>Primed</th>
<th>Un-primed</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>de Koch</td>
<td>0.47</td>
<td>0.09</td>
<td>0.28</td>
</tr>
<tr>
<td>Emergent type</td>
<td>1.22</td>
<td>0.91</td>
<td>1.06</td>
</tr>
<tr>
<td>Non-emergent type</td>
<td>0.16</td>
<td>0.03</td>
<td>0.09</td>
</tr>
<tr>
<td>ssp. spathulata</td>
<td>0.03</td>
<td>0.13</td>
<td>0.08</td>
</tr>
</tbody>
</table>

Mean: 0.47, 0.29, 0.38

Genotype difference: $P<0.001$

I.s.d. ($P = 0.05$): 0.474

Priming difference: Not significant

Genotype x priming differences: Not significant

Figure 37. *Establishing seedlings of the “Emergent” genotype of old man saltbush following direct seeding at Mingenew on 27 August 2009. Photograph taken on 1 December 2009.*
A demonstration trial was conducted, in collaboration with the Saltland Pastures Association, to compare direct seeding of *A. nummularia* using an experimental cone seeder with a commercial “Mallen” niche seeder.

**Materials and methods**

The trial was located on a saline, sandy loam, prone to waterlogging, 25 km south of Wickepin (32°59′59″S, 117°33′11″E). A weed-free seedbed had been prepared at the site with a double knockdown of glyphosate at 2 L/ha (a.i. 680 g/L) six and two weeks prior to sowing.

Two unreplicated 60 m strips, located 70 m apart, with each strip containing two rows, were sown on 7 September 2009. The first strip was sown with an experimental cone seeder, which placed seeds 5-10 mm below the surface into 3 cm deep furrows. This machine also used depth wheels to ensure precise seed placement and trailing press wheels to provide good seed-soil contact. The other strip was sown by a commercial saltbush seeding contractor using a “Mallen” niche seeder (see Figure 38). Sowing rate of the cone seeder was 33.6 seeds/m, while the Niche seeder was configured to deliver 25 seeds in each “placement”, located 2 m apart.

The same commercial seed batch of cultivar de Koch (subsp. *nummularia*) was sown in both machines. Seeds with intact bracts were separated from any debris with a vacuum aspirator and x-rayed using a Faxitron MX-20 x-ray machine. Any seeds lacking embryos were removed from the sample by hand. The remaining seeds were checked for viability using the tetrazolium staining method described by the International Seed Testing Association (Anon. 1999). Only confirmed viable seeds were used for experiments.

One row in each strip was sown with seed primed with kinetin, while the other row was sown with unprimed seed. Primed seeds were soaked in 0.05 mM kinetin for 18 hours, extracted, rinsed under water and patted dry with a paper towel. Seeds were then left to bench dry for two days at 22°C and 55% relative humidity and were then transferred to a 16°C, 25% relative humidity room to further dry for another four days.

Seedling counts were conducted on 24 November (85 days after sowing) within every 2 m section of row sown by the cone seeder and within each 2 m placement sown by the niche seeder.

As the strips sown by each machine were unreplicated and located 70 m apart, direct comparisons between the success of each machine were precluded. However, comparisons between priming treatments within each sowing method were conducted, using paired Student’s t-tests.
Figure 37. Atriplex nummularia direct seeding demonstration strip sown by a commercial “Mallen” niche seeder. Approximately 25 seeds were placed every 2 m.

Results and discussion

Figure 39. Monthly rainfall totals and mean maximum and minimum temperatures from 1 July 2009 to 30 June 2010 at Wickepin, 25 km north of the demonstration site (Data from the Australian Bureau of Meteorology).
Monthly rainfall totals and mean maximum and minimum temperatures from 1 July 2009 to 30 June 2010 are shown for Wickepin in Figure 39. Germination conditions were very favourable. The soil surface was very moist at sowing and remained moist until mid-October, with 39 mm of rain falling in the five weeks after sowing (Figure 39).

Establishment was successful from both machines, with the cone seeder having an establishment rate of 17% of all seed sown and the niche seeder 15% (Table 13). Figure 40 shows the resulting establishment in the cone-seeded strip. Priming with kinetin did not affect establishment of seed sown with the cone seeder, supporting results from both Wagin and Mingenew. However, priming produced a significantly higher established density of seed sown by the niche seeder. Although valid comparisons between the establishment success of seed sown by both machines cannot be made, the establishment percentage of un-primed seeds was lower for the niche seeder, while that of primed seeds was similar between machines. This suggests that *A. nummularia* establishment using a commercial niche seeder might be improved if seeds are primed with a signalling compound.

![Figure 40. Establishment of Atriplex nummularia at Wickepin in the demonstration strip sown by a cone-seeder on 7 September 2009. Photograph taken on 24 November 2009.](image-url)
This project has demonstrated that direct seeding of *A. nummularia* is quite feasible using conventional seeding equipment. The results of this project and from industry experience suggest the following principles for reliable establishment of *A. nummularia* through direct seeding.

1. **Select appropriate paddocks**

   Good site selection is critical to successful establishment. The most appropriate paddocks are those with slightly- to moderately-saline land (ECₐ values at 0-30 cm depth between 2 and 8 dS/m). Productivity is markedly reduced on extremely saline land (ECₐ > 32 dS/m). *Atriplex nummularia* is susceptible to waterlogging, so avoid areas prone to prolonged waterlogging. Sites with 50-100% annual ryegrass and some slender ice plant are generally ideal. If the site has samphire or is bare and scalded, then it is too saline. If the site has substantial sea barleygrass, then it is too waterlogged. Duplex soils with sandy loam over clay are the easiest to establish, as the sandy surface allows salts to leach through the soil following winter rains, creating a favourable environment for germination. More details on appropriate site selection are provided in Barrett-Lennard et al. (2003).

2. **Prepare sites for optimum establishment**

   A weed-free seed bed is essential, as *A. nummularia* seedlings are weak competitors. Plan a year ahead if possible and reduce weed seed-set in the year before sowing by grazing and spray-topping, especially for ice plant and annual ryegrass. Commence control of rabbits and kangaroos if they are a potential problem.

   A good weed control strategy is to use two knockdown sprays (4-6 weeks and 1-2 weeks before seeding). Cultivation prior to sowing can make the surface too uneven for precise sowing. Apply a residual insecticide with the final knockdown herbicide (or at sowing) to control caterpillars, cutworms, aphids and redlegged earth mites.

3. **Sow the best seed**

   Germination of *A. nummularia* seed batches is generally low, and typically varies from 5–20%. Send to an accredited seed laboratory if concerned about the expected germination of a seed batch. The following steps will help maximise use of high seed quality.

   **Ensure seed is subspecies nummularia**

   This project indicates that subspecies *nummularia* is best suited to direct seeding. The alternative subspecies, subsp. *spathulata*, appears to have different requirements for germination and does not readily establish from direct seeding (see Section 6). Other studies in the “Enrich” project of the FFI CRC have also shown that subsp. *nummularia* is much more palatable to grazing sheep than subsp. *spathulata*.

   **Ensure seed is fresh**

   Use seed harvested within the previous six months that has been stored in a cool, dry environment.

   **Use large heavy fruits**

   Large and heavy fruits should be selected for sowing, as they are more likely to contain mature, viable seeds. Small fruits, on the other hand, are more likely to be empty or contain undeveloped embryos. If there is a large range in fruit size, grade off the smaller fruits.
Retain bracts

Do not remove the bracts surrounding the fruit, as establishment success in the field has been higher for seed with bracts, compared to seed with their bracts removed (see Section 4.1).

4. Sow into moisture in late winter to early spring

The ideal time for sowing is a compromise between soil temperatures being warm enough for germination and the likelihood of sufficient moisture being retained for strong root development before summer. Seeds should be sown into a moist seedbed, or if there is a strong likelihood of rain. Although *A. nummularia* is salt tolerant, its germinating seeds are susceptible to salinity and will not germinate if the soil surface is too saline. Rainfall leaches salts from the surface, providing a relatively fresh environment for germination. If the soil surface in a saline environment is dry, it is quite likely to be too saline for germination of *A. nummularia*.

The optimum sowing time for *A. nummularia* appears to be late winter to early spring, with the ideal sowing window being earlier in more northerly districts than more southerly ones. This time of year also allows good control of winter weeds and insects prior to sowing. For example, the best sowing times for different regions in Western Australia are:

- Northern agricultural region - early to late August
- Central and eastern wheatbelt - mid-August to early September
- Southern agricultural region - late August to late September

In areas with more reliable spring and summer rainfall, such as northern New South Wales, the sowing window will be more extended.

On saline land, sowing into moisture in late winter to early spring also means salts are more likely to have been flushed from the soil surface, providing a favourable environment for germination, whereas surface salinity levels increase as spring progresses, due to increasing evaporation rates. However, if the area to be sown is waterlogged, sowing should be deferred until later in spring, or to the following year.

5. Aim to establish at least one plant per 2 m of row

Use a sowing rate of ~10 fruits/m (if germination rate is 15%). This should provide at least one plant every 2 m, allowing for losses of ~60%. Higher rates should be used for seed of lower germination.

6. Set-up the seeder for the best result

A standard Massey Ferguson combine seeder (or something similar) can be used to direct seed old man saltbush, with some minor modifications. The simplest way is to remove tynes not needed for sowing. Small boxes (one for each seeding tyne) can be attached to the seeding box to hold the small quantity of seed required, with hoses attached to seeding tynes. Seeders should also have the following features.

- **Tynes that form furrows**
  
  Tynes should create furrows to capture rainfall and increase seedling survival, particularly in dry springs. Furrow formation also scalps away non-wetting sand and removes weed seeds. Furrows should be formed to minimise soil in-fill and should be broad to increase the area of rainfall capture.

  The key is to sow into moist soil. Furrows of 50 mm depth are sufficient if the soil surface is moist, while deeper furrows can be used if the soil surface is dry or highly non-wetting,
provided furrow collapse and soil in-fill is avoided, as this causes burial of the seed, which can markedly reduce seedling emergence.

**Press wheels**

Press wheels provide good seed contact with soil moisture and reduce in-fill of soil into the furrows. They should press soil in the furrow bottoms and minimise soil in-fill from the sides. Flat-bottomed wheels give the best results.

**Row width**

Calculate the desired width between saltbush rows for each seeding pass and adjust the width of seeding tynes accordingly, removing non-seeding tynes. Typical row widths for alleys of double or triple rows are 1 m. A stand of saltbushes 1 m x 1 m apart can be regarded as dense.

7. **Sow to a depth of 5–10 mm**

*Atriplex nummularia* requires shallow seeding to a depth of 5–10 mm. Seeds sown too deep will not emerge. The simplest method is to drop fruits in the bottom of furrows and press them in with press wheels. When correctly adjusted, this will leave a small proportion of fruits visible on the soil surface. Sowing directly onto the surface is unreliable.

8. **Control weeds and pests (insects, kangaroos and rabbits)**

Summer growing weeds compete strongly with saltbush seedlings for soil moisture. Control weeds with appropriate herbicides to maximise establishment. Monitor the paddock for insect damage, especially over the first eight weeks and control if needed. Good control of kangaroos and rabbits is essential to protect young seedlings.

9. **Defer grazing until seedlings are well established**

The first grazing of *A. nummularia* bushes should be deferred until they are well established and actively growing. This will vary with seasonal conditions and may not be until after the break of the next season. Ensure plants are firmly anchored before introducing animals.
9 ESTABLISHMENT OF OTHER SPECIES

9.1 Rhagodia preissii

The results of Section 5.1 suggest *R. preissii* can be established using the same direct seeding methods as for *A. nummularia*. However, this is based on the success of just one trial in the northern agricultural area of WA. This is the region where *R. preissii* has been promoted, but further trials are required to demonstrate repeatability of establishment in more southerly areas, with cooler soil temperatures, and on other soil types.

9.2 Other Atriplex species

This project was not able to develop reliable establishment packages for other *Atriplex* species including *A. amnicola* and *A. undulata*. The project had limited success with these species, which presently work well with the “Mallen” niche seeder. Further work is needed to understand the triggers for seedling emergence before these species can be direct-seeded with conventional machinery.

9.3 Maireana brevifolia and *M. pyramidata*

Direct sowing of *M. brevifolia* and *M. pyramidata* appears to be problematic in much of southern Australia. The main difficulty is their requirement for warm temperatures (~30°C) to germinate (see Section 3.1). These temperatures do not normally occur until November in southern Australia, by which time the winter growing season has generally ceased. This precludes the direct seeding of these species without the aid of irrigation. An exception to this would be areas with more reliable summer rainfall, such as northern New South Wales. Here, sowing could be deferred until late spring-early summer.

Field observations indicate widespread recruitment of new *M. brevifolia* seedlings from surrounding bushes, most likely after episodic summer rainfall events (P.G.H. Nichols and E.G. Barrett-Lennard, unpublished data). This suggests an alternative and potentially cheap method of establishing *M. brevifolia*, if it is already present in the area, is to encourage natural recruitment of seedlings from seed produced on surrounding bushes. However we need ways to get at least small numbers of viable plants into virgin sites. One way of doing this could be to transplant a low density of nursery-raised seedlings which could then act as a seed source for natural recruitment. It is likely *M. pyramidata* could be established in a similar way. In order to devise strategies to increase recruitment of new seedlings, there is clearly a need for studies to better understand the ecology of *M. brevifolia* and *M. pyramidata* and their cues for germination. It may also be possible to identify populations or genotypes of both species with lower temperature requirements for germination, that make them more suited to direct sowing in late winter or early spring.
10 Conclusions and further work

10.1 Seeding machinery

This project has illustrated the feasibility of establishing *A. nummularia* and (perhaps) *R. preissii* through direct seeding with conventional seeding equipment. This raises the possibility of wide-scale plantings in mildly to moderately saline land and rangeland country. The main principles are to scalp away non-wetting sand and weeds, and sow into furrows with precise depth control (5-10 mm). Furrows are important for harvesting rainfall, especially during dry springs and in low rainfall areas with sporadic rainfall events.

While the principles have been established, it would appear that further machinery development may be required to fine-tune seeding for more reliable establishment. In areas prone to waterlogging, the current system of placing seeds in furrows below the surface may not be ideal for longer-term stand survival. These may become inundated, particularly during winter in subsequent seasons, with a resulting reduction in stand survival. One advantage of the “Mallen” niche seeder is that seeds are placed on mounds above the soil surface, reducing the effects of waterlogging. In such areas machinery design may need to be modified to enable furrow formation on top of mounds.

Some machinery modifications to form broad, shallow furrows may also be beneficial for low rainfall agricultural and rangeland areas. In such areas drought stress is likely to be a common problem and the formation of such furrows would harvest more rainfall to provide moisture to emerging seedlings.

10.2 Seed quality and seeding rates

Poor seed quality is a major limiting factor for successful chenopod establishment. Several commercial seed lots we tested had very low viability levels, with a high proportion of inert matter and empty fruits. This indicates considerable room for higher seed quality within the seed industry. A greater consumer awareness of the large variability in seed quality and encouragement to conduct formal germination testing may help lift industry standards.

Some seed-related areas leading to increased likelihood of establishment success were identified in this project. The first concerns selection of larger (heavier) fruits for sowing, as they are more likely to contain mature, viable seeds. Larger fruits can be selected directly off the bush, while smaller fruits can be graded off in commercially purchased batches (these are likely to have low germination in any case). Secondly, seeds should be stored post-harvest in a cool and dry environment and should ideally be used within six months of harvest.

With *A. nummularia* this project identified the greater suitability of subsp. *nummularia* than subsp. *spathulata* for direct seeding. Other work in the FFI CRC “Enrich” and “Saltbush selection” projects have demonstrated much greater palatability of subsp. *nummularia* than subsp. *spathulata*. It is of concern that there is a general lack of awareness of differences between *A. nummularia* subspecies in the nursery and seed trade and among consumers. A greater distinction needs to be made between these subspecies, in order that the public buys the type that is best suited to their needs.

The identification of genotypic differences for ability to establish from direct seeding within subsp. *nummularia* means there is a possibility of breeding cultivars of *A. nummularia* with enhanced ability to establish from direct seeding. With this in mind, seed of the Emergent type, which has been identified as having a high ability to emerge from direct seeding (see Sections
3.4 and 6), has been provided to the FFI CRC old man saltbush breeding program for incorporation as a breeding objective.

The biggest limitation in determining appropriate seeding rates is seed quality. The proof of concept trials for direct seeding of *A. nummularia* (reported in Section 6) indicate a sowing rate of ~10 fruits/m is feasible to produce an established plant every 2 m. However, this is based on a germination rate of 15% and seedling losses of ~60%. Further work is needed to determine the most appropriate seeding rates, but a good understanding of the quality of the seed lot to be sown is clearly needed before this can be determined.

If seeding rates can be reduced reliably to the order of ~10 seeds per linear metre, sowing costs will be markedly reduced over the “Mallen” niche seeder, which commonly uses 50 seeds per 2 m placement, making it more attractive to sow saltbush over wide areas. Premium seed lines that are likely to be developed through the FFI CRC old man saltbush breeding program will most probably be more expensive than wild seed lots and we need to be able to get the best result with tiny amounts of seed per hectare.

### 10.3 Use of plant signalling chemicals to enhance germination and emergence

The most commonly used saltbush across southern Australia is presently old man saltbush. A strong case cannot be made based on our work for the use of plant signalling compounds to improve the establishment of this species.

Plant signalling chemicals were identified that significantly promoted germination under laboratory conditions in otherwise physiologically dormant species such as *Maireana brevifolia*, *Atriplex semibaccata* and *Rhagodia preissii*. However, this improvement was only transferred to the field for *R. preissii*. Given the inconsistency of results in the field, it is unlikely that the seed industry would adopt this technology, unless delivery of the benefits can be markedly improved.

### 10.4 The role of bracteoles in regulating germination

The field results in this project conflicted with the laboratory results of Stevens et al. (2006), who showed enhanced germination from removal of bracteoles in *A. undulata*. In *A. nummularia*, it appears that the bracteoles absorb moisture and act to regulate germination. As a result, it takes longer for bracted seeds to germinate than naked seeds. However, this appears to give an advantage under field conditions, as the bracteoles only allow the seeds to germinate under optimal conditions, hence increasing the chances of seedling survival. Further work is needed to gain a greater understanding of the role of bracteoles in regulating germination of chenopods. At present we recommend that old man saltbush be sown with the bracteoles intact.
11 ACKNOWLEDGEMENTS

This project was first conceived by Dr Mike Ewing and Dr Warren Mason and initiated as part of the former CRC for Plant-based Management of Dryland Salinity, which made a direct funding contribution. Funding was also provided by Meat & Livestock Australia, Australian Wool Innovation Ltd and Land and Water Australia. We are grateful for the interaction and support of the Saltland Pastures Association. The project team wishes to thank the following farmers and their families for use of their properties for field work: Colin Pearse (Meckering), Chris English (Wagin), Greg and Nathan Astbury (Wickepin), Ian Bagley (Mingenew), and Andrew Lee (Dumbleyung).


