FloraSearch *Atriplex nummularia* trial establishment and breeding strategies.

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Introduction

The rational of establishing woody perennial plants as an ameliorative treatment for dryland salinity is well established. It is also well established that the extent of the problem dictates that a substantial proportion of those plants need to have a commercial foundation if they are to be integrated into farming systems.

Historically, scant research has been done investigating the economic potential of native Australian woody perennials as crop plants for the agricultural regions. Recently three related projects have sought to address this shortcoming by screening the flora for species with good potential for further development. In 1999 the Search Project screened almost 300 Western Australian species for commercial potential. This resulted in a shortlisting of species for further development. In 2002 a similar project, FloraSearch (Phase one) was conducted for south eastern Australia. The national FloraSearch (Phase two) program continues the work of both these projects, and it was here that *Atriplex nummularia* was identified as a high potential candidate for further investigation as a fodder crop, based on its wide distribution and broad application.

Although halophytes are now widely used by farmers to rehabilitate and increase productivity of saline land, little selection and breeding work has been done with this group of plants. *Atriplex nummularia* ranges across almost the entire continent, but despite widespread use on farmland as a grazing plant, both in natural stands and in planted areas, no systematic investigation of the variation in agronomic traits has previously been attempted. Genetic improvement of *A. nummularia* has been restricted to two cultivars and a clone which are available on the open market. The two cultivars "De Kock" and "van Holt" were developed from a land race in South Africa. The commercially available clone 'Eyres Green' (Topline Plant Company, Uraidla, 2003) was selected from a plantation of *A. nummularia* growing near Rudall, South Australia.

In the current FloraSearch project, seed of two subspecies of Atriplex nummularia has been collected from 27 provenances across Australia. Seedlings of these collections are being grown in family/provenance trials to investigate the variation in the species and provide the foundation for a breeding program. The material is replicated on four trial sites, in WA (two sites), SA and NSW.

Trial objectives

The two primary objectives for the breeding strategy are to generate clonally propagated selections within 3 years, and to develop high quality cultivars for seed production within 6 years.

The development of clones is achieved by screening the progeny trials (and subsequent generations of progeny trials) for desirable individuals. These plants can then be clonally propagated, and tested in a range of environments to ensure stability of desired traits prior to release.

The production of high quality seed from cultivars is more complex. The objective in breeding out-crossing perennials is to concentrate desirable alleles in the breeding population. Factors affecting this process include choice of parents, mating system, heritability and selection intensity. Progress in genetic gain can be slow and made even slower if multiple traits are selected for. Nothing is known about the heritability of various traits in *A. nummularia* or about genetic and phenotypic correlations amongst them. If heritability is high and correlations are positive or at least neutral then better progress is likely to be made than with high heritability and negative correlations between some traits.

Primary research objectives should therefore include the estimation of heritability and genetic correlations, as this will directly affect the way the breeding strategy is conducted. Until such information is acquired it is difficult to direct with any certainty what the best strategy may be.

The Breeding Program

Effective breeding programs maintain four general types of population (Eldridge *et al.* 1994):

- **The natural or base population** - Includes wild populations from which parental selection is conducted.
- **Breeding population.** - Consists of the selected parent trees and their progeny - which may be established in a range of progeny or family/provenance trials in which the cycle of mating and selection will be repeated.
- **Propagation population.** - Consists of the best genotypes selected from the breeding population in the form of seedling seed orchards, clonal seed orchards or cuttings multiplication areas. These populations are managed to produce large quantities of genetically superior propagules for deployment in plantations.
- **Production Population.** - The fourth population which the breeding program feeds into. Consists of operational plantations.

Infusions of new material from other sources can be brought into the breeding population in second and later generations as required to offset the effects of inbreeding.

How these populations relate can be seen in Figure 1.

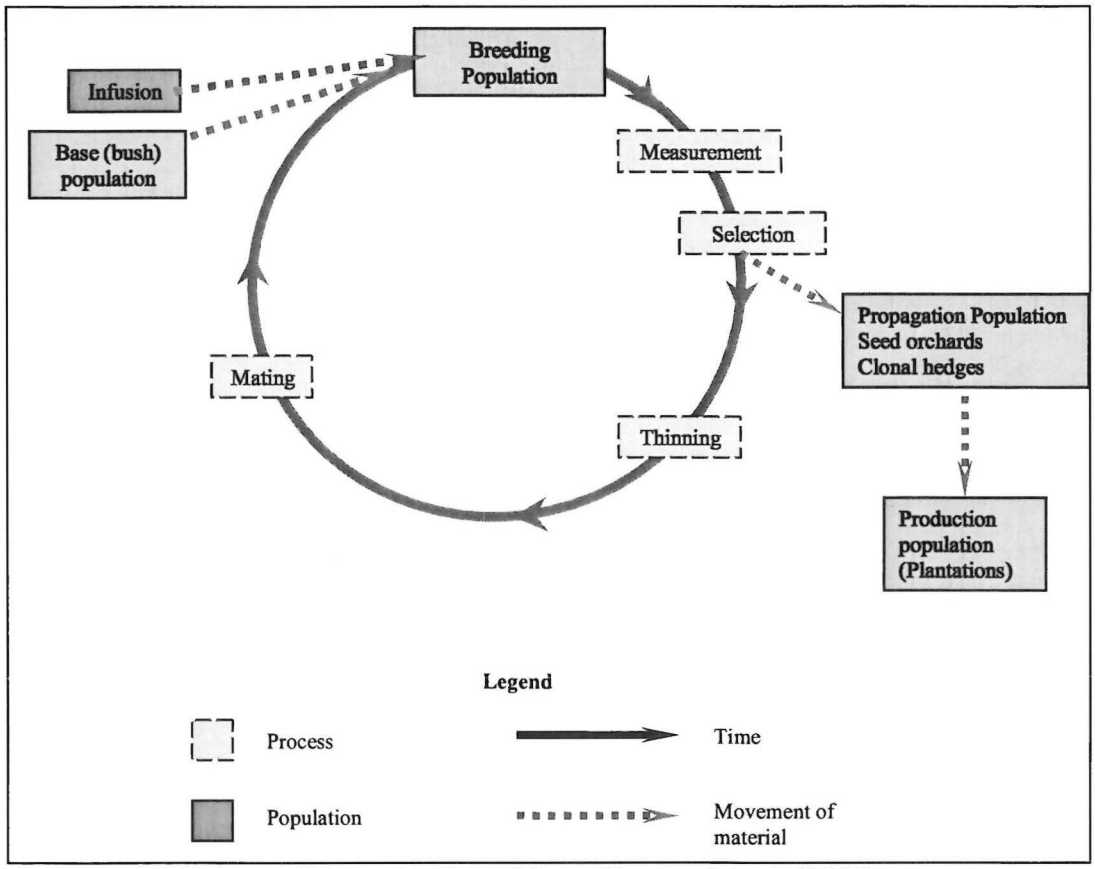


Figure 1. General breeding cycle adapted from Harwood *et al.* (2001)

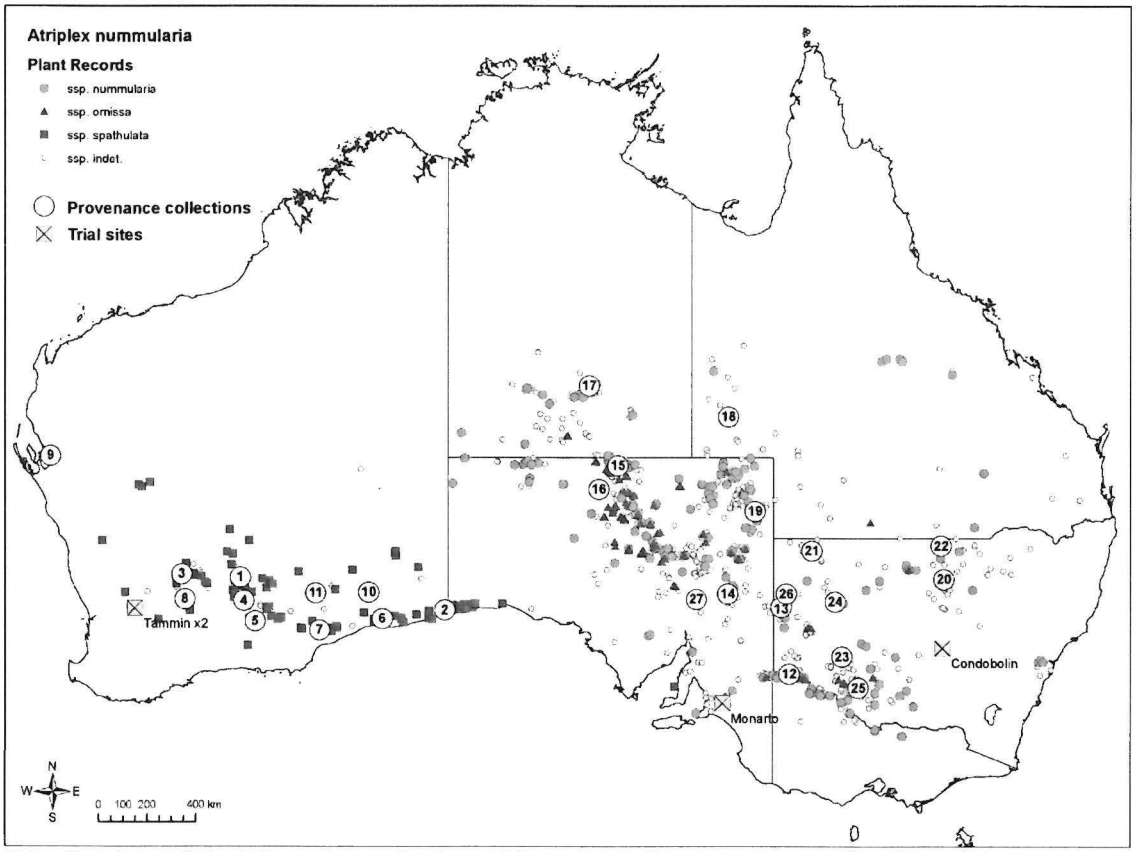
The success of a breeding program depends entirely on the cumulative improvement of the breeding population. Control of inbreeding in advanced generations of the breeding population and ultimately in the resulting production populations is a primary concern facing plant breeders. Rapid improvement is effected by intensity of selection and elimination of inferior families. While this leads to rapid short term gain it decreases genetic diversity, which in turn reduces opportunity for making long term gains (White 2001). Through the use of a large, careful structured breeding population and control over related mating, breeding strategies aim to optimize both short term gains and long term gains.

Establishing the breeding population-collection strategy

Historically there have been a number of approaches to determining how many plants should be collected to represent a provenance. After a review of the literature it was considered that a minimum of 15 plants per provenance should be collected, and beyond that number as many as resources will permit, in this case 20-25 plants per provenance. Plants need to be healthy, carrying an adequate crop of ripe, viable seed. They have to be in a population of sufficient size to allow good separation between plants collected. Attention must be paid to land tenure and distance from other collections.

Based on these guidelines, collectors from the WA Department of Environment and Conservation and the NSW Department of Primary Industries collected material across the range of the species. Of the two subspecies collected, one (subspecies *spathulata*) is largely restricted to Western Australia. It is represented by 11 collections, from near Shark Bay to Eucla. The other subspecies (subspecies *nummularia*) is in all other mainland states, and is represented by 16 collections.

As well as seed, leaf samples were gathered for DNA analysis of each provenance. GPS locations of every plant collected from were taken along with observations of soil type, pH, land form, associated vegetation, and dimensions of the parent plant. The plants were photographed to allow an assessment of form.



Distribution of *Atriplex nummularia* and location of provenance collections. Map courtesy of Trevor Hobbs.

Ecology of the natural populations.

In Western Australia *Atriplex nummularia* subspecies *spathulata* occurs on a broad range of landforms, including quartzite and banded iron formation hills, breakaways, broad valleys, exposed granite areas and calcareous, greenstone and granulite plains.

Soils data gathered with the seed collections showed naturally occurring *Atriplex nummularia* subspecies *spathulata* growing most commonly on sandy clay loams to clay

loams. One site was recorded as a silty loam, and one as a sandy loam. The soils at all sites were alkaline, showing a pH range of approximately 7-9. Soils were consistently reddish on the Munsell classification. Electrical conductivity of these soils, determined with EC1:5 analyses ranged from 100uS to over 5mS.

In Western Australia, *Atriplex nummularia* subspecies *spathulata* occurs in two discrete environments, as an understorey plant in woodlands, and as a dominant plant in chenopod shrublands.

The woodlands are open and dominated by Eucalyptus species, most commonly *Eucalyptus salubris* and *E. salmonophloia,* but also frequently with *E. longicornis, E. griffithsii, E. clelandii, E. corrugata, E. lesouefii, E. oleosa,* and *E. yalatensis.* On the southern Nullarbor co-dominance with Melaleucas is common, in particular *M. cymbifolia.*

In the shrublands, *Atriplex nummularia* frequently co-occurs with other Atriplex species, including *A. bunburyana, A. paludosa, A. hymenotheca* and *A. vesicaria*. Other chenopods in the Mairiana, Enchylaena, Sclerolaena and Rhagodia genera commonly co-occur, as do other shrubs such as Acacia, Eremophila, Olearia, Ptilotus, Zygophyllum, Scaevola and native grasses, particularly Austrostipa and Neurachne species.

In central and eastern Australia, observations accompanying seed collections show *Atriplex nummularia* subspecies *nummularia* to grow predominately on flat alluvial plains, with occasional occurrences on sandy rises and dunefields. The alluvial plains incorporate areas of relict lakes systems, gilgai, flood plains and stream beds.

The most common soil type for this subspecies can be classed as a high pH cracking clay, frequently with some degree of sandy topsoil, with minor occurrences on red and brown earths, and red alluvial soils. They are mostly in the Munsell brown soil range. The soils are generally alkaline, usually in the pH range of 7.5-9, but there are less common locations down to pH5.5, and up to pH10.5. The areas of deeper, lighter soils tend to have a lower pH, and to be towards the red end of the Munsell scale.

A similar plant community pattern to that seen in WA exists with the occurrence of *Atriplex nummularia* subspecies *nummularia*. The species again occurs as either a mid stratum shrub in open woodlands, or as a dominant plant in open, chenopod dominated shrublands.

The woodlands are predominately Eucalyptus or Acacia species, alone or co-occurring. Typical species include *Eucalyptus camaldulensis, E. largiflorens , E. microtheca, Acacia stenophylla, A. salicina, A. pycnantha*, and *A. victoriae. Atalaya hemiglauca* and *Myoporum montanum* are also recorded as significant in the upper stratum of the open woodlands. There is usually an understorey of mixed annual grasses and forbs beneath the Atriplex.

In the more arid part of its range the subspecies occurs as the tallest stratum in mixed shrublands, frequently occurring with other chenopods, notably *Maireana sedifolia*. The shrubs variously have an understorey of annual grasses, annual grasses and forbs, or annual saltbushes and forbs.

The trial plots

After cleaning, all seed was sown in the Kalannie Tree Supplies nursery in Western Australia. All seedlings were tagged in the nursery and repacked in a predetermined planting sequence for transport to the trial sites.

When selecting trial sites consideration was given to the knowledge that *Atriplex nummularia* shows a preference for clay or clay-loam soils, with recognition of the importance of selecting sites which are representative of the areas in which the species is likely to be grown.

Four sites were chosen for the provenance/family trials. Two trials were established on farmland near Tammin in Western Australia. They were chosen because they are representative of WA Wheatbelt soils on which saltbush might be planted, and because of their proximity to experimental sites for which significant site characterization had been conducted (Norman *et al.* 2007). Mean annual rainfall in the area is 317 mm. The first site was on non-saline shallow duplex soil, comprising red sandy loam over clay. This soil type is commonly associated with salmon gum (*Eucalyptus salmonophloia*) and gimlet (*Eucalyptus salubris*). The other site is a saline "morrel" soil, consisting of a shallow duplex of grey alluvial sands over pallid clay (Norman *et al.* 2007). The water table is approximately 1.2 metres deep, and is highly saline. Shallow ground water salinity in adjacent experimental plots ranged from 22000 – 48000mg/L. (Norman *et al.* 2007). The third site was established at Monarto just north of Adelaide in South Australia. The soil at this site consists of sandy loams over red clay, and the mean annual rainfall is 360 mm. The fourth site was established at Condobolin in New South Wales, where the soil is a red clay, and the mean annual rainfall is 439 mm.

All provenances were represented in the trials in Western Australia and Monarto (SA), but insufficient seedlings were available to represent the Oodnadatta provenance in the trial at Condobilin (Table 1). The commercially available clone "Eyres Green" was included in the trials as a control but will not be included in any breeding operations. Trials were established as nine replicate, latinised row-column designs. Families were established in four tree row plots. Spacing was 3 m between rows and 1.5 m within rows.

Trial	Location	N Families	N Reps	N Trees/Plot	Total Trees/Trial	Sum
OMS01	Tammin Non-Saline	520	9	4	18720	
OMS02	Tammin Salt	500	9	4	18000	
OMS03	Monarto	528	9	4	19008	
OMS04	Condobilin	475	9	4	17280	73008

Table 1 Trial locations, Numbers of families, reps and total trees (excluding buffers)

A fundamental question in progeny testing is that of how many progeny per family to establish in order to accurately rank families. Cotterill and James (1984) and Cotterill and Dean (1990) recommend that for traits of low heritability it is necessary to measure at least 20 individuals per family to determine reliable family means. Where the trial serves as a breeding population from which to make selections within families, as many individuals as possible should be planted in order to maximise selection intensity and hence genetic gain from individual selection within families. In addition to improved opportunity for selection, extra seedlings per family provide insurance against mortality within the site.

Williams *et al.* (2002) suggest that a minimum of four replicates should be used for provenance/ progeny trials in order to provide adequate degrees of freedom for the error term used for testing. The number of replicates above this, and seedlings per family plot are dependant on the number of seedlings available and the purpose of the trial. Single tree plots are not recommended owing to the possibility of computational difficulties following mortality. In addition, multiple tree plots allow for estimation of the between tree within plot variance component which gives an indication of genetic variation within seedlots.

Breeding strategy and trial plot management

It is uncertain at this early stage whether both sub-species will be bred together, separately, hybridised from separate breeding populations, or even if one subspecies will be dropped entirely. For the purposes of illustrating the breeding strategy, it will be assumed here that both subspecies will be bred together. Variations to accommodate any of the above scenarios can be implemented.

The first stage of the breeding strategy effectively converts the progeny trial into the first generation main breeding population by preventing poorer individuals contributing genes to the second generation. This is done by selecting the best individuals (an even mix of males and females) from the best 300 families across all trials (assuming GxE is not significant). The trials should be thinned at this point to eliminate the worst provenances and/ or families from the trial, and remove related individuals within family plots.

From this population the best 40 males and 40 females (may select more intensively on the males) are selected to form the nucleus. Control pollinated crosses will be conducted to produce the next generation of the nucleus.

Seed from select individuals in the main population can now be collected and a progeny test established. This progeny test will form the second generation breeding population. The progeny test will also provide a means of checking the effectiveness of forward selection in the original family-provenance trials. This check is important because little is known about the effectiveness of selection in old man salt bush. Control pollinated seed from the nucleus parents is also established in progeny tests.

The second generation main population is subject to measurement, selection and thinning, conducted in the same manner as for the first generation.

Concurrent measurement of the second generation nucleus trials enables performance of the first generation parents to be ranked. Commercial seed orchards are then established using clones of the best unrelated nucleus parents. On the basis of the progeny tests, these individuals are known to produce superior progeny and constitute the first cultivar for seed production. Seed from this first generation clonal seed orchard is used until it is superseded by the next generation of improved clonal seed orchards and so on. This process circumvents the segregation and subsequent decline in performance that can occur from cycling through multiple generations of a conventional cultivar.

The next stage sees intensive selection conducted in the second generation nucleus population to select the best individuals. Controlled crossing is then used to generate the next nucleus population. Seed or pollen from the best 10 individuals in the main population can then be used to introduce new genetic material into the nucleus. Similarly seed from the best individuals in the nucleus is incorporated into the main population.

The improved seed from the clonal seed orchards constitutes a cultivar which is sold with seed certification specifying that growers will not be able to cycle it through more than one generation without it segregating too much and losing some or all genetic gain derived from the breeding process. Consequently growers should buy seed from the breeding program for reliable production.

If forward selection proves to be completely ineffective then a progeny testing stage may need to be introduced between each generation of breeding population and nucleus population. Backward selection can be used to select the best parents for regenerating the next breeding population, but this would increase the time required to cycle through generations.

Table 2 presents a tentative time-line for implementation of the breeding strategy. A degree of flexibility is required. Methodology, direction or timing may need to be adjusted to account for new knowledge as it comes to light.

Table 2 Draft timeline for breeding and establishment of old man saltbush trials

Calender Year	Month	Activity	Comments
2006	July – August	Establish family provenance trials	
2007	September-October	First measurement – Biomass, survival, form and gender. Initiate cuttings experiments	Gender assessment assumes flowering has initiated
2007	November	Data analysis	
2008	May –Dec	Sample trials for nutritional traits. Sample analysis	
2009	January	Data analysis	
2009	February-March	Index selection and thin trials to improve genetic quality – remove close relatives. Control Pollination (CP) for nucleus	CP of nucleus trees is dependant on time of flowering
2010	November-December	Seed collection and sow in Nursery	
2011	June – August	Establish 2nd generation breeding population and nucleus population on 3 sites	May include new seedlots from best provenances as determined in original trials
2012	October-November	Measure 2nd generation trials. Backward selection	
2012	November-December	Strike cuttings for Clonal Orchards	
2013	June July	Establish Clonal Seed Orchards (CSO) Establish clone trials on multiple sites	Establish clone bank for "storage" of clonal material. Becomes a source for clonal multiplication if required

Saltbush measurement protocols

An assessment of the family/provenance trial sites is being conducted at age one (2007). The following range of factors are being assessed for each plant in five replicates of trials in WA and SA.

- Survival and health
- Biomass (using the Adelaide Method)
- Plant height, plant width across the mound, plant width along the mound
- Plant form
- Branch angle
- Internodes in a 10 cm length of branch
- Leaf size
- Sex determination (where possible)
- Proportion of female flowers in monoecious plants
- Leaf angle relative to stem.

This assessment will contribute to the process of selection for the next stage of the breeding program. Additional sampling to determine nutritional traits will be conducted in 2008.

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