Western Botanical

Germination Trial of *Atriplex* sp. Yeelirrie Station

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Cameco Australia

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1. Introduction

Cameco Australia Pty Ltd (Cameco) proposes to develop the Yeelirrie Uranium Project, approximately 700 km northeast of Perth and 70 km southwest of Wiluna. *Atriplex* sp. Yeelirrie Station has been recognised as a rare, new species of *Atriplex* (Chenopodiaceae) comprising two genetically distinct populations in arid Western Australia, described here as the Western and Eastern Populations. The Western and Eastern Populations were found to have similar levels of genetic diversity, but exhibited an unexpected level of genetic differentiation given their proximity (Clarke *et al.* 2012). The Western Population lies wholly within the economic orebody and encompasses two sub-populations that are located in close proximity to each other. The Eastern Population, approximately 30 km east of the Western Population, encompasses 10 sub-populations in close proximity to each other.

The seed biology of this species is largely unknown and of importance in the context of development of the proposed mine. Western Botanical was commissioned by Cameco to conduct a germination trial of *Atriplex* sp. Yeelirrie Station to assess the germinability of seed from old and new fruit. The findings from this trial are presented in this document and will assist future management of *Atriplex* sp. Yeelirrie Station.

2. Methods

Fruit were collected from six *Atriplex* sp. Yeelirrie Station plants, three on the Yeelirrie orebody of the western population and three from the nearby rehabilitation population in the Southern Stockpile area. Old and new fruit (greater and less than one-year old, respectively) were harvested, with new fruit appearing tan coloured compared to the grey, weathered bracts of older fruit.

Fruit were assessed visually and tactilely for seed fill with full fruit used for the germination trial. Experimental design of the germination is presented in Table *1*. Fruit of each plant were tested independently and separately by fruit age (less than one year versus greater than one year old). Two treatments were applied; 1) seed retained within fruit bracts, and 2) seed excised from fruit bracts.

All fruit and seed were tested on agar plates placed in an incubator set at a 12 hour light-dark cycle with temperatures of 29 and 14 °C, respectively. In-bract seed were monitored for germination weekly for 28 days, while excised seed were monitored twice-weekly for 15 days before completion of trial. Germination was considered complete on eruption of both radical and cotyledons.

Table 1. Experimental design for the seed trial of *Atriplex* sp. Yeelirrie Station.

Location	Plant #	Fruit Age	Trial Treatment	# of Replicate Plates
Western Population	Yeel 115	New	In fruit 29/14	6
Western Population	Yeel 115	Old	In fruit 29/14	6
Western Population	Yeel 115	New	Excised (short)	1
Western Population	Yeel 115	Old	Excised (short)	1
Western Population	Yeel 136	New	In fruit 29/14	6
Western Population	Yeel 136	Old	In fruit 29/14	6
Western Population	Yeel 136	New	Excised (short)	1
Western Population	Yeel 136	Old	Excised (short)	1
Western Population	Yeel 140	New	In fruit 29/14	6
Western Population	Yeel 140	Old	In fruit 29/14	6
Western Population	Yeel 140	New	Excised (short)	1
Western Population	Yeel 140	Old	Excised (short)	1
Rehabilitation Population	Yeel 1	New	In fruit 29/14	6
Rehabilitation Population	Yeel 1	Old	In fruit 29/14	6
Rehabilitation Population	Yeel 10	New	In fruit 29/14	6
Rehabilitation Population	Yeel 10	Old	In fruit 29/14	6
Rehabilitation Population	Yeel 89	New	In fruit 29/14	6
Rehabilitation Population	Yeel 89	Old	In fruit 29/14	6

3. Results & Discussion

Seed fill appears moderately variable amongst the six plants sampled (Figure 1), particularly for new fruit, though this may be due to small sample size of plants included in this preliminary trial. If representative of this species, variable seed fill may be due to variable success in wind pollination, microhabitat of individual plants (perhaps water availability within variable self-mulching clay soils of playas), or may reflect a difference amongst individual plants in periodic seed shed from within bracts.

Germination results are summarised in Figure 2. When fruit bracts are present, germinability of seed in old fruit is appreciably higher than in new fruit (39.51% versus 3.35%, respectively). This indicates either, a) a dormancy mechanism in fruit bracts to prevent germination of seed in new fruits, or b) that seed in new fruits are immature and not yet germinable. However, there was no difference in germinability of old and new fruit when seeds were excised from fruit bracts; 82.00% versus 82.67% for old and new fruit, respectively. Immaturity of young seeds is therefore discounted as the cause of low germinability of seed within fruit bracts.

Exogenous dormancy mechanisms (a chemical inhibitor is suggested) conferred by fruit bracts is therefore likely responsible for severely reduced germinability of seed in new fruit. The less severe reduction in germinability of seed in older fruit may indicate a lessening of the exogenous dormancy mechanism, perhaps by leaching of germination inhibitors from fruit bracts. A second possibility is the presence of a less severe physical dormancy conferred by the dry and woody bracts of older fruit.

The scale of the bract dormancy mechanism is appreciable; overall, excising from bracts doubles germinability of seed from old fruit, and increases germinability of seed from new fruit by 24-fold. This is advantageous in an experimental or nursery situation where rapid germination is desirable. However, excising may not be advantageous when field-sowing where staggered germination in response to natural environmental triggers of moisture and temperature is advantageous. Fruit bracts may also confer protection from predation and increased seed dispersal (fruit readily float).

Western Botanical currently holds 2.23 kg (~3.5 million seeds) of *Atriplex* sp. Yeelirrie Station seed collected in 2010/2011. The germination trial for this stored seed (Landcare Services 2011) approximates the results of this current trial: germinability of 73% and 78% for old and new fruit (respectively), versus in bract germination rate of 22% and 0%. Current viability and germinability of this stored seed is not known.

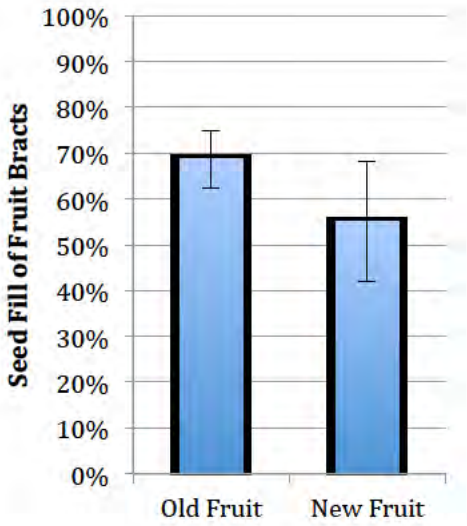

Figure 1. Seed fill of *Atriplex* sp. Yeelirrie Station fruit bracts for old and new fruit (greater and less than one year old, respectively).

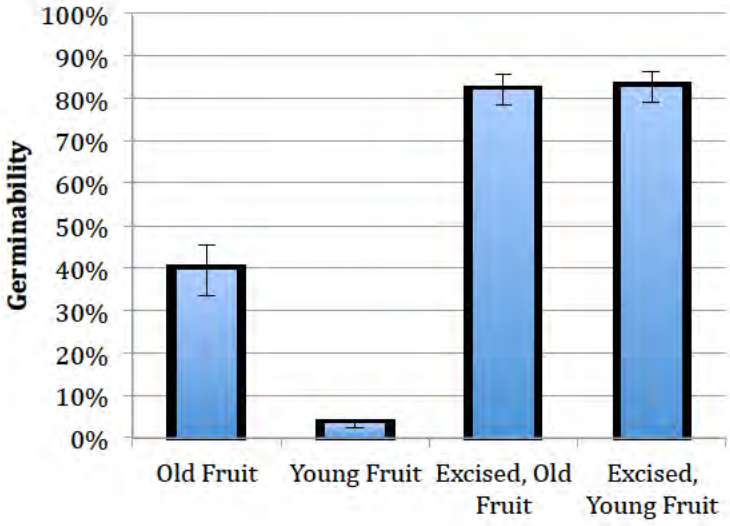

Figure 2. Germinability of *Atriplex* sp. Yeelirrie Station fruit by age (old and new; greater and less than one year old, respectively) and by trial treatment (in bracts and excised from bracts).

4. Conclusion

The results of the germination trial provide information that will assist future management of *Atriplex* sp. Yeelirrie Station. This trial's germinability of 39.51% for seeds within mature fruit is encouraging for establishing both translocation and rehabilitation populations from seed. Key conclusions from the study include:

- Seed fill of *Atriplex* sp. Yeelirrie Station fruit appears moderately variable, perhaps due to plant microhabitat (but may be an artefact of low plant replicates).
- Seed germinates readily when excised from fruit bracts, regardless of fruit age. The low germinability of seed within bracts of new fruit is likely due to dormancy mechanism of fruit bracts rather than seed immaturity.
- Excising seed from fruit bracts to dramatically increase germinability is recommended for ex-situ propagation of *Atriplex* sp. Yeelirrie Station. However, retaining seed within bracts may be more appropriate for in in-situ direct seeding to provide staggered germination and protection from seed predation.
- Western Botanical recommends a more extensive and detailed seed germination study of *Atriplex* sp. Yeelirrie Station, incorporating:
  - A balanced experimental design, with increased replicates of individual plants and the excised seed treatment.
  - Additional testing to clarify the dormancy mechanism due to presence of fruit bracts (i.e. physical, chemical, or both).
  - Additional investigation to determine if any difference exists in seed fill and germinability between fruit of male and female plants.
  - Determination of seed dehiscence triggers from fruits.
  - Emergence trials determine appropriate seed sowing depth and seedling vigour using both excised seed (large quantity in store) and seed within bracts (new collections required).
  - Additional germination trials testing seed that has been stored since 2011.

5. References

Clarke L.J., Jardine D.I., Byrne M., Shepherd K. & Lowe A.J. (2012). Significant population genetic structure detected for a new and highly restricted species of Atriplex (Chenopodiaceae) from Western Australia, and implications for conservation management. *Australian Journal of Botany* 60(1): 32-41.

Landcare Services (2011). *Atriplex sp. Yeelirrie Station Seed Collection Program Report.* Consultant report prepared for URS Australia Pty Ltd and BHP.

6. List of Participants

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