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Seed quality of *Eucalyptus rhomboidea*, *Hakea pendens* and *Marianthus aquilonaris* seed collections collected by Botanica Consulting for Audalia Resources Limited

Introduction

Seed was collected by Botanica Consulting from the Medcalf Project over a period of time whilst carry out site visits. The collection of seed from *Eucalyptus rhomboidea* and *Hakea pendens* where opportunistic and these collections were made in spring 2019. These two collections from Metcalf were cleaned and supplied to DBCA in November 2019. The collections of *Marianthus aquilonaris* was from a collection in 2016 and was collected under a permit from the DBCA for this purpose. This seed was supplied to DBCA in November 2019. A collection of the *Stenanthemum bremerense* was also made in Spring 2019 but the seed material was considered not viable in the initial examination that was carried out by Botanica and Dr Andrew Crawford so was considered not worth testing this species.

Seed collections from three Western Australian conservation listed species (*Eucalyptus rhomboidea*, *Hakea pendens* and *Marianthus aquilonaris*) where received from Botanica Consulting by the Western Australian Seed Bank for quality assessment in November 2019. These collections were small and turned out to be samples of the full collections. The full collections were requested so that an assessment of quality of the complete collection could be undertaken. The full collections were received at the end of November 2019. The samples originally received were re-incorporated into the full collection for *M. aquilonaris* and *H. pendens*. For *E. rhomboidea* the quality of the initial sample which had been partially cleaned was visually quite different to the main collection, so the two portions were kept separate and the quality for each portion was assessed independently.

Collections were weighed (initial collection weight) then dried under standard conditions of 15% relative humidity at 15°C for a minimum of two weeks before quality assessments were undertaken.

Seed quality assessment

Quantification and purity assessment

- After drying (at 15% RH, 15°C) a total collection dry weight was obtained for each collection.
- For *H. pendens* and *E. rhomboidea* (sample 1) as the estimated number of seed was <1000, all seed in the samples was separated from non-seed material then re-weighed and the seed counted.
- For *M. aquilonaris* and *E. rhomboidea* (sample 2) Five random samples of ca. 100 seed were taken from the main collection. Each sample was weighed, the seed separated from non-seed material, then re-weighed.
- The following quality attributes of the collections were calculated:
 - Seeds per gram (sample) = number of seed / sample weight (g)
 - Seeds per gram (pure) = number of seed / weight of pure seed (g)
 - Seed purity (%) = pure seed weight (g) / sample weight (g) x 100
- For *M. aquilonaris* and *E. rhomboidea* (sample 2) the estimated number of seed in the total collection was calculated.
 - Estimated seed = collection weight (g) x seeds per gram (sample)

Germination/viability testing

- Germination tests were conducted on the pure seed fraction obtained for each collection from the quantification process.
- For *H. pendens* and *E. rhomboidea* four replicates of 25 seed were placed into petri dishes containing 7.5g/l agar.

- For seed of *M. aquilonaris* the seed coats were manually scarified using a scalpel. Four replicates of 25 filled seed were then placed into petri dishes containing 7.5g/l agar and 100mg/l gibberellic acid.
- All petri dishes were incubated at 15°C with a 12-hour light /12-hour dark photoperiod.
- Germination was scored weekly with seed classed as germinated when a radicle of at least 2mm in length was observed.
- Germination of *E. rhomboidea* was poor under these germination conditions. A second test was conducted at 20°C.
- Germination of *E. rhomboidea* was slow at 20°C. With germination not complete seven weeks after the test was commenced, a viability stain was used to assess the viability of a 100 seed sample of each collection. Seed were soaked in a 1% w/v solution of 2,3,5-triphenyl tetrazolium chloride at 30°C, in the dark, for 16 hours. Seed were then cut to assess staining. Seed which had stained red were classed as viable, un-stained (white) seed were classed as non-viable.



Figure 1. Seed viability assessment of *Eucalyptus rhomboidea* after a 16 hour soak in tetrazolium chloride. *a.* Empty, non-viable seed; *b.* Filled, non-viable seed; *c.* Filled, viable seed.

Results

Seed quality measurements for the four seed collections submitted for assessment are presented in Table 1. The collections of *Hakea pendens* and *Marianthus aquilonaris* both had high purity and high germination. The purity of the two samples of *Eucalyptus rhomboidea* was low (< 20%).

Low purity is not unusual for eucalypt collections where it can be difficult to separate seed from chaff and non-seed material, however there was a considerable amount of non-seed material including soil that could be simply removed using sieves. Seed of *E. rhomboidea* was readily distinguishable from other material in the samples.

The germination of the *E. rhomboidea* was poor and lower than might be expected for a good eucalypt collection. Initial germination tests on *E. rhomboidea* were conducted at 15°C, the temperature routinely used by the Western Australian Seed Centre for germination testing.

A second germination temperature, 20°C, has been tested but germination has been slow. This test is ongoing, with germination still occurring seven weeks after the test was started. Under optimal germination conditions, germination of a eucalypt should be completed with a month indicating that this temperature was still not optimal for this species.

A viability stain (tetrazolium chloride) was used as an alternate method of determining the viability of the *E. rhomboidea* collections due to the poor germination results. Using this test living seed tissue stains red, whilst non-living tissue does not stain. Staining of *E. rhomboidea* showed that seed of both collections was high, 100% for the first sample and 96% for the second sample.

- The remaining seed in these collections will be stored for a period of five years in the seed vault of the Western Australian Seed Centre (until 1st December 2024) after which time a storage agreement will need to be renegotiated. The seed remains the property of Audalia Resources Limited and will be made available to Audalia Resources Limited when requested.

Species Name	Initial collection weight (g)	Collection dry weight (g)	SAMPLE			PURE		Seed in collection (* estimated)	cut test	Germination (% ± S.E.) (# ongoing)	Tetrazolium viability (%)
			weight/seed (g)	Seed per g	Purity (%)	weight/seed (g)	Seed per g				
<i>Hakea pendens</i>	11.7	11.6	1.48 x10 ⁻²	68	79.5	1.18 x10 ⁻²	85	781	na	99 ± 1	na
<i>Marianthus aquilonaris</i>	20.5	20.0	1.83 x10 ⁻³	546	89.1	1.63 x10 ⁻³	612	10890*	0.97	85 ± 9	na
<i>Eucalyptus rhomboidea</i> (sample 1)	4.5	4.3	6.82 x10 ⁻³	147	17.4	1.18 x10 ⁻³	845	630	na	15°C: 11 ± 2 20°C: 18 ± 6 [#]	100
<i>Eucalyptus rhomboidea</i> (sample 2)	212.5	211.5	3.02 x10 ⁻²	33	2.8	8.35 x10 ⁻⁴	1198	6997*	na	15°C: 3 ± 1 20°C: 12 ± 4 [#]	96

Table 1: Seed quality characteristics of *Hakea pendens*, *Marianthus aquilonaris* and *Eucalyptus rhomboidea* seed collections.



Figure 2. Seed sample of *Marianthus aquilonaris*.

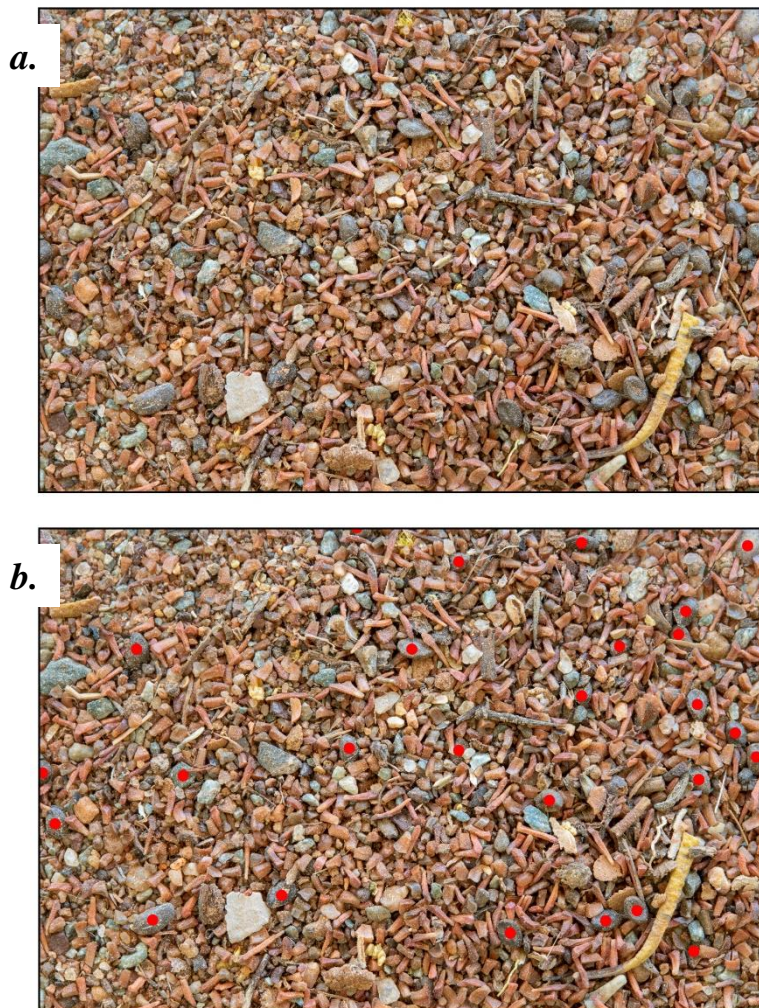


Figure 3. Seed sample of *Eucalyptus rhomboidea* (sample 2). Obvious seed have been marked with a red dot (b).

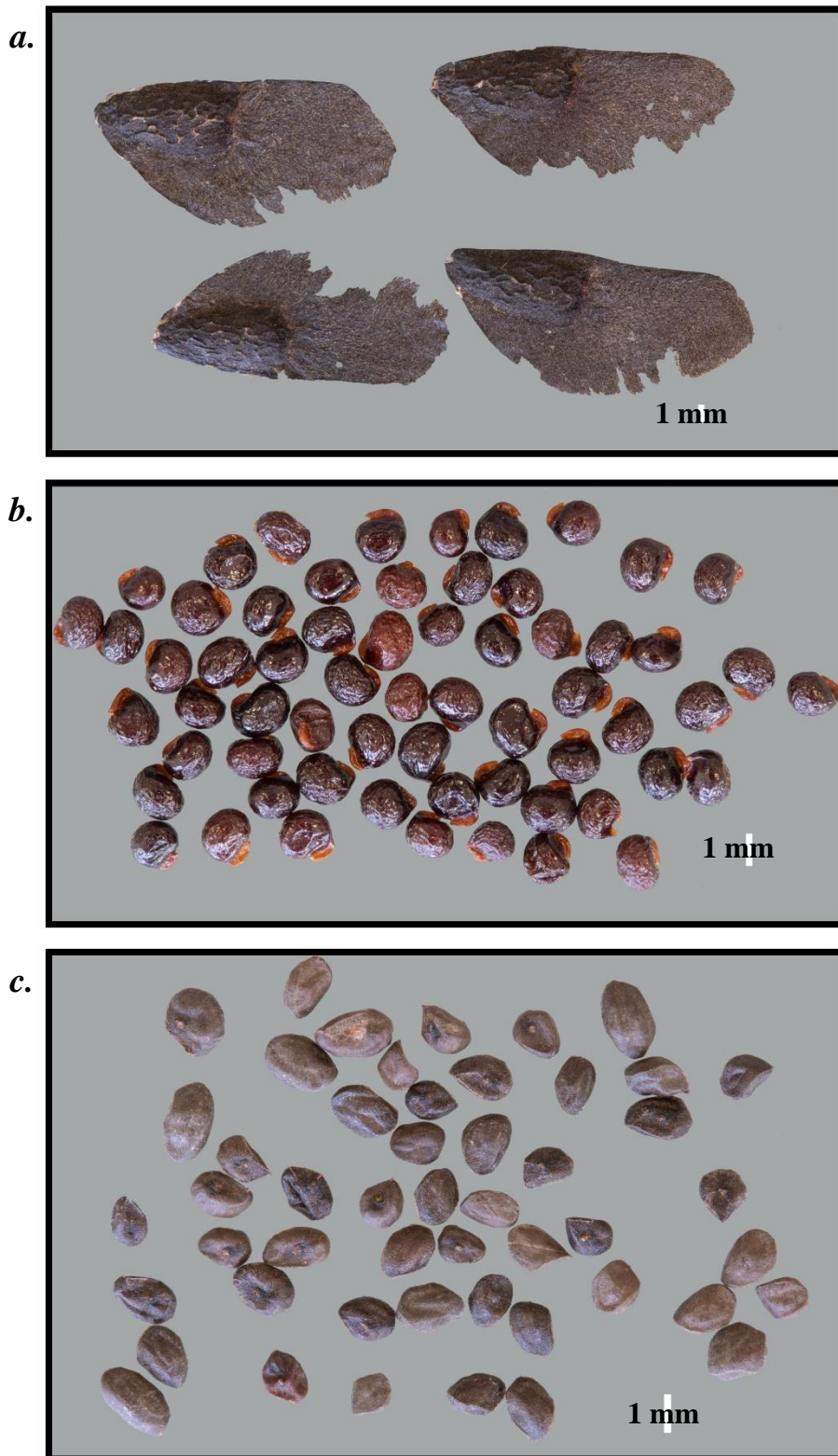


Figure 4. Pure seed of *a. Hakea pendens*, *b. Marianthus aquilonaris* and *c. Eucalyptus rhomboidea*.

References:

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Recommendations

- No further collections of the *Marianthus aquilonaris* and *Hakea pendens* seed as these two species are able to germinate readily.
- Further collections of *Stenanthemum bremerense* are required to carry out germination testing of this species.
- Further collections of *Eucalyptus rhomboidea* across its range to see if any viable seed is present.
- Both collections of seed from *Eucalyptus rhomboidea* and *Stenanthemum bremerense* need to be carried out in late spring as it would be beneficial for Audalia to work closely with the Threatened Flora Seed Centre to ensure that collections meet their standards.
- Further consultation with the Threatened Flora Seed Centre to ensure that if required seed of these four species need to be held in their collection so the seed can be stored for an indefinite time and they can ensure the highest possible viability of the seed.