

AUSTRALIAN FLORA FOUNDATION- Final Report

Establishment of an *ex-situ* collection and seed orchard for the endangered Grampians Globe-pea.

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Introduction

The Grampians Globe-pea *Sphaerolobium acanthos* (Crisp, 1994) is endemic to the Grampians National Park in the state of Victoria, Australia. It was known from three small populations within the park and the global population was believed to be 26 plants. Past herbarium records indicate that this species was once more widespread through the Park (SAC, 2014). Recent searches of previous localities suggest that the species no longer occurs at these sites. *Sphaerolobium acanthos* was shown to be susceptible to *P. cinnamomi*, which occurs near three of the populations (Reiter *et al.* 2004), and is predicted to be at moderate risk to extinction due to *P. cinnamomi*. The plant has been listed under the Flora and Fauna Guarantee Act 1988 and in 2016 was listed as federally endangered under the Environment Protection Biodiversity and Conservation Act 1999. This species has recently been assessed as critically endangered under the IUCN criteria (David Cameron pers.com).

Without the establishment of an ex-situ population, propagation, and conservation translocation *S. acanthos* is likely to become extinct in our lifetime. *Ex-situ* conservation methods for Fabaceae, using both seed germination techniques (Roche *et al.*, 1997; Loyd *et al.*, 2000 and Offord and Meagher, 2009), and micro propagation in Fabaceae and physiologically similar species (Rout, 2005; Cenkci *et al.*, 2008; Toth *et al.*, 2004 and Anthony *et al.*, 2000), offer a sound basis for exploring methods for the ex-situ conservation of *Sphaerolobium acanthos*.

This project aimed to:

- 1) Collect seed and propagation material from the remaining populations
- 2) Determine the optimal germination technique with seed and cuttings.
- 3) Establish an *ex-situ* collection for a seed orchard to be used for conservation translocation.

Methods

Study Species

Sphaerolobium acanthos is an erect, wiry shrub to 1 m tall with numerous spinescent, rigid, scabrous branchlets. The leaves are subulate, 2-3 mm long, initially scattered along stems, but soon falling so that the plant appears more or less leafless. One or two orange flowers are borne on a common peduncle; the stigma is subtended by a ring of hairs. The pod is obovate to ellipsoid. Flowering occurs from November- January (Walsh and Entwisle, 1996).

Seed collection

Seed was collected from the two largest populations, Site 1 and the new population discovered on Site 4 in January 2015-2017. Plants were bagged with stockings post natural pollination in December, and seed was subsequently collected from pods that had naturally dehisced within the stockings.

A small proportion (less than 10%) of seed was collected from each of the remaining plants that produce seed in 2015- 2017. Records of the number of plants remaining in each population were made and add to the small existing knowledge of the species. Approximately 400 seeds were collected.

Collection of seed was not taken from Site 2 as cuttings of each individual (see below) have struck. Seed was unable to be collected from Site 3 of 6 plants on the Northern side of the Park as these plants had been thoroughly eaten by goats.

Cuttings

The second largest population is at Site 2 in the Grampians. Each plant had cutting material removed in August 2015. 75 cuttings were trimmed to have at least one growing node, a sliver was taken from the woody stem, and the stem was then placed in both 3 g/L Indole-3 butyric Acid and 0.1% Indole-3 butyric Acid. The plants were then kept on a heat bed until cuttings struck. They were then transferred to pots and an open nursery area (Figure 1).

Pre- treatment of seed

Pre-treatment of seeds is often used to overcome any dormancy issues. Seeds of *S. acanthos* (N=60) (provenance Site 1 and Site 4), half of which were treated with light sand paper (Grade P40) rubbed gently between two sheets for 2 seconds, with the other half were placed in boiling water under the laminar flow for 15 minutes. For sterilisation we opted for an alteration of the methods used by (Tapingkae et al, 2007) as follows: both treatments were rinsed in 80 % ethanol for five minutes, followed by a 10 minute rinse in 0.05 % NaOCl. After sterilising, seed was washed four times each for five minutes in sterile water and kept overnight in the last wash (24hrs) before sowing on media.

Media for germination of seed

Seeds of *S. acanthos* (Site 1 and Site 4) (N=230) were placed in boiling water under the laminar flow for 15 minutes. Seeds were then rinsed in 80 % ethanol for five minutes, followed by a 10 minute rinse in 0.05 % NaOCl. After sterilising, seed was washed four times each for five minutes in sterile water and kept overnight in the last wash (24hrs) before sowing on media. Sterile seed was placed under the laminar flow with tweezers into each of the media prepared in Table 1. The media (Table 1) were chosen after a review of both native and non-native germination literature for Fabaceae, which have largely used a combination various concentrations of MS, Sucrose and Agar (Jusaitis, 1997; Koné et al, 2015; Kone et al, 2009; Perveen et al, 2013; Raut et al, 2015; Tapingkae et al, 2008; Ugandhar et al, 2012; Yang et al, 2001). As the seed came from three different collections from two provenances each with differing amounts of seed available, only one collection was sown on all five media (between 30- 80 seeds per media), with the remaining smaller seed collections only sown on media 1-2 and media 1-3 respectively.

Table 1: Media used for germination trials of *S. acanthos*.

Media	Agar g/L	MS g/L	Sucrose g/L
1: 0.8% Agar only	8	0	0
2: 0.6% Agar only	6	0	0
3: ½ MS and 0.8% Agar	8	2.2g/L	
4: ½ Strength MS, 3% Sucrose and 0.8% Agar	8	2.2g/L	7.5
5: ¼ Strength MS, 3% Sucrose and 0.8% Agar	8	1.1g/L	7.5

Results

Surveys while seed collection increased total number of known wild plants and populations

This project has discovered an additional population of 6 plants (Site 4) and increased the number of known plants at Site 1 to 50, bringing the known number of individuals in the wild to 70 plants.

Cuttings

Both hormone concentrations worked well and over 90 % of cuttings struck. Of these 16 % survived to flowering adults plants (N=13). These 13 plants are kept as part of a permanent ex-situ collection at South Yarra site of the Royal Botanic Gardens Victoria (Figure 1). These plants matured in the Summer of 2017/18.

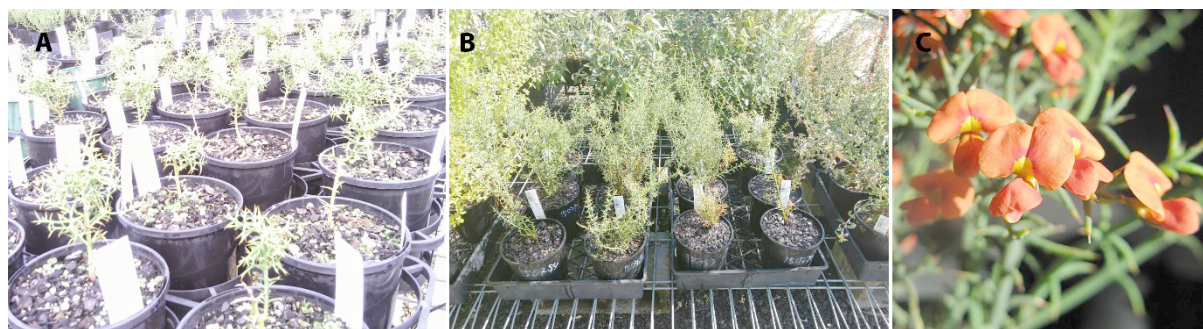


Figure 1: *Ex-situ* collection of plants propagated from cuttings from Site 3 (A) 1st year, (B) 2nd year, (C) flowering in second year.

Pre-treatment of seed

After four weeks 70 % of seed germinated with the boiling water pre-treatment and none had germinated using light abrasion with sandpaper.

Media for germinating seed

Of the five media that were tested for the germination of *S. acanthos* (Table 1), media 3 proved to be the most successful (Figure 2, Table 2) with 57 % of plants germinating on this media within four weeks. All plants were then transferred to petri dishes and grown on in tissue culture tubes (Figure 2). These plants can now be used for further micro propagation from cuttings as they mature in culture and also be de-flasked to enhance the diversity and number of the *ex-situ* collection.

Table 2: *S. acanthos* germination counts after four weeks on differing media.

Media	Germination %
1: 0.8% Agar only	42 % +/- SE 0.1
2: 0.6% Agar only	55 % +/- SE 0.2
3: ½ MS and 0.8% Agar	57 % +/- SE 0.3
4: ½ Strength MS, 3% Sucrose and 0.8% Agar	30 %
5: ¼ Strength MS, 3% Sucrose and 0.8% Agar	23 %

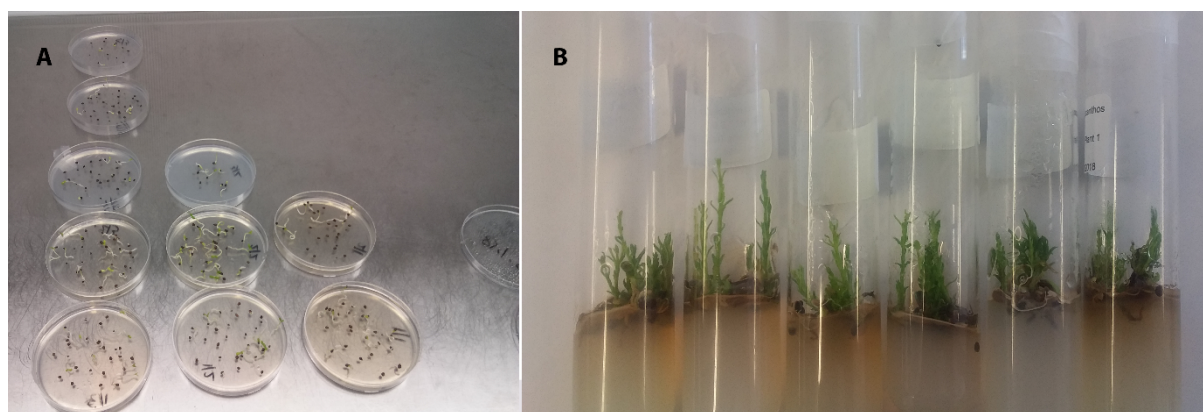


Figure 2: A) *S. acanthos* germination after 1 month on different media B) *S. acanthos* at 4 months with established root system ready for de-flasking or micro-propagation.

Establishing a seed orchard

The established nursery plants will be used to harvest seed with the plants grown from seed in germination trials once they reach maturity.

Lodge seed with the Victorian Conservation Seedbank

Half of the seed collection has been lodged with the Victorian Conservation Seed Bank with representatives from Site 1 and Site 4.

Discussion

This project has succeeded in collecting both seed and cuttings from the three largest remaining populations of *S. acanthos*. This project has discovered an additional population of 6 plants and increased the number of known plants at Site 1 to 50, bringing the known number of individuals in the wild to 70 plants. Both seed and cuttings from each of these populations is now growing to form a permanent *ex-situ* collection. This is a large step towards securing this species from extinction. We have found that, while both cutting and seed germination techniques result in plants for this species, seed germination yields the quickest (establishing root systems within 4 months) and largest number of plants (only 16% of cuttings surviving to maturity).

Standard techniques for germination include scarification and hot water (Roche et al, 1997; Loyd et al, 2000 and Offord and Meagher; 2009). With *S. acanthos* nicking of the seed, a technique sometimes employed with Fabaceae, is difficult due to the very small seeds and likelihood of damaging the embryo. Scarification did not result in germination within four weeks, while the majority of the seed treated with boiling water did and should be used as the pre-treatment for this species with future propagation. *S. acanthos* did not have a strong preference for any of the basic Fabaceae germination media used in the literature, germinating on all five media. However, a 34 % gain in seed germinated within four weeks was found using half MS and no sucrose.

Further research

While we have made significant ground in securing this species into the future, further research on the biology of *S. acanthos* and re-introduction techniques are required to remove this species from the brink of extinction in the wild. We suggest the following key areas for further research.

1. The vectors involved in the pollination of *S. acanthos* are unknown. *Sphaerolobium* in Western Australia are known to be pollinated by insects (Western Australian Herbarium, 2008). It is important as part of the translocation site selection for new populations of this species to uncover what the pollinators are of this species and if they are present at the potential translocation sites. The newly established *ex-situ* collection could be used as bait plants in the field to detect the presence of pollinators.
2. As propagation techniques are now established for this species it will be critical to fill any gaps in the seed and grown plants we have with individuals not sampled in this study. This should include investigating if there is a fitness benefit for the offspring if seed was produced between the existing populations, using the *ex-situ* established plants.
3. Establish re-introduction from seed produced and grown using the above techniques for introduction.

Adjusted Timeline

Due to the difficulty collecting sufficient seed, this project took an additional 18 months to complete. However, this allowed for an *ex-situ* population to be established with representatives in both seed and cuttings from the three largest populations Site 1, Site 3 and Site 4.

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