The Horticultural Potential of an endangered species, the Freycinet Wax Flower from Eastern Tasmania

Final report to the Australian Flora Foundation on AFF Grant 001-09

by

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Abstract

Surveys undertaken on the Freycinet Peninsula found over 100 plants of the critically endangered Freycinet Wax Flower (*Philotheca freyciana*). All plants are restricted to the Hazards, and a single plant is also known from nearby Cape Tourville. The Freycinet Wax Flower is restricted to skeletal soils derived from granitic rocks, and it often occurs in runnels and in vertical crack lines in the granitic terrain. The Freycinet Wax Flower is likely to be slow growing, although both young and mature plants were located, which indicates recruitment is occurring in the wild.

As the species is critically endangered a number of propagation techniques were attempted, including seed, tissue culturing and standard vegetation propagation, to establish an ex situ population. Standard nursery vegetative propagation methods were found to be the most effective, and as a result the Royal Tasmanian Botanical Gardens (RTBG) now holds 70 cutting-produced plants in pots from various genotypes. The project has successfully established an ex situ collection of this critically endangered species at the RTBG.

Tissue culture techniques have successfully resulted in shoot proliferation, but we have been unable to induce root initiation in explants to date. If we can successfully overcome this challenge it will become possible to explore the horticultural potential of this attractive species.

Background

The Freycinet Wax Flower (*Philotheca freyciana*) was described in 2001. This species, was at that time restricted to 3 known plants in the wild, all localised to the Hazards and Cape Tourville on the Freycinet Peninsula in eastern Tasmania. Because of its rarity and restricted distribution it was listed as critically endangered under the Environment Protection and Biodiversity Conservation Act 1999.

This study supported by the Australian Flora Foundation aimed to determine:
(a) the distribution of the species by undertaking surveys in the Freycinet Peninsula and other areas of similar habitat,
(b) commence life history studies to better understand the ecology of the species, and (c) attempt to propagate the species through a variety of propagation techniques.

a. Survey

To extend knowledge of the ecology of this species a systematic search of the Freycinet Peninsula was carried out in 2002, which located over a 100 plants in populations extending

from the summits of various peaks in the Hazards to near sea level. All plants occurred on granite outcrops, often precariously over the edge of cliffs in drip lines and vertical cracks (Fig. 1). The boulder-strewn terrain makes some areas of the Freycinet Peninsula difficult to access. Searches on granitic rocks on nearby Schouten and Maria Islands failed to find any plants of *Philotheca freyciana*.

Fig. 1. Field surveys involved clambering over the granitic rocks of Freycinet Peninsula looking for the Freycinet wax flower. The plants often occurred in cracks and along runnels in the granitic rocks. The rock strewn terrain makes some areas of the Freycinet Peninsula difficult to access. This view shows the picturesque Wine Glass Bay in the background.

From these various surveys undertaken by staff from the Tasmanian Herbarium, Department of Primary Industries, Water and Environment and the Royal Tasmanian Botanical Gardens it was concluded that the species is restricted to the Hazards on Freycinet Peninsula, with plants occurring on Mt Mayson, Mt Amos, Mt Dove and Mt Parsons with a single plant occurring at Cape Tourville. The results of the surveys would support the species retaining its status as being critically endangered.

b. Life History Studies

From the largest population (of about 50 plants), on Mt Mayson, 35 were selected to begin a study of growth and development. All plants were measured for height, width of plant, stem diameter at ground level (Fig. 2), and observations were made as to whether the plants were in bud, flowering, or post anthesis.

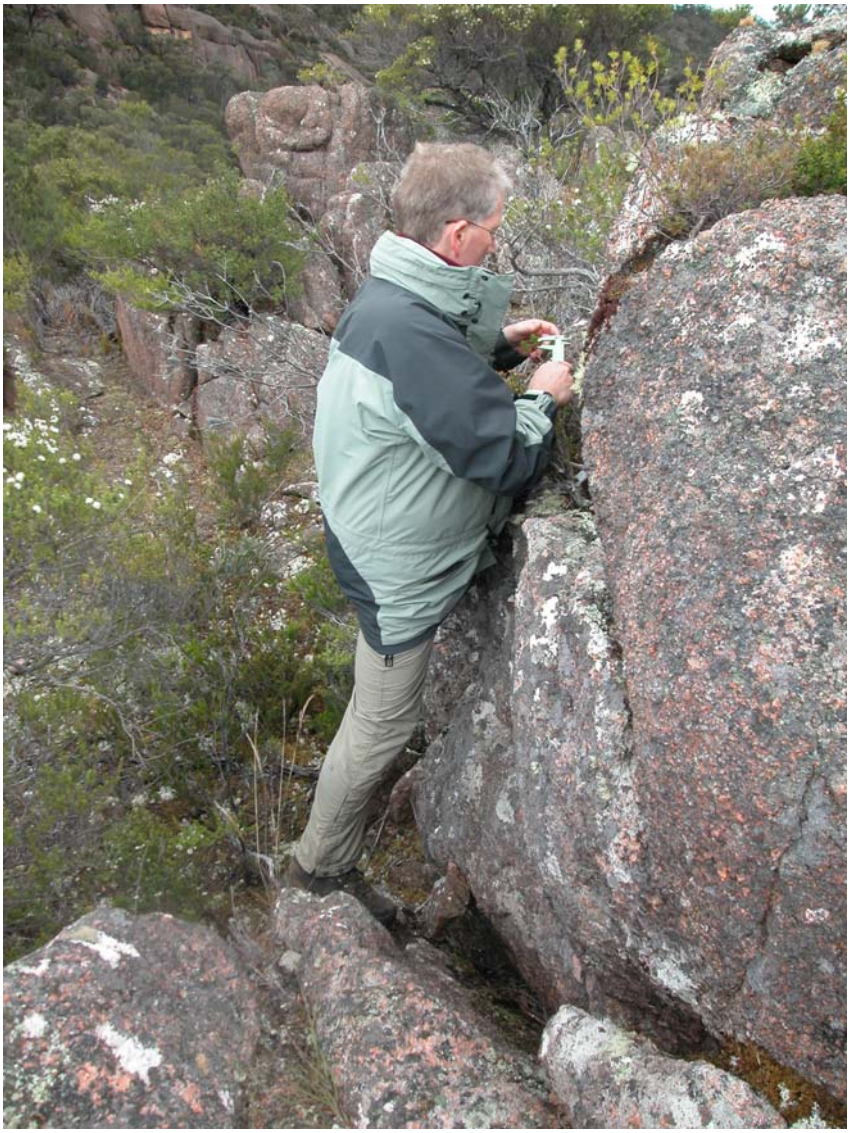

Fig. 2. Measuring stem diameter of Freycinet Wax Flower plants at Mount Mayson.

The plants are generally spindly, consisting of a main stem and are often sparingly branched. The population included young plants, 4 cm in height, through to large, well established plants that were up to 90 cm in height. Resprouting from older stems that had been damaged or eaten by herbivores was observed (Fig. 3). The stems in larger specimens of the plant are up to 1.3 cm in diameter.

Fig. 3. Young leafy shoot of the Freycinet Wax Flower at Mount Mayson, resprouting off an older stem.

The limited observations suggested that the Freycinet Wax Flower was slow growing in the wild. General observations also suggest that the plant has an extended flowering period. The plants were visited by generalist pollinators such as beetles and small flies (Fig. 4).

Fig. 4. Flowering specimen of the Freycinet Wax Flower. Two species of beetles and small flies were seen visiting flowers.

It was hoped the basic data might give an indication of how this plant would perform in a horticultural setting. Unfortunately circumstances only allowed for initial parameter measurements and a follow up survey of six months later.

c. Propagation of *Philotheca freyciana*

Rationale of Propagation Trials

The Freycinet Wax Flower is a compact shrub commonly less than 60 cm in height with attractive 5-petalled white flowers which are pink in bud. It aesthetically has good horticultural potential but at the time of the study the practicality of propagation was unknown.

Vegetative Propagation – cuttings

Cutting material from the two populations at this site was collected in October 2002 and propagation trials have now been completed.

Since this is the first attempt at cutting propagation in this species, a range of wood types was trialled to assess the most suitable. In general, softwood and semi-hardwood cuttings were quite successful, using standard RTBG cutting mix, following treatment with the commercial rooting hormone Clonex Purple™ (Table 1).

Less success was achieved with the hardwood cuttings, but this increased after wounding and treatment with the same rooting hormone (Fig. 5). Until rooting was established, all cutting material was initially held on a heat bed in conjunction with a misting system.

Fig. 5. Cuttings of the Freycinet Wax Flower in standard RTBG native potting mix.

Rooting in some cases was well established about 8-10 weeks after initial propagation and the plants were then potted on, using standard RTBG native potting mix.

The success rate of this method of vegetative propagation would indicate that it is a suitable tool for the *ex-situ* management of this threatened species. The preferential use of softwood and/or semi-hardwood cuttings would probably increase this success rate, and may be the optimal route for commercial production. Seasonal timing of the material may be a critical factor here.

Table 1. Vegetative Propagation Trials

Acc. No	Cutting Type	Cutting Date	Potting On	Tubes July 04
02.0035	7 soft 6 hard	22/1/02	4/9/02	1
02.0042	4 semi-hard 4 soft	22/1/02	20/3/02	3
02.0043	9 semi-hard	22/1/02	20/3/02 3/9/02 12/11/02	1 1 1
02.0496	Soft Semi-hard	28/8/02	23/10/02 12/11/02	9
02.0625	23 soft clonex 30 semi-hard ezi-root	21/10/02 14/1/03	24-25/6/02 6/11/03	21
02.0691	27 semi-hard	11/12/02	26/5/02	6
02.0692	18 semi-hard	11/12/02		7
03.0023 xRTBG	7 semi-hard	14/1/03	24/6/03	6
03.0051 xRTBG	12 semi-hard	29/1/03		10
03.0254	3 semi-hard	7/10/02	28/3/03	1

Propagation from stock plants in the Nursery (03.0023, 03.0051) was the most successful. This would be due to the fact that the stock material was never stressed and cutting material was available for propagation immediately after cutting. In the field the cutting material is wrapped in a moist bag but often it is stored in a fridge or esky for several days before it is propagated from. Once a good stock collection was established, production would be relatively straight forward. Most of the plants flowered in their pots within the first year of production. Four plants have been planted out at the RTBG in dolerite soil and their progress will be monitored.

Seed Propagation

At this stage, seed propagation has had limited success with the main problem being the difficulty in obtaining enough material. In 2002 seed was collected from 3 locations – Cape Tourville (1 genotype) Mt Amos (3 genotypes) and Mt Mayson
(2 genotypes). In total, only 17 seeds were available, and in November 2002 these were treated with boiling water, and allowed to soak overnight. They were then sown in native seed raising mix, placed in a glasshouse on a hot bed.

After 10 weeks there was no sign of activity, but eventually two of the seeds germinated after about 12 weeks, one subsequently survived and continues to grow well.

Clearly, much more seed was required so that further germination trials under different conditions can be undertaken. With this in view, 10 suitable plants were bagged in two populations on Mt Mayson (December 2002) and these were retrieved in May 2003. Only 16 seed were collected; although too few for a statistically significant trial the following treatments were carried out in June 2003.

Germination trials – each treatment was undertaken for 14 days.

1) N=5 Leaching: in calico bag suspended in toilet cistern.

2) N=6 Wet heat: seeds soaked in boiling water.

3) N=5 Smoke / water: soaked in 1:10 Regen Smokemaster.

Seed were then sown in native seed raising mix and placed in a glasshouse on a hot bed. No germination occurred and the sown seed mix was discarded after 1 year.

Tissue Culture

As tissue culture of this species had not been previously attempted, a preliminary trial, using a related species - *P.verrucosa* - was undertaken, in a standard MS tissue culture medium (Murashige & Skoog, 1962). This produced satisfactory results, so in December 2002 fresh material was collected from Mt Mayson. Two populations were sampled – 8 mixed genotypes from the eastern site and 5 mixed genotypes from the west.

In this trial, a standard MS culture medium was used (Table 2) but with differing concentrations of hormones and auxins (Table 3, Fig. 6). Eight treatments were used, with one control, the variables being the concentration of NAA, IAA and BAP, keeping the medium at a uniform pH of 5.8. This experiment, begun in early December 2002, used 180 explants, with a standard light/dark regime at a controlled temperature. Due to the nature of some of the collected material, a fairly high loss rate was anticipated, and to some extent this occurred, with death and contamination affecting some 60% of the cultures.

Fig. 6. Tissue culturing of the Freycinet Wax Flower using various media.

Table 2. Multiplication Media
Multiplication and elongation medium *Philotheca freyciana*

Components	**mg/L**
INORGANIC SALTS	
Macronutrients	
$NH_4 NO_3$	**1650.00**
KNO_3	**1900.00**
$CaCl_2 . 2H_2O$	**440.00**
$MgSO_4.7H_2O$	**370.00**
KH_2PO_4	**170.00**
Micronutrients	
KI	**0.83**
H_3BO_3	**6.20**
$MnSO_4.4H_2O$	**22.30**
$ZnSO_4.7H_2O$	**8.60**
$Na_2MoO_4.2H_2O$	**0.25**
$CuSO_4.5H_2O$	**0.025**
$CoCl_2.6H_2O$	**0.025**
Na_2.EDTA	**37.30**
$FeSO_4.7H_2O$	**27.80**
VITAMINS	
myo-Inositol	**100.00**
Nicotinic Acid	**0.50**
Pyridoxine HCl	**0.50**
Thiamine HCl	**0.10**
OTHER COMPONENTS	
Glycine (Free Base)	**2.00**
Sucrose	**30000.00**
Agar Agar	**7000.00**
Ph	**5.8**
HORMONES	
BAP	**1.0**
IAA	**0.1**

Table 3. Treatments

BAP (μm)	IAA (μm)	NAA (μm)	pH	Tube Colour
1	0.1		5.84	Brown (B)
1	0.6		5.84	Blue (Bl)
1		0.1	5.78	Pink (P)
1	0.1	0.6	5.80	Orange (O)
10	0.6		5.80	Red (R)
10			5.78	Silver (S)
10		0.1	5.83	Green (G)
10		0.6	5.80	Mauve (M)
Control -	-	-	-	Yellow (Y)

Contamination

The overall contamination/death rate was:

19/12/02	8 days post tube up	31%
6/1/03	26 days post tube up	54%
16/1/03	36 days post tube up	60%

Initial Proliferation

Proliferation at 36 days of the remaining 40% (72 tubes) was:

Callus proliferation	27 tubes
Shoot proliferation	17 tubes
No proliferation	20 tubes
Proliferation ambiguous	8 tubes

Shoot Explants Suitable for Rooting Trials

At 4 months (16/4/03) post tube-up, 33 tubes were considered to have shoot material suitable for root development trials (Table 4). It was clear that shooting was more successful in some media than others.

Table 4. Effect of hormone combinations on propagation of Freycinet Wax Flower in tissue culture

BAP (μm)	IAA (μm)	NAA (μm)	Tube Colour	Tubes (# explants)
1	0.1		Brown (B)	8 (13)
1	0.6		Blue (Bl)	8 (62)
1		0.1	Pink (P)	3 (8)
1	0.1	0.6	Orange (O)	4 (8)
10	0.6		Red (R)	-
10			Silver (S)	-
10		0.1	Green (G)	1 (4)
10		0.6	Mauve (M)	4 (11)
Control -	-	-	Yellow (Y)	6 (11)

Originally explants were added to 20 tubes of each medium and the control. The 1μm BAP mix was the most successful with all variations on this having proliferated shoot material suitable for the rooting trials. Eight tubes each out of 20 of the IAA mix were suitable but the 0.6 μm IAA produced enough material for 62 explants to be potted on into the rooting media and the 0.1 μm only produced 13. The 1μm BAP /0.6 μm IAA media clearly carried the highest success rate for production of viable shoot material.

Root Proliferation 16/4/03

For the root proliferation trial solutions of MS and ½ MS media were made up with replicates of the same hormone combinations added to each. There was adequate material for 15 tubes of each of the 8 treatments. Tubes were placed in a standard light/dark regime at a controlled temperature and monitored weekly for root growth. (Table 5).

Table 5. Treatment combinations used for rooting trials on explants from tissue culture

Media	Hormones	pH	Tube Colour	Proliferation
MS	60 μm 2,4-D	5.83	Pink	-
MS	10 μm 2,4-D	5.79	Green	-
MS	30 μm NAA 0.03 μm BAP	5.85	Red	-
MS	5.71 μm IAA 4.9 μm IBA	5.83	Yellow	-
1/2 MS	60 μm 2,4-D	5.81	Orange	-
1/2 MS	10 μm 2,4-D	5.81	Purple	-
1/2 MS	30 μm NAA 0.03 μm BAP	5.82	White	-
1/2 MS	5.71 μm IAA 4.9 μm IBA	5.82	Blue	-

No root proliferation had resulted in any of the media after 2 months and a second trial was undertaken using shoot material derived from the first trial. This trial used 25 tubes of each of the 4 treatments below (Table 6).

Table 6. Treatment combinations used for rooting trials on explants from tissue culture 16/6/03

Media	Treatment	pH	Tube Colour	Proliferation
1/2 MS	activated charcoal	4.00	Purple	-
1/2 MS	9.8 μm IBA 2 wk dark	3.99	Black	-
1/2 MS	9.8 μm IBA	4.00	White	-
1/2 MS	control	4.02	Green	-

Running in parallel with the above 4 treatments, 20 explants from the first rooting trial were treated with Clonex Purple rooting hormone gel and planted in standard cutting mix in the RTBG Nursery on 23/6/03. Five were potted up on 30/8/04 in native tube mix and as of 11/7/05 one survives.

Again there was no root development *in vitro* with any of the above treatments.

Maintenance of Shoot Explants in Tissue Culture

All surviving material was tubed into standard MS media up in March 2003 without added hormones. These have been successfully maintained *in vitro* as shooted explants to now (Fig. 7). A trial using *in vitro* soil medium (Newell *et al.* 2003) was undertaken using approximately 100 explants on August 2nd 2005 in an attempt to generate root proliferation. It is too early to gauge success at this stage.

Fig. 7. Freycinet Wax Flower shooted explant.

Discussion

From the results of the various propagation methods that have been trialled thus far it is clear that from a practical point of view standard nursery vegetative propagation methods are the most effective. Currently the RTBG holds 70 cutting-produced plants in pots (Fig. 8). A limited number have been planted out in a garden bed at the RTBG but they are not thriving probably due to inappropriate edaphic conditions. They are planted in a dolerite clay but their natural habitat is an open granite sand or in drip cracks in the rock.

Fig. 8. One of the successful cutting-produced plants.

Propagation from seed was unsatisfactory in part due to the difficulty in obtaining material in the wild. Of the 2 seeds that germinated one did not get past the first leaf stage but the other still survives. It may now be feasible to collect seed from nursery stock plants as it is not justifiable to continue to collect from a small population of wild plants.

Although shoot proliferation has been achieved in tissue culture the stock is from a more restricted range of genotypes and while suitable for horticultural production, a wider range of genotypes would be needed for conservation outcomes. However, the initiation of root development is yet to be achieved. Although experimental work is continuing if this proves unsuccessful then further propagation should be undertaken using standard nursery procedures. If improved propagation results can be achieved it will be possible to explore the horticultural potential of this attractive species.

References

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