CULTIVATION OF *ORITES MYRTOIDEUS* – THE FIRST 12 MONTHS

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ABSTRACT

Orites myrtoideus (Poepp. & Endl.) Engl. is an evergreen shrub in the family Proteaceae, endemic to the Andes of both Chile and Argentina. With a small distribution and increasing risks, direct conservation action has been recommended. *Ex situ* cultivation and subsequent translocation of populations may be an option for the conservation of this species. In recent documented history this species has been grown *ex situ* on only a small number of occasions. One plant was grown outside at Benmore Botanic Garden for a period of five years and another has been grown at the Royal Botanic Garden Edinburgh as part of the Arid Lands collections. A better understanding of its cultivation requirements has been deemed necessary before *ex situ* collections can be established. This study aims to give a broad overview of the germination and first 12 months of cultivation of this taxon with the aim of informing future *ex situ* cultivation and subsequent restoration initiatives.

INTRODUCTION AND CULTIVATION HISTORY

Orites myrtoideus (Poepp. & Endl.) Engl. (Figs 1 & 2) is known in its native Chile by the name 'radal enano' and has previously had the following synonyms: *Embothrium myrtifolium* Poepp. ex Meisn., *Lomatia alpina* Phil., *Lomatia chilensis* Gay., *Roupala myrtoidea* Poepp. & Endl., *Tricondylus chilensis* (Gay) Kuntze. and *Orites mytoidea* (Poepp. & Endl.) Engl. (IPNI (2015)).

First collected and described by Eduard Friedrich Poeppig in 1829 under the name *Embothrium myrtifolium*, it is one of only two species of *Orites* to occur in South America, the other seven coming from mainland Australia and Tasmania. The type specimen is held in the Missouri Botanical Garden Herbarium, with further specimens held at the Royal Botanic Gardens, Kew (RBG, Kew); the Royal Botanic Garden Edinburgh (RBGE); the Gray Herbarium, Harvard University; the Muséum national d'Histoire naturelle in Paris; and the Berlin-Dahlem Botanical Garden and Botanical Museum. Interestingly, there are no historical herbarium collections at RBGE of this species even though most other early 19th-century herbarium collections from Chile are held here.

Although the plant hunter William Lobb, employed by the Veitch Nurseries, and the botanist and gardener Harold Frederick Comber both collected within its range and habitat, the taxon has only twice been successfully introduced to cultivation in

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Fig. 1 *Orites myrtoideus* (Poepp. & Endl.) Engl. (Nickrent *et al.*, 2006–). Photo: P. Pelser (Phytoimages, 2010).

the UK; both successful introductions have been made by RBGE. The first of these, ICE (Brownless, Gardner & Maxwell) 466, was collected from east of the bridge near Pangue, on the road to Parque Nacional de Laguna del Laja in Chile, and led to a plant (accession 19961648) which survived for a period of five years outside at RBGE's Benmore Garden. A second plant (accession 20120392), from seed collected by P. Baxter & M.F. Gardner No. 115 on 31 March 2012, survives and is held at RBGE.

Accession 19961648 was planted on the Benmore Garden's Chilean Hillside in 2005 but died in 2010. Many seedlings from the same seed collection are reported to have germinated and some were distributed to other gardens, but they gradually died over a period of three to four years.

A third introduction to cultivation has been made at the University of California Botanical Garden at Berkeley but no further information regarding this introduction is known (PlantSearch, 2017).

DESCRIPTION OF THE PLANT AND DISTRIBUTION

An evergreen shrub 0.6 to 2 m tall, often low and spreading, and forming relatively large thickets. Leaves coriaceous, 1.8–3.5 × 0.6–1 cm, petioles 2–4 mm long, oblong to

Fig. 2 Seedhead of *Orites myrtoideus*. Photo: M. Gardner.

elliptical or oblong lanceolate, margin entire, apex obtuse or with a short point. Flowers in dense racemes of 1.5–2.5 cm long, arising from leaf axils: winter and spring. Fruits 1.6–2 × 0.6–0.8 cm, ellipsoid, woody, hairless and cinnamon-coloured: maturing from February to March (Gardner *et al.*, 2006).

Orites myrtoideus has an area of distribution covering a north–south distance of 268.6 km along the Andes mountains from 35°57′00″S to 38°00′00″S. It is found in both Chile and Argentina; however, only one population is known from the latter. In Chile it is restricted to fewer than 15 locations where it inhabits the alpine zone between 760 and 2,100 m in altitude, colonising volcanic substrates. In three localities it is found on rocky outcrops below the treeline and associates with *Nothofagus* spp. in two of these locations and *Araucaria araucana* in the third (Gardner *et al.*, 2006). Fig. 3 shows the distribution of the species.

CONSERVATION STATUS

Orites myrtoideus has not had a recent formal assessment of its conservation status and the Endangered status given is based on a provisional evaluation. This evaluation categorises *O. myrtoideus* as Endangered using the criteria laid out by the International Union for the Conservation of Nature (IUCN) Red List version 3.1 (IUCN, 2012).

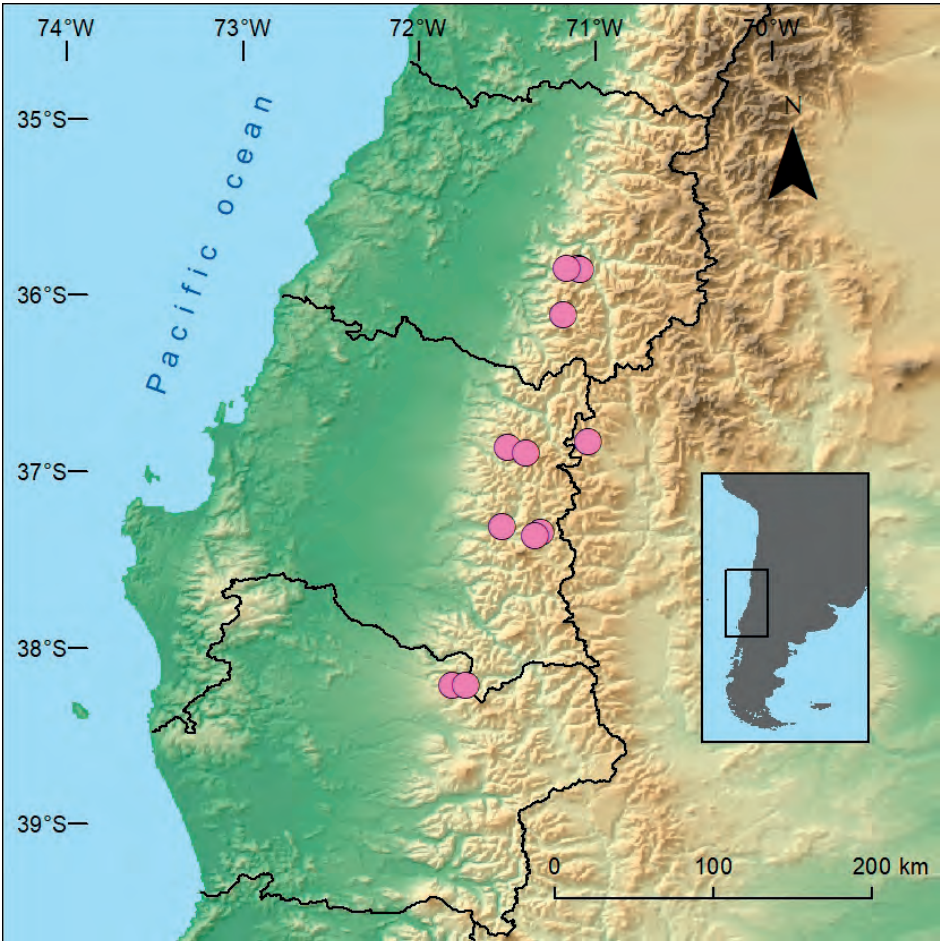

Fig. 3 Map of Chile showing the distribution of *Orites myrtoidea* indicated by purple dots. Map drawn by Vanezza Morales.

The Endangered B2ab(i,iii,v) assessment was based on *Orites myrtoideus* having (B2) an area of occupancy estimated to be less than 500 km^2 in addition to the following characteristics:

a. Having a severely fragmented population or being known to exist at no more than five locations.
b. A continuing decline, observed, inferred or projected, in its (i) extent of occurrence, (iii) area of occupancy and (v) area, extent and/or quality of habitat (IUCN, 2012).

The 1997 IUCN Red List of Threatened Plants assessed the species as Rare due to a small population that was not at the time Endangered or Vulnerable but was seen to have potential future risk. The largest populations are protected within three national parks, but subpopulations of this species have been severely affected by development during the past twelve years. Road building and flooding for hydroelectric dam construction have caused the most notable declines. Recent evidence shows that a current increase in development for hydroelectric schemes will impact this species further (Martin Gardner, pers. comm.).

A recommendation has been made (Gardner *et al.*, 2006) that this species be afforded some level of protection, that subpopulations be monitored closely and that before *ex situ* collections can be established there needs to be a better understanding of its cultivation requirements.

GERMINATION

Seed of *Orites myrtoideus* was acquired from two separate sources during 2017 (Fig. 4). One hundred seeds came from ChileFlora (www.chileflora.com) and a further 160 seeds from the International Conifer Conservation Programme (ICCP) Chile expedition, collected by Peter Baxter, Tom Christian and Martin Gardner, Expedition No. 77, at coordinates -35°51′6.84″S, 71°9′51.71″W and at an altitude of 793 m on 15 February 2017.

Identifiers were assigned to each accession of seed.

Baxter, P., Christian T. & Gardner, M.G. No. 77 – RBGE77

Chile Flora – CF001

Fig. 4 Photograph of seed of *Orites myrtoideus.* Photo: R. Blackhall-Miles.

An informal experiment was set up where multiple batches of seed from each accession were prepared and given individual identifiers (Table 1). Each batch was then treated according to one of the following germination temperature regimes:

1. 12 hours of 5°C at night and 12 hours of 21°C during the day, using a temperature-controlled unit with a day/night fluctuation of 16°C.
2. Constant 21°C bottom heat in a propagator.
3. Room temperature, approximately 18°C.

The remainder of the seed was stored for future research.

Three different seed soak treatments were used to break dormancy:

1. Gibberellic acid (GA_3), as used previously for this purpose with *Orites myrtoideus* (Gardner *et al.*, 2006).
2. Smoke water solution (Kirstenbosch Smoke Primer) (Duncan *et al.*, 2013) and 1 per cent hydrogen peroxide (H_2O_2) (Brits, 1986).
3. Water.

After treatment all seed was sown onto the surface of sterilised fine-milled pine bark and kept in sterilised and sealed transparent food standard tubs so that any sign of germination could be readily observed.

Germination was deemed as having been achieved at the stage of radical production and at this point seedlings were pricked out into individual 100 g, food standard, white polystyrene pots (Table 2). Pricking out at this stage was done to limit the potential negative impact to the very fine root systems of *Proteaceae* seedlings (Duncan *et al.*, 2013) (Fig. 5).

SEEDLING MORTALITY

An elevated level of mortality was noted, with the majority dying within a few weeks of being pricked out (Table 3). Mortality seemed to be linked to root rot possibly caused by damage done during pricking out yet, as this did not take place immediately

CF001.01	Treated with GA_3 for 24 hours and kept in day/night variable temperature unit.
CF001.02	Treated with smoke water for 24 hours and H_2O_2 for a further 24 hours before being kept in a heated propagator at 21° C.
CF001.03	Treated with water for 24 hours and subsequently kept at room temperature, 18°C.
RBGE77.01	Treated with GA_3 for 24 hours and kept in day/night variable temperature unit.
RBGE77.02	Treated with GA_3 for 24 hours and kept in heated propagator at 21°C.

Table 1 Batch treatments and germination conditions.

Accession and batch	Number of seeds sown	Number germinated	% germination
CF001.01	30	3	10%
CF001.02	30	0	0%
CF001.03	20	0	0%
RBGE77.01	30	14	46.67%
RBGE77.02	30	0	0%

Table 2 Germination rates.

Fig. 5 *Orites myrtoideus* seedling. Photo: R. Blackhall-Miles.

Accession and batch	Number germinated	Mortality count	Mortality rate
CF001.01	3	1	33.33%
RBGE77.01	14	9	64.29%

Table 3 Mortality rates.

after pricking out, other factors cannot be ruled out. Proteaceae are known to suffer significant mortality during the earliest stages of growth, with the main factors being drought (Midgely, 2010), heat (Blackhall-Miles & Ram, 2016) phytophthora, botrytis and pythium (Duncan *et al*., 2013).

SUBSTRATE CHOICE

Seedlings were initially pricked out into individual 100 g, food standard, white polystyrene pots into a compost mix of 25 per cent washed sharp sand, 25 per cent horticultural grit, 25 per cent 5 mm pumice and 25 per cent high grade (Melcourt) fine composted pine bark (Fig. 6). Reduced organic matter was chosen for the seedling compost as organic matter can have elevated levels of phosphate (Blackhall-Miles & Ram, 2017) and can reduce drainage, leading to fungal infection (Duncan *et al.*, 2013). The addition of pumice reduced the weight of the compost and increased water retention in a way that would not lead to infection or anaerobic conditions at the root zone. Pumice served a secondary role in providing mineral nutrients to the seedlings through the ability of Proteaceae to produce organic carboxylates at the root tip to access nutrients from rock (Blackhall-Miles & Ram, 2017). Given the volcanic natural environment of *Orites myrtoideus* other volcanic rocks could have been used, such as basaltic scoria.

The decision to use white polystyrene pots was to maintain a steady temperature at the root zone (Blackhall-Miles & Ram, 2016).

Fig. 6 White 100 g polystyrene pots used for seedlings, showing compost mix. Photo: R. Blackhall-Miles.

SUBSEQUENT CULTIVATION

Seedlings were initially grown under controlled conditions with a day/night temperature range of 23°C/13°C with a light level of 1,300 lux and a UVA reading of 25 $\mu W/cm^2$ also on a 12-hour day/night cycle. Air movement was increased over the seedlings using a computer fan with airflow of 0.8 cubic metres per minute to reduce the risk of damping off.

Once the seedlings had produced a minimum of two true leaves (Fig. 7) they were moved to a cold frame to be hardened off for a period of one month before being transferred to a polytunnel. All pots were kept abutted to one another in this polytunnel, whose sides were not rolled down past the level of the tops of the pots. This was to reduce the impact of sunlight heating the root zone (Blackhall-Miles & Ram, 2016). As roots were observed at the drainage holes of the polystyrene seedling containers they were potted on into 1-litre pots. Two plants were sunk directly into larger pots without being removed from the white polystyrene seedling pots, a technique we will call 'pot in a pot'. One of these larger pots was made of terracotta and the other of black plastic (Fig. 8). The remaining five plants were potted into black plastic pots by removing them from their polystyrene seedling pots. The compost mix was the same as that used at pricking out, with the addition of various nutrient additives (see the next section on irrigation and fertiliser). At 12 months from germination the 'pot in a pot' plants had visibly grown more than the others and were showing roots at the drainage holes (Fig. 9). The 'pot in a pot' technique is one used by alpine plant enthusiasts to simulate the root restriction that occurs when plants grow naturally in rock crevices. This may also be a valuable aid to avoid overwatering.

IRRIGATION AND FERTILISER

At the potting-up stage, in addition to the standard compost mix (used for pricking out) dried blood, iron sulphate, potassium sulphate, ground seaweed meal and a proprietary micronutrient feed were added, at half the suggested rate of dosage.

Hand watering has been the only irrigation system employed. This normally takes place at the end of the day, first thing in the morning or during cool weather to reduce the potential threat of phytophthora (Kueh, 2011). Liquid fertiliser has been applied fortnightly throughout the warmer months on rotation with sequestered iron, iron sulphate, potassium sulphate and magnesium sulphate, again at half the suggested rate of dosage. Many Proteaceae species are widely known to suffer if supplied with phosphate, so at no point has additional phosphate been applied (Delgado *et al.*, 2014).

PESTS AND DISEASES

The main pest and disease problem noted for this species so far has been the root rot observed at seedling stage. Botrytis has occurred in the growing tip of seedlings when ventilation has not been sufficient and the air humidity has been high, causing mortality

Fig. 7 Seedlings: (A) first true leaf stage and (B) two true leaves ready to be potted on. Photo: R. Blackhall-Miles.

Fig. 8 Plants with restricted roots in terracotta and plastic 1-litre pots. Photo: R. Blackhall-Miles.

in two cases. Tortrix moth (*Epiphyas postvittana*) caterpillars have also proven to be problematic for this species but have so far not caused any mortality.

CONCLUSION AND RECOMMENDATIONS

This study has attempted to show the horticultural experiences of the initial stages of growth of *Orites myrtoideus,* outlining informal experiments undertaken to that end.

Temperature variation and treatment with the growth hormone GA3 has resulted in the greatest germination success of *Orites myrtoideus*. No germination success has been observed from the traditional smoke water and H_2O_2 method or from soaking them in water. Limited germination has been achieved with the bought seed (batch CF001.01) treated with GA_3. This may be due to factors including the age of the seed, its storage and the seed collection locality. As no further information is known about this accession the germination result cannot be better understood. The 46.67 per cent germination success of batch RBGE77.01 is reflective of the typical germination rate of many Proteaceae species (Blackhall-Miles & Ram, 2016).

Mortality has been a major issue during the early stages of seedling development with a combined mortality rate of 53 per cent. The majority of seedling mortality

Fig. 9 Comparison of plant with restricted roots (right) and unrestricted roots (left). Photo: R. Blackhall-Miles.

was observed during the first month after germination. Two seedlings died at a later stage, after true leaves had emerged. Air circulation, air humidity, soil temperature and watering impact the development of seedling disease and thus trials should be done regarding these areas. Suitable fungicide for both pythium and botrytis could be used to reduce the occurrence of infection.

The importance of nutrition during the initial stages of seedling development has not been considered for this species; however, there is evidence to suggest that Proteaceae seedlings survive on cotyledon reserves for the first 80 to 120 days of development and thus additional nutrient supplementation may not be required (Stock *et al.*, 1990). Given the volcanic and phosphorus-rich natural environment of *Orites myrtoideus*, there may be reason to expect this species to have high demand for mineral nutrition and some phosphorus during its earliest stages of growth (Delgado *et al.*, 2014). Future experiments to investigate the importance of varying levels of nutrition at seedling stage should be conducted to understand its impact on seedling mortality and growth rate.

The 'pot in a pot' technique has shown increased vigour in both plants grown in this manner. This technique has also worked with other members of the Proteaceae that come from rocky habitats (Blackhall-Miles & Ram, unpublished research) and more research is required to better understand the relevance of this to growth in *Orites myrtoideus*. Any further experiments should try to understand if this effect is due to root constriction or better moisture regulation and be conducted over a much larger sample size.

Whilst its home country Chile is actively increasing the land area protected by national parks it is also increasing its road network and hydroelectric power industry: the two greatest threats to *Orites myrtoideus*. *Ex situ* cultivation and future restoration could act as a lifeline for this species. Certainly, more work needs to be done to better understand the cultivation requirements of this threatened member of the Proteaceae.

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