Propagation of *Lomatia tasmanica*©

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***Lomatia tasmanica* W.M.Curtis is a critically endangered endemic plant that is restricted to southwest Tasmania. The single population is made up of less than 500 stems from a triploid clone (Lynch et al., 1998) originating at least 43,600 years ago (Jordan et al., 1991), and as such may be the oldest living plant in the world. The Royal Tasmanian Botanical Gardens has worked in collaboration with the Threatened Species Section of the Nature Conservation Branch since 1994 to secure an ex-situ conservation collection currently being held at the Gardens.**

INTRODUCTION

The genus *Lomatia* of the family Proteaceae, has a southern hemisphere distribution with nine species occurring in Australia and three in South America (Wilson et al., 1995). Of the Australian species, three are endemic to Tasmania, two of which are widespread throughout the state, and one, *L. tasmanica*, that is restricted to a single population of less than 500 stems in the southwest. At the state level, it is listed as endangered under the Tasmanian Threatened Species Protection Act 1995 and federally as critically endangered under the Commonwealth Environment Protection and Biodiversity Conservation Act 1999.

Its common name, King's lomatia, is derived from tin miner and local naturalist, Deny King, who first found the species growing in 1934 (Lynch and Balner, 2004). It was only formally described by Dr. Winifred Curtis of the Tasmanian Herbarium in 1967, 2 years after the King family collected a flower from the wild. It occurs along creek lines in mixed forest with a canopy of *Eucalyptus nitida* (Smithton peppermint) over rainforest elements such as *Nothofagus cunninghamii* (myrtle) and *Phyllocladus asplenifolius* (celery top pine) with an understorey of sclerophyllous shrubs, ferns, and mosses.

Lomatia tasmanica grows as a shrub or small tree of 5 to 8 m in height with broad leathery divided leaves and produces clusters (racemes) of waxy burgundy flowers at the ends of the branches (Fig. 1). In cultivation at the Royal Tasmanian Botanical Gardens (RTBG) flowering generally takes place in December, plants take up to 7 years to bloom, and do not produce flowers every year. In the wild, mature plants are leggy; some with long thin stems with an umbrella-like clump of leaves at the apex, due to the lack of light under the canopy, but those at the Gardens are multi-branched due to repeated cutting for propagation material.

Figure 1. Flower cluster of *Lomatia tasmanica.*

Mature fruit and seed have never been found in cultivation or the wild and this is most likely due to the triploid nature of the clone, making it sterile. Lynch et al. in 1998 found no genetic diversity in a study of 78 plants from the population. Investigation of the chromosome number showed it to have 33 chromosomes compared to the normal diploid number of 22 found in other lomatia and the Tasmanian species, *L. tinctoria,* also investigated in the study. Fossil leaves of *L. tasmanica* found in southwest Tasmania were dated at 43,600 years old (Jordan et al., 1991) and given that the plant is a clone and has only been able to reproduce vegetatively by rhizome, it is possibly the oldest living plant in the world.

Like many members of the Proteaceae family, *L. tasmanica* is susceptible to *Phytophthora cinnamomi* root rot and the disease has been found within 20 m of the first plant in the wild population (Tim Rudman in Threatened Species Section, 2006). This was evident in a field trip the RTBG participated in 2005 to collect propagation material to build up the ex-situ collection. There were many dead and dying banksias across the buttongrass plain adjacent to the wild population and standard practice by government agencies and researchers entering the area are to use a footbath to disinfect shoes and equipment before entering the area.

Another threat to the species is an increased incidence of fire (Lynch and Balmer, 2004). *Lomatia tasmanica* will regenerate vegetatively following fire and many of the extant plants date from a 1934 fire, however some stems predate this (Lynch and Balmer, 2004). An increase in the incidence of firing may not allow the population to re-establish between fire events, and an increase in the ferocity of fire could result in the death of all stems. The population is restricted to an area of only 1.2 km (Lynch et al., 1998), and fire would also open up the vegetation, increasing the chance of infection by *Phytophthora* root rot at the site (Podger et al., 1990).

A further threat to the future survival of *L. tasmanica* is climate change. Jayne Balmer (Balmer, 2009) surveyed the population in both 1993 and 2008 and noted a decline in stem numbers. Given the difficulty of surveying in the poor light and tangled understorey below the rainforest canopy, Balmer found there was a real decline in numbers of between 18% and 32% over the 14-year period. Loss was mainly in the small stems. Possible causes suggested were reduced light level as the rainforest canopy had closed over following the last major fire in 1934, build up of litter, and wood debris and competition from ground storey species. Analysis of the rainfall data for the period showed that rainfall in the 14 years previous to the study at the nearest weather station at Maatsuyker Island was almost 500 mm higher per year than during the study period. The experience in cultivation at the RTBG has shown that *L. tasmanica* is extremely susceptible to changes in moisture and humidity, with rapid death following display indoors. Balmer (2009) noted loss in the wild population was in loose clusters, rather than across the area surveyed, and suggested that this sensitivity to changes in moisture and humidity may have led to the decline in the wild with the decreased rainfall.

In-situ protection of the population is of highest importance as outlined in the Department of Primary Industries and Water Flora Recovery Plan 2006-2010, but the establishment of an ex-situ collection provides some insurance against possible loss in the wild. The RTBG has worked in collaboration with the Threatened Species Section of the Nature Conservation Branch with the aim of securing a conservation collection of 50 plants. The first material for this target was collected by RTBG staff member Mark Fountain from the wild population in 1994. Propagation, given the failure of the species to produce seed, was from cuttings, and this has been the main method of producing new plants to the present day.

Over time cutting propagation has been refined and semi-hardwood tip and stem cuttings dipped with Clonex Purple® have been found to produce the best results. Cuttings are generally taken in January and February and placed in a perlite and washed river sand (1 : 1, v/v) mix and placed under mist, raised slightly above bottom heat. They can take up to 12 months to root and have a strike rate of between 60% and 80%. Leaf cuttings have been trialled, and while they produce roots, only one has ever produced a shoot. Whilst the strike rate is reasonably good, *L. tasmanica* resents root disturbance, and major losses occur when potting on. To overcome this, in part, the cuttings are originally place in individual potting cells, rather than punnets. Problems still exist when potting on to tubes and larger pot sizes and this combined with the sensitivity to changes in moisture and humidity make it very difficult to maintain in pot culture. An example of the sensitivity to root disturbance can be seen in the loss of the last plant propagated in 1994, which survived potting on over time to a 20-in. pot, but died following disturbance to the surface soil to remove a coating of liverworts in 2007. At present the RTBG holds 46 pots of varying sizes in the nursery under shade, the oldest surviving from propagation in 1998 is about 90 cm tall.

As well as being tricky in pot culture, *L. tasmanica* has also proven even more difficult to maintain in the ground. Only specimens that have been grafted on to a rootstock of another Tasmanian species, *L. tinctoria*, have survived in the ground. The grafting technique was developed by local nurseryman Ken Gillanders who has used a side wedge graft. Ken has worked with RTBG propagator Lorraine Perrins over the past few years to produce plants that can hopefully be used as in-ground

stock plants. Grafting is done in February with cuttings of 3 to 4 buds of both species. The graft union is placed below the mix, which is the same as used for traditional cuttings. One of the problems has been matching the size of the scion and rootstock and the RTBG will trial the Chilean species *L. ferruginea*, which is as robust as *L. tasmanica*, next year.

A major problem in securing an ex-situ conservation collection of *L. tasmanica* has been the paucity of material for propagation. As the species is a clone, it is an ideal candidate for proliferation in tissue culture propagation. One advantage of using tissue culture is that large numbers of plants can be produced from small amounts of material once a species has been successfully established in vitro. It has been noted in the RTBG nursery that the leaves of *L. tasmanica* invariably blacken off shortly before the plant dies and this is due to a build up of phenolic compounds in the tissue. This can be a response to injury and has been an ongoing problem with proteas in the cut flower industry (Reid et al., 1989; Bieleski et al., 1992). Unfortunately this has also been a big problem in the work RTBG staff members Alan Macfadyen and Natalie Tapson have carried out on the tissue culture propagation of the species. This research has been undertaken in collaboration with Anthony Koutoulis and Aina Price of the Plant Science Department of the University of Tasmania since late 2004. Cut stems immediately blacken off when material is cut for explants and this increases during disinfestation prior to tissue culture. Various treatments have been trialled to overcome this with an incremental improvement in survivorship in vitro. This work will continue, but again a shortage of material is a problem, and trials can only be carried out once a year. This has generally been done in August when the material on the RTBG plants is relatively soft and before flowering occurs.

Plants of the genus *Lomatia* produce a leathery follicle but no fruit has ever been recorded in *L. tasmanica* in cultivation or in the wild. The flowers of *Lomatia* species are protandrous, the pollen is released from the anthers before the stigma becomes receptive, and this promotes out crossing between individuals. Royal Tasmanian Botanical Gardens staff Lorraine Perrins and Lizzie Ziegler carried out a pollen viability trial in December 2008. The pollen was removed from the stigma of recently open *L. ferruginea* flowers and *L. tasmanica* pollen was placed on the stigmatic surface and the flowers were bagged. Follicles had developed on two of the flowers in May 2009 and germination testing undertaken by James Wood of the Tasmanian Seed Conservation Centre has resulted in the germination of 3 out of 9 seeds. These will be grown on in the nursery to see if hybrids have been generated.

The RTBG has almost reached its target of holding 50 plants of *L. tasmanica* in an ex-situ conservation collection and will continue to build on this. The threats facing the species in the wild are very real, with the proximity of *Phytophthora* root rot to the population, the most pressing at present. As stated, the lack of material for propagation continues to be a problem and the annual nursery propagation cycle will be ongoing to ensure that the target number is maintained. The survival of explants in vitro has been increased from a few days to over 8 months and research will continue with this difficult clone in the hope of mass producing it to help secure its survival into the future.

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