Rescuing the Near Dead: Propagation for Conservation©

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The author reviews recent work on *Mellissia begoniifolia*, *Ramosmania rodriguesii,* and *Zanthoxylum paniculatum*. From these case studies, key issues are explored surrounding requirements for successful propagation to support conservation.

INTRODUCTION

The Temperate and Arboretum Nursery at Royal Botanic Gardens (RBG) Kew has built a specialism of propagating endangered and threatened plants from island floras. The following three case studies illustrate some of the problems and rewards of this work. Propagators need to have the confidence to rise to the challenge and continue to try techniques even on "problem plants".

CASE STUDIES

Mellissia begoniifolia. In November 1998, after a gap of 124 years, a plant last recorded by Melliss in 1875, *Mellissia begoniifolia* (Fig. 1) the St. Helena boxwood, was rediscovered (Melliss, 1875). Mr. Stedson Stroud, an islander out hiking, found a group of plants he did not recognise, only one out of the seven remained alive. Fortunately seed had been set and was immediately harvested. The plant is a short-lived woody perennial distantly related to the Cape gooseberry. Always a rare plant the islanders once used it for tinder to aid fire lighting, however, it was almost certainly grazing by goats that caused its decline.

Propagation from seed was tried on the island but results were poor so 50 seeds were dispatched to RBG Kew. The plant is a member of the Solanaceae, and most species in this family are easy to raise from seed—which also has a long viability. For these reasons I chose to use conventional techniques and *Mellissia* germinated easily on seed compost at 15°C and grew on under glass at 9 to 15°C with 13 plants produced. Further sowings produced more plants and different temperatures were used; however, the cooler regime has been the most successful.

The next problem was how to increase seed supplies without loss of genetic variation. To determine current genetic variability of the 13 individuals, randomly amplified polymorphic DNA (RAPD) genetic fingerprinting was employed (Grant and Culham, 1997). As this is the most accurate method commonly used, small genetic differences between individuals can be detected. Results showed a homogeneous population with no variation between individuals, this meant that a simple crossing programme would be effective at producing seed without fear of loosing genetic information. Seed has now been safely deposited at the Millennium Seed Bank, Wakehurst Place, and a horticultural protocol to guide those who want to grow plants for conservation has been written.

Ramosmania rodriguesii. Two further species with which Kew propagators have been involved are endemic to the tiny island of Rodrigues, approximately 200 miles

Figure 1. *Mellissia begoniifolia.*

from Mauritius in the Indian Ocean. Once a verdant forested jewel in a blue tropical sea; man has taken his toll, the habitat is dryer and forest has given way to savannah and woodland. *Ramosmania rodriguesii* (Fig. 2) known locally as the "Café Maron" was again thought extinct for 40 years until rediscovered in 1980 by a local teacher whilst taking a nature study class (Strahm, 1989). The islanders claim that a tincture made from the bark will cure hangovers and venereal disease; soon, the last remaining plant was under threat with locals taking branches. The International Union for the Conservation of Nature (IUCN) was alerted and they arranged protection for the plant—three concentric fences—and for cuttings to be flown to Kew.

At RBG Kew both conventional and in vitro propagation techniques were tried. Rooting of the conventional apical softwood cutting, treated with rooting hormone Synergol (IBA/NAA at 2500 ppm) and struck under enclosed mist with a bottom heat of 21 to 23°C took 6 weeks. Continued propagation using apical shoots was slow, with one shoot replaced by a single shoot.

In vitro propagation proved problematic, this species also carries a slime mold (a fungus-like organism) which is latent in the plant but becomes apparent in culture that it soon envelops. Although many attempts have been made to control it with antibiotics a clean culture has yet to be produced.

The shoot morphology of *Ramosmania* is unusual as flowering lateral shoots are produced internodally, these are easily rooted but are "programmed" to produce flowering wood and after a while peter out. A breakthrough was achieved when

Figure 2. *Ramosmania rodriguesii.*

nodal shoots were produced on a plant that had been left to grow ever taller; eventually a bend in the stem occurred and with apical dominance removed true side shoots were initiated. Hard pruning can also bring this about. Production of cuttings is now easy and more rapid and we expect to repatriate material shortly.

As no fruit set had occurred, despite hand pollination, Professor Simon Owens worked on the floral biology of this plant. He found that the plant is functionally male as the style is dysfunctional and does not support pollen tube growth (Owens et al., 1993). He suggested that the plant could be either dioecious (separate male and female plants) or hetrostylus (flowers of different style length) but as further collections can't be made this will remain a mystery. Even with these systems for out-breeding, ovary fertilisation may be possible in vitro if the slime mold problem can be controlled.

Zanthoxylum paniculatum. *Zanthoxylum paniculatum* (Fig. 3), another Reunion endemic tree, is also critically endangered with only three known individuals worldwide. One plant survives in the wild, one in a Reunion nursery, and one at RBG Kew. Since 1989 the number of plants known of this species has halved so this is a serious case. RBG Kew's plant has been in cultivation since 1974, since when propagation has been tried both from cuttings and in vitro culture, so far without success. Recently air layering has been attempted but was unsuccessful. A similar story is reported from the Conservatoire Botanique National de Brest which has received material from Reunion. The strongest hope appears to be in grafting this

Figure 3. *Zanthoxylum paniculatum*.

species onto *Z. heterophyllum*, a closely related species from Reunion. It is not certain if *Z. paniculatum* shows dioecy, earlier reports record fruit set on plants 900 yards apart which would point to monoecy and the possibility of selfing plants if they can be persuaded to flower. Time may be running out but whilst plants remain efforts will continue.

KEY ISSUES FOR THE CONSERVATION PROPAGATOR

So what are the key issues for a propagator involved with plant conservation? What are the themes highlighted by these case studies?

The majority of such species are from areas where there is not an established horticultural practise, either commercially or at a botanic garden. There is therefore a high likelihood that species will be new to cultivation or may even be new to science! This means there is no track record to follow and the propagator has to extrapolate from experience with closely related species and use knowledge from plants of similar habitats. With nothing to benchmark your plants against it is important that you ask yourself: is this plant being grown in the best possible way? Or are you just managing to keep it alive?

Usually the volume of propagating material is very limited and so very precious; access to further material may be difficult precluding another attempt. This can dictate a conservative approach to cultivation as the response to composts, fertiliser, environment, cultural techniques, and biocides will be unknown. Breeding systems evolved to maintain a healthy species can work against the propagator when few plants are available. Dioecy (separate male and female plants) or outbreeding systems can negate production by seed. Steep declines in population numbers can produce genetic bottlenecks where genetic variation is reduced to very low degrees. This can affect a species' ability to adapt to new or changing environments. Remember, genes can't be made, only recombined. However, many species are successful as clones, foremost amongst these are the apomicts, so the clonal state can be enduring. Mother plants can be unhealthy, they can have pests or diseases which are manifest or latent, some will be in terminal decline through old age if no reproduction has taken place for some time. Many species also have complex associations with mychorrizal fungi that we still don't understand.

RISING TO THE CHALLENGE!

Now there is an appreciation of the task, solutions can be found. Information on cultivating unusual plants can be difficult to find, older taxonomic works rarely include cultivation information as this was not viewed as proper science—fortunately we live in a more enlightened world now where the synergy of knowledge ensures better sustainability of this important resource. For this reason RBG Kew has established a cultivation protocol developed for its rare and endangered collections, within this is included a section on propagation, an example of which follows:

Example of an RBG Kew Horticultural Protocol:

Author(s): Belinda Parry and Stedson Stroud

Date: 07/11/2000

Genus: *Mellissia*

Species: *begoniifolia* (Roxb.) Hook. f.

Synonyms: Common name: St. Helena boxwood

Cultivar:

Hybrid?: no

Type of hybrid: N.A.

Where cultivated: Temperate and Arboretum nursery, RBG KEW

IUCN Category of Threat: Endangered in the Wild (EW) [IUCN 1994]

Recommended propagation method:

unknown	**leaf cuttings**	**stooling**	***root stock for grafts:**
✓ **seeds**	**herbaceous cutting**	**grafting***	
division	✓ **softwood cuttings**	**bud grafting**	
root cuttings	**hardwood cuttings**	**layering**	
sucker	**micropropagation**	**air layering**	

other: Also, semi-ripe cuttings

Level of difficulty: uncomplicated

Remarks:

Propagation by seeds:

Composition of sowing compost:

fine bark:	0%	**charcoal:**	0%	**perlite:**	0%
coarse bark:	0%	**sharp sand:**	0%	**seramis:**	0%
bracken peat:	0%	**loam:**	0%	**baked clay:**	0%
coir:	0%	**vermiculite:**	0%	**hydroponics:**	0%

slow-release fertiliser: 0% **other:** Same as potting mix (45% coir, 45% silvafibre, 10% loam, 0.5 g $litre^{-1}$ keiserite, 1.5 g $litre^{-1}$ Osmacote 15N : $11P_2O_5$: $13K_2O$: 2 Mg +te) but with added grit (compost and grit, [2 : 1, v/v]) thin layer of grit on top for moisture retention, discouragement of weeds.

quick-release fertiliser: 0%

trace elements: 0%

Remarks: No requirement for extra fertiliser.

Pre-sowing treatment:

Subsequent treatment: As cultivation (under glass 9 to 15°C minimum humidity range 40% to 65% RH)

Germination time: 28 days

Propagation by cuttings:

Composition of rooting compost:

fine bark:	35%	**charcoal:**	0%	**perlite:**	30%
coarse bark:	0%	**sharp sand:**	0%	**seramis:**	0%
bracken peat:	0%	**loam:**	0%	**baked clay:**	0%
coir:	35%	**vermiculite:**	0%	**hydroponics:**	0%

slow-release fertiliser: 0%

quick-release fertiliser: 0%

trace elements: 0%

other: **Remarks:**

Condition of cutting material: soft or semimature actively growing

Remarks:

Rooting conditions: Mist unit, bottom heat 21 to 24°C

Subsequent treatment:

Rooting time: 14 days

PROPAGATOR QUALITIES

The greatest factor critical for meeting the challenge comes from within; it is the attitude of the propagator. It is vital to develop a positive attitude and frame of mind. Too often capable horticulturists and propagators give up because they have failed a few times. Failure must be viewed as a learning exercise, a bit like cracking a code. The approach should be "This plant can be propagated, I just need to find out how." Ask yourself "Have I tried all methods and conditioning strategies?" In choosing strategies, the risk of inaction through being cautious has to be balanced against lost opportunity—plants are living and without reproduction will not last forever, older plants might be more difficult to propagate. Time may not be on your side, every season is different and the physiology of the plant will change so don't give up! Keep an open mind to new techniques—discuss problems with colleagues as their experience might trigger that all-important idea that will bring success.

LITERATURE CITED

Grant, M. and **A. Culham**, 1997. DNA fingerprinting and identification of cultivars. The New Plantsman 4(2):85-87.

Melliss, J.C. 1875. St. Helena: A physical, historical, and topographical description of the island, including its geology fauna, flora and meteorology. L. Reeve & Co. London.

Owens, S.J., A. Jackson, M. Maunder, P. Rudall, and **M.A.T. Johnson.** 1993. The breeding system of *Ramosmania heterophylla*—dioecy or heterostyly? Bot. J. Linn. Soc. 113:77-86.

Strahm, W. 1989. Plant red data book for Rodrigues Koeltz Scientific Books for IUCN, The World Conservation Union.