RAISING THE LIVING DEAD: *RAMOSMANIA RODRIGUESII*

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ABSTRACT

'Living dead' is a term biologists have begun to use to describe those species that are not expected to escape extinction without significant human intervention (Janzen, 2001), such as captive breeding or cultivation. While the number of taxa that fall into this category continues to increase, this paper reports on a species that may beat the odds and escape its fate, after decades of cultivation at the Royal Botanic Gardens, Kew. An account of the historical facts that led to the recovery of the species in question, *Ramosmania rodriguesii*, and the latest update regarding its current situation are provided along with cultivation notes.

THE COMPROMISED ECOSYSTEMS OF THE MASCARENE ISLANDS

French explorer and naturalist François Leguat (1637/1639–1735) was fascinated by the natural history of the island of Rodrigues and in his first account of it he described the island as a paradise (Leguat, 1708). A few decades after colonisation by European settlers, Isaac Bayley Balfour (1853–1922), a Scottish botanist who travelled to the island as a graduate, stated, "The island is covered with a vegetation mainly of social weeds, and destitute of any forest save in unfrequented and inaccessible parts." (Balfour, 1887)

Through millennia of isolation, the Mascarene Archipelago has evolved a unique and rather fascinating flora. However, just a few centuries after being discovered, its distinctive biodiversity was seriously degraded through habitat destruction, introduction of exotic but invasive species and the associated extinctions that followed. Mauritius, Rodrigues and Réunion have lost many native plant species. Of 1,296 native plants, 53 species are extinct and 393 of the surviving species are threatened, according to *The 1997 IUCN Red List of Threatened Plants* (Walter and Gillett, 1998). Little remains of its ebony forests, and eight ebony tree species are virtually extinct. The islands have lost six of their beautiful orchid species, and thirteen more are threatened. Mauritius and Rodrigues have been described as the 'Islands of the Living Dead' by authors Beverly and Stephen Stearns (Animal Welfare Institute, 1983) because at least 30 plant species have ceased reproducing in the wild and are living on the edge of extinction. Rodrigues is the smallest and, perhaps, the youngest of the Mascarene group, where at least eight species of vascular plants are already extinct. Of the 38 surviving endemic species 21 are endangered and at least 10 of those species survive in populations smaller than 20 specimens (Strahm, 1983).

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THE *RAMOSMANIA* CASE

Ramosmania is a genus in the Rubiaceae endemic to Rodrigues, which is represented by one or possibly two species, *Ramosmania heterophylla* and *R. rodriguesii* (Fig. 1) (Verdcourt, 1986). This genus has always been known for being elusive in many ways. Until recently no specimens had been found and after several searches, it was feared extinct. But suddenly, in the mid-1980s, a single specimen was discovered (Moutia, 1984) by a schoolboy, Hedley Manan, who had been encouraged by his teacher, Raymond A-Keeh, to search for rare plants. Tivergandum (1989) assigned the newly found specimen to *Ramosmania rodriguesii*, differentiating it from the extinct *R. heterophylla* mainly on the basis of leaf morphology (following Balfour, 1877).

Since its rediscovery in the mid-1980s, the remaining wild tree had never set seed and the plant was being continuously damaged by locals; three concentric fences have now been erected to prevent vandalism. Some locals believe that the plant can treat hangovers and venereal disease, referring to it as 'Café Marron' (called wild coffee in English) (Strahm, 1983).

Fig. 1 *Ramosmania rodriguesii*. Photo: Andrew McRobb.

Then, in 1986, in a collaboration between a local conservationist, Wendy Strahm, IUCN, Royal Botanic Gardens, Kew (RBG Kew) and the Rodriguean Forestry Service, cuttings were flown to Mauritius and then on to London in the hope of saving this important endemic plant by growing it *ex situ* at Kew. Two lateral branches were sent to the Micropropagation Unit and a single apical cutting was sent to the Temperate Nursery. Tissue cultures failed to establish as all plant tissues carried a microorganism that invaded the culture media. However, the cutting sent to the Temperate Nursery was successfully rooted by Kew horticulturist David Cooke, resulting in the first *ex* *situ* propagation of the species, from which other plants were produced and dozens of cuttings subsequently obtained. Many of these soon came into flower (Cooke, pers. comm.).

However, for more than two decades no fruits (and therefore no seeds) were recorded, either in the wild or in cultivation. Some suspected that the last clone was male or sterile. Closer study showed that the pollen was fertile and the ovules fully developed; however, the style appeared non-receptive. The most likely explanation was that this last plant was a male, though heterostyly could not be ruled out (Owens *et al.*, 1993). Left with an apparently self-incompatible single clone, and without any extant species in the genus with which to hybridise, the genus *Ramosmania* seemed doomed to extinction in the wild, soon acquiring the title of 'The Living Dead'. Despite this, the only clone kept flowering repeatedly.

Eleven cuttings were repatriated to Mauritius in 2001 but any hope of re-establishing the species in the wild seemed to be in vain, since the lack of seed meant that this was a species that was unable to self-propagate in the wild. The only future the plant could have was totally reliant on horticulturists. From the conservationist's point of view it was an extinction waiting to happen – an ever-blooming remembrance of what has been lost and a hopeless case sentenced to a captive or caged environment.

BREAKING THE CURSE AND THUS CHANGING THE FATE

Then, against the odds, in August 2003 a fruit was produced in RBG Kew's Tropical Nursery during some trials aimed at overcoming the supposed self-incompatibility which involved amputating the stigma and depositing pollen in the created wound. The fruit, once ripened, was found to have seven seeds. Since the seeds could be the only part of the plant's tissues free of the invasive fungus, they were sent to the Micropropagation Unit where embryos extracted from them started to grow but, unfortunately, failed to establish (Sarasan, pers. comm.). It was clear that *Ramosmania rodriguesii* was not fully sterile after all. But the enigma remained and seeds were unavailable again.

Repetition of the cut-style technique failed to produce more fruits. Investigation of the environmental conditions to which the specimens were exposed in the weeks prior to the plant producing a fruit provided some vital clues. Environmental factors seemed to be related to this event as the specimens had been exposed to record high temperatures and sunlight exposure. Plants were distributed to different locations at RGB Kew and the floral biological cycle was recorded and compared over a series of some months (Magdalena, 2004). This showed that warmer temperatures, combined with more exposure to sunlight, produced plants that had longer styles on their flowers, especially towards the end of the flowering cycle. Following this observation a group of ten plants was moved next to heating pipes in an area that had more sun exposure. Pollen was then transferred from recently opened flowers to stigmas on older blooms more than 300 separate times (Fig. 2). This led to the development of some fruits and over the course of a year more than a hundred seeds were harvested from several fruits, each containing one to ten seeds. The rate of success was low per flower pollinated, but it did produce viable seeds which resulted in healthy seedlings.

Three and a half years after the first seedlings were germinated, using standard horticultural techniques, the new clones started to flower. This was remarkable as the saplings grew into two clearly different plant types. Some were heavy bloomers and had flowers identical to the self-pollinated parent. They produced viable pollen, but no fruits seemed to develop on them (Fig. 3). More importantly, however, others had few flowers, which were solitary and had shorter corolla tubes and smaller petals but longer styles, rising above the anthers, which, in turn, lacked pollen (Fig. 4). Cross-pollination of these with pollen from the other type led to fruits setting readily (Fig. 5). Surprisingly, these fruits contained an average of 80 seeds each, which were rather larger than those developed by the surviving clone.

Fig. 2 Carlos Magdalena pollinating *Ramosmania rodriguesii* in the nursery at RBG Kew. Photo: Andrew McRobb.

Fifty new saplings are now in cultivation at RBG Kew. Others have been repatriated to the Republic of Mauritius. Using the new females, nearly 1,000 seeds have been produced at RBG Kew, and as of April 2010, 550 of those seeds have been successfully repatriated to Rodrigues and 300 have germinated within 4 weeks of being sown. A few have been planted back into the wild, and it is expected that these will provide clues on how to plan a mass reintroduction. Fruits continue to develop and ripen at RBG Kew and are harvested regularly in order to supply the Mauritian Wildlife Foundation and the Rodriguean Forestry Service nursery's needs while the new seedlings reach maturity and start to produce seeds by themselves. Work towards making this species a common sight once more on its native island has now begun in earnest.

Fig. 3 *Ramosmania rodriguesii* – cross-section of male flower showing short style and pollen-loaded anthers. Photo: Andrew McRobb.

Fig. 4 *Ramosmania rodriguesii* – cross-section of female flowers, showing shorter corolla tube, stigma protruding through anthers and no pollen. Photo: Andrew McRobb.

Fig. 5 *Ramosmania rodriguesii* – fruits. Photo: Andrew McRobb.

THE MANY FORMS OF *RAMOSMANIA RODRIGUESII*

Like many other species from the Mascarene Islands, *Ramosmania rodriguesii* is a heterophyllous species. There is a marked difference between the juvenile and adult forms. Juvenile forms have a single unbranched stem. Leaves are linear, very narrowly elliptic, 300+mm long and 8–22mm broad. Leaf colouration is very unusual, having a mix of dark brown, black, crimson and silver blotches, but no trace of green is seen (Fig. 6). This is believed to be a grazing deterrent against two species of billed grazers – the giant land tortoises and the solitaire, a dodo-like terrestrial bird, both of which are now extinct. Once the plants reach 1–1.5m above the ground they start branching, and the foliage changes dramatically. These adult leaves are elliptic, variable in shape and dark green in cultivation, but occasionally blotched with a bronze colour on Rodrigues.

Adult plants have two types of branches. Apical branches grow slowly and do not flower but grow upwards and produce lateral flowering branches. The lateral branches contain a single stem from which opposite leaves grow in pairs. After each pair of leaves the single flowers (on female plants) (Fig. 7) or the inflorescences (viviparous cymes) on male plants (Fig. 8) grow. After the flower senesces, two new flowers appear from the bases of the spent flower. Up to 12 blooms have been recorded on a single inflorescence, and several inflorescences appear along the length of the stem. Once all the inflorescences are exhausted, the branch dies back to the apical branch but not before several new flowering branches above the old one have started flowering. This enables the male adult form to be a relentless and ever-blooming plant of high horticultural value. Specimens at RBG Kew have been flowering constantly now for more than a decade. As long as the specimens are properly grown, they flower endlessly.


Fig. 6 *Ramosmania rodriguesii* – juvenile leaves. Photo: Andrew McRobb.

Fig. 7 *Ramosmania rodriguesii* – female flowers. Photo: Andrew McRobb.

Fig. 8 *Ramosmania rodriguesii* – close-up of male flower. Photo: Andrew McRobb.

CULTIVATION

Growing environment: *R. rodriguesii* is cultivated at RBG Kew in several moist tropical areas. The environments are kept at a minimum of 19°C. Vents open at 23°C, but temperatures of 30°C have been recorded both at RBG Kew and in the wild without visual damage to the plant. The relative humidity is kept at a constant 70–80 per cent. The plant tolerates shade as well as full sun; however, it seems to flower better in only partial shade or full sun.

Compost: This species also seems to be tolerant of a range of composts. In the nursery plants are grown in an organic, peat-free compost that is neutral to slightly acidic and contains: 10 per cent loam (screened and sterilised), 45 per cent coir, 45 per cent Silvafibre, with 1.5g/l of Osmocote 'Plus'® (15:8:11+2MgO and trace elements) and 0.5g/l of Kieserite (hydrated magnesium sulphate) also added. Plants on display are grown in compost in beds with a high loam and sand content, to which layers of organic mulch can be added at surface level.

Potting: It is advisable to pot on the plants regularly; every six months has been found to be sufficient. Partially reducing the rootball together with a small increase in pot size enables frequent potting without requiring enormous containers in a short period of time. *R. rodriguesii* roots (especially those of seedlings and juvenile plants) have a tendency to grow straight towards the base of the pot. The upper half often does not contain any roots, and the compost in this area tends to decay and degrade. At potting time this compost should be removed and the rootball lightly reduced and then placed in a deeper container that is as narrow as possible to fit the root system comfortably. Saplings need to be staked until fully established in the new pot.

Irrigation: Plants are watered using reverse osmosis water and should be kept moist at all times, whilst waterlogging due to excessive irrigation should be avoided.

Fertiliser: *R. rodriguesii* plants are subject to the same feeding regime as other woody tropical plants at RBG Kew. During the summer they are fed with a standard balanced pot feed (21:7:21) and, every second week, a calcium nitrate fertiliser. During winter the calcium supply is reduced, and a high potassium fertiliser (15:7:30) is used instead of the standard formula (21:7:21).

Pruning/Hygiene: Lateral flowering branches are pruned at the apical branch as they finish flowering and start to senesce as this encourages a neater appearance and obviates the need to leaf pick afterwards. Apical branches generally do not produce secondary apical branching unless damaged or pruned. Naturally, they only tend to branch if they become top heavy and lean to one side. During the time that the new leaders are replacing the previous one, flowering may be reduced.

Spent flowers tend to accumulate on the inflorescences and the leaves, probably as a consequence of lacking rain and wind when cultivated under glass, so ideally they should be removed by hand to avoid a build-up of pests and improve the appearance of the specimens. Hosing the plants with a jet nozzle will remove spent flowers, but this is not recommended in winter as it may cause overwatering of the compost.

Pests: Mealybugs (family Pseudococcidae) are the most common pest of this species. Mature leaves are generally unaffected, but the growing tips can become infested and damaged if populations are not kept in check. This can cause distortion of the leaves and can hinder growth. Pesticides can be effective against mealybugs and these can be applied by spraying. However, systemic insecticides can be more effective in the long term. Biological controls such as the fungal biological control *Verticillium lecanii*, known as Mycotal; alternatively, the Australian ladybird (*Cryptolaemus montrouzieri*) can be used. Since mealybugs are capable of hiding in the stipules and unfurled buds, it is very difficult to totally eradicate them. By using a systemic pesticide and hosing the plants with pressurised water once a week, the levels of this pest can be more easily controlled. Brown scale (*Coccus hesperidum*) is an occasional problem but can be controlled in similar ways to mealybugs.

Western flower thrips (*Frankliniella occidentalis*) are also common. They generally live inside the tubular corolla and feed on the pollen of the male plants. They generally do not affect the appearance of the flowers but can be a problem if pollen from a specific plant is required as the flowers can be depleted by this pest.

PROPAGATION

Propagation can be achieved by both vegetative and sexual means.

Cuttings: Lateral branches cannot be used for this purpose as, even though they may root, they will only produce flowers until they die, rather than apical growth. Thus, stem cuttings from the main apical branch should only be used. Tip cuttings are best, but semi-ripe internodal ones are also suitable if available. Sections of the terminal apical stem should be 100–150mm long. The lateral branches should be removed if they are growing on the lower sections of the cutting, and those that remain above the surface of the rooting media when inserted should be reduced to two or four leaves. Rooting hormones may be used but cuttings can be successfully rooted without them. The propagation compost used at RBG Kew is made of 70 per cent finely shredded coir and 30 per cent medium-sized perlite. Cuttings are slow to root, generally taking at least six weeks.

Micropropagation: All techniques used to propagate the original clone have consistently failed because of contamination by microorganisms that are endogenous in all tissues of the plant. Embryos have been successfully developed, but, to date, attempts to grow them up to weaning stage have failed.

Seeds: When fruits are ripening they change colour from green to yellow, often with peachy tones. At this stage the seed is ready for collection. When fully ripened, the fruits will detach when touched or will simply fall to the ground. Once this has occurred, the outer skin can be cut using a scalpel. With the blade facing outwards, the outer layers can be slowly removed with no risk of damaging the seeds, which are clustered towards the centre of the fruit (Fig. 9). With the help of forceps they should be separated one by one while they are still fresh and moist otherwise they will dry and stick together making the sowing process very difficult (Fig. 10). Once extracted and separated, seeds can be placed on a petri dish, making sure that they do not touch each other, and then placed in a seed-drying container with silica gel, for one week. It is not clear whether the seed is recalcitrant, intermediate or orthodox, but it has been observed that they lose viability quickly. Seeds older than one year do not germinate, or they do so at a very low rate.

After the seed has been dried it is placed in a small container of water at 20–25°C for 24 hours prior to sowing. At RBG Kew seeds of this species are sown as follows: a layer of potting compost 30mm deep is placed at the bottom of the container which is then filled up to within 15mm of the rim with a mix of 50 per cent horticultural silver sand and 50 per cent sieved coir. The surface is then pressed lightly to level the compost, and the seeds are placed on the surface of the media with the aid of forceps, spacing them up to 20mm apart. At this stage they are covered with a fine layer of compost and placed in water to wet the compost thoroughly. They are then placed in a closed cabinet with bottom heat of 23°C.

Germination will occur after four to five weeks and, if the seed is fresh, the success rate should be around 85 per cent. The seedlings should remain in this container until they have developed two or three pairs of true leaves (Fig. 11). Trials have shown that if they are potted up earlier, they damp off. The seedlings germinate in the sterile sand/coir mix without fungal attack. They have a marked tendency to grow directly to the bottom of the pot from day one. This is the reason why a layer of normal (but nutrient-rich) compost is placed at the bottom of the pot so that nutrients are available when the roots reach this layer within a few days. For potting on, see the potting section above.

Fig. 9 *Ramosmania rodriguesii* – cross-section of fruit showing the seeds. Photo: Andrew McRobb.

Fig. 10 Close-up of *Ramosmania rodriguesii* seed. Photo: Andrew McRobb.

Fig. 11 *Ramosmania rodriguesii* seedlings. Photo: Andrew McRobb.

CONCLUSION

The reproductive barrier that prevented the reintroduction and recovery of this species in the past seems to have been completely overcome after several decades of cultivation at RBG Kew. It is likely that the species will be able to perpetuate itself in the wild if the current reintroduction programme continues. The white tubular corolla suggests that the species was pollinated by moths and that fruit and seeds were dispersed by the Rodrigues fruit bat (*Pteropus rodricensis*) as this is the case for two other endemic genera in Rubiaceae on Rodrigues (*Doricera* and *Scyphoclamys*) (Begue, pers. comm.). As its likely pollinators and seed dispersal agents are not extinct and should still be present in its habitat range, a full recovery of the species is feasible. Current DNA work is being carried out at RBG Kew to assess the genetic variability left in the species. Preliminary AFLP fingerprints of the surviving clone and two of the seedlings indicate that some variability occurs (Smith, pers. comm.). This is very encouraging and good news for the future of the species.

REFERENCES

ANIMAL WELFARE INSTITUTE (1983). *Endangered Species Handbook – It's Too Late*. Available online: http://www.endangeredspecieshandbook.org/dinos_plants.php (accessed 23 July 2010).

BALFOUR, I.B. (1877). Aspects of the Phanerogamic Vegetation of Rodrigues. *Botanical Journal of the Linnean Society*, 16, 7–25.

JANZEN, D.H. (2001). Latent extinctions – the Living Dead, in LEVIN, S.A. (ed.). *Encyclopedia of Biodiversity vol. 3*. Academic Press, New York, 689–699.

LEGUAT, F. (1708). *Voyages et aventures de François Leguat et de ses compagnons en deux îles désertes des Indes orientales.* 2 vols. J.L. de Lorme, Amsterdam & London.

MAGDALENA, C. (2004). Ramosmania rodriguesii*: New hope for a doomed genus*. RBG Kew, School of Horticulture. Unpublished.

MOUTIA, S. (1984). Randia heterophylla *Balf. F. is not extinct in Rodrigues*. *Mauritius Institute Bulletin* 10, 123.

OWENS, S.J., JACKSON, A., MAUNDER, M., RUDALL, P. & JOHNSON, M.A.T. (1993). The breeding system of *Ramosmania heterophylla* – dioecy or heterostyly? *Botanical Journal of the Linnean Society*, 113, 77–86.

STRAHM, W. (1983). Rodrigues: can its flora be saved? *Oryx*, 17, 122–125.

TIVERGANDUM, D.D. (1989). *Ramosmania rodriguesii* nouvelle rubiacée endémique de Rodrigues. *Compte Rendu des Séances de la Société de Biogéographie*, 65, 14.

WALTER, K.S. & GILLETT, H.J. (eds) (1998). *The 1997 IUCN Red List of Threatened Plants*. IUCN Publications Unit, Gland, Switzerland and Cambridge, lxiv, 862.