

I would gauge the TSCC volunteer germination group as a successful volunteer program. Although I have had about a fifth of applicants leave after 6 months the bulk of volunteers have been with the seedbank between 5–10 years. The TSCC volunteers are very self-motivated and find their work fascinating and rewarding. I don't shy away from sharing my frustration or excitement about test results and it's great to see that reflected by volunteers, particularly when they finally get some germination activity in a challenging collection. Although the primary goal of wild seedbanks is the *ex-situ* conservation of seed-bearing plants, the germination testing we conduct is equally as important. Testing is key to the functioning of a seedbank (why store seeds if you can't turn them back into plants?) however identifying techniques to germinate wild species has implications for the broader plant conservation community. As such the

RTBG began sharing its germination data with the public since 2008 by placing it online. If you would like to see what we do you can find the TSCC Germination Database on the RTBG website (link below).

References

Martin, A. C. (1946). The comparative internal morphology of seeds. *The American Midland Naturalist*, 36(3), 513-660.

Resources

TSCC Germination Database
<https://gardens.rtbg.tas.gov.au/conservation/tscgerminationdatabase/>

Seedbank Origami: envelopes, trays and boats
https://rtbg.tas.gov.au/wp-content/uploads/2020/07/RTBG_SeedBank_Origami.pdf

Ex situ management including seed orchard establishment for Native Guava (*Rhodomyrtus psidioides*) affected by Myrtle Rust

VERONICA VILER AND CATHERINE A. OFFORD*

Australian PlantBank, Royal Botanic Gardens and Domain Trust, Australian Botanic Garden, Mount Annan NSW 2567.

*Corresponding author: Cathy.Offord@rbgsyd.nsw.gov.au

In 2010, the plant pathogen *Austropuccinia psidii*, commonly referred to as Myrtle Rust, was detected in Australia for the first time on the NSW Central Coast. Over the subsequent decade Myrtle Rust's impact on many native Myrtaceae species has been significant, particularly for *Rhodomyrtus psidioides* (G.Don) Benth. or the Native Guava. Once common from Broken Bay on the NSW coast to south east Queensland and up to 120 km inland, in February 2019 the species was listed as Critically Endangered in NSW. *Rhodomyrtus psidioides* is severely threatened by Myrtle Rust over its entire range and characterised as 'extremely susceptible' to infection (Pegg *et al.* 2014; NSW Scientific Committee 2017). All plant parts have been documented as being affected including leaves, stems, flowers and fruits (Pegg *et al.* 2014; Carnegie *et al.* 2016; NSW Scientific Committee 2017). Damage to new foliage and subsequent failure to replace older leaves progressively weakens the plant, ultimately causing death. How long this process takes remains unclear. *Rhodomyrtus psidioides* readily suckers, however new growth is often rapidly overwhelmed by Myrtle Rust. Flowers and fruits are similarly affected and seldom manage to produce any viable seed, therefore

R. psidioides struggles to reproduce either asexually or sexually in the wild and has suffered serious decline as a result.

Collecting seed or cuttings of *R. psidioides*, along with other Myrtle Rust susceptible species, was flagged as a high priority by Australian Plantbank collectors after the disease emerged in 2010. In the wild, plants were often covered in Myrtle Rust or had deteriorated to the point where taking cutting material was no longer feasible. Seed was often not viable, not filled or in such small numbers that it was impossible to determine seed storage behaviour. As a result, early seed collections were treated as orthodox and frozen at -18°C.

Conventional horticultural wisdom often prescribes ideals: plant material in good condition, pest and disease free, collected at a specific time of year, all supported by known data. The reality of working with many threatened species is that material may be limited and of poor quality, access to plants restricted due to rarity, location or other external factors, information on propagation and cultivation non-existent, and resources limited.



Potted plants of *Rhodomyrtus psidioides* developed flowers and fruit at a young age. Photo: Veronica Viler

Despite the best efforts of staff, by 2015 only 12 seeds were held in the Australian PlantBank, and four whole-plant genotypes in the nursery of the Australian Botanic Garden, Mount Annan (ABGMA). With so little material available, the ability to achieve any meaningful conservation outcomes appeared doubtful.

Later that year a program commenced to preserve the small nursery collection and develop propagation techniques for the species. In the first two years of growth, cutting grown plants became fertile and developed fruit. The resulting seed appeared viable and the idea for a seed production area was conceived.

Managing Myrtle Rust-susceptible collections is especially challenging with a huge number of species and subspecies known to be affected. To date (2020), *A. psidii* has proved capable of infecting 382 native Australian plant species or subspecies (17% of the 2,253 known native Myrtaceae) (pers. comm. R.O.Makinson, July 2020). This meant the potential for outbreaks within the nursery and garden environment was considerable and therefore understanding the disease and current management strategies was essential. The safe use of chemicals permitted for treating Myrtle Rust requires attention to the surrounding environment and a high level of PPE (personal protective equipment).

Local weather conditions, micro-climates and seasonality all play a role in the behaviour of the disease. The lifecycle (sporulation, infection, to re-sporulation) of *A. psidii*

can extend from the usual 2 weeks to 5-6 weeks under cooler conditions (Makinson 2018a, p41; Carnegie and Lidbetter 2012) and the pathogen's latent period (active but asymptomatic infection) can extend to overwintering in some hosts and conditions (Beresford *et al.* 2020). Quarantine and treatment of incoming plant material, diligent monitoring, appropriate cultural methods and sound hygiene practices are all vital to maintaining healthy potted and in-ground collections.

In February 2018, a planting site suitable for seed production was selected in consultation with the ABGMA horticultural staff. A strategy was agreed upon and three genotypes planted. Collections grown for research purposes necessitate more intensive management than those grown purely for ornamental display. Living collections can also provide an opportunity to educate the public and demonstrate the strong links between science and horticulture in conservation.

To optimise seed production and allow for Myrtle Rust control, plants were pruned and kept below two meters high. Watering was restricted to soil soaking to avoid moisture settling on foliage, a key requirement for Myrtle Rust spore germination (Makinson 2018a, p42).

Plants were left to pollinate naturally as the pollination system is open with multiple insect vectors (Williams 2018) and plants were monitored closely by staff. While waiting for the fruit to mature, the unripe crop sustained a large and unexpected loss to predation by birds.



Rhodomyrtus psidioides under netting at the Australian Botanic Garden, Mount Annan, to prevent predation by birds. Photo: Veronica Viler

In April 2019, two maternal seed collections were processed yielding a modest 366 seeds. Seed storage testing for the species was possible for the first time. Germination of fresh viable seed was $\geq 87\%$ and the seed was found to be desiccation tolerant but freezing sensitive, suitable for storage at 4°C rather than -18°C (Sommerville *et al.* 2019).

Germination testing produced several hundred seedlings, some of which were initiated into tissue culture for cryopreservation research, others given to partner botanic gardens as part of a broader conservation strategy for the species and the rest discarded. While it can be difficult to let go of surplus material, especially when it has been challenging to produce, prudent curation of research collections ensures time and resources are not wasted and duplication/distribution of material is a sound risk management strategy.

The following season, developing fruit on all three plants were netted to prevent further predation. Interestingly one individual that had not fruited well previously was the best performer, suggesting mast seeding. In all, three maternal collections were made in 2020, totalling 7,945 seeds. *“Editor’s note: Mast seeding is where there is great variation in seeding among years, with many seeds being produced in some years and none (or very few) produced in others”.*

The challenge for those working with threatened species is to find innovative ways to maximise outcomes with the material and resources available.

Vegetative propagation of *R. psidioides* has proven to be relatively simple via stem cuttings and sucker excavation where reasonable material exists, however at the time of writing, wild seed remains elusive and the future of *Rhodomyrtus psidioides* in the wild uncertain.

The actions described correspond to identified priority actions in the *Draft Action Plan (DAP)* for Myrtle Rust in Australia (Makinson 2018b). The relevant Actions are:

4.1.1, *Secure future options for species in current or projected decline through germplasm capture;* and

4.1.2, *Seed storage, enablement research and determination of alternative germplasm storage options.*

The establishment of reliable seed-production systems and the accumulation of seed in turn enable other DAP actions around screening for rust-resistance traits and eventual selection or breeding of more resistant genotypes.

Acknowledgements

The authors would like to thank Graeme Errington and Dr Karen Sommerville (Australian PlantBank) for their contribution to and review of this case study; Simon Reid and Craig Ward (ABGMA) for their input and commitment to the project; and Bob Makinson for comments on the draft manuscript.



Rhodomyrtus psidioides fruits. Photo: Veronica Viler



Rhodomyrtus psidioides seedlings from cultivated plants in the nursery at the Australian Botanic Garden, Mount Annan. Photo: Veronica Viler

References

- Beresford, R.M., Shuey, L.S. and Pegg, G.S. (2020). Symptom development and latent period of *Austropuccinia psidii* (Myrtle Rust) in relation to host species, temperature, and ontogenetic resistance. *Plant Pathology* 69: 484-494.
- Carnegie, A.J., Kathuria, A., Pegg, G.S., Entwistle, P., Nagel, M. and Giblin, F.R. (2016). Impact of the invasive rust *Puccinia psidii* (Myrtle Rust) on native Myrtaceae in natural ecosystems in Australia. *Biological Invasions* 18: 127-144.
- Carnegie, A.J. and Lidbetter, J.R. (2012). Rapidly expanding host range for *Puccinia psidii* sensu lato in Australia. *Australasian Plant Pathology* 41: 1329
- Makinson, R.O. (2018a). *Myrtle Rust reviewed: the impacts of the invasive pathogen Austropuccinia psidii on the Australian environment*. Plant Biosecurity Cooperative Research Centre, Canberra.
- Makinson, R.O. (2018b). *Myrtle Rust in Australia – a draft Action Plan*. Plant Biosecurity Cooperative Research Centre, Canberra. Available at: <https://www.anpc.asn.au/myrtle-rust/>
- NSW Scientific Committee (2017). *NSW Scientific Committee Preliminary determination Rhodomyrtus psidioides*. Available at: <https://www.environment.nsw.gov.au/resources/threatenedspecies/determinations/PDRhodpsidCR.pdf>
- Pegg, G.S., Giblin, F.R., McTaggart, A.R., Guymmer, G.P., Taylor, H., Ireland, K.B., Shivas, R.G. and Perry, S. (2014). *Puccinia psidii* in Queensland, Australia: disease symptoms, distribution and impact. *Plant Pathology* 63: 1005-1021.
- Sommerville, K.D., Cuneo, P., Errington, G., Makinson, R.O., Pederson, S., Phillips, G., Rollason, A., Viler, V. and Offord, C.A. (2019). Conservation in the wake of Myrtle Rust – a case study on two critically endangered Australian rainforest plants. *Pacific Conservation Biology* <https://doi.org/10.1071/PC19026>
- Williams, G. (2018). Insects associated with flowering of *Rhodomyrtus psidioides* (Myrtaceae): Is this a Myrtle Rust (*Austropuccinia psidii*)-induced plant-pollinator interaction extinction event? *Cunninghamia* 18: 23-27.

Strings Attached: Managing *ex situ* plants highly susceptible to pathogens

REBECCA (BEC) STANLEY¹ AND EMMA BODLEY^{*2}

¹Curator, Auckland Botanic Gardens, 102 Hill Road, Manurewa, Auckland 2015, New Zealand. Rebecca.Stanley@aucklandcouncil.govt.nz

²Botanical Records and Conservation Specialist, Auckland Botanic Gardens, 102 Hill Road, Manurewa, Auckland 2015, New Zealand.

*Corresponding author: Emma.Bodley@aucklandcouncil.govt.nz

Cultivating plants in *ex situ* collections is an important method to support plant conservation. Several recent *ex situ* projects at Auckland Botanic Gardens (ABG) have highlighted some complexities of this approach when a plant is threatened by an invasive pathogen. For managers of wild sites, where threatened plants are declining due to pathogens, removing plants and/or germplasm to the safety of an *ex situ* provider such as a nursery or a botanic garden is a well-established response in threatened plant recovery management. For a recipient nursery, the opportunity to participate in threatened plant recovery is a tangible way to demonstrate a clear role in plant conservation and a rewarding way for staff to contribute to a recovery project. However if the subject plant is host to a devastating environmental pathogen which may infect other crops on site, put collections at risk or adversely affect other projects undertaken on site (including those with other stakeholders), thought must be given to how to manage these issues. When Myrtle Rust (*Austropuccinia psidii*) surfaced as a threat to Myrtaceae, many practices at our nursery were reviewed.

Nursery Biosecurity

Nursery biosecurity is an increasing focus for nurseries and botanic gardens worldwide (Hayden 2020) to prevent the proliferation and movement of invasive pathogens which have the potential to adversely affect wild ecosystems and increase the risk of extinction of highly susceptible plant species. Nurseries provide pathogens with opportunities to infect a range of susceptible hosts or habitats (such as pots), in one location. Biosecurity accreditation schemes in both New Zealand (NZPPI) and Australia (Greenlife Industry Australia) assist nurseries to mitigate the risk through a systems approach to minimise the arrival and proliferation of pests in the nursery setting and mitigate the risk of introducing pathogens into new wild sites through infested nursery raised stock in revegetation programmes (Frankel *et al.* 2020). At its core, nursery biosecurity is based on the principle of preventing the arrival and establishment of a pathogen as the most effective way to maintain nursery hygiene.