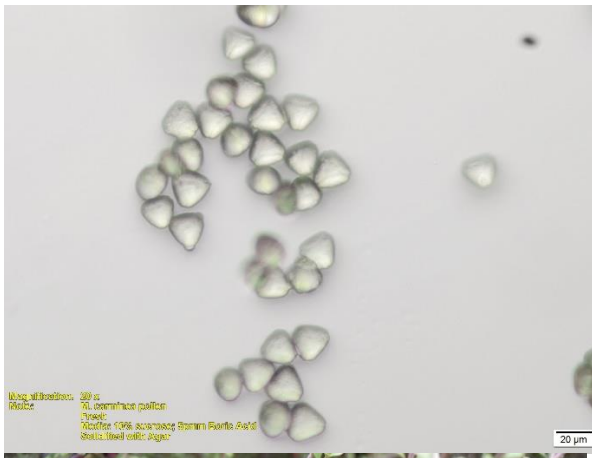
Lions Ōtari Plant Conservation Laboratory

Report: July 2020 – June 2022






Compiled by: Dr Karin van der Walt
Conservation and Science Advisor
Ōtari Native Botanic Garden, Wellington City Council
Karin.vanderwalt@wcc.govt.nz

Overview

The Lions Ōtari Native Plant Conservation Laboratory has been in operation for four years. This dedicated facility provides Otari staff, volunteers, students and external researchers with essential facilities and equipment to study New Zealand native plant species, providing baseline information to inform conservation actions. Being situated in a native botanic garden, the lab provides opportunity for cutting edge research into cryopreservation while at the same time establishing operational protocols for seed germination and long-term seed storage, which has immediate conservation benefits.

As is the case for the rest of the world, Covid-19 caused some upheaval for the Lab especially with lock-down happening at the peak of the seed collection season. This meant some projects such as Kauri seed banking and tissue culture of *Pachycladon,* had to be postponed, while more time sensitive work on *Syzygium maire* were prioritized to some extent. However, with lab time restricted to 2 days a week, much of the time was dedicated to keeping current projects contamination free. The value of seed banking was highlighted during this time, with all seed, pollen, and vegetative material in storage requiring no maintenance over the lockdown and months thereafter.

Work quickly gained momentum again in the spring of 2020 and a seed collection trip to collect *Dactylanthus taylorii* (wood rose) at Pureora Forest escalated to a huge contingent including representatives of all 6 iwi of Wellington travelling to Pureora, bringing back thousands of seed. Summer of 2020 focussed again on the collection of *Syzygium maire* while the main projects for autumn involved Kauri, *Lophomyrtus* and *Neomyrtus.*

In December 2021 the Lions Ōtari lab expanded into a two-bedroom flat adjacent to the Portacom building. At the same time, we received a grant from Te Tahua Taiao Nga Taonga (Lotteries: Environment and Heritage Fund) for a three year project on the conservation of five threatened orchid species from the Wellington Region.

Lions Ōtari Native Plant Conservation Laboratory – Phase 2

The success of the lab, illustrated through project funding, publications, presentations and use by students and external researchers, was used to submit a business case to expand the lab into the adjacent two-bedroom residential flat. Renovations started in July 2021 and were completed by December. At the same time a generous donation was received from Darea Sherratt in memory of Rodney Lewington to purchase two High Efficiency Particulate Air (HEPA) filters. The installation of the HEPA filters means we now have class 7 clean rooms (the same as operating theatres) in the two bedrooms. The living area houses incubators, fridges, microscopes, and other small equipment, while the kitchen is used to prepare media (Figure 1).

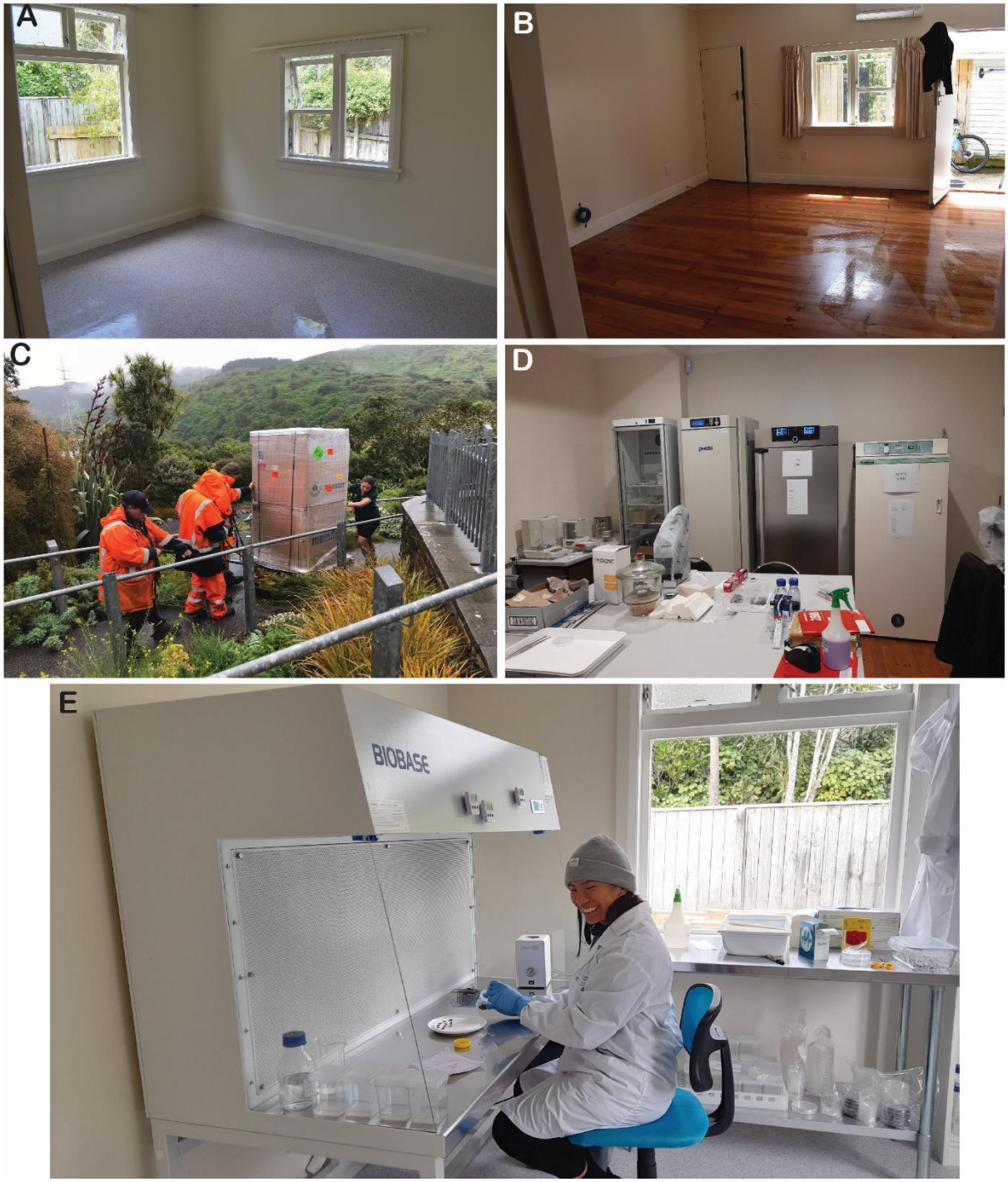


Figure 1: The renovated flat showing one bedroom (A) and the living area (B) with new incubators (C) located in the main living space (D). Apprentice Novelyn Paus doing seed germination in the class 7 clean room (E) made possible by a donation from Darea Sherratt.

Orchid Conservation Project

Another highlight from the Otari lab is the successful application to Te Tahua Taiao Nga Taonga (Lotteries: Environment and Heritage Fund) for a three year project looking into the conservation of five threatened orchid species from the Wellington region. We are delighted that through this funding we are able to employ Jennifer Alderton-Moss on a part time basis

(see more about Jennifer below). The project is led by Ōtari through Karin van der Walt, with Carlos Lehnebach (Te Papa) and Jennifer Alderton-Moss (now also Ōtari) making up the rest of the team (Figure 2). The project will furthermore provide a summer scholarship through Victoria University of Wellington. Since the five species are all threatened, five common surrogate species were also identified (Table 1). These surrogate species will be used to determine protocols so seed from threatened species can be handled effectively (Figure 3).

Figure 2: The orchid conservation project team includes Carlos Lehnebach, Botany Curator at Te Papa (left), Karin van der Walt, Conservation and Science Advisor (Ōtari) and Jennifer Alderton-Moss, Research Technician (Ōtari)

Table 1: The three year orchid conservation project will focus on five threatened species and four more widespread surrogates.

Species	Conservation Status	Surrogate Species
Corybas dienemus	**Nationally Critical**	***C. macranthus***
Drymoanthus flavus	**At risk - Declining**	***D. adversus***
Gastrodia cooperae	**Nationally Critical**	***G. cunninghamii***
Pterostylis irwinii	**Nationally Endangered**	***P. banksii***
P. micromega	**Nationally Critical**	***P. banksii***

Figure 3: The five species used in the orchid conservation project include species from the genera *Corybas, Drymoanthus, Gastrodia* and *Pterostylis*. Photos: C. Lehnebach.

Species highlights

Dactylanthus taylorii (wood rose)

One of the first species the Lions Ōtari lab worked on in 2018 was *Dactylanthus taylorii*. This fascinating plant is New Zealand's only fully parasitic plant, which means it relies on a host tree for all its nutrients. In July 2021, we were delighted to observe the first seeds starting to germinate in the Lions Ōtari Plant Conservation laboratory (Figure 4). *Dactylanthus taylorii* can take between 5 and 12 years to germinate and it is not currently known what the triggers for germination are. To date, there are more than 300 split seeds growing in the incubator (in the lab) and we will be introducing these seeds to host tree roots in June 2022. For *Dactylanthus taylorii* it is also very important that we can effectively collect and store pollen as many of the populations consists of only females and seed production therefore requires hand-pollination using pollen transported from another population. Research has been conducted in the lab to determine pollen germination protocols (to test viability) and pollen is stored at 5°C, -18°C and -196°C (Figure 5).

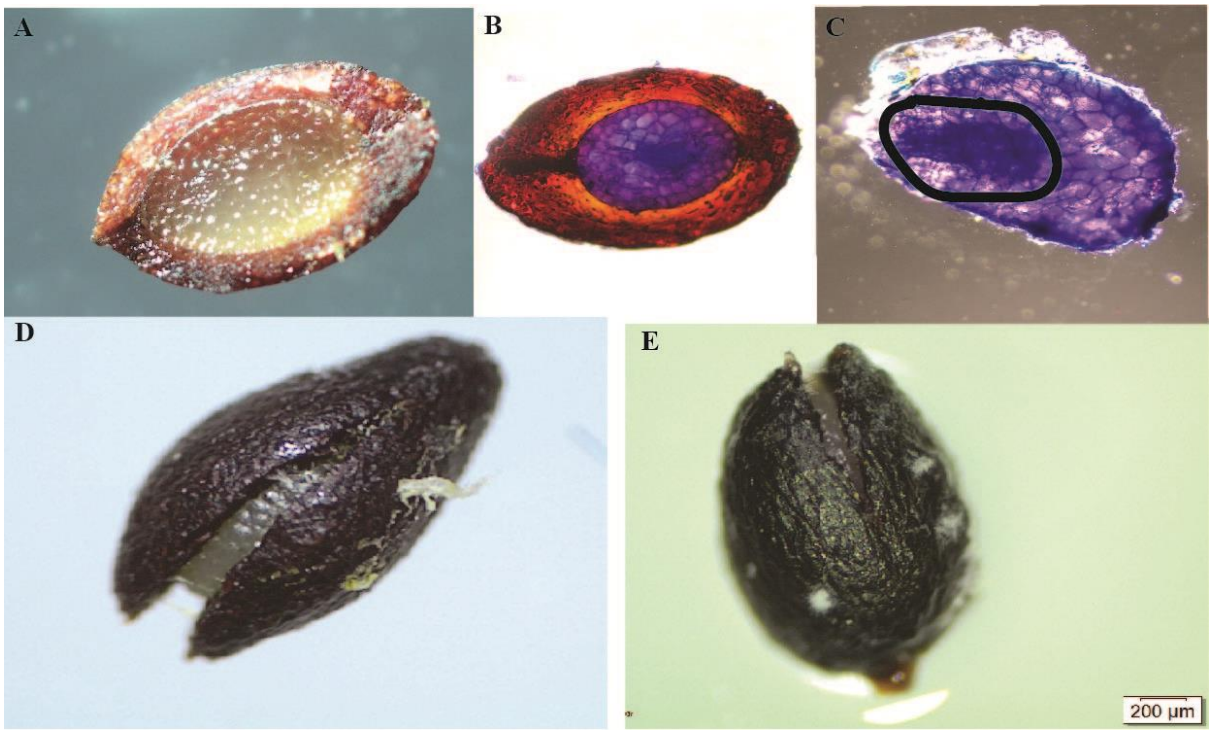


Figure 4: *Dactylanthus taylorii* seed (A) were stained to reveal the embryo (B) encircled (C). The first stage of seed germination, indicated by splitting seed (D & E) was achieved in the Lions Otari Lab in July 2021.

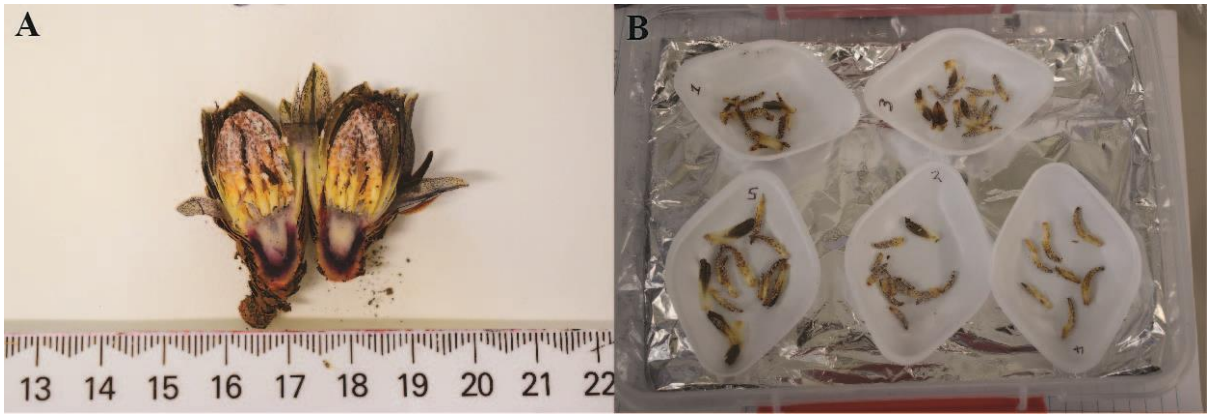


Figure 5: Dactylanthus taylorii flowers were collected (A) to determine pollen conservation protocols (B) which will enable the hand-pollination of isolated populations or individuals.

Agathis australis (Kauri)

Although Kauri is arguably New Zealand's most iconic species, we are not yet sure how to store the seed. A study conducted by Kew Millennium Seed Bank in the 1990's found that some *Agathis* seeds did not respond well to conventional seed banking (low moisture content and storage at -18°C). After discussions with Dr Dani Ballesteros, cryobiologist at Kew, we decided to investigate this problem further using some of our 200 Kauri trees growing at Ōtari. After initial results we can see that seed banking Kauri is not completely straightforward, although the reasons why the seeds lose viability is not yet clear. The Lions Ōtari Plant conservation lab has partnered with Victoria University of Wellington with input from Dr Dani Ballesteros to investigate the seed storage behaviour for Kauri seeds. Seed viability, seedling vigour and biochemical characteristics are compared between seeds stored at 5°C, -18°C and -196°C for various times. Through these experiments we hope to identify when seeds start to deteriorate and what the reasons are (biochemical or structural). Kauri cones, which house the seeds, have very few viable seeds, so for us to obtain sufficient seed for research, we needed to collect and sort through more than 6,000 seeds. The Ōtari volunteers have made this possible in 2021 and 2022 by spending countless hours finding viable seeds (Figure 6).

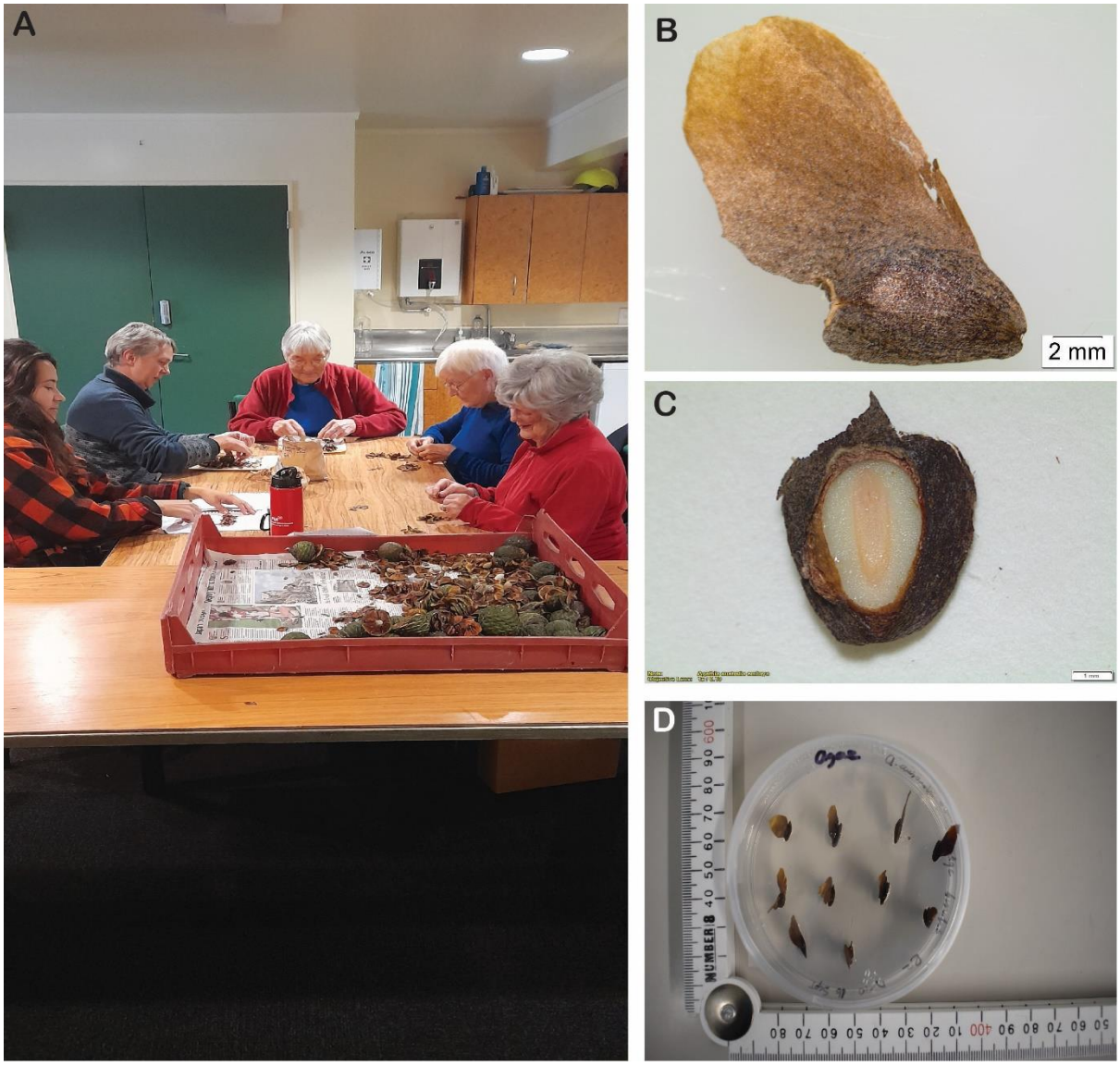


Figure 6: The volunteers at Ōtari sorting processing Kauri seeds (A) to find viable seeds (B) which contains an embryo (C). These seeds are then germinated in the Lions Ōtari lab (D) as part of the overall seed storage project for Kauri seeds.

Students and Staff Update

Karin van der Walt, Conservation and Science Advisor at Ōtari Native Botanic Garden, is responsible for the overall running of the lab and conservation programmes. Her main interest is the long term storage of germplasm (seed, embryos, pollen, cells or shoots) using cryopreservation. Using the lab, which includes all the equipment needed for cryopreservation, Karin completed her PhD (2022) which investigated the ex situ conservation of New Zealand Myrtaceae species. In this report we have three additional contributions, Jennifer tells us about her orchid work, Debra has been using the lab to understand germination in *Ranunculus* and Ryan did his CREST science project on *Lophomyrtus obcordata.*

Jennifer Alderton-Moss (Victoria University of Wellington)

I'm a current* MSc student from Victoria University of Wellington (VUW), and through a summer scholarship was lucky enough to become a (semi-)permanent fixture of the Lions Otari Plant Conservation Laboratory. This is where I developed my love of native orchids, a group containing ~110 species with interesting, and sometimes highly specific, fungal relationships. My work has had me spread between a lab at VUW, the genetics lab at Te Papa, and, my personal favourite, the conservation lab at Otari, where I've been exploring the plant-fungi relationships of orchids. Orchid seeds will typically only germinate in the presence of a suitable fungal partner, or on specially designed nutrient media, however for many orchid species a germination-inducing fungus is yet to be identified. Last year I was given the challenge of working with the Nationally Critical orchid, *Corybas carsei*. My goal was to understand its fungal associations and see if we could use fungi found in its roots to promote germination and boost its dwindling population. At Otari this involved initially extracting and culturing fungal structures from plant roots, and later establishing germination trials with this species, as well as *C. macranthus* (Not Threatened) and *C.* "Remutaka" (Data Deficient). Although this initial study faced problems with poor seed quality and fungal contaminations, it has laid the groundwork for continued conservation efforts.

I am currently preparing for germination trials of three Non-Threatened species: *Earina mucronata*, *Thelymitra longifolia*, and *Gastrodia cunninghamii.* My preparations so far include seed collection, and isolation of potentially symbiotic fungi from a range of orchids. Already, fungi have been grown from the roots of *Caladenia* sp., *Corybas carsei*, *G. cunninghamii*, and *T. longifolia*. These germination trials should begin in mid-March, although orchids can take several months before showing any signs of growth. The information gained from this study (e.g. which fungi help or harm), will guide a second round of germination later in the year. This will include winter flowering *Corybas* species, again including *C. carsei*. The aim of this work is to generate *C. carsei* seedlings to support the sole remaining population, and to refine orchid conservation protocols that can then be applied to other threatened New Zealand taxa.

* We are delighted (and very proud) that Jennifer has submitted her master's thesis in April 2022.

Dr Debra Wotton (Moa's Ark Research)

Germination trials of the critically endangered Castle Hill Buttercup

Castle Hill buttercup (*Ranunculus paucifolius*) is found only on fine limestone scree at Lance McCaskill Nature Reserve, Castle Hill Basin, Canterbury. The sole population of this Nationally Critical species numbers fewer than a hundred plants and is not increasing. Seedlings have not been seen for decades. Plants produce few seeds and only about 10% of them germinate. Germination can also take up to four years.

Castle Hill buttercup seeds are collected for propagation in February when mature, although many seeds disappear before they can be collected. At least some are eaten by birds. Some *Ranunculus* species germinate better when seeds are collected green (seeds are initially green, swell up, then turn brown and dry out when mature). Collecting seeds earlier, while still green, may increase the percentage that germinate and/or reduce seed losses to bird predation.

Over the past year, I have been conducting germination trials in the seed laboratory at Otari-Wilton's Bush to assess the effect of seed collection time on germination for Castle Hill buttercup. I sowed seeds in four treatments: (1) green seeds; (2) chilled (cold-stratified) green seeds; (3) mature seeds; and (4) cold-stratified mature seeds. Green seeds were collected in January 2020 and mature seeds in late February/early March 2020. Germination has been low, but results show that green seeds can germinate. Cold stratification does not appear to be necessary for germination. This work has provided evidence that green seeds are viable, meaning that seeds can be collected earlier, avoiding seed losses to bird predation and other causes, and increasing the number of seeds available for propagation of this critically endangered species.

Ryan Gordon (Onslow College)

In the second half of 2020 I worked with Karin van der Walt on a project based around the conservation of Lophomyrtus obcordata (rohutu). For me this was the research part of my silver CREST project. Silver CREST is run by the royal society and is targeted at Y11-13 students at college. It is independent from school exams and designed to give college students an idea of what a research project looks like – hands

on experience of the scientific method we're taught but never use in school. To achieve silver CREST a student needs to come up with a scientific/technological question they're interested in, conduct experiments around it, and write a report (I'm currently in the middle of writing my report).

Rohutu is currently listed as nationally critical due to the threat of myrtle rust and we were looking at the best environment to seedbank rohutu in. We experimented with different levels of seed desiccation at three different temperatures – 5ºC, -18ºC, and -196ºC. This project was invaluable for me – it has given me experience in the field of science that many students my age would never get, it gave me an idea of what conducting a scientific experiment was really like with proper scientific equipment. Previously the closest I'd got was mixing chemicals together in chemistry and recording an anticipated result.

Publications

Nadarajan J, van der Walt K, Lehnebach CA, Saeiahagh H, Pathirana R. 2020. Integrated ex situ conservation strategies for Endangered New Zealand species. New Zealand Journal of Botany. DOI: 10.1080/0028825X.2020.1754245.

Van der Walt K. 2018. *Ex situ* conservation of Myrtaceae, a response to Myrtle Rust in the Pacific Region. *Samara* **34 (December)**, 13.

Van der Walt K, Nadarajan J, Burritt DJ, Kemp P. 2019. Cryopreservation of zygotic embryos of New Zealand's critically endangered tree, *Syzygium maire* (swamp maire). *Cryobiology* **91,** 161.

Van der Walt K, Kemp P, Sofkova-Bobcheva S, Burritt DJ, Nadarajan J. 2020. Seed development, germination, and storage behaviour of Syzygium maire (Myrtaceae), a threatened endemic New Zealand tree. New Zealand Journal of Botany, DOI: 10.1080/0028825X.2020.1794911.

Van der Walt K, Kemp P, Sofkova-Bobcheva S, Burritt DJ, Nadarajan. 2020. Evaluation of droplet vitrification, vacuum infiltration vitrification and encapsulation-dehydration cryopreservation for *Syzygium maire* zygotic embryos. *CryoLetters*. Article in Press.

Van der Walt, K., Alderton-Moss, J., Lehnebach, C.A. 2022. Cross-pollination and pollen storage to assist conservation of *Metrosideros bartlettii* (Myrtaceae), a critically endangered tree from Aotearoa New Zealand. Pacific Conservation Biology. DOI: https://doi.org/10.1071/PC21054.

Van der Walt, K. Burritt, D.J., Nadarajan, J. 2022. Impacts of rapid desiccation on oxidative status, ultrastructure and physiological functions of *Syzygium maire* (Myrtaceae) zygotic embryos in preparation for cryopreservation. *Plants 11*, 1056. https://doi.org/10.3390/plants11081056.